Animal Signals and Communication 8

Peggy S. M. Hill · Valerio Mazzoni ·
Nataša Stritih-Peljhan ·
Meta Virant-Doberlet ·
Andreas Wessel *Editors*

Biotremology: Physiology, Ecology, and Evolution

Springer

Animal Signals and Communication

Volume 8

This book series provides a wide coverage of animal communication research including signal production and perception, development, behavioural mechanisms, signal function, population effects and the evolution of communication systems. It includes intra- and inter-specific communication, addresses communication in all taxa including humans and covers signals of all sensory modalities - visual, acoustic, chemical, tactile and electrical. Each volume summarises our current understanding of signals and communication in a particular context, this may be in a single taxonomic group or as a topic of current interest across a wide range of taxa. The series is aimed at scientists, university teachers and graduate students.

Peggy S. M. Hill • Valerio Mazzoni •
Nataša Stritih-Peljhan • Meta Virant-Doberlet •
Andreas Wessel
Editors

Biotremology: Physiology, Ecology, and Evolution

Springer

Editors
Peggy S. M. Hill
Department of Biological Science
University of Tulsa
Tulsa, OK, USA

Valerio Mazzoni
Research and Innovation Centre, Fondazione
Edmund Mach
San Michele all'Adige, Trento, Italy

Nataša Stritih-Peljhan
Department of Organisms and Ecosystems
Research
National Institute of Biology (NIB)
Ljubljana, Slovenia

Meta Virant-Doberlet
Department of Organisms and Ecosystems
Research
National Institute of Biology
Ljubljana, Slovenia

Andreas Wessel
Center for Integrative Biodiversity
Discovery
Museum für Naturkunde - Leibniz Institute
for Research on Evolution and
Biodiversity
Berlin, Germany

ISSN 2197-7305 ISSN 2197-7313 (electronic)
Animal Signals and Communication
ISBN 978-3-030-97418-3 ISBN 978-3-030-97419-0 (eBook)
https://doi.org/10.1007/978-3-030-97419-0

© Springer Nature Switzerland AG 2022
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

To Matija Gogala,
A pioneer in studying vibrational
communication, and the founder of the
Slovenian School of Biotremology.

Contents

Part I Studying Vibrational Behavior: Ideas, Concepts and History

Part II The State of the Field: Concepts and Frontiers in Vibrational Behavior

Part III Practical Issues in Studying Vibrational Behavior

Part IV Vibrational Behavior in Less Explored Contexts

Part V Vibrational Behavior in Some Well-Studied Taxa

Part VI Applied Biotremology

Part I

Studying Vibrational Behavior: Ideas, Concepts and History

Chapter 1
Quo Vadis, Biotremology?

Peggy S. M. Hill, Valerio Mazzoni, Nataša Stritih-Peljhan, Meta Virant-Doberlet, and Andreas Wessel

Abstract Since our first collaborative book in 2014, which also included the first suggestion for a name for our new scientific discipline of biotremology, our focus has shifted from studying vibrational communication in a few groups of arthropods, to studying vibrational behavior (so that cues could be included) and now to a focus on physiology, ecology, and evolution. During this time, our scope has dramatically increased, but so have the numbers of publications on biotremology, as have the numbers of biotremologists of all ages, representing all continents on the Earth except for Antarctica. Our range of taxa has also expanded to encompass nematodes to mammals and birds. In this first chapter, our international editorial team, which represents Italy, Slovenia, Germany, and the USA, has taken on the role of writers to introduce each of the other 22 chapters in a preview of what the reader will find within the book. We also have collaborated to address the question in our chapter's title, "Quo vadis, biotremology?" Where are we going with this scientific discipline, and where would we aspire to go, if we had unlimited funding and unlimited years to our lives?

P. S. M. Hill (✉)
Department of Biological Science, The University of Tulsa, Tulsa, OK, USA
e-mail: peggy-hill@utulsa.edu

V. Mazzoni
Department of Sustainable Ecosystems and Bioresources, Research and Innovation Center, Fondazione Edmund Mach, San Michele all'Adige, Italy

N. Stritih-Peljhan · M. Virant-Doberlet
Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia

A. Wessel
Museum of Natural History Berlin, Leibniz Institute for Evolution and Biodiversity Science at Humboldt University Berlin, Berlin, Germany

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_1

1.1 Introduction

"Quo vadis, biotremology?" Authors in our growing research alliance in biotremology continue to ask this question of where we think we are going as a discipline in the short-term, as well as what we hope to explore as a community as we face a more distant future full of promise. As in our two previous volumes, we provide a figure that reveals the growth in the use of the term *biotremology* in publications, based on a Google Scholar search (Fig. 1.1). From being found in one publication in 2014 (not recognized by Google Scholar), growth in the use of the term biotremology increased rather linearly until early in 2018, when there was a clear inflection point with a change in slope of the line. In spring 2018, the slope of the line was similar, but the number of results for the use of the term increased measurably, until in November 2019 there was a leap in numbers due to the

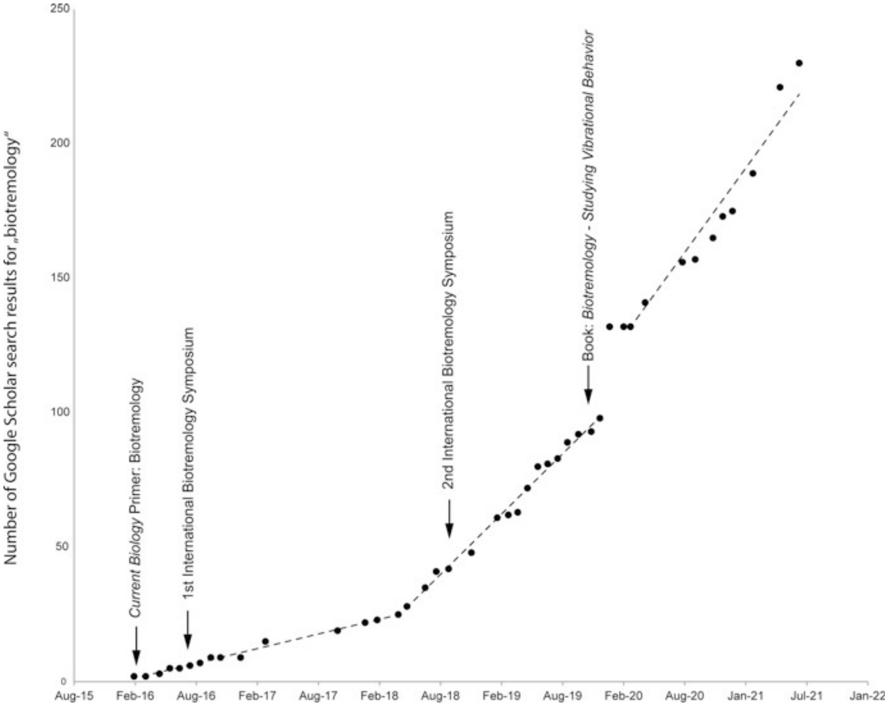


Fig. 1.1 Results of Google Scholar searches for the term *biotremology*, over the period from December 2015 to July 2021. The search results reflect the actual numbers for a full search on a certain day, and did not use the "custom range"-function for a retroactive limitation of search time periods. This method allows for a finer temporal resolution, but includes a "time lag" as the publications do not appear immediately in the database. The regression lines comprise the periods from December 2015 to March 2018, April 2018 to November 2019, and December 2019 to July 2021, respectively. For each of these three time periods, the increase in search results is highly significantly linear

publication of the second volume. In this third free-standing book of chapters on biotremology for the Springer series, *Animal Signals and Communication* (see Cocroft et al. 2014a; Hill et al. 2019), this need to define and make adjustments as we go is evident in our shift away from the 2014 focus on research in the field of animal communication, but almost exclusively arthropod communication. By 2019, we were expanding our collective vision at a great rate after having found a name (Endler 2014) and established biotremology as a broad discipline with boundaries that extend beyond the study of communication in animals to encompass the study of vibrational behavior of all types (Hill and Wessel 2016).

The change in focus was rapid once we faced the realization of at least two facts, which could not be ignored. First, the receiving mechanisms that detect animal signals also detect incidental cues. These bits of information are transduced and transmitted through the nervous system in the same way and, as far as we currently know based on limited publications, may be perceived in the same region of the nervous system. Further, mechanical waveforms that carry information in both signals and cues cannot be fully understood without knowing more about all mechanical waveforms and the physical world where these waveforms also propagate. Substrates filter and change mechanical waves in ways that must be reversed in order to produce functional playback signals, for example. We also know that energy carried in mechanical waveforms is transferred at a boundary between media (such as the soil and air, or water and air) and may propagate through the second medium in a different waveform, as is likely at junctions of spider web threads. We know that most all communication signals and cues that pass between living things are carried as boundary waves (Rayleigh waves in the Earth and bending waves in plants). However, there are many other types of mechanical waves on this planet that are not known at this time to be involved in sound communication or used for vibrational signals and cues. Are they involved in vibrational behavior in ways yet to be imagined and documented? This caused us to broaden our interest in mechanical waves, generally. We then had to consider that other lifeforms, and their interactions that fell outside the animal communication paradigm, as well as the physical environment, could be studied using many of the concepts and tools already in use for studying communication. Yet, as we look to the future to expand our understanding of the scope and depth of biotremology, we are also increasingly aware of how ancient, broadly represented and diverse the interactions studied within biotremology actually are. Therefore, we continue our commitment to ground our current and future investigations in the findings of the pioneers of our past, and even to a world (and beyond) that we now know existed more than 230 million years ago when there were no human scholars, and there was no science, but biotremological interactions were already taking place (Hoch et al. 2006). Thus, we find ourselves as a discipline and a community that embrace work focused on the distant past and the distant future, while we also examine questions at any of the levels of biological organization, from cells to the biosphere. Further, we do this at the pure, practical, and applied levels. The core terminology required to anchor this diversity has to be both durable and flexible, and so we also attempt to adapt new language elements and stabilize others as the discipline evolves, while maintaining cohesion of our shared principles.

As in the two previous Springer books from our biotremology community, we use the structure of the part/section topics to present a portfolio, of sorts, that reflect the scope of research areas that belong under the umbrella of the discipline of biotremology. Most of the topics remain the same through the three volumes, even though the chapters themselves have very different contents; however, after 2014, we added a section on Applied Biotremology. In this third book we changed the section in the previous two books that dealt with detection and orientation to a new section on Vibrational Behavior in Less Explored Contexts. This first chapter serves as an introduction by the Editors to the book, as a whole, as well as to Part I: Studying Vibrational Behavior: Ideas, concepts, and history. We continue our short tradition of including a translation of an important and classic historical text that is only poorly known to the broader biotremology community (see Chap. 2). Here the work of Karl H. C. Jordan on sound production in two families of true bugs is showcased as an early study of both airborne and substrate-borne signal components. In translating from the original German the text from two papers that most directly impacted research in biotremology, Hoch and Wessel also give our community an opportunity to honor, for the first time, the work of Helga Slowioczek. The findings published in these translated passages of Jordan's two papers were taken from Slowioczek's unpublished diploma thesis on work supervised by Jordan. As was conventional at the time, Slowioczek was not included as co-author on either paper, thus this chapter honors her as a pioneer in biotremology for arguably the first time.

1.2 The State of the Field: Concepts and Frontiers in Vibrational Behavior

This part topic has been included in all three of the Springer biotremology books written by our research community. With this section we hope to present something of the research being conducted at the extremes of our discipline, or work that has moved beyond what is known about a group of living things in a way that explores newly identified interactions in vibrational behavior.

The endemic weta of New Zealand (Orthoptera: Anostostomatidae) are not well-known to most biologists, unless the scientists have experience with the New Zealand fauna or perhaps are entomologists with a particular interest in the Orthoptera. Yet, this unique group offers us a gold mine of information that can be used in comparative studies that would, among other things, help to resolve phylogenetic relationships in the Orthoptera. However, their importance to biotremology exceeds even this valuable contribution. For example, the giant weta of the Deinacridinae were the largest animals ever confirmed to produce substrate-borne vibrational signals through tremulation. Strauß and Howard (see Chap. 3) provide us with a primer, introducing the weta and the diversity of their vibrational behavior.

Within this group, mechanisms for production of substrate-borne vibrations via drumming, stridulation and tremulation are all found, and in at least one group multiple examples of organs used in stridulation are also known.

In behavioral ecology studies, so many investigations are grounded in cost-benefit analyses of a functional behavior, especially in exploring sexual selection of a trait/behavior. Energetic costs are often estimated in the absence of data without any physiological testing, or assumed to be greater or lesser in comparative studies. Only a handful of academic publications actually address the issue of energetic costs in biotremology based on the classic respiratory studies that have been conducted from a wide representation of animal life. Kuhelj and Virant-Doberlet (see Chap. 4) report on their work with a model organism, the leafhopper *Aphrodes makarovi*, one of only four species of arthropods for which energetic costs have been studied. Their very careful case study can be used as a standard for future studies in other organisms that use vibrational signals exclusively in mating behavior, or as part of a multimodal system that also includes acoustic components.

The Hawai'ian Islands serve as a laboratory for radiation and evolutionary dynamics of so many species of living things, and this is also true of the planthoppers of Hemipteran Fulgoromorpha. Indeed, before pioneering investigations in the early 1990s, no information was available on behavior of any Hawai'ian planthoppers. Ashe and colleagues (see Chap. 5) summarize the current state of knowledge of vibrational signals of a large number of species of Cixiidae and Delphacidae that were recorded in Hawai'i between 1989 and 1998. Signals are characterized and other details of morphology and natural history are documented. Photographs and graphical representations of the signals are included, and recordings are available from the VibroLibrary at the Museum fur Naturkinde in Berlin.

1.3 Practical Issues in Studying Vibrational Behavior

This section has been a mainstay of all three of our research community's Springer volumes on biotremology. In it we attempt to showcase some technical or theoretical issues specific to working within the science of biotremology. In this section we address three topics that have not been covered in previous volumes and, additionally, are not widely covered in other biotremology literature: noise in the vibrational channel, development of equipment to solve a problem in playback stimulus delivery, and a comparison of current and historical research methods for sending and receiving substrate-borne vibrational signals or cues.

Human activity represents a considerable source of mechanosensory disturbance in the environment, which acts through both airborne and substrate-borne channels. While its acoustic influence on animal communities has received considerable research attention, the interference of anthropogenic noise on animal behavior via the vibrational channel remained largely unstudied. In the review of Roberts and Howard (see Chap. 6) the state-of-the art in the study of vibrational noise is explored by describing noise sources, its impacts on organisms and their strategies for communication in noise, the ways to measure and mitigate anthropogenic noise in the substrates, and suggestions for future studies for this largely understudied area. Twenty-two existing studies, altogether, that deal with vibrational noise in aquatic or terrestrial habitats suggest that much is left to be done to start understanding the actual scope of influences of anthropogenic vibrational noise on the species, population, and ecosystem levels before we will be able to take effective measures in its mitigation and control. In particular need of research are aquatic environments, where the impact of anthropogenic vibration is much less known than in terrestrial ones, starting with an almost unknown sensitivity of aquatic animals to substrate vibrations.

While vibrational signaling through the substrate has been traditionally considered merely a (side) component of acoustic signaling, the development of biotremology facilitated our understanding of the distinction between the acoustic and vibrational communication channels at basically any system level, from signal emission and transmission to detection and neuronal processing. Similarly distinct may be the methodological aspects in studies of these systems, including both signal presentation and recording. Conducting a behavioral playback experiment in a vibrational system is typically much more complex and technically challenging than an experiment including presentation of acoustic stimuli. A review of the more than 20 years of work of Warkentin and coworkers (see Chap. 7) on playback experiments provides an example of highly challenging development of a stimulus delivery system that requires vibrational playback to egg clutches (i.e., embryos), combined with tactile stimuli, in such a way as to mimic conditions occurring in nature during predator attacks. The authors present a step-by-step development of a complex device that enables a high level of control for the simultaneous and independent playback of both modalities, along with a detailed control over their application to individual eggs in a clutch, while maintaining minimal variation across experimental replicates. In addition to stressing differences between studies of vibrational behavior between embryos and adult animals, studies of Warkentin and her group demonstrate how extensive the distinction between approaches and methods in bioacoustics and biotremology studies can be.

Despite considerable progress in biotremology and the increasing recognition of the field among researchers studying animal behavior, an important goal of our community continues to be raising awareness of the vibrational channel as a highly important mode of information exchange among animals. As discussed previously (Cocroft et al. 2014a, b), studying vibrational communication has a large potential for answering behavioral research questions that could not be explained by studying other modalities. Due to the price, however, the specialized research equipment standard for vibration recording and playback is limited mostly to laboratories focused on vibrational communication research, thus precluding the possibility of considering this mode more widely. Nieri and colleagues present the results of tests on the performance of various older, inexpensive alternatives to the specialized equipment used in vibrational recording and playback, in particular in the research of animal interactions through plants (see Chap. 8). Especially for the playback, they demonstrate almost no difference in the performance of the cheap vs. expensive actuators that vary 1000-fold in price, while the use of alternative low-cost sensors is more limited and is suggested to be used in cases where quantitative signal analysis is not required.

1.4 Vibrational Behavior in Less Explored Contexts

The topic of Part IV in this book is new to our portfolio of areas covered in our ever-expanding discipline of biotremology. In this section our chapter authors cover topics not addressed in our two previous Springer books from the biotremology community, whether the chapters be taxon-specific, niche-specific or an introduction to novel research methods.

The lack of an established evolutionary framework is often cited as a weakness of studies in biotremology. We consider that the hundreds of millions of years spent in evolution of vibrational behavior have provided unique challenges to modern researchers in obtaining empirical support from controlled experiments conducted with remnant populations that may, or may not, be representative of the ancestral condition. Conrad presents a review of her sexual selection studies of population divergence and speciation in the red mason bee (see Chap. 9). Of particular interest is the novel solution for modifying the vibrations produced by courting males of the divergent populations in a carefully controlled experimental design.

Ota and Soma review their work on the tap-dancing behavior in the blue-capped cordon bleu finch (see Chap. 10), which is a socially monogamous song bird with multimodal courtship displays. Both males and females of these birds exhibit singing and visual courtship displays. Yet, they also produce a bobbing behavior on their perches that has been described as a sort of tap dance, once high-speed video was viewed at a lower speed so the motion of the feet could be observed. This body of work represents the first and only case describing the presence of substrate-borne vibration in bird multimodal courtship displays. In fact, the previous lack of reports of any sort of vibrational behavior in birds was a major barrier to acceptance of the study of vibrational behavior as a significant component of bioacoustics studies. Thus, this chapter and its findings, after a comprehensive analysis of the substrate-borne component of the multimodal display, represents a portal into a totally new research line in birds, where the realm of biotremology has essentially been ignored/overlooked in the recent past.

Some related, but still distinct, research areas that currently are not incorporated into biotremology are very well developed and have their own research communities and international conferences. Some may continue to exist into the future as distinct entities without external ties to any one related school of thought, while others will find commonalities with biotremology that make joining with this new discipline very attractive. One such research area is that of buzz pollination, which is presented (see Chap. 11) as a potential good fit within biotremology. Buzz pollination represents a type of pollinator-plant mutualism where plants only release their pollen when exposed to vibrations delivered by a bee probing for nectar. The pollen lodges on the hairs covering the bee and can then be deposited on a neighboring plant of the same species as the bee continues to probe for nectar resources. Our current understanding of plant biotremology is still in its infancy, but pioneering studies are being completed that show the plant as more than just a substrate for animal interactions. Inclusion of the study of buzz pollination as a component of biotremology, much as the study of vibration-induced rapid hatching has been (see Chap. 7), will make currently unknown questions available for exploration, or perhaps older, resolved questions can be revisited with fresh eyes.

Nematodes are so abundant on our planet that we have difficulty even estimating how many there are. Most of the interactions known between and among nematodes and other lifeforms have been assumed to be due to chemical stimuli. In 2004, Torr and colleagues challenged and refuted this conventional wisdom with a well-controlled experiment that found parasitic nematodes responded to host-generated vibrations in the soil that allowed them to locate and infect the host (Torr et al. 2004). Yet, most research today still holds to the convention that interactions are chemical. Sugi (see Chap. 12) provides our biotremology community with its first ever chapter on nematodes as he reviews mechanosensory behavior in *Caenorhabditis elegans*, the model organism that is arguably the best-studied nematode of all. Of particular interest is the ability of this nematode, though it has not been described in others, to acquire memories of mechanical stimuli and then modify their mechanosensory behavior based on these experiences, even though any fitness benefits are still untested in the wild.

Lastly in this section on less explored contexts, Stritih-Peljhan and colleagues use an interesting case study approach to compare and contrast the lives of cave-dwelling insects from two distinct orders: the Cixiidae (planthoppers) of the Hemiptera and the Rhaphidophoridae (cave crickets, cave weta, or camel crickets) of the Orthoptera (see Chap. 13). Authors Stritih-Peljhan and Strauß are Orthoptera experts and focus on caves in Slovenia, while Wessel and Hoch are Hemiptera experts and focus on caves in Hawai’i. Cave environments are highly interesting to study due to their strong influence on organisms, including sensory systems and behavior. Yet, they are among the least studied in all fields, including biotremology. Thus, this chapter provides new and provocative insight into the adaptation to life in caves, and particularly the dependence on substrate as it explores the issue of the evolution of vibrational behavior of cave-dwelling species and solutions found from these two insect orders.

1.5 Vibrational Behavior in Some Well-Studied Taxa

This part topic has also been an important part of all the biotremology books published by our research community with Springer. The description of well-studied taxa may surprise some readers new to the biotremology literature. We refer to taxa in which substrate-borne vibrational behavior has been studied over decades, or perhaps a smaller group has been extensively studied with respect to diverse concepts in biotremology. In this volume we also include taxa that are well-studied, in general, but in which vibrational behavior has gained little attention by way of empirically supported vibrational behavior studies. In each case, however, these studies may or may not be well-known outside the biotremology community. Thus, our chapters are reviews and updates of the literature on a variety of taxa of special importance to our science. These include two vertebrate groups (snakes and the Israeli molerat), three groups of social insects (honey bees, ants and social wasps) and caterpillars (larval Lepidoptera).

Han and Young (see Chap. 14) provide us with an extensive review of the literature on detection of and sensitivity to mechanical waveforms in snakes as they present the current state of our knowledge of snake biotremology. Even though snakes are rather well-known throughout the environments where they are found on the planet, our knowledge of ophidian biotremology is shallow to nonexistent across taxa. Strong evidence exists that snakes are able to detect and respond to substrate-borne vibrations, and in some taxa, i.e., rattlesnakes, we can record and measure vibrations induced by the snake's behavior, as well as the incoming vibrations detected by the snake, and characterize them. Yet, something as basic as the pathway from detection of the signal until it projects onto one or more regions of the brain is still very much a black box. Even more challenging for the non-specialist reader is the blending in the literature of descriptions of hearing and vibration sensitivity, much of which is anecdotal and based on natural history observations. In many cases it is not at all clear that the evidence of *hearing* is referring to the pathway we typically reference when we speak of airborne sound waves setting up tympanic membrane vibrations and eventually leading to projection onto some auditory-specific area of the brain. Snakes do not have external ears and so are considered deaf with respect to classic hearing patterns known from other vertebrate classes. Yet the literature includes information on pathways from detection to perception that are bone-conducted, lung-conducted and somatic receptor pathway-conducted. In newer biotremology literature we have used "extra-tympanic" to refer to pathways carrying bone-conducted information from the substrate to a series of bones that eventually lead to the inner ear, i.e., the quadrate bone to columella path. Yet, is this information perceived in the brain as sound or vibration, or does it even matter? Is there some unnamed aggregate class that includes both? There is clearly much yet to be learned. This review chapter is a major contribution to sorting out what is known through scientific study about how snakes are able to use mechanical waves (including pressure/sound waves) to interpret and respond to their environment. The authors have included reviews of the physiological and anatomical bases that support what we would call vibrational behavior in snakes, as well as suggestions on the likely most fertile research investigations that remain to tempt us to know more.

Nevo reviews his 70 years of research, conducted across disciplines, on the Israeli molerat genus, *Spalax* (see Chap. 15). Few stories, especially in vertebrate animals, are as complete as that of *Spalax*, and here we are able to read this story in the words of the single person who spent his entire career in pursuit of understanding the totality of the *Spalax* system. Nevo's pioneering work is broad-ranging and innovative, and the references list for this chapter provides a rich treasure trove of investigations that will be of interest to most biotremologists, regardless of their specialty. In addition, Nevo integrates and showcases the work of his many students from these decades of field and laboratory work. Furthermore, he encourages us to engage in comparative studies of sound and vibration parameters across Israeli species, while expanding to comparative studies of the species in the Near East and North Africa, as well as to use new tools from genomics and transcriptomics to analyze the evolution of communication in these fascinating animals.

Honey bees are considered to be a model animal study system, and communication in the western honey bee has been investigated intensively for over 100 years. Yet, the well-known dance language still is not fully understood at this time within the honey bee communication system. We know that honey bees use multiple sensory modalities in communication: chemical, visual, tactile, acoustic and vibrational. The chemical and vibrational modalities are considered to be the oldest forms of communication, and even though chemical communication has received much more attention, the acoustic component of mechanical vibrations in honey bees was known and described more than 400 years ago. Kirchner, Hager and Krausa make a first and much-needed review of the terminology used in studying honey bee vibrational behavior (see Chap. 16), even if these terms were first used to describe airborne sounds. Further, they examine the current state-of-the-art findings with respect to each of these terms to simplify, standardize, and align the honey bee communication terminology with the biotremology framework for vibrational communication mechanisms. After this review and detailed analysis, Kirchner and colleagues were able to assign the vibrational behavior of honey bees to the categories of either tremulatory or drumming behavior, both of which can be produced without specialized morphological adaptations. All factual information on vibrational behavior in bumblebees and stingless bees, as well as the Asian species of *Apis*, support the strong conclusion that vibrational behavior is ancestral in bees. However, so little has been investigated systematically, even in western honey bees, that only the stop signal and queen tooting and quacking can be classified as true vibrational communication signals at this time.

We are told that most all ants communicate essentially through chemicals found in pheromones, but this is not the whole story. Chemical communication is ubiquitous in ants, without argument, but current evidence suggests that vibrational behavior in the form of stridulation…where individuals rubbing two body parts against each other produce both acoustic and vibrational mechanical waves…can serve to modulate chemical signals and to release other context-specific behaviors. Arguments are made in support of the role of pheromones in ants that vibrational behavior is not at all important in social insects, generally, but especially so in ants. Yet, a long history of empirically supported data reveals that vibrational signals and cues are produced through the mechanisms of drumming, stridulation, and scratching. The literature on stridulation in ants, alone, is almost 150 years old. In Chap. 17 Roces provides a focused review on stridulation in the leaf-cutting ants in the genus *Atta*. He reviews the contributions of Hubert Markl in the 1960s and 1970s, as well as his own pioneering literature, some of it done with Bert Hölldobler, in the 1990s. This is followed by an update of new work from his lab on stridulation by leaf-cutting ants that reveals two unexpected and novel findings. While foraging under natural field conditions, grass-cutting *Atta* use substrate-borne vibrations produced by stridulations in the absence of pheromones for short-range recruitment of helpers. Secondly, while organizing tasks inside the nest, leaf-cutting ants in the genus *Atta* coordinate workers to engage in both digging and waste disposal by attracting them to excavation or unloading sites while stridulating. These contexts were previously unknown and thus represent new knowledge; however, Roces adds that the idea was suggested 120 years ago that stridulation "must be of great service" (see Chap. 17) in maintaining the colony and its excavations. Old ideas, when acted upon, actually can result in new knowledge.

In Chap. 18 Nieri and colleagues review the literature of the three subfamilies of the Vespidae that are considered eusocial wasps (the Stenogastrinae, Polistinae, and Vespinae) to explore the extent of their use of vibrational behavior in a variety of contexts. These wasps have been important in studying the evolution of sociality since the 1940s, in part because of their broad range of social organization. Much as we know a great deal in general about behavior and communication in honey bees and ants, we know a great deal about communication in social wasps. As with ants, chemical communication through the use of pheromones and cuticular hydrocarbons has been studied almost to the exclusion of any other communication modality in social wasps. These wasps exhibit a range of described behaviors that are similar to those that produce substrate-borne vibrations in other animal taxa (tremulations, drumming, and a scraping, which has characteristics similar to stridulation, if one were to consider the substrate to be part of the file and scraper mechanism we see in other arthropods). These described behaviors have also been given many names across wasp species, but few hypotheses have been generated or tested. The wasps even live together as more than one generation in a nest composed of material the adults construct, themselves, and the nest is very well suited structurally to propagate mechanical vibrations throughout. The observed behaviors have led some to conclude that social wasps are a good candidate for the investigation of vibrational behaviors, yet, of the 50 species reported in the literature to perform vibrational behavior, actual vibrations produced by the various behaviors they display have been measured in only four species. Admittedly, social wasps may be a bit more intimidating, even threatening, than a honey bee or an ant. It is yet to be established whether the sting of one wasp is more dangerous to humans than the stings of 10 or more bees, or a hundred ants, but few researchers have chosen to measure the vibrations propagating through the nest material of social wasps. This leaves us with actual functions of these familiar behaviors really not identified for most species of social wasps. Fortunately for us, Nieri and colleagues have sorted out the literature and proposed hypotheses for functions of these myriad observed vibrational behaviors that appear to induce vibrations in a variety of contexts in the lives of social wasps. Our challenge is to use their information to do some data mining.

Biotremology of embryos and/or larvae is a rare focus of current research, whether these young are from vertebrate or invertebrate taxa (but see Chap. 7). Knowledge of this life stage is of primary importance to understanding vibrational behavior in juveniles and adults, but the worlds of individuals in these earliest developmental stages can be vastly different, even if not necessarily less complex, than their succeeding stages. Yack and Yadav (see Chap. 19) review our knowledge of vibratory sensing and communication in caterpillars (larval Lepidoptera), which lags far behind that for adults. Indeed, vibration receptors have not even been identified in any holometabolous insect larvae. So, why would we think that vibrational behavior, or at minimum vibrational sensing, is important in caterpillars, much less required for their survival? These larvae, like most insect eggs, nymphs, and pupae, are obligately substrate-bound. Further, like the social wasps, they exhibit behaviors associated with the ability to detect and discriminate vibration sources, whether these sources are predators, rainfall, other members of their population, or ants with which they maintain a mutualistic relationship. They live in a substrate-bound world and appear to actively respond to and discriminate among diverse bits of information known to propagate through their substrates. Yack and Yadav not only give us the gift of a solid literature review, but they use their own extensive knowledge of larval morphology and physiology to lead us from a primer on vibratory receptors in adults to a discussion of putative receptors in larvae, and where on the body these might be hiding in plain sight, if we find time to look for them.

1.6 Applied Biotremology

Applied biotremology is opening new opportunities in terms of research and innovation for practical applications to agriculture and ecology. Thanks to the enormous potential of multidisciplinary research, many industries, from big multinationals to small startups, are now well connected with the academic world and are supporting the efforts of scientists to find new solutions for manipulation of insect pest behavior through the use of substrate-borne vibrations. At the same time, applied biotremology is attracting experts from many sectors, such as mechanical and electrical engineers, informatics specialists and designers. All this knowledge is converging to create new solutions that result in new devices for field and greenhouse applications, innovative energy supply optimization, and/or algorithms that provide the devices with real-time responses to external conditions.

A wide range of insect pests are being studied in this field, and information on new research on some of them is treated in Part VI of this volume. Behavioral control approaches to control longicorn beetles and stinkbugs are here presented. Compared to other taxa, which are considered as models for biotremology studies, beetles, and Coleopterans in general, have been poorly studied; although, we know that there are species that can detect vibrations via leg chordotonal organs. Takanashi and Nishino (see Chap. 20) review the ability of beetles to sense vibrations, providing a description of the morphology of their femoral chordotonal organs and central projections. Then they discuss how vibrations could be used as a tool of pest control, also presenting a new procedure for vibrational pest management, based on vibration sensitivities in longicorn beetles. Unlike beetles, Pentatomidae have been studied for some decades and now represent model species for research in biotremology. Stinkbugs are important pests for many crops worldwide and many scientists deal with them to find solutions for sustainable control. The massive use of insecticides is no longer an acceptable solution for many governments, which now address funding campaigns toward more environmentally sustainable methods. Laumann et al. (see Chap. 23) explore the available options of behavioral manipulation that derive from the rapidly accumulating knowledge of the mating communication of stinkbugs. Two potential strategies are considered, the first being the contemporary use of vibratory and chemical signals (i.e., pheromones) to attract and trap target pest species, and the other is the transmission through plants of artificial or natural signals to interfere in reproductive behavior (i.e., mating disruption) of the target pest species.

A third chapter is, then, dedicated to vibrational communication of a worldwide economically important taxon, the Psyllids. Avosani et al. (see Chap. 22) review the current state of knowledge of this group of insects and discuss strategies to create behavioral manipulations to deal with them. Psyllids are considered major threats to world agriculture in that they are vectors of economically relevant plant diseases. Psyllids, as well as stinkbugs, are hitchhikers. This means that they are easily transported through human trade and touristic routes, thus are able to colonize new geographic areas as invasive alien species. In this chapter, an overview of what is known about their mating behavior, as well as the potential options to interfere with their behavior by means of vibrational signals, is given. Psyllid intraspecific communication is mediated by vibrational signals in the form of stridulations; recent studies indicate that biotremology approaches could be used for trapping and/or disrupting their communication, thus providing tools for monitoring and control. Examples are given for several important psyllid pests, including the Asian citrus psyllid, *Diaphorina citri*, and the North American potato psyllid, *Bactericera cockerelli*, for which specific devices have been developed that are described in the chapter.

Finally, the use of principles of biotremology to study subterranean arthropods, with ecological and economic implications, is reviewed by Mankin (see Chap. 21). Despite the many technical restraints that make the study of the community of subterranean arthropod herbivores a hard task to accomplish, advancing technology is getting us closer to finding some solutions. It is clear that these insects can have tremendous impacts on above-ground biota, including crops to which they cause economic damage; therefore, the use of vibro-detecting systems could provide the farmers with new tools for pest control. Researchers continue to look deeper into this topic of using knowledge from applied biotremology to find solutions for more efficient and ecologically safer pest control. In this chapter, Mankin reports on some of the latest developments of new systems of monitoring and control of subterranean Cicadoidean, Ensiferan, Scarabaeoid, and Curculionid species. The discussion also includes other taxa, such as Lepidoptera, social insects, and spiders, with respect to multimodal communication.

1.7 What Is Left to Be Learned?

In the earliest days of biotremology, before the discipline even had a name, many people worked in isolation to study a communication modality that allowed them to access questions about the taxon of most interest to them. Without moving into the unknown world of substrate-borne vibration in search of answers for their species or genera, the observations that led to their questions would simply continue to exist as anomalies. . .behaviors outside what is known and expected in the explanatory theory of the current paradigm. For some groups of arthropods, a great deal of information has been gathered across our traditional and most common biological disciplines, such as physiology, anatomy, behavior, ecology, and even genetics and neuroscience. For others, almost nothing is known about vibrational behavior and the communication modality that defines the behavior. For example, there are no studies about mites, which are important both as parasites and predators, and in the case of insects there is still a lot to learn about aphids, thrips, and many other groups of general interest that we expect to make extensive use of vibrational signals and cues.

Other than in some mammals and anuran amphibians, we know very little about the breadth and depth of vibrational behavior in vertebrates, which has unfortunately led some experts in animal communication to suggest that biotremology has little to offer to increase understanding of anomalous behavior in vertebrates. If the modality is so ancient and wide-spread as biotremologists claim, it surely would have been recognized in vertebrates before now, would it not? Yet, when vibrational behavior has been explored in mammals, amphibians, and reptiles, mysteries have been solved, and whole new worlds of questions are opened to the curious. Recent work reported on biotremology in birds (see Chap. 10) illustrates this trend very well, but there still remains much to be learned. It is to be expected that vibrational behavior plays a major role in many multimodal displays where it has been previously ignored.

In the recent past with its focused growth of the biotremology community, we have identified a number of areas where initial research efforts have yielded quick success that, in turn, has stimulated interest and growth, even for applicative purposes. In other areas, even when identified and promoted, progress has been slow. However, biotremology is a brilliant example of how basic research can turn, in a relatively short time, into applied research. The next step is to provide this discipline with the proper nomenclature and terminology in order to place biotremology beside chemical ecology, as a sister discipline to reinforce sustainable pest control. In fact, biologically speaking, vibrational signals for mating communication are not that different from pheromones and for this reason we predict a fruitful future of their use in application. Indeed, in the field of applied biotremology there is really much to do and we are seeing only the tip of the iceberg at this stage. The impressively fast progress in technology associated with cost reductions has led to feasible practical solutions that only a few years ago were considered impossible.

As our community was coalescing from isolated groups, we urged others to extend beyond their achievements to put students to work on unexplored peripheral areas to build on the framework already established by the pioneers. For example, we asked if a particular discovery of vibrational behavior in a species could be explored in the most closely related taxa, or within the original species but in new and different contexts. If the research focus had been on behavior and communication, could others be recruited to work on physiology, neuroscience, community interactions, genetics or even comparative studies? In search of an evolutionary framework for biotremology, especially when considering that such an ancient and widely employed communication modality might be basal for all animals, what tools of modern biological research might be employed by scientists who would never have considered research in animal communication? In a genus such as *Spalax*, which is well-studied in Israel but known across the Near East and North Africa, Nevo (see Chap. 15) has suggested using the tools of genomics and transcriptomics to synthesize a fuller picture of evolution of communication among this range of molerat species. One such paper (Su et al. 2021) became available online in May 2021 that reports on a putative abdominal vibration-related gene from transcriptome analyses in the brown planthopper (*Nilaparvata lugens*). Yet, even though dramatic advances have been made across the research areas under study in biotremology, so much is still to be learned.

In this volume, we have two strong chapters on the biotremology of specific embryos and larvae (see Chaps. 7, 19). Yet, most of our research laboratories working in biotremology have focused on adult animals (but see Endo et al. 2019). Progression of the field now allows those whose primary home disciplines are not biotremology to identify a wealth of research questions that can be answered based on their own expertise, and the research techniques used in biotremology are being demystified, as well as becoming more widely accessible.

One extremely fertile area for research is our understanding of *perception*, or how animals become aware of external vibrational stimuli, or interpret them, within the brain. Almost nothing is known of perception and very little has been done across taxa on distinguishing neural pathways once the receiver organs have been located and described. To improve our understanding of the perception of vibratory signals and cues, i.e., to find out which parameters are important for their detection, recognition, categorization, etc., we need to increase our study efforts also in behavioral physiology to explore these parameters, along with deciphering the underlying sensory neural pathways and processing mechanisms. These pathways and mechanisms have only been studied for a limited number of species, predominantly insects, but even here progress has been much slower than for other sensory modalities. Even further, the actual receiver organs for vibrational stimuli have not been identified in many taxa. One aspect that has remained completely untouched, but would be particularly interesting to explore in insects, is the neural mechanisms of noise reduction and selective attention, which may be considered especially relevant in the plant signaling environment. To fill the gap in our understanding of vibration perception, we not only need to increase interest in neurobiology among biotremologists. Since these topics are not as readily accessible for studies as is behavior, for example, it would be of great benefit to our field of study to (re)increase interest in this sensory modality among bioacousticians, as well. In Orthoptera, the established invertebrate models of hearing, the auditory and vibratory senses have often been studied together in the past. Given the close interrelation of the two modalities, it would be beneficial for both disciplines if the perception of sound and vibration signals in animals were again studied together in the future.

In arthropods, providing information about the direction of the stimulus can be regarded as one of the main tasks of the vibratory system, which most likely underlay escape and prey finding behavior before the use of signals in a social context. The ability to localize the source of vibration had been demonstrated or described for a number of taxa, even when the mechanisms remained unknown, at a time when conventional wisdom held that this was simply not possible. Examples include the pioneering work of Brownell and Farley (1979a, b) with scorpions on sand substrates, Bleckmann and Barth (1984) with a fishing spider on the water surface, Hergenröder and Barth (1983) with a wandering spider, and Salmon and Horch (1972) with ghost crabs. Early investigations with vertebrates were also underway (Hetherington 1985: the bullfrog, Hetherington 1989: the sandswimming lizard). Yet, few species even today, including well-studied ones like the western honey bee (see Chap. 16), have ever been studied for directional vibration sensing. So much information could be gained by investigating relevant directional cues in different species, with potential constraints based on differences due to size or transmission constraints due to characteristics of the substrates on which they vibrate.

Additionally, a great deal could be achieved in advancing the field of biotremology if additional contexts for potential vibrational behavior were investigated in species for which at least one use of vibrational behavior has already been confirmed. Understudied behaviors include typical contexts such as territorial behavior, recruitment of helpers and other social interactions outside mating and parental care, mutualistic or parasitic relationships (especially with ants) and predator detection. Further, examining different developmental stages to investigate vibrational behavior over the lifespan would be very informative, especially in embryos or larvae of taxa already known for vibrational behavior in another life stage. At the same time, we are just now investigating vibrational behavior interactions at the community and ecosystem level, or *vibroscape* (Šturm et al. 2019, 2021). These investigations are revealing to us this previously unexplored world of new vibrational behavior contexts at these levels of organization in natural conditions, with the plethora of signalers, intended and unintended receivers, and noise sources.

References

Bleckmann H, Barth FG (1984) Sensory ecology of a semi-aquatic spider (*Dolomedes triton*). II. The release of predatory behavior by water surface waves. Behav Ecol Sociobiol 14:303–312

Brownell P, Farley RD (1979a) Orientation to vibrations in sand by the nocturnal scorpion *Paruroctonus mesaensis*: mechanisms of target localization. J Comp Physiol A 131:31–38

Brownell P, Farley RD (1979b) Prey-localizing behavior of the nocturnal desert scorpion, *Paruroctonus mesaensis*: orientation to substrate vibrations. Anim Behav 27:185–193

Cocroft R, Gogala M, Hill PSM, Wessel A (2014a) Studying vibrational communication. Springer, Berlin Heidelberg

Cocroft R, Hamel J, Su Q, Gibson J (2014b) Vibrational playback experiments: challenges and solutions. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin Heidelberg, pp 249–274

Endler JA (2014) The emerging field of tremology. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin Heidelberg, pp vii–x

Endo J, Takanashi T, Mukai H, Numata H (2019) Egg cracking vibration as a cue for stink bug siblings to synchronize hatching. Curr Biol 29(1):143–148

Hergenröder R, Barth HG (1983) The release of attack and escape behavior by vibratory stimuli in a wandering spider (*Cupiennius salei* keys). J Comp Physiol A 152:347–358

Hetherington TE (1985) Role of the opercularis muscle in seismic sensitivity in the bullfrog *Rana catesbeiana*. J Exp Zool 235:27–34

Hetherington TE (1989) Use of vibratory cues for detection of insect prey by the sandswimming lizard *Scincus scincus*. Anim Behav 37:290–297

Hill PSM, Wessel A (2016) PRIMER: Biotremology. Curr Biol 26:R187–R191

Hill PSM, Lakes-Harland R, Mazzoni V, Narins PM, Virant-Doberlet M, Wessel A (2019) Biotremology: studying vibrational behavior. Springer Nature, Switzerland

Hoch H, Deckert J, Wessel A (2006) Vibrational signalling in a Gondwanan relict insect (Hemiptera: Coleorrhyncha: Peloridiidae). Biol Lett 2:222–224

Salmon M, Horch KW (1972) Acoustic signalling and detection by semiterrestrial crabs of the family Ocypodidae. In: Winn HE, Olla BL (eds) Behavior of marine animals, vol 1. Plenum Press, New York, pp 60–96

Šturm R, Polajnar J, Virant-Doberlet M (2019) Practical issues in studying natural vibroscape and biotic noise. In: Hill PSM, Lakes-Harland R, Mazzoni V, Narins PM, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational behavior. Springer Nature, Switzerland, pp 125–148

Šturm R, Rexhepi B, López Díez JJ, Blejec A, Polajnar J, Sueur J, Virant-Doberlet M (2021) Hay meadow vibroscape and interactions within insect vibrational community. iScience 24(9):103070

Su Q, Lv J, Li W-X, Sun J-W, Li S-H, Zhang W-Q (2021) Identification of putative abdominal vibration-related genes through transcriptome analyses in the brown planthopper (*Nilaparvata lugens*). Genomics and Proteomics, Comp Biochem Physiol D. https://doi.org/10.1016/j.cbd.2021.100856

Torr P, Heritage S, Wilson MJ (2004) Vibrations as a novel signal for host location by parasitic nematodes. Int J Parasitol 34:997–999

Chapter 2
Sound Production in True Bugs from the Families Acanthosomatidae and Pentatomidae (1958)

Karl H. C. Jordan, Helga Slowioczek, Hannelore Hoch, and Andreas Wessel

Abstract Representatives of the Heteroptera families Acanthosomatidae and Pentatomidae were studied with respect to their sound production. In the course of these studies, we found that the species studied were capable of producing sound, which could be made audible by means of a modified stethoscope. Sounds were emitted predominantly by males, and, in several species, also by females. Sound characteristics and morphological information suggest that the sounds are not of stridulatory origin, but are produced by a hitherto unknown mechanism, i.e., an oscillating membrane. We hypothesize that the sound-producing organ(s) of the Pentatomidae and the Acanthosomatidae may be a simple precursor of the cicada's drumming organ. Behavioral observations revealed that the sounds certainly play a role in mating behavior, and may also be significant for defense; however, sound

---

Hannelore Hoch and Andreas Wessel are Translators.

Editorial Note
In 1958, Karl H. C. Jordan (1888–1972, then Zoological Institute, Technical University, Dresden, Germany) published two articles on the biology of the parent bug *Elasmucha grisea* L. (Insecta: Hemiptera: Acanthosomidae) (Jordan 1958a) and on sound emissions in the Hemiptera families Cydnidae, Pentatomidae, and Acanthosomatidae (Jordan 1958b). Both articles are significant contributions to the methodological and behavioral aspects of biotremology, yet have been largely ignored by the international scientific community, as they are both in German. The results published in both papers were findings obtained in the course of a diploma thesis by Jordan's student Helga Slowioczek. As was the custom of the times, Slowioczek was not offered coauthorship for the publications resulting from her work. Her diploma thesis proper has never been published. It is thus also our intention to honor Helga Slowioczek's contribution to biotremology by providing English translations for those text passages that are of direct impact on this field of research. It should be noted that the terminology used in the two publications does not discriminate between air-borne and surface-borne vibrations.

K. H. C. Jordan · H. Slowioczek
Zoological Institute, Technical University, Dresden, Germany

H. Hoch (✉) · A. Wessel
Museum of Natural History Berlin, Leibniz Institute for Evolution and Biodiversity Science at Humboldt University Berlin, Berlin, Germany
e-mail: Hannelore.Hoch@mfn.berlin

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_2

emissions were also observed in situations where the behavioral context remained unresolved. Frequency and amplitude of the sounds emitted were observed to be temperature-dependent.

2.1 Sound Production in the Acanthosomatid *Elasmucha grisea* L. (Jordan 1958a: pp. 393–395)

2.1.1 The Sound Emission of the Imago

Hitherto, there are no reports of sound emissions of *Elasmucha grisea* in the existing literature. I thus encouraged my student Helga Slowioczek to study Pentatomidae with respect to their sound emissions. In the course of her work, she discovered a special mode of sound production that is entirely different from the stridulation that is common in insects, and in particular, Heteroptera.

With the help of a stethoscope as described by Leston (1954), it is possible to detect sound emissions in a number of Pentatomidae. The Institute for Electrical Engineering of the Technical University Dresden enabled us to produce oscillograms. Following multiple amplifications, the sounds were recorded on magnetic tape, and the sounds played from these tapes were made visible by a loop oscillograph.

Among others, *Elasmucha grisea* belongs to those species of which only the male produces sounds, i.e., female sound emissions could not be registered in any case. The “song” of the male lasted for ca. 45 min, while single calls were observed to be of rather long duration and were emitted in irregular intervals. The first sound type, which has a duration of 1.3 s and a fundamental frequency of 67 Hz, increases to 83 Hz. Modulation of amplitude is periodic at the start, and becomes irregular later.

The second sound type has a duration of 1.0 s, and also increases fundamental frequency from 67 Hz to 83 Hz. Impulse frequency of both sounds equals 21 Hz.

In comparison to the pitch of crickets (5000–17,000 Hz), locusts (6000–8000 Hz) and cicadas (7000–8000 Hz), the frequencies of Pentatomid sounds are extraordinarily low, i.e., 2–3 octaves below the standard pitch (440 Hz, oscillations per second).

The (low) pitch right away excludes sound production via a stridulation apparatus; however, it indicates the existence of an oscillating membrane. Although no morphological structures (underlying sound production) could be identified, the behavior of the animals observed during sound emissions hints to sound production being performed in the first two abdominal segments.

Abdominal tergites 1 and 2 are vividly being moved for- and backwards; additionally, rapid up-and-down movements of the whole abdomen are observed simultaneously. It appears as if at the beginning of a sound, the first two tergites move forward, while during the duration of the sound the up-and-down-movements of the whole abdomen are performed, and that towards the end of a sound, the tergites return to their normal position. The stronger the movements, the more intense the sounds. The most plausible conclusion, therefore, is to assume the oscillations of the abdominal integument [“Abdominalwandung”] are responsible for the sounds. A specialized muscular configuration could not be identified; it is thus plausible to assume that normal dorsoventral and longitudinal abdominal muscles trigger the movements during sound production. The sounds of all Pentatomidae studied so far are very soft and not audible to the human ear without amplification. There is no effective resonance body of the kind we know from cicadas. With some reservations, it can be hypothesized that the sound production apparatus for the Pentatomidae is a simple precursor of the tymbal organ [“Trommelorgan”] of cicadas, which show the organ in highest perfection.

In Acanthosomatidae, the mesothorax bears a lamellae-shaped longitudinal carina that touches, laterodistally, a thorn-shaped protrusion for some of its length. It is conceivable that the abdominal movements cause the distal part of the abdominal longitudinal carina to chafe at the thoracal foliate carina—however, there are no teeth or spines of any kind that would bring forth any sound. Sounds produced in this manner would also be stridulatory. It is worth noting that Pentatomidae that do not bear carinae, as described above [in this section] for *Elasmucha*, are capable of producing very similar sounds.

Now the question arises as to the biological significance of sound production. Unfortunately, it was impossible for my student to perform experiments during mating season in spring, but only from the end of September to October. They [the experiments] are yet no less convincing and give proof that sound production is connected to copulation. In an experiment, a pair [of *Elasmucha grisea*] was placed under the stethoscope. The male initiated calling while unfolding his copulation apparatus, and with his antennae vividly moving. The female was grabbed by the male from the side, but did not at first show any interest in mating, but instead tried to get rid of the male by means of abdominal movements. After 45 min of incessant singing [of the male], the female was finally motivated for mating and permitted copulation.

It has been observed repeatedly that Pentatomidae also mate in the autumn, and occasionally even egg batches can be found. I am not aware of any such late oviposition in *Elasmucha*; it is also not reported in the literature. The long period of parental care and the prolonged nymphal development could perhaps barely be completed before the onset of winter, especially since low temperatures would further decelerate developmental rates.

It is also interesting that males (in the absence of females) emit sounds, however, only when they are in contact with other conspecific males. In contrast, males do not react to contact with other species (e.g., *Palomena prasina* L.) by emitting sounds.

2.2 Sound Production in the Pentatomidae and Acanthosomatidae (Jordan 1958b: pp. 130, 134–143)

2.2.1 Introduction

Hitherto there are only a few studies that report sound emissions in the Cydnidae, Pentatomidae, and Acanthosomatidae. In all cases described, these are stridulatory organs, which produce more or less intense chirping sounds with the help of a file ["Schrilleiste"]—which is not unusual within the suborder Hemiptera-Heteroptera. One of my diploma students, Ms. Helga Slowioczek, has been given the task to study representatives of aforementioned families in respect to their sound emissions, and in the course of this work, (she) found a hitherto unknown/undescribed mode of sound production, which is not caused by stridulation, in the families Pentatomidae and Acanthosomidae.

2.2.2 Methods and Material

Leston's stethoscope is set up as follows: the top of a glass funnel is covered with a paper membrane and, at its lower opening, features a T-piece connected with two tubes that are inserted in the [experimentor's] ears. Leston covers the paper membrane with a glass vial in which a thermometer is inserted in order to measure the temperature.

When studying Pentatomids, however, it will be sufficient to cover the animals [sitting] on a glass plate with the funnel. This will account for softer sounds. However, on the other hand, all ambient noise resulting from the oscillations proper of the paper membrane are avoided. Illumination of the [test] animals with a 40 or 60 W lamp causes the bugs to initiate calling sooner, but a too strong illumination and heat radiation (above 30 °C) cause disturbance and may trigger rigor in the animals.

Owing to the kind support of the Institute of Electrical Engineering of the Technical University Dresden, it has been possible to conduct precise sound recordings, which were recorded on magnetic audiotape after repeated amplification, and could subsequently be used to produce oscillograms. Unfortunately, the oscillograph wasn't available at all times, thus, it wasn't possible to produce oscillograms for all species studied.

The species selected for investigation were placed into a glass container of 46.7 cm^3 volume, the upper opening of which had been sealed off with plastilin, and which was carrying a condenser microphone. In order to obtain a precise/clear/noise-free sound recording, this part of the experimental set-up was brought into an anechoic room. The sounds that were played back from the magnetophone were made visible by means of a galvanometer oscillograph. Current fluctuations, which correspond to the sounds, are transformed into rotations of a small mirror by the galvanometer oscillograph, resulting in the deflection of a light beam that originates from a fixed light source. A light-sensitive stripe of paper is now made to run perpendicular to the direction of the deflection at a speed of 45 cm/sec. This is how the electric current, which equals the sound pressure, is being made visible as a function of time.

In order to analyze the oscillogram, a time marker of known frequency (500 Hertz) was recorded and then used to calculate unknown frequencies.

For example:

1 cm of the film tape equals 12 oscillations of the time marker (500 Hertz)

1 cm of the film tape equals 3.6 oscillations of the time marker pertaining to the unknown markers of the oscillogram

fz = frequency of the time registrator 500 Hz

fx = unknown frequency of the recorded sounds

$$\frac{fz}{fx} = \frac{\text{Number of oscillations of the time registrator per cm}}{\text{Number of oscillations of the unknown frequency per cm}}$$

This means: $fx = fz \cdot \frac{Osc.\ \ x/cm}{Osc.\ \ z/cm}$.

Using the values mentioned above, then

$fx = 500 \cdot \frac{3.6}{dx12.} = 41.7 \cdot 3.6.$

All we need to do is to multiply the number of oscillations per cm by 41.7.

Since 500 oscillations are exerted in 1 s (frequency of the time registrator), while 1 cm equals 12 oscillations of the time marker, it is logical that 1 cm of film tape per second equals the following time duration:

$$\frac{12}{500}\ \text{s} = 0.024\ \text{s}$$

Thus the number of cm of the film tape must be multiplied by 0.024, to calculate the duration of single calls/sounds and intervals.

The frequency of the standard pitch is 440 Hertz. The sounds of the Pentatomidae are within the range of 55–198 Hz, i.e., comparatively low, as compared to other insect sounds.

Species studied by Slowioczek in regard to their sound production with the help of a stethoscope as described by Leston (1954):

Pentatomidae:

Subfamily Pentatominae: *Carpocoris pudicus*, *Palomena prasina*, *Palomena viridissima*, *Dolycoris baccarum*, *Eurydema oleraceum*, *Pentatoma rufipes*, *Aelia acuminata*;

Subfamily Asopinae: *Picromerus bidens*, *Arma custos*;

Acanthosomatidae: *Elasmucha grisea*.

2.2.3 Pentatomidae

2.2.3.1 Behavior of the Species Studied in the Stethoscope: Subfamily Pentatominae

Carpocoris pudicus When placed inside the stethoscope and exposed to light, the animals soon become active and start emitting sounds. Simultaneously, an up-and-down movement of the abdomen is observed, which is in connection with the song. Males as well as females are capable of sound production; however, individuals differ in their motivation to emit sounds. Some are "singing" continuously, while others show an inclination to emit sounds only now and then.

Single humming sounds are audible, which are usually held in the same pitch. The most rapid emission of sounds was 15 sounds within 5 s, which were emitted in regular intervals. In contrast, when only 3–5 sounds were emitted in the same time span, these were in irregular intervals. This was observed among males as much as among females. Two individuals would sing either simultaneously or would alternate. Singing was also initiated when other Pentatomids, e.g., *Palomena prasina* or *Aelia acuminate*, were added into the experimental arena in the stethoscope.

Although sound emissions can be detected throughout the entire vegetation period with the exception of winter quiescence, there is hardly any doubt that sexual behavior is influenced by the sound production. In the mating season, a pair was brought together in the stethoscope. When the male made physical contact with the female, it felt the female with the antennae, while it was constantly emitting sounds. It was only after 10 min that the female stopped walking about and started calling. From then on, male and female called in alternation, with the male taking the more active part. The male usually started with a high-pitch signal/sound that was followed by lower pitched ones, while the female replied with 4–5 sounds that were emitted in short intervals. The alternating singing lasted for 45 min, followed by copulation, during which no sounds were emitted at all. Each sound corresponds to an up-and-down movement of the abdomen, whereby the volume of the sound correlated directly with the amplitude with the movement. After removal of the flight organs, a for- and backward movement of the first and second abdominal segments, which is also in total synchrony with the sounds, can be observed under the stereoscope. During very vivid singing the abdomen shows a distinct vibration.

Palomena prasina Also in this species, both sexes are capable of emitting sounds; the species generally shows high similarity to *Carpocoris pudicus* with respect to behavior. Within 5 s, up to 20 sounds are emitted in regular intervals, while also in this species intervals become irregular when fewer sounds are emitted over the same period. Copulation is always preceded by alternating singing, whereby the male keeps singing continuously. Occasionally this courtship may last up to one hour.

It is also interesting that sounds are seemingly emitted also for reasons of defense. In one specimen of *Palomena*, both pairs of wings were removed in order to facilitate observation of the abdominal movements. A female of *Arma custos*, a well-known predatory species, was placed close to the *Palomena* specimen and immediately started attacking *Palomena*. *Palomena* first replied with lateral movements of the abdomen, which is characteristic of Pentatomidae, while emitting very loud and long sounds that—in connection with the abdominal movements—caused *Arma custos* to cease the attack. Similarly strong sounds were also repeatedly uttered, when one animal climbed across a conspecific individual. From this it can be concluded that sound production does not only have significance for sexual behavior but that it can also be a more or less effective defense reaction.

Palomena viridissima There were no notable differences when compared to *P. prasina*. When a *P. prasina* male was brought in contact with a *P. viridissima* female, the male started singing immediately and approached the female. However, as the female did not react, the male ceased singing soon afterwards. On the basis of these observations, it cannot be decided whether or not the sounds, which to our opinion do not show any differences, are nonetheless species-specific, or whether or not any species-specific olfactory sensations are involved. Unfortunately, no oscillogram of *P. viridissima* was recorded.

Dolycoris baccarum This species exhibited very low sound-emitting activity. In none of the observed cases could a sound emission of the female be detected. Also the number of emitted male sounds was smaller, only 4–5 per 5 s. The up-and-down movement of the abdomen, as well as the anterior-posterior movement of the first two tergites, appeared insignificant. *Dolycoris* seems thus a species in which the sound emission behavior is not very strongly developed.

Eurydema oleraceum Despite numerous observation trials, it has only once been possible to perceive sound emissions, which were generated only by the male and which were very soft and intermittently produced. The courtship song lasted 30 min, after which copulation occurred. When a second male was placed into the observation arena, the copulating male again emitted sounds when it came into physical contact with the other male. Thus also in this species, emissions of defense sounds may be present.

Pentatoma rufipes Unfortunately, it has not been possible to observe sound emissions during the mating season. In the course of the observations in July and August, the song was quite monotonous and consisted of 5–7 sounds in the course of 5 s. On some occasions, no sound emission activity was observed during several hours, followed by bursts of few sounds, again followed by a longer period without sound emissions. The movements of the abdomen totally correspond to those of the other species studied.

Aelia acuminata The song of the males (the only sex observed to emit sounds) features 7–10 sounds within 5 s, in rapid bursts (Fig. 2.1), and is accompanied by a delicate humming sound, which is soft at the start of the sound emissions, then maintains a steady pitch, and eventually softly fades. The insects are motivated to produce sounds not only by the presence of the other sex, but also by other conspecific males and even by specimens of different species.

When animals are placed in the stethoscope during mating season, the male will sing in the way described above [in this section]. However, when animals start touching each other, the song suddenly changes. A high-pitch, longer sound is followed by a number of lower-pitch and shorter sounds, which are emitted in

Fig. 2.1 Oscillogram of the *Aelia acuminata* male mating song. 7–10 sounds in 5 s; duration of the single oscillation sections, 0.04–0.05 s; frequency, 120–125 Hz

short intervals. This unilateral courtship song lasted 15 min. The female was increasingly pacified and eventually permitted copulation. Also in this species, the characteristic abdominal movement is observed in the male.

2.2.3.2 Behavior of the Species Studied in the Stethoscope: Subfamily Asopinae

Picromerus bidens While during September and October, no sound emissions were detected at all, despite numerous observation trials, the song of a male could be successfully detected in the mating season at the end of May. In the course of 5 s ca. 7 sounds, which were very short in duration, were emitted in equal frequencies. Single signal trains tended to alternate with longer intermissions. Recording on tape failed, as the insects in the pressure chamber could not be stimulated to sing. The movements of the abdomen were the characteristic ones, however displaying weak amplitudes.

Arma custos Also in this species, sounds were observed to be produced by males only; the test animals were available for observation only outside the mating season. The specimens emitted long, intermittent sounds in irregular intervals. One male would only sing if harassed by another animal (also those of other species).

Further investigations were conducted with *Eurygaster maura*, *Stollia fabricii* and *Troilus luridus*. None of the mentioned species produced any sound that could be detected. It is, however, plausible to assume that these species are capable of sound production, and that sounds may eventually be detected when observations are made of a higher number of specimens and within the mating season.

2.2.4 Acanthosomatidae

Elasmucha grisea Studies of this species were conducted from the end of September until October, which is outside the usual mating season. The male produces rather long sounds of 1–3 s duration (Fig. 2.2), which are emitted in irregular intervals. In one observed couple, the male would cling to the female laterally, unfolding his copulation apparatus, while the female apparently tried to shake him off by means of

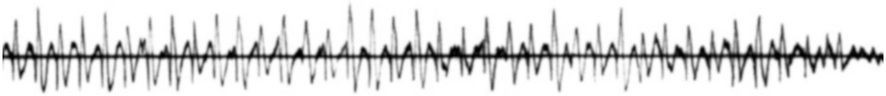


Fig. 2.2 Oscillogram of the *Elasmucha grisea*. Produced sound, long (1–3 s); frequency, 67–83 Hz

lateral abdominal movements. Only after 45 min of continuous singing, the female calmed down, stopped running about and permitted copulation. This observation of copulation outside the regular mating season of *Elasmucha* is a nice proof of the significance of sound emissions for sexual behavior.

That the songs are potentially also serving a defense function can be concluded from the observation that during mutual touching, and even expulsion, conspecific males would also emit sounds; however, they never did so when encountering animals of other species.

In *Elasmucha ferrugata* and *Elasmostethus* no sound emissions were observed; however, the same statement that has been made above (see Sect. 2.2.3.2) for *Eurygaster maura*, *Stollia fabricii* and *Troilus luridus* applies.

2.2.5 Conclusions

Even though at present only a few species of Cydnidae, Pentatomidae, and Acanthosomidae have been studied, we can already say that sound production among these families is rather common. In Cydnidae only stridulation, which is typical for the Heteroptera, has been documented, whereas in the other families stridulation, as well as abdominal sound production, is present; the latter is described in detail for the first time. It is assumed that these modes of sound production are mutually exclusive; however, further investigations are necessary. Also it cannot be concluded with certainty, yet, whether or not in some species females are mute. Sound production by females could be confirmed in only three out of ten species studied (*Carpocoris pudicus*, *Palomena prasina* and *P. viridissima*).

The songs of Pentatomidae and Acanthosomidae are very soft and not audible to the human ear without the aid of artificial devices. Most notable are the low frequencies, which are in the range of 55 Hz and 198 Hz, which is 2–3 octaves below the standard pitch. In comparison to the frequencies of Orthoptera (5000–17,000 Hz) and those of cicadas (7000–8000 Hz), the sounds of Pentatomidae and Acanthosomatidae are of unusually low pitch.

The song consists of single or sounds. In *Carpocoris*, they commence with higher frequencies, increase to a certain degree, then transform into a decreasing pitched sound that ends abruptly. In *Palomena*, in contrast, the sound increases gradually in amplitude and pitch and fades slowly.

In some species, composed sounds are observed that are either "built from single impulses or consist of numerous oscillation sections, which have been generated by periodical consequential decrease of amplitude" (Slowioczek; Editors comment: the quotation refers either to a personal comment by H.S. or to her—unpublished—diploma thesis; H.S. is not cited in the references).

Each oscillogram can be divided into certain sections, which is demonstrated by the example of the sounds emitted by *Carpocoris pudicus* (males and females). For recording, single specimens were placed in front of the microphone, and the sound recording was transformed into an oscillogram as described earlier (see Sect. 2.2.2).

Male

Section	Duration in sec	Frequencies in Hz
A	0.12	198
B	0.18	150
C	0.17	150
D	0.12	150
E	0.17	150
F	0.17	150

Time intervals between oscillation groups are less than a second:

Interval a 0.07 s
Interval b 0.10 s
Interval c 0.08 s
Interval d 0.08 s
Interval e 0.10 s

The intermissions between sounds, which can be observed and which may last over long periods, have been mentioned above (see Sect. 2.2.3).

In each oscillation group, two sections with differing amplitudes can be distinguished. Presumably, the first quarter of the oscillation serves as a build-up of amplitude, which is only then reaching its full range.

Female

Section	Duration in sec	Frequencies in Hz
A	0.03	154
B	0.02	154
C	0.02	154
D	0.03	154
E	0.02	154
F	0.03	154
G	0.03	154
H	0.03	154

All sounds consist of numerous, short impulses, which are always held at a steady pitch.

Time intervals are even smaller than in males:

Interval a 0.009 s
Interval b 0.009 s
Interval c 0.01 s
Interval d 0.02 s
Interval e 0.02 s
Interval f 0.01 s

2.2.6 Topology of Sound-Producing Instruments

All Pentatomidae and Acanthosomatidae studied so far produced sounds even when fore- and hindwings had been removed. We can thus conclude that there are no vibrations of the wings involved in sound production. Further, no stridulatory organs could be found. Apart from the pitch observed, a stridulatory origin of the sounds can be excluded by the following reason. The analysis of the oscillograms by colleagues of the Institute of Electroacoustic Engineering, Technical University Dresden revealed that overtones ["Oberfrequenzen"] which are typical for sounds generated by stridulation were not detected.

Eventually one could take into consideration that sounds could be generated by the oscillation of an air column of sorts. This, however, appears impossible in the face of the low pitch of the sounds recorded. In *Carpocoris*, the species with the highest recorded frequency of 198 Hz, the height of such an air column would have to be significantly larger than the body size of the animal itself. A hollow space can also act as a resonance body if its dimensions are whole multiples of $\frac{\lambda}{4}$.

An example:

Carpocoris pudicus (highest observed frequency: 198 Hz)

$$\frac{\lambda}{4} = \frac{340\ \mathrm{m/s}}{4f} = \frac{340}{4.198} = \mathbf{0.43\ m}$$

Arma custos (lowest observed frequency: 55 Hz)

$$\frac{\lambda}{4} = \frac{330\ \mathrm{m/s}}{4.55f} = \frac{330}{4.55} = \mathbf{1.50\ m}$$

From these calculations, it becomes obvious that the values obtained do not make sense for the body size of a small insect.

Slowioczek further investigated whether or not a chitinous plate capable of oscillating may be present inside the spiracles. This was not the case. In a convincing experiment—sealing the abdominal stigmata with beeswax—most animals ceased singing. A few specimens, however, did produce sounds without the slightest sign of alteration, regardless of the manipulation.

It appears thus logical to assume *sound production by means of oscillations of a membrane*. During all sound emissions observed in Pentatomidae and Acanthosomatidae, sound production was accompanied by up-and-down movements of the abdomen, and—particularly—a forward-backward movement of the first two abdominal tergites. The chitinous exoskeleton of insects is a priori a material very well suited for oscillations, and thus, for sounds. The muscular configuration of the abdominal segments facilitates easy movements of the abdominal tergites. The movement of the entire abdomen does perhaps have some significance in modulating the song.

In *Palomena prasina* one abdominal movement corresponds to one sound. In *Aelia acuminata* the number of abdominal movements equals the number of impulses during courtship song. As in *Carpocoris pudicus* the sequence of impulses is more rapid, and one can observe a distinct vibration of the abdomen. Vivid abdominal movements always generate vivid sound emissions.

Even though there is convincing evidence that sound production indeed occurs as described in this section, anatomical studies are not yet sufficiently progressed to identify a particular muscle as a "sound muscle," as in cicadas.

2.2.7 The Significance of Sound Emissions

In some Pentatomidae species that could also be studied during the mating season, a "courtship song" can be distinctly discriminated against usual sound emissions. The male initiates mating with a song that often may last for an hour before the female is in the mood for copulation. In cases where the female itself is capable of sound production, it will reply to the song of its sexual partner, so that a proper "alternating song" is created, whereby the male, however, always takes on the more active part. In females, where hitherto no sound production has been documented, one could nonetheless observe that they do react to the song of the males, approach them while vividly moving their antennae, and eventually calm down sufficiently to permit copulation. Particularly impressive is the connection between sound production and mating in the example of the Acanthosomatid *Elasmucha grisea*, where a mating was even observed in the fall, because the male produced the courtship song in the narrow experimental cage for an entire 45 min.

Besides the courtship song, so-called "usual sounds" are emitted outside the reproduction period. They can be clearly discriminated from the courtship song. To assess their significance, however, is more complicated. When an additional animal is placed next to a specimen already singing, the added animals will eventually become motivated to also produce sounds. We can therefore assume some sort of communication, without actually being able to assess its subject. Unfortunately, the behavioral studies are particularly difficult for us, as we cannot perceive the sounds without technical aid, so we cannot directly conclude which particular sounds, or sequences of sounds, trigger which reactions.

Inside the stethoscope Pentatomidae are being motivated to emit sounds even by non-conspecific animals. Only conspecific animals, however, will produce alternating songs.

A third possibility for the significance of sound emissions is defense against, or deterrence of, other animals.

When only males belonging to different species are placed inside the stethoscope, they were observed to emit sounds whenever they touched, or climbed on top of each other, or one of the animals landed on its back. In some species (*Palomena viridissima*, *P. prasina*, and *Carpocoris pudicus*) particularly strong sounds could be heard under such circumstances. One could be tempted to assume that the sounds meant ways to deter or scare antagonistic males. However, some observations contradict this assumption: animals do not seem to take any notice of these sounds among themselves, they also do not react to mechanical stimuli by the observer. In one case, where an *Arma custos* (male) preyed upon a non-conspecific animal, the victim, although in principle capable of sound production, did not emit any sounds at all.

Further investigations are necessary, to learn more about the factors that trigger sound emission; it will then be easier to recognize the significance of sound emissions. For now, we only know that animals are more lively and emit sounds more rapidly when kept under warm temperature and exposure to light. The temperature optimum is around 20 and 24 °C. If temperature increases to 30 °C, and even more, the bugs become disturbed, lapse into rigor, and stop emitting sounds altogether.

References

Jordan KHC (1958a) Die Biologie von *Elasmucha grisea* L. (Heteroptera: Acanthosomidae) [the biology of *Elasmucha grisea* L. (Heteroptera: Acanthosomidae)]. Beitr Ent 8:385–397

Jordan KHC (1958b) Lautäußerungen bei den Hemipteren-Familien der Cydnidae, Pentatomidae und Acanthosomidae. [sound emissions in the Hemiptera families Cydnidae, Pentatomidae and Acanthosomidae]. Zool Anz 161:130–144

Leston D (1954) Strigils and stridulation in Pentatomoidea (hem.): some new data and a review. Ent Month Mag 90:49–56

Part II
The State of the Field: Concepts and Frontiers in Vibrational Behavior

Chapter 3
Vibrational Behaviour and Communication in the New Zealand Weta (Orthoptera: Anostostomatidae)

Johannes Strauß and Daniel R. Howard

Abstract Vibrational signalling is an important communication mode in different taxa of Ensifera (long-horned grasshoppers), even though crickets and tettigoniids are mostly thought of as well-established models for the study of acoustic communication in calling and courtship. In the New Zealand weta (Anostostomatidae), pair formation is not mediated by long-range acoustic communication. This chapter summarises the recent research that has identified the different means of substrate-borne vibrational signals used by weta. Notably, diverse behavioural contexts and signalling modes rely on vibrational signals: giant weta (Deinacridinae) mediate male–male agonistic interactions by dorso-ventral tremulation, ground weta (genus *Hemiandrus*) use abdominal drumming on plant substrates in pair formation, and tree weta (genus *Hemideina*) produce vibrational signals by tremulation in aggressive behaviour and by stridulation in defence. Such stridulation produces both airborne sound and substrate vibrations, and as in all other Ensifera studied thus far, the substrate vibrations are detected by mechanosensory organs located in the legs. The sensory elements involved in vibration detection are conserved among weta species with and without tympanal hearing organs, but show specific functional adaptations relating to the different modalities. While the importance and diversity of substrate vibration signals have been established for weta, further characterisation of these signals and sampling of related species will offer insights into the evolutionary divergence of vibrational signalling across the weta groups, and will support studies of the evolution of vibration detection and tympanal hearing in the broader Ensifera.

J. Strauß (✉)
Justus-Liebig-Universität Gießen, Institute for Animal Physiology, AG Integrative Sensory Physiology, Gießen, Germany
e-mail: johannes.strauss@physzool.bio.uni-giessen.de

D. R. Howard
Department of Biological Sciences, College of Life Sciences and Agriculture, University of New Hampshire, Durham, NH, USA

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_3

3.1 Biology and Communication in Weta

3.1.1 Biology and Taxonomy of Anostostomatidae

Vibrational signals and cues are essential to the majority of insect species in mate finding, prey detection, or predator evasion (see reviews by Virant-Doberlet and Čokl 2004; Cocroft and Rodríguez 2005; Hill 2009). Here, we summarise the knowledge on vibrational communication in the New Zealand weta, belonging to the ensiferan Anostostomatidae—the weta and king crickets (Field 2001a; Song 2018). Ensifera, and Orthoptera in general, serve as a model group for communication by airborne sound and vibration as well as for the neuronal mechanisms of signal generation and analysis (e.g., Alexander 1962; Walker 1962; Bailey 1991; Pollack 1998; Robinson and Hall 2002; Hedwig 2014). The New Zealand weta extend our knowledge further in this regard due to their reliance on substrate-borne information in several behavioural contexts.

The New Zealand insect fauna and its taxonomy and behavioural adaptations have been of particular interest from an evolutionary perspective as it evolved isolated for the last ~75 million years (e.g., Gibbs 2006; Trewick and Morgan-Richards 2005, 2009, 2019); although the maintenance of possible land bridges over a longer period is discussed (Buckley et al. 2015). Overall, the orthopteran fauna of New Zealand is unique with respect to the lineages present and in the species diversity, compared to the Australian fauna (Trewick and Morgan-Richards 2019). The weta belong to the Anostostomatidae, which were first identified as a separate ensiferan taxon by Johns (1997), and this Maori term (from *wetapunga*, loosely interpreted as "god of ugly things") is used mainly for the New Zealand species (Field 2001a); although, some species of the relevant genera can occur elsewhere, e.g., in Australia and New Caledonia (Monteith and Field 2001; Cigliano et al. 2021). Anostostomatidae are closely related to Gryllacridiae (raspy crickets) and Stenopelmatidae (Jerusalem crickets) (Fig. 3.1a; Song et al. 2020), together often referred to as the Stenopelmatoidea (see Ingrisch and Rentz 2009; Song et al. 2015, 2020; Vandergast et al. 2017). Some molecular approaches supported the Anostostomatidae as monophyletic (Pratt et al. 2008; Song et al. 2020), but other studies have not reproduced this finding (see Song et al. 2015, including the highly derived Cooloolidae in Anostostomatidae, and Vandergast et al. 2017, supporting two anostostomatid clades). The genus *Transaevum*, occurring in Australia (northern Queensland) (Johns 1997; Monteith and Field 2001), presumably represents the basal anostostomatid lineage (Monteith and Field 2001; Pratt et al. 2008). In addition, different taxonomic subgroups are recognised among the weta: the giant weta (genus *Deinacrida*), the ground weta (genus *Hemiandrus*), the tree weta (genus *Hemideina*), and the tusked weta (*Motuweta* and *Anisoura*) (e.g., Gibbs 2001; Trewick and Morgan-Richards 2019; see Table 3.1, Fig. 3.1b–d). While *Hemideina* and *Deinacrida* were assumed as sister genera (Deinacridinae; Gibbs 2001), more recent molecular studies have not supported the monophyly of *Deinacrida* (giant weta), as two species (*D. talpa*, *D. pluvialis*) were found more closely related to

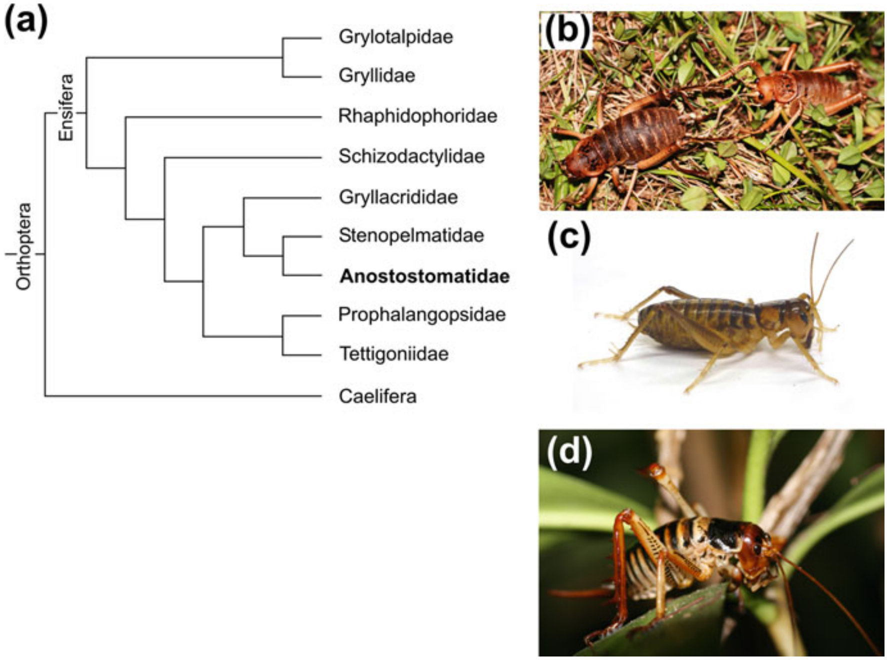


Fig. 3.1 Phylogenetic position of New Zealand weta and representatives of different weta groups. (**a**) Phylogenetic relationship of Anostostomatidae within Ensifera, including Gryllacrididae (raspy crickets), Gryllidae (crickets), Gryllotalpidae (mole crickets), Prophalangopsidae (grigs), Rhaphidophoridae (cave crickets), Schizodactylidae (splay-footed crickets), Stenopelmatidae (Jerusalem crickets), Tettigoniidae (bush crickets and katydids) (phylogeny adapted from Song et al. 2020, Nat Commun 11: 4939). (**b**) New Zealand Cook Strait giant weta *Deinacrida rugosa* (photograph by D. R. Howard). (**c**) Ground weta *Hemiandrus pallitarsis*, female (from Strauß et al. 2017). (**d**) Wellington tree weta *Hemideina crassidens*, female (photograph by D. R. Howard)

Table 3.1 Different groups of Anostostomatidae weta with numbers of identified species

Group	Genus	Described species	Distribution
Giant weta	*Deinacrida*	11 species	New Zealand
Ground weta	*Hemiandrus*	18 species	New Zealand, Eastern Australia
Tree weta	*Hemideina*	7 species	New Zealand, Eastern Australia
Tusked weta	*Motuweta, Anisoura*	3 species	New Zealand

Number of formally described species: Cigliano et al. (2021), Gibbs (2001), Trewick (2021), and Trewick and Morgan-Richards (2019); distribution: Monteith and Field (2001); Cigliano et al. (2021)

Hemideina (tree weta: Trewick and Morgan-Richards 2004). The phylogeny of *Hemiandrus* (ground weta) is also complex, with different branches for New Zealand and Australian species based on molecular sequence data (Pratt et al. 2008). Most species of New Zealand weta are endemic to this isolated Gondwanan land mass (Trewick and Morgan-Richards, 2019) known as Aotearoa, Maori for "land of the long white cloud".

To understand the characteristics and functions of substrate-borne vibration signals in the New Zealand weta, it is informative to couple what is currently hypothesized regarding their evolutionary patterns with what we have observed with respect to the diversity of their ecologies. Most weta are nocturnal or crepuscular, with peak activity during the darkest hours, and some favouring nights with relatively low levels of moon light (Gibbs 1998; Kelly 2006a; Watts and Thornburrow 2011). This is presumably due to co-evolutionary histories shared with a primarily avian and reptilian predator fauna. Due to New Zealand's virtually mammal-free evolutionary history (until human settlement; Gibbs 2006), the weta are presumed to have filled the ecological niche of the small mammals found on other continents (Griffin et al. 2011). Relatedly, most weta exhibit polyphagous habits; both the arboreal tree weta and the ground dwelling giant weta are known to feed on fruit and facilitate seed dispersal in their hosts (Duthie et al. 2006) in addition to feeding on leaves. Ground weta are known for carnivory (Cary 1983), but even the presumably herbivorous tree weta *H. crassidens* preferred a meal of fresh moth over the leaves and fruit of one of its putative botanical hosts (Griffin et al. 2011). The mating systems of New Zealand weta are generally poorly studied outside of the tree weta, most of which exhibit a form of resource defence polygyny. Male Wellington tree weta are trimorphic, and can reach sexual maturity in the eighth-tenth instar (Kelly and Adams 2010). These tenth instar males exhibit megacephaly, using outsized mandibles to defend harems of females via intermale contests at arboreal gallery sites (Kelly 2006b), while smaller males adopt a satellite strategy and seek matings away from the gallery (Field and Jarman 2001). Pair formation in both ground and giant weta are presumed to occur via scramble competition polygyny. In the Cook strait giant weta *D. rugosa*, for example, searching males follow a presumed chemical trail laid down by foraging females, then engage in courtship via a prolonged tactile interchange leading to copulation (Kelly et al. 2008). In the ground weta, *H. pallitarsis*, mobile females search for stationary males, which are known to produce a pre-mating substrate-borne signal for advertisement (Gwynne 2004). Nuptial gifts are known to occur in both tree and ground weta, but have not been observed in the giant weta.

For the different weta groups, diverse signalling modes and behaviours are apparent (e.g., Johns 1997; Field 1993, 2001b; Song 2018) and likely relate to their divergent ecological niches. New Zealand weta are found from sea level to alpine peaks, living on and in the soil, among tussocks and low shrubs, and into the tops of giant ferns (Gibbs 2001). These diverse habitats and their variable signal transmission qualities likely act as physical constraints to the evolution of substrate-borne communication across the group. For weta, the acoustic long-range calling/ courtship signals characterised in crickets and bushcrickets for mate finding by phonotaxis are not developed (Field and Jarman 2001; Robinson and Hall 2002), as all species are apterous; although, stridulatory signals are produced in particular contexts via other means (Field 2001b). Different vibrational signals have been identified and analysed in giant weta, ground weta, and tree weta (see Table 3.3), while vibrational communication in Motuweta has not been studied so far (Howard et al. 2018). The species found in the New Zealand cave weta group belong to the independent lineage of Rhaphidophoridae, or the cave crickets, and not Anostostomatidae (e.g., Trewick and Morgan-Richards 2019). With the focus of the present chapter on the Anostostomatidae, case studies of vibrational signalling by New Zealand cave crickets will be included in a study of cave insects by Stritih-Peljhan et al. (see Chap. 13).

3.1.2 Signalling Modalities in Weta

For some time, weta—as many other Orthoptera—have been studied with a focus mainly on acoustic signalling by stridulation and on the tympanal hearing organs. This is for some obvious reasons, e.g., the largest tympanal membranes amongst members of Ensifera are found in giant weta (with a length of 4 mm in *Deinacrida heteracantha*; Ball and Field 1981; Field 2001d), and there are overall similarities—with relevant differences—of the auditory systems compared to the Tettigoniidae (katydids and bushcrickets) (Field et al. 1980; Ball and Field 1981; Nishino and Field 2003; for a review, see Robinson and Hall 2002). In addition, the stridulatory structures and sounds have been compared among several species of *Hemideina* and *Deinacrida*. Notably, weta seem not to produce acoustic courtship signals by tegminal (wing-wing) stridulation known from crickets and tettigoniids, but mainly in the context of defence using stridulatory structures on other body parts (Field 1993). A broader repertoire of airborne sounds is produced by *Hemideina*, the tree weta (Field and Rind 1992; see Sect. 3.2.4).

Weta produce sound signals by a diversity of stridulatory mechanisms (Field 1978, 1982, 1993, 2001b; Field and Rind 1992; Field and Roberts 2003). They are therefore important to study in the context of the diversification of mechanical communication modes in insects (e.g., Field 1993; Greenfield 2002). Regarding the evolutionary sequence of the origins of communication systems and mechanosensory organs, weta are also a relevant taxon within the Ensifera, since they include groups with and without tympanal hearing organs. In the genus *Transaevum*, as well as Australian species of *Hemiandrus*, and a so far undescribed anostostomatine genus (new genus B; Monteith and Field 2001), the foreleg tibia even has a single antero-dorsal tympanum (Johns 1997; Monteith and Field 2001). For the atympanate *Hemiandrus* species from New Zealand, it is assumed that they are insensitive to airborne sound (Field 2001b; Gwynne 2004); although, neurophysiological studies are needed to confirm this hypothesis. This diversity of sensory structures raises the question of the ancestral communication mode in the group. The recent phylogenomic analysis by Song et al. (2020) indicated that the atympanate *Hemiandrus* are not the most basal group within Anostostomatidae. Together with phylogenetic analysis, information on signalling modes and behavioural adaptations will give insights into the evolution of signalling modalities within the Ensifera.

In this chapter, we discuss the vibrational behaviours and signals used in the distinct weta groups. The behaviour of weta has been studied to some extent (see summaries by Field 2001b; Field and Jarman 2001; Field and Sandlant 2001), and the aim here is to provide an overview specifically of the current knowledge on weta biotremology, and discuss differences and specific underlying adaptations. Therefore, we summarise data from giant weta, ground weta, and tree weta species, and also identify open questions on the weta communication by substrate vibrations.

3.2 Vibrational Behaviour of Weta

3.2.1 Vibrational Signalling in Insects

Insects produce vibrational signals by different behaviours, mainly tremulation (movements or shaking of the body or body parts without making contact with the substrate), drumming (striking body parts against the substrate), stridulation (rubbing of specialised body parts against each other), and tymbal buckling (movement of a tymbal membrane driven by specialised muscles, in Hemiptera) (Hill 2008, 2012, 2014; Čokl and Virant-Doberlet 2009; Yack 2016; Strauß et al. 2021; extensive review in Wessel et al. 2014; and see Chap. 13). Often, the mechanical signals are continuous between airborne sound and substrate vibrations, e.g., stridulation produces acoustic signals, but the movements of body parts also induce vibrations to the substrate (Keuper and Kühne 1983; Stiedl and Kalmring 1989; Hill and Shadley 1997), and it is often not analysed whether only one or both components are detected as signals (Hill 2014). Drumming can also generate sound components together with the behaviourally relevant substrate vibrations (Hill 2012; Caldwell 2014). The different vibrational signalling modes often correlate with substrate types and different signal features, e.g., spectral components (Elias et al. 2004; Elias and Mason 2010; Gordon et al. 2019). Amongst species of weta, vibrational signalling via tremulation, drumming, and stridulation are described, which are involved in intraspecific and interspecific communication contexts. Notably, American species of Anostostomatidae also use drumming or tapping by single hindlegs on the substrate for signal production (Weissman 2001). This type of drumming is so far not described from the New Zealand weta.

3.2.2 Giant Weta Deinacrida

Giant weta occur on the North and South island of New Zealand (Gibbs 1999, 2001; Trewick and Morgan-Richards 2005). They occupy diverse habitats, from temperate rainforests to alpine zones, exhibiting a classic ecological speciation biogeography. Giant weta reside mainly on mixed soil and organic substrates (McIntyre 2001), are wingless, and generally exhibit female-biased size dimorphism. They use stridulation by femoro-abdominal and abdominal tergo-tergal mechanism in heterospecific defence (Field 1982, 1993; Field and Roberts 2003); these stridulatory behaviours produce low amplitude airborne sounds.

While the communication system in giant weta was not understood in detail for some time, recent investigations have demonstrated the importance of vibrational signalling, which has been described in the New Zealand Cook Strait giant weta, *Deinacrida rugosa* (Fig. 3.1b; Howard et al. 2018) and also in *D. mahoenui* (Fig. 3.3a). In a combination of field and laboratory study of *D. rugosa* communication behaviour at Matiu-Somes Island Scientific and Historic Reserve, adult giant weta were shown to employ substrate-borne vibrational signals in certain mating-related contexts (Howard et al. 2018). In the presence of females, pairs of *D. rugosa* males would engage in a bout of alternating substrate-borne vibrations generated via dorso-ventral tremulation (Fig. 3.2a,b). Vibrational pulses consisted of multiple syllables produced at a low dominant frequency (37.00 ± 1.63 Hz) with average durations of 0.95 s in both males and females (Howard et al. 2018). Signal alternations eventually intensified in duration and amplitude, coupled with parallel but generally non-aggressive contact interactions between the males. Eventually one of the duetting males would withdraw from the contest, with the remaining male often continuing to produce vibrations. In these cases, it appeared that the losing male, as opposed to the female being courted, was the target of these signals. This tremulation behaviour was very rarely shown by females, and when produced, their vibrational signals were not different to those of males (Howard et al. 2018). These observations support a role for vibrational signals in this species in establishing dominance in male-dependent social contexts (Fig. 3.2c,d). In *D. rugosa*, a lowland species that generally courts on the ground, vibrational signals appear to have no role in pair formation as they only occur in close contact or in courtship between males courting the same female, and individuals do not perform vibrotaxis. Playback experiments supported this hypothesis, as only male vibrations could be elicited by playing vibration signals in an arena with a mini-shaker in male–male–female settings (Fig. 3.2d).

The transmission environment likely plays a role in the diversification of substrate-borne signalling behaviour in the New Zealand giant weta. In laboratory studies of *D. connectens*, the alpine scree weta, adult weta placed in the same assortment of social combinations as in studies with *D. rugosa* were never observed to produce substrate-borne signals (DRH, personal observation). Males would engage in intense fighting bouts, and would attempt coercive matings with females, and on some occasions with other males. Alpine scree weta occur on mixed rock substrates, which may impose a constraint on the generation and propagation of substrate-borne signals via tremulation. Additional studies on other alpine and/or rock-dwelling species may provide insights into the general applicability of this hypothesis across the group.

Deinacrida mahoenui (Mahoenui giant weta, Fig. 3.3a) is a large, arboreal species of giant weta found on the North Island of New Zealand. Mahoenui giant weta spend the day in tree canopy refugia, coming out after dark to forage and to search for mates. While adults will sometimes move between isolated trees by descending to the ground, and females oviposit in moist soils, most of their activity is thought to occur on woody substrates 3–10 m above the forest floor (Sherley and Hayes 1993). In laboratory studies, *D. mahoenui* adults were placed in upright

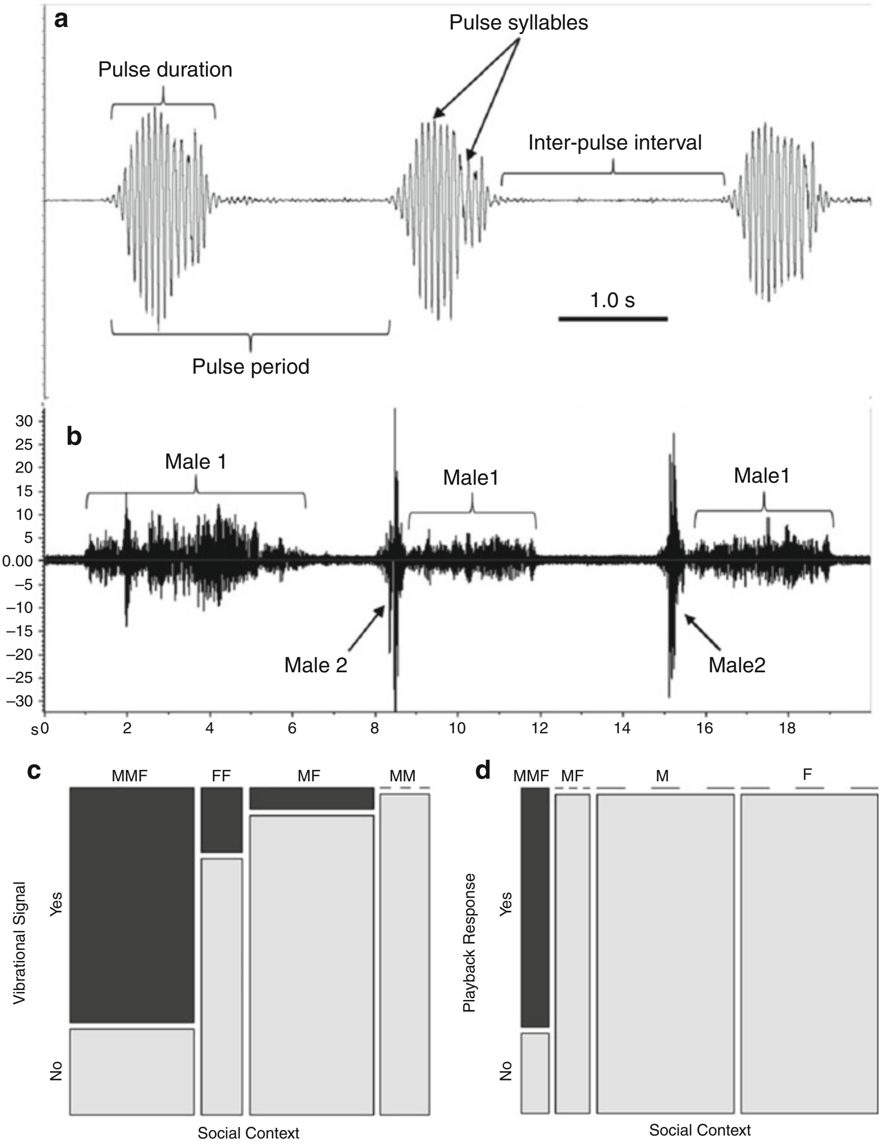


Fig. 3.2 Vibrational signals in the New Zealand Cook Strait giant weta *Deinacrida rugosa*. (**a**) Vibrational pulse recorded from a stationary male after a male–male interaction. Each pulse consists of short pulse syllables. (**b**) Vibration bouts from two males. Note alternating signals that occur in the presence of a female. (**c,d**) Mosaic plot of vibration signal behaviour in different social contexts by constellation of males and females. Data are from (**c**) spontaneous vibration signals and (**d**) responses to vibrational signal playback in arena experiments. Animal constellations are male–male–female (MMF) triads, female–female (FF) dyads, male–female (MF) pairs, individual males (M) and females (F). Adapted from Howard et al. (2018)

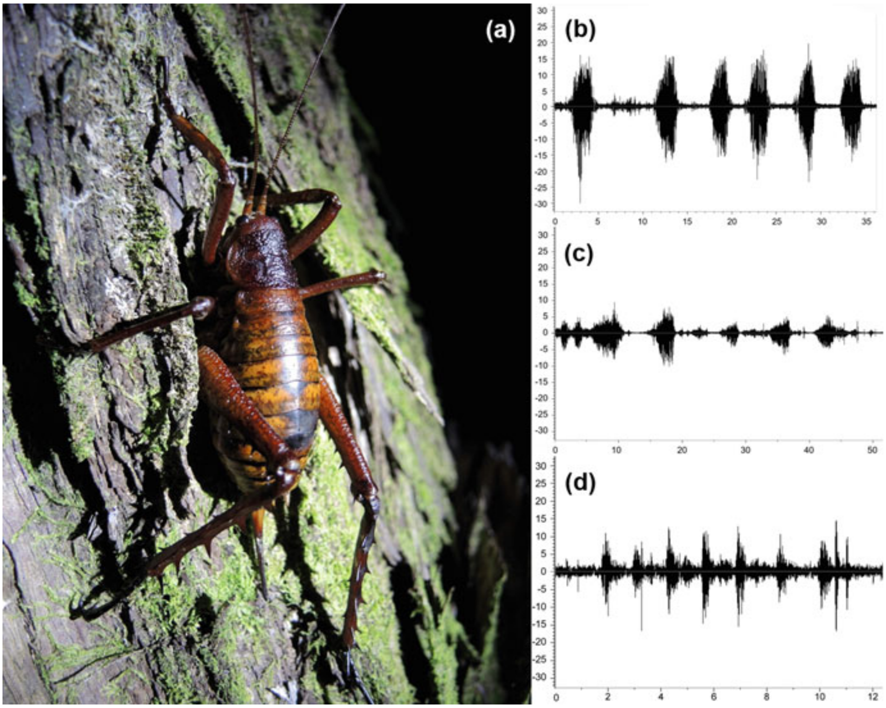


Fig. 3.3 Vibrational signals in the giant weta *Deinacrida mahoenui*. (**a**) Adult female resting on a native manuka tree (Maori kahikātoa, *Leptospermum scoparium*). Adult *D. mahoenui* were observed in laboratory experiments to produce at least three distinctive substrate-borne vibrational signals depending on the social context (unpublished data, DRH). (**b**) Male *D. mahoenui* generated aggressive pulses while in the presence of another male and female, like Cook Strait giant weta (*D. rugosa*). (**c**) Males would also generate a more variable series of pulses with a lower amplitude while in contact with a female during courtship. (**d**) Females were sometimes observed to rhythmically scrape their ovipositor on the substrate while males produced the courtship vibrations prior to copulation

screened behavioural arenas as in ter Hofstede et al. (2015) in male–male–female combinations. Interactions were recorded using laser Doppler vibrometry. *D. mahoenui* adults produced at least three different forms of substrate-borne signals, each unique in its temporal, frequency, and amplitude patterns. Both males and females appeared to produce a form of low amplitude advertisement vibration when not in direct contact with a conspecific. During courtship, however, males produced a slightly different signal that appeared to target the female being courted (Fig. 3.3c), and then produced a third signal type when contesting with a male in a male–male–female combination where all three animals were in contact (Fig. 3.3b). On some occasions, females were observed to respond to the courtship vibration of a male by rhythmically rasping her ovipositor on the substrate, which may represent a vibrational response (Fig. 3.3d). Contrasting sharply with the broken, rocky transmission environment of the alpine scree weta, the woody substrate frequented by Mahoenui giant weta likely affords a much more efficacious signalling condition. Additional studies on the eight other *Deinacrida* species are required to assess whether species' ecology and ecological niche select for substrate-borne signalling across the group.

3.2.3 Ground weta **Hemiandrus**

Among the different groups of weta, the highest number of species has been described for *Hemiandrus*, the ground weta (Taylor Smith et al. 2013; Trewick 2021; Trewick et al. 2021). Additional species await taxonomic description (Trewick and Morgan-Richards 2019). Their distribution in New Zealand is broad, with different habitats of forest, grassland, or shrubs (Chappell et al. 2012). *Hemiandrus* also extend to Eastern Australia with 10 currently undescribed species (Johns 1997, 2001; Gibbs 2001; Monteith and Field 2001), with these Australian species being rain-forest restricted (Monteith and Field 2001). *Hemiandrus* species are notably smaller in size compared to giant, tree and tusked weta (*Motuweta*). They also express a sexual dimorphism with larger females (Chappell et al. 2014). Their femoro-abdominal defence stridulation is seen as ancestral for the New Zealand weta (Field 1993). The *Hemiandrus* species share nocturnal activities for feeding and mating (Gwynne 2004) and stay in burrows in the ground during the day (Taylor Smith et al. 2013). During night activity, insects move on the ground or into the lower vegetation but also trees (Taylor Smith et al. 2013). For vibrational communication, the ground weta species *Hemiandrus pallitarsis* (Fig. 3.1c) is studied in most detail, addressing the signalling behaviour, signal variations, and some mechanosensory organs. Like other New Zealand weta, ground weta are apterous insects (Monteith and Field 2001; Taylor Smith et al. 2013). Some species use stridulation as a secondary defence mechanism, producing a hissing sound, which has been described for few *Hemiandrus* species as `rocking stridulation´ where the animal rocks forward and raise the abdomen against hind legs (Field 2001b; Field and Glasgow 2001). Stridulatory pegs occur on the inner hindleg femur and the anterior three abdominal tergites (Taylor Smith et al. 2016). However, long-range acoustic signalling apparently is not involved in mating behaviour, as all species in the group lack tympana to detect airborne sound (Field 2001b).

Gwynne (2004) investigated ground weta reproductive behaviour with respect to vibrational signals involved in mating for *Hemiandrus pallitarsis* and four undescribed species (*H.* "onokis", *H.* "vicinus", *H.* "promontorius", *H.* "horomaka" ("peninsularis")). Male ground weta drum their abdomens on a plant substrate (generally the leaves of ferns or shrubs) producing substrate-borne vibrational signals in a premating or precopulatory context (Gwynne 2004). The signals consist of drumming bouts containing different pulses; the individual units of a pulse are termed oscillations (Fig. 3.4; Gwynne 2004; Chappell et al. 2012), and the number of pulses in a bout corresponds to the movements of the abdomen (Gwynne 2004). Among the species, the length of abdominal drumming bouts and the number of

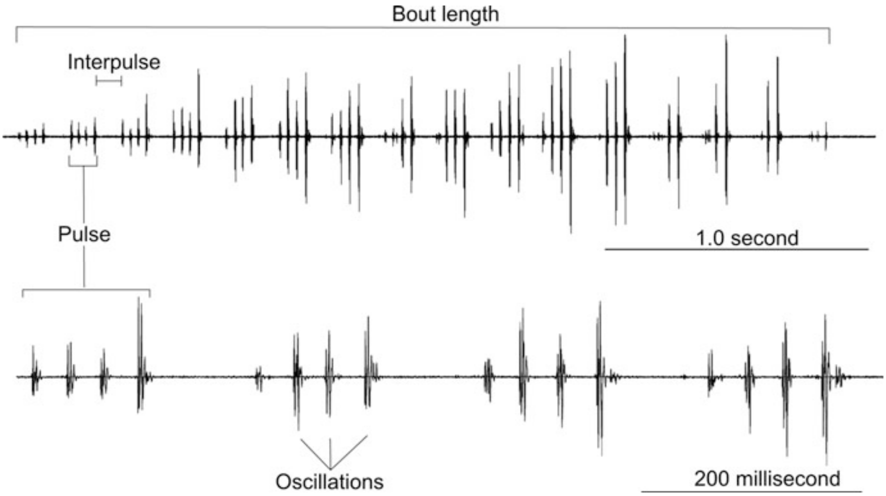


Fig. 3.4 Vibration signals of the ground weta *Hemiandrus pallitarsis* produced by abdominal drumming. Drumming bouts consist of several pulses (upper trace), which are formed by 3–4 oscillations (magnified in lower trace). Provided by Steven A. Trewick, modified from Chappell et al. (2012), with kind permission from Oxford University Press and S. A. Trewick

pulses within each bout differed and are likely species-specific (Gwynne 2004). In *H. pallitarsis*, the bout length and number of pulses in a bout differs among populations, ranging between 2.6–3.3 s, while 3–4 oscillations build a pulse (Gwynne 2004; Chappell et al. 2012). One observation included a *H. pallitarsis* female responding to the male signal by abdominal drumming, resulting in a vibrational duet: in this case, signals differed between sexes as the inter-bout interval was considerably shorter in the female than in the male signals (Gwynne 2004). The drumming behaviour seemed to be restricted to pair formation, as it was not noted during or after copulation (Gwynne 2004). Spontaneous stridulatory movements with single hind legs have also been noted for *Hemiandrus maculifrons* (*Zealandosandrus gracilis*) males maintained in the laboratory, and the possibility of vibrational signal production was considered for the hind legs contacting the substrate (Cary 1981; see Field 2001b).

The variation in drumming signals among populations has been studied in *H. pallitarsis* in the context of species identification, matching two major mitochondrial haplogroups as well as morphogroups with the drumming signals from three North Island populations (Chappell et al. 2012). The signal differences supported intraspecific over interspecific variation in drumming signals, as genetic divergence was not reflected in signal differences (Chappell et al. 2012).

3.2.4 Tree weta Hemideina

Hemideina tree weta occur widely on both islands of New Zealand (Gibbs 2001; Trewick and Morgan-Richards 2009). The name tree weta refers to their use of tunnels or cavities in tree trunks as refugia (galleries); these are produced not by the tree weta but by the tunnelling behaviour of larval beetles (Cerambycidae: *Ochrocydus huttoni*) or butterflies (Hepialidae: *Aenetus virescens*) and subsequently occupied by the weta (Field and Sandlant 2001; Gibbs 2001). Certain species can also inhabit treeless areas by occupying galleries in rocky terrain and logs (*H. ricta*, *H. maori*; Gibbs 2001). Adult male tree weta compete to occupy galleries with several reproductively mature females and fight intruding males (Field 2001c; Field and Sandlant 2001). The intensity of male *Hemideina* defence of these arboreal galleries is associated with the number of adult females in the harem (Kelly 2006a,c).

Five different types of stridulatory behaviours have been described for tree weta, mainly in *Hemideina crassidens* (Fig. 3.1d) and also in related species *H. femorata*, *H. thoracica*, and *H. ricta*: aggression, mating, calling, defence, and disturbance stridulation (Field and Rind 1992; Field 2001b). The stridulatory apparatus in tree weta consists of a file of several parallel ridges on the second abdominal tergite and a field of pegs on the inside of the leg's femur (Field 2001b). The complex stridulatory signals of tree weta, including social signals (calling and mating stridulation), are supposedly derived from an ancestral stridulation behaviour restricted to defence (Field 1993), and are presumed to transfer information via airborne sound. Further study is required to disentangle how the substrate-borne component produced by stridulation may also contribute to information exchange. *Mating stridulation* is the only signal type performed by male weta towards conspecific females, but it does not necessarily precede copulation and often occurs after a failed copulation attempt or rejection by a female. For the intraspecific mating stridulation, all tarsi remain on the ground while moving the abdomen up and down against the hind legs (Field and Rind 1992; Field 2001b). *Calling stridulation* is expressed by male weta in or near the entrance of a gallery; as males may respond to each other, it probably demarks territories in inter-male interactions (Field 2001b). The stridulatory movement patterns have not been observed so far (Field and Rind 1992), but are possibly the same as in mating stridulation (Field 2001b). In *defensive stridulation*, the legs are first raised above the body and moved down along the abdomen, giving broadband sounds (Field and Rind 1992). This defence is mainly used against heterospecific predators rather than conspecifics (Field 2001b). For defensive stridulation, the simultaneous generation of airborne sound and substrate vibration has been shown (McVean and Field 1996; Fig. 3.5). In male agonistic interactions, sound signals produced in *aggression stridulation* by femoro-abdominal stridulation (Field and Rind 1992; Field 2001b) can influence the establishment of dominance of one conspecific individual over another. It is produced during fights, with both individuals stridulating, and winners continuing to stridulate (Field 2001b). However, this seems to be only a minor component of deciding the outcome of aggressive interactions, mainly affecting interactions between males of similar body size

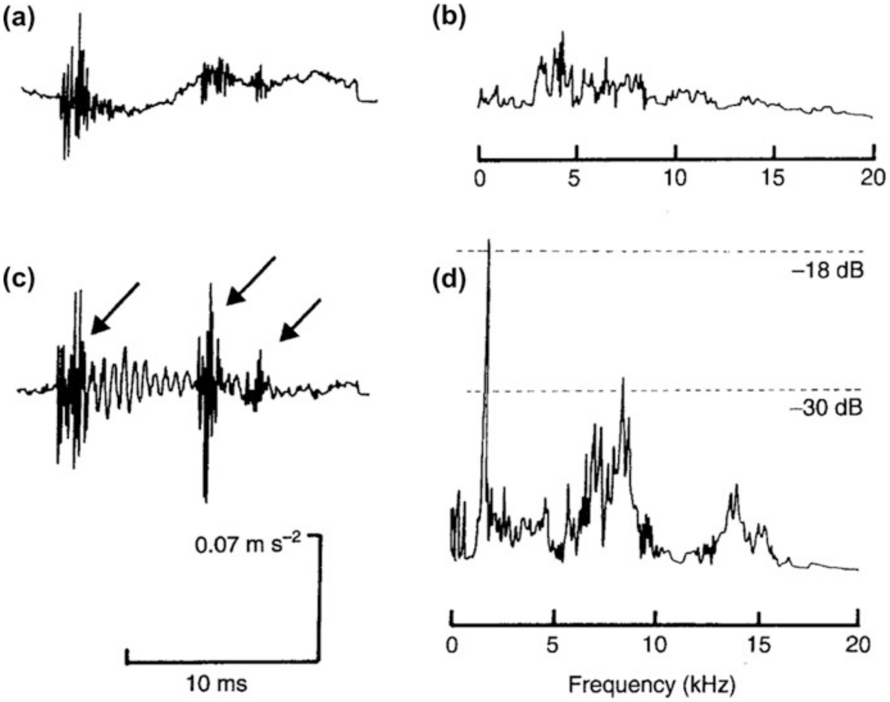


Fig. 3.5 Acoustic and vibrational signals from defensive stridulation of *Hemideina femorata*. The defensive stridulation elicits an acoustic signal: (**a**), oscillogram; (**b**), frequency spectrum, as well as a simultaneous vibrational signal propagated by the manuka tree: (**c**), oscillogram; (**d**), frequency spectrum. The vibrational signal shown here is generated by a single leg rotation and contains three bursts of vibrations (arrows in **c**). In the vibrational power spectrum (**d**), the two peaks at 1.25–1.5 kHz and 7–8 kHz are generated by stridulation while the peak at 13.5 kHz represents background vibrations. Reprinted from McVean and Field (1996), with kind permission from John Wiley & Sons Limited

(Ewers and Cowley 2005). *Disturbance stridulation* is produced when wetas are disturbed in their galleries, by upward abdomen movements similar to mating and aggression stridulation, resulting in sporadic, short sounds (Field 2001b).

So far, the stridulation mechanisms have been studied in more detail than the vibration behaviour. Two aspects of vibrational signalling are relevant in tree weta: tremulation to generate exclusively substrate vibration signals (Field 2001b) and substrate vibrations generated by stridulation in addition to airborne sound (McVean and Field 1996).

3.2.4.1 Tremulation

Tremulation occurs in agonistic interactions between males in both *H. crassidens* and *H. femorata*; although it cannot be ruled out to occur in other species as well (Field 2001b). During the tremulation, the abdomen is moved dorsoventrally but does not contact the stridulatory structures on the femur nor the substratum (Field 2001b). Tremulation has been noted by intruders or residents during fights at the gallery (Field 2001c). Aggressive interactions between males occur at the gallery entrances, and four levels of aggressions were distinguished with increasing aggressive escalation and injury risk (Field 2001c). In low-level aggressive interactions, the gallery resident male can stridulate or tremulate after antennal contact with an intruder (Field 2001c). In higher level aggressive interactions, tremulations have been described when an intruder male tries to remove the resident male from the tree gallery by gripping the resident opponent with the mandibles. The tremulation signals are produced by repeated bursts of movements of the abdomen lasting for about 1 s (Field 2001b). The vibrational signal characteristics for the tremulation behaviour (frequency content, temporal pattern) have not been documented so far. Overall, tremulatory behaviour and its resulting vibrations in tree weta are important in intrasexual (male-male) agonism (Field 2001c); although, recordings taken from inside weta galleries might yield new insights into the function of this behaviour. It was suggested that tremulations induce low-frequency vibrations in the tree, which could be detected by vibration receptor organs in the legs (Field 2001b).

3.2.4.2 Stridulation

In *H. femorata*, defensive stridulation generates airborne sound (Fig. 3.5a,b) but also vibrations transmitted through the stem and branches of Manuka trees (Fig. 3.5c; McVean and Field 1996). The acoustic signals, as well as vibrations induced in substrates, have been documented for their temporal pattern and frequency spectrum, and the vibration component is broad-banded with energy maxima at 1.25–1.5 kHz and 7.5 kHz (Fig. 3.5b,d). These substrate vibrations are detected by other weta by the sensory organs in their legs (see Sect. 3.3.1). Besides the defensive femoro-abdominal stridulation, the social signals (mating and calling stridulation) can likely induce substrate vibrations as well, and deserve additional study.

It has been argued that the matched sensory detection of stridulatory sound and vibrations help the flightless tree weta to detect rival males and orient in the search for galleries in trees. The localisation of a stridulating male and a gallery in a specific tree is more ambiguous from the distance and directionality of acoustic signals, while the simultaneous detection of vibrations along a branch likely indicates more accurately the source of stridulation on the same tree (McVean and Field 1996; Field 2001b). The vibrational signal is also unambiguously indicating a conspecific, as the frequency component is different from the vibrational background noise (Fig. 3.5d). Competing males would thus be more likely to reliably identify a tree with an active gallery based on the multimodal information being transmitted from weta activity (Field 2001b). As noted previously, additional analysis is needed to address the relevance of substrate-borne vibration in the context of multimodality in the New Zealand tree weta.

3.3 Vibration Receptor Mechanisms

3.3.1 Mechanosensory Organs Detecting Vibrations in Insects

Several types of sensilla and sensory organs detect substrate vibrations in insects (Kalmring 1985; Lakes-Harlan and Strauß 2014; Yack 2016). Highly sensitive vibration receptor organs consist of scolopidial sensilla that form so-called chordotonal organs. This type of sensilla is found internally at nearly all body segments and appendages of insects. The individual sensilla consist of 1–4 sensory neurons with further non-neuronal cell types (Field and Matheson 1998). Hair sensilla on the body surface may also detect substrate-borne vibrations (Lakes-Harlan and Strauß 2014; Yack 2016). Certain scolopidial organs located in the insect legs can detect substrate vibrations, as the legs transfer the vibration energy taken up by the tarsi (Kalmring 1985; Hill 2008; Stritih Peljhan and Strauß 2018). The sensory organ that usually has the highest sensitivity to substrate vibrations, and is present in most insects, is the subgenual organ. It is located in the proximal tibia and responds to accelerations of vibration stimuli (Schnorbus 1971; Field and Matheson 1998; Čokl and Virant-Doberlet 2009; Yack 2016; Strauß et al. 2021). The subgenual organ responds best to vibration stimulation along the tibia's main axis (e.g., Rohrseitz and Kilpinen 1997; Strauß and Lakes-Harlan 2017). In tympanate Ensifera, including the weta, the subgenual organ together with adjacent chordotonal organs forms the complex tibial organ (see Nishino and Field 2003). In other orthopteroid insects, including taxa lacking tympana, these sensory organs in the proximal tibia are together also called the subgenual organ complex.

The specific attachment and coupling of the sensory organs to cuticle or the hemolymph space in the legs influence the responsiveness to vibrational stimuli. Important vibration receptor organs are the complex tibial organ in the proximal tibia and the femoral chordotonal organ (Strauß et al. 2021). In tree weta, both sensory organs have been studied in detail with respect to the comparative neuroanatomy (complex tibial organ: Ball and Field 1981; Nishino and Field 2003; femoral chordotonal organ: Matheson and Field 1990; Nishino 2003). Importantly, the physiological responses of the complex tibial organ to vibration stimuli transmitted through the natural substrate have been demonstrated in *Hemideina femorata* (McVean and Field 1996).

3.3.1.1 The Complex Tibial Organ of Anostostomatidae

For Anostostomatidae, most neurobiological studies have focussed on the foreleg tympanal organs of *Hemideina* (tree weta) species (Field et al. 1980; Hill 1980; Ball 1981; Ball and Field 1981; Field 2001d; Nishino and Field 2003; Lomas et al. 2011, 2012). The hearing organ (*crista acustica*) is associated with an array of chordotonal organs, which together form the complex tibial organ (*Hemideina crassidens*: Ball and Field 1981; *Hemideina femorata*: Nishino and Field 2003). These organs are the subgenual organ, the intermediate organ, and the accessory organ. This sensory organisation is also typical for other Ensifera like Tettigoniidae, Prophalangopsidae, and Stenopelmatidae (Strauß and Lakes-Harlan 2009; Strauß 2019). The serial organisation in all three leg pairs has been documented for *Hemideina femorata* (Nishino and Field 2003). The atympanate species of *Hemiandrus* ground weta are putatively insensitive to airborne sound (Field 2001b; Gwynne 2004) but can detect substrate vibrations through the subgenual organ.

The subgenual organ in the tree weta *Hemideina femorata* consists of ~70 scolopidial sensilla, the intermediate organ and the foreleg *crista acustica* contain on average up to 21 and 53 sensilla, respectively, while the accessory organ is the smallest part in the complex tibial organ with maximally 12 sensilla (Nishino and Field 2003; see also Ball and Field 1981). The subgenual sensilla are arranged in a hemicircle (Fig. 3.6a), and their dendrites insert into a cellular mass spanning the hemolymph channel, which is presumably moved in the hemolymph along the proximo-distal direction of the tibia from vibrations transmitted along the leg. In the ground weta *Hemiandrus pallitarsis*, the same organs are present (Figs. 3.6b, 3.7), which contain fewer sensilla than in *Hemideina* (subgenual organ: ~50 sensilla, intermediate organ: 15–19, *crista acustica* homolog: ~12, accessory organ: ~10; Strauß et al. 2017). However, the sensory complex in *Hemiandrus* lacks adaptations relating to sound reception and instead is more suited to detect vibrations transmitted from the substrate (Table 3.2). In particular, the intermediate organ and the *crista acustica* homolog are closely attached to the tibia's inner cuticle, and they may respond preferentially to vibrations transmitted via the cuticular surface of the leg, independently from the subgenual organ (Strauß et al. 2017). In addition, the tracheae are not enlarged as in tree weta forelegs (Fig. 3.6). The intermediate organ of tree weta is compact and attached dorsally to the cuticle by connective strands (Nishino and Field 2003). The functional relevance of these differences in the intermediate organ mass compared to other Ensifera is so far not understood. The intermediate sensilla in tympanate Tettigoniidae are bifunctional, responding to both airborne sound and substrate vibrations (e.g., Kalmring et al. 1994); it will be interesting to study the functional morphology with respect to vibration detection also in different weta. For the tree weta *crista acustica*, the highest number of sensilla is present in the forelegs bearing the tympana (Nishino and Field 2003), while in the atympanate ground weta no such serial gradient is present between the leg pairs (Fig. 3.7; Strauß et al. 2017). The physiology of the *crista acustica*/*crista acustica* homolog in the atympanate legs is so far not investigated. However, the

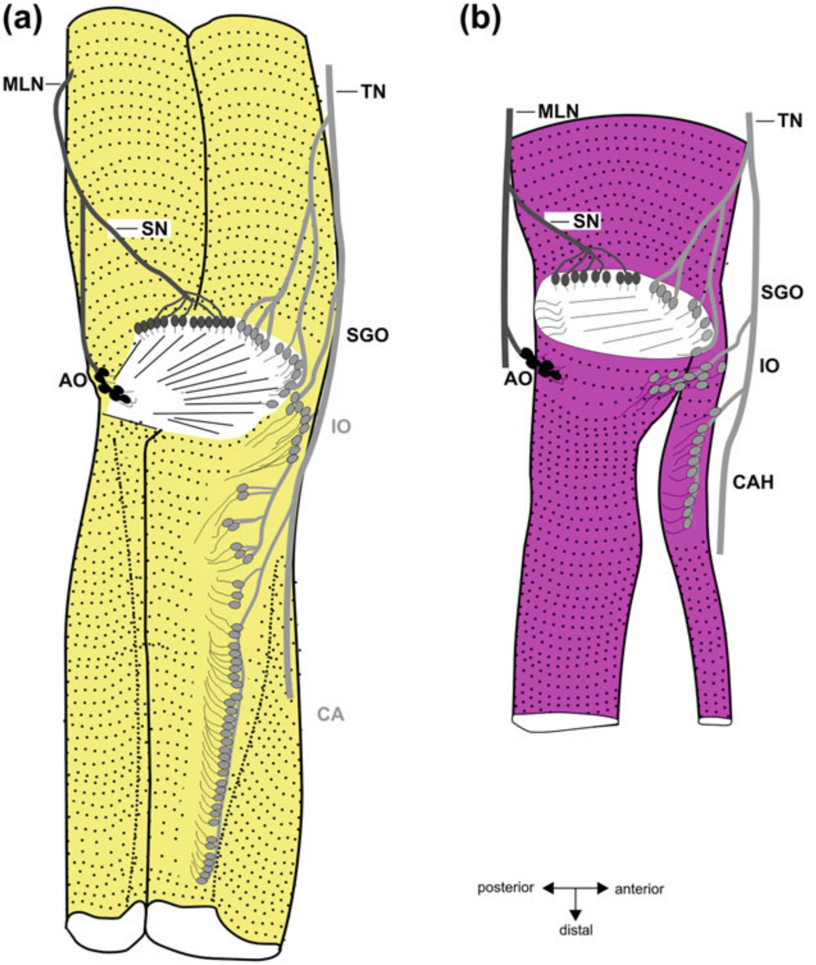


Fig. 3.6 Schematic of the complex tibial organ: (**a**) tree weta *Hemideina crassidens*, (**b**) ground weta *Hemiandrus pallitarsis*. Tibial trachea are shown in (**a**) yellow and (**b**) magenta. Adapted from (**a**) Ball and Field (1981), (**b**) Strauß et al. (2017). Not drawn to scale between both species. Abbreviations: **AO**, accessory organ; **CA**, *crista acustica*; **CAH**, *crista acustica* homolog; **IO**, intermediate organ; **MLN**, main leg nerve; **SGO**, subgenual organ; **SN**, subgenual nerve; **TN**, tympanal nerve. Terminology for nerves based on Ball and Field (1981), see also Schumacher (1973)

similar organisation of the sensory organs in all leg pairs suggests similar sensory functions, and vibration detection is a likely role in the absence of tympanal hearing. The accessory organ in both tree weta and ground weta is placed closely under the hypodermis. The dendrites notably are oriented in the proximal direction of the leg (Nishino and Field 2003; Strauß et al. 2017). This organisation of the accessory organ may support the detection of low-frequency vibrations or cuticular strain (see review in Strauß 2017a). Physiological recordings in another ensiferan insect, the

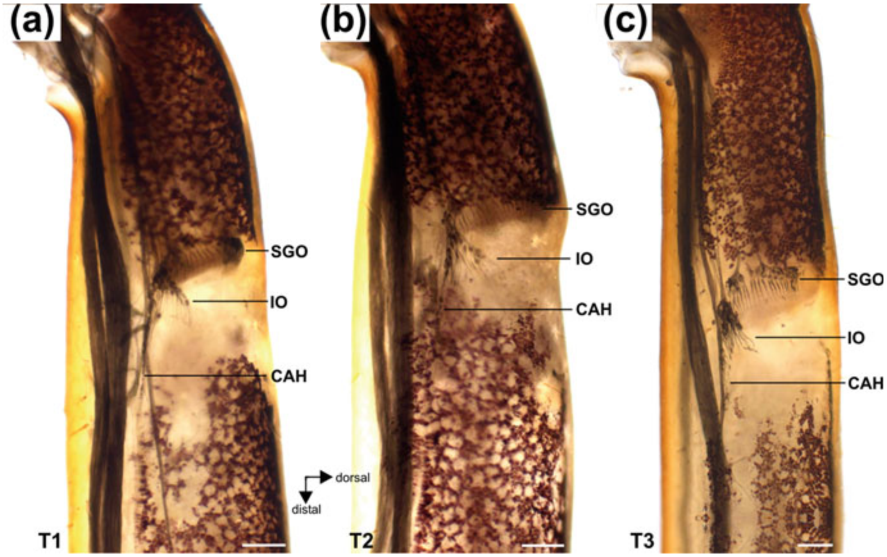


Fig. 3.7 Complex tibial organ of the ground weta *Hemiandrus pallitarsis* in serial organisation (anterior view) (adapted from Strauß et al. (2017). Scale = 100 μm. Abbreviations: **CAH**, *crista acustica* homolog; **IO**, intermediate organ; **SGO**, subgenual organ; **T1**, foreleg; **T2**, midleg; **T3**, hindleg

Table 3.2 Comparison of the complex tibial organs in ground weta and tree weta

	Hemiandrus pallitarsis Ground weta	*Hemideina species* Tree weta
Tympana	No tympanal membranes	Large foreleg tympana
Acoustic vesicles	No tracheal vesicles in tibia	Tracheal vesicles in tibia
SGO sensilla	Medium number of SGO sensilla (~50)	Many SGO sensilla (~70)
CA sensilla foreleg	Low number of CAH sensilla (12)	High number of CA sensilla (50–53)
Serial CA/ CAH gradient	No serial gradient in number of CAH sensilla from foreleg to hindleg	Clear gradient in CA sensilla from foreleg (high) to hindleg (low)
Size of CA/ CAH somata	Similar over CAH	Become smaller in distal CA
Distal CA/ CAH sensilla	No double row of CAH somata	Double row of distal CA somata
IO anatomy	Prominent IO extends dorsally in tibia	IO compact
IO mass	IO/CAH attached jointly at dorsal cuticle over broad area	IO mass attached dorsally by connective strands

Adapted from J. Strauß et al. (2017)

cave cricket *Troglophilus cavicola*, also support a tuning of accessory sensilla to low-frequency vibrations at 50–300 Hz (Stritih-Peljhan et al. 2019). In sum, the functional organisation of the complex tibial organs, especially in the ground weta, suggests different adaptations to vibration detection for the different organs: the subgenual organ apparently detects vibrations transmitted longitudinally in the hemolymph, the intermediate organ and the *crista acustica* homolog likely detect mainly vibrations transmitted over the leg cuticle, and the accessory organ presumably detects low-frequency vibrations at the posterior tibia (Strauß et al. 2017). Further insights on the vibrosensory adaptations of the complex tibial organ could be gained by matching the frequency components of the *Hemiandrus* drumming signal with the physiological tuning to vibration frequencies for the distinct sensory organs.

The distribution of the sensory organs in the complex tibial organ in related Ensifera (Strauß and Lakes-Harlan 2009; Strauß 2017b) also suggests that the number and organisation of sensory organs in giant weta resemble that of tree and ground weta, by having a complex tibial organ consisting of the subgenual organ, the intermediate organ, the *crista acustica*, and the accessory organ. As species of giant weta are highly vulnerable and protected (e.g., Pennisi 2017), comparative data would best be gained on the complex tibial organ using non-invasive µCT methods on available collection material.

3.3.1.2 The Femoral Chordotonal Organ

The femoral chordotonal organ has been studied in the tree weta *Hemideina femorata* for its neuroanatomy (Nishino 2003) and physiology (Field and Rind 1981; Field 2001d). The organ is an important leg proprioceptor that monitors the position and movements of the tibia, as it is attached proximally to the cuticle by connective tissue, and distally to two separate ligaments that connect to a cuticular apodeme. In Orthoptera, this organ contains up to several hundred sensilla located in separate groups of sensilla (scoloparia) (e.g., locust: Field and Pflüger 1989; cricket: Nishino and Sakai 1997). In *H. femorata*, the femoral chordotonal organ consists of a dorsal and a ventral scoloparium. The dorsal scoloparium consists of the dorsal scoloparium neurons (DSN) with 23–27 sensory neurons on average. The ventral scoloparium is divided into a ventral and dorsal group of neurons, while the ventral group has a ventral and a dorsal subgroup (vVG and dVG) with averages of 110–122 and 14–20 sensory neurons (Nishino 2003). The dorsal group of the ventral scoloparium (DG) contains 23-30 sensory neurons (Nishino 2003).

The femoral chordotonal organ is not only sensitive to active or passive leg movements, but also to movements of the tibia induced from the substrate by vibrations. Physiological evidence for the detection of substrate vibrations by the femoral chordotonal organ comes from the dorsally located proximal scoloparium of locusts (Field and Pflüger 1989) and from the stick insect (Stein and Sauer 1999). In tree weta, the sensilla are attached to the cuticle via connective tissue, and their dendrites extend either into the ligaments linked to the receptor apodeme or into connective tissue attached to the ligament (Field 2001d; Nishino 2003).

Additional possible vibration receptor organs like the tibio-tarsal organ or the tarsal-pretarsal organ (Lakes-Harlan and Strauß 2014) are so far not studied physiologically in weta. The tibio-tarsal organ is attached to the muscle apodeme by a large ligament (Field 2001d), presumably indicating a specialisation in proprioception, rather than vibroreception.

3.3.2 Physiological Responses to Substrate Vibrations

While most investigations on weta sensory physiology address the tympanal hearing organs, a physiological approach has also demonstrated the response of the complex tibial organ (subgenual organ) to vibration stimuli from a conspecific on the natural substrate (McVean and Field 1996). This study investigated the transmission of vibration stimuli, which are generated during defence stridulation together with airborne sounds in manuka trees (Fig. 3.5). In addition, physiological recordings from the complex tibial organ afferents in the atympanate midleg of *H. femorata* in the sensory nerve (N5B1; homologous to the tympanal nerve of the foreleg) demonstrated the neuronal response to defined vibration stimuli at specific frequencies, and also to the vibrations elicited by the defensive stridulation of a conspecific stridulating on the same tree log (Fig. 3.8). The specific sensory response showed a linear increase in action potentials in response to increasing vibration amplitudes for stimulus frequencies from 20 Hz-3 kHz (McVean and Field 1996). The vibration threshold was lowest at 20 Hz and 1 kHz, for the latter frequency at 0.015 m/s^2. This sensitivity tunes well with the presence of a strong signal peak around 1 kHz (McVean and Field 1996; see Fig. 3.5d). Signals with an amplitude of 0.34 m/s^{-2} have been estimated to be detectable over a distance of 2–3 m on a tree (McVean and Field 1996).

In the ground weta *H. pallitarsis*, preliminary results on the responses of sensory organs to vibrational stimuli delivered in vertical direction to the midleg tarsi measured by whole-nerve recordings indicated a high sensitivity to vibrations (L. H. Field and J. Strauß, unpublished).

3.3.3 Central Processing of Vibrosensory Inputs

Scolopidial sensilla contain primary sensory neurons that project axons into the central nervous system. The afferents usually terminate in the ipsilateral ganglion of the specific segment (Field and Matheson 1998). The afferents of tree weta (*H. femorata*) have been studied by anterograde axonal tracing. An important primary neuropil in the processing of mechanosensory information is the median ventral association centre (mVAC), which is an evolutionarily conserved neuropil for processing mechanosensory input in insects (Pflüger et al. 1988; Lakes-Harlan et al. 1999; Nishino et al. 2016). In Orthoptera, this mechanosensory centre is usually bimodal, processing both the auditory and vibratory inputs from scolopidial organs (Kalmring 1983; Kalmring and Kühne 1983). For the complex tibial organ,

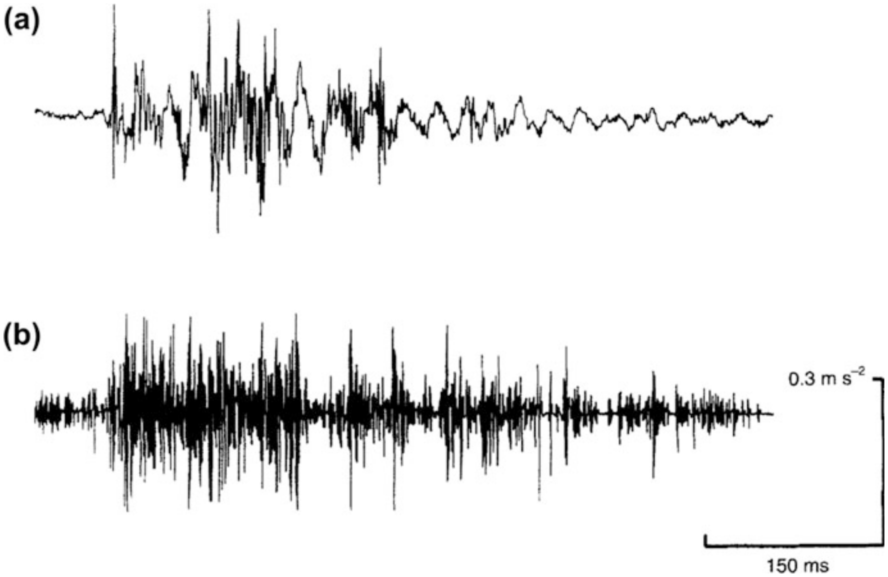


Fig. 3.8 Detection of stridulation-induced vibrations by the leg vibroreceptors in *H. femorata*. (**a**) Signal of substrate vibrations induced in a log by defensive stridulation. (**b**) Simultaneous recording of summed action potentials from the mesothoracic sensory leg nerve (N5B1) in a second weta placed on the same log. Reprinted from McVean and Field (1996), with kind permission from John Wiley & Sons Limited

including the auditory sensilla, and for the femoral chordotonal organ, the afferents from sensory neurons of different organs or groups form complex somatotopic projections in the mVAC, relating to their respective positions in the periphery (Nishino 2003; Nishino and Field 2003). This would allow in the next step the processing of substrate vibrations and airborne sound by distinct interneurons. So far, the central neural network (interneurons) underlying the analysis of these modalities has not been investigated in weta.

The subgenual organ projects prominently into the mVAC for both tree weta (Nishino and Field 2003) and ground weta (Strauß et al. 2017). In tree weta, the projection areas of the accessory organ include the mVAC, supporting a physiological role in detecting low-frequency vibrations in the tree weta *Hemideina femorata* (Nishino and Field 2003). In the femoral chordotonal organ, the projection pattern of sensory afferents is complex and for several neuron groups represents the relative positions of their somata (Nishino 2003). The afferents of the dorsal scoloparium neurons (DSN), the dorsal group of the ventral scoloparium (DG), and also the dorsal subgroup from the ventral group of the ventral scoloparium (dVG) project into the lateral and medial mVAC. Especially the afferents from the dorsal group (DG) and the dorsal scoloparium sensilla overlap with the afferents from the vibrosensory subgenual organ and accessory organ (Nishino 2003). This projection argues for a role in vibration detection for sensilla in these parts of the femoral chordotonal organ, especially since such sensory projections are also found for the homologous sensilla in locusts (the proximal scoloparium of the femoral chordotonal organ), which physiologically respond to vibration stimuli (Field and Pflüger 1989). Since the afferents from the dorsal group of the ventral scoloparium afferents also target motor and auditory neuropils, they are probably less specialised for vibration detection (Nishino 2003).

3.4 Open Questions in Weta Biotremology

Following initial studies on the sense of hearing in weta, more recent studies have also demonstrated the importance of vibrational detection for communication in three groups of weta (Table 3.3). Obviously, the use of these two modalities in communication is not mutually exclusive, and the detection of substrate vibrations induced by stridulation also producing airborne sound highlights how tree weta could use both information sources simultaneously for orientation in the habitat (McVean and Field 1996; Field 2001b). Remarkably, weta show a diverse array of vibrational signalling behaviours including tremulation, drumming, and stridulation. For a small taxon that is rather limited in the number of species, this indicates a rather robust evolutionary diversification of vibrational signalling. This evolutionary diversification likely parallels ecological niche differentiation across the different weta groups. Notably, giant weta present a rare case of insects tremulating directly on soil substrates (see Alexander 1961 for field crickets, Hayashi et al. 2018 for mole crickets), which is likely increased in efficiency due to the large body mass of these insects (Howard et al. 2018). In this group, gigantism is yet another result of the 80 million year evolutionary experiment that is the New Zealand fauna (McIntyre 2001; Gibbs 2006).

At the present moment, the data on vibrational communication systems in New Zealand weta are centred on a few species, and more data need to be accumulated for each weta group. Hence, it may be too early to attempt comparisons

Table 3.3 Comparison of vibrational communication behaviours in New Zealand weta

	Deinacrida Giant weta	*Hemiandrus* Ground weta	*Hemideina* Tree weta
Signal production	Tremulation	Abdominal drumming	Femoro-abdominal stridulation
Substrate	Soil, woody tree limbs	Foliage	Tree trunk
Behavioural context	Intraspecific: Intrasexual agonism, pair formation	Intraspecific: Mate attraction	Intraspecific: Male agonism; Interspecific: Aggression
Vibrational duetting	Male–male, male–female	Male–female	Simultaneous during male–male fights
Vibrotaxis between sexes	*D. rugosa*—No *D. mahoenui*—Possibly	Yes	Possible identification of trees with galleries?

References in the main text

between the distinct groups and infer adaptations for the different signalling systems. So far, the focus of study and the depth of insights gained differ for the different weta groups with respect to their vibrational behaviour and signals. Some obvious aspects can be indicated, which will require further investigation to compare the signalling systems across the different weta. The study of weta biotremology is just building a framework to accommodate proximate and ultimate aspects of vibration communication systems, and currently requires us to gather broader data sets.

First, for all three groups of weta, few species have been studied in detail for vibrational behaviours (*D. rugosa*, *H. pallitarsus, H. crassidens*, *H. femorata*). It will be necessary, as well as interesting, to sample other members of these groups to identify commonalities and specific adaptations in signalling behaviours, substrates, and signal characteristics within and between these groups, including the biogeography and the phylogenetic relatedness.

Second, it is notable that even for species where the vibrational behaviour is described, an extended characterisation of the signals is lacking, which would allow more detailed comparisons. Especially with respect to signal characteristics and sensory detection, the current knowledge is uneven across taxa. For example, in *Hemideina* species, tremulation signals have not been documented, and in *H. pallitarsis*, the frequency spectrum of drums is not analysed. The acoustic analysis of these signals will provide a rich field of investigations. The data obtained so far on signals can direct future investigations to measure similar parameters for better comparisons between species. These should include (but are not limited to) the frequency spectrum/components, dominant frequency, pulse structure, pulse rate and duration, and information on the composition of the signal bouts as in Howard et al. (2018). The signal structures, however, clearly differ between species of giant and ground weta. The giant weta produces bouts consisting of several continuous pulses, each with on average of 22 pulse syllables (Howard et al. 2018; Fig. 3.2a). The *Hemiandrus* signals have a more complex structure, as the individual pulses in a signal bout consist of 3–4 oscillations. In the terminology of Howard et al. (2018), the ground weta oscillations again consist of several syllables (compare Gwynne 2004; Chappell et al. 2012). One important parameter for signal complexity could arise from the use of the continuous, less differentiated vibration signals on soil/detritus substrates in giant weta, and the possible occurrence and characteristics of vibrational signals from species in different habitats, like arboreal species, will give insights on adaptive differentiation of signals (Howard et al. 2018). Third, the communication systems need to be studied in depth in the species' natural habitat, e.g., identifying the signalling distances and signal transmission properties. This will eventually also include sensory and behavioural physiology, to link signals to the sensory and behavioural thresholds, frequency tuning of sensory organs, signal recognition, and the directional discrimination (see Hill 2008, 2009).

In the growing field of biotremology, the interest in the endemic New Zealand insects and their communication will hopefully continue and provide further understanding of the behaviour and mechanisms of vibrational signalling in this primitive group. Such studies will also allow insights into the evolutionary diversification and adaptations of vibrational signalling in Orthoptera, which are well studied for several taxa (see the in-depth discussion in Field 2001b; Stritih and Čokl 2014; Howard et al.

2018). While weta are a fascinating group to study in their own right, such behavioural data will be particularly interesting from a comparative view of the Ensifera at large, in order to better understand the evolution of both sound and vibration signals and their detection mechanisms. Given that in the weta variation is also found in the occurrence of tympanal membranes, from the complete lack of hearing organs to having one or two tympana in the tibia, this highlights the group as a key taxon to reconstruct the evolution of auditory and vibratory structures in the Ensifera.

Acknowledgements We wish to thank Peggy Hill and Andreas Wessel for inviting a contribution on biotremology, and Meta Virant-Doberlet for comments on the manuscript. We are indebted to Steve Trewick for providing the version of Fig. 4, and information on the taxonomy of weta species. We thank Oxford University Press and John Wiley & Sons for kind permission to reprint figures. We also thank Nataša Stritih-Peljhan for helpful comments on an earlier manuscript version. We acknowledge and thank the Te Atiawa, Taranaki, Ngati Tama, and Ngati Ruanui Maori Iwi, along with the Waikato Tainui for approval of research and access to the lands on which much of this described research occurred. Finally, we thank Dr. Larry Field for the research cooperation on *H. pallitarsis* at the Edward Percival Field Station, Kaikoura, New Zealand, and Dr. Corinne Watts for assistance with initial work with *D. mahoenui* at Landcare Research, Hamilton, New Zealand, and UNH students Brooke Woelber, Sarah Dodgin, and Megan Bezdicek for valuable assistance with giant weta field studies. J. S. is supported by a grant from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)—STR 1329/2-1.

References

Alexander RD (1961) Aggressiveness, territoriality, and sexual behavior in field crickets (Orthoptera: Gryllidae). Behaviour 17:130–223

Alexander RD (1962) Evolutionary change in cricket acoustical communication. Evolution 16:443–467

Bailey WJ (1991) The acoustic behaviour of insects. An evolutionary perspective, Chapman and Hall, London

Ball EE (1981) Structure of the auditory system of the weta *Hemideina crassidens* (Blanchard, 1851) (Orthoptera, Ensifera, Gryllacridoidea, Stenopelmatidae). 2. Ultrastructure of the auditory sensilla. Cell Tissue Res 217:345–359

Ball EE, Field LH (1981) Structure of the auditory system of the weta *Hemideina crassidens* (Blanchard, 1851) (Orthoptera, Ensifera, Gryllacridoidea, Stenopelmatidae). 1. Morphol Histol Cell Tissue Res 217:321–343

Buckley TR, Krosch M, Lesche RAB (2015) Evolution of New Zealand insects: summary and prospectus for future research. Austral Entomol 54:1–27

Caldwell MS (2014) Interactions between airborne sound and substrate vibration in animal communication. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin Heidelberg, pp 65–92

Cary PRL (1981) The biology of the weta *Zealandosandrus gracilis* (Orthoptera: Stenopelmatoidea) from the Cass region. M. Sc. Thesis, Zoology, University of Canterbury, Christchurch, New Zealand

Cary PRL (1983) Diet of the ground weta *Zealandosandrus gracilis* (Orthoptera: Stenopelmatidae). New Zeal J Zool 10:295–297

Chappell EM, Trewick SA, Morgan-Richards M (2012) Shape and sound reveal genetic cohesion not speciation in the New Zealand orthopteran, *Hemiandrus pallitarsis*, despite high mitochondrial DNA divergence. Biol J Linn Soc 105:169–186

Chappell EM, Webb DS, Tonkin JD (2014) Notes on sexual size dimorphism, sex ratio and movements of adult ground weta *Hemiandrus maculifrons* (Walker) (Orthoptera: Anostostomatidae). N Z Entomol 37:83–92

Cigliano MM, Braun H, Eades DC, Otte D (2021) Orthoptera species file Version 5.0/5.0. [07.07.2021]. http://Orthoptera.SpeciesFile.org

Cocroft RB, Rodríguez RL (2005) The behavioral ecology of insect vibrational communication. Bioscience 55:323–334

Čokl A, Virant-Doberlet M (2009) Vibrational communication. In: Resh VH, Cardé RT (eds) Encyclopedia of insects, 2nd edn. Academic Press, Amsterdam, pp 1034–1038

Duthie C, Gibbs G, Burns KC (2006) Seed dispersal by weta. Science 31:1575–1575

Elias DO, Mason AC (2010) Signaling in variable environments: substrate-borne signaling mechanisms and communication behavior in spiders. In: O'Connell-Rodwell CE (ed) The use of vibrations in communication: properties, mechanisms and function across taxa. Research Signpost, Kerala, India, pp 25–46

Elias DO, Mason AC, Hoy RR (2004) The effect of substrate on the efficacy of seismic courtship signal transmission in the jumping spider *Habronattus dossenus* (Araneae: Salticidae). J Exp Biol 207:4105–4110

Ewers RM, Cowley G (2005) The role of sound production in determining dominance in agonistic interactions between male tree wetas (*Hemideina crassidens*, Orthoptera: Anostostomatidae). N Z Nat Sci 30:11–17

Field LH (1978) The stridulatory apparatus of New Zealand wetas in the genus *Hemideina* (Insecta: Orthoptera: Stenopelmatidae). J Roy Soc N Z 8:359–875

Field LH (1982) Stridulatory structures and acoustic spectra of New Zealand wetas (Orthoptera: Stenopelmatidae). Int J Insect Morphol Embryol 11:39–51

Field LH (1993) Structure and evolution of stridulatory mechanisms in New Zealand wetas (Orthoptera: Stenopelmatidae). Int J Insect Morphol Embryol 22:163–183

Field LH (2001a) Introduction. In: Field LH (ed) The biology of wetas, king crickets and their allies. CABI Publishing, Wallingford, pp xv–xx

Field LH (2001b) Stridulatory mechanisms and associated behaviour in New Zealand weta. In: Field LH (ed) The biology of wetas, king crickets and their allies. CABI Publishing, Wallingford, pp 271–295

Field LH (2001c) Aggression behaviour in New Zealand tree wetas. In: Field LH (ed) The biology of wetas, king crickets and their allies. CABI Publishing, Wallingford, pp 333–349

Field LH (2001d) Sensory physiology. In: Field LH (ed) The biology of wetas, king crickets and their allies. CABI Publishing, Wallingford, pp 429–458

Field LH, Glasgow S (2001) Defence behavior. In: Field LH (ed) The biology of wetas, king crickets and their allies. CABI Publishing, Wallingford, pp 297–316

Field LH, Jarman TH (2001) Mating behaviour. In: Field LH (ed) The biology of wetas, king crickets and their allies. CABI Publishing, Wallingford, pp 317–332

Field LH, Matheson T (1998) Chordotonal organs of insects. Adv Insect Physiol 27:1–228

Field LH, Pflüger H-J (1989) The femoral chordotonal organ: a bifunctional orthopteran (*Locusta migratoria*) sense organ? Comp Biochem Physiol A 93:729–743

Field LH, Rind FC (1981) A single insect chordotonal organ mediates inter- and intra-segmental leg reflexes. Comp Biochem Physiol 68A:99–102

Field LH, Rind FC (1992) Stridulatory behavior in a New Zealand weta, *Hemideina crassidens*. J Zool 228:371–394

Field LH, Roberts KL (2003) Novel use of hair sensilla in acoustic stridulation by New Zealand giant wetas (Orthoptera: Anostostomatidae). Arthropod Struct Dev 31:287–296

Field LH, Sandlant GR (2001) The gallery-related ecology of New Zealand tree wetas, *Hemideina femorata* and *Hemideina crassidens* (Orthoptera, Anostostomatidae). In: Field LH (ed) The biology of wetas, king crickets and their allies. CABI Publishing, Wallingford, pp 243–257

Field LH, Hill KG, Ball EE (1980) Physiological and biophysical properties of the auditory system of the New Zealand weta *Hemideina crassidens* (Blanchard, 1851) (Ensifera: Stenopelmatidae). J Comp Physiol A 141:31–37

Gibbs G (1998) Raukumara tusked weta: discovery, ecology and management implications. Conservation advisory science notes 218, department of conservation, Wellington NZ

Gibbs GW (1999) Four new species of giant weta, *Deinacrida* (Orthoptera: Anostostomatidae: Deinacridinae) from New Zealand. J Roy Soc N Z 29:307–324

Gibbs GW (2001) Habitats and biogeography of New Zealand's deinacridine and tusked weta species. In: Field LH (ed) The biology of wetas, king crickets and their allies. CABI Publishing, Wallingford, pp 35–55

Gibbs GW (2006) Ghosts of Gondwana. The history of life in New Zealand, Craig Potton Publishing, Nelson, NZ

Gordon SD, Tiller B, Windmill JF, Krugner R, Narins PM (2019) Transmission of the frequency components of the vibrational signal of the glassy-winged sharpshooter, *Homalodisca vitripennis*, within and between grapevines. J Comp Physiol A 205:783–791

Greenfield MD (2002) Signalers and receivers. Oxford University Press, Oxford, Mechanisms and evolution of arthropod communication

Griffin MJ, Trewick SA, Wehi PM, Morgan-Richards M (2011) Exploring the concept of niche convergence in a land without rodents: the case of weta as small mammals. New Zeal J Ecol 35: 302–307

Gwynne D (2004) Reproductive behavior of ground weta (Orthoptera: Anostostomatidae): drumming behavior, nuptial feeding, post-copulatory guarding and maternal care. J Kansas Entomol Soc 77:414–428

Hayashi Y, Yoshimura J, Roff DA, Kumita T, Shimizu A (2018) Four types of vibration behaviors in a mole cricket. PLoS One 13(10):e0204628

Hedwig B (2014) Towards an understanding of the neural basis of acoustic communication in crickets. In: Hedwig B (ed) Insect hearing and communication. Springer, New York, pp 123–141

Hill KG (1980) Physiological characteristics of auditory receptors in *Hemideina crassidens* (Blanchard) (Ensifera: Stenopelmatidae). J Comp Physiol 141:39–46

Hill PSM (2008) Vibrational communication in animals. Harvard University Press, Cambridge MA

Hill PSM (2009) How do animals use substrate-borne vibrations as an information source? Naturwissenschaften 96:1355–1371

Hill PSM (2012) Do insect drummers actually drum? Studying vibrational communication across taxa. Mitt DGaaE 18:603–611

Hill PSM (2014) Stretching the paradigm or building a new? Development of a cohesive language for vibrational communication. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin Heidelberg, pp 13–30

Hill PSM, Shadley JR (1997) Substrate vibration as a component of a calling song. Naturwissenschaften 84:460–463

Howard DR, Schmidt AP, Hall CL, Mason AC (2018) Substrate-borne vibration mediates intrasexual agonism in the New Zealand Cook Strait giant weta (*Deinacrida rugosa*). J Insect Behav 31:599–615

Ingrisch S, Rentz DCF (2009) Orthoptera (grasshoppers, locusts, katydids, crickets). In: Resh VH, Carde RT (eds) Encyclopedia of insects, 2nd edn. Academic Press, Amsterdam, pp 732–743

Johns PM (1997) The Gondwanaland Weta: family Anostostomatidae (formerly in Stenopelmatidae, Henicidae or Mimnermidae): nomenclatural problems, world checklist, new genera and species. J Orthoptera Res 6:125–138

Johns PM (2001) Distribution and conservation status of ground weta, *Hemiandrus* species (Orthoptera: Anostostomatidae). Science for conservation 180. Department of Conservation, Wellington

Kalmring K (1983) Convergence of auditory and vibratory senses at the neuronal level of the ventral nerve cord in grasshoppers; its probable importance for behavior in the habitat. In: Horn E (ed) Multimodal convergences in sensory systems. Fortschr Zool 28:129–141

Kalmring K (1985) Vibrational communication in insects (reception and integration of vibratory information). In: Kalmring K, Elsner E (eds) Acoustic and vibrational communication in insects. Parey, Berlin Hamburg, pp 127–134

Kalmring K, Kühne R (1983) The processing of acoustic and vibrational information in insects. In: Lewis B (ed) Bioacoustics. Academic Press, London, A comparative approach, pp 261–282

Kalmring K, Rössler W, Unrast C (1994) Complex tibial organs in the fore-, mid- and hindlegs of the bushcricket *Gampsocleis gratiosa* (Tettigoniidae): comparison of physiology of the organs. J Exp Zool 270:155–161

Kelly CD (2006a) Movement patterns and gallery use by the sexually dimorphic Wellington tree weta. New Zeal J Ecol 30:273–278

Kelly CD (2006b) Fighting for harems: assessment strategies during male–male contests in the sexually dimorphic Wellington tree weta. Anim Behav 72:727–736

Kelly CD (2006c) Resource quality or harem size: what influences male tenure at refuge sites in tree weta (Orthoptera: Anostostomatidae)? Behav Ecol Soiobiol 16:145–152

Kelly CD, Adams DC (2010) Sexual selection, ontogenetic acceleration, and hypermorphosis generates male trimorphism in Wellington tree weta. Evol Biol 37:200–209

Kelly CD, Bussière LF, Gwynne DT (2008) Sexual selection for male mobility in a giant insect with female-biased size dimorphism. Am Nat 172:417–423

Keuper A, Kühne R (1983) The acoustic behaviour of the bushcricket *Tettigonia cantans* II. Transmission of airborne sound and vibration signals in the biotope. Behav Process 8:125–145

Lakes-Harlan R, Strauß J (2014) Functional morphology and evolutionary diversity of vibration receptors in insects. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin Heidelberg, pp 277–302

Lakes-Harlan R, Stölting H, Stumpner A (1999) Convergent evolution of an insect ear from a preadaptive structure. P Roy Soc Lond B 266:1161–1167

Lomas K, Montealegre-Z F, Parsons S, Field LH, Robert D (2011) Mechanical filtering for narrow-band hearing in the weta. J Exp Biol 214:778–785

Lomas KF, Greenwood DR, Windmill JF, Jackson JC, Corfield J, Parsons S (2012) Discovery of a lipid synthesising organ in the auditory system of an insect. PLoS One 7(12):e51486

Matheson T, Field LH (1990) Innervation of the metathoracic femoral chordotonal organ of *locusta migratoria*. Cell Tissue Res 259:551–560

McIntyre M (2001) The ecology of some large weta species in New Zealand. In: Field LH (ed) The biology of wetas, king crickets and their allies. CABI Publishing, Wallingford, pp 231–242

McVean A, Field LH (1996) Communication by substratum vibration in the New Zealand tree weta, *Hemideina femorata* (Stenopelmatidae: Orthoptera). J Zool 239:101–122

Monteith GB, Field LH (2001) Australian king crickets: distribution, habitats and biology (Orthoptera: Anostostomatidae). In: Field LH (ed) The biology of wetas, king crickets and their allies. CABI Publishing, Wallingford, pp 79–94

Nishino H (2003) Somatotopic mapping of chordotonal organ neurons in a primitive ensiferan, the New Zealand tree weta *Hemideina femorata*: I. Femoral chordotonal organ J Comp Neurol 464: 312–326

Nishino H, Field LH (2003) Somatotopic mapping of chordotonal organ neurons in a primitive ensiferan, the New Zealand tree weta *Hemideina femorata*: II. Complex tibial organ J Comp Neurol 464:327–342

Nishino H, Sakai M (1997) Three neural groups in the femoral chordotonal organ of the cricket *Gryllus bimaculatus*: central projections and soma arrangement and displacement during joint flexion. J Exp Biol 200:2583–2595

Nishino H, Mukai H, Takanashi T (2016) Chordotonal organs in hemipteran insects: unique peripheral structures but conserved central organization revealed by comparative neuroanatomy. Cell Tissue Res 366:549–572

Pennisi E (2017) Saving the ‘god of ugly things’. Science 356:1001–1003

Pflüger H-J, Bräunig P, Hustert R (1988) The organization of mechanosensory neuropiles in locust thoracic ganglia. Philos Trans R Soc Lond B 321:1–26

Pollack GS (1998) Neural processing of acoustic signals. In: Hoy RR, Popper AN, Fay RR (eds) Comparative hearing: insects. Springer, New York, pp 139–196

Pratt RC, Morgan-Richards M, Trewick SA (2008) Diversification of New Zealand weta (Orthoptera: Ensifera: Anostostomatidae) and their relationships in Australasia. Philos Trans R Soc Lond B 363:3427–3437

Robinson DJ, Hall MJ (2002) Sound signalling in Orthoptera. Adv Insect Physiol 29:151–278

Rohrseitz K, Kilpinen O (1997) Vibration transmission characteristics of the legs of freely standing honeybees. Zoology 100:80–84

Schnorbus H (1971) Die subgenualen Sinnesorgane von *Periplaneta americana*: Histologie und Vibrationsschwellen. Z vergl Physiol 71:14–48

Schumacher R (1973) Morphologische Untersuchungen der tibialen Tympanalorgane von neun einheimischen Laubheurschrecken. Z Morphol Tiere 75:267–282

Sherley GH, Hayes LM (1993) The conservation of a giant weta (*Deinacrida* n. sp. Orthoptera: Stenopelmatidae) at Mahoenui, King Country: habitat use, and other aspects of its ecology. N Z Entomol 16:55–68

Song H (2018) Biodiversity of orthoptera. In: Foottit RG, Adler PH (eds) Insect biodiversity: science and society, vol 2. Wiley, Hoboken, pp 245–279

Song H, Amédégnato C, Desutter-Grandcolas L, Heads SW, Huang Y, Otte D, Whiting MF (2015) 300 million years of diversification: elucidating the patterns of orthopteran evolution based on comprehensive taxon and gene sampling. Cladistics 31:621–651

Song H, Béthoux O, Shin S, Donath A, Letsch H, Liu S, McKenna DD, Meng G, Misof B, Podsiadlowski L, Zhou X, Wipfler B, Simon S (2020) Phylogenomic analysis sheds light on the evolutionary pathways towards acoustic communication in Orthoptera. Nat Commun 11:4939

Stein W, Sauer AE (1999) Physiology of vibration-sensitive afferents in the femoral chordotonal organ of the stick insect. J Comp Physiol A 184:253–263

Stiedl O, Kalmring K (1989) The importance of song and vibratory signals in the behaviour of the bushcricket *Ephippiger ephippiger* Fiebig (Orthoptera, Tettigoniidae): taxis by females. Oecologia 80:142–144

Strauß J (2017a) The scolopidial accessory organs and Nebenorgans in orthopteroid insects: comparative neuroanatomy, mechansosensory function, and evolutionary origin. Arthropod Struct Dev 46:765–776

Strauß J (2017b) The scolopidial accessory organ in the Jerusalem cricket (Orthoptera: Stenopelmatidae). Arthropod Struct Dev 46:171–177

Strauß J (2019) What determines the number of auditory sensilla in the tympanal hearing organs of Tettigoniidae? Perspectives from comparative neuroanatomy and evolutionary forces. J Orthoptera Res 28:205–219

Strauß J, Lakes-Harlan R (2009) The evolutionary origin of auditory receptors in Tettigonioidea: the complex tibial organ of Schizodactylidae. Naturwissenschaften 96:143–146

Strauß J, Lakes-Harlan R (2017) Vibrational sensitivity of the subgenual organ complex in female *Sipyloidea sipylus* stick insects in different experimental paradigms of stimulus direction, leg attachment, and ablation of a connective tibial sense organ. Comp Physiol Biochem A 203:100–108

Strauß J, Stritih-Peljhan N, Nieri R, Virant-Doberlet M, Mazzoni V (2021) Communication by substrate-borne mechanical waves in insects: from basic to applied biotremology. Adv Insect Physiol 61:189–307

Strauß J, Lomas K, Field LH (2017) The complex tibial organ of the New Zealand ground weta: sensory adaptations for vibrational signal detection. Sci Rep 7:2031

Stritih N, Čokl A (2014) The role of frequency in vibrational communication of Orthoptera. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin Heidelberg, pp 375–393

Stritih Peljhan N, Strauß J (2018) The mechanical leg response to vibration stimuli in cave crickets and implications for vibrosensory organ functions. J Comp Physiol A 205:687–702

Stritih-Peljhan N, Rühr PT, Buh B, Strauß J (2019) Low-frequency vibration transmission and mechanosensory detection in the legs of cave crickets. Comp Biochem Physiol A 233:89–96

Taylor Smith BL, Morgan-Richards M, Trewick SA (2013) New Zealand weta: (Anostostomatidae: *Hemiandrus*): description of two species with notes on their biology. New Zeal J Zool 40:314–329

Taylor Smith BL, Trewick SA, Morgan-Richards M (2016) Three new ground weta species and a redescription of *Hemiandrus maculifrons*. New Zeal J Zool 43:363–383

ter Hofstede HM, Schöneich S, Robillard T, Hedwig B (2015) Evolution of a communication system by sensory exploitation of startle behavior. Curr Biol 25:3245–3252

Trewick SA (2021) A new species of large *Hemiandrus* ground wētā (Orthoptera: Anostostomatidae) from North Island, New Zealand. Zootaxa 4942:207–218

Trewick SA, Morgan-Richards M (2004) Phylogenetics of New Zealand's tree, giant and tusked weta (Orthoptera: Anostostomatidae): evidence from mitochondrial DNA. J Orthoptera Res 13:185–196

Trewick SA, Morgan-Richards M (2005) After the deluge: mitochondrial DNA indicates Miocene radiation and Pliocene adaptation of tree and giant weta (Orthoptera: Anostostomatidae). J Biogeogr 32:295–309

Trewick SA, Morgan-Richards M (2009) New Zealand, biology. In: Gillespie R, Clague D (eds) Encyclopedia of islands. University of California Press, Oakland CA

Trewick SA, Morgan-Richards M (2019) Wild life New Zealand, 2nd edn. Hand-in-Hand Press, Palmerston North, NZ

Trewick SA, Taylor-Smith BL, Morgan-Richards M (2021) Ecology and systematics of the wine wētā and allied species, with description of four new *Hemiandrus* species. New Zeal J Zool 48: 47–80

Vandergast AG, Weissman DB, Wood DA, Rentz DCF, Bazelet CS, Ueshima N (2017) Tackling an intractable problem: can greater taxon sampling help resolve relationships within the Stenopelmatoidea (Orthoptera: Ensifera)? Zootaxa 4291(1):001–033

Virant-Doberlet M, Čokl A (2004) Vibrational communication in insects. Neotrop Entomol 33: 121–134

Walker TJ (1962) Factors responsible for intraspecific variation in the calling songs of crickets. Evolution 16:407–428

Watts C, Thornburrow D (2011) Habitat use, behavior and movement patterns of a threatened New Zealand giant weta, *Deinacrida heteracantha* (Anostostomatidae: Orthoptera). J Orthoptera Res 20:127–135

Weissman DB (2001) Communication and reproductive behaviour in North American Jerusalem crickets (Stenopelmatus) (Orthoptera: Stenopelmatidae). In: Field LH (ed) The biology of wetas, king crickets and their allies. CABI Publishing, Wallingford, pp 351–373

Wessel A, Mühlethaler R, Hartung V, Kuštor V, Gogala M (2014) The tymbal: evolution of a complex vibration-producing organ in the Tymbalia (Hemiptera excl. Sternorrhyncha). In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin Heidelberg, pp 395–444

Yack J (2016) Vibrational signaling. In: Pollack GS, Mason AC, Popper AN, Fay RR (eds) Insect hearing. Springer International Publishing, Switzerland, pp 99–123

Chapter 4
Energetic Costs of Vibrational Signaling

Anka Kuhelj and Meta Virant-Doberlet

Abstract Costs associated with the production of signals used in sexual communication play a central role in the sexual selection theory. Arthropods relying on substrate-borne vibrations have often been included among examples of acoustic communication; however, taking into account that air-borne and substrate-borne mechanical signals are subject to different selection pressures when they travel through the environment via different transmission media, the costs associated with the production of these two types of mechanical signals are also likely to differ. So far, remarkably little is known about costs associated with substrate-borne vibrational communication. In this chapter, we provide an overview of our current knowledge on energy expenditure associated with the production of vibrational signals and indirect costs of male vibrational signaling. We also discuss some technical challenges encountered when measuring respiration rates and determining a relationship between the effort of vibrational signaling and survival. Our goal is to point out an important gap in our understanding of vibrational communication systems and stimulate further studies in this area.

4.1 Introduction

Communication between partners is an essential part of reproductive behavior (e.g., Shuster and Wade 2003), and as such, it is under strong evolutionary pressures resulting from ecological and sexual selection (Maan and Seehausen 2011; Mendelson and Shaw 2012; Rodríguez et al. 2013; Safran et al. 2013). Signals used in sexual communication enable identification (species, sex, condition, receptivity), as well as the location of the potential partner (e.g., Mendelson Shaw, 2012; Wilkins et al. 2013). Sexual selection (differential reproductive success arising from competition for mates and access to fertilization) via male–male competition and/or

---

A. Kuhelj (✉) · M. Virant-Doberlet
Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia
e-mail: anka.kuhelj@nib.si; meta.virant-doberlet@nib.si

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_4

female choice is recognized as one of the main drivers of the evolution of male secondary sexual characters like signals used in communication (e.g., Wong and Candolin 2005; Irschick et al. 2007; Hunt et al. 2009; Safran et al. 2013; Miller and Svensson 2014). Females usually prefer more complex, longer, and noticeable (e.g., louder) signals, and costs associated with the production of such signals play a central role in the sexual selection theory (e.g., Andersson 1994; Johnstone 1995; Kotiaho 2001; Kokko et al. 2006; Woods et al. 2007). Such costs could either have a direct (i.e., immediate) negative effect through predation or parasitism (e.g., Zuk and Kolluru 1998; Hoskin and Higge 2010; Hughes et al. 2012) or indirect (i.e., delayed) negative effect due to high energy expenditure (Kotiaho 2001; Hunt et al. 2006). It should be emphasized that while it is generally accepted that sexual signaling is associated with energy expenditure, it may not be at the same time also evolutionarily costly in terms of a reduced lifespan of the signaler (e.g., Kotiaho 2000).

Signals produced by mechanical vibrations and transmitted from the signaler to the receiver through the surrounding medium (air, water, solids) via mechanical waves play a key role in communication in the majority of animals (Bradbury and Vehrencamp 2011). Acoustic (air- or water-borne sound) communication has been considered as one of the most widespread forms of animal communication and particularly well suited to play a role in the mate recognition process since it can be used when partners are not in close proximity (e.g., Wilkins et al. 2013). As such, it was also considered as one of the best model systems to study the role of sexual and ecological selection in speciation (e.g., Ryan and Kime 2003; Ophir et al. 2010; Wilkins et al. 2013), and arthropod acoustic signals provided some of the best known examples (e.g., Andersson 1994; Panhuis et al. 2001; Rodríguez et al. 2013; Wilkins et al. 2013). In the literature, arthropods relying on substrate-borne mechanical communication have been traditionally included among examples of acoustic communication (e.g., Panhuis et al. 2001; Wilkins et al. 2013). However, recent establishment of biotremology as an independent discipline within the area of mechanical communication (Hill and Wessel 2016; Hill et al. 2019) helped us to realize that there are important gaps in our knowledge. Among others, we became aware that in contrast to air-borne sound communication, remarkably little is known about the costs associated with substrate-borne vibrational communication. Taking into account that air-borne and substrate-borne mechanical signals are subject to different selection pressures when they travel through the environment via different media and, consequently, likely follow different evolutionary paths (Endler 2014; Hill et al. 2019), the costs associated with the production of these two types of mechanical signals are also likely to differ. Moreover, costly sexual signaling as a form of reproductive effort is also a life history trait (e.g., Kokko et al. 2002; Hunt et al. 2006; Judge et al. 2008) and whether the relationship between signaling effort and survival is negative or positive is likely to depend also on the communication system itself (Shuster and Wade 2003).

In this chapter, we wish to provide an overview of the current knowledge of energetic costs associated with vibrational signaling in order to stimulate further work needed to increase our understanding of the sources of selection on vibrational communication systems. Our intention is not to focus on technical details about methodology for metabolic measurements, since this topic has been the subject of several comprehensive reviews (e.g., Lighton 2008; Lighton and Halsey 2011; Lighton 2017). Since there are so far only a few studies on energetic costs of vibrational signaling, we start by briefly reviewing the information on indirect costs of insect air-borne signaling.

4.2 Energetic Costs of Insect Acoustic Signaling

Production of mechanical signals depends on contractions of muscles attached to vibrating body parts or specialized structures and therefore requires chemical energy needed to carry out physiological processes underlying these contractions (e.g., Prestwich 1994). The energetic costs of signaling are usually estimated by measuring the metabolic rate (i.e., energy used by individual per unit of time), either by the rate of O_2 consumption or CO_2 production (Lighton 2008; Lighton and Halsey 2011). Measuring both respiratory gases simultaneously also allows us to determine whether the respiratory substrate is carbohydrates or lipids (Lee and Loher 1993; Lighton and Halsey 2011).

As summarized in Table 4.1, most studies on energetic costs of insect acoustic signaling have been carried out on relatively big species. Although these studies used different methods to determine the rate of O_2 consumption and/or CO_2 production, all showed that respiration rate during signaling is higher than during resting; however, this increase varied between 1.3 and 65 times, depending on the species. The majority of studies were done on ensiferans (crickets and bushcrickets), which produce air-borne songs by wing stridulation and for which the mechanics of sound production have been well studied (Prestwich and Walker 1981; Gerhardt and Huber 2002; Robinson and Hall 2002, Hartbauer et al. 2012; Erregger et al. 2017). The basic element of ensiferan songs is a pulse, which is produced by a single wing stroke. Results suggest that energy consumption depends on the intensity of the produced sound, as well as on the song structure; trilling produced by continuous wing strokes appears to be energetically more demanding than chirping, where few wing strokes are followed by a pause (Lee and Loher 1993; Prestwich 1994; Erregger et al. 2017). Results also show that metabolic rate is temperature dependent (Table 4.1).

The existing studies suggest that the relationship between the signaling effort and longevity may be species-specific and dynamic. In *Gryllus veletis*, the authors monitored the calling activity of individual crickets continuously throughout their lifetime and showed that the calling effort was not predictive of an individual's lifespan (Fitzsimmons and Bertram 2011). Other studies determined calling effort over periods of several hours, and the results revealed positive correlation between signaling effort and longevity in *G. pennsylvanicus* (Judge et al. 2008), negative correlation in *Teleogryllus commodus* (Hunt et al. 2004, 2006), or no correlation in *Gryllodes sigillatus* (Okada et al. 2011).

Table 4.1 Measuring indirect costs of insects' acoustic signaling

Species	Mass [g]	Size [mm]	Mechanism	Method	O_2/ CO_2	T [°C]	Resting VO_2/ VCO_2 [mL g^{-1} h^{-1}]	Singing VO_2/ VCO_2 [mL g^{-1} h^{-1}]	Factorial metabolic scope	Source
Gryllidea										
Acheta domesticus	0.40	20	Stridulation	Open-flow, zirconia cell GA	O_2	NA	0.413[a]	0.620[a]	1.5	8
Gryllus lineaticeps	0.53	24	Stridulation	Closed-bottle, fuel cell GA	O_2	25	0.464[a]	1.238[a]	2.7	7
Teleogryllus commodus	0.95	30	Stridulation	Manometric	O_2	21	0.309[a]	1.209[a]	3.9	4
				Open-flow, zirconia cell GA	$O_2 + CO_2$	27	0.850[a] NA	2.020[a] NA	1.3 NA	6
Teleogryllus oceanicus	NA	31	Stridulation	Open-flow, zirconia cell GA	$O_2 + CO_2$	27	1.050[a] NA	1.320[a] NA	2.0 NA	6
Anurogryllus muticus	0.40	18	Stridulation	Open-flow, zirconia cell GA	$O_2 + CO_2$	27	1.700[a] NA	10.760[a] NA	6.3 NA	6
				Flow-through, IR diff. GA	CO_2	26	0.572[a]	8.813[a]	15.4	15
Anurogryllus arboreus	0.40	22	Stridulation	Manometric	O_2	20	0.236[a]	3.721[a]	15.8	3
						24	0.315[a]	3.891[a]	12.4	
						26	0.364[a]	3.648[a]	10.0	
Oecanthus celerinictus	0.06	14	Stridulation	Manometric	O_2	16	0.191[a]	2.289[a]	12.0	3
						23	0.437[a]	3.368[a]	7.7	
						28	0.761[a]	4.763[a]	6.3	
Oecanthus quadripunctatus	0.06	14	Stridulation	Manometric	O_2	23	0.476[a]	3.818[a]	8.0	3
						28	0.782[a]	5.089[a]	6.5	

Gryllotalpa australis	1.14	NA	Stridulation	Manometric	O_2	23	0.420^{a}	5.303^{a}	12.6	4
Gryllotalpa monanka	0.98	35	Stridulation	Flow-through, diff. fuel cell GA	O_2	21	0.440^{a}	6.205^{a}	14.1	11
Scapteriscus borellii[b]	0.84	29	Stridulation	Flow-through, diff. fuel cell GA	O_2 + CO_2	25	0.241^{a} 0.227^{a}	2.098^{a} 1.804^{a}	8.7 7.9	10
Scapteriscus vicinus[b]			Stridulation	Flow-through, diff. fuel cell GA	O_2 + CO_2	25	0.288^{a} 0.290^{a}	1.387^{a} 1.297^{a}	4.8 4.5	10
Tettigoniidae										
Euconocephalus nasutus	0.65	38	Stridulation	Manometric	O_2	25	0.280^{a}	18.400^{a}	65.7	1
Neoconocephalus robustus	0.88	63	Stridulation	Manometric	O_2	25	0.760^{a}	15.800^{a}	20.8	1
Requena verticalis	0.37	NA	Stridulation	Closed-bottle, paramag. GA	O_2	21	0.248^{a}	0.399^{a}	1.6	5
Mecopoda elongata	3.30	35	Stridulation	Flow-through, IR diff. GA	CO_2	27	0.380^{a}	1.476^{a}	3.9	13
Mecopoda sp.	3.30	NA	Stridulation	Flow-through, IR diff. GA	CO_2	28	0.497^{a}	5.653^{a}	11.4	15
Plangia graminea	0.71	25	Stridulation	Flow-through, IR GA	CO_2	24	0.279^{a}	0.443^{a}	1.6	14
Docidocercus gigliotosi	NA	35	Stridulation	Flow-through, IR diff. GA	CO_2	NA	NA	0.001^{b}	NA	12
Cicadidae										
Cystosoma saundersii	1.30	47	Tymbal buck.	Manometric	O_2	23	0.323^{a}	6.277^{a}	19.4	2

(continued)

Table 4.1 (continued)

Species	Mass [g]	Size [mm]	Mechanism	Method	O_2/ CO_2	T [°C]	Resting VO_2/ VCO_2 [mL g^{-1} h^{-1}]	Singing VO_2/ VCO_2 [mL g^{-1} h^{-1}]	Factorial metabolic scope	Source
Pyralidae										
Achoria grisella	0.01	13	Tymbal buck.	Flow-through, IR GA	CO_2	24	1.340[a]	3.660[a]	2.7	9

1 Stevens and Josephson (1977); 2 MacNally and Young (1981); 3 Prestwich and Walker (1981); 4 Kavanagh (1987); 5 Bailey et al. (1993); 6 Lee and Loher (1993); 7 Hoback and Wagner (1997); 8 Hack (1998); 9 Reinhold et al. (1998); 10 Prestwich and O'Sullivan (2005); 11 White et al. (2008); 12 Römer et al. (2010); 13 Hartbauer et al. (2012); 14 Doubell et al. (2017); 15 Erregger et al. (2017)

[a] Calculated per wet weight

[b] Species formerly in the genus *Scapteriscus* were transferred to *Neoscapteriscus*

[c] Reported as mL per signal

NA no data available; GA gas analyzer

4.3 Estimation of Indirect Costs of Vibrational Signaling

Energetic costs of vibrational signaling have been so far studied only in four arthropod species, the tenebrionid beetle *Psammodes striatus* (Lighton 1987), the wolf spider *Hygrolycosa rubrofasciata* (Kotiaho et al. 1998a), the bushcricket *Docidocercus gigliotosi* (Römer et al. 2010), and the leafhopper *Aphrodes makarovi* (Kuhelj et al. 2015b). Indicatively, since male drumming in *H. rubrofasciata* is audible to humans, in the original publications on sexual selection and associated costs in this species, the authors either described drumming exclusively as an air-borne signal or mentioned the possibility that it may be perceived as substrate-borne vibrations as less likely (Kotiaho et al. 1996; Mappes et al. 1996; Parri et al. 1997; Kotiaho et al. 1998a,b; Kotiaho et al. 1999; Rivero et al. 2000) and only later acknowledged the importance of the vibrational channel (e.g., Kotiaho et al. 2004; Huber 2005).

As shown in Table 4.2, *P. striatus* and *D. gigliotosi* are relatively large insects, while *H. rubrofasciata* and *A. makarovi* are smaller and comparable in size. Although these four species use different mechanisms to produce vibrational signals, in all of them respiration rate increased during signal emission; however, energy consumption was species-specific. Even in *P. striatus* and *H. rubrofasciata*, which both produce vibrational signals by abdominal percussion, signals were associated with a 2.7 and 22 times increase in comparison with resting, respectively (Lighton 1987; Kotiaho et al. 1998a). In *A. makarovi*, vibrational signals are produced by a tymbal mechanism (Ossiannilsson 1949; Wessel et al. 2014; Kuhelj et al. 2018), and emission of male advertisement calls is around 12 times energetically more demanding than resting, which corresponds to factorial metabolic scopes found in many insects communicating by stridulatory air-borne sounds (see Table 4.1). In contrast, in *D. gigliotosi* vibrational signals produced by abdominal tremulation are around 6.7 times more energetically demanding than air-borne sounds produced by stridulation (Römer et al. 2010).

In *H. rubrofasciata* an *A. makarovi* studies also provided some insight into the costs of vibrational signaling. The signaling effort was determined over short periods and results revealed that in both species indirect costs associated with vibrational signaling have a negative effect on male survival (Mappes et al. 1996; Kotiaho 2000, 2001; Kuhelj et al. 2015b).

4.3.1 *Challenges Encountered When Determining Indirect Costs of Vibrational Signaling*

Besides our perceptional bias in favor of air-borne sound communication and general lack of understanding of the importance of the vibrational channel for animals, there are several other more technical reasons why in comparison with

Table 4.2 Measuring indirect costs of arthropods' vibrational signaling

Species	Mass [g]	Size [mm]	Mechanism	Method	O_2/ CO_2	T [°C]	Resting VO_2/ VCO_2 [mL g^{-1} h^{-1}]	Singing VO_2/ VCO_2 [mL g^{-1} h^{-1}]	Factorial metabolic scope	Source
Arachnida										
Lycosidae										
Hygrolycosa rubrofasciata	0.02	6	Drumming	Flow-through, IR GA	CO_2	25	0.220^a	4.800^a	21.8	2
Orthoptera										
Tettigoniidae										
Docidocercus gigliotosi	NA	35	Tremulation	Flow-through, IR diff. GA	CO_2	NA	NA	0.005^c	NA	3
Heteroptera										
Cicadellidae										
Aphrodes makarovi	0.01	7	Tymbal buck.	Closed-bottle, lifetime-based optode	O_2	25	0.855^b	10.069^b	11.8	4
Coleoptera										
Tenebrionidae										
Psammodes striatus	3	20	Tapping	Flow-through, coulometric	O_2	23	0.219^a	0.592^a	2.7	1

1 Lighton (1987); 2 Kotiaho et al. (1998a); 3 Römer et al. (2010); 4 Kuhelj et al. (2015b)

[a] Calculated per wet weight

[b] Calculated per dry weight

[c] Reported as mL per signal

NA no data available; GA gas analyzer

insect acoustic communication the energetic costs of vibrational signaling so far have received so little attention.

The majority of arthropods relying on vibrational signaling are smaller than 1 cm, and as a result, reliable metabolic rates of individual insects may not be easy to obtain. While in such cases, it is tempting to use more animals simultaneously to increase the amount of O_2 consumed or CO_2 produced, with such measurements we lose information about individual performance, and crucially, crowded conditions may increase stress that, in turn, affects respiration rates. The solution is to use a stop-flow respirometry, where the small animal is placed in a sealed chamber, and O_2 and/or CO_2 concentration is determined after a period of time (Lighton and Halsey 2011). However, designing and manufacturing such custom-made small respiration chambers out of inert material like glass, as well as calibration and testing of such a system (Lighton and Halsey 2011; Kuhelj et al. 2015b), may not be appealing to many biotremologists.

In contrast to the majority of air-borne sound communication systems in insects, where males continuously produce their calling songs up to several hours (e.g., Gerhardt and Huber 2002), in mating systems based on substrate-borne vibrations, male advertisement calls are relatively short discrete events, at most lasting up to a few minutes (e.g., Derlink et al. 2014; Rodríguez and Barbosa 2014, Polajnar et al. 2016). Moreover, while the emission of insect air-borne sounds is audible to humans, and in most cases the movements associated with the production are also visible, the majority of substrate-borne vibrational signals are not perceived by humans, and in many small insects, movements linked to signal production are not discernible. It is not surprising that vibrational signals of *P. striatus*, *D. gigliotosi*, and *H. rubrofasciata* have an audible component and/or their production is associated with visible movements (Lighton 1987; Kotiaho et al. 1998a; Römer et al. 2010). However, in most species it is necessary to register the signaling activity of the animal closed in the chamber continuously in order to reliably associate the drop in O_2 and/or increase in CO_2 concentration with signal production.

While left free on the plant, males usually readily emit advertisement calls spontaneously; however, when they are sealed in a small respiration chamber constructed out of glass and metal, their signaling has to be triggered by playback of female vibrational signals or a male–female duet. Placing a piece of plant tissue into the chamber for the animal to hold onto is not possible, since it would affect the amount of O_2 and CO_2 in the chamber. However, it should be taken into account that stimulation with male–female duet may be perceived by the live male in the chamber as a close proximity of a rival and the competitive situation may affect the respiration rate. To avoid additional stress, the amplitude of the playback should be kept below the amplitude of the signals emitted by a live male in the chamber. Ideally, one should also directly test whether such stimulation has an effect on the respiration rate.

Many communication systems based on vibrational systems are characterized by a male fly/jump/walk-call strategy (Hunt and Nault 1991; De Luca and Cocroft 2011) used to increase the active range of vibrational signals, as well as by male approach to the stationary female. Consequently, when placed in the chamber males will show different levels of locomotor activity. Although in a small chamber the male is not able to move to another position, locomotor activity may be expressed in turning around or trying to climb. In such conditions, it is difficult to reliably determine the respiration rate during resting, and thus continuous filming of animal behavior is needed in order to take into account only the sections when the male is not moving. If males are not alternating the periods of walking and signaling, but are instead signaling during walking (e.g., de Groot et al. 2011), it may not be possible to reliably estimate the energy consumption associated with the production of vibrational signals.

Insect substrate-borne vibrational signals differ enormously in their temporal characteristics and complexity. They are often composed of sections with highly diverse structures, and in which vibrations are produced by different mechanisms that include drumming, tremulation, stridulation, and tymbal buckling (e.g., Gogala et al. 1974; Elias et al. 2003; Virant-Doberlet and Čokl 2004; Cocroft and McNett 2006; Miles et al. 2017). Furthermore, a species-specific vibrational repertoire often includes several structurally different signals associated with different stages of the mating sequence (e.g., Mazzoni et al. 2009; Polajnar et al. 2014; Derlink et al. 2018; Lopez-Diez 2019). Moreover, in many species in which vibrational signals have been recorded, the production mechanisms remain elusive. Although morphological studies of vibration production mechanisms have recently received increased attention (Wessel et al. 2014; Davranoglou et al. 2019a,b), in contrast to insect acoustic signals, the structure of species-specific vibrational signals has been directly associated with physiology and mechanics of signal production only in the stink bug *Nezara viridula* (Čokl 2008) and the treehopper *Umbonia crassicornis* (Miles et al. 2017). Taking into account that in small animals reliable metabolic measurements are already difficult to make due to small changes in O_2 and CO_2 concentration, one can still estimate an overall energetic consumption during the production of a single complex vibrational signal; however, it is not possible to estimate the relative contribution of structurally different sections to energy expenditure.

It should also be taken into account that species-specific reproductive behavior may include multimodal signaling, for example in stink bugs, where sexual communication includes pheromones and vibrational signals (Čokl et al. 2019), and both modalities are linked via feedback loops (Miklas et al. 2003). In such systems it may not be possible to separate energetic costs associated with the production of chemical and vibrational signals.

To determine the indirect costs of vibrational signaling, besides demonstrating that signal production is associated with increased energy expenditure, one should also show that this increase has a negative effect on survival or future reproductive success (Kotiaho 2001). In vibrational communication systems determining the lifetime signaling effort by continuous long-term monitoring of spontaneous signaling would be challenging, due to technical difficulties in simultaneously recording activity of a large number of males. Moreover, in duetting systems present in many species relying on vibrational communication, males increase signaling activity after they establish a duet with the female (Mazzoni et al. 2009; de Groot et al. 2012; Rodríguez et al. 2012). In such systems, signaling effort can be determined in short behavioral tests, where an increased signaling rate is induced by a live female or by playback of a female reply (Mappes et al. 1996; Kuhelj et al. 2015b). Since signaling effort is measured only over short periods, such an approach is missing the information on spontaneous signaling activity outside the trial periods. In addition, since males are approaching the females, such behavioral trials also include searching effort (i.e., energy expenditure during locomotion), which may be an important part of overall energy consumption associated with reproductive behavior (e.g., Lighton et al. 1993; Halsey 2016). Nevertheless, in systems where males have to locate the female, they may also be under selection for mate location ability (Kokko and Rankin 2006).

4.3.2 Case Study of **Aphrodes makarovi**

The leafhopper *A. makarovi* (Hemiptera, Cicadellidae) is one of the model species to study the sources of selection on vibrational communication systems (Virant-Doberlet et al. 2011; Kuhelj et al. 2015b, 2016; Kuhelj and Virant-Doberlet 2017; Virant-Doberlet et al. 2019), and this species provided us with an ideal opportunity to also study the energetic costs of vibrational signaling. In leafhoppers, mate recognition and location is mediated exclusively by vibrational signals (Virant-Doberlet and Čokl 2004). With male size of around 6.5 mm, this species is a relatively big representative of the Cicadellidae (Bluemel et al. 2014). Males initially use fly/jump/walk-call strategy to establish contact with the receptive female, and throughout the mating sequence, males emit just one type of vibrational signal, termed advertisement call, which is relatively long (15–19 s); however, it includes several structurally different sections composed of different elements, termed rumble, chirp, and pulse (Fig. 4.1a). Single males usually sporadically emit isolated calls; however, calling effort increases significantly after establishing a vibrational contact with the female (Fig. 4.2). While searching for the female, the male remains stationary during signaling and is walking on the plant only during female reply (de Groot et al. 2011; Kuhelj et al. 2016). Such periods of high signaling activity and walking require a substantial amount of energy, and during a 15–20 min playback behavioral trial in which a male is also supposed to locate the source of a female reply, males can lose up to 1.1 mg (i.e., around 8%) of their weight (Kuhelj 2015; Kuhelj and Virant-Doberlet 2017). However, on a plant males can replace the used energy without interrupting communication with the female. Leafhoppers are phloem feeders, and they can keep their feeding stylets inserted into plant tissue while emitting vibrational signals. During behavioral trials successfully completed in the allotted time, they can even gain weight (Kuhelj 2015; Kuhelj and Virant-Doberlet 2017).

In the presence of a rival, males overlap the female reply with a masking signal (Kuhelj et al. 2015b; Kuhelj and Virant-Doberlet 2017), which is structurally composed of continuously repeated pulses and can be as long as the male advertisement call (Fig. 4.1b). In behavioral contexts like rivalry and distress, individuals also

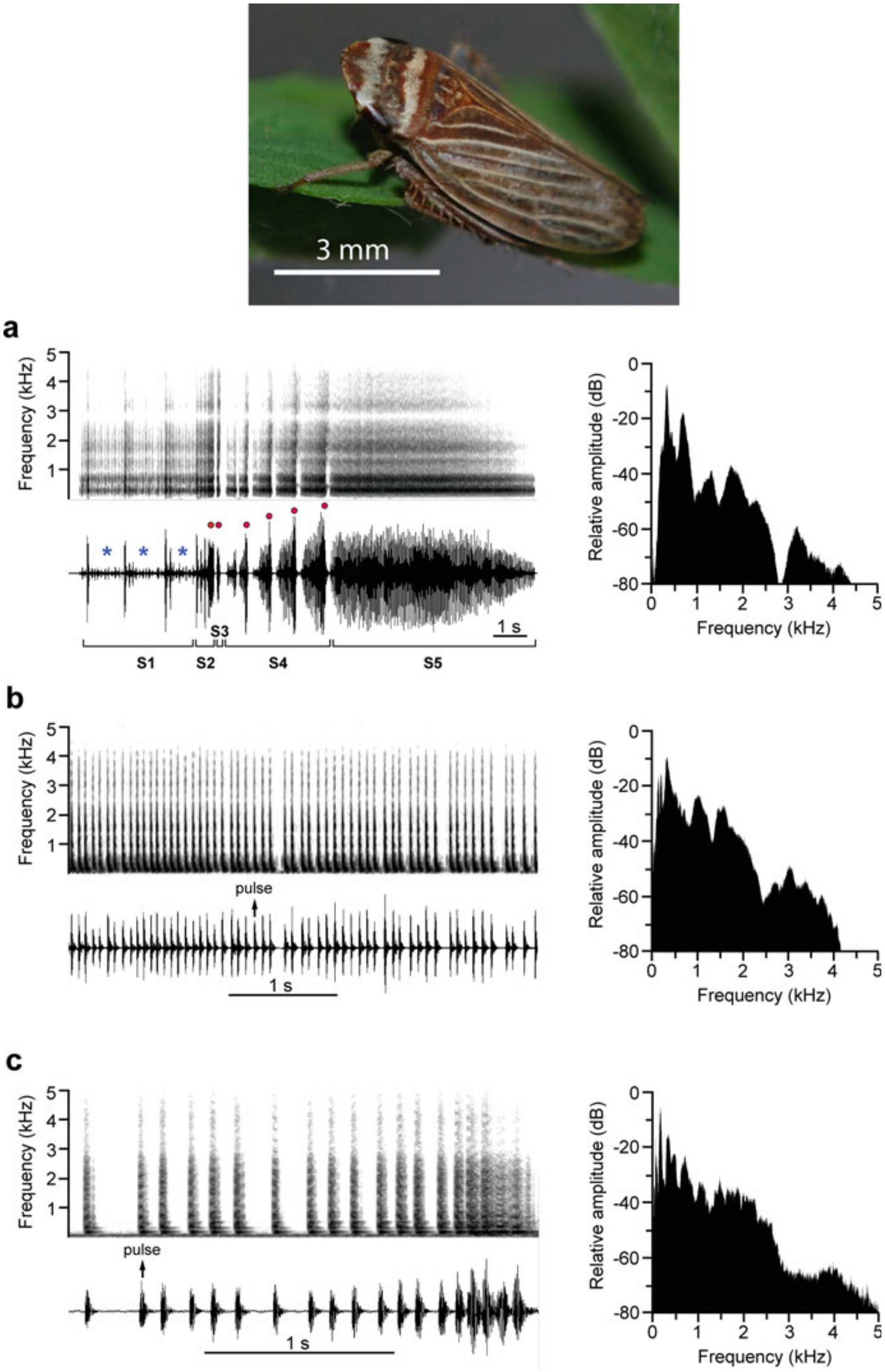


Fig. 4.1 Representative vibrational signals emitted by males of the leafhopper *Aphrodes makarovi* (shown at top) as recorded with a laser vibrometer from the plant. (**a**) Advertisement call: S1 -S5,

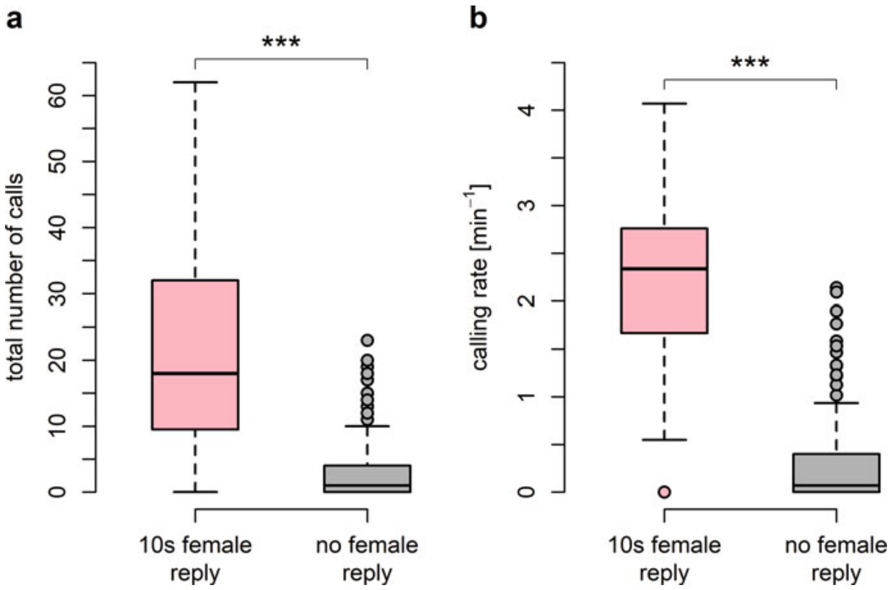


Fig. 4.2 Comparison of signaling activity of *Aphrodes makarovi* males in the presence of a female reply of average duration (10 s) and absence of a female reply. (**a**) Differences in the number of advertisement calls emitted during the trial; (**b**) calling rate per min of trial; box and whisker plots show the median (black line), the 25–75% interquartile range (boxes), the lowest and the highest data points still within 1.5 of interquartile range (whiskers) and outliers (circles). $***p < 0.001$, Wilcoxon rank sum test. Adapted from: Kuhelj et al. (2015b)

emit pulse signals; however, their duration is much shorter, up to 3.5 s, even though they consist of pulses that are longer than those in advertisement calls and masking signals (Fig. 4.1c).

We assessed the resting respiration rate and energetic costs of vibrational signaling by estimating O_2 consumption using stop-flow respirometry and an optical sensor for measuring O_2 concentration (Kuhelj et al. 2015b). Individual males were placed in the custom-made glass respiration chamber for 30 min at 24.5 °C (Fig. 4.3). To induce signaling, males were stimulated with a male–female duet, while vibrational signals and male behavior in the chamber were continuously recorded by a laser vibrometer and a video camera, respectively. During the measurements the males were restless, and the periods when they were not moving were short. To estimate the resting respiration rate, we took into account the 15–30 s periods (i.e., similar to the advertisement call duration) in which males were not showing any other activity (signaling or moving). Equally long periods in which

◄

Fig. 4.1 (continued) structurally different sections of advertisement call (after de Groot et al. 2012); blue asterisks –rumble', red circles-chirp; (**b**) masking signal; (**c**) pulse signal. The spectrogram is shown above the corresponding waveform; frequency spectra are shown on the right. Adapted from: Kuhelj et al. (2015b)

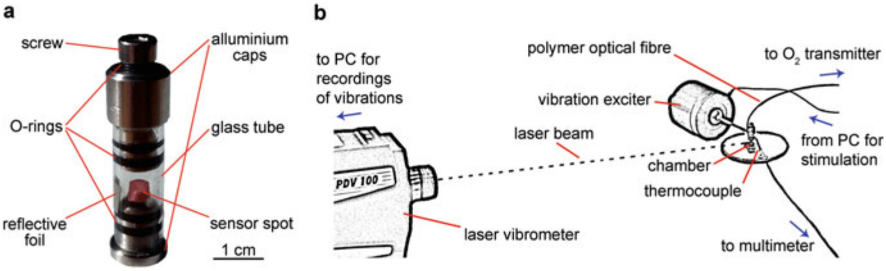


Fig. 4.3 Experimental setup for metabolic rate measurements carried out on *Aphrodes makarovi*. (**a**) Custom made air-tight chamber; (**b**) experimental setup for measuring O_2 consumption with simultaneous stimulation and registration of vibrational signals. Not shown is a video camera recording the behavior of the male in the chamber. Adapted from Kuhelj et al. (2015b)

males were moving their legs were used to estimate the energy consumption during locomotion. The O_2 concentration in the chamber was measured with the rate of one sample per 2 s, and this also allowed us to determine the respiration rate during production of a single advertisement call and masking signal (Table 4.3, Fig. 4.4).

The calculated respiration rate, taking into account the changes in the O_2 concentration, male weight, air temperature, and pressure (Lampert 1984; Kuhelj et al. 2015b), showed that the respiration rate was significantly affected by the total signaling time (Fig. 4.4). The majority of males emitted more than one type of vibrational signal, and perhaps not surprisingly, all males sealed in the chamber emitted pulse signals; however, only five males emitted advertisement calls. Directly comparing the respiration rates during emission of single advertisement calls and masking signals with resting and moving periods of comparable durations shows that during locomotion and signaling the male requires significantly more energy than during resting (Fig. 4.5). Due to the relatively low number of emitted advertisement calls and masking signals, a reliable comparison between locomotion and signaling is difficult. While the results show that the emission of advertisement calls is also 2–3 times more energetically demanding than locomotion, they suggest that the energetic costs of producing masking signals do not differ from costs of locomotion and emission of advertisement calls.

In laboratory conditions, male mortality increases after 3–4 weeks even in the absence of predation (Chiykowski 1970), and we investigated the indirect costs of vibrational signaling by studying the effect of signaling effort on male survival. In playback experiments, which simulated a natural mating behavior and where males had to locate the source of female reply in the allotted time, we determined the calling effort of individual males once a week throughout their adult life until death.

Results show that a higher calling rate has a significant positive effect on the probability of locating the female; however, longevity was negatively correlated with all measured parameters of the calling effort (number of emitted calls per trial, calling rate per minute of trail, and calling time during the trail), and males that were signaling more in early life died sooner (Kuhelj et al. 2015b; Fig. 4.6). According to

Table 4.3 Respiration rate calculations from Fig. 4.4. Bold rows show final calculations of respiration rates under standard and experimental conditions

Values & formulas	Segment 1	Segment 2	Segment 3
$m_{(Aphrodes)}$ [g] (dry)	0.0039	0.0039	0.0039
% $O_{2\ (1)}$	18.917	18.910	18.859
% $O_{2\ (2)}$	18.912	18.851	18.854
t [h]	0.0033	0.0033	0.0033
V(chamber) [m^3]	2.58E-07	2.58E-07	2.58E-07
T(chamber) K	297.65	297.65	297.65
P [$kg\ m^{-1}\ s^{-2}$]	101,900	101,900	101,900
$M(O_2)$ [kg Mol-1]	0.032	0.032	0.032
R [$kg * m^2\ s^{-2}\ Mol^{-1}\ K^{-1}$]	8.3145	8.3145	8.3145
T_{st} [K]	273.15	273.15	273.15
P_{st} [$kg\ m^{-1}\ s^{-2}$]	100,000	100,000	100,000
$w_{(1)} = \%\ O_{2\ (1)} / 100$	0.1892	0.1891	0.1886
$w_{(2)} = \%\ O_{2\ (2)} / 100$	0.1891	0.1885	0.1885
$m(O_2) = (P * V * M * w_{(1)}/ (R * T) * 1000 * 1000$ [mg]	0.0643	0.0643	0.0641
$rr_{mg} = ((w_{(1)} * m(O_2) - w_{(2)} * m(O_2))/ w_{(1)})/ (t * m_{(Aphrodes)})$ [$mg\ O_2\ h^{-1}\ g^{-1}$]	1.4260	15.1676	1.2964
$\rho(O_2) = (P_{st} * M)/ (R * T_{st})$ [$mg\ mL^{-1}$]	1.409	1.409	1.409
$Rr = rr_{mg}/ \rho(O_2)$ [$mL\ O_2\ h^{-1}\ g^{-1}$] at STPD*	**1.01**	**10.76**	**0.92**
$\rho(O_2) = (P * M)/ (R * T)$ [$mg\ mL^{-1}$]	1.318	1.318	1.318
$Rr = rr_{mg}/ \rho(O_2)$ [$mL\ O_2\ h^{-1}\ g^{-1}$] at experimental conditions	**1.08**	**11.51**	**0.98**

$m_{(Aphrodes)}$ = male body mass; % $O_{2\ (1)}$ & % $O_{2\ (2)}$ = O_2 concentration at the beginning and the end of the segment; t [h] = time interval; V(chamber) = chamber volume; T(chamber) = ambient temperature; P = air pressure; $M(O_2)$ = molar mass of O_2, R = gas constant; T_{st} = standard temperature = 273.15 K; P_{st} = standard pressure conditions = 100,000 Pa; $w_{(1)}$ & $w_{(2)}$ = proportion of O_2 in the chamber at the beginning and the end of the segment; $m(O_2)$ = mass of O_2 in the chamber; rr_{mg} = respiration rate in mg of O_2 per hour per animal; $\rho(O_2)$ = O_2 density; rr = respiration rate in mL of O_2 per hour per animal weight
STPD* = standard temperature and pressure and dry (no humidity)

the average longevity of 50 days, males were arbitrarily divided into a short- and a long-lived group (< 50 and > 50 days, respectively). Throughout their lives, males that lived longer than 50 days emitted significantly fewer calls per trial than short-lived males and, in particular, invested less in calling in early life (Figs. 4.7, 4.8). Moreover, in contrast to short-lived males, their signaling activity increased in the late period.

But can we correlate the constraints imposed by high energetic demands of vibrational signals production with the observed mating behavior in this species? When sexual communication is based on a coordinated reciprocal exchange of signals (i.e., duet), females can express their preference, or choice, either by selectively replying to preferred males and /or emitting longer replies in response to more attractive advertisement calls (e.g., Tauber et al. 2001; Villarreal and Gilbert 2014;

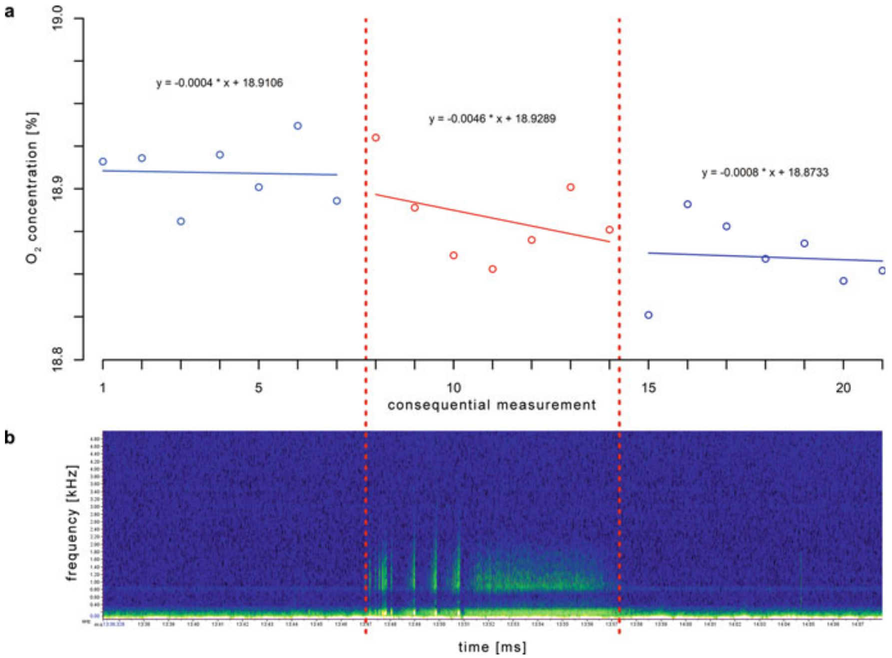
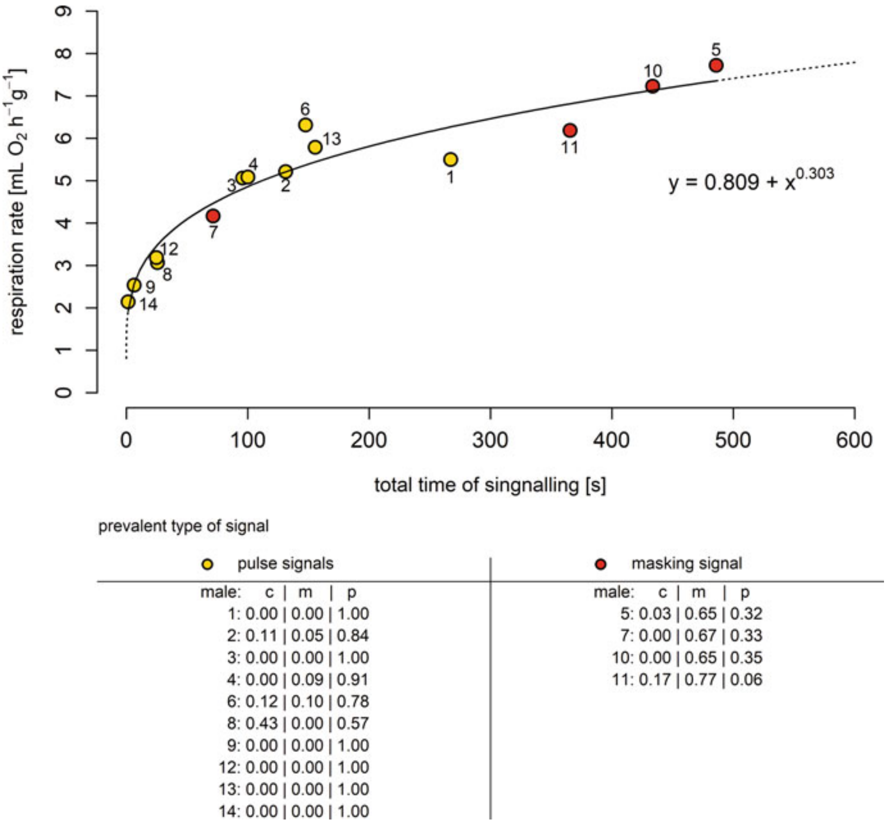


Fig. 4.4 Representative measurements of O_2 concentration in closed-bottle system in *A. makarovi*. (**a**) Measurements of O_2 concentration in the chamber collected at a rate 1 sample per 2 s (individual circles); in blue, are shown periods when male did not show any activity, while in red is shown the period when the male emitted one advertisement call. Linear regression equations shown correspond to the measurements. Note the difference in the rate of O_2 consumption in the signaling and inactive periods. (**b**) A spectrogram of advertisement call emitted by the male in the chamber and registered simultaneously with O_2 measurements on the glass wall; calculations of respiration rate based on these measurements are shown in Table 4.3

Rodríguez and Barbosa 2014). In *A. makarovi*, a sex-specific, precisely coordinated male–female duet is always initiated by a male, and his success in finding the female directly depends on the effort he invests in inducing her reply (Kuhelj et al. 2015b, 2016). In this species, the duration of a female vibrational reply is highly variable (5–60 s) and is often even longer than the triggering male call (de Groot et al. 2012). Longer female replies should provide males with better searching cues (Kuhelj et al. 2015a), and as predicted, results of playback experiments showed that female reply duration is negatively correlated with the male calling effort invested in finding the female (Kuhelj et al. 2016). However, the experiments involving live trios (two males and a female) showed that in such a situation male–male competition had important effects on male mating success (Kuhelj and Virant-Doberlet 2017). In contrast to insects emitting air-borne sounds (e.g., Bertram et al. 2013; Callander et al. 2013), *A. makarovi* males did not increase their calling effort, but instead invested more in competitive behavior (interference by masking signals and



prevalent type of signal

○ pulse signals male:	c	m	p	● masking signal male:	c	m	p
1:	0.00	0.00	1.00	5:	0.03	0.65	0.32
2:	0.11	0.05	0.84	7:	0.00	0.67	0.33
3:	0.00	0.00	1.00	10:	0.00	0.65	0.35
4:	0.00	0.09	0.91	11:	0.17	0.77	0.06
6:	0.12	0.10	0.78				
8:	0.43	0.00	0.57				
9:	0.00	0.00	1.00				
12:	0.00	0.00	1.00				
13:	0.00	0.00	1.00				
14:	0.00	0.00	1.00				

Fig. 4.5 Respiration rates of *Aphrodes makarovi* males (1–14) obtained in 30 min measurements in relation to the total signaling time. The best model fitted by nonlinear least square analysis was $y = 0.809 + x^{0.303}$. According to this model, the resting respiration rate is at 0.809 mL O_2h^{-1} g^{-1}, which corresponds well to the average O_2 consumption obtained during resting (0.943 mL O_2h^{-1} g^{-1}). Inset shows for each individual the proportions of each type of vibrational signal within the total signaling time. c: advertisement calls; m: masking signals; p: pulse signals. Modified from Kuhelj et al. (2015b)

exploitation of female replies to rival’s calls, i.e., satellite behavior). The females replied equally to both males and mated with the first male that located them, even with the males that were only exploiting female replies to approach them and were during the trials never calling themselves. However, mating with the first male that locates her may be a strategy to mate with the male in better condition, tentatively supported by the observation that, in general, males that reached the females first were heavier at the beginning of the trial. Since they did not have to replace the used energy by feeding during the trial, they could move more freely, which may be a crucial factor when competing with a rival. Moreover, they could also invest more in

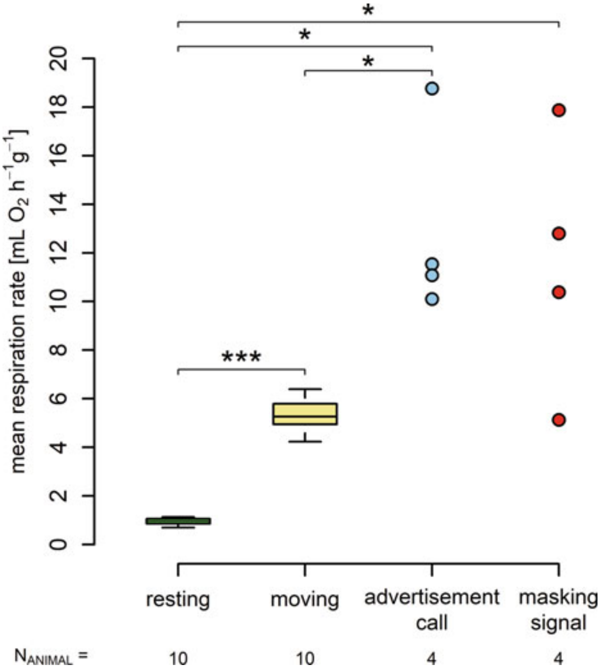


Fig. 4.6 Comparison of respiration rates in *Aphrodes makarovi* males associated with 15–30 s long periods of resting and moving, as well as with the emission of advertisement calls and masking signals. Box and whisker plots show the median (black line), the 25–75% interquartile range (boxes), the lowest and the highest data points still within 1.5 of interquartile range (whiskers) and outliers (circles). *indicates significant differences (Wilcoxon rank sum test for multiple comparisons with Bonferroni correction, $*p < 0.05$, $***p < 0.001$). $N =$ number of males. When $N < 6$, raw data are shown

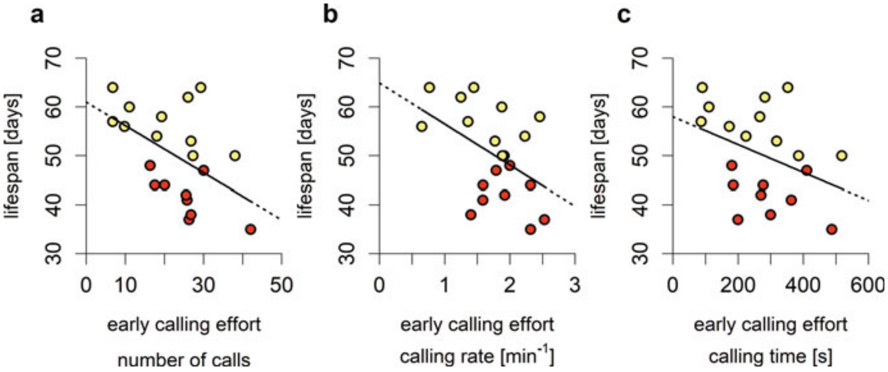


Fig. 4.7 The relationship between longevity of *Aphrodes makarovi* males and calling effort in early life (weeks 2–5) expressed as: (**a**) the mean number of calls emitted during the trial; (**b**) the mean calling rate per minute of trial; (**c**) the mean calling time during the trial. The lines represent the common regression slopes. a: Pearson's correlation coefficient $(r) = -0.503$ $(p = 0.024)$; b: $r = -0.472$ $(p = 0.036)$; c: $r = -0.382$ $(p = 0.096)$. Short- and long-lived males are shown as red and yellow circles, respectively. Modified from Kuhelj et al. (2015b)

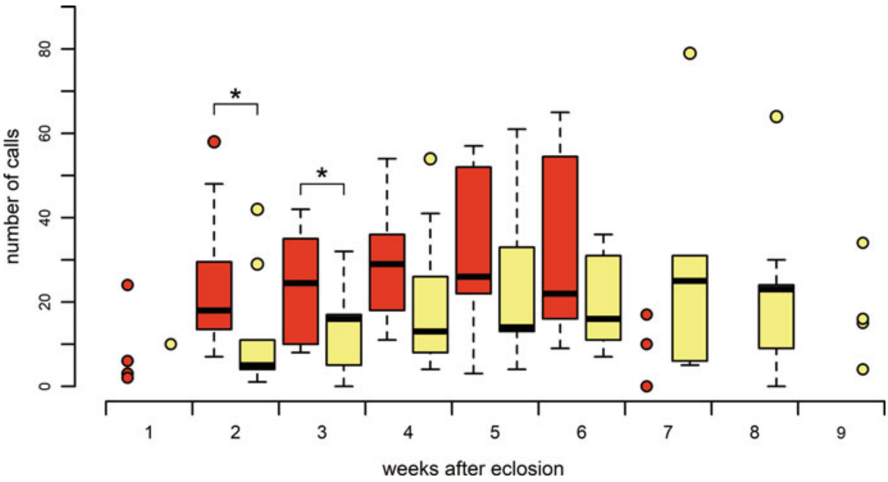


Fig. 4.8 Calling effort of short- (red box) and long-lived (yellow box) *Aphrodes makarovi* males by age. The number of emitted advertisement calls in each week of adult life is shown. Box and whisker plots show the median (black line), the 25–75% interquartile range (boxes), the lowest and the highest data points still within 1.5 of interquartile range (whiskers) and outliers (circles). *indicates significant differences between calling activity of short- and long-lived males in a particular week (Wilcoxon rank sum test, $p < 0.05$). When $N < 6$, raw data are shown. Adapted from Kuhelj et al. (2015b)

energetically demanding masking signals used to confound the searching rival with the second source (Kuhelj et al. 2015b).

In summary, the studies of selection pressures on the vibrational communication system in *A. makarovi* so far have revealed that males face direct costs imposed by eavesdropping predators (Virant-Doberlet et al. 2011), as well as indirect costs due to energetically demanding signaling (Kuhelj et al. 2015b). However, studies also revealed that males are highly attentive to female vibrational replies and also show high plasticity in adapting their signaling behavior (Kuhelj et al. 2016). Yet, male mating success depends on complex interactions underlying male–male competition (Kuhelj and Virant-Doberlet 2017).

4.4 Concluding Remarks

The need to increase the diversity of species included in the study of sexual selection has been stressed recently (Zuk et al. 2014; Monaghan 2015). Traditionally, sexual selection studies focused primarily on male sexual displays, male–male competition, and female choice (e.g., Darwin 1871; Andersson 1994; Bonduriansky 2001); however, more recent evidence suggests that sexual selection acts on both sexes (Bonduriansky 2001; Clutton-Brock 2007, 2009). At present, we are only beginning to examine the possibility of male–female reciprocal interactions during the reproductive process (e.g., Rodríguez 2015), and vibrational communication systems, in which duetting appears to be prevalent, offer ideal opportunities to study simultaneously the sources of selection on male and female vibrational signals and signaling behavior. Taking into account the prevalence of vibrational communication among animals and its long evolutionary history, more detailed studies of such systems are likely to provide new insights and understanding of the behavioral, physiological, and evolutionary dynamics involved in mating systems.

As it is evident from this chapter, our understanding of energy consumption associated with the production of a vibrational signal and its relationship with the signaling effort and mortality is limited to only two species, and hopefully, our review will stimulate more work in this area, not only in connection with sexual selection, but also in revealing potential effects of climate change (Brandt et al. 2018; Jocson et al. 2019; Macchiano et al. 2019; Rosenthal and Elias 2019). We also wish to emphasize that a more comprehensive approach to investigating energetic costs of vibrational signaling should include more detailed physiological studies on signal production mechanisms.

Acknowledgments We would like to thank Peggy Hill for her support and infinite patience. The work received funding from the Slovenian National Research Agency (research program P1-0255, research project J1-8142, as well as PhD fellowship 1000-11-310197 and postdoc research project Z1-8144 awarded to AK).

References

Andersson M (1994) Sexual selection. Princeton University Press, Princeton NJ

Bailey WJ, Withers PC, Endersby M, Gaull K (1993) The energetic costs of calling in the bushcricket *Requena verticalis* (orthoptera: Tettigoniidae: Listroscelidinae). J Exp Biol 178: 21–37

Bertram SM, Harrison SJ, Thomson IR, Fitzsimmons LP (2013) Adaptive plasticity in wild cricket's acoustic signalling. PLoS One 8:e69247

Bluemel JK, Derlink M, Pavlovcic P, Russo IRM, King RA, Corbett E, Sherrard-Smith E, Blejec A, Wilson MR, Stewart AJA, Symondson WOC, Virant-Doberlet M (2014) Integrating vibrational signals, mitochondrial DNA and morphology for species determination in the genus *Aphrodes* (Hemiptera: Cicadellidae). Syst Entomol 39:304–324

Bonduriansky R (2001) The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biol Rev 76:305–339

Bradbury JW, Vehrencamp SL (2011) Principles of animal communication. Sinauer Associates, Sunderland MA

Brandt EE, Kelley JP, Elias DO (2018) Temperature alters multimodal signaling and mating success in an ectotherm. Behav Ecol Sociobiol 72:191

Callander S, Kahn AT, Hunt J, Backwell PRY, Jennions MD (2013) The effects of competitors on calling effort and life-span in male field crickets. Behav Ecol 24:1251–1259

Chiykowski LN (1970) Notes on the biology of the leafhopper *Aphrodes bicincta* (Homoptera: Cicadellidae) in the Ottawa area. Can Entomol 102:750–758

Clutton-Brock T (2007) Sexual selection in males and females. Science 318:1882–1885

Clutton-Brock T (2009) Sexual selection in females. Anim Behav 77:3–11

Cocroft RB, McNett GD (2006) Vibratory communication in treehoppers (Hemiptera: Membracidae). In: Drosopoulos S, Claridge MF (eds) Insect sounds and communication: physiology, behaviour, ecology and evolution. Taylor and Francis, Boca Raton FL, pp 307–317

Čokl A (2008) Stink bug interaction with host plants during communication. J Insect Physiol 54: 1113–1124

Čokl A, Blassioli-Moraes MC, Laumann RA, Žunič A, Borges M (2019) Stinkbugs – multisensory communication with chemical and vibratory signals transmitted through different media. In: Hill PSM, Lakes-Harlan R, Mazzoni V, Narins PM, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational behavior. Springer, Heidelberg, pp 91–122

Darwin C (1871) The descent of man and selection in relation to sex. John Murray, London, pp 214–423

Davranoglou LR, Cicirello A, Taylor GK, Mortimer B (2019a) Planthopper bugs use a fast cyclic elastic recoil mechanisms for effective vibrational communication at small body size. PLoS Biol 17:e3000155

Davranoglou LR, Mortimer B, Taylor GK, Malenovský I (2019b) On the morphology and possible function of two putative vibroacoustic mechanisms in derbid planthoppers (Hemiptera: Fulgoromorpha: Derbidae). Arthropod Struct Dev 52:100880

de Groot M, Čokl A, Virant-Doberlet M (2011) Search behaviour in two hemipteran species using vibrational communication. Cent Eur J Biol 6:756–769

de Groot M, Derlink M, Pavlovčič P, Prešern J, Čokl A, Virant-Doberlet M (2012) Duetting behaviour in the leafhopper *Aphrodes makarovi* (Hemiptera: Cicadellidae). J Insect Behav 25: 419–440

De Luca PA, Cocroft RB (2011) The influence of age on male mate-searching behaviour in thornbug treehoppers. Ethology 117:1–11

Derlink M, Pavlovčič P, Stewart AJA, Virant-Doberlet M (2014) Mate recognition in duetting species: the role of male and female vibrational signals. Anim Behav 90:181–193

Derlink M, Abt I, Mabon R, Julian C, Virant-Doberlet M, Jacquot E (2018) Mating behavior of *Psammotettix alienus* (Hemiptera: Cicadellidae). Insect Sci 25:148–160

Doubell M, Grant PBC, Esterhuizen N, Bazelet CS, Addison P, Terblanche JS (2017) The metabolic costs of sexual signalling in the chirping katydid *Plangia graminea* (Serville) (orthoptera: Tettigoniidae) are context dependent: cumulative costs add up fast. J Exp Biol 220:4440–4449

Elias DO, Mason AC, Maddison WP, Hoy RR (2003) Seismic signals in a courting male jumping spider (Araneae: Salticidae). J Exp Biol 206:4029–4039

Endler JA (2014) The emerging field of tremology. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Heidelberg, pp vii–x

Erregger B, Kovac H, Stabentheiner A, Hartbauer M, Römer H, Schmidt AKD (2017) Cranking up the heat: relationships between energetically costly song features and the increase in thorax temperature in male crickets and katydids. J Exp Biol 220:2635–2644

Fitzsimmons LP, Bertram SM (2011) The calling songs of male spring field crickets (*Gryllus veletis*) change as males age. Behaviour 148:1045–1065

Gerhardt HC, Huber F (2002) Acoustic communication in insects and anurans. University of Chicago Press, Chicago

Gogala M, Čokl A, Drašlar K, Blaževič A (1974) Substrate-borne sound communication in Cydnidae. J Comp Physiol 94:25–31

Hack MA (1998) The energetics of male mating strategies in field crickets (orthoptera: Gryllinae: Gryllidae). J Insect Behav 11:853–867. https://doi.org/10.1023/A:1020864111073

Halsey LG (2016) Terrestrial movement energetics: current knowledge and its application to the optimising animal. J Exp Biol 219:1424–1431

Hartbauer M, Stabentheiner A, Römer H (2012) Signalling plasticity and energy saving in a tropical bushcricket. J Comp Physiol A 198:203–217

Hill PSM, Wessel A (2016) Biotremology. Curr Biol 26:R181–R191

Hill PSM, Virant-Doberlet M, Wessel A (2019) What is biotremology? In: Hill PSM, Lakes-Harlan R, Mazzoni V, Narins PM, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational behavior. Springer, Heidelberg, pp 15–25

Hoback WW, Wagner WE (1997) The energetic cost of calling in the variable field cricket, *Gryllus lineaticeps*. Physiol Entomol 22:286–290

Hoskin CJ, Higge M (2010) Speciation via species interactions: the divergence of mating traits within species. Ecol Lett 13:409–420

Huber BA (2005) Sexual selection research on spiders: Progress in biases. Biol Rev 80:363–385

Hughes NK, Kelley JL, Banks PB (2012) Dangerous liaisons: the predation risk of receiving social signals. Ecol Lett 15:1326–1339

Hunt J, Brooks R, Jennions MD, Smith MJ, Bentsen CL, Bussl̀ere LF (2004) High-quality male field crickets invest heavily in sexual display but die young. Nature 432:1024–1027

Hunt J, Jennions MD, Spyrou N, Brooks R (2006) Artificial selection on male longevity influences age-dependent reproductive effort in the black field cricket *Teleogryllus commodus*. Am Nat 168:E72–E86

Hunt J, Breuker CJ, Sadowski JA, Moore AJ (2009) Male-male competition, female mate choice and their interaction: determining total sexual selection. J Evol Biol 22:13–26

Hunt RE, Nault LR (1991) Roles of interplant movement, acoustic communication and phototaxis in mate-location behavior of the leafhopper *Graminella nigrifrons*. Behav Ecol Sociobiol 28: 315–320

Irschick DJ, Herrel A, Vanhooydonck B, Van Damme R (2007) A functional approach to sexual selection. Funct Ecol 21:621–626

Jocson DI, Smeester M, Leith NT, Macchiano A, Fowler-Finn KD (2019) Temperature coupling of mate attraction signals and female mate preferences in four populations of *Enchenopa* treehoppers (Hemiptera: Membracidae). J Evol Biol 32(10):1046–1056

Johnstone RA (1995) Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. Biol Rev 70:1–65

Judge KA, Ting JJ, Gwynne DT (2008) Condition dependence of male life span and calling effort in a field cricket. Evolution 62:868–878

Kavanagh MW (1987) The efficiency of sound production in two cricket species, *Gryllotalpa australis* and *Teleogryllus commodus* (orthoptera: Grylloidea). J Exp Biol 130:107–119

Kokko H, Rankin DJ (2006) Lonely hearts or sex in the city? Density dependent effects in mating systems. Philos T Roy Soc B 361:319–334

Kokko H, Brooks R, McNamara JM, Houston AI (2002) The sexual selection continuum. P Roy Soc Lond B 269:1331–1340

Kokko H, Jennions MD, Brooks R (2006) Unifying and testing models of sexual selection. Annu Rev Ecol Evol Syst 37:43–66

Kotiaho JS (2000) Testing the assumptions of condition handicap theory: costs and condition dependence of a sexually selected trait. Behav Ecol Sociobiol 48:188–194

Kotiaho JS (2001) Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. Biol Rev 76:365–376

Kotiaho JS, Alatalo RV, Mappes J, Parri S (1996) Sexual selection in a wolf spider: male drumming activity, body size, and viability. Evolution 50:1977–1981

Kotiaho JS, Alatalo RV, Mappes J, Nielsen MG, Parri S, Rivero A (1998a) Energetic costs of size and sexual signalling in a wolf spider. P Roy Soc Lond B 265:2203–2209

Kotiaho JS, Alatalo RV, Mappes J, Nielsen MG, Parri S, Rivero A (1998b) Male mating success and risk of predation in a wolf spider: a balance between sexual and natural selection. J Anim Ecol 67:287–291

Kotiaho JS, Alatalo RV, Mappes J, Nielsen MG, Parri S (1999) Sexual signalling and viability in wolf spider (*Hygrolycosa rubrofasciata*): measurements under laboratory conditions. Behav Ecol Sociobiol 46:123–128

Kotiaho JS, Alatalo RV, Mappes J, Parri S (2004) Adaptive significance of synchronous chorusing in an acoustically signalling wolf spider. P Roy Soc Lond B 271:1847–1850

Kuhelj A (2015) Sexual competitors in the communication strategy of the southern green stink bug (*Nezara viridula*, Pentatomidae) and the leafhoppers of the genus *Aphrodes* (Cicadellidae). PhD Thesis, University of Ljubljana, in Slovene

Kuhelj A, Virant-Doberlet M (2017) Male-male interactions and male mating success in the leafhopper *Aphrodes makarovi*. Ethology 123:425–433

Kuhelj A, de Groot M, Blejec A, Virant-Doberlet M (2015a) The effect of timing of female vibrational reply on male signalling and searching behaviour in the leafhopper *Aphrodes makarovi*. PLoS One 10:e0139020

Kuhelj A, de Groot M, Pajk F, Simčič T, Virant-Doberlet M (2015b) Energetic cost of vibrational signalling in a leafhopper. Behav Ecol Sociobiol 69:815–828

Kuhelj A, de Groot M, Virant-Doberlet M (2016) Sender-receiver dynamics in leafhopper vibrational duetting. Anim Behav 114:139–146

Kuhelj A, Škorjanc A, Vittori M, Žnidaršič N, Hoch H, Wessel A, Virant-Doberlet M (2018) Vibrational signal production in *Aphrodes makarovi*. In: Hill PSM, Mazzoni V, Virant-Doberlet M (eds) Abstract book, 2nd International symposium on biotremology, p 24. Available from https://eventi.fmach.it/biotremology2018/Book-of-Abstracts

Lampert W (1984) The measurement of respiration. In: Downing JA, Rigler FH (eds) A manual on methods for the assessment of secondary productivity in fresh waters. Blackwell, Oxford, pp 413–468

Lee H-J, Loher W (1993) The mating strategy of the male short-tailed cricket *Anurogryllus muticus* de Geer. Ethology 95:327–344

Lighton JRB (1987) Cost of tokking: the energetics of substrate communication in the Tok-Tok beetle, *Psammodes striatus*. J Comp Physiol B 157:11–20

Lighton JRB (2008) Measuring metabolic rates: a manual for scientists. Oxford University Press, Oxford

Lighton JRB (2017) Limitations and requirements for measuring metabolic rates: a mini review. Eur J Clin Nutr 71:301–305

Lighton JRB, Halsey LG (2011) Flow-through respirometry applied to chamber systems: pros and cons, hints and tips. Comp Biochem Physiol A 158:265–275

Lighton JRB, Weier JA, Feener DH (1993) The energetics of locomotion and load carriage in the desert harvester ant *Pogonomyrex rugosus*. J Exp Biol 181:49–61

Lopez-Diez JJ (2019) Reproductive strategy of the leafhopper *Orientus ishidae* Matsumura (Hemiptera: Cicadellidae). Thesis, University of Ljubljana

Maan ME, Seehausen O (2011) Ecology, sexual selection and speciation. Ecol Lett 14:591–602

Macchiano A, Sasson DA, Leith NT, Fowler-Finn KD (2019) Patterns of thermal sensitivity and sex-specificity of courtship behavior differs between two sympatric species of *Enchenopa* treehopper. Front Ecol Evol 7:361

MacNally R, Young D (1981) Song energetics of the bladder cicada, *Cystosoma saundersii*. J Exp Biol 90:185–196

Mappes J, Alatalo RV, Kotiaho J, Parri S (1996) Viability costs of condition-dependent sexual male display in a drumming wolf spider. P Roy Soc Lond B 263:785–789

Mazzoni V, Prešern J, Lucchi A, Virant-Doberlet M (2009) Reproductive strategy of the Nearctic leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae). Bull Entomol Res 99:401–413

Mendelson TC, Shaw KL (2012) The (mis)conception of species recognition. Trends Ecol Evol 27: 421–427

Miles CI, Allison BE, Losinger MJ, Su QT, Miles RN (2017) Motor and mechanical bases of the courtship call of the male treehopper, *Umbonia crassicornis*. J Exp Biol 220:1915–1924

Miklas N, Lasnier T, Renou M (2003) Male bugs modulate pheromone emission in response to vibratory signals from conspecifics. J Chem Ecol 29:561–574

Miller CW, Svensson EI (2014) Sexual selection in complex environments. Annu Rev Entomol 59: 427–445

Monaghan P (2015) Behavioral ecology and the successful integration of function and mechanism. Behav Ecol 25:1019–1021

Okada K, Pitchers WR, Sharma MD, Hunt J, Hosken DJ (2011) Longevity, calling effort and metabolic rate in two populations of cricket. Behav Ecol Sociobiol 65:1773–1778

Ophir AG, Schrader SB, Gillooly JF (2010) Energetic cost of calling: general constraints and species-specific differences. J Evol Biol 23:1564–1569

Ossiannilsson F (1949) Insect drummers. A study on the morphology and function of the sound-producing organ of Swedish Homoptera-Auchenorrhyncha. Opuscula Entomologica, Supplementum X:1–146

Panhuis TM, Butlin R, Zuk M, Tregenza T (2001) Sexual selection and speciation. Trends Ecol Evol 16:364–371

Parri S, Alatalo RV, Kotiaho J, Mappes J (1997) Female choice for male drumming in the wolf spider *Hygrolycosa rubrofasciata*. Anim Behav 53:305–312

Polajnar J, Eriksson A, Rossi Stacconi MV, Lucchi A, Anfora G, Virant-Doberlet M, Mazzoni V (2014) The process of pair formation mediated by substrate-borne vibrations in a small insect. Behav Process 107:68–78

Polajnar J, Maistrelo L, Bertarella A, Mazzoni V (2016) Vibrational communication of the brown marmorated stink bug (*Halyomorpha halys*). Physiol Entomol 41:249–259

Prestwich KN (1994) The energetics of acoustic signaling in anurans and insects. Am Zool 34:625–643

Prestwich KN, O'Sullivan K (2005) Simultaneous measurement of metabolic and acoustic power and the efficiency of sound production in two mole cricket species (orthoptera: Gryllotalpidae). J Exp Biol 208:1495–1512

Prestwich KN, Walker TJ (1981) Energetics of singing in crickets: effect of temperature in three trilling species (orthoptera: Gryllidae). J Comp Physiol B 143:199–212

Reinhold K, Greenfield MD, Jang Y, Broce A (1998) Energetic cost of sexual attractiveness: ultrasonic advertisement in wax moths. Anim Behav 55:905–913

Rivero A, Alatalo RV, Kotiaho JS, Mappes J, Parri S (2000) Acoustic signalling in a wolf spider: can signal characteristics predict male quality? Anim Behav 6:187–194

Robinson DJ, Hall MJ (2002) Sound signalling in orthoptera. Adv Insect Physiol 29:151–278

Rodríguez RL (2015) Mating is a give-and-take of influence and communication between the sexes. In: Peretti AV, Aisenberg A (eds) Cryptic female choice in arthropods. Springer, Heidelberg, pp 479–496

Rodríguez RL, Barbosa F (2014) Mutual behavioral adjustment in vibrational duetting. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Heidelberg, pp 147–169

Rodríguez RL, Haen C, Cocroft RB, Fowler-Finn KD (2012) Males adjust signalling effort based on female mate-preference cues. Behav Ecol 24:1218–1225

Rodríguez RL, Boughman JW, Gray DA, Hebets EA, Höbel G, Symes LB (2013) Diversification under sexual selection, the relative roles of mate preference strength and the degree of divergence in mate preferences. Ecol Lett 16:964–974

Römer H, Lang A, Hartbauer M (2010) The signaller's dilemma: a cost-benefit analysis of public and private communication. PLoS One 5:e13325

Rosenthal MF, Elias DO (2019) Nonlinear changes in selection on a mating display across a continuous thermal gradient. P Roy Soc B 286:20191450

Ryan MJ, Kime NM (2003) Selection on long distance calls. In: Simmons MA, Fay RA, Popper AN (eds) Acoustic communication. Springer, Heidelberg, pp 225–274

Safran RJ, Scordato ESC, Symes LB, Rodríguez RL, Mendelson TC (2013) Contributions of natural and sexual selection to the evolution of premating reproductive isolation: a research agenda. Trends Ecol Evol 28:643–650

Shuster SM, Wade MJ (2003) Mating systems and mating strategies. Princeton University Press, Princeton NJ

Stevens ED, Josephson RK (1977) Metabolic rate and body temperature in singing katydids. Physiol Zool 50:31–42

Tauber E, Cohen D, Greenfield MD, Pener MP (2001) Duet singing and female choice in the bushcricket *Phaneroptera nana*. Behaviour 138:411–430

Villarreal SM, Gilbert C (2014) Male *Scudderia pistillata* katydids defend their acoustic duet against eavesdroppers. Behav Ecol Sociobiol 68:1669–1675

Virant-Doberlet M, Čokl A (2004) Vibrational communication in insects. Neotrop Entomol 33: 121–134

Virant-Doberlet M, King RA, Polajnar J, Symondson WOC (2011) Molecular diagnostics reveal spiders that exploit prey vibrational signals used in sexual communication. Mol Ecol 20:2204–2216

Virant-Doberlet M, Kuhelj A, Polajnar J, Šturm R (2019) Predator-prey interactions and eavesdropping in vibrational communication networks. Front Ecol Evol 7:203

Wessel A, Mühlenthaler R, Hartung V, Kuštor V, Gogala M (2014) The tymbal: evolution of a complex vibration-producing organ in Tymbalia (Hemiptera exl. Sternorrhycha). In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Heidelberg, pp 395–444

White CR, Matthews PG, Seymour RS (2008) In situ measurement of calling metabolic rate in an Australian mole cricket, *Gryllotalpa monanka*. Comp Biochem Physiol A Mol Integr Physiol 150(2):217–221

Wilkins MR, Seddon N, Safran RJ (2013) Evolutionary divergence in acoustic signals: causes and consequences. Trends Ecol Evol 28(3):156–166

Wong BBM, Candolin U (2005) How is female mate choice affected by male competition? Biol Rev 80:1–13

Woods WA, Hendrickson H, Mason J, Lewis SM (2007) Energy and predations costs of firefly courtship signals. Am Nat 170:702–708

Zuk M, Bastiaans E, Langkilde T, Swanger E (2014) The role of behaviour in the establishment of novel traits. Anim Behav 92:333–344. https://doi.org/10.1016/j.anbehav.2014.02.032

Zuk M, Kolluru GR (1998) Exploitation of sexual signals by predators and parasitoids. Q Rev Biol 73:415–438

Chapter 5
The Hawaiian Planthoppers (Hemiptera: Auchenorrhyncha: Fulgoromorpha) and Their Courtship Songs

Manfred Asche, Hannelore Hoch, Caitlin O'Connell-Rodwell, and Andreas Wessel

Abstract The Hawaiian islands are among the most isolated archipelagos in the world. Previous research has revealed what are now classic examples for adaptive radiation and rapid evolution of diversity in geologically young, geographically dynamic, and ecologically rich environments in various groups of organisms, many of them pertaining to insects. An important group of Hawaii's endemic insect fauna are the Fulgoromorpha (Hemiptera). Most Hemiptera communicate by surface-borne vibrational signals in order to locate and recognize potential mating partners. Here we document the vibrational courtship calls of 6 Cixiidae (ca. 10% of the Hawaiian species) and 34 endemic and 4 immigrant Delphacid species (ca. 25% of Hawaii's species), and provide information on their distribution and host plant associations. The vibrational signals of Hawaii's planthoppers constitute a hitherto neglected aspect of the diversity of Hawaiian organisms. We point out threats to the conservation of Hawaii's planthoppers and indicate potential research opportunities for further study of signal evolution.

5.1 Introduction

The Hawaiian archipelago provides nearly ideal conditions to study evolutionary processes. The Hawaiian islands are the most isolated major island group in the world, being more than 4000 km from the nearest continent, and 1600 km from the nearest other island groups. The linear arrangement of the six major islands is in chronological order; geological age is gradually decreasing from Kauai (ca. 5 Ma:

M. Asche · H. Hoch (✉) · A. Wessel
Museum für Naturkunde, Leibniz-Institute for Research on Evolution and Biodiversity, Humboldt-Universität zu Berlin, Berlin, Germany
e-mail: manfred.asche@mfn-berlin.de; hannelore.hoch@mfn.berlin

C. O'Connell-Rodwell
Eaton Peabody Lab, Mass Eye & Ear, Harvard Medical School, Boston, MA, USA
e-mail: ceoconnell@stanford.edu

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_5

Clague and Dalrymple 1987) to the Island of Hawaii (ca. 1 Ma: Sherrod et al. 2007), as the islands were sequentially formed as the Pacific tectonic plate moved in a northwesterly direction over a volcanic hot spot. Hawaii Island is still over the hot spot and has two active volcanoes, Mauna Loa and Kilauea. The high altitude of the major islands and their position in the trade wind zone result in a steep rainfall gradient between windward and leeward sides. Due to their isolated position within the Pacific, only a fraction of plant and animal taxa that are found on the adjacent continents was able to cross the Pacific and successfully establish viable populations. Subsequently, these taxa adapted to the wide variety of ecological conditions, and underwent rapid, explosive speciation and adaptive radiation, giving rise to many endemic species. The conveyor-belt fashion in which the islands were formed produced “replicas” of these processes on each island, often resulting in similar species communities (e.g., Simon 1987; Shaw and Gillespie 2016). Most of the flora and fauna of Hawaii are endemic, with endemism as high as 90–99% for terrestrial taxa (Carlquist 1980). The biota of Hawaii have been described as naturally depauperate and secondarily enriched (Mueller-Dombois 1981). More than 150 distinct natural ecosystems (i.e., a discrete group of interacting species in a common area) are found in the Hawaiian islands, and more than 100 different plant communities have been recognized (Howarth and Mull 1992).

The Hawaiian Islands harbor a rich and diverse arthropod fauna (Nishida 2002), of which the ca. 5400 endemic insect species constitute a major element. The native insect fauna presumably derived from only about 400 primary colonizing species (Howarth 1990), which equals only one successful arrival every 75,000 years (Howarth and Mull 1992).

Native insects are found in virtually all terrestrial ecosystems of Hawaii, including high-stress environments such as the nearly vegetation-free, dry Aeolian zone (above 3000 m a.s.l.) where they feed on wind-borne organic debris, and in the numerous lava tubes, where they feed on roots of epigean plants or live as predators and scavengers. Well-known examples of large insect radiations (> 100 species) in Hawaii are the Diptera, the Coleoptera, the Lepidoptera, and Hymenoptera (for an overview on taxa see information in Howarth and Mull 1992).

The Fulgoromorpha, or planthoppers, constitute an important element of the Hawaiian insect fauna. Out of the 18 Fulgoromorpha taxa in family rank occurring worldwide, only two have representatives which are native to Hawaii: the Delphacidae and the Cixiidae. The Flatidae, the Derbidae, the Tropiduchidae, and the Issidae species present in Hawaii are more recent immigrant species (Zimmerman 1948; Asche 2000b; Gnezdilov and Bartlett 2022).

Currently, 211 native Hawaiian planthopper species (numbers combined from Asche 1997; Hoch and Howarth 1999) are recognized. The vast majority of species were described in the first half of the nineteenth century. Apart from Giffard’s (1925) review on Hawaiian Cixiidae, the only comprehensive treatment of the Hawaiian Auchenorrhyncha (Fulgoromorpha and Cicadomorpha) to date was provided by Zimmerman (1948). For a more detailed account of the history of planthopper research on Hawaii, and the history of Hawaiian Entomology see e.g., Zimmerman 1948; Howarth and Mull 1992; Asche 1997; Evenhuis 2007). Since Zimmerman’s

Fig. 5.1 Planthopper habitat types. (**a**) mountainous wet forest, Kauai, Alakai swamp; (**b**) lowland dry forest, Hawaii Island, Manuka Natural Area Reserve. Photographs by H. Hoch

(1948) overview, only a few authors have contributed to the taxonomy and systematics of Hawaiian planthoppers (J. W. Beardsley, R.G. Fennah, M. Asche, H. Hoch, F.G. Howarth: see references).

Although planthoppers as phytophagous, sap-sucking insects are elements of nearly all Hawaiian terrestrial ecosystems (Figs. 5.1 and 5.2), very little was known about their ecology and evolution. Even host plant information does not exist for many species. Hawaiian planthoppers are apparently oligophagous or monophagous on native Hawaiian plant species (in over 70 genera), mostly on ferns and woody dicots (Fig. 5.3) (see Asche 1997, and references therein). Only recently Hiller et al. (2019) presented a study on the evolution of niche differentiation in Hawaiian arthropods, including species of the delphacid genus *Nesosydne* (see Sect. 5.3.2.5). Some of the introduced planthopper species are important pests of crops in Hawaii, such as the Australasian delphacid *Perkinsiella saccharicida* Kirkaldy, 1903 on sugarcane (Zimmerman 1948).

Prior to the study on the evolutionary dynamics of the troglobitic cixiid *Oliarus polyphemus* from lava tubes on the Island of Hawaii (Howarth et al. 1990; Hoch and Howarth 1993) and a study on species differentiation in the *Acacia koa*-feeding species of the *Nesosydne koae* species group (O'Connell 1991) no information on the behavior of any of the Hawaiian planthoppers was available. More recently, Goodman et al. (2015) attempted to identify the factors shaping diversification of

Fig. 5.2 Planthopper habitat types (continued). (**a**) mountainous cloudswept forest, Oahu, Mt. Ka'ala, with M.A. collecting on *Gunnera petaloïdea*; (**b**) mountainous fern forest, Maui, Hale'akala, Halemau Trail; (**c**) coastal vegetation, Oahu, Ka'ena Point; (**d**) Maui, Hale'akala canyon, *Argyroxiphium sandwicense*. Photographs by H. Hoch

vibrational signals among allopatric and sympatric populations of *Nesosydne chambersi* from Hawaii Island.

Planthoppers, like most Auchenorrhyncha (except the Cicadidae, which produce audible sound), communicate by vibrational signals that are transmitted through the substrate, which is usually the host plant (for more information on signal production see Ossiannilson 1949; Wessel et al. 2014; Davranoglou et al. 2019). The signals predominantly serve mate recognition and location and act as prezygotic reproduction barriers; patterns are species-specific (see Claridge 1985, and references therein). The three studies mentioned above revealed a high degree of diversification

Fig. 5.3 Host plants of Hawaiian planthoppers. (**a**) *Metrosideros polymorpha*, Hawaii Island, Kilauea Iki crater; (**b**) *Sadleria cyatheoides*, Hawaii Island, Kilauea, fern forest; (**c**) *Acacia koae*, Hawaii Island, Kilauea, Crater Rim Trail; (**d**) *Geranium cuneatum*, Hawaii Island, Mauna Loa Trail. Photographs by H. Hoch

between populations in the species complexes studied (Hoch and Howarth 1993; O'Connell 1991; Goodman et al. 2015).

In the course of completed and ongoing systematic revisions of Hawaiian cixiid (Hoch 2006) and delphacid taxa (Asche 1997, 1998, 2000a, b), recordings of vibrational communication signals of representatives of both families were obtained during several field trips between 1989–1998. Here we summarize the current state of knowledge of vibrational signals of 44 species of Cixiidae (6) and Delphacidae (34 native, 4 immigrant), along with information on their systematics, distribution, and ecology. An overview of the species recorded, their locality, and host-plants is provided by Table 5.1.

Table 5.1 Overview of vibrational signals of Hawaiian planthopper species (Cixiidae and Delphacidae) as documented in the VibroLibrary of the Animal Sound Archive (Tierstimmenarchiv, Museum für Naturkunde, Berlin)

	Species	Signals recorded from populations on	Host plants (if not indicated otherwise, hostplant records are from Zimmerman 1948)	VibroLibrary identifier of signal recordings (original recording numbers in brackets)
Cixiidae	*Iolania perkinsi* Kirkaldy	Hawaii Island	Ferns (*Cibotium*, *Sadleria*)	vibro:Iolania_perkinsi_Hoc0024_01 (H95-rec.03); vibro:Iolania_perkinsi_Hoc0066_07 (H97-rec.15)
	Oliarus filicicola Kirkaldy	Hawaii Island	Fern spp.	vibro:Oliarus_filicicola_Hoc0023_01 (H95-rec.01); vibro:Oliarus_filicicola_Hoc0033_01 (H95-rec.17); vibro:Oliarus_filicicola_Hoc0033_03 (H95-rec.18); vibro:Oliarus_filicicola_Hoc0066_13 (H97–18)
	Oliarus hevaheva Kirkaldy	Hawaii Island	Fern-*Metrosideros*-forest	vibro:Oliarus_hevaheva_Hoc0030_01 (H95-rec.09); vibro:Oliarus_hevaheva_Hoc0066_09 (H97-rec.16)
	Oliarus kaonohi Kirkaldy	Oahu	Ferns	vibro:Oliarus_kaonohi_Hoc0065_05 (H97-rec.08)
	Oliarus lorettae Hoch & Howarth	Hawaii Island	*Sida fallax* (Hoch and Howarth 1999)	vibro:Oliarus_lorettae_Hoc0027_01 (H95-rec.06); vibro:Oliarus_lorettae_Hoc0028_01 (H95-rec.07); vibro:Oliarus_lorettae_Hoc0031_03 (H95-rec.12); vibro:Oliarus_lorettae_Hoc0032_01 (H95-rec.13); vibro:Oliarus_lorettae_Hoc0032_03 (H95-rec.14); vibro:Oliarus_lorettae_Hoc0032_05 (H95-rec.15)
	Oliarus polyphemus species group	Hawaii Island	*Metrosideros polymorpha* (Howarth 1972, Fennah 1973)	For information on signal recordings see Hoch and Hoch and Howarth (1993), Wessel et al. (2013), Strithi et al., Chap. 13, this book
Delphacidae	*Emoloana sporobolicola* (Kirkaldy)	Oahu	*Sporobolus* spec.	vibro:Emoloana_sporobolicola_Hoc0069_01 (H98-rec.01)
		Maui	*Sporobolus* spec.	vibro:Emoloana_sporobolicola_Hoc0072_01 (H98-rec.14)
		Hawaii Island	*Sporobolus* spec.	vibro:Emoloana_sporobolicola_Hoc0067_01 (H97-rec.19)

	Aloha campylothecae Muir	Oahu	*Campylotheca*, *Dubautia*[a], *Carex* near *Bidens*	vibro:Aloha_campylothecae_Hoc0070_01 (H98-rec.04); vibro:Aloha_campylothecae_Hoc0071_05 (H98-rec.09); vibro:Aloha_campylothecae_Hoc0071_07 (H98-rec.10)
	Aloha swezeyi Muir	Kauai	*Campylotheca*, *Bidens*[a]	vibro:Aloha_swezeyi_Hoc0077_05 (H89/90-rec.03)
		Oahu	*Campylotheca*, *Bidens*[a]	vibro:Aloha_swezeyi_Hoc0065_01 (H97-rec.06); vibro:Aloha_swezeyi_Hoc0071_07 (H98-rec.10)
	Dictyophorodelphax mirabilis Swezey	Oahu	*Chamaesyce celastroides*	vibro:Dictyophorodelphax_mirabilis_Hoc0082_03 (H89/90-rec.15); vibro:Dictyophorodelphax_mirabilis_Hoc0071_03 (H98-rec.08); vibro:Dictyophorodelphax_mirabilis_Hoc0071_11 (H98-rec.12)
	Leialoha suttoniae Muir	Kauai	*Myrsine (Suttonia) sandwicensis*	vibro:Leialoha_suttoniae_Hoc0064_07 (H97-rec.04)
	Leialoha pacifica (Kirkaldy)	Kauai	No information	vibro:Leialoha_pacifica_Hoc0071_01 (H98-rec.07 (a))
	Leialoha sp. aff. lehuae (Kirkaldy)	Kauai	*Metrosideros polymorpha*	vibro:Leialoha_Hoc0071_01 (H98-rec.07 (b))
	Nesothoë munroi Muir	Hawaii Island	*Dodonaea*	vibro:Nesothoë_munroi_Hoc0024_03 (H95-rec.04)
	Nesothoë nov. spec. 1 (undescribed)	Hawaii Island	*Antidesma platyphyllum* (Asche, personal observation)	vibro:Nesothoë_munroi_Hoc0024_03 (H98-rec.32)
	***Nesosydne koae* species group = *Nesosydne* s.str.**			
	Nesosydne koae Kirkaldy	Kauai	*Acacia koa*	vibro:Nesosydne_koae_Hoc0064_01 (H97-rec.01); vibro:Nesosydne_koae_Hoc0079_01 (H89/90-rec.05); vibro:Nesosydne_koae_Hoc0077_03 (H89/90-rec.06)
		Lanai	*Acacia koa*	vibro:Nesosydne_koae_Hoc0066_01 (H97-rec.12)

(continued)

Table 5.1 (continued)

	Species	Signals recorded from populations on	Host plants (if not indicated otherwise, hostplant records are from Zimmerman 1948)	VibroLibrary identifier of signal recordings (original recording numbers in brackets)
		Hawaii Island	*Acacia koa*	vibro:Nesosydne_koae_Hoc0066_03 (H97-rec.13); vibro:Nesosydne_koae_Hoc0067_07 (H97-rec.22); vibro:Nesosydne_koae_Hoc0073_07 (H98-rec.23); vibro:Nesosydne_koae_Hoc0077_01 (H89/90-rec.01); vibro:Nesosydne_koae_Hoc0082_05 (H89/90-rec.16); vibro:Nesosydne_koae_Hoc0082_07 (H89/90-rec.17)
	Nesosydne rubescens Kirkaldy	Oahu	*Acacia koa*	vibro:Nesosydne_rubescens_Hoc0071_09 (H98-rec.11)
		Hawaii Island	*Acacia koa*	vibro:Nesosydne_rubescens_Hoc0067_11 (H97-rec.24)
	Nesosydne geranii (Muir)	Maui	*Geranium arboreum*	vibro:Nesosydne_geranii_Hoc0071_13 (H98-rec.13)
	Nesosydne spec. 1 (undescribed)	Hawaii Island	*Geranium cuneatum* (Asche, personal observation)	vibro:Nesosydne_spec_Hoc0073_11 (H98-rec.25); vibro:Nesosydne_spec_Hoc0074_05 (H98-rec.29); vibro:Nesosydne_spec_Hoc0075_03 (H98-rec.33)
	Nesosydne spec. 2 (undescribed)	Hawaii Island	*Myoporum sandwicensis* (Asche, personal observation)	vibro:Nesosydne_spec_Hoc0068_01 (H97-rec.25)
	Nesosydne naenae (Muir)	Kauai	*Dubautia paleata*[a]	vibro:Nesosydne_naenae_Hoc0064_05 (H97-rec.03); vibro:Nesosydne_naenae_Hoc0069_05 (H98-rec.03)
		Oahu	*Dubautia sp.*[a]	vibro:Nesosydne_naenae_Hoc0064_09 (H97-rec.05); vibro:Nesosydne_naenae_Hoc0065_03 (H97-rec.07)

	Nesosydne spec. 3, nr. *nigriceps* Muir (undescribed)	Lanai	*Pittosporum confertiflorum* (Asche, personal observation)	vibro:Nesosydne_naenae_Hoc0065_03 (H97-rec.09); vibro:Nesosydne_spec_Hoc0065_09 (H97-rec.10)
	Nesosydne spec. 4 (undescribed)	Molokai	*Sadleria, Cibotium* ferns (Asche, personal observation)	vibro:Nesosydne_spec_Hoc0073_01 (H98-rec.20)
	***Nesosydne gunnerae* species group**			
	Nesosydne amaumau (Muir)	Maui	Sadleria fern	vibro:Nesosydne_amaumau_Hoc0072_05 (H98-rec.16)
	Nesosydne spec. 5 (undescribed)	Oahu	*Cheirodendron*/*Pelea* (Asche, personal observation)	vibro:Nesosydne_spec_Hoc0069_03 (H98-rec.02)
	***Nesosydne cyathodis* species group**			
	Nesosydne eeke (Muir)	West Maui	*Argyroxiphium caliginis*[a]	vibro:Nesosydne_eeke_Hoc0072_11 (H98-rec.19)
	***Nesosydne aku* species group**			
	Nesosydne chambersi Muir	Hawaii Island	*Dubautia ciliolata*[a]	vibro:Nesosydne_chambersi_Hoc0073_13 (H98-rec.26); vibro:Nesosydne_chambersi_Hoc0075_05 (H98-rec.34)
	Nesosydne ipomoeicola Kirkaldy	Hawaii Island	Polyphagous, e.g., on *Ipomoea*, *Sadleria*	vibro:Nesosydne_ipomoeicola_Hoc0067_03 (H97-rec.20); vibro:Nesosydne_ipomoeicola_Hoc0073_03 (H98-rec.21); vibro:Nesosydne_ipomoeicola_Hoc0074_03 (H98-rec.28 (a)); vibro: Nesosydne_ipomoeicola_Hoc0074_07 (H98-rec.30)
	Nesosyne osborni Muir	Maui	*Dubautia ciliolata*[a]	vibro:Nesosyne_osborni_Hoc0072_03 (H98-rec. 15); vibro:Nesosyne_osborni_Hoc0072_07 (H98-rec.17)

(continued)

Table 5.1 (continued)

	Species	Signals recorded from populations on	Host plants (if not indicated otherwise, hostplant records are from Zimmerman 1948)	VibroLibrary identifier of signal recordings (original recording numbers in brackets)
	Nesosydne umbratica Kirkaldy	Hawaii Island	Polyphagous, e.g., on *Pipturus*	vibro:Nesosydne_umbratica_Hoc0066_11 (H97-rec.17); vibro:Nesosydne_umbratica_Hoc0067_05 (H97-rec.21); vibro:Nesosydne_umbratica_Hoc0073_05 (H98-rec.22); vibro:Nesosydne_umbratica_Hoc0074_03 (H98-rec.28 (b))
	Nesosydne nephelias Kirkaldy	Lanai	*Hedyotis terminalis*	vibro:Nesosydne_nephelias_Hoc0065_11 (H97-rec.11)
	Nesosydne imbricola species group			
	Nesosydne raillardiae Kirkaldy	Hawaii Island	*Dubautia* spp., e.g., *D. scabra*[a]	vibro:Nesosydne_raillardiae_Hoc0066_05 (H97-rec.14); vibro:Nesosydne_raillardiae_Hoc0073_09 (H98-rec.24)
	***Nesosydne argyroxiphii* species group**			
	Nesosydne argyroxiphii Kirkaldy	Maui	*Argyroxiphium* spec.[a]	vibro:Nesosydne_argyroxiphii_Hoc0072_09 (H98-rec.18)
	Immigrant species			
	Perkinsiella saccharicida Kirkaldy	Kauai	*Saccharum officinarum* (sugarcane)	vibro:Perkinsiella_saccharicida_Hoc0080_01 (H89/90-rec.11); vibro:Perkinsiella_saccharicida_Hoc0070_03 (H98-rec.05)

	Tarophagus colocasiae (Matsumura)	Hawaii Island	*Colocasia esculenta* (Taro) (Asche and Wilson 1989a, b)	vibro:Tarophagus_colocasiae_Hoc0074_01 (H98-rec.27)
	Syndelphax disonymus (Kirkaldy)	Kauai	*Cynodon dactylon* (Asche 2000b)	vibro:Syndelphax_disonymus_Hoc0064_03 (H97-rec.02)
	Toya dryope (Kirkaldy)	Kauai	*Cynodon dactylon* (Asche 2000b)	vibro:Toya_dryope_Hoc0070_05 (H98-rec.06)

---

[a] Host-plants belonging to the silversword alliance (Asteraceae), the group of plants which is utilized by the largest number of *Nesosydne* s.l. species

5.2 Materials and Methods

The majority of recordings presented here were made in the laboratory, either at the facilities of the University of Hawaii at Manoa, and of Bishop Museum, Honolulu, or at temporary set-ups at various accommodations on Hawaii Island, Kauai, Maui and Lanai, using the magneto-dynamic system designed by Strübing & Rollenhagen (1988) (for detailed description, see also Chap. 13). Wherever possible, the presumed host-plant was used as a substrate; alternatively, small pieces of balsa wood or thin slices of *Acacia koa* wood were used. Usually, one male and one female of a given species were placed into a small cage containing substrate to which the magnet was attached. For signal recording, we used stereo-cassette recorders Philips D 6920 AV MK 2 and Sony TC-D 5 M (Hoch and Howarth 1993).

General signal structure (time-dependent amplitude modulation) is visualized using WaveLab 8 (64 bit) sound analysis software. Recorded signals and metadata (e.g., pertaining to precise location) are documented in the VibroLibrary (Frommolt et al. 2019) of the Animal Sound Archive (Tierstimmenarchiv) at Museum für Naturkunde Berlin. Voucher specimens are currently accommodated in the research collections of H. Hoch, M. Asche, and A. Wessel, Museum für Naturkunde Berlin, and will eventually be deposited at Bishop Museum, Honolulu.

5.3 Results

5.3.1 Cixiidae

Within the Cixiidae, two lineages have originally colonized the Hawaiian Islands, and given rise to a total of 69 endemic species and 24 subspecies (see Asche 1997; Hoch and Howarth 1999; Hoch 2006): *Iolania* with 6 species (Hoch 2006) and *Oliarus* with 63 (56 epigean, 7 cavernicolous) species, and 24 subspecies (Zimmerman 1948; Fennah 1973; Hoch and Howarth 1999). Cixiid species are found in a variety of habitats; however, specific hostplants are hard to assess since nymphs have been found, "…in Hawaii beneath stones, in rotting tree fern stumps and fronds, in tree fern ground litter, in rotting wood and under the bark of trees" (Zimmerman 1948: 94).

5.3.1.1 *Iolania* Kirkaldy, 1902

Endemic to Hawaii, the two *Iolania* species originally described from Australia (Muir 1931) are no longer regarded congeners (Asche 1997). Four of the six species are single-island endemics: *I. kraussohana* on Kauai, *I. koolauensis* on Oahu, *I. mauiensis* on East and West Maui, and *I. perkinsi* on Hawaii Island. Only two species have a wider distribution: *I. lanaiensis* on Lanai and Molokai and

I. oahuensis on Oahu, Molokai, Lanai, and West Maui (Hoch 2006). Vibrational signals were recorded from *Iolania perkinsi* Kirkaldy, 1902 (Fig. 5.4a) from Hawaii Island (Table 5.1.). For signal characteristics see Fig. 5.9a.

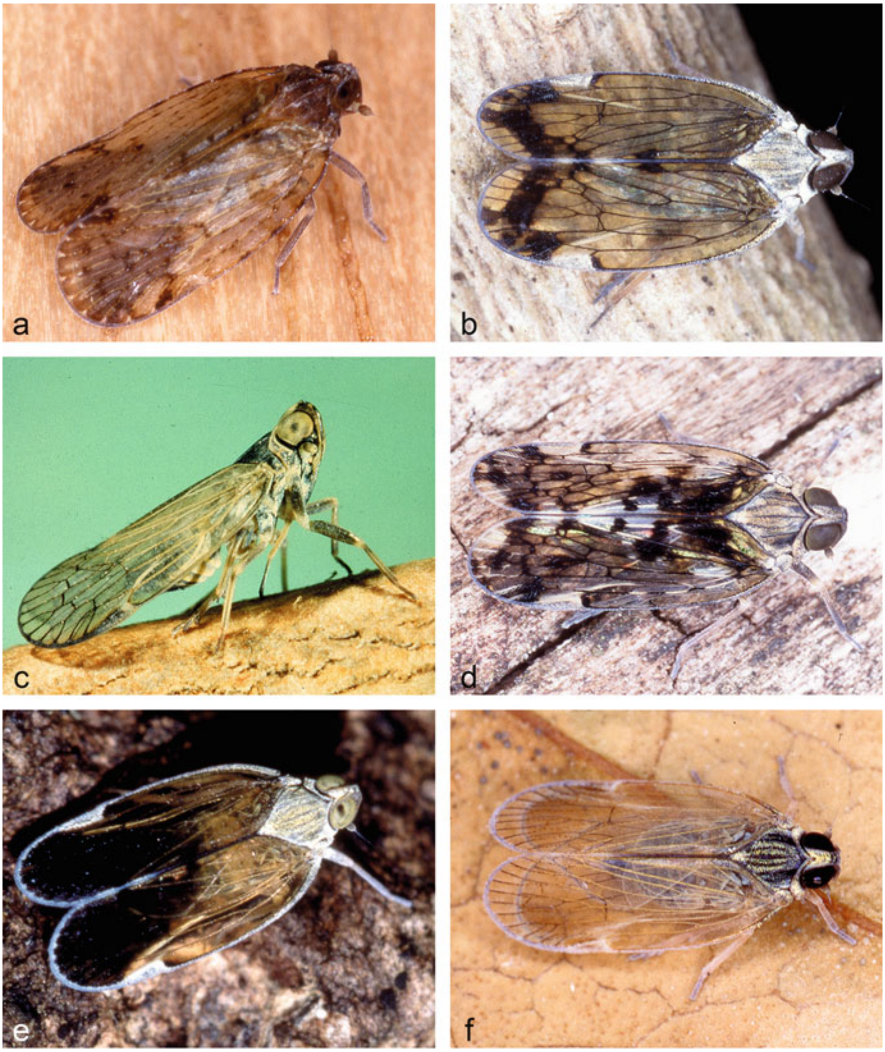


Fig. 5.4 Diversity of Hawaiian Cixiidae. (**a**) *Iolania perkinsi*, Hawaii Island; (**b**) *Oliarus* spec. Indet., Hawaii Island; (**c**) *Oliarus filicicola*, Hawaii Island; (**d–f**) *Oliarus* spp. indet., Hawaii Island. Photographs (**a**–**b**, **b**–**f**) by H. Hoch and (**c**) by W.P. Mull (with kind permission)

5.3.1.2 *Oliarus* Stål, 1862

The Hawaiian "*Oliarus*" species are not congeneric with *Oliarus* sensu *strictu* (Hoch 2005), or with any other "*Oliarus*" from elsewhere. Within the Cixiidae, the genus *Oliarus* is a typical "dumping ground" for species in the tribe Pentastirini, and must be regarded as polyphyletic. *Oliarus* species have been described from both the Old and New World, as well as from several archipelagoes in the Pacific, such as the Marquesas, the Society Islands, the Austral and Cook Islands, and from the Galápagos (Holzinger et al. 2002). The subgenus *Nesoliarus*, erected by Kirkaldy (1909) to separate the Hawaiian *Oliarus* species as a "group by themselves" was later synonymized with *Oliarus* by Zimmerman (1948). In their review of Cixiidae, Holzinger et al. (2002) used *Nesoliarus* for the Hawaiian species, however, without taxonomically formalizing it. For the time being, we shall thus continue the use of "*Oliarus*." The Hawaiian *Oliarus* species most likely form a monophyletic clade, i.e., are descendants of a single colonizing species, which is likely to have reached Hawaii from North America (Asche 1997). No closer relationships have hitherto been recognized to any of the "*Oliarus*" from other Pacific Islands.

Oliarus species are found on all islands and are documented from a wide range of habitats, epigean species (Fig. 5.4b–f) feeding on native vegetation, from lowland dry forest to montaneous rainforests (see information in Zimmerman 1948), as well as from lava tubes on Molokai, Maui, and Hawaii Island (see Chap. 13).

Vibrational signals were recorded from the epigean species *O. filicicola* Kirkaldy, *O. hevaheva* Kirkaldy, and *O. kaonohi* Kirkaldy (for signal characteristics see Fig. 5.9b.), as well as from the cave-dwelling *O. lorettae* Hoch and Howarth (Table 5.1). For vibrational signals of various populations of the cave-dwelling *O. polyphemus* Fennah species group see Hoch and Howarth 1993, Wessel et al. 2014 (see Chap. 13).

5.3.2 *Delphacidae*

Currently, 154 species and 2 subspecies of delphacids have been reported from Hawaii, 143 of which (93.5%) are endemic (Asche 1997, 2000a). Except for the grass-feeding species, originally placed into *Kelisia*, now in *Emoloana* (see Sect. 5.3.2.1) all endemic species were formerly placed into a separate tribe, the Alohini. Asche (1985, 1997), however, provided evidence that the Alohini are in fact polyphyletic. The Hawaiian delphacid species are thus part of the Delphacini Muir. Also several of the genera into which the Hawaiian delphacid species were placed are now considered polyphyletic, e.g., *Nesosydne* s.l., *Aloha* s.l., *Nothorestias* (see Asche 1997). In contrast to continental areas where most delphacid species feed on monocots, i.e., grasses and sedges, the vast majority of Hawaiian delphacids are associated with ferns and dicots, such as trees, shrubs, vines, herbs (Zimmerman 1948). Many species are oligo- or even monophagous (Asche 1997), e.g., in

Nesosydne s.l. 88% of species were found using plants within a single family, and 77% using a single plant species (Roderick 1997). The group of plants which is utilized by the largest number of *Nesosydne* s.l. species (at least 15: see Roderick 1997) is the so-called *silversword-alliance* (Asteraceae), which has undergone extensive adaptive radiation on the Hawaiian Islands (Carr 1987), including some of the most spectacular of Hawaii's endemic plants, e.g., *Argyroxiphium sandwicense* ssp. *macrocephalum*, the Haleakala silversword from Maui. The evolution of the relationships between *Nesosydne* species and plant species within the silversword alliance has been subject to several studies (Roderick 1997; Goodman et al. 2012, 2015).

Due to the lack of robust phylogenies for most of the Hawaiian delphacids, it is difficult to determine the number of original ancestral taxa which colonized the Hawaiian Islands (Asche 1997), the more as putative relatives from continental regions or neighboring island groups have not been identified.

Nevertheless, several monophyletic groups are recognized: *Emoloana* (Asche 2000a), *Aloha* (partim) (Asche 1997), *Dictyophorodelphax* (Asche 1997), *Leialoha* + Nesothoë

(Asche 1997), *Nesodryas* (Asche 1998), *Nesosydne* (partim) (Asche 1997). Each represents an independant lineage colonizing the Hawaiian Archipelago. Vibrational signals were recorded from a total of 34 endemic delphacid species, belonging to the taxa *Emoloana*, *Aloha*, *Dictyophorodelphax, Leialoha*, *Nesosydne* s. str., and 5 „*Nesosydne*"s.l. lineages, as well as from four immigrant species (Table 5.1).

5.3.2.1 *Emoloana* Asche, 2000

The genus comprises at least 6 species, which are the only native grass-feeding Hawaiian delphacids (Asche 1997, 2000a). Originally placed in the genus *Kelisia* Fieber, *Emoloana* species are found on all major Hawaiian islands, feeding on native grasses such as *Eragrostis* spp., *Sporobolus virginicus*, *Deschampsia australis*, *Vincentia angustifolia*, *Gahnia* (Zimmerman 1948). Vibrational signals were recorded from *Emoloana sporobolica* (Kirkaldy) populations from Oahu, Maui, and Hawaii Island.

Emoloana sporobolicola (Kirkaldy, 1910).

Kauai, Oahu, Maui, Hawaii (Zimmerman 1948), Molokai (a). Most widely distributed species in the genus. Populations display a high degree of variation in morphology and are ecologically diverse, as the species is associated with at least 5 grass species, and occurs from dry lowland biotopes, e.g., on Oahu, to moist montane biotopes, such as the Haleakala canyon rim on Maui, or the Kilauea region on Hawaii Island (Asche 2000a).

Asche (1997) suggested a biosystematic study including the analysis of vibrational communication signals to determine whether *E. sporobolicola* is a single widespread species, or consists of several reproductively isolated units. Vibrational signals were obtained from populations from Oahu, Maui, and Hawaii Island (see Table 5.1).

Remarks:

Although only few male calls could be recorded from each of the populations on Oahu, Maui, and Hawaii Islands, there are indications that there may be divergence in signal structure among populations. Further investigations are needed to explore whether these differences are indicative of incipient allopatric speciation.

5.3.2.2 *Aloha* Kirkaldy, 1904 (Partim)

Vibrational signals were recorded from *Aloha campylothecae* Muir from Oahu, and *A. swezeyi* Muir (populations from Kauai and Oahu). For signal characteristics of *Aloha swezeyi* see Fig. 5.9c.

5.3.2.3 *Dictyophorodelphax* Swezey, 1907

The five species of this endemic genus are characterized by a conspicuous prolongation of the head (Fig. 5.5). *Dictyophorodelphax* species are documented from Oahu, Lanai, Maui (Zimmerman 1948) and Kauai (Beardsley 1956). The configuration of their genital morphology and the association of all species with the native *Chamaesyce* (Euphorbiaceae) species supports the assumption of their monophyly

Fig. 5.5 *Dictyophorodelphax mirabilis*, Oahu. Photograph by W.P. Mull (with kind permission)

and descent from a single colonizing species (Asche 1997). Vibrational signals were recorded from *Dictyophorodelphax mirabilis* Swezey from Oahu.

5.3.2.4 *Leialoha (*Kirkaldy) Muir, 1915 + *Nesothoë* Kirkaldy, 1908

Vibrational signals were recorded from *Leialoha suttoniae*, *L. pacifica*, and *L. sp. aff. Lehuae*, all from Kauai, as well as from *Nesothoë munroi* and one yet undescribed *Nesothoë* species, both from Hawaii Island. For signal characteristics of *Leialoha sp. aff. Lehuae*, see Fig. 5.9d.

5.3.2.5 *Nesosydne* Kirkaldy, 1907

With 82 species (Asche 1997) represented on all islands, *Nesosydne* s.l. is the largest delphacid group on the Hawaiian Islands. The species currently accommodated in this genus, however, are morphologically heterogeneous. Hitherto not a single character could be identified as synapomorphic for all Hawaiian species. Thus, *Nesosydne* is most likely polyphyletic (Asche 1997). Within „*Nesosydne* "sensu *lato*, several distinct species groups can be recognized (see below), based on the configuration of male genital characters (Asche 1997, Asche, unpublished data). Each of these groups is likely to be monophyletic, representing independent lineages colonizing the Hawaiian Islands. Thus only the species here assigned to the *Nesosydne koae* species group are to be regarded *Nesosydne* sensu *strictu;* for all other species groups listed below new genera will have to be established (Asche, in preparation).

Nesosydne koae—species group (= *Nesosydne* sensu stricto)

The assumption of monophyly of this group is based on several characters of the male genitalia which can be interpreted as synapomorphic. Not all species feed on *Acacia koa*, but also on species of the genera *Geranium*, *Dubautia*, *Pittosporum*, *Myoporum* as well as on fern species (see Table 5.1) and are represented on all main islands. A biosystematic analysis of the *Acacia koae*—feeding *Nesosydne* species group on the basis of morphological as well as on behavioral characters revealed a higher degree of speciation than was previously assumed (O'Connell 1991).

Vibrational signals documented here (see Table 5.1) were recorded from *Nesosydne koae* (populations from Kauai, Lanai, and Hawaii Island), *N. rubescens* (populations from Oahu, and Hawaii Island), *N. geranii* (population from Maui), *N. naenae* (population from Kauai and Oahu; for signal characteristics see Fig. 5.9f), and from 4 yet undescribed species (2 from Hawaii Island, one from Lanai, and one from Molokai) (see Table 5.1).

Song patterns in all species were found to be sexually dimorphic: male calls are usually complex and species-specific, while female calls are comparatively simple and show little interspecific differences.

For signal characteristics of *Nesosydne koae* see Figs. 5.9e and 5.10. Males of *Nesosydne koae* (Fig. 5.6a,b) emit calling signals of extraordinarily complex time-

Fig. 5.6 (**a, b**) *Nesosydne koae*, Hawaii Island, Kilauea, Crater Rim Trail: (**a**) female; (**b**) male; (**c–e**) *Nesosydne* spec. 1 (undescribed), Hawaii Island, Mauna Loa (trail to Red Hill cabin): (**c**) male and two females on hostplant, *Geranium cuneatum*; (**d**) male; (**e**) female; (**f**) *Nesosydne raillardiae*, Hawaii Island; (**g**) *Nesosynde cyathodis*, Hawaii Island. Photographs by H. Hoch

amplitude patterns, if not the most complex calling signal of all planthopper species. All specimens tested also showed a high motivation for calling, started calling almost immediately after being placed in the experimental arena, and kept calling for longer periods of time (up to 1 hour).

Evolutionary implications:

Based on a phylogeny integrating male genital characters as well as characters of the vibrational courtship songs, a reconstruction of ancestral song structure was attempted (O'Connell 1991). Interestingly, the most ancestral species, *Nesosydne koae*, maintains the highest complexity of the male call (Fig. 5.10), consisting of three separate sections: the first section (A) consists of a regularly repeated series of pulses, and the second (B) of a series of complex and irregularly repeated phrases of pulse groups. The third section (C) is made up of one of four different types of phrases or pulses, or variations of these types. One of these types consists of a continuous burst as opposed to a series of pulses. In contrast, the call structure of derived species is much simpler than that of the ancestral species, consisting of one repeated pulse or phrase. Interestingly, *Nesosydne* populations with ancestral and derived call structure occur sympatrically. Based on call patterns, O'Connell (1991) identified what appeared to be a hybrid zone of the ancestral and derived species within Hakalau Reserve on the Island of Hawaii. She crossed the two putative parent species in the lab and recorded the songs of the hybrid. Based on the resulting song patterns, she hypothesized that the simpler songs of the derived species may have evolved from the third section of the complex ancestral song. Different "section three" morphology of the call may prevent derived species from „recognizing "their own species song in the sympatric ancestor, thus maintaining a reproductive barrier between the two sympatric species in most cases. These results may provide further evidence in support of Kaneshiro's (1976) asymmetrical mate preference model which assumes relaxed sexual selection in founder populations (Kaneshiro 1989).

Nesosydne s.l. gunnerae—species group

The seven species of this group are distributed on Oahu (2 species), Maui (4 species), and Lanai (1 species) (Asche, unpublished). Vibrational signals were recorded from *Nesosydne amaumau* (Muir) from Maui, and from one yet undescribed species from Oahu (see Table 5.1).

Nesosydne s.l. cyathodis species group

Vibrational signals were recorded from *Nesosydne eeke* (Muir) from West Maui. For signal characteristics see Fig. 5.9g.

Nesosydne s.l. aku—species group

Vibrational signals were recorded from *Nesosydne chambersi* Muir (see also Goodman et al. 2015), *N. ipomoeicola* Kirkaldy (Figs 5.7e,f), and *N. umbratica* Kirkaldy from Hawaii Island, *N. osborni* Muir from Maui, and *N. nephelias* Kirkaldy from Lanai. For signal characteristics of *Nesosydne osborni* see Fig. 5.9h.

Nesosydne chambersi has been subject to various studies exploring the diversification of vibrational signals among populations in relation to geographic and ecological parameters (Goodman et al. 2012, 2015). The results of these studies provide evidence that in a geologically dynamic area like Hawaii Island, geographic isolation precedes ecological specialization. Allopatric lineages subsequently may undergo adaptive radiation and host specialization, with divergence being driven by

Fig. 5.7 (**a, b**) *Nesosydne aku*, male, Hawaii Island, on its host plant *Cyanea tritomantha*; Hawaii Island, Ola'a forest; (**c–e**) *Nesosydne chambersi*, Hawaii Island; (**a**) two males and one female on host plant *Dubautia ciliolata*; (**d**) female, (**e**) male; **f**, **g**) *Nesosydne ipomoeicola,* Hawaii Island: (**f**) male, (**b**) female. Photographs by H. Hoch

genetic drift, or by natural or sexual selection. The latter will act on call characteristics, and eventually lead to reproductive isolation among populations specializing on closely related, but structurally differing *Dubautia* species (Goodman et al. 2012, 2015). For signal characteristics see Fig. 5.9i.

Nesosydne s.l. imbricola—species group

Vibrational signals were recorded from *Nesosydne raillardiae* Kirkaldy (Fig. 5.6f) from Hawaii Island. For signal characteristics see Fig. 5.9j.

Nesosydne s.l. argyroxiphii—species group

Vibrational signals were recorded from *Nesosydne argyroxiphii* Kirkaldy from Maui.

5.3.3 Immigrant Species

Hitherto 11 adventive delphacid species have been reported from the Hawaiian Islands (Beardsley 1990; Asche and Wilson 1989a, b; Asche 2000b), most probably introduced accidentally. Some are important pests on crops, such as *Perkinsiella saccharicida* on sugarcane. Ironically, it was this pest species that gave impetus to entomological research in Hawaii, starting with the recruitment of entomologists and the foundation of the Division of Entomology at the Experiment Station of the Hawaiian Sugar Planters'Association in Honolulu, in order to identify biological control agents to minimize damage by *P. saccharicida*. Zimmerman (1948: 237) states that „The story of biological control of the sugarcane leafhopper in Hawaii belongs high in the annals of entomological history".

Vibrational signals were recorded from *Perkinsiella saccharicida* Kirkaldy, *Syndelphax disonymus* (Kirkaldy)—see Asche 2000b, and *Toya dryope* (Kirkaldy) from Kauai, and *Tarophagus colocasiae* (Matsumura) from Hawaii Island.

Perkinsiella saccharicida Kirkaldy, 1903 (Fig. 5.8a,b) is widely distributed in Africa, Australia, North America, Oceania and Southern Asia, and was introduced in Hawaii (ITIS 2020).

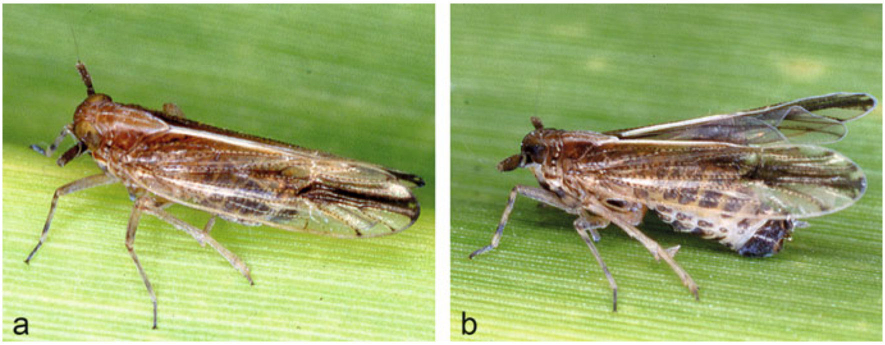


Fig. 5.8 (**a**, **b**) *Perkinsiella saccharicida*, Kauai, Kekaha: (**a**) male in repose, (**b**) male wing movements during signal emission. Photographs by H. Hoch

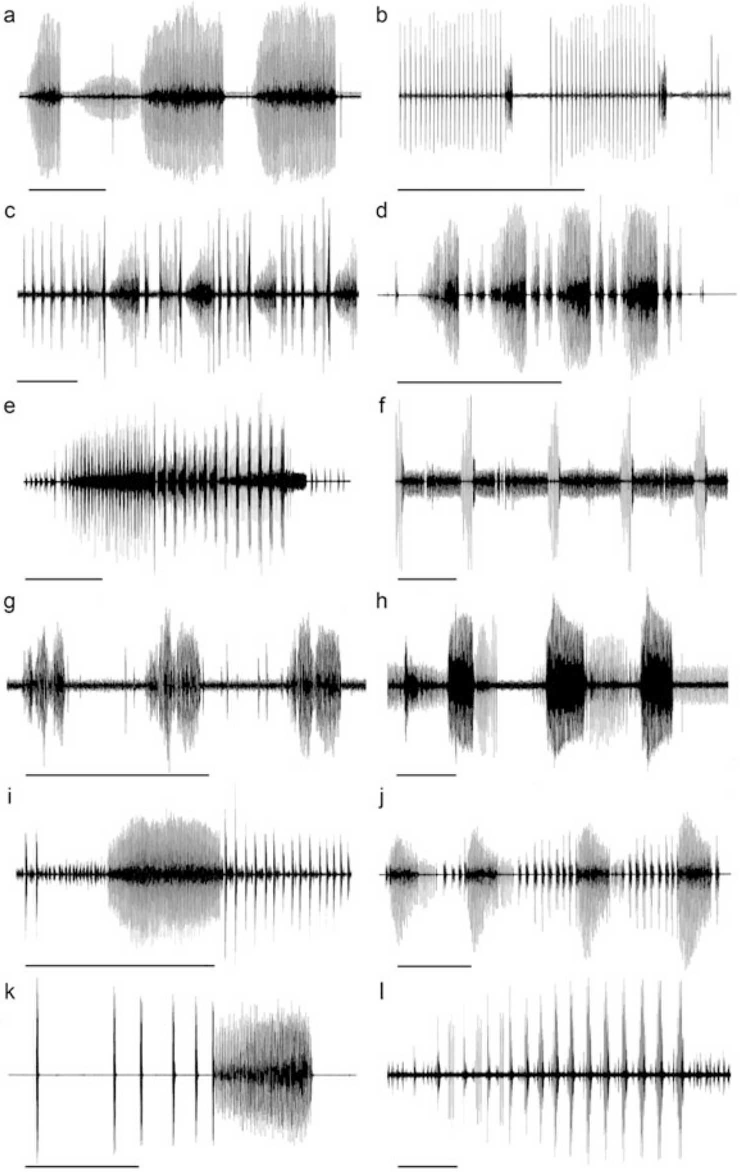


Fig. 5.9 Male vibrational courtship calls of select Hawaiian Cixiidae and Delphacidae species (oscillograms of time-amplitude patterns). Locality, host-plant information, and VibroLibrary identifiers are available (see Table 5.1). (**a**) *Iolania perkinsi*; (**b**) *Oliarus kaonohi*; (**c**) *Aloha swezeyi*; (**d**) *Leialoha lehuae*; e) *Nesosydne koae* (Hawaii Island); (**f**) *Nesosydne naenae*; (**g**) *Nesosydne eeke*; (**h**) *Nesosydne osborni*; (**i**) *Nesosydne chambersi*; (**j**) *Nesosydne raillardiae*; (**k**) *Perkinsiella saccharicida*; (**l**) *Toya dryope*. Scale bars equal 5 sec. Photographs by H. Hoch

Recordings were obtained from specimens collected from sugarcane (*Saccharum offininarum*) on Kauai. For signal characteristics see Fig. 5.9k.

For signal characteristics of *Toya dryope* (Kirkaldy, 1907) see Fig. 5.9l.

5.4 Conclusions

Hawaii's vibroscape, made up of the vibrational signals of planthoppers and presumably many other groups of organisms has been largely unexplored in the past. The vibrational signals of Hawaii's planthoppers explore a hitherto neglected aspect of the diversity of Hawaiian organisms, and add yet another dimension to the knowledge of Hawaii's endemic biota.

In the course of our study, we were able to document vibrational signals of at least 6 Cixiidae, 34 endemic and 4 immigrant species of Delphacidae, i.e., in total ca. 10% of the Cixiidae and ca. 25% of the Delphacidae occurring in Hawaii. Our studies provide evidence that the group's enormous morphological and ecological diversity is paralleled by an extraordinary diversity of their vibrational communication signals. What applies to Hawaii's biota in general ("naturally depauperate and secondarily enriched": Mueller-Dombois 1981), apparently also applies to its vibroscape.

The vibrational signals of the species recorded were observed to be omitted in the context of male-female interactions, and can thus be interpreted as courtship calls. All signals show highly structured time-amplitude patterns and are most likely species-specific. Although for many species only few specimens recordings could be obtained, there are indications for incipient speciation (e.g., *Emoloana sporobolicola*, see Sect. 5.3.2.1).

The results presented here, however, offer a mere glimpse of the diversity of Hawaiian planthoppers which may or may have existed. It remains a task for the future to assess the range of variation of the vibrational signals, within and among populations, among species and among islands. Referring to *Neosydne* alone, Zimmerman (1948: 174, 175) states: „In spite of the large number of species known, many remain to be described. ...... There may be more than 150 species living in Hawaii today, and it is probable that the number of species which have become extinct since the occupation of the islands by man is large". Indeed, no new species of *Nesosydne* have been described since Muir (1922), and many may have become extinct since their discovery. The largest (4.5 mm!) *Nesosydne* species, *Nesosydne gigantea* (Muir, 1921), described from Oahu's Koolau mountains, is only known from the type series; it has never been recollected. The major threats to Hawaii's planthoppers comes from the decline or even extinction of native vegetation (Fig. 5.11a–c), be it from extensive land use, urbanization, agriculture, recreational purposes, from disturbance by feral pigs, or from competition between native hosts and non-native plant-species (Cuddihy and Stone 1990). An example for a planthopper species at high risk of extinction is *Nesosydne aku* (Muir, 1921) (Fig. 5.7a,b), which is specific on its endemic host *Cyanea tritomantha*. The plant is restricted to wet forest in higher altitudes on Hawaii Island, but apparently, many of the sites where it occurs have been damaged by feral pigs (Wagner et al. 1999: 466), reducing the planthopper's habitat. Another example of vanishing planthopper habitat is the Kilauea caldera area on Hawaii Island: here, the introduced grass species *Pennisetum clandestinum* (*Kikuyu* grass, introduced from Africa for feed on island cattle ranches) is now widespread (Stone and Pratt 1994), while native

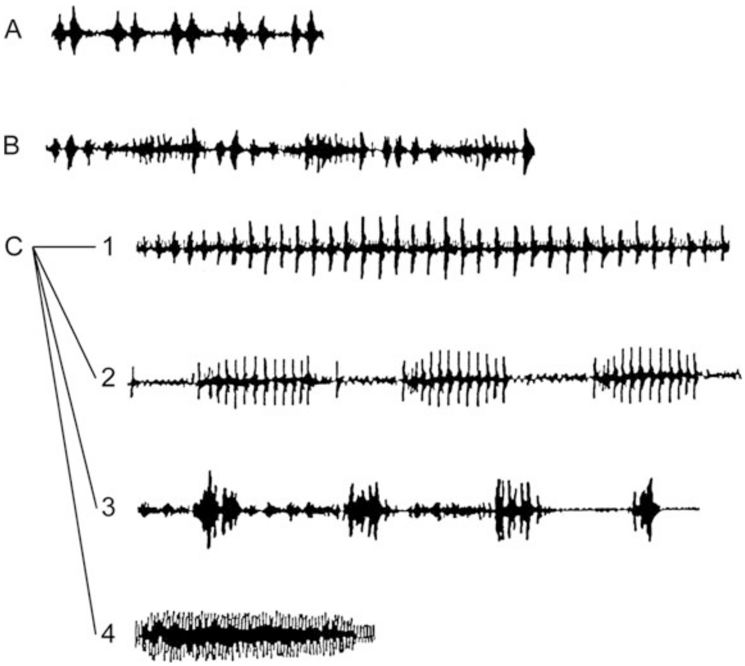


Fig. 5.10 Call structure of *Nesosydne koae*, Hawaii Island. A single call consists of three separate sections (**A**, **B**, **C**). Section C shows the highest degree of variation: signal pattern 4 was common to all individuals tested, patterns 1, 2, and 3 were distinctive to three separate populations (from O'Connell 1991: 57, Fig. 8)

grasses such as *Sporobolus,* host to the endemic *Emoloana* species*,* appear to have become comparatively rare.

The few studies which have hitherto been dedicated to specific species, or species-groups mentioned above (*Oliarus polyphemus*, *Iolania*, *Emoloana*, *Nesosdryas*, *Nesosydne koae*, *Nesosydne*—silversword alliance) provided excellent models for the study of speciation and generated valuable insights in interactions between the dynamic geological and biological factors that underlie the evolutionary processes on young oceanic islands.

Systematic revision of the two largest planthopper groups, *Oliarus* s.l. in the Cixiidae, and *Nesosydne* s.l. in the Delphacidae, and the analyses of the phylogenetic relationships within the Hawaiian taxa as well as to putative continental relatives is long overdue and might unveil a huge potential for further studies on the origin of Hawaiian planthopper diversity. The same certainly applies to the other Auchenorrhyncha taxon which has undergone extensive speciation in Hawaii: the Cicadomorpha, or leafhoppers. The largest native genus is *Nesophrosyne* Kirkaldy, 1907 of which Zimmerman (1948: 37) believes that "... it includes well over 100 species." *Nesophrosyne* has been virtually untouched by scientific investigation

Fig. 5.11 Major threats to Hawaiian planthopper diversity: (**a**) urban sprawl, Oahu, Honolulu; (**b**) non-native grasses, Hawaii Island, Kilauea; (**c**) land use for recreational purposes: golf course, Lanai. Photographs by H. Hoch

since then, however, may provide a unique chance to test hypotheses pertaining to parallel evolution of entirely different lineages in similar environments.

Acknowledgments We would like to thank Bishop Museum and the University of Hawaii at Manoa, Honolulu, for hosting us during various research stays, especially Scott E. Miller, Ken Y. Kaneshiro, and Hampton L. Carson, for their support, encouragement, and valuable discussions. *Mahalo nui loa* to all the people who made our fieldwork possible by granting permits, access to their properties, by pointing out interesting sites and rare plants, and by helping with collections. The work presented here could not have been conducted without the help of many people, e.g., Frank G. Howarth, Fred D. Stone, William P. Mull, Jon Giffin, Betsy Gagné, Steve Montgomery, David Preston, Raina Takumi, and Randy Bartlett, to name only a few. Funding was provided by various grants of the German Research Council (DFG) to M.A. and H.H. The M.Sc. research project of C.O-R. was supported by the Hawaii Bishop Research Institute and a University of Hawaii Seed grant. We dedicate this contribution to Kenneth Y. Kaneshiro (see Fig. 5.1a), University of Hawaii, Manoa, Honolulu, in acknowledgment for his outstanding contribution to Hawaiian evolutionary and conservation biology, and to the late William P. Mull, formerly of Volcano village, Hawaii Island, naturalist and *aikane nui* (good friend), who introduced us to the magic of a Hawaiian forest and who took great pleasure in listening in on the silent sounds of Hawaiis creatures.

References

Asche M (1985) Zur Phylogenie der Delphacidae Leach, 1815 (Homoptera Cicadina Fulgoromorpha). Marburger Entomol Publ 2(1–2):1–912

Asche M (1997) A review of the systematics of Hawaiian planthoppers (Hemiptera: Fulgoroidea). Pac Sci 51(4):366–376

Asche M (1998) A review of the planthopper genus *Nesodryas* Kirkaldy and related taxa (Homoptera, Fulgoroidea, Delphacidae). Proc Hawaiian Entomol Soc 33:89–124

Asche M (2000a) *Emoloana*, a new genus for the endemic grass-feeding Hawaiian Delphacidae (Homoptera Fulgoroidea). Proc Hawaiian Entomol Soc 34:71–113

Asche M (2000b) New state records of immigrant planthoppers in Hawaii (Homoptera: Fulgoroidea). Proc Hawaiian Entomol Soc 34:205–207

Asche M, Wilson MR (1989a) The three taro planthoppers: species recognition in *Tarophagus* (Hemiptera: Delphacidae). Bull Entomol Res 79:285–298

Asche M, Wilson MR (1989b) Addenda (Vol. 79). Bull Entomol Res 79(4):no page number

Beardsley JW (1956) A new *Dictyophorodelphax* from Kauai (Homoptera: Delphacidae). Proc Hawaiian Entomol Soc 16:1–23

Beardsley JW (1990) Notes on immigrant delphacid planthoppers in Hawaii (Homoptera: Fulgoroidea). Proc Hawaiian Entomol Soc 30:121–129

Carlquist S (1980) Hawaii: a natural history. Pacific Tropical Botanic Garden, Lawai, Kauaii, Hawaii

Carr GD (1987) Beggar's ticks and tarweeds: masters of adaptive radiation. TREE 2(7):192–195

Clague DA, Dalrymple GB (1987) The Hawaiian-emperor volcanic chain. Part 1: geologic evolution. In: Decker RW, Wright TL, Stauffer PH (eds) Volcanism in Hawaii. US Govt Printing Office, Washington, pp 5–54

Claridge MF (1985) Acoustic signals in the Homoptera: behaviour, taxonomy, and evolution. Annu Rev Entomol 30:297–317

Cuddihy LW, Stone CP (1990) Alteration of native Hawaiian vegetation: effects of humans, their activities and introductions. University of Hawaii Press, Honolulu

Davranoglou LR, Cicirella A, Taylor GK, Mortimer B (2019) Planthopper bugs use a fast, cyclic recoil mechanism for effective vibrational communication at small body size. PLoS Biol 17(3): e3000155. https://doi.org/10.1371/journal.pbio.3000155

Evenhuis NL (ed) (2007) Barefoot on lava: the journals and correspondence of naturalist R.C.L. Perkins in Hawai'i, 1892-1901. Bishop Museum Bulletins in Zoology:1–412

Fennah RG (1973) The cavernicolous fauna of Hawaiian lavatubes. 4. Two new blind *Oliarus* (Fulgoroidea: Cixiidae). Pac Insects 15:181–184

Frommolt K-H, Hoch H, Wessel A (2019) Call for the establishment of a VibroLibrary at the animal sound archive Berlin. In: Hill PSM, Lakes-Harlan R, Mazzoni V, Narins PM, Virant-Doberlet-M, Wessel A (eds) Biotremology: studying vibrational behavior. Springer Nature, Cham, pp 479–483

Giffard WM (1925) A review of the Hawaiian Cixiidae, with descriptions of species (Homoptera). Proc Hawaiian Entomol Soc 6:51–171

Gnezdilov V, Bartlett CR (2022) First record of the family Issidae (Hemiptera, Auchenorrhyncha, Fulgoroidea) from the Hawaiian Islands. Biodiv Data J 10:e80135. https://doi.org/10.3897/BDJ.10.e80135

Goodman KR, Welter SC, Roderick GK (2012) Genetic divergence is decoupled from ecological diversification in the Hawaiian *Nesosydne* planthoppers. Evolution 66-9:2798–2814. https://doi.org/10.1111/j.1558-5646.2012.01643.x

Goodman KR, Kelley JP, Welter SC, Roderick GK, Elias DO (2015) Rapid diversification of sexual signals in Hawaiian *Nesosydne* planthoppers (Hemiptera: Delphacidae): the relative role of neutral and selective forces. J Evol Biol 28:415–427

Hiller AE, Koo MS, Goodman KR, Shaw KL, O'Grady PM, Gillespie RG (2019) Niche conservatism predominates in adaptive radiation: comparing the diversification of Hawaiian arthopods using ecological niche modelling. Biol J Linn Soc 20:1–14

Hoch H (2005) On the identity of the type species of the planthopper genus *Oliarus* Stål, 1862, *Oliarus walkeri* (Stål, 1859) (Hemiptera: Cixiidae). Zootaxa 1056:53–60

Hoch H (2006) Systematics and evolution of *Iolania* (Hemiptera: Fulgoromorpha: Cixiidae) from Hawaii. Syst Ent 31:302–320

Hoch H, Howarth FG (1993) Evolutionary dynamics of behavioral divergence among populations of the Hawaiian cave-dwelling planthopper *Oliarus polyphemus* (Homoptera: Fulgoroidea: Cixiidae). Pac Sci 47(4):303–318

Hoch H, Howarth FG (1999) Multiple cave invasions by species of the planthopper genus *Oliarus* in Hawaii (Homoptera: Fulgoroidea: Cixiidae). Zool J Linnean Soc 127:453–475

Holzinger WE, Emeljanov AF, Kammerlander I (2002) The family Cixiidae Spinola, 1839 (Hemiptera: Fulgoromorpha) – a review. Denisia 04, zgl. Katalog des OÖ. Landesmuseums Neue Folge Nr:113–138. Available online: 2002-holzinger-et-al-cixiidae-review.pdf (oekoteam.at)

Howarth FG (1972) Cavernicoles in lava tubes on the island of Hawaii. Science 175(4019):325–326

Howarth FG (1990) Hawaiian terrestrial arthropods: an overview. Bishop Museum Occas Pap 30: 4–26

Howarth FG, Mull WP (eds) (1992) Hawaiian insects and their kin. University of Hawaii Press, Honolulu

Howarth FG, Hoch H, Asche M (1990) Duets in darkness: species-specific substrate-borne vibrations produced by cave-adapted cixiid planthoppers in Hawaii (Homoptera: Fulgoroidea). Mem Biospeol 17:77–80

ITIS (2020) Retrieved January 31, 2020, from the Integrated Taxonomic Information System, ITIS, on-line database: http://www.itis.gov

Kaneshiro KY (1976) Ethological isolation and the phylogeny in the *planitibia* subgroup of Hawaiian D*rosophila*. Evolution 30:740–745

Kaneshiro KY (1989) Natural hybridization in D*rosophila*, with special reference to species from Hawaii. Canadian J Zool 68:1800–1805

Muir FAG (1922) New and little known Hawaiian Delphacidae. Proc Hawaiian Ent Soc 5(1): 91–101

Muir FAG (1931) Description and records of Fulgoroidea from Australia and the South Pacific Islands, no. 1. Australian Mus Records 18(2):63–83

Mueller-Dombois D (1981) Island ecosystems: what is unique about their ecology? In: Mueller-Dombois D, Bridges KW, Carson HL (eds) Island ecosystems: biological organization in selected Hawaiian communities, US/IBP synthesis series, vol 15. Hutchinson Ross Publishing Co., PA, pp 485–501

Nishida GM (2002) Hawaiian Terrestrial Arthropod Checklist, 4th ed. Bishop Museum technical report, 22. Hawaii Biological Survey, Honolulu

O'Connell C (1991) Species barriers and breakdowns in Hawaiian Planthoppers. Thesis, University of Hawaii at Manoa

Ossiannilsson F (1949) Insect drummers. A study on the morphology and function of the sound-producing organ of Swedish Homoptera Auchenorrhyncha with notes on their sound-production. Opusc Entomol Suppl 10:1–146

Roderick GK (1997) Herbivorous insects and the Hawaiian silversword alliance: coevolution or cospeciation? Pac Sci 51(4):440–449

Shaw KL, Gillespie RG (2016) Comparative phylogeography of oceanic archipelagos: hotspots for inferences of evolutionary process. P Natl Acad Sci USA 113(29):7986–7993

Sherrod DR, Sinton JM, Watkins SE, Brunt KM (2007) Geological map of the state of Hawai'i. Myers, MD (ed) U.S. geological survey open-file report 2007–1089, 1st ed. US Geological Survey, Reston VA

Simon C (1987) Hawaiian evolutionary biology: an introduction. TREE 2(7):175–178

Stone CP, Pratt LW (1994) Hawai'i's plants and animals. Biological sketches of Hawaii Volcanoes National Park. University of Hawaii Press, Honolulu

Strübing H, Rollenhagen T (1988) Ein neues Aufnehmersystem für Vibrationssignale und seine Anwendung auf Beispiele aus der Familie Delphacidae (Homoptera-Cicadina) – a new recording system for vibratory signals and its application to different species of the family Delphacidae (Homoptera-Cicadina). Zool Jb, Abt Allg Zool Physiol Tiere 92:245–268

Wagner WL, Herbst DR, Sohmer SH (1999) Manual of the flowering plants of Hawaii, vol 1. University of Hawaii Press, Honolulu

Wessel A, Hoch H, Asche M, von Rintelen T, Stelbrink B, Heck V, Stone FD, Howarth FG (2013) Founder effects initiated rapid species radiation in Hawaiian cave planthoppers. P Natl Acad Sci USA 110(23):9391–9396

Wessel A, Mühlethaler R, Hartung V, Kuštor V, Gogala M (2014) The tymbal – evolution of a complex vibration-producing organ in the Tymbalia (Hemiptera excl. Sternorrhyncha). In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, New York Heidelberg, pp 395–444

Zimmerman EC (1948) Homoptera Auchenorrhyncha. In: Insects of Hawaii, vol 4. University of Hawai'i Press, Honolulu

Part III
Practical Issues in Studying Vibrational Behavior

Chapter 6
Substrate-Borne Vibrational Noise in the Anthropocene: From Land to Sea

Louise Roberts and Daniel R. Howard

Abstract Here we provide an overview of work related to anthropogenically produced substrate-borne vibrational noise. We review the marine and terrestrial vibrational noise literature base, focusing upon the species studied, the increasing research attention, and the findings of latest papers. We highlight the key sources of vibrational noise, how noise may be measured and mitigated (both by humans and by animal receivers), and how we can test for the potential impacts of noise sources. We present two case studies of previously untested species, the first relating to vibrational sensitivity of barnacles, and the second relating to activity patterns of the Madagascar hissing cockroach under vibrational noise. Currently it is difficult to draw firm conclusions on the effects of vibrational noise, given the few studies in both environments. However, effects seen to date include interference with signaling, pair formation and parental care, in addition to activity changes, and an increase of stress-related behaviors. Notably the aquatic research base lags behind the terrestrial, with the vibrational sensing capabilities of most benthic organisms largely unknown currently. We highlight vibrational noise as an area that requires more research attention both on the land and in the sea.

6.1 Introduction

Acoustic noise is present in all animal habitats and has in part shaped the communication systems of terrestrial species that employ some form of sound to communicate (Forrest 1994; Wiley 2006; Hammond et al. 2013). Anthropogenic acoustic noise, however, is relatively novel and has increased in prevalence since the industrial revolution. Airborne and water-borne acoustic noise is known to influence

---

L. Roberts (✉)
Department of Entomology, Cornell University, Geneva, NY, USA
e-mail: louise.roberts@cornell.edu

D. R. Howard
Department of Biological Sciences, Center for Acoustic Research and Education, University of New Hampshire, Durham, NH, USA

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_6

animal behavior in a variety of ways and can change community interactions and composition. These forms of noise have consequently received considerable attention from biologists. However, many animals rely on vibrations within solid substrates as a source of information either as environmental cues that correlate with a condition of interest or as signals in communication. The activity of humans is adding noise to natural vibroscapes (Roberts and Elliott 2017), and there appears to be a substantial overlap in the frequency range of anthropogenic and biological vibrations (Virant-Doberlet et al. 2014), yet for the most part these have gone relatively unnoticed and unstudied.

In the aquatic environment many activities produce vibrations within the sediment (see summary Box 6.1), yet research attention still focuses upon the waterborne acoustic energy of anthropogenic noise sources rather than measurement of substrate vibration produced. The problem is further exacerbated by a lack of data regarding the vibrational detection abilities of benthic organisms, see the review by Roberts and Elliott (2017). Yet there is evidence to suggest that substrate-borne energy adversely impacts the benthos (Berghahn et al. 1995; Kastelein 2008; Fitzgibbon et al. 2017; Roberts et al. 2017; Day et al. 2017, 2019; Roberts and Laidre 2019a, b). In the terrestrial environment, the presence of substrate-borne vibrations caused by human activity are likely more widespread, given the increased accessibility of substrates to humans, but their effects on animal behavior again remain poorly studied. Human activities produce substrate-borne vibration, including those generated by activities related to transportation, construction, and energy development and production (Kurzweil 1979; Kim and Lee 2000; Popper and Hastings 2009).

In this chapter, we summarize this area of biotremology, highlighting vibrational noise studies and the species that have been tested between both land and aquatic environments, and incorporating two case studies of previously untested species. We will discuss the sources of noise (with focus upon anthropogenic noise, while touching upon biotic and natural abiotic sources) and how these can be measured, and then end with discussion of mitigation strategies (be it by the animal or by man) and future research directions. It should be noted that while we attempt to address both environments in each section, research in the aquatic sphere is considerably behind that of terrestrial research. Hence, while we currently understand that aquatic animals can detect and use vibrational cues, studies on vibrational communication in aquatic species remain rare. As such, text addressing vibrational communication largely applies to terrestrial species only.

6.1.1 Defining Vibrational Noise

Noise in a general sense is any external stimulus that holds the potential to limit signal or cue detection (Forrest 1994; Wiley 2006). However, for the purpose of this chapter we define the term “vibrational noise” as being any anthropogenically produced substrate-borne vibrational signature, be it complex (i.e., an actual source) or “simple” (i.e., a reproduced vibration within the frequency and amplitude range of an anthropogenic source). If a vibrational noise is reproduced via playback, or otherwise, we append “playback” to indicate that the signature is not identical to the original. We use the term “biotic noise” for a natural signature that is not anthropogenically produced. We append “acoustic” to noise when the noise is detected with pressure or pressure-difference receivers, and carried as sound via compressional P-waves. The focus of this section is thus on noise produced as vibrational waves transmitted through a solid substrate, rather than noise carried as sound through a fluid (see Hill and Wessel 2016 for a recent review of the importance of this distinction).

Box 6.1 Key Sources of Vibrational Noise in Terrestrial and Aquatic Environments

The vibration produced by an activity may be impulsive or continuous, may vary with frequency content (e.g., being a mix of frequencies, broadband or tonal), amplitude, and occurrence. Vibrational noise sources may also be highly mobile or be a stationary single-point source. The vibration produced may radiate predominantly through the substrate, and through the air or the water, or most likely be a combination of all (Hill 2008; Hazelwood 2012; Miller et al. 2016).

Terrestrial	Aquatic
Transportation – Light non-commercial vehicle traffic – Commercial truck traffic – Construction vehicle activity – Train traffic	Commercial fishing – BOTTOM trawling – Dredging – Traps and pots Waterway maintenance – Dredging – Sediment deposition Construction of offshore and inshore platforms and structures – Foundation building, e.g., pile driving, anchoring – Cable laying and trench digging Transportation (not directly contacting the substrate) – Light non-commercial – Medium to large non-commercial – Freight
Energy development and production – Oil and gas exploration (seismic tests) – Oil and gas extraction (operating well pumps) – Oil transport via subsoil pipeline – Construction of associated structures (e.g., by pile driving) – Mining (blasting/extraction) – Wind energy production (vibration from turbines)	

6.2 Which Species Have Been Studied in Relation to Vibrational Noise?

When vibrational noise produced by a source matches or overlaps the spectral characteristics of a signal or cue, information masking can occur, and the receiver may no longer completely discriminate the signal/cue from background vibrational noise (Forrest 1994; Wollerman 1999; Brumm and Slabbekoorn 2005). The presence of additional vibration can also limit perception of relevant stimuli by demanding the attention of an animal, distracting them from attending to signals or cues in the environment (Chan et al. 2010; Purser and Radford 2011). Noise is an inescapable component of animal environments, produced by biotic sources, geophysical factors, and anthropogenic sources (Pijanowski et al. 2011), and its prevalence has selected for traits that allow animals to maintain effective communication and cue recognition in noisy conditions (Wiley and Richards 1978; Endler 1992; Wiley 2006, 2015). Natural forms of acoustic noise have been shown to shape signal frequency (Kirschel et al. 2009; Röhr et al. 2016), auditory physiology (Schmidt et al. 2011), and other aspects of communication behavior (Schwartz and Wells 1983; Greenfield 1988). The ecological effects of noise are especially important in the context of a changing Anthropocene, as human activity patterns have significantly increased ambient noise levels and introduced novel forms of noise across the landscape (Barber et al. 2010; Shannon et al. 2016). Noise pollution can influence animal behavior in ways that are difficult to predict (see Raboin and Elias 2019 for a recent review on vibrational noise effects on insects), and has led to concerns that this novel selective pressure could lead to short-term declines in fitness and long-term shifts in population dynamics (Francis and Barber 2013).

In order to understand the interaction between vibrational noise and the aquatic and terrestrial spheres, the literature was searched extensively for published works relating to vibrational noise. Note that studies with animals exposed to an acoustic noise are excluded from the search results unless sediment vibration was quantified. Although we acknowledge these experiments are likely to produce vibration within the substrate, vibration must be measured to be included here. In all cases the search aimed to find publications that tested the effects of vibrational noise rather than those that aimed to test vibrational perception/detection. For a review of the known sensitivity thresholds (and vibration exposures) of bivalves and crustaceans, please see Roberts and Elliott (2017), and for terrestrial environments, see Lakes-Harlan and Strauß (2014) and Stritih and Čokl (2014).

6.2.1 *Aquatic Review*

The aquatic review collated $n = 8$ published works directly testing the effects of vibrational noise (to the authors' knowledge) (Table 6.1), with the earliest in 1992. For the purpose of the count the studies of Roberts and Laidre (2019a, b) and Roberts

Table 6.1 Summary of collated studies regarding vibrational exposures created within aquatic sediments (laboratory or field-based)

	Species tested	Source type	Frequency range (Hz)	Citation
Mollusks	*Cerastoderma edule*, common cockle	Vibration box in laboratory tank	Unspecified	Kastelein (2008)
	Pecten fumatus, common scallop	In situ seismic air gun	c.a. 20	Day et al. (2017)
	Mytilus edulis, blue mussel	In situ pile driving medium scale	25–35	Roberts et al. (2017, in prep.)
Arthropods	*Crangon*, brown shrimp	In situ roller mimic fishing gear	50–500	Berghahn et al. (1995)
	Pagurus acadianus, Acadian hermit crab	In situ small-scale pile driving	60–400	Roberts and Laidre (2019a, b)
	Jasus edwardsii, southern rock lobster	In situ seismic air gun	c.a. 20	Fitzgibbon et al. (2017), Day et al. (2019)
	Pagurus bernhardus, common hermit crab	In situ pile driving medium scale	25–35	Roberts et al. (2017, in prep.)

et al. (2017, in prep.) are considered as two rather than four since the papers are in linked pairs. Of the papers found, four use actual anthropogenic sources, with another two attempting to mimic such sources on a small scale.

Few studies have tested how vibrational noise pollution affects benthic species, and these are to date focused upon invertebrates (Table 6.1). Most notable are the recent experiments of an Australian research group who have investigated the responses of invertebrates to seismic surveys in field conditions (Fitzgibbon et al. 2017; Day et al. 2017, 2019). The exposure source, which was a seismic air gun used specifically for the experiments (rather than in an actual seismic survey), was measured using dual or tri-axial geophone systems on the seabed near the exposed animals, in addition to acoustic measurements. This makes these experiments somewhat unique in the literature base to date, firstly in use of a fully controllable "real" source, in the measurement of seabed vibration and the focus upon benthic invertebrate responses. Day et al. (2017) exposed the scallop *Pecten fumatus* by deploying animals in cages and measuring mortality, behavioral responses such as recessing reflexes (time to burrow into the substrate), and testing hemolymph. Mortality was shown to increase with the number of passes of the air gun above the animals, and to increase over time. Video observations did not show elevated swimming behavior, but did indicate increased burial in the sediment and "flinching" of the valves (also observed in Roberts et al. 2015) prior to the audible sound, indicating that this was linked to seabed vibration. There were indications of compromised homeostasis and the potential for immunodeficiency. The authors attribute the results to the seabed vibration portion of the signal. Similar experiments were undertaken with the rock lobster *Jasus edwardsii* (Fitzgibbon et al. 2017; Day et al. 2019), using an air gun source quantified upon the seabed with geophones.

Increased statocyst damage and increased righting reflexes were demonstrated in exposed lobsters compared to controls (Day et al. 2019), but detailed measures of hematological homeostasis indicated that seismic surveys had little impact biochemically (Fitzgibbon et al. 2017). Interestingly, all three of these papers, while measuring seabed vibration, do not present the velocity measurements in detail, nor discuss the implications of seabed vibration to these benthic animals. Nonetheless the papers are great progress for the research area.

The remaining literature directly linking seabed vibration to responses is by the author (LR) and as such will not be dwelt upon here. The proceedings paper of Roberts et al. (2017) exposed caged blue mussels *Mytilus edulis* and the marine hermit crab *Pagurus bernhardus* to a pile driver in semi-field conditions. The pile driving sound and vibration were quantified using a water-borne particle motion sensor, a hydrophone, and a tri-axial geophone on the seabed. The measures of the source are to be included in a full paper of the work (Roberts et al., in prep.). The exposed animals exhibited changes in oxygen consumption (*M. edulis*) and behavior, such as environmental sampling and anti-predator responses (both species). Roberts and Laidre (2019a, b) exposed free-ranging hermit crabs to a small-scale vibratory source that aimed to mimic the characteristics of a pile driver. The stimulus was measured on the seabed with a tri-axial geophone system, although the water-borne portion of the stimulus could not be measured. A chemical cue was used to attract hermit crabs to the experimental area, a cue which had previously proven to be indicative of new shell (a “home”). Numbers of crabs arriving at the chemical cue were counted before and after either a control period or a period of vibrational noise. It was found that significantly fewer crabs were attracted to the chemical cue during vibrational noise exposure, indicating that noise can affect the use of chemical information and therefore has a cross-modal impact.

Other papers should be included here that focus upon anthropogenic vibration but use smaller scale vibrational sources. Kastelein (2008) tested responses of common cockles *Cerastoderma edule* to a small vibrating motor in the laboratory, with the concern being the cockle fishery being impacted by vibrational noise. The cockles were observed to retract the two siphons and close the valves in response to vibration, but the vibration levels were not disclosed. Berghahn et al. (1995) directly tested anthropogenic interactions with the sediment, but in specific relation to the efficiency of brown shrimp fishery gear, rather than vibrational noise. Brown shrimp *Crangon* were exposed to a single roller of fishing gear, water pulses, and shakers to determine whether fishing gear increased the tailflip response (which aids fishery catches). Thus, the vibrational sensitivity and responsivity to vibration was tested but discussed in a different context. Flatfish were also tested for sensitivity to vibration (but not to the gear itself). It was found that shrimp responded to gear vibrations with flicking of the second antennae, and with tailflips, in some cases. The vibrations of the gear in the intertidal were measured in the field and related to behavioral responses.

Three additional works, which do not directly test the effects of vibrational noise but are related, should be highlighted here. For example, the papers of the author (Roberts et al. 2015, 2016a) focus predominantly on the sensitivity of *P. bernhardus*

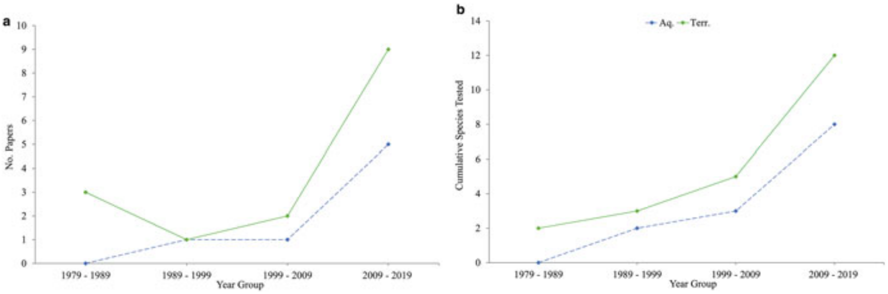


Fig. 6.1 Summary of Tables 6.1 and 6.2. (**a**) Number of papers regarding noise published between 1979 and 2019; (**b**) Cumulative number of species tested across the same time period

and *M. edulis* to sinusoidal vibrations in the laboratory, but then link these sensitivities to collated measurements of vibrational noise upon the seabed. They demonstrate that these species are likely able to detect anthropogenic seabed vibrations at distances from source, which is a first for the research area. Finally, Kowalewski et al. (1992) tested whether a generalized vibrational noise produced by a shaker system could inhibit the settlement of juvenile Zebra mussel (*Dreissena polymorpha*). It was found that noise at very high exposure levels could cause mortality, rather than inducing settlement behavioral changes, but it would be interesting to test this further with exposure levels linked to actual noise sources. Due to the indirect noise applications, these three studies are not included in Table 6.1 or Fig. 6.1.

6.2.2 Terrestrial Review

The terrestrial review revealed $n = 14$ works since 1983 (Table 6.2) with six testing actual anthropogenic sources. Interestingly, some of the first studies to evaluate these effects did so in the context of vibrations produced during transport and/or handling of domestic farm animals. In most cases, farm animals (pigs, chickens, and dairy cattle) exposed to vibrational noise exhibited stress-related behaviors or other physiological indicators of stress (Stephens and Rader 1983; Stephens et al. 1985; Hagstrum and Flinn 1993; Scott 1994; Kauke and Savary 2010; Gebresenbet et al. 2011). Briese et al. (1984) observed that the embryonic development of lab mice exposed to combinations of airborne and vibrational noise was significantly perturbed, likely due to stress-related constriction of the female parent's blood vessels supporting placental function. More recently, several studies have shown that agricultural pest insect behavior and reproduction can be influenced by exposure to experimentally induced noise. Mazzoni et al. (2009, 2017) and Eriksson et al. (2012) showed that the application of plant-borne vibration can interfere with the signaling behavior and subsequent pair formation in both the American grapevine

Table 6.2 Summary of collated studies regarding vibrational noise in the terrestrial environment (laboratory or field)

Group	Species	Source type	Freq. range (Hz)	Citation
Insects	*Nicrophorus marginatus*, burying beetle	Replication of wind turbine rotating blades in laboratory experiments	25–500	Phillips et al. (2020)
	Scaphoideus titanus, American grapevine leafhopper	Synthesized playbacks in the laboratory	White noise 0–8000	Mazzoni et al. (2009)
	Homalodisca vitripennis, glassy-winged sharpshooter	Synthesized playbacks in the laboratory	80, 240	Mazzoni et al. (2017)
	Scaphoideus titanus, American grapevine leafhopper	Synthesized playbacks in the field	50–300	Eriksson et al. (2012)
Arachnids	*Araneus diadematus,* European garden spider	Ambient traffic and wind noise, white noise	Unspecified	Wu and Elias (2014)
Annelids	*Diplocardia* earthworms	"Grunting" technique	Unspecified <500	Mitra et al. (2009)
Mammals	*Dipodomys stephensi,* Stephens' kangaroo rat	Traffic noise recording playbacks	Approximated to traffic levels; unspecified <500	Shier et al. (2012)
	Bos taurus, domestic dairy cattle	Vibration during truck transport	1.3–23	Gebresenbet et al. (2011)
	Bos taurus, domestic dairy cattle	Sound/vibration playbacks replicating milking parlor	Unknown; acceleration only specified	Kauke and Savary (2010)
	Sus scrofa domesticus, domestic pig	Sound/vibration playbacks replicating truck transport	Unknown; given in dB	Stephens and Rader (1983)
	Sus scrofa domesticus domestic pig	Sound/vibration playbacks replicating truck transport	Unknown; given in dB	Stephens et al. (1985)
	Mus musculus, laboratory mice	Synthesized playbacks	Unknown; given in dB	Briese et al. (1984)
Birds	Domestic chicken	Vibration playbacks replicating truck transport	1–18	Scott (1994)
	Domestic chicken	Vibration playbacks with thermal stress	2	Abeyesinghe et al. (2001)

leafhopper and the glassy-winged sharpshooter (leafhoppers). In these cases, vibrational noise masked critical sexual signals and resulted in lower levels of successful courtship and reproduction.

Wu and Elias (2014) found that traffic in developed urban areas generated vibrations greater than ambient in webs of the European garden spider, *Araneus diadematus*, and showed experimentally that vibrational noise could affect prey detection thresholds. The authors concluded, however, that these human-generated sources were minor compared to those induced naturally by winds. Mitra et al. (2009) showed that *Diplocardia* earthworms could be induced to emerge from the soil shortly after experimental exposure to vibrational noise, and proposed that the low-frequency seismic noise mimicked cues from rainfall or fossorial predators. Shier et al. (2012) observed that the frequency range of traffic vibrational noise overlapped with the foot drumming signals produced by the endangered Stephens' kangaroo rat, *Dipodomys stephensi,* and documented that individuals would respond to the replicated traffic noise by foot drumming in return, likely lacking the ability to discriminate between conspecific noise and traffic noise. By replicating in controlled experimental settings the temporal pattern of seismic vibration produced by the rotating blades of a wind turbine, Phillips et al. (2020) found that exposure to vibrational noise influenced biparental care behaviors in the Nicrophorinae burying beetle *Nicrophorus marginatus*. Beetles in noisy conditions took longer to bury the carcass and produced fewer offspring, highlighting the potential fitness costs of seismic noise. These studies were some of the first to support the assertion that vibrational noise pollution produced by humans may influence terrestrial animal fitness, as has been found for other forms of acoustic noise pollution (Kight and Swaddle 2011).

Occasionally, unexamined effects of vibrational noise appear as anecdotes in the literature. Barth (1988) observed the presence of leaf vibrations induced by a passing truck while studying spider responses to rain and wind vibrational noise. The author noted that the focal spider could distinguish between the vibrations produced by the truck and those produced by a walking prey item. Similarly, Duarte et al. (2019) found that calling crickets would cease calling in response to the vibrational noise generated by passing mining trucks. While the authors measured and described the attributes of ambient airborne noise, the results that show a more pronounced effect on the signal attributes of ground-dwelling field crickets than tree crickets point intriguingly to seismic noise influences. Several studies and reviews document the sensitivity of orthopterans to substrate-borne vibrational noise (Dambach 1989; Friedel 1999; Stritih and Čokl 2014), but rarely do investigators address the potential for human-generated vibrational noise to influence reproductive behavior. Additional research on this topic deserves attention. Other studies may propose to examine the effects of airborne noise on animal behavior, but overlook the possibility of seismic disturbance occurring in concert with airborne sound. Rabanal et al. (2010) assessed the responses of rainforest mammals to dynamite blasts occurring during oil exploration. They found that elephants and apes avoided areas near blast sites, but they attributed this response to the airborne sound produced by dynamite explosions and made no mention of potential disturbance from seismic vibrations.

Wrege et al. (2010) found that African elephants shifted to mostly nocturnal patterns of activity during these same types of mining-related seismic disturbances. Given their well-known use of seismic signals in communication, it is quite possible that the seismic vibrations may have, in part, caused the avoidance responses. Davis et al. (2018) exposed monarch butterfly larvae to airborne traffic noise and observed increased heart rates over short exposure durations. Although not explicitly tested for or discussed, these larvae likely responded to the substrate-borne vibrations of the leaf rather than airborne pressure waves or particle motion. Similarly, Gurule-Small and Tinghitella (2018) exposed field crickets during development to airborne traffic noise and found that, as adults, female phonotactic performance was negatively influenced. Given that juvenile crickets' auditory systems do not fully develop until late instar stages, the observed effects likely result from the airborne stimulus inducing vibrations either in the substrate or in the juvenile crickets themselves. Likely many studies that seek to measure the influence of anthropogenic airborne noise on animal behavior or ecology may unintentionally be documenting the effects of substrate-borne vibration.

Given the relatively recent progress of biotremology as a research area and scientific discipline, and the focus upon water-borne or airborne sounds that we as humans can detect rather than substrate-borne vibrations, it is perhaps unsurprising that vibrational noise has received little research attention to date. However, interest in vibrational noise is slowly increasing (Fig. 6.1a), and the cumulative number of tested species is increasing especially in the last 10 years (Fig. 6.1b). In the aquatic environment the logistics of measuring vibration upon the seabed are challenging, which has led to relatively shallow-water experiments, to date. In addition to this, the non-charismatic nature of many sediment dwelling animals underwater likely has an impact upon research attention (Solan et al. 2016), in addition to the small size of many and their often-hidden lifestyles. Underwater sound in many cases is now regulated, measured, and reported (Popper et al. 2014), but there are no such regulations for seabed vibration. In the terrestrial world, perhaps the exploration into the depths of acoustic communication and airborne noise has meant vibrational noise has been largely overlooked.

6.3 Biotic Vibrational Noise and the Ambient Vibroscape

Vibrational noise is not confined to sources produced by humans, and thus, animals may have pre-adaptions to avoid masking and disturbance effects. In the aquatic environment, water currents, sedimentation, transportation, and accumulation of debris may directly create vibrations within the sediment. Adding to this, wind, rain, and waves on the surface may indirectly translate into sediment vibrations, depending on the depth of the water. Wave energy on the shoreline crashing upon the sediment produces vibrations in the intertidal and subtidal areas; however, there are few vibrational measurements of this. Recent measurements of waves crashing upon a Costa Rican beach were found to have peak vibrational energy at 30 Hz with spectral comparisons between high and low shore indicating a difference in the 400–3000 Hz range (LR personal communication, in prep.). Acoustic recordings of waves on the shore indicate waves produce 40–300 Hz energy, with peak energy at 60–100 Hz (Ellers 1995). In the terrestrial environment, wind and rain are the most common natural sources of vibrational noise (Cocroft and Rodríguez 2005; Virant-Doberlet et al. 2014). When raindrops hit a substrate, or when wind rustles the leaves of a plant, low-frequency vibrations are produced that have the potential to interfere with perception of vibrational cues or signals. The vibrations produced by wind and rain vary with substrate type, but are generally broadband, with most energy concentrated at frequencies at or below 100 Hz (Barth 1988; McVean and Field 1996; Casas et al. 1998; Tsubaki et al. 2014; Halfwerk et al. 2016). According to Tishechkin (2007), the average wind speed in an area is not strongly related to the intensity or frequency of vibration generated in plant substrates, but rather windspeed within the microclimate and the structure of the plant are more influential. Different plant species show different filtering properties for wind vibrational noise (Saxena and Kumar 1980; Barth 1988). Additionally, different parts of a single plant can have different filtering properties, which could alter the spectral characteristics of wind or rain vibrational noise (McNett and Cocroft 2008). Wind blowing through vegetation also has the potential to induce vibrations in the soil (Narins et al. 1997), which may create seismic noise for subterranean animals. We know that rain also produces vibrations in the soil, and these vibrations are easily detectable as cues for burrowing toads (Dimmitt and Ruibal 1980). Thus, rain vibration in soil has the potential to affect communication in other subterranean animals.

The substrate-borne vibrations produced by other animals can also add to the ambient vibroscape (Roberts and Elliott 2017) and could potentially cause sensory interference. The vibrations measured at close range (ca. < 30 cm) to the hermit crab *Pagurus bernhardus* walking on a sandy marine sediment are <500 Hz (LR personal communication), and groups of their terrestrial relatives *Coenobita compressus* produce vibrations with peak energy around 100–300 Hz (LR personal communication). However, the movements and seismic productions of most aquatic animals are entirely unknown currently, although some benthic fish produce water-borne sounds that may translate into the sediment (Janssen 1990). On land, animals chewing or walking on a leaf produce substrate-borne vibrations that are typically higher in frequency than those induced by wind or rain (Barth 1988; Pfannenstiel et al. 1995; Castellanos and Barbosa 2006). Remarkably, *Semiothisa aemulataria* caterpillars are able to distinguish between the incidental vibrations of harmless herbivores and those of insect predators, and potentially even between different types of predators (Castellanos and Barbosa 2006). Vibrational noise may also come from animals signaling in the air, as the signal is transferred across the boundary with solid substrates and can be detected by vibration-sensitive animals directly from the solid (Gordon and Uetz 2012).

Often multiple conspecific and heterospecific signalers are present within the active space of a receiver. Hemipteran insects that rely on vibrational courtship signals for successful copulation must be able to communicate effectively in transmission environments where many other related signalers are present and contributing to the noisescape (Hunt and Morton 2001; Mazzoni et al. 2009; de Groot et al. 2010, 2011). The signals of conspecifics are often spectrally similar, and thus individuals must co-opt alternative mechanisms other than frequency distinction to avoid sensory interference.

6.4 Strategies for Effective Communication in Vibrational Noise

In general, acoustically communicating animals are thought to cope with the masking effects of noise by partitioning the soundscape temporally, spectrally, or spatially (Schmidt and Balakrishnan 2015). Substrate-borne vibrations associated with weather tend to fluctuate throughout the day in a predictable manner, and it has been suggested that *Enchenopa binotata* treehoppers could shift their calling periods to the morning and evening to avoid vibrational noise from wind (Tishechkin 2007). However, diel timing of vibrational communication events as a noise-avoidance strategy has yet to be explicitly tested. The most commonly observed response of signalers to masking vibrational noise is a cessation of signaling (Polajnar and Čokl 2008; McNett et al. 2010; de Groot et al. 2010, 2011). This limits communication in the moment but may allow individuals to avoid wasting energy in futile signal production.

For shorter timescales, gap detection may offer relief from vibrational noise disruption. Gap detection has been seen in vibrationally communicating insects that produce choruses (Hunt and Morton 2001). This allows signalers to avoid the signal disruption by conspecific competitors by restricting signals to quiet gaps. For vibrational noise that is unpredictable, it has been suggested that insects could also use gap detection to signal within windows of low or no noise (Cocroft and Rodríguez 2005; Tishechkin 2013). Indeed, McNett et al. (2010) showed that male *Enchenopa binotata* "*Ptelea*" treehoppers preferentially signal in wind-free gaps. However, if wind velocity was high, communication disruption extended into wind-free gaps (McNett et al. 2010). Thus, gap detection alone is not always an optimal solution to periodic or sporadic vibrational noise.

Box 6.2 How Can We Measure Vibrational Noise?

Geophones, accelerometers, and laser Doppler vibrometers may be used in both aquatic and terrestrial environments; however, these sensors were made predominantly for land-based applications, making some adaptation a requirement prior to use underwater. Waterproofed geophones, being more rugged and robust than accelerometers, are utilized for the measurement of vibration upon the seabed. These can be deployed on sledges or weighted and lowered to the seabed. In laboratory settings accelerometers are suitable for use in

(continued)

Box 6.2 (continued)
aquatic tanks, many being waterproofed already, and can be purchased with water-resistant cables that simply require the connectors to be shielded from water ingress. Other sensors may be potted within a hard-setting compound such as epoxy, a process which requires some courage depending on how expensive the sensor is! Laser Doppler vibrometry may be used inside aquatic tanks, although few studies have done this to date. Along with the substrate-borne part of the stimuli, measurements of the airborne and water-borne portion must also be undertaken where at all possible. In water, hydrophones are used to measure acoustic pressure and microphones for the air counterpart. Water-borne particle motion is more difficult, but may be measured in certain acoustic conditions using a dual-hydrophone method or with a particle motion sensor (Nedelec et al. 2016; Popper and Hawkins 2018), but there are currently no commercially available sensors for this purpose.

Another way to avoid masking noise known from acoustics is through spectral partitioning of the soundscape (Sueur 2002; Schmidt et al. 2012). Vibrational signals may avoid masking if they fall outside the frequency range of noise. Caldwell et al. (2010b) identified the presence of high frequencies (>100 Hz) and an initial period of building intensity as typical characteristics of rain noise, making it distinct from the predator-induced vibrations used as cues by red-eyed tree frog embryos. Other studies have found, however, that wind and rain produce low frequencies compared to the relevant cues for invertebrate animals (Barth 1988; Castellanos and Barbosa 2006). Regardless, these studies have found differences in substrate-borne vibrational profiles between noise and relevant cues that could possibly be exploited by animals for cue discrimination.

Green stinkbug females (*Nezara viridula*) have been shown to shift the frequency of their vibrational calling signal, which has a peak around 100 Hz, by as much as 10 Hz in response to masking noise playbacks (Polajnar and Čokl 2008; Čokl et al. 2015). This plasticity in signaling behavior has likely evolved to ameliorate the disruptive effects of masking by other signaling conspecifics. This is likely an effective mechanism for increasing signal-to-noise ratio for this species. However, this type of plasticity in signal behavior is uncommon in other species whose signal characteristics are likely limited by the rigid morphology of having the vibration-production structure imbedded in the cuticle (Lampe et al. 2012).

Still other strategies for vibrational noise avoidance may exist that resemble those used by animals who communicate acoustically in air or water. Some animals may increase the intensity of their signal in an attempt to increase the signal-to-noise ratio, a strategy known as the Lombard effect (Scheifele et al. 2005). Female frogs are known to isolate the calls of individual males from the masking chorus noise through "spatial release from masking" (Schwartz and Gerhardt 1989). Vibrational receivers could potentially do something similar, although vibrations within a single plant stem propagate essentially in one direction (Casas et al. 2007). Therefore, this strategy may have limited utility. Another strategy may be for the signaler or receiver to simply change physical positions to less windy areas to improve signal transmission and avoid vibrational noise interference (Tishechkin 2007). If ecological strategies fail, however, animal populations respond to these selective pressures, resulting in adaptive changes to signaling behavior, signal frequency, or shifts in receiver sensory tuning (Wiley 2006). Evolutionary responses to human-generated vibrational noise represent a research frontier in dire need of exploration.

6.5 How Can We Test the Potential Effects of Vibrational Noise?

As with acoustic noise, the effects of vibrational noise upon a terrestrial or aquatic animal depend upon the properties of the source itself (which influence, e.g., the intensity level, duration, repetition, spectral range of emitted vibrations; see summary Box 6.2), the levels received, the background levels of vibration, and the detection abilities of the receiver (Chapman and Hawkins 1973; Tasker et al. 2010; Hawkins and Popper 2014; Hawkins et al. 2014a). As such, testing for potential effects of anthropogenic vibration can be a challenge, given the complexity of anthropogenic sources and the scenario specificity of each occurrence and the difficulties of replicating them accurately with a wider applicability. In addition to this, animals must be exposed under realistic conditions that differ little from those naturally experienced or exposed in ways that minimize undue behavioral changes.

6.5.1 Playbacks Versus Actual Sources

The ideal approach is to expose animals to actual anthropogenic substrate-impacting sources (Berghahn et al. 1995; McCauley et al. 2017; Day et al. 2019), but in many cases this is expensive, requires a large-scale approach, and may require permits to work around construction sites that, in turn, may constrain timings of experiments. Another approach is to mimic a source manually on a small scale (Roberts et al. 2017; Roberts and Laidre 2019a), or to replicate sources using transducer or shaker playbacks (McGregor 1992; see Table 6.3).

Playback allows the exposure source to be fully adjustable and controlled, allowing, for example, dose–response curves to be produced associating the intensity level of the stimulus to a particular response. A discussion of substrate-borne playbacks has been covered in great detail in Cocroft et al. (2014) and Elias and Mason (2014); hence here we largely discuss additional considerations for playbacks in the aquatic environment. Few acoustic playback experiments in the aquatic environment have used complex signatures due to difficulties of sound projector arrays being able to reproduce these in terms of frequency, waveform, and overall

Table 6.3 Considerations and measurements to ensure a ‘good’ vibrational playback experiment, combined from the recommendations of McGregor (1992), Popper (2005), Cocroft et al. (2014), Elias and Mason (2014)

Feature	Considerations
Test vibrations	Fully calibrated source (e.g., duration, intensity, frequency range, s/n ratio)
	Received vibration quantified
	Sources defined and decided by biotremologists
	Frequency profile of the signature matched to original recording
	Temporal and amplitude characteristics matched to original
	Distortion and transmission loss calculated
Playback equipment	Speaker/transducer resonance within the setup quantified
	Coupling of the setup to the substrate and axes of vibration understood
	Fidelity of equipment (e.g., s/n ratio, frequency range, amplitude calibration)
Playback procedure	Quantitative design to ensure statistically valid results (avoiding pseudoreplication)
	Control trials, and control/base animals, blind analysis
	Transducer and observer position
Test animals	Subjects location in relation to territorial boundaries (if applicable)
	Predators and conspecifics
	Time of day
	Proximity to important resources (mates, food, etc.)
	Health and acclimatization of subjects if enclosed
	Sensitivity for the species known (ideally)
	Choice of substrate: natural or artificial, and multiple exemplars
Environmental conditions	Time of year (influences ambient vibration intensity, flora, behavior of subjects and other species)
	Background noise sources measured/controlled where possible
	Accessibility for playback equipment to the study site
	Weather (same influence as above)
	Time of day (degradation effects)
	Environmental measurements (salinity, moisture, temperature, etc.)

sound field. To overcome this, special transducer systems have been created (Hawkins et al. 2014b), adapted to replicate signatures accurately in the water column in terms of pressure and particle motion. It is likely that the same issues are encountered when reproducing substrate-borne anthropogenic signatures in aquatic sediments, due to the effects of the substrate upon wave propagation as discussed in Elias and Mason (2014). Tactile speakers, which reduce water/airborne sound and increase substrate-borne vibration, are the ideal tool and have been employed in land playbacks successfully (Lewis et al. 2006; O’Connell-Rodwell et al. 2006; Márquez et al. 2017). However, adapting this approach to an aquatic environment has not, to the authors’ knowledge, been undertaken to date, although weatherproof tactile speakers with boat amplifiers have been used in damp beach environments (LR personal communication). Another method for reproducing a vibrational signature is to use a shaker system—the few examples of vibrational noise playbacks in terrestrial substrates have used these to great success (Hill and Shadley 2001; O'Connell-Rodwell et al. 2007; Morales et al. 2008; Caldwell et al. 2010a). However, even though shakers have been used in aquatic laboratory tanks using stinger rods or membranes (Mosher 1972; Barth 1980; Heinisch and Wiese 1987; Kowalewski et al. 1992; Berghahn et al. 1995; Breithaupt 2002; Roberts et al. 2015, 2016a) and shaker tables (Mooney et al. 2010), a shaker system would likely need to be very powerful to produce enough vibration in an aquatic environment, in addition to needing to be fully submergible and remotely operated.

In the laboratory, tanks used for playbacks should be fitted to reduce external vibrations (damping infrastructure, gaskets, or suspension) (Mosher 1972; Karlsen 1992; Mooney et al. 2010) and to isolate the exposure stimulus. Another technique is to suspend a small water tank within another tank to reduce external vibrations (Kastelein 2008; Roberts et al. 2017). In a laboratory tank, an acoustic sound field is affected by the reflectance of the tank walls (in Hill et al. 2019; see Sect. 6.8 and references therein on this concern), which create standing waves of differing frequencies (Parvulescu 1964; Rogers et al. 2016). This means that it is difficult to replicate an acoustic noise or biological source accurately in the laboratory, and the received sound may not be representative of the original source frequencies (Parvulescu 1964; Rogers et al. 2016). Substrate-borne waves may similarly be affected by such factors, an area to be investigated further.

Many aquatic bioacoustic studies involving vibrational noise have been undertaken using captive or caged animals, allowing observation of the animals throughout the exposures either by confining them to cages or pens (Engås et al. 1995, 1998; Schwarz and Greer 1984). This approach has been tried for sources quantified in the sediment (Roberts et al. 2017; Day et al. 2019), but behavior of captive organisms is not always consistent with animals in the wild (Benhaïma et al. 2012). Alternatively animals could be tagged or observed passively (such as acoustically) to monitor responses to exposures (Mueller-Blenkle et al. 2010) or be attracted to a specific area using a bait or cue (Roberts et al. 2016b; Neo et al. 2018; Roberts and Laidre 2019a). Species with specific home ranges, like reef fish, are more easily observed (e.g., Wardle et al., 2001), an approach used on land to monitor animal behavior during playbacks at specific locations such as watering holes (O'Connell-Rodwell et al. 2006, 2007), within burrows (Hill and Shadley 2001), or upon single plants (Morales et al. 2008; Caldwell et al. 2010a). The sessile lifestyle of many invertebrates, and the territorial nature of some benthic fish, e.g., the sound-producing Lusitanian toadfish (Vasconcelos et al. 2010), in aquatic systems means that this monitoring approach may be feasible in some cases.

6.6 How Might Vibrational Noise Be Mitigated?

Mitigation of anthropogenic noise is the minimization, elimination, or control of the impacts of an anthropogenic stressor (Harwood 2002). While the aim of such processes is clearly to reduce the impact as much as possible, mitigation measures must also be economically viable and be accompanied by evidence that they will be successful (Ducrotoy and Elliott 2008; Hawkins and Popper 2012), since measures are likely to pose a cost to the noise producer. There are principally two types of mitigation method: changing the source itself or using biological knowledge to minimize the effects. In regard to changing the source itself, a number of options exist, including stopping the emission completely or using alternative technology, minimizing the output (e.g., with engineering devices to reduce), or trading the intensity for duration, or the size of the whole process for duration (e.g., with pile driving, increasing the number of strikes but decreasing the hammering force, or using many smaller piles instead of larger ones). Alternatively, mitigation techniques can use biological information, such as excluding vibrational noise-producing activities from specifically biologically sensitive areas or at specific times, driving animals away or physically excluding certain species from areas, or restricting activity to sighting-free periods for large animals. However, in order to mitigate, the vibrational intensity levels that have an effect must be understood, with knowledge of the intensities encountered and the thresholds for behavioral, physiological, and physical damage so that noise-producers can work within these. As such, vibrational noise exposure criteria would need to be produced in a similar way to underwater sound exposure criteria (Southall et al. 2007; Popper et al. 2014). It is clear from this review that we are a long way from being able to set these.

6.6.1 Difficulties of Mitigating Vibrational Noise

Mitigation often involves a standard treatment or practice that is broadly applicable to a given environment. Vibrational noise mitigation is full of challenges, but these three remain as major barriers to a sustainable solution:

1. Lack of available biological and experimental information on which to base mitigation continues to challenge us because of the abundant variation in living things, even within a species, e.g., behavior varies with size, physiology, individual, age, species, context, environmental parameters, and motivation (Ellison et al. 2011; Hawkins and Popper 2012; Hawkins et al. 2014a; Miller et al. 2016).
2. Impacts of vibrational noise vary with, for example, background intensity levels, propagation conditions, and the noise properties at the source (intensity level, frequency composition, duration, and repetition rate) (Kastelein 2008; Götz et al. 2009). It is difficult to measure the effects since every vibration has distinct characteristics, varying in intensity, frequency content, pattern of occurrence, and movement (stationary or mobile).
3. Effects of vibrational noise, as with other anthropogenic stressors, may be cumulative or in combination with other influences (e.g., light changes, chemical inputs, airborne or water-borne sound; Halfwerk and Slabbekoorn 2015).

Agencies, organizations, and/or foundations charged with evaluating the ecological, environmental, and human health effects of noise should invest funding to generate the science needed to develop coherent policies related to mitigating substrate-borne vibrational noise. Whether considering terrestrial or aquatic environments, vibrational noise remains a research and policy frontier, deserving, if not demanding, attention.

6.7 Future Research Directions

In the aquatic environment, the study of vibrational noise would benefit from more understanding of the anthropogenic sources to which organisms are exposed. This means quantification in terms of duty cycle, exposure length, frequency range, and amplitude range within the seabed, and at various distances from operations to understand how these vary across different substrates and different environmental conditions. Measurement of underwater acoustic noise is commonplace but vibration in the seabed is often omitted, yet could greatly inform modeling of noise sources. Indeed, neither water-borne stimuli nor substrate-borne stimuli should be considered separately, and the interactions between the two need to be further explored. In the case of pile driving, for example, measurements of the sound produced by a range of hammer types and pile diameters are required in addition to measurements of the efficacy of mitigation measures such as ramp-up.

In addition to this, there are many other benthic organisms where vibrosensory thresholds are undescribed, as discussed 60 years ago and more recently (Frings 1964; Frings and Frings 1967; Roberts and Elliott 2017). This species deficit must be addressed on all levels—from macro- to meiofauna, from sessile to infaunal animals, from benthic vertebrate to invertebrates. If effects or responses to vibrational noise are observed, approaches need to be implemented to determine whether short-term behavioral effects translate to longer term and population level effects on an ecosystem level (Elliott and Quintino 2007; Roberts and Elliott 2017). Further, thresholds for other potential effects, such as physical damage to tissues or injury, and physiological changes, should be investigated.

In terrestrial environments, numerous potential vibrational noise sources remain unevaluated with respect to the following: (1) their characterization (intensity, temporal patterns, spectral ranges) and (2) the identification of which species may be impacted by the sources. This biological relevance question likely has a significant spatio-temporal component that requires careful examination. Part of this challenge is related to deficits in our current understanding of the vibrosensory system thresholds in many terrestrial animals, and how this relates to the different levels of behavior (mechanism, function, ontogeny, evolution). Further, community-level interactions may be influenced by vibrational noise, and this remains unstudied. The growing field of agroacoustics has made recent advances in testing how experimentally induced noise can potentially complement insect pest management (IPM) approaches in commercial agrosystems. Additional research is needed to understand how targeted pest insect populations may adapt to vibrational noise exposure over time, how vibrations may influence beneficial insects, and how the plants themselves respond to vibrational noise exposure.

Finally, we must recognize that laboratory work cannot replicate natural conditions and so must be repeated or translated in larger field studies in more natural vibration conditions. Further, effects of vibrational noise, as with other anthropogenic stressors, may be cumulative or in combination with other influences (Crain et al. 2008; Halpern et al. 2008; Hawkins and Popper 2012; Spiga et al. 2012). For example, pile driving not only creates vibrational noise, but the end-product may be a new physical structure in the ocean, which may induce local environmental changes such as artificial light and chemical variations. Other vibrations are intentionally produced, such as for seismic surveys, or are incidental to human processes such as the transport of goods (shipping) or the construction of a wind farm (piling). Indeed, a comprehensive management of multiple environmental stressors is necessary to evaluate impact upon the marine environment (Halpern et al. 2008; Elliott 2014).

6.7.1 Case Studies

Here, we present new preliminary data aimed to stimulate further progress of the field of vibrational noise studies. The sensitivities of many marine animals to substrate-borne vibration are not well understood; hence, we provide data regarding the sensitivity of the barnacle *Balanus crenatus* to sinusoidal waves (Case study 1). In Case Study 2, we provide data regarding the activity patterns of the cockroach *Gromphadorhina portentosa* after exposure to vibrational noise.

6.7.1.1 Case Study 1: Filling in the Data Gaps: Sensitivity of *Balanus crenatus* to Sinusoidal Vibrations

Vibrational threshold experiments were undertaken with *Mytilus edulis* (L., family Mytilidae), as detailed in Roberts et al. (2015), to understand the precise sensitivity to substrate-borne stimuli. Encrusting on the valves of many of the test subjects were *Balanus crenatus* (L., family Balanidae), a common sublittoral barnacle species that typically resides on hard substrata (Newman and Abbott 1980). Sensitivity to vibration has not been documented in detail in the literature for *B. crenatus*, but for details of potential reception systems, see Cragg and Nott (1977) and Zhadan (2005).

A shaker system was used to generate sinusoidal waves (5–410 Hz) within the substrate of a marine tank, with the resultant waves measured for threshold calculation in the vertical plane (m s^{-2}, 1 k/s sampling rate; Brüel & Kjær type 4333 piezo-electric accelerometer, sensitivity 20.60 mV/g; Brüel & Kjær Charge Amplifier type 2635; ADInstruments PowerLab module and CHART software v5.5.6). Signal analysis was undertaken as described in Roberts et al. (2015). The tank

(400 × 600 mm) had a water depth of 150 mm, and a substrate depth of 30 mm. At one end of the tank a small circular arena (100 × 50 mm) contained the test organisms, being a single mussel with encrusting *B. crenatus*, viewed from above via a web camera. A base of black plastic around the base of the mussel increased the visibility of the barnacle cirri in the camera observations, which focused upon one barnacle per group. Threshold was determined using the staircase method (Cornsweet 1962) inside a tank constructed with vibration damping material.

The presentations of the stimulus and the amplitude adjustments were made in response to the mussel responses rather than the barnacle. As such, the thresholds of ad hoc observations here were not calculated with the same accuracy as the mussel thresholds (Roberts et al. 2015). The threshold at each frequency was calculated using average acceleration (m s^{-2}) where a response was observed, rather than a 50% response level. It is of note that the vibrational stimulus received at the shell of the barnacle itself was not measured, but was measured next to the test arena.

Results and Discussion The shell of the barnacle, *B. crenatus* was noted to open and close during vibration exposure, both independently of the associated mussel valve and in association with the mussel host. A response was defined as activity cessation or an increased beating of the cirri, which is a notable activity change from normal baseline cirral beating.

At the greatest amplitudes, barnacles retracted the cirri simultaneously with the closure of the associated mussel valve, making it difficult to ascertain if the response was to the stimulus itself or to the mussel valve closure. In other cases, barnacles responded independently, beating the cirri faster during the exposure, with individuals responding typically to 2–3 of the 7 stimuli presented (responses seen at all 5–410 Hz frequencies; $n = 11$). Since numbers of barnacles observed, and those responding, were low (threshold calculated from 2–6 individuals), the data here should be considered preliminary (Fig. 6.2). There were predominant peaks at 90 and 210 Hz, with sensitivity at 20, 40, and 410 Hz more stable in the region of 0.1 m s^{-2}. The data indicate a threshold range of 0.1–1 m s^{-2} across all frequencies.

In response to vibrations in the current work, most barnacles exhibited full retraction of the cirri, briefly (<2 s) at the onset of the cue, before resuming beating behavior. In some cases, the cirri were observed to beat faster for a short period before returning to “normal” rhythm. Cirral activity can be divided into four or five classes, depending on the dominant function (respiratory or feeding) as described in detail by Crisp and Southward (1961). These behaviors range from “testing” behavior, where the valves are open, the operculum is moving but cirri are withdrawn, to full extension of the cirri without beating, known as “extension.” “Normal” beating behavior, without pauses, typically settles into a regular rhythm, consisting of activity with a short rest period in between (Southward and Crisp 1965). The rhythm appears to vary according to the natural environment of the barnacle, with the Balanidae, for example, exhibiting faster beating (perhaps according to water current variation) (Southward and Crisp 1965). It is likely that two types of cirral activity observed in the current study were the “normal” beating activity: (1) a strong movement of the operculum and cirri beating, and (2) the “fast beating,” when the

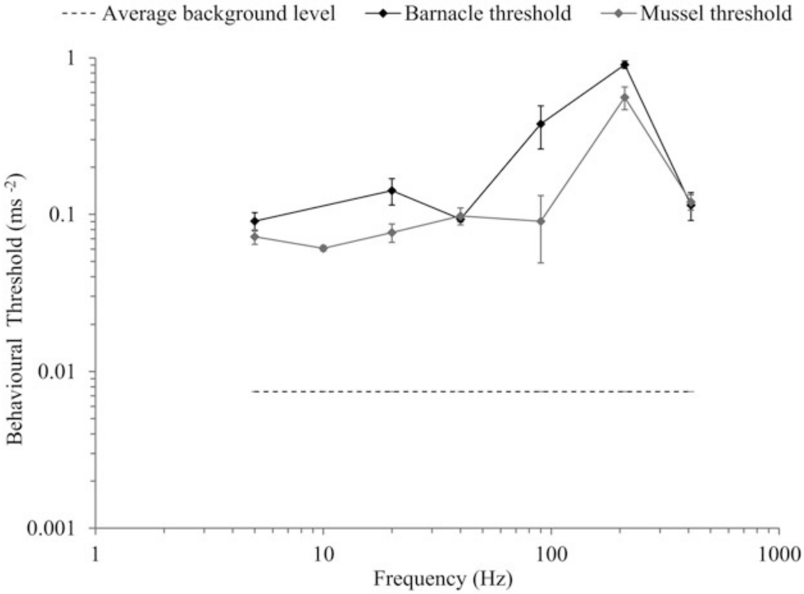


Fig. 6.2 Average behavioral threshold (m s^{-2}, RMS) of the Acorn barnacle *Balanus crenatus* (*n* = 11, ±SE) (encrusting on the Blue mussel *Mytilus edulis* valves) to sinusoidal vibration in the vertical axis (5–410 Hz). This preliminary data indicates a similar sensitivity to *M. edulis* (*n* = 15, ±SE; gray line). Average background noise levels are denoted by a dotted line

valves stay open, and cirri sweep rapidly without withdrawal into the shell. With the current experimental setup, it was difficult to see the movement of the operculum, although cirral movement was clear. In some cases, onset of vibration initiated a period of faster beating for a short time. This may be because the vibration affected water currents within the arena, or it may have been incidental to the stimulus, and be part of normal removal of internal acid buildup, which occurs sporadically (Southward and Crisp 1965). A "shock" reaction is described as full operculum closure with full retraction of the cirri (Crisp and Southward 1961), which was often observed in this work. This response has been observed in response to vibrational cues. While *B. crenatus* was clearly sensitive to the stimulus, the precise sensitivity of adult barnacles to sound and vibration does not appear to have been reported in the literature, although observations of retraction at vibration onset have been described (Crisp and Southward 1961; Southward and Crisp 1965). It is of note that only one barnacle was observed per group in the current study, but "neighborly" responses may also have influenced the observed responses.

As crustacea, barnacles may detect vibrations in a similar way to the postlarvae of decapod crabs, despite the lack of a specific statocyst receptor. Much of the literature on the sensitivity of barnacles to vibration is focused solely upon the larval stages (Branscomb and Rittschof 1984; Guo et al. 2011, 2012; Choi et al. 2013). Sound may be advantageous to barnacle cyprids, acting as an attractant to habitats in a similar way to bivalve and crab larvae (Simpson et al. 2004; Montgomery et al. 2006). Work has previously focused upon the inhibition of settlement upon man-made marine structures, due to the widespread biofouling threat barnacles pose to man-made marine structures. Focus has been upon the cyprid stage, which is known to explore the substratum before metamorphosing into the sessile adult. For example, settlement of *Amphibalanus variegatus* and *Elminus* sp. has been reduced by exposure to vibrations of 70–445 Hz (Choi et al. 2013), with the higher frequencies being more successful, indicating a sensitivity of the cyprids to such frequencies. Similarly, attachment of *Balanus amphitrite* may be prevented using a biofouling-prevention oscillator, with 30 Hz being better than other frequencies at preventing attachment (Branscomb and Rittschof 1984). At the opposite end of the spectrum, ultrasound has been equally effective at preventing settlement (Guo et al. 2011), for example sinusoidal energy at 23, 63, and 102 kHz. At low amplitudes ultrasound significantly affected exploratory behavior and reduced basal areas in those that metamorphosed upon the substratum, although Guo et al. (2012) were unable to repeat such results. The above studies indicate that the larval stage of barnacles may detect and be affected by sound; this has been attributed to sensitive sensory organs used for exploration (Rittschof et al. 1998; Maruzzo et al. 2011). However, in the case of ultrasound it may in fact be the side effects of the waves (the creation of cavitation bubbles at the water-solid boundary) causing damage, rather than the characteristics of the wave itself.

Vibration use might be advantageous to adult barnacles, which have been observed to retract in response to tactile and vibrational stimuli (Crisp and Southward 1961). Since intertidal predators, such as dog whelk (*Nucella lapillus*) and sea stars (e.g., *Asterias rubens*), would be moving across barnacle colonies when feeding at high tide, by detecting their approach early and retracting into the shell, *B. crenatus* could avoid mortality. By responding to vibrations, the barnacles may also be in tune with the tidal cycles upon the seashore, which would increase feeding success. Additional study is required to further understand the role that vibrations may play in barnacle life.

6.7.1.2 Case Study 2: Filling in the Data Gaps—The Effects of Vibrational Noise on Activity Patterns in a Terrestrial Invertebrate, *Gromphadorhina portentosa*

The Madagascar hissing cockroach, *Gromphadorhina portentosa*, a colony-living terrestrial insect, produces an airborne signal during courtship, agonistic encounters, and when disturbed (Clark and Moore 1995; Guerra and Mason 2005); some of these stereotypical behaviors may relate to information exchange or extraction from substrate-borne signals or cues in some behavioral contexts. In a series of laboratory experiments we examined how exposure to vibrational noise influences the daily activity patterns in this nocturnal species. To document the species' natural circadian patterns under noise-free control conditions, we placed individual animals in a TriKinetics locomotor activity monitor (LAM). The device collects discrete event data each time an individual breaks the LAM photo eye array in the center of the 50 mm × 20 mm containment tube inhabited by the subjects. Activity event data were binned using TriKinetics Filescan110 software, and Actograms were created using the R package ShinyR-DAM. A Kruskal–Wallis test with Dunn's post hoc comparisons was used to determine differences in activity levels among the 12 two-hour time blocks, and between vibrational noise and control treatments ($N = 32$ adult individuals each). In controls, peak activity was observed between 2000 and 2400 h, with significant increases in activity in the first four dark hours (Kruskal–Wallis = 58.87, d.f. = 11, $P < 0.0001$), and thus, noise playbacks occurred during these 4 h.

Substrate-borne vibration stimuli consisted of a train of 500 ms vibration pulses (brown noise, generated and band pass filtered in Adobe Audition version 3.0 to generate energy only between 10 and 300 Hz) with 5-s inter-pulse intervals. An electromagnetic shaker (AuraSound, Inc. AST-2B-04 50-Watt bass shaker) was bolted to the underside of a custom raised platform on the top of which was affixed the LAM used in control trials. The shaker was powered by an ART SLA4 4-Channel 140-Watt amplifier (ART ProAudio, Niagara Falls, NY) and was calibrated before each trial with a Polytech PDV-100 laser Doppler vibrometer (Polytec GmbH, Waldbronn, Germany) to an amplitude of 15.0 mm/sec. Control and vibrational noise treatments lasted 5 days, with the first day being used as an acclimation period. All trials were conducted in a hemi-anechoic chamber maintained at 23 °C with a 14:10 L:D photoperiod.

Madagascar hissing cockroaches in vibrational noise conditions exhibited significantly higher activity levels than those in control conditions (Kruskal-Wallis = 197.31, d.f. = 11, $P < 0.0001$). While animal activity across the diel period remained similar in pattern as in controls, with cockroaches maintaining peak activity the first 4 h of dark, median animal activity level was significantly elevated (Fig. 6.3). Additional study is required to understand the metabolic, physiological, reproductive, and life span costs associated with these noise-induced activity shifts, and to understand the persistence of the effects.

6.8 Concluding Remarks

The presence of natural noise selects for appropriate adaptations in animal communication systems that allow for effective signal transmission (Wiley and Richards 1978; Wiley 2006, 2017). However, vibrational noise generated by human activity is a relatively novel and recent phenomenon that is increasing rapidly with the growth of human populations (Barber et al. 2010). In general, anthropogenic disturbances are novel selective pressures that allow little time for species to adapt via selection (Sih et al. 2010), although rapid evolution in the Anthropocene is not uncommon (Palumbi 2001). Indeed, rapid evolution in response to anthropogenic change may be possible for some animals (Rabin and Greene 2002; Lampe et al. 2012). For animals with long generation times, however, behavioral plasticity may be a more

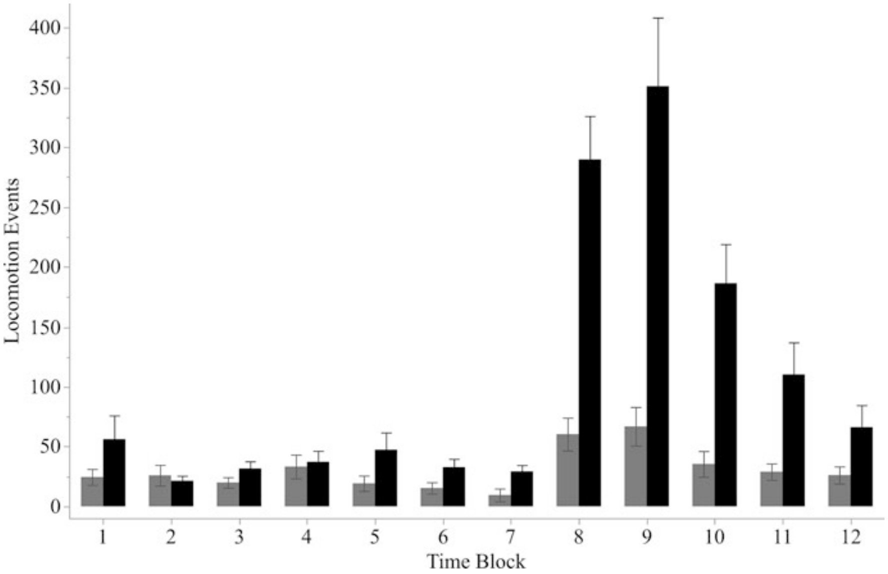


Fig. 6.3 Comparison of median daily activity patterns in the Madagascar hissing cockroach, *Gromphadorhina portentosa*. The species exhibits nocturnality in quiet control conditions (gray bars) with most locomotor activity concentrated during the dark blocks (8–12). When exposed to vibrational noise during the two peak periods of activity (blocks 8–9), cockroaches exhibited elevated activity in all periods except 2–4 (black bars)

effective mechanism for coping with anthropogenic change in a short timeframe (Sih et al. 2010; Tuomainen and Candolin 2011). Behavioral changes associated with airborne and water-borne acoustic noise pollution have been well documented (Slabbekoorn and Peet 2003; Foote et al. 2004; Miksis-Olds and Wagner 2011; Orci et al. 2016) and reviewed in Barber et al. (2010) and Shannon et al. (2016). It appears that many animals are still able to survive and reproduce in acoustic noise, though the required behavioral changes could lead to reduced fitness, altered community composition, and consequently altered ecological processes (Habib et al. 2007; Francis et al. 2009; Slabbekoorn et al. 2010; Francis and Barber 2013; Read et al. 2013; Bunkley et al. 2017).

Regarding noise in the vibrational realm, given the low amount of studies and species studied in this area to date (summarized in this chapter), it is currently not possible to draw firm conclusions on its effects. On land, effects to date include interference with signaling, pair formation and parental care, in addition to activity changes, and an increase of stress-related behaviors (see references in Table 6.2). In water, studies have shown increases in mortality, increases in startle responses, differences in activity levels and behaviors relating to sensing the environment, and internal biochemical changes (see references in Table 6.1). However, the aquatic research is far behind the terrestrial, with the detection abilities of most benthic species unknown (Roberts and Elliott 2017) and the extent to which vibrational cues are used being largely neglected to date. In total, our search found 22 published papers between the two environments, with a total of 20 species tested across different substrate types. It is clear, then, that there is still much to be learnt regarding vibrational noise, which may expose animals from the land to the sea.

Acknowledgments LR would like to recognize and thank those in the underwater noise community who continue to discuss and highlight seabed vibration where few people are doing so currently—particularly Anthony Hawkins and Arthur Popper; Richard Hazelwood, Thomas Breithaupt, Krysia Mazik, and Michael Elliott. DRH thanks Mia Phillips, Nicole Abate, Jessica Briggs, Alicyn Evans, Sophia Bloomer, Sarah Blatchley, and Carrie Hall for assistance in the field and laboratory with collecting data and literature on the effects of vibrational noise on terrestrial animals.

References

Abeyesinghe SM, Wathes CM, Nicol CJ, Randall JM (2001) The aversion of broiler chickens to concurrent vibrational and thermal stressors. Appl Anim Behav Sci 73(3):199–215

Barber JR, Crooks KR, Fristrup KM (2010) The costs of chronic noise exposure for terrestrial organisms. Trends Ecol Evol 25(3):180–189

Barth FG (1980) Campaniform sensilla: another vibration receptor in the crab leg. Naturwissenschaften 67(4):201–202

Barth FG (1988) Spiders of the genus *Cupiennius* Simon 1891 (Araneae, Ctenidae). Oecologia 2: 194–201

Benhaïma D, Péanb S, Lucas G, Blanca N, Chatain B, Bégout M-L (2012) Early life behavioural differences in wild caught and domesticated sea bass (*Dicentrarchus labrax*). Appl Anim Behav Sci 141(1–2):79–90

Berghahn R, Wiese K, Lüdemann K (1995) Physical and physiological aspects of gear efficiency in North Sea brown shrimp fisheries. Helgoländer Meeresuntersuchungen 49(1–4):507–518

Branscomb ES, Rittschof D (1984) An investigation of low frequency sound waves as a means of inhibiting barnacle settlement. J Exp Mar Biol Ecol 79(2):149–154

Breithaupt T (2002) Sound perception in aquatic crustaceans. In: Wiese K (ed) The crustacean nervous system. Springer, Berlin, pp 449–548

Briese V, Fanghänel J, Gasow H (1984) Effect of pure sound and vibration on the embryonic development of the mouse. Zbl Gynakol 106(6):379–388

Brumm H, Slabbekoorn H (2005) Acoustic communication in noise. Adv Stud Behav 35:151–209

Bunkley JP, McClure CJW, Kawahara AY, Francis CD, Barber JR (2017) Anthropogenic noise changes arthropod abundances. Ecol Evol 7:2977–2985

Caldwell MS, Johnston GR, McDaniel JG, Warkentin KM (2010a) Vibrational signaling in the agonistic interactions of red-eyed treefrogs. Curr Biol 20(11):1012–1017

Caldwell MS, McDaniel JG, Warkentin KM (2010b) Is it safe? Red-eyed treefrog embryos assessing predation risk use two features of rain vibrations to avoid false alarms. Anim Behav 79(2):255–260

Casas J, Bacher S, Tautz J, Meyhöfer R, Pierre D (1998) Leaf vibrations and air movements in a leafminer-parasitoid system. Biol Control 11(2):147–153

Casas J, Magal C, Sueur J (2007) Dispersive and non-dispersive waves through plants: implications for arthropod vibratory communication. Proc R Soc B 274(1613):1087–1092

Castellanos I, Barbosa P (2006) Evaluation of predation risk by a caterpillar using substrate-borne vibrations. Anim Behav 72(2):461–469

Chan A-H, Giraldo- Pérez P, Smith S, Blumstein DT (2010) Anthropogenic noise affects risk assessment and attention: the distracted prey hypothesis. Biol Lett 6:458–461

Chapman CJ, Hawkins AD (1973) A field study of hearing in the cod, *Gadus morhua* (L.). J Comp Physiol A 85:147–167

Choi CH, Scardino AJ, Dylejko G, Fletcher E, Juniper R (2013) The effect of vibration frequency and amplitude on biofouling deterrence. Biofouling 29(2):195–202

Clark DC, Moore AJ (1995) Variation and repeatability of male agonistic hiss characteristics and their relationship to social rank in *Gromphadorhina portentosa*. Anim Behav 50(3):719–729

Cocroft RB, Rodríguez RL (2005) The behavioral ecology of insect vibrational communication. Bioscience 55(4):323–334

Cocroft RB, Hamel J, Su Q, Gibson J (2014) Vibrational playback experiments: challenges and solutions. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 249–274

Čokl A, Laumann RA, Žunič Kosi A, Blassioli-Moraes MC, Virant-Doberlet M, Borges M (2015) Interference of overlapping insect vibratory communication signals: an *Eushistus heros* model. PLoS One 10:e0130775

Cornsweet TN (1962) The staircase-method in psychophysics. Am J Psychol 75:485–491

Cragg SM, Nott JA (1977) The ultrastructure of the statocysts in the pediveliger larvae of *Pecten maximus* (L.) (Bivalvia). J Exp Mar Biol Ecol 27(1):23–36

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. Ecol Lett 11(12):1304–1315

Crisp DJ, Southward AJ (1961) Different types of cirral activity of barnacles. Philos Trans R Soc Lond B 243:271–307

Dambach M (1989) Vibrational response. In: Huber F, Moore TE, Loher W (eds) Cricket behavior and neurobiology. Cornell University Press, Ithaca, pp 178–197

Davis AK, Schroeder H, Yeager I, Pearce J (2018) Effects of simulated highway noise on heart rates of larval monarch butterflies, *Danaus plexippus*: implications for roadside habitat suitability. Biol Lett 14:20180018

Day RD, McCauley RD, Fitzgibbon QP, Hartmann K, Semmens JM (2017) Exposure to seismic air gun signals causes physiological harm and alters behavior in the scallop *Pecten fumatus*. Proc Natl Acad Sci USA 114(40):E8537–E8546

Day RD, McCauley RD, Fitzgibbon QP, Hartmann K, Semmens JM (2019) Seismic air guns damage rock lobster mechanosensory organs and impair righting reflex. Proc Roy Soc B Bio 286(1907):20191424

de Groot M, Čokl A, Virant-Doberlet M (2010) Effects of heterospecific and conspecific vibrational signal overlap and signal-to-noise ratio on male responsiveness in *Nezara viridula* (L.). J Exp Biol 213:3213–3222

de Groot M, Čokl A, Virant-Doberlet M (2011) Species identity cues: possibilities for errors during vibrational communication on plant stems. Behav Ecol 22(6):1209–1217

Dimmitt MA, Ruibal R (1980) Environmental correlates of emergence in spadefoot toads (*Scaphiopus*). J Herpetol 14(1):21–29

Duarte MHL, Caliari EP, Scarpelli MDA, Lobregat GO, Young RJ, Sousa-Lima RS (2019) Effects of mining truck traffic on cricket calling activity. J Acoust Soc Am 146:656–664

Ducrotoy J-P, Elliott M (2008) The science and management of the North Sea and the Baltic Sea: natural history, present threats and future challenges. Mar Pollut Bull 57:8–21

Elias DO, Mason AC (2014) The role of wave and substrate heterogeneity in vibratory communication: practical issues in studying the effect of vibratory environments in communication. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin Heidelberg, pp 215–247

Ellers O (1995) Discrimination among wave-generated sounds by a swash-riding clam. Biol Bull 189(2):128–137

Elliott M (2014) Integrated marine science and management: wading through the morass. Mar Pollut Bull 86(1–2):1–4

Elliott M, Quintino V (2007) The estuarine quality paradox, environmental homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. Mar Pollut Bull 54(6): 640–645

Ellison WT, Southall BL, Clark CW, Frankel AS (2011) A new context-based approach to assess marine mammal behavioral responses to anthropogenic sounds. Conserv Biol 26(1):21–28

Endler JA (1992) Signals, signal conditions, and the direction of evolution. Am Nat 139:S125–S153

Engås A, Misund OA, Soldal AV, Horvei B, Solstad A (1995) Reactions of penned herring and cod to playback of original, frequency-filtered and time-smoothed vessel sound. Fish Res 22(3–4): 243–254

Engås A, Haugland EK, Øvredal JT (1998) Reactions of cod (*Gadus morhua,* L.) in the pre-vessel zone to an approaching trawler under different light conditions. Hydrobiologia 371(372): 199–206

Eriksson A, Anfora G, Lucchi A, Virant-Doberlet M, Mazzoni V (2012) Exploitation of insect vibrational signals reveals a new method of pest management. PLoS One 7:e32954

Fitzgibbon QP, Day RD, McCauley RD, Simon CJ, Semmens JM (2017) The impact of seismic air gun exposure on the haemolymph physiology and nutritional condition of spiny lobster, *Jasus edwardsii*. Mar Pollut Bull 125(1–2):146–156

Foote AD, Osborne RW, Hoelzel AR (2004) Whale-call response to masking boat noise. Nature 428(6986):910

Forrest TG (1994) From sender to receiver: propagation and environmental effects on acoustic signals. Am Zool 34:644–654

Francis CD, Barber JR (2013) A framework for understanding noise impacts on wildlife: an urgent conservation priority. Front Ecol Environ 11(6):305–313

Francis CD, Ortega CP, Cruz A (2009) Noise pollution changes avian communities and species interactions. Curr Biol 19(16):1415–1419

Friedel T (1999) The vibrational startle response of the desert locust *Schistocerca gregaria*. J Exp Biol 202:2151–2159

Frings H (1964) Problems and prospects in research on marine invertebrate sound production and reception. In: Tavolga WN (ed) Marine bioacoustics. Pergamon Press, Oxford, pp 155–173

Frings H, Frings M (1967) Underwater sound fields and behavior of marine invertebrates. In: Tavolga WN (ed) Marine bioacoustics. Pergamon Press, Oxford, pp 261–282

Gebresenbet G, Aradom S, Bulitta FS, Hjerpe E (2011) Vibration levels and frequencies on vehicle and animals during transport. Biosyst Eng 110(1):10–19

Gordon SD, Uetz GW (2012) Environmental interference: impact of acoustic noise on seismic communication and mating success. Behav Ecol 23(4):707–714

Götz T, Hastie G, Hatch L, Raustein O, Southall BL, Tasker M, Thomsen F (2009) Overview of the impacts of anthropogenic underwater sound in the marine environment. OSPAR Commission Biodiversity Series. https://qsr2010.ospar.org/media/assessments/p00441_Noise_background_document.pdf

Greenfield MD (1988) Interspecific acoustic interactions among katydids *Neoconocephalus*: inhibition-induced shifts in diel periodicity. Anim Behav 36(3):684–695

Guerra PA, Mason AC (2005) Information on resource quality mediates aggression between male Madagascar hissing cockroaches, *Gromphadorhina portentosa* (Dictyoptera: Blaberidae). Ethology 111(6):626–637

Guo S, Lee HP, Khoo BC (2011) Inhibitory effect of ultrasound on barnacle (*Amphibalanus amphitrite)* cyprid settlement. J Exp Mar Biol Ecol 409(1–2):253–258

Guo S, Lee HP, Teo SLM, Khoo BC (2012) Inhibition of barnacle cyprid settlement using low frequency and intensity ultrasound. Biofouling 28(2):131–141

Gurule-Small GA, Tinghitella RM (2018) Developmental experience with anthropogenic noise hinders adult mate location in an acoustically signalling invertebrate. Biol Lett 14(2):20170714

Habib L, Bayne EM, Boutin S (2007) Chronic industrial noise affects pairing success and age structure of ovenbirds *Seiurus aurocapilla*. J Appl Ecol 44(1):176–184

Hagstrum DW, Flinn PW (1993) Comparison of acoustical detection of several species of stored-grain beetles (Coleoptera: Curculionidae, Tenebrionidae, Bostrichidae, Cucujidae) over a range of temperatures. J Econ Entomol 86(4):1271–1278

Halfwerk W, Slabbekoorn H (2015) Pollution going multimodal: the complex impact of the human-altered sensory environment on animal perception and performance. Biol Lett 11(4):20141051

Halfwerk W, Ryan MJ, Wilson PS (2016) Wind- and rain-induced vibrations impose different selection pressures on multimodal signaling. Am Nat 188(3):279–288

Halpern BS, McLeod KL, Rosenberg AA, Crowder LB (2008) Managing for cumulative impacts in ecosystem-based management through ocean zoning. Ocean Coast Manag 51(3):203–211

Hammond PS, Macleod K, Berggren P, Borchers DL, Burt L, Cañadas A, Desportes G, Donovan GP, Gilles A, Gillespie D, Gordon J, Hiby L, Kuklik I, Leaper R, Lehnert K, Leopold M, Lovell P, Øien N, Paxton CGM, Ridoux V, Rogan E, Samarra F, Scheidat M, Sequeira M, Siebert U, Skov H, Swift R, Tasker ML, Teilmann J, Van Canneyt O, Vázquez JA (2013) Cetacean abundance and distribution in European Atlantic shelf waters to inform conservation and management. Biol Conserv 164:107–122

Harwood J (2002) Mitigating the effects of acoustic disturbance in the oceans. Aquat Conserv 12(5):485–488

Hawkins AD, Popper AN (2012) Effects of noise on fish, fisheries, and invertebrates in the U.S. Atlantic and Arctic from energy industry sound-generating activities. A literature synthesis. U.S. Department of the Interior, Bureau of Ocean Energy Management. Contract M11PC00031 153. https://tethys.pnnl.gov/sites/default/files/publications/Hawkins-and-Popper-2012.pdf

Hawkins AD, Popper AN (2014) Assessing the impact of underwater sounds on fishes and other forms of marine life. Acoust Today 10(2):30–41

Hawkins AD, Pembroke AE, Popper AN (2014a) Information gaps in understanding the effects of noise on fishes and invertebrates. Rev Fish Biol Fisher 25:39–64

Hawkins AD, Roberts L, Cheesman S (2014b) Responses of free-living coastal pelagic fish to impulsive sounds. J Acoust Soc Am 135(5):3101–3116

Hazelwood RA (2012) Ground roll waves as a potential influence on fish: measurement and analysis techniques. In: Popper AN, Hawkins A (eds) The effects of noise on aquatic life. Springer, New York, pp 449–452

Heinisch P, Wiese K (1987) Sensitivity to movement and vibration of water in the North Sea shrimp *Crangon crangon* L. J Crustacean Biol 7(1):401–413

Hill PSM (2008) Vibrational communication in animals. Harvard University Press, Cambridge MA

Hill PSM, Shadley JR (2001) Talking back: sending soil vibration signals to lekking prairie mole cricket males. Am Zool 41(5):1200–1214

Hill PSM, Wessel A (2016) Biotremology. Curr Biol 26(5):R187–R191

Hill PSM, Mazzoni V, Narins P, Virant-Doberlet M, Wessel A (2019) Quo vadis, biotremology? In: Hill PSM, Lakes-Harlan R, Mazzoni V, Narins P, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational behavior. Springer Nature, Cham, pp 3–14

Hunt RE, Morton TL (2001) Regulation of chorusing in the vibrational communication system of the leafhopper *Graminella nigrifrons*. Integr Comp Biol 41(5):1222–1228

Janssen J (1990) Localization of substrate vibrations by the mottled sculpin *(Cottus bairdi)*. Copeia 1990(2):349–355

Karlsen HE (1992) Infrasound sensitivity in the plaice (*Pleuronectes platessa*). J Exp Biol 171:173–187

Kastelein RA (2008) Effects of vibrations on the behaviour of cockles (Bivalve molluscs). Bioacoustics 17(1–3):74–75

Kauke M, Savary P (2010) Effect of noise and vibration in milking parlour on dairy cow. Agrarforsch Schweiz 1(3):96–101

Kight CR, Swaddle JP (2011) How and why environmental noise impacts animals: an integrative, mechanistic review. Ecol Lett 14(10):1052–1061

Kim D-S, Lee J-S (2000) Propagation and attenuation characteristics of various ground vibrations. Soil Dyn Earthq Eng 19(2):115–126

Kirschel ANG, Blumstein DT, Cohen RE, Buermann W, Smith TB, Slabbekoorn H (2009) Birdsong tuned to the environment: green hylia song varies with elevation, tree cover, and noise. Behav Ecol 20(5):1089–1095

Kowalewski J, Patrick PH, Christie AE (1992) Effect of acoustic energy on the zebra mussel (*Dreissena polymorpha*). In: Nalepa TF, Scholesser DW (eds) Zebra mussels biology, impacts and control. Lewis Publishers, Boca Raton, FL

Kurzweil LG (1979) Ground-borne noise and vibration from underground rail systems. J Sound Vib 66(3):363–370

Lakes-Harlan R, Strauß J (2014) Functional morphology and evolutionary diversity of vibration receptors in insects. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, Heidelberg, pp 277–302

Lampe U, Schmoll T, Franzke A, Reinhold K (2012) Staying tuned: grasshoppers from noisy roadside habitats produce courtship signals with elevated frequency components. Funct Ecol 26(6):1348–1354

Lewis ER, Narins PM, Jarvis JU, Bronner G, Mason MJ (2006) Preliminary evidence for the use of microseismic cues for navigation by the Namib golden mole. J Acoust Soc Am 119(2): 1260–1268

Márquez R, Beltrán JF, Llusia D, Penna M, Narins PM (2017) Synthetic rainfall vibrations evoke toad emergence. Curr Biol 26(24):R1270–R1271

Maruzzo D, Conlanb S, Aldred N, Clare AS, Høeg JT (2011) Video observation of surface exploration in cyprids of *Balanus amphitrite*: the movements of antennular sensory setae. Biofouling 27(2):225–239

Mazzoni V, Lucchi A, Čokl A, Prešern J, Virant-Doberlet M (2009) Disruption of the reproductive behaviour of *Scaphoideus titanus* by playback of vibrational signals. Entomol Exp Appl 133(2): 174–185

Mazzoni V, Gordon SD, Nieri R, Krugner R (2017) Design of a candidate vibrational signal for mating disruption against the glassy-winged sharpshooter, *Homalodisca vitripennis*. Pest Manag Sci 73(11):2328–2333

McCauley RD, Day RD, Swadling KM, Fitzgibbon QP, Watson RA, Semmens JM (2017) Widely used marine seismic survey air gun operations negatively impact zooplankton. Nat Ecol Evol 1: 0195. https://www.nature.com/articles/s41559-017-0195

McGregor PK (1992) Playback and studies of animal communication. Springer, New York

McNett GD, Cocroft RB (2008) Host shifts favor vibrational signal divergence in *Enchenopa binotata* treehoppers. Behav Ecol 19(3):650–656

McNett GD, Luan LH, Cocroft RB (2010) Wind-induced noise alters signaler and receiver behavior in vibrational communication. Behav Ecol Sociobiol 64(12):2043–2051

McVean A, Field LH (1996) Communication by substratum vibration in the New Zealand tree weta, *Hemideina femorata* (Stenopelmatidae: Orthoptera). J Zool 239(1):101–122

Miksis-Olds JL, Wagner T (2011) Behavioral response of manatees to variations in environmental sound levels. Mar Mamm Sci 27(1):130–148

Miller JH, Potty GR, Kim HK (2016) Pile-driving pressure and particle velocity at the seabed: quantifying effects on Crustaceans and groundfish. In: Popper A, Hawkins A (eds) The effects of noise on aquatic life II. Springer, New York, pp 719–728

Mitra O, Callaham M, Smith ML, Yack JE (2009) Grunting for worms: seismic vibrations cause *Diplocardia* earthworms to emerge from the soil. Biol Lett 5:16–19

Montgomery JC, Jeffs A, Simpson SD, Meekan M, Tindle C (2006) Sound as an orientation cue for the pelagic larvae of reef fish and crustaceans. Adv Mar Biol 51:143–196

Mooney TA, Hanlon RT, Christensen-Dalsgaard J, Madsen PT, Ketten DR, Nachtigall PE (2010) Sound detection by the longfin squid (*Loligo pealeii*) studied with auditory evoked potentials: sensitivity to low-frequency particle motion and not pressure. J Exp Biol 213:3748–3759

Morales MA, Barone JL, Henry CS (2008) Acoustic alarm signalling facilitates predator protection of treehoppers by mutualist ant bodyguards. P Roy Soc B Biol 275:1935–1941

Mosher JI (1972) The responses of *Macoma balthica* (bivalvia) to vibrations. Proc Malac Soc Lond 40(2):125–131

Mueller-Blenkle C, McGregor PK, Gill AB, Andersson, MH, Metcalfe J, Bendall V, Sigray P, Wood D, Thomsen F (2010) Effects of pile-driving noise on the behaviour of marine fish. COWRIE Ref: Fish 06–08/Cefas Ref: C3371. https://tethys.pnnl.gov/sites/default/files/publications/Mueller-Benkle_et_al_2010.pdf

Narins PM, Lewis ER, Jarvis JJ, O'Riain J (1997) The use of seismic signals by fossorial southern African mammals: a neuroethological gold mine. Brain Res Bull 44(5):641–646

Nedelec SL, Campbell J, Radford AN, Simpson SD, Merchant ND (2016) Particle motion: the missing link in underwater acoustic ecology. Methods Ecol Evol 7(7):836–842

Neo YY, Hubert J, Bolle LJ, Winter HV, Slabbekoorn H (2018) European seabass respond more strongly to noise exposure at night and habituate over repeated trials of sound exposure. Environ Pollut 239:367–374

Newman WA, Abbott DP (1980) Cirripedia. In: Morris RH, Abbott EC, Haderlie DP (eds) Intertidal invertebrates of California. Stanford University Press, Stanford, CA, pp 504–535

O'Connell-Rodwell CE, Wood JD, Rodwell TC, Puria S, Partan SR, Keefe R, Shriver D, Arnason BT, Hart LA (2006) Wild elephant (*Loxodonta africana*) breeding herds respond to artificially transmitted seismic stimuli. Behav Ecol Sociobiol 59(6):842–850

O'Connell-Rodwell C, Wood J, Kinzley C, Rodwell T, Poole J, Puria S (2007) Wild African elephants (*Loxodonta africana*) discriminate between familiar and unfamiliar conspecific seismic alarm calls. J Acoust Soc Am 122(2):823–830

Orci KM, Petróczki K, Barta Z (2016) Instantaneous song modification in response to fluctuating traffic noise in the tree cricket *Oecanthus pellucens*. Anim Behav 112:187–194

Palumbi SR (2001) Humans as the world's greatest evolutionary force. Science 293(5536): 1786–1790

Parvulescu A (1964) The acoustics of small tanks. In: Tavolga WN (ed) Marine bioacoustics. Pergamon Press, Oxford, pp 7–13

Pfannenstiel RS, Hunt RE, Yeargan KV (1995) Orientation of a hemipteran predator to vibrations produced by feeding caterpillars. J Insect Behav 8:1–9

Phillips ML, Chio G, Hall CL, ter Hofstede HM, Howard DR (2020) Seismic noise influences brood size dynamics in a subterranean insect with biparental care. Anim Behav 161:15–22

Pijanowski BC, Villanueva-Rivera LJ, Dumyahn SL, Farina A, Krause BL, Napoletano BM, Gage SH, Pieretti N (2011) Soundscape ecology: the science of sound in the landscape. Bioscience 61(3):203–216

Polajnar J, Čokl A (2008) The effect of vibratory disturbance on sexual behaviour of the southern green stink bug *Nezara viridula* (Heteroptera, Pentatomidae). Cent Eur J Biol 3(2):189–197

Popper AN (2005) What do we know about pile driving and fish? In: Proceedings of the 2005 Internation Conference on Ecology and Transportation, pp 26–28. https://trid.trb.org/Results?q=&serial=%222005%20International%20Conference%20on%20Ecology%20and%20Transportation%20(ICOET%202005)%22#/View/1355622

Popper AN, Hastings MC (2009) The effects of anthropogenic sources of sound on fishes. J Fish Biol 75(3):455–489

Popper AN, Hawkins AD (2018) The importance of particle motion to fishes and invertebrates. J Acoust Soc Am 143:470–488

Popper AN, Hawkins AD, Fay D, Mann RR, Bartol S, Carlson S, Coombs T, Ellison WT, Gentry R, Halvorsen MB, Lokkeborg S, Rogers P, Southall BL, Zeddies DG, Tavolga WN (2014) ASA S3/SC1.4 TR-2014 sound exposure guidelines for fishes and sea turtles: a technical report prepared by ANSI-accredited standards committee S3/SC1 and registered with ANSI. Springer, Cham

Purser J, Radford AN (2011) Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks (*Gasterosteus aculeatus*). PLoS Biol 6(2):e17478

Rabanal LI, Kuehl HS, Mundry R, Robbins MM, Boesch C (2010) Oil prospecting and its impact on large rainforest mammals in Loango National Park, Gabon. Biol Conserv 143(4):1017–1024

Rabin LA, Greene CM (2002) Changes to acoustic communication systems in human-altered environments. J Comp Psychol 116(2):137–141

Raboin M, Elias DO (2019) Anthropogenic noise and the bioacoustics of terrestrial invertebrates. J Exp Biol 222:jeb178749

Read J, Jones G, Radford AN (2013) Fitness costs as well as benefits are important when considering responses to anthropogenic noise. Behav Ecol 25(1):4–7

Rittschof D, Forward RB, Cannon G, Welch JM, McClary M, Holm ER, Clare AS, Conova S, McKelvey LM, Bryan P, Van Dover CL (1998) Cues and context: larval responses to physical and chemical cues. Biofouling 12(1–3):31–44

Roberts L, Elliott M (2017) Good or bad vibrations? Impacts of anthropogenic vibration on the marine epibenthos. Sci Total Environ 595:255–268

Roberts L, Laidre ME (2019a) Finding a home in the noise: cross-modal impact of anthropogenic vibration on animal search behaviour. Biol Open 8:1–7

Roberts L, Laidre ME (2019b) Noise alters chemically-mediated search behavior in a marine hermit crab: studying cross-modal effects on behavior. Proc Meetings Acoust 37:070001

Roberts L, Cheesman S, Breithaupt T, Elliott M (2015) Sensitivity of the mussel *Mytilus edulis* to substrate-borne vibration in relation to anthropogenically generated noise. Mar Ecol Prog Ser 538:185–195

Roberts L, Cheesman S, Elliott M, Breithaupt T (2016a) Sensitivity of *Pagurus bernhardus* (L.) to substrate-borne vibration and anthropogenic noise. J Exp Mar Bio Ecol 474:185–194

Roberts L, Pérez-Domínguez R, Elliott M (2016b) Use of baited remote underwater video (BRUV) and motion analysis for studying the impacts of underwater noise upon free ranging fish and implications for marine energy management. Mar Pollut Bull 112(1–2):75–85

Roberts L, Harding HR, Voellmy I, Bruintjes R, Simpson SD, Radford AB, Breithaupt T, Elliott M (2017) Exposure of benthic invertebrates to sediment vibration: from laboratory experiments to outdoor simulated pile-driving. Proc Meetings Acoust 27:1

Rogers P, Hawkins AD, Popper AN, Fay RR, Gray MD (2016) Parvulescu revisited: small tank acoustics for bioacousticians. In: Hawkins AD, Popper AN (eds) The effects of noise on aquatic life II. Springer, New York, pp 933–941

Röhr DL, Paterno GB, Camurugi F, Juncá FA, Garda AA (2016) Background noise as a selective pressure: stream-breeding anurans call at higher frequencies. Org Divers Evol 16(1):269–273

Saxena KN, Kumar H (1980) Interruption of acoustic communication and mating in a leafhopper and a planthopper by aerial sound vibrations picked up by plants. Experientia 36(8):933–936

Scheifele PM, Andrew S, Cooper RA, Darre M, Musiek FE, Max L (2005) Indication of a Lombard vocal response in the St. Lawrence River beluga. J Acoust Soc Am 117:1486–1492

Schmidt AKD, Balakrishnan R (2015) Ecology of acoustic signalling and the problem of masking interference in insects. J Comp Physiol A 201(1):133–142

Schmidt AKD, Riede K, Römer H (2011) High background noise shapes selective auditory filters in a tropical cricket. J Exp Biol 214:1754–1762

Schmidt AKD, Römer H, Riede K (2012) Spectral niche segregation and community organization in a tropical cricket assemblage. Behav Ecol 24(2):470–480

Schwartz JJ, Gerhardt HC (1989) Spatially mediated release from auditory masking in an anuran amphibian. J Comp Physiol A 166(1):37–41

Schwartz JJ, Wells KD (1983) The influence of background noise on the behavior of a Neotropical treefrog *Hyla ebraccata*. Herpetologica 39(2):121–129

Schwarz AL, Greer GL (1984) Responses of Pacific herring, *Clupea harengus pallasi*, to some underwater sounds. Can J Fish Aquat Sci 41(8):1183–1192

Scott GB (1994) Effects of short-term whole body vibration on animals with particular reference to poultry. World Poultry Sci J 50(1):25–38

Shannon G, McKenna MF, Angeloni LM, Crooks KR, Fristrup KM, Brown E, Warner KA, Nelson MD, White C, Briggs J, McFarland S, Wittemyer G (2016) A synthesis of two decades of research documenting the effects of noise on wildlife. Biol Rev 91:982–1005

Shier DM, Lea AJ, Owen MA (2012) Beyond masking: endangered Stephen's kangaroo rats respond to traffic noise with footdrumming. Biol Conserv 150(1):53–58

Sih A, Stamps J, Yang LH, McElreath R, Ramenofsky M (2010) Behavior as a key component of integrative biology in a human-altered world. Integr Comp Biol 50(6):934–944

Simpson SD, Meekan MG, McCauley RD, Jeffs A (2004) Attraction of settlement-stage coral reef fishes to reef noise. Mar Ecol Prog Ser 276(1):263–268

Slabbekoorn H, Peet M (2003) Birds sing at a higher pitch in urban noise. Nature 424:267

Slabbekoorn H, Bouton N, van Opzeeland I, Coers A, ten Cate C, Popper AN (2010) A noisy spring: the impact of globally rising underwater sound levels on fish. Trends Ecol Evol 25(7): 419–427

Solan M, Hauton C, Godbold JA, Wood CL, Leighton TG, White P (2016) Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates mediate ecosystem properties. Sci Rep 6:1–9

Southall BL, Bowles AE, Ellison WT, Finneran JJ, Gentry RL, Greene CR, Kastak D, Ketten DR, Miller JH, Nachtigall PE, Richardson W, Thomas J, Tyack P (2007) Marine mammal noise exposure criteria: initial scientific recommendations. Aquat Mamm 33(4):411–509

Southward AJ, Crisp DJ (1965) Activity rhythms of barnacles in relation to respiration and feeding. J Mar Biol Assoc UK 45(1):161–185

Spiga I, Cheesman S, Hawkins A, Perez-Dominguez R, Roberts L, Hughes D, Elliott M, Nedwell J, Bentley M (2012) Understanding the scale and impacts of anthropogenic noise in the marine environment. SoundWaves Consortium Technical Review (ME5205). https://research.ncl.ac.uk/soundwaves/links/pubblications/REVIEW%20new.pdf

Stephens DB, Rader RD (1983) Effects of vibration, noise and restraint on heart rate, blood pressure and renal blood flow in the pig. J R Soc Med 76(10):841–847

Stephens DB, Bailey KJ, Sharman DF, Ingram DL (1985) An analysis of some behavioural effects of the vibration and noise components of transport in pigs. Q J Exp Physiol 70(2):211–217

Stritih N, Čokl C (2014) The role of frequency in vibrational communication of orthoptera. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 375–393

Sueur J (2002) Cicada acoustic communication: potential sound partitioning in a multispecies community from Mexico (Hemiptera: Cicadomorpha: Cicadidae). Biol J Linn Soc 75(3): 379–394

Tasker ML, Amundin M, Andre M, Hawkins A, Lang W, Merck T, Scholik-Schlomer A, Teilmann J, Thomsen F, Zakharia M (2010) Underwater noise and other forms of energy. MSFD Task Group 11, Joint Research Centre European Commission, Luxembourg. https://ec.europa.eu/environment/marine/pdf/10-Task-Group-11.pdf

Tishechkin DY (2007) Background noises in vibratory communication channels of Homoptera (Cicadinea and Psyllinea). Russ Entomol J 16:39–46

Tishechkin DY (2013) Vibrational background noise in herbaceous plants and its impact on acoustic communication of small Auchenorrhyncha and Psyllinea (Homoptera). Entomol Rev 93(5):548–558

Tsubaki R, Hosoda N, Kitajima H, Takanashi T (2014) Substrate-borne vibrations induce behavioral responses in the leaf-dwelling cerambycid, *Paraglenea fortunei*. Zool Sci 31(12):789–794

Tuomainen U, Candolin U (2011) Behavioural responses to human-induced environmental change. Biol Rev 86(3):640–657

Vasconcelos RO, Simões JM, Almada VC, Fonseca PJ, Amorim MCP (2010) Vocal behavior during territorial intrusions in the Lusitanian toadfish: boatwhistles also function as territorial 'keep-out' signals. Ethology 116(2):155–165

Virant-Doberlet M, Mazzoni V, de Groot M, Polajnar J, Lucchi A, Symondson WOC, Čokl A (2014) Vibrational communication networks: eavesdropping and biotic noise. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 249–274

Wardle CS, Carter TJ, Urquhart GG, Johnstone ADF, Ziolkowski AM, Hampson G, Mackie D (2001) Effects of seismic air guns on marine fish. Cont Shelf Res 21(8–10):1005–1027

Wiley RH (2006) Signal detection and animal communication. Adv Stud Behav 36:217–247

Wiley RH (2015) Noise matters: the evolution of communication. Harvard University Press, Cambridge, MA

Wiley RH (2017) How noise determines the evolution of communication. Anim Behav 124:307–313

Wiley RH, Richards DG (1978) Physical constraints on acoustic communication in the atmosphere: implications for the evolution of animal vocalizations. Behav Ecol Sociobiol 3(1):69–94

Wollerman L (1999) Acoustic interference limits call detection in a Neotropical frog *Hyla ebraccata*. Anim Behav 57(3):529–536

Wrege PH, Rowland ED, Thompson BG, Batruch N (2010) Use of acoustic tools to reveal otherwise cryptic responses of forest elephants to oil exploration. Conserv Biol 24(6): 1578–1585

Wu C-H, Elias DO (2014) Vibratory noise in anthropogenic habitats and its effect on prey detection in a web-building spider. Anim Behav 90:47–56

Zhadan PM (2005) Directional sensitivity of the Japanese scallop *Mizuhopecten yessoensis* and swift scallop *Chlamys swifti* to water-borne vibrations. Russ J Mar Biol 31:28–35

Chapter 7
Research Approaches in Mechanosensory-Cued Hatching

Karen M. Warkentin, Julie Jung, and J. Gregory McDaniel

Abstract Mechanosensory-cued hatching (MCH) is widespread, diverse, and important for survival in many animals. Disturbance by predators elicits escape-hatching. Agitation by hosts stimulates parasite hatching. Sibling movements and parental vibrations synchronize hatching. Abiotic vibrations inform embryos of habitat conditions. Tests for MCH often use manual disturbance or mechanical vibrations from lab mixers; controlled vibration playbacks to embryos are rare. Our research with terrestrial embryos of red-eyed treefrogs illustrates how challenges and requirements of playbacks differ with embryos vs. post-hatching animals. Most vibrations salient to embryos are generated by direct forcing of eggs or egg masses, not transmitted via other substrates; thus they are shaped by egg and clutch mechanics. Most are highly variable incidental cues, not stereotyped signals, yet embryos distinguish among vibration sources. The necessary robustness of decision rules for incidental cues means even imperfect playbacks may be sufficient for initial studies of embryo behavior. Improvements in playback quality must address constraints of embryo development and behavior, as well as mechanics. We describe a series of playback systems we designed for red-eyed treefrog embryos, their merits and limitations, and what experiments with each have revealed about escape-hatching behavior. We discuss the iterative development of a new system for rearing eggs in trays and coupling them to shakers for motion, tactile, and bimodal mechanosensory playbacks, and the advances in understanding behavioral ontogeny this system has enabled. Mechanosensory-cued hatching offers excellent, untapped

---

K. M. Warkentin (✉)
Department of Biology, Boston University, Boston, MA, USA

Smithsonian Tropical Research Institute, Panama City, Panama
e-mail: kwarken@bu.edu

J. Jung
Department of Biology, Boston University, Boston, MA, USA
e-mail: jungj@bu.edu

J. G. McDaniel
Department of Mechanical Engineering, Boston University, Boston, MA, USA
e-mail: jgm@bu.edu

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_7

opportunities for playback experiments to advance our understanding of embryo behavior and how animals use incidental cues to inform behavior.

7.1 Introduction

As with other sensory modalities, most research on how animals use vibration to inform behavior examines communication signals. These signals evolved, and were selected for their effects on receivers, in the context of interactions with conspecifics—especially mate choice, competition for mates, and parental care (Cocroft et al. 2014b). A smaller subset of research examines how animals use incidentally generated biotic and abiotic vibrations to inform behavior in a variety of contexts, including predator-prey interactions (e.g., Pfannenstiel et al. 1995; Bacher et al. 1997; Brownell and Van Hemmen 2001; Castellanos and Barbosa 2006; Oberst et al. 2017; Roberts 2018), weather-cued behavior (Marquez et al. 2016), and behavioral synchronization with conspecifics (Doody et al. 2012). Of course, incidental cues may also elicit the use of communication signals (Guedes et al. 2012), and eavesdropping on communication signals can inform predator-prey interactions (Virant-Doberlet et al. 2019).

In addition, most work on vibrational communication and incidental cues focuses on vibrations that are generated in natural substrates (ground, plants, etc.) and are transmitted a substantial distance through these substrates from source to receiver. This transmission path alters the properties of the vibrations in complex ways, including frequency filtering, frequency-dependent transmission rate, attenuation, and resonance (Hill 2008; Cocroft et al. 2014b). The complexities of vibration transmission through substrates pose specific challenges, both for animals generating and receiving signals (Caldwell 2014) and for experimentalists recording signals and presenting them to animals in playback experiments (Cocroft et al. 2014a).

Animals also use mechanosensory cues—including vibration—to inform hatching timing (Warkentin 2005; Warkentin 2011b). In most cases, these cues are incidentally generated by predators (Warkentin 2005; Doody and Paull 2013), hosts (Whittington and Kearn 1988, 2011; Wang et al. 2012), siblings (Endo et al. 2019; Noguera and Velando 2019), or abiotic sources (Roberts 2001). However, some embryos hatch in response to specific types of physical disturbance by parents (Mukai et al. 2012, 2014; Goyes Vallejos et al. 2018), which may be evolved, stereotyped signals. In contrast to vibrational communication, it appears that many mechanosensory cues that embryos use to inform hatching behavior are not transmitted over distances through substrates. Often, they are generated by forces applied directly either to individual eggs or to groups of eggs that are physically in contact or enclosed within a shared, maternally-produced structure, such as an egg case or gelatinous egg mass (e.g., predators manipulating eggs while feeding, or siblings hatching). In this context, the mechanosensory cues available to embryos include a combination of vibrations generated by forcing the egg itself and those transmitted from egg to egg or through a surrounding maternal structure, as well as direct and indirect tactile cues (e.g. from predator contact and from eggs rubbing together as they are moved), and perhaps pressure cues (if egg shape is deformed). Embryos in eggs do not simply perceive vibrations generated elsewhere; they are often part of the forced and vibrating structure. The structural mechanics that shape such mechanosensory cues will, therefore, be largely determined by the maternally produced structures of both individual eggs and larger egg masses. Thus, evolutionary changes in egg and clutch structure will affect the information available to embryos. Moreover, the fitness costs and benefits of information transmission to embryos may impose selection on the maternal traits that produce these structures. In addition, the sensory morphology and physiology of embryos, through which they perceive cues available *in ovo*, change rapidly as they develop (Gottleib 1973; Romagny et al. 2012; Jung et al. 2019, 2020), sometimes in complex ways with cross-modal interactions (Carlsen and Lickliter 1999; Shimojo and Shams 2001). Sensory systems at the onset of their function are still poorly understood relative to later life stages.

Mechanosensory-cued hatching (MCH) offers excellent research opportunities to examine how animals use incidental, non-stereotyped cues to make important behavioral decisions, to understand how and why development changes behavior, and to understand the sensory and cognitive world of the earliest stages of animal life. Scientists working on embryo behavior have used many creative approaches to study MCH. However, few have employed the power of vibration playback experiments, presenting controlled sets of natural or synthetic vibrations to examine how vibration properties affect behavior (Table 7.1). Conducting playback experiments with embryos poses technical challenges that must be solved to ask the biological questions of interest. Some of these challenges also apply to the study of communication signals transmitted through substrates. For instance, in both cases investigators must compensate for nonlinearities in the transfer functions of shakers, or other vibration playback actuators, and changes in these nonlinearities when the actuator is connected to the substrate being shaken (Cocroft et al. 2014a). Other challenges are more specific to research on embryo behavior, such as how to physically couple eggs to an actuator for playback and how to optimize playback stimuli without inducing hatching or altering the subsequent behavior of test embryos. Moreover, some of the questions asked, and associated experimental designs and technical standards required to answer them, differ between studies of stereotyped signals and of incidental cues, as indeed do the strategies that animals can use to parse information and make behavioral decisions in these two contexts (Warkentin and Caldwell 2009).

We first briefly review research on MCH across taxa, highlighting the kinds of approaches used and questions addressed. We then discuss vibration playbacks to red-eyed treefrog embryos in more detail, as a case study to address issues in the design of playback systems for the study of MCH. We particularly address the challenges and solutions in developing a new playback system to expand the range of questions that can be investigated in this system. While our own research focus is on terrestrial frog eggs in gelatinous clutches that must distinguish mechanosensory cues from predators vs. benign abiotic sources, similar or parallel issues are likely to

Table 7.1 Research approaches in mechanosensory-cued hatching (MCH)

Taxa	Disturbance source	Forcing/ transmission	Experimental manipulation of mechanosensory cues to eggs	Results and interpretation	References
1. Manipulated connection/isolation from vibration source					
Stink bug, *Halyomorpha halys*	Siblings	Within egg mass	Manipulated vibration transmission pathway	Hatching cue is substrate-borne	Endo et al. (2019)
2. Manually applied physical disturbance					
Parasitic flatworm, *Diclidophora luscae*	Host—Pouting	Water turbulence	Agitated egg bundles with needle, water jets from pipette	Physical disturbance elicited rapid hatching; role in host-cued hatching	Whittington and Kearn (1988)
Parasitic flatworm, *Branchotenthes octohamatus*	Host—Spotted ray	Water turbulence	Tapped dish containing eggs, water jets from pipette	Most eggs hatched when disturbed; two other species showed no response	Glennon et al. (2006)
Mosquitoes, *Aedes caspius, A. vittatus*	Rain	Water vibrations	Finger drumming on containers of eggs	Rainlike vibrations increased hatching of submerged eggs in both species	Roberts (2001)
California grunion, *Leurethes tenuis*	Waves	Egg tumbling	Swirled eggs in seawater, developmental series	Environment alters hatching period, hatch timing affects post-hatching traits	Smyder and Martin (2002), Martin et al. (2011)
7 species of treefrogs, Phyllomedusidae	Predators (snakes)	Direct forcing of eggs, mass	Intermittent jiggling and rolling with forceps; live snake attacks	MCH occurs in all species tested; species vary in response to snakes	Gomez-Mestre et al. (2008)
Red-eyed treefrog, *Agalychnis callidryas*	Predators (snakes)	Direct forcing of eggs, mass	Intermittent jiggling and rolling with forceps, developmental series	Low genetic variation for MCH onset, little correlation with undisturbed hatching timing	Gomez-Mestre and Warkentin (2013)

Red-eyed treefrog, *Agalychnis callidryas*	Predators (snakes)	Direct forcing of eggs, mass	Intermittent jiggling with blunt metal probe, developmental series	MCH begins later than hypoxia-cued hatching; not limited by hatching ability; mediated by vestibular and lateral line mechanoreceptors	Warkentin et al. (2017); Jung et al. (2019, 2020)
Delicate skink, *Lampropholis delicata*	Predators, siblings?	Direct forcing, egg-to-egg	Prodded with bamboo skewer; rolled, pinched with fingers	Very rapid MCH, explosive exit from egg, escape sprint	Doody and Paull (2013)
Mole salamander, *Ambystoma talpoideum*	Predators?	Direct forcing of eggs, mass	Probed with dull pencil	Physical disturbance elicited rapid early hatching	Doody (2018)
3. Exposed to vibrating laboratory equipment					
California grunion, *Leurethes tenuis*	Waves	Egg tumbling	Agitated in fluid on rheostatically controlled shaker	Agitation in fluid induces hatching, regardless of oxygen level	Griem and Martin (2000)
Parasitic nematode, *Strelkovimermis spiculatus*	Host—Mosquito larvae	Water motion	Metal “larvae” animated with magnetic stirrer	Vibration increases hatching response to host chemicals; no hatching with just vibration	Wang et al. (2012)
Pig-nosed turtle, *Carettochelys insculpta*	Siblings	Within egg mass	Isolated eggs in sand placed on lab shaker	Shaken eggs hatch rapidly, days before controls; sibling cue to hatch is vibrational	Doody et al. (2012)
Delicate skink, *Lampropholis delicata*	Predator, siblings?	Direct forcing, egg-to-egg	Isolated eggs placed on orbital shaker, short daily exposure	Eggs exposed to shaking hatched earlier in development	Doody and Paull (2013)
Burrower bug, *Adomerus rotundus*	Mother	Shaking egg mass	Pestle mixer to animate dead female on clutch, on/off pattern	Maternal vibration of clutch induces hatching, facilitates synchrony	Mukai et al. (2012)
Shield bug, *Parastrachia japonensis*	Mother	Shaking egg mass	Pestle mixer on thread suspending clutch, intermittent vs. continuous vibration	Intermittent maternal vibration of clutch induces hatching, facilitates synchrony	Mukai et al. (2014)

(continued)

Table 7.1 (continued)

Taxa	Disturbance source	Forcing/ transmission	Experimental manipulation of mechanosensory cues to eggs	Results and interpretation	References
Desert locust, *Schistocerca gregaria*	Siblings	Within egg mass	Vortex mixer used to briefly vibrate isolated eggs	Vibration stimulates hatching, facilitates synchrony	Nishide and Tanaka (2016)
4. Playback of controlled vibration stimuli (electrodynamic shakers and vibrational speakers)					
Red-eyed treefrog, *Agalychnis callidryas*	Predators and benign abiotic disturbances	Direct forcing of eggs, mass	Shaker connected to gelatinous egg mass via metal tines among eggs; played recorded vibrations, edited recordings, synthetic stimuli	Snake vibrations elicit more hatching than rain vibrations; embryos use many vibrational properties non-redundantly to assess risk, adjust sampling with cost of information	Warkentin (2005), Warkentin et al. (2006, 2007), Caldwell et al. (2009, 2010b)
Red-eyed treefrog, *Agalychnis callidryas*	Predators and benign abiotic disturbances	Direct forcing of eggs, mass	Shaker connected to a plastic tray holding up to 15 eggs in individual funnels; played synthetic stimuli to different developmental stages	Adaptive ontogenetic changes in information use, as predicted from developmental change in missed cue–false alarm trade-off	Warkentin et al. (2019), Jung et al. (2021)
Stink bug, *Halyomorpha halys*	Siblings	Within egg mass	Eggs glued to coverslips set on platform on shaker; played recorded vibrations	Egg cracking vibration induces hatching, facilitates synchrony	Endo et al. (2019)
Migratory locust, *Locusta migratoria*	Siblings	Within egg mass	Vibrational speaker connected via a set of wires to individual eggs mounted on sand; played recorded vibrations	Embryo vibrations recorded 0–2.5 h before hatching accelerate hatching; vibrations recorded 8–10 h before hatching delay hatching	Sakamoto et al. (2019)

pertain in research on the MCH of many other animals within different maternally produced structures.

7.2 Research on Mechanosensory-Cued Hatching Across Taxa

Mechanosensory-cued hatching is widespread and may be common. As part of research focused on other questions, observations often suggest that embryos respond to physical disturbance or vibrations, without experimentally ruling out other sensory modalities. For instance, anecdotal field observations of frog embryos hatching in response to attempted egg collection indicate a response to physical disturbance (Brown and Iskandar 2000). In some insects, experimental isolation of embryos from siblings or parents disrupts synchronous hatching, and observations suggest that cues are vibrational (Endo and Numata 2017; Nishide et al. 2017). A recent laboratory study found that bird embryos exposed to alarm calls somehow transmit information about risk to other eggs in the nest, and documented higher vibration rates of call-exposed eggs as a possible mechanism (Noguera and Velando 2019). Such studies offer excellent starting points and motivation for more focused research on MCH.

One straightforward method to assess the sensory modality of hatching cues is to manipulate the connection between an embryo and the putative vibration source (Table 7.1.1). For instance, Endo et al. (2019) hypothesized that vibrations from siblings help synchronize the hatching of stink bug eggs. They compared hatching synchrony in pairs of eggs that were naturally attached, glued together after separation, attached 1 mm apart on a piece of paper, 1 mm apart with a connecting piece of mechanical pencil lead, and on different pieces of paper. The greater synchrony of eggs in contact or connected by pencil lead, and lack thereof with physical separation, even with close proximity, supports that cues are substrate-borne. Variants of this method, breaking and reconnecting a vibration transmission channel, have also been useful in work with post-embryonic stages (e.g., Guedes et al. 2012).

A second, more widely used, experimental approach is manually applied physical disturbance. Investigators have directly applied forces to both aquatic and terrestrial eggs using a variety of hand-held instruments (needles, probes, forceps, etc.; Table 7.1.2) and even just fingers (Doody and Paull 2013). These direct disturbance stimuli are simple to apply and effectively induce hatching of many vertebrate and invertebrate species (Table 7.1.2), clearly demonstrating the importance of mechanosensory cues. The combination of egg-motion and contact (tactile) stimulation may be particularly relevant in the context of predator-induced escape-hatching (Warkentin 1995; Doody and Paull 2013; Warkentin et al. 2017). However, the extent to which embryos rely on each sensory modality within such complex cues is unclear. With aquatic eggs, investigators have also indirectly moved or vibrated eggs by swirling, tapping, or drumming on containers of eggs, or manually applying water jets (Table 7.1.2), mimicking the turbulence created by host animals, tumbling in waves, or the patter of rain. Both direct and indirect manual disturbance cues have been used not only to evaluate the nature of hatching cues, but also to determine the period of hatching plasticity, to assess how hatching responses vary among and within species, to examine the hatching process, and to determine post-hatching consequences of hatching at different stages (Table 7.1.2) (Warkentin 1995, 1999; Touchon et al. 2013; Willink et al. 2014).

A variety of common types of laboratory equipment generate vibrations, albeit with limited flexibility of vibration properties. Several investigators have successfully used vibrations from laboratory equipment to test the role of mechanosensory cues in hatching (Table 7.1.3). Griem and Martin (2000) used a rheostatically controlled shaker to agitate grunion embryos, which develop in beach sand above the high tide line, determining that physical disturbance by waves—not hypoxia—induces hatching when spring tides flood the eggs. Wang et al. (2012) made artificial metal mosquito larvae and animated them using a magnetic stirrer to assess hatching stimuli for a mosquito-parasitic nematode, revealing that water vibrations from simulated host movement increase the response to host chemical cues. Doody and collaborators used laboratory shakers to test the role of vibrations in hatching of both pig-nosed turtles, which hatch synchronously when flooded, and delicate skinks, which hatch rapidly in response to manual physical disturbance; in both cases, the shaker stimulus accelerated hatching, demonstrating that vibrations alone can elicit hatching (Doody et al. 2012; Doody and Paull 2013). Following a daily exposure protocol similar to Doody and Paull (2013), Nishide and Tanaka (2016) used a vortex mixer to stimulate isolated desert locust eggs, supporting that vibrations play a role in the synchronous hatching of egg masses. Mukai and collaborators (Mukai et al. 2012, 2014) used a pellet pestle mixer to shake the egg masses of burrower bugs and shield bugs, turning the mixer on and off to roughly imitate the temporal pattern in which mothers shake their clutches. In both species, embryos responded to this stimulus by hatching synchronously; in shield bugs, a continuous vibration stimulus was ineffective in inducing or synchronizing hatching (Mukai et al. 2014). Like manual disturbance, vibrating equipment may enable the assessment of cue modality as well as other elements of hatching responses.

It is likely that neither manual physical disturbances (Table 7.1.2) nor standard laboratory mixers and shakers (Table 7.1.3) precisely replicate the vibrational properties of natural hatching stimuli. In most cases these natural stimuli have never been recorded, nor have the detailed vibrational properties of effective artificial stimuli been reported. Moreover, some imprecision is unavoidable in manually applied stimuli, and laboratory shakers are limited in the range of vibrations they can produce. Nevertheless, in many species, such cues elicit hatching behavior. In some cases, based on knowledge of their study system or simply by luck, investigators may have chosen the piece of available lab equipment or method of manual disturbance that best mimicked the properties of natural vibrations salient to the embryos they study. However, it also seems likely that, at least in some species, the range of vibrational stimuli effective in eliciting hatching is substantially broader than the range of stimuli effective in eliciting behaviors such as courtship, mating, or territorial aggression. In many cases embryos use incidental cues, which are inherently more variable than communication signals, to inform hatching timing. The best decision rules will depend on their background vibrational environment, which might elicit false alarms, and the consequences of both false alarms and missed cues (Warkentin and Caldwell 2009). An expansive or permissive response could be favored if irrelevant background vibrations are rare, weak, or easily distinguished; if costs of false alarms are low; and/or if costs of missed cues are very high. Indeed, embryos that fail to respond to cues indicating imminent consumption by a predator or the transient availability of a host often die (Warkentin 2000; Gomez-Mestre and Warkentin 2007; Whittington and Kearn 2011), and failing to synchronize hatching with clutch-mates can be similarly lethal, exposing animals to sibling cannibalism (Mukai et al. 2018).

Vibration playback experiments (Table 7.1.4) offer a powerful and flexible approach to study mechanosensory-cued hatching (MCH) responses in more depth, investigating how precise or permissive they are, the ways different cue properties affect embryo behavior, and a wide range of other questions. Such methods are commonly employed in studies of vibration-mediated behavior more generally (Hill 2008; Cocroft et al. 2014b) but have rarely been applied to hatching. To our knowledge, only three research groups have performed vibration playback experiments on embryos: Warkentin and collaborators working with red-eyed treefrogs, Endo et al. (2019) working with stink bugs, and Sakamoto et al. (2019) working with locusts (Table 7.1.4). These researchers used electrodynamic shakers or vibrational speakers for direct forcing of eggs attached to the actuator platform by custom-made interfaces. A common starting point for playback experiments is to record vibrational cues (or signals) and the background vibrational environment. We began by recording vibrations from egg clutches in undisturbed conditions, and during snake attacks, rainstorms, and wind (Warkentin 2005; Caldwell et al. 2009, 2010a). We used these recordings directly in playback experiments and also to inform the construction of synthetic stimuli to ask a variety of questions (Table 7.1.4, Sects. 7.3.1 and 7.3.5, Fig. 7.15). Endo et al. (2019) recorded vibrations from eggs caused by neighboring eggs hatching. They used an electrodynamic shaker to play exemplars of a single egg-cracking vibration to eggs glued to glass coverslips and set on a metal platform. They found that this elicited a substantial hatching response, while background vibrations from the same recordings elicited little hatching. Sakamoto et al. (2019) recorded vibrations from eggs at different times before hatching. They used a vibrational speaker attached to a set of wires to play these vibrations to eggs mounted in sand, finding accelerated hatching in response to vibrations recorded shortly before hatching and delayed hatching in response to vibrations recorded long before hatching. These methods for stinkbugs and locusts might be productively adapted for vibration playback experiments with other terrestrial insect eggs to enable deeper exploration of vibration-cued embryo behavior. Our methods might be readily adapted for other terrestrial frog eggs and potentially, with modification, for other terrestrial vertebrate eggs.

We now know that MCH is widespread, offering great opportunities for research into the perceptual world of embryos, the development of behavior, and how animals use incidental, non-stereotyped cues to make important behavioral decisions. Relatively simple methods can be used to test for plasticity in hatching timing, determine if embryos use mechanosensory cues, examine how hatching responses vary with developmental stage or context, and assess how hatching timing affects post-hatching life (Table 7.1.1-3). However, a deeper understanding of how embryos make decisions based on mechanosensory cues requires greater experimental control of cue properties. Red-eyed treefrog embryos have been very tractable for such research and our work with these eggs offers an example of the iterative development of playback methods for asking different kinds of questions, as well as some of the challenges involved in such work.

7.3 Red-Eyed Treefrogs as a Case Study for Research in Mechanosensory-Cued Hatching

My decision to attempt vibration playback experiments with red-eyed treefrog embryos, *Agalychnis callidryas*, and the approach I took (Warkentin 2005), was based on several key findings from prior work. These embryos escape from snake attacks on their arboreal, gelatinous egg clutches by hatching rapidly and prematurely, falling into the water below, and they face a risk trade-off because less developed hatchlings are at higher risk of predation by aquatic predators (Warkentin 1995). Manually jiggling eggs can elicit hatching, suggesting physical disturbance of clutches is sufficient, and visual and chemical cues from snakes are unnecessary; however, not all forms of egg-jiggling, nor all natural physical disturbances, induce hatching (Warkentin 1995). I had seen hatching-competent embryos buffeted in torrential rainstorms without hatching, suggesting they discriminate among disturbances. In addition, I had never observed embryos hatch while snakes were crawling on plants, even near enough to tongue-flick eggs, only after a snake began biting and pulling at the clutch. Thus it appeared the cue involved direct physical disturbance of the egg mass, rather than vibrations transmitted through plants. In my initial pilot experiments, placing leaves with *A. callidryas* egg clutches on various pieces of vibrating lab equipment, no embryos hatched—unlike results from some other species (Table 7.1.3). Thus, before I could design experiments to examine embryo responses to vibrational cues, I needed to assess the properties of natural vibrations among which embryos discriminate.

I decided to record vibrations from accelerometers embedded within the gelatinous clutches—as if they were eggs—rather than attached to their leaf substrate, because vibrations of the eggs themselves seemed critical. Implicit in this approach was the assumption that the vibration measured by the accelerometer would resemble or represent that experienced by eggs. I, therefore, worked with the smallest accelerometers available to minimize mass-loading the egg clutch: initially Endevco 25B (0.2 g, San Juan Capistrano, CA) and later also AP19 (0.14 g, AP Technology, Oosterhout, Netherlands). This is a far from standard application of accelerometry (see details in Sect. 7.3.4.6) but nonetheless seemed to be the best technology available. Because laser Doppler vibrometry requires continuous line-of-sight contact with a reflective target, it would be disrupted by both feeding snakes and falling rain—the two most important natural vibration sources—coming between the clutch and laser. Moreover, snakes displace eggs in multiple, uncontrolled directions by distances that would move a reasonably-sized spot out of the laser beam.

7.3.1 A Brief History of Tine-Based Vibration Playbacks to **Agalychnis callidryas** *Egg Clutches*

For playbacks, it seemed important to present vibrations directly to eggs in clutches, rather than through intervening plant substrate, which required developing new playback methods. When I started working on vibration playbacks to eggs, the robustness of hatching responses to highly variable snake attacks (ca. 80% escape success across four snake species with very different feeding behavior, Warkentin unpublished) gave me hope that even imperfect playback methods, if applied with appropriate controls, might provide useful information about embryo behavior. My initial attempt to use a playback system based on a modified pen motor from a chart recorder quickly revealed that the motor had insufficient strength to effectively vibrate *A. callidryas* egg masses. Thus I began working with an electrodynamic minishaker (Brüel & Kjær model 4810).

My first effective playbacks used a hand-made, tine-based minishaker–clutch interface (MCI) to present vibrations to embryos in their gelatinous egg mass (Fig. 7.1a–c). The MCI was constructed from a piece of 18-gauge galvanized wire bent into tight loops to produce blunt tines that could be inserted into the clutch, between eggs, and glued to a metal shaft threaded for attachment to a minishaker (Warkentin 2005). I hung the shaker upside-down from a wooden frame and attached leaves with clutches to a heavy stand below it, so shaker activation moved the tines—and eggs—up and down, while their leaf stayed put (Fig. 7.1a). This system enabled me to shake eggs in different patterns and revealed variation in their hatching responses to different stimuli. For instance, playbacks of recorded snake attacks elicited more hatching than did playbacks of rain, revealing that embryos discriminate among stimuli (Warkentin 2005). Moreover, manipulating the temporal patterns of recordings by consolidating or breaking up periods of silence altered embryo responses to both types of stimuli, showing that temporal patterns affect the hatching response (Warkentin 2005). These important aspects of embryo behavior were evident even though the amplifier I used for those early playbacks attenuated low frequencies, which we now know are important hatching cues, and I neither quantified this low-frequency roll-off nor pre-filtered stimuli to compensate for it. However, this playback infidelity likely contributed to the relatively low range of hatching responses (ca. 0–40%; Warkentin 2005) compared to

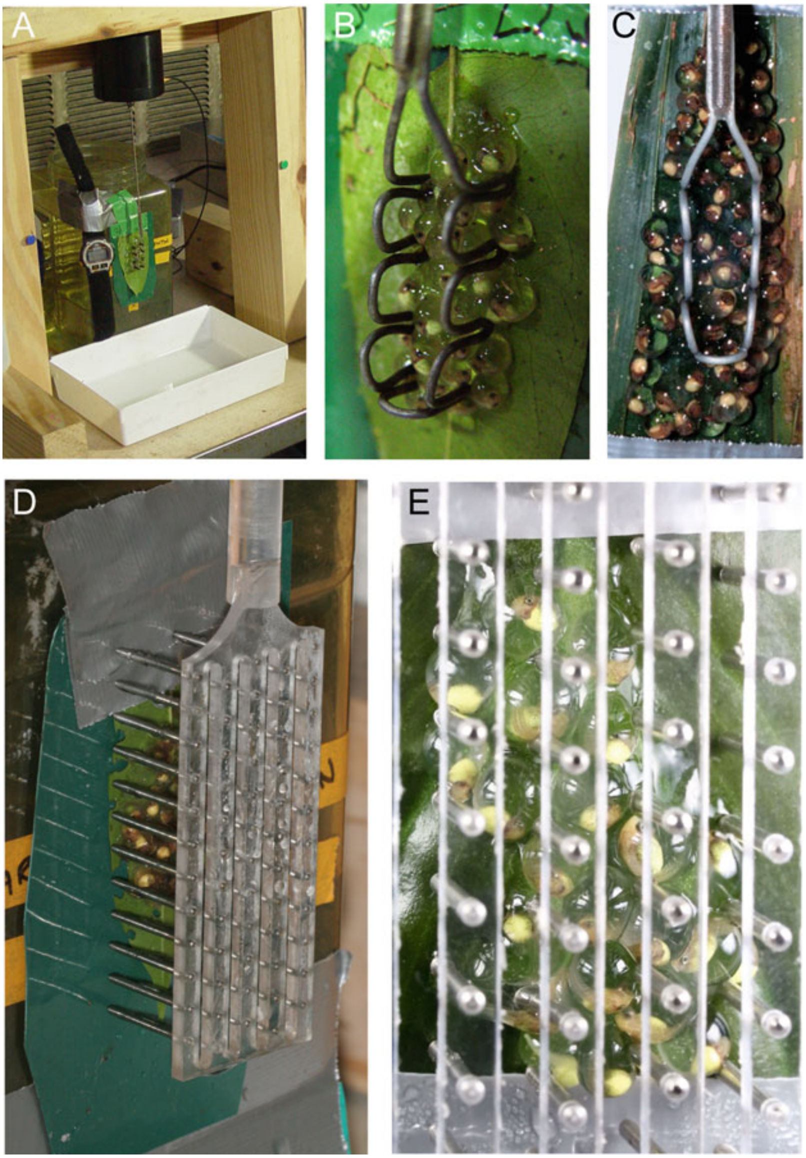


Fig. 7.1 Tine-based playback systems used to test *Agalychnis callidryas* embryo responses to vibrational cues. (**a–c**) Original minishaker–clutch interface (MCI) showing (**a**) setup of egg clutch on stand, shaker hanging from wooden frame, and water to receive hatchlings; (**b**) MCI tines in a small clutch; and (**c**) MCI tines in a larger clutch. (**d**) The second MCI, with a larger field of smaller, more closely spaced tines, delivered more uniform stimuli across a broader range of clutch sizes. (**e**) View of embryos through the tines of the second MCI. Both tine-based systems deliver an inseparable combination of motion and tactile cues in playback

later work with better, custom-made amplifiers and compensation for nonlinearities in the frequency transfer function (e.g., Caldwell et al. 2009; ca. 0–100% hatching).

Another clear limitation of the first playback system was that the motion of eggs in playbacks appeared to vary with their distance from tines. Moreover, across clutches of different sizes (Fig. 7.1b, c) the proportion of embryos near the tines varied. Thus, so did both the mean intensity of direct forcing, from the tines, and the extent to which the tines damped the natural ring-down after each vibration we played. In an effort to reduce this variation, and vibrate entire clutches more uniformly, my collaborators Michael Caldwell and vibrations engineer J. Gregory McDaniel designed a second MCI, affixing 1.5 mm diameter blunt-ended stainless steel tines (extracted from a comb) to an acrylic plate, mounted on an acrylic rod. Tines were 6 mm apart in 5 offset columns, 6.5 mm apart, creating a 75 mm high by 31 mm wide tine field, and sections of the acrylic plate were cut out between tine columns to improve egg visibility (Fig. 7.1d, e) (Warkentin et al. 2006). This enabled us to present much more consistent vibration throughout the tine field, across a larger range of clutch sizes, and to reduce free vibrations of the clutch to improve playback fidelity in the time domain.

Improving fidelity in the frequency domain was a greater challenge. Playbacks of substrate-borne vibrations are subject to changes in frequency spectra due to both nonlinearities in playback systems and filtering by substrates (Cocroft et al. 2014a). In addition, coupling playback devices to substrates can alter device output, in substrate-dependent ways. The usual solution to this problem is to determine the response properties of the playback device coupled to the specific substrate system (e.g., individual stem, leaf, patch of soil, etc.) to achieve substrate-specific control of playback fidelity. Similarly, one can compensate for frequency filtering by the substrate to produce desired vibrations at a particular substrate location (Cocroft et al. 2014a). Generally, this process requires playing vibrations into the substrate, recording and analyzing the vibration produced at the relevant location(s), and iteratively adjusting the input to achieve desired output prior to introducing a test animal.

Attaching our shakers to elastic, gelatinous egg clutches via an MCI clearly changes their output frequency response, and clutches vary substantially in number of eggs, egg size, jelly thickness, and other structural properties. However, because our test subjects, the embryos, are an integral part of the structure to be vibrated, we cannot perfect playbacks prior to introducing them. Subjecting egg clutches to the necessary playback and recording process to fine-tune stimuli can elicit hatching and, even if embryos remain unhatched, their prior exposure to vibrations could alter behavioral responses to subsequent playbacks. Thus we used a series of naturally varied egg masses to develop a mean transfer function for our shaker–MCI–egg clutch playback system. We then used that mean function to adjust playback stimuli for presentation to experimental clutches (Caldwell et al. 2009, 2010a). We did not attempt to generate precisely identical vibrations in structurally varied experimental clutches, nor to perfectly replicate the unique, clutch-specific vibrations that would occur if identical forcing was applied to structurally different clutches – for instance by hypothetical, physically identical snake bites or raindrops. Our rather more modest goal was simply to have sufficient control of playback properties to answer some biologically interesting questions. This, we achieved.

We used our MCI-based playback system in a series of experiments, presenting a range of recorded vibrations, edited natural stimuli, and systematically varied synthetic stimuli. For instance, using simple noise bursts in rhythmic temporal patterns, we learned that vibration duration and inter-vibration interval both affect hatching, as two non-redundant elements of a composite cue, with a single hatching peak around 0.5 s vibration/1.5 s silence and decreasing hatching as either element departs from these values (Warkentin et al. 2006). We found that low frequencies stimulate hatching, and higher frequencies inhibit it, which appears to be adaptive considering the preponderance of low frequencies and absence of high frequencies in predator-attack vibrations and the presence of high frequencies during rain (Caldwell et al. 2009, 2010a). We determined that earlier vibrations can either decrease or increase the response to subsequent vibrations. The period of intensity build-up at the start of rainstorms contributes to embryos' tolerance of heavier rain without hatching (Caldwell et al. 2010a), and a few short vibrations—in themselves insufficient to induce hatching—followed by a period of silence can increase the hatching response to subsequent "scary" vibrations (Jung 2021). Unsurprisingly, higher amplitude playbacks elicit more hatching; however, this amplitude-response function is steeper for playbacks of snake-attack recordings and shallower for playbacks of rain recordings (Caldwell 2010). Moreover, for stimuli that ultimately elicit the same proportion of hatching, the cycle length of the pattern affects the timing of hatching onset (Warkentin et al. 2007). This suggests that embryos adjust how much information they use for their hatching decision based on the cost of information sampling; in attacks, longer cycles likely entail more predation risk per unit of temporal pattern information (Warkentin et al. 2007; Warkentin and Caldwell 2009).

7.3.2 "Good Enough" Biotremology Methods for Biological Questions About Incidental Cues

An important lesson from our research on vibration-cued hatching of *A. callidryas* embryos is that the level of playback fidelity required depends on the biological question. This point will be relevant for other studies of behavior where playback optimization may be constrained, such as with embryos of other taxa. Very simple methods of physical disturbance can enable assessment of cue modality (Table 7.1.2-3), while just slightly more refined methods may allow initial tests of discrimination among certain kinds of mechanosensory stimuli (Warkentin 2005; Mukai et al. 2014). Relatively imperfect playbacks may be most useful with animals that use highly variable incidental cues from predators, or other natural sources, to make important behavioral decisions, and therefore need robust mechanisms—insensitive to both such natural variation and to certain limitations on playback fidelity—to identify and/or discriminate among sources of vibration. For some biological questions, investigators need to develop methods to present stimuli that are within identifiable ranges of natural stimulus variation and adequately represent differences between biologically meaningful stimulus categories. This performance requirement may be substantially lower than the level of playback fidelity required to understand how animals respond to small variations in highly stereotyped communication signals. Incidental vibrational cues are likely to be broadly important in animal behavior including, but not limited to, embryo self-defense. The paucity of information about such cues, as well as the system-specific challenges of developing playback methods for work with vibrational and other physical-disturbance cues, suggests the use of simple "good enough" methods to ask the first, most basic questions, before investing in improvements to playback fidelity for study systems that prove interesting and tractable. An appreciation of the diversity of methods that may be sufficient to address certain kinds of initial questions could encourage an expansion of exploratory biotremological research into a broader range of organismal systems as well as the publication of biologically interesting results on new study systems. As suggested for acoustic playback experiments (Wiley 2003), we can make substantial progress using appropriate controls, interpretation, and reporting of what might be less-than-ideal experiments.

7.3.3 Constraints of Tine-Based Playbacks for Studying Mechanosensory-Cued Hatching

While our tine-based playbacks enable us to rear and test embryos in their natural clutch environment, they have limitations. First, tine playbacks expose embryos to somewhat variable, uncontrolled, and inseparable combinations of motion and tactile cues; i.e., tines move eggs and directly push some of them, and eggs may rub against each other as the clutch is shaken (see Fig. 7.1). This combination of motion and tactile cues also occurs naturally in predator attacks. However, it is not clear from tine playbacks to what extent the embryos' hatching response depends on each mechanosensory modality, or if this varies with stimulus properties or embryo development. Second, both the visual barrier of the MCI between eggs and observer and the complex structure and sheer number of eggs in natural clutches (on average 40, but often as high as 80) limits our ability to keep track of individuals or make behavioral observations *in ovo* during playbacks (Fig. 7.1e).

Third, the inevitable motion and tactile stimulation that occurs when setting up clutches for tine playbacks can induce hatching. We established an acclimation period (5 min after any setup-induced hatching) and a criterion (<25% hatched in setup) to determine if a clutch is useable for playback. As embryos develop, more clutches fail the criterion, and well before the peak of spontaneous hatching it becomes impossible for even skilled *A. callidryas* biotremologists to set up clutches for playback. Therefore, we focused our tine-based playbacks on a fairly narrow subset of *A. callidryas* embryonic development at age 5 days, about mid-way through their period of hatching competence. During this period, about 1–2 days before the peak of spontaneous hatching, embryos are highly responsive to some physical disturbances, and on average 80% escape in snake attacks (Warkentin 1995; Gomez-Mestre and Warkentin 2007; Gomez-Mestre et al. 2008). They are also highly discriminating. We were able to elicit a broad range of hatching responses, from ca. 0–100%, in playbacks and to measure discrimination among stimuli using relatively low numbers of egg clutches per stimulus—usually 8–13 but sometimes as low as five—so that experiments could be completed in reasonable time periods (Warkentin et al. 2006; Caldwell et al. 2010b). The lower hatching rates of less-developed embryos make larger sample sizes necessary to distinguish responses to different stimuli, while more-developed embryos simply hatch in setup before we can present any controlled stimulus. This obvious and inescapable developmental variation raised intriguing questions about ontogenetic changes in embryo responses to mechanosensory cues, while also making it impossible to answer those questions using tine-based playbacks.

7.3.4 Developing a New System for Playbacks to Agalychnis callidryas *Eggs*

7.3.4.1 Design Concept and Requirements

In 2012, Warkentin and McDaniel decided to develop a new playback system for *A. callidryas* embryos to ask questions that are intractable with tine-based playbacks. Our idea was to raise groups of eggs, from before the onset of mechanosensory-cued hatching (Warkentin et al. 2017), in a standardized, replicable physical structure that we could attach to a shaker at the desired age for playbacks without having to touch individual eggs. We also wanted to separate, and independently control, motion and tactile cues as well as improve our ability to observe and measure the behavior of individual embryos.

Thus, for motion-only playbacks we needed a structure to hold eggs, a method to keep embryos healthy and developing normally in it until tested, and a structure and method to rapidly, smoothly, and robustly couple the egg-holders to a shaker for playbacks. For a second stage, we needed a device to touch eggs, also coupled to a shaker, and a way to combine the motion and tactile playback technologies for bimodal playbacks. Our design constraints included the biology and mechanics of *A. callidryas* eggs, the performance of our shakers, and the capabilities of our human operators. Overall, we needed to meet seven performance requirements for our new egg-rearing and playback system.

1. Support normal, healthy embryo development with logistically feasible levels of manual maintenance (i.e., egg checks a few times per day)
2. Allow embryos to hatch normally and fall into a pool of water both during pre-testing development/maintenance and during playback trials

3. Enable attachment of the egg-holding structure to a shaker for playback while minimizing the hatching induced by setup, across a broad range of developmental stages
4. Maintain the coupling of eggs to their egg-holding-structure at reasonably high playback amplitudes across the relevant frequency range (0–500 Hz)
5. Enable playback of motion cues to eggs with adequate reliability and control for experimental goals
6. Enable addition of a second component to present tactile cues, with or without concurrent motion cues
7. Be operable by reasonably careful undergraduate interns, after a short period of training

7.3.4.2 Iterations Toward Solutions: Solve a Problem, Reveal Another Problem

Our methods development process involved iterative periods of device design and construction at Boston University, working with the Engineering Products Innovation Center (EPIC), alternating with field seasons at the Smithsonian Tropical Research Institute, in Gamboa, Panama, where we tested devices with eggs and could perform certain, limited, kinds of device modifications. We considered using hydrated jelly bases to mimic clutches but decided to try plastic in hopes of easier maintenance and greater standardization.

The first versions of egg-holders, "egg boxes," were rectangular blocks of acrylonitrile butadiene styrene plastic (ABS), with shallow cylindrical holes drilled to hold eggs. We laid them flat to place eggs in holes, first placing a drop of water in each hole. As eggs absorbed the water, they would lodge into the hole and adhere, while still partially protruding. Then we could stand the box vertically and eggs would remain mounted (Fig. 7.2). In this position, we could spray eggs-in-boxes to maintain hydration and excess water would run off; this naturally occurs when egg clutches are hanging from leaves, which we have long mimicked in the laboratory (Warkentin 2002, 2005). To present motion cues, we hung a minishaker upside-down from a wooden frame, as with tine playbacks (Fig. 7.1a). However, instead of an MCI with tines, we attached a rod with a coupling to attach to the top of each box of eggs. This enabled eggs to be moved up and down, and embryos to hatch toward the observer, then fall into a container of water below.

We tested many variations of hole depth and diameter for holding eggs (Fig. 7.2a). It was surprisingly hard for hatched tadpoles to get out of the holes, so embryos that hatched before testing could become trapped and die unless eggs-in-boxes were checked multiple times daily. Making holes shallower reduced this problem, but if holes were too shallow eggs would fall out. Determining the precise dimensions of holes that could work, and the size range of eggs that functioned with those hole dimensions, required multiple steps of trial and error. These egg boxes constrained us to work within a narrow range of initial egg sizes and to limit changes in size once eggs were in boxes. However, we could achieve that reasonably well

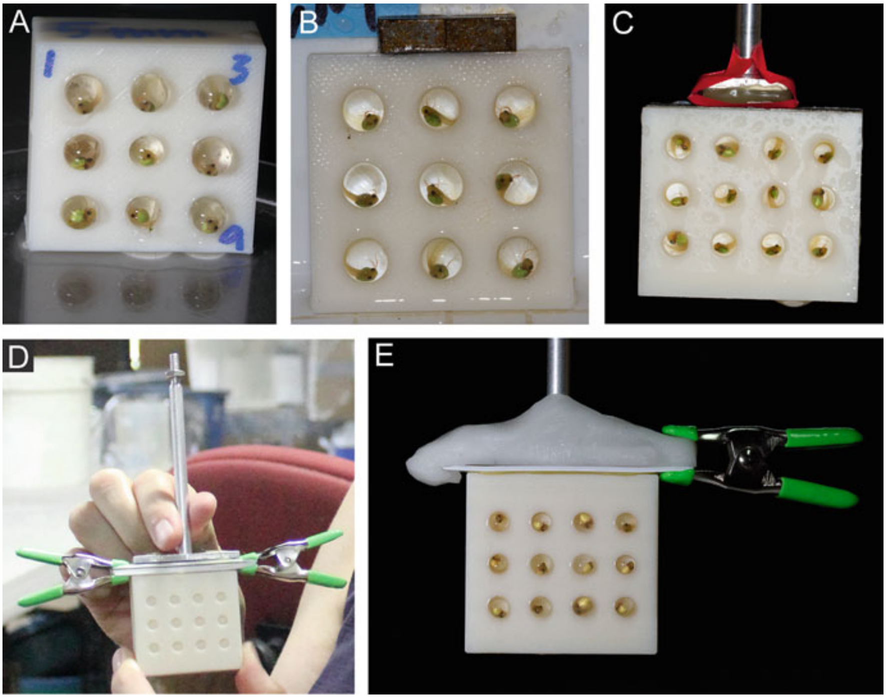


Fig. 7.2 Examples of iterative modifications of vertically oriented egg-holding devices (egg boxes) and systems to attach them to a minishaker. (**a**) We began testing plastic blocks for holding eggs in cylindrical holes of different depths and diameters, then (**b**) added metal for a magnet-based connection and (**c**) tried variants of magnetic coupling strength and padding to make the connection process smoother. (**d**) In the field, we modified a 2-screw connection system into a 2-clamp system, then (**e**) a 1-clamp system, which enabled playbacks that revealed the limits of egg-to-plastic adhesion

with careful hydration of clutches before transferring eggs to boxes and sufficiently frequent checks of eggs in boxes.

Our initial versions of coupling systems were magnetic (Fig. 7.2b, c). We glued a piece of metal to the top of each egg-box and attached a magnet to the end of the minishaker interface rod. We tried two variants of metal pieces on the boxes, multiple methods of softening or damping the vibrational "clunk" of attachment with intervening materials, and multiple methods of handling the box during attachment to limit vibration. If the magnet-metal coupling was too weak, it would rattle during playback. If it was too strong, it was not possible to avoid a hatching-inducing vibration during attachment. After failing repeatedly to simultaneously solve both problems, we gave up on magnets.

The next version of coupling, tested in 2014, was screw-based. We affixed a flat aluminum plate to the end of the rod, with a hole on each side. The matching egg-boxes each had two small plastic screws protruding from the top, and we used plastic nuts to attach the tray to the shaker interface. This gave a vibrationally robust coupling, with no rattling, but attaching a box of eggs for playback took much longer than with the magnet-based coupling. Even though the vibrations created by screwing on the nuts were substantially weaker than the clunk of (sufficiently strong) magnetic couplings, they elicited unacceptably high levels of hatching.

We then modified our shaker interface, in the field, to create a third, clamp-based type of coupling. We glued a rectangular plastic plate, the same depth as our egg-boxes but wider, to the aluminum plate at the end of the rod, and glued matching plastic plates to the top of the egg-boxes. Then, to attach an egg-box to the shaker interface, we simply aligned the two plastic plates and clamped their protruding flanges together, using rubber-tipped spring clamps positioned on either side (Fig. 7.2d). This gave a robust coupling, with no rattling during playback. It also enabled substantially smoother setup, and less setup-induced hatching, than either the magnetic or screw-based systems. However, it still took longer to attach the egg-box than we wanted, and induced more hatching. Our goal of testing eggs throughout their plastic hatching period, into the range of spontaneous hatching, would require further reducing and simplifying the motions involved in setup to minimize hatching-inducing vibrations.

The fourth kind of coupling we tested introduced asymmetry into the system (Fig. 7.2e). We kept the spring-clamped flanges on the left side but used ThermoMorph plastic (thermomorph.co.uk) to construct a slot for the flange on the right side. This asymmetry substantially simplified the motion involved in setup; we just had to slide the flange into its slot then clamp it on one side, which was easier, faster, and smoother than clamping both sides. Moreover, the mass of the flange-slot material protruding from one side reasonably balanced the spring clamp on the other, to minimize torque on the shaker. Again, the coupling was robust enough for rattle-free playback, and finally, the embryos tolerated the setup process well enough that we could expand our testing of the system, playing a broader range of test vibration files. At this point we learned that certain vibration frequencies within the range of interest, played at high but reasonable amplitudes, caused some eggs to oscillate such that they decoupled from their mounting hole, slid down, and fell from the box, potentially bumping other eggs in passing but without necessarily hatching in the process. Therefore, we needed to modify our egg-box design, again.

To construct an egg-holding device that would hold eggs more securely for playback, while still enabling the easy escape of hatchlings, in 2015 we designed "egg hotels" (Fig. 7.3). The basic structure was still a vertically positioned plastic block, but rather than blind-ended cylindrical pockets drilled into the face of the block, we designed angled cones that perforated the block to hold eggs (Fig. 7.3a, b). Thus, we enlisted gravity to help maintain eggs in position, and hatched tadpoles could slide down the cone to exit at the back. Rather than machine these more complex structures, we used a 3D printer to produce a set of prototype variants, with different cone dimensions and angles, for testing with eggs. We also modified our field-constructed asymmetrical attachment system into the first version of a cone-and-clamp coupling. We made an extension at the top-right of each egg-hotel with a horizontal hole through it, in the plane of the plate, that we filled with Thermo Morph to precisely match the end of an aluminum cone attached to the end of the shaker

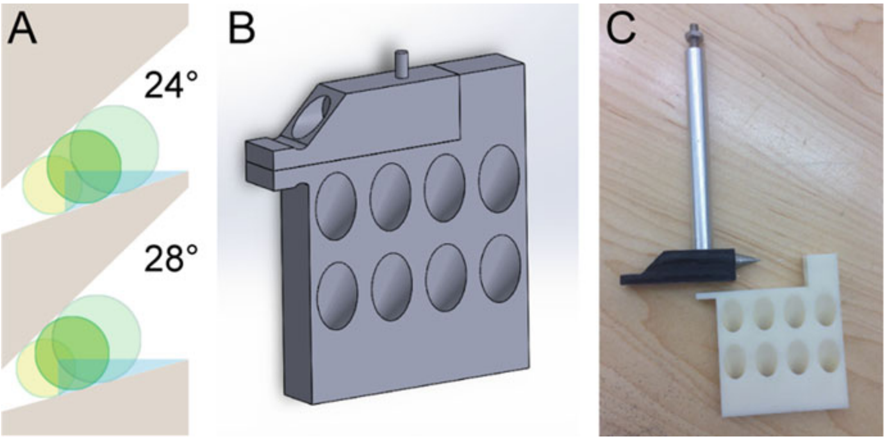


Fig. 7.3 (**a**, **b**) Design and (**c**) prototype egg-hotel to hold eggs in cones, shake them vertically, and allow hatchlings to slide out the back. We constructed and tested several variants with different cone dimensions and angles (schematic of two shown in **a**) but none worked well. The attachment system design (**b**, **c**) was the first field-tested iteration of a cone-and-clamp system, very similar to the ones we still use

interface rod (Fig. 7.3c). As a self-correcting paired structure, designed to yield a single-position end point across a range of starting points and attachment-motion variants, the cone made mounting the egg-hotel on the shaker-interface much easier and smoother, with greater tolerance for human error. As before, we clamped the flanges together with a rubber-tipped spring clamp. The flange part of the shaker-interface was part of a plastic housing surrounding the cone-ended aluminum cylinder. Given the interface asymmetry, an initial machined aluminum interface was too imbalanced; constructing part of it from plastic reduced the mass on the left side to adequately compensate for the left-positioned mass of the spring clamp.

Back in Panama for the 2015 field season, we rapidly learned two things. First, 3D-printed plastic wicks water out of frog eggs, presumably via micro-capillary action. Second, in attempting to keep eggs adequately hydrated, we found that eggs housed within the angled cones were also at risk of submergence and hypoxia, through water accumulating in the cone mouth. Even within the same egg-hotel, some individual eggs could dry out while others drowned. We briefly experimented with modifications of the cone shapes to avoid excessive water accumulation and lining cones with ThermoMorph to avoid the wicking effect. However, maintaining healthy embryo development in 3D-printed plastic was too difficult, and we soon abandoned the egg-hotels. Nonetheless, both cone-shaped spaces to accommodate different egg sizes and tunnels allowing hatchlings to exit at the rear of the egg-holding structure were useful design concepts. Therefore, we hand-drilled and carved some funnel-shaped holes into “blank” pieces of ABS plastic, cut to fit our cone-and-clamp shaker interface, as prototypes. While facilitating egg-rearing and hatching across a greater egg-size range, these devices did not solve the problem of eggs falling out of place when shaken.

On July 20, 2015, Greg McDaniel suggested we rotate the entire system 90°—turning the vertical egg-holding structures into horizontal "egg trays"—so that gravity would help keep eggs in their funnels. Although cantilevering an egg-tray on the end of a rod attached to a shaker seemed a potentially risky endeavor (for the shaker) we determined to support both the rod and tray using pieces of foam to avoid torque on the shaker's mounting platform, suspending the eggs over a small water bath between foam pieces. We found that this change solved the final problem that stood between us and new kinds of experiments in embryo behavior. After 3.5 years of methods development, fabricating 12 iterations of egg-holding devices and 6 iterations of attachment systems, making multiple minor modifications to devices, and testing usage variants in our effort to make different systems work, we had finally developed a playback system good enough to collect the behavioral data we wanted.

7.3.4.3 A Functional Tray-Based System for Motion Playbacks to Groups of Individual Eggs

Egg Tray Design and Manufacture The heart of the new playback system is the egg-tray, housing 15 *A. callidryas* eggs in individual funnel-shaped spaces in a grid of three rows of five eggs (Figs. 7.4, 7.5, and 7.6), first described in (Warkentin et al. 2019). Replicate trays were precision-cut from 12.7 mm thick sheets of ABS plastic, using a Sharp SV-2412 vertical machining center, then we added ThermoMorph plastic, hand-molded to fit the aluminum cone of the shaker attachment. Tray dimensions are given in Fig. 7.4 for 2016 trays ($N = 85$); 2015 trays ($N = 45$) were identical except for slightly larger 4.5 mm diameter tunnels. The tray design was produced as a Solidworks CAD file and processed with GibbsCam, a virtual machining CAM program, to produce the G-code to control the Sharp machining center at Boston University's Engineering Products Innovation Center (EPIC).

Dimensions machined by the SV-2412 are within 50 μm, but ABS sheet thickness varies more. We measured thickness and weighed a random subset of 20 trays from each set (2015, thickness: 12.63 ± 0.44 mm mean ± SD, mass: 51.20 ± 1.60 g; 2016, thickness 12.81 ± 0.22 mm, mass: 52.27 ± 0.89 g). Thickness explains most of the variance in mass ($r^2 = 0.98$) and the 2015 trays with the larger 4.5 mm tunnels are ca. 1 g lighter. To contextualize this variation, adding 15 eggs to a tray would add about 0.7–1.7 g, depending on egg size (4.5–6 mm); egg size varies somewhat due to maternal investment and more due to hydration. Then as eggs hatched, the mass would decrease accordingly. In addition, water droplets, which may be present on the tray surface if eggs were recently misted, could add about 0.6 g. Thus, replicate egg-trays for playbacks vary slightly in mass, in part due to variation among the trays and at least as much due to variation in the number and state of eggs. However, compared with the over five-fold variation in egg number among clutches used for tine playbacks (15–80 eggs) and the additional variation in clutch mass due to jelly

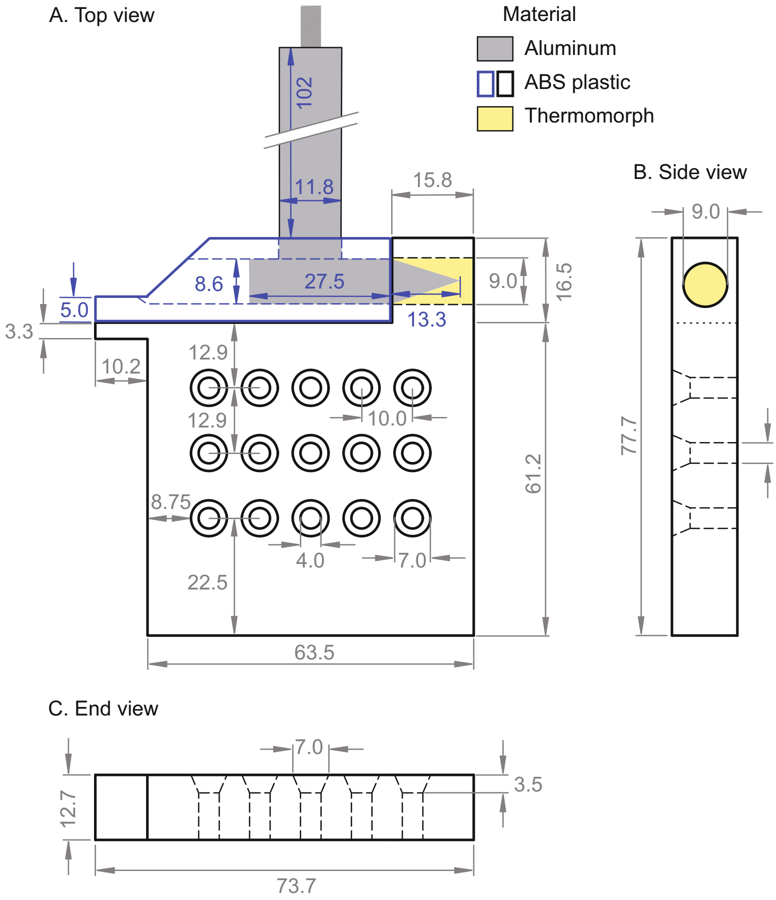


Fig. 7.4 Design schematic for egg-tray and minishaker–tray interface (MTI) for motion playbacks to *Agalychnis callidryas* embryos. The tray and plastic portion of the MTI are made of the same ABS plastic; diagram is color-coded to distinguish parts. All dimensions are in mm

volume and egg- and jelly-hydration level, egg-trays are substantially more uniform (compare Figs. 7.1b, c vs. 7.5c).

Maintaining Eggs in Trays Until Playbacks Developing an efficient and effective system for maintaining eggs in trays was critical for our goal of studying embryo responses to mechanosensory cues across a broad range of developmental stages, since many—perhaps all—forms of risk, stress, or suboptimality in the embryos' environment elicit hatching (Warkentin 1995, 2000, 2002; Warkentin et al. 2001; Warkentin and Caldwell 2009; Salica et al. 2012; Moskowitz et al. 2016; Vasquez et al. 2016; Tippett and Warkentin 2017; Snyder et al. 2018; Guevara Molina et al. 2020). Red-eyed treefrog egg clutches contain a mass of jelly that absorbs and stores water. As eggs develop, they move to the surface of the jelly, where they are exposed

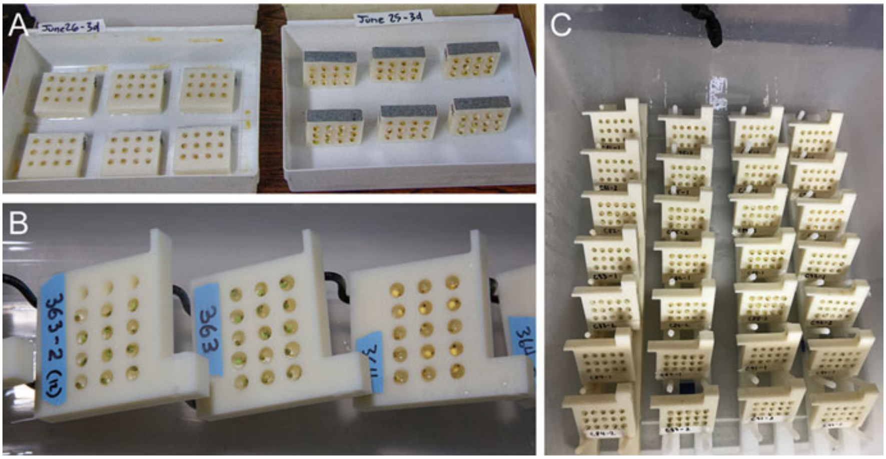


Fig. 7.5 Maintenance of *Agalychnis callidryas* embryos in egg-holding devices. (**a**) Early versions of egg-boxes were laid horizontally until eggs adhered, then maintained vertically in shallow trays of water and manually misted to maintain hydration. (**b**) Egg-trays on a manually constructed rack. (**c**) An egg-humidor housing 28 egg-trays on standardized racks; note nozzle of automatic misting system at top of image. Eggs are transferred to trays at age 3 days, before physical disturbance induces hatching, and develop normally in trays until playback

to the air; *A. callidryas* requires at least 15% air-exposed surface for normal embryo development (Warkentin et al. 2005). Eggs lose water through evaporation to the air but can absorb it from the jelly, buffering against dehydration. We keep eggs on their clutches as long as possible and transfer them to trays in the evening at age 3 days, shortly before the onset of mechanosensory-cued hatching (Warkentin et al. 2017). Once eggs are in trays, removed from the clutch jelly, they become more dependent on frequent external hydration. The vertical orientation of our initial egg-holding devices (egg-boxes) allowed excess water to run off when we sprayed eggs and did not require us to rotate box position when connecting them for playback (Fig. 7.5a). However, the horizontally oriented egg-tray playback system created a challenge in egg care.

Maintaining trays vertically required us to rotate them 90° for playback, which induced hatching. In addition, during the developmental period between egg-transfer and playback, eggs were more likely to slide out of funnels than out of blind-ended cylindrical holes. Placing the trays horizontally created two problems: hatchlings would get stuck in tunnels if trays were on the substrate, and even if they were elevated, water could pool in funnels with smaller eggs, reducing air-exposure and generating developmental variation. We solved these problems with racks. We constructed prototype racks by hand-bending plastic-covered wire and affixing it with ThermoMorph to bases weighted with glass marbles, to determine appropriate angles and spacing for trays (Fig. 7.5b). More standardized racks were then manufactured at EPIC from two 2-cm square ABS plastic rails, each 47 cm long, connected by 15 mm diameter plastic rods to hold the rails spaced about 2 cm apart.

Each rail was drilled at 6 cm intervals to support 8 pairs of plastic pegs, 6.7 mm in diameter, that protrude vertically from the rail to 4.4–5.9 cm; peg height varies among racks and pegs on the two rails are about 4 cm apart. Egg-trays set between peg-pairs are held at an angle of 40°–45° to vertical, allowing water to run off while eggs remain stable in their individual funnel positions. The racks also elevate trays 2 cm from the container bottom, which we flood with aged tap water to catch hatchlings. For playbacks, trays must be rotated to horizontal; however, this 45°–50° rotation induces less hatching than did rotating trays a full 90°.

Once we solved the problem of water pooling in egg-funnels, we wanted to mist eggs a lot—much more frequently than was convenient with the manual misting we had long used for intact egg clutches. We constructed a set of "egg humidors" from plastic storage bins (30 cm high, bottom dimensions 38 × 47.5 cm) with a section of the lid replaced with fine screen for ventilation. We fitted each humidor with a nozzle from a Mist King (www.mistking.com) automatic misting system, which we set to spray a fine mist of rainwater into the humidor on a predetermined schedule; about 20 s per hour is good under most conditions. These automatic humidors greatly facilitate the care of eggs in trays, and we now use them for clutches as well, to ensure excellent egg-hydration before traying eggs. Maintaining egg clutches, which we mount on plastic cards and stand upright in plastic cups (Warkentin 2002, 2005), in the automatic humidors also requires removing excess water from cups at least twice daily and changing clutch positions within the humidor periodically, as the spray is not uniform throughout the bin. Once eggs are in trays, we generally do not move them again until their playback. We attach the Mist King piping to support structures to minimize the transfer of vibrations to humidors, and use strategically placed foam to further damp vibrations from pumping water; however, it is not feasible to completely eliminate these vibrations under our field conditions. Therefore, we turn off the spray system during playback sessions. Undisturbed/spontaneous hatching in *A. callidryas*, in egg clutches and in trays, occurs gradually over a few days (Warkentin 1995; Warkentin et al. 2019); thus we expect a reduction in the number of embryos available for testing as they develop. For eggs to be tested late in development, turning off the misting in the afternoon at age 5 days seems to reduce the number that hatch that night, leaving more for testing at 6 days. In addition, the onset of darkness stimulates hatching (Güell and Warkentin 2018), so leaving lights on over the humidors can also reduce hatching.

One of the primary reasons we developed the egg-tray playback system was to facilitate research on developmental changes in behavior. In parallel with developing these methods, we also developed and tested a new, fine-scale developmental staging system for *A. callidryas* (Warkentin 2017), dividing the period of hatching competence into 14 stages. This system will be presented in detail elsewhere (Warkentin in preparation). After each playback, we record the developmental stage of a subset of animals from each tray, using this system. Based on over 2500 tray-reared individuals examined for developmental stage to date, and over 1400 measured, it is clear that embryos develop normally in trays (Fig. 7.6c, e). They also develop at the same rate as do their siblings on clutches. For 21 clutches with eggs in trays, we kept a subset of siblings on the clutch, also in the humidor system. After

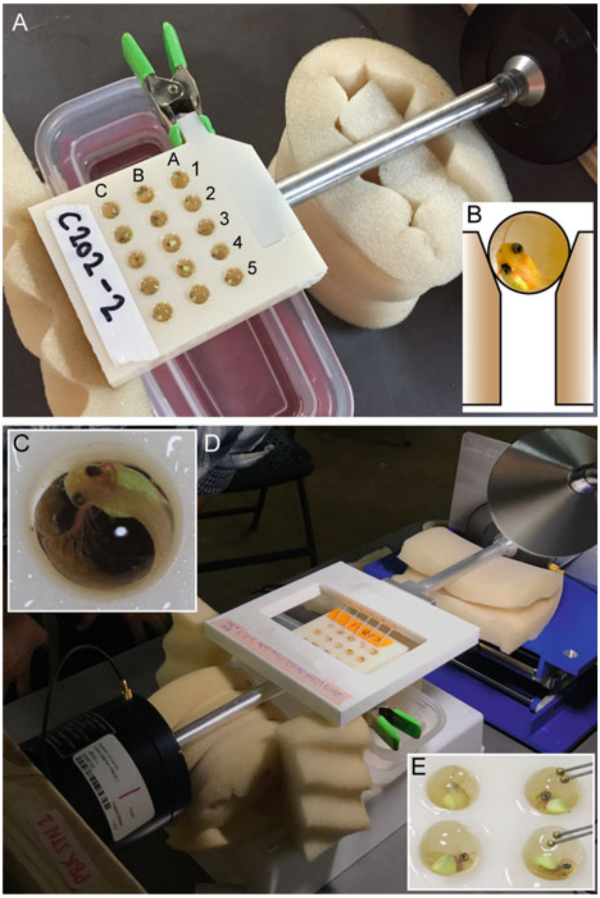


Fig. 7.6 Tray-based playback system for presenting motion and tactile cues to *Agalychnis callidryas* embryos. (**a**) Egg-tray mounted on minishaker–tray interface (MTI) for playback, showing supporting foam and water container to receive hatchlings. Egg positions are individually numbered by row and column, A1–C5, to facilitate data collection. (**b**) Each egg rests in a funnel-shaped space, and hatchlings can slide through to the water below; graphic is from Warkentin et al. (2019). (**c**) Embryos develop normally and their behavior is easily visible. (**d**) Tactile + motion playback setup, showing tactile playback device attached to minishaker to the right, mounted on a scissor jack to raise and lower the pin-heads that contact embryos. The MTI and egg-tray are attached to the shaker on the left, with all cantilevered components supported by foam. (**e**) Close-up of pin–egg contact. Tactile playback pins (right) contact eggs in rows **a** and **c**, leaving row **b** as non-contact controls

playbacks and post-playback testing for hatching-competence (Warkentin et al. 2019), we staged a subset of 3 hatchlings per tray and, at the same time, staged 2–3 (mean $N = 2.86$) of their siblings remaining on the clutch. In both individual trays and clutches, the mean stage range was 0.53 stages (range 0–2 stages). Stages were not significantly different between eggs in trays and their siblings on clutches (difference 0.17 ± 0.14 stages, mean ± SE; Wilcoxon signed rank test, $P = 0.11$).

Connecting Egg-Trays for Playback: The Minishaker–Tray Interface The minishaker–tray interface (MTI) is a critical element of our playback system (schematic with dimensions Fig. 7.4, photograph Fig. 7.6a). Each MTI was constructed from an aluminum alloy 6061-t6 cylinder, with one end shaped into a cone that fits precisely into the cone-seat extension on the trays because it was used to hand-mold the ThermoMorph component. The cylinder is enclosed in an ABS plastic housing matched on two sides to the connecting tray surfaces, with the exposed aluminum cone to the right. A 105 mm long aluminum rod, fitted at both ends with screw connections, is attached to the cylinder through a close-fitting hole in the housing, enabling the MTI to be attached to a minishaker. For playbacks, we simply slide an egg-tray onto the MTI cone and clamp the two flanges together with a rubber-tipped spring clamp (51 mm long, 8.66 g). The matching shapes of cone and cone-seat ensure that, wherever the cone-tip enters its seat within the 9 mm diameter entrance hole, the tray will rapidly and smoothly reach the same final position. This ensures a robust and standardized coupling of trays to the MTI, while minimizing hatching-inducing vibrations during setup.

The MTIs we now use (shown in Figs. 7.4 and 7.6) are the fourth variant of the cone-and-clamp system. The first, machined entirely from aluminum, was immediately rejected without field-testing due to mass imbalance. The second used a press-fit plastic housing (Fig. 7.3c) that cracked at the shaft connection after 3 months. We hand-molded a third housing from ThermoMorph, in the field in Panama, which functioned well (see Warkentin et al. 2019, Fig. 7.2, for a photograph). We subsequently slightly modified the design of the machined plastic housing to strengthen the side with the shaft connection and reduce strain; these have now been in use for over 3 years.

The asymmetric design of our MTI-tray system greatly facilitates our ability to do playback experiments with embryos late in their plastic hatching period, well into the range of spontaneous hatching. However, it also created a balancing challenge. The MTI shaft, connecting to the shaker, is centered in the tray, aligned with the middle column of eggs. The spring clamp on the flanges is a left-positioned mass, imposing a torque that would tend to unscrew the shaft from the shaker. To compensate for this, both the trays and MTI have greater mass to the right (Figs. 7.4 and 7.6). To assess the balance (net torque) of the MTI-tray system, we supported the rod at two points using custom-fitted smooth plastic rings, within which it rotated easily. We supported the center of eggless trays with a vertically positioned thin metal plate, aligned with the rod under the center row of egg-funnels. Supported thus, trays immediately tip down to the left. By placing a series of small masses at measured distances to the right of the center, we determined the net torque to be 0.0014 Nm. For context, this is over two orders of magnitude less than the 0.23 Nm minimum locking torque of the 10–32 Helicoil inserts in the minishaker platforms, to which the MTI attaches; i.e., this imbalance does not unscrew the MTI. Moreover, to additionally counter the small torque from the slight lateral imbalance in the MTI-tray system, we support the full width of the free edge of the tray with a rectangular block of open-cell foam (Fig. 7.6a, d).

For tray playbacks, we mount minishakers (4810 or LDS V203, Brüel & Kjær, Nærum, Denmark) horizontally (Fig. 7.6a, d). We constructed stands from 2 × 4″ lumber, with a vertical surface drilled for mounting shakers. We place the shaker stand on a rubber mat and weight it with a cement block for stability. The B & K 4810 shakers have a single central mounting hole in the base. Thus, in setting up for a playback session, we can mount a shaker on its stand, attach the MTI, attach an empty tray to the MTI, and level the tray by rotating the shaker around its attachment point. The shaker performance appears to be similar at different angles. The LDS V203 has two attachment points on the sides to connect it to a trunnion, on which it can be tipped to horizontal in a single plane. It also has three mounting holes in the base, which we used to mount it on our wooden shaker stand. Maintaining its alignment, when rotated, as if it were on the trunnion is critical for normal performance of this shaker in non-upright positions. Thus, to level the MTI for egg-tray mounting and playback requires fine adjustments to the exposed length of the MTI shaft's attachment screw using washers and nuts.

To avoid torque on horizontally mounted shakers, from the cantilevered mass of MTI and egg-tray, we position a piece of open cell foam under the MTI shaft and, when a tray is connected, place a second piece of foam under the free end of the tray. The center of the tray, holding eggs, is suspended over a container of water to catch any tadpoles that hatch. Foam pieces are sized such that the restoring force from very slight compression of the foam opposes the weight of the MTI and tray, supporting the system in a neutral position without impeding its horizontal motion (Fig. 7.6a). We use bubble levels to check the setup with empty trays each session, prior to conducting playbacks with eggs.

7.3.4.4 A Tactile Playback Device for Direct-Contact Cues

Once the basic egg-tray, egg-maintenance, and MTI connection system met our performance requirements for asking behavioral questions (Warkentin et al. 2019), we designed a second interface to present tactile (direct contact/rubbing) cues to eggs in trays. Our goal was to do playbacks in two mechanosensory modalities, separately or in combination. Developing this device also required a few iterations; here we present only the functional version.

The tactile interface was constructed of a rectangular ABS plastic frame attached to an aluminum alloy 6061-t6 shaft, threaded for connection to a minishaker (photo, Fig. 7.6d; schematic with dimensions, Fig. 7.7). Two interior edges of the frame were cut at an angle and drilled with 5 small holes spaced at 1 cm to match the egg funnels in trays. Into each hole, we glued a pair of stainless steel insect pins (0.4 mm diameter, 37 mm long, Bioquip 1208S1) with their rounded nylon heads aligned to contact the eggs in rows A and C of trays (Figs. 7.6d, e and 7.7). To smoothly lower the pin-heads onto eggs for playback, we use an 8″ square aluminum scissor jack (P/N 3588–3, New Star Environmental, Roswell, GA). The 7.6 cm base of a custom-

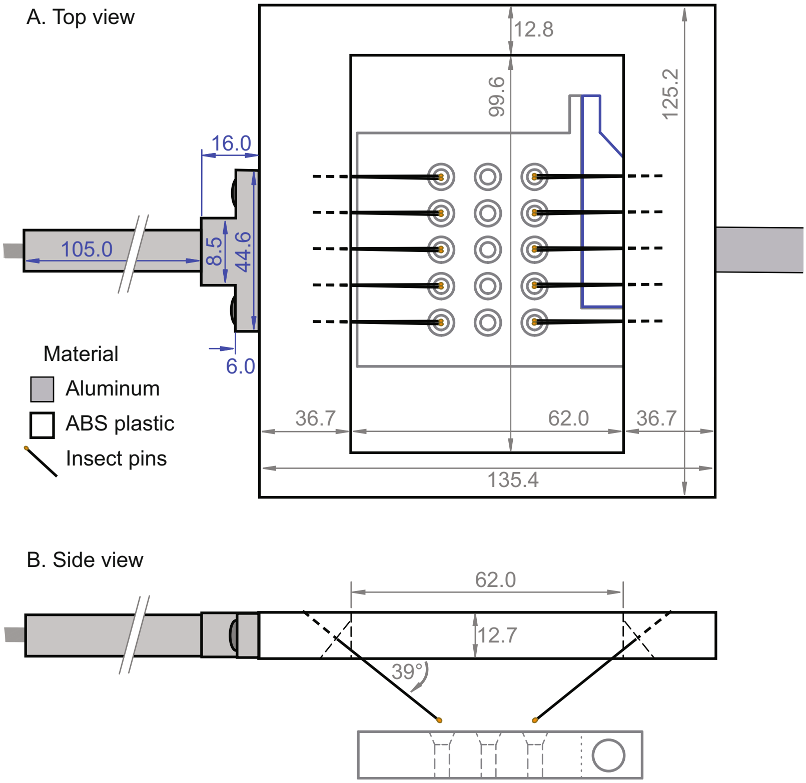


Fig. 7.7 Design schematic for tactile + motion system for playbacks to *Agalychnis callidryas* embryos. The tactile interface holds a set of insect pins, whose heads can be lowered onto eggs in the outer rows of a tray positioned below it. Shaking the pin-frame presents a tactile stimulus without moving eggs. Shaking the tray, with pins in contact, presents both motion and tactile stimuli. Raising the pin-frame disconnects it from eggs, enabling presentation of motion-only stimuli. All dimensions are in mm

milled aluminum L-bracket is bolted onto the platform of the scissor jack; its 10 cm vertical flange has a hole centrally positioned at 4.5 cm above the platform to attach a B & K 4810 shaker (Fig 7.6d). The scissor jack platform extends 4.5 cm beyond the shaker, accommodating the foam we use to support the shaft of the tactile interface and level it in line with the axis of shaker motion. We level the tactile interface at right angles to the axis of motion by rotating the shaker around its attachment point. For tactile playbacks, we lower the frame so that the pin-heads contact as many eggs as possible (up to 10), adding two additional pieces of foam to support the free end of the frame (Fig. 7.6d). Due to egg size variation, even with both tray and pin-frame perfectly leveled, it is not always possible to position pins to contact all eggs in rows A and C without pressing excessively on the largest, highest positioned eggs. Thus, in all playbacks, we record which eggs are and are not exposed to tactile contact. By design, eggs in the middle row (B1–B5) are always non-contact, serving as an internal control within each tray.

7.3.4.5 Activating the Playback Systems

We activate the shakers by playing vibration files in Audacity (www.audacityteam.org) on Macbook laptop computers via external sound cards (MSE-U33HB, Onkyo, Japan or, more recently, Focusrite Scarlett 2i2, focusriteplc.com) and custom-designed amplifiers (E. Hazen, Boston University Electronics Design Facility). To compensate for frequency filtering in the playback system, we construct transfer functions by recording playbacks of a frequency series of sine waves, using accelerometers mounted on trays and, for tactile playbacks, on the pin frame. We use the inverse of these functions to produce draft stimulus files using a custom MatLab script (Caldwell et al. 2009). Then we play and record these draft stimuli, making additional iterative adjustments as needed to achieve desired output. We have used both 0.14 g AP19 and 2.0 g AP32 accelerometers (AP Technology International, Oosterhout, The Netherlands), with APC7, B & K 1704, and PCB 480E09 (PCB Piezoelectronics, Depew, NY) signal conditioners. The B & K 1704 and PCB 480E09 also require charge-to-voltage converters (AP5000/10 or PCB 422E51). We are not equipped, and have not attempted, to record directly from the pin-heads or from test embryos in playbacks.

7.3.4.6 Rainforest Accelerometry and Limits to Measurement Precision

We work in a tropical rainforest environment, often in over 90% humidity, using equipment that is not rated to function under these conditions. We began by recording vibrations from inside gelatinous egg clutches, outside in the rain and during snake attacks (Warkentin 2005), then added recordings from clutches during playbacks and modal testing (Caldwell et al. 2009, 2010a; Caldwell 2010). To avoid hatching during setup, we insert accelerometers into clutches at age 3 days, before the onset of mechanosensory-cued hatching (Warkentin et al. 2017), and need them to function for recordings up to 2 days later. Therefore, we pot our miniature accelerometers in a thin layer of flowable silicone to seal out water. This has largely maintained their functionality under our testing conditions, with occasional failure solved by desiccation and re-sealing.

Our miniature accelerometers are monoaxial, with little transverse sensitivity; AP Technology reports <5% for the AP19 and < 3% for the AP32. Thus, the accuracy of amplitude measurement depends on axis alignment with the direction of motion, and misalignment results in lower measured amplitudes. For in-clutch measurements, using the smallest available accelerometers, we have attempted to align the axis of maximum sensitivity roughly with the vertical (usually longest) axis of the clutch; however, both eggs and accelerometers sometimes shift position between mounting and testing. Moreover, while rain often falls down, it also blows at other angles, and snakes attack clutches from all directions. Our recordings of these natural disturbances, therefore, do not necessarily capture maximum accelerations. Nonetheless, it is clear that both snake and rain vibrations vary substantially in amplitude, and this variation does not impair embryos' ability to distinguish between them (Warkentin 1995, 2005; Warkentin and Caldwell 2009; Caldwell 2010).

To construct transfer functions and set playback levels for tine-playbacks, we recorded from accelerometers (AP19 or Endevco 25B) embedded in clutches, removing two of the MCI tines to accommodate accelerometer placement among eggs without direct tine contact (Caldwell et al. 2009). We have also recorded from the MCI frame, with the tines in a clutch. To attach a potted accelerometer to the MCI, we encase it in a small piece of plasticine and stick that to the acrylic columns holding the tines, as accelerometer wax does not adhere well to the silicone potting material, aligning the axis of sensitivity with the vertical axis of playback motion. This means the side of the accelerometer, not its base, is against the acrylic, with intervening thin layers of silicone and plasticine. This makes axis alignment less precise than it would be if we were attaching the flat base of a naked accelerometer to a flat surface. In practice, we find that measured amplitudes vary somewhat if we disconnect and re-mount the accelerometer, indicating imperfections in alignment (see Figs. 7.8 and 7.10). Thus, we can achieve greater precision of amplitude matches among stimuli within experiments (with all levels set or measured without

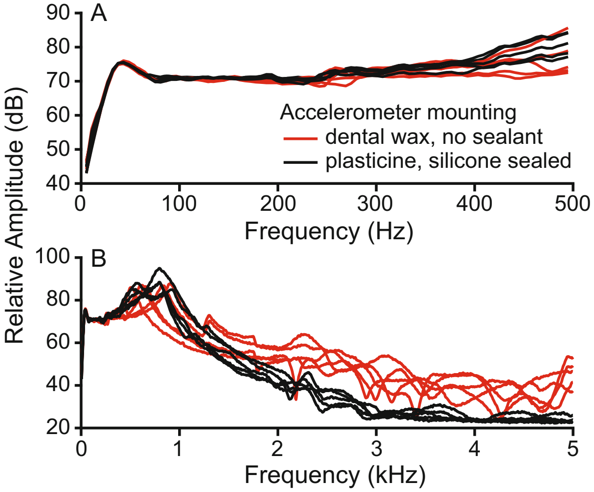


Fig. 7.8 Effect of accelerometer mounting method on recorded frequencies. Spectra are from recordings using the same AP32 accelerometer, attached in the same position on the same egg tray, playing uncompensated white noise to show the transfer function (non-linear frequency response) of the egg-tray playback system. (**a**) Low frequencies, relevant to hatching decisions of *Agalychnis callidryas*, show little variation among replicate accelerometer attachments ($N = 10$) or across attachment methods (line colors). (**b**) Higher frequencies show more variation among replicate attachments and lower representation of very high frequencies using the silicone-sealed, plasticine-mounted accelerometer

re-mounting the accelerometer) than we can for absolute amplitude measurements. Surprisingly, although our ability to align accelerometers for in-clutch measurements is worse, those measurements appear to be somewhat less sensitive to alignment imperfections, perhaps because clutch motion is three-dimensional even when force is applied in a single dimension.

In our testing, accelerometer calibrations (using B & K type 4294 or Wilcoxon Model REF2500 calibrator exciters) do not differ for unsealed vs. silicone-sealed accelerometers, or with wax vs. plasticine mounting; although, the variance among measurements of the same calibration excitation is higher with sealed, plasticine-mounted accelerometers than with the same accelerometer wax-mounted. For example, calibration recordings for one AP32 accelerometer ($N = 10$ wax-mounted, 11 plasticine-mounted recordings) showed greater variance with plasticine mounting (Levene's test, $F_{1,19} = 4.795$, $P = 0.04$) but no difference in mean values (Welch's test, $F_{1,14.5} = 0.326$, $P = 0.58$).

In our work with red-eyed treefrog embryos, we are most concerned with frequencies below 500 Hz. Frequencies below about 200 Hz can induce hatching, and those below 100 Hz strongly stimulate hatching, while higher frequencies within this range reduce the hatching response to concurrently presented low frequencies (Caldwell et al. 2009, 2010a). To assess the effect of our non-standard accelerometer mounting methods, we recorded playbacks of synthetic white noise (uncorrected for equipment transfer functions) from a single egg tray, using the same AP32 accelerometer attached by its base to the end of the tray opposite the shaker, in alignment with the central axis of the tray, either with a thin layer of dental wax or using plasticine, after silicone potting. The frequency response was consistent across repeated attachments, using the same or different methods, below about 200 Hz. Above 250 Hz, the response varied with re-attachment and above 1.5–2 kHz the silicone-sealed, plasticine-mounted accelerometer recorded lower amplitudes than it had when wax-mounted prior to potting (Fig. 7.8). We suspect that potting and plasticine-mounting may reduce the transfer of these high frequencies; although, it is also possible that the added mass of mounting material altered the transfer function of the playback system at these frequencies. Assuming the former, it is possible that our accelerometer recordings from egg clutches also underrepresent high frequencies (e.g., in rain); however, playbacks of synthetic high-frequency stimuli, even at relatively high amplitudes, do not elicit hatching (Caldwell et al. 2009).

7.3.4.7 Egg-Tray Playback System Performance

The egg-tray playback system enables us to do playback experiments at previously intractable developmental stages, facilitates behavioral observations of individual embryos, and enables us to present motion cues alone, without tactile cues. It also increases our control of motion stimuli presented to individual eggs, and their consistency across replicate playbacks, given the greater structural consistency among trays of eggs than among clutches. This is evident in the time domain (Fig. 7.9), as there is minimal ringdown of the egg-tray-MTI system after each

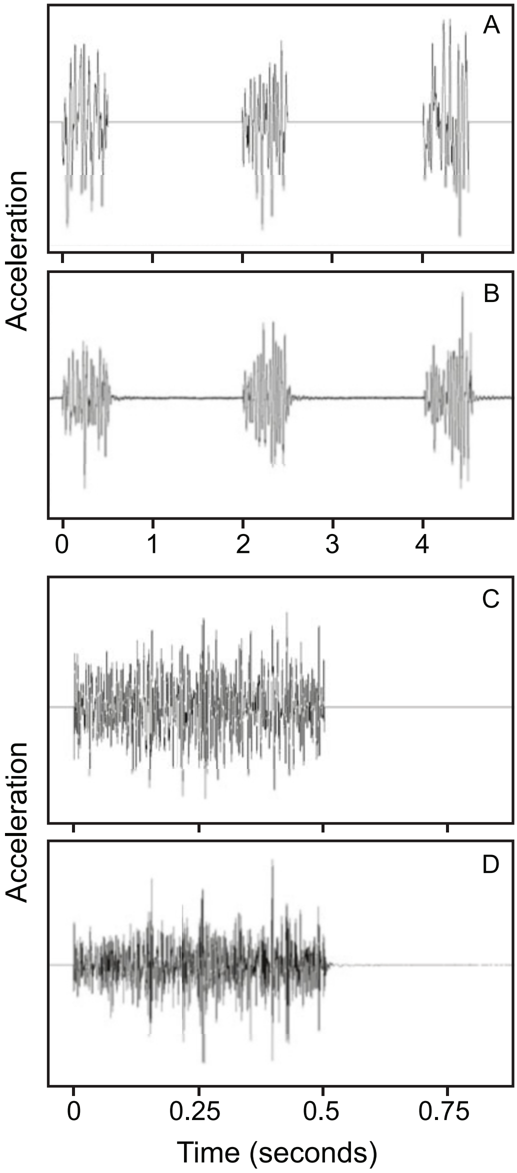


Fig. 7.9 Waveforms of example stimuli (**a**, **c**) and corresponding playbacks (**b**, **d**) recorded from a potted AP19 accelerometer mounted with plasticine in the B3 position of empty egg-trays. Stimuli were 0.5 s bursts of low frequency (**a**) or broad spectrum (**c**) noise. The egg-tray playback system has excellent time-domain representation of vibrational pulse patterns, which we know strongly affect the hatching response of *Agalychnis callidryas* embryos (Warkentin 2005; Warkentin et al. 2006). Figure is from supplementary material in Warkentin et al. (2019)

vibration pulse presented. We have not recorded vibrations directly from eggs, but highspeed video (see supplementary material in Warkentin et al. 2019) indicates that the coupling of eggs-to-funnels in the trays is excellent, so that eggs move along with their tray and do not jostle about in the funnel.

To assess the magnitude of variation in vibration that might occur due to the slight differences in mass among individual trays as well as the number and size of eggs

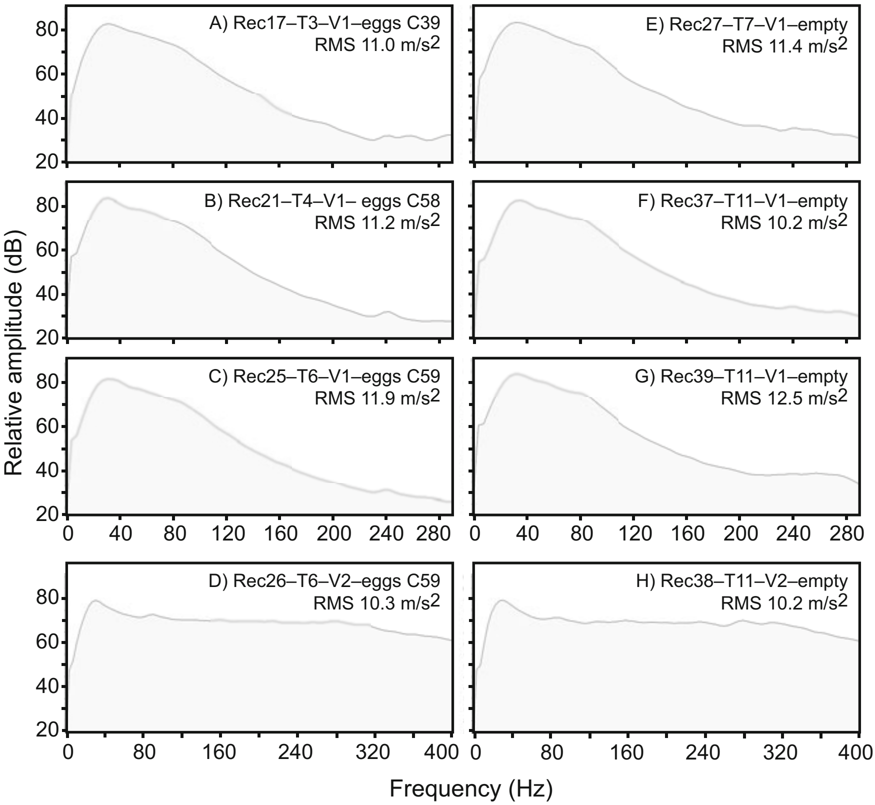
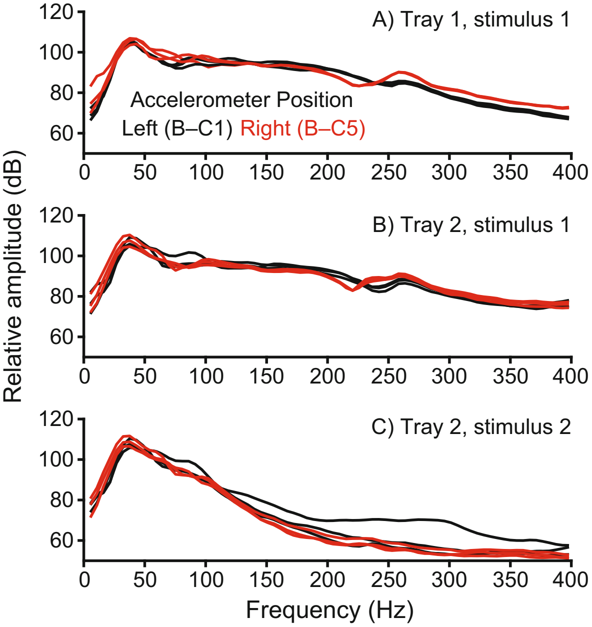


Fig. 7.10 Example frequency spectra and RMS amplitudes from playbacks of two test vibration files recorded from egg-trays. Vibrations were 30 s periods of continuous noise that differed in frequency spectra (V1 low, V2 broad). Each tray either contained 15 eggs (**a–d**) or was empty (**e–h**), with each full tray containing eggs from a different 4-day-old *Agalychnis callidryas* clutch (2018–C39, C58, C59). Vibrations were recorded using a silicone-potted AP19 accelerometer mounted on its side in the label zone of the tray (see Fig. 7.6) using plasticine. Spectra illustrate the level of consistency of frequency content across repeated presentation of the same vibration file via different trays, containing different numbers of eggs, and with slight changes in setup (e.g., adjusting foam position), as well as the repeatable differences between playbacks with different frequency content (note the two frequency scales). Amplitude variation among presentations using different trays (compare **c**, **f**, trays 6 and 11) was within the range of variation from simply remounting the accelerometer on the same tray (**f**, **g**, tray 11). Recordings 37 (**f**) and 38 (**h**) are of different stimuli played via the same tray, without remounting the accelerometer, showing the amplitude matching across stimuli. Recording 39 (**g**) was after remounting the accelerometer, showing the range of amplitude imprecision from variation in accelerometer axis alignment; note that measured frequency spectra also vary slightly with accelerometer remounting

within them and variations in foam placement, we recorded test stimuli played through different trays, either empty or filled with 15 eggs, moving and replacing the foam between recordings (Fig. 7.10). Measured amplitude and frequency

Fig. 7.11 Frequency spectra of vibrations recorded from left and right positions in egg trays, remounting an AP19 accelerometer three times in each position on the tray, without remounting the tray on the MTI or adjusting foam, for two different egg-trays (and foam placements) and two stimuli (S1: broader spectrum, and S2: narrower spectrum of low frequencies)



variation across trays and foam setups was within the range of variation due to simply remounting the accelerometer on the same tray.

To assess the extent to which the asymmetry in the MTI-tray system and/or asymmetric mass-loading of trays (e.g., if eggs were only present on one side) might generate systematic differences between tray motion at different egg positions, we recorded from AP19 accelerometers on the right and left sides of trays, plasticine-mounted between B1C1 and B5C5 egg positions. There is, as expected, some variation in both amplitude and frequency spectra simply from remounting the accelerometer in the same position, on the same tray (Figs. 7.8, 7.10, and 7.11). Recordings of the same test file do not differ in amplitude between right and left sides of the tray (Mixed model ANOVA with tray as a random effect, Position effect: $F_{1,14} = 0.02348$, $P = 0.64$; Fig. 7.11). At the low frequencies that are most important for *A. callidryas* escape-hatching response, we have found no evidence for systematic asymmetries in frequency representation. Figure 7.11a, b suggest some asymmetry at higher frequencies that could generate variation in the stimulus provided to different eggs within a tray. This within-stimulus frequency variation (i.e., playback infidelity) is much smaller than the frequency variation found within important classes of natural disturbance, such as rainstorms, and seems unlikely to be behaviorally relevant for *A. callidryas* embryos (Caldwell et al. 2009; Caldwell 2010). However, if we were interested in assessing embryo responses to small variations at these higher frequencies, further iterations of playback system refinement might be necessary.

Natural variation in vibrational information in incidental cues, due to structural variation among clutches and attachment substrates as well as variation in forcing, presumably selects for robustness in embryos' risk assessment strategies. Thus, with reasonable replication, similar variation in playback experiments should not impair our ability to detect differences in the response of embryos to different stimuli. Indeed, thus far it has not.

7.3.4.8 Bimodal Tactile + Motion Playback System Performance

Our fundamental goal in designing the tactile playback device was to enable us to independently control tactile and motion cues to eggs, presenting just motion cues via trays, just tactile cues via pins, or bimodal mechanosensory cues by lowering pins to contact eggs and activating tray motion. Of course, in tactile playbacks some vibrational energy does transfer into the tray via the pin–egg contact in rows A and C. To assess how much, we recorded amplitude-matched tactile and motion stimuli, presented from two B & K 4810 shakers (Fig. 7.6d), using a potted AP19 accelerometer plasticine-mounted in the non-contact zone (row B) of trays and on the pin-frame, during unimodal and bimodal playbacks. Amplitudes measured from directly shaken structures—trays in motion and bimodal playbacks and pin-frame in tactile playbacks—have been indistinguishable across the 3 stimulus types, and vibrations recorded from the non-contact zone of trays during tactile playbacks have been consistently much lower. Figure 7.12 shows levels from 3 replicate trays, holding eggs from different sibships, with 7–9 eggs in contact for tactile and bimodal stimuli. Direct shaking amplitudes were 15.0 ± 0.3 m/s^2 (mean ± SE; ANOVA, Stimulus $F_{2,4} = 0.009$, $P = 0.99$; Tray $F_{2,4} = 2.836$, $P = 0.17$) while indirect vibration amplitude was 0.6 ± 0.05 m/s^2 (direct vs. indirect: ANOVA, $F_{1,11} = 561$,

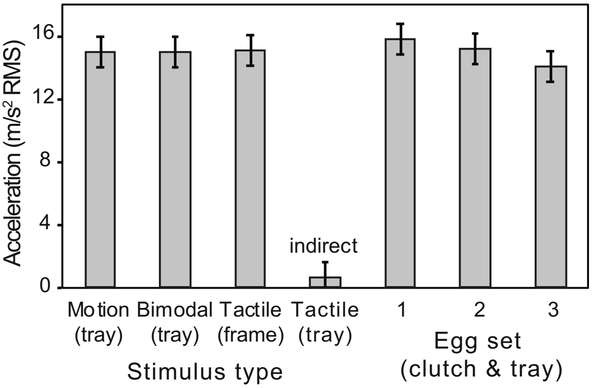


Fig. 7.12 Comparison of playback amplitude measured from non-contact zone of trays and from pin-frame in motion, bimodal, and tactile playbacks to three sets of eggs. Vibration amplitudes of directly shaken structures (tray, pin-frame) were indistinguishable across stimuli and replicate trays, while indirect vibration of trays via eggs in tactile playback was much lower. Data are mean ± SE

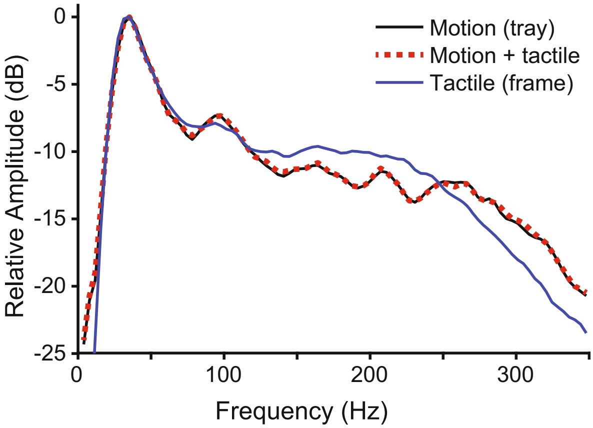


Fig. 7.13 Example frequency spectra from recordings of the same stimulus played by shaking a tray without pin contact (motion) and with pin contact (bimodal motion + tactile) and shaking the frame holding pins, in contact with 7 eggs (tactile). Pin contact has little or no effect on tray motion. The playback stimulus was designed to have a low-frequency peak and some energy through ca. 200 Hz, with decreasing energy at higher frequencies. Spectra from tray and pin-frame are well matched for the desired low-frequency peak and range through 100 Hz, but the high-frequency drop-off, of less relevance for inducing hatching, differs slightly

$P = 4.08\text{e-}10$). In frequency-domain comparisons of motion vs. bimodal playbacks, pin contact does not appear to measurably alter the frequency response of the system (Fig. 7.13). For the same playback stimulus, comparing motion and bimodal playbacks vs. tactile playbacks (Fig. 7.13) reveals an excellent match of the desired low-frequency peak and a less-perfect match of the higher-frequency drop-off. Depending on the needs of particular experiments, this could potentially be improved by further iterations of transfer function measurement and stimulus file design.

7.3.5 *Using Egg-Tray Playbacks to Study Developmental Changes in Embryo Behavior*

It was clear, from our inability to set up older clutches for tine playbacks, that embryos late in their plastic hatching period hatch readily in response to stimuli that rarely elicit hatching of younger, less developed embryos. But what causes this change in behavior? Might it simply reflect sensory system development? Is it an adaptive response to developmentally changing risk trade-offs? Do embryos become generally more responsive to all types of disturbance, or does the ontogenetic change vary with stimulus properties? The egg-tray playback system has enabled us to address these and other new questions, performing experiments that were simply not possible with the tine-based system.

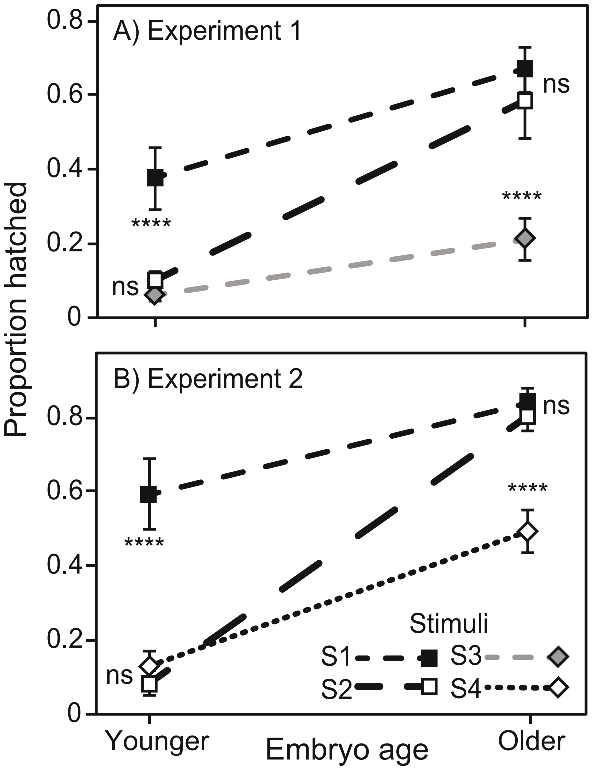


Fig. 7.14 The hatching response to egg-tray motion-only playbacks changes developmentally in a stimulus-dependent manner. We presented four stimuli representing three temporal patterns, indicated by dash dimensions (short, medium and long dashes indicate fast, medium, and slow patterns, respectively), and two frequency spectra, indicated by line color (black: low frequency; gray: higher, broad band). Younger embryos show little hatching response to a low-frequency, slow-tempo stimulus, requiring both medium-tempo and low-frequency properties to decide to hatch. Older embryos show a strong hatching response to both medium- and slow-tempo, low-frequency stimuli but, like younger embryos, hatch less in response to both a broad-band stimulus and a fast-tempo stimulus, in which the property indicative of low risk is rapidly evident. The data are mean proportion of eggs hatched ± SE across trays. Figure is from Warkentin et al. (2019)

Our first egg-tray playback experiments demonstrated that motion alone, without concurrent tactile cues, is sufficient to elicit a hatching response (Warkentin et al. 2019). They also revealed that information sampling and decision strategies change developmentally, in ways that are predictable from the developmentally decreasing cost of false alarms (as the chance of hatchling survival increases) and the consistently high cost of missing egg-predator cues (Fig. 7.14). Specifically, younger embryos gather more information before deciding to hatch, and use more cue properties as indicators of safety. Older embryos gather less information and stop using slow temporal patterns—which are costly to assess—as indicators of safety, but continue to avoid hatching if the benign nature of a vibrational cue is rapidly apparent (Warkentin et al. 2019). Subsequent experiments revealed that the response of embryos to ambiguous vibrational cues—neither clearly benign nor clearly indicative of risk—also changes developmentally. Younger embryos treat ambiguous cues as benign, erring toward missed cues, while older embryos treat ambiguous cues as threatening, erring toward false alarms (Jung et al. 2021). Experiments comparing embryo responses to tactile, motion, and bimodal playback stimuli indicated that younger embryos treat motion and tactile cues as non-redundant information sources; i.e., bimodal playbacks elicit more hatching than either unimodal stimulus (Fouilloux et al. 2019). In contrast, older embryos hatch at similar rates to motion, tactile, and bimodal cues, indicating functional redundancy (Fouilloux et al. 2019). All of these developmental changes in risk assessment and escape-hatching behavior are consistent with predictions based on the hypothesis of ontogenetic adaptation to changing trade-offs (Wiedenmayer 2009; Warkentin et al. 2019).

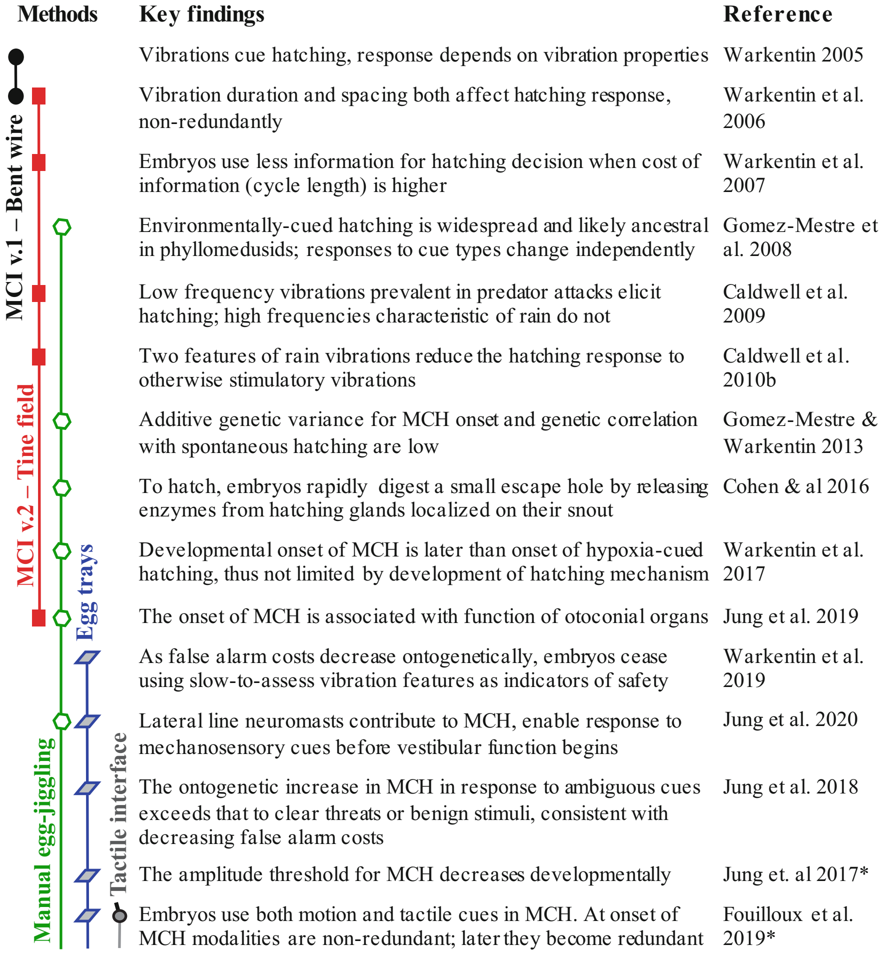
In addition to adaptive ontogenetic changes, it is clear that mechanosensory system development affects the hatching response to physical disturbance, including motion-only cues in playbacks and more complex mechanosensory cues in manual egg-jiggling (Warkentin et al. 2017; Jung et al. 2018, 2019, 2020; Jung 2021). The hatching response to hypoxia begins developmentally earlier than the response to egg-jiggling, and the existence of a period when hatching-competent embryos fail to flee from predators or respond to jiggling or playbacks suggests a sensory constraint (Warkentin et al. 2017). The onset of hatching in response to physical disturbance is strongly associated with a marker of vestibular function, the vestibulo-ocular reflex (VOR), supporting that the developing otoconial organs in the ears are important motion sensors mediating escape-hatching (Jung et al. 2019). In addition, after the onset of mechanosensory-cued hatching (MCH), the minimum stimulus amplitude that induces hatching in motion-only playbacks decreases substantially as embryo ears develop and grow (Jung et al. 2018; Jung 2021). However, otic mechanoreceptors cannot be the only sensors mediating MCH, as a weak response to egg-jiggling is present before the onset of VOR (Jung et al. 2019). Lateral line neuromasts are present before ears begin to function (Cohen et al. 2019) and also contribute to embryonic risk assessment (Jung et al. 2020). Experimentally blocking neuromast function substantially reduces the hatching response to jiggling cues both before and just after VOR onset, and slows the response to these cues later in development (Jung et al. 2020). In contrast, embryos' response to motion-only playbacks is unaffected by neuromast blocking, thus appears to be mediated by ears alone (Jung et al. 2020). Moreover, both the hatching responses of embryos to tactile cues without egg motion (Fouilloux et al. 2019) and the fact that a very small number of embryos lacking both otoconial organ and lateral line function hatched in response to egg-jiggling (Jung et al. 2020) suggest that a third mechanosensory system, cutaneous mechanoreceptors, can also contribute to MCH. Thus, it appears that red-eyed treefrogs facing the threat of egg predation may use all possible mechanosensory systems to inform their behavioral decisions.

7.4 Conclusions and Future Directions for Research on Mechanosensory-Cued Hatching

Hatching is a dramatic life history transition and, often, an active embryo behavior. From flatworms and insects to frogs and turtles, animal embryos use mechanosensory cues and signals to inform their hatching timing. These include a broad range of incidental cues, from egg motion and contact in direct attacks by egg-predators to vibrations transmitted through the clutch from hatching siblings, from vibrations generated by rainfall to disturbances caused by the motion of nearby hosts or tumbling in waves. Embryos also respond to parental vibrations that may be evolved, stereotyped signals. MCH offers excellent opportunities to advance our understanding of adaptive embryo behavior and, more broadly, of animal information use and behavioral decisions based on both incidental cues and stereotyped signals.

Our research with MCH in red-eyed treefrog embryos has revealed some of the complexity of information use underlying cued hatching behavior (Fig. 7.15). These embryos assess multiple frequency and temporal properties of cues, using multiple mechanosensory systems, for their hatching decision. The relative importance of different sensors changes developmentally, as does the way embryos combine information across modalities. In addition, changing risk trade-offs appear to have shaped ontogenetic changes in the decision rules embryos apply to the information they receive. A variety of experimental approaches have been productive with these embryos, from simple manual egg-jiggling to vibration playback experiments using recorded and synthetic stimuli presented via a series of different shaker–egg interfaces. These interfaces offer different levels of stimulus control, with greater investment in device design and testing required for higher levels of control. Thus, our research illustrates both the kinds of advances that can be made using relatively simple devices and low-fidelity playbacks and the greater breadth and complexity of questions that can be addressed after investing time and effort to improve playback systems (Fig. 7.15).

The constraints on playback optimization that pertain for studies of hatching can be very different from those encountered in most biotremology research. The eggs under study can themselves be major structural components of the physical system to be vibrated, in which case the behavior of interest—hatching—destroys the system; i.e., the testing is fundamentally destructive. This may impose limitations on the precise consistency of stimuli presented to different individuals, if variation among eggs or masses alters the response of the playback system. However, for the many species in which embryos use variable incidental cues, hatching decision rules are likely to be robust to some range of natural variation in cues. In such cases, the behavioral robustness may to some extent relax fidelity requirements for playbacks, enabling substantial research progress using stimuli that are simply "good enough" to represent relevant natural categories, for instance by using mean transfer functions. The evidence that, in many species, embryos hatch in response to manual disturbance and/or vibrations from lab mixers also suggests that precise replication



Key findings	Reference
Vibrations cue hatching, response depends on vibration properties	Warkentin 2005
Vibration duration and spacing both affect hatching response, non-redundantly	Warkentin et al. 2006
Embryos use less information for hatching decision when cost of information (cycle length) is higher	Warkentin et al. 2007
Environmentally-cued hatching is widespread and likely ancestral in phyllomedusids; responses to cue types change independently	Gomez-Mestre et al. 2008
Low frequency vibrations prevalent in predator attacks elicit hatching; high frequencies characteristic of rain do not	Caldwell et al. 2009
Two features of rain vibrations reduce the hatching response to otherwise stimulatory vibrations	Caldwell et al. 2010b
Additive genetic variance for MCH onset and genetic correlation with spontaneous hatching are low	Gomez-Mestre & Warkentin 2013
To hatch, embryos rapidly digest a small escape hole by releasing enzymes from hatching glands localized on their snout	Cohen & al 2016
Developmental onset of MCH is later than onset of hypoxia-cued hatching, thus not limited by development of hatching mechanism	Warkentin et al. 2017
The onset of MCH is associated with function of otoconial organs	Jung et al. 2019
As false alarm costs decrease ontogenetically, embryos cease using slow-to-assess vibration features as indicators of safety	Warkentin et al. 2019
Lateral line neuromasts contribute to MCH, enable response to mechanosensory cues before vestibular function begins	Jung et al. 2020
The ontogenetic increase in MCH in response to ambiguous cues exceeds that to clear threats or benign stimuli, consistent with decreasing false alarm costs	Jung et al. 2018
The amplitude threshold for MCH decreases developmentally	Jung et. al 2017*
Embryos use both motion and tactile cues in MCH. At onset of MCH modalities are non-redundant; later they become redundant	Fouilloux et al. 2019*

Fig. 7.15 Sequence of research on the mechanosensory-cued hatching (MCH) of *Agalychnis callidryas*, using five different methods to present cues to embryos, with key findings from each study. Symbols on lines indicate methods employed in each study; some investigations combined multiple cue-presentation methods. Asterisks indicate unpublished research that has been presented and is available in the form of conference abstracts or posters deposited in online repositories

of natural vibrational stimuli may be unnecessary for many initial investigations into MCH.

Given the number and breadth of species that exhibit MCH and the fact that, to our knowledge, the power of playback experiments has to date only been employed with embryos of three species (red-eyed treefrogs, stink bugs, and migratory locusts), there is substantial scope for advances in this field. In many cases, we know very little about the stimulus properties that are effective in eliciting hatching or the extent to which MCH is a general or specific response. This could be explored with relatively simple playbacks. We might expect response specificity to vary among species with the amount of variation in their natural hatching stimuli; for instance, hatching in response to parental vibrations might require very specific patterns, matching stereotyped signals, whereas responses to disturbance by potential hosts might be less specific. In addition, response specificity seems likely to vary with the background vibrational environment and cost of false alarms. For instance, for predator-induced hatching, if embryos are frequently exposed to irrelevant background vibrations and face high false alarm costs, selection for discrimination among stimuli may be strong. In contrast, if embryos are rarely exposed to irrelevant background vibrations, or face low false alarm costs, a general hatching response to any disturbance may be sufficient. Nonetheless, even without selection against false alarms favoring discrimination, embryos' sensory abilities likely constrain the range of effective hatching stimuli. Although for some species we have information about fitness consequences of missed cues and false alarms (e.g., Martin et al. 2011; Warkentin 2011a, b; Whittington and Kearn 2011), there is little or nothing known about the background vibrational environment or amount of variation in natural hatching cues for most species. Assessing these features of the sensory environments of embryos could clarify the information-processing challenges they may face.

We also know very little about the sensory systems that mediate MCH or even, in many cases, which elements of physical disturbance cues are most relevant. For some embryos, it is clear that substrate-borne vibrations cue hatching (e.g., stink bugs; Endo et al. 2019). For others, effective stimuli involve substantial whole-egg motion (e.g., grunion; Griem and Martin 2000). Direct physical disturbance, by predators or conspecifics, often provides both motion and tactile stimuli, and embryos may use either or both as hatching cues. Determining which mechanosensory cues embryos use for hatching decisions, and the sensors that enable their perception, are crucial elements of understanding the mechanisms underlying this important behavior.

While substantive advances in understanding MCH are likely to be possible using simple technology, answering many important questions will require more controlled playback systems. Depending on the level of control needed to address the questions of interest, and the sensitivity of embryos to handling, this may require solving system-specific challenges at the intersection of ecological developmental biology and vibrations engineering, drawing on expertise from both disciplines. Our experience also suggests that an iterative approach may be necessary, as some issues may not be evident until others have been solved. For species with interesting MCH and readily available, experimentally tractable embryos, such investment into methods development can enable rich and multifaceted explorations of information use and behavioral decisions *in ovo*.

References

Bacher S, Casas J, Wackers F, Dorn S (1997) Substrate vibrations elicit defensive behaviour in leafminer pupae. J Insect Physiol 43:945–952

Brown RM, Iskandar DT (2000) Nest site selection, larval hatching, and advertisement calls of *Rana arathooni* from southwestern Sulawesi (Celebes) island, Indonesia. J Herpetol 34:404–413

Brownell PH, Van Hemmen JL (2001) Vibration sensitivity and prey-localizing behaviour of sand scorpions. Am Zool 41:1229–1240

Caldwell MS (2010) The use of vibrational information by red-eyed treefrogs for communication and antipredator defense. PhD Dissertation, Boston University, Boston

Caldwell MS (2014) Interactions between airborne sound and substrate vibration in animal communication. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 64–92

Caldwell MS, McDaniel JG, Warkentin KM (2009) Frequency information in the vibration-cued escape hatching of red-eyed treefrogs. J Exp Biol 212:566–575

Caldwell MS, McDaniel JG, Warkentin KM (2010a) Is it safe? Red-eyed treefrog embryos assessing predation risk use two features of rain vibrations to avoid false alarms. Anim Behav 79:255–260

Caldwell MS, Johnston GR, McDaniel JG, Warkentin KM (2010b) Vibrational signaling in the agonistic interactions of red-eyed treefrogs. Curr Biol 20:1012–1017

Carlsen R, Lickliter R (1999) Augmented prenatal tactile and vestibular stimulation alters postnatal auditory and visual responsiveness in bobwhite quail chicks. Dev Psychobiol 35:215–225

Castellanos I, Barbosa P (2006) Evaluation of predation risk by a caterpillar using substrate-borne vibrations. Anim Behav 72:461–469

Cocroft RB, Hamel J, Su Q, Gibson JS (2014a) Vibrational playback experiments: challenges and solutions. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 249–276

Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) (2014b) Studying vibrational communication. Springer, Berlin

Cohen KL, Seid MA, Warkentin KM (2016) How embryos escape from danger: the mechanism of rapid, plastic hatching in red-eyed treefrogs. J Exp Biol 219:1875–1883

Cohen KL, Piacentino ML, Warkentin KM (2019) Two types of hatching gland cells facilitate escape-hatching at different developmental stages in red-eyed treefrogs, *Agalychnis callidryas* (Anura: Phyllomedusidae). Biol J Linn Soc 126:751–767

Doody JS (2018) *Ambystoma talpoideum* (mole salamander). Environmentally cued hatching. Herpetol Rev 49:722–723

Doody JS, Paull P (2013) Hitting the ground running: environmentally cued hatching in a lizard. Copeia 2013:159–164

Doody JS, Stewart B, Camacho C, Christian K (2012) Good vibrations? Sibling embryos expedite hatching in a turtle. Anim Behav 83:645–651

Endo J, Numata H (2017) Effects of embryonic responses to clutch mates on egg hatching patterns of Pentatomidae (Heteroptera). Physiol Entomol 42:412–417

Endo J, Takanashi T, Mukai H, Numata H (2019) Egg-cracking vibration as a cue for stink bug siblings to synchronize hatching. Curr Biol 29:143–148

Fouilloux C, Jung J, Ospina-L AM, Snyder R, Warkentin KM (2019) Do developmental changes in fitness trade-offs predict how embryos use mechanosensory cues for escape-hatching decisions? https://doi.org/10.6084/m9.figshare.9758372.v2

Glennon V, Chisholm LA, Whittington ID (2006) Three unrelated species, 3 sites, same host – monogenean parasites of the southern fiddler ray, *Trygonorrhina fasciata*, in South Australia: egg hatching strategies and larval behaviour. Parasitology 133:55–66

Gomez-Mestre I, Warkentin KM (2007) To hatch and hatch not: similar selective trade-offs but different responses to egg predators in two closely related, syntopic treefrogs. Oecologia 153: 197–206

Gomez-Mestre I, Warkentin KM (2013) Risk-induced hatching timing shows low heritability and evolves independently of spontaneous hatching in red-eyed treefrogs. J Evol Biol 26:1079–1089

Gomez-Mestre I, Wiens JJ, Warkentin KM (2008) Evolution of adaptive plasticity: risk-sensitive hatching in neotropical leaf-breeding treefrogs. Ecol Monogr 78:205–224

Gottleib G (1973) Introduction to behavioral embryology. In: Gottleib G (ed) Behavioral embryology. Academic Press, New York, pp 3–45

Goyes Vallejos J, Grafe TU, Wells KD (2018) Prolonged parental behaviour by males of *Limnonectes palavanensis* (Boulenger 1894), a frog with possible sex-role reversal. J Nat Hist 52:2473–2485

Griem JN, Martin KLM (2000) Wave action: the environmental trigger for hatching in the California grunion *Leuresthes tenuis* (Teleostei: Atherinopsidae). Mar Biol 137:177–181

Guedes RNC, Matheson SM, Frei B, Smith ML, Yack JE (2012) Vibration detection and discrimination in the masked birch caterpillar (*Drepana arcuata*). J Comp Physiol A 198:325–335

Güell BA, Warkentin KM (2018) When and where to hatch? Red-eyed treefrog embryos use light cues. PeerJ 6:e6018

Guevara Molina EC, Ribeiro Gomes F, Warkentin KM (2020) The VTMax of embryos: interacting effects of warming and dehydration on hatching behavior in red-eyed treefrogs, *Agalychnis callidryas* (Anura: Phyllomedusidae). Integr Comp Biol 60:E333–E333

Hill PSM (2008) Vibrational communication in animals. Harvard University Press, Cambridge MA

Jung J (2021) Developmental changes in vibration sensing and vibration-cued hatching decisions in red-eyed treefrogs. PhD Dissertation, Boston University, Boston

Jung J, McDaniel JG, Warkentin KM (2017) Ontogeny of vibration-cued escape hatching in red-eyed treefrogs: two reasons older embryos hatch more. Integr Comp Biol 57:E82–E82

Jung J, Guell BA, Warkentin KM (2018) Inner ear development across onset and improvement of escape-hatching ability in red-eyed treefrogs: a confocal and μCT analysis. Integr Comp Biol 58:E348–E348

Jung J, Kim SJ, Pérez Arias SM, McDaniel JG, Warkentin KM (2019) How do red-eyed treefrog embryos sense motion in predator attacks? Assessing the role of vestibular mechanoreception. J Exp Biol 222:jeb206052

Jung J, Serrano-Rojas SJ, Warkentin KM (2020) Multimodal mechanosensing enables treefrog embryos to escape egg-predators. J Exp Biol 223:jeb236141

Jung J, McDaniel JG, Warkentin KM (2021) Escape-hatching decisions show adaptive ontogenetic changes in how embryos manage ambiguity in predation risk cues. Behav Ecol Sociobiol 75:141

Marquez R, Beltran JF, Llusia D, Penna M, Narins PM (2016) Synthetic rainfall vibrations evoke toad emergence. Curr Biol 26:R1270–R1271

Martin K, Bailey K, Moravek C, Carlson K (2011) Taking the plunge: California grunion embryos emerge rapidly with environmentally cued hatching (ECH). Integr Comp Biol 51:26–37

Moskowitz NA, Vasquez AM, Warkentin KM (2016) Embryo decisions and developmental changes in metabolism across the plastic hatching period of red-eyed treefrogs. Integr Comp Biol 56:E337–E337

Mukai H, Hironaka M, Tojo S, Nomakuchi S (2012) Maternal vibration induces synchronous hatching in a subsocial burrower bug. Anim Behav 84:1443–1448

Mukai H, Hironaka M, Tojo S, Nomakuchi S (2014) Maternal vibration: an important cue for embryo hatching in a subsocial shield bug. PLoS One 9:e87932

Mukai H, Hironaka M, Tojo S, Nomakuchi S (2018) Maternal hatching synchronization in a subsocial burrower bug mitigates the risk of future sibling cannibalism. Ecol Evol 8:3376–3381

Nishide Y, Tanaka S (2016) Desert locust, *Shistocerca gregaria*, eggs hatch in synchrony in a mass but not when separated. Behav Ecol Sociobiol 70:1507–1515

Nishide Y, Suzuki T, Tanaka S (2017) Synchrony in the hatching of eggs in the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae): egg condition influences hatching time in the laboratory and under simulated field temperatures. Appl Entomol Zool 52:599–604

Noguera JC, Velando A (2019) Bird embryos perceive vibratory cues of predation risk from clutch mates. Nat Ecol Evol 3:1225–1232

Oberst S, Bann G, Lai JCS, Evans TA (2017) Cryptic termites avoid predatory ants by eavesdropping on vibrational cues from their footsteps. Ecol Lett 20:212–221

Pfannenstiel RS, Hunt RE, Yeargan KV (1995) Orientation of a hemipteran predator to vibrations produced by feeding caterpillars. J Insect Behav 8:1–9

Roberts DM (2001) Egg hatching of mosquitoes *Aedes caspius* and *Ae. vittatus* stimulated by water vibrations. Med Vet Entomol 15:215–218

Roberts DM (2018) Predator feeding vibrations encourage mosquito larvae to shorten their development and so become smaller adults. Ecol Entomol 43:534–537

Romagny S, Darmaillacq AS, Guibe M, Bellanger C, Dickel L (2012) Feel, smell and see in an egg: emergence of perception and learning in an immature invertebrate, the cuttlefish embryo. J Exp Biol 215:4125–4130

Sakamoto H, Tanaka S, Hata T (2019) Identification of vibrational signals emitted by embryos of the migratory locust *Locusta migratoria* (Orthoptera: Acrididae) that induce synchronous hatching. Eur J Entomol 116:258–268

Salica MJ, Vonesh JR, Warkentin KM (2012) Egg clutch dehydration induces early hatching in red-eyed treefrogs. Integr Comp Biol 52:E323–E323

Shimojo S, Shams L (2001) Sensory modalities are not separate modalities: plasticity and interactions. Curr Opin Neurobiol 11:505–509

Smyder EA, Martin KLM (2002) Temperature effects on egg survival and hatching during the extended incubation period of California grunion, *Leuresthes tenuis*. Copeia 2002:313–320

Snyder RK, Ospina-L AM, Warkentin KM, Snyder R (2018) When does flooding induce hatching? Behavioral decisions of red-eyed treefrog embryos under moderate hypoxia. Integr Comp Biol 58:E422–E422

Tippett CM, Warkentin KM (2017) How not to die if its too dry: a comparison of spontaneous and dehydration-induced hatching in red-eyed treefrogs. Integr Comp Biol 57:E168–E168

Touchon JC, McCoy MW, Vonesh JR, Warkentin KM (2013) Effects of plastic hatching timing carry over through metamorphosis in red-eyed treefrogs. Ecology 94:850–860

Vasquez AM, Moskowitz NA, Warkentin KM (2016) Embryo decisions, metabolism, and development when arboreal eggs are flooded. Integr Comp Biol 56:E385–E385

Virant-Doberlet M, Kuhelj A, Polajnar J, Sturm R (2019) Predator-prey interactions and eavesdropping in vibrational communication networks. Front Ecol Evol 7:203

Wang Y, Lutfi Z, Dong LM, Suman DS, Sanad M, Gaugler R (2012) Host cues induce egg hatching and pre-parasitic foraging behaviour in the mosquito parasitic nematode, *Strelkovimermis spiculatus*. Int J Parasitol 42:881–886

Warkentin KM (1995) Adaptive plasticity in hatching age: a response to predation risk trade-offs. Proc Natl Acad Sci U S A 92:3507–3510

Warkentin KM (1999) The development of behavioral defenses: a mechanistic analysis of vulnerability in red-eyed tree frog hatchlings. Behav Ecol 10:251–262

Warkentin KM (2000) Wasp predation and wasp-induced hatching of red-eyed treefrog eggs. Anim Behav 60:503–510

Warkentin KM (2002) Hatching timing, oxygen availability, and external gill regression in the tree frog, *Agalychnis callidryas*. Physiol Biochem Zool 75:155–164

Warkentin KM (2005) How do embryos assess risk? Vibrational cues in predator-induced hatching of red-eyed treefrogs. Anim Behav 70:59–71

Warkentin KM (2011a) Plasticity of hatching in amphibians: evolution, trade-offs, cues and mechanisms. Integr Comp Biol 51:111–127

Warkentin KM (2011b) Environmentally cued hatching across taxa: embryos respond to risk and opportunity. Integr Comp Biol 51:14–25

Warkentin KM (2017) Development of red-eyed treefrog embryos: a staging table for integrative research on environmentally cued hatching. Integr Comp Biol 57:E175

Warkentin KM, Caldwell MS (2009) Assessing risk: embryos, information, and escape hatching. In: Dukas R, Ratcliffe JM (eds) Cognitive ecology II. University of Chicago Press, Chicago, pp 177–200

Warkentin KM, Currie CC, Rehner SA (2001) Egg-killing fungus induces early hatching of red-eyed treefrog eggs. Ecology 82:2860–2869

Warkentin KM, Gomez-Mestre I, McDaniel JG (2005) Development, surface exposure, and embryo behavior affect oxygen levels in eggs of the red-eyed treefrog, *Agalychnis callidryas*. Physiol Biochem Zool 78:956–966

Warkentin KM, Caldwell MS, McDaniel JG (2006) Temporal pattern cues in vibrational risk assessment by red-eyed treefrog embryos, *Agalychnis callidryas*. J Exp Biol 209:1376–1384

Warkentin KM, Caldwell MS, Siok TD, D'Amato AT, McDaniel JG (2007) Flexible information sampling in vibrational assessment of predation risk by red-eyed treefrog embryos. J Exp Biol 210:614–619

Warkentin KM, Cuccaro Diaz J, Güell BA, Jung J, Kim SJ, Cohen KL (2017) Developmental onset of escape-hatching responses in red-eyed treefrogs depends on cue type. Anim Behav 129:103–112

Warkentin KM, Jung J, Rueda Solano LA, McDaniel JG (2019) Ontogeny of escape-hatching decisions: vibrational cue use changes as predicted from costs of sampling and false alarms. Behav Ecol Sociobiol 73:51

Whittington ID, Kearn GC (1988) Rapid hatching of mechanically-disturbed eggs of the monogenean gill parasite *Diclidophora luscae*, with observations on sedimentation of egg bundles. Int J Parasitol 18:847–852

Whittington ID, Kearn GC (2011) Hatching strategies in monogenean (platyhelminth) parasites that facilitate host infection. Integr Comp Biol 51:91–99

Wiedenmayer CP (2009) Plasticity of defensive behavior and fear in early development. Neurosci Biobehav Rev 33:432–441

Wiley RH (2003) Is there an ideal behavioural experiment? Anim Behav 66:585–588

Willink B, Palmer MS, Landberg T, Vonesh JR, Warkentin KM (2014) Environmental context shapes immediate and cumulative costs of risk-induced early hatching. Evol Ecol 28:103–116

Chapter 8
Inexpensive Methods for Detecting and Reproducing Substrate-Borne Vibrations: Advantages and Limitations

Rachele Nieri, Sabrina C. J. Michael, Carlos F. Pinto, Omar N. Urquizo, Heidi M. Appel, and Reginald B. Cocroft

Abstract There is increasing appreciation of the role of vibration-mediated interactions among animals, and between animals and plants. However, growth of the field is limited by the widespread assumption that this research requires expensive, specialized equipment for detecting and reproducing vibrations. In this chapter, we demonstrate that this assumption is not entirely justified for plant-borne vibrations. We compare the performance of industry-standard equipment for vibration recording and playback with inexpensive alternatives. For conducting high-fidelity playback experiments, there is no difference in the performance of actuators that vary a thousand-fold in price, as long as the appropriate software is used. For obtaining accurate recordings of plant-borne vibrations, the utility of the low-cost alternative sensors we tested is constrained by their frequency response, which is not flat and which can vary between measurements. These readily available sensors are thus primarily useful for detecting and monitoring vibrational signals, rather than for quantitative description or for conducting playback experiments. However, although inexpensive alternatives do not replace calibrated vibration sensors for quantifying the physical properties of signals, they do open up new possibilities for investigation, such as characterizing natural vibrational soundscapes using many sensors at once.

R. Nieri (✉)
Department of Horticulture, Oregon State University, Corvallis, OR, USA
e-mail: rachele.nieri@unitn.it

S. C. J. Michael · R. B. Cocroft
Division of Biological Sciences, University of Missouri, Columbia, MO, USA
e-mail: sabrina.michael@mail.missouri.edu; cocroftr@missouri.edu

C. F. Pinto · O. N. Urquizo
Universidad Mayor, Real y Pontificia de San Francisco Xavier de Chuquisaca, Sucre, Bolivia

H. M. Appel
Department of Environmental Sciences, University of Toledo, Toledo, OH, USA
e-mail: Heidi.Appel@utoledo.edu

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_8

8.1 Introduction

The number of animal taxa that use substrate-borne mechanical waves to communicate is substantial, particularly among invertebrates. The percentage of species relying on vibrational communication is estimated to be 90% among insects and even higher among spiders (Barth 2002; Cocroft and Rodriguez 2005). Plants also perceive mechanical stimuli (Braam 2004; Telewski 2006) and have recently been found to respond appropriately to incidental vibrations produced by feeding herbivores or flying pollinators (Appel and Cocroft 2014; Body et al. 2019; Veits et al. 2019). However, despite the widespread use of mechanical waves as signals in nature (Hill and Wessel 2016), the study of how vibrations influence biotic interactions remains limited by access to suitable equipment for recording and playing back vibrations.

For vibrational stimuli, the "gold standard" devices for recording are laser Doppler vibrometers, which allow non-contact measurement of the velocity of a moving surface. The other standard sensors are accelerometers, which measure acceleration when in contact with a moving surface. Lasers and accelerometers provide repeatable measurements that (with appropriate data acquisition hardware and software) can be expressed in real-world units of velocity and acceleration. Laser vibrometers are costly sensors, on the order of tens of thousands USD, and individually calibrated accelerometers are less costly but still on the order of a thousand USD. In contrast, more inexpensive vibration sensors include phonograph cartridges and piezoelectric disks or film, among others. Piezo disks, which typically cost less than 1 USD, have been used very successfully for detection and control of insect pests on large woody hosts (Dunn 2006; Mankin 2019) and they may be useful for a wider variety of applications. We also test one relatively inexpensive accelerometer (less than 100 USD) that has been used for vibrational communication research (e.g., Cocroft 1999) and which has a flat frequency response over a range useful for many substrate-borne insect signals.

For reproducing vibrations, the "gold standard" device is the mini-shaker, which is constructed like a speaker with a moving coil and magnet but designed to couple the vibration to a solid structure rather than to the air. Most mini-shakers are also relatively expensive, on the order of thousands of USD. A variety of inexpensive devices have also been used for reproducing vibrations, including loudspeakers modified to reduce airborne sound and to couple the speaker motion to a surface, electromagnets that drive a magnet attached to a surface, and piezoelectric disks. The advantages and disadvantages of inexpensive vibration sensors and actuators have been little explored in the literature.

In this chapter, we conduct playbacks and recordings to illustrate the advantages and disadvantages of several inexpensive methods for vibration recording and playback. Our survey is not exhaustive but instead focuses on several devices that are readily available. As a standard for comparison, we use a laser vibrometer or calibrated accelerometer for recording, and a Brüel & Kjær mini-shaker for playback. Our aim is to provide a non-exhaustive list of inexpensive tools that can be used to investigate the role of vibrational signals in a growing number of taxa and contexts. Because our research deals mainly with plant-dwelling insects and plants, the focus of this study will be on small animals using plants as a substrate. However, many of our considerations can be applied to larger-scale systems. For playbacks, we first demonstrate that the use of an expensive device is not sufficient for conducting high-fidelity playback experiments, providing a cautionary note against uncritical use of the equipment. We then show that inexpensive alternatives perform equally well in reproducing plant-borne vibrations. For sensors, we show that while inexpensive sensors are suitable for detecting and monitoring vibrational signals, their limitations include the lack of a flat frequency response and a lack of consistency in the output. As a result, while high-fidelity vibrational playbacks can be done with readily available and inexpensive devices, access to a calibrated vibration transducer such as an accelerometer is still necessary for studies in which precise control over the stimulus is needed. On the other hand, the availability of inexpensive, easily replaced vibration sensors opens up a new range of research avenues.

8.2 Is Use of an Industry-Standard Device Sufficient for Reliable Playbacks?

To conduct a reliable playback experiment, the experimenter needs adequate control over the properties of the played-back signal so that, for example, the characteristics of a played-back natural signal match those of the original recording. The primary way that playback experiments fail to achieve this goal is that the played-back signal differs from the original in its frequency spectrum and its amplitude (Cocroft et al. 2014). Better control over the experimental stimulus requires that the researcher calculate the filter imposed by the playback system, design a compensation filter, and adjust the amplitude of the pre-filtered stimulus. Software for achieving these tasks is available (Michael et al. 2019). We first show that simply recording a signal and playing it back is not sufficient, even when using a laser vibrometer and a shaker. We also show that a given compensation filter is accurate only for the location on the plant where it was calculated, and only until the shaker is detached from the substrate, even if it is replaced in the same location.

8.2.1 Mini-Shaker: Methods

The experimental setups for comparing playback and recording devices are shown in Fig. 8.1. For testing the mini-shaker (Type 4810, Brüel and Kjær Sound & Vibration A/S, Nærum, Denmark) and other playback devices, we chose three stimuli representative of those used in playback experiments on plants: band-limited noise (50–10,000 Hz); an advertisement signal of the treehopper *Umbonia crassicornis*

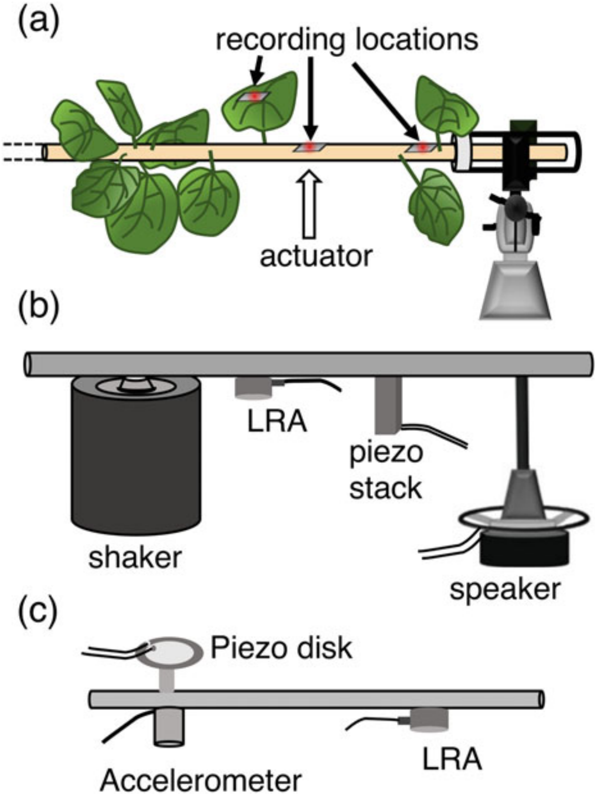


Fig. 8.1 Experimental apparatus used for recording and playback: (**a**) setup for testing playback devices; (**b**) playback devices; (**c**) setup for testing sensors

(Hemiptera: Membracidae) recorded on the stem of a woody host, *Albizia julibrissin* (Mimosaceae); and vibrations produced by a caterpillar, *Vanessa cardui* (Lepidoptera: Nymphalidae), feeding on a soybean leaf (Fabaceae: *Glycine max*). The male signal and the feeding vibrations were recorded with a laser vibrometer (PDV-100, Polytec Inc., Auburn, MA, USA). The mini-shaker was driven from the computer via an amplifier (Behringer HA8000 8-channel High-Power Headphones Mixing and distribution amplifier, Behringer USA, Bothell, WA USA). To record the vibrations produced by the mini-shaker, the laser signal was sent to an audio interface (Celesonic US-20x20, Tascam, TEAC America Inc., Santa Fe Springs, CA USA) whose inputs we calibrated using a Tenma 72-2580 digital oscilloscope (Newark Element 14, Chicago, IL, USA).

We performed the compensation-and-calibration step using the Matlab (Mathworks, Natick, MA USA) script described in detail by Michael et al. (2019), which is free and available online. We used a frequency range of 50–10,000 Hz for designing the frequency filter. The target amplitudes for the playbacks were set to match the peak velocity of the original signals: 0.4 and 3.5 mm/s for the *U. crassicornis* male call and the *V. cardui* feeding vibrations, respectively. The target amplitude for the white noise was arbitrarily set at 1 mm/s.

We tested the mini-shaker when it was unloaded and when it was attached to a plant stem. The plant stem was a 45-cm-long branch cut from a *Hibiscus* sp. and placed in a water tube (stem diameter at the attachment site 5 mm). To couple the plant stem to the shaker, which remained in an upright position on the vibration table, we positioned the stem horizontally using clamps so that it lightly but securely contacted the tip of a metal screw attached to the mounting stud of the shaker. We added a small quantity of wax between the stem and the screw. We measured plant vibration in three locations (Fig. 8.1a): on the stem immediately opposite the site of attachment to the shaker; on the stem 10 cm from the attachment site; and on a leaf whose petiole attached to the stem 2.5 cm from the mini-shaker.

8.2.2 Mini-Shaker: Results and Discussion

There is a basic mismatch between a laser vibrometer and a mini-shaker: the shaker is designed to have a flat frequency response with respect to acceleration, while the laser's output is proportional to velocity. Accordingly, a laser recording of broadband noise played back through a shaker will show the expected 6 dB/octave falloff (Fig 8.2a). So while it would seem straightforward to record an insect signal with a laser vibrometer and play it back with a mini-shaker, the played-back signal will differ substantially from the original (Fig 8.2b). This mismatch can be corrected with a compensation filter, however, and the resulting signal closely matches the original (Fig. 8.2a, b).

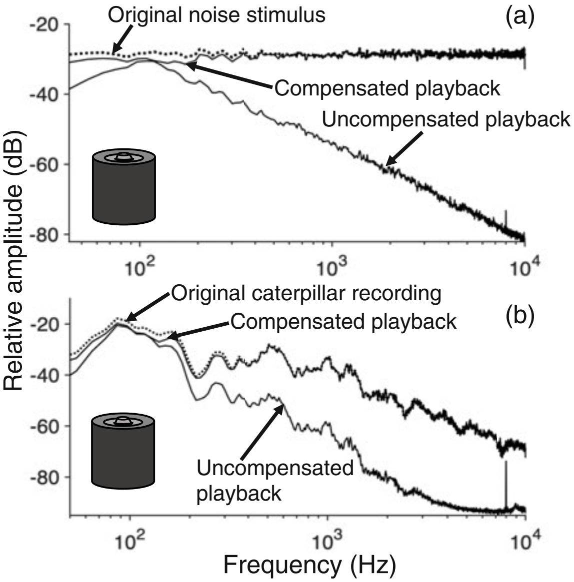


Fig. 8.2 Amplitude spectra illustrating the performance of the mini-shaker with and without pre-filtering of the stimulus using a compensation filter. Stimuli were (**a**) band-limited noise and (**b**) feeding vibrations of a butterfly larva (*Vanessa cardui*)

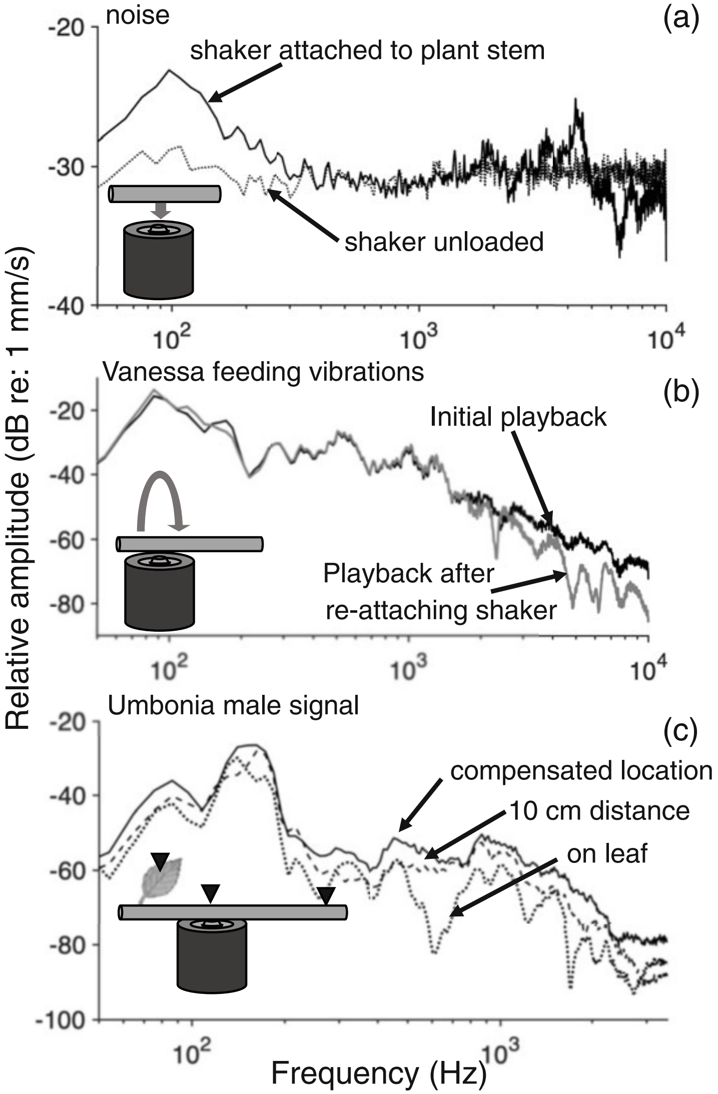


Fig. 8.3 Amplitude spectra illustrating the effect of (**a**) adding a load to the shaker without calculating a new compensation filter; (**b**) detaching and re-attaching the mini-shaker without calculating a second compensation filter and (**c**) recording the stimulus at the location of the mini-shaker and at two other locations on the plant stem

Once the shaker is attached to a substrate, in this case a plant stem, a new compensation filter is needed (Fig. 8.3a). Furthermore, for precise control over the stimulus, this filter must be re-calculated each time the shaker is detached from and re-attached to the substrate, even if it is re-attached in the same location (Fig. 8.3b).

And note that this compensation filter is only accurate for the location where it is calculated; once the signal propagates through the plant, it will be subject to additional filtering (Fig. 8.3c).

8.3 Alternative Playback Devices

A variety of devices have been used as actuators for vibrational playback experiments. These include not only the mini-shaker described above, but also small speakers modified as described in Michael et al. (2019), linear resonant actuators (LRAs; Losinger 2016), piezoelectric actuators (Appel and Cocroft 2014), and piezoelectric disks (Mankin 2019). Our goal was to illustrate the utility of a range of playback devices with costs ranging from less than 1 USD (piezoelectric disk), less than 10 USD (LRAs), less than 1000 USD (piezoelectric actuator and driver) to well over 1000 USD (mini-shaker).

8.3.1 Playback Devices: Methods

We used the same plant stem as with the mini-shaker to examine the output of four other devices: two sizes of LRAs (8 mm × 3.2 mm, 9 mm × 3.4 mm; Fyber Labs, Inc., Kirkland, WA, USA); an 8-ohm speaker modified as described in Michael et al. (2019); and a piezoelectric actuator (Thorlabs AE0505D18F actuator; Thorlabs, Inc., Newton, NJ, USA). To drive the LRAs and speaker we used the Behringer HA8000 amplifier, and for the piezoelectric actuator we used a Thorlabs MDT693B controller.

We used wax to attach the LRAs and to secure the attachment between the piezoelectric actuator and modified speaker to the stem. The piezoelectric actuator was supported on a magnetic base stand, and the speaker was placed directly on the anti-vibration table. We tested each playback device using the same playback files and setup we used for the mini-shaker (Fig. 8.1a, b).

8.3.2 Playback Devices: Results and Discussion

Although the playback devices differed greatly in size, design, and cost, all were capable of matching the desired frequency spectrum to within ±3 dB over a 65–10,000 Hz range (Fig. 8.4a; the LRA 9 mm and the modified speaker exceeded that deviation at 50 Hz). All of the devices also successfully reproduced the insect-generated vibrations (Fig. 8.4b, c), with an average deviation from the original recording of $\leq$1 dB and maximum deviations of ±3 dB with the exception of the 9 mm LRA (maximum deviation of 5 dB) and the modified speaker (maximum

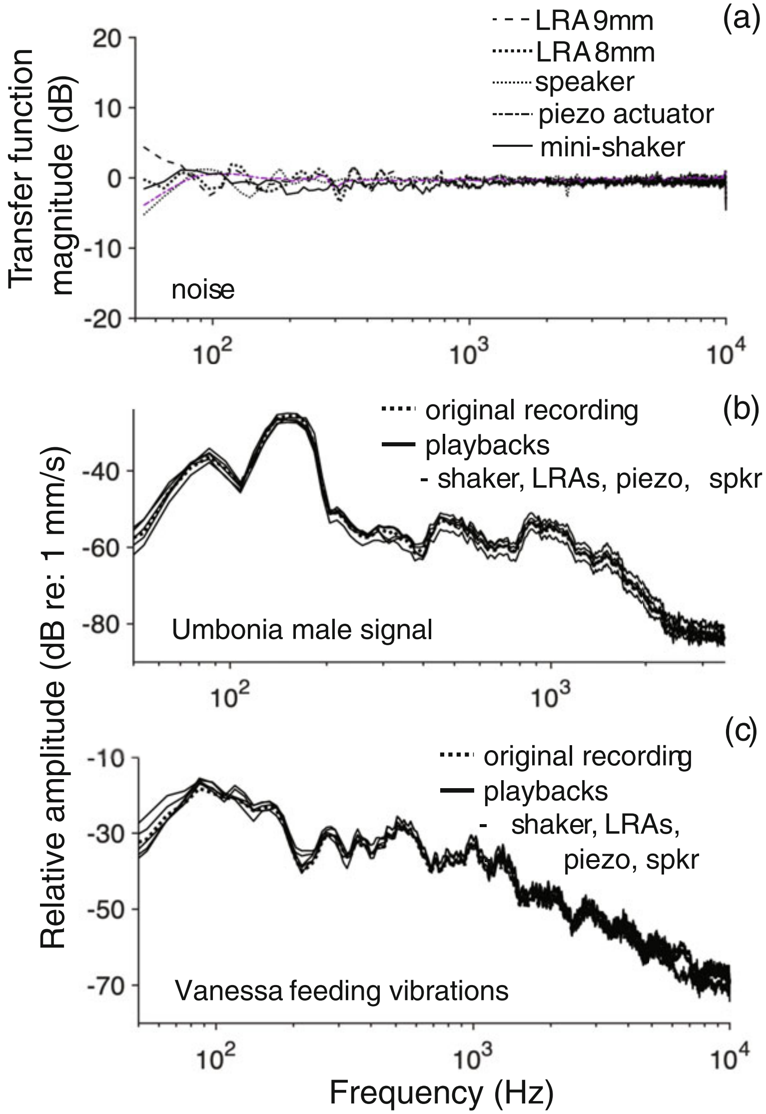


Fig. 8.4 Relative performance of the five tested playback actuators: (**a**) transfer function between original noise stimulus and the five actuators, each of which received its own compensation filter; (**b**) amplitude spectra of the original recording of a treehopper signal and playbacks of that signal using each of the five actuators; (**c**) amplitude spectra of the original recording of caterpillar feeding vibrations and playbacks of that recording using each of the five actuators

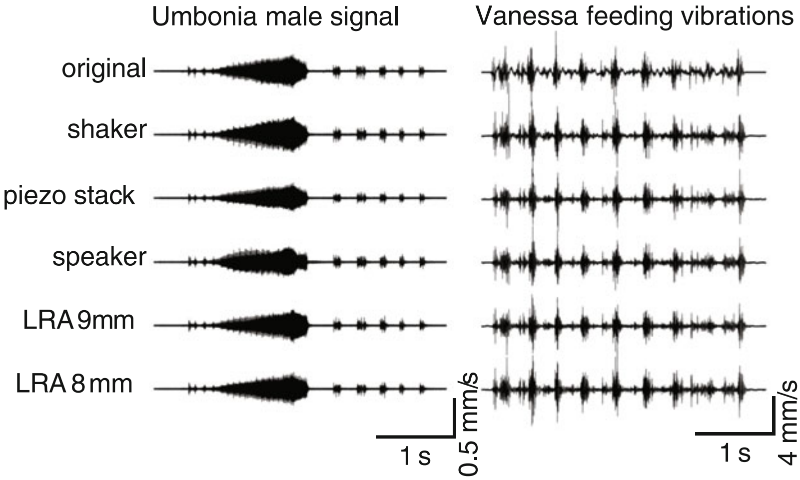


Fig. 8.5 Waveforms of the treehopper signal and caterpillar feeding vibrations, including the original recording and compensated playback of that recording with the five actuators

deviation of 4 dB). Examination by eye of the waveforms of the played-back signals (Fig. 8.5) suggests that qualitatively, the waveforms produced by the shaker and the LRAs were most similar to the original, while the waveform produced by the modified speaker (at least for the *Umbonia* male signal) was less similar to the original. However, cross-correlating the original waveform with the recorded playbacks of the *Umbonia* signal does not support this impression; the cross-correlation coefficients, standardized to a maximum of 1.0, were: shaker (0.94), piezoelectric stack (0.91), modified speaker (0.95), LRA 9 mm (0.95), LRA 8 mm (0.97). In spite of the 1000-fold cost difference between an LRA and a mini-shaker, then, for purposes of reproducing an insect mating signal on a plant, the two are equivalent.

8.4 Inexpensive Vibration Sensors

The industry-standard methods for measuring vibration are laser vibrometers and accelerometers. However, there are a multitude of ways of converting mechanical vibrations into electrical signals. Among the many methods that have been used in studies of vibrational communication are phonograph cartridges (Henry et al. 1999), loudspeaker membranes (Moraes et al. 2005), and piezoelectric disks (Mankin 2019). Piezoelectric disks have been incorporated into an apparatus for detecting insect vibrations in trees with high sensitivity and low noise (Dunn 2006), and this work has led to new methods of controlling the behavior of harmful insects (Hofstetter et al. 2019). New methods for vibration detection continue to be developed, such as computer vision processing of high-speed audio (Davis et al. 2014).

Here we compare the output of an individually calibrated accelerometer with the output of three sensors: a 2.7 cm piezo disk, a ceramic-element phonograph cartridge, and a Knowles BU-1771 accelerometer (Knowles Electronics LLC, Itasca, IL, USA).

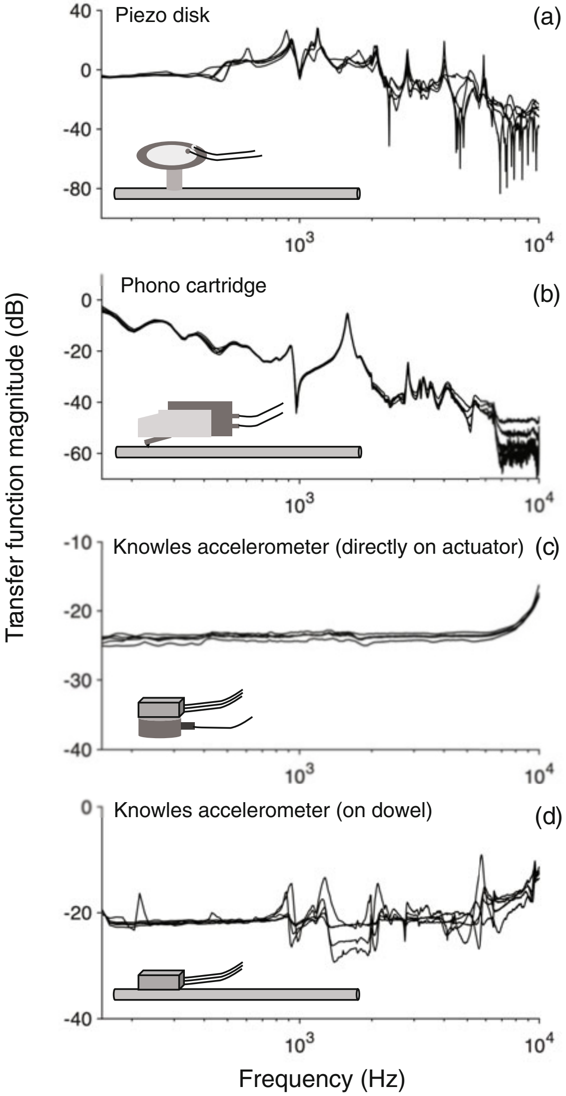
Our goal was to characterize the frequency response of the sensors over the range of 50–10,000 Hz and to assess the repeatability of their output. In particular, we wanted to capture variability in the sensors' frequency response across measurements, since the manner in which a sensor is attached will influence its frequency response, and this influence can be greater for non-standard sensors.

8.4.1 Vibration Sensors: Methods

We played back and recorded broadband noise (50–10,000 Hz) through a dowel (6.25 mm diameter) held in a PanaVise. We used a 9 mm × 3.2 mm LRA as a playback device and calculated the compensation filter using an accelerometer (Vibrametrics 9002A with P5000 Power supply, Mistras Group, Princeton Junction, NJ, USA; sensitivity is 100 mV/G and we used 10x gain) attached to the dowel 10 cm from the LRA. We attached each sensor in turn to the dowel opposite the accelerometer (Fig. 8.1c). We modified the piezo disk by gluing a short length of dowel (8 mm long × 6 mm diameter) to the center of the metal disk on the opposite side from the ceramic wafer; a consistent means of attaching a piezo disk to the substrate can improve the sensitivity and repeatability of the measurements (D Dunn, personal communication). We attached the piezo disk to the stem with wax. We positioned the phonograph cartridge by attaching it to a dowel held by a Panavise, such that the tip of the stylus lightly but firmly contacted the stem opposite the accelerometer. We tested the Knowles accelerometer as with the other sensors, attaching it with wax to the dowel. In addition, to assess the influence of attaching the sensor to a curved surface of the dowel rather than to a flat surface, we also conducted a second test of the dowel in which one surface of the Knowles was attached to the LRA and the other was attached to the Vibrametrics accelerometer. The piezoelectric disk and phono cartridge require a high-impedance input, provided by the audio interface (Behringer U-PHORIA UMC202HD audio interface, Behringer USA, Bothell, WA USA). The interface was connected to a Macintosh computer. The Knowles accelerometer was powered by a custom-made amplifier and power supply (circuit available from the authors on request).

To estimate the repeatability of the measurements from each sensor, after the first recording we detached the sensor, re-attached it in approximately the same location, and again recorded the played-back noise for a total of 5 sets of recordings per sensor. For each measurement, we obtained the transfer function between the signal acquired by the Vibrametrics accelerometer and the signal acquired by the sensor.

Fig. 8.6 Comparison of the frequency response of three vibration sensors, including (**a**) a piezo disk, (**b**) a ceramic phonograph cartridge, (**c**) a Knowles accelerometer attached directly to the flat surface of an actuator and (**d**) a Knowles accelerometer attached to the dowel



8.4.2 Vibration Sensors: Results and Discussion

The frequency responses of the piezoelectric disk and phonograph cartridge are complex and are not identical each time the sensor is attached to the substrate (Fig. 8.6a, b). The frequency response of the Knowles accelerometer (when attached to the surface of the LRA) is flat up to ~7000 Hz, after which the response is influenced by the resonant frequency of the sensor (Fig. 8.6c). However, after attaching this sensor to the dowel, its frequency response was more variable (Fig. 8.6d).

The complexity and the variability in the frequency response of these sensors between uses is a constraint on their utility for studies of vibration-mediated interactions. These issues with the frequency responses of the tested sensors have at least three causes. The first is that we have re-purposed the sensor for a new use, and phonograph cartridges provide an illustrative example. They are designed to reproduce the waveforms inscribed in the groove of a phonograph record; as the stylus travels through the groove, the downward pressure applied to the stylus is a constant, determined by the weight of the arm that holds it, and the waveform inscribed in the vinyl causes side-to-side movement of the stylus that is transferred to the piezoelectric element inside the cartridge. Placing the stylus in contact with a plant stem is a novel use of the sensor that does not provide the same kind of motion it was designed to detect, nor does the method used to hold the cartridge in place provide the repeatable pressure on the stylus that a phonograph player would.

The second cause of complexity and variability in frequency response is illustrated by piezoelectric disks. While these ubiquitous sensors have a range of uses, the metal disk to which the piezo element is attached has resonant peaks that vary with the size of the disk and the way it is attached to the substrate. The addition of a dowel to facilitate a more consistent point of attachment between the disk and the substrate did not in this case eliminate variability in measurements, especially at higher frequencies.

The third cause of a lack of repeatability in the sensors' frequency response is the manner in which we attached them to the substrate. Results from the Knowles accelerometer reveal that when this sensor is firmly secured to a flat surface each time it is used, it can provide a reliably flat frequency response. However, attaching it to a dowel introduced variability in frequency response from one use to the next. For studies where repeatable measurements are important, however, the unit can be glued onto the substrate (Cocroft 1999), which takes more time but provides a more secure coupling between sensor and substrate.

8.5 General Discussion

The measurements made here of the performance of various vibration playback and recording sensors illustrate a few general points. Here we discuss the opportunities provided by the devices we tested, as well as their limitations.

8.5.1 Inexpensive Playback Devices: Advantages and Limitations

The data presented in this chapter illustrate that a range of devices can produce playbacks that closely match the frequency spectrum of the original signal, as long as the user calculates a compensation filter, applies it to the stimuli, and calibrates the amplitude of the playback. Because the compensation filter adjusts the relative amplitude of different frequencies in the signal relative to each other, stimuli containing only a single frequency do not require a compensation filter, only amplitude calibration. Especially for playback experiments where higher throughput is required and which involve multiple channels (e.g., Appel and Cocroft 2014), there is a substantial cost savings from using actuators like the ones tested here, with no reduction in playback quality.

The main limitation of the piezoelectric actuators and LRAs tested here, relative to the shaker, is the total displacement possible. For the shaker, the maximum peak-to-peak displacement is 4 mm, while for the piezo actuator the maximum peak-to-peak displacement is 16 μm. We did not measure peak displacement for the LRA, but for the playback of caterpillar feeding vibrations the maximum peak-to-peak displacement (obtained by integrating the laser signal) was 8 μm. For the plant-borne vibrations of the feeding and signaling insects we have worked with, however, peak-to-peak displacements are small (on the order of 1–10 μm) and all of the devices we used can produce playbacks with that amplitude. For modified loudspeakers, millimeter-scale displacements are possible with larger speakers. So the limited peak-to-peak displacement of most of the actuators we tested is not a disadvantage for playback of relatively low-amplitude vibrations like the ones produced by insects on plants; and for playback of higher-amplitude vibrations, modified speakers of the appropriate size should be able to produce the needed displacement. The need for larger total displacement is most likely to arise when reproducing low-frequency signals produced by relatively large signalers.

An additional advantage of the LRAs for playback is that, for suitable structures such as woody stems, the actuator can be attached directly to the substrate. In contrast, for all of the other devices tested, the actuator must be supported on a flat tabletop (mini-shaker) or otherwise held in place (piezo actuator, modified speaker). The ability to attach an actuator directly to the substrate without external support is a distinct advantage in some circumstances, such as for conducting a playback at a particular location on a large woody hostplant. However, very light plant structures such as leaves, petioles, or the stems of small herbaceous plants cannot support the weight of an actuator, so an external positioning system is needed.

8.5.2 Inexpensive Vibration Sensors: Advantages and Limitations

The availability of sensitive, low-cost vibration sensors such as piezo disks overcomes some serious limitations of expensive, dedicated equipment such as laser vibrometers and calibrated accelerometers. Although lasers and calibrated accelerometers have been used in the field, lasers and most accelerometers are not weather-resistant, and the need to avoid damaging difficult-to-replace sensors limits the kind of work that can be done. In contrast, monitoring vibrational signals in natural environments is eminently feasible, as the sensors can be waterproofed for outdoor use and replaced as needed. Furthermore, their low cost makes them useful for applications requiring many sensors at a time, as long as quantitative characterization of the true frequency spectrum or amplitude is not needed. The only limiting factor is the number of channels of data acquisition. Sensors such as piezo disks are also very suitable for use in science outreach activities. Piezo disks can be waterproofed and mounted on a clip for easy attachment to vegetation, and the cable can be plugged into an amplified speaker for a very simple "vibrational prospecting" kit.

Additional work will be required to overcome the main limitations of inexpensive vibration sensors—i.e., their complex frequency response and the variability in output between repeated uses. If attachment methods can be developed that maximize the repeatability of the output of these sensors, then it would be possible to design a compensation filter to convert the output to closely match the output that would have been provided by a calibrated sensor. It is likely, however, that attachment methods that yield more repeatable measurements (such as using adhesive rather than wax) will require more advanced preparation, leading to a tradeoff between the quality and the ease of the measurement. And of course, the method of attachment to the substrate is a source of variation in output for any sensor, including the most expensive dedicated equipment.

Any of the sensors we tested here can be used to calibrate reliable playback experiments, under certain conditions (see Cocroft et al. 2014 for a more extended discussion of this point). One such condition would be if the original signal is recorded at the location where the playback subject will be, and the playback is calibrated on the same substrate without detaching the sensor. Another would be if the frequency range of the playback stimuli fell within a frequency range where the sensor does yield consistent measurements. A third would be if the sensor could be attached in the same manner between locations, as by adhesive contact of the entire sensor (in the case of a piezo disk or film) or by embedding the sensor within a medium such as soil. The second two conditions would need to be tested further to ensure that the measurements were indeed consistent.

For sensors then, our current information indicates that for experimental work with vibrations, it remains important to have at least one calibrated vibration sensor, such as an accelerometer. If a low-cost sensor can be identified that does provide consistent measurements from one use to the next, this would be extremely useful.

LRAs (which can also be used as sensors) offer some promise in this regard, though their output when used as sensors is low and their consistency from one measurement to the next has not been demonstrated. Lower-cost accelerometers, such as the Knowles accelerometer tested here, provide another potential solution, as long as their sensitivity, frequency range, and mass are appropriate for the questions addressed. However, these accelerometers are not individually calibrated and once they are attached to a cable, their sensitivity and resonant frequency can vary from one unit to the next, making it advisable to calibrate their output using a factory-calibrated sensor.

8.6 Conclusion

The availability of low-cost playback devices and vibration sensors provides new opportunities for the study of vibration-mediated interactions. For playback devices, inexpensive solutions are available that perform as well as expensive, dedicated devices. For sensors, the limitations of the devices we tested mean that for some research purposes, dedicated equipment such as individually calibrated accelerometers or laser vibrometers are necessary. At the same time, the availability of low-cost sensors opens up avenues for exploration in directions that the use of more delicate, less replaceable sensors does not support, and use of these sensors has the potential to advance the field in significant ways.

Acknowledgments We thank Barb Sonderman for providing plant material for our measurements of playback devices. We thank Ron Miles and Michael Losinger for introducing the authors and the vibrational communication community more generally to the use of LRAs for playbacks. We gratefully acknowledge the help and patience of our editors, Nataša Stritih-Peljhan and Peggy Hill. The work was supported by the National Science Foundation grant IOS-1359593 to HMA and RBC, Oregon State University Postdoctoral Association Professional Development Award to RN, Comisión Nacional de Ciencia y Tecnología postdoctoral project 3160356 to CFP, and International Science Programs at Uppsala University project BOL-01 to CFP.

References

Appel HM, Cocroft RB (2014) Plants respond to leaf vibrations caused by insect herbivore chewing. Oecologia 175:1257–1266. https://doi.org/10.1007/s00442-014-2995-6

Barth FG (2002) A Spider's world: senses and behavior. Springer, Berlin

Body MJA, Neer WC, Vore C, Lin C-H, Vu D, Cocroft RB, Appel HA (2019) Caterpillar chewing vibrations cause changes in plant hormones and volatile emissions in *Arabidopsis thaliana*. Front Plant Sci 10:810. https://doi.org/10.3389/fpls.2019.00810

Braam J (2004) In touch: plant responses to mechanical stimuli. New Phytol 165:373–389. https://doi.org/10.1111/j.1469-8137.2004.01263.x

Cocroft RB (1999) Offspring-parent communication in a subsocial treehopper (Hemiptera: Membracidae; *Umbonia crassicornis*). Behaviour 136:1–21

Cocroft RB, Rodriguez RL (2005) The behavioral ecology of insect vibrational communication. Bioscience 55:323–334

Cocroft RB, Hamel J, Su Q, Gibson J (2014) Vibrational playback experiments: challenges and solutions. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 249–274

Davis A, Rubinstein M, Wadhwa N, Mysore GJ, Durand F, Freeman WT (2014) The visual microphone: passive recovery of sound from video. ACM Trans Graphic 33:1–79

Dunn D (2006) Liner notes of the sound of light in trees. www.acousticecology.org/dunn/solitnotes.html

Henry CS, Martinez Wells ML, Simon CM (1999) Convergent evolution of courtship songs among cryptic species of the *Carnea* group of green lacewings (Neuroptera: Chrysopidae: Chrysoperla). Evolution 53:1165–1179

Hill PSM, Wessel A (2016) Biotremology. Curr Biol 26:R187–R191. https://doi.org/10.1016/j.cub.2016.01.054

Hofstetter RW, Aflitto N, Bedoya CL, Yturralde K, Dunn D (2019) Vibrational behavior in bark beetles: applied aspects. In: Hill PSM, Lakes-Harlan R, Mazzoni V, Narins P, Virant-Doberlet-M, Wessel A (eds) Biotremology: studying vibrational behavior. Springer Nature, Cham, Switzerland, pp 415–435

Losinger MJ (2016) Form, function and social context of substrate-borne vibrational signals in the treehopper, *Umbonia crassicornis*. Dissertation, State University of New York, Binghamton. https://www.researchgate.net/profile/Michael_Losinger/publication/315678893_Form_function_and_social_context_of_substrate-borne_vibrational_signals_in_the_treehopper_Umbonia_crassicornis/links/58dae469aca272d801fa9965/Form-function-and-social-context-of-substrate-borne-vibrational-signals-in-the-treehopper-Umbonia-crassicornis.pdf

Mankin RW (2019) Vibrational trapping and interference with mating of *Diaphorina citri*. In: Hill PSM, Lakes-Harlan R, Mazzoni V, Narins P, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational behavior. Springer Nature, Cham, Switzerland, pp 399–412

Michael SCJ, Appel HA, Cocroft RB (2019) Methods for replicating leaf vibrations induced by insect herbivores. In: Gassmann W (ed) Plant innate immunity: methods and protocols. Springer Nature, Cham, Switzerland, pp 141–157

Moraes MCB, Laumann RA, Cokl A, Borges M (2005) Vibratory signals of four neotropical stink bug species. Physiol Entomol 30:175–188

Telewski FW (2006) A unified hypothesis of mechanoperception in plants. Am J Bot 93:1466–1476. https://doi.org/10.3732/ajb.93.10.1466

Veits M, Khait I, Obolski U, Zinger E, Boonman A, Goldshtein A, Saban K, Seltzer R, Ben-Dor U, Estlein P, Kabat A, Peretz D, Ratzerkorfer I, Krylov S, Chamovitz D, Sapir Y, Yovel Y, Hadany L (2019) Flowers respond to pollinator sound within minutes by increasing nectar sugar concentration. Ecol Lett 22:1483–1492. https://doi.org/10.1111/ele.13331

Part IV
Vibrational Behavior in Less Explored Contexts

Chapter 9
Sexual Selection in the Red Mason Bee: Vibrations, Population Divergence, and the Impact of Temperature

Taina Conrad

Abstract Solitary bees are usually considered superior pollinators to the honeybee and with so many different species, there are ample options to choose from. Regardless, there is still a lack of knowledge when it comes to their communication and especially the use of vibrational signals. *Osmia bicornis* shows a complex mating behavior including thorax vibrations, which we have intensely studied. We were able to show that these vibrational signals actually play a vital role in the mating by encoding not only a signal for the male's physical fitness but also a signal associated with their region of origin—both of which are being used by the female to choose a suitable male. Furthermore, we have found that the vibrations produced by a male are influenced by changes in temperature, which leads to different males having an advantage. In view of climate change, this could lead to important population changes and should be considered as a factor also when looking at different bee species. Overall we show that vibrational signals can be very important in solitary bees and should not be neglected in future studies.

9.1 Introduction

The "buzzing" of a bee is certainly widely recognized, so much so that it is not only known to scientists and non-scientists alike, but it even has found its place in songs and literature. However, while there are estimated to be more than 30,000 bee species worldwide (Michener 2000), vibrational communication has only been studied in very few bee species to date.

So far most evidence of vibrational communication in Apoidea has been found in the eusocial species such as the honeybee, bumblebees, and stingless bees, though this is probably due to lack of research rather than the non-occurrence of this type of communication (Hill 2008).

T. Conrad (✉)
Evolutionary Animal Ecology, University of Bayreuth, Bayreuth, Germany
e-mail: taina.conrad@uni-bayreuth.de

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_9

One of the main reasons for this lack of research may be the focus on chemical communication in the Hymenoptera over the past decades, which has been very fruitful and does play a major role in a lot of bee species (Ayasse et al. 2001). However, as the field of biotremology emerges and more and more work is being done, we have come to realize that more species use vibrations as a means of communication than previously thought and that thoracic oscillations are actually widespread among bees. Thanks to the work of Michelsen, Tautz, Kirchner, and others over the past four decades, we now know about the importance of substrate vibrations in the waggle dance of the honeybee; although, a lot of questions still remain unanswered (Michelsen 2014). Additionally, our knowledge of the use of biotremology in the foraging of stingless bees has greatly increased through the work of Hrncnir and Barth over the last 20 years. Unlike in the waggle dance in honeybees the vibrational signals in stingless bees do not convey information about the direction of the food source but rather quality and net gain of the food source. So the informational content of these signals can be quite diverse (Hrncir and Barth 2014). Buzz pollination is a third area that has been intensely studied and has led to some exciting new insights concerning the frequency and acceleration used by bumblebees during foraging. We are looking at an exciting co-evolution between plants and bees driven by vibrational signals (Vallejo-Marín 2018).

As early as 1986, Ole Larsen mentioned that vibrations are widespread and might convey important messages between the sexes during mating (Larsen et al. 1986). It is therefore remarkable that almost no research on the role of biotremology during mating and/or sexual selection has been done since then. Considering the substantial number of solitary bees displaying complex mating behaviors that include “buzzes” in one way or another, this is an area that promises to be rewarding if explored and will allow us to better understand the purpose of these signals. While bees are the most important insect pollinators, we have come to realize that honeybees are actually not the best choice in a lot of cases. Wild bees are generally considered superior pollinators (Valido et al. 2019), so it is of vital importance to increase our knowledge in this area. Life history information, especially knowledge of communication, mating, and speciation, will improve our ability to use these bees as efficient pollinators and, of course, protect them and their habitats.

9.2 Mating Behavior of the Red Mason Bee

The reproductive biology of the red mason bee, *Osmia bicornis,* has been studied already in the past (Seidelmann 1991, 1995). *Osmia bicornis* usually lays its eggs into holes it finds in dead wood. However, anything of the right size may be used, even old bullet caps (O’Toole 2000). It is one of the first bees to occur in spring and normally emerges in late March or early April, which leads to its use as a pollinator for agricultural plants like apple trees (Westrich 1989). *Osmia bicornis* is a strongly proterandric species in which the males emerge first to be present in great numbers all through the emergence of the females during spring time (Seidelmann 1995). The males either wait in front of the nests or around flowers for the appearance of potential females for mating (Ayasse et al. 2001). Since the gender ratio is shifted in favor of the males (approx. 1.2 ♂:1♀), the pre-condition for female choice is met (Andersson 1994).

Contrary to the males, females only mate once and the mating can be described in three phases: precopulation, copulation, and postcopulation (Seidelmann 1995). During the precopulatory courtship, the male embraces the female by sitting on her back, holding the female's mesothorax with its first and second pairs of legs. The male's antennae are pointing toward the upper-front, while the female's antennae point toward the side (Seidelmann 1995). The male then engages in a series of behaviors in order to persuade the female to mate. He vibrates his thorax, rubs himself against the female, and passes his antennae repeatedly over those of the female. Meanwhile, he also moves his forelegs over the female's compound eyes (Seidelmann 1995). After this complex mating behavior, which can last from only a few seconds to up to an hour, the male moves back on the female's back and tries to insert his genitalia into the genital chamber, while using his antennae for a tremolo on the female's face (Seidelmann 1995). The female may then reject the male by physically pushing him off her back or bending her abdomen away from him (Conrad et al. 2010). In the former case, the male will fly off in search of another female, while in the latter case the male will go back to his mating behavior and try again at a later time.

9.3 Female Choice in *Osmia bicornis*

Charles Darwin noticed the strong sexual dimorphism we find in many species (Darwin 1871). The males can actually be so different from females that on many occasions they have been thought to be two entirely different species until their actual mating was observed. Looking at the magnificent tail of the peacock, it is not hard to understand that something other than natural selection is at play here. Obviously, the peacock is truly handicapped by his tail, especially if he is attacked by a predator. The explanation for this can of course be found in sexual selection (Dimijian 2005).

In many cases, sexual selection is based on a male's fitness, its freedom from parasites, or its genetic relatedness to the female, all of which are evaluated through various different male traits (Clarke and Faulkes 1999; Kose and Møller 1999). In the barn swallow, for example, the white spots on the tail are directly correlated with parasite infestation and thus are used by females as an honest signal for male health (Kose and Møller 1999). All of these male traits can be of various different types, and sometimes even hard to spot or to distinguish from traits resulting from selection by other natural selection (Brown 1975; Endler 1986). In the case of the peacock, it is due to female choice that the male's tail developed in such a way. Female choice is just one kind of sexual selection in which the female chooses its mate according to a set of traits the male possesses. Although male signals like color, size, or odor are probably more commonly known in regards to sexual selection, in many cases, female choice is based on vibrational signals, for example in blood-sucking bugs (Roces and Manrique 1996) and treehoppers (Rodríguez et al. 2004). Various parts of the male signal may be responsible for female choice, such as base frequency, length of the whine section, pulse rate, and number of pulses as is seen in the case in the treehopper *Enchenopa binotata* (Rodríguez et al. 2004).

Considering the aforementioned gender ratio and the complex mating behavior in *O. bicornis* one would expect that female choice is at play in this species. We, therefore, set out to find the male characteristics a female might use to evaluate the male's suitability as a mate. In a series of behavioral experiments, coupled with chemical and molecular analyses, we tested if size, odor, relatedness, and vibrations differed between those males that were accepted by the females and those that were rejected. Size was determined by interocular distance, as that correlates with overall size in bees. Odor was determined by gas chromatography and relatedness between a mating pair was estimated using microsatellite analyses. We recorded the vibrations using a laser vibrometer aimed at the thorax of a male during pre-copulation. Pairs were established by introducing one female at a time into a flight cage with about 40–50 males. After a bit of scramble competition one male gained the position on the female's back and started his mating behavior. Usually, the other males would then fly off and leave the pair alone. All males were marked with a white dot on their thorax to better reflect the laser used to record vibrational behavior.

Interestingly there was no significant difference in size between accepted and rejected males (Mann–Whitney U test, $P > 0.05$). However, we observed a significant difference in the variances of accepted and rejected males, with the variance of rejected males being much higher than that of the accepted males (Ansari–Bradley Test, $P < 0.05$). This indicates a more consistent choice by females for males of an intermediate size (Conrad et al. 2010). Since in most species larger males are preferred, this points toward a disadvantage for males that are too large in this species. One such disadvantage could be that larger males have trouble with temperature regulation, as is the case in the sphecid wasp *Bembix rostrata* (Larsson 1991).

Our other results also showed clear differences between accepted and rejected males for odor and relatedness, showing that certain odors are preferred, which are possibly used to gain information about relatedness. We also found that females follow optimal outbreeding, in that they avoid mating with brothers but also males too distantly related to them.

Our vibrational recordings showed that male vibrations occur in a series of trains (periods of vibrations) with longer breaks in between. Each train, in turn, consists of a series of bursts or pulses, which can either occur with short breaks between them or without any distinguishable breaks at all. We measured pulse length, break length, and dominant frequency and found that only the pulse length differed significantly between accepted and rejected males (Mann–Whitney *U* test, $P < 0.05$; Fig. 9.1), leading us to the conclusion that males who were able to vibrate longer, which of course is energetically costly, were the ones preferred by the females—presumably as they are the stronger individuals. Since often vibrational signals, especially

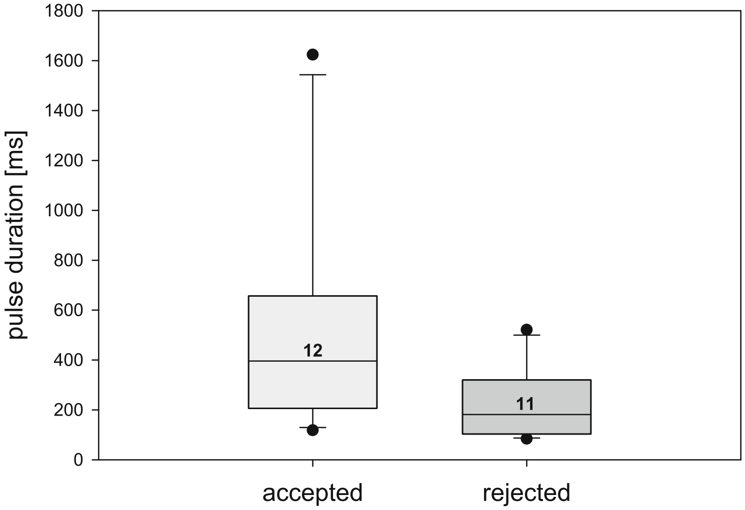


Fig. 9.1 Pulse duration of vibrations in accepted and rejected males. The median, quartiles, and outliers (circles) are shown. The two groups differ significantly (Mann–Whitney U Test, $P < 0.05$)

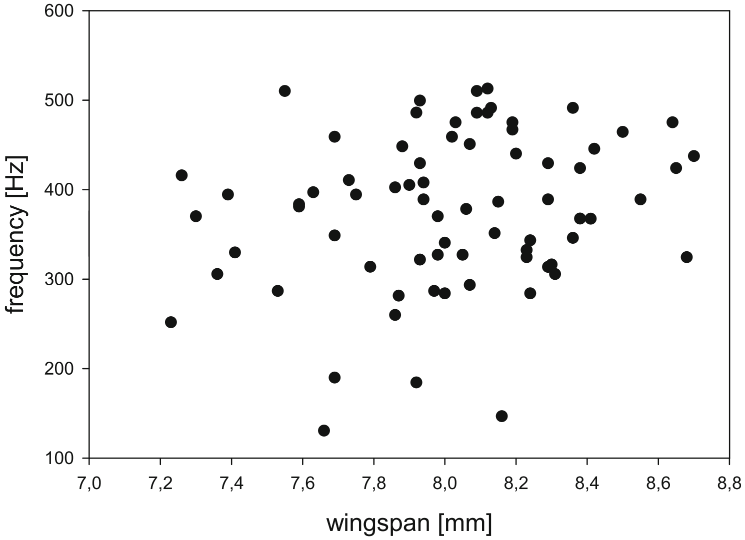


Fig. 9.2 Correlation between dominant frequency and wingspan in *O. bicornis* males. There is no significant correlation (Spearman's rho, $P > 0.05$). Number of individuals tested: $n = 73$

frequency, are directly related to size with bigger males producing lower frequencies, we also ran a correlation between size and vibrations but found no link between the two (Spearman's rho, $P > 0.05$; Fig. 9.2). Overall our results confirmed for us that female choice is present in *O. bicornis* (Conrad et al. 2010). Numerous traits of the males are used in this choice and are therefore under sexual selection.

9.4 The Role of Vibrations as Isolation Barriers in *Osmia bicornis* Populations in Europe

Traits under sexual selection can be a driving force of speciation if female preference differs between different forms of trait development (Lande 1981; Boake 2002; Coyne and Orr 2004; Andersson and Simmons 2006) and a Fisherian runaway process may consequently divide the two populations. It is possible that in certain populations one male in dozens could actually mate with the majority of females if by chance he develops a trait preferred by the females. In consequence, very few males would be fathering the next generation of offspring and passing on their genes and phenotypic traits. That could explain why we find sexual selection to act so fast in changing populations (Turner and Burrows 1995; Gavrilets and Boake 1998). A Fisherian runaway process is especially likely in situations where there is already a pre-existing bias in the females, like in the swordtail fish group. Here, females from species that do not have swords still prefer them when confronted with a choice (Basolo 1990).

There are various examples of sexual selection in insects playing a potential role in speciation, like in the well-known Hawaiian *Drosophila* (Hoy et al. 1988), where different songs have led to the evolution of over 600 species, or the Australian scorpionflies of the genus *Harpobittacus*. Two species of scorpionflies, both found sympatrically in Australia, fail to mate in the lab due to different male sex pheromones known to have evolved under sexual selection (Bornemissza 1966).

There are also many reports of intraspecific differences in vibrational signals between populations and their influence in mate choice (Gillham 1992; Claridge and DeVrijer 1993; Ryan et al. 1996; Čokl et al. 2000). In the southern green stink bug, *Nezara viridula*, for example, there are distinct differences in female calls between populations and males are able to recognize not only conspecific calls but also females from their own population, which are preferred (Miklas et al. 2003).

It is clear today that vibrational signals are very often species-specific and can be used for species recognition, which may lead to discrimination of individuals of different populations. Slight signal differences involved in the mating process have already been recognized as a possible first-step toward reproductive isolation and can play a role in speciation (Darwin 1871; West-Eberhard 1983; Andersson 1994; Panhuis et al. 2001; Coyne and Orr 2004), especially where female choice leads to the selection of male traits. It is therefore highly likely that vibrational communication plays a part in speciation by sexual selection.

In bees, prezygotic isolation barriers are predominantly found to prevent interspecific mating. There are many reports of species-specific odors in bees, especially in the female sex pheromone, which function as isolating barriers, since they only attract con-specific males (Bergman and Bergström 1997; Ayasse et al. 2001). In other bees, however, heterospecific mating is limited by behavioral differences or diverging flight times (Ayasse et al. 2001), like in the cave-dwelling *Apis nuluensis*. Here, isolation from other *Apis* species is achieved by a different flight period of the males (Koeniger and Koeniger 2000). Surprisingly, research into vibrational signals

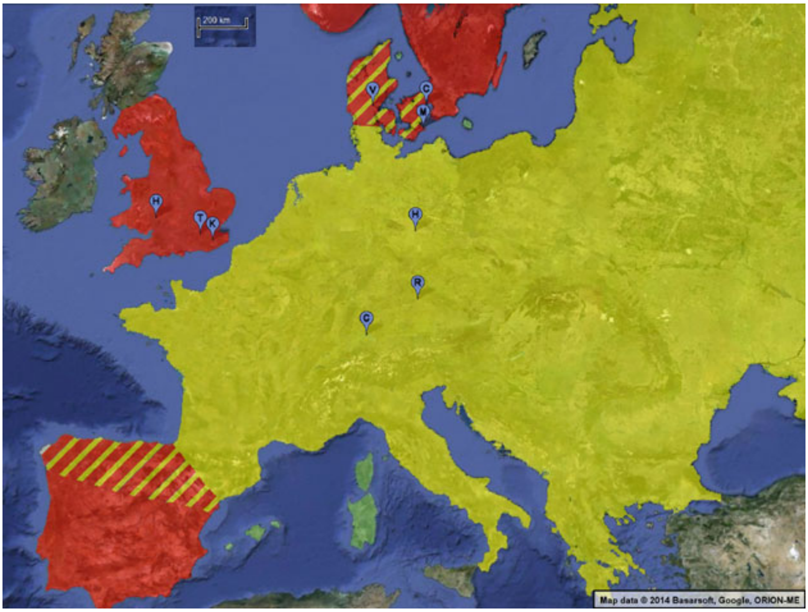
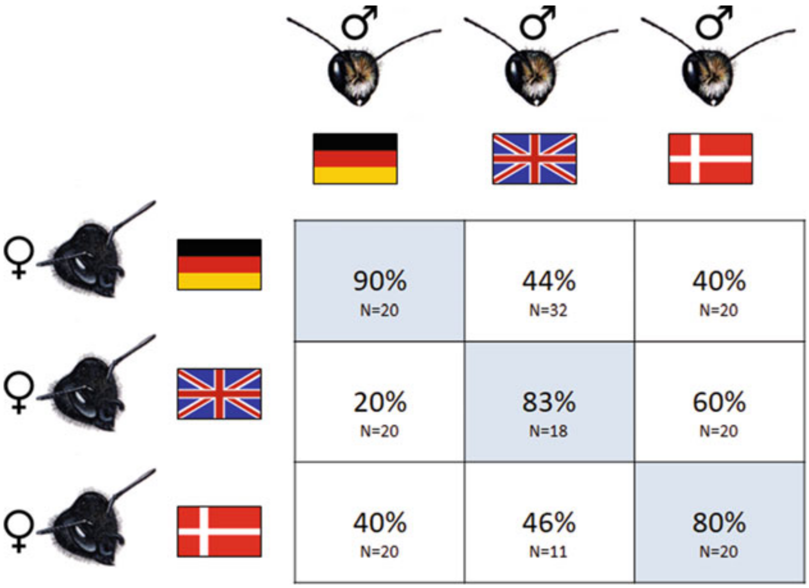


Fig. 9.3 Map showing the distribution of the two main subspecies of *O. bicornis* (red = *O. bicornis rufa*, yellow = *O. bicornis cornigera*) in Europe as suggested by Peters (1978). The location of the populations used for our analyses are marked in England (Kent, Hereford, and Tonbridge), Germany (Halle, Regensburg, and Constance), and Denmark (Copenhagen, Vejle, and Møn)

being used for species recognition or isolation barriers in bees was lacking entirely until our research.

According to Peters (1978), *O. bicornis* is found throughout Europe mainly in two subspecies, *O. bicornis rufa* and *O. bicornis cornigera,* which are distinguishable only by the difference in coloration at the tip of the abdomen. Only on the balearic islands a third subspecies, *O. bicornis fracticornis,* can be found. The subspecies *O. bicornis cornigera* can be found allopatrically mainly in central Europe, whereas *O. bicornis rufa* is found allopatrically around the edges of Europe. However, there are two overlapping regions in which both subspecies are found sympatrically—one in northern Spain and one in Denmark, where hybrids are supposed to occur (Peters 1978) (Fig. 9.3).

Since the classification into these subspecies is solely based on one morphological trait, it is worthwhile to take a closer look at their behavior. The aim of our following research was, therefore, to first ascertain if the subspecies described by Peters (1978) do still mate with each other, and to then investigate the role of the male's vibrational signals in the female's choice. In order to establish if *O. bicornis* from different populations in Europe did indeed still mate with each other, or if some sort of isolation barriers were already in place, we first conducted cross-matings with bees from the allopatrically occurring *Osmia* from England and Germany, as well as bees from Denmark, where both subspecies are supposed to occur sympatrically.



	♂ 🇩🇪	♂ 🇬🇧	♂ 🇩🇰
♀ 🇩🇪	90% N=20	44% N=32	40% N=20
♀ 🇬🇧	20% N=20	83% N=18	60% N=20
♀ 🇩🇰	40% N=20	46% N=11	80% N=20

Fig. 9.4 Mating success of *O. bicornis* in cross-matings between England, Germany, and Denmark. Within country success differed significantly from between country success (GLM pairwise, *q*-values, $P < 0.05$)

Our results clearly showed that there is no pre-copulatory isolation in place as all of the different male/female combinations had at least some successful matings. However, the females clearly preferred to mate with males from their own country, i.e., the region of origin (Fig. 9.4). We, therefore, concluded that there is a female preference for the "own" population and we are potentially witnessing the beginning of an ongoing separation process (Conrad and Ayasse 2015).

The next question was obviously which characteristics of a male the female uses to recognize their region of origin. Although odor has been shown to be used by various bees in the past (Ayasse et al. 2001), we were interested to see if vibrations might be used in *Osmia* and how much they might factor into the female's decision. However, to test whether or not vibrations are the key, we had to design a set-up in which it was possible to change the vibrations a male produces without influencing any other parameters, such as odor or visual cues, e.g., the movement of the antennae or front legs. Because males are mounted on top of the female and the only way to establish rejection or acceptance is during a copulation attempt, this also had to happen on a live male without it being too invasive to hinder him in his usual mating behavior. We were able to develop an innovative new bioassay to test this by using small strong magnets as mini-vibrators and gluing them onto the males' thoraxes. We began by establishing mating pairs and then placed the pair on top of an inductor that was connected to a frequency generator equipped with a pre-recorded signal of a male. The signal was then transferred to the magnet through the electromagnetic field of the inductor, leading to vibrations in the desired frequency and modulation (Fig. 9.5). Fortunately, most males stopped emitting their own signals during this

Fig. 9.5 Setup of the bioassay. The pre-copulatory pair was placed on an iron core within the inductor. The signal of a successful male, produced by a frequency generator and then amplified, was then transmitted to the magnet on the back of the male via the electromagnetic field within the inductor

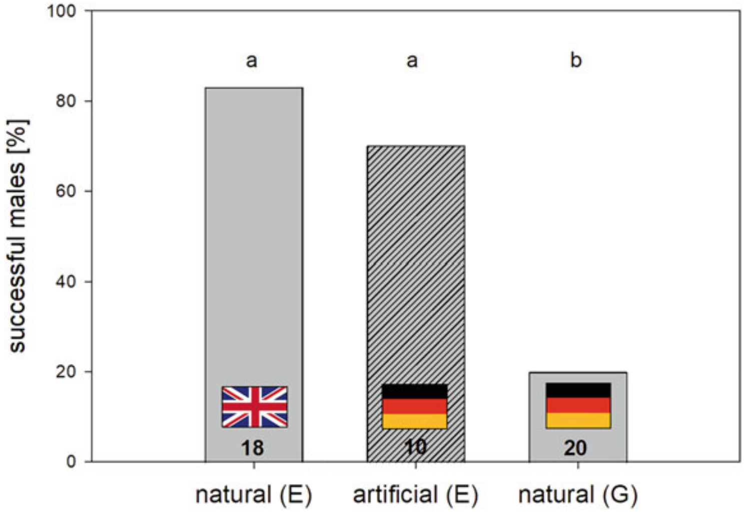


Fig. 9.6 Mating success of *O. bicornis* males with English females. Comparison of mating success of *O. bicornis* males of different origin (shown by flags) with a natural or an imposed artificial signal (E, English; G, German), which tried to mate with English *O. bicornis* females. The sample sizes are shown beneath each flag. Significant differences are marked by different letters (Waldchi-square, $\chi^2 = 13.804$, $df = 2$, $P < 0.05$; q-values <0.05)

process but continued their usual mating behavior, including copulation attempts. This enabled us to impose the signal of successful English males onto German males and vice versa. We used this combination as it was least successful in the previous cross-mating experiments (Conrad and Ayasse 2015).

When we changed the signal of a German bee to that of an English bee, while coupling it with an English female, the success rate of the males increased until it was not significantly different from that of the natural English males (Fig. 9.6). The same was true for English bees that had a German signal imposed on them, as they coupled with a German female. Since this could be a positive effect of the inductor set-up itself we also tried a control during which we changed a German male into one with an English signal while mating with a German female. As expected, the success rate decreased significantly (2 × 2 contingency table, $df = 1$, $P < 0.05$), confirming that the vibrational signal is used by the females to make their choices in this case (Conrad and Ayasse 2015). These experiments showed that females in *O. bicornis* are indeed able to use the vibrational signals emitted by the males to choose a male from their own country/region of origin, meaning that the signal does not only encode fitness, as we showed before, but also information about their origin.

However, our attempts to identify which part of the signal encodes this information have so far been inconclusive, as there are no discernible differences between frequency, pulse length, modulation range, or pulse pattern between English and German populations in Europe. Surprisingly, there is a difference in frequency and modulation range between German and Danish bees (Conrad and Ayasse 2019). We currently suspect that another aspect of the signal, which we have not been able to determine yet, may be responsible for this remarkable behavior. Another explanation is that the differences are extremely subtle and a much higher sample size is needed to identify them.

It is important to keep in mind that animals may be able to perceive differences in signals that researchers are currently unable to detect. There are many examples of this, yet only a few of them are published so far (pers. comm.). In experiments with the elm leaf beetle, for example, the beetle can distinguish between the odors of two differently treated elms. However, the difference was undetectable for the researchers (Büchel et al. 2013).

9.5 The Influence of Temperature on Vibrations

While there is still a, thankfully dwindling, minority of climate change deniers, it has become a sad reality that we are facing today (Parmesan and Yohe 2003). Fortunately, temperature changes and their effect on natural systems have moved into the focus of research to increase our knowledge of the challenges we might be facing. Many plant and animal species from various different taxonomical groups are already known to be affected in one way or another (Root et al. 2003; Pörtner and Knust 2007; Kearney et al. 2009; Sentis et al. 2013). Unfortunately, a disproportionate amount of research in insects has focused on more popular species from the Lepidoptera, Diptera, or Orthoptera, while orders with much higher species richness have been neglected (Andrew et al. 2013). Among the abiotic factors influencing an animal's life, temperature changes are of particular importance, not only because of climate change but also because they universally affect almost every organism to some degree during their life span (Chapman 1982). With insects being poikilotherms, they end up especially affected. More importantly, we know that in addition to the influence on bodily functions, temperature changes also affect communication systems, as has been shown in electric fish (Feng 1976), fireflies (Carlson et al. 1976), anurans (Zweifel 1968; Gayou 1984), and orthoptera (Walker 1975; Pires and Hoy 1992). However, studies on the effect of temperature on communication signals in animals and their consequence are scarce and often solely based on field work, as opposed to lab experiments under controlled conditions.

Research on the consequences temperature changes can have on acoustic signals in insects so far focuses on the Orthoptera, which have specialized sound-producing organs. In anurans and crickets, the studies show that temporal parameters of the song are particularly altered by temperature (Gayou 1984; Pires and Hoy 1992), leading to an increase in pulse rate with raising temperatures (Gayou 1984).

When female choice is at play, there are usually two ways a female can deal with a change in temperature when it comes to the male. One option is to take the change in temperature into account when evaluating the male's signal and then interpret it accordingly. As a result, female preference actually changes with temperature. We find that strategy, for example, in odor signaling in moths (Linn et al. 1988) and in acoustic signaling in crickets (Pires and Hoy 1992). The second possibility is that female preference stays the same regardless of temperature. In this case, those males whose signals are not affected by temperature would be chosen over those that cannot keep up their usual signal and thus have the advantage (Fig. 9.7).

There have always been high temperature fluctuations during the flight time of *Osmia* bees in spring. *Osmia bicornis* need temperatures around 15 °C to emerge from their nest. In recent years, temperature fluctuations during March, April, and June have led to temperatures of 25 °C regularly, and sometimes even more (Seidelmann 1991; Conrad personal observation). We, therefore, investigated the possible variations in male vibrational signals and female preference at different temperatures. For this, we once again recorded mating pairs in a controlled environment in a climate chamber, at either 17 °C–21 °C as a low-temperature setting, or 22 °C–26 °C as a high-temperature setting. Looking at the results we found a significant difference in pulse duration and dominant frequency for the rejected males but surprisingly not for the accepted males (Mann–Whitney U-Test, $P < 0.05$; Figs. 9.8 and 9.9). We, therefore, concluded that female preference for male vibratory signals does not change with temperature. However, at least some males are obviously unable to produce the desired signal, particularly at low temperatures. This means that males, who are able to produce the appropriate vibrations, may be able to mate much earlier during the day when temperatures are still low, which is actually a common situation in solitary bees. In my experience, the bees start flying as soon as the temperature reaches 15 °C and then stop activity when conditions become too hot (Conrad et al. 2017). Nevertheless, one can easily imagine that this clear advantage for "early risers" in males will diminish more and more as global temperatures rise. The bees will then either face a disruption in their mate choice, leading presumably to a loss of their temperature adaptations, or they might have to move to higher latitudes or elevations to avoid higher temperatures. With habitat loss already being a global issue, however, this might not be an option. This might ultimately lead to unforeseeable evolutionary changes in this species (Conrad et al. 2017).

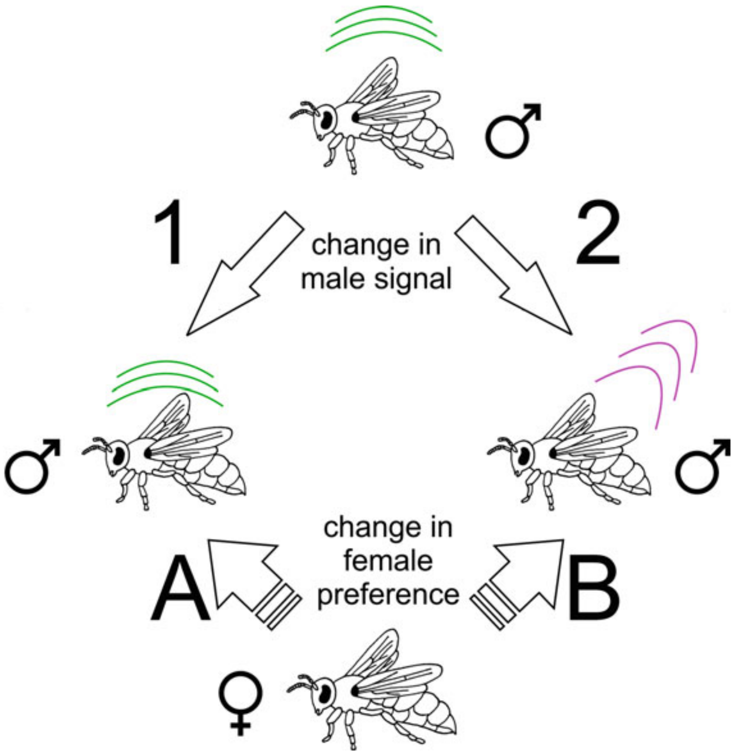


Fig. 9.7 Possible changes in male signals and female preference due to changes in temperature. There are two possible outcomes for a male signal subjected to temperature changes: either the signal stays the same (**1**) or the signal changes (**2**). Female preference can either stay the same (**a**) or females can adapt to a change in temperature with a change in preference for a, now changed, male signal (**b**)

9.6 Conclusion

Vibrational signals are obviously a vital part of the communication between male and female in *O. bicornis*. Not only is a representation of the male's physical fitness encoded in the signal but we have shown that information on the region of origin is also contained in these vibrations. This suggests once again the high potential vibrational signals have for encoding information, even if they look "simple" at first glance. With our studies on the red mason bee, we have barely scratched the surface when it comes to potential vibrational communication in solitary bees. With 30,000 bee species worldwide, I strongly believe it is worth taking a closer look at how different species use biotremology not only during their mating behavior but also in other behavioral contexts. The more we know about these important pollinators, the better we will undoubtedly understand how to protect and use them in regard to the challenges we face in the future. Rising temperature, an increasing

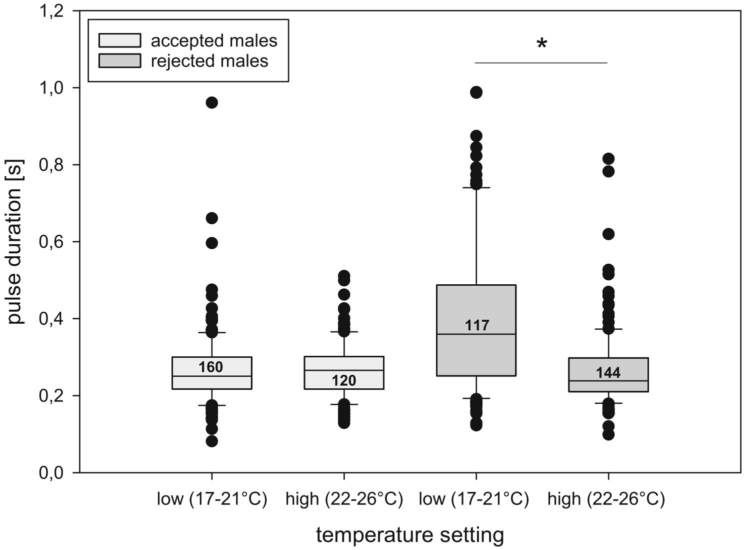


Fig. 9.8 Comparison of the pulse duration during different temperature settings between accepted and rejected males. The medians, quartiles, outliers (circles) and sample sizes (numbers) are shown. Significant differences are marked by an asterisk (* Mann–Whitney U-Test, $P < 0.05$). Rejected males showed significantly longer pulse durations at lower temperatures

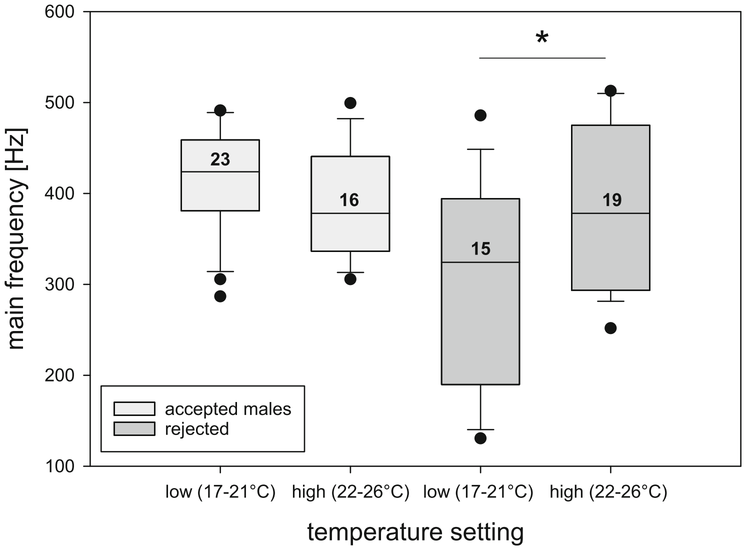


Fig. 9.9 Comparison of the dominant frequency during different temperature settings between accepted and rejected males. The medians, quartiles, outliers (circles) and sample sizes (numbers) are shown. Significant differences are marked by an asterisk (* *t*-Test, $P < 0.05$). Rejected males showed significantly lower dominant frequencies at lower temperatures

world population, and insufficient sustainable agriculture mean that this kind of research goes far beyond the purely intellectual search for knowledge. Finally, I hope that our methodology of using magnets and inductors proves useful in other experiments, in which vibrating the ground an animal stands on is not sufficient, but where the animal itself is actually the substrate.

Acknowledgments I am grateful to my husband Markus Conrad for comments on the manuscript. I would also like to thank my PhD supervisor Prof. Manfred Ayasse for making this work possible and for introducing me to the bees. Big thanks to everyone who has provided me with bees over the years: Robin Dean, Dr. Karsten Seidelmann, Dr. Erhard Strohm, Sabine Rademacher, Mike Hermann, Henrik F Brødsgard, Anja Amtoft Wynns, and Adam Bates. Valerie Littlewood and Iris Thiede-Conrad I would like to thank for permission for the use of their artwork (Figs. 9.4 and 9.5) Special thanks to Dr. Axel Schmid for the initial idea of using magnets in the bioassay, Dr. Sebastian Breckerbohm for help with the physics, and Carina Stöker and Melanie Marquardt for their help collecting data. This research was funded by the German Federal Environmental Foundation (DBU).

References

Andersson M (1994) Sexual selection. Princeton University Press, Princeton, NJ

Andersson M, Simmons LW (2006) Sexual selection and mate choice. Trends Ecol Evol 21:296–302

Andrew NR, Hill SJ, Binns M, Bahar MH, Ridley EV, Jung M-P, Fyfe C, Yates M, Khusro M (2013) Assessing insect responses to climate change: what are we testing for? Where should we be heading? PeerJ 1:e11. https://doi.org/10.7717/peerj.11

Ayasse M, Paxton RJ, Tengö J (2001) Mating behavior and chemical communication in the order hymenoptera. Annu Rev Entomol 46:31–78. https://doi.org/10.1146/annurev.ento.46.1.31

Basolo AL (1990) Female preference predates the evolution of the sword in swordtail fish. Science 250:808–810. https://doi.org/10.1126/science.250.4982.808

Bergman P, Bergström G (1997) Scent marking, scent origin, and species specificity in male premating behavior of two Scandinavian bumblebees. J Chem Ecol 23:1235–1251. https://doi.org/10.1023/B:JOEC.0000006461.69512.33

Boake CRB (2002) Sexual signaling and speciation, a microevolutionary perspective. Genetica 116:205–214. https://doi.org/10.1023/A:1021224207727

Bornemissza GF (1966) Observations on the hunting and mating behaviours of two species of scorpion flies (Bittacidae Mecoptera). Aust J Zool 14:371. https://doi.org/10.1071/ZO9660371

Brown JL (1975) The evolution of behavior. Norton, New York

Büchel K, Austel N, Mayer M, Gershenzon J, Fenning T, Meiners T (2013) Smelling the tree and the forest: Elm background odours affect egg parasitoid orientation to herbivore induced terpenoids. BioControl 59:1–15. https://doi.org/10.1007/s10526-013-9544-9

Carlson AD, Copeland J, Raderman R, Bulloch AGM (1976) Role of interflash intervals in a firefly courtship (*Photinus macdermotti*). Anim Behav 24:786–792. https://doi.org/10.1016/S0003-3472(76)80009-7

Chapman RF (1982) The insects: structure and function. Harvard University Press, Cambridge, MA

Claridge MF, DeVrijer PWF (1993) Reproductive behavior: the role of acoustic signals in species recognition and speciation. In: Denno RF, Perfect TJ (eds) Planthoppers, their ecology and management. Chapman & Hall, New York

Clarke FM, Faulkes CG (1999) Kin discrimination and female mate choice in the naked mole-rat *Heterocephalus glaber*. Proc R Soc B Lond 266:1995–2002

Čokl A, Virant-Doberlet M, Stritih N (2000) The structure and function of songs emitted by southern green stink bugs from Brazil, Florida, Italy and Slovenia. Physiol Entomol 25:196–205. https://doi.org/10.1046/j.1365-3032.2000.00187.x

Conrad T, Ayasse M (2015) The role of vibrations in population divergence in the red mason bee, *Osmia bicornis*. Curr Biol 25:2819–2822. https://doi.org/10.1016/j.cub.2015.08.059

Conrad T, Ayasse M (2019) The differences in the vibrational signals between male *O. bicornis* from three countries in Europe. J Low Freq Noise Vibr Act Control 38:871–878. https://doi.org/10.1177/1461348418816263

Conrad T, Paxton RJ, Barth FG, Francke W, Ayasse M (2010) Female choice in the red mason bee, *Osmia rufa* (L.) (Megachilidae). J Exp Biol 213:4065–4073. https://doi.org/10.1242/jeb.038174

Conrad T, Stöcker C, Ayasse M (2017) The effect of temperature on male mating signals and female choice in the red mason bee, *Osmia bicornis* (L.). Ecol Evol 1:8966–8975. https://doi.org/10.1002/ece3.3331

Coyne JA, Orr HA (2004) Speciation. Sinauer Associates, Sunderland, MA

Darwin C (1871) The descent of man and selection in relation to sex. Murray, London

Dimijian GG (2005) Evolution of sexuality: biology and behavior. Baylor Univ Med Center Proc 18:244–258

Endler JA (1986) Natural selection in the wild. Princeton University Press, Princeton, NJ

Feng AS (1976) The effect of temperature on a social behavior of weakly electric fish *Eigenmannia virescens*. Comp Biochem Phys A 55:99–102. https://doi.org/10.1016/0300-9629(76)90074-8

Gavrilets S, Boake CRB (1998) On the evolution of premating isolation after a founder event. Am Nat 152:706–716. https://doi.org/10.1086/286201

Gayou DC (1984) Effects of temperature on the mating call of *Hyla versicolor*. Copeia 1984:733–738. https://doi.org/10.2307/1445157

Gillham MC (1992) Variation in acoustic signals within and among leafhopper species of the genus *Alebra* (Homoptera, Cicadellidae). Biol J Linn Soc 45:1–15. https://doi.org/10.1111/j.1095-8312.1992.tb00628.x

Hill PSM (2008) Vibrational communication in animals. Harvard University Press, London

Hoy RR, Hoikkala A, Kaneshiro K (1988) Hawaiian courtship songs: evolutionary innovation in communication signals of *Drosophila*. Science 240:217–219. https://doi.org/10.1126/science.3127882

Hrncir M, Barth FG (2014) Vibratory communication in stingless bees (Meliponini). In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 349–374

Kearney M, Shineb R, Porter WP (2009) The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. Proc Natl Acad Sci USA 106:3835–3840

Koeniger N, Koeniger G (2000) Reproductive isolation among species of the genus *Apis*. Apidologie 31(2):313–339

Kose M, Møller AP (1999) Sexual selection, feather breakage and parasites: the importance of white spots in the tail of the barn swallow (*Hirundo rustica*). Behav Ecol Sociobiol 45:430–436

Lande R (1981) Models of speciation by sexual selection on polygenic traits. Proc Natl Acad Sci USA 78:3721–3725

Larsen ON, Gleffe G, Tengö J (1986) Vibration and sound communication in solitary bees and wasps. Physiol Entomol 11:287–296. https://doi.org/10.1111/j.1365-3032.1986.tb00416.x

Larsson FK (1991) Some take it cool, some like it hot: a comparative study of male mate searching tactics in two species of Hymenoptera (Colletidae and Sphecidae). J Therm Biol 16:45–51

Linn CE, Campbell MG, Roelofs WL (1988) Temperature modulation of behavioural thresholds controlling male moth sex pheromone response specificity. Physiol Entomol 13:59–67

Michelsen A (2014) Mechanical signals in honeybee communication. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 333–347

Michener CD (2000) The bees of the world. John Hopkins University Press, Baltimore, MD

Miklas N, Čokl A, Renou M, Virant-Doberlet M (2003) Variability of vibratory signals and mate choice selectivity in the southern green stink bug. Behav Process 61:131–142. https://doi.org/10.1016/s0376-6357(02)00186-9

O'Toole C (2000) The red mason bee: taking the sting out of bee-keeping: a practical guide to managing *Osmia rufa* as a pollinator in gardens, allotments, and orchards. Osmia Publications, Banbury, UK

Panhuis TM, Butlin R, Zuk M, Tregenza T (2001) Sexual selection and speciation. Trends Ecol Evol 16:364–371. https://doi.org/10.1016/s0169-5347(01)02160-7

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42. https://doi.org/10.1038/nature01286

Peters DS (1978) Systematik und Zoogeographie der west-paläarktischen Arten von *Osmia* sstr, *Monosmia* und *Orientosmia*. Senckenberg Biol 58:287–346

Pires A, Hoy RR (1992) Temperature coupling in cricket acoustic communication. J Comp Physiol A 171:69–78

Pörtner HO, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315:95–97. https://doi.org/10.1126/science.1135471

Roces F, Manrique G (1996) Different stridulatory vibrations during sexual behaviour and disturbance in the blood-sucking bug *Triatoma infestans* (Hemiptera: Reduviidae). J Insect Physiol 42:231–238

Rodríguez RL, Sullivan LE, Cocroft RB (2004) Vibrational comunication and reproductive isolation in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). Evolution 58:571–578

Root TL, Price JT, Hall KR, Schneider SH (2003) Fingerprints of global warming on wild animals and plants. Nature 421:57–60. https://doi.org/10.1038/nature01309

Ryan MA, Cokl A, Walter GH (1996) Differences in vibratory sound communication between a Slovenian and an Australian population of *Nezara viridula* (L.) (Heteroptera: Pentatomidae). Behav Process 36:183–193. https://doi.org/10.1016/0376-6357(95)00026-7

Seidelmann K (1991) Ausgewählte Aspekte der Populationsökologie der Roten Mauerbiene, *Osmia rufa* (L.), untersucht in Stammzuchten. Diploma thesis, Martin-Luther University, Halle-Wittenberg, Germany

Seidelmann K (1995) Untersuchungen zur Reproduktionsbiologie der Roten Mauerbiene, *Osmia rufa* (L., 1758). PhD thesis, Martin-Luther University Halle-Wittenberg, Germany

Sentis A, Hemptinne J-L, Brodeur J (2013) Effects of simulated heat waves on an experimental plant-herbivore-predator food chain. Glob Chang Biol 19:833–842. https://doi.org/10.1111/gcb.12094

Turner GF, Burrows MT (1995) A model of sympatric speciation by sexual selection. Proc R Soc B Lond 260:287–292. https://doi.org/10.1098/rspb.1995.0093

Valido A, Rodríguez-Rodríguez MC, Jordano P (2019) Honeybees disrupt the structure and functionality of plant-pollinator networks. Sci Rep 9:4711. https://doi.org/10.1038/s41598-019-41271-5

Vallejo-Marín M (2018) Buzz pollination: studying bee vibrations on flowers. New Phytol. https://doi.org/10.1111/nph.15666

Walker T (1975) Effects of temperature on rates in poikilotherm nervous systems: evidence from the calling songs of meadow katydids (Orthoptera: Tettigoniidae:Orchelimum) and reanalysis of published data. J Comp Physiol A 101:57–69. https://doi.org/10.1007/BF00660119

West-Eberhard MJ (1983) Sexual selection, social competition, and speciation. Q Rev Biol 58: 155–183

Westrich P (1989) Die Wildbienen Baden-Württembergs I, II. Eugen Ulmer Verlag, Stuttgart

Zweifel RG (1968) Effects of temperature, body size, and hybridization on mating calls of toads, *Bufo americanus* and *Bufo woodhousii fowleri*. Copeia 1968:269–285

Chapter 10
Vibrational Signals in Multimodal Courtship Displays of Birds

Nao Ota and Masayo Soma

Abstract Birds often combine vocalization and body movements for communication with other individuals, but our knowledge of this multimodal communication is primarily limited to the vocal and visual signals. Since birds often stay and communicate upon a substrate that seems to be efficient in the transmission of substrate-borne vibrations (e.g., courtship bobbing on a branch), it is rather surprising that vibrational signals have been almost completely overlooked in past studies of their communication. In the blue-capped cordon-bleu (*Uraeginthus cyanocephalus*), a socially monogamous songbird, both sexes perform courtship displays that are characterized by singing and simultaneous visual displays. We previously found that their courtship bobbing includes rapid tap dance-like behavior, which is assumed to produce vibrations that propagate through the perch and non-vocal sounds in addition to visual signals. In this chapter, we introduce our previous findings of this multimodal courtship display as well as quantitative analyses of substrate-borne vibrations produced by cordon-bleus. We will also discuss the potential of vibrational signals for bird communication by describing their possible functions and evolutionary scenarios. We believe that investigating vibrational communication in birds can provide fruitful insights into the role of vibrations in the evolution of complex communication systems.

N. Ota (✉)
Department of Behavioural Neurobiology, Max Planck Institute for Ornithology, Seewiesen, Germany
e-mail: nota@orn.mpg.de

M. Soma
Department of Biology, Faculty of Science, Hokkaido University, Sapporo, Japan
e-mail: masayo.soma@sci.hokudai.ac.jp

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_10

10.1 Introduction

To obtain a clear understanding of complex communication systems, it is of great importance to investigate its various modalities and components. Birds often combine vocalization (i.e., song) with body movements (i.e., dance) during courtship. Several studies reported that dances are coordinated with songs in male songbirds (Cooper and Goller 2004; Dalziell et al. 2013; Ullrich et al. 2016). Such coordination of multimodal signals presumably contributes to enhancing signal efficacy while sexually stimulating females (O'Loghlen and Rothstein 2010).

Past studies of multimodal communication in birds typically focused on a combination of vocal and visual signals; whereas, less attention has been paid to other modalities and components. However, several studies reported that body movements of birds can produce various types of signals in addition to vocal and visual. For instance, male manakins (Pipridae) produce non-vocal sounds by stridulating using wing feathers during courtship (Bostwick 2000; Bostwick and Prum 2005). Hummingbirds (Trochilidae) also produce non-vocal sounds from the wing and tail feathers during courtship flights (Clark et al. 2011, 2018). Mourning doves (*Zenaida macroura*; Coleman 2008) and crested pigeons (*Ocyphaps lophotes*; Hingee and Magrath 2009) are known to produce non-vocal sounds from the wing feathers during flight in the context of predator avoidance. The Java sparrow (*Lonchura oryzivora*) produces bill-clicking sounds that are coordinated with song note sequences (Soma and Mori 2015). The dark eyed junco (*Junco hyemalis*) produces an odor signal by bill wiping against its perch or other available substrates and releasing residual preening oils and waxes on the bill (Whittaker et al. 2015). These communicative components have been relatively neglected compared to visual and vocal signals in previous bird communication studies. We still have very little knowledge about these signal production mechanisms and their functions.

Vibrational communication has been found in a wide range of taxa (e.g., insects, spiders, mammals, reptiles, amphibians, fishes; review in Hill 2009). Nevertheless, to our knowledge, there are no behavioral studies that have quantitatively investigated the role of substrate-borne vibrations in bird communication. This is surprising because both the ecology and behavior of birds seem to be suitable for vibrational communication. For instance, songbirds often perch on a thin branch and show specific body movements such as bobbing and bill wiping for courtship (c.f., courtship displays in Estrildid finches; Goodwin 1982). Such body movements would generally function as visual signals, but vibrations can also be simultaneously transmitted via the perching substrate. In fact, spiders use vibrations produced by bird pecking behavior as a predation cue (Lohrey et al. 2009). Even vocalizations can generate substrate vibrations in addition to airborne sounds (Caldwell 2014). Although we still have no direct evidence of the vibrational communication occurring among birds, a recent study reported that bird embryos use substrate-borne vibrations to get information about predation risks (Mariette and Buchanan 2019; Noguera and Velando 2019).

While substrate-borne vibrations have been predominantly overlooked in bird communication, we previously uncovered a novel behavioral mechanism of dance display in songbirds that seems to contribute to the production of non-vocal sounds and vibrations. In this chapter, we describe our past findings of the unique multimodal dance display in a socially monogamous songbird, blue-capped cordon-bleus (Estrildidae: *Uraeginthus cyanocephalus*; hereinafter "cordon-bleus"). We expect that the multimodal courtship display of cordon-bleus has great potential to advance our knowledge of vibrational signals and their functions in birds. In addition, we examined the relationship between vibrational amplitude and dance performances, which to our knowledge is the first quantitative analysis of substrate-borne vibrations in bird communication behavior. We also introduce the results of phylogenetic comparative analysis in Estrildid finches—including cordon-bleus—to shed light on the evolutionary processes of vibrational signals in their courtship communication. Based on these implications, we discuss the potential roles of vibrational signals in bird communication and propose future research directions.

10.2 Behavioral Analyses of Multimodal Mutual Courtship Display in Cordon-Bleus

The blue-capped cordon-bleu is a socially monogamous and gregarious songbird. They have sexually dimorphic plumage (i.e., males have blue-caps) but both sexes perform conspicuous courtship display in the same manner. During the display cordon-bleus hold a piece of nesting material while bobbing up and down and singing (Goodwin 1982; Ota et al. 2015). Interestingly, cordon-bleu courtship bobbing produces rhythmical airborne sounds (Fig. 10.1b, c). Such conspicuous non-vocal sounds are generally not observed when other songbirds perform courtship bobbing (personal observations in Java sparrows, see also Sect. 10.4). Therefore, we expected that the cordon-bleu dance display involved some special behavioral mechanism to produce non-vocal sounds.

As a result of observations of cordon-bleu dance display using high-speed camera recordings (300 frames/s), we discovered that their visual courtship display includes rapid step-dancing, which is similar to human tap-dancing (Fig. 10.1; Ota et al. 2015). Within a single bobbing motion, cordon-bleus hop with their heads pointed upwards and stamp their feet several times (Fig. 10.1; Ota et al. 2015). These movements are so rapid that they are invisible to the human naked eye.

We have conducted behavioral analyses of these dance displays, using both normal- and high-speed movies, and found that the performances vary both among and within individuals (Fig. 10.2; Ota et al. 2015). No significant sex differences were detected in dance performances, while the number of steps in one bobbing did differ significantly between individuals (Ota et al. 2015). This implies that female cordon-bleus have the ability to perform dance displays as complex as those of males and that the dance display may convey individual information other than sex, such as

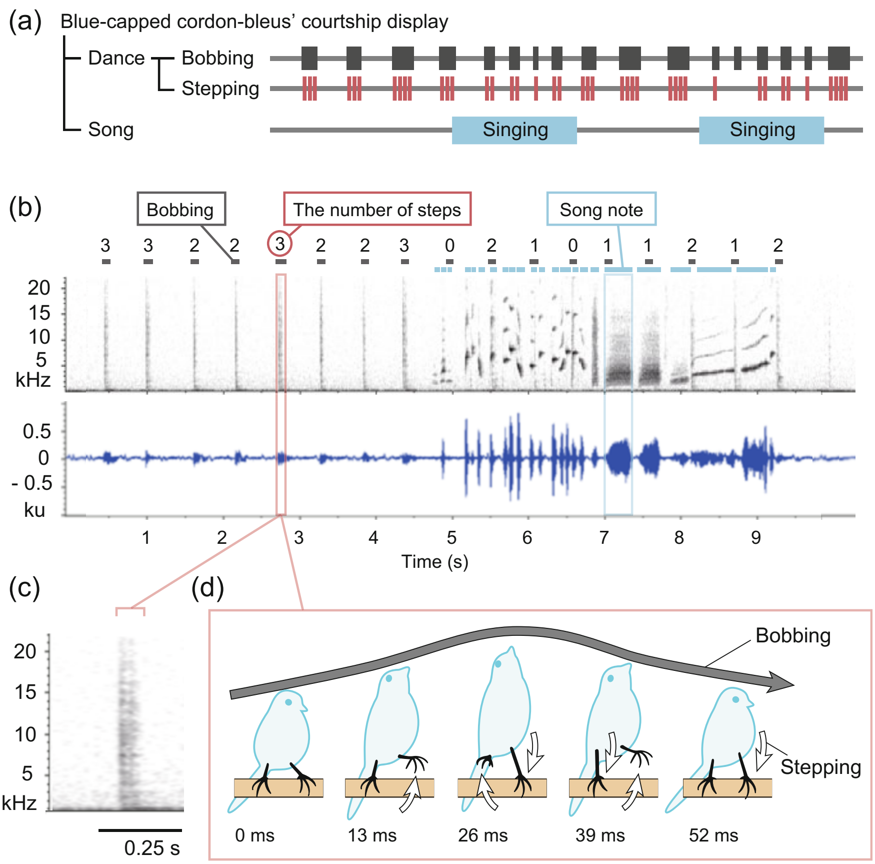


Fig. 10.1 (**a**) An example of cordon-bleu courtship display sequence. Bobbing and stepping occur simultaneously, and singing at certain times. (**b**) Spectrogram and waveform of cordon-bleu courtship display; numbers at the top of the spectrogram indicate how many steps were performed to produce non-vocal sounds. (**c**) Enlarged section of the step sound spectrogram; even though cordon-bleus usually perform stepping behavior several times per bobbing, the sound appears as a single pulse on the spectrogram. (**d**) Diagram of step behavior producing non-vocal sounds. (**b–d** modified from Ota et al. 2017 under authors' copyright)

physical abilities and breeding motivation. The result was surprising because we expected that the male performances would be more exaggerated than those of females, as is observed in plumage (Goodwin 1982) and song (Geberzahn and Gahr 2011).

Dance performances also varied at the within-individual level, according to the social condition and singing activity (Fig. 10.2; Ota et al. 2015). Cordon-bleus intensified their dance performances when their mate remained nearby. Specifically, both the number of steps in one bobbing and bobbing tempo (number of bobs/sec) were increased when a mate stayed on the same perch. Dance performances were also altered depending on singing behavior. During singing, cordon-bleus performed

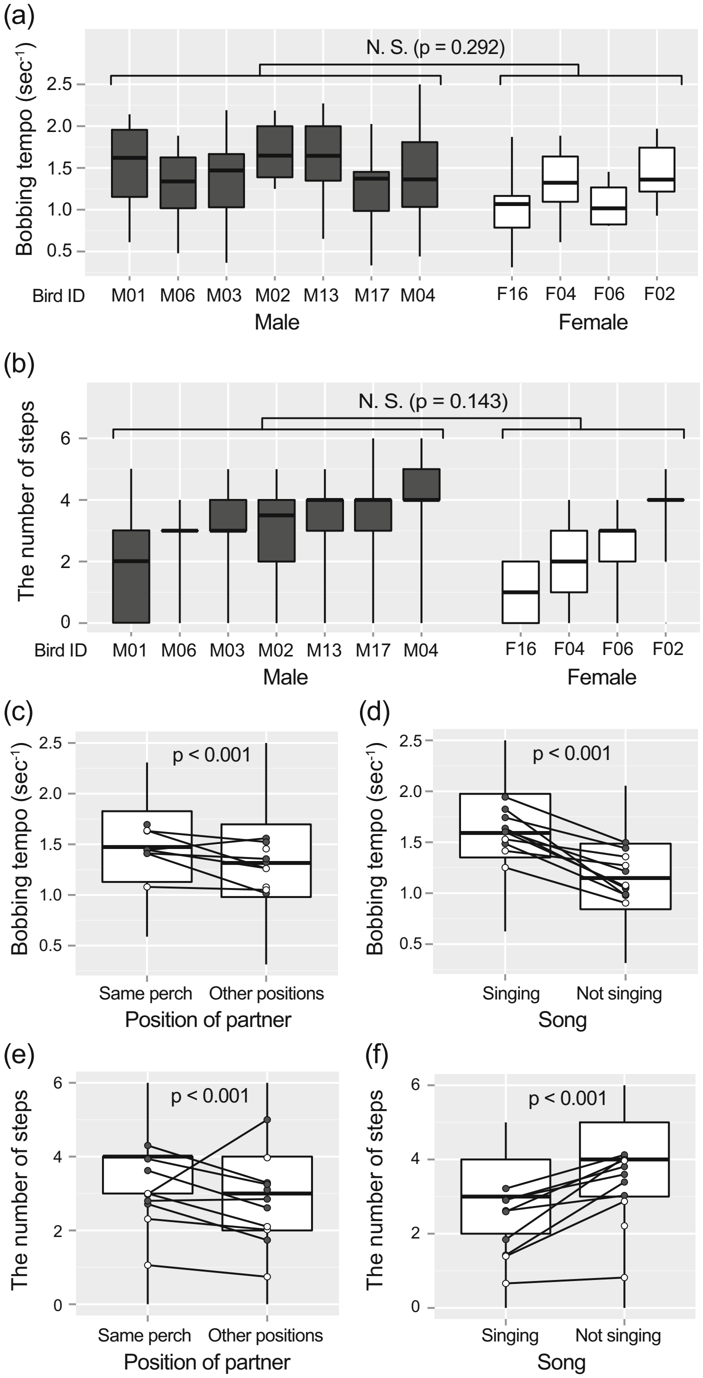


Fig. 10.2 Individual differences of (**a**) bobbing tempo and (**b**) number of steps per bob (shaded bars: males, white bars: females). Bobbing tempo depended on whether (**c**) partner was on the same perch,

fewer numbers of steps within a single bobbing but increased the bobbing tempo. This context-dependent adjustability seems to enable cordon-bleus to exchange multimodal signals, including substrate-borne vibrations, effectively between males and females.

Cordon-bleu courtship displays are unique in at least two ways. First, this specific tap dance-like display has not yet been reported in songbirds and presumably produces non-vocal sounds and/or substrate-borne vibrations in addition to song. It is novel that "songbirds" produce multimodal complex signals by dancing, since they were previously assumed to use songs as a primary sexual communication tool, as the name suggests (e.g., Catchpole and Slater 2008). Second, our findings that both sexes of cordon-bleus produce multimodal courtship displays are puzzling from the pervasive view of courtship display as an epigamic male trait evolved through sexual selection (Andersson 1994). The courtship display of cordon-bleus exhibits features that are analogous to dance displays of male manakins in that it includes acrobatic movements and often production of non-vocal sounds (Bostwick and Prum 2005; DuVal 2007; Fusani and Schlinger 2012). However, manakins are a lekking and non-song-learning species, so they differ from cordon-bleus in vocal learning ability and behavioral mutuality. Song duetting is a well-known example of mutual courtship display of males and females in songbirds (Hall 2004), but the female dance display and its multimodalities have been overlooked in past communication studies.

The complex courtship display of cordon-bleus raises interesting questions concerning the evolution and functions of multimodal courtship signals, including substrate-borne vibrations. Our findings emphasize that, even in songbirds, dance display plays an important role for sexual communication and the body movements can produce substrate-borne vibrations and non-vocal sounds in addition to visual signals.

10.3 How Can Vibrations Serve as a Signal in Cordon-Bleus?

In order for a signal to be successful in communication, there are two main requirements for the signal design (Guilford and Dawkins 1993). Firstly, the signal must be transmitted successfully through the medium (e.g., air, water, signaling substrate) to be perceived by receivers. Secondly, the signal must reflect certain information, such as the signaler's quality and motivation. In Sect. 10.3, we will

---

Fig. 10.2 (continued) and (**d**) birds were singing. Similarly, the number of steps depended on whether (**e**) the partner was on the same perch, and (**f**) birds were singing. (**a**–**f**) All box plots show medians, quartiles, and minimum and maximum values. (**c**–**f**) Mean individual values (closed circles: males, open circles: females) and their within-individual changes are indicated (modified from Ota et al. 2015 under authors' copyright) discuss and examine how substrate-borne vibrations can work effectively as a signal in fluctuating environments and what information can be transmitted via vibrations by describing our findings in cordon-bleus.

10.3.1 Signal Efficacy of Substrate-Borne Vibrations

Since non-sound vibrations are generally transmitted through a substrate (Hill 2009), substrate properties can be critical factors for successful transmission and accurate reception of vibrations (Elias and Mason 2014). For instance, wolf spiders (*Schizocosa ocreata*) produce more visual versus vibrational signals when on surfaces precluding the transmission of substrate-borne vibrations (Gordon and Uetz 2011). Male jumping spiders (*Phidippus clarus*) have higher mating success when a male and a female are present on substrates that are effective for producing propagating vibrations (Elias et al. 2004).

Ecological environments of birds, while not in flight (e.g., on a branch or the ground), seem to be generally effective for producing vibrations via the substrate; although, it varies widely depending on species and should be carefully considered. Information concerning ecology and behavior of cordon-bleus in the wild is limited, but according to the literature their habitat comprises arid areas with bushes and trees (Goodwin 1982). Ota (2020) recently conducted fieldwork in Tanzania to observe courtship displays of wild cordon-bleus. The field observations revealed that cordon-bleus performed a tap-dance-like display upon trees and often shook branches (Ota 2020). The results implied that wild environments of cordon-bleus are likely to meet the criteria that allow for the production of substrate-borne vibrations (see Sect. 10.6.1).

Positions of signal receivers (i.e., potential mates) are also important for successful vibration transmission. Receivers must remain on the same substrate as signal senders in order to perceive substrate vibrations. Cordon-bleus, as well as other Estrildid finches, are gregarious and court in close proximity, particularly when a male and a female perch side-by-side (Goodwin 1982; Ota et al. 2015; Ota 2020). Partners remaining nearby to a signal sender are usually sexually motivated and show specific responses for courtship display (e.g., bill wiping, tail flicking, angling their tails toward their partner; Goodwin 1982; Ota et al. 2018). Within such a close distance, vibrational signals can be exerted with higher efficacy; thus, vibrations via perch may be effective for expressing the motivation of a signal sender toward a potential partner.

10.3.2 Information Within Vibrations: Quantitative Analyses of Vibrational Amplitude during Dance Display

Generally speaking, sexual signals are favored by mate choice or intrasexual competition when they can serve as honest indicators of individual condition (Andersson 1994). We previously found that cordon-bleus exhibit individual differences in the number of steps performed within a single bobbing (Ota et al. 2015). We also revealed that the amplitude of non-vocal sounds increased depending on the number of steps (Fig. 10.3; Ota et al. 2017). The amplitude range of non-vocal sounds during courtship was within the range of song amplitude, and was louder than other non-courtship context foot movements (Ota et al. 2017). This suggests that non-vocal sounds during courtship are not simply byproduct sounds from movement. These results support the idea that cordon-bleus produce non-vocal sounds to convey individual information.

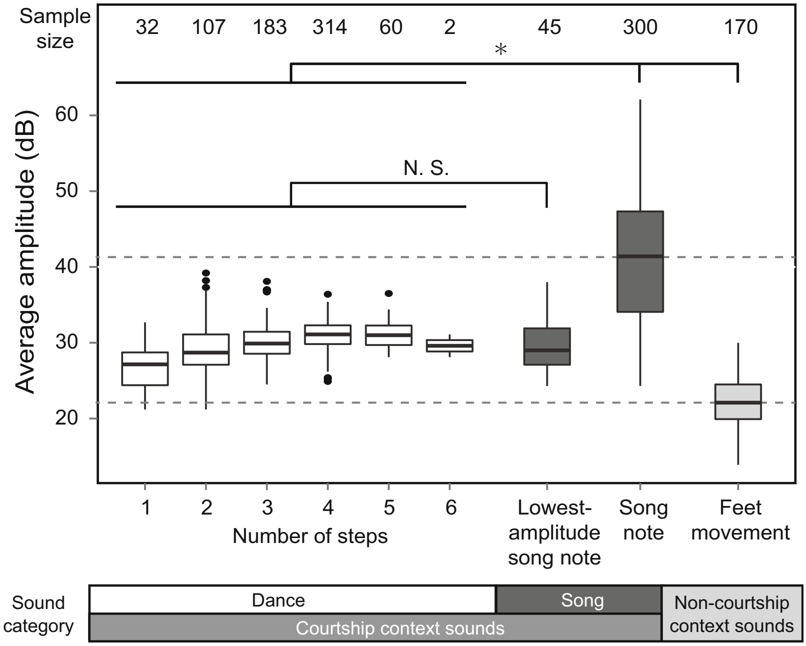


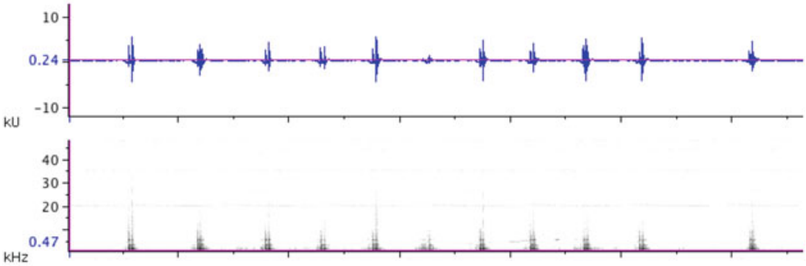
Fig. 10.3 Effect of the number of steps on sound amplitude, plotted as a function of the number of steps compared with that of the lowest-amplitude song notes (c.f. Ota et al. 2017), arbitrarily chosen song notes, and foot movement sounds. Stepping sounds were significantly louder than foot movement sounds but quieter than that of song notes. Although amplitude of arbitrarily chosen song notes was clearly higher than step sound amplitude, significance disappeared when comparing step sound amplitude with that of the lowest-amplitude song notes. Numbers above represent sample size; box plots show medians and quartiles; outliers are plotted as points (modified from Ota et al. 2017 under authors' copyright)

Fig. 10.4 Recording box conditions. Accelerometers were equipped under branches. Photo by authors

Likewise, we expected that substrate-borne vibrations would also reflect some traits of dance display for signaling individual information (Ota et al. 2017). To test this, we measured vibrational amplitude using accelerometers and compared it to the number of steps in the cordon-bleu courtship display. We introduced a male and a female in a recording box containing two perches with accelerometers equipped under each perch (Fig. 10.4; adapted by Reinhard Biller, MPIO Seewiesen). To digitize the vibrational signals, we used a multi-channel A/D converter (Fast Track Ultra 8R; M-Audio, Cumberland, RI, USA) connected to a PC. Each of the resulting digitized signals were recorded in parallel using multi-channel software (16-bit, 44,100 Hz; ASIO Rec, adapted by Markus Abels, MPIO Seewiesen). In addition to recording the vibrations, we filmed dance display using normal- (Q3HD ZOOM, Japan; NTSC, 30 frames/s) and high-speed (GZ-E355 Victor, Japan; 300 frames/s) cameras, enabling us to observe airborne sounds and substrate-borne vibrations simultaneously (Fig. 10.5). Cordon-bleus in the recording box were provided with finch seed mixture, cuttlebone, water, and cucumber ad libitum; nests and nesting materials were always available in their cages. All procedures were in accordance with German National Laws and approved by the Government of Upper Bavaria.

We measured vibrational amplitude of a male cordon-bleu dance display using Raven Pro 1.4 (www.birds.cornell.edu/raven) and counted the number of steps during a single bobbing session from a high-speed movie. Since dance performances vary depending on whether cordon-bleus sing or not (Ota et al. 2015), we checked sound data using a normal-speed camera to confirm if cordon-bleus sing while dancing. To assess whether dance performances influence sound amplitude, we investigated the effects of the number of steps and singing activity on vibrational amplitude using a linear model (LM). All statistical analyses were performed using R 3.3.0 (www.r-project.org).

(a) Recorded by an accelerometer



(b) Recorded by a microphone

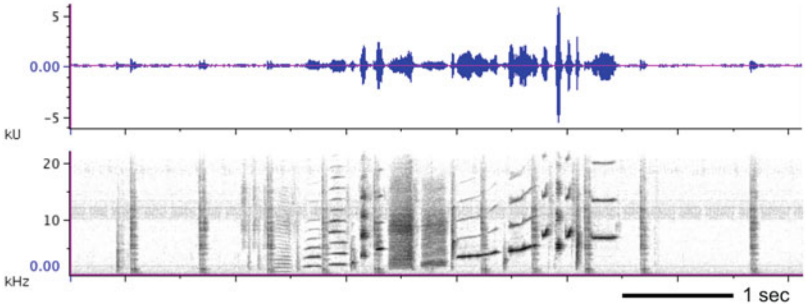


Fig. 10.5 Spectrograms and waveforms from accelerometer (**a**) and microphone within a normal-speed camera (**b**)

As a result, we found vibrational amplitude increased depending on the number of steps (Fig. 10.6 and Table 10.1). Although further behavioral experiments are still needed (see Sect. 10.6.1), the results support the idea that substrate-borne vibrations, as well as non-vocal sounds, can serve as a signal to convey individual information (Ota et al. 2017). Interestingly, vibrational amplitude observed during singing was decreased compared to that observed when birds were not singing, even if the number of steps were equal, while the positive correlation between vibrational amplitude and number of steps was observed both during singing and not singing (Fig. 10.6 and Table 10.1). The low vibrational amplitude during singing can be explained from the perspective of motor constraints because performing multiple behavioral components (i.e., dance and song) is assumed to be energetically demanding (reviewed in Mitoyen et al. 2019).

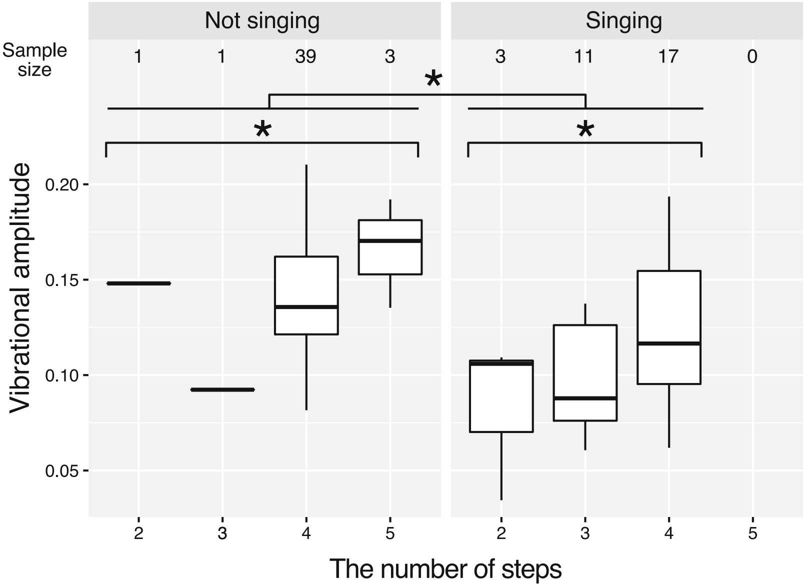


Fig. 10.6 Effects of the number of steps on vibrational amplitude either while not singing (left) or during singing (right). Numbers above represent sample size; box plots show medians and quartiles. * $p < 0.05$

Table 10.1 Effects of the number of steps in one bobbing and singing activity on vibrational amplitude: a result of LM analyses. Significant p-values are indicatd in bold

Coefficient		SE	*t*	*p*
(Intercept)	0.060	0.028	2.164	0.034
The number of steps	0.020	0.007	2.942	**0.004***
Singing activity	−0.019	0.008	−2.225	**0.029***

*$p < 0.05$

10.4 Possible Roles of Vibrational Signals

Multimodal signals can be divided into two primary types, depending on if they include the same information or not (Johnstone 1996; Partan and Marler 2005). If multimodal signals convey the same information, they function as a redundant signal for increase of the detectability and accuracy of signals. Redundant signals have also been labeled "backup signals" since they can work as coverage for each other in fluctuating environments (Johnstone 1996). Second, if multimodal signals convey different messages, respectively, and/or express new information by simultaneous production, they function as non-redundant signals. Non-redundant signals can contribute to gaining more information and achieving effective transmission.

Although the two hypotheses appear potentially contradictory, they are not necessarily mutually exclusive. The redundant signal hypothesis is likely to be applicable to multimodal signals produced by a single behavioral component (e.g., visual signal, substrate-borne vibrations, and non-vocal sounds produced by dancing), while the non-redundant signal hypothesis seems to be helpful for elucidating multimodal signals produced by multiple behavioral components (e.g., simultaneous song and dance). Based on these two hypotheses, here we discuss what information can be conveyed during courtship and how vibrational signals can work in the multimodal communication of cordon-bleus. Additionally, we will discuss to whom and for what substrate-borne vibrations and other multimodal signals are produced.

10.4.1 Vibrations as a Redundant Signal

We have revealed that substrate-borne vibrations and non-vocal sound amplitude reflect the number of steps performed per bobbing in cordon-bleus (Ota et al. 2017; see Sect. 10.3.2). These results strongly suggest that the multimodal signals produced by dance display convey the same information and work as redundant signals. Vibrations may contribute to accelerating detection of signalers and enhance responses from receivers. For example, vibrations improve identification and location of signal/cue senders in insects (Devetak 2014). Simultaneous production of multimodal signals can enhance receiver responses when compared to unimodal signal production in spiders (Uetz et al. 2009).

Furthermore, producing redundant multimodal signals has an advantage in fluctuating environments since effective range and environmental conditions of signals vary depending on modalities (Partan 2017). For instance, male wolf spiders produce more substrate-borne vibrations in dark conditions while employing more visual signals in the light when they are on substrates that attenuate vibrational signals (Taylor et al. 2005; Gordon and Uetz 2011). Visual and acoustic signals generally have a broader effective range compared to vibrational signals. Tap dance-like movements (estimated rate of 25 and 50 Hz; Ota et al. 2015) are likely to be visible for cordon-bleus since the limit of flicker fusion frequency in birds is over 100 Hz under appropriate light conditions (Jones et al. 2007). Non-vocal sound amplitude is close to song amplitude so that non-vocal sounds can be heard by surrounding signal receivers (Ota et al. 2017). Vibrations might function as a backup for visual and acoustic signals when a location is shaded and/or the sound channel is noisy.

10.4.2 Vibrations as a Non-redundant Signal

While multimodal signals produced by dancing are likely to convey the same information as redundant signals, different information may be added when the dance display overlaps with song. The ability to generate one signal is often constrained by simultaneous production of other signals, and multimodal signaling in itself can be an indication of signaler ability (Hebets and Papaj 2005; Mitoyen et al. 2019).

Simultaneous performing of song and dance requires precise temporal coordination and is likely physically demanding in cordon-bleus; therefore, it may provide additional information of signaler quality. Cordon-bleu dance performances vary depending on singing activity (Sect. 10.2; Fig. 10.2; Ota et al. 2015). Fewer steps during singing can be explained from the perspective of motor constraints because dance displays and singing are both physically demanding. In contrast, increased bobbing tempo cannot be explained by simple trade-offs between singing and dancing activities. We are still not sure if cordon-bleus adjust bobbing tempo deliberately or are constrained during singing, but such changes appear to contribute to better song–dance coordination. Cordon-bleus usually sing songs several times per courtship sequence (Fig. 10.1; Ota et al. 2015). Repetitive production of a well-coordinated song and dance can be an indicator of physical ability and an important factor for mate choice (Mowles and Ord 2012). In this case, substrate-borne vibrations may help to increase accuracy and/or speed for perception of the quality of song–dance coordination.

10.4.3 Targets and Functions of Vibrational Signals

Interestingly, our past study (Ota et al. 2018) implies that cordon-bleu multimodal signals convey different messages to multiple signal receivers. We found that both male and female cordon-bleus produce multimodal signals (i.e., song accompanied by dance) toward a paired partner more often when an additional audience is present, compared to without an audience. Multimodal courtship display was usually directed to a paired partner even if the audience was the opposite sex of the signal sender and thus could be a potential partner (Ota et al. 2018). Although mutual courtship displays are generally thought to function in private communication between a male and female, the result suggests that multimodal mutual courtship display of cordon-bleus works in a broader social context than previously thought.

While multimodal and multicomponent courtship display has multifaceted functions toward multiple individuals, substrate-borne vibrations might be effective for within-pair communication, such as pair bonding and its maintenance. Vibrational signals are perceived only by individuals that remain on a single substrate with the signal sender (see Sect. 10.3.1), a requirement not needed for perceiving visual and acoustic signals (Fig. 10.7). As such, substrate-borne vibrations are generally

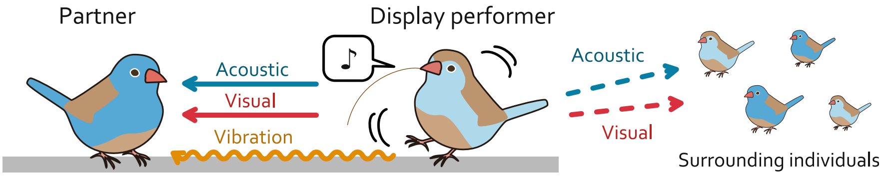


Fig. 10.7 Expectation of the target individuals and effective ranges of multimodal signals. Vibrations can be transmitted via the substrate to a potential partner standing nearby on the same perch, while visual and acoustic signals can be perceived by other surrounding individuals

transmitted only to a sexually motivated partner that stays near a signal sender. Therefore, we expect that these vibrations play an important role in expressing the loyalty toward a partner, which can lead to stable pair bonding.

10.5 Evolutionary Aspects of Dance Display for Vibrational Signaling in Estrildid Finches

To our knowledge, our finding in cordon-bleus (see Sect. 10.2–10.4) is the first and only case describing the presence of substrate-borne vibration in bird multimodal courtship displays. However, considering that vibrational communication in birds was generally overlooked in the past, there remains the possibility that other avian species also use vibrations as a communication channel.

Interspecific comparisons can give us a clue to understand the evolution of vibrational communication in birds. Cordon-bleus belong to the family Estrildidae, which includes several model songbird species, such as zebra and Bengalese finches (*Taeniopygia guttata*, *Lonchura striata* var. *domestica*), as well as several popular pet species (e.g., Gouldian finches, *Erythrura gouldiae*; Java sparrows, *Lonchura oryzivora*), from which we can easily access display behaviors. Table 10.2 lists the courtship components and possible included multimodal signals. According to our personal observations, those Estrildid species appear not to exhibit tap dance-like displays equivalent to cordon-bleus. In particular, they do perform bobbing during courting (Ullrich et al. 2016), but without alternate multiple stepping motions (as shown in Fig. 10.1). This implies that Estrildid species other than cordon-bleus might utilize substrate-borne vibration by bobbing/stepping on the same perch with a prospective partner, but that their signal intensity would fall short of that of cordon-bleus.

Estrildid courtship dance shows great interspecific variation with regard to the presence/absence of each dance element listed in Table 10.2. Previous phylogenetic comparative studies have tried to uncover the factors that could explain the evolution of dance complexity, which was determined based on the number of dance element types of each sex (Soma and Garamszegi 2015; Gomes et al. 2017; Soma 2018). According to the study, males and females of the same species present similar

Table 10.2 Possible multimodal signals in Estrildid courtship display[a]

Courtship components	Visual	Sound	Substrate-borne Vibration	Odor
Body movements:				
Bowing[1]	+			
Horizontal body movement[2]	+			
Wing movement	+			
Tail movement	+			
Erection of feathers	+			
Bobbing/stepping[3,4]	+	+	+	
Nest material holding[1,5]	+			+?
Bill wiping[6]	+	+	+?	+
Showing mouth	+			
Bill-clicking[7]		+		
Vocalization: Song		+		

+ = present, +? = suspected to be present

[a] These components are described in Goodwin (1982), Restall (1996). Other additional references for specific components are numbered in the list and indicated as follows: [1] Zanollo et al. 2013, [2] Ullrich et al. 2016, [3] Ota et al. 2015, [4] Ota et al. 2017, [5] Soma 2018, [6] Whittaker et al. 2015, [7] Soma & Mori 2015

courtship dance complexity, and species of larger body size and intraspecific brood parasitism tend to exhibit more complex courtship dances (Soma and Garamszegi 2015). These findings could be interpreted as indicating complex mutual courtship between sexes may have evolved in response to increased reproductive cost, due to severe within-species competition from brood parasitism.

10.5.1 *Phylogenetic Comparative Analyses of Bobbing/Stepping Behavior*

To characterize the evolutionary history of bobbing/stepping displays, we performed ancestral state reconstructions similar to previous research (Soma and Garamszegi 2015). Relying on the literature descriptions of Estrildid finch courtship behaviors (Goodwin 1982; Restall 1996) and the phylogenetic relationships among them (Restall 1996), we investigated the evolution of bobbing/stepping involved in courtship display. As a result, we found that considerable proportions of Estrildid species are reported in the literature to show bobbing and/or stepping as a part of courtship display, and their common ancestor thus was likely to exhibit bobbing/stepping (Fig. 10.8).

We also conducted a new phylogenetic comparative analysis in order to elucidate the factors associated with the presence of bobbing/stepping displays. Specifically, we used the same set of life-history variables as our previous study (Soma and Garamszegi 2015) and tested their effects on the presence of bobbing/stepping,

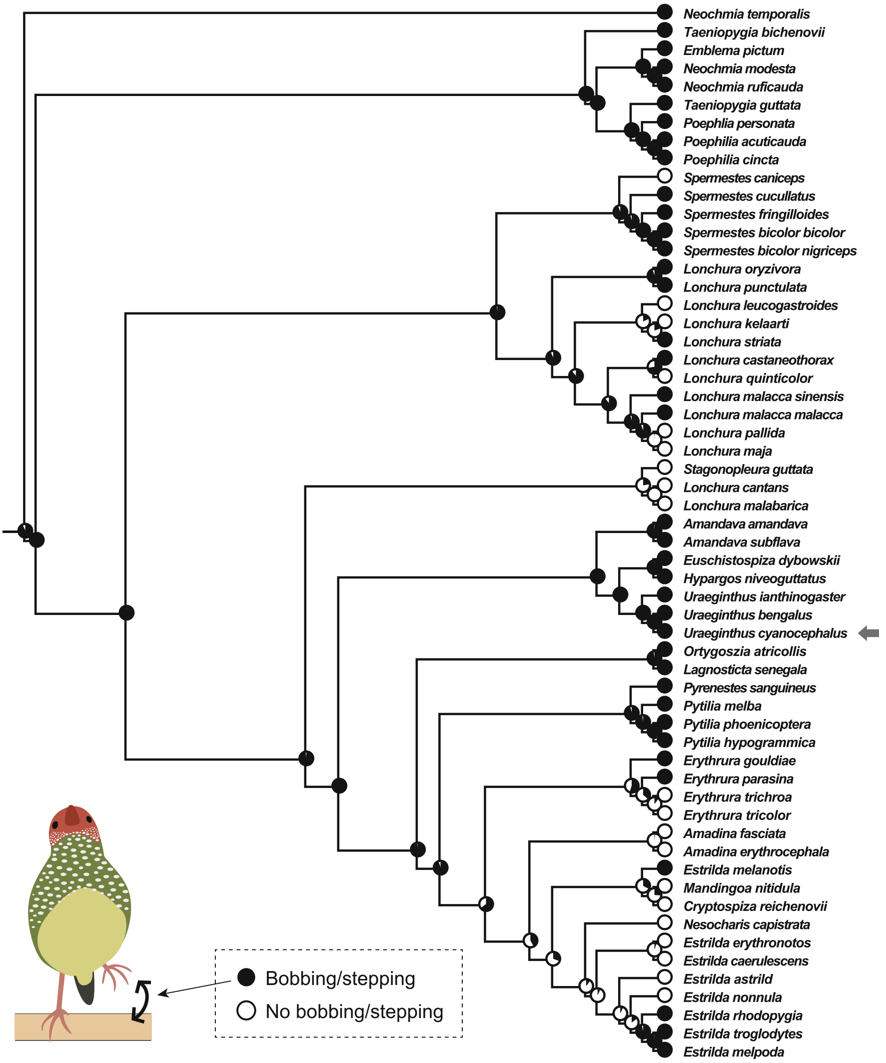


Fig. 10.8 Ancestral reconstruction of male bobbing/stepping involved in courtship display of Estrildid finches. Maximum-likelihood reconstruction of ancestral states for the evolution of bobbing/stepping is shown along the phylogenetic tree, created based on the information published in a previous study (Arnaiz-Villena et al. 2009). Presence of the behavior is based upon literature descriptions of courtship behaviors of Estrildid finches (Goodwin 1982; Restall 1996), and is displayed by black filled circles. Open circles indicate that either absence or presence of the behavior was not confirmed. Phylogenetic signals for presence of male bobbing/stepping were moderately strong and statistically significant ($\lambda = 0.503$, $p = 0.026$)

Table 10.3 Effects of life-history traits on the presence of bobbing/stepping courtship in males and females. Significant p-values are indicatd in bold

	Male bobbing/stepping				Female bobbing/stepping			
Coefficient		SE	z	p	Coefficient	SE	z	p
(Intercept)	1.603	3.199	0.501	0.616	−6.598	3.762	−1.754	0.079
Body size	0.032	0.165	0.192	0.848	0.522	0.216	2.414	**0.016***
Coloniality	0.197	0.435	0.452	0.651	−0.537	0.544	−0.989	0.323
Clutch size	−0.649	0.493	−1.317	0.188	−0.128	0.508	−0.252	0.801
Interspecific brood parasitism	1.615	0.687	2.353	**0.019***	1.619	0.816	1.983	**0.047***
Intraspecific brood parasitism	0.399	0.906	0.440	0.660	1.268	0.907	1.399	0.162
Log research effort[a]	0.609	0.562	1.083	0.279	1.032	0.605	1.706	0.088

[a] Log research effort (log number of publications) is included as an explanatory variable because the available information on dance behaviors can be biased by the intensity of studies targeting the species

*$p < 0.05$

while controlling for phylogenetic species-relatedness using a phylogenetic Generalized Linear Model (Ho and Ané 2014). Interestingly, a variable associated with reproductive cost turned out to have an influence on presence of the display (Table 10.3). Species with interspecific brood parasitism are statistically significantly more likely to exhibit bobbing/stepping display in both males and females (Table 10.3). Some Estrildid finches in Africa, including cordon-bleus, are targets of interspecific brood parasitism by birds belonging to the genus Vidua (Sorenson et al. 2004), where pairing partners may require exaggerated multimodal courtship signals to ensure commitment. In addition, we also found that female bobbing/stepping was exhibited in species using female songs (note that some Estrildid species lack female song; Soma and Garamszegi 2015). Although the roles of female song within Estrildid finches are not well understood (but see Gahr and Güttinger 1986), presumably bobbing/stepping and singing in females may have evolved for the same purpose, such as mate defense or attraction (Langmore 1998). These insights support the hypothesis that vibrations produced by bobbing/stepping contribute to pair bonding and its maintenance (see Sect. 10.4.3).

10.6 Future Directions

Throughout this chapter, we described the potential for vibrational communication in birds, particularly cordon-bleus. Bird courtship display has a long history of study and has greatly advanced our knowledge of sexual selection. Yet despite this historical interest, critical gaps remain in our understanding of how and why birds use substrate-borne vibrations in their communication. Although we introduced several implications regarding tap dance-like display as a possible example of vibrational communication, further studies would still be required to understand the detailed communicative functions of vibrational signals.

Since cordon-bleu courtship display includes conspicuous body movements and it is relatively easy to quantify the behavior, we strongly believe that cordon-bleus could play a vital role in future studies of bird vibrational communication. However, it would also be important to investigate the behaviors of other birds in order to gain insights into the evolution of vibrational communication.

10.6.1 Behavioral Experiments with Cordon-Bleus

Although our studies imply that cordon-bleus produce substrate-borne vibrations that can serve as a signal, we still have no direct evidence concerning the communication system. Thus far, we cannot completely deny the possibility that these vibrations are simply a byproduct of a vigorous motion used as a visual signal. To understand the role of vibrational signals in cordon-bleu courtship display, the responses of signal receivers should be examined depending on the availability and amplitude of substrate-borne vibrations. In addition, it would be fruitful to test if perch choice of signal senders strategically produces vibrational signals during courtship. These experiments are feasible by controlling perch structure and material.

Another lucrative area for further study is the investigation of the wild behaviors of cordon-bleus and their ecological environment. Environment has a great influence on animal signaling behavior (Stevens 2013), as is known for birds modulating songs under noisy conditions (i.e., Lombard effect; Brumm and Zollinger 2011). Understanding habitats is critical for confirming vibrational communication as the signal efficacy is highly variable depending on substrate properties. Cordon-bleus are typically found in arid areas containing bushes and trees in Africa (Goodwin 1982; Ota 2020). Though not conclusive due to the lack of quantitative evaluation of signal efficacy, the thin and shaky branches are likely to be ideal for producing non-vocal sounds and substrate-borne vibrations (Ota 2020). While attention is typically paid only to elaborate body movements themselves when investigating acrobatic dance displays of birds, focusing on ecological environments and the evaluation of signal efficacy could also be a promising avenue to reveal detailed and possibly unexpected signaling mechanisms and preferences.

Behavioral mutuality is also an interesting perspective for elucidation of the functions and evolutionary processes of substrate-borne vibrational communication in cordon-bleus. Mutual multimodal courtship display in Estrildid finches is at play in various social conditions and appears to affect pair bonding and its maintenance (see Sects. 10.4.3 and 10.5). In particular, substrate-borne vibrations can play an important role in within-pair communication due to a small effective range (see Sect. 10.3.1). To confirm this, the reproductive consequences of mutual multimodal courtship displays and the role of substrate-borne vibrations should be examined. It would be testable by conducting long-term observation of paired cordon-bleus.

10.6.2 Mechanisms for Production and Reception of Vibrational Signals

Investigating how birds produce and receive vibrational signals is essential to elucidate their vibrational communication. Although we have revealed behavioral mechanisms for producing substrate-borne vibrational signals in cordon-bleus, the neuromuscular mechanisms used to perform the rapid tap-dancing display are still veiled in mystery. In manakins, for instance, only species that perform fast wing displays during courtship have faster twitch speeds of forelimb muscles compared to species lacking fast wing displays (Fuxjager et al. 2016). Since such rapid tap-dancing display is found only in cordon-bleus among Estrildid finches, so far (see Sect. 10.5), it is unsurprising that cordon-bleus have no reported anatomical and physiological adaptations for producing substrate-borne vibrations and non-vocal sounds.

Herbst corpuscles are known to be a touch receptor used to detect vibrations in birds (McIntyre 1980). They are sensitive to the wide range of 20–2000 Hz (McIntyre 1980), which covers the frequency of the cordon-bleu tap dance display (estimated rate of 25 and 50 Hz; Ota et al. 2015). Herbst corpuscles are found on most of the body surfaces of birds, including limbs, wings, and bills (Wild 2014) and are often situated close to the follicles of several feather types (McIntyre 1980). For instance, facial bristle feathers near the bill are accompanied by Herbst corpuscles in New Zealand birds and are assumed to have a tactile function and to be used for foraging and gathering information during flight (Cunningham et al. 2011). Herbst corpuscles found along, as well as between, the tibia and fibula in ducks are likely to act as a warning device by detecting substrate-borne vibrations generated by the approach of predators (Dorward and McIntyre 1971). While mechanisms of vibration reception are generally investigated, and in a noncommunicative context, as mentioned above in this section, Kane et al. (2018) found that filoplumes coupled to peafowl crest feathers are driven by airborne vibrations of playback sounds from social display(s). These mechanisms can be used for reception and/or proprioception of substrate-borne vibrational signals produced by the cordon-bleu tap-dancing display.

10.6.3 Insights from Other Bird Species

The prevalence of vibrational signals in birds may be underestimated, and hidden methods of bird communication could remain. It is quite possible that Estrildid finches other than cordon-bleus also produce substrate-borne vibrational signals by bobbing and bill wiping (Table 10.2). Courtship displays of Estrildid finches are generally documented in detail (Goodwin 1982) but this information is usually based on observations of captive birds without quantitative analyses. What we already confirmed is that in addition to blue-capped cordon-bleus, the red-cheeked cordon-bleu (*Uraeginthus bengalus*) perform a tap dance-like display (Ota et al. 2015). We expect that other *Uraeginthus* species, such as the blue-breasted cordon-bleu (*Uraeginthus angolensis*), the violet-eared waxbill (*Uraeginthus granatinus*), and purple grenadier (*Uraeginthus ianthinogaster*), can also perform tap-dancing displays to produce substrate-borne vibrational signals; however, these should be carefully tested.

Many birds other than Estrildid finches also perform elaborate courtship displays that can produce substrate-borne vibrational signals. Several male manakins repeatedly perform take-off and landing on branches while females often observe the courtship display nearby (DuVal 2007; Jones et al. 2014; Ribeiro et al. 2019). Palm cockatoos (*Probosciger aterrimus*) use a modified stick to strike a hollow tree limb repeatedly during courtship (Wood 1984; Heinsohn et al. 2017). Woodpeckers (Aves: Picidae) rapidly and repeatedly hammer their bill against a hard substrate (Stark et al. 1998), and this display can serve as a sexual signal (Miles et al. 2018). Within such displays, substrate-borne vibrations can be an effective sexual signal in addition to visual and airborne acoustic signals.

Investigating vibrational communication systems in various birds from a broad perspective could bring us closer to understanding how this complex communication works and has evolved. While non-sound vibrations have received little consideration in bird communication, substrate-borne vibrational signals might be more widespread and common among birds than we think.

Acknowledgments We are grateful to Mr. Markus Abels and Mr. Reinhard Biller for help with the development of equipment. We thank Dr. Manfred Gahr for providing the experimental equipment and birds and for constructive comments. We also thank Ms. Ayumi Mizuno for helpful comments on the manuscript. This work is supported by JSPS Overseas Research Fellowships and Kawai Foundation for Sound Technology & Music.

References

Andersson M (1994) Sexual selection. Princeton University Press, Princeton

Arnaiz-Villena A, Ruiz-del-Valle V, Gomez-Prieto P, Reguera R, Parga-Lozano C, Serrano-Vela I (2009) Estrildinae finches (Aves, Passeriformes) from Africa, South Asia and Australia: a molecular phylogeographic study. Open Ornithol J 2:29–36

Bostwick KS (2000) Display behaviors, mechanical sounds, and evolutionary relationships of the club-winged manakin (*Machaeropterus Deliciosus*). Auk 117:465–478

Bostwick KS, Prum RO (2005) Courting bird sings with stridulating wing feathers. Science 309(5735):736

Brumm H, Zollinger SA (2011) The evolution of the Lombard effect: 100 years of psychoacoustic research. Behaviour 148:1173–1198

Caldwell MS (2014) Interactions between airborne sound and substrate vibration in animal communication. In: Cocroft R, Gogala M, Hill PSM, Wessels A (eds) Studying vibrational communication. Springer, Berlin, pp 65–92

Catchpole C, Slater P (2008) Bird song: biological themes and variations. Cambridge University Press, Cambridge

Clark CJ, Elias DO, Prum RO (2011) Aeroelastic flutter produces hummingbird feather songs. Science 333(6048):1430–1433

Clark CJ, McGuire JA, Bonaccorso E, Berv JS, Prum RO (2018) Complex coevolution of wing, tail, and vocal sounds of courting male bee humming birds. Evolution 72(3):630–646

Coleman SW (2008) Mourning dove (*Zenaida macroura*) wing-whistles may contain threat-related information for con- and hetero-specifics. Naturwissenschaften 95:981–986

Cooper BG, Goller F (2004) Multimodal signals: enhancement and constraint of song motor patterns by visual display. Science 303(5657):544–546

Cunningham SJ, Alley MR, Castro I (2011) Facial bristle feather histology and morphology in New Zealand birds: implications for function. J Morphol 272(1):118–128

Dalziell AH, Peters RA, Cockburn A, Dorland AD, Maisey AC, Magrath RD (2013) Dance choreography is coordinated with song repertoire in a complex avian display. Curr Biol 23(12):1132–1135

Devetak D (2014) Sand-borne vibrations in prey detection and orientation of antlions. In: Cocroft R, Gogala M, Hill PSM, Wessels A (eds) Studying vibrational communication. Springer, Berlin, pp 319–330

Dorward PK, McIntyre AK (1971) Vibration-sensitive receptors in the interosseous region of the duck's hind limb. J Physiol 219(1):77–87

DuVal EH (2007) Cooperative display and lekking behavior of the lance-tailed manakin (*Chiroxiphia Lanceolata*). Auk 124(4):1168–1185

Elias DO, Mason AC (2014) The role of wave and substrate heterogeneity in vibratory communication: practical issues in studying the effect of vibratory environments in communication. In: Cocroft R, Gogala M, Hill PSM, Wessels A (eds) Studying vibrational communication. Springer, Berlin, pp 215–247

Elias DO, Mason AC, Hoy RR (2004) The effect of substrate on the efficacy of seismic courtship signal transmission in the jumping spider *Habronattus dossenus* (Araneae: Salticidae). J Exp Biol 207(23):4105–4110

Fusani L, Schlinger BA (2012) Proximate and ultimate causes of male courtship behavior in golden-collared manakins. J Ornithol 153:S119–S124

Fuxjager MJ, Goller F, Dirkse A, Sanin GD, Garcia S (2016) Select forelimb muscles have evolved superfast contractile speed to support acrobatic social displays. elife 5:e13544

Gahr M, Güttinger HR (1986) Functional aspects of singing in male and female *Uraeginthus bengalus* (Estrildidae). Ethology 72(2):123–131

Geberzahn N, Gahr M (2011) Undirected (solitary) birdsong in female and male blue-capped cordon-bleus (*Uraeginthus cyanocephalus*) and its endocrine correlates. PLoS One 6(10): e26485

Gomes ACR, Funghi C, Soma M, Sorenson MD, Cardoso GC (2017) Multimodal signalling in Estrildid finches: song, dance and colour are associated with different ecological and life history traits. J Evol Biol 30(7):1336–1346

Goodwin D (1982) Estrildid finches of the world. Cornell University Press, Ithaca, NY

Gordon SD, Uetz GW (2011) Multimodal communication of wolf spiders on different substrates: evidence for behavioural plasticity. Anim Behav 81(2):367–375

Guilford T, Dawkins MS (1993) Receiver psychology and the evolution of animal signals. Anim Behav 42(1):1–14

Hall ML (2004) A review of hypotheses for the functions of avian duetting. Behav Ecol Sociobiol 55:415–430

Hebets EA, Papaj DR (2005) Complex signal function: developing a framework of testable hypotheses. Behav Ecol Sociobiol 57:197–214

Heinsohn R, Zdenek CN, Cunningham RB, Endler JA, Langmore NE (2017) Tool-assisted rhythmic drumming in palm cockatoos shares key elements of human instrumental music. Sci Adv 3(6):e1602399

Hill PSM (2009) How do animals use substrate-borne vibrations as an information source? Naturwissenschaften 96:1355–1371

Hingee M, Magrath RD (2009) Flights of fear: a mechanical wing whistle sounds the alarm in a flocking bird. Proc R Soc Lond B 276(1676):4173–4179

Ho LST, Ané C (2014) A linear-time algorithm for gaussian and non-gaussian trait evolution models. Syst Biol 63(3):397–408

Johnstone RA (1996) Multiple displays in animal communication: 'backup signals' and 'multiple messages'. Philos Trans R Soc Lond B 351:329–338

Jones MP, Pierce KE, Ward D (2007) Avian vision: a review of form and function with special consideration to birds of prey. J Exotic Pet Med 16(2):69–87

Jones MA, Duval EH, Boyle WA (2014) Individual and temporal variability in the courtship behavior of Whiteruffed Manakins (*Corapipo altera*), a species with facultative cooperative displays. Auk 131(4):727–742

Kane AS, Van BD, Dakin R (2018) Biomechanics of the peafowl's crest reveals frequencies tuned to social displays. PLoS One 13(11):e0207247

Langmore NE (1998) Functions of duet and solo songs of female birds. Trends Ecol Evol 13(4): 136–140

Lohrey AK, Clark DL, Gordon SD, Uetz GW (2009) Antipredator responses of wolf spiders (Araneae: Lycosidae) to sensory cues representing an avian predator. Anim Behav 77(4): 813–821

Mariette MM, Buchanan KL (2019) Good vibrations in the nest. Nat Ecol Evol 3:1144–1145

McIntyre AK (1980) Biological seismography. Trends Neurosci 3(9):202–205

Miles MC, Schuppe ER, Ligon RMI, Fuxjager MJ (2018) Macroevolutionary patterning of a complex display reveals how sexual selection elaborates signals under constraint. Proc R Soc Lond B 285:20172628

Mitoyen C, Quigley C, Fusani L (2019) Evolution and function of multimodal courtship displays. Ethology 125(8):503–515

Mowles SL, Ord TJ (2012) Repetitive signals and mate choice: insights from contest theory. Anim Behav 84(2):295–304

Noguera JC, Velando A (2019) Bird embryos perceive vibratory cues of predation risk from clutch mates. Nat Ecol Evol 3(8):1225–1232

O'Loghlen AL, Rothstein SI (2010) It's not just the song: male visual displays enhance female sexual responses to song in brown-headed cowbirds. Condor 112(3):615–621

Ota N (2020) Tap dancers in the wild: field observations of multimodal courtship displays in socially monogamous songbirds. Sci Nat 107:30

Ota N, Gahr M, Soma M (2015) Tap dancing birds: the multimodal mutual courtship display of males and females in a socially monogamous songbird. Sci Rep 5:16614

Ota N, Gahr M, Soma M (2017) Songbird tap dancing produces non-vocal sounds. Bioacoustics 26(2):161–168

Ota N, Gahr M, Soma M (2018) Couples showing off: audience promotes both male and female multimodal courtship display in a songbird. Sci Adv 4(10):eaat4779

Partan SR (2017) Multimodal shifts in noise: switching channels to communicate through rapid environmental change. Anim Behav 124:325–337

Partan SR, Marler P (2005) Issues in the classification of multimodal communication signals. Am Nat 166(2):231–245

Restall R (1996) Munias and Mannikins. Yale University Press, New Haven

Ribeiro PHL, Guaraldo AC, Macedo RH, Manica LT (2019) Variation within and between courts in visual components of swallow-tailed manakin (*Chiroxiphia caudata*) display. J Ornithol 160(2): 485–496

Soma M (2018) Sexual selection in Estrildid finches, with further review of the evolution of nesting material holding display in relation to cooperative parental nesting. Jpn J Anim Psychol 68(2): 121–130

Soma M, Garamszegi LZ (2015) Evolution of courtship display in Estrildid finches: dance in relation to female song and plumage ornamentation. Front Ecol Evol 3:4

Soma M, Mori C (2015) The songbird as a percussionist: syntactic rules for non-vocal sound and song production in Java sparrows. PLoS One 10(5):e0124876

Sorenson MD, Balakrishnan CN, Payne RB (2004) Clade-limited colonization in brood parasitic finches (*Vidua* spp.). Syst Biol 53(1):140–153

Stark R, Dodenhoff D, Johnson E (1998) A quantitative analysis of woodpecker drumming. Condor 100(2):350–356

Stevens M (2013) Sensory ecology, behaviour, and evolution. Oxford University Press, Oxford

Taylor PW, Roberts JA, Uetz GW (2005) Flexibility in the multi-modal courtship of a wolf spider, *Schizocosa ocreata*. J Ethol 23(1):71–75

Uetz GW, Roberts JA, Taylor PW (2009) Multimodal communication and mate choice in wolf spiders: female response to multimodal versus unimodal signals. Anim Behav 78(2):299–305

Ullrich R, Norton P, Scharff C (2016) Waltzing *Taeniopygia*: integration of courtship song and dance in the domesticated Australian zebra finch. Anim Behav 112:285–300

Whittaker DJ, Reichard DG, Drouilly M, Battle K, Ziegenfus C (2015) Avian olfactory displays: a hypothesis for the function of bill-wiping in a social context. Behav Ecol Sociobiol 69(1): 159–167

Wild JM (2014) The avian somatosensory system: a comparative view. In: Scanes CG (ed) Sturkie's avian physiology. Elsevier, New York, pp 55–69

Wood GA (1984) Tool use by the palm cockatoo *Probosciger aterrimus* during display. Corella 8(4):94–95

Zanollo V, Griggio M, Robertson J, Kleindorfer S (2013) Males with a faster courtship display have more white spots and higher pairing success in the diamond firetail, *Stagonopleura guttata*. Ethology 119(4):344–352

Chapter 11
Blooms and Buzzing Bees: Bridging Buzz Pollination and Biotremology

Paul Anthony De Luca and Mario Vallejo-Marín

Abstract Approximately 6% of the world's flowering plant species have specialised stamen morphologies that require mechanical stimulation (vibration) by bees in order to release pollen concealed within. This has given rise to the study of the phenomenon of buzz pollination. Although buzz pollination sits squarely within the discipline of biotremology, this link rarely has been made explicit. Our aim in this chapter is to bridge the gap that historically has existed between buzz pollination research and the discipline of biotremology. We will discuss what we know about bee-induced floral vibrations and compare them to other kinds of plant-borne vibrational signals. We will also highlight how certain experimental approaches developed by biotremology researchers have helped buzz pollination investigators better understand the complex behavioural and ecological interactions occurring between buzz pollinated plants and their bee visitors. We will then provide an overview of research methodologies for buzz pollination scientists and describe some of the more commonly used experimental approaches for recording and playback of bee-induced floral vibrations. By highlighting the many common themes existing between studies in buzz pollination and biotremology we hope to stimulate others to explore the many exciting new research avenues in this unique biotic interaction.

P. A. De Luca (✉)
School of Chemistry, Environmental & Life Sciences, The University of The Bahamas, Nassau, Bahamas
e-mail: paul.deluca@ub.edu.bs

M. Vallejo-Marín
Biological and Environmental Sciences, Faculty of Natural Sciences, University of Stirling, Stirling, UK
e-mail: mario.vallejo@stir.ac.uk

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_11

11.1 Introduction

The floral vibrations produced by some bees when visiting specialised types of flowers have puzzled researchers for more than a century (Teppner 2018). The relationship between flowers in which pollen is tightly protected inside floral structures that restrict pollen access and bees that use thoracic vibrations to remove pollen from these flowers has given rise to the phenomenon of buzz pollination (Buchmann 1983). The last few years have seen renewed and rapidly rising interest in the study of buzz pollination using a variety of approaches, including mechanical studies of floral and bee vibrations, phylogenetic analyses, plant-pollinator ecological investigations in the field, and bee behaviour (reviewed in Vallejo-Marín 2019). In this chapter, we provide an overview of the intimate and complex association between bees and flowers as it relates to the production of vibrations, and how plants respond to these vibrations through pollen release. Our aim is twofold: first, to make explicit the connection buzz pollination has to the discipline of biotremology, which, despite the many common themes that link them, have rarely been considered jointly (Hill 2008; Vallejo-Marín 2019) and second, to provide an overview of recording and playback methodologies used in buzz pollination research to assist scientists new to this field who want to become familiar with some of the more commonly used experimental approaches. Accordingly, we hope to emphasise the important contributions that buzz pollination can make to the discipline of biotremology and highlight how the study of buzz pollination is enriched by the experimental methods and perspectives developed by biotremology researchers. In doing so, we aim to encourage the development of exciting new research avenues on buzz pollination.

11.1.1 What Is Buzz Pollination?

Buzz pollination is a type of pollination that captures the interaction between a specific bee behaviour (the production of vibrations to extract pollen, sometimes called “sonication”) and the pollination of certain types of flowers (often, but not exclusively with poricidal floral morphologies) (Buchmann 1983). The terms “buzz pollination” and “floral sonication” are derived from the fact that there is an audible (i.e., airborne) sound that occurs as vibrations radiate off the body of the bee (and perhaps also the flower) into the surrounding air (Buchmann and Hurley 1978). It is important to note, however, that the airborne sound is just an incidental by-product since the relevant component of the bee’s action is vibrational in nature. Strictly speaking then, it may be more correct to refer to the actual behaviour as “floral vibration” (Vallejo-Marín 2019). Regardless, there is considerable ambiguity in the literature in the use of terminology to describe buzz pollination and its components, and often sonication and buzz pollination are used as synonyms. Future work will benefit from clearly distinguishing the bee behaviour of producing substrate-borne plant vibrations (sonication or floral vibration) from the interaction (buzz

Fig. 11.1 Buff-tailed bumblebee (*Bombus terrestris audax* L.) buzz pollinating a buffalo bur flower (*Solanum rostratum* Dunal). (**a**) Bee approaching the flower. (**b**) Bee vibrating the central anther cone. A bee typically grasps one or more anthers in her mandibles and presses the ventral part of her body tightly against them when producing vibrations. Photos by M Vallejo-Marín

pollination) that arises between vibrating bees and flowers with specialised morphologies (poricidal flowers) (Vallejo-Marín 2019).

During buzz pollination, a female pollen-foraging bee (Fig. 11.1a) mechanically shakes a flower (or part of it) in order to release pollen concealed within a poricidal structure (Buchmann 1983; De Luca and Vallejo-Marín 2013). Typically, these structures are anthers, but in some flower species the anthers are kept within other floral parts such as corolla tubes, and thus in these cases the bee will vibrate the corolla in order to extract the pollen (Macior 1968; Corbet and Huang 2014). A female bee typically adopts a characteristic "C" body posture (Fig. 11.1b) when vibrating a flower (King 1993). She will grasp one or more anthers (or the corolla) with her mandibles, curl the ventral part of her body around them while in direct physical contact and then rapidly contract her indirect thoracic flight muscles. Vibrations are transmitted through the head, mandibles, and ventral thoracic and abdominal sclerites into the anthers, which cause the pollen grains inside to rapidly gain energy and exit in a large cloud through apical pores or slits (Buchmann and Hurley 1978; King and Buchmann 2003). The bee will then groom herself to collect the pollen, which will be transported back to the nest to feed developing larvae. The pollen that cannot be fully groomed from the body may then be incidentally transferred to the stigma of another conspecific flower the bee visits, thus facilitating fertilisation for the plant.

Floral vibration behaviour is exclusively associated with bees (Anthophila; but see Buchmann et al. (1978) for a possible exception from a hoverfly, *Copestylum mexicanum* Macquart, mimicking a carpenter bee). Among bees, floral vibration behaviour is widespread and has been reported in 74 genera distributed among six families, which encompasses 58% of the approximately 20,000 described species (Cardinal et al. 2018). There are some notable exceptions, such as honeybees (*Apis mellifera* L.), which have never been reported to vibrate flowers for pollen collection (Buchmann 1983). Bees use floral vibrations to extract pollen from a variety of flowers, including those with non-poricidal morphologies (e.g., *Pedicularis*, *Rhododendron*, *Rosa*) (Buchmann 1983; De Luca and Vallejo-Marín 2013). Bees probably use floral vibrations as a mechanism to extract pollen when other strategies, such as gathering pollen with the legs, are inefficient or ineffective (Buchmann 1985; Russell et al. 2016; Papaj et al. 2017). Among flowering plants, roughly 6% of the 352,000 described species distributed among >72 families possess poricidal stamen morphologies that probably require mechanical stimulation to release pollen and are thus buzz pollinated. Buzz pollination also occurs in species of agricultural importance such as tomatoes (*Solanum lycopersicum*), blueberries (*Vaccinium* spp.) and kiwifruit (*Actinidia deliciosa*) (Buchmann 1983; De Luca and Vallejo-Marín 2013).

11.1.2 How Does Buzz Pollination Fit into Biotremology?

The vibrations produced by bees are transmitted to a flower via direct physical contact and are thus imparted as substrate-borne vibrations. This places buzz pollination studies directly within the sphere of the discipline of biotremology. However, for the most part, even though buzz pollination researchers rarely cite biotremology studies (the discipline of 'biotremology' was officially named relatively recently (Endler 2014; Hill and Wessel 2016)), we use the term synonymously with 'vibrational communication', which is how this field was traditionally referenced (Cocroft et al. 2014a). We conducted a survey of journal articles that specifically examined vibrational aspects of buzz pollination behaviour—published after the seminal Buchmann (1983) buzz pollination review and leading up to the present--and found 24 relevant papers (The author will share the list upon request). Of these, only 5% of the references within each paper were to biotremology (vibrational communication) studies (average number of references per paper = 33.29 (range: 9–63); average number of biotremology studies cited per paper = 1.67 (range: 0–12)). Furthermore, nearly 60% (14/24) of these papers contained no biotremology references at all. Our survey highlights the communication gap between buzz pollination research and biotremology, as a discipline.

Buzz pollination, however, represents a rapidly bourgeoning field within the discipline of biotremology. In recent years, botanists, pollination ecologists and animal behaviourists, many of whom are new to the discipline of biotremology, are beginning to utilise experimental approaches developed in the context of examining vibrational communication to explore questions about plant-insect mutualisms, co-evolutionary relationships between plants and pollinators, and the role of pollinators in mediating plant community structure at various ecological levels. For the remainder of this chapter, we will discuss what we know about bee-induced floral vibrations and compare them to other kinds of plant-borne vibrational signals. We will also highlight how certain experimental approaches developed by biotremology researchers have helped buzz pollination investigators better understand the complex behavioural and ecological interactions occurring between buzz pollinated plants and their bee visitors.

11.2 Production and Characteristics of Bee Vibrations

Floral vibrations, as with other types of mechanical waves, can be characterised by their duration, frequency, and amplitude characteristics (Fig. 11.2). In this section, we discuss how these characteristics vary in floral vibrations, and we also compare them to other kinds of plant-borne vibrations that are used in arthropod vibrational communication. A summary comparing buzz pollination and communication vibrations is provided in Table 11.1.

11.2.1 Duration

Floral vibrations consist of one or multiple ‘buzzes’ or pulses produced in rapid succession (Fig. 11.2a). The duration of a single floral vibration ‘buzz’ usually lasts between 0.1 and 3.0 s (De Luca and Vallejo-Marín 2013). An individual bee will often produce several pulses within a single visit to a flower, and in these cases, there is often considerable variation in the durations of each pulse (and in the time interval between successive pulses). During floral visitation, a bee often quickly changes its position in consecutive buzzes, grasping and releasing an anther with its mandibles and usually moving from the base of the anther to its tip and probing different anthers (Vallejo-Marín, M. personal observation). Why this behaviour occurs is currently unclear, but it may allow the bee to maximise pollen release or assess pollen availability. Previous work has also shown that bees change the length of their floral vibrations depending on pollen reward levels (Buchmann and Cane 1989; De Luca et al. 2013; Russell et al. 2016) and this dynamic adjustment may allow them to maximise pollen release (De Luca et al. 2013). Floral vibrations thus lack the stereotyped higher order temporal patterns such as chirp trains or trills often exhibited in communication vibrations, which are used in species identification or sex-specific behavioural interactions (Čokl and Virant-Doberlet 2003; Cocroft and Rodrìguez 2005). The temporal variation in floral vibrations observed in bees is

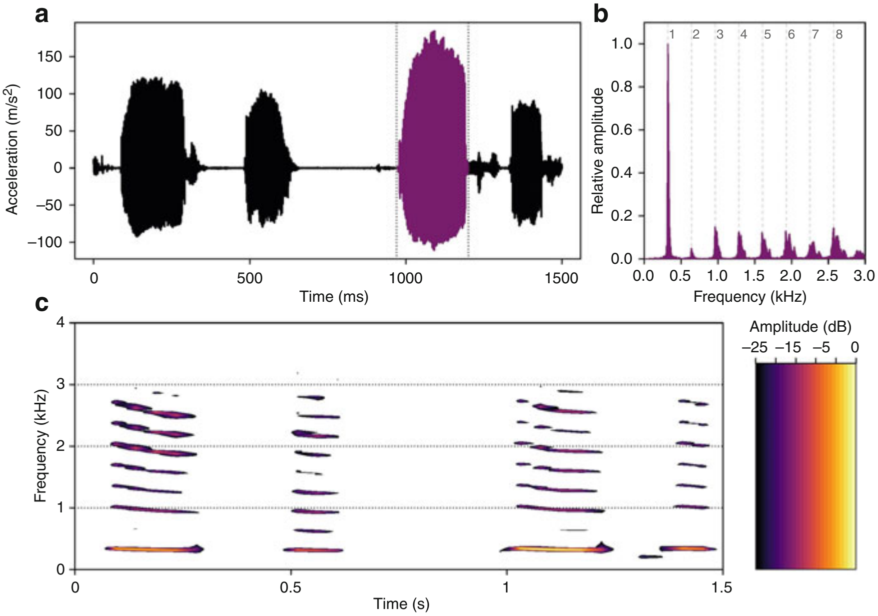


Fig. 11.2 (**a**) Oscillogram (waveform) of four consecutive floral vibrations (buzzes) from a buff-tailed bumblebee (*Bombus terrestris audax* L.) worker on a watermelon nightshade flower (*Solanum citrullifolium* A. Braun), recorded with an accelerometer that was placed at the flower's receptacle. (**b**) Frequency spectrum of the third vibration (indicated in purple inside the dashed lines in (**a**). The fundamental frequency in this vibration is also the dominant frequency (321 Hz). The first eight harmonics are shown with numbered dashed lines. (**c**) Spectrogram showing the magnitude of vibrations (relative amplitude, dB) across a range of frequencies (0–4 kHz). Lighter areas (yellow) represent frequencies of higher amplitude (data from Pritchard and Vallejo-Marín 2020)

therefore likely to be due to dynamic responses to the interaction with flowers of varying characteristics and resource (pollen) levels.

11.2.2 Frequency

Bees generate floral vibrations through the contraction of the thoracic indirect flight muscles. Since the flight muscles are asynchronous, they can contract several times for each nervous stimulation (King et al. 1996). Floral vibrations produced by bees tend to have relatively pure-tone fundamental frequencies ranging between 100 and 400 Hz (De Luca and Vallejo-Marín 2013). The fundamental frequency is also the dominant frequency (i.e., has the greatest relative energy, Fig. 11.2b), as it is common for floral vibrations to exhibit harmonics (i.e., integer multiples of the fundamental) extending up to 8 kHz (Fig. 11.2c) (Buchmann et al. 1978; King and Lengoc 1993). However, the energy of harmonic frequencies is substantially lower

Table 11.1 Comparison of the functions and consequences of vibrational properties on plant substrates in animal communication versus buzz pollination

Vibration component	Property	Animal communication	Buzz pollination
Temporal			
	Duration	• Species recognition (Henry 1980; Čokl and Virant-Doberlet 2003; Rodríguez et al. 2004). • Courtship (De Luca and Morris 1998; Henry et al. 1999, Eberhard et al. 2019). • Duetting interactions (Čokl et al. 2004; Henry and Martinez-Wells 2006; Rodríguez and Cocroft 2006). • Agonistic interactions (Miranda 2006).	• Length of a single floral vibration 'buzz' affects the amount of pollen released from flowers (De Luca et al. 2013; Russell et al. 2016). • May be related to energetic costs incurred by vibrating bees (De Luca and Vallejo-Marín 2013).
	Timing pattern	• Higher order temporal elements (pulse trains, chirps, trills) important in many behavioural contexts (Hunt and Morton 2001; Miklas et al. 2001; Čokl and Virant-Doberlet 2003; Rodríguez et al. 2006, Eberhard et al. 2019).	• Timing of individual buzzes probably less important for pollen release; influenced instead by pollen availability cues received by buzzing bee (Buchmann and Cane 1989; Russell et al. 2015).
Spectral			
	Frequency (general)	• Species recognition (Henry 1980; Rodríguez et al. 2004). • Courtship (Wells and Henry 1992; Čokl and Virant-Doberlet 2003; Cocroft and Rodrìguez 2005). • Frequency of insect vibratory signals not associated with body size within treehopper (Membracidae) species, but negatively correlated with size between closely related treehopper species, and among species in different orders (Cocroft and De Luca 2006).	• Frequency may be important for pollen release if bees are able to produce vibrations at the natural frequency of flowers. However, there is limited evidence that frequency affects pollen release within the natural range observed in bees (De Luca et al. 2013; Rosi-Denadai et al. 2018; Switzer et al. 2019). • Fundamental frequency of floral vibrations correlated with bee size in some taxa (Corbet and Huang 2014; Switzer and Combes 2017) but not in others (De Luca et al. 2014, 2019). • Physiological trade-off between frequency and amplitude may limit ability of bees to generate high frequency, high amplitude vibrations (De Luca et al. 2013; Rosi-Denadai et al. 2018).

(continued)

Table 11.1 (continued)

Vibration component	Property	Animal communication	Buzz pollination
	Low frequencies (<500 Hz)	• Generated by muscles in thorax or abdomen (Henry 1980; Mitomi et al. 1984; Čokl and Virant-Doberlet 2003; Miles et al. 2017). • Low frequency signals tend to be pure tone (Čokl and Virant-Doberlet 2003; Rodríguez et al. 2004). • Frequency modulation occurs but its function is unclear (Rodríguez et al. 2006; De Luca and Cocroft 2009).	• Generated by thoracic indirect flight muscles. • Fundamental frequencies are pure tone and do not exceed ~400 Hz (De Luca and Vallejo-Marín 2013; Switzer and Combes 2017; De Luca et al. 2019). • Frequency modulation occurs but its function is unclear (Burkart et al. 2011).
	High frequencies (>500 Hz)	• Frequency multiplier mechanism often exists (tymbals, stridulation) to produce broadband signals up to 5 kHz (Ossiannilsson 1949; Michelsen et al. 1982; Mitomi et al. 1984; Virant-Doberlet and Čokl 2004; Cocroft and Rodrìguez 2005).	• No frequency multiplier mechanism. • High-frequency harmonics (up to 8 kHz) exist but their function is unclear (King 1993; King and Lengoc 1993; Arceo-Gómez et al. 2011).
	Substrate filtering effects	• Significant effects of substrate on frequency transmission. Dispersive nature of vibrations means higher frequencies travel faster but also attenuate more quickly. Has implications for the ability of a receiver to gauge distance and/or direction to a signaller through evaluation of received frequencies (Michelsen et al. 1982; Čokl et al. 2004; Cocroft and Rodrìguez 2005; Casas et al. 2007; McNett and Cocroft 2008).	• Probably less important for floral buzzes because pure tone vibrations are not affected by substrate filtering (Cocroft et al. 2014b). • Bee is in direct contact with anthers, therefore significant frequency filtering is unlikely (Arroyo-Correa et al. 2019).
Amplitude			
	Acceleration	• Studied with respect to plant transmission effects on signal quality (Cocroft et al. 2014b).	• Important for pollen release by affecting magnitude of anther vibration. Varies widely within and among bee species (King 1993; King and Buchmann 1996, 2003; Arroyo-Correa et al. 2019; Switzer et al. 2019).
	Velocity	• Studied with respect to plant transmission effects on signal quality (Michelsen et al. 1982; Miklas et al. 2001; Čokl and	• Important for pollen release by affecting magnitude of anther vibration. Varies widely within and among bee species

(continued)

Table 11.1 (continued)

Vibration component	Property	Animal communication	Buzz pollination
		Virant-Doberlet 2003; Čokl et al. 2004; Casas et al. 2007). • Younger male treehoppers (*Umbonia crassicornis*) produce mate attraction signals with greater peak velocity (De Luca and Cocroft 2009). • Recruitment communication in stingless bees (*Melipona seminigra*) influenced by velocity of mouth-to-mouth contact vibrations (Hrncir et al. 2006).	(Buchmann and Hurley 1978; De Luca et al. 2013; Nunes-Silva et al. 2013; Rosi-Denadai et al. 2018).
	Displacement	• Sub-genual organs in honeybees (*Apis mellifera*) respond to displacement component of substrate vibrations (Kilpinen and Storm 1997).	• Maximum displacement of a bee's thorax when sonicating affects amplitude of floral buzzes. Has implications for size-related differences in ability of bees to produce large amplitude vibrations to maximise pollen release (Buchmann and Hurley 1978; Harder and Barclay 1994; King and Buchmann 2003; Corbet and Huang 2014).
	Substrate damping effects	• Significant effects of substrate on amplitude. Thickness, stiffness and density of plant substrate will affect magnitude of damping (Bell 1980; Michelsen et al. 1982; McVean and Field 1996; Čokl and Virant-Doberlet 2003; Cocroft et al. 2014b). • Differences in amplitude damping between plant parts (stem, branches, leaves) has implications for optimal signalling locations on plants (Čokl et al. 2004; Casas et al. 2007; McNett and Cocroft 2008). • Amplitude gradients may be used to determine location of a signaller (Gibson and Cocroft 2018).	• Flowers likely differ in their capacity to damp bee-induced vibrations. Variation in species-specific anther characteristics (thickness, stiffness, size of poricidal slits/pores) probably important for translating vibrations into effective pollen release (Buchmann and Hurley 1978; King and Buchmann 1995, 1996; Vallejo-Marín 2019). • When recording floral vibrations at a location other than the anthers it is recommended to calculate a plant-specific amplitude coupling factor (King 1993; Arroyo-Correa et al. 2019).

and thus, they are not thought to be functionally significant (King 1993; but see Arceo-Gómez et al. 2011). It is interesting to note that floral vibration fundamental frequencies seem to have an upper limit around 400 Hz (Burkart et al. 2011; De Luca and Vallejo-Marín 2013; Switzer and Combes 2017; Rosi-Denadai et al. 2018; De

Luca et al. 2019), even though it is possible for asynchronous flight muscle to contract at rates exceeding 500 Hz (Pringle 1949; Josephson et al. 2000; Tercel et al. 2018). Whether this limitation is a result of a physiological constraint for bees, or because frequencies above 400 Hz are not necessary for pollen ejection, certainly warrants further investigation.

For arthropods that communicate using plant-borne vibrations, signals are produced in a variety of ways (drumming, stridulation, tremulation, tymbals) using muscles located in the thorax and/or abdomen (Ossiannilsson 1949; Zeigler and Stewart 1977; Henry 1980; Morris 1980; Rovner and Barth 1981; Mitomi et al. 1984; Čokl et al. 2000; Miles et al. 2017; Eberhard et al. 2019). Fundamental frequencies of these signals are like floral vibrations, ranging between 50 and 500 Hz (Virant-Doberlet and Čokl 2004). Taxa that have been well studied include insect groups such as Hemiptera (true bugs), Neuroptera (lacewings), Orthoptera (crickets and katydids), Mantophasmatodea (heelwalkers), and Plecoptera (stoneflies), and arachnid groups such as Araneae (spiders). However, unlike bees, many other arthropods also possess some form of frequency-multiplier mechanism. This usually takes the form of a stridulatory device or tymbal and enables the production of vibrational signals with much higher broad band carrier frequencies that may extend up to 5 kHz (Michelsen et al. 1982; Čokl and Virant-Doberlet 2003; Elias et al. 2006). One function of these higher frequency vibrations may be related to the ability to localise a signaller on a plant. Generally, higher frequencies attenuate (lose energy and dissipate) faster than lower frequencies, and thus an individual may be able to discriminate the distance and direction of a signaller through evaluation of the specific frequencies it receives (Čokl and Virant-Doberlet 2003; Cocroft and Rodrìguez 2005; Gibson and Cocroft 2018).

11.2.3 Amplitude

The amplitude of a floral vibration refers to the magnitude (strength or intensity) of the vibrational wave as it propagates within a medium. It can be expressed as displacement, velocity or acceleration (Fig. 11.3). In addition, different summary statistics can be used to express magnitude. For example, peak amplitude (PA) refers to the maximum absolute value of a vibration, peak-to-peak (PK-PK) amplitude refers to the difference between the highest (peak) and lowest (trough) absolute values, and root mean square (RMS) amplitude refers to the square root of the peak amplitude (Speaks 1999). Buzz pollination studies that have measured amplitude usually report either peak velocity or peak acceleration (De Luca and Vallejo-Marín 2013). In simple sinusoidal vibrations (as floral vibrations are), knowledge of fundamental frequency and any of the three forms of amplitude (e.g., velocity) allows calculation of the other two (e.g., acceleration and displacement) (Vallejo-Marín 2019). The transducer a researcher uses to record vibrations determines which amplitude component should be reported. For example, phonograph cartridges are sensitive to displacement, laser Doppler vibrometers to velocity, and piezo-electric

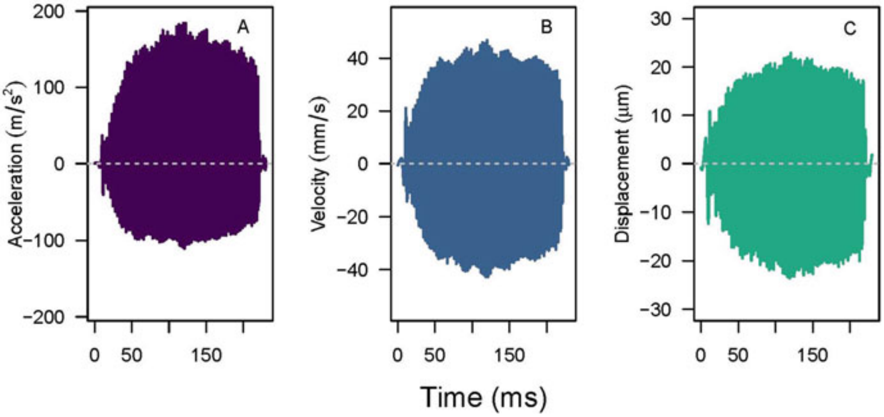


Fig. 11.3 Amplitude waveform of a floral vibration plotted as (**a**) acceleration, (**b**) velocity, and (**c**) displacement. Velocity and displacement values were obtained by numerical integration of the acceleration waveform. The vibration was produced by a buff-tailed bumblebee (*Bombus terrestris audax* L.) worker and recorded with an accelerometer positioned at the receptacle of a watermelon nightshade (*Solanum citrullifolium* A. Braun) flower (data from Pritchard and Vallejo-Marín 2020)

accelerometers to acceleration (Cocroft and Rodrìguez 2005). Amplitude is an extremely important parameter in buzz pollination research because it indicates the magnitude of a floral vibration, and therefore the forces that are transmitted to the flower that ultimately affect pollen release (Buchmann and Hurley 1978; King and Lengoc 1993; Harder and Barclay 1994; King and Buchmann 1996; De Luca et al. 2013; Rosi-Denadai et al. 2018; Switzer et al. 2019).

For vibrationally communicating arthropods on plants, amplitude is an important signal property. Amplitude gradients experienced by an individual at different points on a plant may allow it to localise a signaller (Čokl and Virant-Doberlet 2003; Čokl et al. 2004; Gibson and Cocroft 2018), and peak amplitude may even provide information about individual signaller characteristics such as age or condition (De Luca and Cocroft 2009; De Luca 2015). Vibration amplitude is strongly influenced by substrate properties such as the thickness and stiffness of stems, branches and leaves, and it is also affected by the heterogeneity of the signalling environment, i.e., the complex three-dimensional structure of a plant (Michelsen et al. 1982; Casas et al. 2007; Cocroft et al. 2014b; Gibson and Cocroft 2018). These effects may be less important in floral vibrations, where bees directly contact floral structures containing pollen or when the distance travelled by the vibration is very short (e.g., between adjacent anthers). However, it is a much more complex issue in animal communication where there usually is some measurable distance between a sender and receiver. The extent to which floral structures affect the vibrations imparted by a bee is just beginning to be understood (King 1993; King and Buchmann 1995; Arroyo-Correa et al. 2019), and more work is needed to understand the effects of changes in the transmission properties of flowers for pollen release during buzz pollination.

11.2.4 Behavioural Considerations in the Production of Floral Vibrations

Floral vibrations differ from vibrations produced in other behavioural contexts such as flight and defence (Macior 1968; De Luca et al. 2014; Pritchard and Vallejo-Marín 2020). In *Bombus terrestris* L., floral vibrations have higher frequency, velocity and acceleration than defence vibrations, which are also produced without wing deployment (Pritchard and Vallejo-Marín 2020), suggesting that bees can modulate the characteristics of the vibrations they produce on flowers. Floral vibrations produced by bees contain both innate and learned components, but the extent to which bees can actively modulate vibrations to match specific foraging conditions is unclear. Naïve bees quickly begin producing floral vibrations on buzz pollinated flowers (Morgan et al. 2016), and the basic motor routines of buzz pollination behaviour seem to be innate (Russell et al. 2016). Yet, properties of their floral vibrations are modified significantly as naïve bees gain foraging experience. Studies have shown that duration, frequency and amplitude can all change with increased foraging experience, albeit in species-specific ways. For example, in *B. impatiens* Cresson both duration and amplitude increase over the first 100 vibrations on *Solanum houstonii* Dunal flowers (Russell et al. 2016), while in *B. terrestris* individuals that gained experience foraging on *S. rostratum* Dunal flowers show a decrease in both frequency and amplitude of their vibrations over ten foraging bouts (Morgan et al. 2016). In an ingenious recent study, naïve *B. impatiens* were allowed to forage on synthetic flowers where pollen release could be controlled by the experimenter. Here, larger individuals exhibited greater flexibility in producing vibrations that varied in frequency and amplitude in response to different pollen availability conditions, suggesting that size influences how bees modulate buzz pollination behaviour (Switzer et al. 2019). Experienced bees also demonstrate flexibility in behaviour when visiting rewarding vs unrewarding buzz pollinated flowers by adjusting the duration of vibrations (Buchmann and Cane 1989), or by actively switching between buzz pollination and scrabbling (collecting pollen without vibrating anthers) (Russell et al. 2017).

For vibrationally communicating insects, within-individual changes in signal properties have been documented, but these are thought to be more the result of age or condition-dependent effects than learning or experience per se (Kumar and Saxena 1985; Zeigler and Stewart 1985; Moreira 1993; De Luca and Cocroft 2009; Eberhard et al. 2019). However, communication signals may be actively modified through information gained from a receiver, as in male wolf spiders, which increase the rate at which vibrational courtship signals are produced in response to female receptivity cues (Sullivan-Beckers and Hebets 2014). Accordingly, in both animal communication and buzz pollination, within-individual changes in the production of vibrations appear to be influenced by a combination of ontogenetic, ecological and social factors, affecting both physical and behavioural aspects of the way the vibrations are modulated for the specific conditions being encountered.

11.3 Recording and Playback of Bee Vibrations on Flowers

11.3.1 Practical Aspects of Recording Floral Vibrations

For an experimenter there are two clear (and obvious) locations from which to record floral vibrations: the bee and the anthers (or corolla tubes). However, in practice, both locations have proven to be technically challenging. A buzzing bee does not typically remain stationary as it vibrates a flower and so attempting to measure directly from a moving target (for example with a laser vibrometer) is often difficult (but see Nunes-Silva et al. 2013). Similarly, measuring vibrations directly from anthers is problematic because they are usually concealed by a bee as it curls its body around them when vibrating. To get around these constraints, researchers using an appropriate vibration transducer often measure floral vibrations from another part of the flower that is more easily accessible. Two often used locations are the base (e.g., pedicel, calyx or receptacle) (Arroyo-Correa et al. 2019; Switzer et al. 2019) or the petals (De Luca et al. 2013, 2018). In the absence of a vibration transducer, however, one commonly used alternative is to record the airborne (acoustic) component of a floral vibration with a microphone (e.g., Macior 1968; Burkart et al. 2011; Corbet and Huang 2014; De Luca et al. 2014). The hypothesis here is that the buzzing sound constitutes a faithful reproduction of the substrate-borne vibration and therefore can be used as a proxy in situations where using a vibration transducer is not practical (Burkart et al. 2011). Do floral vibrations recorded from the flower, or airborne buzzing sounds recorded with a microphone, faithfully reproduce what bees impart into anthers? We explore this (see Sect. 11.3.1.1) when we discuss practical aspects of measuring and reproducing floral vibrations, using equipment and methodologies borrowed from the field of biotremology (see Chap. 8, for a comparison of the performance among industry-standard equipment for vibration recording and playback and some inexpensive alternatives). As in Sect. 11.2, we compare recording and playback methodologies between arthropod vibrational communication and buzz pollination studies, and we provide a summary of our comparisons in Table 11.2.

11.3.1.1 Types of Transducers

There are two types of recording devices that are commonly used in buzz pollination studies: (1) transducers that are sensitive to the vibrational (plant-borne) component, and (2) microphones that are sensitive to the acoustic (airborne) sounds of floral vibrations. In this section, we will provide an overview of each type and highlight the methodological advantages and disadvantages of each.

Vibration Transducers The relevant mechanical component of the action of a buzz pollinating bee is vibrational in nature. Accordingly, using an appropriate vibration transducer remains the best method for recording these vibrations. The first type we will discuss are laser Doppler vibrometers. These devices are a non-contact method

Table 11.2 Comparison of experimental methods for recording and playback of plant-borne vibrations in animal communication versus buzz pollination

			Animal communication	Buzz pollination	Pros/Cons for buzz pollination?
1. Recording		**Vibrational components that can be measured**			
Airborne transducer					
	Acoustic microphone	1. Duration 2. Frequency 3. Relative amplitude (dB SPL)	Airborne sounds that originate as substrate-borne signals are extremely weak and not used to characterise vibrational signal components (Michelsen et al. 1982).	Airborne sounds of floral buzzes are often very audible to the human ear. Sounds of floral buzzes may serve as good proxies for vibrational components of temporal and some spectral properties (Burkart et al. 2011; Corbet and Huang 2014; Switzer and Combes 2017; De Luca et al. 2018, 2019; Rosi-Denadai et al. 2018).	Pros: (1) Ideal in field settings for monitoring large numbers of flowers. (2) Can be quickly repositioned to track individual bees moving through a patch of flowers. (3) Price not prohibitive (excellent digital recorders can be purchased for under USD$1000). Cons: (1) Airborne sound amplitude does not correspond to the true vibrational amplitude of floral buzzes. However, relative sound amplitude can be used to compare among a sample of buzzes recorded under identical conditions.
Vibration transducer					
	Piezo-electric accelerometer	1. Duration 2. Frequency	Popular method for recording communication vibrations. For optimal recording accelerometer	Excellent method for recording floral vibrations. Typically affixed to base of flower with	Pros: (1) Ideal in field settings as device can be quickly repositioned if needed.

		3. Acceleration (m/s^2)	is placed as close as possible, and in same plane, as signalling animal (Cocroft et al. 2014b).	wax or via a modified insect pin (Arroyo-Correa et al. 2019; Switzer et al. 2019; Pritchard and Vallejo-Marín 2020).	(2) Absolute amplitude (acceleration) measurements easily obtained in calibrated accelerometers if connected to voltage recorder, or by reference to a calibrated source. (3) Price not prohibitive (many models available for under USD $1000). Cons: (1) Extra mass loading on flower needs to be minimised. (2) Coupling factor of plant needs to be determined to account for plant damping effects on vibration transmission.
	Laser doppler vibrometer	1. Duration 2. Frequency 3. Velocity (mm/s)	Ideal recording method because it is non-contact. Can be focused on the signalling animal or the substrate. For maximum sensitivity laser beam is positioned perpendicular to direction of wave propagation (Dierkes and Barth 1995; McNett et al. 2006; Casas et al. 2007; De Luca and Cocroft 2009).	Can be focused on the bee or flower (King and Buchmann 2003; De Luca et al. 2013; Nunes-Silva et al. 2013; Rosi-Denadai et al. 2018; Pritchard and Vallejo-Marín 2020).	Pros: (1) Best method for recording floral vibrations because it avoids mass loading of substrate. (2) Absolute amplitude measurements easily obtained. Cons: (1) Laser reflection on bee body can be poor, particularly if thorax is hairy, and may require the use of reflective tags. (2) Hard to accurately target rapidly moving bees. (3) Poor laser reflection results in low signal-to-noise ratios, hence mainly limited to laboratory studies since laser beam

(continued)

Table 11.2 (continued)

			Animal communication	Buzz pollination	Pros/Cons for buzz pollination?
					is extremely sensitive to environmental sources of vibrational noise. (4) Price can be prohibitive (~USD$30,000).
	Phonograph cartridge	1. Duration 2. Frequency 3. Displacement (mm)	Extremely sensitive to motion of vibrating surface. For optimal recording stylus is placed as close as possible, and in same orientation, as signalling animal (Hunt and Nault 1991; Hunt and Morton 2001).	To our knowledge has not been used to record bee floral vibrations. However, could be utilised using similar methods as an accelerometer.	Pros: (1) Appropriate for obtaining displacement measurements. (2) Price not prohibitive (under ~USD$1000). Cons: (1) Never been used in floral vibration research. (2) Lack of repeatability of results can be problematic due to poor contact of stylus with substrate. (3) Coupling factor of substrate would need to be determined to account for plant damping effects.
2. Playback		**Vibrational components that can be tested**			
Modified loudspeaker		1. Duration 2. Frequency 3. Velocity (mm/s)	Used to assess plant chemical responses to herbivory (Michael et al. 2019), and to replicate vibrational communication signals (Čokl et al. 2005; Rebar et al. 2012).	Can be affixed directly to anther via modified insect pin or metal claw (Corbet et al. 1988; Rodrigues et al. 2018).	Pros: (1) Price not prohibitive (under ~USD$1000). (2) Step by step instructions to build a complete playback apparatus are available (Rodrigues et al. 2018). Cons: (1) Would need to

					characterise frequency and amplitude characteristics of speaker prior to use.
Electrodynamic vibrator (shaker)		1. Duration 2. Frequency 3. Acceleration (m/s^2)	Used to replicate vibrational communication signals (Cocroft 1996; De Luca and Morris 1998; Hebets et al. 2008).	Can be affixed directly to anther via tape or glue, metal rod, hook or modified forceps (Harder and Barclay 1994; King and Buchmann 1996; De Luca et al. 2013; Rosi-Denadai et al. 2018).	Pros: (1) Frequency and amplitude characteristics of device already determined by manufacturer. (2) Easy to set up and use. Cons: (1) Price can be prohibitive (~USD$3000). (2) Adequate coupling of shaker to flower would need to be devised to ensure vibrations are transmitted in a biologically realistic manner.

that utilises a laser beam, which reflects off the vibrating surface to provide frequency and amplitude (measured as velocity) information about the vibration. Laser vibrometers are best suited for laboratory studies but portable models (e.g., Polytec PDV100; Tustin, CA, USA) are available and can be transported to the field and powered by a battery. Lasers such as the PDV100 offer both digital and analogue ports that enable recordings to be stored as either digital files (typically in S/P-DIF format) or as a voltage time series, both of which preserve the absolute amplitude information of the vibration. During recording, the laser should be situated so that the beam is perpendicular to the direction of wave propagation for maximum sensitivity (McNett et al. 2006). Signal to noise ratios are highest with good reflection of the laser from the measured surface, and thus it is usually necessary to mount a small piece of reflective tape on the vibrating surface to increase reflectance of the laser beam. For high fidelity recordings the laser beam must be in sharp focus on the vibrating surface; therefore, strong external disturbances on the vibrating surface of interest will usually result in noisy and unusable recordings. For example, wind-induced vibrations on plants in the field are a significant source of noise that can mask vibrations (McNett et al. 2010). In the laboratory where environmental conditions can be controlled, laser vibrometry is a powerful method for obtaining floral vibrations.

The second type of vibration transducer commonly used in buzz pollination research is the piezo-electric accelerometer, which measures the acceleration of a vibrating surface. Accelerometers are attached directly to the vibrating object, and in buzz-pollination studies, they are commonly attached to the pedicel, calyx or receptacle of the flower. Typically, glue or beeswax is used to affix an accelerometer to the vibrating surface, but an alternative method is to glue an insect pin to the accelerometer and then attach (insert or firmly contact) the pin on the plant structure of interest (Arroyo-Correa et al. 2019; Switzer et al. 2019; Pritchard and Vallejo-Marín 2020). In contrast to the laser vibrometer, the main drawback of accelerometers is that they impose an extra weight on the vibrating surface. The additional weight of the accelerometer (and associated cables connecting the accelerometer with the signal recorder) can affect the transmission properties of the substrate being investigated (Cocroft and Rodrìguez 2005). Using lightweight models is desirable in order to minimise the effects of the extra mass loading (Cocroft and Rodrìguez 2005). In mechanical engineering, a rule of thumb is to use accelerometers that weigh less than 5% of the mass of the vibrating object, but with floral weights commonly in the range of less than a gram (e.g., *Solanum*) even miniature piezo-electric accelerometers (0.2–0.8 g) represent a significant fraction of the coupled system.

An advantage over laser vibrometers is that accelerometers are much easier to set up and reposition; therefore, they tend to work better under field conditions. As with lasers, environmental sources of noise such as wind can induce unwanted vibrations in a flower, which can potentially mask floral vibrations. Output from an accelerometer is analogue (a change in voltage over time), which can be converted to acceleration using the calibration reference information specific to the type and model being used (see Arroyo-Correa et al. 2019; Switzer et al. 2019; Pritchard and Vallejo-Marín 2020). Regardless of the type of vibration transducer, it is important to consider how the transmission path between the source of the vibration and the sensor may affect vibrational properties. The material and mechanical properties of the substrate can affect both the frequency and magnitude of the vibrations. Factors such as plant characteristics, and the distance between a signaller and recording device, can have unpredictable effects on vibration amplitude and thus need to be accounted for (see Sect. 11.2.3; Cocroft et al. 2014b). For example, a researcher might be interested in assessing the vibrations produced by different species of bees buzzing a flower by placing a sensor somewhere in the flower (e.g., the pedicel). Because the vibrations measured on the flower will depend not only on the bee species but on how the vibrations are changed as they pass through the flower, it is necessary to account for this potentially confounding floral effect. A solution is to empirically estimate the plant's 'coupling factor', which can be done by stimulating anthers with a vibration of known amplitude and then measuring its value with the transducer positioned at the desired recording location. The difference in the amplitude ratio between the two locations is then used to calculate the coupling factor and estimate the vibrations produced at the source (bee) (King 1993; Arroyo-Correa et al. 2019). However, it is important to remember that this is just a rough approximation, as replicating exactly the way in which a bee manipulates a flower during buzzing will be difficult. Yet, this may be the best experimental approach at this time. Very few studies have estimated the coupling factor of different types of flowers (e.g., King 1993), but it seems that even closely related plant taxa have statistically different coupling factors (Arroyo-Correa et al. 2019).

Microphones An alternative method of recording buzz pollination vibrations is to focus on the acoustic component of floral vibrations using a microphone. Here, the signal of interest is the airborne *buzzing* sound that accompanies the production of floral vibrations (Buchmann 1983). This method has been used for over 50 years (Macior 1968), and it continues to be a popular choice for researchers, particularly in the field (Burkart et al. 2011; Corbet and Huang 2014; Switzer and Combes 2017; De Luca et al. 2019). The advantage of this approach is ease of use: modern handheld digital recorders are easy to carry and move around, and so rather than waiting (often patiently!) by a flower that was chosen in advance for a bee to visit with a laser or accelerometer, a researcher can instead actively follow a foraging bee as it moves from flower to flower. This permits the collection of a large sample of recordings in a relatively short amount of time compared to what can be obtained with vibration transducers. For the optimal recording of airborne buzzing sounds, the microphone should be positioned perpendicular to the dorsal surface of the bee's thorax (where the sound radiates most intensely) and be as close as possible without disturbing the natural behaviour of the bee. The distance between the bee and the microphone should be recorded in the data in order to later ensure that the sample was taken outside the near field, but also to be able to calculate attenuation with distance and control for that with multiple samples. Recordings of airborne buzzing sounds have been shown to faithfully reproduce spectral (frequency) and temporal (duration) patterns of floral vibrations, thus their use as proxies for their corresponding vibrational components can be argued (De Luca et al. 2018). However, measures of acoustic power (e.g., dB SPL) are not strongly correlated with vibration amplitude (measured as peak velocity) and should not be used (De Luca et al. 2018). Relative measures of acoustic power have been used in comparative studies, and are appropriate, if the signals being compared are recorded in exactly the same way (De Luca et al. 2014; Morgan et al. 2016). This means ensuring that the distance and orientation of bees to the microphone is kept the same, and that the microphone gain setting is kept identical between recordings.

One consideration of using microphones is background acoustic noise, which can often mask the sounds of buzz pollination vibrations. Sources can include wind, passing vehicles, machinery and animals (especially nearby singing birds); therefore, researchers should be aware of these potential confounding influences and compensate accordingly either during recording (e.g., using wind screens to reduce wind noise) or during analysis (e.g., using high pass filters to reduce low-frequency noise below 100 Hz). Filters used post-data collection may be of limited use if a recording contains noise at frequencies that overlap those of floral vibrations (i.e., within the 100–400 Hz range). We refer readers to Sueur (2018) for an excellent and detailed introduction to sound analysis, including the use of digital filtering.

Another consideration of using microphones is that on some occasions identifying the correct fundamental frequency of a floral vibration can be difficult if the researcher only focuses on the dominant frequency value in a recording. Recordings made with a vibration transducer (e.g., laser or accelerometer) show the fundamental frequency as the dominant frequency. However, acoustic recordings of floral vibrations may sometimes result in a harmonic frequency being dominant (Fig. 11.4). This difference is probably associated with several factors, including the difference in transmission properties of mechanical vibrations in air vs solid mediums, the sensitivity of a microphone brand to a specific frequency range, and because the effect the size of a vibrating object has on the sound (airborne) frequencies that radiate most efficiently from it (Michelsen and Nocke 1974; Michelsen et al. 1982; Bennet-Clark 1989, 1998). Therefore, although the overall spectral pattern (fundamental and associated harmonics) of a floral vibration recorded with an acoustic microphone could be used as a proxy for that recorded with a vibration transducer in some situations, the specific vibration frequency that transmits *best* in the flower often does not match the sound frequency that transmits *best* in the air (Michelsen et al. 1982). Accordingly, researchers using acoustic recordings should examine frequency spectra carefully to ensure that a higher frequency harmonic (>500 Hz) is not used to approximate the fundamental within plant tissue simply because it is the dominant frequency recorded in air. However, the main point to make (in our view) is that acoustic recordings can be a proxy (with the described limitations) only for the bee's behaviour, the buzzing, and not the mechanical response of the flower itself.

Videography Some of the earliest experimental investigations into buzz pollination were conducted using high-speed cameras (Macior 1964). This approach has enabled researchers to construct detailed ethograms describing the sequence of

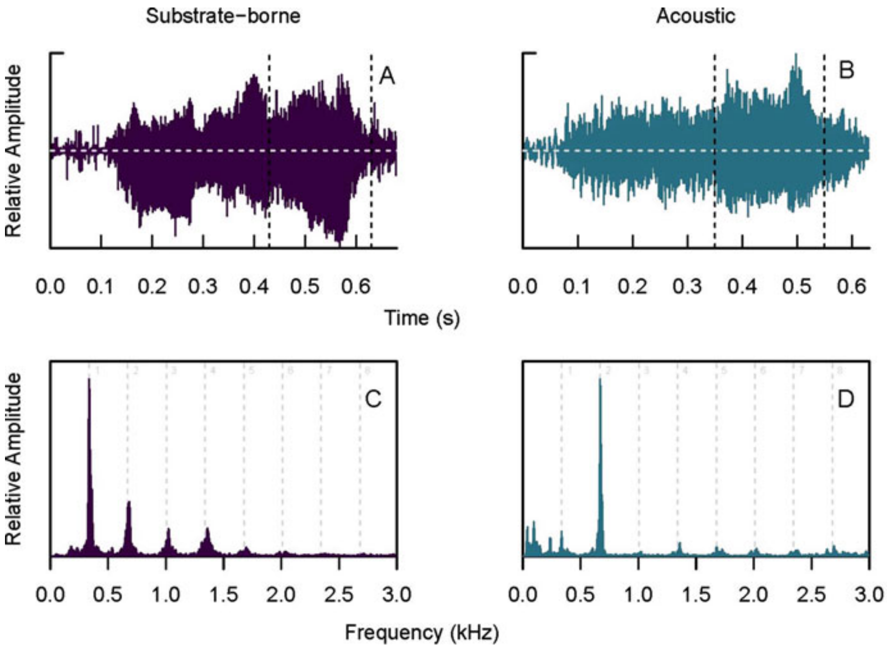


Fig. 11.4 Example of a floral vibration simultaneously recorded with a Polytec PDV100 laser Doppler vibrometer (**a**, **c**) and a Zoom H4 acoustic microphone (**b**, **d**) from an Eastern bumblebee (*Bombus impatiens* Cresson) worker on a ‘diente de burro’ nightshade (*Solanum houstonii* Dunal) flower. The microphone was positioned 14 cm from the flower and the laser beam was directed on one of the flower’s petals, 5 mm from the base of the anthers. Top panels (**a**, **b**) show the entire floral vibration in the time domain, and the bottom panels (**c**, **d**) show the frequency domain of the section marked with black vertical dashed lines in the top panels. Vertical dashed lines in **c** and **d** indicate the position for the fundamental (1) and harmonic (2–8) frequencies calculated from the laser signal. In the spectrum from the laser recording (bottom left panel) the fundamental (339 Hz) is also the dominant frequency. In contrast, in the acoustic recording (bottom right panel) the dominant frequency is at the second harmonic (738 Hz) (data from De Luca PA, unpublished)

behaviours involved in floral sonication (Macior 1968; Russell et al. 2016). Furthermore, when video is combined with acoustic recording it enables researchers to connect physical movements of the bee to changes in buzzing properties (e.g., fluctuations in duration or frequency) as the vibration is imparted into the flower (Switzer et al. 2016).

11.3.2 Playback of Vibrations on Flowers

Characterising properties of bee-induced floral vibrations represents just one side of the buzz pollination story. The other equally important side concerns the effect bee vibrations have on pollen release (Buchmann and Hurley 1978; Harder and Barclay

1994; King and Buchmann 1995, 1996). Although we know a great deal about causes of variability in bee vibrations, we are still in the beginning stages of understanding the factors affecting vibratile ejection of pollen from anthers. The reason is perhaps practical—recording vibrations from bees is comparatively easier to do than performing the kinds of intricate playback experiments needed to adequately evaluate how bee vibrations influence pollen release. Accordingly, this is where knowledge of the challenges involved in the playback of vibrational communication signals can help buzz pollination researchers. The good news is that recent biotremology studies have specifically dealt with evaluating the most common playback issues on plant substrates. We have reviewed the few studies that have examined the effect of floral vibrations on pollen release (see Sect. 11.3.2.1) and then evaluated the equipment and methodologies that are available for investigating this phenomenon and provide some recommendations for performing well-designed playback experiments (see Sect. 11.3.2.2).

11.3.2.1 Review of Experimental Studies to Date

Only a handful of experimental studies have directly assessed how variability in the properties of floral vibrations influences pollen ejection. Buchmann and Hurley (1978) developed a biophysical model that examined how pollen grains inside an anther might behave when the anther was vibrated. A key result was that both vibration frequency and amplitude (expressed as velocity) were important predictors of how quickly pollen grains gain energy and are expelled through apical pores in the anther tips. Accordingly, subsequent experiments have attempted to reproduce bee vibrations on anthers to quantify pollen ejection. Buchmann et al. (1978) used a tuning fork to vibrate the anthers of two *Solanum* species. Although significant quantities of pollen were ejected, no attempt was made to modulate the duration or amplitude of the vibrations emanating from the tuning fork to approximate what a bee might naturally produce. Also, the tuning fork generated a 512 Hz pure tone, which is more than 100 Hz higher than the maximum floral vibration frequencies reported for bees (De Luca and Vallejo-Marín 2013; De Luca et al. 2019). Five subsequent studies were more systematic in their methodology and provide more compelling results. Here, researchers generated artificial stimuli that varied in key properties such as frequency, amplitude and duration, and applied them to anthers of various flowers (i.e., *Actinidia*, *Dodecatheon*, *Rhododendron* and several *Solanum* species), using either a loudspeaker to which a fine wire was glued (Corbet et al. 1988), or a vibration exciter (King and Lengoc 1993; Harder and Barclay 1994; King and Buchmann 1995, 1996). Three of these studies varied amplitude and found more pollen was ejected as amplitude increased (measured as either displacement or acceleration). However, results were equivocal with respect to frequency. Two studies showed more pollen was released with stimuli that contained fundamental frequencies well above what bees naturally produce (i.e., 400–1000 Hz) (Corbet et al. 1988; Harder and Barclay 1994), while two reported greater pollen ejection within the range used by bees (i.e., 100–400 Hz) (King and Buchmann 1995, 1996).

These studies verified that amplitude and frequency were indeed important functional properties of floral vibrations, as varying them affected the quantity of pollen that was ejected from anthers. However, the methodologies in these papers were sometimes vague regarding the number of stimuli used, and it was not made abundantly clear how values for some properties (e.g., duration) were adjusted between different playback exemplars. A study by De Luca et al. (2013) adopted the same experimental approach but the researchers here greatly expanded the number of stimuli that were used, while describing how stimuli were generated. They created 294 different stimuli that simultaneously varied in frequency, duration and amplitude (in seven discrete steps, i.e., mean ± 1, 2, 3 SD) based on measurements taken from 54 workers of *Bombus terrestris* L. bumblebees foraging on *Solanum rostratum* Dunal flowers. Results of the playback experiment revealed that amplitude and duration were positively correlated with pollen release (with amplitude having four times the influence as duration). However, varying frequency had a weak significant quadratic effect on the amount of pollen ejected. They also found significant correlational effects (e.g., a positive interaction between amplitude and duration), suggesting that pollen ejection is a complex process that likely involves different vibrational properties interacting with one another. Most recently, Rosi-Denadai et al. (2018) evaluated pollen ejection in tomatoes (*S. lycopersicum* L.) using 40 artificially generated stimuli that varied in amplitude and frequency (duration was kept constant at 2 s across stimuli). Here, frequency was varied across a wider range (100–1600 Hz) than what bees naturally produce, but their results indicated no single frequency was optimal to maximise pollen release. Rather, variation in amplitude had the greatest effect on the quantity of pollen extracted from anthers, with higher amplitudes releasing more pollen. The results of these eight studies established the important functional role that floral vibrations play in affecting pollen release and highlighted the relative importance of properties such as amplitude, duration and frequency. However, we still have much to learn about how flowers respond to bee-induced vibrations. For example, little comparative data currently exists for flowers with different stamen morphologies (e.g., *Solanum* vs. *Pedicularis*) regarding their responses to floral vibrations. Accordingly, more experiments investigating the biophysical responses of anthers (and of pollen grains) are needed to help us better understand how vibrations transmitted from the bee to the anther translate into useful work to affect pollen release. This will require an integrative approach combining analyses of bee behaviour and floral biomechanics, and how these factors are connected through buzz pollination.

11.3.2.2 Descriptions of Playback Systems and Experimental Approaches

When designing a playback experiment, selecting the right equipment to correctly address the question is always a prime concern. There are several vibration playback systems that are available, and the only limitation for researchers is likely to be cost. The most important methodological consideration for buzz pollination researchers is constructing an apparatus that transmits bee vibrations to anthers in a *biologically realistic* manner. All the studies discussed in the previous section utilised different methods to excite anthers that we further explain in this section.

Vibration Exciters The use of an electrodynamic vibrator (also called a mini-shaker) is by far the most common device that has been used in vibrational playback experiments. Electrodynamic shakers such as the popular Brüel & Kjær (B & K) 4810 shaker (Brüel & Kjær, Naerum, Denmark) are relatively small and can be positioned in any spatial orientation with appropriate clamps. These types of vibrators can handle a wide range of frequencies and amplitudes and thus are quite versatile. The main technical challenge is constructing an appropriate accessory piece to connect to the mounting base to adequately transmit vibrations to flowers in a manner that realistically approximates the action of a buzzing bee. This has ranged from simply attaching anthers directly to the shaker base with tape (King and Lengoc 1993; Harder and Barclay 1994; King and Buchmann 1996), fashioning metal hooks that wrap around the base of anthers (Rosi-Denadai et al. 2018), or using featherweight forceps that grip anthers analogous to the way a bee grips them in her mandibles (De Luca et al. 2013).

Loudspeakers Another method of transmitting vibrations to flowers is with an audio loudspeaker. They are easily modified into a substrate-borne transducer by removing the membrane and affixing a metal wire or pin to the centre moving coil, which is then pressed against the anthers (Corbet et al. 1988). Those interested in utilising this approach are referred to Rodrigues et al. (2018) for further information. The authors provide detailed step-by-step instructions for constructing an affordable playback apparatus using a loudspeaker that is powered by a laptop and uses freely available software (e.g., Audacity: http://audacity.sourceforge.net/) to generate stimuli.

11.3.3 Calibrating Stimuli

Regardless of the playback system used, it is imperative to ensure that playback stimuli are correctly calibrated in the properties of interest prior to conducting the experiment. This will usually entail performing a preliminary test in which stimuli are applied to the substrate (e.g., anthers) and the resulting vibrations are recorded and inspected for accuracy. Our opinion is that amplitude constitutes the most important parameter for attention in playback studies of floral vibrations. Accordingly, experimenters should ensure that the appropriate amplitude component (acceleration, velocity or displacement) is faithfully reproduced. Recall that alternative recording transducers are sensitive to different amplitude components, and so if vibrations are recorded with an accelerometer it is recommended that playback stimuli are calibrated to reproduce correct acceleration values, and if a laser vibrometer is used then stimuli should be calibrated to velocity. To our knowledge, phonograph cartridges (which are sensitive to displacement) have never been used to record floral vibrations, and so we have no experience in their use as a method of calibrating playback stimuli. However, Cocroft et al. (2014b) discuss their use in vibrational communication studies and so we refer interested readers to that chapter for more information.

One difference in vibrational playback studies between buzz pollination and animal communication concerns the calibration of frequency. Plants, just as other substrates, act as filters that may drastically alter the frequency components of a broad band signal (Michelsen et al. 1982). This is important in animal communication studies because many vibrational signals often contain a range of frequencies, and so it is crucial to ensure that initial playback stimuli are conditioned, or matched to the specific plant filtering properties. By the time these playback stimuli reach the intended receiver, any alteration in the initial playback frequencies due to plant-substrate filtering (which cannot be controlled) will result in the correct signal properties required for a realistic proxy for a naturally occurring signal. Thus, playback *signal in* will serve as a reasonable proxy for natural *signal out*. A variety of compensation methods are available (reviewed in Cocroft et al. 2014b), including recently developed software tools that make their implementation straightforward (Michael et al. 2019), which ensures that playback signals containing a range of frequencies are correctly transmitted to a plant. In contrast, floral vibrations may be less affected by these filtering effects as they consist of pure tones (fundamental and a few harmonics) that decay in magnitude with distance, and thus are less likely to be adversely affected by substrate filtering effects when compared to broad band communication vibrations (Cocroft et al. 2014b). Moreover, since the experimental protocol for anther stimulation involves *direct* contact of the playback transducer to the anthers, there is little opportunity for floral vibrations to be altered before they reach their intended location.

11.4 Budding Buzz Pollination: Conclusions and Future Directions

The field of buzz pollination has made great strides in recent years, benefitting from the diverse viewpoints and approaches of researchers actively investigating the many facets of this specialised pollination syndrome. Although the majority of studies have focused on proximate causes, describing the behaviour and mechanisms governing the production of floral vibrations, there is rapidly growing interest in utilising more integrative approaches to examine ultimate (evolutionary) causes of bee-flower interactions, most notably the intersection between bee and floral characteristics and pollen release (Rosi-Denadai et al. 2018; Arroyo-Correa et al. 2019; Switzer et al. 2019). At the forefront of this is the inclusion of a biotremology perspective with its solid theoretical grounding and proven experimental methodology. In this context, studies of buzz pollination have excellent potential to contribute more broadly to other nascent fields, including mechanical ecology, the interface between mechanics and ecology at the organismal level (Bauer et al. 2020). Accordingly, as we conclude this chapter, we propose some future avenues of inquiry that are critical for expanding knowledge of buzz pollination, and that we hope will be enthusiastically embraced by researchers who study this unique biotic interaction.

First, there need to be more studies that address evolutionary hypotheses on the adaptive basis of buzz pollination for both bees and flowers. Specifically, we would like to see experimental tests estimating the fitness consequences of bee and floral traits under natural conditions. This may include examining the co-evolution of plant-pollinator characteristics (Solis-Montero and Vallejo-Marin 2017) or performing comparative analyses within a phylogenetic context to explore the adaptive significance of bee behaviour and poricidal floral morphologies (Cardinal et al. 2018). Second, new knowledge that builds on the groundwork previously established on the biomechanics of buzz pollination will be critical in broadening our understanding of the physical and behavioural factors governing the release of pollen from anthers, arguing that the vibrations induced in the flower are a complex of different wave forms (bending, Rayleigh, longitudinal, pressure) that could only be studied by a combination of 3-D (three dimensional)-measurements and computer simulations based on these measurements. Only then will we have a chance to understand how the bee-induced vibrations in the flower in turn affect the bee's behaviour (see Sect. 11.2.4)—a really new research avenue in buzz pollination research! Experimental techniques already developed by biotremology researchers that integrate different recording methods (high-speed videography and laser vibrometry) and use computer simulation modelling (see Mhatre et al. 2018) offer an unparalleled approach for linking bee behaviour and vibration production with anther mechanical responses. Third, we call for more large-scale ecological studies linking the presence of sonicating bees to plant community structure. A key gap in current knowledge of buzz pollination concerns the role bees have played in driving the structure and assemblage of buzz pollinated plant communities. Although some studies have examined how bees affect the distribution of a single buzz pollinated species (Larson and Scheme 1999a, b), scaling up to the community level has rarely been addressed (Mesquita-Neto et al. 2017). Pollinators are known to filter plant community composition as a result of preferences for certain floral traits (Pellissier et al. 2012), and this may also occur in the buzz-pollination syndrome since many floral traits are hypothesised to be the product of selection resulting from the action of sonicating bees (Dulberger et al. 1994; Marazzi et al. 2007; Vallejo-Marín et al. 2010). Such community-level investigations may be particularly timely, as recent declines in bee abundances observed around the globe (Colla and Packer 2008; Cameron et al. 2010; Goulson et al. 2015) may have drastic consequences for buzz-pollinated plant communities.

Acknowledgments We thank Peggy Hill for inviting us to contribute to this chapter about buzz pollination and its important links to the discipline of biotremology. PAD appreciates the generosity of the University of The Bahamas for supporting this work. MVM was supported by a Leverhulme Trust Research Grant (RPG-2018-235). Kristen Brochu read an earlier version of this work and provided helpful comments that improved its clarity. This chapter was also greatly improved from the insights and suggestions offered by Peggy Hill. We dedicate this chapter to Dr. Reginald B. Cocroft, whose pioneering work examining vibrational communication in treehoppers inspired a new generation of biotremology researchers, including us. Thank you, Rex.

References

Arceo-Gómez G, Martinez ML, Parra-Tabla V, Garcıa-Franco JG (2011) Anther and stigma morphology in mirror-image flowers of *Chamaecrista chamaecristoides* (Fabaceae): Implications for buzz pollination. Plant Biol 13(Suppl 1):19–24

Arroyo-Correa B, Beattie C, Vallejo-Marín M (2019) Bee and floral traits affect the characteristics of the vibrations experienced by flowers during buzz-pollination. J Exp Biol. 222:jeb198176. https://doi.org/10.1242/jeb.198176

Bauer U, Poppinga S, Müller UK (2020) Mechanical ecology--Taking biomechanics to the field. Integr Comp Biol icaa018. https://doi.org/10.1093/icb/icaa018

Bell PD (1980) Transmission of vibrations along plant stems: Implications for insect communication. Journal of the New York Entomological Society 88:210–216

Bennet-Clark HC (1989) Songs and the physics of sound production. In: Huber F, Moore TE, Loher W (eds) Cricket Behavior and Neurobiology. Cornell University Press, Ithaca, London, pp 227–261

Bennet-Clark HC (1998) Size and scale effects as constraints in insect sound communication. Philos Trans R Soc Lond B 353:407–419

Buchmann SL (1983) Buzz pollination in angiosperms. In: Jones CE, Little RJ (eds) Handbook of experimental pollination biology. Van Nostrand Reinhold Company, New York, pp 73–113

Buchmann SL (1985) Bees use vibration to aid pollen collection from non-poricidal flowers. J Kansas Entomol Soc 58:517–525

Buchmann SL, Cane JH (1989) Bees assess pollen returns while sonicating *Solanum* flowers. Oecologia 81:289–294

Buchmann SL, Hurley JP (1978) Biophysical model for buzz pollination in Angiosperms. J Theor Biol 72:639–657

Buchmann SL, Jones CE, Colin LJ (1978) Vibratile pollination of *Solanum douglasii* and *S. xanti* (Solanaceae) in Southern California. Wasmann Journal of Biology 35:1–25

Burkart A, Lunau K, Schlindwein C (2011) Comparative bioacoustical studies on flight and buzzing of neotropical bees. Journal of Pollination Ecology 6:118–124

Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswold TL (2010) Patterns of widespread decline in North American bumble bees. P Natl Acad Sci USA 108:662–667

Cardinal S, Buchmann SL, Russell AL (2018) The evolution of floral sonication, a pollen foraging behavior used by bees (Anthophila). Evolution 72:590–600. https://doi.org/10.1111/evo.13446

Casas J, Magal C, Sueur J (2007) Dispersive and non-dispersive waves through plants: Implications for arthropod vibratory communication. P Roy Soc Lond B 274:1087–1092

Cocroft RB (1996) Insect vibrational defence signals. Nature 382:679–680

Cocroft RB, De Luca PA (2006) Size-frequency relationships in insect vibrational signals. In: Drosopolous S, Claridge MF (eds) Insect Sounds and Communication: Physiology, behaviour, ecology and evolution. CRC Press, Boca Raton, pp 109–120

Cocroft RB, Rodrìguez RL (2005) The behavioral ecology of insect vibrational communication. BioScience 55:323–334

Cocroft RB, Gogala M, Hill PSM, Wessel A (2014a) Fostering research progress in a rapidly growing field. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying Vibrational Communication, vol 3. Springer-Verlag, Berlin, pp 3–12

Cocroft RB, Hamel J, Su Q, Gibson J (2014b) Vibrational playback experiments: Challenges and solutions. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying Vibrational Communication, vol 3. Springer-Verlag, Berlin, pp 249–274

Čokl A, Virant-Doberlet M (2003) Communication with substrate-borne signals in small plant-dwelling insects. Annu Rev. Entomol 48:29–50

Čokl A, Virant-Doberlet M, Stritih N (2000) The structure and function of songs emitted by southern green stink bugs from Brazil, Florida, Italy and Slovenia. Physiol Entomol 25:196–205

Čokl A, Presern J, Virant-Doberlet M, Bagwell GJ, Millar JG (2004) Vibratory signals of the harlequin bug and their transmission through plants. Physiol Entomol 29:372–380

Čokl A, Zorovic M, Zunic A, Virant-Doberlet M (2005) Tuning of host plants with vibratory songs of *Nezara viridula* L (Heteroptera: Pentatomidae). J Exp Biol 208:1481–1488

Colla SR, Packer L (2008) Evidence for decline in eastern North American bumblebees (Hymenoptera: Apidae), with special focus on *Bombus affinis* Cresson. Biodiversity and Conservation 17:1379–1391

Corbet SA, Huang SQ (2014) Buzz pollination in eight bumblebee-pollinated *Pedicularis* species: Does it involve vibration-induced triboelectric charging of pollen grains? Ann Bot 114:1665–1674. https://doi.org/10.1093/aob/mcu195

Corbet SA, Chapman H, Saville N (1988) Vibratory pollen collection and flower form: Bumblebees on *Actinidia*, *Symphytum*, *Borago* and *Polygonatum*. Funct Ecol 2:147–155

De Luca PA (2015) Mass correlates with increased mating success for older but not younger males in thornbug treehoppers. Ann Entomol Soc Am 108:222–228

De Luca PA, Cocroft RB (2009) Age-related changes in an insect mating signal have no effect on female choice. Behav Ecol Sociobiol 63:1787–1798

De Luca PA, Morris GK (1998) Courtship communication in meadow katydids: Female preference for large male vibrations. Behaviour 135:777–794

De Luca PA, Vallejo-Marín M (2013) What's the buzz about? The ecology and evolutionary significance of buzz-pollination. Curr Opin Plant Biol 16:429–435

De Luca PA, Bussière LF, Souto-Vilaros D, Goulson D, Mason AC, Vallejo-Marín M (2013) Variability in bumblebee pollination buzzes affects the quantity of pollen released from flowers. Oecologia 172:805–816

De Luca PA, Cox DA, Vallejo-Marín M (2014) Comparison of pollination and defensive buzzes in bumblebees indicates species-specific and context-dependent vibrations. Naturwissenschaften 101:331–338

De Luca PA, Giebink N, Mason AC, Papaj DR, Buchmann SL (2018) How well do acoustic recordings characterize properties of bee (Anthophila) floral sonication vibrations? Bioacoustics. https://doi.org/10.1080/09524622.2018.1511474

De Luca PA, Buchmann SL, Galen C, Mason AC, Vallejo-Marín M (2019) Does body size predict the buzz-pollination frequencies used by bees? Ecol Evol 9:4875–4887

Dierkes S, Barth FG (1995) Mechanism of signal production in the vibratory communication of the wandering spider *Cupiennius getazi* (Arachnida, Araneae). J Comp Physiol A 176:31–44

Dulberger R, Smith MB, Bawa KS (1994) The stigmatic orifice in *Cassia*, *Senna*, and *Chamaecrista* (Caesalpinaceae): Morphological variation, function during pollination, and possible adaptive significance. Am J Bot 81:1390–1396

Eberhard MJB, Metze D, Küpper SC (2019) Causes of variability in male vibratory signals and the role of female choice in Mantophasmatodea. Behav Process 166:103907

Elias DO, Lee N, Hebets EA, Mason AC (2006) Seismic signal production in a wolf spider: Parallel versus serial multi-component signals. J Exp Biol 209:1074–1084

Endler JA (2014) Foreward: The emerging field of tremology. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying Vibrational Communication. Springer, Berlin Heidelberg, pp vii–x

Gibson JS, Cocroft RB (2018) Vibration-guided mate searching in treehoppers: directional accuracy and sampling strategies in a complex sensory environment. J Exp Biol 221(6):jeb175083

Goulson D, Nicholls E, Botias C, Rotheray EL (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347:1–16

Harder LD, Barclay MR (1994) The functional significance of poricial anthers and buzz pollination: controlled pollen removal from *Dodecatheon*. Funct Ecol 8:509–517

Hebets EA, Elias DO, Mason AC, Miller GL, Stratton GE (2008) Substrate-dependent signalling success in the wolf spider, *Schizocosa retrorsa*. Anim Behav 75:605–615

Henry CS (1980) The importance of low-frequency, substrate-borne sounds in lacewing communication (Neuroptera: Chrysopidae). Ann Entomol Soc Am 73:617–621

Henry CS, Martinez-Wells ML (2006) Testing the ability of males and females to respond to altered songs in the duetting green lacewing, *Chrysoperla plorabunda* (Neuroptera: Chrysopidae). Behav Ecol Sociobiol 61:39–51

Henry CS, Wells MLM, Simon CM (1999) Convergent evolution of courtship songs among cryptic species of the *Carnea* group of green lacewings (Neuroptera: Chrysopidae: Chrysoperla). Evolution 53:1165–1179

Hill PSM (2008) Vibrational Communication in Animals. Harvard University Press, Cambridge

Hill PSM, Wessel A (2016) Biotremology. Curr Biol 26:R187–R191

Hrncir M, Schmidt VM, Schorkopf DLP, Jarau S, Zucchi R, Barth FG (2006) Vibrating the food receivers: A direct way of signal transmission in stingless bees (*Melipona seminigra*). J Comp Physiol A 192:879–887

Hunt RE, Morton TL (2001) Regulation of chorusing in the vibrational communication system of the leafhopper *Graminella nigrifrons*. Am Zool 41:1222–1228

Hunt RE, Nault LR (1991) Roles of interplant movement, acoustic communication, and phonotaxis in mate-location behavior of the leafhopper *Graminella nigrifrons*. Behav Ecol Sociobiol 28: 315–320

Josephson RK, Malamud JG, Stokes DR (2000) Asynchronous muscle: A primer. J Exp Biol 203: 2713–2722

Kilpinen O, Storm J (1997) Biophysics of the subgenual organ of the honeybee, *Apis mellifera*. J Comp Physiol A 181:309–318

King MJ (1993) Buzz foraging mechanism in bumble bees. J Apic Res 32:41–49

King MJ, Buchmann SL (1995) Bumble bee-initiated vibration release mechanism of *Rhododendron* pollen. Am J Bot 82:1407–1411

King MJ, Buchmann SL (1996) Sonication dispensing of pollen from *Solanum laciniatum* flowers. Funct Ecol 10:449–456

King MJ, Buchmann SL (2003) Floral sonication by bees: Mesosomal vibration by *Bombus* and *Xylocopa*, but not *Apis* (Hymenoptera: Apidae), ejects pollen from poricidal anthers. J Kansas Entomol Soc 76:295–305

King MJ, Lengoc L (1993) Vibratory pollen collection dynamics. Transactions of the American Society of Agricultural Engineers 36:135–140

King MJ, Buchmann SL, Spangler HG (1996) Activity of asynchronous flight muscle from two bee families during sonication (buzzing). J Exp Biol 199:2317–2321

Kumar H, Saxena KN (1985) Certain environmental factors influencing the acoustic communication in the sexual behaviour of the leafhopper *Amrasca devastans* (Distant) (Homoptera: Cicadellidae). Appl Entomol Zool 20:199–209

Larson BM, Scheme B (1999a) The ecology of pollen limitation in buzz-pollinated *Rhexia virginica* (Melastomataceae). J Ecol 87:371–381

Larson BM, Scheme B (1999b) The pollination ecology of buzz-pollinated *Rhexia virginica* (Melastomataceae). Am J Bot 86:502–511

Macior LW (1964) Experimental study of floral ecology of *Dodecatheon meadia*. Am J Bot 51:96–108

Macior LW (1968) Pollination adaptation in *Pedicularis groenlandica*. Am J Bot 55:927–932

Marazzi B, Conti E, Endress PK (2007) Diversity in anthers and stigmas in the buzz-pollinated genus *Senna* (Leguminosae, Cassiinae). Int J Plant Sci 168:371–391

McNett GD, Cocroft RB (2008) Host shifts favor vibrational signal divergence in *Enchenopa binotata* treehoppers. Behav Ecol 19:650–656

McNett GD, Miles RN, Homentcovschi D, Cocroft RB (2006) A method for two-dimensional characterization of animal vibrational signals transmitted along plant stems. J Comp Physiol A 192:1245–1251

McNett GD, Luan LH, Cocroft RB (2010) Wind-induced noise alters signaler and receiver behavior in vibrational communication. Behav Ecol Sociobiol 64:2043–2051

McVean A, Field LH (1996) Communication by substratum vibration in the New Zealand tree weta, *Hemideina femorata* (Stenopelmatidae: Orthoptera). J Zool 239:101–122

Mesquita-Neto JN, Costa BKP, Schlindwein C (2017) Heteranthery as a solution to the demand for pollen as food and for pollination—legitimate flower visitors reject flowers without feeding anthers. Plant Biol 19:942–950

Mhatre N, Sivalinghem S, Mason AC (2018) Posture controls mechanical tuning in the black widow spider mechanosensory system. bioRxiv. https://doi.org/10.1101/123456

Michael SCJ, Appel HA, Cocroft RB (2019) Methods for replicating leaf vibrations induced by insect herbivores. In: Gassmann W (ed) Plant innate immunity: methods and protocols. Humana Press, New York, pp 141–158

Michelsen A, Nocke H (1974) Biophysical aspects of sound communication in insects. Adv Insect Physiol 10:247–296

Michelsen A, Fink F, Gogala M, Traue D (1982) Plants as transmission channels for insect vibrational songs. Behav Ecol Sociobiol 11:269–281

Miklas N, Stritih N, Čokl A, Virant-Doberlet M, Renou M (2001) The influence of substrate on male responsiveness to the female calling song in *Nezara viridula*. J Insect Behav 14:313–332

Miles CI, Allison BE, Losinger MJ, Su QT, Miles RN (2017) Motor and mechanical bases of the courtship call of the male treehopper *Umbonia crassicornis*. J Exp Biol 220:1915–1924

Miranda X (2006) Substrate-borne signal repertoire and courtship jamming by adults of *Ennya chrysura* (Hemiptera: Membracidae). Ann Entomol Soc Am 99:374–386

Mitomi M, Ichikawaa T, Okamoto H (1984) Morphology of the vibration-producing organ in adult rice brown planthopper, *Nilaparvata lugens* (STAL): Homoptera: Delphacidae. Appl Entomol Zool 19:407–417

Moreira GRP (1993) Reproductive biology of the stream-dwelling stonefly, *Agnetina capitata* (Pictet) (Plecoptera: Perlidae). Dissertation, Cornell University, Ithaca, NY

Morgan T, Whitehorn PR, Lye GC, Vallejo-Marín M (2016) Floral sonication is an innate behaviour in bumblebees that can be fine-tuned with experience in manipulating flowers. J Insect Behav 29:233–241

Morris GK (1980) Calling display and mating behaviour of *Copiphora rhinoceros* Pictet (Orthoptera: Tettigoniidae). Anim Behav 28:42–51

Nunes-Silva P, Hrncir M, Shipp L, Kevan P, Imperatriz-Fonseca VL (2013) The behaviour of *Bombus impatiens* (Apidae, Bombini) on tomato (*Lycopersicon esculentum* Mill., Solanaceae) flowers: Pollination and reward perception. J Pollinat Ecol 11:33–40

Ossiannilsson F (1949) Insect drummers. A study on the morphology and function of the sound-producing organ of Swedish Homoptera Auchenorrhyncha. Opuscula Entomol Suppl X:1–146

Papaj DR, Buchmann SL, Russell AL (2017) Division of labor of anthers in heterantherous plants: flexibility of bee pollen collection behavior may serve to keep plants honest. Arthropod Plant Interactions 11:307–315

Pellissier L, Alvarez N, Guisan A (2012) Pollinators as drivers of plant distributions and assemblage into communities. In: Patiny S (ed) Evolution of plant-pollinator relationships. Cambridge University Press, New York, pp 392–413

Pringle JWS (1949) The excitation and contraction of the flight muscles of insects. J Physiol (Lond) 108:226–232

Pritchard DJ, Vallejo-Marín M (2020) Floral vibrations by buzz-pollinating bees achieve higher frequency, velocity and acceleration than flight and defence vibrations. J Exp Biol. https://doi.org/10.1242/jeb.220541

Rebar D, Hoebel G, Rodriguez RL (2012) Vibrational playback by means of airborne stimuli. J Exp Biol 215:3513–3518

Rodrigues EV, Riguette JR, Pereira HRC, Tesch JA, Silva AG (2018) An affordable apparatus for fine-controlled emulation of buzzing frequencies of bees for the testing hypothesis in buzz interactions. Ecol Evol 8:7667–7672

Rodríguez RL, Cocroft RB (2006) Divergence in female duetting signals in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). Ethology 112:1231–1238

Rodríguez RL, Sullivan LE, Cocroft RB (2004) Vibrational communication and reproductive isolation in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). Evolution 58:571–578

Rodríguez RL, Ramaswamy K, Cocroft RB (2006) Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feeding insects. Proc R Soc Lond B 273: 2585–2593

Rosi-Denadai CA, Araújo PCS, de Oliveira Campos LA, Cosme L Jr, Guedes RNC (2018) Buzz-pollination in neotropical bees: genus-dependent frequencies and lack of optimal frequency for pollen release. Insect Sci. https://doi.org/10.1111/1744-7917.12602

Rovner JS, Barth FG (1981) Vibratory communication through living plants by a tropical wandering spider. Science 214:464–466

Russell AL, Golden RE, Leonard AE, Papaj DR (2015) Bees learn preferences for plant species that offer only pollen as a reward. Behav Ecol 27:731–740

Russell AL, Leonard AS, Gillette HD, Papaj DR (2016) Concealed floral rewards and the role of experience in floral sonication by bees. Anim Behav 120:83–91

Russell AL, Buchmann SL, Papaj DR (2017) How a generalist bee achieves high efficiency of pollen collection on diverse floral resources. Behav Ecol 28:991–1003. https://doi.org/10.1093/beheco/arx058

Solis-Montero L, Vallejo-Marin M (2017) Does the morphological fit between flowers and pollinators affect pollen deposition? An experimental test in a buzz-pollinated species with anther dimorphism. Ecol Evol 7:2706–2715. https://doi.org/10.1002/ece3.2897

Speaks CE (1999) Introduction to sound: acoustics for the hearing and speech sciences. Singular Publishing Group, San Diego CA

Sueur J (2018) Sound analysis and synthesis with R. Springer, Cham, Switzerland

Sullivan-Beckers LE, Hebets EA (2014) Tactical adjustment of signalling leads to increased mating success and survival. Anim Behav 93:11–17

Switzer C, Combes S (2017) Bumblebee sonication behavior changes with plant species and environmental conditions. Apidologie 48:223–233. https://doi.org/10.1007/s13592-016-0467-1

Switzer C, Hogendoorn K, Ravi S, Combes S (2016) Shakers and head bangers: differences in sonication behavior between Australian *Amegilla murrayensis* (blue-banded bees) and North American *Bombus impatiens* (bumblebees). Arthropod Plant Interactions 10:1–8

Switzer C, Russell AL, Papaj DR, Combes SA, Hopkins R (2019) Sonicating bees demonstrate flexible pollen extraction without instrumental learning. Curr Zool. https://doi.org/10.1093/cz/zoz013

Teppner H (2018) The first records of vibratory pollen-collection by bees. Phyton Annales Rei Botanicae 57:135–141

Tercel MP, Veronesi F, Pope TW (2018) Phylogenetic clustering of wingbeat frequency and flight-associated morphometrics across insect orders. Physiol Entomol 43:149–157

Vallejo-Marín M (2019) Buzz pollination: studying bee vibrations on flowers. New Phytol. https://doi.org/10.1111/nph.15666

Vallejo-Marín M, Da Silva EM, Sargent RD, Scheme B (2010) Trait correlates and functional signficance of heteranthery in flowering plants. New Phytol 188:418–425

Virant-Doberlet M, Čokl A (2004) Vibrational communication in insects. Neotrop Entomol 33: 121–134

Wells MM, Henry CS (1992) The role of courtship songs in reproductive isolation among populations of green lacewings of the genus *Chrysoperla* (Neuroptera: Chrysopidae). Evolution 46:31–42

Zeigler DD, Stewart KW (1977) Drumming behavior of eleven nearctic stonefly (Plecoptera) species. Ann Entomol Soc Am 70:495–505

Zeigler DD, Stewart KW (1985) Age effects on drumming behavior of *Pteronarcella badia* (Plecoptera) males. Entomol News 96:157–160

Chapter 12
Mechanosensory Behaviour and Biotremology in Nematodes

Takuma Sugi

Abstract Nematodes account for approximately 80% of all animals on earth, which, inevitably, has diverse effects on ecosystems. Among diverse nematode species, *Caenorhabditis elegans* has been used as a powerful animal model through which to investigate mechanosensory behaviour and its underlying neural and molecular mechanisms. Furthermore, physical interactions between *C. elegans* and other nematode species and other animals, which have potentially become a good model for understanding biotremology, are discussed. In this chapter, I mainly review the mechanosensory behaviour of nematodes, especially *C. elegans*, and the perspectives on biotremology.

12.1 Introduction

Nematoda is a diverse animal phylum inhabiting a broad range of environments. Estimates of the number of nematode species have been reported ranging from 30,000 to over 1 million. Nematodes constitute approximately 10% of the global animal biomass (Bar-On et al. 2018). Therefore, nematodes are one of the most ubiquitous phyla in the animal kingdom.

Nematodes are broadly categorized into two main groups, free-living and parasitic groups. The majority of nematodes are non-parasitic and free-living. Free-living nematodes feed on organisms in their environment; although, some are phoretically associated with other invertebrates. Parasitic types feed off of a host, and some also live within the host, which can include other animals and plants. In the wild, nematodes are most often found in the dauer stage–a stress-resistant, developmentally arrested stage that is entered in response to an adverse environment (Barrière and Félix 2005). The nematode dauer larva can exhibit nictation behaviour in which the worm stands on its tail and waves its head to interact with other animals and

---

T. Sugi (✉)
Program of Biomedical Science, Graduate School of Integrated Sciences for Life, Hiroshima University, Hiroshima, Japan
e-mail: sugit@hiroshima-u.ac.jp

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_12

plants (Campbell and Gaugler 1993; Lee et al. 2011). Therefore, nematodes have many chances to physically interact with other animals and plants. In terms of biotremology, responses to vibration and acoustic waves are considered to be potentially important environmental cues for physical interactions with environments, but they remain an enigma. However, the basic behavioural responses to these cues and their underlying mechanisms have been long studied in the laboratory in the context of mechanosensory behaviour.

Caenorhabditis elegans, a 1 mm long free-living nematode, has been one of the best model animals to study the mechanism underlying mechanosensory behaviour and has been used for just over 50 years (Brenner 1974). The advantage of *C. elegans* is its simple morphology; the adult hermaphrodite is composed of only 959 somatic cells, including 302 neurons, and these cell numbers do not vary among individuals. All the wiring and connectivity of these neurons have been completely reconstructed from electron micrographs (White et al. 1986). In terms of behavioural studies, this simple system allows us to perform cell-specific genetic experiments in which a gene can be expressed in the targeted cells to investigate a mechanism linking a gene function with a specific cell (Mello et al. 1991). Therefore, in *C. elegans*, there are extensive amounts of accumulated data for mechanosensory behaviour (Schafer 2014; Goodman and Sengupta 2019).

In this review, I mainly focus on the mechanosensory behaviour of nematodes, specifically focusing on *C. elegans,* and discuss this behaviour in the context of biotremology. Finally, I address future perspectives, especially related to research in the wild.

12.2 Neural Circuit and Molecule

12.2.1 Mechanosensory Neural Circuit

C. elegans exhibits clear and simple behavioural responses to mechanical stimuli, such as touch stimulus and nonlocalized vibration produced by tapping on a cultivated Petri plate. Worms usually move forward via undulation. When anterior and posterior parts of the body receive a touch stimulus, the worm shows a backward escape response and forward acceleration, respectively. The neural circuits driving touch-evoked behaviours have been identified by forward genetic screenings of mechanosensory abnormal mutant alleles, called *mec*, and laser ablation experiments (Fig. 12.1) (Chalfie and Sulston 1981; Chalfie et al. 1985). The two mechanosensory neurons ALM and AVM respond to anterior touch stimuli, causing a backward escape response through the downstream interneurons AVD and AVA. Thus, this sub-circuit is the backward-driving circuit. In contrast, the mechanosensory neuron PLM is activated in response to posterior touch stimuli, leading to forward acceleration through the interneurons PVC and AVB. This sub-circuit is known as the forward-driving circuit. The optogenetic activation or inhibition of these sub-circuits

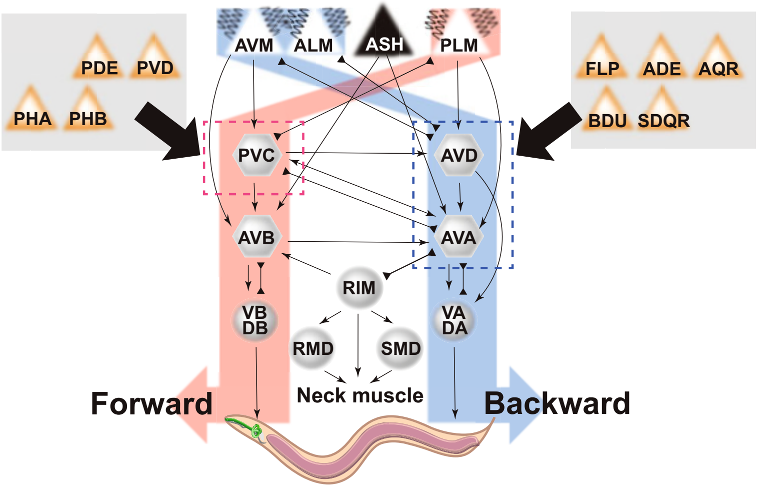


Fig. 12.1 Mechanosensory neural circuit diagram. Triangles, hexagons and circles represent sensory neurons, interneurons and motor neurons, respectively. Sub-circuits involved in forward and backward movements are indicated in magenta and light blue, respectively. The neurons involved in the harsh touch sensation are illustrated in orange triangles; the posterior PDE and PVD neurons and the anus PHA and PHB neurons contribute to forward movement through the PVC neurons (read dashed box); whereas, the FLP, ADE, AQR, BDU and SDQR neurons are involved in backward movement through the AVD and AVA neurons (blue dashed box). The PVD and FLP neurons are multidenderitic neurons. The polymodal neuron ASH is indicated by black triangles. Wavy lines around the AVM, ALM and PLM neurons indicate that they can be activated by nonlocalized mechanical stimuli such as vibration

has proven necessary and sufficient to drive the forward acceleration or backward escape response (Leifer et al. 2011; Stirman et al. 2011).

In the case of a harsh touch stimulus, many neurons are involved in the touch-evoked behavioural responses. The representative neurons are polymodal PVD and FLP neurons, both of which are multidendritic neurons (Fig. 12.1) (Albeg et al. 2011). The PVD neurons tile the body surface as a receptive field; whereas, the FLP neurons cover only the head and neck regions. A nose touch stimulus upon head-on collision with another animal evokes the backward escape response, which depends not only on FLP neurons but also ASH polymodal neurons, OLQ neurons and IL1 neurons (Hart et al. 1995). The FLP, OLQ and CEP neurons are electrically coupled to a single hub interneuron, RIH, through gap junctions. This sub-circuit plays an essential role in nose touch-dependent behavioural responses (Chatzigeorgiou and Schafer 2011). Recent laser ablation experiments indicate several additional neurons to process harsh touch: ADE, AQR, BDU, SDQ, PHA, PHB, PQR and PDE neurons (Li et al. 2011).

In contrast to these local touch stimuli, nonlocalized stimulation, such as a vibration in the growth substrate, e.g., a nematode growth medium plate, activates ALM, AVM, PLM and PVM touch neurons at the same time (Wicks and Rankin

1995). Worms that receive a nonlocalized mechanical stimulus usually execute the backward escape response as a result of the competitive activations between the forward- and backward-driving circuits, in which the activation of backward-driving circuits predominates. More rarely, worms show forward accelerations. The neural circuits and behaviours in response to mechanosensory stimuli have been well described in *C. elegans* through laser ablation and genetic analyses (Bozorgmehr et al. 2013).

12.2.2 Mechanosensory Molecules

In the case of *C. elegans*, due to its morphological simplicity, it is possible to perform cell-specific genetic experiments in vivo, which offers the opportunity to directly examine the specific relationship between each gene and both neurons and behaviour. Two types of ionotropic mechanoreceptor families, DEG/ENaC channels and TRP channels, have been well characterized in terms of mechanosensation, as studied in other organisms (Geffeney and Goodman 2012; Ranade et al. 2015). In *C. elegans*, the DEG/ENaC channels MEC-4 and MEC-10 were characterized a long time ago. MEC-4 and MEC-10 were identified in forward genetic screens for gentle touch-defective mutants (Chalfie and Sulston 1981). MEC-4 shows specific expression in the gentle touch neurons ALM, AVM, PLM and PVM; whereas, MEC-10 is additionally expressed in the FLP and PVD neurons (Driscoll and Chalfie 1991; Huang and Chalfie 1994; Suzuki et al. 2003; Chatzigeorgiou and Schafer 2011; Arnadóttir et al. 2011). Worms carrying *mec-4* null mutations exhibit defects in behavioural and mechanoreceptor current responses to gentle touch (Chalfie and Au 1989; O'Hagan et al. 2005). Therefore, MEC-4 has been thought to directly perceive mechanical stimuli as a mechanosensor; although, reconstitution experiments in heterologous cells remain unestablished thus far. MEC-10 has not been reported to form homomeric channels when expressed in oocytes. Instead, MEC-10 associates with MEC-4 proteins to form a channel complex (Chen et al. 2015). MEC-2 and MEC-6 are also known to associate with MEC-4 and MEC-10 to synergistically increase channel activity (Arnadóttir et al. 2011).

One of the TRP channels, TRP-4, which is the orthologue of the mammalian TRPN protein, has been known to function in several dopaminergic touch receptor neurons, CEP, ADE and PDE, and proprioceptive DVA interneurons (Li et al. 2006). TRP-4 is needed in CEP, ADE and PDE neurons for locomotor slowing behaviour in the presence of food, and nose touch avoidance behaviour, and in DVA for the control of body-bend amplitude (Li et al. 2006; Kindt et al. 2007a; Kang et al. 2010). In contrast to the crucial role of TRP4 in mechanosensation, other TRP channels, TRPA-1 and OSM-9, which are the orthologues of mammalian TRPA and TRPV, respectively, appear unlikely to function directly as a mechanosensor (Kindt et al. 2007b; Chatzigeorgiou et al. 2010; Geffeney et al. 2011). For example, OSM-9 may act as an amplifier of mechanotransduction currents in touch neuron ASH.

Another candidate involved in mechanosensation is Piezo proteins, mechanotransduction channels involved in touch sensing in mammals and flies (Coste et al. 2010; Ranade et al. 2015). *C. elegans* contains an orthologue of the Piezo protein, but its role in mechanosensation remains unclear. In mammals, the transmembrane channel-like (TMC) family proteins TMC1 and TMC2 have also been known to play a role in cochlear hair cell mechanotransduction (Ranade et al. 2015). Recently, Chatzigeorgiou et al. revealed that *C. elegans* TMC-1 is required for sodium chemosensation in polymodal ASH neurons, indicating that it acts as a sodium sensor (Chatzigeorgiou et al. 2013). The *tmc-2* gene is also expressed in mechanosensory neurons, but it remains unknown whether TMC-1 and TMC-2 are directly involved in mechanosensation.

12.3 Physiological Studies

12.3.1 Investigations of Behavioural and Neural Responses Specific to Parameters of Mechanosensory Stimuli

Classically, neural and behavioural responses in nematodes have been examined through body touch stimulation by stroking the worm body with an eyebrow hair (Chalfie and Sulston 1981) and tap-evoked vibration of the growth medium (Gannon and Rankin 1995), until the early 2000s. These classic techniques have largely contributed to the current understanding of each behavioural response and the underlying neural circuitry (see Sect. 12.2.1). However, understanding mechanosensory responses and their underlying mechanisms has progressed with advances in the techniques to control the parameters of mechanosensory stimuli such as frequency, amplitude and stimulus duration (Fig. 12.2). Because a single sensory modality is composed of many parameters, information from even a single sensory modality is extremely diverse. Indeed, animals often exhibit remarkably different, even opposite, behavioural patterns based on stimulus parameter differences, regardless of the stimulus occurring through the same sensory modality (Yoshida et al. 2012).

Park et al. developed a technique to apply defined mechanical load profiles to *C. elegans* and measure applied displacement using piezoresistive microcantilevers as force-displacement sensors coupled to a feedback system (Park et al. 2007). Then, they combined this force-feedback system with in vivo whole-cell patch-clamp recording, called FALCON (Feedback-controlled application of mechanical loads combined with in vivo neurophysiology) (Eastwood et al. 2015). Using this system, they show that current amplitude in the ALM neuron increases with indentation, rather than force, and that fast stimuli evoke larger currents than slower stimuli. Furthermore, indentation decreases with force frequency, and the peak current and sensitivity reach maximal values at 150 and 725 Hz, respectively. This low-frequency filtering might allow TRNs to ignore body movements that involve

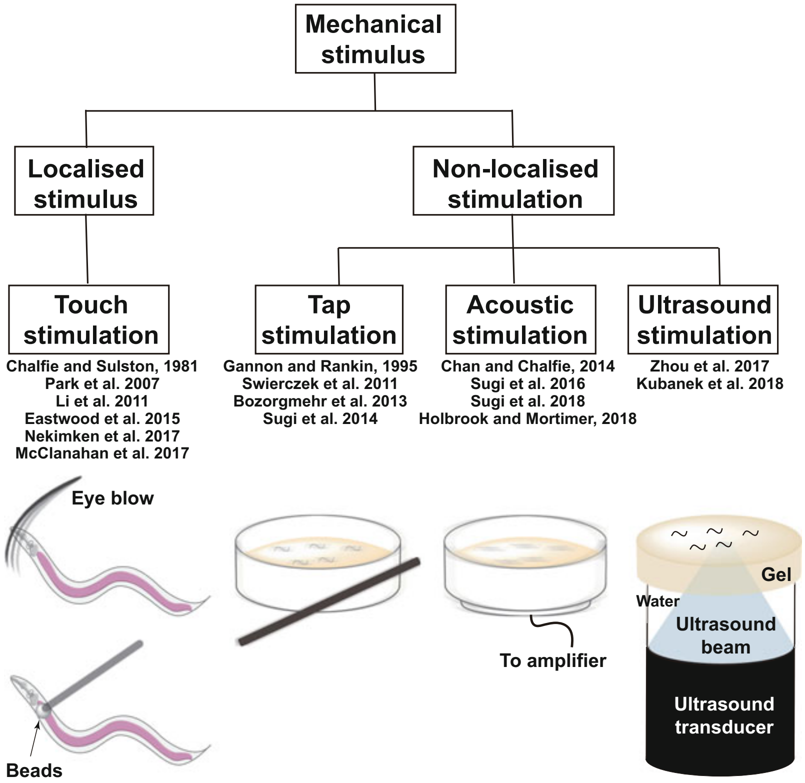


Fig. 12.2 Overview of mechanosensory experiments. Methods for quantifying behavioural responses to each type of mechanical stimulus are described. Representative papers for each method are also shown as references. The figure of ultrasound stimulation was modified from Kubanek et al. (2018). (No permission required by publisher/copyright holder)

undulation frequencies on the order of 0.5 Hz when crawling on nematode growth medium plates in the absence of food (Cronin et al. 2005; Fang-Yen et al. 2010). Finally, a model linking body indentation to mechanoelectrical transduction channel activation was described to reproduce the experimental results.

Recent developments in microfabrication methods have made it possible to combine force parameter control techniques with microfluidic systems. For example, Nekimken et al. established a microfluidic device that allows the precise application of pneumatic pressure to a worm trapped in the microfluidic device (Nekimken et al. 2017). The combination of this system and calcium imaging experiments revealed that the application of a 275 kPa step or ramp stimulus resulted in no detectable response from ALM, AVM and PVM neurons, but strong activation in these neurons was observed after the application of a buzzing stimulus (75 kPa, 10 Hz sine superimposed on a 275 kPa step stimulus). Applying the maximum pressure of 450 kPa also induced calcium transients after both step and ramp stimuli. At nearly the same time, similar pneumatically actuated systems coupled with calcium imaging were established as a fully automated microfluidic platform. McClanahan et al. also developed a multiplexed hydraulic microfluidic device in which each hydraulic valve can deliver a touch stimulus to each worm trapped in each of 64 sinusoidal channels (McClanahan et al. 2017).

Nonlocalized vibration was originally evoked by tapping a Petri plate using a solenoid tapper or a ROBO cylinder (Swierczek et al. 2011; Bozorgmehr et al. 2013; Sugi et al. 2014). Recently, a method was developed to control the frequency and amplitude of the vibration of an agar surface on which many worms are cultivated. In particular, Chen et al. used a speaker to evoke an acoustic vibration in a Petri plate of worms (Chen and Chalfie 2014). Similar to this, another method was also constructed using a piezoelectric sheet speaker (Sugi et al. 2016, 2018). This method has allowed the vibration properties to be changed at the nanoscale displacement level. Calcium imaging experiments using this system revealed that the activity of AVA interneurons at 630 Hz frequency is higher than those at 250, 400, 800 and 1000 Hz. Furthermore, Holbrook and Mortimer showed that *C. elegans* exhibits a different behavioural response to sustained 23 Hz vibration compared with usual short-term nonlocalized mechanical stimuli; worms decreased the incidence of backward movements and increased total distance travelled during sustained vibration; whereas, opposite behavioural patterns were observed in usual mechanical stimuli (Holbrook and Mortimer 2018). This experiment was performed for worms on nematode growth medium unseeded with the food source *Escherichia coli*, but in other studies, behavioural responses are often measured on a medium seeded with the food source. The difference in experimental conditions should be considered in the future.

Recently, pulsed ultrasound has been shown to elicit a backward escape response in *C. elegans* (Zhou et al. 2017; Kubanek et al. 2018). The application of ultrasound generated by interdigital transducers on a piezoelectric substrate indicated that the duration of stimulation is important for eliciting the backward response and that polymodal ASH neurons are activated under this stimulation (Zhou et al. 2017). The application of focused ultrasound to worms also indicated that gentle touch receptor neurons contribute to the detection of ultrasound stimuli (Kubanek et al. 2018). The molecular mechanisms underlying these ultrasound sensations remain elusive, and genetic analysis tools in *C. elegans* will likely pave the way to understanding these mechanisms in the future.

12.3.2 Habituation Memories

C. elegans can memorize previously experienced physical stimuli such as mechanical stimuli (Timbers and Rankin 2011; Schmid et al. 2014) and temperature (Mori and Ohshima 1995; Sugi et al. 2011; Sasakura and Mori 2013). Habituation to a tap stimulus is one of the most traditional paradigms in *C. elegans* (Wicks and Rankin 1995; Rose et al. 2003; Bozorgmehr et al. 2013). After training with repeated mechanical tap stimulation, worms habituate to the stimuli and exhibit a decrease

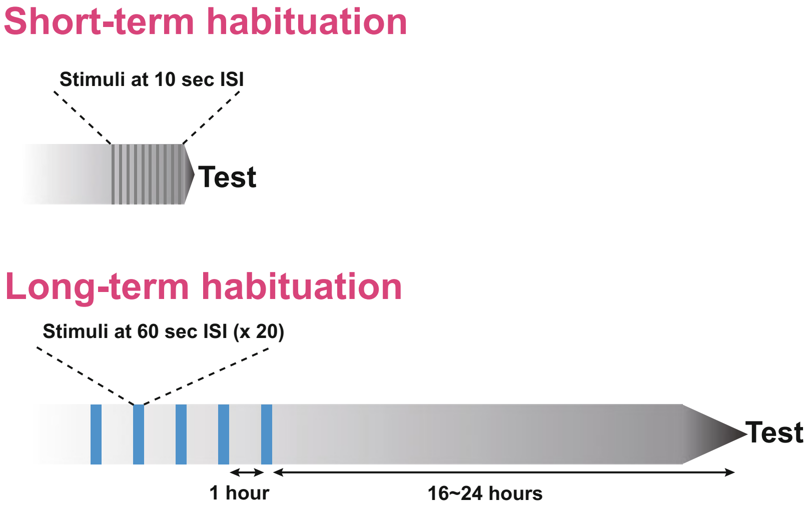


Fig. 12.3 Protocols for inducing short-term and long-term habituation. In short-term habituation, worms receive 10–20 nonlocalized mechanical stimulations with 10 s ISI, and their behaviours are then tested. In long-term habituation, worms are usually trained with four or five blocks of 20 nonlocalized stimulations delivered with a 60 s ISI. Then, a behavioural test is performed 18–24 h after training. The blocks are separated by a 1-h rest period. ISI = interstimulus interval

in the magnitude of the backward escape response. The retention time of memory is dependent on the interstimulus interval (ISI) of the training protocol; worms trained with a short ISI (~10 s) and a long ISI (~60 s) between stimulations exhibit short-term and long-term memories, respectively (Fig. 12.3). Long-term memories last for 24 h after training. The neural basis for this behavioural plasticity has also been investigated by genetic approaches. The *C. elegans* orthologue of the glutamate vesicular transporter EAT-4 is expressed in the touch neurons ALM, AVM and PLM. Worms carrying a mutation in the *eat-4* gene exhibit more rapid habituation to a 10 s ISI than wild-type worms, suggesting that neurotransmitter release from touch neurons contributes to short-term habituation (Rankin and Wicks 2000). During long-term habituation elicited with a 60 s ISI, the expression level of the glutamate receptor GLR-1 significantly decreases in the AVA interneuron that drives the backward response (Rose et al. 2003). In addition, several papers have indicated that habituation memory formation requires the transcription factor cAMP response element-binding protein (CREB), whose phosphorylation is a hallmark for determining which neurons are recruited to a given memory trace in animals ranging from invertebrates to mammals (Lonze and Ginty 2002; Nishida et al. 2011; Kim et al. 2013). The previous studies have suggested that the AVA interneuron is the site of action for habituation memory formation (Timbers and Rankin 2011; Sugi et al. 2014), but future studies are needed to examine the relationship between the transcriptional activity of CREB and the expression of glutamate receptors for long-term habituation.

12.3.3 Biological Relevance of Mechanosensory Behaviours and Biotremology

Many *C. elegans* researchers have increasingly paid attention to physical interactions with other organisms and population-level behaviours (Frézal and Félix 2015). For example, Maguire et al. examined the ecological significance of the touch response in *C. elegans* and, thereby, showed that mutants that are defective in suppressing head movements in response to touch are more often caught by a predacious fungus, *Drechslerella doedycoides*, than wild-type worms (Maguire et al. 2011). Therefore, the touch-evoked backward response contributes to withdrawal from the trap of a predator. However, the ecological significance of the behavioural response to nonlocalized mechanical stimuli remains unknown. As often described in the research field of biotremology, many organisms respond to the surface wave elicited by their prey or predator (Brownell 1977; Hill and Wessel 2016; Mortimer 2017). Interestingly, some parasitic nematodes, such as *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*, exhibit jumping behaviours after nictation for efficient dispersal and host-seeking (Reed and Wallace 1965; Campbell and Kaya 1999; Hallem et al. 2011). From the view of biotremology, these observations raise the hypothesis that jumping frequencies might be modified by the surface wave. Additionally, it is interesting to examine whether nictation behaviour could be affected by nonlocalized mechanical stimulation.

Several population-level behaviours have been reported also in *C. elegans*. One of the representative phenomena is the aggregation by worms that carry a natural variant in *npr-1* gene (de Bono and Bargmann 1998). The *npr-1* encodes a neuropeptide Y-like G protein-coupled receptor: solitary stains possess the valine at position 215 of the NPR-1 protein; whereas, socially aggregating strains have phenylalanine. Since the discovery of this natural variant, the neural and genetic mechanisms underlying this aggregation behaviour have been intensively studied and summarized in another review (de Bono and Maricq 2005). Recently, Ding et al. also investigated this phenomenon in a physical aspect using agent-based simulations and found that cluster-edge reversals, a density-dependent switch of crawling speeds, and taxis towards neighbouring worms are the key behavioural rules for the aggregation (Ding et al. 2019). Another swarming behaviour has been reported also in some species of nematodes, particularly parasitic nematodes, to survive desiccation for extended periods. Sugi et al. reproduced this swarming behaviour using *C. elegans* and demonstrated that dauer worms can collectively form dynamical networks (Sugi et al. 2019). The experimental and simulation analyses of this phenomenon in the context of active matter physics indicated that the physical rules underlying this dynamical network formation are for local alignments of worms after collisions and their smooth turning.

The magnitude of the activation of the backward-driving neuron AVA is small at lower vibration frequencies in the nonlocalized mechanical stimulation of *C. elegans* (Sugi et al. 2018). This result might be reasonable because the wingbeat frequencies shown by many animals, such as honey bees and fruit flies, occur at approximately 200 Hz (Altshuler et al. 2005; Fry et al. 2005). In addition, there still remain many open questions. To date, the mechanosensory behaviours of dauer worms have been less frequently examined than those of fed worms. Considering that the nematode's dominant state in the wild is the dauer state (Barrière and Félix 2005), the mechanosensory behaviour of dauer worms should be examined. Likewise, the biological relevance of ultrasound detection also remains unclear and understanding this ability may require out-field study in the future. It also has been largely unclear whether nematodes other than *C. elegans* can modify their mechanosensory behaviours based on previous experiences and how those memory acquisition abilities contribute to the survival of nematodes in the wild. These open questions should be examined in the wild as much as possible. Such studies will enable filling the gaps between mechanosensory behaviours understood in laboratories and biotremology in the future.

Acknowledgements This work was supported by JSPS KAKENHI Grant-in-Aid for Scientific Research (B) (grant number JP18H02483) and on Innovative areas "Science of Soft Robot" project (grant nmuber JP18H05474), by the PRESTO from Japan Science and Technology Agency (grant number JPMJPR12A9) and by the PRIME from Japan Agency for Medical Research and Development (grant number 19gm6110022h001).

References

Albeg A, Smith CJ, Chatzigeorgiou M, Feitelson DG, Hall DH, Schafer WR, Miller Iii DM, Treinin M (2011) *C. elegans* multi-dendritic sensory neurons: morphology and function. Mol Cell Neurosci 46:308–317. https://doi.org/10.1016/j.mcn.2010.10.001

Altshuler DL, Dickson WB, Vance JT, Roberts SP, Dickinson MH (2005) Short-amplitude high-frequency wing strokes determine the aerodynamics of honeybee flight. Proc Natl Acad Sci USA 102:18213–18218. https://doi.org/10.1073/pnas.0506590102

Arnadóttir J, O'Hagan R, Chen Y, Goodman MB, Chalfie M (2011) The DEG/ENaC protein MEC-10 regulates the transduction channel complex in *Caenorhabditis elegans* touch receptor neurons. J Neurosci 31:12695–12704. https://doi.org/10.1523/JNEUROSCI.4580-10.2011

Bar-On YM, Phillips R, Milo R (2018) The biomass distribution on earth. Proc Natl Acad Sci USA 115:6506–6511. https://doi.org/10.1073/pnas.1711842115

Barrière A, Félix M-A (2005) High local genetic diversity and low outcrossing rate in *Caenorhabditis elegans* natural populations. Curr Biol 15:1176–1184. https://doi.org/10.1016/j.cub.2005.06.022

Bozorgmehr T, Ardiel EL, McEwan AH, Rankin CH (2013) Mechanisms of plasticity in a *Caenorhabditis elegans* mechanosensory circuit. Front Physiol 4:88. https://doi.org/10.3389/fphys.2013.00088

Brenner S (1974) The genetics of *Caenorhabditis elegans*. Genetics 77:71–94

Brownell PH (1977) Compressional and surface waves in sand used by desert scorpions to locate prey. Science 197:479–482

Campbell JF, Gaugler R (1993) Nictation behaviour and its ecological implications in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). Behaviour 126:155–169. https://doi.org/10.1163/156853993x00092

Campbell JF, Kaya HK (1999) How and why a parasitic nematode jumps. Nature 397:485–486. https://doi.org/10.1038/17254

Chalfie M, Au M (1989) Genetic control of differentiation of the *Caenorhabditis elegans* touch receptor neurons. Science 243(4894):1027–1033

Chalfie M, Sulston J (1981) Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. Dev Biol 82:358–370

Chalfie M, Sulston JE, White JG, Southgate E, Thomson JN, Brenner S (1985) The neural circuit for touch sensitivity in *Caenorhabditis elegans*. J Neurosci 5:956–964

Chatzigeorgiou M, Schafer WR (2011) Lateral facilitation between primary mechanosensory neurons controls nose touch perception in *C. elegans*. Neuron 70:299–309. https://doi.org/10.1016/j.neuron.2011.02.046

Chatzigeorgiou M, Yoo S, Watson JD, Lee W-H, Spencer WC, Kindt KS, Hwang SW, Miller DM, Treinin M, Driscoll M, Schafer WR (2010) Specific roles for DEG/ENaC and TRP channels in touch and thermosensation in *C. elegans* nociceptors. Nat Neurosci 13:861–868. https://doi.org/10.1038/nn.2581

Chatzigeorgiou M, Bang S, Hwang SW, Schafer WR (2013) *tmc-1* encodes a sodium-sensitive channel required for salt chemosensation in *C. elegans*. Nature 494:95–99. https://doi.org/10.1038/nature11845

Chen X, Chalfie M (2014) Modulation of *C. elegans* touch sensitivity is integrated at multiple levels. J Neurosci 34:6522–6536. https://doi.org/10.1523/JNEUROSCI.0022-14.2014

Chen Y, Bharill S, Isacoff EY, Chalfie M (2015) Subunit composition of a DEG/ENaC mechanosensory channel of *Caenorhabditis elegans*. Proc Natl Acad Sci USA 112:11690–11695. https://doi.org/10.1073/pnas.1515968112

Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, Dubin AE, Patapoutian A (2010) Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. Science 330:55–60

Cronin CJ, Mendel JE, Mukhtar S, Kim Y-M, Stirbl RC, Bruck J, Sternberg PW (2005) An automated system for measuring parameters of nematode sinusoidal movement. BMC Genet 6:5–19. https://doi.org/10.1186/1471-2156-6-5

de Bono M, Bargmann CI (1998) Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans*. Cell 94:679–689

de Bono M, Maricq AV (2005) Neuronal substrates of complex behaviors in *C. elegans*. Annu Rev Neurosci 28:451–501. https://doi.org/10.1146/annurev.neuro.27.070203.144259

Ding SS, Schumacher LJ, Javer AE, Endres RG, Brown AE (2019) Shared behavioral mechanisms underlie *C. elegans* aggregation and swarming. eLife 8:1181. https://doi.org/10.7554/eLife.43318

Driscoll M, Chalfie M (1991) The *mec*-4 gene is a member of a family of *Caenorhabditis elegans* genes that can mutate to induce neuronal degeneration. Nature 349:588–593. https://doi.org/10.1038/349588a0

Eastwood AL, Sanzeni A, Petzold BC, Park S-J, Vergassola M, Pruitt BL, Goodman MB (2015) Tissue mechanics govern the rapidly adapting and symmetrical response to touch. Proc Natl Acad Sci USA 112:E6955–E6963. https://doi.org/10.1073/pnas.1514138112

Fang-Yen C, Wyart M, Xie J, Kawai R, Kodger T, Chen S, Wen Q, Samuel ADT (2010) Biomechanical analysis of gait adaptation in the nematode *Caenorhabditis elegans*. Proc Natl Acad Sci USA 107:20323–20328. https://doi.org/10.1073/pnas.1003016107

Frézal L, Félix M-A (2015) *C. elegans* outside the Petri dish. eLife 4:381. https://doi.org/10.7554/eLife.05849

Fry SN, Sayaman R, Dickinson MH (2005) The aerodynamics of hovering flight in *Drosophila*. J Exp Biol 208:2303–2318. https://doi.org/10.1242/jeb.01612

Gannon TN, Rankin CH (1995) Methods of studying behavioral plasticity in *Caenorhabditis elegans*. Methods Cell Biol 48:205–223

Geffeney SL, Goodman MB (2012) How we feel: ion channel partnerships that detect mechanical inputs and give rise to touch and pain perception. Neuron 74:609–619. https://doi.org/10.1016/j.neuron.2012.04.023

Geffeney SL, Cueva JG, Glauser DA, Doll JC, Lee TH-C, Montoya M, Karania S, Garakani AM, Pruitt BL, Goodman MB (2011) DEG/ENaC but not TRP channels are the major mechanoelectrical transduction channels in a *C. elegans* nociceptor. Neuron 71:845–857. https://doi.org/10.1016/j.neuron.2011.06.038

Goodman MB, Sengupta P (2019) How *Caenorhabditis elegans* senses mechanical stress, temperature, and other physical stimuli. Genetics 212:25–51. https://doi.org/10.1534/genetics.118.300241

Hallem EA, Dillman AR, Hong AV, Zhang Y, Yano JM, DeMarco SF, Sternberg PW (2011) A sensory code for host seeking in parasitic nematodes. Curr Biol 21:377–383. https://doi.org/10.1016/j.cub.2011.01.048

Hart AC, Sims S, Kaplan JM (1995) Synaptic code for sensory modalities revealed by *C. elegans* GLR-1 glutamate receptor. Nature 378:82–85. https://doi.org/10.1038/378082a0

Hill PSM, Wessel A (2016) Primer: biotremology. Curr Biol 26:R187–R191

Holbrook RI, Mortimer B (2018) Vibration sensitivity found in *Caenorhabditis elegans*. J Exp Biol 221. https://doi.org/10.1242/jeb.178947

Huang M, Chalfie M (1994) Gene interactions affecting mechanosensory transduction in *Caenorhabditis elegans*. Nature 367:467–470. https://doi.org/10.1038/367467a0

Kang L, Gao J, Schafer WR, Xie Z, Xu XZS (2010) *C. elegans* TRP family protein TRP-4 is a pore-forming subunit of a native mechanotransduction channel. Neuron 67:381–391. https://doi.org/10.1016/j.neuron.2010.06.032

Kim J, Kwon J-T, Kim H-S, Han J-H (2013) CREB and neuronal selection for memory trace. Front Neural Circuits 7:44. https://doi.org/10.3389/fncir.2013.00044

Kindt KS, Quast KB, Giles AC, De S, Hendrey D, Nicastro I, Rankin CH, Schafer WR (2007a) Dopamine mediates context-dependent modulation of sensory plasticity in *C. elegans*. Neuron 55:662–676. https://doi.org/10.1016/j.neuron.2007.07.023

Kindt KS, Viswanath V, Macpherson L, Quast K, Hu H, Patapoutian A, Schafer WR (2007b) *Caenorhabditis elegans* TRPA-1 functions in mechanosensation. Nat Neurosci 10:568–577. https://doi.org/10.1038/nn1886

Kubanek J, Shukla P, Das A, Baccus SA, Goodman MB (2018) Ultrasound elicits behavioral responses through mechanical effects on neurons and ion channels in a simple nervous system. J Neurosci 38:3081–3091. https://doi.org/10.1523/JNEUROSCI.1458-17.2018

Lee H, Choi M-K, Lee D, Kim H-S, Hwang H, Kim H, Park S, Paik Y-K, Lee J (2011) Nictation, a dispersal behavior of the nematode *Caenorhabditis elegans*, is regulated by IL2 neurons. Nat Neurosci. https://doi.org/10.1038/nn.2975

Leifer AM, Fang-Yen C, Gershow M, Alkema MJ, Samuel ADT (2011) Optogenetic manipulation of neural activity in freely moving *Caenorhabditis elegans*. Nat Methods 8:147–152. https://doi.org/10.1038/nmeth.1554

Li W, Feng Z, Sternberg PW, Xu XZS (2006) A *C. elegans* stretch receptor neuron revealed by a mechanosensitive TRP channel homologue. Nature 440:684–687. https://doi.org/10.1038/nature04538

Li W, Kang L, Piggott BJ, Feng Z, Xu XZS (2011) The neural circuits and sensory channels mediating harsh touch sensation in *Caenorhabditis elegans*. Nat Commun 2:315. https://doi.org/10.1038/ncomms1308

Lonze BE, Ginty DD (2002) Function and regulation of CREB family transcription factors in the nervous system. Neuron 35:605–623

Maguire SM, Clark CM, Nunnari J, Pirri JK, Alkema MJ (2011) The *C. elegans* touch response facilitates escape from predacious fungi. Curr Biol 21(15):P1326–P1330. https://doi.org/10.1016/j.cub.2011.06.063

McClanahan PD, Xu JH, Fang-Yen C (2017) Comparing *Caenorhabditis elegans* gentle and harsh touch response behavior using a multiplexed hydraulic microfluidic device. Integr Biol (Camb) 82:1–10. https://doi.org/10.1039/C7IB00120G

Mello CC, Kramer JM, Stinchcomb D, Ambros V (1991) Efficient gene transfer in *C.elegans*: extrachromosomal maintenance and integration of transforming sequences. EMBO J 10:3959–3970

Mori I, Ohshima Y (1995) Neural regulation of thermotaxis in *Caenorhabditis elegans*. Nature 376: 344–348. https://doi.org/10.1038/376344a0

Mortimer B (2017) Biotremology: do physical constraints limit the propagation of vibrational information? Anim Behav 130:165–174

Nekimken AL, Fehlauer H, Kim AA, Manosalvas-Kjono SN, Ladpli P, Memon F, Gopisetty D, Sanchez V, Goodman MB, Pruitt BL, Krieg M (2017) Pneumatic stimulation of *C. elegans* mechanoreceptor neurons in a microfluidic trap. Lab Chip 17:1116–1127. https://doi.org/10.1039/C6LC01165A

Nishida Y, Sugi T, Nonomura M, Mori I (2011) Identification of the AFD neuron as the site of action of the CREB protein in *Caenorhabditis elegans* thermotaxis. EMBO Rep 12:855–862. https://doi.org/10.1038/embor.2011.120

O'Hagan R, Chalfie M, Goodman MB (2005) The MEC-4 DEG/ENaC channel of *Caenorhabditis elegans* touch receptor neurons transduces mechanical signals. Nat Neurosci 8:43–50. https://doi.org/10.1038/nn1362

Park S-J, Goodman MB, Pruitt BL (2007) Analysis of nematode mechanics by piezoresistive displacement clamp. Proc Natl Acad Sci USA 104:17376–17381. https://doi.org/10.1073/pnas.0702138104

Ranade SS, Syeda R, Patapoutian A (2015) Mechanically activated ion channels. Neuron 87:1162–1179. https://doi.org/10.1016/j.neuron.2015.08.032

Rankin CH, Wicks SR (2000) Mutations of the *Caenorhabditis elegans* brain-specific inorganic phosphate transporter eat-4 affect habituation of the tap-withdrawal response without affecting the response itself. J Neurosci 20:4337–4344

Reed EM, Wallace HR (1965) Leaping locomotion by an insect-parasitic nematode. Nature 206: 210–211

Rose JK, Kaun KR, Chen SH, Rankin CH (2003) GLR-1, a non-NMDA glutamate receptor homolog, is critical for long-term memory in *Caenorhabditis elegans*. J Neurosci 23:9595–9599

Sasakura H, Mori I (2013) Behavioral plasticity, learning, and memory in *C. elegans*. Curr Opin Neurobiol 23:92–99. https://doi.org/10.1016/j.conb.2012.09.005

Schafer WR (2014) Mechanosensory molecules and circuits in *C. elegans*. Pflûgers Archiv Eur J Physiol 467:39–48. https://doi.org/10.1007/s00424-014-1574-3

Schmid S, Wilson DA, Rankin CH (2014) Habituation mechanisms and their importance for cognitive function. Front Integr Neurosci 8:97. https://doi.org/10.3389/fnint.2014.00097

Stirman JN, Crane MM, Husson SJ, Wabnig S, Schultheis C, Gottschalk A, Lu H (2011) Real-time multimodal optical control of neurons and muscles in freely behaving *Caenorhabditis elegans*. Nat Methods 8:153–158. https://doi.org/10.1038/nmeth.1555

Sugi T, Nishida Y, Mori I (2011) Regulation of behavioral plasticity by systemic temperature signaling in *Caenorhabditis elegans*. Nat Neurosci 14:984–992. https://doi.org/10.1038/nn.2854

Sugi T, Ohtani Y, Kumiya Y, Igarashi R, Shirakawa M (2014) High-throughput optical quantification of mechanosensory habituation reveals neurons encoding memory in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 111:17236–17241. https://doi.org/10.1073/pnas.1414867111

Sugi T, Okumura E, Kiso K, Igarashi R (2016) Nanoscale mechanical stimulation method for quantifying *C. elegans* mechanosensory behavior and memory. Ann Sci 32:1159–1164. https://doi.org/10.2116/analsci.32.1159

Sugi T, Igarashi R, Nishimura M (2018) Noninvasive mechanochemical imaging in unconstrained *Caenorhabditis elegans*. Materials (Basel) 11(6):1034. https://doi.org/10.3390/ma11061034

Sugi T, Ito H, Nishimura M, Nagai KH (2019) *C. elegans* collectively forms dynamical networks. Nat Commun 10:1–9. https://doi.org/10.1038/s41467-019-08537-y

Suzuki H, Kerr R, Bianchi L, Frøkjaer-Jensen C, Slone D, Xue J, Gerstbrein B, Driscoll M, Schafer WR (2003) In vivo imaging of *C. elegans* mechanosensory neurons demonstrates a specific role for the MEC-4 channel in the process of gentle touch sensation. Neuron 39:1005–1017

Swierczek NA, Giles AC, Rankin CH, Kerr RA (2011) High-throughput behavioral analysis in *C. elegans*. Nat Methods 8:592–598. https://doi.org/10.1038/nmeth.1625

Timbers TA, Rankin CH (2011) Tap withdrawal circuit interneurons require CREB for long-term habituation in *Caenorhabditis elegans*. Behav Neurosci 125:560–566. https://doi.org/10.1037/a0024370

White JG, Southgate E, Thomson JN, Brenner S (1986) The structure of the nervous system of the nematode *Caenorhabditis elegans*. Philos Trans R Soc Lond B 314:1–340

Wicks SR, Rankin CH (1995) Integration of mechanosensory stimuli in *Caenorhabditis elegans*. J Neurosci 15:2434–2444

Yoshida K, Hirotsu T, Tagawa T, Oda S, Wakabayashi T, Iino Y, Ishihara T (2012) Odour concentration-dependent olfactory preference change in *C. elegans*. Nat Commun 3(739). https://doi.org/10.1038/ncomms1750

Zhou W, Wang J, Wang K, Huang B, Niu L, Li F, Cai F, Chen Y, Liu X, Zhang X, Cheng H, Kang L, Meng L, Zheng H (2017) Ultrasound neuro-modulation chip: activation of sensory neurons in *Caenorhabditis elegans* by surface acoustic waves. Lab Chip 17:1725–1731. https://doi.org/10.1039/C7LC00163K

Chapter 13
Speleotremology: Ecology and Evolution of Vibrational Communication in Cavernicolous Insects

Nataša Stritih-Peljhan, Johannes Strauß, Andreas Wessel, and Hannelore Hoch

Abstract Colonization of underground habitats requires specific adaptations to cope with conditions that are strongly different from those in surface environments, most notably in the absence of visual and other environmental cues, as well as a less complex community structure. The ability to orientate, to recognize and to locate potential mating partners in permanent darkness is crucial to survive, to produce offspring, and to establish viable populations. Communication systems that are effective for a variety of taxa in surface environments, such as substrate-borne vibrational signals, may or may not be favored by selection during the evolutionary process of adaptation to caves, apparently depending on the preferred substrate of the animals studied. As examples for these selective regimes, we discuss two case studies on intraspecific communication in the cave environment that have been studied in depth, one from the Hemiptera (Auchenorrhyncha: Fulgoromorpha: Cixiidae) and one from the Orthoptera (Ensifera: Rhaphidophoridae). Behavioral and bioacoustic approaches show the complex signaling behaviors involving substrate vibrations in the cave species that occupy specialized and rare habitats favoring vibration transmission. The common rock substrate of caves, on the other hand, provides an unfavorable habitat for vibrational communications, in which vibration signals may not be used effectively by insects. This ecological setting may

---

N. Stritih-Peljhan (✉)
Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia
e-mail: natasa.stritih-peljhan@nib.si

J. Strauß
AG Integrative Sensory Physiology, Institute for Animal Physiology, Justus-Liebig-Universität Gießen, Giessen, Germany
e-mail: johannes.strauss@physzool.bio.uni-giessen.de

A. Wessel · H. Hoch
Museum of Natural History Berlin, Leibniz Institute for Evolution and Biodiversity Science at Humboldt University Berlin, Berlin, Germany
e-mail: Hannelore.Hoch@mfn.berlin

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_13

eventually even lead to an evolutionary regression of the sensory structures involved in vibration detection.

13.1 Introduction

Animals use a set of signals to orientate in their environment, to locate food or prey, to avoid predators, and to recognize and locate potential mating partners. The nature of these signals may be chemical, optical, and mechanical (tactile, acoustic, or vibrational), or a combination of these stimuli. In highly structured surface environments, such as, e.g., forests that are rich in biotic and abiotic cues like diurnally and annually changing light and temperature conditions, presence of air movements, and complex, species-rich community structure (i.e., diverse vegetation, herbivores, predators, scavengers), it may take input from all sensory channels to perceive the situation-relevant cues in order to generate an adequate behavioral response. In contrast, in subterranean environments, such as caves, with their usually steep gradient of diminishing light and air movement, diurnal and annual temperature fluctuation, seasonal humidity levels, scarcity and unpredictability of food, and less complex organismic communities, single sensory modalities may become less important, or even oblivious, e.g., eyes, while others may take on the pivotal role in distinguishing predators from conspecifics, or recognizing potential mating partners and their potential fitness.

The question of why an organism switches from a life on the surface to a permanent life underground has long intrigued evolutionary biologists. Inherent to cave environments are ecological and physiological challenges, such as reduction of food and visual cues, which organisms have to overcome to colonize subterranean habitats. In contrast, caves may provide independence from seasonal climatic changes, and lower predator and parasite pressure. Many animal taxa have successfully dealt with these trade-offs and have evolved to spend part of, or their whole, lifecycle underground, while developing varying degrees of morphological, ecological, and behavioral adaptations (for an overview of cave organisms, see Juberthie and Decu 1994–2001).

Two major hypotheses to explain the evolution of cave organisms have been brought forward: the climatic relict hypothesis (CRH) and the adaptive shift hypothesis (ASH). Under the climatic relict hypothesis, deteriorating climatic conditions on the surface (e.g., in the course of glaciation) led to the extirpation or extinction of surface populations, thus “trapping” troglophile populations in subterranean environments. These populations subsequently evolved troglomorphic features, eventually becoming restricted to the cave environment. The CRH thus considers cave adaptation as a regressive evolutionary trend, and assumes allopatric speciation (Barr 1968). The CRH was developed mainly from observations in temperate regions, and fails to explain the existence of troglobitic faunas on young oceanic islands and in the tropics. Following the discovery of rich and diverse cavernicolous fauna in Hawaii (with many closely related taxa still extant on the surface) in the early

1970s (Howarth 1972), the adaptive shift hypothesis challenged the traditional perception of cave adaptation, which essentially proceeds on the assumption of allopatric origin of cavernicolous taxa, by alternatively explaining cave adaptation through parapatry. According to the ASH, cave adaptation is an active process, much like that underlying adaptive radiations on the surface. During cave adaptation a given organism with certain exaptations (morphological, physiological, behavioral) builds a new ecological niche by forming novel relations with the biotic and abiotic factors of the environment in the process of permanent colonization (for the concept of the ecological niche applied here, see Günther 1950 and Sudhaus 2008). A number of extrinsic and intrinsic factors underlying adaptive shifts into cave habitats have been identified, among which the presence of cavernous landforms, the presence of exploitable food resources and mating behavior, i.e., the ability to locate mates and reproduce underground, are most critical (Howarth 1982; Howarth et al. 2019).

Traditionally, cave-dwelling organisms were classified ecologically as *accidentals* (trogloxenes) that only occasionally enter the caves, *facultative cavernicoles* (troglophiles) that spend part of their life cycle in caves, or *obligate cavernicoles* (troglobites) that are restricted to caves. In order to achieve a more detailed classification of cavernicoles, Sket (2008) and more recently Howarth and Moldovan (2018) developed a catalog of characteristics, pertaining to morphology, behavior, and ecology, with some being discussed below in this section. Consistent patterns of morphological changes (troglomorphies) have been identified in different taxa of animals that adapted to cave habitats in both constructive and regressive manners (Christiansen 2012; Protas and Jeffery 2012; Soares and Niemiller 2020).

Arthropods that have colonized subterranean environments usually display morphological, physiological, and behavioral alterations that can be identified in comparison to their epigean relatives (e.g., Howarth and Moldovan 2018). These include reduction of eyes, bodily pigment, and—in the case of insects—wings, as well as specializations of certain traits, e.g., enhancement of extraoptic senses, including the elongation of antennae and legs, tarsal structures especially suited for walking on wet, rocky surfaces, and a smooth body surface to enhance crawling through narrow voids and cracks (Hoch and Howarth 1989b; Christiansen 2012).

Cave environments may be expected to influence communication behavior significantly due to the change in the available stimulus modalities and environmental transmission, compared to epigean habitats. In conditions of near or complete darkness, the chemosensory channel (olfaction, contact chemoreception) and the mechanosensory channel (audition, substrate vibration reception, touch) are generally available to cave animals for detection of signals and cues underlying mate and food finding, or avoiding danger. For cavernicolous insects, and arthropods in general, the increased role of mechanical senses, particularly in the detection of tactile stimuli and air currents, is obvious from the elongation of appendages, such as antennae, sensory bristles, and legs equipped with a variety of mechanoreceptors (Cloudsley-Thompson 1988; Decu and Juberthie 2004; Taylor 2008; Christiansen 2012). The communication by airborne sound is not known for cave insects and is rare among cavernicolous animals in general, presumably due to unfavorable acoustic properties of the underground habitats (Howarth and Moldovan 2018). However, it has been proposed that cavernicolous animals may be specialized to detect substrate vibrations alongside chemical signals (Taylor 2008), and that they may widely use vibration signals for communication, especially at short distances (Howarth and Moldovan 2018).

The actual data on communication of cavernicolous species, particularly insects, are scarce. In this chapter, we present two case studies on the intraspecific communication in cave insects, which have been studied in depth, providing arguments that vibrational communication appears to be restricted to specialized and rare underground substrate niches, favorable to vibration signal emission and transmission. In unfavorable rocky substrates, which predominate in cave habitats, we show that vibration signals may not be used by insects, eventually leading to an evolutionary regression of both vibrational signaling and the underlying sensory structures.

13.2 Evolutionary Strategies Pertaining to Vibrational Communication During Cave Adaptation: The Case Studies of Hemiptera (Auchenorrhyncha: Fulgoromorpha: Cixiidae) and Orthoptera (Ensifera: Rhaphidophoridae)

In this chapter, we compare two insect taxa with cave-dwelling representatives, planthoppers (Hemiptera: Cixiidae), and cave crickets (Ensifera: Rhaphidophoridae), for their evolutionary histories and adaptations, with special attention to the ecological, behavioral, and physiological context of their vibrational signaling. We also present the current state of knowledge on adaptation in the sensory organs for detecting vibration in both groups, with some general introduction to the anatomy and physiology of these organs in insects. The two insect taxa studied differ not only in phylogenetic position and in their evolutionary origin, but also in degree of troglomorphy, habitat, and feeding type (see Table 13.1). Epigean species in both taxa use vibrational communication as part of their mate recognition system, and here we will explore how this communication channel is affected during the evolutionary process of cave colonization.

Table 13.1 Parameters characterizing the taxa studied

Taxon	Hemiptera: Fulgoromorpha	Ensifera	
	Cixiidae: *Oliarus polyphemus*	Rhaphidophoridae: *Troglophilus neglectus*, *T. cavicola*	Rhaphidophoridae: *Dolichopoda araneiformis*
Distribution	Hawaiian Islands	Western Balkans	Coastal South-western Balkans
Type of cave	Lava tubes	Karstic caves	Karstic caves
Ecological classification	Troglobite	Subtroglophile	Troglobite (most populations)
Troglomorphies	Compound eyes absent, reduced body pigmentation, flightless	Appendages moderately long, body pigmentation relatively light[a]	Appendages very long, body pigmentation very light
Special adaptations to the cave habitat	Alteration of body shape, wax fringe along tegminal margins, alteration of leg and tarsal spinulation	Modified energy metabolism (Lipovšek et al. 2016)	Aseasonality of life cycle (Bernardini and Di Russo 2004)
Feeding type	Herbivore (rhizophagous)	Omnivore	Omnivore
Close epigean relative	Extant	Absent	Absent
Presumed origin	Parapatric	Allopatric	Allopatric
Presumed age	Maximum 250,000 years, however, presumably less than 25,000 years (Wessel et al. 2013)	1–4 Myr for Rhaphidophoridae (Allegrucci and Sbordoni 2019); *Troglophilus* estimated more ancient than *Dolichopoda* (Allegrucci et al. 2017).	0.8–2.4 Myr (Allegrucci et al. 2005)
Communication channel(s)	Vibratory	Tactile, olfactory, vibratory	Tactile

[a] No closely related epigean species known; whether these characters represent elongation and reduction, respectively, is unclear

13.2.1 Cave Planthoppers Rely on Vibrational Communication for Mate Localization in the Cave Environment

13.2.1.1 Evolution, Biology, and Ecology of Hawaiian *Oliarus*

The Fulgoromorpha are distributed worldwide, comprising about 14,000 species (Bourgoin 2019). Cavernicolous species have been reported from four (out of 18) families (Cixiidae, Delphacidae, Kinnaridae, Meenoplidae). In the Cixiidae, cave-dwelling species are known to occur in many parts of the world: Hawaii, the Galápagos, Australia, New Zealand, Mexico, Argentina, Brazil, Madagascar, Réunion, the Macaronesian Islands (Azores, Canary Islands), Sicily and Croatia (see Hoch 2013, and references therein), and the Iberian Peninsula (Hoch, unpublished data).

Very little is known about the biology and behavior of most of these species, with the exception of the Cixiidae (*Oliarus polyphemus* s.l. from Hawaii, and *Solonaima* from Australia). Here we focus on the Hawaiian species of *Oliarus*, as detailed information on ecology, life history, and reproductive behavior is available only for this group.

In Hawaii, the genus *Oliarus* is distributed with 58 species and 24 subspecies (Asche 1997) on all major islands. Epigean *Oliarus* species display well-developed compound eyes, lateral ocelli, vivid body and wing coloration, and functional tegmina and wings. The Hawaiian *Oliarus* are most likely monophyletic (Asche 1997), i.e., all extant species are descendants of a single primary colonizing species to islands, with subsequent adaptive radiation to a wide range of habitats, ranging from coastal biotopes and lowland mesic forests to mountainous rain forests (see Chap. 5). Several lineages within *Oliarus* have separately colonized subterranean environments: three on Maui, one on Molokai, at least three on Hawaii Island (Hoch and Howarth 1999) (Fig. 13.1). Of these, morphologically similar populations of *Oliarus polyphemus* s.l. occur in lava tubes of the major volcanic systems (Mauna Loa, Kilauea, Hualalai, Mauna Kea).

Oliarus polyphemus differ strongly in external morphology from their epigean relatives: they display troglomorphies often observed in obligately cavernicolous insects—they are completely blind, flightless, and unpigmented (Fig. 13.1a–c). In addition to these reductive morphological traits, specializations that probably evolved in response to particular challenges of their underground habitat have been recognized: alteration of overall body shape that may facilitate movements through narrow spaces (Hoch and Howarth 1989b; Hoch 2000), wax fringes along tegminal margins that may repel potential predators, and specialized configuration of leg spinulation and pretarsal claws optimized for walking on wet, rocky surfaces (Howarth 1983; Hoch and Howarth 1989b; Hoch 2000). *Oliarus polyphemus* is restricted to underground environments, and within the lava tubes both nymphs and adults are usually found in the deep cave zone (Howarth 1983), where stable conditions prevail: permanent darkness, constant temperature (equivalent to annual average temperature on the surface), and constant high humidity. In the lava tube ecosystem, *Oliarus polyphemus* are primary consumers, feeding on roots of *Metrosideros polymorpha* (Myrtaceae), an indigenous shrub/tree that is a pioneer on young lava flows and produces long roots that dangle into the lava tubes, forming "root curtains" (McDowell 2002) (Fig. 13.1d). According to Howarth (1983), the exploitation of these roots as a novel food resource has been the driving force for the evolution of *Oliarus polyphemus,* resulting in an adaptive shift from the surface to the cave environment.

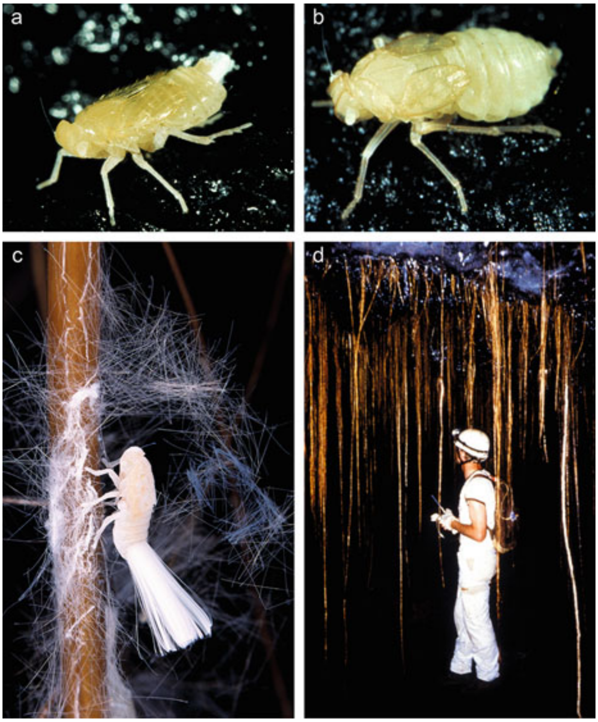


Fig. 13.1 Cavernicolous *Oliarus* from Hawaii: (**a**) *Oliarus polyphemus* (adult female), (**b**) *Oliarus waikau* (Maui) (adult male), (**c**) *Oliarus polyphemus* (nymph), (**d**) cave interior with root curtains. Photographs by W.P. Mull (**a**, **b** with kind permission) and H. Hoch (**c**, **d**)

13.2.1.2 Vibrational Signaling in Planthoppers

The Hemiptera (except the Sternorrhyncha) are characterized by a highly complex sound- and vibration-producing organ, the tymbal. Wessel et al. (2014) gave a detailed account of the current state of knowledge on the morphology and evolution of the tymbal. The authors hypothesized that all these organs are derivatives of the vibration-producing organ of the last common ancestor of the Hemipteran subgroups, except Sternorrhyncha, and proposed for this taxon the name Tymbalia. They postulated several criteria for this "tymbalian tymbal" organ:

> If we want to describe in short the "close similarity in the basic plan" (Pringle 1957, p. 154) of the tymbalian tymbal organs, we must refer first and foremost to a homologous set of muscles (I a dlm + II a dlm + I a dvm + III vlm + II a vlm, see Fig. 20.5), working together in order to produce vibrations for communication purposes. In many taxa, we find that these muscles are combined with more or less specialized integumental parts (the "tymbal plates") that transform the muscle actions into narrow band signals with harmonics, pure tones, or high-pitch pulses. These transformations occur by vibrating membranes or click mechanics or a combination of both. Vibrating or "clicking" sclerites can be found at the lateral and/or dorsal parts of the first two abdominal segments. In some taxa, the signals may even be produced by the vibration/distortion of internal structures (apodemes) in combination with whole abdomen vibrations (see Gogala 2006). Abdominal vibrations (tremulation) may generally facilitate amplitude amplification in small species. In many taxa known to produce vibrational signals, there is as yet no knowledge about the precise mechanism (see Table A.1) and surprises are to be expected. (Wessel et al. 2014, p. 421)

Since then, the biomechanical basis of vibration production across most taxa of the Fulgoromorpha has been analyzed by Davranoglou et al. (2019). All taxa studied (except for the Delphacidae partim and the Derbidae partim) display a similar morphological configuration of skeletal and muscular parts, located “dorsally on each side of the body at the junction between the metathorax and the abdomen, spanning the first two abdominal segments” (Davranoglou et al. 2019, p. 3). To generate vibrations, muscles and sclerites are interacting in such a way that kinetic energy is stored and released by a reciprocal elastic recoil mechanism.

13.2.1.3 Material and Methods

Sampling and Rearing (See also Hoch and Howarth 1993) In order to obtain unmated males and females for behavioral studies, V. instar nymphs of *Oliarus polyphemus* s.l. were collected from populations in various lava tubes, and transferred to the laboratory along with samples of roots of their host plant *Metrosideros polymorpha* under simulated cave conditions (see Howarth 1979). In the laboratory, *M. polymorpha* was substituted with fresh soybean sprouts, which were readily accepted by the nymphs. Several (5–7) immatures were kept in small rearing cages lined with moist filter paper and a small supply of soybean sprouts. Filter paper and soybean sprouts were changed every few days to prevent build-up of mold. The nymphs were kept under conditions resembling those of their natural habitat (complete darkness, temperature: 18 °C) in the facilities of the Hawaiian *Drosophila* Stock Center, University of Hawaii at Manoa, Honolulu. Freshly molted adults were separated and kept individually for behavioral experiments and signal recordings.

Recording of Vibrational Communication Signals Recordings were made in the field and in the laboratory, using the magneto-dynamic system designed by Strübing and Rollenhagen (1988). The design was timely as the MD-system was the first device for recording vibrational signals that was comparatively inexpensive, easily portable, yet sufficiently robust for use in the field.

The system operates on the principle of electromagnetic induction (Hoch and Howarth 1993) and consists of a small magnet attached to the host plant, or the substrate on which the vibration-emitting insect is located, and an induction coil, which is installed close, but without physical contact, to the magnet. The vibrational signals emitted by the insect and transferred to the substrate presumably via the legs cause mechanical oscillations of the magnet. These are then transformed into electrical oscillations by the induction coil, and are recorded on tape. In the process, these low-intensity signals are amplified by a factor of about 1000, and thus become audible to the human ear.

The majority of recordings were made in the laboratory, either at the facilities of the University of Hawaii at Manoa, or of Bishop Museum, Honolulu, or at temporary set-ups at various accommodations on Hawaii Island. As the natural substrate, roots of *Metrosideros polymorpha* were not readily available in the laboratory setting, and the substitute, soybean sprouts, proved to be unsuited for standardized laboratory

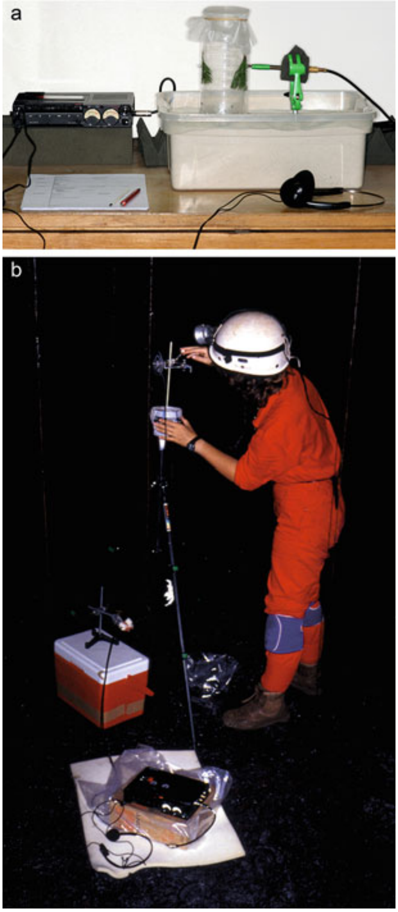


Fig. 13.2 Experimental set-ups (**a**) in the lab and (**b**) in the cave (Pahoa Experiment). Photographs by G. Kunz (**a** with kind permission) and M. Asche (**b**)

experiments, fresh *Metrosideros* leaves, if available (Wessel et al. 2013), or a small (1 cm × 5 cm × 1 mm) piece of balsa wood, were used as a substrate (Hoch and Howarth 1993). For each experiment, one male and one female were placed into a small cage containing the balsa wood to which the magnet was attached (Fig. 13.2a). Recordings were obtained from individuals from a total of 19 populations/lava tubes of Mauna Loa, Kilauea, and Hualalai volcanic systems (Wessel et al. 2013).

To test the efficiency of vibrational communication in the natural habitat, the MD-system was set up in a lava tube (Pahoa cave, Fig. 13.2b) that is part of the Kilauea volcanic system on Hawaii Island, which accommodates a large and stable population of *O. polyphemus*. Roots of the host tree, *Metrosideros polymorpha*, dangle into the cave, forming "root curtains." While young-instar nymphs (stages I–IV) are frequently observed feeding on these roots, V. instar nymphs and adults (the latter being the dispersal stage) are often also seen away from the roots, wandering on rocky surfaces of the ceiling, wall, and floor. Thus, both roots and rock surfaces were conceivable transmission substrates. In the experiment, both a living

Metrosideros root (length ca. 2.50 m, diameter ca. 3–4 mm) and the rock of the cave wall served as test substrates. In the experiment, pre-recorded signals of *O. polyphemus* were played back to the test substrates as vibrations using a modified loudspeaker (pin attached to the membrane). Recordings were made at increasing distances between source of vibrational signal (loudspeaker) and the magnet/coil (2 cm–2.50 m). For signal recording we used stereo-cassette recorders, either Philips D 6920 AV MK 2, or Sony TC-D 5 M (Hoch and Howarth 1993), and a Sony TCD-D8 digital audio tape (DAT) recorder (using TDK DA-RXG DAT tapes) (Wessel et al. 2013).

Analysis of Signals To determine the degree of variation within and between populations from different lava tubes the following call parameters were analyzed: general song pattern (time-dependent amplitude modulation), call duration, number of pulses per call, and interpulse interval. For details of signal processing and statistical analyses, see Hoch and Howarth (1993), as well as Wessel et al. (2013).

13.2.1.4 Mating System of Epigean Planthopper Species

Unlike their well-known cicada relatives (Cicadidae), which are large in body size and communicate predominantly by airborne signals, planthoppers are characterized by small body size (usually a few millimeters), which poses a physical limit to the production of airborne sound (Hasse 1974; Mebes 1974). In order to communicate over longer distances, small insects would thus need to use very high frequency (ultrasonic) sound. However, these frequencies are not suitable in structurally highly complex habitats, such as dense vegetation, where most epigean planthopper species live. Planthoppers thus—like the majority of the Hemiptera—instead communicate by low frequency, surface-borne vibrations (100–2000 Hz, with maximum energy at 100–500 Hz: de Vrijer 1984). The substrate along which the vibrational signals are transmitted is usually living tissue of the host plant, and vibrations produced by a signaling individual may be detected over a distance of 1–2 m (Michelsen et al. 1982). Although visual cues are most likely one of the components of courtship behavior, there is evidence from all species studied that vibrational signals are pivotal for the recognition and location of potential mating partners; whereas chemical communication, by cuticular or by volatile pheromones, apparently plays a minor, if any, role (Aldrich 1996). The sexual behavior patterns of planthoppers can be rather complicated (e.g., Strübing 1960; Booij 1982; Claridge 1985; den Bieman 1986; de Vrijer 1986), and involve a variety of call patterns responding to particular situations, e.g., distant communication between potential sexual partners by spontaneous calling (of either the male or the female), searching and then courtship directly prior to copulation. Male signals are usually more differentiated, pertaining to their time-amplitude pattern, than those of females (e.g., Strübing 1960; Booij 1982; de Winter and Rollenhagen 1990; Claridge 1990), and in many cases accompanied by wing-flapping, which may provide additional visual stimuli for the female (Strübing 1960; Ichikawa 1976; Booij 1982). Call structures (time-amplitude patterns) are species-specific and function as a premating reproductive barrier (see Claridge 1985, 1990; Tishechkin and Vedenina 2016).

From the few species studied so far, we observed that—as expected—the surface-dwelling *Oliarus* species from Hawaii communicate by substrate-borne vibrations (see Chap. 5). Visual cues are also likely to constitute a component of courtship behavior, as adult Hawaiian *Oliarus* often display vivid color patterns of the body and wings, and have well-developed compound eyes.

13.2.1.5 Mating System of Cavernicolous Planthoppers

General Courtship Pattern In the course of our behavioral observations, we found that the troglobitic *Oliarus polyphemus* populations have maintained the communication system of their epigean relatives, and communicate by substrate-borne vibrational signals. Troglobitic *Oliarus*, however, rely entirely on vibrational signals to recognize and locate potential mating partners—being eyeless and living in permanent darkness excludes visual stimuli from the repertoire of potential cues. The general pattern of sexual behavior was similar in all populations studied (see also Hoch and Howarth 1993; Hoch 2000). In the majority of male–female interactions, the female emitted spontaneous calls, i.e. the female performed the initial step of courtship behavior. In cases where these initial calls remained unanswered, the female eventually stopped calling. In cases where a nearby male answered these calls, the female would remain in place, and emit calls at regular intervals. The male would then orientate toward the calling female (in a way that can best be described as making “warm-cold” decisions), calling less often than the female and at irregular intervals. In all observed cases courtship persisted for about an hour before copulation commenced. Copulations lasted from 36–57 min. During and after copulation no calls were emitted. In none of the observed male–female interactions, which displayed the courtship pattern described, was a song-active female observed to reject a responding male, or to show escape reaction during male mating attempts (Hoch and Howarth 1993). Escape reactions, however, were observed in cases when physical contact between the test male and female occurred at random. These observations demonstrate that vibrational signals serve in recognition not only of conspecific individuals, but also of their sex and suitability as mating partners.

Efficiency of Vibrational Communication in the Cave Environment In our field experiment (see Sect. 13.2.1.3) we tested root vs rock substrate for their vibration transmission characteristics. On the living root of *Metrosideros polymorpha*, biologically relevant vibrational signals could be detected easily at distances up to at least 2.50 m away from the source of vibration. In contrast, rocky substrates proved to have poor transmitting characteristics—even intense banging with a forceps in close proximity (a few centimeters) to the recording unit did not result in a vibrational signal on the order of magnitude of planthopper-generated signals. Thus, living root tissue is an especially well-suited substrate for the transmission of low-frequency vibrations: signals may be transmitted along root curtains through

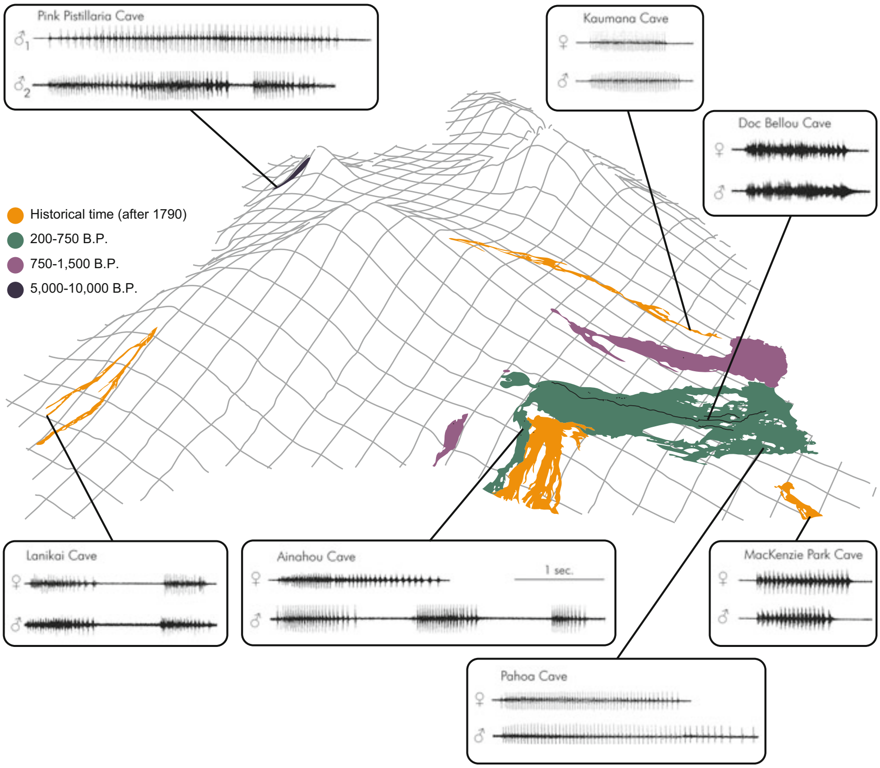


Fig. 13.3 Song pattern diversity of *O. polyphemus* populations in the different lava tube systems on Hawaii Island. The colors code for the age of the lava flows. Waveforms to the same time scale, see 1 s bar on Ainahou Cave waveform

interconnected rootlets branching off the main stems (Hoch and Howarth 1993; Hoch 2000).

Evolutionary Implications Interestingly, the *Oliarus polyphemus* populations studied showed a strong differentiation of vibrational signal characteristics (see Fig. 13.3), as well as in morphometric parameters. This supports the assumption that *Oliarus polyphemus* is in fact a complex of closely related, yet reproductively isolated, species (Hoch and Howarth 1993; Wessel and Hoch 1999). All species occur in very similar environments (deep cave zone, feeding on *Metrosideros polymorpha* roots, similar structure of root communities). The high level of phenotypic and genetic differentiation observed between populations from geographically proximate, geologically young caves (some less than 100 years old) suggests that a) these populations are reproductively isolated, and that b) dispersal events are rare and accidental, accounting for a low migration rate (Wessel et al. 2013). It appears thus plausible that repeated founder events preceded the establishment of new populations. Given the young age of the lava flows colonized and the high degree of differentiation between populations, the Hawaiian *Oliarus polyphemus* lineage exhibits one of the highest speciation rates among arthropods (Wessel et al. 2013).

Alteration of call Structure During Cave Adaptation Time-amplitude patterns of vibrational courtship signals in epigean leaf- and planthoppers have been observed to be extraordinarily complex (e.g., Strübing 1960; Claridge 1985; de Vrijer 1986; see also Chap. 5). In comparison, the calls of troglobitic *Oliarus polyphemus* s.l. individuals are far less complex, consisting of more or less homogenous pulse trains (Hoch and Howarth 1993; Hoch 2000; Hoch and Wessel 2006; Wessel et al. 2013). In contrast to the few epigean *Oliarus* species studied, both males and females displayed similar call structures. The variability of calls (in e.g., duration, interpulse interval) was observed to be comparatively small within a given population, while it was considerably larger among *Oliarus polyphemus* populations from different lava tubes (see also above: “Evolutionary implications”).

13.2.2 Rhaphidophoridae Provide Evidence for Negative Selection on Vibrational Signaling in Caves

13.2.2.1 Biology, Distribution, and Ecology of Rhaphidophoridae

Rhaphidophoridae (also called cave crickets, cave weta, or camel crickets) comprise a globally distributed lineage of Orthoptera (Ensifera), known for their vast reliance on cave habitats (Di Russo and Sbordoni 1998; Allegrucci and Sbordoni 2019). Out of about 750 species currently known (Cigliano et al. 2019), the majority is adapted to spend at least part of their lives in caves. Others are mostly ground forest dwellers, but also include specialized arboreal and sand-treader species.

Rhaphidophoridae have a wide geographic distribution across temperate and tropical regions of both Earth’s hemispheres (Di Russo and Sbordoni 1998; Cigliano et al. 2019). They are considered an old lineage of Orthoptera that originated in the early Cretaceous period before separation of the continental masses (Allegrucci and Sbordoni 2019). Since the early Tertiary, these cave cricket ancestors supposedly adapted to living in wet forests (Hubbell and Norton 1978) and entered the caves as refugia with cooling of the climate at the end of this period, between 4 and 1 Myr ago (Sbordoni et al. 1976; Allegrucci et al. 2005, 2011). Studies on the northern hemisphere species indicate allopatric speciation, mostly determined by the divergence of populations in isolated cave systems (see Allegrucci and Sbordoni 2019, and the references, therein).

The dependence of Rhaphidophoridae on cave habitats varies largely. Many species use caves only as daily or seasonal refugia as they forage and lay eggs in the forest (e.g., Pehani et al. 1997; Taylor et al. 2005; Lavoie et al. 2007), while some complete the whole life cycle in caves; though even in such cases, trophic migrations outside caves may occasionally occur (De Pasquale et al. 1995; Di Russo and Sbordoni 1998). The majority of cavernicolous rhaphidophorids would thus suit the definition of "subtroglophile," which refers to a species that is "inclined perpetually or temporarily to inhabit a subterranean habitat, but is bound to the surface for some biological function (e.g., feeding)" (Sket 2008, p. 1). (By contrast to these periodically cavernicolous species, the "eutroglophiles" are essentially epigean species that are able to maintain permanent subterranean populations; Sket 2008).

All rhaphidophorid species are wingless and non-hearing, and those dwelling in, or migrating to, the surface habitats are exclusively nocturnal. They show a range of troglomorphic adaptations seen in the transition from robust, short-legged epigean representatives with strong pigmentation, to slender ones with highly elongated appendages, pale coloration, and partially or rarely even completely reduced eyes (e.g., Lavoie et al. 2007; Rampini et al. 2013). Due to such ecological and phenotypic variation, Rhaphidophoridae provide a most suitable model to study the evolution of communication and of sensory systems following adaptation to life in caves. In addition, since they may present the most primitive lineage of the Ensifera (Desutter-Grandcolas 2003; Song et al. 2020), they are highly relevant for comparative evolutionary studies in a broader taxonomic context as well (e.g., Stritih and Stumpner 2009; Strauß et al. 2014).

13.2.2.2 Vibrational Signaling in Rhaphidophoridae

In the Ensifera, the majority of species strongly rely on acoustic and vibrational signaling for mate attraction and courtship. These species produce vibratory signals alone, or simultaneously with airborne sound, utilizing different ways of stridulation, drumming on the substrate, and tremulation (reviewed in Stritih and Čokl 2014). Despite their relevance for comparative analysis, mating behavior and mechanisms of communication scarcely have been studied in Rhaphidophoridae (Table 13.2). This may be attributed to their lack of (apparent) production of mechanical signals, negative phototactic behavior, and a high level of arousal that the cavernicolous species may show on horizontal surfaces (Kastberger 1984), making them difficult to observe and manipulate behaviorally. Most data or indications of the acoustic and vibrational behavior of these Ensifera thus originate from general descriptions (including personal and anecdotal reports) in publications focusing broadly on their biology, and often dated more than 50 years ago. Thus, their mating behavior only rarely has been systematically studied, and specialized equipment was used to register their vibrational signals from the substrate only in the study by Stritih and Čokl (2012) (see Sect. 13.2.2.3).

In the following (see this sect., *Stridulation*, *Drumming*, *Tremulation*), the modes of signaling are described and discussed for both epigean and cavernicolous Rhaphidophoridae, the comparison being necessary to understand the evolution of vibrational communication systems underground. Data on mating behavior are available at this time for species from seven genera, in five (out of ten) subfamilies of Rhaphidophoridae, and suggest the presence of vibratory signaling across different lineages. However, this is noted primarily in the epigean and less cave-adapted representatives (Table 13.2). Being supported further by comparative study in

Table 13.2 References describing sexual behavior of Rhaphidophoridae, with indications to the presence and context of vibrational signaling, together with the species' family affiliation, geographic distribution, and the preferred mating habitat

Species	Subfamily/distribution	Vibrational behavior	Signaling context	Mating habitat	References
Troglophilus neglectus	Troglophilinae (Europe)	Abdominal and body tremulation	Courtship, post-copulation	Forest ground	Stritih and Čokl (2012)
Troglophilus cavicola	Troglophilinae (Europe)	Body tremulation	Post-copulation	Caves	Stritih and Čokl (2012)
Dolichopoda sp.	Dolichopodinae (Europe)	No indication	Courtship	Caves	Boldyrev (1913)
Pallidoplectron turneri	Macropathinae (New Zealand)	No indication Tremulation	Courtship	Caves	Richards (1961) M. Fea, personal comm.
Pachyrhamma waitomoensis	Macropathinae (New Zealand)	No indication Body tremulation	Courtship	Caves	Richards (1961) Video, courtesy of M. Fea[a]
Hadenoecus subterraneus	Ceuthophilinae (North America)	No indication	Courtship	Caves	Hubbell and Norton (1978)
H. cumberlandicus	Ceuthophilinae (North America)	No indication	Courtship	Caves	Hubbell and Norton (1978)
Ceuthophilus guttulosus	Ceuthophilinae (North America)	"Abdomen twitching"	Courtship	Forest ground	Eades (1964)
Ceuthophilus latens	Ceuthophilinae (North America)	"Thrusting his cerci"	Courtship	Forest ground	Turner (2015)
Pristoceuthophilus marmoratus	Ceuthophilinae (North America)	"Body vibration" Abdominal vibration[b]	Courtship	Forest ground	Haley and Gray (2012) Video[b]
Tachycines asynamorus	Aemodogryllinae (SE Asia)	"Short body shocks" "Swinging of the body"	Courtship	Forest ground	Boldyrev (1912), Gerhardt (1913)
Daihinibaenetes arizonensis	Ceuthophilinae (North America)	"Kicking the sand with hind legs"	Agonistic/territorial	Sand dunes	Weissmann (1997), anecdotal report
Gammarotettix sp.	Gammarotettiginae (North America)	"Drums against branches and leaves"	?	Trees	Tinkham and Rentz (1969), anecdotal report

[a] Video material, underlying publication of Fea and Holwell (2018) (courtesy of M. Fea). The male body vibrates slightly during close approach of a female

[b] Online video of copulation of *P. marmoratus* (https://www.youtube.com/watch?v=GVgVmw762dw); note the faint abdominal vibrations of the male while courting the female

Troglophilus (see Sect. 13.2.2.3), such signaling was postulated to be ancestral for Rhaphidophoridae and to have been reduced in the course of adaptations to cavernicolous life (Stritih and Čokl 2012).

Stridulation Stridulation refers to the production of acoustic and/or vibrational signals by mechanical energy resulting from rubbing of body parts equipped with specialized morphological structures against each other. For Rhaphidophoridae, contradicting data exist on the presence of femoro-abdominal stridulation or stridulation structures. In earlier summaries, the trait was reported as present for the genus *Troglophilus* (Chopard 1938; Kevan 1955), and was also listed as absent or "not demonstrated" for the family (Ander 1939; Rentz 1980). More recently, Desutter-Grandcolas (2003) considered stridulation as present, but later stated that it may at best be polymorphic in the family (Desutter-Grandcolas, personal communication 2011). Nevertheless, no indication of stridulation has been found in the rhaphidophorid species of various subfamilies that were observed behaviorally so far (including that of the genus *Troglophilus*; Table 13.2), which strongly suggests that this character may be considered absent in Rhaphidophoridae.

Drumming Drumming with hind legs on the sandy substrate was reported for the captured males of the sand-treader species *Daihinibaenetes arizonensis* (Ceuthophilinae: Daihiniini) while establishing territories (Weissmann 1997). Abdominal drumming against branches and leaves was mentioned as an observation in *Gammarotettix* (Gammarotettiginae), an arboreal Rhaphidophoridae (Tinkham and Rentz 1969). Such conspicuous vibrational signaling, which also produces airborne sound, is clearly absent in the cavernicolous Rhaphidophoridae (Table 13.2).

Tremulation In several publications, mating behavior of Rhaphidophoridae was reported to include signaling by tremulation of the males, i.e., body vibration without contacting the substrate, or was described with sufficient detail to allow inferring the presence (or absence) of such behavior (Boldyrev 1912, 1913; Gerhardt 1913; Turner 2015; Richards 1961; Eades 1964; Hubbell and Norton 1978; Haley and Gray 2012; Stritih and Čokl 2012; M. Fea, personal communication; Table 13.2). One may find in the early publications a reference to body or abdominal "twitching," "thrusting," "shocks," or "swinging" (see Table 13.2), while the terms vibration or tremulation have only been used to describe their behavior recently (Haley and Gray 2012; Stritih and Čokl 2012). Of these studies, Stritih and Čokl (2012) provided the most detailed description of courtship, copulation, and post-copulation behavior, along with a record of the accompanying vibratory signals induced in the substrate (see Sect. 13.2.2.3).

13.2.2.3 Mating Behavior and Vibrational Signaling in *Troglophilus*

Ecology of the Study Species Most extensive studies of vibrational communication and signal detection systems have been conducted in the European (Balkan) species

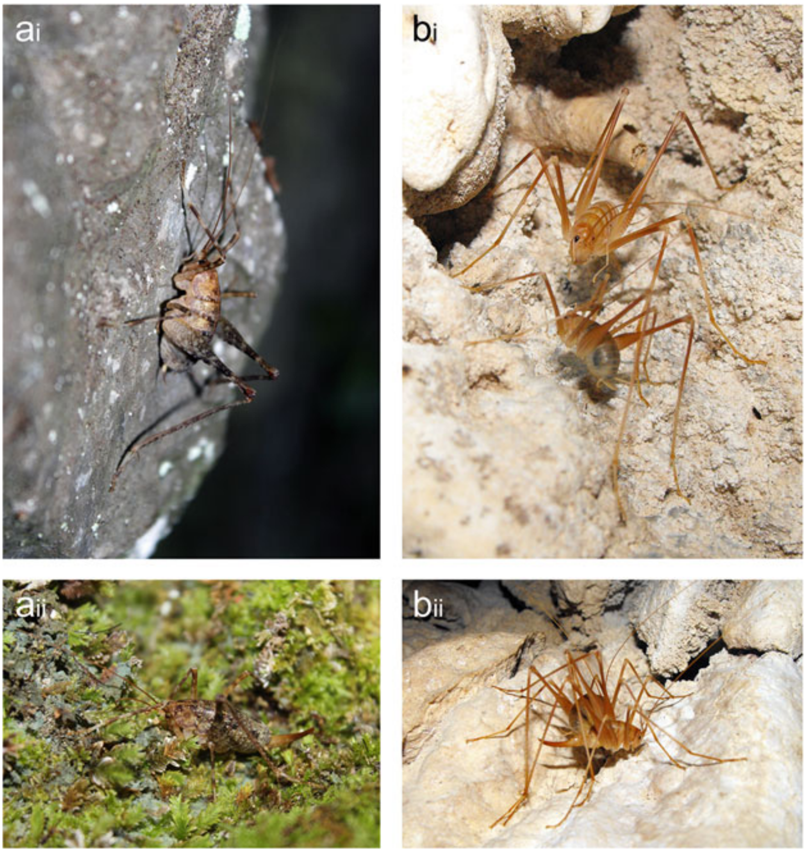


Fig. 13.4 Cave crickets in their natural habitat. (**a**$_i$) A *Troglophilus neglectus* male during daily sojourn close to the entrance of a cave (in NW Slovenia). (**a**$_{ii}$) A female *T. neglectus* transferred to the moss-covered substrate outside the cave, so that its cryptic greenish-brown coloration is notable. This species forages, mates, and lays eggs in the forest. (**b**$_i$) A male-female pair of *Dolichopoda araneiformis* in a cave (in SW Dalmatia) during an encounter and (**b**$_{ii}$) while in copula. The entire life cycle of this species is completed in caves, which they may leave only occasionally for feeding. The elongated appendages and pale body coloration indicate a high level of cavernicolous adaptation. Photographs by N. Stritih-Peljhan

Troglophilus neglectus and *T. cavicola*, along with the analysis of the peculiar olfactory signaling present in *T. neglectus* (reviewed in Stritih-Peljhan 2018). As with other genus members, these species may be designated as "sylvicole cryptobionts," which use caves for overwintering and as daily protection against summer aridity (Karaman et al. 2011). During summer, the animals are found close to cave entrances (Novak and Kuštor 1983; Pehani et al. 1997), as well as in other desiccation-protecting shelters in the forest, like litter, under rocks, or in rotten logs, which they leave at nights for foraging (Pehani et al. 1997; Karaman et al., 2011; Fig. 13.4a).

While in general the two species are ecologically highly similar, they differ in the time and place of sexual maturation and mating: in *T. cavicola*, this phase commences immediately after winter diapause and largely still in deep cave parts, while in *T. neglectus* it takes place in summer and apparently largely outside the hypogean habitat, long after individuals have left their overwintering sites (Pehani et al. 1997; Novak and Kuštor 1983; Stritih and Čokl 2012). This difference is reflected physiologically in a different use of energy reserves during overwintering of these species (Lipovšek et al. 2016), and may be considered a primary cause of their divergent behavioral evolution, affecting both vibrational signaling during male–female interactions and signaling via volatile pheromones in male agonistic contests (Stritih and Čokl 2012; Stritih 2014; Stritih and Žunić Kosi 2017; Stritih-Peljhan 2018). Here we present the methods and results of the vibrational communication study.

Materials and Methods The animals were collected as nymphs in different caves in Slovenia, where they are among the most abundant arthropods, also largely occurring sympatrically (Novak and Kuštor 1982). They were kept in the laboratory separated by sex, in high humidity and under a seasonal day-night regime. All behavioral and vibration recording was conducted in the laboratory in the night phase (under red illumination), using video-recording and laser-Doppler vibrometry (for details, see Stritih and Čokl 2012). Several iterations of improvement in the recording set-up were needed before we were able to observe mating with adequate frequency. Due to over-excitement of animals it was not possible to induce spontaneous behavior in the pairs placed on a loudspeaker membrane, which was to serve as an arena and a reference signaling substrate, at the same time. On the other hand, in terraria furnished with natural materials (moss, bark, and stones), observing neither single male–female pairs nor larger groups of animals yielded the expected results, as their level of activity was extremely low. Successful recordings were finally conducted using a set of 3–5 terraria that we observed in parallel, continuously over the night phase, each terrarium containing 3 females and 2 males. Such groups not only showed the highest level of activity but also enabled us to analyze both male–female and male–male interactions. As the females being approached were often situated in the overhang of bark, which was leaning against the terrarium wall, we were able to record undisturbed male vibratory signals from the upper bark surface, exactly at the signaler's position. Some instances of mating also occurred on stones and moss, with recordings made adjacent to the pair, thus allowing us to calculate relative differences in signal amplitudes between the substrates (see Fig. 13.3). In over 1 month of observations conducted with each species, we recorded courtship vibratory signals from 17 *T. neglectus* males (12 from bark, 1 from moss, 1 from stone—undetectable by the laser vibrometer, and 1 from terrarium net cover) and post-copulation vibratory signals from 12 *T. neglectus* (11 bark, 1 stone) and 8 *T. cavicola* (all bark) male individuals. We detected no vibratory signals emitted by females in any of the species.

Tremulation Signaling in Troglophilus In *T. neglectus*, the males search for and approach the female for mating purposes, following cues that are so far unknown (Stritih 2014). They initiate courtship with a brief antennation of the female, the antennation being followed by male emission of a series of short (mean duration 0.5–0.8 s), amplitude-modulated vibration pulses with a relatively regular rate (mean 0.4–0.6 Hz; Fig. 13.5a). These signals are produced by slight abdominal tremulation (hardly visible to the naked eye), and are followed by rhythmical abdominal stroking of the female's body, which ultimately induces her mounting for copulation. Tremulation pulses are extremely low-frequency substrate vibrations, with the dominant frequency typically in the range below 100 Hz, and most energy content below 200 Hz. In the courtship of *T. cavicola* such vibratory signaling is absent, while the partners spend significantly longer in mutual antennal fencing, apparently compensating in a tactile way for the lack of vibratory information (Fig. 13.5b).

After the completed copula, the males of both species exhibit vigorous tremulation of the whole body, which takes place regardless of female presence, and produces high-amplitude vibration signals that are about 0.6–1.1 s long and are emitted with an irregular and progressively decreasing rate (Stritih and Čokl 2012; Fig. 13.5a). Their temporal and spectral characteristics do not differ between the species, and spectrally they closely resemble the courtship signals of *T. neglectus*. The function of such post-copulation signaling, also observed in some Tettigoniidae (Kernan et al. 2018), is unknown.

On bark and moss, male courtship signals of *T. neglectus* were well detectable (with the mean velocity peaks at 0.184 and 0.063 mm/s, respectively), while on rock their intensity was below the laser vibrometer sensitivity and, apparently, also below the vibration detection threshold of the species (Fig. 13.6). This finding is in agreement with the absence of such signals in the courtship of *T. cavicola* that mates in caves, and further indicates that walls and ceilings of the limestone caves—the typical roosting place of *Troglophilus* and other cavernicolous Rhaphidophoridae—exert a strong negative selection on the use of vibratory signals in the hypogean habitat. Post-copulatory signals, on the other hand, occur on rock at intensities just above the vibration detection limit of *Troglophilus* (Fig. 13.6), in line with the presence of such signaling in both investigated species.

13.2.2.4 Evolution of Vibrational Signaling in Rhaphidophoridae in the Cave Environment

From comparative data, courtship tremulation of *T. neglectus* males may be considered as evolutionarily ancestral (Stritih and Strauß 2015), as it is also seen in the behavior of ground-dwelling epigean rhaphidophorids (Table 13.2) that are supposed to represent an ancestral habitat condition (Hubbell and Norton 1978). However, in the absence of signal records from other species, the potential diversification of such signals is unknown for Rhaphidophoridae, precluding more detailed evolutionary insights. Drumming on the substrate in *Daihinibaenetes* and *Gammarotettix* (see Sect. 13.2.2.2), which are specialized to life on sand dunes and trees, may be considered a derived character in the family.

Due to the constraining influence for signal transmission on rock (see Sect. 13.2.2.3, *Tremulation signaling* and Fig. 13.6) tremulation signaling was apparently

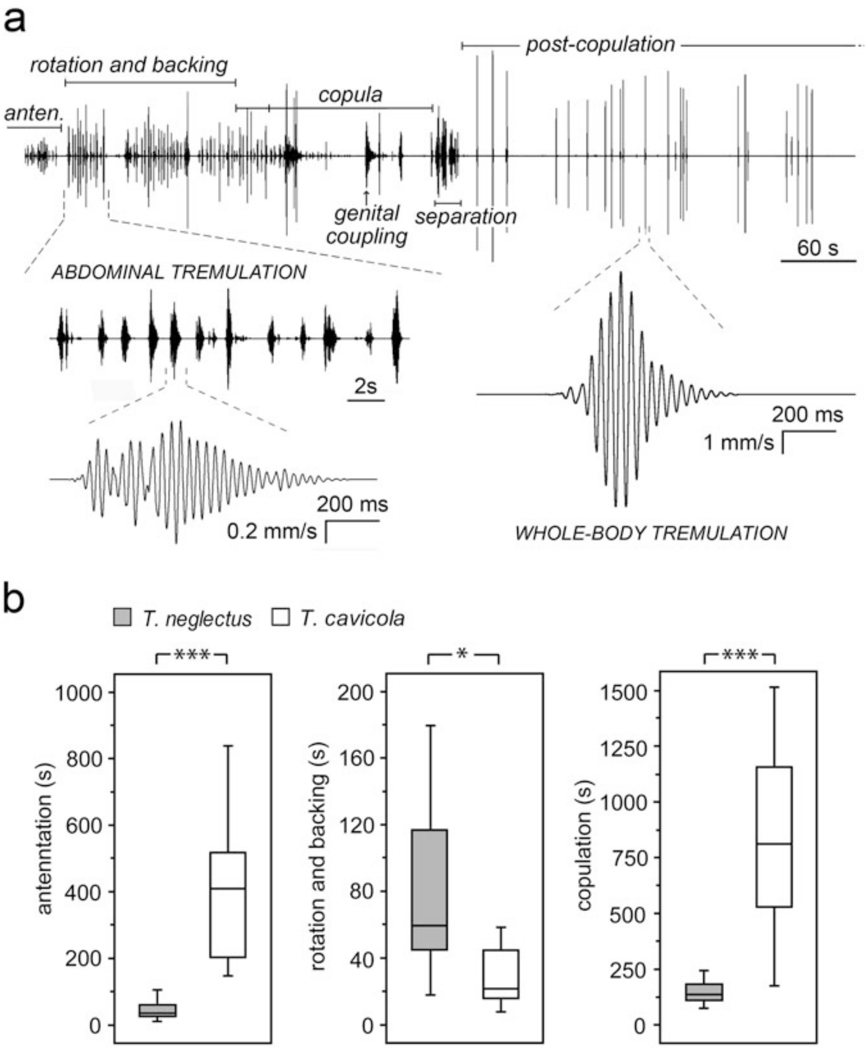


Fig. 13.5 Characteristics of mating behavior in *Troglophilus.* (**a**) Vibration signal records from bark supporting the mating pair of *T. neglectus*. Enlargements show oscillograms of signals produced by abdomen and whole-body tremulation during courtship and after copulation, respectively. Note that after the partners' separation, recording sensitivity has been 5-times reduced (compare also the scales in signal enlargements). The dominant frequency is similar for both signal types and substrate dependent, ranging between ~30 and 110 Hz. (**b**) Duration (plots show median and interquartile ranges) of main phases of the mating process compared between *T. neglectus* and *T. cavicola*. Asterisks indicate the degrees of statistical significance (for the details, see Stritih and Čokl 2012). Vibrational signaling in courtship is expressed during rotation and backing in *T. neglectus* only. Modified after Stritih and Čokl (2012)

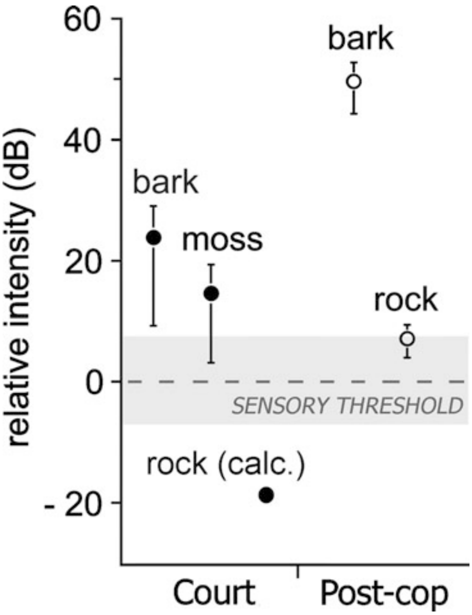


Fig. 13.6 Intensity of courtship and post-copulation signals of *T. neglectus* (closed and open symbols, respectively; mean with SD) when emitted on different test substrates. For the rock, the intensity of courtship signals (that was below the laser vibrometer sensitivity) was calculated (considering the intensity difference of post-copulation signal between bark and rock) to be below the species' physiological detection range. The dashed line shows the mean velocity threshold minimum (considered 0 dB) of the most sensitive vibratory ventral nerve cord interneurons of *T. neglectus* covering the frequency range below 200 Hz, and the gray range indicates the range of these minimal thresholds. The diagram is modified after Stritih and Strauß (2015), and considers the interneuron data from Stritih (2009) and behavioral data from Stritih and Čokl (2012)

reduced in *T. cavicola* and possibly also in other rhaphidophorids that reproduce in caves (Stritih and Čokl 2012). In line with this hypothesis is the absence of description of any movements indicative of tremulation during mating behavior of the highly cave-adapted species of *Hadenoecus* and *Dolichopoda* (Boldyrev 1913; Hubbell and Norton 1978; Table 13.2). While the lack of such observations also might have resulted from the inconspicuousness of tremulation signaling or the lack of the observer's focus on such signaling, it is important to note that at least for *Dolichopoda*, Boldyrev (1913) apparently was aware of such behavior; the author has previously described it for *Tachycines asynamorus*, an epigean rhaphidophorid (Boldyrev 1912; Table 13.2). Further support for the proposed regressive evolution of vibrational communication in caves comes from the comparative neuroanatomy of vibrosensory organs in the legs of ecologically diverse *Troglophilus neglectus* and *Dolichopoda araneiformis*. The latter, as a highly cave-adapted species, shows a significantly lower number of vibratory sensilla, indicating sensory reduction (Strauß and Stritih 2017; see Sect. 13.2.3).

Recently, evidence confounding our hypothesis was provided for the New Zealand *Pachyrhamma waitomoensis* (video records, courtesy of Murray Fea), with the males clearly trembling while courting females on a cave wall. While this species is not a strict troglobite but exhibits regular foraging exits (Fea and Holwell 2018), it regularly mates in cave aggregations (Richards 1961; Fea and Holwell 2018). This leaves the function of male tremulations and the sensory channel for their detection as intriguing questions. M. Fea (personal communication) suggests that *P. waitomoensis* may have filled many more forest niches in their recent evolutionary history, which became reduced to the cave refugium only after many small carnivorous mammals were introduced to New Zealand. This would explain the existence of vibratory signaling that our studies suggested to be inefficient in caves.

13.2.3 Sensory Cavernicolous Adaptations

13.2.3.1 Vibroreceptor Organs of Insects

Sensory organs involved in detecting substrate vibrations with high sensitivity in insects are often chordotonal organs, consisting of scolopidial sensilla (Čokl et al. 2006; Lakes-Harlan and Strauß 2014). The chordotonal organs are internal mechanoreceptors located within the body or the appendages, and important vibroreceptor organs are located in the legs, which by their direct contact with the substrate are suitable to receive and transmit vibration stimuli (see Stritih-Peljhan and Strauß 2018 on *Troglophilus neglectus*). The main vibration receptor organ in the leg usually is the subgenual organ, located in the tibia below the femur-tibia joint, and it is broadly tuned to substrate vibrations. In orthopteroid insects, the subgenual organ is accompanied by additional organs, together forming the subgenual organ complex in the proximal tibia (Field and Matheson 1998; Lakes-Harlan and Strauß 2014; Strauß 2017). Further vibrosensitive organs in the leg include the femoral chordotonal organ (sensilla in the proximal or dorsal scoloparium respond to sinusoidal vibrations), the tibio-tarsal chordotonal organ (Young 1970), and the tarso-pretarsal chordotonal organ (Čokl et al. 2006).

13.2.3.2 Vibroreceptor Organs of Cave Insects

For Auchenorrhyncha, only a few cicada species have been studied with respect to the leg mechanoreceptor/vibration receptor organs (Debaisieux 1938; Nishino et al. 2016; Alt and Lakes-Harlan 2018), which have a rather simple subgenual organ with only two scolopidial sensilla (Nishino et al. 2016; Alt and Lakes-Harlan 2018). The effects of vibrational signaling in cave habitats and possible sensory changes have not been studied. Since vibrational communication is highly effective on living plant roots, there is no regressive evolution of the subgenual organs to be expected.

An additional vibration-receiving organ was described by Vondráček (1949) in non-cicadoid Auchenorrhyncha. According to his observations, this organ is located in the second abdominal segment and consists of a bundle of scolopidia that extend from the lateral portions of the second abdominal tergum (interpreted by Vondracek as "tympanic membranes" or "tympana" to a central ventral sclerite of the intersegmental membrane between sternites I and II). Wessel et al. (2014) hypothesized that this organ might be homologous with the cicadid auditory organ, however, under the assumption that the ventral intersegmental membrane I-II act as the tympanum (vs the lateral portions of the second abdominal tergum, as suggested by Vondracek). Several studies combining histological sections and μCT analysis are currently under way to test these hypotheses (Bräunig and Ehlers, unpublished data).

In Rhaphidophoridae, data on the subgenual organ complex are available for a troglophilic species, *Troglophilus neglectus* (Jeram et al. 1995; Strauß et al. 2014; Strauß and Stritih 2016), and a troglobitic species, *Dolichopoda araneiformis* (Strauß and Stritih 2017). In both species, the subgenual organ complex consists of the subgenual organ, the intermediate organ, and the accessory organ. The overall organization, attachments, and innervation of the sensory structures are similar, and the subgenual organ contains the highest number of sensilla in both species (Fig. 13.7). The subgenual organ forms a hemi-circle of sensilla in the proximal tibia, with dendrites orienting distally in the leg into a tissue spanning the hemolymph channel (Jeram et al. 1995). The intermediate organ is located distally at the anterior tibia, and covered by a tectorial membrane connected to the dorsal cuticle (Jeram et al. 1995; Strauß et al. 2014). The accessory organ is found at the posterior tibia at the level of the subgenual organ with the sensilla's dendrites oriented proximally (Strauß and Stritih 2016), and likely responds to cuticle strain or low-frequency vibrations (Stritih-Peljhan et al. 2019).

The vibrational sensilla and their sensitivity are investigated in depth in *T. neglectus* and *T. cavicola* (review: Stritih-Peljhan 2018). Eleven types of vibrosensitive sensilla from the peripheral sensory organs are identified based on morphology and physiology of their receptor neurons (Stritih and Stumpner 2009; Buh 2011; Stritih and Čokl 2014; Stritih-Peljhan et al. 2019). The sensilla belong mainly to the subgenual organ complex, with classes of sensilla responding best to frequencies below and above 1000 Hz, and highest sensitivities for acceleration at 0.02–1.0 m/s^2 (Stritih and Čokl 2014). In *T. cavicola*, one sensillum type tuned to low frequency vibrations between 50–300 Hz was found, which likely originates from the accessory organ (Stritih-Peljhan et al. 2019). In comparison to Ensifera with tympanal hearing organs, the responses to sound stimuli were restricted to low frequencies at high amplitudes (Jeram et al. 1995; Čokl et al. 1995).

13.2.3.3 Sensory Reduction in *Dolichopoda araneiformis*

Ecology of the Study Species Most species in the European (Mediterranean) genus *Dolichopoda* are ecologically strictly dependent upon caves, especially in the

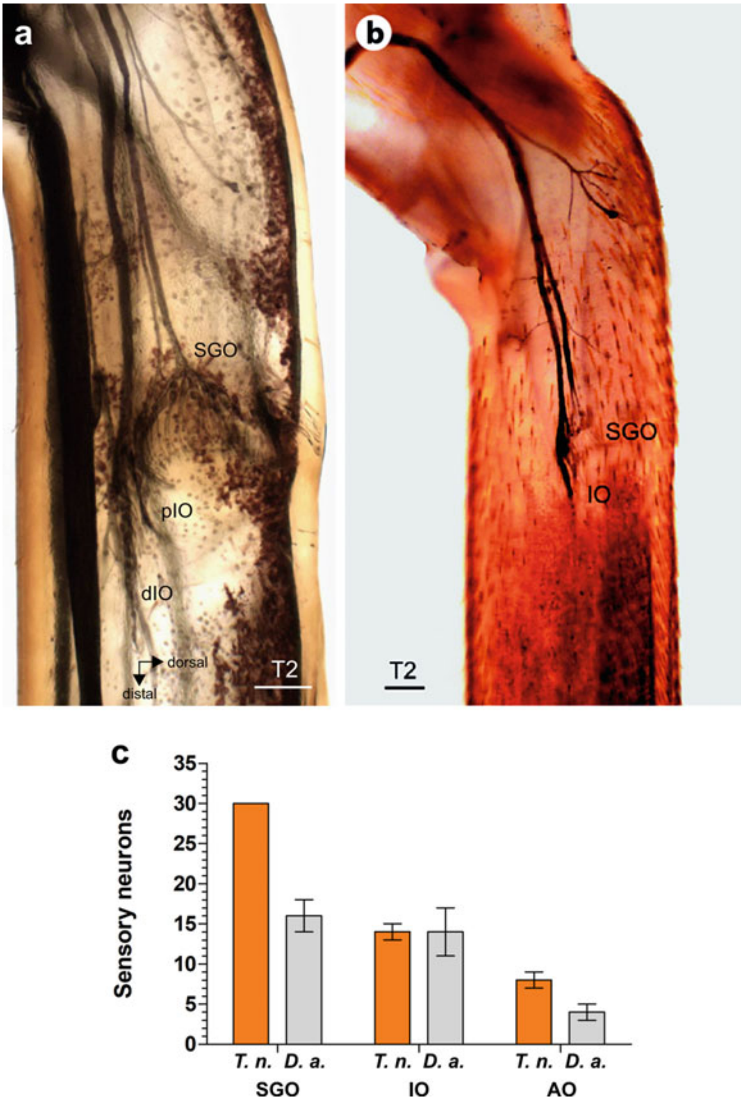


Fig. 13.7 Neuroanatomy of the subgenual organ complex in two species of cave crickets (midleg). Staining of the sensory complex in (**a**) *Troglophilus neglectus* and (**b**) *Dolichopoda araneiformis* shows the subgenual organ (SGO) and intermediate organ (IO). In *T. neglectus*, the IO shows two groups of sensilla, the proximal intermediate organ (pIO) and distal intermediate organ (dIO). (**c**) Comparison of the number of sensory neurons (mean ± standard deviation) in the different sensory organs of *T. neglectus* (orange) and *D. araneiformis* (gray); data from Jeram et al. (1995); Strauß and Stritih (2016, 2017). Note that for the subgenual organ in *T. neglectus*, no standard deviation was given in the original report (Jeram et al. 1995). The average sensillum numbers in *T. neglectus* were similar between leg pairs, and sensillum numbers for *D. araneiformis* were calculated based on data from all leg pairs. Scales = 100 μm. (**a**) Modified from Strauß et al. (2014). (**b**) Reprinted from Strauß and Stritih (2017), with permission (Copyright © 2017 Karger Publishers, Basel, Switzerland)

southern part of their range, associated with the dry and hot Mediterranean climate (Di Russo et al. 2014; Fig 13.4b). In natural caves, these species show a non-seasonal life cycle with individuals mating, hatching, and growing throughout the year (Di Russo et al. 1987; Carchini et al. 1994; Bernardini and Di Russo 2004), and depend almost entirely on cave resources (Carchini et al. 1994; De Pasquale et al. 1995). Only in their recent evolutionary history have some populations colonized more superficial caves, adapting their life cycle to the seasonal temperate regimes (Di Russo et al. 1987).

Sensory Neuroanatomy of the Subgenual Organ Complex In *D. araneiformis*, advanced morphological traits of cave adaptations are apparent, like very long, slender legs, resulting in an increased ratio of hind femur to pronotum length in comparison to related species (Di Russo et al. 2014; Strauß and Stritih 2017). For the subgenual organ and the accessory organ in the subgenual organ complex, the numbers of scolopidial sensilla are only ~50% of those in *T. neglectus* (Fig. 13.7c). Given that *Dolichopoda* cave crickets invaded caves from epigean niches (Allegrucci et al. 2011), this difference indicates an evolutionary regression of the vibrosensory elements in *D. araneiformis* (Strauß and Stritih 2017), which in turn likely results from the limited use of vibrational signals due to poor transmission of vibrations on rocky substrate (Fig. 13.6). It also suggests that the sensilla for rather low-frequency vibrations (<700 Hz), present in the subgenual and the accessory organ, are affected specifically by the regression, but not the high-frequency vibrational sensilla of the intermediate organ. This, however, is a surprising finding since the transfer of vibration over the rock may be expected to be most efficient at lower frequencies (see Elias et al. 2004). So far, the anatomy of other sensory organs, such as the femoral chordotonal organ, or aspects of vibration detection, like the stimulus transmission over the leg or the vibrational thresholds of the subgenual organ complex, have not been investigated in these highly cave-adapted insects.

13.3 Conclusions

In both taxa studied, the cave crickets as well as the cave planthoppers, we observe reductive trends pertaining to vibrational communication during the evolutionary process of cave adaptation. In the largely troglobitic cave crickets, such as *Dolichopoda araneiformis*, as well as in the troglophilic species that mate in caves, like *Troglophilus cavicola*, the overall importance of vibrational signals is reduced. Individuals of the troglobitic planthopper *Oliarus polyphemus* depend on vibrational signaling as perhaps the only communication channel to recognize and locate potential mating partners; however, here signal complexity appears reduced in comparison to epigean planthopper taxa.

In the case of the troglobitic planthopper *Oliarus polyphemus*, vibrational signaling is fundamental to intraspecific communication. Vibrational signals are transmitted over distances of several meters along living roots (see Sect. 13.2.1.5). In contrast, in the case of cave crickets, epigean species communicate via the substrate utilizing tremulation at short distances and drumming signals presumably also at longer distances (i.e., in a territorial context), which may be considered their ancestral and derived signaling modes in the epigean habitats, respectively (Stritih and Strauß 2015). In the cave environment, the shift to a novel mating substrate, i.e., the rocks of cave walls and ceiling inhabited by cave crickets, is likely to have caused selection against vibrational signaling and detection of substrate vibration. This is supported also by the substantial reduction in the number of scolopidial sensilla in the main vibrosensory system (the subgenual organ complex) in the legs of strongly cave-adapted *D. araneiformis* when compared to much less cave-adapted *T. neglectus*, which forages and reproduces in the forest. In line with these data, the reduced efficiency of vibration signals emitted on rock has been demonstrated in the courtship of jumping spiders (Elias et al. 2004), and is further indicated by the absence of vibrational signaling in other troglobitic insects, such as stoneflies (López-Rodríguez and Tierno de Figueroa 2012), which otherwise strongly rely on vibrational communication (e.g., Stewart and Zeigler 1984).

Thus, it is evident that the preferred substrate (rock vs plant roots) of the species studied strongly influences selection on the vibrational communication channel. What is striking, however, is the observed reduction of signal complexity in the cave planthopper.

O. polyphemus is eyeless and lives in permanent darkness, thus cannot process visual cues, and since pheromones have not been documented to play a role in mating behavior of planthoppers, most likely also receives no chemical cues. Consequently, all information on potential mating partners—conspecificity, sex, and genetic fitness—must be encoded in the vibrational signals by the sender. In addition, this information must be evaluated by the receiver with respect to distance and direction. It should therefore be expected that signals ought to be highly complex, time-amplitude patterns.

One way to explain this phenomenon is the energy economy, which is commonly invoked as a selective agent in the reductive evolution of cave animals. As many cave organisms are strongly food-limited, it has been postulated that the reduction of energy-consuming morphological or behavioral traits will have a selective advantage (Culver 1982). However, this is not the case in *Oliarus polyphemus*. As stated above in this section, the exploitation of a novel food resource (roots) is likely to have been the driving force for the adaptive shift into the cave environment, and roots of *Metrosideros polymorpha* are ample and abundant throughout the year. Instead, a scenario as described by Hutchinson (1958) may account for the observed reduction of signal complexity. In Hawaii, cavernicolous *Oliarus* species occur largely allopatrically, and in any given cave the species present is the only cavernicolous planthopper (with one exception: see Hoch and Howarth 1993). Consequently, competitive pressure by sympatric allies to maintain high signal complexity in order to bring together the sexes of the same species does not exist (ethological release hypothesis). Thus, maintaining a signal with minimal complexity may have been sufficient to serve the purpose of mate recognition and location (see also Hoch 2000, p. 217).

13.4 Perspectives

In order to better understand the evolutionary trends and their presumed underlying causes observed in cave crickets and cave planthoppers presented in this chapter, it is necessary to study the role of vibrational communication in additional taxa, which have undergone, or are about to undergo, cave adaptation. Taxa ideally suited as models should a) be monophyletic, b) comprise species/populations displaying varying degrees of cave adaptation, and c) contain extant epigean species, in parapatric situations.

In the Cixiidae, the monophyletic genus *Solonaima* (Queensland, Australia) comprises epigean species as well as several cavernicolous species that display various degrees of troglomorphy (Hoch and Howarth 1989b). Preliminary studies on the courtship behavior of cavernicolous *Solonaima* species revealed that one of the less troglomorphic, presumed eutroglophile species, *Solonaima pholetor*, displays a much higher degree of signal reduction than the strongly troglomorphic, troglobitic *Solonaima baylissa* (Hoch 2000, p. 216f). Interestingly, in the cave where *S. pholetor* occurs, it is the only cave-dwelling cixiid species; whereas, *S. baylissa* populations coexist with another cave-dwelling, yet not closely related, species of the genus *Undarana* (Hoch and Howarth 1989a). These findings support the assumption that reduction of signal complexity may indeed be attributed to ecological release, but more information on more taxa is needed to understand the process of evolutionary transformation of vibrational communication during cave adaptation.

Another promising taxon to investigate behavioral and genetic divergence between surface and cave populations is the European cixiid species *Trigonocranus emmeae*. Adults of *T. emmeae* display remarkable intraspecific variation with respect to the overall size of their compound eyes, number of ommatidia, length of forewings, and bodily pigmentation (Hoch et al. 2013). *Trigonocranus emmeae*—like other Cixiidae—also communicate by substrate-borne vibrations, and it is expected that phenotypic variation is equaled by variation of the vibrational signals, offering a model system to address questions of behavioral differentiation among macropterous and brachypterous individuals, as well as symmetry of mating success among similar and different phenotypes. Hoch and colleagues hypothesized that "under an assumed scenario of disruptive selection (either through the availability of a novel habitat or food resources, or by ecological/climatic deterioration on the surface) *T. emmeae* is a promising candidate for giving rise to obligately cavernicolous species, equally conceivable in either allopatry or sympatry" (Hoch et al. 2013, p. 160).

In Rhaphidophoridae, a further suitable example to be studied is the highly species-rich and ecologically diverse genus *Ceuthophilus* of North-West America. This genus contains epigean, troglophilic and highly cave-adapted representatives, which have been well investigated with respect to their ecology and also phylogeny (e.g., Taylor et al. 2005, 2007; Lavoie et al. 2007; Weckstein et al. 2016). Their mating behavior has been investigated so far in two epigean forest species, *C. latens*

(Turner 2015) and *C. guttulosus* (Eades 1964); in both publications one may find behavioral indications on male-produced abdominal vibratory tremulation signals during close-range courtship (see Table 13.2). It would be interesting not only to describe and compare characteristics of sexual behavior and (the presence of) vibratory signals between these and further *Ceuthophilus* species that vary in reliance on the cave habitat, but also to assess the potential of their putative vibratory emissions to inform the partner of the quality and/or conspecificity of the signaler. The latter quantification was not possible in *Troglophilus*, where one of the study species relies on tactile courtship communication, entirely.

In contrast to the cixiid leafhoppers, however, speciation in the cavernicolous Rhaphidophoridae is thought to be allopatric, related to the divergence of populations in isolated cave systems, as shown by the examples studied from different continents (Caccone and Sbordoni 1987; Allegrucci et al. 2011; Allegrucci and Sbordoni 2019). Since this precludes the comparison of cave populations to their direct epigean ancestors, the interpretation of what is ancestral or derived has to be considered with care. In the Ensifera, however, the Hawaiian genus *Caconemobius* (Gryllidae) complies with the criteria listed in the first paragraph of this section (Howarth et al. 2019). Here, marine littoral crickets colonized young lava flows and gave rise to several lineages, which adapted to underground environments utilizing organic material that had accumulated underground as a food resource (Howarth et al. 2019).

While the neuroanatomical and physiological basis for the production and perception of vibrational signals is comparatively well known in the Ensifera, corresponding information for the planthoppers, especially on vibration receptor organs, is scarce (see Wessel et al. 2014). Combining classical methods such as histology with state-of-the-art CT methods can generate high-resolution 3D visualizations, providing information on neuroanatomy. Refined physiological procedures will hopefully add to our understanding of how vibrations are produced and perceived. It is expected that integration of behavioral, anatomical, physiological, and genetic data will help to decode the information contained in vibrational signals.

Acknowledgements Cave cricket study: We thank Andrej Čokl and Meta Virant-Doberlet for their constant support of the work on *Troglophilus* cave crickets in Ljubljana, which they had initiated in the 1990s. Recording cave cricket behavior and signals would not have been possible without a great deal of assistance by the students Dajira Omerćehajić, Živa Justinek, and Nadja Pohl. We wish to thank Reinhard Lakes-Harlan for providing both an intellectual and laboratory home for comparative studies on insect sensory organs in Gießen. The work was financially supported by the Slovenian Research Agency (the research core funding P1–0255 and the research project J1–0823, for NSP). JS is supported by a grant from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, STR 1329/2-1).

Planthopper study: We would like to express our sincere *mahalo* to the many people and organizations who have helped to make the study on Hawaiian cave planthoppers possible by granting research permissions (Hawaii Volcanoes National Park, US Geological Survey, Department of Land and Natural Resources), and to landowners who trustfully permitted access to the caves on their properties, and to everyone who provided information on the location of lava tube entrances. Bishop Museum, and the University of Hawaii at Manoa, Honolulu, kindly and generously hosted us during research visits. We are deeply indebted to our colleagues, friends, and long-time caving buddies Francis G. Howarth, Portland, Oregon, formerly of Bishop Museum, and the late Fred D. Stone, formerly of Kurtistown, Hawaii Island, for sharing their enthusiasm as well as their expertise with us, and for providing their unconditional support during all phases of the project. Manfred Asche, Museum für Naturkunde, Berlin, not only accompanied the project from day one intellectually and logistically, he proved to be the much needed rock whenever the going (in- and outside the caves) got rough. Without him, our studies on Hawaiian cave planthoppers would not have been possible. Financial support was provided by various grants of the German Research Council (DFG) and The Nature Conservancy of Hawaii.

Data repository The original recordings of vibrational signals of Hawaiian planthoppers are deposited in the Animal Sound Archive (Tierstimmenarchiv) of the Museum of Natural History Berlin (Museum für Naturkunde Berlin); digital copies of the recordings and source documentation form the basis of a newly established VibroLibrary at the Animal Sound Archive (see Frommolt et al. 2019).

References

Aldrich JR (1996) Sex pheromones in Homoptera and Heteroptera. In: Schaefer CW (ed) Studies on hemipteran phylogeny. Entomological Society of America, Lanham, MD, pp 199–233

Allegrucci G, Sbordoni V (2019) Insights into the molecular phylogeny of Rhaphidophoridae, an ancient, worldwide lineage of Orthoptera. Mol Phylogenet Evol 138:126–138

Allegrucci G, Todisco V, Sbordoni V (2005) Molecular phylogeography of *Dolichopoda* cave crickets (Orthoptera, Rhaphidophoridae): a scenario suggested by mitochondrial DNA. Mol Phylogenet Evol 37:153–164

Allegrucci G, Trucchi E, Sbordoni V (2011) Tempo and mode of species diversification in *Dolichopoda* cave crickets (Orthoptera, Rhaphidophoridae). Mol Phylogenet Evol 60:108–121

Allegrucci G, Ketmaier V, Di Russo C, Rampini M, Sbordoni V, Cobolli M (2017) Molecular phylogeography of *Troglophilus* cave crickets (Orthoptera, Rhaphidophoridae): a combination of vicariance and dispersal drove diversification in the East Mediterranean region. J Zool Syst Evol Res 55:310–325

Alt JA, Lakes-Harlan R (2018) Sensing of substrate vibrations in the adult cicada *Okanagana rimosa* (Hemiptera: Cicadidae). J Insect Sci 18(3):16

Ander K (1939) Vergleichend-anatomische und phylogenetische Studien über die Ensifera (Saltatoria). Opuscula Ent Suppl 2:1–306

Asche M (1997) A review of the systematics of Hawaiian planthoppers (Hemiptera: Fulgoroidea). Pac Sci 51:366–376

Barr TC (1968) Cave ecology and the evolution of troglobites. Evol Biol 2:35–102

Bernardini C, Di Russo C (2004) A general model for the life cycle of *Dolichopoda* cave crickets (Orthoptera: Rhaphidophoridae). Eur J Entomol 101:69–73

Boldyrev B (1912) Begattung und Spermatophoren bei *Tachycines asynamorus* Adel. (Orthoptera, Stenopelmatidae). Rev Rus Entomol 12:552–570

Boldyrev BT (1913) Über Begattung und die Spermatophoren bei Locustodea und Gryllodea. Rev Rus Entomol 13:484–490

Booij CJH (1982) Biosystematics of the *Muellerianella* complex (Homoptera, Delphacidae), interspecific and geographic variation in acoustic behaviour. Z Tierpsychol 58:31–52

Bourgoin T (2019) FLOW (Fulgoromorpha lists on the web): a world knowledge base dedicated to Fulgoromorpha. Version 8, updated 27 December 2019. http://hemiptera-databases.org/flow/

Buh B (2011) Morphological and functional characterization of vibratory receptor neurons in cave crickets of the genus *Troglophilus* (Orthoptera, Rhaphidophoridae). Graduation thesis, University of Ljubljana

Caccone A, Sbordoni V (1987) Molecular evolutionary divergence among north American cave crickets. I. Allozyme variation. Evolution 41:1198–1214

Carchini G, Rampini M, Sbordoni V (1994) Life cycle and population ecology of the cave cricket *Dolichopoda geniculata* (costa) from Valmarino cave (Central Italy). Int J Speleol 23:203–218

Chopard L (1938) La Biologie des Orthoptères. Lechevalier, Paris

Christiansen K (2012) Morphological adaptations. In: White WB, Culver DC (eds) Encyclopedia of caves, 2nd edn. Elsevier, Amsterdam, pp 517–528

Cigliano MM, Braun H, Eades DC, Otte D (2019) Orthoptera species file. Ver 5.0./5.0. [02.08.2019]. http://orthoptera.speciesfile.org/

Claridge MF (1985) Acoustic signals in the Homoptera: behavior, taxonomy, and evolution. Annu Rev Entomol 30:297–317

Claridge MF (1990) Acoustic recognition signals: barriers to hybridisation in Homoptera Auchenorrhyncha. Can J Zool 68:1741–1746

Cloudsley-Thompson JL (1988) Evolution and adaptation of terrestrial arthropods. Springer, Heidelberg

Čokl A, Kalmring K, Rössler W (1995) Physiology of atympanate tibial organs in forelegs and midlegs of the cave-living ensifera, *Troglophilus neglectus* (Raphidophoridae, Gryllacridoidea). J Exp Zool 273:376–388

Čokl A, Virant-Doberlet M, Zorović M (2006) Sense organs involved in vibratory communication of bugs. In: Drosopoulos S, Claridge MF (eds) Insect sounds and communication. Physiology, behaviour, ecology and evolution. CRC Press, Boca Raton, FL, pp 71–80

Culver DC (1982) Cave life: evolution and ecology. Harvard University Press, Cambridge, MA

Davranoglou L-R, Cicirella A, Taylor GK, Mortimer B (2019) Planthopper bugs use a fast, cyclic recoil mechanism for effective vibrational communication at small body size. PLoS Biol 17(3): e3000155. https://doi.org/10.1371/journal.pbio.3000155

De Pasquale L, Cesaroni D, Di Russo C, Sbordoni V (1995) Trophic niche, age structure and seasonality in *Dolichopoda* cave crickets. Ecogeography 18:217–224

De Vrijer PWF (1984) Variability in calling signals of the planthopper *Javesella pellucida* (F.) (Homoptera: Delphacidae) in relation to temperature, and consequences for species recognition during distant communication. Neth J Zool 34(3):388–406

De Vrijer PWF (1986) Species distinctiveness and variability of acoustic calling signals in the planthopper genus *Javesella* (Homoptera: Delphacidae). Neth J Zool 36:162–175

de Winter AJ, Rollenhagen T (1990) The importance of male and female acoustic behaviour for reproductive isolation in *Ribautodelphax* planthoppers (Homoptera: Delphacidae). Biol J Linn Soc 40:191–206

Debaisieux P (1938) Organes scolopidiaux des pattes d'Insectes. II. La Cellule 47:77–202

Decu V, Juberthie C (2004) Insecta: Coleoptera (beetles). In: Gunn J (ed) Encyclopedia of caves and karst science. CRC Press, New York, pp 965–974

den Bieman CFM (1986) Acoustic differentiation and variation in planthoppers of the genus *Ribautodelphax* (Homoptera, Delphacidae). Neth J Zool 36:461–480

Desutter-Grandcolas L (2003) Phylogeny and the evolution of acoustic communication in extant Ensifera (Insecta, Orthoptera). Zool Scr 32:525–561

Di Russo C, Sbordoni V (1998) Gryllacridoidea. In: Juberthie C, Decu V (eds) Encyclopaedia Biospeologica. Moulis, Bucharest, pp 979–988

Di Russo C, Vellei A, Sbordoni V (1987) Life cycle and age structure of *Dolichopoda* populations (Orthoptera, Raphidophoridae) from natural and artificial cave habitats. Boll Zool 54:337–340

Di Russo C, Rampini M, Cobolli M (2014) The cave crickets of Greece: a contribution to the study of southern Balkan Rhaphidophoridae diversity (Orthoptera), with the description of a new species of *Troglophilus* Krauss, 1879. Biodivers J 5:397–420

Eades DC (1964) General biology and geographic variation of *Ceuthophilus guttulosus* Walker (Orthoptera: Gryllacrididae: Rhaphidophorinae). Trans Am Entomol Soc 90:73–110

Elias DO, Mason AC, Hoy RR (2004) The effect of substrate on the efficacy of seismic courtship signal transmission in the jumping spider *Habronattus dossenus* (Araneae: Salticidae). J Exp Biol 207:4105–4110

Fea M, Holwell G (2018) Exaggerated male legs increase mating success by reducing disturbance to females in the cave weta *Pachyrhamma waitomoensis*. Proc R Soc B 285:20180401

Field LH, Matheson T (1998) Chordotonal organs of insects. Adv Insect Physiol 27:1–228

Frommolt K-H, Hoch H, Wessel A (2019) Call for the establishment of a VibroLibrary at the animal sound archive Berlin. In: Hill PSM, Lakes-Harlan R, Mazzoni V, Narins PM, Virant-Doberlet-M, Wessel A (eds) Biotremology: studying vibrational behavior. Springer Nature, Cham, pp 479–483

Gerhardt U (1913) Copulation und Spermatophoren von Grylliden und Locustiden I. Zool Jb Syst Geograph Biol Tiere 35:415–532

Gogala M (2006) Vibratory signals produced by Heteroptera-Pentatomorpha and Cimicomorpha. In: Drosopoulos S, Claridge MF (eds) Insect sounds and communication: physiology, behaviour, ecology and evolution. CRC Press, New York, pp 275–295

Günther K (1950) Ökologische und funktionelle Anmerkungen zur Frage des Nahrungserwerbes bei Tiefseefischen mit einem Exkurs über die ökologischen Zonen und Nischen. In: Grüneberg H, Ulrich W (eds) Moderne Biologie. Peters, Berlin, pp 55–93

Haley EL, Gray DA (2012) Mating behavior and dual-purpose armaments in a camel cricket. Ethology 118:49–56

Hasse A (1974) Lauterzeugungsmechanismus und Lautrezeption von Kleinzikaden. Naturwissenschaften 61:81–82

Hoch H (2000) Acoustic communication in darkness. In: Wilkens H, Culver D, Humphreys WF (eds) Subterranean ecosystems, ecosystems of the world 30. Elsevier Science B.V., Amsterdam, pp 211–219

Hoch H (2013) *Trirhacus helenae* sp.n., a new cave-dwelling planthopper from Croatia (Hemiptera: Fulgoromorpha: Cixiidae). Dtsch Entomol Z 60:155–161

Hoch H, Howarth FG (1989a) Reductive evolutionary trends in two new cavernicolous species of a new Australian cixiid genus (Homoptera Fulgoroidea). Syst Entomol 14:179–196

Hoch H, Howarth FG (1989b) Six new cavernicolous cixiid planthoppers in the genus *Solonaima* from Australia (Homoptera Fulgoroidea). Syst Entomol 14:377–402

Hoch H, Howarth FG (1993) Evolutionary dynamics of behavioral divergence among populations of the Hawaiian cave-dwelling planthopper *Oliarus polyphemus* (Homoptera: Fulgoroidea: Cixiidae). Pac Sci 47:303–318

Hoch H, Howarth FG (1999) Multiple cave invasions by species of the planthopper genus *Oliarus* in Hawaii (Homoptera: Fulgoroidea: Cixiidae). Zool J Linnean Soc 127:453–475

Hoch H, Wessel A (2006) Communication by substrate-borne vibrations in cave planthoppers. In: Drosopoulos S, Claridge MF (eds) Insect sounds and communication: physiology, behaviour, ecology and evolution. CRC Press, Boca Raton, FL, pp 187–197

Hoch H, Mühlethaler R, Wessel A (2013) Acoustic communication in the subtroglophile planthopper *Trigonocranus emmeae* Fieber, 1876 (Hemiptera: Fulgoromorpha: Cixiidae: Oecleini). Acta Mus Morav Sci Biol 98:155–162

Howarth FG (1972) Cavernicoles in lava tubes on the island of Hawaii. Science 175:325–326

Howarth FG (1979) An inexpensive constant temperature chamber for field and laboratory use. Environ Entomol 8:236–237

Howarth FG (1982) Bioclimatic and geologic factors governing the evolution and distribution of Hawaiian cave insects. Entomol Gen 8:17–26

Howarth FG (1983) Ecology of cave arthropods. Annu Rev Entomol 28:365–389

Howarth FG, Moldovan OT (2018) The ecological classification of cave animals and their adaptations. In: Moldovan OT, Kovács L, Halse S (eds) Cave ecology, ecological studies 235. Springer Nature, Cham, Switzerland, pp 41–67

Howarth FG, Hoch H, Wessel A (2019) Adaptive shifts. In: White WB, Culver DC, Pipan T (eds) Encyclopedia of caves, 3rd edn. Academic Press, Amsterdam, pp 47–55

Hubbell TH, Norton RM (1978) The systematics and biology of the cave-crickets of the north American tribe Hadenoecini (Orthoptera Saltatoria: Esifera: Rhaphidophoridae: Dolichopodinae). Misc Publ Mus Zool Univ Michigan 156:1–124

Hutchinson GE (1958) Concluding remarks. Cold Spring Harb Symp Quant Biol 22:415–427

Ichikawa T (1976) Mutual communication by substrate vibration in the mating behavior of planthoppers (Homoptera: Delphacidae). Appl Entomol Zool 11:8–23

Jeram S, Rössler W, Cokl A, Kalmring K (1995) Structure of atympanate tibial organs in legs of the cave-living Ensifera, *Troglophilus neglectus* (Gryllacrididae, Raphidophoridae). J Morphol 223:109–118

Juberthie C, Decu V (eds) (1994–2001) Encyclopaedia Biospeologica, Vol I–III. Société Internationale de Biospéologie, International Society for Subterranean Biology, Moulis & Bucarest

Karaman I, Hammouti N, Patičević D, Kiefer A, Horvatović M Seitz A (2011) The genus *Troglophilus* Krauss, 1879 (Orthoptera: Rhaphidophoridae) in the West Balkans. Zool J Linnean Soc 163:1035–1063

Kastberger G (1984) Gating of locomotor activity in the cave-cricket, *Troglophilus cavicola*. Physiol Entomol 9:297–314

Kernan C, Hamel J, Iwan A, ter Hofstede H (2018) Calling, courtship, and post-mating tremulations in a Neotropical katydid. In: Hill PSM, Mazzoni V, Virant-Doberlet M (eds) Abstract Book, 2nd International Symposium on Biotremology, p 60. https://eventi.fmach.it/biotremology2018/Book-of-Abstracts

Kevan DKM (1955) Méthodes inhabituelles de production de son chez les Orthoptères. In: Busnel RG (ed) Colloque Sur L'Acoustique Des Orthoptères. INRA, Paris, pp 103–141

Lakes-Harlan R, Strauß J (2014) Functional morphology and evolutionary diversity of vibration receptors in insects. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, New York, pp 277–302

Lavoie KH, Helf KL, Poulson TL (2007) The biology and ecology of north American cave crickets. J Cave Karst Stud 69:114–134

Lipovšek S, Novak T, Janžekovič F, Pabst MA (2016) Role of the fat body in the cave crickets *Troglophilus cavicola* and *Troglophilus neglectus* (Rhaphidophoridae, Saltatoria) during overwintering. Arthr Struct Dev 40:54–63

López-Rodríguez MJ, Tierno de Figueroa JS (2012) Life in the dark: on the biology of the cavernicolous stonefly *Protonemura gevi* (Insecta, Plecoptera). Am Nat 180:684–691

McDowell WM (2002) An ecological study of *Metrosideros polymorpha* gaud. (Myrtaceae) roots in lava tubes. Thesis, University of Hawai'i at Manoa, USA

Mebes H-D (1974) Zur Biophysik der Schallerzeugung bei Kleinzikaden: the biophysics of sound production of leafhoppers. Forma et Functio 7:95–118

Michelsen A, Fink F, Gogala M, Traue D (1982) Plants as transmission channels for insect vibrational songs. Behav Ecol Sociobiol 11:269–281

Nishino H, Mukai H, Takanashi T (2016) Chordotonal organs in hemipteran insects: unique structures but conserved central organization revealed by comparative neuroanatomy. Cell Tissue Res 366:549–572

Novak T, Kuštor V (1982) Contribution à la connaissance de la biomasse et du bilan énergétique de la faune des entrées de grottes en Slovénie (Yugoslavie). Mém Biospéol 8:27–32

Novak T, Kuštor V (1983) On *Troglophilus* (Rhaphidophoridae, Saltatoria) from North Slovenia (YU). Mém Biospéol 10:183–189

Pehani Š, Virant-Doberlet M, Jeram S (1997) The life cycle of the cave cricket *Troglophilus neglectus* Krauss with a note on *T. cavicola* Kollar (Orthoptera: Rhaphidophoridae). Entomologist 116:224–238

Pringle JWS (1957) The structure and evolution of the organs of sound-production in cicadas. Proc Linn Soc Lond 167:144–159

Protas M, Jeffery WR (2012) Evolution and development in cave animals: from fish to crustaceans. Wiley Interdiscip Rev Dev Biol 1:823–845

Rampini M, Di Russo C, Pavesi F, Cobolli M (2013) New records of Aemodogryllinae (Orthoptera, Rhaphidophoridae) from caves of Laos with description of *Eutachycines cassani* Chopard male. J Trop Asian Entomol 2:37–43

Rentz DCF (1980) A new family of ensiferous Orthoptera from the coastal sands of Southeast Queensland. Memoirs Queensland Mus 20:49–63

Richards AM (1961) The life history of some species of Rhaphidophoridae (Orthoptera). Trans R Soc N Zeal 1:121–137

Sbordoni V, de Matthaeis E, Cobolli Sbordoni M (1976) Phosphoglucomutase polymorphism and natural selection in populations of the cave cricket *Dolichopoda geniculata*. Z Zool Syst Evol 14:292–299

Sket B (2008) Can we agree on an ecological classification of subterranean animals? J Nat Hist 42: 1549–1563

Soares D, Niemiller ML (2020) Extreme adaptations in caves. Anat Rec 303:15–23

Song H, Béthoux O, Shin S, Donath A, Letsch H, Liu S, McKenna DD, Meng G, Misof B, Podsiadlowski L, Zhou X, Wipfler B, Simon S (2020) Phylogenomic analysis sheds light on the evolutionary pathways towards acoustic communication in Orthoptera. Nat Commun 11: 4939

Stewart KW, Zeigler DD (1984) The use of larval morphology and drumming in Plecoptera systematics, and further studies of drumming behavior. Ann Limnol 20:105–114

Strauß J (2017) The scolopidial accessory organs and Nebenorgans in orthopteroid insects: comparative neuroanatomy, mechanosensory function, and evolutionary origin. Arthropod Struct Dev 46:765–776

Strauß J, Stritih N (2016) The accessory organ, a scolopidial sensory organ, in the cave cricket *Troglophilus neglectus* (Orthoptera: Ensifera: Rhaphidophoridae). Acta Zool 97:187–195

Strauß J, Stritih N (2017) Neuronal regression of internal leg vibroreceptor organs in a cave-dwelling insect (Orthoptera: Rhaphidophoridae: *Dolichopoda araneiformis*). Brain Behav Evol 89:104–116

Strauß J, Stritih N, Lakes-Harlan R (2014) The subgenual organ complex in the cave cricket *Troglophilus neglectus* (Orthoptera: Rhaphidophoridae): comparative innervation and sensory evolution. R Soc Open Sci 1:140240

Stritih N (2009) Anatomy and physiology of a set of low-frequency vibratory interneurons in a nonhearing Ensiferan (*Troglophilus neglectus*, Rhaphidophoridae). J Comp Neurol 516:519–532

Stritih N (2014) Signaling by protrusive scent glands in cave crickets, *Troglophilus neglectus* Krauss (Orthoptera: Rhaphidophoridae), is primarily involved in male-male agonism. J Insect Behav 27:317–332

Stritih N, Čokl A (2012) Mating behaviour and vibratory signalling in non-hearing cave crickets reflect primitive communication of Ensifera. PLoS One 7:47646

Stritih N, Čokl A (2014) The role of frequency in vibrational communication of Orthoptera. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 375–393

Stritih Peljhan N, Strauß J (2018) The mechanical leg response to vibration stimuli in cave crickets and implications for vibrosensory organ functions. J Comp Physiol A 204:687–702

Stritih N, Strauß J (2015) Tremulation signalling and sensory neuroanatomy of cave crickets (Rhaphidophoridae: *Troglophilus*) are consistent with ancestral vibrational communication in Ensifera. Mitt Dtsch Ges Allg Angew Entomol 20:333–336

Stritih N, Stumpner A (2009) Vibratory interneurons in the non-hearing cave cricket indicate evolutionary origin of sound processing elements in Ensifera. Zoology 112:48–68

Stritih N, Žunič Kosi A (2017) Olfactory signaling of aggressive intent in male-male contests of cave crickets (*Troglophilus neglectus*; Orthoptera: Rhaphidophoridae). PLoS One 12(11): e0187512

Stritih-Peljhan N (2018) Cave cricket genus *Troglophilus* as a model for studying function and evolution of sensory systems and behaviour. Acta Entomol Slov 26:131–150

Stritih-Peljhan N, Rühr PT, Buh B, Strauß J (2019) Low-frequency vibration transmission and mechanosensory detection in the legs of cave crickets. Comp Biochem Physiol A 233:89–96

Strübing H (1960) Paarungsverhalten und Lautäusserung von Kleinzikaden, demonstriert an Beispielen aus der Familie der Delphacidae (Homoptera, Auchenorrhyncha). Proc XXI Int Congr Entomol Wien 11:12–14

Strübing H, Rollenhagen T (1988) Ein neues Aufnehmersystem für Vibrationssignale und seine Anwendung auf Beispiele aus der Familie Delphacidae (Homoptera-Cicadina) – a new recording system for vibratory signals and its application to different species of the family Delphacidae (Homoptera-Cicadina). Zool Jahrb Allg Zool 92:245–268

Sudhaus W (2008) Von der Evolutionsmorphologie zur Evolutionsökologie. Mitt Dtsch Ges Allg Angew Entomol 16:451–466

Taylor SJ (2008) Cave adapted insects. In: Capinera JL (ed) Encyclopedia of entomology. Springer, Dordrecht, Netherlands, pp 803–806

Taylor SJ, Krejca JK, Denight ML (2005) Foraging range and habitat use of *Ceuthophilus secretus* (Orthoptera: Rhaphidophoridae), a key trogloxene in Central Texas cave communities. Am Midl Nat 154:97–114

Taylor SJ, Weckstein JD, Takiya DM, Krejca JK, Murdoch JD, Veni G, Johnson KP, Reddell JR (2007) Phylogeography of cave crickets (*Ceuthophilu*s spp.) in Central Texas: a keystone taxon for the conservation and management of federally listed endangered cave arthropods. Ill Nat Hist Surv Tech Rep 2007(58):1–45

Tinkham E, Rentz D (1969) Notes on the bionomics and distribution of the genus *Stenopelmatus* in Central California with description of a new species. Pan-Pac Entomol 45:4–14

Tishechkin DY, Vedenina VY (2016) Acoustic signals in insects: a reproductive barrier and a taxonomic character. Entomol Rev 96:1127–1164

Turner CL (2015) Breeding habits of *Ceuthophilus latens*, the camel cricket. Bull Wis Nat Hist Soc 13:32–41

Vondráček K (1949) Příspěvek k poznání zvukového ústrojí u samcu křísu/contribution to the knowledge of the sound-producing apparatus in the males of the leafhoppers (Homoptera-Auchenorrhyncha). Acta Acad Sci Nat Moravo-Siles (Brno) 21:1–36

Weckstein JD, Johnson KP, Murdoch JD, Krejca JK, Takiya DM, Veni G, Reddell JR, Taylor SJ (2016) Comparative phylogeography of two codistributed subgenera of cave crickets (Orthoptera: Rhaphidophoridae: *Ceuthophilus* spp.). J Biogeogr 43:1450–1463

Weissmann MJ (1997) Natural history of the giant sand treader camel cricket, *Daihinibaenetes giganteus* Tinkam (Orthoptera: Rhaphidophoridae). J Orthoptera Res 6:33–48

Wessel A, Hoch H (1999) Remane's statistic species criterion applied to Hawaiian cave planthoppers. Reichenbachia 33:27–35

Wessel A, Hoch H, Asche M, von Rintelen T, Stelbrink B, Heck V, Stone FD, Howarth FG (2013) Rapid species radiation initiated by founder effects in Hawaiian cave planthoppers. Proc Natl Acad Sci USA 110:9391–9396

Wessel A, Mühlethaler R, Hartung V, Kuštor V, Gogala M (2014) The Tymbal: evolution of a complex vibration-producing organ in the Tymbalia (Hemiptera excl. Sternorrhyncha). In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, New York, pp 395–444

Young D (1970) The structure and function of a connective chordotonal organ in the cockroach leg. Philos Trans R Soc Lond B 256:401–426

Part V

Vibrational Behavior in Some Well-Studied Taxa

Chapter 14
Ophidian Biotremology

Dawei Han and Bruce A. Young

Abstract Snakes evince the ability to detect substrate-borne mechanical waves (through a variety of substrates) and surface mechanical waves; exactly how these specialized vertebrates accomplish this remains largely unknown. Behavioral and neurophysiological studies in snakes have struggled to differentiate the modalities, mechanisms, and central pathways for the airborne and ground-borne detection of mechanical waves. The snake cochlea is the best-known component of this sensory system; previous studies have shown that the snake cochlea has a rather consistent frequency response range, some intriguing differences in sensitivity, and a mechanical coupling to the middle ear ossicle. How pressure waves reach the middle ear ossicle/cochlea is not clear; whether or not there are pathways (perhaps utilizing the lung) to the cochlea that bypass the ossicle, and the relative role of the snake's vestibular system in the detection of mechanical waves (if any), remain a mystery. The pathway by which neural signals transduced in the cochlea reach higher brain centers has not been determined in snakes. Perhaps most intriguing, we do not know how pressure stimuli encoded at the cochlea are integrated with stimuli encoded elsewhere on the snake's body.

14.1 Introduction

The abundance of popular accounts of snake behavior, coupled with confusion and contradiction in the scientific literature on snakes, has created confusion regarding aspects of the sensory biology of snakes. Behavioral and physiological studies have established that snakes can detect mechanical pressure waves propagating in air, water, and through soil. Abundant anecdotal, and limited behavioral, evidence suggest that snakes can perceive surface-borne mechanical waves. Physiological

---

D. Han
Department of Biology, University of Maryland, College Park, MD, USA

B. A. Young (✉)
Department of Anatomy, Kirksville College of Osteopathic Medicine, Kirksville, MO, USA
e-mail: byoung@atsu.edu

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_14

studies have shown that snakes can detect vibrational stimuli applied to the surface of their head and/or body. In the past, and even currently, some workers have referred to all of these forms of stimuli perception as hearing. Other workers have argued that snakes are only capable of "vibration detection," but have not provided a specific definition of "vibration." Recently, Hill and Wessel (2016) have tried to standardize terminology based on waveforms that would use "sound" and "hearing" only with respect to information being carried as pressure waves, while all other mechanical waves used in information transfer via surface- or substrate-borne waves would be considered to be vibrational waves, even though pressure waves are also mechanical and vibrational. Further complicating this is the scant neurophysiological evidence from snakes that has failed to resolve the distinction, if any exists, at the level of receptor organ, neural pathway, or central processing, among perception of all sorts of mechanical waveform detection.

The older "natural history" literature contains numerous references to hearing in snakes (see Young 2003). These older references are almost evenly split between claims that snakes are completely deaf and accounts of snakes being attracted to particular sounds (often musical instruments). By the early 1900s a scientific, though not a popular, consensus seemed to emerge regarding snake hearing: Smith (1943) remarked, "... that they can hear very well is indisputable"; Gadow (1901) noted that, "..Snakes can hear very well"; and Wall (1921) prefaced his exposition on vibration detection by stating that "...Snakes hear well." It is interesting that despite this agreement, in the interval leading up to the physiological establishment of snake "hearing" (Wever and Vernon 1960; Hartline 1971a; Wever 1978), no clear examples of ophidian biotremology were presented.

Perhaps the most detailed example of biotremology in snakes involves the defensive foot drumming performed by a variety of rodent species (e.g., Randall 2001). This defensive behavior, which is most commonly performed in the presence of rattlesnake predators, maybe just one part of a more complex defensive behavior (e.g., Swaisgood et al. 2003; Rundus et al. 2007). The foot drumming produces seismic vibrations that are detected by the snake and may alter the snake's predatory behavior (e.g., Randall and Matocq 1997; Whitford et al. 2019).

Sand vipers of the genus *Cerastes* can successfully ambush prey while buried in fine sand (e.g., Subach et al. 2009). Experimental analysis demonstrated that *Cerastes* is able to localize substrate-borne vibrations produced by rodent footfalls. Disruption of non-auditory sensory systems did not significantly influence the accuracy of the strike, and the buried snakes would strike at artificial footfalls (Young and Morain 2002).

The anaconda (*Eunectes*) will strike prey in the water, waiting in ambush with all of the body, and the majority of the head, submerged (Barrio Amoros and Manrique 2008). Laboratory analysis revealed that when in this foraging posture *Eunectes* would respond to substrate-borne vibrations and would direct predatory strikes at submerged speakers playing recordings of mammals swimming (Young 2007). Vincent et al. (2005) found that *Acrochordus* would preferentially respond to a stimulus of surface wave vibrations, and recent work has reported underwater "hearing" in a sea snake (Chapuis et al. 2019).

With the breadth of evidence from behavioral studies spanning multiple species, it is easy to conclude that snakes can undoubtedly detect some sort of mechanical waves. It is much more strenuous, however, to unveil the mechanism underlying biotremology in snakes, a group of animals with an apparent lack of external receivers. In the following sections, we will review the anatomical and physiological foundations, as well as recent progress and future directions, for the study of ophidian biotremology.

14.2 Anatomical Basis for the Detection of Mechanical Waves in Snakes

Popular literature has often cited snakes as "deaf," most likely due to their superficial lack of hearing organs. Although lacking external ear openings, snakes have middle and inner ears (Fig. 14.1). The middle ear lies underneath layers of skin and muscle and is reduced to a single ossicle, the stapes or columella auris. The Eustachian tube is absent. Wever (1978) described a flattened middle ear cavity along the course of the columella, bound by a thin membrane. The space is devoid of fluid, but whether it functions the same as the gaseous middle ear cavity is unknown.

The distal end of the stapes is attached to the quadrate. The quadrate of the snake links the supratemporal to the articular portion of the mandible. In colubrids, the cartilaginous surfaces of the columella and the quadrate directly about each other and are joined by connective tissue. In boids, a number of intervening cartilages lie

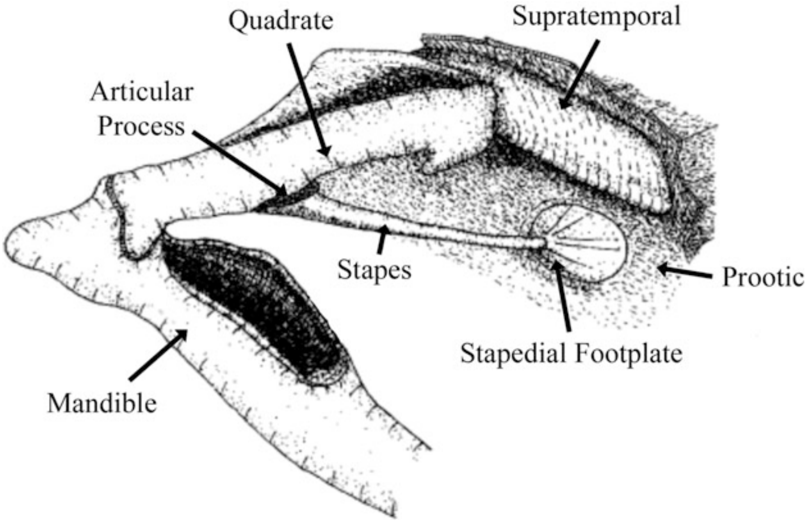


Fig. 14.1 Diagram of the middle ear of a colubrid snake (modified from Wever 1978, with permission)

between the two processes and altogether form a small capsule, providing more surfaces of sliding contact. In *Typhlops pusillus*, a member of blind snakes (Scolecophidae), the columella is joined to the quadrate by a heavy ligament (Wever 1978). These cartilaginous elements, often referred to as extrastapes or extracolumella, are thought to have derived from different lizard homologs (Rieppel 1980).

The proximal end of the stapes terminates with an expanding footplate and rests on the fenestra ovalis on the otic capsule. A unique feature among modern squamates, a crest of bone called the crista circumfenestralis rises to surround the stapedial footplate (Baird 1970). This structure could be further divided into three main components: crista prootica, crista tuberalis, and crista interfenestralis (Rieppel and Zaher 2001). Variation in the height, degree of protrusion, and amount of coverage occur among different species. Palci and Caldwell (2014) suggested that each element of the crista circumfenestralis may have different developmental origins, and each component should be assessed individually.

The linkage from mandible to otic capsule via the quadrate and columella is essential for vibration detection in snakes. However, this anatomical setup dictates that snakes face the problem of impedance mismatch when presented with airborne sounds. In mammals and birds, the thin tympanum is induced to vibrate by airborne pressure waves, and a set of middle ear ossicles transforms tympanic motion to fluid motion in the cochlea; snakes have lost this adaptation, and skin, muscle, and the quadrate bone lie over the inner ear. Despite some arguments that the quadrate to footplate ratio could act to offset this limitation (Wever 1978), the ophidian ear is not well-equipped to detect airborne sound.

The inner ear of snakes is similar to that of other reptiles, consisting of the three semicircular canals, the saccule, utricle, and cochlea. The cochlear duct in snakes is divided into limbic and lagenar regions (Miller 1968). The limbus is a round-oval to elongated-oval tube that lies directly ventral to the saccule, and supports the main auditory sensory organ, the basilar papilla. The stapedial footplate and fenestra ovalis lies close to the cochlea, and movement of the footplate generates perilymphatic pressure waves in the cochlear duct. Snakes lack the round window that most terrestrial vertebrates use to balance fluid movement from the oval window, but instead have a re-entrant fluid circuit. Displacement of perilymphatic fluid passes through scala vestibuli and scala tympani to excite hair cells on the basilar papilla, then returns to the stapes through the perilymphatic labyrinth. In the snake papilla, the hair cells are arranged in irregular rows, most of which are abneurally oriented. The basilar papilla varies greatly in length (0.1–1.5 mm) and hair cell number (50–1500) and appears to be more correlated with habitat rather than taxonomy (Miller 1968; Wever 1978). Burrowing species tend to have the largest papillae, followed by terrestrial species, with arboreal species possessing the smallest. In general, the hair cells of the snake papilla are less densely packed than those of lizards but are greater than those of turtles and amphisbaenians (Baird 1970; Miller 1978). The lagena lies ventral to the cochlea, and the lagena macula is covered by an otolithic membrane, suggesting that its role is vestibular instead of auditory.

Despite having a larger size compared to the auditory papilla, the function of the lagena in snakes is poorly understood.

The vestibulocochlear nerve (VIII) in snakes, like that of other reptiles, is composed of anterior and posterior divisions. The purely vestibular anterior division originates from the cristae of the anterior and lateral ampullae, as well as the utricular macula. The posterior division is mixed, receiving vestibular inputs from the saccular macula and the posterior ampulla, and auditory inputs from the basillar papilla and lagena macula (Carr et al. 2017). The branch of the VIIIth nerve innervating the auditory papilla enters the brainstem, but little is currently known about its central projections in snakes. In an early comparative study of vertebrate auditory neuroanatomy, Holmes (1902) examined the brainstem of four species of snakes and found that their cochlear nuclei resemble those of lizards, and are characterized by a small and ill-defined nucleus dorsalis. Weston (1936) documented the reptile vestibular system, and in passing also noted that the snake cochlear nuclei followed a lizard pattern, with the absence of a nucleus laminaris. Perhaps the most comprehensive study was from Miller (1980), where he used degeneration of the VIIIth nerve to study cochlear nuclei in the brainstem and performed comparative studies on six species of snakes across four families. He described four nuclei: nucleus angularis (NA), nucleus magnocellularis medialis (NM), nucleus magnocellularis lateralis, and nucleus laminaris (NL), which are the same as that of lizards (Miller 1975). However, in species such as *Cylindrophis rufus*, nucleus laminaris was deemed "indistinct," and in some species, the two magnocellular nuclei are "merged." Miller also noted that the cochlear nuclei of snakes and lizards were much less well-developed compared to *Caiman crocodilus* (Leake 1974), which he used as his guide. Comparative neuroanatomy, like all of comparative biology, depends on the context; crocodilians are too specialized and too phylogenetically removed to be considered a good outgroup to snakes (Modesto and Anderson 2004). This weak comparative foundation, coupled with Miller's admission that he struggled to find the crocodilian pattern in the snakes' neuroanatomy, have led subsequent workers to treat Miller's findings with caution. Later Defina and Kennedy (1983) surveyed 17 species and found similar variation as in Miller (1980) in regard to nucleus angularis and nucleus magnocellularis. They also noted the ambiguity between cochlear and vestibular nuclei in many species.

There are no studies regarding the organization of the snake auditory midbrain and its projections to the telencephalon. Studies in lizards show that the central nucleus of the torus semicircularis can be subdivided into three components and receives distinct afferent inputs from the auditory brainstem. The lateral subdivision receives inputs from NL and NA, the ventral subdivision receives inputs from the superior olive and nucleus lemniscus, and the dorsomedial subdivision lacks ascending inputs. The central nucleus then projects to the nucleus medialis in the dorsal thalamus (Foster and Hall 1978; Yan et al. 2010). Terminations from the nucleus medialis could be further traced to the medial dorsal ventricular ridge and the striatum (Foster and Hall 1978; Bruce and Butler 1984). Given the specializations of their peripheral auditory system, whether the snake's central auditory pathway has the same three components and pathways is questionable and requires further examination using modern tracing techniques.

The torus semicircularis is best known as an auditory processing center (see review in Bass et al. 2005); however, it also receives other ascending sensory input such as that from the somatosensory system (fish: Echteler 1984; Yamamoto et al. 2010; frogs: Muñoz et al. 1995, 1997; lizards: Foster and Hall 1978; turtles: Kunzle and Woodson 1982; crocodilians: Pritz and Stritzel 1989; birds: Wild 1995; mammals: Gruters and Groh 2012). In general, somatosensory projection can be found in the external shell region overlaying the auditory central core nuclei, and development of the shell region precedes that of the core region (Zeng et al. 2007a, b, 2008, 2009). In snakes, spinal projections have been shown to terminate in the external layers of the posterior midbrain in snakes (Schroeder 1985). The torus is relatively small in the caudal midbrain compared to lizards, but anterior to the torus, Senn (1969) describes a unique ophidian structure termed the paratorus, which is larger than the torus and differs in cytoarchitecture. Further studies revealed that the paratorus receives projections from the nucleus dorsalis myelencephali in the caudal medulla, which relays inputs from the ipsilateral spinal cord (Schroeder 1985). These anatomical implications, combined with physiological evidence for “somatic hearing” (Hartline 1971a, b; see Sect 14.3), suggest that the somatosensory system may contribute significantly to vibration detection in snakes.

14.3 Physiological Basis for Ophidian Biotremology

The earliest attempt at assessing auditory physiology in snakes comes from Adrian (1938). He failed to obtain responses from VIIIth nerve recordings in response to airborne sound; however, “tactile stimuli and vibration of the supporting surface gave an irregular discharge” from the nerve. Wever and Vernon (1960) were the first to demonstrate that snakes are capable of responding to vibrational or airborne sound stimuli with cochlear microphonic recordings, and that the quadrate bone, which contributes to the cranial kinesis for which snakes are widely known, is an essential component in these scenarios. Removal of the quadrate leads to notable declines in response thresholds. Further findings, detailed in Wever (1978), showed that to evoke cochlear potentials, the quadrate, to which the columella was attached, was not required to move in a swaying motion about one of its joints. Instead, the articulations were loose enough for it to vibrate as a whole. Wever (1978) refuted the common view that snakes can only detect attenuated airborne sound that transmits through the substrate, as greater sensitivity was observed when airborne sound was presented ipsilateral to the recording site. If snakes only detected attenuated airborne sound through the substrate, then both ipsilateral and contralateral sound stimuli should elicit the same response. He also explored the motion of the columella during sound detection, as connective tissue enclosing the articulation of the distal end of the columella with the quadrate enables firm attachment with a degree of freedom along the longitudinal direction. He demonstrated that driving the columella longitudinally in and out of the oval window had lower vibration thresholds than moving the shaft laterally. However, this does not indicate axial motion is the one used in natural circumstances; more than likely it is a combination of both.

Another important contribution from Wever and Vernon (1960) and Wever (1978) was their comparative approach; sensitivity curves from 18 species of snakes across 6 families were obtained. In general, snakes showed highest sensitivity to stimulus frequencies of 200–400 Hz, declining toward the edges of the range. No differences were found based on phylogenetic background or habitat preference; although, there was a significant correlation between hair cell number and sensitivity.

Hartline and Campbell (1969) individually evaluated how well snakes could respond to airborne sound and substrate-borne vibration by performing extracellular recordings in the torus semicircularis. Snakes were sensitive to airborne sound or substrate-borne vibration delivered to areas along the body, extending beyond midsection, indicating a mechanism by which snakes could detect peripheral stimuli. Note that in this study, airborne sound was delivered quantitatively, with response thresholds representing those of Wever and Vernon (1960) and Wever (1978), but substrate-borne vibration was only evaluated qualitatively by tapping the table or stimulating underneath with a speaker under the platform on which the snake was placed. To probe the neural basis for peripheral detection, Hartline and Campbell (1969) transected the spinal cord of *Pituophis* and obtained responses when airborne sounds of 140 Hz at 80 dB SPL were delivered. Midbrain responses were abolished after bilateral destruction of the inner ear, even when airborne sound stimuli increased to 100 dB. In another species, *Crotalus viridis*, severing of the spinal cord also did not eliminate responses to mid-body airborne sound and substrate-borne vibration stimuli. The authors concluded that the spinal cord does not contribute to airborne sound or substrate-borne vibration detection in snakes. However, their experiments only disproved the necessity of the spinal cord for midbrain responses. Without eliminating contributions of the inner ear and recording with only the spinal cord intact, the sufficiency of the spinal cord cannot be ruled out.

Perhaps the authors realized the fallacy in their conclusion. Hartline (1971a) explored the responses of the torus to airborne sound and ground-borne vibration by addressing the inner ear and the spinal cord separately. Contrary to the previous report (Hartline and Campbell 1969), the spinal cord, or the somatic system, was evidenced to mediate responses in the auditory midbrain. The response thresholds to airborne sound pressure and substrate-borne vibration after the destruction of the inner ear with only the spinal cord intact were generally greater than that with ear intact but spinal cord transected. For the somatic system, the shape of the response threshold curve was flatter without a distinct best frequency (Fig. 14.2). Not surprisingly, stimuli delivered directly to the head or columella failed to elicit a response. The topography of sites from which a somatic response could be recorded also differed from that of the auditory system. In a follow-up paper, Hartline (1971b) demonstrated that the latency of the somatic system was consistently lower (by as much as 10 ms) than that of the auditory system. Mechanoreceptors such as cutaneous vibration receptors found by Proske (1969) may serve as peripheral

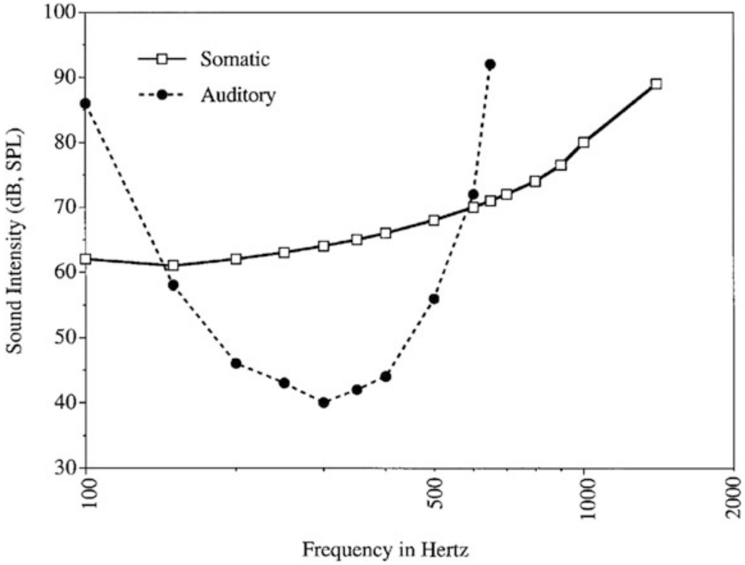


Fig. 14.2 Threshold to airborne sound for snakes (from Young 2003). Somatic responses are more narrowly tuned compared to auditory responses

detectors; however, systematic differences of intensity-latency curves at different frequencies indicate a multi-receptor contribution (Hartline 1971b).

Hartline (1971a) noted that recordings sites for somatosensory responses were located more superficially than those of the auditory system and covered a larger surface area of the torus. In these cases, it is likely that Hartline (1971a, b) was recording from the nucleus laminaris, or shell region, of the torus semicircularis, which receives input from the somatosensory system in many vertebrates. Recordings from Khachunts (1982) show that turtles also have a superficial region of the torus that responded primarily to somatosensory stimuli, and was layered over one that responds to auditory stimuli, as Hartline (1971a, b) illustrated in snakes. In cats (Aitkin et al. 1978, 1981), both electrical stimulation to the dorsal column and acoustic stimuli to the ear were presented while recording from the external nucleus of the shell nuclei (mammalian homolog of the torus). Twenty percent of the cells responded to spinal stimulation alone, and 54% responded to bimodal stimulation. It has been postulated that somatosensory inputs in the IC may initiate reflexive head orientation toward sound (Thompson and Masterton 1978) or aid in suppressing self-generated sound (Jain and Shore 2006). In addition, Aitkin et al. (1978) noted that the cells in ICX that responded to auditory stimuli had broader tuning curves compared to those of units in ICC. The somatic receptive fields for bimodal cells were located in the forelimb or hindlimb of cats, suggesting connections between auditory space and limb position. Response threshold curves for somatic responses in snakes also are more broadly tuned than those of the auditory system (Hartline

1971a, b). Hartline (1971b) mentioned in passing that while generally more broadly tuned, intensity-amplitude curves for single unit somatic recordings were more variable than auditory curves. Combining the studies in mammals and snakes, it is enticing to postulate that snakes could use the somatic system for substrate-borne vibration localization; however, as with any untested hypothesis, this idea requires further systematic evaluation. Moreover, the specific peripheral receptor that facilitates somatic vibration detection has yet to be identified.

Hartline (1971a) also proposed that snakes use their lung to amplify airborne sound. Over a given distance, intensity of locally applied airborne sound decreased more when measured external to the snake, compared to that measured from inside the lung. In spine-severed snakes, injecting saline into the lung greatly reduced auditory responses in the midbrain to local airborne sound stimuli, suggesting that the lung is involved in the transmission of acoustic energy from the periphery to the ear. Teleost fish use internal air cavities to detect sound pressure underwater. In some species, specially adapted bony Weberian ossicles, or even gas-filled vesicles, proximal to the ear are involved; however, the swim bladder is adequate to enhance pressure detection, though the mechanism for this is still obscure (e.g., Blaxter 1977; Chapman and Sand 1974). Amphibians use the lung to enhance sound as well (see review, Capshaw and Soares 2016), but the pathway through which sound travels remains unknown, with suggestion through the endolymphatic sac that extends from the vertebral canal to the inner ear, through soft tissue, or the amphibian opercularis system. In snakes, it was recently shown that head vibrations induced by threshold-level airborne sound pressure matched those induced by threshold-level substrate-borne vibrations, indicating detection of airborne sound is mediated via sound-induced head vibrations (Christensen et al. 2012). Therefore, if the lung were to act the way Hartline (1971a) suggested, it must facilitate displacement of the skull that in turn stimulates the auditory system. For this reason, we are skeptical of the conclusions drawn by Hartline (1971a). Injection of saline should affect responses to control general-field stimuli if the lung was translating vibrations to skull displacement; instead response amplitude to general-field stimuli showed no changes after the air sac became occluded. Moreover, Hartline (1971a) severed the spinal cord in this experiment but did not specify the precise location of transection. If lung mechanoreceptors were still innervated, and contribute to somatic hearing, one would expect to see complete abolishment of responses to local application of airborne sound or substrate-borne vibration after occluding the lung with water; whereas, responses to global sound stimuli would not be affected, as these would be the results of direct head vibrations and are auditory in nature. It is likely the lung plays a role in peripheral detection of pressure stimuli; however, the neural "interpretation" of this signal requires additional scrutiny. Hartline's work, and much of the discussion of it that have followed, were predicated on the assumption that airborne pressure reception was "audition" and substrate-borne pressure reception was "vibration detection" but there is little evidence that there are distinct neural pathways for these sensations within the snake brain.

14.4 Areas for Future Study

White-faced Capuchin monkeys produce snake-specific alarm calls (Coss et al. 2019) but the frequency of the alarm calls fall well beyond the range of snake audition, and there is no obvious behavior on the part of the snake to suggest that interspecific acoustic communication happens within this behavioral interaction (Coss et al. 2019). However, the increasing recognition of the importance of substrate-borne vibration detection in snakes (Christensen et al. 2012), and the "coupling" between airborne and substrate-borne vibrations (e.g., Cress et al. 1980; Narins et al. 2009; Caldwell 2014), suggests that there are numerous undescribed or poorly described examples of biotremology in snakes.

The mating calls of cicadas include not only high-frequency high-intensity airborne components but also substrate-borne components that extend into the hearing range of snakes (Stölting et al. 2002). These substrate-borne vibrations are generally produced while the cicada is in a tree, and these vibrations (e.g., McVean and Field 1996; Hill 2009) can propagate through plant tissue. Copperheads (*Agkistrodon contortrix*) forage on cicadas (e.g., Lagesse and Ford 1996), and have been observed climbing trees housing calling cicadas (Fitch 1960). Recently molted cicadas may be located using chemotaxis (e.g., Beaupre et al. 2001), but the potential for copperheads to biotremologically "eavesdrop" on the mating call of the cicada warrants careful study.

Though more speculative, a biotremology perspective may expose new forms of intraspecific communication in snakes. Low-frequency body oscillations are often produced by snakes (especially males) during courtship and copulation (e.g., Noble 1937; Shetty and Shine 2002; Senter et al. 2014). These oscillations have been described, but the influence of the oscillations on the receiver has yet to be determined. In other snakes, body oscillations are performed as part of a defensive response. Interestingly, some of these species, like *Xenopeltis,* are semi-fossorial (Flower 1899; Smith 1943), and inhabit muddy soil through which these body oscillations could potentially propagate as substrate-borne mechanical waves. In this scenario, there is a frequency match between the "signal" producer (the oscillations), the preferred propagation frequency, and frequency of substrate-borne vibration detection in snakes (Wever 1978); although, there is no evidence to date of a behavioral response to such a "signal" (Young et al. 2013).

Biotremology offers a refreshing perspective on snakes; not only does it help focus on ground-borne vibration detection (which in snakes is more sensitive than airborne hearing; Christensen et al. 2012), but it is not limited to vestibulocochlear reception. Snakes have mechanoreceptors termed sensilla or tubercles that project beyond the surface of the scales (e.g., Jackson 1977; Crowe-Riddell et al. 2019). The distribution of the sensillae differs among different groups of snakes (e.g., Underwood 1967), yet all sensillae appear to have the same basic morphology (e.g., von During and Miller 1979). The ophidian sensilla (Fig. 14.3) bear a superficial resemblance to the (better known) crocodilian integumentary scale organs (Leitch and Catania 2012). Recent work has shown that the crocodilian integumentary scale

Fig. 14.3 Scale sensillae (white spots) on the supraocular scale of a king cobra (*Ophiophagus hannah*), micrograph by B. Young using a Leica M80 dissecting microscope. Scale bar is 0.5 mm

organ is a true multi-modal receptor (Di-Poï and Milinkovitch 2013); the same resolution has yet to be applied to the ophidian scale sensillae. The scale sensillae of several groups of aquatic/marine snakes have been proposed to respond to substrate-borne vibrations (Povel and Van Der Kooij 1997; Westhoff et al. 2005), though this functional role has not been well established. Mechanoreception has also been proposed for the sensillae of terrestrial forms (e.g., Noble 1937; Proske 1969).

Acknowledgments The authors wish to thank Catherine Carr for her considerate guidance, and Peter Kondrashov for his continued support. D.H. was supported by NIDCD 000436.

References

Adrian ED (1938) Effect of sound on reptiles. J Physiol 92:9–11

Aitkin LM, Dickhaus H, Schult W, Zimmermann M (1978) External nucleus of inferior colliculus: auditory and spinal somatosensory afferents and their interactions. J Neurophysiol 41:837–847

Aitkin LM, Kenyon CE, Philpott P (1981) The representation of the auditory and somatosensory systems in the external nucleus of the cat inferior colliculus. J Comp Neurol 196:25–40

Baird IL (1970) The anatomy of the reptilian ear. In: Gans C, Parsons TS (eds) Biology of the Reptilia, vol 2. Academic Press, London, pp 193–275

Barrio Amoros C, Manrique R (2008) Observations on the natural history of the green anaconda (*Eunectes murinus* Linnaeus, 1758) in the Venezuelan Llanos: an ecotourism perspective. Iguana 15:93–101

Bass AH, Rose GJ, Pritz MB (2005) Auditory midbrain of fish, amphibians, and reptiles: model systems for understanding auditory function. In: Winer J, Schreiner C (eds) The inferior colliculus. Springer, New York, pp 459–492

Beaupre SJ, Roberts KG, Senter P, Hartmann PA, Al E (2001) *Agkistrodon contortrix contortrix* chemotaxis, aboreality, and diet. Herpetol Rev 32:44

Blaxter JHS (1977) The effect of swimbladder deflation on pressure sensitivity in the saithe *Pollachius virens*. J Mar Biol Assoc UK 57:1057–1064

Bruce LL, Butler AB (1984) Telencephalic connections in lizards. II. Projections to anterior dorsal ventricular ridge. J Comp Neurol 229:602–615

Caldwell MS (2014) Interactions between airborne sound and substrate vibration in animal communication. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 65–92

Capshaw G, Soares D (2016) Hearing in plethodontid salamanders: a review. Copeia 104:157–164

Carr CE, Christensen-Dalsgaard J, Edds-Walton P, Köppl C, Tang Y, Young BA, Willis KL (2017) Evolutionary trends in hearing in nonmammalian vertebrates. In: Kaas J (ed) Evolution of nervous systems. Elsevier, New York, pp 291–308

Chapman CJ, Sand O (1974) Field studies of hearing in two species of flatfish *Pleuronectes platessa* (L.) and *Limanda limanda* (L.) (family Pleuronectidae). Comp Biochem Physiol A 47:371–385

Chapuis L, Kerr CC, Collin SP, Hart NS, Sanders KL (2019) Underwater hearing in sea snakes (Hydrophiinae): first evidence of auditory evoked potential thresholds. J Exp Biol 222: jeb198184

Christensen CB, Christensen-Dalsgaard J, Brandt C, Madsen PT (2012) Hearing with an atympanic ear: good vibration and poor sound-pressure detection in the royal python, *Python regius*. J Exp Biol 215:331–342

Coss RG, Cavanaugh C, Brennan W (2019) Development of snake-directed antipredator behavior by wild white-faced capuchin monkeys: III. The signaling properties of alarm-call tonality. Am J Primatol. https://doi.org/10.1002/ajp.22950

Cress D, Lundien J, Flohr M (1980) Coupling of airborne sound into the earth: frequency dependence. J Acoust Soc Am 67:1502–1506

Crowe-Riddell JM, Williams R, Chapuis L, Sanders KL (2019) Ultrastructural evidence of a mechanosensory function of scale organs (sensilla) in sea snakes (Hydrophiinae). R Soc Open Sci 6. https://doi.org/10.1098/rsos.182022

Defina A, Kennedy M (1983) The cochlear nuclei in colubrid and boid snakes: a quantitative study. J Morphol 178:285–301

Di-Poï N, Milinkovitch MC (2013) Crocodylians evolved scattered multi-sensory micro-organs. EvoDevo 4. https://doi.org/10.1186/2041-9139-4-19

Echteler SM (1984) Connections of the auditory midbrain in a teleost fish, *Cyprinus carpio*. J Comp Neurol 230:536–551

Fitch H (1960) Autecology of the copperhead. Univ Kansas Publ Mus Nat Hist 13:85–288

Flower SS (1899) Notes on a second collection of reptiles made in the Malay peninsula and Siam, from November 1896 to September 1898, with a list of the species recorded from those countries. Proc Zool Soc Lond 67:600–697

Foster RE, Hall WC (1978) The organization of central auditory pathways in a reptile, *Iguana iguana*. J Comp Neurol 178:783–831

Gadow H (1901) Amphibia and reptiles. MacMillan, New York

Gruters KG, Groh JM (2012) Sounds and beyond: multisensory and other non-auditory signals in the inferior colliculus. Front Neural Circuits 2012:1–39

Hartline PH (1971a) Physiological basis for detection of sound and vibration in snakes. J Exp Biol 54:349–371

Hartline PH (1971b) Mid-brain responses of the auditory and somatic vibration systems in snakes. J Exp Biol 54:373–390

Hartline PH, Campbell HW (1969) Auditory and vibratory responses in the midbrains of snakes. Science 163:1221–1223

Hill PSM (2009) How do animals use substrate-borne vibrations as an information source? Naturwissenschaften 96:1355–1371

Hill PSM, Wessel A (2016) Biotremology. Curr Biol 26:R187–R191

Holmes G (1902) On the comparative anatomy of the Nervus Acusticus. Trans R Irish Acad B 32: 101–104

Jackson MK (1977) Histology and distribution of cutaneous touch corpuscles in some leptotyphlopid and colubrid snakes (Reptilia, Serpentes). J Herpetol 11:7

Jain R, Shore S (2006) External inferior colliculus integrates trigeminal and acoustic information: unit responses to trigeminal nucleus and acoustic stimulation in the Guinea pig. Neurosci Lett 395:71–75

Khachunts AS (1982) Electrophysiological characteristics of representation of auditory and somatosensory systems in the turtle midbrain. Neurophysiology 14:191–198

Kunzle H, Woodson W (1982) Mesodiencephalic and other target regions of ascending spinal projections in the turtle, *Pseudemys scripta elegans*. J Comp Neurol 212:349–364

Lagesse LA, Ford NB (1996) Ontogenetic variation in the diet of the southern copperhead, *Agkistrodon contortrix*, in northeastern Texas. Texas J Sci 48:48–54

Leake PA (1974) Central projections of the statoacoustic nerve in *Caiman crocodilus*. Brain Behav Evol 10:170–196

Leitch DB, Catania KC (2012) Structure, innervation and response properties of integumentary sensory organs in crocodilians. J Exp Biol 215:4217–4230

McVean A, Field LH (1996) Communication by substratum vibration in the New Zealand tree weta, *Hemideina femorata* (Stenopelmatidae: Orthoptera). J Zool 239:101–122

Miller MR (1968) The cochlear duct of snakes. Proc Calif Acad Sci 35:425–476

Miller MR (1975) The cochlear nuclei of lizards. J Comp Neurol 159:375–406

Miller MR (1978) Scanning electron microscope studies of the papilla basilaris of some turtles and snakes. Am J Anat 151:409–435

Miller MR (1980) The cochlear nuclei of snakes. J Comp Neurol 192:717–736

Modesto SP, Anderson JS (2004) The phylogenetic definition of reptilia. Syst Biol 53:815–821

Muñoz A, Muñoz M, González A, Donkelaar HJT (1995) Anuran dorsal column nucleus: organization, immunohistochemical characterization, and fiber connections in *Rana perezi* and *Xenopus laevis*. J Comp Neurol 363:197–220

Muñoz A, Muñoz M, González A, Ten Donkelaar HJ (1997) Spinal ascending pathways in amphibians: cells of origin and main targets. J Comp Neurol 378:205–228

Narins PM, Losin N, O'Connell-Rodwell CE (2009) Seismic and vibrational signals in animals. In: Squire L (ed) Encyclopedia of neuroscience. Academic Press, Oxford, pp 545–549

Noble GK (1937) The sense organs involved in the courtship of *Storeria*, *Thamnophis* and other snakes. Bull Am Mus Nat Hist 73:673–725

Palci A, Caldwell MW (2014) The upper cretaceous snake *Dinilysia patagonica* Smith-Woodward, 1901, and the crista circumfenestralis of snakes. J Morphol 275:1187–1200

Povel D, Van Der Kooij J (1997) Scale sensillae of the file snake (Serpentes: Acrochordidae) and some other aquatic and burrowing snakes. Netherlands J Zool 47:443–456

Pritz MB, Stritzel ME (1989) Reptilian somatosensory midbrain: identification based on input from the spinal cord and dorsal column nucleus. Brain Behav Evol 33:1–14

Proske U (1969) Nerve endings in skin of the Australian black snake. Anat Rec 164:259–265

Randall JA (2001) Evolution and function of drumming as communication in mammals. Am Zool 41:1143–1156

Randall JA, Matocq MD (1997) Why do kangaroo rats (*Dipodomys spectabilis*) footdrum at snakes? Behav Ecol 8:404–413

Rieppel O (1980) The sound-transmitting apparatus in primitive snakes and its phylogenetic significance. Zoomorphology 96:45–62

Rieppel O, Zaher H (2001) The development of the skull in *Acrochordus granulatus* (Schneider) (Reptilia: Serpentes), with special consideration of the otico-occipital complex. J Morphol 249: 252–266

Rundus AS, Owings DH, Joshi SS, Chinn E, Giannini N (2007) Ground squirrels use an infrared signal to deter rattlesnake predation. Proc Natl Acad Sci USA 104:14372–14376

Schroeder DM (1985) The nucleus dorsalis myelencephali of snakes: relay nucleus between the spinal cord and the posterior colliculus (paratorus). Brain Res 336:359–362

Senn DG (1969) The saurian and ophidian colliculi posteriores of the midbrain. Acta Anat 74:114–120

Senter P, Harris SM, Kent DL (2014) Phylogeny of courtship and male-male combat behavior in snakes. PLoS One 9. https://doi.org/10.1371/journal.pone.0107528

Shetty S, Shine R (2002) The mating system of yellow-lipped sea kraits (*Laticauda colubrina*: Laticaudidae). Herpetologica 58:170–180

Smith MA (1943) The fauna of British India, Ceylon and Burma, including the whole of the Indo-Chinese sub-region. Reptilia and Amphibia, vol 3 - Serpentes. Taylor & Francis, London

Stölting H, Moore TE, Lakes-Harlan R (2002) Substrate vibrations during acoustic signalling in the cicada *Okanagana rimosa*. J Insect Sci 2:1–7

Subach A, Scharf I, Ovadia O (2009) Foraging behavior and predation success of the sand viper (*Cerastes vipera*). Can J Zool 87:520–528

Swaisgood RR, Rowe MP, Owings DH (2003) Antipredator responses of California ground squirrels to rattlesnakes and rattling sounds: the roles of sex, reproductive parity, and offspring age in assessment and decision-making rules. Behav Ecol Sociobiol 55:22–31

Thompson GC, Masterton RB (1978) Brain stem auditory pathways involved in reflexive head orientation to sound. J Neurophysiol 41:1183–1202

Underwood G (1967) A contribution to the classification of snakes. Trustees of the British Museum of Natural History, London

Vincent SE, Shine R, Brown GP (2005) Does foraging mode influence sensory modalities for prey detection in male and female filesnakes, *Acrochordus arafurae*? Anim Behav 70:715–721

von During M, Miller MR (1979) Sensory nerve endings of the skin and deeper structures. In: Gans C (ed) Biology of the reptilia, vol 9. Academic Press, New York, pp 407–441

Wall F (1921) Ophidia Taprobanica or the snakes of Ceylon. H.R. Cottle, Colombo

Westhoff G, Fry BG, Bleckmann H (2005) Sea snakes (*Lapemis curtus*) are sensitive to low-amplitude water motions. Zoology 108:195–200

Weston JK (1936) The reptilian vestibular and cerebellar gray with fiber connections. J Comp Neurol 65:93–199

Wever EG (1978) The reptile ear. Princeton University Press, Princeton, NJ

Wever EG, Vernon JA (1960) The problem of hearing in snakes. J Aud Res 1:77–83

Whitford MD, Freymiller GA, Clark RW (2019) Managing predators: the influence of kangaroo rat antipredator displays on sidewinder rattlesnake hunting behavior. Ethology 125:450–456

Wild JM (1995) Convergence of somatosensory and auditory projections in the avian torus semicircularis, including the central auditory nucleus. J Comp Neurol 358:465–486

Yamamoto N, Kato T, Okada Y, Somiya H (2010) Somatosensory nucleus in the torus semicircularis of cyprinid teleosts. J Comp Neurol 518:2475–2502

Yan K, Tang YZ, Carr CE (2010) Calcium-binding protein immunoreactivity characterizes the auditory system of *Gekko gecko*. J Comp Neurol 518:3409–3426

Young BA (2003) Snake bioacoustics: toward a richer understanding of the behavioral ecology of snakes. Q Rev Biol 78:303–325

Young BA (2007) Response of the yellow anaconda (*Eunectes notaeus*) to aquatic acoustic stimuli. In: Henderson RW, Powell R (eds) Biology of boas and pythons. Eagle Mountain, Utah, pp 199–205

Young BA, Morain M (2002) The use of ground-borne vibrations for prey localization in the Saharan sand vipers (*Cerastes*). J Exp Biol 205:661–665

Young BA, Mathevon N, Tang Y (2013) Reptile auditory neuroethology: what do reptiles do with their hearing? In: Köppl C, Manley GA, Popper AN, Fay RR (eds) Insights from comparative hearing research. Springer handbook of auditory research, vol 49. Springer, New York, pp 323–346

Zeng SJ, Li J, Zhang XW, Zuo MX (2007a) Distinction of neurochemistry between the cores and their shells of auditory nuclei in tetrapod species. Brain Behav Evol 70:1–20

Zeng SJ, Xi C, Zhang XW, Zuo MX (2007b) Differences in neurogenesis differentiate between core and shell regions of auditory nuclei in the turtle (*Pelodiscus sinensis*): evolutionary implications. Brain Behav Evol 70:174–186

Zeng SJ, Tian CP, Zhang XW, Zuo MX (2008) Neurogenic development of the auditory areas of the midbrain and diencephalon in the *Xenopus laevis* and evolutionary implications. Brain Res 1206:44–60

Zeng SJ, Lin YT, Tian CP, Song KJ, Zhang XW, Zuo MX (2009) Evolutionary significance of delayed neurogenesis in the core versus shell auditory areas of *Mus musculus*. J Comp Neurol 515:600–613

Chapter 15
Evolution of Communication Systems Underground in a Blind Mammal, *Spalax*

Eviatar Nevo

Abstract The evolution of mammals to life underground, which has been unfolding globally for over 50 million years, is one of nature's best-studied long-term evolutionary experiments. The *Spalax ehrenbergi* superspecies in Israel described here has been studied inter- and multidisciplinarily since 1948 as a long-term evolutionary model project of active Pleistocene speciation, primarily peripatrically and chromosomally. Four species, with $2n = 52$, 54, 58, and 60, respectively, have been shown to be good biological species (*Spalax galili, S. golani, S. carmeli,* and *S. judaei*). Southwards they form an adaptive evolutionary speciation cline with each species adapted to a different climatic regime, from the humid and cold north of Israel to the dry warm northern Negev desert. Recently we described the fifth *Spalax* species, originating genically and not chromosomally from *Spalax galili* ($2n = 52$) on volcanic basalt on which it speciated sympatrically ~228,000 years ago. *Spalax* species are blind and live most of their lives underground, yet they are able to maintain photoperiodicity. Major replacements of biological sensory modalities include olfaction, vocalization, and vibratory seismic communication. The vocal dialects and the vibratory seismic dialects provide ethological premating reproductive isolating signals that separate the chromosomal species, complementary to meiotic postzygotic reproductive isolation. Here I review the evolution of the adaptive low-frequency, speciational *short-distance* vocal dialects, and the vibrational *long-distance* seismic communication, described structurally, functionally, and evolutionarily. Convergence with African bathyergid mole rats is emphasized. The adaptive progression of the subcutaneous regressive eyes responding underground to photoperiodic perception, and the adaptive networked ingenuity of the vocal and vibratory communication modalities are highlighted evolutionarily.

E. Nevo (✉)
Institute of Evolution, University of Haifa, Haifa, Israel
e-mail: nevo@research.haifa.ac.il

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_15

15.1 Evolution of Mammals to Life Underground

The evolution of mammals to life underground is one of nature's best-studied long-term experiments, an unfolding global evolutionary experiment that is over 50 million years old. The global adaptive convergence of subterranean mammals currently involves three orders: rodents, insectivores, and marsupials. This global evolutionary process resulted by stepwise climatic cooling and drought, followed by biotic extinction in the transition from the middle Eocene to the early Oligocene, a period of about 10 million years of profound change in Earth's geology, climate, and biota. The Earth changed from the Mesozoic "hot house" to the Neogene (Miocene to present) "cold house," i.e., from a warm, equable, mostly subtropical world that persisted from the Mesozoic to the beginning of the present glaciated world, currently warming globally due to human activities. The ecological theater of open country biota that emerged progressively in the Cenozoic, following the Eocene-Oligocene transition, was associated with increasing aridity, colder climate, and terrestrialism. This climatic change set the stage for a rapid evolutionary playoff of recurrent Neogene adaptive radiation of unrelated mammals on all continents into the subterranean ecotope (Fig. 15.1; Nevo 1999).

The subterranean ecotope is relatively simple, specialized, and predictable, with relatively constant temperature and humidity, and low to medium productivity. By contrast, however, it consists of *multiple stressor*s, including darkness, hypoxia, hypercapnia, energetic loads, food scarcity, and extensive pathogenicity (Nevo 2013). This ecotope involves the herbivorous (rodents) and insectivorous (insectivores and marsupials) niches. All subterranean mammals *partly* share molecular and organismal convergent adaptations to their common and unique ecology, with much variation even among subterranean rodents, described here. By contrast, they display divergent adaptations to their different niches, herbivory and insectivory, and to their different phylogenies. The remarkable *adaptive* evolution of subterranean mammals involves *structural and functional*, *regressiv*e and *progressive* variations associated with their underground ecotope. Remarkably, multiple *convergent* evolutionary patterns occurred in subterranean mammals due to similar ecological *constraints* and *stressors* underground at both the molecular and organismal levels, causing evolutionarily distant organisms to *adaptively converge*.

15.2 Spalacidae, Focusing on the Genus *Spalax*, the Blind Mole Rats in Israel

The Spalacidae are Eurasian, primarily East-Mediterranean subterranean rodents (Fig. 15.2), highly adapted for life underground. Morphologically, they are cylindrical, powerful, heavy-bodied animals, with short limbs and claws and projecting incisors. The head and body length ranges from 130 to 310 mm, with a minute stub tail, not visible externally. Average weight ranges from less than 100 to 570 g,

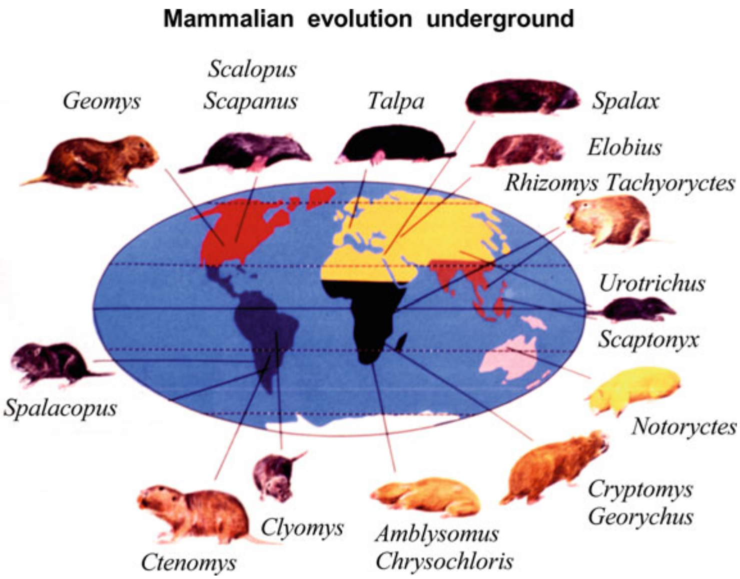


Fig. 15.1 Distribution of subterranean mammals across the planet. Palearctic region: *Talpa* (Talpidae, insectivores), *Spalax* (Spalacidae, rodents, SE Europe, Turkey, Near East, North Africa) and *Elobius* (Arvicolidae, rodents, Asia); Ethiopian: *Chrysochlorus, Amblysomus* (Chrysochlorides, insectivores, South Africa), *Tachyoryctes* (Rhizomyidae, rodents, East Africa); Oriental: *Scaptonyx* and *Urotrichus* (Talpidae, insectivores, East Asia), and *Rhizomys* (Rhizomyidae, rodents); Australian: *Notoryctes* (Notoryctidae, marsupial moles, Australia); Nearctic: *Scalopus* and *Scapanus* (Talpidae, insectivores) and *Geomys* (Geomyidae, rodents); Neotropical: *Spalacopus* (Octodontidae, rodents), *Ctenomys* (Ctenomyidae, rodents), and *Clyomys* (Echimyidae, rodents). Different colors mark the different zoogeographical regions (from Burda, with permission)

varying geographically with climate, soil type, and habitat productivity. Pelage varies from dark-brown to yellowish-gray, partly correlated with soil color (Heth et al. 1988a), suggesting differential predation above ground (Heth 1991; Šklíba et al. 2016). The broad and flat head is used in seismic communication. The incisors are long and procumbent, used in chisel-tooth digging, and their enamel thickness varies with soil type (Flynn et al. 1987). The cheek teeth are rooted, with a Z- or S-shaped enamel pattern that is species and soil specific (Butler et al. 1993).

15.3 The Mole Rat Eye: Structure, Function and Evolution

Spalacids are distinguished from all other rodents, including other subterranean rodents, by the absence of an external eye opening (Fig. 15.2). The subcutaneous vestigial eyes lost their vision, but retained and even expanded photoperiodic perception by a minute but functional retina, thus displaying mosaic eye evolution

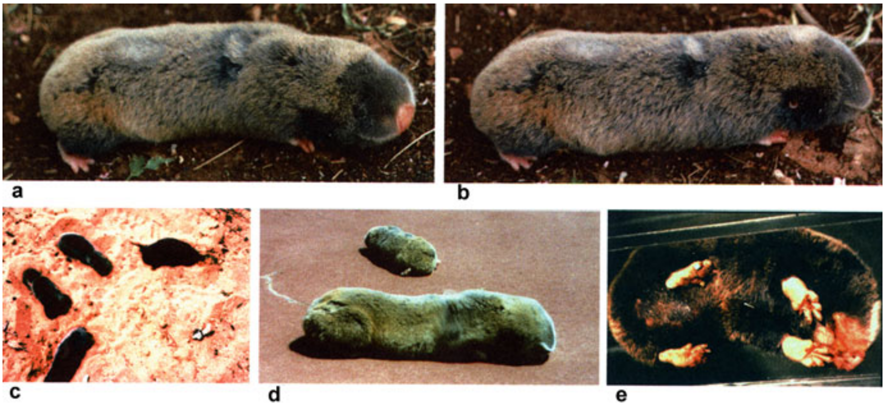


Fig. 15.2 *Spalax* mole rats. (**a**, **b**) *Spalax eherenbergi* superspecies in Israel. Note the distinct nose pad, no external eye and pinna, with only an ear opening; (**c**) *Spalax eherenbergi* superspecies, $2n = 60$, new species from El-Hamam, North Africa (Nevo et al. 1991b, 1992; Nevo 2007); unlike most *Spalax*, animals, at El Hamam, north Egypt, they are *social* and *pacifistic*; (**d**) Comparison of the largest, *S. microphthalmus* from Ukraine, and smallest, *S. judaei*, $2n = 60$, from Israel. The Ukraine *Spalax* is fivefold larger, exhibiting the Bergmann ecological rule (Photo K. Rybalko); (**e**) *Spalax* turned to show the front and rear legs (Photo by E. Nevo)

(Hendriks et al. 1990; Sanyal et al. 1990; Cooper et al. 1993; Nevo 1998a). We first established that the mole rat is blind (Haim et al. 1983). Yet removal of the eyes disturbs photoperiodic perception in mole rats (Pevet et al. 1984), and the circadian rhythm (Nevo et al. 1982a) is entrainable by light (Rado and Terkel 1989). The first anatomical study of the eye was conducted by Cei (1946). To elucidate the possible remaining *function* of the vestigial eyes, we studied their development and ultrastructure (Sanyal et al. 1990; Fig. 15.3). After the initial normal formation of the lens vesicle and optic cup, many degenerative and abnormal developments occur. A dominant feature is the hypertrophy of the iris-ciliary body in a heavily pigmented mass, capping the front of the eye. By contrast, retinal histogenesis progresses relatively normally, resulting in differentiated photoreceptor, neuronal, and ganglion cell layers in the adult eye (Fig. 15.3). Immunologically, the presence of opsin could be demonstrated in the photoreceptor cells (de Grip et al. 1992).

Evaluation of the visual pathway through the visual evoked potentials showed that the mole rat does not respond to flash stimuli and can thus be considered to be effectively blind, while clear potentials could be recorded in the rat (Fig. 15.4). Nevertheless, mole rats cannot see patterns or movement, but can differentiate between darkness and light. We demonstrated photoperiodic perception in mole rats (Haim et al. 1983) by studying thermoregulatory responses to changes in photoperiod, acclimating cold-sensitive individuals to short photoperiod (8L:16 D) at an ambient temperature (Ta) of 22 °C. They increased their thermoregulatory capacity in cold conditions when compared to individuals that were acclimated to a photoperiod of 12L:12D at the same Ta. Acclimation of cold-resistant individuals to

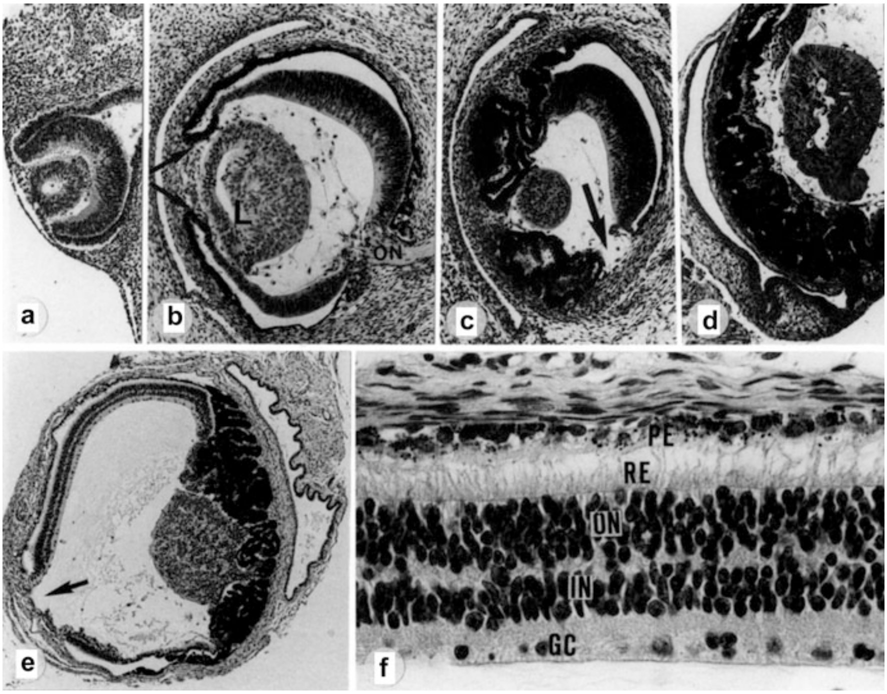


Fig. 15.3 Light micrographs showing cross-sections of the developing eye of the mole rat *Spalax ehrenbergi* superspecies. (**a**) Optic cup and lens vesicle initially develop normally (×100). (**b**) Eye at a later embryonic stage. Note appearance of iris-ciliary body rudiment, and development of the lens nucleus (L). ON, optic nerve (×100). (**c**) Eye at still later fetal stage. Note massive growth of the iris-ciliary body complex and the colobomatous opening (arrow) (×100). (**d**) Early postnatal stage. The iris-ciliary body complex completely fills the chamber. The lens is vacuolarized and vacuolated (×100). (**e**) Adult eye. Eyelids are completely closed, and a pupil is absent. Note atrophic appearance of the optic discretion (arrow) (×65). (**f**) Higher magnification of the adult retina. The different retinal layers are retained: PE, pigment epithelium; RE, receptor layer; ON, outer nuclear layer; IN, inner nuclear layer; GC, ganglion cell layer (×500) (Sanyal et al. 1990)

a Ta = 17 °C, but with a photoperiod of (16L:8D), caused a decrease in thermoregulatory capacity.

Spalax pinnae are rudimentary (Fig. 15.2), but the middle ear ossicles (Burda et al. 1989; Burda et al. 1990b), and particularly the cochlea (Bruns et al. 1988), are uniquely structured among mammals. These are adapted to underground low-frequency and a *rich* repertoire of *short-distance* vocal communication (Heth et al. 1986; Nevo et al. 1987a; Heth et al. 1988b) and *long-distance* seismic communication by head thumping onto the ceiling (Heth et al. 1987; Rado et al. 1987). Head thumping (a type of drumming behavior) is a major underground communication modality between these highly solitary and territorial animals (Nevo et al. 1991a), and it varies between species (Heth et al. 1991). Adaptive morphological differentiation exists in the body and head parameters (Nevo et al. 1988a) and in the skin of the head (Klauer et al. 1997). Olfaction is an important

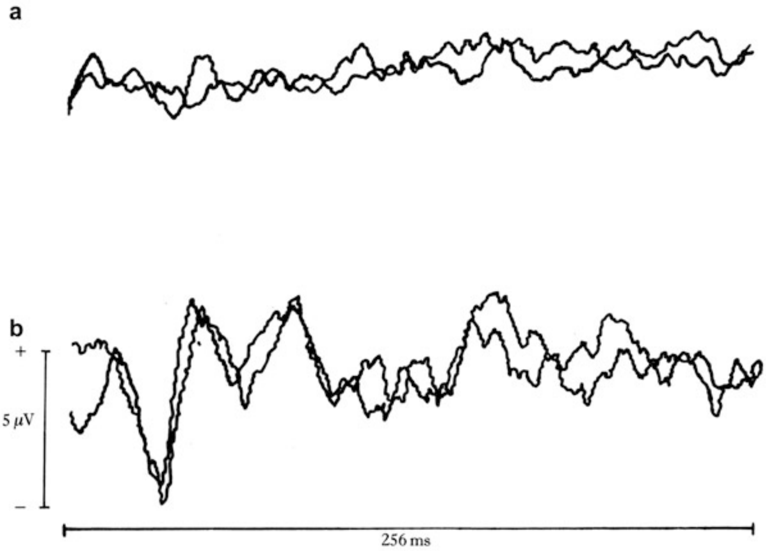


Fig. 15.4 Electrophysiological demonstration. Visually evoked potentials from a mole rat *Spalax eherenbergi* superspecies (**a**), and a laboratory rat, *Rattus norvegicus* (**b**), recorded by using the same stimulus and recording configuration. Stimulus was a short flash. Two replications of each recording are superimposed to assess reproducibility. Note the clearly reproducible recording from the laboratory rat, in contrast to the lack of any reproducible component in the mole rat recording (Haim et al. 1983)

communication modality (Nevo and Heth 1976), in reproduction through sexual pheromones (Nevo et al. 1987a, b; Menzies et al. 1992; Heth et al. 1996) and in food identification (Heth et al. 1992, 1996). Tactile cues are also important but as yet unquantified (Burda et al. 1990a, b). Mole rats are confined most of their lives to sealed underground tunnels (Nevo 1961), but evidence for some above ground activity has been noted (Heth 1991; Šklíba et al. 2016). They are chisel-tooth diggers using fore- and hind-feet to push earth out behind, packing and bulldozing excavated earth with their broad and flat heads to form the external small nutritional mounds, and in badly drained soils also the huge breeding mounds (Nevo 1961) (Figs. 15.5 and 15.6). The head is also used for thumping onto the ceiling in seismic communication. Specialized jaws and strong muscles aid the teeth in loosening the soil, using specialized incisors whose enamel microstructure is both adaptive to soil structure and holds phylogenetic significance (Flynn et al. 1987).

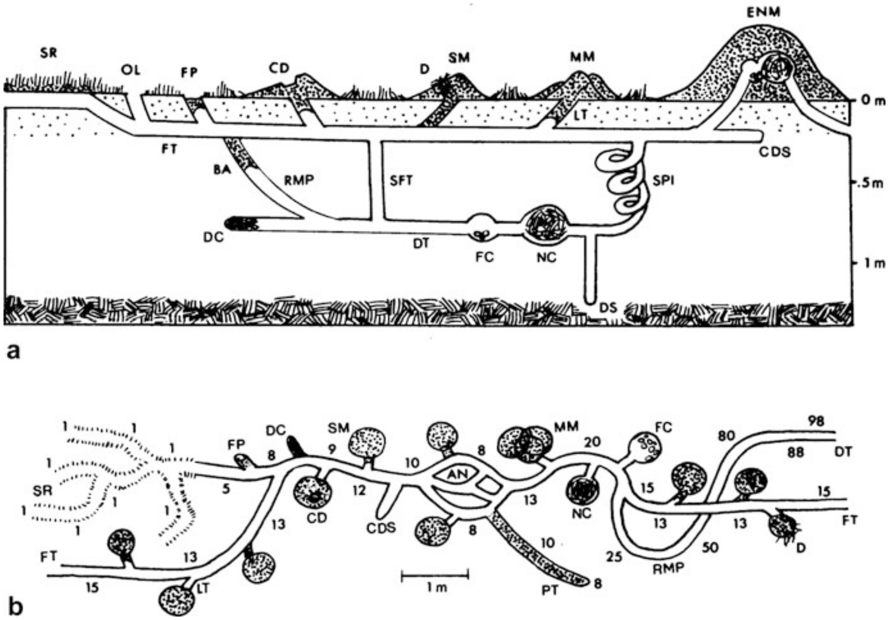


Fig. 15.5 Burrow system of subterranean mammals (A synthetic diagram: All features are not present in any one system and position of structures may vary within groups). (**a**) Sagittal view with a section of burrow excavated through soil horizons; (**b**) Aerial view with depths of burrow features indicated in cm. AN = Anastomosing; BA = Barricade; CD = Central Depression; CDS = Cul-de-sac; D = Detritus; DC = Defecation chamber; DS = Down Shaft; DT = Deep Tunnel; ENM = Elevated Nest Mound, "Breeding Mound;" FC = Food Chamber; FP = Flush Plug; FT = Foraging Tunnel; LT = Lateral Tunnel; MM = Multiple Mound; NC = Nest Chamber; OL = Open Lateral; PT = Plugged Tunnel; RMP = Ramp; SFT = shaft; SM = Surface Mound; SPI = Spiral; SR = Surface Ridge (from Hickman 1990)

15.4 Adaptation and Evolution of Tunnel Architecture

Structure, adaptation, and evolution of tunnel system architecture in subterranean mammals have been reviewed in Nevo (1999). The subterranean ecotope is structurally simple. It is essentially a sealed system, microclimatically relatively stable, i.e., permanent and predictable, with structures related to feeding, defecation, and breeding (Figs. 15.5 and 15.6). In badly drained soils, especially in valleys and plains with black clay soils, the uniquely impressive breeding mounds with an exquisite big dome-like structure are built by females, once a year, only after the first rains. The mounds are where females store food for the winter, and raise the 3–7 pups, primarily with a major peak of parturition in the first 2 weeks of February, and a minor peak in early March (Nevo 1961). Smaller *conical-shaped* male mounds are dispersed among the *dome-shaped* breeding mounds of females, each animal having its own territory. In January, males explore the breeding mounds of females underground, forming linear straight lines of small typical male mounds. Mating occurs in

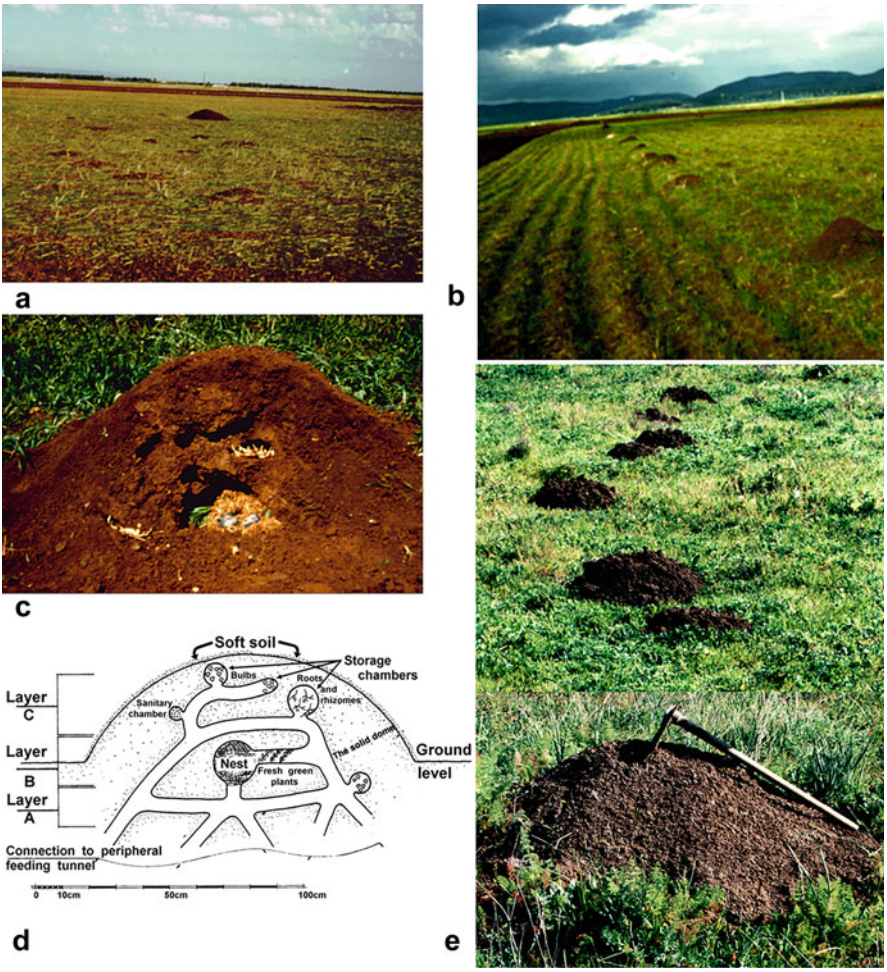


Fig. 15.6 (**a**) Breeding mound of the blind mole rat, *Spalax carmeli* ($2n = 58$) of the *Spalax ehrenbergi* superspecies, near Akko (Acre), Israel, surrounded by regular, small, nutritional mounds. (**b**) Linear alignment of *breeding mounds* rising on a slight ridge that serves to avoid high water levels and bad drainage. (**c**) Cross-section in a breeding mound. Note the nest in the dome-like structure built by the female, with 3–7 pups. Note also several storage food chambers, each with specific bulbs, onions, corms and roots, pruned by the female to keep them ungerminated, thereby preserved for winter food. (**d**) Diagrammatic sections of a breeding mound, showing three layers, with the nest, storage food chambers with bulbs, corms and roots, and sanitary (defecation) chambers. (**e**) Small nutritional mounds radiating from a big breeding mound, with a hoe on top for scale (from Nevo 1961; photos by E. Nevo)

the breeding mound of the females, possibly as described in Nevo (1969), after which the male leaves to find other females. Females store the food of each geophyte species or root in a separate cache, collect green leaves for enriching the diet before parturition, and after about 34 days of pregnancy, give birth. Females raise the 3–7 naked pups, alone in the nest. The young are born in the nest totally naked, are raised only by the female, and develop pelage covering the tiny little black eyes. Dispersal of young after about a month in the nest occurs in March–early April. Activity patterns underground were initially described using radiotracking by Kushnirov et al. (1998), and later extended by Šklíba et al. (2016).

15.5 *Spalax ehrenbergi* Superspecies in Israel: Origin, Structure, Adaptive Evolution and Speciation in Israel: (1) Climatic, Chromosomal, and *Peripatric*, and (2) Edaphic, Genic, and *Sympatric*

Subterranean mole rats of the *Spalax ehrenbergi* superspecies in Israel represent an active case of allopatric (or peripatric) ecological speciation, *primarily* climatically, chromosomally, and *peripatrically* (Fig. 15.7) (Nevo 1991; Nevo et al. 2001). S*econdarily* (not shown in Fig. 15.7), allopatric speciation occurs edaphically and genically, while *sympatric speciation* has also been reported (Hadid et al. 2013; Li et al. 2015, 2016; Lövy et al. 2015; Šklíba et al. 2016; Lövy et al. 2017, 2019).

The unraveling of the underground evolution of blind mole rats in Israel initiated a long-term research program, started in 1948 and continuing for 70+ years by Nevo and colleagues (Nevo 2018), which started when their breeding mounds were first discovered (Nevo 1961; Fig. 15.6). This long-term research program (Nevo 2018) has yielded some 400 articles and two books (for the publication list see http://evolution@haifa.ac.il), describing their adaptive evolution to increasing aridity southward, where the four chromosomal species radiated. *Spalax* became an ideal multidisciplinary evolutionary model for both *adaptive evolution* and *peripatric speciation* (Nevo 1989), and recently the first *sympatric speciation* has been discovered in *Spalax galili*, $2n = 52$ (Hadid et al. 2013; Li et al. 2015, 2016; Zhao et al. 2016; Lövy et al. 2015, 2017, 2019). The classic species *Spalax ehrenbergi* turned out to be a dynamically climatically and edaphically evolving superspecies, which now includes, at least in Israel and Jordan, nine species: five in Israel and four in Jordan. By adding other Near Eastern countries, including Egypt, Lebanon, Syria, Iraq, and southern Turkey, the *Spalax* (*Nannospalax*) *ehrenbergi* superspecies might rise significantly in species number. The four chromosomal species earlier described in Israel ($2n = 52, 54, 58, 60$; Wahrman et al. 1969a, b, 1984; Nevo 1991) have been described later as good biological species (Nevo et al. 2001) despite the hybrid zones separating them (Nevo and Bar-El 1976). They are considered good species due to their adaptive climatic evolution and have been named formally (Nevo et al. 2001): *Spalax galili* ($2n = 52$), which radiated in the cool-humid upper Galilee Mountains; *Spalax golani* ($2n = 54$) in the cool semi-humid Golan Heights; *Spalax carmeli* ($2n = 58$) in the warm-humid Lower Galilee Mountains, central Yizreel valley and Coastal Plains; and finally, the xeric *Spalax judaei* ($2n = 60$) in the mountains of Samaria, Judea, northern Negev, and the southern part of the Jordan Valley, and

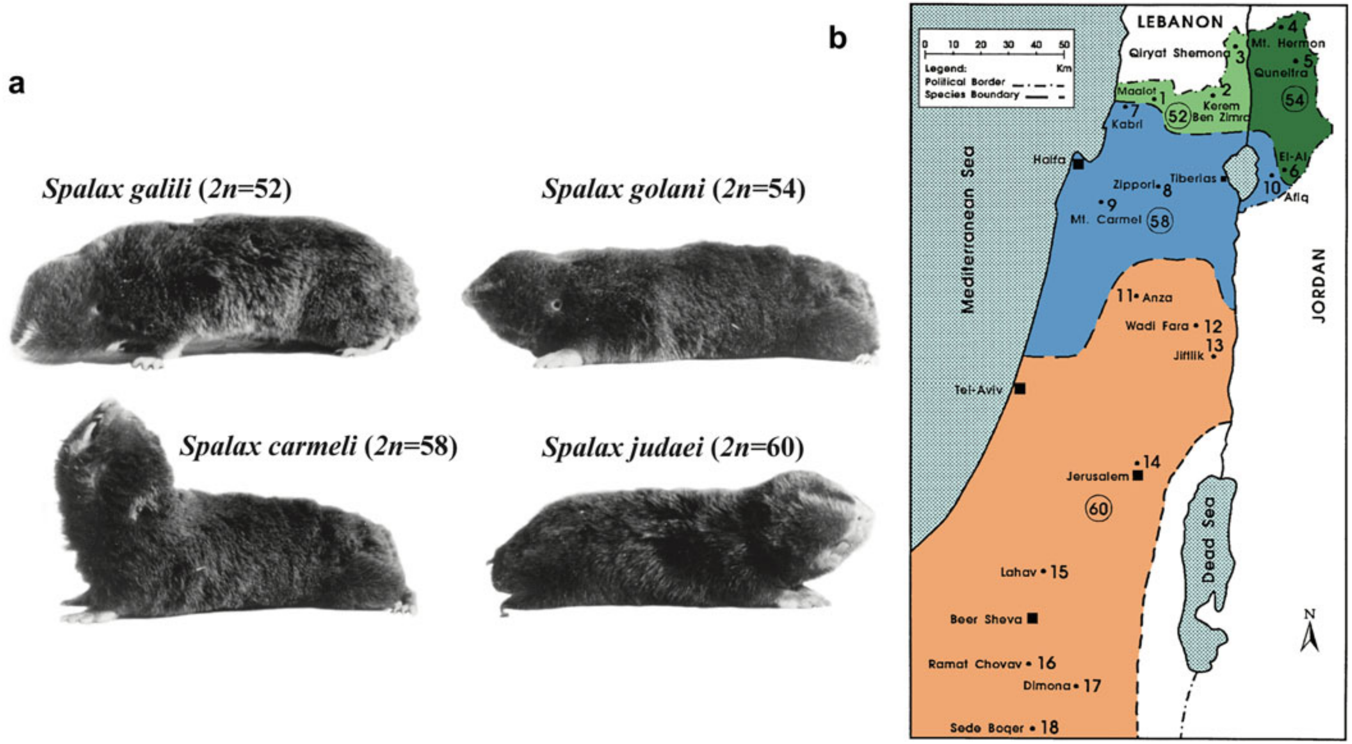


Fig. 15.7 (**a**) The four chromosomal species (morphologically undistinguishable sibling species) of the *Spalax ehrenbergi* superspecies (Nevo et al. 2001): *Spalax galili*, $2n = 52$; *S. golani*, $2n = 54$; *S. carmeli*, $2n = 58$; *S. judaei*, $2n = 60$). (**b**) The ecogeographical, climatic distribution of the four chromosomal species in Israel from the north, which is cool and humid ($2n = 52$), to the northeast, cool and semi-humid ($2n = 54$), through the central region, warm and humid ($2n = 58$), to the warm and dry northern Negev desert ($2n = 60$). The four species form a climatic cline, with a southward transect ($2n = 52, 54, 58, 60$), following first a short eastward trend ($2n = 52 \rightarrow 54$), then the main southward, increasingly xeric, climatic transect ($2n = 52, 54 \rightarrow 58 \rightarrow 60$) of the peripatric speciation cline, where each species is associated with a specific climatic regime. Later, in the first decade of 2000, we identified a fifth species on the Pleistocene basalt, which has speciated sympatrically, *genically* and *not chromosomally* ($2n = 52$), and tentatively called it *Spalax galili* basalt. See references in Sect. 15.5 (Image is a personal figure of E. Nevo)

Coastal Plain (Fig. 15.7). Their adaptive radiation in Israel from the Early to late Pleistocene is closely associated with increasing aridity, progressive deforestation and emergence of savanna, hence with distinct climatic diversity in the Mediterranean, steppic, and northern Negev desert climatic regimes. Recently, we identified the first sympatric speciation in *Spalax galili* ($2n = 52$) in the Pleistocene volcanic basalt in the Eastern Upper Galilee mountains, which occurred *genically* and not *chromosomally*. We temporarily called the new species *Spalax galilee* basalt (Hadid et al. 2013; Li et al. 2015, 2016; Lövy et al. 2015, 2017, 2019) since it evolved from a chalk-dwelling progenitor that speciated sympatrically when it invaded the volcanic Pleistocene basalt. Earlier we discovered relatively little allozymic genetic differentiation between the chromosomal species (Nevo and Cleve 1978), but later identified larger genomic differentiation when we sequenced the genomes of all species (Li and Nevo, unpublished). Remarkably, *Spalax* is extremely hypoxia-resistant (Arieli and Nevo 1991) and cancer-resistant (Gorbunova et al. 2012) and might transform cancer medical treatment.

Reproductive isolation among the four chromosomal species of *S. ehrenbergi* (Heth and Nevo 1981) comprises both post-mating, chromosomal incompatibilities (Wahrman et al. 1984) and species-specific olfactory "recognition," premating isolating mechanisms (Nevo et al. 1976; Nevo and Heth 1976; Heth and Nevo 1981). In Sect. 15.6 I primarily discuss two of the communication systems supporting ethological premating isolating mechanisms, and focus on their structure, function, and evolution (Nevo 1990, 1991, 1998a, b).

15.6 Communication Systems Supporting Ethological Premating Isolating Mechanisms

Positive mate preference and assortative mating provide the basis for sexual isolation in the *S. ehrenbergi* superspecies. Mating behavior of *S. ehrenbergi* consists of three phases: agonistic, courtship, and copulation (Nevo 1969). Aggression was significantly higher in heterospecific than in homospecific matings. Estrous females significantly prefer their chromosomal mates (Nevo and Heth 1976). However, females of older species (i.e., *S. galili*, $2n = 52$, *S. golani*, $2n = 54$, and *S. carmeli*, $2n = 58$) select their mates much better than females of the recent derivative of chromosomal speciation in Israel, *S. judaei*, $2n = 60$ (Heth and Nevo 1981; Beiles et al. 1984).

Five quantifiable nonvisual ethological mechanisms of mate choice (olfaction, vocalization, seismic communication, aggression, and bacular variability) contribute in blind *Spalax* (Haim et al. 1983), singly and in combination, to maximize species-specific recognition signals within species, thereby perfecting reproductive isolation. Tactile behavior between partners may also contribute to effective mate selection, but it is hard to record either qualitatively or quantitatively. Olfaction communication is very effective in *Spalax* (Nevo et al. 1976; Heth et al. 1992, 1996). Estrous females of either *S. galili*, $2n = 52$, or *S. carmeli*, $2n = 58$, significantly prefer homochromosomal odors of urines, presumably discriminating among species-specific pheromones (Nevo et al. 1987b; Menzies et al. 1992). Vocal communication also plays an important role in the subterranean life of mole rats (Capranica et al. 1974). The four chromosomal species ($2n = 52, 54, 58, 60$) vary in their mating dialects, which appear to be *species-specific* in their physical structures, thereby providing an effective acoustic premating isolating mechanism as was revealed in laboratory female discrimination tests (Nevo et al. 1987a). Seismic communication was described in *S. ehrenbergi* superspecies (Heth et al. 1987; Rado et al. 1987) and its somatosensory mechanism was elucidated (Nevo et al. 1991a). Since *Spalax judaei*, $2n = 60$, has a species-specific seismic communication signal (Heth et al. 1991), it may enrich premating reproductive isolation mechanisms. Baculum and phalic morphologies vary significantly between *Spalax* species and might produce differential responsiveness in females, thereby contributing to prezygotic reproductive isolation (Simson et al. 1993), as hypothesized by Patterson and Thaeler, Jr. (1982). High interspecific aggression between geographically contiguous species also plays an important role in premating reproductive isolation by maximizing species identification (Guttman et al. 1975; Nevo et al. 1975, 1986a). Remarkably, aggression has also been identified recently in incipient sympatric speciation of fruit-flies *Drosophila melanogaster* in Evolution Canyon, Mount Carmel, Israel, the "Israeli Galapagos" (Palavicino-Maggio et al. 2019). An overview of structural and functional aspects of vocalization and seismic (vibratory) communication modalities follows (see Sects. 15.7–15.15), for both speciation and adaptation of the four chromosomal species of subterranean blind mole rats in Israel (*Spalax galili*, $2n = 52$, *S. golani*, $2n = 54$, *S. carmeli*, $2n = 58$, and *S. judaei*, $2n = 60$). These species display two major types of communications: *acoustic* and *seismic*. Their evolutionary roles are subdivided into (i) *Vocal*, primarily short range, and (ii) *Seismic,* or vibrational communication modalities, primarily long-range (Nevo 1990; Nevo et al. 1991a).

15.7 Vocal Communication: Physical Structure of the Courtship Call

Members of the *Spalax ehrenbergi* superspecies in Israel are territorial and solitary (in contrast to *Spalax ehrenbergi* new species (Nevo et al. 1991b) in El-Hamam north Egypt, which are both social and pacifistic (Nevo et al. 1992; Nevo 2007) and are highly vocal, particularly during physical, agonistic, and mating contacts. Their vocal repertoire consists of at least six call types: attack, crying, invitation, courtship, release (distress) and threat calls. Each occurs in a different behavioral context (Capranica et al. 1974). The major call studied is the distinct "purring," designated as a "courtship" call, that blind mole rats of the *S. ehrenbergi* superspecies in Israel emit during mating (Nevo 1969) (Fig. 15.8). This call has a communicative function. Its production during the preliminary (and more aggressive) stages of mating causes

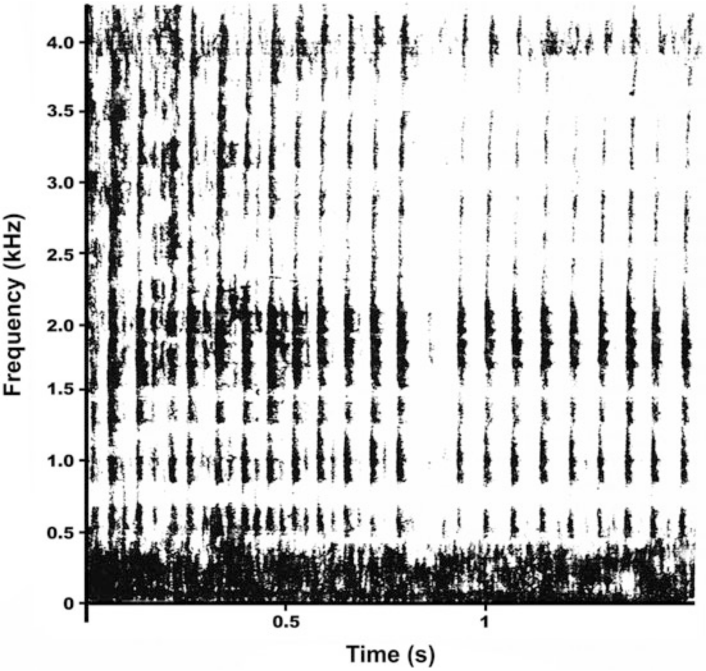


Fig. 15.8 Sound spectrogram of the "courtship" call of the mole rat *Spalax eherenbergi* superspecies (Heth et al. 1988b)

receptive females to reduce their agonistic behavior, which presumably tests the physical potential of the male, so that copulation with the chosen male can be achieved. Moreover, receptive females can discriminate between recorded conspecific and heterospecific courtship calls (Nevo et al. 1987a). Courtship calls were analyzed in mole rats, *Spalax ehrenbergi* superspecies (Heth et al. 1988b; Fig. 15.8). The mean of the main frequency was 568.0 ± 36.9 Hz, and the pulse repetition rate was 23.7 ± 2.8/s. Calls are noisy, of broad frequency bands and regular repetition bands, with energy spread primarily from 0.5 to 4.5 kHz. The low-frequency of this call is adaptive to life in the underground tunnels (see Sect. 15.8).

15.8 Adaptive Optimal Sound for Vocal Communication in Tunnels of *Spalax ehrenbergi* Superspecies

The adaptive value of sound signal characteristics for transmission in the underground tunnel ecotope of the *Spalax ehrenbergi* superspecies was tested in the field, using mole rat tunnels (Heth et al. 1986). We analyzed the propagation of synthetic calls with various frequencies through natural tunnels along different distances. The sound propagation proved efficient only across short distances of a few meters. The least sound attenuation occurred at low frequencies. The 440 Hz sound was transmitted better than the lower (220 Hz) or higher (880, 1760, 3520 Hz) tested frequencies. These characteristics matched perfectly with the mole rat features of vocalization and hearing, thus reflecting the operation of natural selection for adaptive vocal communication in the underground tunnel ecotope. The best frequency of transmission in solid materials is between hundreds and a few hundreds of Hz.

15.9 Ecogeographic Dialects in Blind Mole Rats: Role of Vocal Communication in Active *Spalax* Speciation

Vocal dialects were expected in the speciation of the four chromosomal species of the *Spalax ehrenbergi* superspecies. We compared and contrasted physical courtship call structure of 59 subterranean mole rats belonging to the *S. ehrenbergi* superspecies in Israel, comprising 11 populations of the four species: *S. galili*, $2n = 52$, *S. golani*, $2n = 54$, *S. carmeli*, $2n = 58$ and *S. judaei*, $2n = 60$. We also conducted behavioral auditory discrimination tests of 144 females of the four species in the laboratory (Nevo et al. 1987a). The results indicated that each chromosomal species has a vocal dialect *significantly different from all others*; although, the call of *S. judaei*, $2n = 60$, the last derivative of speciation in Israel, is not yet fully differentiated (Fig. 15.9). Females of *S. galili*, $2n = 52$, *S. golani*, $2n = 54$ and *S. carmeli*, $2n = 58$ preferred their homospecific mates' calls, whereas females of *S. judaei*, $2n = 60$, did not. We concluded that call differentiation builds up *gradually*, in allopatry, or peripatry (Nevo 1990) and provides an efficient ethological reproductive premating isolation mechanism between the emerging species in the active speciation of mole rats in Israel (Heth and Nevo 1981; Nevo 1990). We next analyzed ear structure and hearing sensitivity of Israeli mole rats of the four chromosomal species.

15.10 Inner and Middle Ear Structures and Audiograms of Israeli *Spalax*

The inner ear of *Spalax* is structurally unique and adaptive to the low frequencies characterizing its vocalization underground. The inner ear of *Spalax* was described long ago (Szakall 1903). The ossicular chain of the middle ear of *Spalax hungaricus* Nhr. was briefly described by Fleischer (1973), but no special structures were indicated. Biophysical arguments suggest that the acoustic communication and vocal orientation underground is governed by low frequencies. We have shown that, indeed, structure and function coevolved in unison.

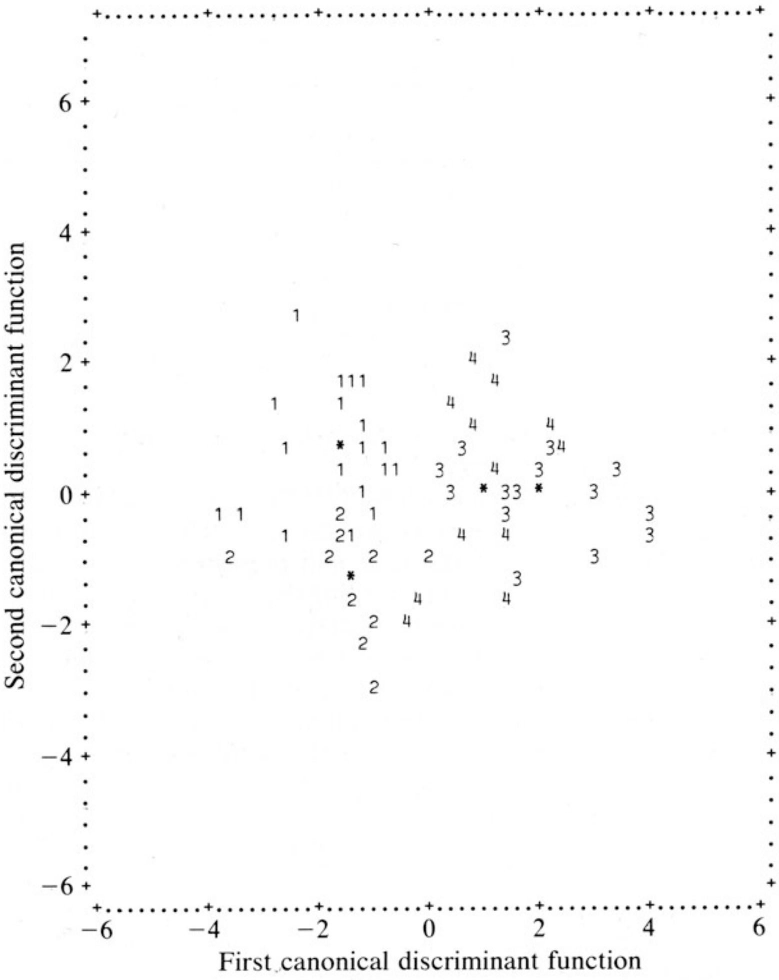


Fig. 15.9 Stepwise discriminant analysis of the courtship calls of the four chromosomal species of *Spalax ehrenbergi* superspecies, based on three call parameters: (i) number of harmonics; (ii) pulse rate and (iii) main frequency, chosen by the computer program. The program maximizes the overall F statistics among the four species and reveals the discriminatory power of the variables used. Group centroid •: 1, $2n = 52$; 2, $2n = 54$; 3, $2n = 58$; 4, $2n = 60$ (Nevo et al. 1987a)

15.10.1 Cochlea Structure

We described the unique organization of the *Spalax ehrenbergi* superspecies cochlea among mammals (Bruns et al. 1988). The cochlea is subdivided into different subsystems, where in the apical subsystem the fluid space and the organ of Corti differ remarkably from that in the basal subsystem, a feature as yet unknown in other

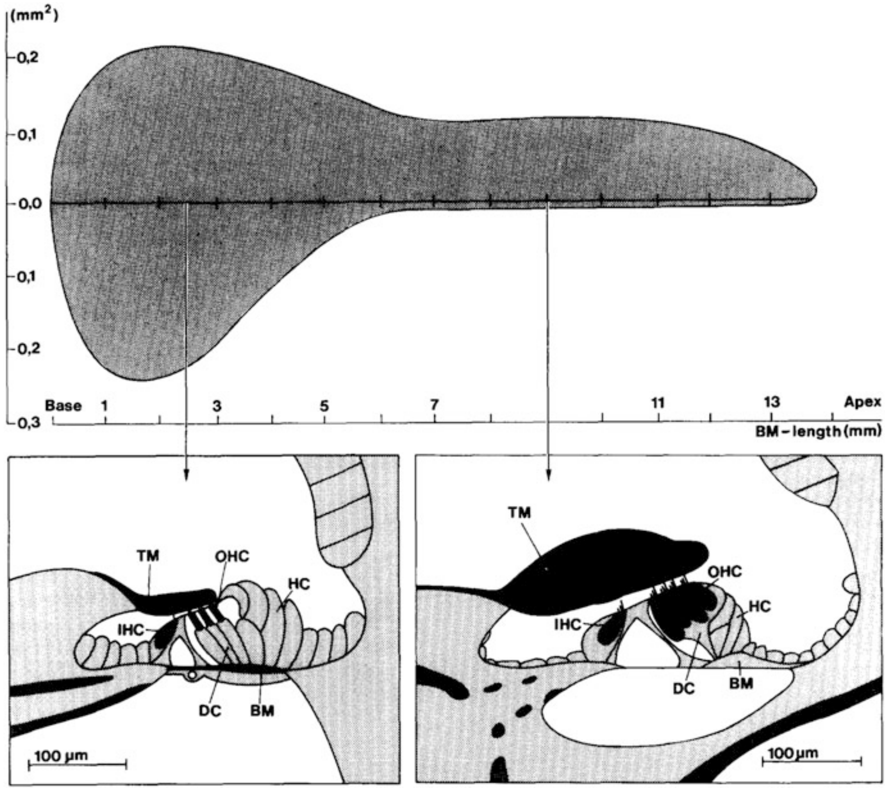


Fig. 15.10 Cochlea structure of *Spalax ehrenbergi* superspecies. Above: Cross-sectional area of the cochlear fluid spaces in the mole rat, and below, the basilar membrane, which is uncoiled and shown as a horizontal line. Below: Radial sections of the organ of Corti on the basal (left) and apical (right) subsystems. Positions along BM are indicated by arrows. Abbreviations: BM = basilar membrane, DC = Deiter's cell, HC = Hensen's cell, IHC = inner hair cell, OHC = outer hair cell, TM = tectorial membrane (from Bruns et al. 1988)

mammals (Fig. 15.10). The basilar membrane was relatively and absolutely longer (12.6 mm) than in larger generalized rats of the genus *Rattus* (9.4–12.1 mm). The *Spalax* cochlea had 1.25 more turns, was 3.3 mm longer, and had 2100 more cochlear neurons than *Rattus.* Among rodents, a comparatively long basilar membrane occurs in fossorial gerbils and African subterranean mole rats, *Cryptomys hottentotus* (Müller and Burda 1989).

The mean ratio between the number of outer and inner hair cells (OHC, IHC) in the organ of Corti found in *Spalax* (4.15:1) was higher than the mammalian average (3.65:1, sd 0.25:1; Burda et al. 1989). A lower ratio than the average may be found in generalized murids. In all mammalian species studied so far, the ratio was subject to species-specific intra-cochlear changes. Usually, higher values of the ratio were found in the apical cochlear regions. The minimal values were found at different species-specific locations along the organ of Corti. The ratio remained almost constant in *Spalax* for about 65% of the length of the basilar membrane, and dropped along the apical 35% of the length, reaching its minimal value at the very apex. The population of cochlear receptors in *S. ehrenbergi* superspecies was qualitatively and quantitatively analyzed (Burda et al. 1989).

15.11 Adaptive Subterranean Differentiation of Middle Ear Structures and Cochlear Receptors in *S. ehrenbergi* Superspecies in Israel

The eardrum and auditory ossicles of the middle ear of *S. ehrenbergi* were studied qualitatively and quantitatively by Burda et al. (1989). Convergence in some features was identified between the middle ear of *Spalax* and many other specialized herbivore and insectivore subterranean mammals (Talpidae, Chrysochloridae, Geomyidae, Bathyergidae), yet not found in generalized mammals (e.g., Soricidae, Muridae). These adaptive morphological convergences to life underground include, for example, a flat incudomalleolar joint, a large incus (compared to the malleus), the crus breve includes almost paralleling the manubrium of the malleus, a free-swinging malleus with a reduced goniale and without orbicular apophysis.

The following conclusions could be drawn from the findings of adaptive ear structures and the low-frequency vocal communication underground, based on comparative studies, and analogies with other receptor systems (Burda et al. 1989, 1990a, b, and references therein): (i) A longer basilar membrane, and thus also a higher receptor number, may be necessary to ensure at least a certain degree of resolution capabilities in a low-frequency range. (ii) A higher density of receptors in the apical cochlear regions may be correlated with the best sensitivity of hearing restricted to low frequencies. (iii) The mean values of the width of the triad of OHC and those of the ratio between OHC and IHC indicate *general tuning of hearing to low frequencies*. It was suggested that auditory analysis of *temporal parameters* may be more relevant for *Spalax* than exact *frequency* information (Bruns et al. 1988). It is possible to speculate that some structural peculiarities, e.g., relatively high density of OHC and low density of IHC, are particularly correlated with better temporal, and poorer frequency, resolution of hearing (Burda et al. 1989).

15.12 Species-Specific Ear Characteristics

Various structures of the middle and inner ear were morphologically analyzed in the four chromosome species of Israeli *S. ehrenbergi* superspecies (Burda et al. 1990a, b). Particularly significant were non-allometric differences in the area of the eardrum and the basilar membrane between some pairwise combinations of species. These differences were not directly correlated with differences in body or

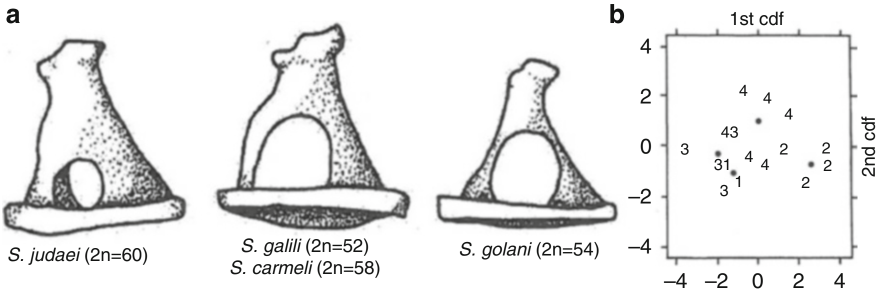


Fig. 15.11 (**a**) Comparison of the basic types of the stapes characteristics (but not exclusively specific) for the respective chromosome species of the *Spalax ehrenbergi* superspecies: *massive* stapes in *Spalax judaei*, $2n = 60$, *intermediate* one in *Spalax galili*, $2n = 52$ and *Spalax carmeli*, $2n = 58$, and slender *small* stapes in *Spalax golani*, $2n = 54$. (**b**) Discriminant analysis of the four chromosomal species using means of three selected ear variables (see also table in Burda et al. 1990a, b). Legend: 1, $2n = 54$, 2, $2n = 52$, 3, $2n = 58$, 4, $2n = 60$. • = Species centroid. First cdf = first canonical discriminant function, second cdf = second canonical discriminant function

skull size. For example, though *S. judaei*, $2n = 60$, is the smallest species in body size as compared to all other mole rat species in Israel, enabling it to better adaptively thermoregulate in the desert (Nevo et al. 1986b), it tends to have longer basilar membranes, higher density and higher total numbers of cochlear sensory receptors, more massive stapes, larger eardrum areas, and higher final transformation ratios than other species. All these traits may suggest, if the frequency tuning of hearing is comparable, a tendency to adaptively improve the auditory sensitivity in the *S. ehrenbergi* superspecies southwards towards the Negev desert, where the territories are relatively larger due to food scarcity. Similarly, ear structure differentiation may indicate that the hearing in the northern species, *S. galili*, $2n = 52$ and *S. golani*, $2n = 54$, is less sensitive than in the central species, *S. carmeli*, $2n = 58$, and the southernmost xeric species, *S. judaei*, $2n = 60$. The discriminant analysis involving ear structure ear drum area, stapedial footplate area, and basilar membrane length revealed that individuals of the four chromosomal species were clearly distinct, and were mostly correctly classified into their species cluster (Fig. 15.11b).

The differentiation of ear structures is quantitative and gradual rather than categorical. Our results of ear structure differentiation between species thus corroborate conclusions of previous morphometric (Nevo et al. 1988a) and other studies, claiming that speciation in *Spalax* is gradual rather than punctuated. It is assumed that the described differentiation of ear structures may result in functional differentiation of hearing qualities, and thus may be adaptively selected in nature in accordance with the different habitats occupied by each species. We hypothesized that the morphometric trend in increasing parameters of some components (e.g., ear drum area, basilar membrane length and number of receptors) is correlated with call differentiation (Nevo et al. 1987a). Ear structure differentiation may enhance hearing sensitivity, which may be related to increased requirements for, and higher involvement of, acoustic communication in arid regions, where the productivity is low, territories are large, and the population density of mole rats is low (Nevo et al. 1982a, b). Interestingly, the brain size is also significantly increasing in *S. judaei*, in contrast to the decreasing body size (Nevo et al. 1988b; Pirlot and Nevo 1989). The increase in brain size due to stress in the desert, possibly in association with scarce food resources and larger territory in the desert, may also affect a better magnetic orientation (Marhold et al. 2000) of *S. judaei*, $2n = 60$. The higher $2n$ in the desert appears to also be adaptive due to increase in genetic polymorphism selected under stress, uplifting the potential for adaptive evolution (Nevo 1998b). Thus, remarkably and importantly, evolution is networked adaptively, affecting similarly vocalization and seismic communication, hearing sensitivity and call transmission in the tunnel.

15.13 Hearing Sensitivity

The audiograms based on cochlear microphonics and on evoked potential recordings from the midbrain and brain stem of S. *ehrenbergi* superspecies reveal a hearing range from 0.1 kHz–10 kHz with the best sensitivity between 0.5 and 1 kHz (Fig. 15.12). The hearing range of *Rattus* reaches from 0.25 to 80 kHz, with a region of highest sensitivity between 9 and 32 kHz (Kelly and Masterton 1977). By contrast, *Spalax* possesses an *extremely low-frequency range* (Fig. 15.12). The upper-frequency limit of *Spalax* revealed by the electrophysiological audiograms is below that in man, and even below that in the largest terrestrial mammal, the Indian elephant (Masterton et al. 1969; Heffner and Heffner 1983). The same is true for the best frequency, which is about 4 kHz in man (Masterton et al. 1969) and about 1 kHz in the elephant. The auditory capabilities of *Spalax*, based on the

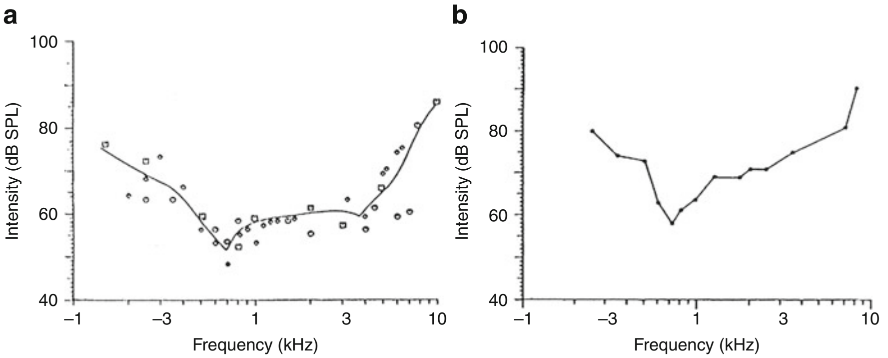


Fig. 15.12 (**a**) *Spalax ehrenbergi superspecies* audiograms displaying its hearing range and sensitivity. Averaged isopotential curve was revealed by evoked potential from the midbrains and brainstems of three animals. Absolute threshold of evoked potentials was about 10–15 dB below the isopotential curve. The symbols indicate isopotential points of different individuals at about twice the background noise level. (**b**) Cochlear microphonic isopotential curve. The curve shows almost the same course as the recordings from the brain (from Bruns et al. 1988)

isopotential curve, match better those of the average of reptiles (Manley 1971) than those of the average mammal. In comparison with *Rattus,* the frequency range of *Spalax* is two octaves smaller, but the basilar membrane is 3.3 mm longer. On the average, the basilar membrane length in *Spalax* is almost twice that of the basilar membrane length per octave. This difference between *Rattus* and *Spalax* is consistent with the low-frequency hearing and an expansion of the biologically relevant frequency bands of mole rats (Bruns et al. 1988, and references therein). In analogy to the high frequency "acoustic" fovea in bats, emitting pure-tone echolocating signals, the almost constant width of the basilar membrane in the apical subsystem of *Spalax* could be interpreted as a low-frequency "acoustic fovea."

The frequency range, and the best frequency region of hearing sensitivity, in *Spalax* is in line with findings of the courtship call structure (Heth et al. 1988b; Fig. 15.8) and with the best propagation frequency underground (Heth et al. 1986). Based on the ear structures and electrophysiological audiograms, we hypothesized that the broadband signal stimulates a broad region of the organ of Corti and thus could enhance general sensitivity. Likewise, temporal parameters might be more relevant than exact frequency information. Notably, we have established that substrate vibration directly stimulates the brain somatosensorily (Nevo et al. 1991a), reducing the importance of the ear in long-range communication, in contrast to the hypothesis suggested by (Rado et al. 1987).

15.14 Seismic Communication in *Spalax*, a Blind Subterranean Mammal: A Major Somatosensory Mechanism in Adaptive Evolution Underground

Seismic communication in *Spalax* is through low-frequency and structured substrate-borne vibrations that are generated by head thumping onto the tunnel ceiling. These substrate-borne vibrations travel long distances underground, within and between territories, and are important in the evolution of subterranean mole rats of the *Spalax ehrenbergi* superspecies (Nevo et al. 1991a). This is true both *intraspecifically* in adaptation (Heth et al. 1987, 1991; Rado et al. 1987) and *interspecifically* in speciation (Heth et al. 1987). The thumps' sequence is structured, consisting of pulse groups separated by inter-pulse group intervals. Each pulse group involves one or more thumps or pulses (Fig. 15.13).

We have shown experimentally, by separating substrate-borne vibrational and airborne vocal stimuli, and recording potentials evoked in the brain, that the mechanism of seismic communication is *somatosensory*, and is basically independent of the auditory mechanism (Nevo et al. 1991a) (Fig. 15.14). The auditory system functions in *short-range airborne* vocal communication, as described earlier. Vibration stimuli, or seismic communication, function at *long-range and are transmitted directly to the brain for neural processing*. Seismic, vibrational communication appears to be a major somatosensory channel of long-distance communication in

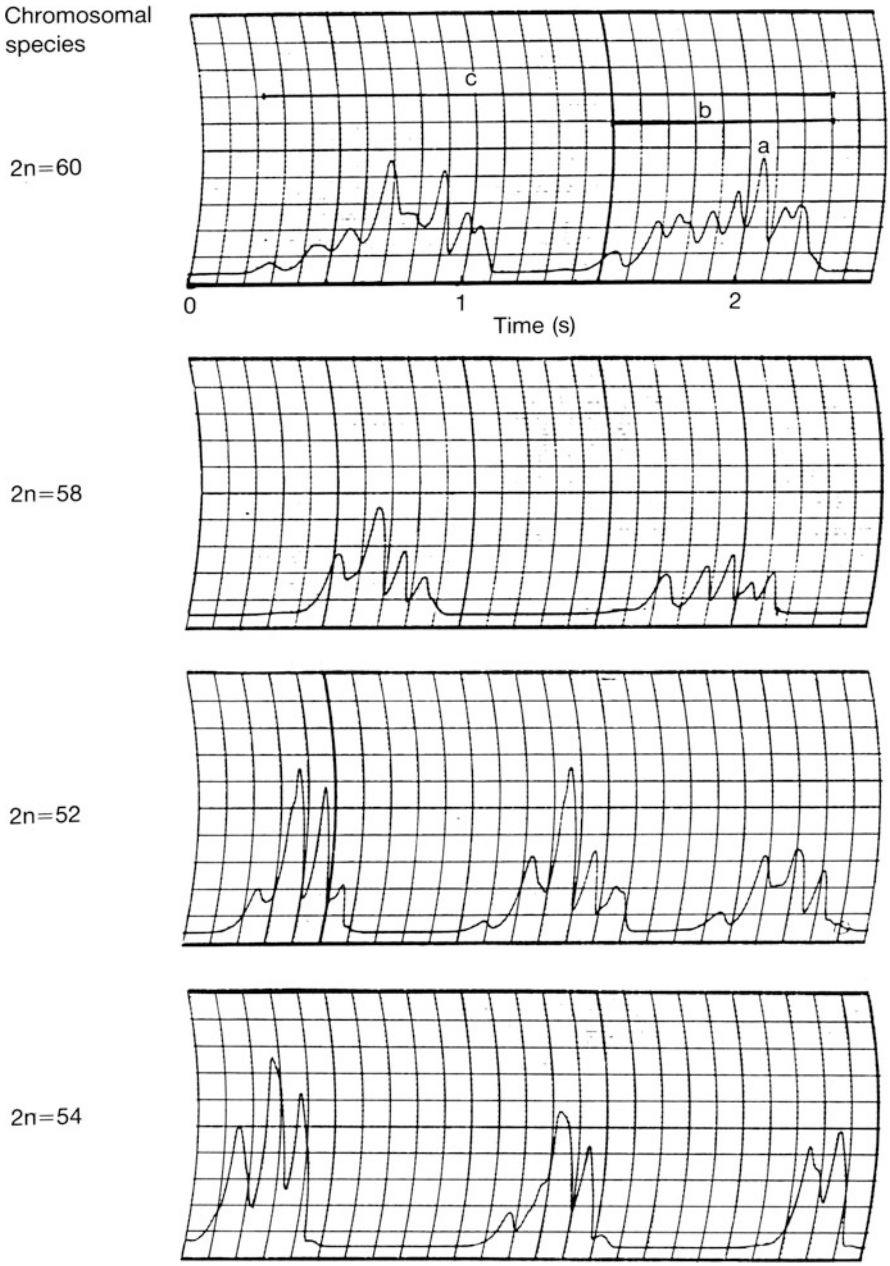


Fig. 15.13 Seismic communication in the *Spalax ehrenbergi* superspecies. A random sample of the thumping structure of signals of mole rats belonging to the four chromosomal species: *Spalax galili*, $2n = 52$; *S. golani*, $2n = 54$; *S. carmeli*, $2n = 58$; and *S. judaei*, $2n = 60$. (**a**) Each peak represents a thump or a pulse; (**b**) sequential pulses or a pulse group (PG); (**c**) series of PGs, with inter-PG intervals equal or shorter than 2 s or a section (from Heth et al. 1991)

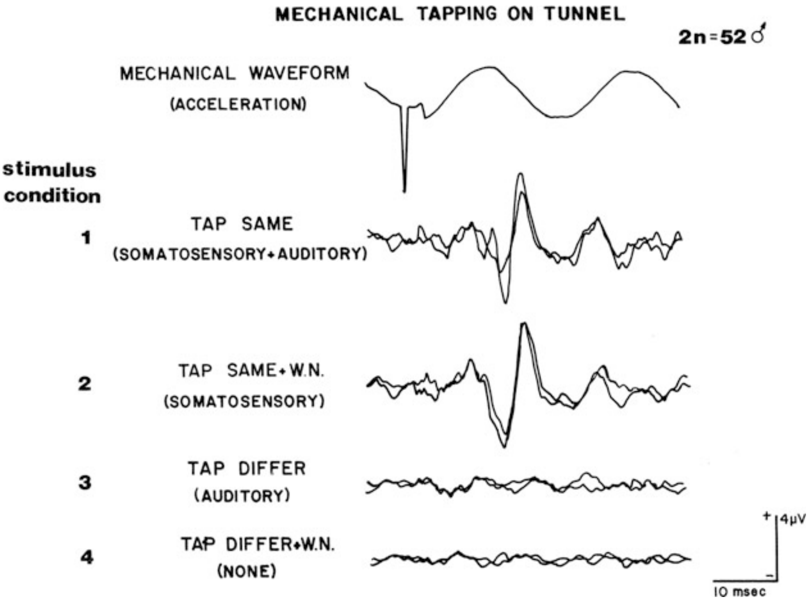


Fig. 15.14 The somatosensory mechanism of seismic communication in *Spalax*. Stimulus waveform (top trace) and evoked potentials to different stimulus conditions produced by mechanical tapping on tunnels. *Tap Same* indicates tapping on the same tunnel the mole rat (*Spalax galili*, 2*n* = 52) was in; *Tap Differ* stands for a separate tunnel facing but detached from the tunnel in which the animal was; *W.N.* stands for white noise masking. Stimulus conditions included the following: (1) Mixed auditory and somatosensory stimulation (second trace from top); (2) Only somatosensory stimulation (third trace from top); (3) Auditory stimulation (second trace from bottom); and (4) Neither auditory nor somatosensory stimulation (bottom trace) (Nevo et al. 1991a)

the evolution of *Spalax*, complementing the short-distance vocal communication (Nevo et al. 1991a).

15.15 Species-Specific Seismic Signals

We have examined seismic signaling and vibrational somatosensory perception in the four parapatric species of the *S. eherenbergi* superspecies (Heth et al. 1991) (Figs. 15.13 and 15.14). In 47 out of 59 mole rat encounters (80%), one or both mole rats responded with thumping, and in 24 of these cases, the pairs were dueting during the encounter. Seismic signals also were frequently transmitted between combatants in fights (Nevo et al. 1986a). The average number of thumps (pulses) per pulse group, PG, and the average duration of PG increased in the following order: *S. golani*, $2n = 54 < $ *S. galili*, $2n = 52 < $ *S. carmeli*, $2n = 58 < $ *S. judaei*, $2n = 60$. Remarkably, however, whereas the first three species were similar, the fourth species, *S. judaei*, $2n = 60$, had significantly higher values. Thumping frequencies showed the opposite order. The results indicated a trend of increasing number of thumps in PG and slower rate of thumping southwards, from the northern ($2n = 54, 52$) to the central ($2n = 58$) and the southern species ($2n = 60$). The unique and significant ($P < 0.001$) thumping structure could be considered a premating isolating mechanism distinguishing $2n = 60$ from its ancestor, $2n = 58$ (Fig. 15.13).

The amplitudes of the evoked potentials in response to the seismic vibrations differed significantly between species. The $2n = 52$ species had the lowest, and $2n = 60$ had the highest somatosensory response among the chromosomal species (Heth et al. 1991). The higher somatosensory and auditory sensitivities of the xeric *S. judaei*, $2n = 60$, appear to correlate with the larger territories of this species due to sparser food resources in both steppic and desert environments, where it is distributed. Thus, both acoustic and seismic stimuli might have evolved as by-products of adaptive ecological differentiation in accordance with the Darwin-Muller-Mayr theory of the origin of premating reproductive isolation (Mayr 1988).

15.16 Conclusions and Prospects

15.16.1 Conclusions

The evolution of subterranean mammalian rodents started ~50 mya. The blind mole rat *Spalax* represents an extreme case of subterranean mammals, highlighting adaptive evolution to life underground, displaying regressive, progressive, and convergent phenotypic and genotypic evolution. We initiated our studies on the blind mole rat in Israel in 1948, and continue them now, studying *Spalax* as an evolutionary model of adaptation and speciation. *Spalax ehrenbergi* superspecies in Israel involves five species: four parapatric chromosomal species that speciated peripatrically and adapted to increasing aridity southward: *Spalax galili*, $2n = 52$, S.*golani*, $2n = 54$, *S. carmeli*, $2n = 58$, and *S. judaei*, $2n = 60$. Recently, we identified that *Spalax galili* speciated sympatrically from its chalk progenitor to a basalt derivative species. The studies reviewed here integrate multidisciplinarily in blind mole rats of the *Spalax eherenbergi* superspecies we identified in Israel the existence of primarily two major evolutionary processes of adaptive communication systems: vocal, *short distance*, courtship calls, and *long-distance* vibratory, seismic communication thumpings onto the tunnel ceiling by the flat head. Both the vocal and vibratory (seismic) communication systems are adaptive to life underground, both in adaptive evolution and in climatic speciation. Both signals, call and vibrational (seismic), appear to be adaptive to life underground. Both signals are optimally transmitted in the subterranean ecotope through the evolution of *low-frequency signals*. Remarkably, both signals are divergent among the four chromosomal species, providing *effective ethological premating* reproductive isolation. The evolution of all call perspectives, the physical structure of the middle ear ossicles, the cochlea structure, the low-frequency and functional hearing sensitivity are all evolutionarily networked to generate and receive the signals that are *species-specific*.

The cochlea of *Spalax* is unique among mammals in its adaptation to optimal acoustic transmission of low frequencies underground, complemented by adaptations in the middle ear structures. These display the intimate coevolution of structure and function in the twin processes of adaptation and speciation.

15.16.2 Prospects

First and foremost, it will be very desirable to compare and contrast all vocal and vibratory parameters between the four chromosomal peripatric species and the first sympatric speciation on the basalt of *Spalax galili* ~ 228,000 ya. This genic, non-chromosomal sympatric speciation occurred when blind mole rats moved from the carbonaceous domain (chalk and limestone) to the siliceous domain of basalt, which is cooler, more humid and hypoxic/hypercapnic, richer in geophytes and worse in drainage than the abutting chalk progenitor population habitat (Hadid et al. 2013; Li et al. 2015, 2016; Lövy et al. 2015, 2017, 2019; Šklíba et al. 2016; Zhao et al. 2016). Second, it will be exciting to compare and contrast the Israeli *Spalax* species with the four chromosomal species in Jordan, all with $2n = 60$ (Nevo et al. 2000), as well as with the north African isolates in Egypt and Libya, and also in other Near Eastern countries, in which *Spalax ehrenbergi* superspecies occurs (Lebanon, Syria, Southern Turkey, and Iraq). Finally, until now very few of the hundreds of subterranean mammals in 11 subterranean families across the globe have been analyzed for vocal, and even for the existence of seismic communication, and it could be fascinating to extend these analyses globally.

Finally, and most importantly, it will be of great interest to analyze *genomically* and *transcriptomically* the underlying genomics of the communication systems described here. The genomic revolution (Shapiro 2011) can now highlight both the *coding* (genes coding for proteins) and *noncoding* genome elements. Most of the genome elements are *noncoding for proteins* (also generalized as the repeatome), were thought wrongly to be "junk DNA," and are known now to function primarily as regulators of gene function.

Acknowledgments I thank all my colleagues and students who participated in the *Spalax* saga for their devotion and enthusiasm. Special thanks are extended to Ruth Ben David and Kexin Li for their devoted technical assistance. This work, including all my works since 1985, could not have been implemented without the financial support of the Ancell-Teicher Research Foundation for Genetics and Molecular Evolution. I thank the Foundation wholeheartedly for its generous financial support.

References

Arieli R, Nevo E (1991) Hypoxia survival between two mole rat species (*Spalax ehrenbergi*) superspecies of humid and arid habitats. Comp Biochem Physiol 3:543–545

Beiles A, Heth G, Nevo E (1984) Origin and evolution of assortative mating in actively speciating mole rats. Theor Pop Biol 26:265–270

Bruns V, Müller M, Hofer W, Heth G, Nevo E (1988) Inner ear structure electrophysiological audiograms of the subterranean mole rat, *Spalax ehrenbergi*. Hear Res 33:1–9

Burda H, Bruns V, Nevo E (1989) Middle ear and cochlear receptors in the subterranean mole-rat, *Spalax ehrenbergi*. Hear Res 39:225–230

Burda H, Bruns V, Müller M (1990a) Sensory adaptations in subterranean mammals. Prog Clin Biol Res 335:269

Burda H, Nevo E, Bruns V (1990b) Adaptive differentiation of ear structures in subterranean mole-rats of the *Spalax ehrenbergi* superspecies in Israel. Zool Jahrb Abt Syst Geog Biol Tiere 117: 369–382

Butler P, Nevo E, Beiles A, Simson S (1993) Variations of molar morphology in the *Spalax ehrenbergi* superspecies: adaptive and phylogenetic significance. J Zool 229:191–216

Capranica RR, Nevo E, Moffat AJM (1974) Vocal repertoire of a subterranean rodent (*Spalax*). J Acoust Soc Am 55(2). https://doi.org/10.1121/1.3438017

Cei G (1946) Ortogenesi parallela e degradazione degli organi della vista negli spalacidi. Monit Zool Ital 55:69–88

Cooper HM, Herbin M, Nevo E (1993) Ocular regression conceals adaptive progression of the visual system in a blind subterranean mammal. Nature 361:156–159

de Grip WJ, Janssen JJM, Foster RG, Korf H-W, Rothschild KJ, Nevo E, de Caluwé GLJ (1992) Molecular analysis of photoreceptor protein function. In: Hargrave PA, Hofmann KP, Kaupp UB (eds) Signal transduction in photoreceptor cells. Springer, Berlin, pp 42–59

Fleischer G (1973) Studien am Skelett des Gehororgans der Saugetiere einschliesslich des Menchen. Saugetierkd Mitt 21:131–239

Flynn LJ, Nevo E, Heth G (1987) Incisor enamel microstructure in blind mole rats: adaptive and phylogenetic significance. J Mammal 68:500–507

Gorbunova V, Hine C, Tian X, Ablaeva J, Gudkov AV, Nevo E, Seluanov A (2012) Cancer resistance in the blind mole rat is mediated by concerted necrotic cell death mechanism. Proc Natl Acad Sci USA 109:19392–19396

Guttman R, Naftali G, Nevo E (1975) Aggression patterns in three chromosome forms of the mole rat, *Spalax ehrenbergi*. Anim Behav 23:485–493

Hadid Y, Tzur S, Pavlíček T, Šumbera R, Šklíba J, Lövy M, Fragman-Sapir O, Beiles A, Arieli R, Raz S, Nevo E (2013) Possible incipient sympatric ecological speciation in blind mole rats (*Spalax*). Proc Natl Acad Sci USA 110:2587–2592

Haim A, Heth G, Pratt H, Nevo E (1983) Photoperiodic effects on thermoregulation in a blind subterranean mammal. J Exp Biol 107:59–64

Heffner RS, Heffner HE (1983) Hearing in large mammals: horses (*Equus caballus*) and cattle (*Bos taurus*). Behav Neurosci 97:299

Hendriks W, Sanyal S, Nevo E (1990) The eye of the blind mole rat (*Spalax ehrenbergi*): regressive evolution at the molecular level. Prog Clin Biol Res 335:383–395

Heth G (1991) Evidence of aboveground predation and age determination of the preyed, in subterranean mole rats (*Spalax ehrenbergi*) in Israel. Mammalia 55:529–542

Heth G, Nevo E (1981) Origin and evolution of ethological isolation in subterranean mole rats. Evolution 35:259–274

Heth G, Frankenberg E, Nevo E (1986) Adaptive optimal sound for vocal communication in tunnels of a subterranean mammal (*Spalax ehrenbergi*). Experientia 42:1287–1289

Heth G, Frankenberg E, Raz A, Nevo E (1987) Vibrational communication in subterranean mole rats (*Spalax ehrenbergi)*. Behav Ecol Sociobiol 21:31–33

Heth G, Beiles A, Nevo E (1988a) Adaptive variation of pelage color within and between species of the subterranean mole rat (*Spalax ehrenbergi*) in Israel. Oecologia 74:617–622

Heth G, Frankenberg E, Nevo E (1988b) “Courtship” call of subterranean mole rats (*Spalax ehrenbergi*): physical analysis. J Mammal 69:121–125

Heth G, Frankenberg E, Pratt H, Nevo E (1991) Seismic communication in the blind subterranean mole-rat: patterns of head thumping and of their detection in the *Spalax ehrenbergi* superspecies in Israel. J Zool 224:633–638

Heth G, Nevo E, Ikan R, Weinstein V, Ravid U, Duncan H (1992) Differential olfactory perception of enantiomeric compounds by blind subterranean mole rats (*Spalax ehrenbergi*). Experientia 48:897–902

Heth G, Beauchamp GK, Nevo E, Yamazaki K (1996) Species, population and individual specific odors in urine of mole rats (*Spalax ehrenbergi*) detected by laboratory rats. Chemoecology 7: 107–111

Hickman GC (1990) Adaptiveness of tunnel systems features in subterranean mammals. In: Nevo E, Reig OA (eds) Evolution of subterranean mammals at the organismal and molecular levels. Wiley Liss, New York, pp 185–210

Kelly JB, Masterton B (1977) Auditory sensitivity of the albino rat. J Comp Physiol Psychol 91: 930–936

Klauer G, Burda H, Nevo E (1997) Adaptive differentiations of the skin of the head in a subterranean rodent, *Spalax ehrenbergi*. J Morphol 233:53–66

Kushnirov D, Beolchini F, Lombardini F, Nevo E (1998) Radiotracking studies in the blind mole rat, *Spalax ehrenbergi* supespecies (abstract # 381) Euro-American Mammal Congress, 19–24 July 1998, Santiago de Compostella (Spain)

Li K, Hong W, Jiao H, Wang G-D, Rodriguez KA, Buffenstein R, Zhao Y, Nevo E, Zhao H (2015) Sympatric speciation revealed by genome-wide divergence in the blind mole rat *Spalax*. Proc Natl Acad Sci USA 112:11905–11910

Li K, Wang L, Knisbacher BA, Xu Q, Levanon EY, Wang H, Frenkel-Morgenstern M, Tagore S, Fang X, Bazak L, Buchumenski BI, Zhao Y, Lovy M, Li X, Han L, Frenkel Z, Beiles A, Cao YB, Wang ZL, Nevo E (2016) Transcriptome, genetic editing, and microRNA divergence substantiate sympatric speciation of blind mole rat, *Spalax*. Proc Natl Acad Sci USA 113: 7584–7589

Lövy M, Šklíba J, Hrouzková E, Dvořáková V, Nevo E, Šumbera R (2015) Habitat and burrow system characteristics of the blind mole rat *Spalax galili* in an area of supposed sympatric speciation. PLoS One 10:e0133157

Lövy M, Šklíba J, Šumbera R, Nevo E (2017) Soil preference in blind mole rats in an area of supposed sympatric speciation: do they choose the fertile or the familiar? J Zool 303:291–300

Lövy M, Šumbera R, Heth G, Nevo E (2019) Presumed ecological speciation in blind mole rats: does soil type influence mate preferences? Ethol Ecol Evol. https://doi.org/10.1080/03949370.2019.1646809

Manley G (1971) Some aspects of the evolution of hearing in vertebrates. Nature 230:506–509

Marhold S, Beiles A, Burda H, Nevo E (2000) Spontaneous directional preference in a subterranean rodent, the blind mole-rat, *Spalax ehrenbergi*. Folia Zool 49:7–18

Masterton B, Heffner H, Ravizza R (1969) The evolution of human hearing. J Acoust Soc Am 45: 966–985

Mayr E (1988) The why and how of species. Biol Philos 3:431–441

Menzies RA, Heth G, Ikan R, Weinstein V, Nevo E (1992) Sexual pheromones in lipids and other fractions from urine of the male mole rats, *Spalax ehrenbergi*. Physiol Behav 52:741–747

Müller M, Burda H (1989) Restricted hearing range in a subterranean rodent, *Cryptomys hottentotus* (Bathyergidae). Naturwissenschaften 76:134–135

Nevo E (1961) Observations on Israeli populations of the mole rat *Spalax erhrenbergi* Nehring 1898. Mammalia 25:127–144

Nevo E (1969) Mole rat *Spalax ehrenbergi*:Mating behavior and its evolutionary significance. Science 163:484–486

Nevo E (1989) Modes of speciation: the nature and role of peripheral isolates in the origin of species. In: Giddings LV, Kanishero KY, Anderson WW (eds) Genetics, speciation and the founder principle. Oxford University Press, Oxford, pp 205–236

Nevo E (1990) Evolution of nonvisual communication and photoperiodic perception in speciation and adaptation of blind subterranean mole rats. Behaviour 114:249–276

Nevo E (1991) Evolutionary theory and processes of active speciation and adaptive radiation in subterranean mole rats, *Spalax ehrenbergi* superspecies, in Israel. Evol Biol 25:1–125

Nevo E (1998a) Evolution of a visual system for life without light: optimization via tinkering in blind mole rats. In: Weibel ER, Taylor CR, Bolis C (eds) Principles of animal design: the optimization and symmorphosis debate. Cambridge University Press, Cambridge, pp 288–298

Nevo E (1998b) Molecular evolution and ecological stress at global, regional and local scales: the Israeli perspective. J Exp Zool 282:95–119

Nevo E (1999) Mosaic evolution of subterranean mammals: regression, progression, and global convergence. Oxford University Press, Oxford

Nevo E (2007) Evolution of pacifism and sociality in blind mole-rats. In: Wolf JO, Sherman PW (eds) Rodent societies: an ecological & evolutionary perspective. University of Chicago Press, Chicago, pp 293–302

Nevo E (2013) Stress, adaptation, and speciation in the evolution of the blind mole rat, *Spalax*, in Israel. Mol Phylogenet Evol 66:515–525

Nevo E (2018) The evolution of the blind mole rat in Israel: 70 years of a multidisciplinary research project. Gamla Et 69 (in Hebrew)

Nevo E, Bar-El H (1976) Hybridization and speciation in fossorial mole rats. Evolution 30:831–840

Nevo E, Cleve H (1978) Genetic differentiation during speciation. Nature 275:125–126

Nevo E, Heth G (1976) Assortative mating between chromosome forms of the mole rat, *Spalax ehrenbergi*. Cell Mol Life Sci 32:1509–1511

Nevo E, Naftali G, Guttman R (1975) Aggression patterns and speciation. Proc Natl Acad Sci USA 72:3250–3254

Nevo E, Bodmer M, Heth G (1976) Olfactory discrimination as an isolating mechanism in speciating mole rats. Experientia 32:1511–1512

Nevo E, Guttman R, Haber M, Erez E (1982a) Activity patterns of evolving mole rats. J Mammal 63:453–463

Nevo E, Heth G, Beiles A (1982b) Population structure and evolution in subterranean mole rats. Evolution 36(6):1283–1289

Nevo E, Heth G, Beiles A (1986a) Aggression patterns in adaptation and speciation of subterranean mole rats. J Genet 65:65–78

Nevo E, Beiles G, Heth G, Simson S (1986b) Adaptive differentiation of body size in speciating mole rats. Oecologia 69:327–333

Nevo E, Heth G, Beiles A, Frankenberg E (1987a) Geographic dialects in blind mole rats: role of vocal communication in active speciation. Proc Natl Acad Sci USA 84:3312–3315

Nevo E, Heth G, Auffray J, Beiles A (1987b) Preliminary studies of sex-pheromones in the urine of actively speciating subterranean mole rats of the *Spalax-ehrenbergi* superspecies in Israel. Phytoparasitica 15:163–164

Nevo E, Tchernov E, Beiles A (1988a) Morphpmetrics of speciating mole rats: adaptive differentiation in ecological speciation. J Zool Syst Evol 26:286–314

Nevo E, Pirlot P, Beiles A (1988b) Brain size diversity in adaptation and speciation of subterranean mole rats. J Zool Syst Evol Res 26:467–479

Nevo E, Heth G, Pratt H (1991a) Seismic communication in a blind subterranean mammal: a major somatosensory mechanism in adaptive evolution underground. Proc Natl Acad Sci USA 88: 1256–1260

Nevo E, Simson S, Heth G, Redi C, Filippuci G (1991b) Recent speciation of subterranean mole rats of the *Spalax eherenbergi* superspecies in the El-Hamam isolate, northern Egypt (Abstract). In: 6th International Colloquium on the Ecology and Taxonomy of Small African Mammals. August 11–16, 1991. Mitzpe Ramon, Israel, p 43

Nevo E, Simson S, Heth G, Beiles A (1992) Adaptive pacifistic behaviour in subterranean mole rats in the Sahara desert, contrasting to and originating from polymorphic aggression in Israeli species. Behaviour 123:70–76

Nevo E, Ivanitskaya E, Filippucu MG, Beiles A (2000) Speciation and adaptive radiation of subterranean mole rats, *Spalax ehrenbergi* superspecies in Jordan. Biol J Linn Soc Lond 69: 263–281

Nevo E, Ivanitskaya E, Beiles A (2001) Adaptive radiation of blind subterranean mole rats: naming and revisiting the four sibling species of the *Spalax ehrenbergi* superspecies in Israel: *Spalax galili* (2n = 52), *S. golani* (2n = 54), *S. carmeli* (2n = 58) and *S. judaei* (2n = 60). Backhuys Publishers, Leiden

Palavicino-Maggio CB, Trannoy S, Holton KM, Song X, Li K, Nevo E (2019) Aggression and courtship differences found in *Drosophila melanogaster* from two different microclimates at evolution canyon. Israel Sci Rep UK 9:4084

Patterson BD, Thaeler CS Jr (1982) The mammalian baculum: hypotheses on the nature of bacular variability. J Mammal 63:1–15

Pevet P, Heth G, Hiam A, Nevo E (1984) Photoperiod perception in the blind mole rat (*Spalax ehrenberg*i, Nehring): involvement of the Harderian gland, atrophied eyes, and melatonin. J Exp Zool 232:41–50

Pirlot P, Nevo E (1989) Brain organization and evolution in subterranean mole rats. J Zool Syst Evol Res 27:58–64

Rado R, Terkel J (1989) Circadian activity of the blind mole rat, *Spalax ehrenbergi*, monitored by radio telemetry, in seminatural and natural conditions. Environ Qual Ecosyst Stability 4:391–400

Rado R, Levi N, Hauser H, Witcher J, Alder N, Intrator N, Wollberg Z, Terkel J (1987) Seismic signalling as a means of communication in a subterranean mammal. Anim Behav 35:1249–1251

Sanyal S, Jansen H, de Grip W, Nevo E, De Jong W (1990) The eye of the blind mole rat, *Spalax ehrenbergi*. Rudiment with hidden function? Invest Ophth Vis Sci 31:1398–1404

Shapiro AJ (2011) Evolution: a view from the 21st century. FT Press Science, Upper Saddle River, NJ

Simson S, Lavie B, Nevo E (1993) Penial differentiation in speciation of subterranean mole rats *Spalax ehrenbergi* in Israel. J Zool 229:493–503

Šklíba J, Lövy M, Koeppen SC, Pleštilová L, Vitámvás M, Nevo E, Šumbera R (2016) Activity of free-living subterranean blind mole rats *Spalax galili* (Rodentia: Spalacidae) in an area of supposed sympatric speciation. Biol J Linn Soc Lond 118:280–291

Szakall J (1903) Das Gehororgan der ungarischen Blindmaus (*Spalax hungaricus* Nhrg). Naturwiss Ber Ungarn 21:135–158

Wahrman J, Goiten R, Nevo E (1969a) Mole rat *Spalax*: evolutionary significance of chromosome variation. Science 164:82–84

Wahrman J, Goiten J, Nevo E (1969b) Geographic variation of chromosome forms in *Spalax*, a subterranean mammal of restricted mobility. In: Benirschke K (ed) Comparative mammalian cytogenetics. Springer, New York, pp 30–48

Wahrman J, Richler C, Gamperl R, Nevo E (1984) Revisiting *Spalax*: mitotic and meiotic chromosome variability. Israel J Zool 33:15–38

Zhao Y, Tang J-W, Yang Z, Cao Y-B, Ren J-L, Ben AY, Li K, Chen X-Q, Du J-Z, Nevo E (2016) Adaptive methylation regulation of *p53* pathway in symoatric speciation of blind mole rats, *Spalax*. Proc Natl Acad Sci USA 113:2146–2151

Chapter 16
Vibrational Behaviour in Honeybees

Wolfgang H. Kirchner, Felix A. Hager, and Kathrin Krausa

Abstract In the darkness of the nests of the western honeybee, *Apis mellifera*, communication among colony members is primarily based on chemical and mechanical signals. In this chapter, we review what is known about vibrational communication in honeybees. We first focus on the signals produced by *A. mellifera* queens and workers and then widen the scope and review the sparse literature on vibrational signalling in Asian honeybee species. In most of the studies on acoustic communication in honeybees, airborne sounds rather than substrate vibrations were recorded, thus our knowledge about the actual generation of substrate vibrations is limited. We then discuss what is known about transmission and perception of substrate vibrations that travel in the nests of honeybees through the wax comb. Finally, we report about attempts to make use of sound and vibration signals for the assessment of the status of managed honeybee colonies.

16.1 Introduction

Honeybee colonies consist of thousands of individuals that form an entity, on which every single member relies to survive. To be successful as a colony requires united and coordinated behaviour of colony members, which is based on their ability to

---

W. H. Kirchner (✉)
Faculty of Biology and Biotechnology, Ruhr University Bochum, Bochum, Germany
e-mail: Wolfgang.H.Kirchner@ruhr-uni-bochum.de

F. A. Hager
Faculty of Biology and Biotechnology, Ruhr University Bochum, Bochum, Germany

Present Address: Research and Development, Bayer Crop Science, Monheim, Germany
e-mail: Felix.Hager@bayer.com

K. Krausa
Faculty of Biology and Biotechnology, Ruhr University Bochum, Bochum, Germany

Present Address: International Centre of Insect Physiology and Ecology, Nairobi, Kenya
e-mail: kkrausa@icipe.org

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_16

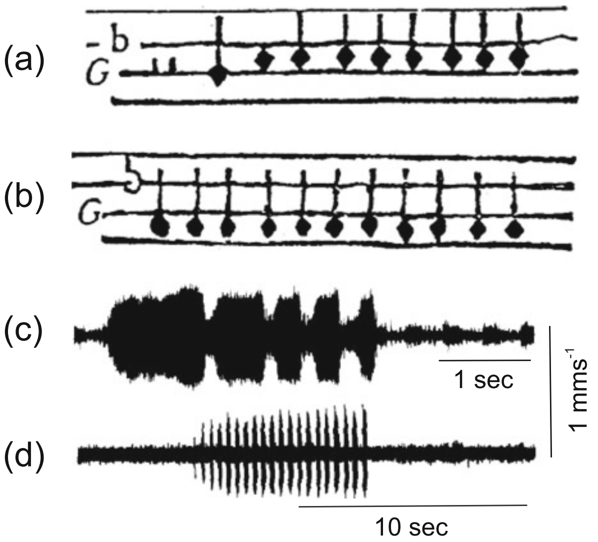


Fig. 16.1 (**a**) Tooting and (**b**) quacking as noted by Butler (1609) in musical notation. (**c**) Tooting and (**d**) quacking recorded as vibration velocity of the comb surface. Adapted from Butler (1609) and Michelsen et al. (1986b)

communicate. The honeybee is a very well-studied model organism. Its famous dance language is often referred to as one of the most complex communication systems in the animal kingdom, involving various sensory modalities. Given that Karl von Frisch had already earned a Nobel Prize for his discoveries on honeybee communication in 1973 (Cronholm, 1973), it may be a surprise that we still do not entirely understand this communication system. It is increasingly recognized that communication involves multiple sensory modalities. The study of such complex communication systems asks for a patient reductionist study of the communication modes and a subsequent analysis of their interactions.

The oldest modes of communication are chemical and vibrational, yet chemical signals have gained much more attention than vibratory signals. Honeybees produce vibrational signals in the contexts of foraging and reproduction. Sounds produced by queens were probably the first insect sound and vibration signals that were “recorded”. As early as the year 1609, Charles Butler published a book that was remarkable in many respects: “The Feminine Monarchie”, the first scientific book on honeybee biology.[1] He documented the auditory component of vibrational signals emitted by young queens, in his words “The Bees Musicke” Butler 1609), using the musical notation of his times (Fig. 16.1). It took almost 350 years before it was experimentally shown that queen signals are transmitted through the combs and

---

[1] Amongst many new observations and conclusions, Butler discovered that the single individual surrounded by a retinue of worker bees is not a king, a male, as previously generally assumed, but is in fact a female, the queen. It might have made the unthinkable idea of a female monarch easier to consider that just in the period of time in which Butler made his observation the British Empire was ruled by Elizabeth I. Butler observed and carefully described the life cycle of honeybees including the swarming behaviour.

perceived as substrate vibrations (Hansson 1945). Since then, the equipment to measure vibrations has much improved, up to the point where we use laser Doppler vibrometry that allows us to measure the vibration's temporal structure and amplitudes without touching the vibrating structure.

Biotremology has only recently begun to diverge from bioacoustics because it became increasingly evident that substrate-borne vibrations have many peculiarities when compared to sound vibrations (Cocroft et al. 2014; Hill and Wessel 2016). Despite their differences, both disciplines share many experimental approaches. Early studies recorded the airborne sound that is emitted by bees. However, in some cases, it turned out that substrate-borne vibrations rather than sound are crucial for signal perception. We here focus on the behaviour of honeybees that causes substrate-borne vibrations and review the evidence for their communicative value.

16.2 Vibrational Behaviour in Honeybees

Vibrational communication is defined as the process of conveying information through signals or cues travelling through the substrate (Hill 2014; Hill and Wessel 2016). Vibrational signals are selected by natural selection. They are produced to convey information that may be perceived by a receiver, which in response alters its behaviour in such a way that promotes the fitness of the sender. Yet, substrate-borne vibrations may be merely incidental events and the by-product of routine activities that may serve as cues (Hill 2008). However, arguments can be made that these vibrational cues from the substrate are also subject to natural selection if the incidental events are common, and have an effect on fitness, such as those involving predator and prey species (Hill et al. 2019). We believe that a clear terminology is required to sort and unify the diverse terminology used in the study of honeybee vibrational communication. We here follow the standardization of terms proposed by Hill (2014) to align the findings with the biotremological framework. In this sense, we assign the vibrational behaviour possessed by honeybees either to tremulatory behaviour, i.e., body motions without any percussive impact with the substrate, or drumming behaviour, i. e., percussive strikes of the substrate with body parts (Hill 2014).

16.2.1 Queen Tooting and Quacking

The first electromechanical recordings of queen tooting and quacking were presented using a classical Kay Sonagraph (Wenner 1962a). A detailed analysis of queen signals has been published by Michelsen et al. (1986b) using laser vibrometry, allowing for the first time not only the measurement of temporal structures but also amplitudes of undisturbed signals.

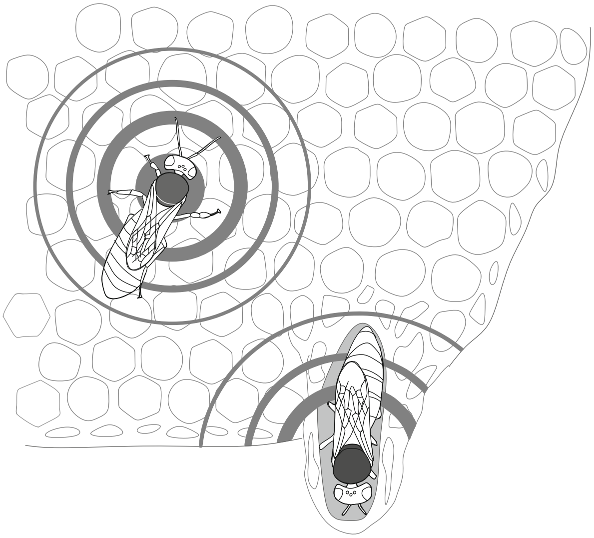


Fig. 16.2 Tremulatory signals of honeybee queens. Emerged virgin queen (left) produces a tooting signal and a virgin queen still confined in the cells (right) responds with quacking. Drawing by K. Krausa

Tooting is emitted by young queens that have emerged from their cells and walk over the comb (Fig. 16.2). As a reply young queens that are still inside the cells produce quacking (Huber 1792). The queens press their thorax against cell walls, contract their wing muscles and thereby tremulate, i. e., cause the substrate to vibrate with non-percussive body motions (Simpson 1964). Tooting and quacking consist of several pulses and have a typical temporal structure (Fig. 16.1). The first tooting pulse lasts for more than 1 s and rises in amplitude and frequency (Table 16.1). The following pulses are shorter and show an initial rise in amplitude. Quacking consists of shorter pulses of 0.2 s duration. Quacking pulses lack a rise in frequency and amplitude (Michelsen et al. 1986b). The fundamental frequency of tooting (300–500 Hz) is higher than that of quacking (200–300 Hz), and the fundamental rises in both signals substantially with the queen's age (Michelsen et al. 1986b). Quacking amplitudes in the combs that are recorded close to the emitter can be as high as 6 mms^{-1}, corresponding to displacement amplitudes of up to 5000 nm (Michelsen et al. 1986b).[2]

If tooting amplitudes are high enough, worker bees exhibit a freezing response (Hansson 1945; Michelsen et al. 1986b). Queens that are still confined in their cells, stop cutting open the cells when tooting is emitted. This leads to a considerable emergence delay (Grooters 1987). When the first emerged young queen eventually leaves the nest with a swarm, one or more of the quackers emerge from their cells and become tooters. Finally, the workers allow one queen to kill all her competitors (Simpson 1964; Simpson and Cherry 1969; Bruinsma et al. 1981; Grooters 1987; Gilley 2001). The rearing of several queens and the regulation of virgin queen

---

[2] Amplitudes are throughout the text, table and figures in this chapters generally peak-to-peak amplitudes.

Table 16.1 Vibrational Behaviour in Honeybees. Note that amplitude is given only if substrate-borne vibrations were measured

Signal (synonyms)	Context	Sender	Receiver	Recording method	Pulse duration [s]	Number of pulses	Carrier frequency [Hz]	Substrate vibration amplitude [$\text{mms}^{-1}{}_{\text{pp}}$]	References
Tooting (piping)	Swarming	Emerged virgin queen	Unemerged queens, workers	Musical notation		8–10			Butler (1609)
				Microphone		5–23	370–540		Hansson (1945)
				Microphone	First: 1–2 Subsequent: 0.2–0.3	6–7	1300–1400[a]		Wenner (1962a)
				LDV	First: 1 Subsequent: 0.25	5–10	300–500	Up to 2.5	Michelsen et al. (1986b)
Quacking (piping)	Swarming	Unemerged queen	Emerged virgin queens, workers	Musical notation		11			Butler (1609)
				Microphone		29–94	270–380		Hansson (1945)
				Microphone	0.15	9	1800[a]		Wenner (1962a)
				LDV	0.15		200–300	3–6	Michelsen et al. (1986b)
Stop signal (Piep-Laut, begging signal)	Recruitment, danger, competition at a food source	Dance follower, Tremble dancer	Waggle dancers, workers	Microphone	0.1–0.2	1	300–400		Esch (1964)
				LDV	Up to 0.1	1	320	Up to 3	Michelsen et al. (1986a)
				LDV	0.05–0.2	1	350–450	Up to 1	Kirchner (1993)

(continued)

Table 16.1 (continued)

Signal (synonyms)	Context	Sender	Receiver	Recording method	Pulse duration [s]	Number of pulses	Carrier frequency [Hz]	Substrate vibration amplitude [$\mathrm{mms}^{-1}{}_{\mathrm{pp}}$]	References
				Microphone	0.23	1	270–540		Thom et al. (2003)
				Microphone	0.17	1	330		Lau and Nieh (2010)
Waggle dance vibrations	Recruitment	Waggle dancer		Microphone			200		Wenner (1962b)
				Microphone	0.012–0.018		200–290		Esch (1961b)
				Microphone	0.02		250–300		Michelsen et al. (1986a)
				LDV				Not detect.	
				Microphone	0.1–0.2		250–270		Waddington and Kirchner (1992)
				LDV			244	0.05–0.13	Nieh and Tautz (2000)
				LDV	0.013–0.017		213–235	270–390[b]	Hrncir et al. (2011)
DAV (vibration dance, vibration signal, shaking)	Activation of receiver, task allocation, house-hunting, worker competition	Worker		Accelerometer	4–5	8–27	500–800	0.06–0.1	Ramsey et al. (2018)
Low-frequency vibrations	Motivational state of sender	Worker		LDV	0.6		31–43	5–10[b]	Hrncir et al. (2019)

[a] High carrier frequencies might be an artefact due to measurement equipment, see Michelsen et al. (1986b)

[b] Thorax vibrations

emergence via vibrational communication could be adaptive because it ensures that the colonies have some spare queens in case a young queen gets lost on her mating flight or turns out to be infertile. A number of open questions remain, and neither the ultimate nor proximate causes of queen tremulatory signals have been investigated sufficiently to date.

16.2.2 Vibrational Behaviour of Worker Bees

16.2.2.1 Stop Signal

The stop signal is probably the best-studied tremulatory signal of worker bees. While studying the honeybee's dance language, Esch (1964) noted that workers produce clearly audible sound pulses when following dances. Later it turned out that the airborne sound is emitted by the comb, which is vibrated when bees press their thorax against it and contract their dorsoventral thoracic muscles (Fig. 16.3). Bees vibrate the comb with amplitudes of 1500 nm at around 320 Hz (Table 16.1). The amplitude is a factor of 3–4 above the background noise (Michelsen et al. 1986a). The threshold of vibration perception indicates that bees at some centimetres' distance should be able to detect the substrate-borne vibrations (Michelsen et al. 1986a; see Sect. 16.4).

Esch (1964) suggested that dance followers use the signal to beg for food. Other studies confirmed that other bees stop moving when the signal is emitted (von Frisch 1967; Michelsen et al. 1986a). However, it took almost 30 years until the signal's message and meaning were studied and Esch's assumptions were found to be wrong. The signals are not only produced by dance followers but also by tremble and waggle dancers (Kirchner 1993; Nieh 1993). Contrary to what would be expected from the signal's original interpretation, it rarely elicits trophallaxis but causes

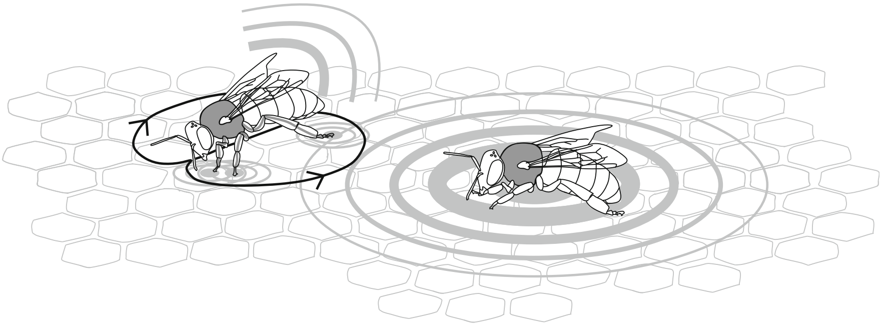


Fig. 16.3 Waggle dancers produce thoracic and wing vibrations that induce strong airborne nearfield sound signals. In addition, vibrations are transmitted through the legs on the comb (left). Workers produce stop signals by pressing the oscillating thorax on the comb (right). As a response the dancer often stops dancing and leaves the dance floor. Drawing by K. Krausa

waggle dancers to leave the dance floor (Kirchner 1993; Nieh 1993; Pastor and Seeley 2005). Furthermore, playback of the vibrational signal strongly reduces the duration of waggle dances and recruitment success (Kirchner 1993). The signal's message and meaning were reconsidered, and it was consequently named "stop signal" (Nieh 1993). Most evidence suggests that the stop signal is a negative feedback component (Seeley 1992). We now understand that the signal affects the behaviour of bees in many contexts: it modulates the tremble dance (Seeley 1992; Thom et al. 2003), decreases recruitment to a food source in the presence of danger or competition at a feeding station (Lau and Nieh 2010; Nieh 2010; Jack-McCollough and Nieh 2015; Kietzman et al. 2017), and provides cross-inhibition during a swarm's nest site selection process (Seeley et al. 2012; Laomettachit et al. 2016). It appears that the stop signal balances the positive feedback of the waggle dance.

Overall, the signal allows a sensitive and quick adjustment of response from the colony as a unit to changing conditions (Kietzman and Visscher 2015). After some years of study, we have an idea which message is transmitted and what it means for the signal recipients. However, our knowledge on the transmission and perception of the stop signal is still limited and needs further investigation.

16.2.2.2 Tremulations Emitted by Waggle Dancers

The dance language is often said to be one of the most complex communication systems in the animal kingdom as it provides nestmates with information about the location as well as quality of resources kilometres away from the nest. Successful foragers advertise the previously visited food source by dancing. During the dance's waggle phase, they produce pulsed thoracic vibrations. In contrast to bees emitting stop signals, waggle dancers do not press their bodies against the substrate but stand on their legs, vibrate their wings and emit airborne sound (Fig. 16.3). Esch (1961a, b) and Wenner (1962b) independently discovered these sounds and found that signal duration and the number of pulses correlate with the distance of the food source and might provide the dance followers with distance information. Later, it turned out that the signal's temporal patterns and amplitude are in addition modulated according to food profitability (Waddington and Kirchner 1992; Hrncir et al. 2011).

In early studies, only airborne sounds emitted by dancers were recorded. Esch (1961a, b) argued that sounds are perceived by Johnston's organs located in the bee's antennae, Wenner (1962b) stated that the mechanical waves could be perceived through tactile interactions as well as airborne sound or substrate vibration. Up to now, the relative significance of these three sensory pathways is not fully understood.

Using laser vibrometry Michelsen et al. (1986a) searched for substrate vibrations produced by waggle dancers and could not find any detectable substrate-borne waves (Table 16.1). Later Nieh and Tautz (2000) recorded comb vibrations correlated with the airborne sound emitted by the dancer. The vibration amplitudes (90 nm at 223 Hz) are close to the bee's physiological perception threshold (see

Sect. 16.4). However, it must be questioned whether these substrate-borne vibrations are of communicative value because their amplitude does not exceed background noise (Michelsen et al. 1986a). Despite some indirect evidence for the significance of substrate waves generated by waggle dancers (Sandeman et al. 1996; Tautz 1996; Tautz et al. 1996; Tautz et al. 2001), there is at this time no direct experimental evidence supporting the idea that dance information is transmitted through comb vibrations. This leaves the question open whether substrate vibrations emitted during waggle dancing are a highly masked signal or just a by-product of sound signal emission.

Some studies addressed the role of tactile interactions between dancers and dance followers. Dancers and dance followers perform tactile interactions (Tautz and Rohrseitz 1998; Gil and De Marco 2010). Brief antennal contacts between dancer and dance follower might help to gain directional information about the food source (Rohrseitz and Tautz 1999) and additionally enable the bees to estimate the length of the waggle phase (Gil and De Marco 2010).

The sound emitted by dancing bees turned out to be of high particle velocity amplitude close to the dancer (Michelsen et al. 1987). The airborne nearfield sound can be perceived through Johnston's organ in the antennae (Towne and Kirchner 1989; Kirchner et al. 1991; Dreller and Kirchner 1993a; Hasegawa and Ikeno 2011). And there are at least three direct lines of evidence strongly supporting that information is indeed transmitted through the air. Experiments in which the airborne sound signal was manipulated using a wing-mutant bee emitting sounds of higher frequency and lower amplitude revealed that the mutant recruits nestmates less effectively than wild-type bees (Kirchner and Sommer 1992). Experiments in which signal perception was manipulated showed that bees with ablated antennae are recruited much less than untreated controls (Dreller and Kirchner 1993b). Experiments in which the waggle dance is simulated using a robotic dancer indicate recruitment into the direction indicated by the emission of dance sounds and tail wagging movements of the artificial dancer (Michelsen et al. 1989, 1992). However, Greggers et al. (2013) found that the wing vibrations of dancing bees do not only elicit airborne nearfield sounds, but also an electric field that can induce the antenna to vibrate and might act synergistically to airborne transmission of sound.

16.2.2.3 Dorsoventral Abdominal Vibration (DAV)

Workers that walk on the comb occasionally stop and shake their body. They grasp another bee, move their body dorsoventrally for 1–2 s and thereby shake the nestmate. A bee can produce 20 or more of these movements per minute and may continue doing so for several minutes and contact hundreds of different recipients scattered throughout the nest (Schneider 1986; Nieh 1998; Seeley et al. 1998). Queens, workers and drones are signal recipients (Allen 1959a, b; Painter-Kurt and Schneider 1998a, b; Boucher and Schneider 2009; Slone et al. 2012). Occasionally bees shake their body without contacting a nestmate and drum with their abdomen on the comb (Fig. 16.4).

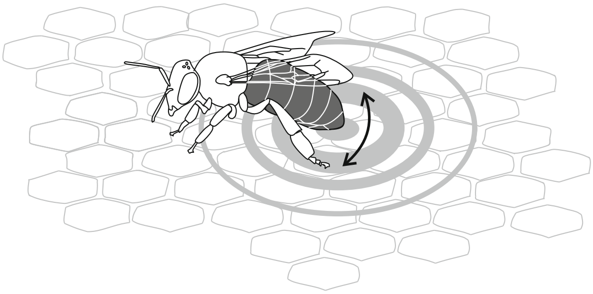


Fig. 16.4 Dorsoventral abdominal vibrations produced by a worker bee. Drawing by K. Krausa

Shaking a nestmate and drumming of the abdomen on the comb are both predominantly referred to as vibration signal (Painter-Kurt and Schneider 1998a, b; Visscher et al. 1999; Lewis and Schneider 2000; Schneider et al. 2001; Lewis et al. 2002; Donahoe et al. 2003; Hyland et al. 2007; Cao et al. 2007, 2009; Gilbert et al. 2010; Skaggs et al. 2014; for a list of other frequently used terms see Schneider and Lewis 2004). From a biotremological perspective, however, this term is misleading because it does not refer to substrate vibrations but vibrations of the bees' body. Therefore, we prefer the term dorsoventral abdominal vibration (DAV) introduced by Milum (1955).

Studies indicate that DAVs have a modulatory signal function. The signal is directed toward diverse recipients, causes a general increase in activity that alters responsiveness to other stimuli, and thus influences the performance of many different tasks simultaneously (Schneider and Lewis 2004). More recent studies suggest that DAVs are a component in the cascade of communication signals that orchestrate house-hunting and colony relocation decisions (Pierce et al. 2006; Gilbert et al. 2010), may help to adjust task allocations to the different stages of colony development (Gahl 1975; Hyland et al. 2007; Cao et al. 2007, 2009) and are associated with the reproductive competition among workers in queenless colonies (Skaggs et al. 2014).

For a long time, the DAVs were only studied using visual methods, which failed to detect the substrate impact, and were incorrectly classified as a form of tremulation (Hill 2014). Recently, Ramsey et al. (2018) published the first study of their physical nature by combining visual and vibrational measurements. It became apparent that detection of a substrate-borne component of DAV is entirely restricted to those instances where honeybees drum in the immediate vicinity of the accelerometer (2–3 cm radius), and directly onto the comb rather than on a nestmate (Ramsey et al. 2018). When honeybees perform DAVs, they move their abdomen vigorously and rhythmically in a direction orthogonal to the plane of the honeycomb. In doing so, they drum on the substrate at a frequency of 10–22 Hz (pulse repetition rate). A series of drums lasts about 0.9–1.5 s (Visscher et al. 1999; Ramsey et al. 2018). Each drum elicits substrate vibrations of 800 Hz with a mean acceleration of 0.3–0.4 ms^{-2} (Ramsey et al. 2018). It is doubtful that the produced substrate-borne vibrations can be perceived by the bees because the signal-to-noise ratio is low (Ramsey et al. 2018), and the threshold curve of vibration perception suggests that the measured amplitude is too weak to be perceived (see Sect. 16.4). When a DAV is emitted directly on a queen cell, it might be perceived by the confined queens. This, however, needs further investigation.

16.2.2.4 Low-Frequency Vibrations

Very recently Hrncir et al. (2019) investigated irregular thoracic vibrations generated by workers of different activity level-groups. Twitches of the asynchronous flight muscles elicit low-frequency vibrations of the thorax (up to 50 Hz). Velocity amplitudes and main frequency components increased with the signaler's activity level. Nectar foragers, moreover, modulate their low-frequency thoracic vibrations during trophallaxis according to the collected food quality. These vibrations might provide information on the motivational status of honeybee workers. The highest recorded vibrational amplitudes measured on the thoraxes were 12 mms^{-1}. Bees producing low-frequency thoracic vibrations do not press their thorax on the comb. Vibrations are rather transmitted through the legs and consequently attenuated (see Sect. 16.3). The damping effect of the legs has never been tested on natural nest material but is believed to be too strong to result in detectable substrate vibrations (Hrncir et al. 2019).

16.2.2.5 Worker Piping

Worker piping, as it was described in the context of swarming cannot be referred to as a vibrational signal. Nevertheless, we mention and describe it here, to avoid future misunderstandings.

After leaving the nest, a swarm of bees congregates mostly in a nearby place, often hanging under branches. Thousands of bees cling to each other while only some have contact to the branch. Shortly before the swarm takes off to move into a new nest, workers start to pipe (Lindauer 1955; Esch 1964; Seeley et al. 1979; Camazine et al. 1999; Seeley and Tautz 2001; Seeley and Visscher 2003; Visscher and Seeley 2007; Schlegel et al. 2012). Piping bees press their thorax against the bees beneath and vibrate their muscles. In this way, bees are vibrated through direct body contact. At the same time an audible sound is emitted. So far only the airborne component of piping has been studied (Seeley and Tautz 2001; Schlegel et al. 2012).

Since pipes are emitted shortly before the swarm flies to the new nest location, it was assumed that they trigger coordinated take-off (Camazine et al. 1999). Seeley and Tautz (2001) investigated the pipes' meaning experimentally. Bees that perceived the pipes via direct body contact warmed themselves to a flight-ready body temperature. In contrast, bees that were caged to prevent direct contact with the emitting bee did not respond in the same way. This suggests that the signal's message is "get ready for take-off" or "warm-up for flight". The results indicate that the signal is transmitted through direct body contact, i.e., tactile stimulation. A significance in a biotremological sense is doubtful because the vast majority of the bees does not have any contact to the substrate but only to other bees.

16.2.3 Vibrational Behaviour of Asian Honeybees

The genus *Apis* comprises about 11 species that predominantly occur in Asia (Michener 2000). *A. mellifera* has been studied nearly exclusively. It is the only species that made its way out of Asia into Europe, Africa and more recently into the America's and Oceania.

The first recordings of acoustical signals of Asian honeybees were made by Towne (1985), who found that *Apis cerana* dancers emit sounds and stop signals pretty much like western honeybees (*A. mellifera*). While the giant honeybee *Apis dorsata* produces dance sounds at low frequencies around 100 Hz (Kirchner and Dreller 1993), the dwarf honeybee *Apis florea* (Towne 1985) and the giant honeybee of the Himalayan ranges *Apis laboriosa* (Kirchner et al. 1996) do not emit sounds when dancing.

Stop signals have been recorded in *A. dorsata*, *A. cerana*, *A. florea* (Towne 1985) and *A. laboriosa* (Kirchner et al. 1996). All these recordings were made using microphones. Recently stop signalling by *A. cerana* was investigated in more detail using more appropriate measurement equipment. The production of stop signals in *A. cerana* can be triggered by predator attacks upon foragers at a food source and inhibits waggle dancing inside the hive (Tan et al. 2016). Furthermore, the amplitude of vibrations is modulated according to the extent of danger experienced at a food source. The stop signal amplitude is higher when the attacker is the more dangerous *Vespa mandarinia* than the less dangerous *Vespa velutina*. In line with this, it was found that higher amplitude signals led to greater inhibitory effects than lower amplitudes (Dong et al. 2019).

The stop signal produced in this context was interpreted as a referential alarm signal (Tan et al. 2016; Dong et al. 2019). Given that vibration amplitude decreases quickly and relatively unpredictably while travelling through the inhomogeneous substrate (see Sect. 16.3), it is questionable whether it can reliably carry referential information. Based on amplitudes alone, a signal receiver, therefore, can hardly tell whether variations are due to a modulation by the signaler or due to varying distance from the vibration source. On the colony level, however, amplitude modulated signals could be adaptive. Signals with a high amplitude could simply inform more receivers about the presence of danger than signals with a lower amplitude.

Another context in which Asian bees might make use of substrate vibrations is nest defence. When nests are disturbed, bees produce an initial piping that elicits hissing sounds and shimmering movements that might be aposematic. This behaviour is found in *A. cerana* (Koeniger and Fuchs 1972; Fuchs and Koeniger 1974; Kawakita et al. 2018, 2019), *A. dorsata* (Kastberger et al. 2013) and *A. florea* (Sen Sarma et al. 2002). So far, it was not investigated whether piping and hissing induce substrate vibrations that could carry information.

Apis cerana is the only species of Asian honeybees in which the threshold of substrate vibration perception has been studied. In comparison with the vibrational perception threshold of *A. mellifera* (see Sect. 16.4), *A. cerana* responds to a similar frequency range. *A. cerana* is most sensitive at vibrations of 550 Hz. An amplitude of 0.6 ms^{-2} is sufficient to elicit a freezing response (Dong et al. 2019).

16.3 Transmission of Vibrations

In contrast to other insect species, no anatomically specialized structures for vibrational signal production were found in honeybees. Tremulations in honeybees result from rhythmic oscillations of the thorax. Flight is the most important context in which bees produce thoracic vibrations. If they fold their wings over the abdomen, they are decoupled (Snodgrass 1956). Muscle contractions with folded wings result in vibrations with a higher main frequency component compared to flight vibrations (Nachtigall 2003).

Honeybees that vibrate their flight muscles sometimes press their thorax directly on the comb and thereby directly transmit vibrations to the substrate (Esch 1964; Simpson 1964; Michelsen et al. 1986a,b; Pratt et al. 1996; Thom et al. 2003). If the thorax is not pressed on the comb, i.e., in direct contact with the substrate, thoracic vibrations may also be transmitted through the bee's legs. The amplitudes of substrate vibrations induced through the legs (Nieh and Tautz 2000) are much smaller compared to vibrations measured on the thorax (Hrncir et al. 2011). To better understand the transmission of thoracic vibrations to the substrate, sophisticated measurements with two laser vibrometers simultaneously pointing on the thorax and on the comb are required. These measurements have so far not been conducted in honeybees. Therefore, we make use of insights from stingless bees: Foragers of *Melipona seminigra* are of similar size as honeybees and produce pulsed thoracic vibrations in the context of foraging. The amplitude of thoracic vibrations is strongly attenuated by more than 48 dB when transmitted from the forager onto the substrate (Hrncir et al. 2006a).

Honeybees may also generate substrate vibrations by drumming the abdomen on the comb when performing dorsoventral abdominal vibrations (Ramsey et al. 2018). The induced vibrations, however, are so weak that it is questionable whether they can be detected by nestmates (see Sect. 16.2.2.3).

Contrary to acoustic signals that propagate through the air, vibrational signals are transmitted through different media with very different physical properties. Vibrational signals produced by honeybees travel through combs. Bees prefer to dance on the lower comb in the area next to the entrance (Lindauer 1952; Seeley 1994). In these lower parts of the frames, bees commonly remove wax between the comb and the frame (Sandeman et al. 1996). Measurements of the comb's physical properties suggest that vibrational signals produced on a frameless area travel over a much greater distance than those produced on framed combs. Impedance at the edge of solid substrates, i.e., where combs are not connected to frames is lower compared to the centre of combs (Michelsen et al. 1986a; Sandeman et al. 1996). Although it is questionable that substrate vibrations can be perceived from the waggle dance (see Sect. 16.2.2.2), it is worth noting that dancers on combs with open, empty cells recruit three times as many nestmates to a food source as dancers on capped brood cells (Tautz 1996). Dances on open cells are not significantly more attractive than dances on sealed cells, but dancers on open cells attract 90% of their dance follower bees from a range of within 27 mm, dancers on sealed cells from within 18 mm (Tautz and Rohrseitz 1998). These results may indicate that the bees manipulate the comb in a way that might favour vibrational communication.

Honeycombs are highly complex and variable. This is reflected in difficulties in obtaining impedance values for the comb in general because of extreme variations (Michelsen et al. 1986a; Sandeman et al. 1996). On open combs vibrations from 10 to 150 Hz and above 250 Hz were found to be attenuated; whereas vibrations between 150 and 250 Hz were amplified (Sandeman et al. 1996). Measurements of the comb's physical properties are still very limited, and further studies are highly desirable.

16.4 Perception of Vibrations

The first indication for vibration sensing in honeybees arose from a behavioural study of Hansson (1945), adopting an approach successfully used by von Frisch (1919), who studied the bees' olfactory sense. Hansson (1945) trained honeybees to discriminate a sound-emitting food chamber from a mute chamber. The bees were able to associate sound with food reward only when they approached the feeding chamber walking on a platform in front of the chamber but were unable to learn when they had to fly to the entrance of the feeding chamber. He correctly concluded that a sense of vibrations was used to solve the task.

Honeybees have a variety of mechanosensory receptors all over their body (Thurm 1964). The reception of substrate-borne vibrations is primarily attributed to the subgenual organ. This chordotonal organ is located in the tibia of each leg (Schön 1911; McIndoo 1922; Autrum and Schneider 1948). More recently, the biophysics of the subgenual organ was studied in more detail. The organ oscillates with the hemolymph and, due to inertia, lags behind the movement of the leg, which mechanically stimulates the receptor cells (Kilpinen and Storm 1997; Storm and Kilpinen 1998). Autrum and Schneider (1948) were the first to measure the sensitivity of the sensory cells electrophysiologically. The sensitivity thresholds differ between front, mid and hind legs (Fig. 16.5). The highest sensitivity of 13 nm was found in the hind leg at a frequency of 2500 Hz. Calibrations of vibrational amplitudes were made using light microscopy for the range of amplitudes down to the limit of resolution. The threshold determined by Autrum and Schneider (1948) is therefore based on extrapolation over two orders of magnitude. Additional electrophysiological recordings by Kilpinen and Storm (1997) and Rohrseitz and Kilpinen (1997) basically confirm the data reported by Autrum and Schneider (1948) and

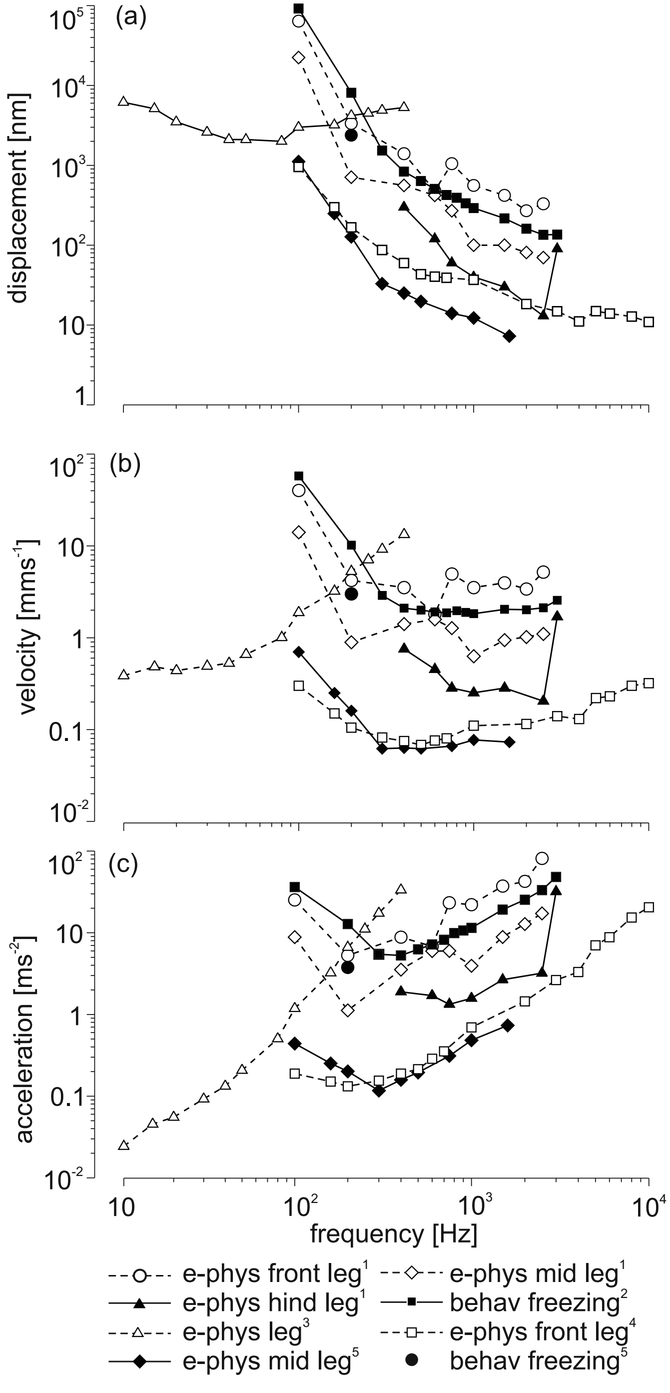


Fig. 16.5 Vibrational perception thresholds of honeybees (*Apis mellifera*) as (**a**) displacement, (**b**) velocity and (**c**) acceleration. Values were extracted from graphs, recalculated and redrawn after [1]Autrum and Schneider (1948); [2]Michelsen et al. (1986a); [3]Sandeman et al. (1996); [4]Kilpinen and Storm (1997); [5]Rohrseitz and Kilpinen (1997)

furthermore revealed that the subgenual organ is more sensitive to vertical vibrations than horizontal. This suggests that the leg position is crucial for vibration sensing, as it was recently discussed by (Strauß et al. 2019). Sandeman et al. (1996) recorded extracellular recordings of bee legs and showed a receptor sensitivity to low frequencies. The authors, however, might have recorded responses of different receptors compared to the other studies (Fig. 16.5).

The threshold of vibration perception was also studied in a more natural context by observing the freezing response of bees inside their hive. The freezing effect can be observed in response to tooting queens. When the comb is vibrated with suitable frequency and sufficiently high amplitude virtually all of thousands of bees on the comb stop instantaneously.[3] Frings and Little (1957) used the freezing response of worker honeybees to determine the frequency range of responses to substrate vibrations. The absolute vibrational amplitudes were not determined, instead the input voltage of the loudspeaker was used as a measure of amplitude. This early study underestimated the frequency range to which bees are sensitive. Thresholds of freezing responses to comb vibrations of defined amplitudes were recorded by Michelsen et al. (1986a) and by Rohrseitz and Kilpinen (1997). Honeybees appear to be most sensitive to vibrations in the range of 200–3000 Hz. At 300 Hz freezing is elicited as a response to vibrations of about 3 mms^{-1} (Michelsen et al. 1986a).

Because the relationship between acceleration, velocity and displacement amplitudes is frequency dependent, the very same thresholds seem to be lowest at high frequencies if we look at displacement amplitudes, but lowest at much lower frequencies if we consider acceleration amplitudes (Fig. 16.5). In a high-frequency range, model calculations suggest that the subgenual organ behaves like an overdamped system and that the sensory cells are displacement sensitive (Kilpinen and Storm 1997; for review of the functional morphology of the subgenual organ, see Lakes-Harlan and Strauß 2014). When comparing the vibrational perception threshold curves from all available studies, it appears that the sensitivity is quite constant over a wide range of frequencies when amplitudes are expressed as velocities (Fig. 16.5). This suggests that the sense organ is basically a velocity receptor over a wide range of frequencies.

16.5 Biotremology and Digitized Apiculture

The assessment of the colonies' status with respect to the number of adult workers, number of brood, pollen, honey storage, and health so far requires visual inspections by beekeepers. Particularly if swarming needs to be prevented, beekeepers have to

---

[3] The behaviour reminds the human observer of the fairy tale of the Sleeping Beauty (Grimm brothers) in which everybody living in the princess" castle freezes immediately and falls asleep for a hundred years when the princess pricks her finger on the needle of the spinning wheel. In honeybees the effect lasts less than a hundred years–bees restart moving after some few seconds.

inspect the colonies' status regularly. First attempts to reduce the required labour were made by Woods (1959), who introduced the "Apidictor". Based on the analysis of sounds produced in a hive, the "Apidictor" attempted to predict swarming. In recent years many efforts have been undertaken to establish more advanced monitoring systems for apiaries that might reduce the amount of labour required (reviewed by Kridi et al. 2016; Gil-Lebrero et al. 2017; Ramsey et al. 2017, 2018; Debauche et al. 2018; Howard et al. 2018; Seritan et al. 2018; Bencsik and Newton 2019; Henry et al. 2019; Hunter et al. 2019).

Sensors that measure temperature, humidity, sound, gases and vibrations are employed to monitor hive condition and predict colony development. Since vibrations are ubiquitous in honeybee colonies, they may indicate colony status. In the context of swarming, for example, it might be possible to predict a swarm's take-off (Bencsik et al. 2011). Furthermore, a correlation between vibrational amplitude and the brood cycle was found. Vibrations closely match the worker bee brood cycle. The maxima of the vibration amplitudes most probably correspond to the intervals between brood-rearing, i.e., when cells are empty (Bencsik et al. 2015).

In a recent study, it was stated that the stop signal and drumming induced by DAVs can be measured automatically (Ramsey et al. 2017). However, it became apparent that the detection of substrate-borne vibrational signals is entirely restricted to the immediate vicinity of sensors. Unless many sensors are used, these measurements cover only a very restricted area of the nest. It is doubtful that such limited measurements are suitable to reliably predict the colonies' state. To establish a long-term automated recording and analysis of vibrational signals may, combined with other colony parameters, become a sensitive tool for non-invasive and remote assessment of colony state. However, further studies are required to make reliable predictions that actually help beekeepers.

16.6 Conclusions

Whichever communication context is subject to research, we are increasingly confronted with the fact that multiple sensory modalities are employed. Honeybees make use of chemical, visual, tactile, acoustic and vibratory signals that contribute to their complex communication system. The relative importance of sensory modalities is largely unknown because it is often difficult to study them independently. This is particularly true for the dance language. Sophisticated experiments such as those employing robotic bees (Michelsen et al. 1992; Landgraf et al. 2018) considerably contributed to a systematic analysis of sensory channels.

It is now apparent that while dancing, two or more mechanical, plus one electric, channels might be involved, simultaneously. In addition, dancing bees emit chemical signals that are involved in the recruitment of foragers (Thom et al. 2007).

Communication takes place when a sender encodes information into a signal, sends it via a medium to a receiver that decodes information and as a response alters its behaviour (Maynard Smith and Harper 2003; Scott-Phillips 2008). To decipher a behaviour's message and meaning is challenging but a mandatory prerequisite to calling it a signal. So far, we can only refer to the stop signal and queen tooting and quacking as vibrational signals. In contrast, the messages and meanings of vibrations induced when performing DAV, tremulations while dancing, and irregular thoracic vibrations have not been studied sufficiently to name them vibrational signals.

With respect to signal transmission and perception, a great number of questions remains to be answered. The ability of bees to discriminate vibrational signals based on frequencies, amplitudes and temporal structure needs to be studied in much more detail. Orientation inside of the hive is basically dependent on chemical and mechanical cues. It is observed that workers are attracted by queen quacking signals. Like many other insects (Hager and Kirchner 2019), honeybees could orient vibrotactically in this context. So far directional vibration sensing in honeybees has not been studied.

Little is known about the evolution of vibrational communication. This is probably because the basis, on which hypotheses about the evolution of communication could be built, is not yet solid enough. Vibrational communication in *Apis* bees has nearly exclusively been studied in western honeybees. The Asian species merit much more attention in this respect. Vibrational signals are found in most bumble bees (Bombini) and stingless bees (Meliponini), the closest relatives of the genus *Apis* (Apini) (Hrncir et al. 2006b, Krausa et al. 2017). The fact that vibrational communication is employed by bumblebees and stingless bees indicates that communication via vibrations is ancestral. However, hardly any species has been studied comprehensively.

Although honeybees have been studied for hundreds of years, we are far away from a complete understanding of their biology and their communication system, in particular. It is still like von Frisch stated long ago (as reported by Hölldobler 1983; Lindauer 1987 and others): The life of the bee is like a magic well: The more you draw from it, the more there is to draw.

References

Allen MD (1959a) The occurrence and possible significance of the 'shaking' of honeybee queens by workers. Anim Behav 7:66–69

Allen MD (1959b) The 'shaking' of worker honeybees by other workers. Anim Behav 7:233–240

Autrum H, Schneider W (1948) Vergleichende Untersuchungen über den Erschütterungssinn der Insekten. Z vergl Physiol 31:77–88

Bencsik M, Newton MI (2019) Honey bee vibration monitoring using the 805M1 accelerometer. Proceedings of the 5th international electronic conference on sensors and applications. Proceedings 4(1):42

Bencsik M, Bencsik J, Baxter M, Lucian A, Romieu J, Millet M (2011) Identification of the honey bee swarming process by analysing the time course of hive vibrations. Comput Electron Agr 76: 44–50

Bencsik M, Le Conte Y, Reyes M, Pioz M, Whittaker D, Crauser D, Delso NS, Newton MI (2015) Honeybee colony vibrational measurements to highlight the brood cycle. PLoS One 10: e0141926

Boucher M, Schneider SS (2009) Communication signals used in worker–drone interactions in the honeybee, *Apis mellifera*. Anim Behav 78:247–254

Bruinsma O, Kruijt IP, van Dusseldorp W (1981) Delay of emergence of honey bee queens in response to tooting sounds. Proc K Ned Akad Wet C 84:381–387

Butler C (1609) The feminine Monarchie or a treatise concerning bees, and the due ordering of them. Joseph Barnes, Oxford

Camazine S, Visscher PK, Finley J, Vetter RS (1999) House-hunting by honey bee swarms: collective decisions and individual behaviors. Insect Soc 46:348–360

Cao TT, Hyland KM, Malechuk A, Lewis LA, Schneider SS (2007) The influence of the vibration signal on worker interactions with the nest and nest mates in established and newly founded colonies of the honey bee, *Apis mellifera*. Insect Soc 54:144–149

Cao TT, Hyland KM, Malechuk A, Lewis LA, Schneider SS (2009) The effect of repeated vibration signals on worker behavior in established and newly founded colonies of the honey bee, *Apis mellifera*. Behav Ecol Sociobiol 63:521–529

Cocroft RB, Gogala M, Hill PSM, Wessel A (2014) Fostering research progress in a rapidly growing field. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, Heidelberg, pp 3–12

Cronholm, B (1973) Award ceremony speech. NobelPrizeorg Nobel Media AB 2019 Fri 6 Dec 2019. <https://www.nobelprize.org/prizes/medicine/1973/ceremony-speech/>

Debauche O, Moulat ME, Mahmoudi S, Boukraa S, Manneback P, Lebeau F (2018) Web monitoring of bee health for researchers and beekeepers based on the internet of things. Procedia Comput Sci 130:991–998

Donahoe K, Lewis LA, Schneider SS (2003) The role of the vibration signal in the house-hunting process of honey bee (*Apis mellifera*) swarms. Behav Ecol Sociobiol 54:593–600

Dong S, Tan K, Zhang Q, Nieh JC (2019) Playbacks of Asian honey bee stop signals demonstrate referential inhibitory communication. Anim Behav 148:29–37

Dreller C, Kirchner WH (1993a) Hearing in honeybees: localization of the auditory sense organ. J Comp Physiol A 173:275–279

Dreller C, Kirchner WH (1993b) How honeybees perceive the information of the dance language. Naturwissenschaften 80:319–321

Esch H (1961a) Ein neuer Bewegungstyp im Schwänzeltanz der Bienen. Naturwissenschaften 48: 140–141

Esch H (1961b) Über die Schallerzeugung beim Werbetanz der Honigbiene. Z vergl Physiol 45:1–11

Esch H (1964) Beiträge zum Problem der Entfernungsweisung in den Schwänzeltänzen der Honigbiene. Z vergl Physiol 48:534–546

Frings H, Little F (1957) Reactions of honey bees in the hive to simple sounds. Science 125:122

Fuchs S, Koeniger N (1974) Schallerzeugung im Dienst der Verteidigung des Bienenvolkes (*Apis cerana*, Fabr.). Apidologie 5:271–287

Gahl RA (1975) The shaking dance of honey bee workers: evidence for age discrimination. Anim Behav 23:230–232

Gil M, De Marco RJ (2010) Decoding information in the honeybee dance: revisiting the tactile hypothesis. Anim Behav 80:887–894

Gilbert S, Lewis LA, Schneider SS (2010) The role of the vibration signal during nest-site selection by honey bee swarms. Ethology 117:254–264

Gil-Lebrero S, Quiles-Latorre FJ, Ortiz-López M, Sánchez-Ruiz V, Gámiz-López V, Luna-Rodríguez JJ (2017) Honey bee colonies remote monitoring system. Sensors 17:s17010055

Gilley DC (2001) The behaviour of honey bees (*Apis mellifera ligustica*) during queen duels. Ethology 107:601–622

Greggers U, Koch G, Schmidt V, Dürr A, Floriou-Servou PD, Göpfert MC, Menzel R (2013) Reception and learning of electric fields in bees. P Roy Soc B 280:20130528

Grooters HJ (1987) Influences of queen piping and worker behavior on the timing of emergence of honey bee queens. Insect Soc 34:181–193

Hager FA, Kirchner WH (2019) Directionality in insect vibration sensing: behavioral studies of vibrational orientation. In: Hill P, Lakes-Harlan R, Mazzoni V, Narins P, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational behavior. Animal signals and communication, vol 6. Springer, Cham

Hansson A (1945) Lauterzeugung und Lautauffassungsvermögen der Bienen. Opusc Entomol Suppl 6:1–124

Hasegawa Y, Ikeno H (2011) How do honeybees attract nestmates using waggle dances in dark and noisy hives? PLoS One 6:e19619

Henry E, Adamchuk V, Stanhope T, Buddle C, Rindlaub N (2019) Precision apiculture: development of a wireless sensor network for honeybee hives. Comput Electron Agr 156:138–144

Hill PSM (2008) Vibrational communication in animals. Harvard University Press, London

Hill PSM (2014) Stretching the paradigm or building a new? Development of a cohesive language for vibrational communication. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, Heidelberg, pp 13–30

Hill PSM, Wessel A (2016) Biotremology. Curr Biol 26:R181–R191

Hill PSM, Virant-Doberlet M, Wessel A (2019) What is biotremology? In: Hill PSM, Lakes-Harlan R, Mazzoni V, Narins PM, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational behavior. Springer, Berlin, Heidelberg, pp 15–25

Hölldobler B (1983) Karl von Frisch and the beginning of experimental behavioural ecology. Forts Zool 31:1–3

Howard D, Duran O, Hunter G (2018) A low-cost multi-modal sensor network for the monitoring of honeybee colonies/hives. Intell Environ 2018:69–78

Hrncir M, Schmidt VM, Schorkopf DLP, Jarau S, Zucchi R, Barth FG (2006a) Vibrating the food receivers: a direct way of signal transmission in stingless bees (*Melipona seminigra*). J Comp Physiol A 192:879–887

Hrncir M, Barth FG, Tautz J (2006b) Vibratory and airborne-sound signals in bee communication (hymenoptera). In: Drosopoulos S, Claridge MF (eds) Insect sound and communication – physiology, behaviour, ecology and evolution. CRC Press, Taylor & Francis Group, Boca Raton, pp 421–436

Hrncir M, Maia-Silva C, Mc Cabe SI, Farina WM (2011) The recruiter's excitement – features of thoracic vibrations during the honey bee's waggle dance related to food source profitability. J Exp Biol 214:4055–4064

Hrncir M, Maia-Silva C, Farina WM (2019) Honey bee workers generate low-frequency vibrations that are reliable indicators of their activity level. J Comp Physiol A 205:79–86

Huber F (1792) Nouvelles observations sur les abeilles. Paschoud, Geneva

Hunter G, Howard D, Gauvreau S, Duran O, Busquets R (2019) Processing of multi-modal environmental signals recorded from a 'smart' beehive. Proc Inst Acoust 41:337–348

Hyland KM, Cao TT, Malechuk AM, Lewis LA, Schneider SS (2007) Vibration signal behaviour and the use of modulatory communication in established and newly founded honeybee colonies. Anim Behav 73:541–551

Jack-McCollough RT, Nieh JC (2015) Honeybees tune excitatory and inhibitory recruitment signalling to resource value and predation risk. Anim Behav 110:9–17

Kastberger G, Weihmann F, Hoetzl T (2013) Social waves in giant honeybees (*Apis dorsata*) elicit nest vibrations. Naturwissenschaften 100:595–609

Kawakita S, Ichikawa K, Sakamoto F, Moriya K (2018) Hissing of *a. cerana japonica* is not only a direct aposematic response but also a frequent behavior during daytime. Insect Soc 65:331–337

Kawakita S, Ichikawa K, Sakamoto F, Moriya K (2019) Sound recordings of *Apis cerana japonica* colonies over 24 h reveal unique daily hissing patterns. Apidologie 50:204–214

Kietzman PM, Visscher PK (2015) The anti-waggle dance: use of the stop signal as negative feedback. Front Ecol Evol 3:1–5

Kietzman PM, Visscher PK, Lalor JK (2017) Stop signaling by foragers not encountering danger at a food source. Insect Soc 64:307–309

Kilpinen O, Storm J (1997) Biophysics of the subgenual organ of the honeybee, *Apis mellifera*. J Comp Physiol A 181:309–318

Kirchner WH (1993) Vibrational signals in the tremble dance of the honeybee, *Apis mellifera*. Behav Ecol Sociobiol 33:169–172

Kirchner WH, Dreller C (1993) Acoustical signals in the dance language of the giant honeybee, *Apis dorsata*. Behav Ecol Sociobiol 33:67–72

Kirchner WH, Sommer K (1992) The dance language of the honeybee mutant diminutive wings. Behav Ecol Sociobiol 30:181–184

Kirchner WH, Dreller C, Towne WF (1991) Hearing in honeybees: operant conditioning and spontaneous reactions to airborne sound. J Comp Physiol A 168:85–89

Kirchner WH, Dreller C, Grasser A, Baidya D (1996) The silent dances of the Himalayan honeybee, *Apis laboriosa*. Apidologie 27:331–339

Koeniger N, Fuchs S (1972) Kommunikative Schallerzeugung von *Apis cerana* Fabr. im Bienenvolk. Naturwissenschaften 4:169

Krausa K, Hager FA, Kiatoko N, Kirchner WH (2017) Vibrational signals of African stingless bees. Insect Soc 64:415–424

Kridi DS, de Carvalho CGN, Gomes DG (2016) Application of wireless sensor networks for beehive monitoring and in-hive thermal patterns detection. Comput Electron Agr 127:221–235

Lakes-Harlan R, Strauß J (2014) Functional morphology and evolutionary diversity of vibration receptors in insects. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Heidelberg, pp 277–302

Landgraf T, Bierbach D, Kirbach A, Cusing R, Oertel M, Lehmann K, Greggers U, Menzel R, Rojas R (2018) Dancing honey bee robot elicits dance-following and recruits foragers. arXiv:1803.07126

Laomettachit T, Termsaithong T, Sae-Tang A, Duangphykdee O (2016) Stop-signaling reduces split decisions without impairing accuracy in the honeybee nest-site selection process. J Insect Behav 29:557–577

Lau CW, Nieh JC (2010) Honey bee stop-signal production: temporal distribution and effect of feeder crowding. Apidologie 41:87–95

Lewis LA, Schneider SS (2000) The modulation of worker behavior by the vibration signal during house hunting in swarms of the honeybee, *Apis mellifera*. Behav Ecol Sociobiol 48:154–164

Lewis LA, Schneider SS, Degrandi-Hoffman G (2002) Factors influencing the selection of recipients by workers performing vibration signals in colonies of the honeybee, *Apis mellifera*. Anim Behav 63:361–367

Lindauer M (1952) Ein Beitrag zur Frage der Arbeitsteilung im Bienenstaat. Z vergl Physiol 34: 299–345

Lindauer M (1955) Schwarmbienen auf Wohnungssuche. Z vergl Physiol 37:263–324

Lindauer M (1987) Introduction. In: Menzel R, Mercer A (eds) Neurobiology and behavior of honeybees. Springer, Berlin, Heidelberg, pp 1–6

Maynard Smith J, Harper D (2003) Animal Signals. Oxford University Press

McIndoo NE (1922) The auditory sense of the honeybee. J Comp Neurol 34:173–199

Michelsen A, Kirchner WH, Lindauer M (1986a) Sound and vibrational signals in the dance language of the honeybee, *Apis mellifera*. Behav Ecol Sociobiol 18:207–212

Michelsen A, Kirchner WH, Andersen BB, Lindauer M (1986b) The tooting and quacking vibration signals of honeybee queens: a quantitative analysis. J Comp Physiol A 158:605–611

Michelsen A, Towne WF, Kirchner WH, Kryger P (1987) The acoustic near field of a dancing honeybee. J Comp Physiol A 161:633–664

Michelsen A, Andersen BB, Kirchner WH, Lindauer M (1989) Honeybees can be recruited by a mechanical model of a dancing bee. Naturwissenschaften 76:277–280

Michelsen A, Andersen BB, Storm J, Kirchner WH, Lindauer M (1992) How honeybees perceive communication dances, studied by means of a mechanical model. Behav Ecol Sociobiol 30: 143–150

Michener CD (2000) The bees of the world. Johns Hopkins University Press, Baltimore, MD

Milum VG (1955) Honey bee communication. Amer Bee J 95:97–104

Nachtigall W (2003) Insektenflug. Springer Verlag, Berlin, Heidelberg, New York

Nieh JC (1993) The stop signal of honey bees: reconsidering its message. Behav Ecol Sociobiol 33: 51–56

Nieh JC (1998) The honey bee shaking signal: function and design of a modulatory communication signal. Behav Ecol Sociobiol 42:23–36

Nieh JC (2010) A negative feedback signal that is triggered by peril curbs honey bee recruitment. Curr Biol 20:310–315

Nieh JC, Tautz J (2000) Behaviour-locked signal analysis reveals weak 200-300 Hz comb vibrations during the honeybee waggle dance. J Exp Biol 203:1573–1579

Painter-Kurt S, Schneider SS (1998a) Age and behavior of honey bees, *Apis mellifera* (hymenoptera: Apidae), that perform vibration signals on workers. Ethology 104:457–473

Painter-Kurt S, Schneider SS (1998b) Age and behaviour of honey bees, *Apis mellifera* (hymenoptera: Apidae), that perform vibration signals on queens and queen cells. Ethology 104:475–485

Pastor KA, Seeley TD (2005) The brief piping signal of the honey bee: begging call or stop signal? Ethology 111:775–784

Pierce AL, Lewis LA, Schneider SS (2006) The use of the vibration signal and worker piping to influence queen behaviour during swarming in honey bees, *Apis mellifera*. Ethology 113:267–275

Pratt SC, Kühnholz S, Seeley TD, Weidenmüller A (1996) Worker piping associated with foraging in undisturbed queenright colonies of honey bees. Apidologie 27:13–20

Ramsey M, Bencsik M, Newton MI (2017) Long-term trends in the honeybee 'whooping signal' revealed by automated detection. PLoS One 12:e0171162

Ramsey M, Bencsik M, Newton MI (2018) Extensive vibrational characterisation and long-term monitoring of honeybee dorso-ventral abdominal vibration signals. Sci Rep 8:14571

Rohrseitz K, Kilpinen O (1997) Vibrational transmission characteristics of the legs of freely standing honeybees. Zoology 100:80–84

Rohrseitz K, Tautz J (1999) Honey bee dance communication: waggle run direction coded in antennal contacts? J Comp Physiol A 184:463–470

Sandeman DC, Tautz J, Lindauer M (1996) Transmission of vibration across honeycombs and its detection by bee leg receptors. J Exp Biol 199:2585–2594

Schlegel T, Visscher PK, Seeley TD (2012) Beeping and piping: characterization of two mechano-acoustic signals used by honey bees in swarming. Naturwissenschaften 99:1067–1071

Schneider SS (1986) The vibration dance activity of successful foragers of the honeybee, *Apis mellifera* (hymenoptera: Apidae). J Kansas Entomol Soc 59:699–705

Schneider SS, Lewis LA (2004) The vibration signal, modulatory communication and the organization of labor in honey bees, *Apis mellifera*. Apidologie 35:117–131

Schneider SS, Painter-Kurt S, Degrandi-Hoffman G (2001) The role of the vibration signal during queen competition in colonies of the honeybee, *Apis mellifera*. Anim Behav 61:1173–1180

Schön A (1911) Bau und Entwicklung des tibialen Chordotonalorgans bei der Honigbiene und bei Ameisen. Zool Jb (Anat) 31:439–472

Scott-Phillips TC (2008) Defining biological communication. J Evol Biol 21:387–395

Seeley TD (1992) The tremble dance of the honey bee: message and meanings. Behav Ecol Sociobiol 31:375–383

Seeley TD (1994) Honey bee foragers as sensory units of their colonies. Behav Ecol Sociobiol 34: 51–62

Seeley TD, Tautz J (2001) Worker piping in honey bee swarms and its role in preparing for liftoff. J Comp Physiol A 187:667–676

Seeley TD, Visscher PK (2003) Choosing a home: how the scouts in a honey bee swarm perceive the completion of their group decision making. Behav Ecol Sociobiol 54:511–520

Seeley TD, Morse RA, Visscher PK (1979) The natural history of the flight of honey bee swarms. Psyche 86:103–113

Seeley TD, Weidenmüller A, Kühnholz S (1998) The shaking signal of the honey bee informs workers to prepare for greater activity. Ethology 104:10–26

Seeley TD, Visscher PK, Schlegel T, Hogan PM, Franks NR, Marshall JAR (2012) Stop signals provide cross inhibition in collective decision-making by honeybee swarms. Science 335:108–111

Sen Sarma M, Fuchs S, Werber C, Tautz J (2002) Worker piping triggers hissing for coordinated colony defence in the dwarf honeybee *Apis florea*. Zoology 105:215–223

Seritan GC, Enache BA, Argatau FC, Adochiei FC, Toader S (2018) Low cost platform for monitoring honey production and bees health. In: IEEE International Conference on Automation, Quality and Testing, Robotics (AQTR). IEEE, Cluj-Napoca. https://doi.org/10.1109/AQTR.2018.8402704

Simpson J (1964) The mechanism of honey-bee queen piping. Z vergl Physiol 48:277–282

Simpson J, Cherry SM (1969) Queen confinement, queen piping and swarming in *Apis mellifera* colonies. Anim Behav 17:271–278

Skaggs R, Jackson JC, Toth AL, Schneider SS (2014) The possible role of ritualized aggression in the vibration signal of the honeybee, *Apis mellifera*. Anim Behav 98:103–111

Slone JD, Stout TL, Huang ZY, Schneider SS (2012) The influence of drone physical condition on the likelihood of receiving vibration signals from worker honey bees, *Apis mellifera*. Insect Soc 59:101–107

Snodgrass RE (1956) Anatomy of the honey bee. Comstock, Ithaca and London

Storm J, Kilpinen O (1998) Modelling the subgenual organ of the honeybee, *Apis mellifera*. Biol Cybern 78:175–182

Strauß J, Stritih-Peljhan N, Lakes-Harlan R (2019) Determining vibroreceptor sensitivity in insects: the influence of experimental parameters and recording techniques. In: Hill PSM, Lakes-Harlan R, Mazzoni V, Narins PM, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational behavior. Springer, Berlin, Heidelberg, pp 209–233

Tan K, Dong S, Li X, Liu X, Wang C, Li J, Nieh JC (2016) Honey bee inhibitory signalling is tuned to threat severity and can act as a colony alarm signal. PLoS Biol 14:e1002423

Tautz J (1996) Honeybee waggle dance: recruitment success depends on the dance floor. J Exp Biol 199:1375–1381

Tautz J, Casas J, Sandeman D (2001) phase reversal of vibratory signals in honeycomb may assist dancing honeybees to attract their audience. J Exp Biol 204:3737–3746

Tautz J, Rohrseitz K (1998) What attracts honeybees to a waggle dancer? J Comp Physiol A 183: 661–667

Tautz J, Rohrseitz K, Sandeman DC (1996) One-strided waggle dance in bees. Nature 382:32

Thom C, Gilley DC, Tautz J (2003) Worker piping in honey bees (*Apis mellifera*): the behavior of piping nectar foragers. Behav Ecol Sociobiol 53:199–205

Thom C, Gilley DC, Hooper J, Esch HE (2007) The scent of the waggle dance. PLoS Biol 5:e228

Thurm U (1964) Mechanoreceptors in the cuticle of the honey bee: fine structure and stimulus mechanism. Science 145:1063–1065

Towne WF (1985) Acoustic and visual cues in the dances of four honey bee species. Behav Ecol Sociobiol 16:185–187

Towne WF, Kirchner WH (1989) Hearing in honey bees: detection of air-particle oscillations. Science 244:686–688

Visscher PF, Seeley TD (2007) Coordinating a group departure: who produces the piping signals on honeybee swarms? Behav Ecol Sociobiol 61:1615–1621

Visscher PK, Shepardson J, McCart L, Camazine S (1999) Vibration signal modulates the behavior of house-hunting honey bees (*Apis mellifera*). Ethology 105:759–769

von Frisch K (1919) Über den Geruchssinn der Bienen und seine blÜtenbiologische Bedeutung. Zool Jb (Physiol) 37:1–238

von Frisch K (1967) The dance language and orientation of bees. Belknap Press of Harvard University Press, Cambridge MA

Waddington KD, Kirchner WH (1992) Acoustical and behavioural correlates of profitability of food sources in honey bee round dances. Ethology 92:1–6

Wenner AM (1962a) Communication with queen honey bees by substrate sound. Science 138:446–448

Wenner AM (1962b) Sound production during the waggle dance of the honey bee. Anim Behav 10: 79–95

Woods EF (1959) Electronic prediction of swarming in bees. Nature 184:842–844

Chapter 17
Vibrational Communication Outside and Inside the Nest in Leaf-Cutting Ants

Flavio Roces

Abstract Ants live in complex societies that organize their activities, as all animal societies, mostly by means of communication. While chemical communication via pheromones is ubiquitous in ants, increasing evidence points at the use of stridulatory vibrations not only as modulators of chemical communication signals but also as releasers of context-specific behaviors. Leaf-cutting ants, particularly those of the genus *Atta*, have extensively been investigated concerning both physiological and behavioral aspects of their vibrational communication, and they therefore provide the most comprehensive example of the use of vibrational signals as organizers of social behavior in ants. In this chapter, I summarize pioneer, early studies on signal production, response sensitivity thresholds, and function of stridulation in *Atta* leaf-cutting ants. I then follow with more recent laboratory and field investigations highlighting different contexts, both outside and inside the nest, in which leaf-cutting ants employ mechanical communication to coordinate their behaviors. These encompass cutting behavior and recruitment of nestmates, leaf transport, leaf processing inside the nest, nest excavation, and underground waste disposal. It will be argued that the response to stridulatory signals in leaf-cutting ants, despite their elementary and unitary character, strongly depends on the social context in which the receivers are situated.

17.1 Introduction

Ant colonies are highly organized societies without central control, which rely on communication signals from different modalities for the organization of their tasks (Hölldobler and Wilson 1990). It has often been argued that vibrations are less common than other communication signals, particularly pheromones. This view may be true, yet rests on the facts that research on pheromones and particularly ant

---

F. Roces (✉)
Department of Behavioral Physiology and Sociobiology, Biocenter, University of Würzburg, Würzburg, Germany
e-mail: roces@biozentrum.uni-wuerzburg.de

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_17

pheromones has a long tradition (Karlson and Butenandt 1959; Wilson and Bossert 1963), and also that only few studies have experimentally addressed the use of vibrational signals in ants, as compared to the bulk of literature on pheromone communication (Morgan 2008). Ants are well known to communicate via tactile displays, pheromones, and the substrate-borne components of their mechanical signals, thus making them tempting model systems for the emerging field of Biotremology (Hill and Wessel 2016). Communication via mechanical signals is in fact common in social insects (Hunt and Richard 2013), and not only new examples in different social insect species accumulated over the years but also new contexts for which the specific use and function of vibrational communication were clarified.

For ants in general, three mechanisms of sound production have been described so far: knocking or "drumming", scratching, and stridulation (e.g., Sharp 1893; Markl and Fuchs 1972; Rohe and Rupprecht 2001), and the produced mechanical signals mediate alarm, food recruitment, interruption of mating, and may also modulate the receiver's response to communication signals of other modalities. First reports of sound-producing ants trace back to the 1880s (Forbes 1881; Peal 1881) and first detailed morphological descriptions of both the stridulatory apparatus and chordotonal organs in ants were also published at that time (Lubbock 1877; Graber 1881). The presence of chordotonal organs and the ability to produce human-audible sounds led to the hypothesis that ants may be sensitive to both the airborne- and substrate-borne components of the produced signals. Experimental studies, however, have so far provided no evidence that ants respond to airborne vibrations (Fielde and Parker 1904; Autrum 1936; Haskins and Enzmann 1938). More recent claims that ants can hear stridulatory signals produced by nestmates as near-field sound (Hickling and Brown 2000) were challenged by calculations of the amplitude of the near-field particle oscillation around a stridulating ant, and by comparisons with the sensitivity threshold of the ant's sensory receptors described so far. The calculated amplitude was at least 50 times lower than the sensitivity threshold, a fact that precludes the perception of near-field sounds (Roces and Tautz 2001). So far, there is no compelling evidence that ants may use airborne sounds for communication, as is the case for substrate-borne drumming (Markl and Fuchs 1972) and stridulatory vibrations (Roces et al. 1993).

Leaf-cutting ants form one of the most complex insect societies, characterized by extremely large colony sizes, marked polymorphism and task allocation among workers, and their underground agriculture, i.e., the maintenance of gardens of a symbiotic fungus that represents the main food source for the developing brood (Weber 1972; Fröhle and Roces 2009; Roces and Bollazzi 2009). To organize their social behaviors, leaf-cutting ants rely very strongly on chemical signals, particularly during recruitment to newly discovered plants (Kleineidam et al. 2007; Hölldobler and Wilson 2011). In fact, the very first ant trail pheromone chemically identified was that of the leaf-cutting ant *Atta texana* (Tumlinson et al. 1971), with workers showing graded responses to changes in pheromone concentration (Robinson et al. 1974). Besides chemical communication, the production of stridulatory vibrations by leaf-cutting ants was recognized already at the end of the 19th and the beginning of the twentieth century in *Atta cephalotes* and *Atta fervens* (actually *Atta texana*), respectively (Sharp 1893; Wheeler 1903), although their function remained initially elusive until the discovery of one of their functions, namely alarm communication (Markl 1965).

Together with Bert Hölldobler, we have reviewed several aspects of the behavioral ecology of stridulatory communication in leaf-cutting ants several years ago (Hölldobler and Roces 2001), particularly emphasizing the production of vibrational signals during foraging and their use in the context of multimodal communication. Since then, a few studies on stridulation in leaf-cutting and other fungus-growing ants have been published. It is not because of the admittedly reduced number of following studies on the topic that a review on vibrational communication in leaf-cutting ants appears worth writing. More exciting appears to be the fact that recent studies revealed the use of stridulatory signals during foraging under natural field conditions and also inside the nest during the organization of both collective nest excavation and underground disposal of colony refuse. Leaf-cutting ants emerged, as I outline in this chapter, as the ant group for which studies on vibrational communications highlighted both mechanistic aspects such as signal production and sensitivity thresholds, as well as adaptive aspects such as a number of functions of stridulatory vibrations and the context-specificity of responses to them.

17.2 Vibrational Communication during Foraging

17.2.1 Stridulation as Short-Range Recruitment Signal

Ants stridulate by raising and lowering their gasters, so that a cuticular file located on the first gastric tergite is rubbed against a scraper situated on the preceding third abdominal segment (postpetiole), whereby a series of so-called “chirps” synchronized with the gaster movements are produced (Spangler 1967). In *Atta cephalotes* leaf-cutting ants, a single chirp is produced while the gaster is moving up, which is much stronger than the chirp produced during its downward movement (Markl 1968; Roces et al. 1993), as observed in other fungus-growing ants of the genus *Trachymyrmex* (Carlos et al. 2014), and also in seed-harvester ants (Spangler 1967). Sometimes the scraper may not contact the cuticular file during the downward movement, so that no chirp would be produced (Markl 1968; Carlos et al. 2014). Each chirp is composed of a sequence of clicks that results from the impact of the scraper against each ridge of the cuticular file (*pars stridens*), which comprises, depending of ant body size, ca. 40–100 ridges (Markl 1968; Kermarrec et al. 1976; Carlos et al. 2014). The duration of each single upwards and downwards chirp, therefore, appears to directly depend on the number of ridges impacted on the file during the stridulatory movements, since there is so far no evidence that ants modulate the duration of each single chirp by changing the speed of their gaster movements (which is exactly what blood-sucking bugs do to produce distinct, context-specific vibrational signals by rubbing the tip of their proboscis against the groove of the prosternal file: single chirps of alarm stridulations are roughly four times longer than those of male-deterring stridulations; Roces and Manrique 1996). Nor that the observed variability in chirp duration and chirp repetition rate in leaf-cutting ants (Carlos et al. 2018), as it will be outlined below, conveys information for recipient workers.

Leaf-cutting ant workers stridulate whenever they are prevented from moving freely. This may occur, for instance, when part of the colony is confined by a cave-in of the nest: buried workers stridulate and attract nestmates, which subsequently engage in rescue digging (Markl 1965, 1967). Stridulation was therefore considered to serve primarily as an alarm and underground distress signal, and although early studies have discussed the evolution of stridulatory communication in ants for this particular context (Markl 1973), a recent detailed phylogenetical comparison regarding the presence of the stridulatory organ among arboreal and ground-nesting ant genera disproved the view that stridulation in ants first evolved as an underground signal to alert nestmates for rescue digging (Golden and Hill 2016).

In the 1990s, during my postdoctoral stage at the Department of Behavioral Physiology and Sociobiology of the University of Würzburg, headed by Bert Hölldobler, we discovered a hitherto unknown function of stridulation in ants, which also illustrated the significance of context-specific responses to communication signals: *Atta cephalotes* workers stridulated when they cut an attractive leaf. Nearby workers responded to the stridulatory vibrations transmitted through the plant material by orienting towards the source of the vibrations. As it often occurs in science, the discovery of stridulation during cutting was accidental. At that time, I was particularly interested in the organization of foraging behavior in leaf-cutting ants, and addressed questions related to fragment-size selection, communication signals, and the energetics of leaf-cutting (Roces and Hölldobler 1994; Roces and Lighton 1995). To characterize the mandible movements during cutting, I videotaped ants on a small rotating platform while cutting pieces of scented Parafilm as "pseudoleaves," a simple method I developed to standardize the mechanical and chemical properties of the material to be cut (Roces 1990). Fascinated by the use of a very strong macro lens and high-speed recording, I was curious not only about the mandible movements, but also about the position of the legs and body axis while cutting, and displaced the focus of the camera lens back and forth regularly. I noted that a number of workers displayed dorsoventral motions with their gasters while cutting, with a pattern nearly identical to that performed by restrained stridulating workers. We verified that leaf-cutting ants produced stridulation signals while cutting by employing noninvasive laser-Doppler vibrometry. For that, we offered several leaves of privet individually pinned to the foraging table of a laboratory colony of *Atta cephalotes*, and as soon as a worker started to cut at the leaf edge, we carefully removed the leaf from the nest table and pinned it on a vibration-buffered table, while the ant continued with its cutting behavior. The laser beam was focused on a small white spot (2 mm diameter), painted on the leaf ca. 2 cm away from the cutting site. When a worker stridulated during cutting, vibrational signals were recorded on the leaf surface, which also traveled along the stem for several centimeters. They consisted of long series of chirps, similar to those observed during

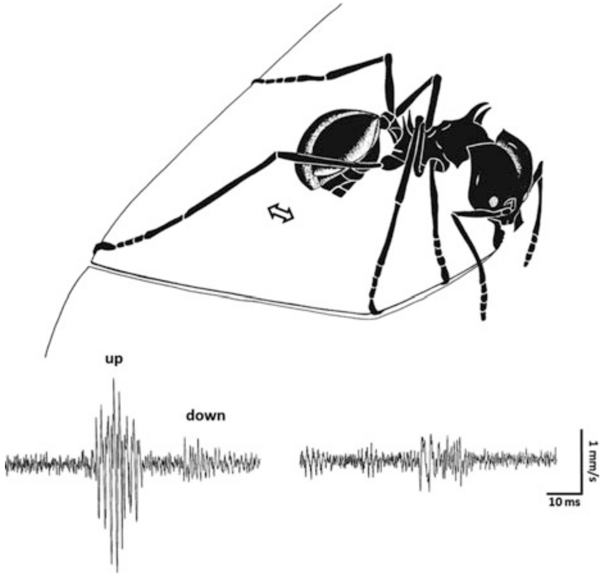


Fig. 17.1 Drawing of an *Atta* worker stridulating by moving its gaster up and down while cutting a leaf. The arrow denotes the gaster movements during signal production (top). Stridulatory signals produced by a single worker of the leaf-cutting ant *Atta cephalotes* engaged in cutting, recorded as velocity of the leaf's vibration via laser-Doppler-vibrometry (bottom). Measurements were performed on the leaf surface, approximately 2 cm away from the cutting site. On the left, substrate-borne vibrations transmitted mostly through the mandibles while cutting. "Up" and "down" denote the direction of the gaster's movement. On the right, vibrations transmitted into the substrate only through the legs, when the worker finished its cut and stood on the leaf. Drawing by Griselda Roces, with permission. Bottom part of the figure from Roces et al. (1993), modified

alarm vibrations in the same species (Markl 1968; Masters et al. 1983), repeated at a rate that varied between 2 and 20 chirps per second (Roces et al. 1993). Stridulations, markedly attenuated as compared to those recorded while cutting (Fig. 17.1), could also be recorded after the workers finished the cut and stood freely on the leaf with their loads.

Not all ants stridulated while cutting. However, the probability to stridulate, yet not the repetition rate of the signal, strongly depended on the quality of the leaf being cut. When presented with leaves of two different grades of toughness, i.e., thin and thick privet leaves, which strongly differed in their acceptance, significantly more ants stridulated while cutting the more attractive thin leaves. When the quality of the two kinds of leaves was increased by coating them with sugar, almost all workers stridulated while cutting, irrespective of the different mechanical properties of the leaves (Roces et al. 1993).

The observed relationship between leaf quality and stridulation prompted the idea that stridulations by foraging leaf-cutting ants may attract workers to the cutting site, acting therefore as short-range recruitment signals. This idea was explored in the laboratory by presenting foraging workers with a choice between two stems of

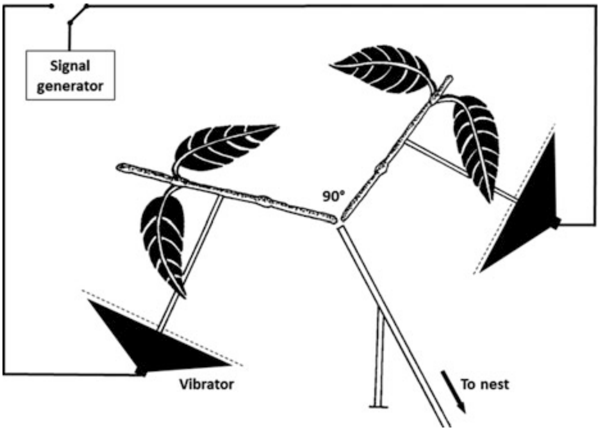


Fig. 17.2 Set-up to present workers in the context of foraging with a binary choice between two stems of privet. Single stems could be marked with pheromones or not, depending on the posed question, and/or vibrated using play backs of either synthetic or natural stridulatory signals, which were transmitted to the vibrators. A shield in front of them precluded air movements that might disturb ants. Drawing by Griselda Roces (with permission; modified from Roces et al. 1993)

privet: a “stridulating” (test) stem and a “silent” (control) one, and by recording their choice when walking towards a harvesting site. Each stem was attached to a membrane of a loudspeaker that served as a vibrator, and stridulations were played back to one of the sides, which was changed after a single worker was tested (Fig. 17.2). Putative chemical cues that could affect the workers’ choice were excluded. It was observed that significantly more ants chose the vibrating stem as compared to the silent side, clearly indicating that substrate-borne stridulatory vibrations alone, in the absence of recruitment pheromones, can act as short-range recruitment signals (Roces et al. 1993).

An additional line of evidence based on field studies also indicated that stridulatory vibrations alone can serve as short-range recruitment signals. The grasslands of the South American Gran Chaco are inhabited by a number of ant species. Colonies of the grass-cutting ant *Atta vollenweideri* build conspicuous conical or ellipsoidal-shaped mounds that are exposed to seasonal floods (Pielström and Roces 2014) and maintain a system of superficial trunk trails that lead to harvesting areas where workers mainly cut grass fragments (Röschard and Roces 2003a) for the maintenance of their underground fungus gardens. To address the question of whether workers engaged in cutting grass fragments stridulate and attract nestmates to join at the cutting site, we monitored colonies located at the Biological Reserve “El Bagual” (Formosa, Argentina) and recorded stridulations produced by workers while cutting the grass *Paspalum intermedium*, a densely tufted, yearlong green and perennial grass that may reach up to 100 cm in height. During foraging, workers climb on a single grass blade until reaching the grass tip, turn down and up 2–3 times after having walked very short distances, and finally start to cut across the grass width, which results in the selection of a longish fragment that is either carried directly to

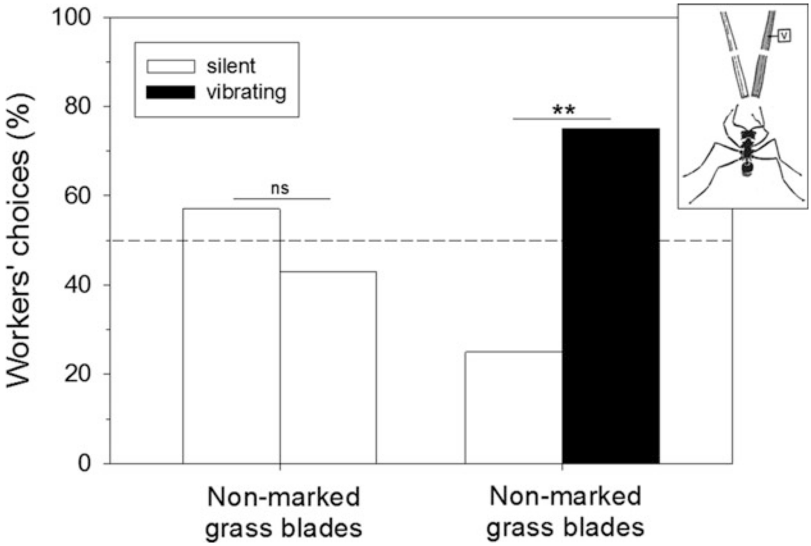


Fig. 17.3 Number of recruited workers of the leaf-cutting ant *Atta vollenweideri* that chose between a silent and a vibrating grass blade in the field, as shown in the drawing on the right upper corner. On the left, an experiment with the two grass blades silent and not marked with trail pheromone, to control for potential side biases in the setting ($n = 60$). On the right, non-marked grass blades were also offered, one of them vibrating using a playback of natural stridulatory signals ("V" in the drawing denotes the vibrator used for playbacks) ($n = 70$). Statistics: "**" indicates that the distribution of single choices differs statistically from the ratio 1:1, after a log-likelihood G-test for goodness of fit to the ratio 1:1; "ns" indicates that choices were not significantly different from the ratio 1:1. Drawing by Malu Obermayer, with permission

the nest or dropped to the ground for further transport (Röschard and Roces 2003b). It is unlikely that workers deposit a pheromone while walking up a long grass blade before the initiation of a cut, as no typical trail-marking behavior, i.e., dragging or tipping the gaster on the grass blade while walking, can be observed. By gently attaching an accelerometer to the grass blade being cut, stridulations produced by roughly 90% of workers engaged in cutting were recorded. Vibrations produced by the mechanism of stridulating traveled along the blades for distances up to 80 cm, attenuating at an average of 0.6 dB/cm. Playbacks of the recorded stridulations were alone sufficient to attract nearby workers, which readily climbed the vibrating grass blade, in a choice experiment. As in the laboratory experiments described in this section, single workers approaching a *Paspalum* plant adjacent to a trunk trail, i.e., those in the foraging context, were confronted with a choice between a vibrating and a silent grass blade, both experimentally removed from a distant plant and therefore not pheromone-marked. Stridulations were played back via a vibrator attached to one of the blades (Fig. 17.3; unpublished results). It was observed that significantly more ants chose the vibrating grass blade, providing the first evidence that substrate-borne stridulatory vibrations are used under natural conditions and can act as short-range recruitment signals alone, in the absence of recruitment pheromones.

17.2.2 Stridulatory Signals in the Presence of Pheromones

The use of stridulatory signals during food recruitment is also known for other ant species, in which vibrations act as modulators of chemical signals and not as releasers of specific behaviors (Hölldobler 1999). Ants of the genera *Aphaenogaster* and *Messor* lay pheromone trails and produce stridulatory vibrations during recruitment of nestmates to attractive food sources. The chemical recruitment is enhanced by the presence of stridulatory vibrations (Markl and Hölldobler 1978; Hahn and Maschwitz 1985; Baroni-Urbani et al. 1988), but there is no evidence that vibrations alone trigger a recruitment response, as is the case in leaf-cutting ants.

The extent to which pheromones and stridulatory vibrations may act in conjunction in leaf-cutting ants was explored in the laboratory. The experimental set-up (Fig. 17.2; see Sect. 17.2.1) enabled us to combine the two communication modalities, stridulatory vibrations, and trail pheromone, and also to provide them as competing signals in a binary choice. Facing a choice between a "silent" stem marked with synthetic trail pheromone (a solution of 0.05 ng/μl 4-methylpyrrole-2-carbolylic acid in hexane, applied to produce a pheromone trail with 1 μl of the solution over 5 cm) and an unmarked "stridulating" stem, significantly more workers preferred the chemically-marked, yet silent stem (Hölldobler and Roces 2001). Similar results were obtained for an artificial trail laid with a pheromone solution diluted by one order of magnitude, which is known to still attract foragers (Robinson et al. 1974), and also for natural trails laid on the experimental stem by a single loaded forager, indicating that the use of the synthetic main component of the *Atta* trail pheromone does not invalidate the approach. When both stems were marked with trail pheromone but only one was vibrating, however, the "stridulating" stem was markedly preferred (Hölldobler and Roces 2001). Clearly, outgoing *Atta* foragers rely more on recruitment pheromones than on substrate-borne stridulations, yet the response to the recruitment pheromone is much higher when the chemical signal is combined with vibrational signals. Under natural conditions, pheromone trails may lead ants, for instance to a tree crown, but it appears unlikely that trails may end up in the close vicinity of particular leaves to be cut. Successful workers stridulating while cutting may therefore attract nearby nestmates solely via the stridulatory vibrations transmitted through the plant, which may be locally used to orient to the actual cutting site.

While the above examples refer to the combined use of vibrations and pheromones in the foraging context, the two communication modalities are also used in the alarm context (Markl 1967), in which workers release alarm pheromones and stridulate whenever they are prevented from moving freely, for instance during a nest cave-in. But even in the foraging context, alarm pheromones may be released and impact on the response of ants to stridulatory vibrations. Hager et al. (2017) recently explored the influence of an alarm pheromone on directional sensitivity to vibrations in leaf-cutting ants. They used Citral as alarm compound, a monoterpene from the mandibular gland of *Atta sexdens* leaf-cutting ants that deters workers (Blum et al. 1968; Roces 1994), yet the focus of their elegant study was on the mechanisms underlying the directional vibration sensing. The authors discovered that workers use time differences as small as 0.1 ms in the arrival of a vibration between contralateral legs to turn and orient to the signal source. Regarding the influence of alarm pheromone perception on directional sensitivity, ants exposed to a low dose of Citral still preferred the side from which a vibrational signal arrived first. However, ants showed no side preferences, i.e., no time-of-arrival-based directional sensitivity, for higher doses of Citral. Ants in the foraging context, therefore, appear to ignore time-based directional cues when exposed to high doses of an alarm pheromone (Hager et al. 2017). The fact that ants still showed directional sensitivity when exposed to a low dose of Citral may not necessarily indicate that vibrations and alarm pheromones serve as multimodal communication signals in this context; it is possible that such a low Citral dose is below threshold and therefore elicits no change in the expected response of workers.

We have asked a different question regarding the effect of Citral as an alarm pheromone on the ants' response to stridulations, which extends the findings by Hager et al. (2017) outlined in this section. Namely, whether the presence of Citral changes the context from foraging to alarm and prompts workers to orient to a source of stridulatory vibrations in a choice situation. We used again the experimental choice set-up (Fig. 17.2; see Sect. 17.2.1) to present workers with a choice between stems marked with Citral that were combined with presence or absence of stridulatory vibrations, as follows. First, we explored whether foraging workers coming from the nest would orient to a source of Citral and show alarm behaviors, as expected if the presence of the Citral leads to a context change. Ants presented with a choice between two non-vibrating stems, one marked with Citral and the other non-marked, clearly preferred the marked one (Fig. 17.4, left; unpublished results). Workers ran over the marked stem with increased velocity and opened mandibles, showing that Citral was perceived and elicited a change of contexts, from foraging to alarm. Secondly, a control series with both non-vibrating stems marked with Citral evinced no side bias in the experimental set-up (Fig. 17.4, middle; unpublished results). Finally, when the two stems were marked with Citral and only one of them vibrated, workers strongly preferred the vibrating one (Fig. 17.4, right; unpublished results), which indicates that in the context of alarm, elicited by the perception of Citral, workers show clear directional alarm responses to a source of stridulatory vibrations.

If alarm stridulations can be released at the cutting site and so communicate danger, the question arose whether workers would be able to differentiate such alarm stridulations from cutting stridulations, i.e., those produced, for instance, by nearby workers engaged in cutting that may have not noticed the danger. So far, there is no evidence that ants can extract information from the repetition rate of stridulations, or its temporal modulation. Alarm and cutting stridulations do not differ in the structure of their single chirps, yet in their repetition rate. While alarm stridulations are monotonically repeated at almost invariant rates around 5 to 7 chirps per second (Markl 1965, 1968), cutting stridulations occur most often when the mandibles are closing, reaching rates up to 10–15 chirps/s over 2–3 seconds, and not at all in the brief pauses between the single bites (Tautz et al. 1995).

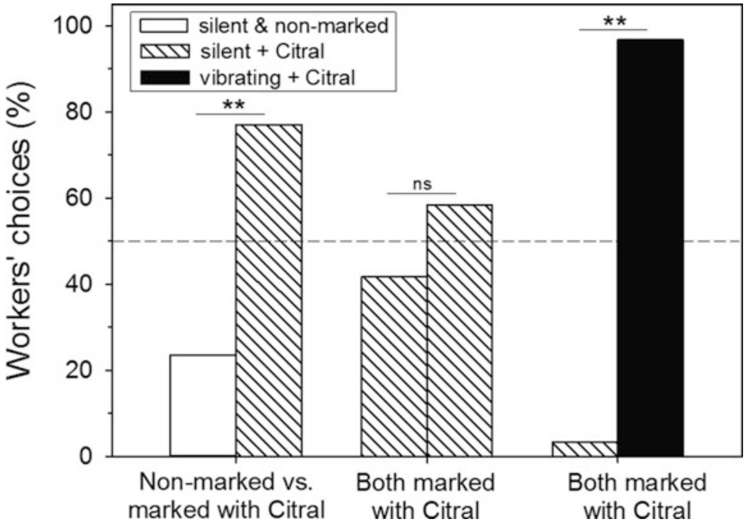


Fig. 17.4 Number of recruited workers of the leaf-cutting ant *Atta cephalotes* that chose between two stems of privet in a laboratory setting. These were marked with alarm pheromones and/or vibrated, depending on the posed question. On the left, an experiment presenting a non-marked vs. a Citral-marked stem, both silent ($n = 60$). In the middle, both stems were silent and marked with Citral, to control for potential side biases in the setting ($n = 60$). On the right, both stems were marked with Citral, and one of them vibrated via playbacks of natural stridulatory signals ($n = 60$). Statistics: "**" indicates that the distribution of single choices differs statistically from the ratio 1:1, after a log-likelihood G-test for goodness of fit to the ratio 1:1; "ns" indicates that choices were not significantly different from the ratio 1:1

To investigate whether foraging ants may distinguish between a source of alarm stridulation and one of cutting stridulations, we used the choice set-up (Fig. 17.2; see Sect. 17.2.1) and presented workers with a choice between them, using play backs from natural stridulations. Workers showed no preference for the offered stridulations when both stems were marked with synthetic trail pheromone (Fig. 17.5, left; unpublished results). The same was the case when both stems were not marked with trail pheromone; although, a slight, yet not statistically significant tendency towards a preference for cutting stridulations can be recognized (Fig. 17.5, right; unpublished results). Direct observations provided no evidence for alarm behaviors, such as hectic runs with opened mandibles, in those ants, roughly one-half of which chose the stem with alarm vibrations, i.e., ants did not appear to have changed their context upon their perception. Taken together, the differences in the signal repetition rate between alarm and stridulatory vibrations, at least for ants in the foraging context, convey no context-specific information. Interestingly, *Crematogastar* ants also produce different stridulatory vibrations in the contexts of alarm and fluid feeding, and vibrations in the feeding context even differ depending on the size of the food droplet (Masoni et al. 2021). Future playback experiments focusing on the behavior of receiver ants, as discussed by the authors, will elucidate whether stridulations

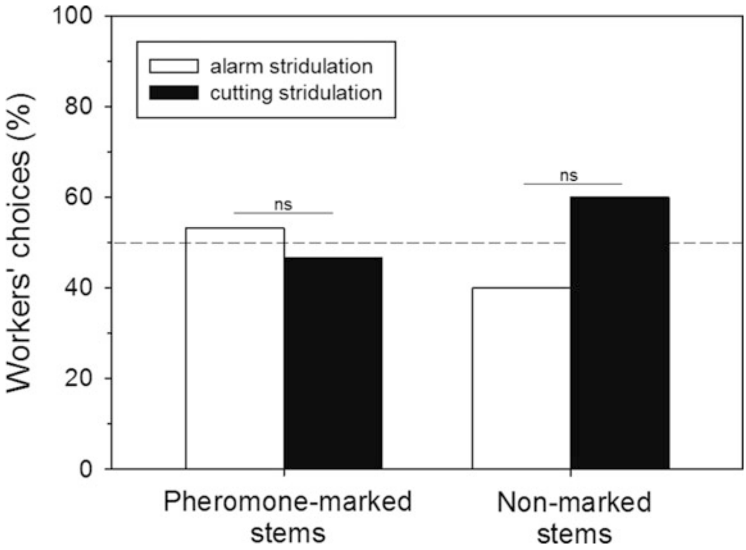


Fig. 17.5 Number of recruited workers of the leaf-cutting ant *Atta cephalotes* that chose between two stems of privet in a laboratory setting. These offered playbacks of either alarm stridulations (single chirps repeated at a rate of 5–7 chirps/s) or cutting stridulations, the repetition rate of which is strongly modulated over time, reaching up to 10–15 chirps/s. See Sect. 17.2.2 for further details. On the left, both stems were marked with synthetic trail pheromone (a solution of 0.05 ng/μl 4-methylpyrrole-2-carbolylic acid in hexane, applied to produce a pheromone trail with 1 μl of the solution over 5 cm) ($n = 60$). On the right, the stems were not marked ($n = 60$). Statistics: "**" indicates that the distribution of single choices differs statistically from the ratio 1:1, after a log-likelihood G-test for goodness of fit to the ratio 1:1; "ns" indicates that choices were not significantly different from the ratio 1:1

indeed provide contextual information and function as graded food-recruitment signals.

17.2.3 Stridulation as Communication Signal Between Leaf Carriers and Hitchhikers

In a foraging column of leaf-cutting ants, minim workers, which are unable to cut leaf fragments because of their small body size, often "hitchhike" on leaf fragments carried by foragers back to the nest. At the cutting site and along the foraging trail, minim workers usually walk around or stand with opened mandibles near workers engaged in cutting, and often investigate loaded nestmates by briefly climbing onto the carrier and its load. It has been demonstrated that they defend loaded workers against parasitic Phorid flies that attempt to oviposit on the ants' bodies (Eibl-Eibesfeldt and Eibl-Eibesfeldt 1967; Feener and Moss 1990).

In the laboratory, we investigated the cues used by potential hitchhikers of the leaf-cutting ant *Atta cephalotes* to locate leaf carriers and explored whether vibrational signals produced by carriers may attract minim workers for hitchhiking. Three different lines of evidence demonstrated that hitchhikers and leaf carriers communicate by using plant-borne stridulatory vibrations produced by the latter (Roces and Hölldobler 1995). Firstly, the repetition rate of the stridulations produced by foragers as they maneuvered the leaf fragment into the carrying position immediately before carriage, when hitchhiking usually takes place, significantly increased as compared to the rate while cutting. Even workers that did not stridulate during cutting were observed to stridulate as they loaded up the fragment. Secondly, stridulations played back on leaves, in the absence of cutting workers, were highly attractive for minim workers, which spent significantly longer times on "stridulating" than on "silent" leaves. Finally, hitchhiking occurred more often in leaf carriers that foraged on stridulating than on silent leaves, indicating that the presence of stridulations motivated minims to search for loaded workers in order to climb and to be carried back to the nest.

In Sect. 17.2.2 we indicated that even though the repetition rate of the chirps differs between alarm and cutting stridulations, foraging workers showed no preference for one of them when facing a choice. It is an open question whether the temporal distribution of the chirps during stridulations encoded information, the meaning of which could be decoded by the recipient ant. If this would be the case, the observed higher repetition rate of stridulations produced immediately after the cut in the context of hitchhiking, up to three times (Roces and Hölldobler 1995), may convey information tailored to hitchhikers, an idea that remains to be investigated.

As depicted in Fig. 17.1, stridulations produced by workers standing on a leaf can only be transmitted to the substrate through the worker's legs, showing considerable attenuation in comparison with those transmitted mostly through the mandibles when a worker is actually cutting a leaf fragment. Their amplitudes average 20 nm, 4–5 times lower than those recorded during the cutting activity (Roces et al. 1993), yet they are above the sensitivity threshold measured electrophysiologically in leg nerves of *Atta* workers (1.3 nm in forelegs of minor workers, which are 4–5 times more sensitive than middle and hind legs; Markl 1970). Stridulations by walking foragers can therefore only be detected from a maximal distance of 2–3 cm. Interestingly, minim workers are on average 3–4 times more sensitive to substrate-borne vibrations than larger workers (Markl 1970), making them considerably more responsive to leg-transmitted vibrations than their larger nestmates.

17.2.4 Stridulation: Mechanical Support during Cutting or Communication Signal?

It has been hypothesized that stridulatory vibrations may aid soil manipulation in ants engaged in digging, by loosening aggregated soil particles while workers press their mandibles into the soil (Spangler 1973). The question arose, whether stridulations may also mechanically facilitate the cutting process, irrespective of their function as signals, in the manner of a vibrating knife that accelerates and thereby stiffens the material to be cut. To answer this question, we performed a detailed video analysis of the temporal relation between mandible movements and stridulations recorded via laser vibrometry (Tautz et al. 1995). Stridulation generated complex vibrations of the mandibles, particularly of the cutting mandible that is pulled against the leaf tissue, and which appeared to stiffen the material to be cut. Ants did not stridulate continuously; stridulation occurred most often when the cutting mandible was moved through the plant tissue. Force measurements of detached mandibles pushed against tender and tough leaves clearly indicated that vibrations facilitated a smoother cut through tender leaf tissue (Tautz et al. 1995). Interestingly, the vibratome-mode of cutting leaves inspired applied research on bionic applications for minimally invasive surgery, with the development of a surgical cutting tool with flexible ultrasonic transmission (Qiao et al. 2002); although, the feasibility of such an approach remains to be verified. A recent study on the stridulatory organ in *Atta cephalotes* (Yao et al. 2018), which extends and complements a very detailed, 50-years-old study on biophysical aspects of vibrational signaling in the same species (Markl 1968), provides detailed information about the mechanical properties of the file-scraper device and also theoretical arguments for a future development of bionic vibrating surgical instruments.

We have speculated, based on our results on mechanical facilitation and on the evidence that stridulations act as recruitment signals, on the evolution of stridulatory vibrations in leaf-cutting ants, arguing that stridulations may have been first used to mechanically facilitate the cutting of leaves, and subsequently may have evolved via ritualization to serve as short-range recruitment signals (Tautz et al. 1995). However, a number of observations were in conflict with the idea that the use of stridulation is related to the mechanical problem of leaf cutting. Increasing the sugar content of the leaves, for example, without changing their physical traits, i.e., increasing leaf palatability, led to a significant increase in the probability to stridulate while cutting, as indicated above (Roces et al. 1993). Secondly, tender leaves, the cutting of which is mechanically facilitated by the stridulations, are highly preferred by leaf-cutting ants anyway, and significantly more nestmates are recruited when ants harvest tender leaves (Roces and Hölldobler 1994). Admittedly, these observations could be interpreted in a converse way, as follows: leaf-cutting ants actually stridulate to attract nestmates, and the mechanical facilitation during cutting of tender leaves represents a by-product of recruitment communication.

We designed an experimental study to distinguish between these two competing hypotheses, i.e., foragers stridulate to mechanically support their cutting behavior, or the mechanical facilitation represents an epiphenomenon derived from the use of stridulation as a recruitment signal. For that, leaf-cutting ant foragers were presented with tender leaves of invariant mechanical properties, and the production of stridulation was evaluated in the following situations, which were aimed to modulate the thresholds at which recruitment communication is initiated. Firstly, leaves were smeared with plant secondary compounds to reduce their palatability. Secondly, harvesting deprivation was simulated, since lack of leaves over several days is known to increase the intensity of chemical recruitment (Roces and Hölldobler 1994). Finally, workers were given unfamiliar leaves after a period of feeding with familiar leaves. Unfamiliar leaves, when palatable, are strongly preferred by leaf-cutting ants (Cherrett 1972) and this "novelty effect" markedly increases the intensity of chemical recruitment (Roces and Hölldobler 1994). If stridulation is primarily used for communication irrespective of the mechanical support during cutting, the occurrence of stridulation would directly depend on the workers' foraging motivation, i.e., it would be modulated by leaf palatability, colony starvation, etc., as known for chemical recruitment signals (Roces 1993; Roces and Núñez 1993).

As in the analysis of hitchhiking behavior (see Sect. 17.2.3), three different lines of evidence supported the hypothesis that leaf-cutting ants stridulate during cutting in order to recruit nestmates and that the observed mechanical facilitation of cutting represents an epiphenomenon of recruitment communication (Roces and Hölldobler 1996). Firstly, by keeping constant the mechanical properties of the leaves, and by reducing their palatability by coating them with tannin, it was shown that the number of stridulating workers decreased with decreasing leaf palatability, an observation that is inversely comparable to the positive effect on stridulation caused by a sugar coating (Roces et al. 1993). Secondly, no workers at all stridulated while cutting tender leaves after intense feeding and "harvesting satiation", when no recruitment of nestmates appears to be necessary. The percentage of stridulating workers increased over the period of harvesting deprivation, reaching its maximum of 100% five days after the last feeding event. Thirdly, whatever kind of leaves used to initially feed the colony until satiation, significantly more workers stridulated when cutting unfamiliar leaves of similar physical features. Such a novelty effect caused by attractive, unfamiliar leaves strongly supports the idea that stridulation is produced as a recruitment signal, independent of the leaves' mechanical properties, and that the observed mechanical facilitation during cutting is likely an epiphenomenon of recruitment communication (Roces and Hölldobler 1996).

17.3 Vibrational Communication inside the Nest

17.3.1 Stridulatory Signals and the Organization of Collective Digging

Leaf-cutting ants of the genus *Atta* excavate the largest and likely most complex nests among ants (Jonkman 1980; Moreira et al. 2004; Bollazzi et al. 2012), with several thousands of underground chambers, mostly for rearing their fungus garden, and for waste disposal. The external nest mound does not solely result from a passive accumulation of the excavated soil, because workers import material to reinforce and stabilize the construction, and in some species, they also build structures on the top of several central nest openings that function as ventilation turrets (Kleineidam et al.

2001). Both the underground cavities and the turrets are structures that emerge from collective digging and building activities that are decentrally organized.

How individual ants coordinate their activities to create functional structures is poorly understood, and only a few studies have experimentally addressed the mechanisms underlying the organization of collective building in leaf-cutting ants (Bollazzi and Roces 2007, 2010; Fröhle and Roces 2009; Cosarinsky and Roces 2012; Römer and Roces 2014, 2015; Halboth and Roces 2017).

One well-known mechanism involved in the organization of collective responses in social insects is the stigmergy, a term coined by Grassé (1959) during his pioneer studies on nest building in termites. Stigmergy describes the process by which an animal responds to the product of earlier building work done by nestmates, without the need of a worker-worker interaction, and its response then amplifies the former stimulus that subsequently triggers a stronger response, and so on. As such, stigmergy represents a form of indirect communication among workers via the structure being built, without the involvement of communication signals.

While stigmergic responses may occur during nest building in leaf-cutting ants, we asked whether communication via vibrational signals mediated the organization of their collective digging. Steffen Pielström, a brilliant doctoral student in my lab, explored the occurrence of stridulation in digging workers of the leaf-cutting ant *Atta vollenweideri*. He designed a number of ingenious laboratory experiments to investigate whether stridulatory vibrations guide workers when searching for a site to initiate their excavations, and if workers in the social context of nest enlargement stridulate depending on their space demands and potential needs for additional recruitment of nestmates. He discovered that beyond the use of vibrational signals in the contexts of food recruitment and alarm communication (see Sects. 17.2.1 and 17.2.2), leaf-cutting ant workers stridulate while engaged in digging and attract nestmates to join excavation activity at the site (Pielström and Roces 2012; commented by Sendova-Franks 2012).

It was observed that isolated workers readily stridulated while excavating in moist soil, producing chirps not only while actually working the material with their mandibles, but also in advance, before they started to mechanically interact with the soil (Pielström and Roces 2012). When leaving the site carrying a soil pellet, most workers ceased stridulating. Signals produced by single workers were, as expected, damped over distance, and the measured attenuation rate suggested that signals might be detected up to a distance of 6 cm. The possibility that digging stridulation is used as recruitment signal to attract other workers was explored in a choice experiment offering different locations where workers may initiate digging. Results clearly indicated that workers were more likely to dig close to a source of stridulatory vibrations than at alternative sites, with a probability that positively correlated with the intensity of the vibrations produced at that location (Pielström and Roces 2012).

In the social context of nest enlargement, workers excavating a tunnel that were allowed to suddenly break into an existing chamber gradually discontinued their signal production when nest space was available; although, the removal of excavated soil pellets continued at a constant rate for at least 3 hours after the decrease in stridulation rate. It is unclear whether excavation continued while workers decreased their stridulation rate, or whether digging activity decreased yet soil transport continued because of prior accumulation of soil pellets inside the chamber (Pielström and Roces 2012). In any case, stridulations recorded in the social context of regular nest excavation to relocate and maintain the symbiotic fungus support the idea that they are used under natural conditions to coordinate, and likely control, the dynamics of collective nest building.

To investigate whether the stridulation by digging workers may be related to the mechanical properties of the material to be removed, stridulations produced by *Atta vollenweideri* workers digging in soils of different moistures were recorded (Pielström and Roces 2014). It was observed that stridulation rates were slightly yet significantly lower, the higher the soil water content. It is unclear whether the observed decrease in repetition rates has a communicative meaning, or it occurs because of a different handling of moist materials by digging workers. As discussed in Sects. 17.2.2 and 17.2.3, the repetition rate of stridulatory vibrations appears to convey no specific information, at least in the foraging context, which makes it unlikely that the slight differences in repetition rates between dry and moist soils have a meaning for recruited workers. Regarding a potential mechanical aid during excavation, as suggested by Spangler (1973) for seed-harvester ants, we discussed different possible interpretations for the observed relationship between soil moisture and stridulation rates (Pielström and Roces 2014). Firstly, if we assume that workers may adjust their stridulatory behavior based on the actual mechanical properties of the soil, lower stridulation rates in moist soils, with poor transmission properties, may be adaptive because workers would stridulate less when the environment is poorly suitable for vibrational recruitment. Conversely, high stridulation rates in dry soils could be a reaction to materials harder to excavate in, so that workers may increase their efforts to recruit additional workers. Finally, the effect of soil moisture on stridulatory behavior could also be interpreted without any communicative function, i.e., excavation of dryer, harder soils would require more stridulatory vibrations to mechanically support the removal of soil particles, a possibility that cannot be ruled out and remains, as the other possibilities, as an open question for future research (Pielström and Roces 2014).

Taken together, stridulation signals produced while digging are used for communication as short-range recruitment signals that attract nestmates, which react by digging close to stridulating ants and therefore amplify the digging process (Pielström and Roces 2012). Results revealed a new context in which stridulations are used by leaf-cutting ants, and provided the first experimental evidence that communication signals are used to spatially organize collective nest building in social insects.

17.3.2 Stridulatory Signals and Underground Waste Disposal

Fungus cultivation by colonies of leaf-cutting ants produces copious amounts of waste, which is mostly composed of decaying fungus and plant material, discarded leaf fragments, and dead ants. There are two species-specific modes of waste disposal in leaf-cutting ants, either outside the nest in above ground piles, or in underground chambers. Colonies of *Atta laevigata* dispose of their waste in large underground chambers, with workers showing preferred values of temperature and air humidity across the soil profile, yet not of CO_2 levels, for the deposition of their waste (Römer et al. 2019). Once the first waste particles are disposed of at a suitable underground free space, for instance inside a tunnel end or an empty cavity, workers that are carrying additional waste particles orient towards waste volatiles and drop their loads at the site in a stigmergic response (Römer and Roces 2019), resulting in the deposition and accumulation of large amounts of refuse at a single location.

The task of underground waste disposal is expected to be tightly associated with the task of digging, which creates empty space for waste deposition. Free space may trigger waste deposition at the site, causing a concomitant reduction in space that may prompt workers to dig and generate additional space. Such a hypothetical regulatory feedback loop may control the final size of the waste chamber, as it is known for the control of the size of fungus chambers in leaf-cutting ants (Fröhle and Roces 2009; Römer and Roces 2014, 2015). The question arose, whether waste-carrying workers may also locate a dumpsite to drop their loads by responding to the stridulatory vibrations produced by workers engaged in excavation, besides the use of environmental and olfactory cues for orientation.

Baris Düdükcü, a bright Master's student in my lab, recently investigated the organization of underground waste disposal by the leaf-cutting ant *Atta laevigata*, focusing on the question of whether waste-carrying workers orient towards a digging site and drop their waste particles there because of the presence of stridulatory vibrations produced by digging workers (Düdükcü 2018). In laboratory colonies, waste removal was triggered by initially adding waste particles in the close vicinity of a fungus garden. Workers readily picked up waste particles and walked towards a deposition site in an empty plastic chamber, the base of which offered a binary choice between one side providing stridulatory vibrations, and the other side being silent. The base of the deposition chamber consisted of two adjacent, identical plastic plates separated by a thin segment of rubber, which prevented vibrations from one side from traveling to the adjacent side. Below each side of the deposition chamber, an empty box, which served as a control, or a box partially filled with moist clay as digging chamber, were tightly attached. Workers from a separate fungus chamber gained access to the covered box containing clay and initiated digging there, with their stridulations being transmitted through the box walls to the "upper floor", i.e., to the base of the deposition chamber. Consequently, the deposition chamber represented for waste-carrying workers a uniform empty space, yet one side of its base vibrated, and the other side was silent. By scoring individual choices made only by the first waste-loaded workers in independent assays, to avoid social influences and stigmergic responses, Düdükcü (2018) showed that significantly more depositions of waste particles, approximately 90% of them, occurred on the side of the deposition chamber where stridulations were provided. Stridulations evidently attracted waste-loaded workers and appear therefore to help coordinate the activities of workers engaged in two associated tasks, namely digging and waste disposal, thus providing evidence for their function in a hitherto unknown context inside the underground nest.

17.4 Behavioral Contexts and the Evolution of Stridulatory Communication in Leaf-Cutting Ants

Since the pioneer and detailed studies on stridulation in leaf-cutting ants by Markl (1965, 1967, 1968, 1970), it has been hypothesized that stridulation in ants first evolved as an underground alarm signal to attract nestmates for rescue digging (Markl 1973). Markl's hypothesis was based on a correlative study of nesting ecology, mode of colony foundation, and presence of a stridulatory organ in workers of all ant subfamilies. It is important to indicate that stridulation is not ubiquitous among ants; it is only found in ants belonging to four (out of 17) subfamilies: Myrmeciinae, Myrmicinae, Ponerinae, and Pseudomyrmecinae. By studying 1354 ant species belonging to 205 genera, Markl (1973) argued that a hypothesis correlating the evolution of stridulation production with nesting ecology in ants may explain major patterns of the occurrence of the stridulatory organ in ants, yet he recognized that many exceptions did not fit into the outlined arguments, and therefore advanced his arguments as a working hypothesis. As indicated in Sect. 17.2.1, a recent detailed phylogenetic study comparing the occurrence of stridulatory organs in arboreal and ground-dwelling ants did not provide supportive evidence for the hypothesis that stridulation in ants first evolved as an underground signal to alert nestmates for rescue digging. Workers of a large proportion of ant genera considered primarily arboreal have a stridulatory organ, and those from a number of completely subterranean ant genera possess no organ (Golden and Hill 2016).

The above arguments make it therefore unlikely that the stridulatory organ in ants first evolved to mechanically facilitate digging, without any communicative function, as proposed by Spangler (1973). Not only do leaf-cutting ants stridulate while digging for communicative purpose (see Sect. 17.3.1; Pielström and Roces 2012, 2014), but also *Solenopsis* fire ants were observed to stridulate during nest excavation in the laboratory (Rauth and Vinson 2006). Although different functions were hypothesized for the stridulations of fire ants (i.e., the mechanical facilitation and compression of soil particles, the potential assessment of the tunnel wall thickness, much like an acoustic imaging tool does, and communication to attract nestmates to the digging site; Rauth and Vinson 2006), no one of these has so far been experimentally demonstrated.

Besides the context of alarm and rescue digging, stridulation in leaf-cutting ants is used in several additional contexts to attract nestmates to the sender ant, as described in this chapter, and may therefore serve different functions. We have demonstrated that in *Atta* leaf-cutting ants, stridulations are used as a short-range recruitment signal, both under controlled laboratory conditions and in the field, and that trail and alarm pheromones modulate the response of receiver ants to them. In addition, stridulation produced by leaf carriers serves as a communication signal to attract minor nestmates as hitchhikers. Inside the nest, leaf-cutting ants stridulate while digging and attract nestmates to the site, and waste carriers orient to stridulating nestmates to find digging sites to dispose of their waste particles. In the alarm context, Attine ants (genus *Trachymyrmex*) were observed to stridulate when attacked by army ants (LaPolla et al. 2002), and newly-mated queens of the leaf-cutting ant *Acromyrmex striatus* appear to stridulate while walking on the floor searching for a suitable place to initiate the founding of a nest (Diehl-Fleig and Lucchese 1992), perhaps as aposematic signaling to deter potential predators (Masters 1979). Finally, leaf-cutting ants also stridulate inside the nest during further cutting, licking, and shredding of the collected plant tissue to be incorporated into their fungus gardens. The signals recorded on the incorporated plant fragments during those processes showed high variability in both signal amplitude and repetition rates. Considering that only a few records of single workers stridulating while performing a given task were obtained, and that in most of them 3–5 workers processed the leaf fragment at the same time and may have stridulated simultaneously, it appears difficult to correlate a given signal amplitude with a specific behavior, as aimed by the authors (Carlos et al. 2018). Understanding the use of stridulatory vibrations in the context of leaf processing and fungus tending remains a challenge for future research.

In the foraging context, it has recently been reported that leaf-cutting ants appear to use their vibrational sense to detect substrate-borne waves produced by the impact of rain droplets on the substrate, and upon perception of such “rain signals,” walk faster to the nest in order to avoid losing the carried fragments because of potential wetting (Farji-Brener et al. 2018). This behavior is intriguing, since loaded ants returning to the nest are expected to link the perception of likely varying substrate-borne vibrations with a specific threat, such as the rain, not experienced directly. In field colonies, researchers monitored the speed of single loaded workers before, during, and after a simulation of rainfall generated by dropping water from a watering can onto a large leaf, the stem of which was buried 10 cm beside the foraging trail. It was assumed, yet not measured, that the vibrations generated on the leaf surface would travel through both the leaf stem and the soil, and be perceived as substrate-borne vibrations by workers on their way to the nest. Intriguingly, loaded workers increased their walking speed up to 30%, yet not during the actual simulation of rainfall, but thereafter. Since ants typically respond to disturbances with increases in walking speed, it remains an open question whether the observed increase in speed after the simulation represented a specific response aimed at returning earlier to the nest to avoid rainfall, or a non-specific alarm response triggered by the vibrations. Regrettably, measurements were not validated, for instance with control records of walking speed, in workers confronted with vibrational stimuli not related to rainfall, such as alarm stridulations, or in workers before and during actual rainfalls. A crucial control experiment would have also recorded putative changes in speed of unloaded workers running towards the foraging patch, and not to the nest. If outgoing workers respond to vibrations as indicators of rain, they would either turn back to the nest or maintain, but not necessarily increase, their speed toward the foraging patch. A potential increase in speed, on the other hand, would indicate that the perceived vibrations caused an unspecific disturbance. In absence of such controls, we cannot be certain that leaf-cutting ant foragers perceive substrate-borne vibrations as an indicator of an actual rain, as argued by the authors. Leaf-cutting ants do indeed behave anticipatory to rainfalls, for instance by increasing their foraging rates, yet as a direct response to a decrease in barometric pressure, which often drops before rains (Sujimoto et al. 2019).

In trying to outline the scenario in which the use of stridulation may have evolved, we had postulated that stridulation in leaf-cutting ants first evolved to mechanically support leaf-cutting, and subsequently as a communication signal (Tautz et al. 1995). Such a hypothesis was particularly tempting, yet like many evolutionary hypotheses, it was based on a plausible correlation rather than experimental proof. If we argue that the use of stridulation as a mechanical aid has been favored during evolution, stridulations should provide benefits for instance as a reduction of the time- and/or energy-costs of cutting, since leaf cutting is an energetically very expensive behavior. Even though the mandibular vibrations generated by stridulation reduce force fluctuations during the cut of tender leaves, the total force employed remained the same with or without vibrations, and stridulating ants did not cut leaves for instance faster than non-stridulating ants (Tautz et al. 1995). In addition, the metabolic rate of workers cutting tender leaves was similar for stridulating and non-stridulating ants (Roces and Hölldobler 1996). It is therefore unlikely that any potential energetic improvement (due to stridulation) on cutting mechanics would significantly decrease the impressive costs of leaf-cutting. The metabolic rate of the mandibular muscles measured during leaf cutting is extremely high (leaf-cutting costs are 31-times higher than costs of basal metabolism), and it approaches that for the insect flight muscle, the metabolically most active animal tissue known (Roces and Lighton 1995). Based on these arguments and experimental evidence, we later rejected our original hypothesis that stridulatory communication signals derived from a vibratory mechanism that mechanically aided the process of leaf-cutting, and hypothesized that ants stridulate to attract nestmates, with the mechanical facilitation of cutting being a byproduct of recruitment communication (Roces and Hölldobler 1996).

Stridulation in leaf-cutting ants is employed in a number of different contexts, yet the evolution of its function remains elusive. The early discovery that buried *Atta* workers stridulate and attract nestmates for rescue digging when part of the colony is confined by a cave-in of the nest (Markl 1965) led to the arguments that vibrational signals used in alarm and defense may have later acquired a function as a recruitment signal in the foraging context. Considering that leaf-cutting ants also stridulate during nest excavation (Pielström and Roces 2012), equally likely would be the hypothesis that the original function of stridulation was a communicative one to organize underground nest-building activities. Interestingly, the idea that stridulation may be fundamental to organizing collective ant behaviors inside the nest was advanced roughly 120 years ago by Wheeler (1903), who argued: "*Even more remarkable is the stridulation in a colony of* Atta fervens *(=* texana*), the Texan' leaf-cutting ant.*" And further added: "*The contact-odor sense, important as it undoubtedly is, must obviously have its limitations in the dark subterranean cavities in which the ants spend so much of their time, especially when the nests are very extensive like those of* Atta*. Under such conditions stridulation and hearing must be of great service in maintaining the integrity of the colony and its excavations.*" Hopefully, additional studies on the use of stridulatory vibrations in leaf-cutting and other ants may uncover additional contexts in which these rather elementary communication signals are used, explore their use as modulatory signals that lower the response threshold for other stimuli, and outline a scenario to understand their evolution.

Acknowledgments I initiated my studies on vibrational communication in leaf-cutting ants in 1992 as a postdoc fellow at the Department of Behavioral Physiology, Biocenter, University of Würzburg. I am deeply indebted to Bert Hölldobler for generous support, encouragement, and discussions over the years, and for having established a very active, diverse, and highly reputed department focusing on social insect research, in which I was free to develop my own projects as a young researcher. I also thank Jürgen Tautz for having introduced me to the methods of bioacoustics. I am particularly indebted to my former Ph.D. student Steffen Pielström, who discovered and explored the amazing use of vibrational signals during the organization of collective nest digging in leaf-cutting ants, and for his comments on the manuscript. I also thank Baris Düdükcü, who discovered the significance of stridulations by digging workers for the spatial organization of underground waste disposal, and for his comments. Thanks are also due to Annette Laudahn and Adrienne Gerber-Kurz for maintenance of the laboratory colonies, to my sister Griselda Roces for the beautiful drawings presented in the first two figures of this chapter, to Rainer Christian Rosenbaum for helping with the layout of the first figure, and to Malu Obermayer for the nice ant drawing of Fig. 17.3. Our own field work reported here was performed at the Reserva Ecológica El Bagual (Alparamis SA - Aves Argentinas) in eastern Chaco, Province of Formosa, Argentina. I am very much indebted to the ornithologist Alejandro G. Di Giacomo, his field assistants, and especially the Götz family for providing facilities at the Biological Station, daily help during our stays and invaluable logistical support throughout years of ant research. Financial support was provided by the DAAD and the DFG, Germany.

References

Autrum H (1936) Über Lautäusserungen und Schallwahrnehmung bei Arthropoden 1. Untersuchungen an Ameisen. Eine allgemeine Theorie der Schallwahrnehmung bei Arthropoden. Z vergl Phys 23:332–373

Baroni-Urbani C, Buser MW, Schilliger E (1988) Substrate vibration during recruitment in ant social organization. Insect Soc 35:241–250

Blum MS, Padovani F, Amante E (1968) Alkanones and terpenes in the mandibular glands of *Atta* species (hymenoptera: Formicidae). Comp Biochem Physiol 26:291–299

Bollazzi M, Roces F (2007) To build or not to build: circulating dry air organizes building responses for climate control in the leaf-cutting ant *Acromyrmex ambiguus*. Anim Behav 74:1349–1355

Bollazzi M, Roces F (2010) Control of nest water losses through building behavior in leaf-cutting ants (*Acromyrmex heyeri*). Insect Soc 57:267–273

Bollazzi M, Forti L, Roces F (2012) Ventilation of the giant nests of *Atta* leaf-cutting ants: does underground circulating air enter the fungus chambers? Insect Soc 59:487–498

Carlos AA, Barbero F, Casacci LO, Bonelli S, Bueno OC (2014) Bioacoustics of *Trachymyrmex fuscus*, *Trachymyrmex tucumanus*, and *Atta sexdens rubropilosa* (hymenoptera: Formicidae). J Acoust Soc Am 136:2074–2074

Carlos AA, Diniz EA, Verza da Silva S, Bueno OC (2018) Vibration of the plant substrate generated by workers' stridulation during fungus garden cultivation in *Atta laevigata* (smith) (hymenoptera: Formicidae). EntomoBrasilis 11:107–112

Cherrett JM (1972) Chemical aspects of plant attack by leaf-cutting ants. In: Harborne JB (ed) Phytochemical ecology. Academic Press, London, pp 13–24

Cosarinsky MI, Roces F (2012) The construction of turrets for nest ventilation in the grass-cutting ant *Atta vollenweideri*: import and assembly of building materials. J Insect Behav 25:222–241

Diehl-Fleig E, Lucchese MEP (1992) Nest foundation by *Acromyrmex striatus* (hymenoptera, Formicidae). In: Billen J (ed) Biology and evolution of social insects. Leuven University Press, Leuven, pp 51–54

Düdükcü B (2018) Wie graben Blattschneiderameisen ihre Kammern? Untersuchungen zum kontextabhängigen Grabeverhalten. Master Thesis, University of Würzburg, Germany

Eibl-Eibesfeldt I, Eibl-Eibesfeldt E (1967) Das Parasitenabwehren der Minima-Arbeiterinnen der Blattschneider-Ameise (*Atta cephalotes*). Z Tierpsych 24:278–281

Farji-Brener AG, Dalton MC, Balza U, Courtis A, Lemus-Domínguez I, Fernández-Hilario R (2018) Working in the rain? Why leaf-cutting ants stop foraging when it's raining. Insect Soc 65:233–239

Feener DH Jr, Moss KAG (1990) Defense against parasites by hitchhikers in leaf-cutting ants: a quantitative assessment. Behav Ecol Sociobiol 26:17–29

Fielde AM, Parker GH (1904) The reactions of ants to material vibrations. Proc Acad Nat Sci Phila 56:642–650

Forbes HO (1881) Sound-producing ants. Nature 24:101–102

Fröhle K, Roces F (2009) Underground agriculture: the control of nest size in fungus-growing ants. In: Theraulaz G, Solé R, Kuntz P (eds) From insect nests to human architecture – workshop on engineering principles of innovation in swarm-made architectures. European Centre for Living Technology, Venice, pp 95–104

Golden TMJ, Hill PSM (2016) The evolution of stridulatory communication in ants, revisited. Insect Soc 63:309–319

Graber V (1881) Die chordotonalen Sinnesorgane und das Gehör der Insecten. Arch Mikrosk Anat 20:506–640

Grassé P-P (1959) La reconstruction du nid et les coordinations interindividuelles chez *Bellicositermes natalensis* et *Cubitermes sp*. La théorie de la stigmergy: Essai d'interprétation du comportement des termites constructeurs. Insect Soc 6:41–84

Hager FA, Kirchner L, Kirchner WH (2017) Directional vibration sensing in the leafcutter ant *Atta sexdens*. Biol Open 6:1949–1952

Hahn M, Maschwitz U (1985) Foraging strategies and recruitment behaviour in the European harvester ant *Messor rufitarsis* (F.). Oecologia 68:45–51

Halboth F, Roces F (2017) The construction of ventilation turrets in *Atta vollenweideri* leaf-cutting ants: carbon dioxide levels in the nest tunnels, but not airflow or air humidity, influence turret structure. PLoS One 12:e0188162

Haskins CP, Enzmann EV (1938) Studies of certain sociological and physiological features in the Formicidae. Ann N Y Acad Sci 37:97–162

Hickling R, Brown RL (2000) Analysis of acoustic communication by ants. J Acoust Soc Am 108: 1920–1929

Hill PSM, Wessel A (2016) Primer – Biotremology. Curr Biol 26:R187–R191

Hölldobler B (1999) Multimodal signals in ant communication. J Comp Phys A 184:129–141

Hölldobler B, Roces F (2001) The behavioral ecology of stridulatory communication in leafcutting ants. In: Dugatkin LS (ed) Model Systems in Behavioral Ecology - integrating conceptual, theoretical, and empirical approaches. Princeton University Press, Princeton, pp 92–109

Hölldobler B, Wilson EO (1990) The ants. Belknap Press, Harvard University Press, Cambridge

Hölldobler B, Wilson EO (2011) The leafcutter ants – civilization by instinct. Norton & Company, New York

Hunt JH, Richard FJ (2013) Intracolony vibroacoustic communication in social insects. Insect Soc 60:403–417

Jonkman JCM (1980) The external and internal structure and growth of nests of the leaf-cutting ant *Atta vollenweideri* Forel, 1893 (Hym.: Formicidae). Part II. The internal nest structure and growth. Z ang Ent 89:217–246

Karlson P, Butenandt A (1959) Pheromones (ectohormones) in insects. Annu Rev Entomol 4:39–58

Kermarrec A, Mauléon H, Antun AA (1976) La stridulation de *Acromyrmex octospinosus* Reich. (Formicidae, Attini): biométrie de l'appareil stridulateur et analyse du signal produit. Insect Soc 23:29–47

Kleineidam C, Ernst R, Roces F (2001) Wind-induced ventilation in the giant nests of the leaf-cutting ant *Atta vollenweideri*. Naturwissenschaften 88:301–305

Kleineidam C, Rössler W, Hölldobler B, Roces F (2007) Perceptual differences in trail-following leaf-cutting ants relate to body size. J Insect Physiol 53:1233–1249

LaPolla JS, Mueller UG, Mueller M, Seid M, Cover S (2002) Predation by the army ant *Neivamyrmex rugulosus* on the fungus-growing ant *Trachymyrmex arizonensis*. Insect Soc 49:251–256

Lubbock J (1877) On some points in the anatomy of ants. Mon Microsc J 18:121–142

Markl H (1965) Stridulation in leaf-cutting ants. Science 149:1392–1393

Markl H (1967) Die Verständigung durch Stridulationssignale bei Blattschneiderameisen. I. Die biologische Bedeutung der Stridulation. Z vergl Physiol 57:299–330

Markl H (1968) Die Verständigung durch Stridulationssignale bei Blattschneiderameisen. II. Erzeugung und Eigenschaften der Signale. Z vergl Physiol 60:103–150

Markl H (1970) Die Verständigung durch Stridulationssignale bei Blattschneiderameisen. III. Die Empfindlichkeit für Substratvibrationen. Z vergl Physiol 69:6–37

Markl H (1973) The evolution of stridulatory communication in ants. In: Proceedings VII Congress IUSSI, London, pp 258–265

Markl H, Fuchs S (1972) Klopfsignale mit Alarmfunktion bei Rossameisen (*Camponotus*, Formicidae, hymenoptera). Z vergl Physiol 76:204–255

Markl H, Hölldobler B (1978) Recruitment and food-retrieving behavior in *Novomessor* (Formicidae, hymenoptera). II: vibration signals. Behav Ecol Sociobiol 4:183–216

Masoni A, Frizzi F, Nieri R, Casacci LP, Mazzoni V, Turrilazzi S, Santini G (2021) Ants modulate stridulatory signals depending on the behavioral context. Sci Rep 11:5933

Masters WM (1979) Insect disturbance stridulation: its defensive role. Behav Ecol Sociobiol 5:187–200

Masters WM, Tautz J, Fletcher NH, Markl H (1983) Body vibration and sound production in an insect (*Atta sexdens*) without specialized radiating structures. J Comp Physiol A 150:239–249

Moreira AA, Forti LC, de Andrade APP, Boaretto MAC, Lopes JFS (2004) Nest architecture of *Atta laevigata* (F. smith, 1858) (hymenoptera: Formicidae). Stud Neotropical Fauna Environ 39:109–116

Morgan ED (2008) Chemical sorcery for sociality: exocrine secretions of ants (hymenoptera: Formicidae). Myrmecol News 11:79–90

Peal SE (1881) Sound-producing ants. Nature 24:484

Pielström S, Roces F (2012) Vibrational communication in the spatial organization of collective digging in the leaf-cutting ant *Atta vollenweideri*. Anim Behav 84:743–752

Pielström S, Roces F (2014) Soil moisture and excavation behaviour in the Chaco leaf-cutting ant (*Atta vollenweideri*): digging performance and prevention of water inflow into the nest. PLoS One 9(4):e95658

Qiao F, Roces F, Schilling C, Wurmus H (2002) Cutting with flexible ultrasound transmission for minimally invasive surgery with biological inspiration. In: Actuator 2002. Bremer Actuator, Bremen, pp 668–671

Rauth SJ, Vinson SB (2006) Colony wide behavioral contexts of stridulation in imported fire ants (*Solenopsis invicta* Buren). J Insect Behav 19:293–304

Robinson SW, Moser JC, Blum MS, Amante E (1974) Laboratory investigations of the trail-following responses of four species of leaf-cutting ants with notes on the specificity of a trail pheromone of *Atta texana* (Buckley). Insect Soc 21:87–94

Roces F (1990) Leaf-cutting ants cut fragment sizes in relation to the distance from the nest. Anim Behav 40:1181–1183

Roces F (1993) Both evaluation of resource quality and speed of recruited leaf-cutting ants (*Acromyrmex lundi*) depend on their motivational state. Behav Ecol Sociobiol 33:183–189

Roces F (1994) Odour learning and decision-making during food collection in the leaf-cutting ant *Acromyrmex lundi*. Insect Soc 41:235–239

Roces F, Bollazzi M (2009) Information transfer and the organization of foraging in grass- and leaf-cutting ants. In: Jarau S, Hrncir M (eds) Food exploitation by social insects: ecological, behavioral, and theoretical approaches. CRC Press: Contemporary Topics in Entomology Series, Boca Raton, FL, pp 261–275

Roces F, Hölldobler B (1994) Leaf density and a trade-off between load-size selection and recruitment behavior in the ant *Atta cephalotes*. Oecologia 97:1–8

Roces F, Hölldobler B (1995) Vibrational communication between hitchhikers and foragers in leaf-cutting ants (*Atta cephalotes*). Behav Ecol Sociobiol 37:297–302

Roces F, Hölldobler B (1996) Use of stridulation in foraging leaf-cutting ants: mechanical support during cutting or short-range recruitment signal? Behav Ecol Sociobiol 39:293–299

Roces F, Lighton JRB (1995) Larger bites of leaf-cutting ants. Nature 373:392–393

Roces F, Manrique G (1996) Different stridulatory vibrations during sexual behaviour and disturbance in the blood-sucking bug *Triatoma infestans* (Hemiptera: Reduviidae). J Insect Physiol 42:231–238

Roces F, Núñez JA (1993) Information about food quality influences load-size selection in recruited leaf-cutting ants. Anim Behav 45:135–143

Roces F, Tautz J (2001) Ants are deaf. J Acoust Soc Am 109:3080–3082

Roces F, Tautz J, Hölldobler B (1993) Stridulation in leaf-cutting ants: short-range recruitment through plant-borne vibrations. Naturwissenschaften 80:521–524

Rohe W, Rupprecht R (2001) Klopfen und Kratzen als Alarmsignale bei westmalayischen Drüsenameisen (Hymenoptera: Formicidae: Dolochoderinae). Entomol Gen 25:81–96

Römer D, Roces F (2014) Nest enlargement in leaf-cutting ants: relocated brood and fungus trigger the excavation of new chambers. PLoS One 9(5):e97872

Römer D, Roces F (2015) Available space, symbiotic fungus and colony brood influence excavation and lead to the adjustment of nest enlargement in leaf-cutting ants. Insect Soc 62:401–413

Römer D, Roces F (2019) Waste deposition in leaf-cutting ants is guided by olfactory cues from waste. Sci Nat 106:3

Römer D, Bollazzi M, Roces F (2019) Leaf-cutting ants use relative humidity and temperature but not CO_2 levels as cues for the selection of an underground dumpsite. Ecol Entomol 44:502–511

Röschard J, Roces F (2003a) Fragment-size determination and size-matching in the grass-cutting ant *Atta vollenweideri* depend on the distance from the nest. J Trop Ecol 19:647–653

Röschard J, Roces F (2003b) Cutters, carriers and transport chains: distance-dependent foraging strategies in the grass-cutting ant *Atta vollenweideri*. Insect Soc 50:237–244

Sendova-Franks A (2012) Vibrational communication in digging ants – in focus. Anim Behav 84: 739–741

Sharp D (1893) On stridulation in ants. Trans Entomol Soc London 1893:199–213

Spangler HG (1967) Ant stridulations and their synchronization with abdominal movement. Science 155:1687–1689

Spangler HG (1973) Vibration aids soil manipulation in hymenoptera. J Kansas Entomol Soc 46: 157–160

Sujimoto FR, Costa CM, Zitelli CHL, Bento JMS (2019) Foraging activity of leaf-cutter ants is affected by barometric pressure. Ethology 126:290–296

Tautz J, Roces F, Hölldobler B (1995) Use of a sound-based vibratome by leaf-cutting ants. Science 267:84–87

Tumlinson JH, Silverstein RM, Moser JC, Brownlee RG, Ruth JM (1971) Identification of the trail pheromone of a leaf-cutting ant, *Atta texana*. Nature 234:348–349

Weber NA (1972) Gardening ants - the Attines. The American Philosophical Society, Philadelphia

Wheeler WM (1903) Ethological observations on an American ant (*Leptothorax emersoni* Wheeler). J Psychol Neurol 2:64–78

Wilson EO, Bossert WH (1963) Chemical communication among animals. Recent Prog Hormone Res 19:673–716

Yao G, Feng L, Zhang D, Jiang X (2018) Morphology and mechanical properties of vibratory organs in the leaf-cutting ant (*Atta cephalotes*). J Bionic Engin 15:722–730

Chapter 18
Biotremology of Social Wasps: The Next Step to Understand Wasps' Social Life

Rachele Nieri, Alessandro Cini, Marco Valerio Rossi Stacconi, Irene Pepiciello, Valerio Mazzoni, and Rita Cervo

Abstract Vibrational communication, the use of the surface-borne component of mechanical waves, is widespread in arthropods, but its role is almost unknown for the majority of species. The preponderance of other communication modalities has often masked the occurrence of vibrational communication, even when observations suggested that substrate-borne waves were involved in the communication of the species. Social wasps are a good example of this knowledge gap: their social life has been considered to be based on chemical communication since the early 1970s and other modalities have been overlooked. Only recently, some attention has been paid to visual signals and vibrational communication is still largely neglected in this group. However, it has been confirmed that vibrations mediate crucial aspects of social life in some species. This chapter aims to provide an overview of our current, often scarce, incomplete, and disorganized knowledge on the production and use of substrate-borne waves in social wasps. Their putative and demonstrated functions in wasps' societies are then discussed. We hope to demonstrate that biotremology, the study of vibrational communication, represents a necessary next step toward a more complete understanding of social life of wasps.

R. Nieri (✉) · M. V. Rossi Stacconi
Department of Horticulture, Oregon State University, Corvallis, OR, USA
e-mail: rachele.nieri@unitn.it; marcovalerio.rossistacconi@fmach.it

A. Cini
Dipartimento di Biologia, Università degli Studi di Firenze, Sesto Fiorentino, Italy

Centre for Biodiversity and Environment Research, University College London, London, UK

I. Pepiciello · R. Cervo
Dipartimento di Biologia, Università degli Studi di Firenze, Sesto Fiorentino, Italy
e-mail: rita.cervo@unifi.it

V. Mazzoni
Research and Innovation Center, Fondazione Edmund Mach, San Michele all'Adige, Trento, Italy
e-mail: valerio.mazzoni@fmach.it

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_18

18.1 Introduction

People dislike social wasps, such as hornets and yellowjackets, for their disturbance and danger to human life; moreover, their ecological role and economic impact are underrated, and, as a consequence, the scientific effort to study these insects is low (Sumner et al. 2018). However, social wasps play a crucial role in regulating their ecosystem both as predators and as pollinators (Sumner et al. 2018; Thomson 2019). Thus, understanding their social life is crucial to increasing the awareness and conservation efforts for this group of insects.

By definition eusocial species live in colonies where two or more generations of adults overlap and show reproductive division of labor, that is the presence in the colony of non-reproductive individuals who cooperate in brood care and colony maintenance (workers) and of one or a few individuals who reproduce (the reproductives) (Wilson 1971). Eusociality has been documented in only three subfamilies in the family Vespidae: Stenogastrinae, Polistinae, and Vespinae (Hunt 2007). Their highly variable and plastic social structures differentiate them from other eusocial species, such as some ant species and honey bees. Their wide range of social organization, from the barely eusocial stenogastrines to the highly eusocial colonies of some of the swarm-founding polistines, has established them as a key group in which to study the evolution of sociality (Pardi 1996; O'Donnell 1998). This has held true since the early years of sociobiology in the 1940s, and they have been extensively studied ever since. Nevertheless, many critical aspects of their communication are yet to be understood.

Living in colonies poses a series of challenges to the members of the insects' societies, which are mainly related to the collaborative resource utilization, collective defensive actions, and the reproductive division of labor (Leonhardt et al. 2016). The latter adds an additional level of complexity to wasps' societies; in fact, Stenogastrinae and independent-founding Polistinae are considered primitively eusocial, that is most females can potentially mate and lay eggs, but only one of them, the dominant female, secures reproduction, while the others remain in the nest as workers (Jeanne 2003; Hunt 2007). Thus, the dominant individual must impose its dominance over the other females and maintains it throughout the colony cycle.

Intraspecific communication plays a pivotal role in overcoming all these challenges, and chemicals have been considered to govern wasp social life (Richard and Hunt 2013; Leonhardt et al. 2016). For instance, the chemical blend (mainly hydrocarbons) that covers the cuticle of these insects has been shown to distinguish both nestmates from alien individuals and the dominant female from subordinates (Bruschini et al. 2010); the alarm pheromone released by the venom gland recruits nestmates and coordinates a collective defense when the colony is threatened (Jeanne 1981; Fortunato et al. 2004; Bruschini et al. 2008).

As chemicals are extremely important in the social life of wasps, their study might have biased a comprehensive investigation of communication. Other modalities have been left in the shadow, as in the case of visual signals (Cervo et al. 2015) and vibrations. The mechanical modality is extremely suitable for communication in the colony life of wasps. First, all intracolony activities take place in the nest, which is made by the wasps themselves, who mix organic and inorganic material with their saliva and manipulate it into the nest structure. Both the structure (single or multiple combs, with or without envelope) and the materials of their nests have always been considered suitable for substrate-borne wave propagation (Schaudinischky and Ishay 1968). Secondly, behaviors that may induce vibrations in the nest's structure (i.e., vibrational behaviors) have been reported for several species in all three subfamilies, Stenogastrinae, Vespinae, and Polistinae (Jeanne and Suryanarayanan 2011; Turillazzi 2012) (Fig. 18.1). These behaviors have been identified with several names (e.g., oscillatory behavior, drumming, vibration, waving, wagging, scraping), and in some cases, a correlation to other social traits has been found, such as to the dominant or subordinate role in Polistinae and Vespinae, respectively (Ishay and Schwartz 1973; Jeanne 2009). Yet, just a few studies measured the substrate-borne waves associated with such behaviors, and in a minority of those, a functional hypothesis has been tested (Ishay 1975; Ishay and Nachsen 1975; Brennan 2007; Suryanarayanan et al. 2011; Pepiciello et al. 2018; Taylor and Jeanne 2018). Thus, for most of these behaviors a function has yet to be identified.

Despite social wasps being good candidates for the use of vibrational communication, biotremology is still a niche topic in this group. In this chapter, we give an overview of the current knowledge on the occurrence of substrate-borne waves and vibrational communication in all three subfamilies of social wasps. We take into consideration how wasps effectively transmit vibrations to the nest structure, how receivers on the nest perceive them, and the transmission properties of different nests. Then we discuss their functions, either putative or tested, and we classify vibrations induced by social wasps based on the context in which they are usually displayed. By the end of the chapter, we hope to convey the message that vibrational communication in wasps deserves a higher consideration than it has received so far.

18.2 How and Where: Mechanisms of Production, Perception, and Transmission of Vibrations in Wasps

18.2.1 Production of Vibrations

Insects have many mechanisms for transmitting vibrations to the substrate on which they are standing. Some of them do not require specialized structures or organs, but employ peculiar vibrational behaviors, such as tremulation, drumming, and scraping (Hill 2009).

There are several behaviors of social wasps that likely induce vibrations in the substrate, none of which require specialized structures. However, they have rarely been studied as vibrational signal mechanisms: descriptions are often incomplete and the measurement of the vibrations is lacking for most of the species. They have been

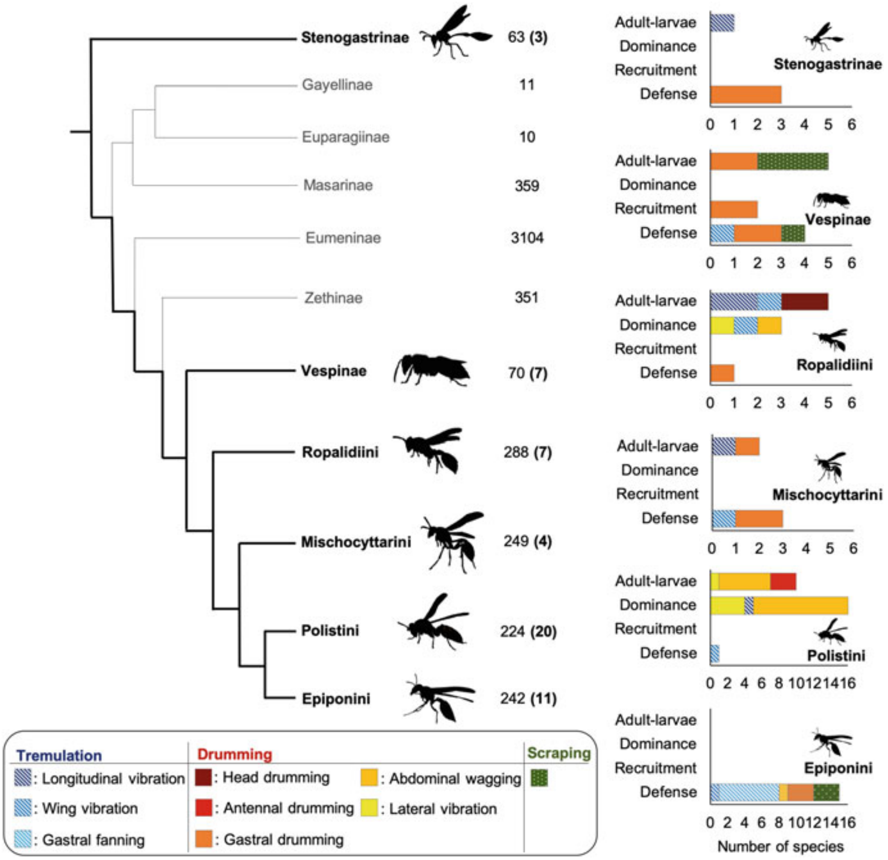


Fig. 18.1 Cladogram of social wasp taxa and presence of behaviors inducing vibrations in the nest. Groups containing solitary species are in gray. The subfamily Polistinae is divided into four tribes. The number next to the taxon name is the total number of species; the number in brackets is the number of species in which at least one vibrational behavior has been described. Histograms report the context in which the vibrational behavior has been observed or to which it has been associated. The cladogram has been drawn by the authors based on data from Hines et al. (2007) and Piekarski et al. (2018)

described merely based on the basis of the behavioral patterns associated with the production of vibrations as drumming, beating, rattling, body oscillations (waving, wagging, vibration), and scraping. Plus, the terminology used so far to describe these behaviors is inconsistent. To facilitate further studies of the biotremology of social wasps, we propose to categorize the behaviors that have been reported to induce substrate-borne waves by social wasps based on the mechanisms of the vibrations' production: tremulation, drumming, and scraping (Fig. 18.2).

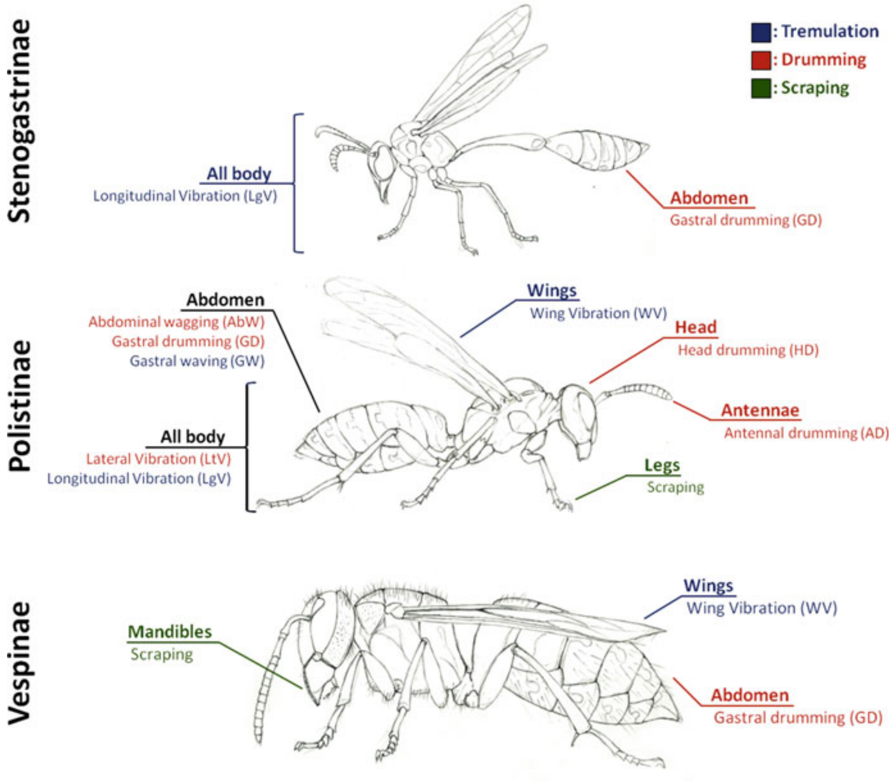


Fig. 18.2 Scheme of the behaviors inducing substrate-borne waves into the substrate for each subfamily of social wasps. Drawings by Marco Valerio Rossi Stacconi

18.2.1.1 Tremulation

Tremulation consists in a general shaking of the whole body or parts of it (i.e., wings and abdomen) while the wasp is standing or walking on the nest. The trembling of the body causes the nest to shake jointly with the wasp. An example of tremulation is the "longitudinal vibration" (LgV) of *Polistes metricus*, which consists in a backward and forward movement of the wasp along her longitudinal axis. A behavior similar to LgV has been reported in two other genera of Polistinae, *Belonogaster petiolata* (Keeping 1992), *Belonogaster juncea juncea* (Tindo et al. 1997), *Mischocyttarus drewseni* (reported as "pecking"; Jeanne 1972), and one species of Stenogastrinae, *Eustenogaster fraterna* (Francescato et al. 2002).

In some cases, wasps contract flight muscles while standing on the comb. As a result of the contractions, the wings are rapidly vibrated as if the individual is ready to fly away from the nest. The "wing vibration" (WV) has been observed in *Vespa orientalis* (Ishay and Sadeh 1982), *Ropalidia revolutionalis* (Ito 1987), *Ropalidia marginata* (Sen and Gadagkar 2006), *Polistes dominula* (formerly *P. gallicus* and

P. dominulus, Castro and Dvořák 2009; Bruschini et al. 2005), *Polybia occidentalis* (Jeanne 1981), and *Mischocyttarus cerberus* (Togni and Giannotti 2010).

In seven species of the Epiponini tribe, *Polybia occidentalis, P. dimidiata, P. dimorpha, Agelaia yepocapa*, *Apoica albomacula, Brachygastra augusti*, and *Synoeca septentrionalis*, workers have been reported to perform "gaster-flagging" (GF), which consists in waving the uplifted abdomen side to side while standing on the nest surface (Jeanne 1981; O'Donnell et al. 1997). In these species, the oscillating movement of the gaster may potentially induce vibrations in the nest, particularly when multiple individuals perform GF simultaneously, but vibrations of the nest surface have never been measured.

18.2.1.2 Drumming

Among all families of social wasps, drumming is the mechanism most widely used to induce vibrations into the nest. It consists of the wasps rhythmically striking the nest surface with parts of the body, such as the head, the abdomen, or the antennae.

Depending on the body part contacting the nest, the direction of the movement (parallel or perpendicular to the nest), and the intensity of the movement, the following five behaviors have been described: "head drumming" (HD), "antennal drumming" (AD), "gastral drumming" (GD), "abdominal wagging" (AbW), and "lateral vibration" (LtV). When inspecting cells containing larvae, adults have been documented to repeatedly beat the cell rim with either the head (HD) or the antennal flagella (AD). HD is peculiar to *Ropalidia revolutionalis* (Hook and Evans 1982) and *R. fasciata* (Ito 1983), whereas AD was described for the first time in *Polistes fuscatus* (F.) (Rau 1928; Pratte and Jeanne 1984), and it has been observed in *Polistes snelleni* (Yamane 1971) and *Polistes carnifex* (Corn 1972).

Among social wasps, striking the abdomen on the nest surface is more common than the head or its appendages. The GD consists of the wasp moving the abdomen perpendicularly to the nest to hit the comb, and it is performed by species of all the three subfamilies of social wasps. The part of the gaster used to hit the substrate varies depending on the species, from the distal tip of the abdomen to its ventral section. Among Vespinae, GD was originally named "awakening dance" (Ishay and Schwartz 1973). It is always performed on the back of the comb and has been reported in *Vespa orientalis* (Ishay and Schwartz 1973), *Vespula germanica* (Ishay and Brown 1975; Ishay and Nachsen 1975), *Vespula consobrina* (Akre et al. 1982), *Vespula vulgaris*, and *Vespula maculifrons* (Ross 1982). Only one genus of Stenogastrinae, *Eustenogaster*, has been documented to perform behaviors inducing vibrations into the substrate. GD has been observed in three species: *E. calyptodoma* (Hansell 1987), *E. eximia* (Krombein 1991), and *E. fraterna* (Francescato et al. 2002). Among Polistinae, GD occurs in *Mischocyttarus labiatus* (Litte 1981), in *M. cerberus* (Togni and Giannotti 2010), in *M. drewseni* (Jeanne 1972), in *Ropalidia opifex* (Fortunato et al. 2004), in *Parachartergus colobopterus* (Jeanne and Keeping 1995), and in *Synoeca septentrionalis, Synoeca virginea*, and *Angiopolybia pallens* (West-Eberhard 1982). Two species of obligate social parasites among *Polistes*, *P. sulcifer* and *P. semenowi* perform GD after usurpation of the nest of congeneric host species (Cervo 1990; Zacchi et al. 1996).

Among the Polistini, the most common behavior inducing vibrations is AbW, which consists of the wasp swinging the gaster side to side while being stationary or walking on the face of the comb. Video and vibration recordings of AbW suggest that vibrations are induced by the wasp striking the nest surface with the ventral side of the gaster while wagging (see Sect. 18.2.1.4). The term "abdominal wagging" is actually incorrect, as it is the gaster to be moved (i.e., rearmost tagma, starting at the wasp "waist" or petiole), whereas "abdomen" includes the gaster and one or two anterior segments that in these Hymenoptera are fused with the thorax. AbW should thus be better called "gaster or gastral wagging." However, to assure continuity with previous literature and for a broader understanding also in a non-expert audience, we prefer to keep the term "abdominal wagging." This behavior has been described in several species of *Polistes*, including *P. dominula* (Heldmann 1936; Pardi 1942; Röseler and Röseler 1989; Brillet et al. 1999; Brennan 2007), *P. flavus, P. chinensis*, and *P. canadensis* (West-Eberhard 1969), *P. exclamans* (West-Eberhard 1969; Hermann et al. 1975), *P. versicolor* (de Souza and Prezoto 2012), *P. versicolor* var. *vulgaris* (Esch 1971), *P. carnifex* (Corn 1972), *P. annularis* (Hermann and Dirks 1975; Hughes et al. 1987), *P. metricus* (Gamboa et al. 1978; Gamboa and Dew 1981), *P. major* (West-Eberhard 1982), *P. instabilis* (Hughes and Strassmann 1988), *P. stigma* (Suzuki 1996), *P. fuscatus* (Harding and Gamboa 1998), *P. biglumis* and *P. atrimandibularis* (Mignini and Lorenzi 2015), *P. jokahamae* (Yoshimura et al. 2019), *P. gallicus*, *P. nimphus*, *P. semenowi*, and *P. sulcifer* (Cervo personal observation). However, AbW is also present in the ropalidiine *B. juncea* (Tindo et al. 1997), and the epiponine *Polybia atra* (Hase 1935).

A minority of species display a behavior similar in its movement to AbW, but that is generally described as more intense, the "lateral vibration" (LtV) of *P. metricus* (Gamboa and Dew 1981). In LtV the wasp shakes the entire body in a horizontal plane with respect to the nest's surface while stationary. This behavior has been described as very similar to AbW, but they can be distinguished by duration, frequency of body oscillation, and intensity (Gamboa and Dew 1981). In fact, the shaking of the nest is so intense that in some cases it can be distinctly seen moving and it produces an audible sound. Vibrations induced by LtV have never been recorded, and there is no video evidence that the abdomen touches the nest, but considering the similitude with AbW and the sound described, the mechanism of production of vibrations is likely to be drumming. A movement similar to the LtV of *P. metricus* has also been observed in *Polistes erythrocephalus* (West-Eberhard 1982), *P. canadensis* (West-Eberhard 1986), *P. fuscatus* (Savoyard et al. 1998), and *Ropalidia cyathiformis* (Gadagkar and Joshi 1984).

18.2.1.3 Scraping

Scraping the nest surface is another example of a mechanism to induce vibrations in the substrate that is made possible by the rigid exoskeleton. The individual scratches the nest with a part of the body and generates a signal that may consist of two components: substrate-borne waves and sound.

Guard wasps of *Asteloeca ujhelyii* are a subgroup of workers that usually stand on the outside of the nest defending it from intruders (Nascimento et al. 2005). While standing on the nest's envelope, some workers have been observed scraping the nest surface with the tarsal claws of their forelegs. Substrate-borne waves were recorded when a dissected wasp's leg was moved repeatedly along the surface of the nest envelope. Similarly, workers of *Synoeca virginea* (Overal 1982) and *S. septentrionalis* (O'Donnell et al. 1997) have been observed scraping their mandibles on the nest when attacked.

Larvae of social wasps have softer bodies compared to the adults and they are constrained inside a cell where their movements are restricted. Thus, they are not the perfect candidate to emit vibrations. However, larvae of *Vespa crabro* and *V. orientalis* have been reported to concurrently generate sounds and induce substrate-borne waves into the nest (Ishay and Landau 1972; Ishay and Schwartz 1973; Barenholz-Paniry and Ishay 1988). While contracting their soft bodies in an antero-posterior direction, the larvae scrape the interior wall of the cell with their sclerotized mandibles inducing the nest to vibrate. A similar behavior is also performed by larvae of *Vespa velutina* (Cervo, Cini, and Pepiciello personal observation).

18.2.1.4 Spectral and Temporal Features of Vibrations

Despite numerous descriptions of vibration-inducing behaviors that have been reported among social wasps, although with a confusing terminology (see Sect. 18.2.1), direct measurement of substrate-borne waves associated with such behaviors is scarce. Knowing the structure of a signal is a crucial aspect of the study of animal communication. First, because it gives us more information about how the signal is produced, thus clarifying terminology and classification, as in the case of the AbW. Second, because it helps to understand how the receiver actually perceives the signal, which is crucial to test the function with playback experiments. Vibrational signals are substrate-borne waves and their specific features can be described in terms of spectral (i.e., the frequency pattern, the energy distribution according to the frequency) and temporal parameters (i.e., duration of a signal, interval between signal emissions). In this regard, it must be noted that in earliest papers, the term "frequency" was referred to the frequency of oscillation describing the wasp's body movement (e.g., Gamboa and Dew 1981; Savoyard et al. 1998). Nowadays, it is consolidated to refer to the frequency as a parameter associated with the wave (i.e., substrate-borne vibrations) produced by behavior of the sender. The oscillation of the wasp may determine different parameters of the associated waves, depending on the mechanism of production. For instance, if the abdomen strikes the nest, as in the AbW, the oscillation would determine the interval between pulses (Fig. 18.3).

There are several instruments available to record substrate-borne vibrations and measure their parameters (see Chap. 8). Most devices need to be in contact with the

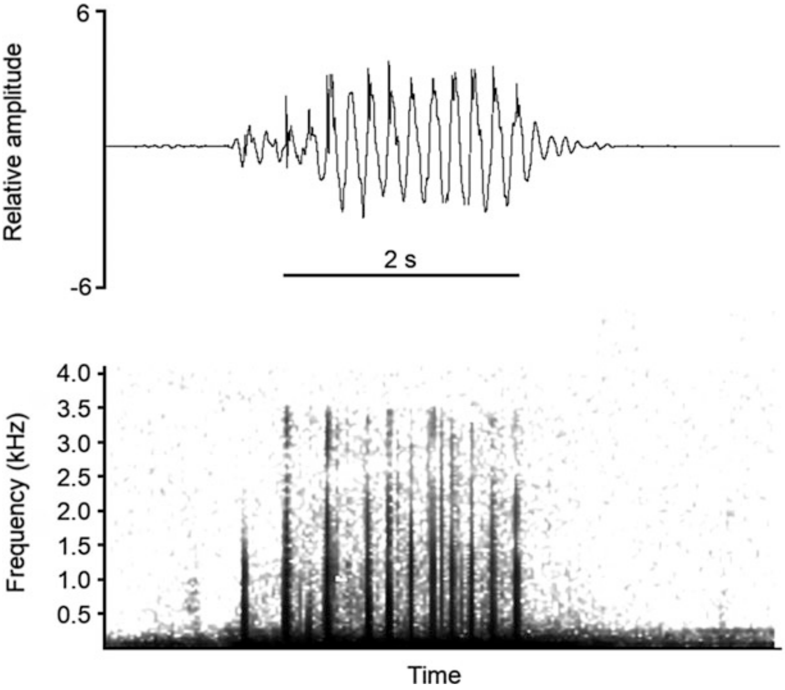


Fig. 18.3 Oscillogram (above) and spectrogram (below) of substrate-borne waves induced into the nest by abdominal wagging (AbW) of *P. dominula* and recorded by means of a laser vibrometer focused on the back side of the nest

substrate (e.g., accelerometer, piezo electric, cartridge), but some, such as the laser Doppler vibrometer, record the displacement of the substrate without adding any weight to it. One downside of contact instruments is that when coupled with the substrate, the weight of the device modifies the structure of the substrate itself and it may distort the original vibration, thus giving imperfect information. To date, laser Doppler vibrometers are considered to be one of the few methodologies capable of recording the displacement of the substrate without affecting the signals' structure. Another important parameter of vibrational signals is the intensity of vibrations. Most instruments listed earlier detect the relative amplitude of vibrations, but only calibrated instruments can measure the absolute amplitude. For this reason, usually an accelerometer or a laser vibrometer is needed to measure the acceleration (m/s^2) or the velocity (m/s) of the substrate's displacement, respectively.

To date, only the vibrations of *V. orientalis*, *V. germanica*, the AbW of *P. dominula*, and the AD of *P. fuscatus* have been measured with instruments capable of recording the substrate-borne component (Table 18.1). From these few studies, we know that vibrational signals of an adult hornet (GD) or paper wasp (AD and AbW) are characterized by a series of pulses with a broadband spectrum profile (Ishay 1975; Ishay and Nachsen 1975; Brennan 2007; Suryanarayanan et al. 2011) (Fig. 18.3). Most of the energy of GD is included in the frequency range of

Table 18.1 List of social wasps in which at least one behavior that may induce vibrations in the nest structure has been reported. The context of the behavior is reported when present in the literature. The vibration sensor and playback device are reported only for the species in which the vibrations transmitted to the nest have been recorded and /or tested with a playback experiment

Group	Species	Behavior	Context	References	Vibration sensor	Playback device
Stenogastrinae	*Eustenogaster calyptodoma*	GD	De	Hansell (1987)		
	Eustenogaster eximia	GD	De	Krombein (1991)		
	Eustenogaster fraterna	GD; LgV	De; A-L	Francescato et al. (2002)		
Vespinae	*Vespula consobrina*	GD	A-L	Akre et al. (1982)		
	Vespula germanica	GD	R	Ishay and Brown (1975), Ishay and Nachsen (1975), Taylor and Jeanne (2018)	Accelerometer	Speaker
	Vespula maculifrons	GD	De	Ross (1982)		
	Vespa crabro	S	A-L	Autrum and Schneider (1948), Ishay and Schwartz (1973)		
	Vespa orientalis	WV; GD; S	De; R; A-L	Schaudinischky and Ishay (1968), Ishay and Landau (1972), Ishay and Schwartz (1973), Ishay et al. (1974), Ishay (1975), Ishay and Sadeh (1982), Barenholz-Paniry and Ishay (1988)	Accelerometer; cartridge	“Home-made vibration transmitter”
	Vespa velutina	S	A-L	Cervo, Cini and Pepiciello pers. obs.		
	Vespula vulgaris	GD	De	Ross (1982)		
Ropalidiini	*Belonogaster juncea*	LgV; GD	Do; A-L	Tindo et al. (1997)		
	Belonogaster petiolata	LgV	A-L	Keeping (1992)		
		LtV	Do	Gadagkar and Joshi (1984)		

	Ropalidia cyathiformis					
	Ropalidia fasciata	HD	A-L	Jeanne (1975), Ito (1983), Ito (1986)		
	Ropalidia marginata	WV	A-L	Sen and Gadagkar (2006)		
	Ropalidia opifex	GD	De	Fortunato et al. (2004)		
	Ropalidia revolutionalis	WV; HD	Do; A-L	Ito (1987), Hook and Evans (1982)		
Mischocyttarini	*Mischocyttarus cassununga*			Santos et al. (2007)		
	Mischocyttarus cerberus	WV; GD	De	Togni and Giannotti (2010)		
	Mischocyttarus drewseni	GD	De	Jeanne (1972)		
	Mischocyttarus labiatus	GD	De	Litte (1981)		
Polistini	*Polistes annularis*	AbW	Do; A-L	Hermann and Dirks (1975), Hughes et al. (1987)		
	Polistes atrimandibularis	AbW	Do; A-L	Mignini and Lorenzi (2015)		
	Polistes biglumis	AbW	Do; A-L	Mignini and Lorenzi (2015)		
	Polistes canadensis	LtV	Do	West-Eberhard (1969), West-Eberhard (1986)		
	Polistes carnifex	AbW; AD	Do; A-L	Corn (1972)		
	Polistes chinensis	AbW	Do	West-Eberhard (1969)		

(continued)

Table 18.1 (continued)

Group	Species	Behavior	Context	References	Vibration sensor	Playback device
	Polistes dominula	WV; AbW	De; Do; A-L	Heldmann (1936), Pardi (1942), Röseler and Röseler (1989), Brillet et al. (1999), Bruschini et al. (2005), Jha et al. (2006), Brennan (2007), Pepiciello et al. (2018)	Accelerometer; laser vibrometer	Electrodynamic shaker
	Polistes erythrocephalus	LtV; AbW	Do			
	Polistes exclamans	AbW	Do	West-Eberhard (1969), Hermann et al. (1975)		
	Polistes flavus	AbW	Do	West-Eberhard (1969)		
	Polistes fuscatus	LtV; AbW; AD	Do; A-L	Rau (1928), Pratte and Jeanne (1984), Harding and Gamboa (1998), Savoyard et al. (1998), Suryanarayanan et al. (2011), Jandt et al. (2017)	Piezoelectric polyvinylidene fluoride foil	Solenoid; piezoelectric polyvinylidene fluoride foil
	Polistes instabilis	AbW	Do	Hughes and Strassmann (1988), Jha et al. (2006)		
	Polistes jokahamae	AbW	Do	Yoshimura et al. (2019)		
	Polistes major major	AbW	Do	West-Eberhard (1982)		
	Polistes metricus	LtV; LgV; AbW	Do; A-L	Gamboa et al. (1978), Gamboa and Dew (1981)		
	Polistes semenowi	GD		Zacchi et al. (1996)		
	Polistes snelleni	AD	A-L	Yamane (1971)		
	Polistes stigma	AbW	Do	Suzuki (1996)		
	Polistes sulcifer	GD		Cervo (1990, 2006)		

	Polistes versicolor	AbW		Esch (1971), de Souza and Prezoto (2012)		
Epiponini	*Asteloeca ujhelyii*	S	De	Nascimento et al. (2005)		
	Agelaia yepocapa	GF	De	O'Donnell et al. (1997)		
	Apoica albomacula	GF	De	O'Donnell et al. (1997)		
	Brachygastra augusti	GF	De	O'Donnell et al. (1997)		
	Parachartergus colobopterus	GD	De	Jeanne and Keeping (1995)		
	Polybia dimidiata	GF	De	O'Donnell et al. (1997)		
	Polybia occidentalis	WV; GF	De	Jeanne (1981)		
	Polybia dimorpha	GF	De	O'Donnell et al. (1997)		
	Polybia atra	AbW	De	Hase (1935)		
	Synoeca septentrionalis	GF; GD; S	De	West-Eberhard (1982), O'Donnell et al. (1997)		
	Synoeca virginea	GD; S	De	Overal (1982), West-Eberhard (1982)		

Behaviors: *LtV* Lateral vibration, *LgV* Longitudinal vibration, *WV* Wing vibration, *GF* Gastral fanning, *AbW* Abdominal wagging, *GD* Gastral drumming, *AD* Antennal drumming, *HD* Head drumming, *S* Scraping. Contexts: *De* Defense, *R* Recruitment, *Do* Dominance, *A-L* Adult–larvae

80–2000 Hz, with a dominant frequency of 500 Hz and 125 Hz in *V. germanica* and *V. orientalis*, respectively (Ishay 1975; Ishay and Nachsen 1975). In contrast, the dominant frequencies of AD and AbW are 16 Hz and 50 Hz, respectively (Suryanarayanan et al. 2011; Pepiciello et al. 2018).

Among arthropods, vibrational signals produced by individuals drumming on several different substrates are usually characterized by a broadband spectrum profile (Elias and Mason 2010). This mechanism theoretically generates a broadband signal that at the source contains all frequencies at equal intensities; the filtering properties of the substrate are responsible for the final spectrum profile of the signal (Hebets et al. 2008). Wasps' drumming vibrations are consistent with the vibrational signals of other arthropods. The differences in the dominant frequencies within different species performing GD, and between AbW and AD, may be due to the differences in nest structure, either of the material or the geometry, and the peculiar way each species strikes the surface.

Until recorded with an accelerometer, AbW was considered to transmit vibrations into the nest just by tremulation (i.e., without contact between the abdomen and the comb) (Brennan 2007). However, spectral analysis (Fig. 18.3) supports the hypothesis that an oscillatory behavior, such as AbW, can also be considered as a particular form of drumming, in that they both transmit similar broadband spectrum profiles in the nest structure when the body of the wasp comes in contact with the comb. Additionally, the simultaneous recording of AbW with an accelerometer and a video camera revealed that each pulse is induced by the beating of the abdomen on the nest (Brennan 2007). Sometimes, during the oscillation, the abdomen does not touch the nest; in these cases, the characteristic pulses of AbW are not picked up by the accelerometer.

As expected for broadband signals, the temporal features seem to be a better candidate to distinguish drumming behaviors among species and within a species, but associated in different contexts (Elias and Mason 2014). In Vespinae, the interval between pulses of GD is shorter in *V. germanica* than in *V. orientalis*, and in the latter, different rhythms distinguish scraping from GD, as well as GD performed by workers in different contexts, e.g., to stimulate the queen to resume her laying activity or to increase the activity of larvae and workers (Ishay et al. 1974; Ishay and Brown 1975).

The frequency profile of vibrations induced by scraping is similar to the spectrum profile of drumming. In the case of Vespinae larvae, most of the energy of the signals is in the range of 100–2000 Hz, and the temporal distinction of discrete pulses is determined by the rhythmic contraction of the larva inside the cell (Ishay and Landau 1972; Ishay and Schwartz 1973). The leg scraping of Polistinae guard wasps induces vibrations with a dominant frequency of 500 Hz, which is maintained even when the velocity of scraping varies (Nascimento et al. 2005).

Unfortunately, vibrations induced in the nest material by tremulation (LtV, LgV, and WV) have not been measured yet. But from what we know of other insects using this mechanism of production, we can expect these behaviors to induce substrate-borne waves with most of the energy concentrated in a narrow range of low frequencies (Hill 2009).

18.2.2 Perception of Vibrations

To communicate via substrate-borne waves, insects usually rely on mechanical receptors that detect particle motion perpendicular to the direction of propagation of waves (reviewed in Hill 2009; and Lakes-Harlan and Strauß 2014). Among social Hymenoptera, most of the knowledge of mechanical receptors comes from studies on *Apis mellifera*, but the few studies on solitary and social wasps unveiled the mechanism of perception of vibrations that is also found in Vespidae (Staudacher et al. 2005; Hunt and Richard 2013).

Social wasps have both external (i.e., hair sensilla) and internal (i.e., scolopidial sensilla) mechanical receptors (Hunt and Richard 2013). All of them are able to detect the displacement of the organ with respect to the environment and translate it into an electrical impulse that is processed by the nervous system. However, probably not all of them are involved in vibrational communication (Lakes-Harlan and Strauß 2014).

To perceive vibrational signals propagated through the nest, social wasps must be able to detect the displacement of the comb's surface perpendicular to their legs. In order to do so, they likely rely on complex chordotonal organs, such as the subgenual organ in the proximal tibia and the Johnston's organ in the antennal pedicel (Fig. 18.4). The detection of the substrate's displacement requires the stimulus and the receptor to be coupled, meaning that the part of the body in which the receiving organ is located must be in contact with the vibrating surface, either directly or indirectly. Since the legs are always in contact with the substrate, the subgenual organ is considered the primary organ for perceiving the vibrations in many insects, and it is also likely the principal receiving mechanism in adult social wasps (Hunt and Richard 2013). The Johnston's organ might play a role in the perception of substrate-borne waves as well, since the highly mobile antennae can easily come in contact with the substrate.

The sensitivity of the subgenual organ has been tested in *V. crabro* (Autrum and Schneider 1948), and its morphology has been described in three polistine species: *Agelaia pallipes, Polybia paulista,* and *Mischocyttarus cassununga* (Santos et al. 2007). In the latter study, the number of scolopidia in the subgenual organ is shown to be around 40. This is consistent with the number of scolopidia observed in other social Hymenoptera, such as ants (Howse 1964; Menzel and Tautz 1994) and honey bees (Kilpinen and Storm 1997). The sensitivity of the subgenual organ in *V. crabro* seems to be tuned to the substrate-borne waves produced by the species. In fact, most of the energy of the vibrations emitted by the larvae is around 160–200 Hz and the lower threshold of the subgenual organ is at about 80 Hz, while its sensitivity decreases rapidly until 1000 Hz (Ishay and Schwartz 1973).

The Johnston's organ has been described in Polistinae, and it perceives the displacement of the flagellum with respect to the pedicel of the antenna (Santos et al. 2007). This means that during inspection of the nest or the brood, when the wasp inserts its head in the cell, the flagellum is very likely to touch the cell's wall from which it could easily then perceive the vibrations.

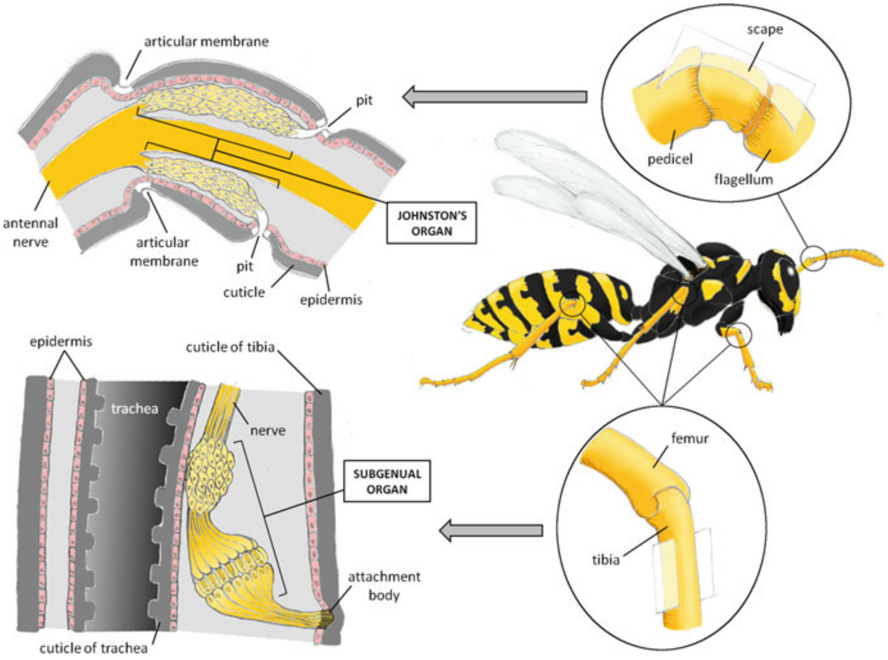


Fig. 18.4 Drawing of a wasp with highlighted locations of mechanoreceptors. Sections are of Johnston's organ and the subgenual organ. Drawings are adapted from Hunt and Richard (2013) and Santos et al. (2007). Drawings by Marco Valerio Rossi Stacconi

On the contrary, there is no evidence so far that social wasps perceive airborne vibrations (sounds). Tympanic organs, which are chordotonal organs specialized in the detection of sounds, are considered absent in all Hymenoptera (Hunt and Richard 2013). An "acoustic box," found in the center of the head of Vespinae (absent in Polistinae), has been hypothesized to be used for gravity and sound perception (Ishay and Shimony 1986). However, the function of this organ has yet to be tested. Even if the dedicated organs are not present, we cannot exclude the possibility that social wasps may be able to detect airborne vibrations. For instance, in *A. mellifera* several ingenious studies revealed that workers are not able to perceive the compressional waves of sounds, but they are sensitive to airflows associated with them (Michelsen 2014), even if the mechanism that enables the workers to perceive the airflow is still unknown.

18.2.3 Transmission of Vibrations

The substrate in which vibrational signals are induced has a key role in biotremology. The geometry and material of the substrate determine the type of waves that are transmitted by the insect and how they are filtered (Elias and Mason

2014). In social wasps, all behaviors involved in transmission of vibrations into the substrates are performed on the nest: on the combs or on the envelope enclosing them. Thus, understanding how substrate-borne waves propagate in the nest structure is crucial in the biotremology of social wasps.

The architecture of nests in social wasps is extremely variable, and it has been studied from an evolutionary point of view in relation to predatory and parasitic pressure (Jeanne 1975). The transmission properties of the nests have been poorly investigated, but the structural similarities with other social insects, and some peculiar characteristics, suggest that it may be a suitable substrate for propagation of vibrational signals. As in the hive of honey bees, the comb of social wasps is made of adjacent hexagonal cells. This structure creates a continuum in the substrates represented by the rim of the cells, which, at least in Vespinae, are thicker compared to the cell walls (Schaudinischky and Ishay 1968). In honey bees, this web made of cell rims enables a very good transmission of vibrations (Michelsen et al. 1986). Moreover, in most wasps' nests, the comb is free on all sides. In the honey bee's comb, the impedance of the comb is lower when the cells are open, and closer to the border of the comb, when it is free to move, as in wild combs that are attached just by one side. Likely for this reason, in commercial hives in which combs are framed, honey bees free the comb from the frame in the area where they usually perform the dances (Sandeman et al. 1996). In the nest of social wasps, the propagation of vibrations may be different compared to the honey bee's comb for the following four characteristics: (1) the comb is usually free on all sides, (2) the surface is striped with a rough pattern, (3) when more than one comb is present, these combs are interconnected, and (4) an external envelope encloses the comb (Fig. 18.5). The first two characteristics are shared by most social wasps, whereas the third and fourth are peculiar to Vespinae and only found in some species of Polistinae (Kojima 1982; Wenzel 1998; de Seixas Felizardo et al. 2018). With the exception of Stenogastrinae and some epiponines, most social wasps' nests are attached to the supporting structure through one or more thin stalks, the petioles (Fig. 18.5). The main function is to defend the nest from intruders, such as ants, but it enables the nest to be entirely suspended and free from restrictions. In this way, we can expect the outer side of the comb to amplify the vibrations as happens in the hive of honey bees when the comb is free from the frame (Schaudinischky and Ishay 1968; Sandeman et al. 1996).

The building material is made by mixing only organic (vegetable fiber) or mineral materials (mud) or a combination of the two with saliva (Ganor et al. 1986). This manufactured material is laid horizontally in the cells' walls in consecutive layers, creating a rough surface. Thus, the stiffness is parallel to the comb's face along the rim of the cells, facilitating the transmission of substrate-borne waves, and the walls are striped with irregularities that enable the production of vibrations by scraping of the surface (Schaudinischky and Ishay 1968).

In the species in which several combs constitute the nest, the transmission of vibrations from one comb to the others can be ensured by thin interconnecting columns made of the same material as the combs, or by the external envelope that, in some species, connects all the combs along their sides (Schaudinischky and Ishay 1968; Jeanne 1975). In a minority of species, multiple combs that are not attached to

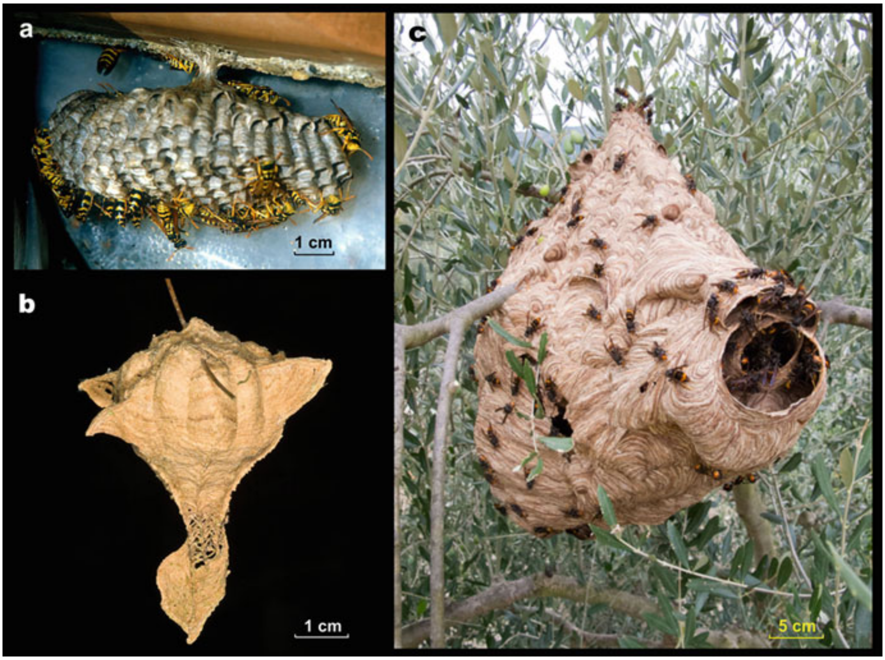


Fig. 18.5 Three examples of social wasps' nests and features that may enhance vibrational communication: (**a**) nest of *Polistes nimpha*, (**b**) nest of *Eustenogaster fraterna*, and (**c**) nest of *Vespa velutina*. In (**a**) the comb is free on all sides, in (**b**) and (**c**) the nest is enclosed in an envelope, in all three nests the surface is striped with a rough pattern. Photographs by Rita Cervo (**a** and **c**) and David Baracchi (**b**), with permission

one another can be part of the same colony (i.e., satellite nests), such as in the case of *R. fasciata* that performs HD (Jeanne 1975; Ito 1983, 1986). Vibrations are mainly transmitted when substrates are contiguous, but low frequencies can be transmitted also to substrates separated by an air gap up to 10 cm (Eriksson et al. 2011; Gordon et al. 2019). Thus, in species with satellite nests, vibrations can be transmitted to colony members present on either the same or close combs.

The external envelope may provide two additional advantages to vibration transmission, improving the use of vibrations as signals for communicative purposes. The envelope isolates the nest from airborne noises and provides a continuous substrate that encloses the entire colony and that may facilitate the exchange of information among all colony members (Schaudinischky and Ishay 1968). Moreover, in some Stenogastrinae, which lack the petiole, the envelope is the main surface to transmit and amplify substrate-borne waves (Hansell 1987; Turillazzi 2012).

Since all the colony activities and nestmate interactions take place on the nest, we expect wasps to take advantage of this self-made substrate and that the signals should match its transmission properties (Elias and Mason 2014). It is likely that wasps' mechanisms of vibration production evolved to match the transmission properties of the substrate. In hornets, the absorption coefficient of the comb is complementary to the spectrum profile of all three observed vibrational behaviors, i.e., the scraping of the larvae and the two drumming behaviors performed by workers (the "awakening dance" and the "dance facing the queen") (Ishay 1975). But this is likely to be a consequence of the production mechanisms, drumming and scraping, which induce broadband vibrations into the substrate (Hebets et al. 2008). Further understanding can be found by investigating other mechanisms of production, such as tremulation, which may induce vibrations with a narrower frequency profile into the substrate.

18.3 Why? Functions of Vibrational Signals in Social Wasps

Over the years, many, and sometimes contrasting, hypotheses on the function of vibrations in wasps' societies have been proposed. Most of the contradictions emerged as the hypotheses were based on correlative studies. In the last decade, some hypotheses have been tested using the playback of previously recorded vibrations, which have provided a clearer understanding of the communicative role of vibrations in social wasps. Nevertheless, the function of most vibrational behaviors in social wasps is yet to be determined, because of the abundance of the behaviors that induce vibrations and the variety of contexts in which they occur. With the aim of providing an overview of the functions that vibrations have in wasps' societies, we discuss the vibrational behaviors associated with the following four challenges faced by social wasps: defense, recruitment, dominance, and larvae–adult communication (Fig. 18.1). For each category, we address the proposed hypotheses, correlated evidence, and, when available, direct testing of functions.

18.3.1 Defense

When a colony is threatened by a predator, the members present on the nest use warning behaviors to both warn the intruder before a direct attack and coordinate a collective response (Starr 1990). Predators, either invertebrates or vertebrates, are warned by the visual and acoustic components, whereas the release of an alarm pheromone is used to recruit other members of the colony toward the attacker (Jeanne 1981; Fortunato et al. 2004). However, most warning behaviors also have a vibrational component, such as with wing vibrations, which are widespread in Polistinae and Vespinae (Ishay and Sadeh 1982; Bruschini et al. 2005; Togni and Giannotti 2010), leg scraping (Nascimento et al. 2005), abdomen drumming (Litte 1981; Ross 1982; West-Eberhard 1982; Hansell 1987; Krombein 1991; Jeanne and Keeping 1995; Francescato et al. 2002; Fortunato et al. 2004; Togni and Giannotti 2010), and mandible scraping (Barenholz-Paniry and Ishay 1988).

Vibrations are rapidly transmitted throughout the nest; thus, they can warn all nestmates standing on the nest of the imminent danger, regardless of their position or activity within the colony. In the termite *Macrotermes natalensis*, substrate-borne waves have been shown to give information on the direction from which the threat is coming and to be used for coordination of group defense (Hager and Kirchner 2014). When a predator attacks a colony, soldiers drum their heads on the walls of the nest, producing a vibrational alarm signal. In response to the alarm signal, workers retreated into the nest, whereas more soldiers were recruited toward the surface where the predator would be found. Termites make use of tropotactic directional vibration sensing to orient to the source of vibrations.

There is evidence in all three subfamilies of social wasps that vibrations can function as a defense-related signal. Among Stenogastrinae, females of *Eustenogaster* spp. often usurp a pre-occupied nest, so the resident female guards the entrance of the nest by standing next to the opening on the inside of the envelope (Hansell 1987; Francescato et al. 2002). Whenever an alien female approaches or lands on the nest, the resident female performs GD to warn the alien female that the nest is occupied. The alien performs GD too after landing on the nest, but if it does not leave the nest after perceiving the GD from the resident female, the confrontation can escalate into a fight. Interestingly, among Polistinae, *Parachartergus colobopterus* seems to have lost the alarm pheromone and replaced it with a form of GD, the gaster tapping (Jeanne and Keeping 1995). Similarly, the leg scraping of *Asteloeca ujhelyii* workers has been hypothesized to inform nest mates about the approach of a wasp to the nest entrance (Nascimento et al. 2005). In hornets, when a colony of *V. orientalis* is threatened, larvae scrape the walls of the cells and workers perform WV; immediately after the onset of WV, every activity on the nest stops (Ishay and Sadeh 1982; Barenholz-Paniry and Ishay 1988). Also among Polistinae WV is correlated with the defense of the colony; however, in this subfamily the vibrational component of WV is not sufficient to elicit a defense response from the colony members. Instead, WV is associated with the release of the alarm pheromone; therefore, in Polistinae, vibrations induced by WV seem to be a by-product of a pheromonal release (Jeanne 1981).

Nevertheless, in order to assess the role of vibrations in coordinating defense strategies in social wasps, experimental tests that quantify the response of colony members to the vibrational component of warning behaviors are needed.

18.3.2 Activation and Recruitment of Nestmates

In social wasps, the work is regulated among members of the colony. The hypothesis that vibrations regulate the activity in the nest was formulated for the first time in the 1970s, based on the observation of workers of *V. orientalis* performing GD (originally named "dance of awakening") (Ishay and Schwartz 1973). When workers' GD vibrations were played back into the nest, the activity in the nest increased: larvae began to scrape their cell walls and workers either resumed attending larvae or attempted foraging flights (Ishay et al. 1974).

Recently, it has been confirmed that in *V. germanica* substrate-borne waves act as a recruitment signal. The playback of the sole vibrational component of GD led to increased movement in the nest, trophallaxis, and worker departures from the nest (Taylor and Jeanne 2018).

In some Polistinae, vibrations are correlated with an increase in general activity of colony members. For instance, LtV is related to an increase in activity and, in particular, of other vibrational behaviors in colonies of *P. fuscatus* (Savoyard et al. 1998). In *P. dominula* and *P. instabilis*, the activity on the nest increases after AbW (Jha et al. 2006). However, this response has yet to be demonstrated to be caused by vibrational stimuli alone.

18.3.3 Dominance

Social structure varies widely across wasp taxa. In Vespinae, reproductive females and workers are clearly distinct, both morphologically and physiologically, whereas in Stenogastrinae and Polistinae there is a more flexible division of labor and all females can potentially lay eggs (Hunt 2007). Among Polistinae, since potentially all females can reproduce, a linear dominance is established; the dominant individual assumes the role of queen and lays eggs, while all other females are subordinates with a lower-ranking position and act as workers (i.e., non-reproductive females that help in maintaining the colony) (Pardi 1948). This structure is established and maintained by means of dominance interactions, which are more aggressive at the beginning of the colony cycle and ritualized once the dominant individual has established its role (Pardi 1948; Jandt et al. 2014). At this stage, the dominant female diverges from the subordinates both in terms of behavior and physiological features; she performs "dominance behaviors" toward subordinates and workers (Pardi 1942) and develops a unique profile of cuticular hydrocarbons (Dapporto et al. 2007). Over the years, many tremulatory and drumming behaviors have been reported to be part of the suite of "dominant behaviors." In particular, LtV, LgV, and AbW are usually performed more frequently by the dominant female, and they have been associated with adult–adult aggressive interactions in several species (Ropalidiini: Gadagkar and Joshi 1984; Ito 1987; Tindo et al. 1997; Polistini: West-Eberhard 1969, 1982, 1986; Corn 1972; Gamboa and Dew 1981; Hughes et al. 1987; Hughes and Strassmann 1988; Suzuki 1996; Savoyard et al. 1998; Brillet et al. 1999; Mignini and Lorenzi 2015). Even in non-aggressive species such as *P. jokahamae,* only the queen performs AbW in association with higher oviposition frequency (Yoshimura et al. 2019).

All "dominance behaviors" are considered to be involved in the maintenance of the reproductive monopoly by the dominant female (Pardi 1948; Jandt et al. 2014). The vibrational component of these behaviors has been hypothesized to be one of the mechanisms by which the dominant individual achieves and maintains the monopoly, both by communicating to the subordinates of the presence on the nest of an active dominant female (direct influence) and by inducing a physiological change in the subordinates to ensure their non-reproductive status (indirect influence) (Jeanne 2009). Whether vibrations have either a direct or indirect influence on subordinates and workers has yet to be tested. The direct influence is difficult to test, because during dominant interactions visual, chemical, and vibrational signals occur together in signaling status, and it is difficult to disentangle the relative role of each of them on fertility, as well as experimentally decouple fertility from dominance. However, the indirect effect of mechanical stimulation has been demonstrated on larval physiology (see Sect. 18.3.4 for details) (Suryanarayanan et al. 2011). Thus, it is possible that vibrational signals associated with "dominance behaviors" also affect the reproduction of workers (Jeanne 2009).

An interesting insight into the role of vibrational stimuli in dominance can be found by studying obligate social parasites and their hosts. Three species of the *Polistes* genus are obligate social parasites. They lack the worker caste and need to usurp a colony from a closely related species to obtain parental care for their brood and thus reproduce (Cervo 2006). The female parasite usurps a nest and eventually replaces the dominant female (which can either stay on the nest as a subordinate or abandon the colony) and begins laying eggs. Workers of the usurped colony then rear the brood of the parasite. The parasite is able to integrate herself into the social structure of the host by performing "dominance behaviors" and modifying her own chemical profile to match that of the host colony (Lorenzi 2006). However, at least in one species, the control over the reproduction of the host is limited (Cini et al. 2014). It is possible that one or more components of the "dominant behaviors" of social parasites may differ from its host. Social parasitism has proven to be a useful tool to reveal mechanisms and evolution of insect societies that are still unknown (Cini et al. 2019), and it may be the case also for vibrations associated with "dominant behaviors." In fact, all three species of obligate social parasites perform AbW in the days following the usurpation of the nest, and *P. semenowi* and *P. sulcifer* also display GD (Cervo 2006; Mignini and Lorenzi 2015). A recent study on the host–parasite model *P. biglumis*–*P. atrimandibularis* supports the use of AbW in the host, *P. biglumis,* as an indication of dominant status (Mignini and Lorenzi 2015). Interestingly, when the parasite *P. atrimandibularis* took the dominant role on the nest, the parasite performed AbW at a rate similar to the dominant female before the intrusion, whereas the subdued female performed AbW less frequently than in non-parasitized colonies. Unfortunately, the AbW and associated vibrations were not measured in this study or in any other host–parasite system to date. The comparison between the host and the parasite vibrations could unveil to what extent dominance is expressed by the vibrational component of "dominant behaviors."

18.3.4 Adult–Larvae Communication

Adults are not the only members of a wasp colony. The immature brood of eggs, larvae, and pupae are often present in the nest, which literally surrounds them within the cell walls. Interactions between larvae and adults can be crucial in social wasps; they often exchange saliva (trophallaxis), which provides essential nutrients to the adults in Vespinae (Ishay and Ikan 1968), and larvae rely on the adults to be fed. Therefore, communication between larvae and adults in the colony is fundamental. The exchange of vibrations is bidirectional: from larvae to adults and from adults to larvae.

Larvae use vibrations to communicate their feeding status to workers and to warn them of an approaching threat. The "hunger signal" of *V. orientalis* larvae is one of the first wasp vibrational signals for which the function has been identified and tested with a playback experiment (Ishay et al. 1974). Ishay and collaborators demonstrated that the scraping of the mandibles on the cell's wall generated by starved larvae attracts workers to the vibrations' source. The attraction was clearly related to feeding. In fact, workers brought food and droplets of saliva to the cell in which the shaker emitting the playback was positioned. Scraping is also performed by larvae of *V. crabro* and *V. velutina*, in which it likely has the same function as in *V. orientalis*. However, it has never been recorded in species of *Vespula* (*V. germanica:* Schaudinischky and Ishay 1968; Ishay and Brown 1975; *V. pensylvanica* and *V. atropilosa:* Akre et al. 1976), and it is not reported in any Stenogastrinae and Polistinae species. In *V. orientalis*, when the colony is disturbed by any threat, larvae scrape the walls of the cells using a different rhythm from that of the "hunger signal." Such behavior has been hypothesized to transmit the alarm inside the nest (Barenholz-Paniry and Ishay 1988).

Adults also use vibrational stimuli to communicate to larvae. For instance, in Vespinae, GD triggers larvae to emit the "hunger signal" (Ishay et al. 1974). In Polistinae, several correlative data showed that larvae may be one of the recipients of adults' vibrational signals: (1) AbW appears when larvae reach at least the third instar, (2) AbW and LtV are performed also in the absence of other adults on the nest, and (3) LtV, AbW, and AD are performed while adults are feeding the larvae (Brillet et al. 1999; Brennan 2007).

A proposed function of vibrations induced by LtV and AbW was that they signal the larvae when to release or withhold their saliva during feeding interactions (Harding and Gamboa 1998; Savoyard et al. 1998). This hypothesis has been recently rejected in *P. dominula*, in which a playback experiment demonstrated that AbW does not have any direct effect on the larvae's release or withholding of saliva (Pepiciello et al. 2018). However, the study showed that AbW elicits an increase in larval movements, which likely attracts the attention and care of the adults, thus providing the first direct demonstration of an adult–larvae vibrational signal in *P. dominula*.

Another function of signals directed at larvae is caste determination, as explained by the "mechanical switch hypothesis" (Jeanne 2009). For many years, the nourishment level of larvae has been considered the only mechanism determining the physiological differences between workers and reproductive females in Polistinae (i.e., low nourishment levels would determine a worker phenotype) (Judd et al. 2015). The "mechanical switch hypothesis" predicts that vibrational stimuli modulate the biochemical pathways that direct the larval development toward a worker phenotype, so that vibrations would have a modulatory effect on the nourishment level and an indirect effect on caste determination (Jeanne 2009). The correlation between the beginning of vibrational behavior occurrence and third instar larvae in the nest supports this hypothesis, as the third instar is the stage at which developmental paths diverge (Jeanne 2009). To date, the "mechanical switch hypothesis" has been verified only for vibrations induced by AD in *P. fuscatus* (Suryanarayanan et al. 2011). In this study, the females emerging from the nests that were subjected to a vibrational playback similar to AD had a more pronounced "worker" phenotype (i.e., low percentage of fat stores) compared to the females that emerged from control nests (i.e., more gyne-like levels of fat stores). Additionally, a novel transcriptional experiment demonstrated that either vibrations or nourishment level alone can influence the gene expression in *P. fuscatus* offspring, but their interaction defines the caste of colony members (Jandt et al. 2017). The validity of the "mechanical switch hypothesis" has yet to be tested for other *Polistes* species and their vibrations.

It cannot be excluded that the "mechanical switch hypothesis" may be valid also for species of other subfamilies, in which the hypothesis has not yet been tested, but vibrations are known to be associated with the feeding of larvae, such as LgV in *E. fraterna* (Francescato et al. 2002) and GD in *V. consobrina* (Akre et al. 1982). Both these species have a primitive social structure similar to Polistinae, in which workers and reproductive females do not differ morphologically. Taking into consideration just Vespinae and Polistinae, it has been hypothesized that vibrations were the rudimentary mechanism for caste biasing in the common ancestor of these two subfamilies, and for this reason, vibrational behaviors associated with brood attendance have been observed only in species with primitive caste differentiation (Jeanne and Suryanarayanan 2011). According to this evolutionary hypothesis, such a rudimentary mechanism would have been replaced by ritualized behaviors in less derived taxa, such as Polistinae, and by more specialized mechanisms, such as pheromones and differences in food quality, in the more derived species of Vespinae. However, this evolutionary model neglects the occurrence of similar vibrations in at least one species of Stenogastrinae. Additionally, according to the most recent molecular studies, sociality evolved twice in social wasps, once in Stenogastrinae and once in Vespinae + Polistinae (Piekarski et al. 2018). So, testing the "mechanical switch hypothesis" also in Stenogastrinae could have an important role in further understanding the evolution of sociality in wasps.

18.4 Perspectives

This chapter highlights that the use of substrate-borne waves is widespread and central to the integration of social life in the wasps. Vibrational communication occurs in all three subfamilies of Vespidae, and in at least two of them, it has been demonstrated to play a crucial role in the organization and maintenance of sociality. In both Vespinae and Polistinae, vibrations mediate adult–brood communication in both directions. Additionally, in Vespinae vibrations are a signal for the recruitment of nestmates, and in Polistinae, vibrations modulate the effect of nourishment on caste determination. Because the number of studies testing the role of vibrations in wasps is so low, much low-hanging fruit remains to be picked. The development of informed, competing hypotheses on function, followed up by well-designed experiments to test them, will yield rapid gains in understanding. We suggest that further work, in particular in the four areas discussed in this section, will bring a better understanding of the social life of wasps and of biotremology of social insects in general.

18.4.1 Measurement of the Surface-Borne Component of Vibrational Behaviors

More than 50 species have been reported to perform vibrational behaviors, but the vibrations associated with these behaviors have been measured in only four of them. Moreover, for one mechanism of production, tremulation, no attempt has yet been made to measure the surface-borne component (Ishay 1975; Ishay and Nachsen 1975; Brennan 2007; Suryanarayanan et al. 2011). The description of spectral and temporal features of substrate-borne waves is essential to being able to compare different behaviors in the same species and across species. This lack of knowledge is probably the main cause of confusion and redundancy in the terminology used so far to describe vibrational behaviors of wasps. Furthermore, any investigation on the communicative role of vibrations requires a functional test, for which a description of the temporal and spectral features of the signal is essential.

Some aspects of the life of social wasps are still poorly investigated and have not yet been associated with the production of substrate-borne waves. Yet, it is possible that some vibrational behaviors have yet to be described. For instance, males in social insects are often neglected, despite the importance and complexity of mating behaviors (e.g., males can establish leks, perform impressive sexual displays, and have alternative mating tactics) (Beani et al. 2014). In many polistine species, males have been observed to scrape either the substrate or the female with the abdomen, legs, or mandibles before and during mating; these behaviors have always been hypothesized to be associated with the release of pheromones (Turillazzi and Cervo 1982; Beani and Calloni 1991a, b; Beani 1996; Ayasse et al. 2001). However, the role of male pheromones has not been demonstrated in social wasps, whereas visual cues have been found to play a role in identification of genders and female choice, at least in Polistinae (Ayasse et al. 2001; Izzo and Tibbetts 2012; Cappa et al. 2016). It is possible that vibrations also mediate male territoriality or male–female interaction.

18.4.2 Mechanisms of Perception and Propagation into the Substrate

The knowledge on both the mechanisms of perception and propagation of vibrations into the nest comes from a minority of species or from the comparison with closely related species. Even though it has been shown that wasps' nests are a suitable substrate for the propagation of vibrations (Schaudinischky and Ishay 1968), it should be recognized that any substrate filters substrate-borne waves, affecting their spectral features in particular (Elias and Mason 2014). Thus, a better understanding of both the material and the structure of the substrate is needed to identify the key characteristics of the putative signal that may convey information. Similarly, understanding the mechanism by which receivers perceive a stimulus can be useful to identify what communication modality is mainly used by an animal. For instance, the inability of honey bees to perceive compressional waves excluded sounds from the possible communication modalities of the species (Michelsen 2014). Mechanoreceptors of social wasps have been poorly investigated. On one hand, the mechanism of perception still needs to be identified, as in the case of larvae. They lack legs and their antennae are too short to easily come in contact with the nest; it is possible that they rely on hair sensilla to perceive vibrations, but it has never been verified. On the other hand, physiological studies on the sensitivity of known mechanoreceptors in wasps are needed to identify the spectral and temporal features of vibrations that may play a major role in communication.

A better understanding of how vibrations are transmitted in the nest structure and perceived by the receivers will also clarify how social insects avoid interference caused by other individuals signaling or moving on the nest (i.e., biotic noise). In social wasps, from a few tens to hundreds of individuals can be present simultaneously in a colony, depending on the species. Additionally, most vibrational behaviors (i.e., drumming and scraping) induce vibrations in the nest surface that have a spectrum similar to that induced by any generic wasp's movement, such as walking. Thus, social wasps must have evolved behavioral and/or physiological strategies to avoid biotic noise (Virant-Doberlet et al. 2014). Vespinae are known to use two behavioral strategies that decrease noise interference: they avoid signals when other substrate-borne waves are induced into the nest (either abiotic or biotic) and they synchronize signaling (Ishay et al. 1974; Ishay and Brown 1975; Barenholz-Paniry and Ishay 1988). Additionally, it is possible that the temporal component of the signals (the total length and the interval between pulses) increases their detectability with respect to incidental vibrations. However, other strategies to reduce noise interference have yet to be investigated.

18.4.3 Playback Tests to Assess the Communicative Function of the Surface-Borne Component

To switch from correlational evidence to a direct test of signal function, all components of a signal should be decoupled and singularly tested on the receivers to quantify their behavioral changes. Such changes can be easily recognized and measured when the signal modifies the behavior of the receiver, as in the case of releaser pheromones, but it may be more difficult when the signal acts on the physiology of the receiver, as for primer pheromones (Richard and Hunt 2013). In social wasps, playback tests enabled the verification of the role of vibrational stimuli in recruitment and adult–brood communication in some species, and gave opportunities for similar tests in other species (Ishay et al. 1974; Suryanarayanan et al. 2011; Jandt et al. 2017; Pepiciello et al. 2018; Taylor and Jeanne 2018). Furthermore, these studies highlighted the findings that substrate-borne waves can act both in a direct and indirect way on the receivers' behavior, similarly to the primer and releaser mechanisms known for pheromones.

Two contexts in which vibrational behaviors are displayed—defense and dominance—have yet to be verified as relying on vibrational communication. Even though, in both contexts, wasps use chemical and visual communication, animal communication is usually complex and often involves multicomponent signals (Higham and Hebets 2013; Richard and Hunt 2013; Cervo et al. 2015). Thus, it is likely that vibrational stimuli modulate the effect of chemicals. For instance, in ants, the vibrational component of stridulation modulates the communicative function of the pheromone released during stridulation (Kirchner 1997). Playback tests of specific functional hypotheses are probably the sole means available to disentangle the overlap of different communication modalities and the role of each one.

18.4.4 Mechanism of Action of Vibrations on Insect Physiology

Mechanical stimuli are known to affect the physiology of insects: for instance, mechanical stress modifies the biogenic-amine levels, larval growth, and tissue damage in the red flour beetle, and substrate-borne waves modify the quantity of the juvenile hormone in honey bee workers (Hirashima et al. 1993; Schneider et al. 2004; Jinham et al. 2012). However, the mechanisms of action present at the molecular level are still unknown. The study of the effect of mechanical stimuli on a wasp's transcriptome and phenotype opens the way to further investigate the causal relation of this phenomenon (Jandt et al. 2017).

Caste determination is crucial in the evolution of sociality and thus of social wasps, and it may be modulated by vibrations not only in the tribe Polistini but also in other subfamilies as proposed by Jeanne and Suryanarayanan (2011). Social wasps are a suitable model to further investigate the processes associating vibrations, physiology, and social behavior.

Acknowledgments We are particularly grateful to Robert Jeanne and Peggy Hill for their helpful suggestions and comments on the early version of this chapter. We appreciate Rachel A. Blood for the English revision of the manuscript and David Baracchi for providing the picture of a nest of *Eustenogaster fraterna*.

References

Akre RD, Garnett WB, MacDonald JF, Greene A, Landolt P (1976) Behavior and colony development of *Vespula pensylvanica* and *V. atropilosa* (Hymenoptera: Vespidae). J Kansas Entomol Soc 49:63–84

Akre RD, Reed HC, Landolt PJ (1982) Nesting biology and behavior of the blackjacket, *Vespula consobrina* (Hymenoptera: Vespidae). J Kansas Entomol Soc 55:373–405

Autrum H, Schneider W (1948) Vergleichende Untersuchungen über den Erschütterungssinn der Insekten. Z vergl Physiol 31:77–88. https://doi.org/10.1007/BF00333879

Ayasse M, Paxton RJ, Tengö J (2001) Mating behavior and chemical communication in the order Hymenoptera. Annu Rev Entomol 46:31–78. https://doi.org/10.1146/annurev.ento.46.1.31

Barenholz-Paniry V, Ishay JS (1988) Synchronous sounds produced by *Vespa orientalis* larvae. J Acoust Soc Am 84:841–846. https://doi.org/10.1121/1.396653

Beani L (1996) Lek-like courtship in paper-wasps: ‘a prolonged, delicate, and troublesome affair’. In: Turillazzi S, West-Eberhard MJ (eds) Natural history and evolution of paper-wasps. Oxford University Press, Oxford, pp 113–125

Beani L, Calloni C (1991a) Male rubbing behavior and the hypothesis of pheromonal release in polistine wasps (Hymenoptera Vespidae). Ethol Ecol Evol 3:51–54. https://doi.org/10.1080/03949370.1991.10721910

Beani L, Calloni C (1991b) Leg tegumental glands and male rubbing behavior at leks in *Polistes dominulus* (Hymenoptera: Vespidae). J Insect Behav 4:449–462. https://doi.org/10.1007/BF01049330

Beani L, Dessì-Fulgheri F, Cappa F, Toth A (2014) The trap of sex in social insects: from the female to the male perspective. Neurosci Biobehav R 46:519–533

Brennan BJ (2007) Abdominal wagging in the social paper wasp *Polistes dominulus*: behavior and substrate vibrations. Ethology 113:692–702. https://doi.org/10.1111/j.1439-0310.2007.01373.x

Brillet C, Semenoff Tian-Chansky S, Le Conte Y (1999) Abdominal waggings and variation of their rate of occurrence in the social wasp, *Polistes dominulus* Christ. I Quantitative analysis. J Insect Behav 12:665–686

Bruschini C, Cervo R, Turillazzi S (2005) Defensive responses to visual and vibrational stimulations in colonies of the social wasp *Polistes dominulus*. Ethol Ecol Evol 17:319–326

Bruschini C, Cervo R, Protti I, Turillazzi S (2008) Caste differences in venom volatiles and their effect on alarm behaviour in the paper wasp *Polistes dominulus* (Christ). J Exp Biol 211:2442–2449

Bruschini C, Cervo R, Turillazzi S (2010) Pheromones in social wasps. In: Litwack G (ed) Pheromones. Academic Press, Burlington, VT, pp 447–492

Cappa F, Beani L, Cervo R (2016) The importance of being yellow: visual over chemical cues in gender recognition in a social wasp. Behav Ecol 27:1182–1189

Castro L, Dvořák L (2009) New and noteworthy records of vespid wasps (Hymenoptera: Vespidae) from the palearctic region (II). Boletin Sociedad Entomológica Aragonesa 44:295–304

Cervo R (1990) Il parassitismo sociale nei *Polistes*. PhD thesis, University of Florence, Italy

Cervo R (2006) *Polistes* wasps and their social parasites: an overview. Ann Zool Fenn 43:531–549

Cervo R, Cini A, Turillazzi S (2015) Visual recognition in social wasps. In: Aquiloni L, Tricarico E (eds) Social recognition in invertebrates. Springer International Publishing, Cham, pp 125–145

Cini A, Nieri R, Dapporto L, Monnin T, Cervo R (2014) Almost royal: incomplete suppression of host worker ovarian development by a social parasite wasp. Behav Ecol Sociobiol 68:467–475. https://doi.org/10.1007/s00265-013-1661-z

Cini A, Sumner S, Cervo R (2019) Inquiline social parasites as tools to unlock the secrets of insect sociality. Philos T Roy Soc B 374:20180193. https://doi.org/10.1098/rstb.2018.0193

Corn ML (1972) Notes on the biology of *Polistes carnifex* (Hymenoptera, Vespidae) in Costa Rica and Colombia. Psyche (Stuttg) 79:150–157. https://doi.org/10.1016/0140-1750(80)90011-1

Dapporto L, Romana Dani F, Turillazzi S (2007) Social dominance molds cuticular and egg chemical blends in a paper wasp. Curr Biol 17:R504–R505. https://doi.org/10.1016/J.CUB.2007.05.002

de Seixas Felizardo SP, dos Santos IPV, Silveira OT (2018) Notes on the nest of the social wasp *Pseudopolybia langi* (Hym., Vespidae, Polistinae). Rev Bras Entomol. https://doi.org/10.1016/j.rbe.2018.02.002

de Souza AR, Prezoto F (2012) Regulation of worker activity in the social wasp *Polistes versicolor*. Insect Soc 59:193–199. https://doi.org/10.1007/s00040-011-0204-8

Elias DO, Mason AC (2010) Signaling in variable environments: substrate-borne signaling mechanisms and communication behavior in spiders. In: O'Connell-Rodwell CE (ed) The use of vibrations in communication: properties, mechanisms and function across taxa. Transworld, Kerala, pp 25–46

Elias DO, Mason AC (2014) The role of wave and substrate heterogeneity in vibratory communication: practical issues in studying the effect of vibratory environments in communication. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer-Verlag, Berlin, Heidelberg, pp 215–247

Eriksson A, Anfora G, Lucchi A, Virant-Doberlet M, Mazzoni V (2011) Inter-plant vibrational communication in a leafhopper insect. PLoS One 6:e19692. https://doi.org/10.1371/journal.pone.0019692

Esch H (1971) Wagging movements in the wasp *Polistes versicolor vulgaris* Bequaert. Z vergl Physiol 72:221–225. https://doi.org/10.1007/BF00297781

Fortunato A, Dani FR, Sledge MF, Fondelli L, Turillazzi S (2004) Alarm communication in *Ropalidia* social wasps. Insect Soc 51:299–305. https://doi.org/10.1007/s00040-004-0744-2

Francescato E, Gerace L, Turillazzi S, Massolo A, Landi M, Hashim R (2002) Colony membership, division of labor, and genetic relatedness among females of colonies of *Eustenogaster fraterna* (Hymenoptera, Vespidae, Stenogastrinae). J Insect Behav 15:153–170. https://doi.org/10.1023/A:1015489532040

Gadagkar R, Joshi NV (1984) Social organisation in the Indian wasp *Ropalidia cyathiformis* (fab.) (Hymenoptera: Vespidae). Z Tierpsychol 64:15–32. https://doi.org/10.1111/j.1439-0310.1984.tb00350.x

Gamboa GJ, Dew HE (1981) Intracolonial communication by body oscillations in the paper wasp, *Polistes metricus*. Insect Soc 28:13–26. https://doi.org/10.1007/BF02223618

Gamboa GJ, Heacock BD, Wiltjer SL (1978) Division of labor and subordinate longevity in foundress associations of the paper wasp, *Polistes metricus* (Hymenoptera: Vespidae). J Kansas Entomol Soc 51:343–352

Ganor E, Barenholz-Paniry V, Ishay JS (1986) Cell wall structure and composition in the comb built by hornets and wasps (Vespinae, Hymenoptera). J Morphol 189:215–224. https://doi.org/10.1002/jmor.1051890211

Gordon SD, Tiller B, Windmill JFC, Krugner R, Narins PM (2019) Transmission of the frequency components of the vibrational signal of the glassy-winged sharpshooter, *Homalodisca vitripennis*, within and between grapevines. J Comp Physiol A 205(5):783–791. https://doi.org/10.1007/s00359-019-01366-w

Hager FA, Kirchner WH (2014) Directional vibration sensing in the termite *Macrotermes natalensis*. J Exp Biol 217:2526–2530. https://doi.org/10.1242/jeb.103184

Hansell M (1987) Elements of eusociality in colonies of *Eustenogaster calyptodoma* (Sakagami & Yoshikawa) (Stenogastrinae, Vespidae). Anim Behav 35:131–141. https://doi.org/10.1016/S0003-3472(87)80218-X

Harding BJ, Gamboa GJ (1998) The sequential relationship of body oscillations in the paper wasp, *Polistes fuscatus* (Hymenoptera: Vespidae). Great Lakes Entomol 31:191–194

Hase VA (1935) Uber den "Verkehr" am Wespennest, nach Beobachtungen an einer tropischen Art. Naturwissenschaften 23:780–783

Hebets EA, Elias DO, Mason AC, Miller GL, Stratton GE (2008) Substrate-dependent signalling success in the wolf spider, *Schizocosa retrorsa*. Anim Behav 75:605–615. https://doi.org/10.1016/J.ANBEHAV.2007.06.021

Heldmann G (1936) Über das Leben auf Waben mit mehreren überwinterten Weibchen von *Polistes gallica* L. Biol Zbl 56:389–401

Hermann HR, Dirks TF (1975) Biology of *Polistes annularis* (Hymenoptera: Vespidae) I. Spring behavior Psyche (Stuttg) 82:97–108

Hermann HR, Barron R, Dalton L (1975) Spring behavior of *Polistes exclamans* (Hymenoptera: Vespidae: Polistinae). Entomol News 86:173–178

Higham JP, Hebets EA (2013) An introduction to multimodal communication. Behav Ecol Sociobiol 67:1381–1388. https://doi.org/10.1007/s00265-013-1590-x

Hill PSM (2009) How do animals use substrate-borne vibrations as an information source? Naturwissenschaften 96:1355–1371. https://doi.org/10.1007/s00114-009-0588-8

Hines H, Hunt JH, O'Connor TK, Gillespie JJ, Cameron SA (2007) Multigene phylogeny reveals eusociality evolved twice in vespid wasps. P Natl Acad Sci USA 104(9):3295–3299

Hirashima A, Nagano T, Eto M (1993) Stress-induced changes in the biogenic-amine levels and larval growth of *Tribolium castaneum* Herbst. Biosci Biotech Bioch 57:2085–2089. https://doi.org/10.1271/bbb.57.2085

Hook AW, Evans HE (1982) Observations on the nesting behaviour of three species of *Ropalidia* Guérin-Méneville (Hymenoptera: Vespidae). J Aust Entomol Soc 21:271–275

Howse PE (1964) An investigation into the mode of action of the subgenual organ in the termite, *Zootermopsis angusticollis* Emerson, and in the cockroach, *Periplaneta americana* L. J Insect Physiol 10:409–424. https://doi.org/10.1016/0022-1910(64)90065-4

Hughes CR, Strassmann JE (1988) Age is more important than size in determining dominance among workers in the primitively eusocial wasp, *Polistes instabilis*. Behaviour 107:1–14

Hughes CR, Beck MO, Strassmann E (1987) Queen succession in the social wasp, *Polistes annularis*. Ethology 76:124–132

Hunt JH (2007) The evolution of social wasps. Oxford University Press, Oxford

Hunt JH, Richard FJ (2013) Intracolony vibroacoustic communication in social insects. Insect Soc 60(4):403–417

Ishay J (1975) Frequencies of the sounds produced by the oriental hornet, *Vespa orientalis*. J Insect Physiol 21:1737–1740. https://doi.org/10.1016/0022-1910(75)90233-4

Ishay J, Brown MB (1975) Patterns in the sounds produced by *Paravespula germanica* wasps. J Acoust Soc Am 57:1521–1525

Ishay JS, Ikan R (1968) Gluconeogenesis in the oriental hornet *Vespa orientalis* F. Ecology 49:169–171

Ishay J, Landau EM (1972) *Vespa* larvae send out rhythmic hunger signals. Nature 237:286–287

Ishay J, Nachsen D (1975) On the nature of the sounds produced within the nest of the wasp *Paravespula germanica* F. Insect Soc 22:213–218

Ishay JS, Sadeh D (1982) The sounds of honey bees and social wasps are always composed of a uniform frequency. J Acoust Soc Am 72:671–675

Ishay J, Schwartz A (1973) Acoustical communication between the members of the oriental hornet (*Vespa orientalis*) colony. J Acoust Soc Am 53:640–649. https://doi.org/10.1121/1.1913368

Ishay J, Shimony TB (1986) Tympanic organ in social wasps (Vespinae). Ital J Zool 20:381–400. https://doi.org/10.1080/00269786.1988.10736569

Ishay J, Motro A, Gitter S, Brown MB (1974) Rhythms in acoustical communication by the oriental hornet, *Vespa orientalis*. Anim Behav 22:741–744

Ito Y (1983) Social behaviour of a subtropical paper wasp, *Ropalidia fasciata* (F.): field observations during founding stage. J Ethol 1:1–14

Ito Y (1986) Social behaviour of *Ropalidia fasciata* (Hymenoptera: Vespidae) females on satellite nests and on a nest with multiple combs. J Ethol 4:73–80

Ito Y (1987) Social behaviour of the Australian paper wasp, *Ropalidia revolutionalis* (de Saussure) (Hymenoptera: Vespidae). J Ethol 5:115–124

Izzo AS, Tibbetts EA (2012) Spotting the top male: sexually selected signals in male *Polistes dominulus* wasps. Anim Behav 83:839–845. https://doi.org/10.1016/j.anbehav.2012.01.005

Jandt JM, Tibbetts EA, Toth AL (2014) *Polistes* paper wasps: a model genus for the study of social dominance hierarchies. Insect Soc 61:11–27. https://doi.org/10.1007/s00040-013-0328-0

Jandt JM, Suryanarayanan S, Hermanson JC, Jeanne RL, Toth AL (2017) Maternal and nourishment factors interact to influence offspring developmental trajectories in social wasps. P Roy Soc B 284:20170651. https://doi.org/10.1098/rspb.2017.0651

Jeanne RL (1972) Social biology of the Neotropical wasp *Mischocyttarus drewseni*. Bull Mus Comp Zool Harv Univ 144:63–150

Jeanne RL (1975) The adaptiveness of social wasp nest architecture. Q Rev Biol 50:267–287. https://doi.org/10.1086/408564

Jeanne RL (1981) Alarm recruitment, attack behavior, and the role of the alarm pheromone in *Polybia occidentalis* (Hymenoptera: Vespidae). Behav Ecol Sociobiol 9:143–148. https://doi.org/10.1007/BF00293585

Jeanne RL (2003) Social complexity in the Hymenoptera, with special attention to the wasps. In: Kikuchi T, Azuma N, Higashi S (eds) Genes, behaviors and evolution of social insects. Hokkaido University Press, Japan, pp 81–130

Jeanne RL (2009) Vibrational signals in social wasps: a role in caste determination? In: Gadau J, Fewell J (eds) Organization of insect societies. Harvard University Press, Cambridge, MA, pp 241–263

Jeanne RL, Keeping M (1995) Venom spraying in *Parachartergus colobopterus*: a novel defensive behavior in a social wasp (Hymenoptera: Vespidae). J Insect Behav 8(4):433–442

Jeanne RL, Suryanarayanan S (2011) A new model for caste development in social wasps. Commun Integr Biol 4:373–377. https://doi.org/10.4161/cib.15262

Jha S, Casey-Ford RG, Pedersen JS, Platt TG, Cervo R, Queller DC, Strassmann JE (2006) The queen is not a pacemaker in the small-colony wasps *Polistes instabilis* and *P. dominulus*. Anim Behav 71(5):1197–1203. https://doi.org/10.1016/j.anbehav.2005.11.005

Jinham AP, Kiruba S, Kumaran JTT, Das SSM (2012) Efficacy of audible sound waves in inflicting tissue damage and mortality in *Tribolium castaneum* (Coleoptera: Tenebrionidae) larvae. Agric Trop Subtrop 45(1):32–36. https://doi.org/10.2478/v10295-012-0005-8

Judd TM, Teal PEA, Hernandez EJ, Choudbury T, Hunt JH (2015) Quantitative differences in nourishment affect caste-related physiology and development in the paper wasp *Polistes metricus*. PLoS One 10:1–18. https://doi.org/10.1371/journal.pone.0116199

Keeping MG (1992) Social organization and division of labour in colonies of the polistine wasp, *Belonogaster petiolata*. Behav Ecol Sociobiol 31:211–224

Kilpinen O, Storm J (1997) Biophysics of the subgenual organ of the honeybee, *Apis mellifera*. J Comp Physiol A 181:309–318. https://doi.org/10.1007/s003590050117

Kirchner WH (1997) Acoustical communication in social insects. In: Lehrer M (ed) Orientation and communication in arthropods. Birkhäuser, Basel, pp 273–300

Kojima J-I (1982) Nest architecture of three *Ropalidia* species (Hymenoptera: Vespidae) on Leyte Island, the Philippines. Biotropica 14:272–280

Krombein KV (1991) Biosystematic studies of Ceylonese wasps, XIX: natural history notes in several families (hymenoptera: Eumenidae, Vespidae, Pompilidae and Crabronidae). Smithsonian contributions to zoology, number 515. Smithsonian Institution Press, Washington, DC. https://doi.org/10.5479/si.00810282.515

Lakes-Harlan R, Strauß J (2014) Functional morphology and evolutionary diversity of vibration receptors in insects. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, Heidelberg, pp 277–302

Leonhardt SD, Menzel F, Nehring V, Schmitt T (2016) Ecology and evolution of communication in social insects. Cell 164:1277–1287. https://doi.org/10.1016/J.CELL.2016.01.035

Litte M (1981) Social biology of the polistine wasp *Mischocyttarus labiatus*: survival in a Colombian rain forest. Smithsonian contributions to zoology, number 327. Smithsonian Institution Press, Washington, DC. https://doi.org/10.5479/si.00810282.327

Lorenzi MC (2006) The result of an arms race: the chemical strategies of *Polistes* social parasites. Ann Zool Fenn 43:550–563

Menzel JG, Tautz J (1994) Functional morphology of the subgenual organ of the carpenter ant. Tissue Cell 26:735–746. https://doi.org/10.1016/S0074-7696(08)60848-7

Michelsen A (2014) Mechanical signals in honeybee communication. In: Cocroft R, Gogala M, Hill P, Wessel A (eds) Studying vibrational communication. Springer, Berlin, Heidelberg, pp 333–347

Michelsen A, Kirchner WH, Lindauer M (1986) Sound and vibrational signals in the dance language of the honeybee, *Apis mellifera*. Behav Ecol Sociobiol 18:207–212. https://doi.org/10.1007/BF00290824

Mignini M, Lorenzi MC (2015) Vibratory signals predict rank and offspring caste ratio in a social insect. Behav Ecol Sociobiol 69:1739–1748. https://doi.org/10.1007/s00265-015-1986-x

Nascimento FS, Hrncir M, Tolfiski A, Zucchi R (2005) Scraping sounds produced by a social wasp (*Asteloeca ujhelyii*, hymenoptera: Vespidae). Ethology 111:1116–1125. https://doi.org/10.1111/j.1439-0310.2005.01124.x

O'Donnell S (1998) Reproductive caste determination in eusocial wasps (hymenoptera: Vespidae). Annu Rev Entomol 43:323–346

O'Donnell S, Hunt JH, Jeanne RL (1997) Gaster-flagging during colony defense in Neotropical swarm-founding wasps. J Kansas Entomol Soc 70:175–180

Overal WL (1982) Acoustical behavior and variable nest architecture in *Synoeca virginea* (hymenoptera: Vespidae). J Georgia Entomol Soc 17:1–4

Pardi L (1942) Ricerche sui Polistini. V. La poliginia iniziale in *Polistes gallicus* (L.). Bollettino dell'Istituto di Entomologia della Università di Bologna 14:1–106

Pardi L (1948) Dominance order in *Polistes* wasps. Physiol Zool 21:1–13. https://doi.org/10.1086/physzool.21.1.30151976

Pardi L (1996) *Polistes*: analysis of a society. In: Turillazzi S, West-Eberhard MJ (eds) Natural history and evolution of paper-wasps. Oxford University Press, Oxford, pp 1–17

Pepiciello I, Cini A, Nieri R, Mazzoni V, Cervo R (2018) Adult-larvae vibrational communication in paper wasps: the role of abdominal wagging in *Polistes dominula*. J Exp Biol 221:jeb186247. https://doi.org/10.1242/jeb.186247

Piekarski PK, Carpenter JM, Lemmon AR, Lemmon EM, Sharanowski BJ (2018) Phylogenomic evidence overturns current conceptions of social evolution in wasps (Vespidae). Mol Biol Evol 35:2097–2109. https://doi.org/10.1093/molbev/msy124

Pratte M, Jeanne RL (1984) Antennal drumming behavior in *Polistes* wasps (hymenoptera: Vespidae). Ethology 66:177–188

Rau P (1928) Trophallaxis in *Polistes pallipes*. Psyche (New York) 35:153–156. https://doi.org/10.1155/1928/61898

Richard FJ, Hunt JH (2013) Intracolony chemical communication in social insects. Insect Soc 60: 275–291

Röseler PF, Röseler I (1989) Dominance of ovariectomized foundresses of the paper wasp, *Polistes gallicus*. Insect Soc 36:219–234. https://doi.org/10.1007/BF02226305

Ross KG (1982) Gastral vibrations in laboratory *Vespula vulgaris* and *V. maculifrons* colonies (hymenoptera: Vespidae). Fla Entomol 65:187–188

Sandeman DC, Tautz J, Lindauer M (1996) Transmission of vibration across honeycombs and its detection by bee leg receptors. J Exp Biol 199:2585–2594

Santos GMT, Alves AA, Mendonça FAS (2007) Morfologia de estruturas sensoriais em pernas e antenas de *Agelaia pallipes* (Olivier), *Polybia paulista* (Ihering) e *Mischocyttarus cassununga* (Ihering) (Hymenoptera: Vespidae). Neotrop Entomol 36:868–873. https://doi.org/10.1590/s1519-566x2007000600007

Savoyard JL, Gamboa GJ, Cummings DLD, Foster RL (1998) The communicative meaning of body oscillations in the social wasp, *Polistes fuscatus* (Hymenoptera, Vespidae). Insect Soc 45: 215–230. https://doi.org/10.1007/s000400050082

Schaudinischky L, Ishay JS (1968) On the nature of the sounds produced within the nest of the oriental hornet *Vespa orientalis* F. (Hymenoptera). J Acoust Soc Am 44:116–124

Schneider SS, Lewis LA, Huang ZY (2004) The vibration signal and juvenile hormone titers in worker honeybees, *Apis mellifera*. Ethology 110:977–985. https://doi.org/10.1111/j.1439-0310.2004.01030.x

Sen R, Gadagkar R (2006) Males of the social wasp *Ropalidia marginata* can feed larvae, given an opportunity. Anim Behav 71:345–350. https://doi.org/10.1016/j.anbehav.2005.04.022

Starr CK (1990) Holding the fort: Colony defense in some primitively social wasps. In: Evans DL, Schmidt JO (eds) Insect defenses: adaptive mechanisms and strategies of prey and predators. SUNY Press, Albany, NY, pp 421–463

Staudacher EM, Gebhardt M, Dürr V (2005) Antennal movements and mechanoreception: neurobiology of active tactile sensors. Adv Insect Physiol 32:49–205. https://doi.org/10.1016/S0065-2806(05)32002-9

Sumner S, Law G, Cini A (2018) Why we love bees and hate wasps. Ecol Entomol 43:836–845. https://doi.org/10.1111/een.12676

Suryanarayanan S, Hermanson JC, Jeanne RL (2011) A mechanical signal biases caste development in a social wasp. Curr Biol 21:231–235. https://doi.org/10.1016/j.cub.2011.01.003

Suzuki T (1996) Natural history and social behaviour of the cofoundresses in a primitively eusocial wasp, *Polistes stigma* (Fabricius) (Hymenoptera: Vespidae), in India: a case study. Japanese J Syst Entomol 64:35–55

Taylor BJ, Jeanne RL (2018) Gastral drumming: a nest-based food-recruitment signal in a social wasp. Sci Nat 105:1–9. https://doi.org/10.1007/s00114-018-1550-4

Thomson DM (2019) Effects of long-term variation in pollinator abundance and diversity on reproduction of a generalist plant. J Ecol 107:491–502. https://doi.org/10.1111/1365-2745.13055

Tindo M, Francescato E, Dejean A (1997) Abdominal vibrations in a primitively eusocial wasp *Belonogaster juncea juncea* (Vespidae: Polistinae). Sociobiology 29:255–261

Togni OC, Giannotti E (2010) Colony defense behavior of the primitively eusocial wasp, *Mischocyttarus cerberus* is related to age. J Insect Sci 10:1–14. https://doi.org/10.1673/031.010.13601

Turillazzi S (2012) The biology of hover wasps. Springer, Berlin, Heidelberg

Turillazzi S, Cervo R (1982) Territorial behaviour in males of *Polistes nimpha* (Christ) (Hymenoptera, Vespidae). Z Tierpsychol [now Ethology] 58(2):174–180. https://doi.org/10.1111/j.1439-0310.1982.tb00315.x

Virant-Doberlet M, Mazzoni V, de Groot M, Polajnar J, Lucchi A, Symondson WOC, Čokl A (2014) Vibrational communication networks: eavesdropping and biotic noise. In: Studying vibrational communication. Springer, Berlin, Heidelberg, pp 93–123

Wenzel JW (1998) A generic key to the nests of hornets, yellowjackets, and paper wasps worldwide (Vespidae: Vespinae, Polistinae). Am Mus Novit 3224:1–40

West-Eberhard MJ (1969) The social biology of Polistinae wasps. Univ of Michigan Mus of Zool Miscellaneous Publ 140:5–101

West-Eberhard MJ (1982) Communication in social wasps: predicted and observed patterns, with a note on the significance of behavioral and ontogenetic flexibility for theories of worker "altruism". In: de Haro A, Espalader X (eds) La Communication Chez les Sociétés d'Insectes: Colloque International U.I.E.I.S.-Section Française, 16–18 de Septembre, Barcelona, pp 13–36

West-Eberhard MJ (1986) Dominance relations in *Polistes canadensis* (L.), a tropical social wasp. Monit Zool Ital 20:263–281

Wilson EO (1971) The insect societies. Harvard University Press, Cambridge, MA

Yamane S (1971) Daily activities of the founding queens of two *Polistes* wasps, *P. snelleni* and *P. biglumis* in the solitary stage (hymenoptera: Vespidae). Kontyû 39:203–217

Yoshimura H, Yamada J, Yamada YY (2019) The queen of the paper wasp *Polistes jokahamae* (hymenoptera: Polistinae) is not aggressive but maintains her reproductive priority. Sociobiology 66(1):166–178. https://doi.org/10.13102/sociobiology.v66i1.3577

Zacchi F, Cervo R, Turillazzi S (1996) How *Polistes semenowi*, obligate social parasite, invades the nest of its host, *Polistes dominulus* (Hymenoptera, Vespidae). Insect Social Life 1:125–130

Chapter 19
Vibratory Sensing and Communication in Caterpillars

Jayne E. Yack and Chanchal Yadav

Abstract Over the past few decades, scientists have discovered that insects live in complex vibratory environments that they both create and exploit for their survival. Yet, this growing body of literature is focused predominantly on adults, leaving a significant knowledge gap on vibratory sensing and communication in juveniles. There is burgeoning yet scattered evidence indicating that juveniles use vibrations in a wide range of contexts. In this chapter, we endeavored to synthesize the literature on vibratory sensing and communication in caterpillars (larval Lepidoptera). Caterpillars are obligate substrate-bound insects that are exposed to a wide range of vibrations generated by biotic and abiotic sources. This chapter shows that caterpillars across diverse taxonomic groups use vibrations in a variety of contexts, including predator detection and risk assessment, detection of abiotic events such as wind and rain, recruitment and spacing, territorial defense, and maintaining relationships with ants. While it is clear that many caterpillars are capable of detecting and discriminating between vibration sources, vibration receptors have not yet been identified in caterpillars, or any other holometabolous insect larvae. We discuss potential vibration receptors in larvae based on our knowledge of adult receptors and larval morphology and physiology. The vibratory landscapes of juvenile insects, including eggs, larvae, nymphs, and pupae, remain poorly understood. Yet, most juveniles are substrate-bound, and therefore, it is likely that vibrations play an important role in their survival. We recommend further investigations on vibratory sensing and communication in juveniles, from documenting the species using vibrations to discovering the sensory organs involved in detecting and processing vibratory information.

J. E. Yack (✉) · C. Yadav
Department of Biology, Carleton University, Ottawa, ON, Canada
e-mail: jayneyack@cunet.carleton.ca; ChanchalYadav@cmail.carleton.ca

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_19

19.1 Introduction

Vibratory sensing and communication in insects have been studied for several decades, and we are now gaining an appreciation for the ubiquity and importance of this sensory modality. At the outset, we would like to define what we mean by "vibratory." In the broadest sense, acoustic signals and events are defined as the transmission of vibrations through an elastic medium, including air, water, or solids. Vibrations transmitted through solids such as plants or the ground are commonly referred to as "vibrations" or "substrate vibrations," whereas air- and water-borne vibrations are commonly referred to as "sounds." This is the terminology that we will adopt in this chapter, but for further discussion of the nomenclature and physical characteristics of vibrations and sounds, see Hill (2008, 2014) and Hill and Wessel (2016). At least 18 insect orders have been reported to include species that use vibratory communication and sensing (Cocroft and Rodriguez 2005). Vibrations are used in a wide variety of contexts, including detection of passive cues generated from biotic sources (e.g., approaching predators) or abiotic sources (e.g., wind, rain) to complex communication signals used for reproduction, territorial displays, recruitment, and other social interactions. There are many reviews on the topic of vibratory sensing and communication. Some of these focus broadly on insects or other invertebrates (e.g., Markl 1983; Gogala 1985; Virant-Doberlet and Čokl 2004; Cocroft and Rodriguez 2005; Hill 2008; Čokl and Virant-Doberlet 2009; Cocroft and Hamel 2010; Randall 2014; Yack 2016). Other reviews focus on specific taxonomic groups that use vibrations, or functions of vibrations, e.g., spiders (Barth 1997, 2002; Baurecht and Barth 1992; Elias and Mason 2010), Neuroptera (Stewart 1997; Devetak 1998), Hemiptera (Gogala et al. 1974; Claridge 1985; Cocroft and McNett 2006; Shestakov 2015), termites (Hager et al. 2019), Mantophasmatodea (Eberhard and Eberhard 2013; Eberhard and Picker 2019), predator–prey interactions (Casas and Magal 2006; Virant-Doberlet et al. 2019), courtship (Rodríguez 2019), and group-living (Cocroft 2001). Research on insect vibratory sensing and communication until now has focused on adults, and specifically, their reproductive behaviors, with little consideration to juveniles. By juveniles, we mean eggs, nymphs of hemimetabolous insects, and larvae and pupae of holometabolous insects.

There is a growing number of examples demonstrating or suggesting that juveniles belonging to several insect orders use vibrations in a variety of contexts, including but not restricted to the following: (1) egg hatching (Fig. 19.1a), e.g., Hemiptera (Mukai et al. 2014; Endo et al. 2019), Orthoptera (Nishide and Tanaka 2016; Tanaka et al. 2018); (2) coordinating social grouping activities and recruitment (Figs. 19.1b, c), e.g., Hymenoptera (Hograefe 1984; Fletcher 2007, 2008), Lepidoptera (Yadav et al. 2017), Coleoptera (Cocroft 2001), Hemiptera (Cocroft 2001; Ramaswamy and Cocroft 2009; Hamel and Cocroft 2012); (3) manipulating ants in parasitic or mutualistic interactions (Fig. 19.1d), e.g., Lepidoptera (Devries 1990; Travassos and Pierce 2000); (4) obtaining food, e.g., Hymenoptera (Ishay et al. 1974; Hograefe 1984), Diptera (McIver and Beech 1986); (5) territorial and

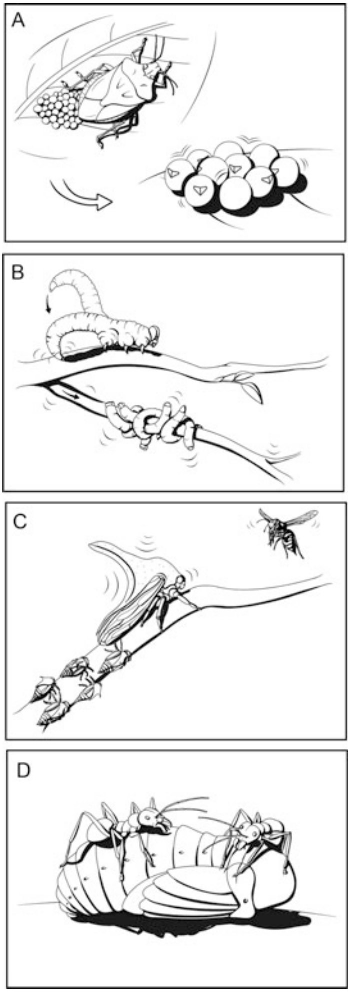


Fig. 19.1 Vibratory sensing and communication has been reported for all juvenile insect stages. Examples include the following: (**a**) Eggs/embryos. Eggs of the brown marmorated stink bug, *Halymorpha halys,* synchronize their hatching by responding to vibrations caused by egg cracking by siblings in a clutch (see Endo et al. 2019). (**b**) Nymphs. Nymphs of the treehopper, *Umbonia crassicornis* (Membracidae), produce vibrational signals to elicit their mother's protective antipredator behavior (see Cocroft 1999). (**c**) Larvae. Larvae of the sawfly, *Perga affinis*, produce vibrations by tapping and contracting to coordinate grouping (see Fletcher 2007). (**d**) Pupae. Pupae of the common imperial blue butterfly, *Jalmenus evagoras* (Lycaenidae), produce vibrations to attract and maintain the presence of attendant ants in butterfly–ant mutualism (see Travassos and Pierce 2000)

spacing behavior, e.g., Lepidoptera (Yack et al. 2001, 2014; Fletcher et al. 2006; Scott et al. 2010; Guedes et al. 2012); (6) detecting and avoiding predators and parasitoids, e.g., Lepidoptera (Djemai et al. 2001; Castellanos and Barbosa 2006; Low 2008), Coleoptera (Kojima et al. 2012), Hemiptera (Gish et al. 2012). We propose that vibratory detection and communication is widespread among juveniles. However, due to a number of factors, including the (typically) small body size of juveniles, that vibrations may be communicated at close range in small groups of juveniles, and that plant-borne vibrations of small insects may not be easily detected by human researchers without specialized instrumentation, these vibratory events may have been overlooked by scientists. Given that juveniles are flightless and substrate-bound, and purportedly require mechanisms for communication and detection of predators and parasitoids, it seems imminent that vibrations play a prominent role in their sensory ecology. We suspect that research on vibratory communication in juveniles lags far behind that for adults, and requires further investigation. In this chapter, we explore the topic of juvenile vibratory sensing and communication by focusing on caterpillars, the larvae of Lepidoptera.

19.2 Caterpillar Sensory Ecology

As caterpillars are often viewed as the “feeding stage” of adult Lepidoptera, sometimes the complexity of their behaviors is overlooked. Caterpillars in fact exhibit highly varied and sometimes complex behaviors that require sensing and interacting with their environments. Many species must protect themselves from physical elements such as rain, light, and heat, by building shelters and finding cover (Lill and Marquis 2007; Greeney et al. 2012). They need to navigate their environments to locate and identify host plants (Fitzgerald and Peterson 1988; Zalucki et al. 2002), create leaf mines (Connor and Taverner 1997), locate pupation sites (Hagstrum and Subramanyam 2010; Grof-Tisza et al. 2015) and shelter sites (Lill and Marquis 2004; Abarca et al. 2014), and in rare cases, locate prey (Montgomery 1983). As caterpillars are prey and hosts for a variety of predators and parasitoids, they have evolved mechanisms to detect and respond behaviorally to these threats in a variety of ways (Lederhouse 1990; Wagner 2005; Greeney et al. 2012). Many species form social groups for purposes of constructing communal shelters, foraging in groups, or cooperating in defense (Despland and Le Huu 2007; Costa 2010; Despland 2019). As such, they require mechanisms to form and maintain groups, construct communal shelters, and coordinate social behaviors. Some butterfly larvae form parasitic or mutualistic relationships with ants, and have evolved mechanisms to communicate with, and manipulate their hosts (Schönrogge et al. 2017; Cassacci et al. 2019). Given these highly varied demands on their sensory systems, it is helpful to first review what is understood about the sensory modalities of caterpillars.

Sensory receptors in caterpillars used to monitor the external environment include those that detect chemical, light, tactile, and acoustic stimuli. Chemoreception is the best understood of all the sensory modalities in caterpillars. Taste and olfactory receptors occur primarily on the head (antennae and mouthparts) (Schoonhoven and Dethier 1966; Schoonhoven and van Loon 2002; Roessingh et al. 2007; Shields and Martin 2012). Gustatory receptors play a key role in food evaluation, host selection, and feeding preferences (Schoonhoven 1987; Schoonhoven and van Loon 2002; de Boer 2006). Olfactory chemoreceptors are utilized in tasks such as detecting trail pheromones (Roessingh 1989), and orientation to host plants (Piesik et al. 2009, 2013). The visual system comprises simple light receptors (stemmata) located on the head region. These can form crude images and discriminate colors (Warrant et al. 2003), potentially functioning in orienting to pupation sites (Starnecker 1996), and feeding sites (Rieske and Townsend 2005). Tactile receptors occur on different parts of the body, and function in maintaining processions (Roessingh 1989; Fitzgerald 2003), and detecting and escaping from predators (Castellanos et al. 2011). The

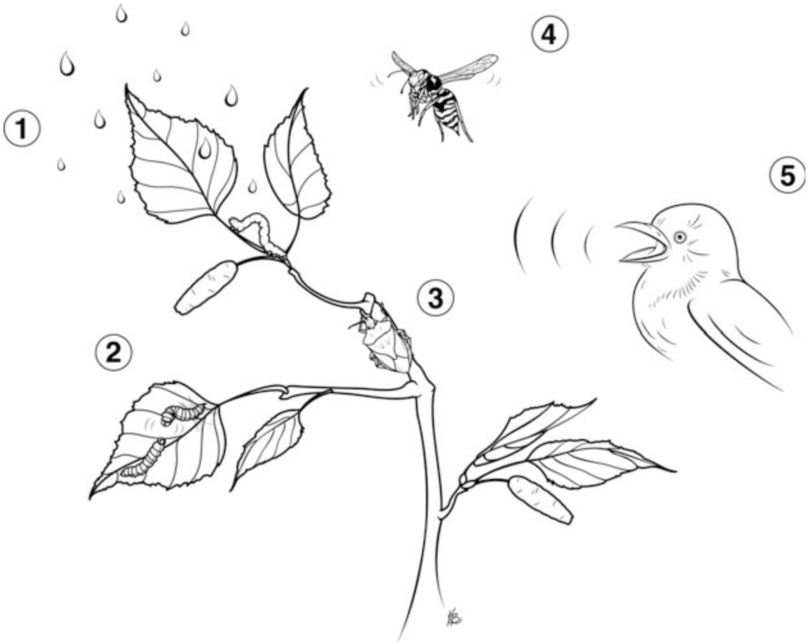


Fig. 19.2 Potential sources of vibrational information for a caterpillar. (**1**) Abiotic sources including rain droplets and wind could inform caterpillars of weather conditions, or mask important vibratory information. (**2**) Other caterpillars may produce incidental vibrations by locomotion (e.g., crawling) or chewing, or by actively signaling (e.g., for recruitment or advertising territory). (**3**) Caterpillars that are prey or hosts may detect incidental cues produced by an approaching predator or parasitoid directly through the substrate. (**4**, **5**) Vibrations may be transmitted to the plant indirectly from air-borne sounds produced by a flying predator, such as a wasp, or a calling predator, such as an insectivorous bird singing in the vicinity

acoustic sensory modalities, including mechanoreceptors that detect both air- or solid-borne vibrations, are poorly understood in caterpillars. In some species, long hairs called trichoid sensilla have been shown to detect near-field sounds and function in detecting flying insect predators and parasitoids (Markl and Tautz 1975; Tautz 1977, 1978; Tautz and Markl 1978; Taylor and Yack 2019). There is increasing evidence that many caterpillar species use solid-borne vibrations. Being substrate-bound, either living on or inside plant material, vibratory sensing and communication may be an important mechanism for communication and risk assessment. Vibrations that are potentially relevant to caterpillars could be transmitted through three routes: from solid to solid, from solid to air to solid, or from air to solid (Fig. 19.2). Vibrations have been noted or proposed to function in a number of different contexts including territorial behavior, recruitment and social interactions, mutualistic or parasitic relationships with ants, and predator detection. The remainder of this chapter focuses on what we know, and what we do not know, about vibratory detection and communication in caterpillars.

19.3 Vibratory Detection and Communication in Caterpillars

19.3.1 Territorial Behavior

Several species of Lepidoptera larvae use substrate-borne vibrations in territorial contests over a resource, such as a feeding site or leaf shelter. Leaf shelters are commonly observed in larval Lepidoptera, providing benefits such as protection from enemies, improved microclimate, and feeding sites (e.g., Hunter and Willmer 1989; Sagers 1992; Berenbaum et al. 1993; Eubanks et al. 1997; Fukui 2001; Lill and Marquis 2007; Abarca et al. 2014). Given the benefits and energetic costs associated with shelters, caterpillars exhibit competition to defend them from intruders (Sigmon 2015). Vibrations have been shown to function in both detecting intruders and generating signals to defend territories. At present, vibratory-mediated territoriality has been most thoroughly studied in species belonging to the superfamily Drepanoidea.

Vibratory-mediated territoriality is widespread in larvae belonging to the superfamily Drepanoidea, and specifically, those in the family Drepanidae (Bowen et al. 2008; Scott et al. 2010; Scott and Yack 2012). The species that has been most thoroughly studied is the masked birch caterpillar (*Drepana arcuata*). Late instar masked birch caterpillars construct and occupy silken leaf shelters on birch (*Betula papyrifera*). Shelters can take more than 3 hours to construct, consuming valuable time and energy. When approached by a conspecific intruder, resident larvae produce a combination of three distinct vibrations by drumming and scraping their mandibles and scraping specialized anal "oars" against the leaf's surface. In staged interactions between residents and intruders, Yack et al. (2001) demonstrated that resident larvae won territorial acoustic disputes 86.8% of the time. A resident larva confronted with an intruding conspecific typically stops feeding, backs into its leaf shelter, and begins signaling. Residents were observed to increase the rate of signaling and varied the use of particular signals in relation to its distance between itself and the intruder (Yack et al. 2001). Signaling rates increased with the amount of time a caterpillar spent on a leaf, as well as with silk accumulation (Yack et al. 2014). Conversely signal rates decreased with shelter removal (Yack et al. 2014). Residents use vibrations to detect approaching intruders, and can distinguish among vibrations caused by a conspecific intruder, wind, and rain (Guedes et al. 2012). Comparative studies show that vibratory-mediated territoriality is taxonomically widespread within the Drepanidae, and it is proposed that the types and intensity of signals vary among species depending on the amount of energy investment in shelters (Scott et al. 2010). A phylogenetic study showed that vibratory-mediated territoriality is a derived trait in species belonging to the subfamilies Drepaninae and Thyatirinae, but is absent in the ancestral Cyclidiinae (Scott et al. 2010). Phylogenetic mapping showed that vibratory signaling evolved as a form of ritualized aggression, and that movements associated with physical fighting (crawling, biting) evolved into ritualized signals to avoid damage incurred by physical fighting (Scott et al. 2010).

In addition to the abovementioned Drepanoidea species, the use of vibrations during competition for resources has been reported for species in the superfamilies Gracillarioidea, Gelechioidea, and Tortricoidea. The cherry leaf roller, *Caloptilia serotinella* (Gracillariidae, Gracillarioidea), produces three distinct vibratory signals: scraping, plucking, and vibrating in response to a conspecific intruder entering a resident's leaf shelter (Fletcher et al. 2006). Scraping, a form of stridulation, is produced by lateral head movements, while the mandibles are held open and scraped against the leaf surface. Vertical movements of the head and thorax produce the plucking signal; either the mandibles or the thorax pulling up on the leaf are thought to produce the signal. "Vibrating" is produced when the larva remains in a fixed position on the leaf and oscillates the thorax horizontally, and while doing so, the thoracic legs scrape the leaf. The "vibrating" signal is presumed to be a combination of tremulation and stridulation. The signals differed in usage when interacting with a conspecific: the most frequently used is scraping followed by plucking and vibrating. The distance between the resident and the intruder also determined which signal was used; at farther distances vibrating was observed, while at closer distances scraping was observed. Vibrational signaling in *Diurnea fagella* (Oecophoridae) larvae has been suggested to play a role in defending resources (Hunter 1987). These larvae occupy leaf shelters where they feed and reside. When disturbed, they scrape a "hook," located on the distal end of a modified third thoracic leg, back and forth on the leaf's surface. Although the signals were recorded as airborne sounds with microphones, it is most likely they are transmitted as vibrations through the leaf. Further research is required to ascertain the precise functional significance of signaling in *D. fagella* larvae, but it has been proposed to function primarily in territorial contests with conspecifics. Another example of vibration-mediated territorial defense was reported for *Sparganothis pilleriana* (Tortricoidea) (Russ 1969). Caterpillars form a leaf shelter on grape leaves and defend them against conspecifics and heterospecifics by rearing up and striking (drumming) the webbing on the leaf repeatedly. Vibration signals were recorded by placing a microphone directly on the substrate. Signals increased in intensity when conspecific intruders remained near the leaf shelter. Fighting ensued until one of the contestants fled. In another study of territorial encounters in shelter building Gelechiidae and Oecophoridae caterpillars (Sigmon 2015) "audible sounds" were noted during territorial encounters when a caterpillar repeatedly tapped (drummed) its head on the leaf surface. However, vibration recordings were not conducted and the role of vibratory communication was not formally tested. Vibratory-mediated territorial disputes may be widespread among the many lepidopteran larvae that invest time and energy in building and maintaining shelters, and should be further investigated.

19.3.2 Group Formation and Maintenance

Several caterpillars form groups at different stages of their development (Costa and Pierce 1997; Fitzgerald and Costa 1999; Zalucki et al. 2002; Costa 2006) for purposes of collective predator defense, thermoregulation, and feeding (e.g., Costa and Pierce 1997; Prokopy and Roitberg 2001; Reader and Hochuli 2003; Costa 2006; Despland 2019). The sensory mechanisms used to form, maintain, and coordinate activities within these groups, however, are not well understood. There are some examples of chemical-mediated recruitment in processionary species (e.g., Costa 2006; Matthews and Matthews 2009; Pescador-Rubio et al. 2011). Vibration-mediated recruitment and group formation would seem to be an optimal means of communication, as signals are short and therefore more difficult for predators to detect than chemical signals. At present, there is one example in larval Lepidoptera. Early instar caterpillars of the hooktip moth caterpillar, *Drepana arcuata*, live in small groups of 2–5 on birch leaves (Yadav and Yack 2018). Upon hatching, neonates begin shelter construction on the edge of a birch leaf, and generate vibratory signals while feeding using specialized oar-like structures, called anal oars, located on the last abdominal segment. These vibratory signals are used by early instars for recruitment by advertising feeding sites and shelter locations to conspecifics (Yadav et al. 2017). To the best of our knowledge, there are no other examples of vibratory recruitment and group maintenance in caterpillars, but there are examples in other holometabolous larvae including sawflies (Hograefe 1984; Fletcher 2007, 2008) and beetles (Greenfield 2002; Cocroft and Hamel 2010). Given the importance of social groups in caterpillars, we recommend that more species be investigated for the role of vibrations in forming and maintaining groups.

19.3.3 Myrmecophilous Larvae

One of the most fascinating examples of vibratory communication in caterpillars occurs in myrmecophilic (ant loving) species belonging to the butterfly families Lycaenidae and Riodinidae. Myrmecophilous larvae "break the codes" of their ant host's communication system to gain access to rich resources, as well as shelter and protection offered by the ant colony. Communication with ants is multimodal, involving chemical, tactile, and vibroacoustic sensory modalities. General reviews on the ecology and evolution of caterpillar–ant interactions include those by Hinton (1951), Fiedler (1991), Pierce et al. (2002), and Casacci et al. (2019). Reviews focusing more specifically on the role of vibratory communication include those by Devries (1991), Travassos and Pierce (2000), Riva et al. (2016), and Schönrogge et al. (2017). Specific, and non-mutually exclusive, examples of caterpillar–ant interactions involve vibroacoustic signals that include the following: (1) attracting ants to be carried into the chamber; (2) begging for food; (3) signaling to strengthen the social status with the colony, and (4) mimicking queen signals. Although riodinid and lycaenid larvae independently evolved the capacity to produce vibratory signals, vibroacoustic signals in larvae of both families have evolved in the context of establishing or enhancing ant symbioses (DeVries 1991). Here, we briefly summarize vibratory-mediated myrmecophilia in larvae of Riodinidae and Lycaenidae.

In Riodinidae larvae at least 19 species have been reported to produce vibratory calls and all of these species are associated with ants (Devries 1991). Most species that produce vibrations do so using a stridulatory mechanism comprising two structures: vibratory papillae and epicranial granulations (Devries 1990, 1991). The vibratory papillae consist of a pair of rod-like appendages on the prothorax. The papillae strike against the head (on various types of textured surfaces referred to as epicranial granulations) through oscillations of the head. The resulting substrate vibrations appear to communicate with ants as demonstrated by DeVries (1990). Larvae that had their papillae removed lost their ability to call and failed to attract ant attendants. After molting, they regained the structures as well as their ability to call.

In Lycaenidae, at least 30 species of larvae have been reported to produce vibratory calls, and all are associated with ants (Devries 1991), but it is proposed that the ability to produce vibrations may be universal in larvae of this family (Riva et al. 2016). A variety of different vibration signals characterized as pulses, drumming, grunts, and hisses have been noted (Travassos and Pierce 2000; Schönrogge et al. 2017). However, the specific mechanisms of signal generation in Lycaenidae larvae are not well understood. One mechanism of call production in Lycaenidae suggested by Hill (1993) involves a file of teeth on the posterior side of the fifth abdominal segment grating against an opposing stridulatory plate on the posterior side of the sixth segment. In other species, sound production is thought to occur through a forced air mechanism (Schurian and Fiedler 1991). Travassos and Pierce (2000) characterized three types of vibrational signals in the Australian lycaenid, *Jalmenus evagoras*. Grunts, hisses, and drums occurred in different contexts and are proposed to play specific roles in communicating with ants. One of the best studied myrmecophilic larvae are *Maculinea* species that are obligate parasites of *Myrmica* ants. *Maculinea* species mimic vibrations of their hosts to attract ants, exploit nest resources, and elevate their social status by mimicking vibration signals of queens (reviewed in Sala et al. 2014; Casacci et al. 2019).

Vibratory-mediated relationships between caterpillars and ants are highly complex and there is much left to learn. Vibration signals should be recorded and characterized for more species, although such studies are difficult to perform inside ant colonies. The mechanisms of vibration production remain unknown for most of the Lycaenidae species. Another challenge is to disentangle the contributions of multiple modes of communication, including chemical and tactile, from vibratory signals. Finally, like for other holometabolous larvae, there is nothing known about how signals are received by the caterpillars.

19.3.4 Avoiding Predators and Parasitoids

Caterpillars are attacked by a wide variety of enemies, including vertebrate predators (e.g., birds, lizards, rodents, frogs) and invertebrate predators and parasitoids (e.g., wasps, flies, mantids, dragonflies) (Jennings et al. 1991; Heinrich 1993; Montllor and Bernays 1993; Greeney et al. 2012) (Fig. 19.2). Sources of vibrations produced by predators or parasitoids that could be used by caterpillars include the following: (1) incidental vibration cues transmitted directly through a plant from the predator to prey, e.g., adult Japanese sawyer beetles (*Monochamus alternatus*) exhibit antipredator behaviors in response to low-frequency vibrations simulating a predator (Takanashi et al. 2016) and adult termites (*Coptotermes acinaciformis*) avoid predatory ants by eavesdropping on their walking vibrations (Oberst et al. 2017); (2) incidental vibration cues transmitted indirectly through the plant from the flight sounds of an approaching vertebrate or invertebrate predator (Caldwell 2014); (3) incidental vibrations transmitted indirectly to the prey from the calling songs of predators such as birds (Caldwell 2014; Yack 2016); and (4) drumming vibration echolocation signals of parasitoids used to locate their hosts (Henaut 1990; Broad and Quicke 2000). All of the abovementioned vibrations could be used by caterpillars to assess predation risk. Currently, however, there are only a few experimental examples showing this. Larvae of the apple leaf miner *Phyllonorycter malella* (Gracillariidae) were shown to detect and respond to vibrations produced by a parasitic wasp *Sympiesis sericeicornis* (Hymenoptera: Eulophidae) (Meyhöfer et al. 1997). During a foraging bout on a mine, *S. sericeicornis* produces characteristic vibrations during the insertion of its ovipositor into the mine. Vibrations are detected by the leaf miner larvae, which stop feeding upon detecting vibrations and resume only after the parasitoid has left the mine (Meyhöfer et al. 1997). Late instar larvae of the masked birch caterpillar (*Drepana arcuata*) detect the low-frequency crawling vibrations of predatory stink bugs (*Podisus sp.*) and are capable of discriminating among vibrations of biotic and abiotic factors, and different types of intruders (Guedes et al. 2012). Larvae of *Semiothisa aemulataria* (Geometridae) responded differently to vibrations produced by various predators and abiotic factors (Castellanos and Barbosa 2006). Larvae responded differentially to different invertebrate predators (stink bugs, wasps) by producing silk escape threads of different lengths, and it was suggested that this could be a result of the differing amplitude of high-frequency components (Castellanos and Barbosa 2006).

Caterpillars have also been reported to generate vibrations in response to enemies. Larvae of the Tupelo leafminer, *Antispila nysaefoliella*, emit vibratory signals when disturbed (Low 2008). Two signals produced by the larvae are referred to as the “tick” and the “rattle.” Ticks are produced by rhythmic back and forth movements of the abdomen that are likened to movements of a clock pendulum. Rattles are produced by shorter, more rapid movements of the abdomen likened to a rattle of a rattlesnake, which vibrate the abdomen rapidly, resembling a tremulatory mechanism. Ticks are described as being slow and rhythmic and rattles are very rapid and occur in bursts that punctuate the ticking. Modified sclerotized structures, referred to as ridges and bumps, occur on the dorsal surface of the abdomen and are proposed to contribute to generating these vibrations. However, the study did not identify whether these structures impact the leaf surface, or whether vibrations were generated by stridulation or tremulation. These signals are proposed to disrupt foraging in parasitic wasps. Another example of signaling in the presence of a predator was reported in late instars of the masked birch caterpillar, *Drepana arcuata* (Drepanidae). When approached by a stink bug predator (*Podisus sp.*), larvae residing inside silk shelters remain still when first detecting the predator, but when pursued, the caterpillar initiates rapid drumming and scraping signals that appeared to thwart the impending attack (Guedes et al. 2012).

19.3.5 Other Potential Functions

In addition to using vibrations for the abovementioned functions, caterpillars have been reported to distinguish between biologically relevant signals or cues and background vibratory noise caused by abiotic factors such as wind and rain (Castellanos and Barbosa 2006; Guedes et al. 2012). Other proposed functions include using vibrations in coordinating group activities such as taking turns feeding and advertising when a caterpillar is about to move in the shelter (Yack lab, unpublished), and identifying individuals for purposes of leadership or task roles. Other possible functions include coordinated molting, navigation (e.g., echolocation), and synchronizing hatching times in neonates.

19.4 Vibration-Sensitive Sensilla and Sensory Organs

While there are many examples of caterpillars demonstrating behavioral evidence of vibratory sensing, vibration-sensitive sensory organs have not yet been identified in any larval insect to the best of our knowledge. Before speculating on the types and locations of putative receptors in larvae, we provide a brief overview of what is currently known for vibratory sensilla in adults (see also Field and Matheson 1998; Yack 2004, 2016; Lakes-Harlan and Strauss 2014; Strauss et al. 2019). In adult insects, mechanosensitive sensilla associated with vibration detection include scolopidia, sensory hairs (i.e., trichoid sensilla), and campaniform sensilla. These sensilla may function as vibration receptors, individually, or may be grouped into sensory organs. Scolopidia are internal mechanoreceptive sensilla that occur throughout the body of Insecta and Crustacea. Scolopidia can be very sensitive to vibratory stimuli and, depending on their position in the body and association with peripheral structures, have been shown to function as proprioceptors (detecting self-induced movements) or exteroreceptors (detecting gravitational forces, air- or water-borne sounds, or solid-borne vibrations) (Field and Matheson 1998; Yack 2004).

Groups of scolopidia are called chordotonal organs. Chordotonal organs identified as being specialized for vibration reception in adult insects include subgenual organs, Johnston's organs, and femoral chordotonal organs. Subgenual organs are the best known vibration receptors in insects and occur in the proximal region of the leg tibia. Johnston's organs occur in the second antennal segment (pedicel) and in some insects function as a vibration receptor when the antennae are placed in direct contact with the substrate. Femoral chordotonal organs occur in the proximal region of the femur and have been shown to detect vibrations in some stick insects and beetles. Trichoid sensilla are long hair-like mechanoreceptors that are widely dispersed over the insect body, and have been proposed or shown to function as vibration receptors in some adult insects, spiders, and scorpions (Keil 1997; Barth 1998; Lakes-Harlan and Strauss 2014). Campaniform sensilla are dome-like structures embedded in the cuticle of insects and respond to stress on the cuticle. There is neurophysiological evidence that they respond to vibrations in the legs of locusts (Kühne 1982), but at present their function as vibration receptors has not been confirmed in any insect. In summary, all vibration-sensitive sensilla confirmed to date in adult insects are either scolopidia or trichoid sensillae, and these can be organized into different vibration sensory organs that vary in their complexity and location on the body. All share a common feature in that they are in direct or indirect contact with the substrate, usually via the legs (Lakes-Harlan and Strauss 2014; Yack 2016). Based on this information, we can speculate on putative vibration receptors in larval insects. A few structures have been proposed based primarily on morphological evidence. These are described below in this section, according to body location.

The antennae have been suggested to detect vibrations in caterpillars based on morphological features and neurophysiological responses (Dethier 1941). The antennae of larval Lepidoptera are located on the ventral surface of the head near the base of the mandibles; each antenna is three-segmented and possesses different mechanoreceptors, including trichoid and campaniform sensilla (Dethier 1941). The antennae from several different lepidopteran species were observed to move in their sockets and respond neurophysiologically when stimulated with a glass probe, and it was proposed that they are capable of detecting both tactile and vibratory stimuli. Therefore, if the antennae are in contact with the substrate during vibratory communication, they might function as vibration receptors.

Structures associated with the thorax and abdomen in caterpillars that have been suggested to function in vibration reception include setae and scolopidia located on the thorax, abdomen, and prolegs. Larvae belonging to the superfamilies Pyraloidea and Gelechioidea possess individual setae, which are connected to internal chordotonal organs via an apodeme, on various locations on the thorax and abdomen (Hasenfuss 1992). The setae and the apodemum are proposed to function as a system of levers transmitting substrate-borne vibrations to the chordotonal organ. Larvae of these two families usually construct tubular silk webs; when the larvae are inside the webs the setae are bent and remain in contact with the threads of silk in the wall (Hasenfuss 1992). The function of the setal-apodemum system has yet to be tested experimentally. Another location to look for vibration receptors in larval insects are the prolegs, as they are usually in direct contact with the substrate. Cuticular hairs (trichoid sensilla) occur on the prolegs of many caterpillar species (Stehr 1987), but the functions of these setae are not well understood. Prolegs of the masked birch caterpillar, *Drepana arcuata* (Drepanoidea), previously described (See Sect. 19.3.4) to use complex vibratory signals for territorial communication, predator detection, and recruitment, possess setae and scolopidia in their prolegs (Rosi-Denadai 2018; Yack lab, unpublished). One particularly prominent thick and rather stiff seta that is innervated by a bipolar sensory neuron is in direct contact with the leaf substrate. Preliminary neurophysiological recordings from the proleg peripheral nerve indicate that these structures are capable of detecting solid-borne vibrations (Rosi-Denadai 2018; Yack lab, unpublished). Another possible location for vibration receptors is on the abdominal body wall. In larvae of cerambycid beetles groups of scolopidia are oriented in ray-like structures (called pleural discs) in the abdominal region and are thought to play a role in orientation by detecting vibrations (Hess 1917; Saliba 1972). In Saliba's study (1972), beetle larvae were observed to re-orient themselves when exposed to the vibrations produced by conspecifics gnawing on wood. Whether the re-orientation was in response to vibration detection by scolopidia of the pleural discs was not confirmed.

Given the wide range of vibrations that caterpillars and other larval insects are capable of detecting, and the (sometimes) complex behaviors associated with vibratory detection and communication, we expect to see corresponding anatomical and physiological complexity mirrored in their vibratory sensory organs. The next steps toward identifying vibratory receptors in caterpillars and other larval insects should include physical or neuropharmacological ablations of putative receptors, coupled with behavioral testing and neurophysiological recordings.

19.5 Conclusion and Future Directions

While the importance of vibrational communication and sensing is well established in adult insects, we are only beginning to appreciate this sensory modality in juveniles. In this chapter, we narrowed our focus to the vibratory "world" of caterpillars. Larval Lepidoptera are important insects both ecologically and economically. Far from being merely the "feeding stages" of adult moths and butterflies, these larvae exhibit complex behaviors associated with feeding, social interactions, defense, and shelter acquisition. However, little is known about their sensory ecology, and particularly how they use sounds and vibrations for their survival. As substrate-bound organisms, it is likely that plant-borne vibrations play a crucial role in their livelihood. In this chapter we have identified a wide range of contexts whereby caterpillars use vibratory signals and events. The best documented examples are those of vibratory-mediated territorial interactions and mutualistic or parasitic relationships with ants. Vibrations have also been confirmed to function in discriminating between different types of predators and recruiting members to social

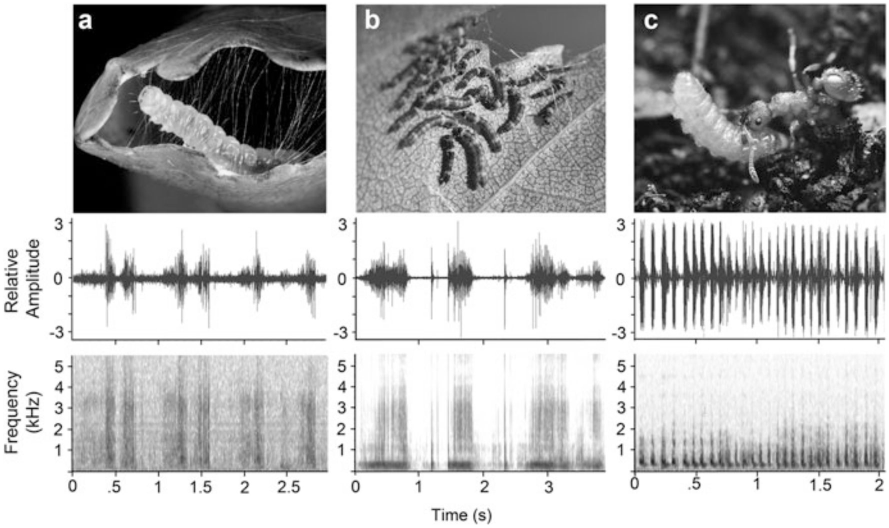


Fig. 19.3 Caterpillars communicate using vibrations in a variety of contexts, as shown in the following examples. (**a**) Territoriality. The cherry leaf roller, *Caloptilia serotinella* (Gracillariidae), defends its leaf shelter by scraping its mandibles on the leaf, plucking, and vibrating (see Fletcher et al. 2006). (**b**) Social recruitment and coordination. Social (early instar) caterpillars of the hook tip moth, *Drepana arcuata* (Drepanidae), generate complex vibrations including mandible scraping, mandible drumming, buzz scraping, and anal scraping on birch leaves to recruit conspecifics to a social group, and possibly to coordinate social activities such as feeding and shelter building within the group (see Yadav et al. 2017). (**c**) Social parasitism. Parasitic larvae of the butterfly *Maculinea alcom* uses vibrations to call to its ant host, *Myrmica scabrinotis*, prior to being adopted into the ant colony (see Sala et al. 2014)

groups, and have been implicated in assessing physical conditions. Despite our increased awareness that caterpillars engage in complex vibratory interactions, we know virtually nothing about how they detect and process vibrations. Also, there is a lack of research on the physical characteristics of their natural vibratory landscapes. Further investigations on the vibratory sensory modalities of caterpillars will no doubt lead to novel insights into the complex vibratory environments of other substrate-bound organisms, and may have practical applications for monitoring and managing pests, and inspiring biomimetic devices.

Acknowledgments We are grateful to Christian Nathan and Conrado Rosi-Denadai for help in reviewing literature for parts of the paper, to Kaylen Brzezinski for the line drawings (Figs. 19.1 and 19.2), and to Francesca Barbero and Michele Zaccagno for contributing a photo and recording of *M. alcom* (Fig. 19.3). This research was funded by the Natural Science and Engineering Council of Canada (2014-05947), the Canadian Foundation for Innovation (9555), and an Early Researcher Award (ERO7-04-1-44) to JEY, and Ontario Graduate Scholarship to CY.

References

Abarca M, Boege K, Zaldívar-Riverón A (2014) Shelter-building behavior and natural history of two pyralid caterpillars feeding on *Piper stipulaceum*. J Insect Sci 14:39

Barth FG (1997) Vibratory communication in spiders: adaptation and compromise at many levels. In: Lehrer M (ed) Orientation and communication in arthropods. Birkhauser Verlag, Basel (Switzerland), pp 247–272

Barth FG (1998) The vibrational sense of spiders. In: Hoy RR, Popper AN, Fay RR (eds) Comparative hearing: insects. Springer handbook of auditory research, vol 10. Springer, New York, pp 228–278

Barth FG (2002) A Spider's world: senses and behavior. Springer Verlag, Berlin, Heidelberg

Baurecht D, Barth FG (1992) Vibratory communication in spiders. J Comp Physiol A 171:231–243

Berenbaum MR, Green ES, Zangerl AR (1993) Web costs and web defense in the parsnip webworm (Lepidoptera: Oecophoridae). Environ Entomol 22:791–795

Bowen JL, Mahony SJ, Mason AC, Yack JE (2008) Vibration-mediated territoriality in the warty birch caterpillar *Drepana bilineata*. Physiol Entomol 33:238–250

Broad GR, Quicke DLJ (2000) The adaptive significance of host location by vibrational sounding in parasitoid wasps. P Roy Soc Lond B 267:2403–2409

Caldwell MS (2014) Interactions between airborne sound and substrate vibration in animal communication. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Animal signals and communication, vol 3. Springer, Berlin, Heidelberg, pp 65–92

Casacci LP, Bonelli S, Balletto E, Barbero F (2019) Multimodal signaling in myrmecophilous butterflies. Front Ecol Evol 7:454

Casas J, Magal C (2006) Mutual eavesdropping through vibrations in a host parasitoid interaction: from plant biomechanics to behavioural ecology. In: Drosopoulos S, Claridge MF (eds) Insect sounds and communication: physiology, behaviour, ecology, and evolution. Taylor and Francis Group, Boca Raton, FL, pp 263–271

Castellanos I, Barbosa P (2006) Evaluation of predation risk by a caterpillar using substrate-borne vibrations. Anim Behav 72:461–469

Castellanos I, Barbosa P, Zuria I, Tammaru T, Christman MC (2011) Contact with caterpillar hairs triggers predator-specific defensive responses. Behav Ecol 22:1020–1025

Claridge MF (1985) Acoustic signals in the Homoptera: behavior, taxonomy, and evolution. Annu Rev Entomol 30:297–317

Cocroft RB (1999) Offspring-parent communication in a subsocial treehopper (Hemiptera: Membracidae: *Umbonia crassicornis*). Behaviour 136:1–21

Cocroft RB (2001) Vibrational communication and the ecology of group-living, herbivorous insects. Am Zool 41:1215–1221

Cocroft RB, Hamel JA (2010) Vibrational communication in the "other insect societies": a diversity of ecology, signals and signal functions. In: O'Connell-Rodwell CE (ed) The use of vibrations in communication: properties, mechanisms and function across taxa. Transworld Research Network, Kerala, pp 47–68

Cocroft RB, McNett GD (2006) Vibratory communication in treehoppers (Hemiptera: Membracidae). In: Drosopoulos S, Claridge MF (eds) Insect sounds and communication: physiology, behaviour, ecology, and evolution. Taylor and Francis Group, Boca Raton, FL, pp 305–318

Cocroft RB, Rodriguez RL (2005) The behavioral ecology of insect vibrational communication. Bioscience 55:323–334

Čokl A, Virant-Doberlet M (2009) Vibrational communication. In: Resh VH, Carde RT (eds) Encyclopedia of insects. Elsevier/Academic Press, Amsterdam, pp 1034–1038

Connor EF, Taverner MP (1997) The evolution and adaptive significance of the leaf-mining habit. Oikos 79:6–25

Costa JT (2006) The other insect societies. The Belknap Press of Harvard University Press, Cambridge, MA

Costa JT (2010) Social evolution in 'other' insects and arachnids. In: Breed MD, Moore J (eds) Encyclopedia of animal behavior. Academic Press, Cambridge, MA, pp 231–241

Costa JT, Pierce NE (1997) Social evolution in the Lepidoptera: ecological context and communication in larval societies. In: Choe JC, Crespi BJ (eds) The evolution of social behavior in insects and arachnids. Cambridge University Press, Cambridge, pp 407–422

de Boer G (2006) The role of the antennae and maxillary palps in mediating food preference by larvae of the tobacco hornworm, *Manduca sexta*. Entomol Exp Appl 119:29–38

Despland E (2019) Caterpillars cooperate to overcome plant glandular trichome defenses. Front Ecol Evol 7:199

Despland E, Le Huu A (2007) Pros and cons of group living in the forest tent caterpillar: separating the roles of silk and of grouping. Entomol Exp Appl 122:181–189

Dethier VG (1941) The function of the antennal receptors in lepidopterous larvae. Biol Bull 80: 403–414

Devetak D (1998) Detection of substrate vibration in Neuropteroidea: a review. Acta Zool Fennica 209:87–94

DeVries PJ (1990) Enhancement of symbioses between butterfly caterpillars and ants by vibrational communication. Science 248:1104–1106

DeVries PJ (1991) Call production by myrmecophilous riodinid and lycaenid butterfly caterpillars (Lepidoptera): morphological, acoustical, functional, and evolutionary patterns. Am Mus Novit 3025:1–23

Djemai I, Casas J, Magal C (2001) Matching host reactions to parasitoid wasp vibrations. P Roy Soc B 268:2403–2408. https://doi.org/10.1098/rsbp.2001.1811

Eberhard MJ, Eberhard SH (2013) Evolution and diversity of vibrational signals in Mantophasmatodea (Insecta). J Insect Behav 26:352–370. https://doi.org/10.1007/s10905-012-9352-6

Eberhard MJB, Picker MD (2019) Vibrational communication in heelwalkers (Mantophasmatodea). In: PSM H, Lakes-Harlan R, Mazzoni V, Narins PM, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational behavior. Springer Nature Switzerland, Cham, pp 293–307

Elias DO, Mason AC (2010) Signaling in variable environments: substrate-borne signaling mechanisms and communication behavior in spiders. In: O'Connell-Rodwell CE (ed) The use of vibrations in communication: properties, mechanisms and function across taxa. Transworld, Kerala, pp 25–46

Endo J, Takanashi T, Mukai H, Numata H (2019) Egg-cracking vibration as a cue for stink bug siblings to synchronize hatching. Curr Biol 29:143–148. https://doi.org/10.1016/j.cub.2018.11.024

Eubanks MD, Nesci KA, Petersen MK, Liu ZW, Sanchez HB (1997) The exploitation of an ant-defended host plant by a shelter building herbivore. Oecologia 109:454–460

Fiedler K (1991) Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionoidea). Bonner Zoologische Monographien 31:1–210

Field LH, Matheson T (1998) Chordotonal organs of insects. Adv Insect Physiol 27:1–228

Fitzgerald TD (2003) Role of trail pheromone in foraging and processionary behavior of pine processionary caterpillars *Thaumetopoea pityocampa*. J Chem Ecol 29:513–532

Fitzgerald TD, Costa JT (1999) Collective behavior in social caterpillars. In: Detrain C, Deneubourg JL, Pasteels JM (eds) Information processing in social insects. Birkhäuser, Basel, pp 379–400

Fitzgerald TD, Peterson SC (1988) Cooperative foraging and communication in caterpillars. Bioscience 38:20–25

Fletcher LE (2007) Vibrational signals in a gregarious sawfly larva (*Perga affinis*): group coordination or competitive signalling? Behav Ecol Sociobiol 61:1809–1821

Fletcher LE (2008) Cooperative signaling as a potential mechanism for cohesion in a gregarious sawfly larva, *Perga affinis*. Behav Ecol Sociobiol 62:1127–1138

Fletcher LE, Yack JE, Fitzgerald TD, Hoy RR (2006) Vibrational communication in the cherry leaf roller caterpillar *Caloptilia serotinella*. J Insect Behav 19:1–18. https://doi.org/10.1007/s10905-005-9007-y

Fukui A (2001) Indirect interactions mediated by leaf shelters in animal-plant communities. Popul Ecol 43:31–40

Gish M, Dafni A, Inbar M (2012) Young aphids avoid erroneous dropping when evading mammalian herbivores by combining input from two sensory modalities. PLoS One 7(4):e32706. https://doi.org/10.1371/journal.pone.0032706

Gogala M (1985) Vibrational communication in insects (biophysical and behavioural aspects). In: Kalmring K, Elsner N (eds) Acoustic and vibrational communication in insects. Paul Parey, Berlin, pp 117–126

Gogala M, Čokl A, Drašlar K, Blaževič A (1974) Substrate borne sound communication in Cydnidae (Heteroptera). J Comp Physiol 94:25–31. https://doi.org/10.1007/BF00610155

Greeney HF, Dyer LA, Smilanich AM (2012) Feeding by lepidopteran larvae is dangerous : a review of caterpillars' chemical, physiological, morphological, and behavioral defenses against natural enemies. Invertebr Surviv J 9:7–34

Greenfield MD (2002) Signalers and receivers: mechanisms and evolution of arthropod communication. Oxford University Press, New York

Grof-Tisza P, Holyoak M, Antell E, Karban R (2015) Predation and associational refuge drive ontogenetic niche shifts in an arctiid caterpillar. Ecology 96:80–89

Guedes RNC, Matheson SM, Frei B, Smith ML, Yack JE (2012) Vibration detection and discrimination in the masked birch caterpillar (*Drepana arcuata*). J Comp Physiol A 198:325–335

Hager FA, Krausa K, Kirchner WH (2019) Vibrational behavior in termites (Isoptera). In: PSM H, Lakes-Harlan R, Mazzoni V, Narins PM, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational behavior. Springer Nature Switzerland, Cham, pp 309–327

Hagstrum DW, Subramanyam B (2010) Immature insects: ecological roles of mobility. Am Entomol 56:230–241

Hamel JA, Cocroft RB (2012) Negative feedback from maternal signals reduces false alarms by collectively signalling offspring. P Roy Soc B 279:3820–3826

Hasenfuss I (1992) Morphology, evolution, and taxonomic importance of supposed web- vibration receptors in the larvae of butterflies (Lepidoptera: Pyraloidea and Gelechioidea). Entomologica Generalis 18:43–54

Heinrich B (1993) How avian predators constrain caterpillar foraging. In: Stamp NE, Casey TM (eds) Caterpillars: ecological and evolutionary constraints on foraging. Chapman and Hall, New York, pp 224–247

Henaut A (1990) Study of the sound produced by *Pimpla instigator* (Hymenoptera: Ichneumonidae) during host selection. Entomophaga 35:127–139

Hess WN (1917) The chordotonal organs and pleural discs of cerambycid larvae. Ann Entomol Soc Am 10:63–74

Hill CJ (1993) The myrmecophilous organs of *Arhopala madytus* Fruhstorfer (Lepidoptera: Lycaenidae). J Aust Entomol Soc 32:283–288

Hill PSM (2008) Vibrational communication in animals. Harvard University Press, Cambridge, MA, London

Hill PSM (2014) Stretching the paradigm or building a new? Development of a cohesive language for vibrational communication. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, Heidelberg, pp 13–30

Hill PSM, Wessel A (2016) Biotremology. Curr Biol 26:R181–R191. https://doi.org/10.1016/j.cub.2016.01.054

Hinton HE (1951) Myrmecophilous lycaenidae and other Lepidoptera--a summary. Proc Trans South Lond Ent Nat History Soc 1949–1950:111–175

Hograefe T (1984) Subtrat-stridulation bei den koloniebildended Blattwespenlarven von *Hemichroa crocea* (Geoff.) (Hymenoptera: Tenthredinidae). Zool Anz 213:234–241

Hunter MD (1987) Sound production in larvae of *Diurnea fagella* (Lepidoptera: Oecophoridae). Ecol Entomol 12:355–357

Hunter MD, Willmer PG (1989) The potential for interspecific competition between two abundant defoliators on oak: leaf damage and habitat quality. Ecol Entomol 14:267–277

Ishay J, Motro A, Gitter S, Brown MB (1974) Rhythms in acoustical communication by the oriental hornet, *Vespa orientalis*. Anim Behav 22:741–744

Jennings DT, Crawford HR Jr, Hunter ML Jr (1991) Predation by amphibians and small mammals on spruce budworm (Lepidoptera: Tortricidae). Great Lakes Entomologist 24:69–74

Keil TA (1997) Functional morphology in insect mechanoreceptors. Microsc Res Tech 39:506–531

Kojima K, Ishikawa Y, Takanashi T (2012) Pupal vibratory signals of a group-living beetle that deter larvae: are they mimics of predator cues? Commun Integr Biol 5:262–264

Kühne R (1982) Neurophysiology of the vibration sense in locusts and bushcrickets: response characteristics of single receptor units. J Insect Physiol 28:155–163

Lakes-Harlan R, Strauss J (2014) Functional morphology and evolutionary diversity of vibration receptors in insects. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication, Animal signals and communication, vol 3. Springer, Berlin, Heidelberg, pp 277–302

Lederhouse RC (1990) Avoiding the hunt: primary defenses of lepidopteran caterpillars. In: Evans D, Schmidt J (eds) Insect Defenses. State University of New York Press, Albany NY, pp 175–189

Lill JT, Marquis RJ (2004) Leaf ties as colonization sites for forest arthropods: an experimental study. Ecol Entomol 29:300–308

Lill JT, Marquis RJ (2007) Microhabitat manipulation: ecosystem engineering by shelter-building insects. In: Cuddington KMD, Byers JE, Hastings A, Wilson WG (eds) Ecosystem engineers: concepts, theory, and applications in ecology. Elsevier Press, San Diego, CA, pp 107–138

Low C (2008) Seismic behaviours of a leafminer, *Antispila nysaefoliella* (Lepidoptera: Heliozelidae). Fla Entomol 91:604–609

Markl H (1983) Vibrational communication. In: Huber F, Markl H (eds) Neuroethology and Behavioural physiology: roots and growing points. Springer-Verlag, Heidelberg, pp 332–353

Markl H, Tautz J (1975) The sensitivity of hair receptors in caterpillars of *Barathra brassicae* L. (Lepidoptera, Noctuidae) to particle movement in a sound field. J Comp Physiol 99:79–87

Matthews RW, Matthews JR (2009) Chemical communication. In: Matthews RW, Matthews JR (eds) Insect behavior. Springer, Dordrecht, pp 217–259

McIver S, Beech M (1986) Prey finding behavior and mechanosensilla of larval *Toxorhynchites brevipalpis* Theobald (Diptera: Culicidae). Int J Insect Morphol Embryol 15:213–225

Meyhöfer R, Casas J, Dorn S (1997) Vibration-mediated interactions in a host-parasitoid system. P Roy Soc Lond B 264:261–266

Montgomery SL (1983) Carnivorous caterpillars: the behavior, biogeography and conservation of *Eupitheoia* (Lepidoptera: Geometridae) in the Hawaiian islands. GeoJournal 76:549–556

Montllor CB, Bernays EA (1993) Invertebrate predators and caterpillar foraging. In: Stamp NE, Casey TM (eds) Caterpillars: ecological and evolutionary constraints on foraging. Chapman and Hall, New York, pp 170–202

Mukai H, Hironaka M, Tojo S, Nomakuchi S (2014) Maternal vibration: an important cue for embryo hatching in a subsocial shield bug. PLoS One 9(1):e87932. https://doi.org/10.1371/journal.pone.0087932

Nishide Y, Tanaka S (2016) Desert locust, *Schistocerca gregaria*, eggs hatch in synchrony in a mass but not when separated. Behav Ecol Sociobiol 70:1507–1515. https://doi.org/10.1007/s00265-016-2159-2

Oberst S, Bann G, Lai JC, Evans TA (2017) Cryptic termites avoid predatory ants by eavesdropping on vibrational cues from their footsteps. Ecol Lett 20:212–221. https://doi.org/10.1111/ele.12727

Pescador-Rubio A, Stanford-Camargo SG, Páez-Gerardo LE, Ramírez-Reyes AJ, Ibarra-Jiménez RA, Fitzgerald TD (2011) Trail marking by caterpillars of the silverspot butterfly *Dione juno huascuma*. J Insect Sci 11(1):55

Pierce NE, Braby MF, Heath A, Lohman DJ, Mathew J, Rand DB, Travassos MA (2002) The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annu Rev Entomol 47:733–771. https://doi.org/10.1146/annurev.ento.47.091201.145257

Piesik D, Rochat D, van der Pers J, Marion-Poll F (2009) Pulsed odors from maize or spinach elicit orientation in European corn borer neonate larvae. J Chem Ecol 35:1032–1042

Piesik D, Rochat D, Delaney KJ, Marion-Poll F (2013) Orientation of European corn borer first instar larvae to synthetic green leaf volatiles. J Appl Entomol 137:234–240

Prokopy RJ, Roitberg BD (2001) Joining and avoidance behavior in nonsocial insects. Annu Rev Entomol 46:631–635

Ramaswamy K, Cocroft RB (2009) Collective signals in treehopper broods provide predator localization cues to the defending mother. Anim Behav 78:697–704

Randall JA (2014) Vibrational communication: spiders to kangaroo rats. In: Witzany G (ed) Biocommunication of animals. Springer, Dordrecht, pp 103–133

Reader T, Hochuli DF (2003) Understanding gregariousness in a larval lepidopteran: the roles of host plant, predation, and microclimate. Ecol Entomol 28:729–737

Rieske LK, Townsend LH (2005) Orientation and dispersal patterns of the eastern tent caterpillar, *Malacosoma americanum* F. (Lepidoptera: Lasiocampidae). J Insect Behav 18:193–208

Riva F, Barbero F, Bonelli S, Balletto E, Casacci LP (2016) The acoustic repertoire of lycaenid butterfly larvae. Bioacoustics 26:77–90. https://doi.org/10.1080/09524622.2016.1197151

Rodríguez RL (2019) Copulatory courtship with vibratory signals. In: PSM H, Lakes-Harlan R, Mazzoni V, Narins PM, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational behavior. Springer Nature Switzerland, Cham, pp 79–89

Roessingh P (1989) The trail following behaviour of *Yponomeuta cagnagellus*. Entomol Exp Appl 51:49–57

Roessingh P, Xu S, Menken SB (2007) Olfactory receptors on the maxillary palps of small ermine moth larvae: evolutionary history of benzaldehyde sensitivity. J Comp Physiol A 193:635–647

Rosi-Denadai C (2018) How do caterpillars detect vibration? Proleg sensory hairs as vibration receptors in *Drepana arcuata* (Drepanidae) and *Trichoplusia ni* (Noctuidae). Carleton University, Dissertation. Available from: https://curve.carleton.ca/8de0920f-b83f-40d8-aa81-e8b2f44e9cda

Russ K (1969) Beiträge zum Territorialverhalten der Raupen des Springwurmwicklers, *Sparganothis pilleriana* Schiff (Lepidoptera: Tortricidae). Pflanzenschutzberichte 40:1–9

Sagers CL (1992) Manipulation of host plant quality herbivores keep leaves in the dark. Funct Ecol 6:741–743

Sala M, Casacci LP, Balletto E, Bonelli S, Barbero F (2014) Variation in butterfly larval acoustics as a strategy to infiltrate and exploit host ant colony resources. PLoS One 9(4):e94341. https://doi.org/10.1371/journal.pone.0094341

Saliba LJ (1972) Gallery orientation of cerambycid larvae. The Entomologist 105:300–304

Schönrogge K, Barbero F, Casacci LP, Settele J, Thomas JA (2017) Acoustic communication within ant societies and its mimicry by mutualistic and socially parasitic myrmecophiles. Anim Behav 134:249–256. https://doi.org/10.1016/j.anbehav.2016.10.031

Schoonhoven LM (1987) What makes a caterpillar eat? The sensory code underlying feeding behavior. In: Chapman RE, Bernays EA, Stoffolano JG (eds) Perspectives in chemoreception and behavior. Springer Verlag, New York, pp 69–97

Schoonhoven LM, Dethier VG (1966) Sensory aspects of host-plant discrimination by lepidopterous larvae. Arch Néerl Zool 16:497–530

Schoonhoven LM, van Loon JJA (2002) An inventory of taste in caterpillars: each species its own key. Acta Zool Acad Sci Hung 48:215–263

Schurian KG, Fiedler K (1991) Einfache Methoden zur Schallwahrnehmung bei Bläulings-larven (Lepidoptera: Lycaenidae). Entomol Z 101:393–412

Scott JL, Yack JE (2012) Vibratory territorial signals in caterpillars of the poplar lutestring, *Tethea or* (Lepidoptera: Drepanidae). Eur J Entomol 109:411–417

Scott JL, Kawahara AY, Skevington JH, Yen S-H, Sami A, Smith ML, Yack JE (2010) The evolutionary origins of ritualized acoustic signals in caterpillars. Nat Commun 1:1–9

Shestakov LS (2015) A comparative analysis of vibrational signals in 16 sympatric species (Pentatomidae, Heteroptera). Entomol Rev 95:310–325

Shields VDC, Martin TLC (2012) The structure and function of taste organs in caterpillars. In: Lynch EJ, Petrov AP (eds) The sense of taste. Nova Science, New York, pp 147–166

Sigmon E (2015) Interspecific variation in aggressive fighting behavior of shelter-building caterpillars. J Insect Behav 28:403–416

Starnecker G (1996) Color preference for pupation sites of the butterfly larvae *Inachis io* and the significance of the pupal melanization reducing factor. Naturwissenschaften 83:474–476

Stehr FW (1987) Order lepidoptera. In: Stehr FW (ed) Immature insects, vol 1. Kendall/Hunt Publishing Company, Dubuque, IA, pp 288–596

Stewart KW (1997) Vibrational communication in insects. Epitome in the language of stoneflies? Am Entomol Summer 1997:81–91

Strauss J, Stritih-Peljhan N, Lakes-Harlan R (2019) Determining vibroreceptor sensitivity in insects: the influence of experimental parameters and recording techniques. In: PSM H, Lakes-Harlan R, Mazzoni V, Narins PM, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational behavior. Springer Nature Switzerland, Cham, pp 209–233

Takanashi T, Fukaya M, Nakamuta K, Skals N, Nishino H (2016) Substrate vibrations mediate behavioral responses via femoral chordotonal organs in a cerambycid beetle. Zool Lett 2:18

Tanaka S, Sakamoto H, Hata T, Sugahara R (2018) Hatching synchrony is controlled by a two-step mechanism in the migratory locust *Locusta migratoria* (Acrididae: Orthoptera): roles of vibrational stimuli. J Insect Physiol 107:125–135

Tautz J (1977) Reception of medium vibration by thoracal hairs of caterpillars of *Barathra brassicae* L. (Lepidoptera, Noctuidae). I. Mechanical properties of the receptor hairs. J Comp Physiol 118:13–31

Tautz J (1978) Reception of medium vibration by thoracal hairs of caterpillars of *Barathra brassicae* L. (Lepidoptera, Noctuidae). II. Response characteristics of the sensory cell. J Comp Physiol 125:67–77

Tautz J, Markl H (1978) Caterpillars detect flying wasps by hairs sensitive to airborne vibration. Behav Ecol Sociobiol 4:101–110

Taylor CJ, Yack JE (2019) Hearing in caterpillars of the monarch butterfly (*Danaus plexippus*). J Exp Biol 222:jeb211862. https://doi.org/10.1242/jeb.211862

Travassos MA, Pierce NE (2000) Acoustics, context and function of vibrational signaling in a lycaenid butterfly ant mutualism. Anim Behav 60:13–26

Virant-Doberlet M, Čokl A (2004) Vibrational communication in insects. Neotrop Entomol 33: 121–134

Virant-Doberlet M, Kuhelj A, Polajnar J, Šturm R (2019) Predator-prey interactions and eavesdropping in vibrational communication networks. Front Ecol Evol 7:203. https://doi.org/10.3389/fevo.2019.00203

Wagner D (2005) Caterpillars of eastern North America: a guide to identification and natural history. Princeton University Press, Princeton NJ

Warrant EJ, Kelber A, Kristensen NP (2003) Eyes and vision. In: Kristensen NP (ed) Handbook of zoology, part 36, Lepidoptera, moths and butterflies, vol 2: morphology, physiology and development, vol IV. Walter de Gruyter, Berlin, New York, pp 325–359

Yack JE (2004) The structure and function of auditory chordotonal organs in insects. Microsc Res Tech 63:315–337

Yack JE (2016) Vibrational signaling. In: Pollack GS, Mason AC, Popper AN, Fay RR (eds) Insect hearing. Springer Verlag, Berlin, pp 99–123

Yack JE, Smith ML, Weatherhead PJ (2001) Caterpillar talk: acoustically mediated territoriality in larval Lepidoptera. P Nat Acad Sci USA 98:11371–11375

Yack JE, Gill S, Drummond-Main C, Sherratt TN (2014) Residency duration and shelter quality influence vibratory signalling displays in a territorial caterpillar. Ethology 120:354–364

Yadav C, Yack JE (2018) Immature stages of the masked birch caterpillar, *Drepana arcuata* (Lepidoptera: Drepanidae) with comments on feeding and shelter building. J Insect Sci 18(1):18

Yadav C, Guedes RNC, Matheson SM, Timbers TA, Yack JE (2017) Invitation by vibration: recruitment to feeding shelters by vibrating caterpillars. Behav Ecol Sociobiol 71:51

Zalucki M, Clarke AR, Malcolm SB (2002) Ecology and behaviour of first-instar larval Lepidoptera. Annu Rev Entomol 47:361–393

Part VI
Applied Biotremology

Chapter 20
Exploitation of Vibration Sensing for Pest Management in Longicorn Beetles

Takuma Takanashi and Hiroshi Nishino

Abstract Many insects exhibit sensitivities to substrate-borne vibrations. Some beetles detect vibrations via leg chordotonal organs and respond with predator avoidance or sexual communication. Because vibrations modify insect behaviors, vibrations could be exploited for physical pest control to reduce insect damage to plants. Here we review the abilities of beetles to sense vibrations and the use of vibrations as a pest management option for the longicorn beetle *Monochamus alternatus*, a vector of the lethal pine wilt disease, and other longicorn beetles. More specifically, we report new findings describing leg chordotonal organs and behavioral control methods using vibrations in *M. alternatus* and *Moechotypa diphysis*, a pest of mushroom bed logs. These beetles show freezing and startle responses when exposed to low-frequency vibration pulses. We characterize the morphologies of the femoral chordotonal organs and their central projections, and describe a new procedure for vibrational pest management, based on vibration sensitivities in longicorn beetles. For this method, a prototype vibration exciter that generates vibrations with large amplitudes is attached to a tree, and the vibrations from the exciter disrupt beetle feeding and walking by initiating startle and freezing responses by beetles. We believe that vibrations can be applied to plants to reduce future damage by various pests that are sensitive to vibrations.

20.1 Introduction

Many insects are sensitive to vibrations transmitted through substrates (Greenfield 2002; Hill 2008). These insects evolved chordotonal organs in their legs that are responsible for detecting vibrations (Field and Matheson 1998; Hill 2008), and many

---

T. Takanashi (✉)
Forestry and Forest Products Research Institute, Tohoku Research Center, Morioka, Japan
e-mail: takanasi@affrc.go.jp

H. Nishino
Research Institute for Electronic Science, Hokkaido University, Sapporo, Japan
e-mail: nishino@es.hokudai.ac.jp

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_20

exhibit various behaviors and communications in response to vibrations (Hill 2008; Takanashi et al. 2019). For instance, larvae of the group-living beetle *Trypoxylus dichotomus* freeze in response to vibrations produced by approaching moles in the soil (Kojima et al. 2012a, b). Furthermore, they also freeze in response to vibrations that pupae of the beetle produce by drumming on their abdomen; as a result, pupal cells are protected from damage caused by approaching larvae (Kojima et al. 2012c). In the brown marmorated stink bug, *Halyomorpha halys*, egg-cracking vibrations promote synchronous hatching in a clutch of eggs in contact with each other on host plants (Endo et al. 2019).

In order to control pest insects, it may be possible to utilize their ability of sensing vibrations to disrupt communications and various behaviors, which would represent an environmentally friendly alternative to the synthetic pesticides that are currently used (Polajnar et al. 2015; Takanashi et al. 2019). A successful example of vibrational pest management is communication disruption in the American grapevine leafhopper, *Scaphoideus titanus* (Eriksson et al. 2012; Polajnar et al. 2016; Nieri and Mazzoni 2018). The mating frequency of this insect in field cages was decreased by disturbance vibrations at 300 Hz produced by an electromagnetic shaker placed in grapevines (Eriksson et al. 2012). Validation of the amplitude threshold for efficacy in mating disruption in a vineyard (Polajnar et al. 2016) was followed by pilot studies on mating disruption (Nieri and Mazzoni 2018). Similarly, communication disruption with vibrations has been reported in the Neotropical brown stink bug *Euschistus heros* (Laumann et al. 2018) and the Asian citrus psyllid *Diaphorina citri* (Lujo et al. 2016). Hosomi (1996) has reported a case of behavioral disruption in which vibrations at 5 to 40 Hz produced by a mechanical knocker suppressed feeding of the longicorn beetle *Apriona japonica* on fig trees. As noted by Takanashi et al. (2019), the vibration exciter hardware and software controlling the spectral, temporal, and amplitude characteristics of the vibrations are important for efficient disruption of communications and behaviors.

Longicorn beetles (Cerambycidae: Coleoptera) comprise more than 3600 species worldwide and include forest and agricultural pests that damage trees (Wang 2017). The beetles include invasive species spread by international trade and vectors of pathogens of serious tree diseases (Kobayashi et al. 1984; Wang 2017). Beetle adults feed on tree bark, and mate and oviposit on the host trees (Wang 2017). Because longicorn beetle larvae bore inside the trees for feeding, they can be difficult to control with pesticides. Long-range vibrational communication for mate localization, which is well known in hemipteran insects, has not yet been found in longicorn beetles (Takanashi et al. 2019). However, various behaviors, such as freezing and walking, are induced by vibrations (Takanashi et al. 2016, 2019).

In this chapter, we report recent findings on leg chordotonal organs and behavioral control methods using vibrations in two longicorn beetle pests, *Monochamus alternatus* and *Moechotypa diphysis*. We also report on previous findings on *Paraglenea fortunei*, a longicorn beetle that feeds on ramie and other plants (Tsubaki et al. 2014; Takanashi et al. 2016), and *M. alternatus*. *Monochamus alternatus* is the vector of the pine wilt nematode *Bursaphelenchus xylophilus*, which kills pine trees by causing water deficiency (Kobayashi et al. 1984; Kikuchi et al. 2011; Yazaki et al.

Fig. 20.1 A female adult of *Monochamus alternatus* on a host pine twig

2018) (Fig. 20.1). *Monochamus alternatus* is distributed in Asia, including Japan, and the damage caused by this species is ca. 400,000 m^3 of pine trees per a year in Japan (Kobayashi et al. 1984; Forestry Agency 2018). *Moechotypa diphysis* is an invasive pest of Shiitake mushroom (*Lentinula edodes*) bed logs from oak trees in Japan (Furukawa and Nobuchi 1996). We discuss new technologies for vibration exciters and the potential use of vibrational pest management against longicorn beetles and other pests.

20.2 Vibration Sense Organs

20.2.1 Chordotonal Organs

Chordotonal organs in insects are internal proprioceptors that measure positions and movements of limb joints or the body wall, and they are distributed ubiquitously in body appendages/segments (Field and Matheson 1998). As schematized in Fig. 20.2a, the sensing unit of a chordotonal organ is a bipolar sensory neuron, in which stretch-sensitive mechanosensory channels are distributed in the sensory cilia of the dendrite. The dendritic tip is inserted in the scolopale cap supported by the scolopale rod, which is further connected to a chord-like attachment cell enriched with microtubules.

The number of sensory neurons varies among distinct chordotonal organs located in different body positions in the same species. Some proprioceptive chordotonal organs are able to detect fast and small displacements (i.e., vibrations) transmitted through attachment cells (e.g., Field and Pflüger 1989; Pflüger and Field 1999). The vibrational sensitivities of chordotonal organs are usually higher than those of other

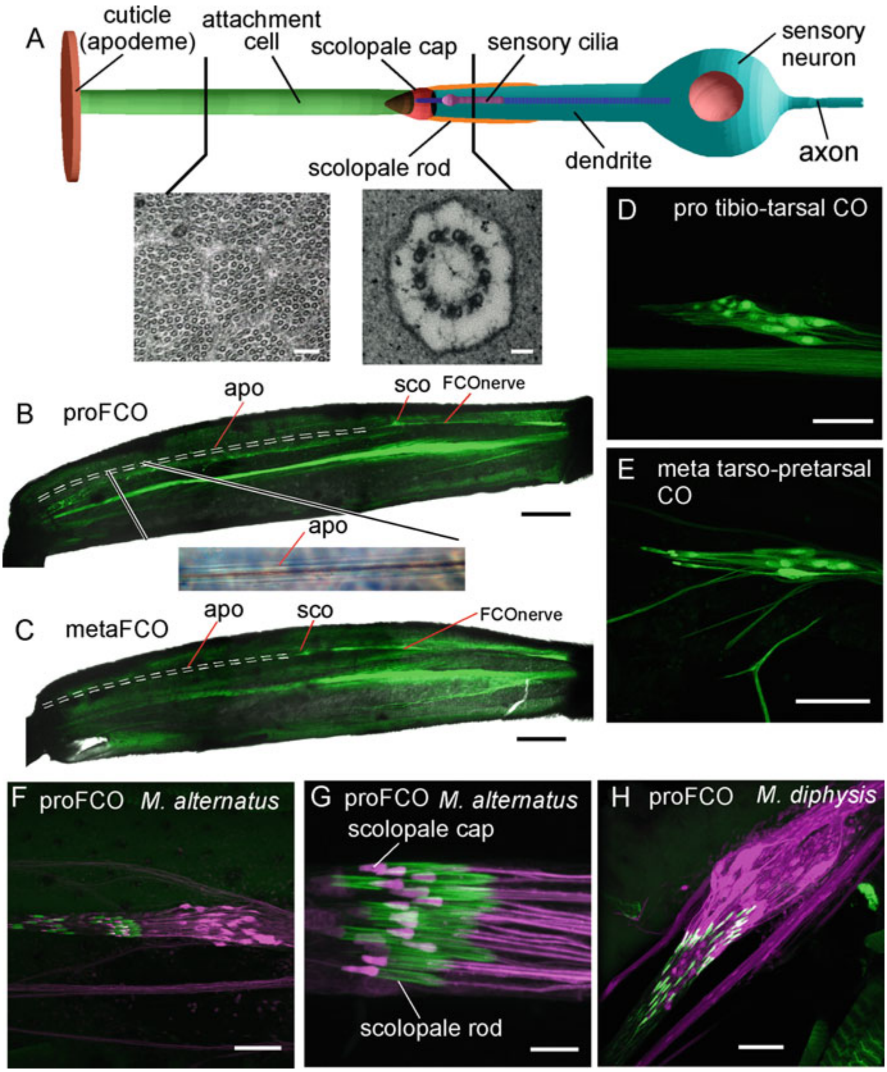


Fig. 20.2 Leg chordotonal organs in longicorn beetles. (**a**) The basic structure of a chordotonal organ (CO) showing the ciliated dendrite with 9 × 2 + 0 axoneme configuration (right inset, derived from the tree weta, *Hemideina femorata*) and the microtubule-rich attachment cell (left inset, derived from *H. femorata*). (**b**, **c**) Low-magnification images of pro- and metathoracic femoral chordotonal organs (FCOs) in *Monochamus alternatus*, showing that the scoloparium (sco) containing sensory neurons is connected to the long cord-like cuticular apodeme (apo). The scoloparium is located more distally in the metathoracic FCO than in the pro- and mesothoracic FCOs. Adapted from photos of Takanashi et al. (2016). (**d**, **e**) Tibio-tarsal CO and tarso-pretarsal COs contain approximately 16 and 10 sensory neurons, respectively, and have cell bodies of varied sizes. (**f–h**) Differential labeling with rhodamine and phalloidin showing sensory neurons (magenta) and scolopale rods (green) in the prothoracic FCOs in *M. alternatus* (**f**, **g**) and *Moechotypa diphysis* (**h**). Scale bars = 100 nm in left inset; 50 nm in right inset; 500 μm in **b** and **c**; 50 μm in **d**, **e**, **f**, **h**; 10 μm in **g**

types of sense organs such as campaniform sensilla and hair sensilla, which detect low-frequency vibrations (Kühne 1982; Ai et al. 2010).

The chordotonal organs in coleopterans have been one of the least studied sense organs because the thick exoskeleton hinders dissection and direct observations of these organs. Our extensive survey using retrograde labeling of nerve trunks originating from thoracic ganglia in *M. alternatus* revealed that in the periphery of the ganglion, there are no subgenual organs or tympanal organs specialized for detecting substrate vibrations or airborne sounds, respectively (Takanashi et al. 2016). This view is in agreement with a previous report on coleopteran species (Schneider 1950).

By backfill staining of the leg nerve of longicorn beetles, we identified on each of the six legs a femoral chordotonal organ (FCO), a tibio-tarsal chordotonal organ, and a tarso-pretarsal chordotonal organ (Fig. 20.2b–e; Takanashi et al. 2016), all three of which potentially detect substrate vibrations (Field and Pflüger 1989; Goodwyn et al. 2009). Among these chordotonal organs, the FCO contained far higher numbers of sensory neurons (Fig. 20.2d–h; Takanashi et al. 2016). We further investigated whether the FCO is the primary vibration detector in the legs.

20.2.2 Morphologies of Femoral Chordotonal Organs

The FCOs of *M. alternatus* share fundamental structures with those in other insect species (Takanashi et al. 2016). The sensory neurons are distally connected via a bundle of attachment cells to the cuticular apodeme, which extends from the dorsal region of the joint pivot of the tibia (apo, Fig. 20.2b, c; Shelton et al. 1992). Therefore, the tibial flexion and extension are converted to distal and proximal apodemal displacements and, in turn, evoke mechanical distortion on the dendrites of sensory neurons. Sensory neurons are functionally specialized for detecting position, velocity, and/or acceleration in particular ranges of femoro-tibial angles; this functional specialization is called range fractionation (Hofmann et al. 1985; Matheson 1990; Büschges 1994; Sauer and Stein 1999).

The main body of the FCO that embeds sensory neurons is called the scoloparium (sco, Fig. 20.2b, c; Takanashi et al. 2016). The FCO in each leg of *M. alternatus* has a single scoloparium, and no clear anatomical subdivision is detectable within it. The scoloparial location somewhat differs between legs: it is located in the proximal third of the femur for pro- and mesothoracic FCOs (Fig. 20.2b) but in the proximal half for the metathoracic FCO (Fig. 20.2c; Takanashi et al. 2016). The different lengths of the apodeme resulting from distinct scoloparial locations may affect the resonant frequency at which each apodeme transmits substrate vibrations, but their functional differences remain to be studied.

The number of sensory neurons embedded in the scoloparium is nearly identical between pro-, meso-, and metathoracic FCOs (Takanashi et al. 2016). As in FCOs of other insects, two neighboring sensory neurons are paired to extend dendrites into a common scolopale cap; these paired neurons are referred to as heterodynal (Fig. 20.2g; Field and Matheson 1998). The number of sensory neurons in the scoloparium is, therefore, countable by checking the number of scolopale caps or rods. The scolopale rods are rich with actin filaments, so they can be visualized by phalloidin staining (Fig. 20.2f–h; Nishino et al. 2016). For example, the number of scolopale rods in the prothoracic FCO of *M. alternatus* is 37, and the estimated number of sensory neurons is therefore 74 (Fig. 20.2f). In *M. diphysis*, the number of scolopale rods in the prothoracic FCO is 41, so there are an estimated 82 sensory neurons (Fig. 20.2h). We found FCOs of the longicorn beetle *Mesosa longipennis*, a pest of oak and conifer trees, in similar locations on all legs; although the exact number of sensory neurons in each FCO has not yet been determined.

20.2.3 Central Projections of Sensory Neurons in the Femoral Chordotonal Organs

Since the FCO of *M. alternatus* is innervated by a long sensory nerve diverged from the main leg nerve (FCO nerve, Fig. 20.2b, c; Takanashi et al. 2016), by immersing the cut end of the FCO nerve into a dye-filled capillary, we could trace sensory axonal fibers projecting to the central nervous system. The FCO nerve contains sensory axons of the FCO, in addition to those of hair sensilla located in the antero-dorsal surface of the femur. Bilateral labeling of FCO nerves in the pro-, meso-, and metathoracic legs revealed extensive projections of FCO axons from lateral to medial in the ipsilateral ganglion (Fig. 20.3a–c). These projections, resembling those of FCOs in other insects (Field and Pflüger 1989; Nishino 2003), were entirely segregated from those of hair sensilla, which nearly exclusively occupy the ventral association center (Fig. 20.3d, f; Pflüger et al. 1981; Newland 1991).

Most characteristically, the FCO axon terminals enter the medio-ventral association center specialized for vibratory/auditory processing (Fig. 20.3d, e), in addition to the motor association neuropil, the lateral association center located more laterally to the medio-ventral association center (Fig. 20.3d; Pflüger et al. 1981; Pflüger et al. 1988; Mücke and Lakes-Harlan 1995). The medio-ventral association center is conserved among different insect orders (Boyan 1993). Retrograde labeling of nerve trunks originating from thoracic ganglia indicated that in longicorn beetles, the FCO is the primary source that feeds axons to the medio-ventral association center region (Nishino, unpublished observation). There is no indication that single neurons project exclusively to the medio-ventral association center, but they appear to possess side branches to other areas, including the lateral association center. Thus, the overall morphologies of single sensory axons resemble those of subgenual organs in orthopteran insects (Mücke and Lakes-Harlan 1995; Stein and Sauer 1999; Nishino and Field 2003; Stritih Peljhan et al. 2019).

Together with the lack of anatomical subdivision in the FCO of *M. alternatus* (Takanashi et al. 2016), we presume that individual sensory neurons might be bifunctional, detecting low-frequency vibrations as well as mediating some

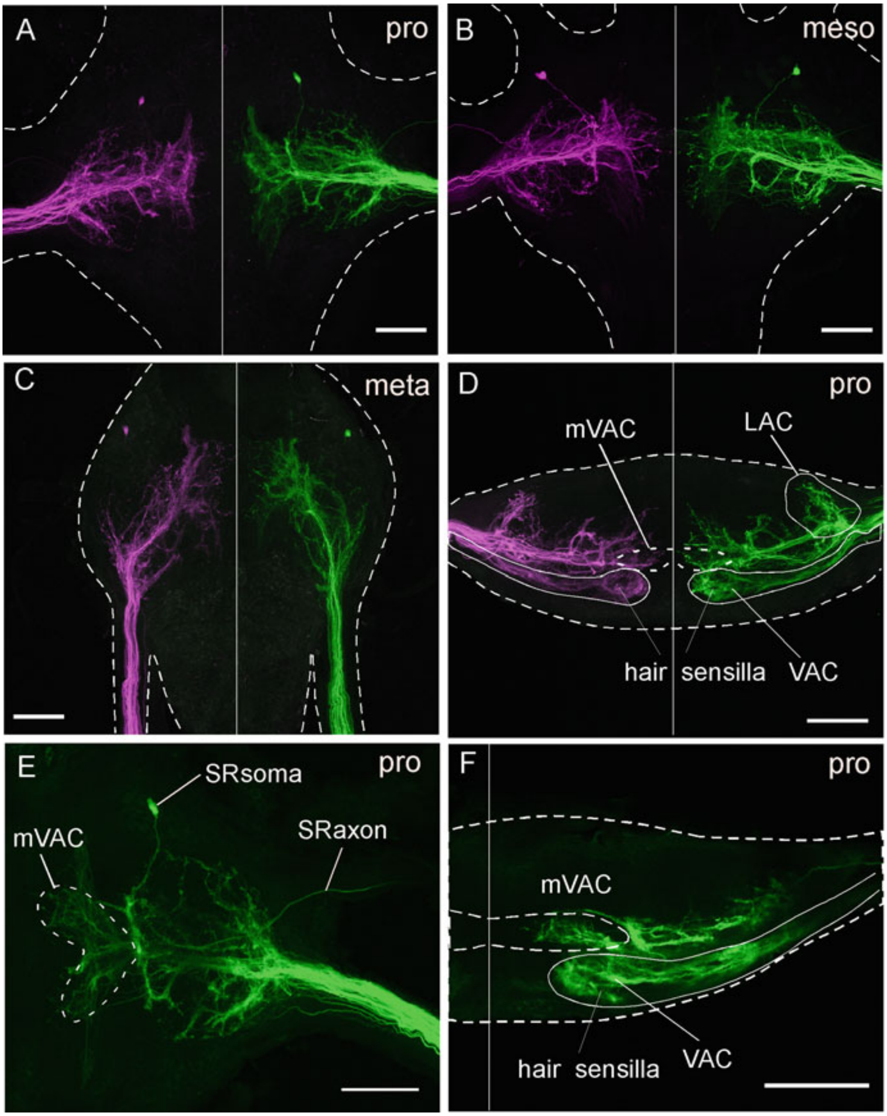


Fig. 20.3 (**a-c**) Central projections of femoral chordotonal organs in *Monochamus alternatus*. Bilateral labeling of femoral chordotonal organ (FCO) nerves with different fluorescent dyes (rhodamine, FITC) in pro-, meso-, and metathoracic legs show mirror-image projections on either side of a midline (solid line). (**d**, **f**) Transverse sections show extensive projections of FCO afferents included in the lateral association center (LAC) and the medio-ventral association center (mVAC) of the ipsilateral ganglion, which are entirely segregated from those of hair sensilla projecting exclusively to the ventral association center (VAC). (**e**) A presumed strand receptor (SR) with a cell body in the central nervous system (Bräunig 1982) is present in each hemisphere of the ganglion. The ganglion was outlined by broken lines in (**a-d**) and (**f**). Scale bars = 100 μm

proprioceptive feedbacks, such as resistance reflex in tibial extensor/flexor muscles (Field and Burrows 1982; Sauer and Stein 1999).

20.3 Behavioral Control with Vibrations

20.3.1 Freezing and Startle Responses and Related Sense Organs

Freezing and startle responses to vibrations are found in *M. alternatus*, *P. fortunei*, and the house longhorn beetle *Hylotrupes bajulus* (Breidbach 1986; Tsubaki et al. 2014; Takanashi et al. 2016). Beetles respond to vibrations while walking by freezing. A startle response—that is, small movement of the legs and antennae—is induced when vibrations are applied to beetles under quiescence. This response is similar to the vibration-induced startle response in the locust *Schistocerca gregaria* under quiescence (Friedel 1999). *Monochamus alternatus* and *P. fortunei* show high sensitivity to frequencies of 20–500 Hz, with response thresholds of 2–20 m/s^2 (Fig. 20.4). In *P. fortunei*, vibrations from approaching conspecifics are far enough above the behavioral thresholds to induce the responses, which allow them to recognize approaching conspecifics or predators (Tsubaki et al. 2014; Takanashi et al. 2019). In addition to these two responses, *M. alternatus* begins walking in response to vibrations at 100 Hz (Takanashi et al. 2016).

To confirm that FCOs detect vibrations, the scoloparia attached to the apodemes of all six femora of beetles were removed with microscissors to produce

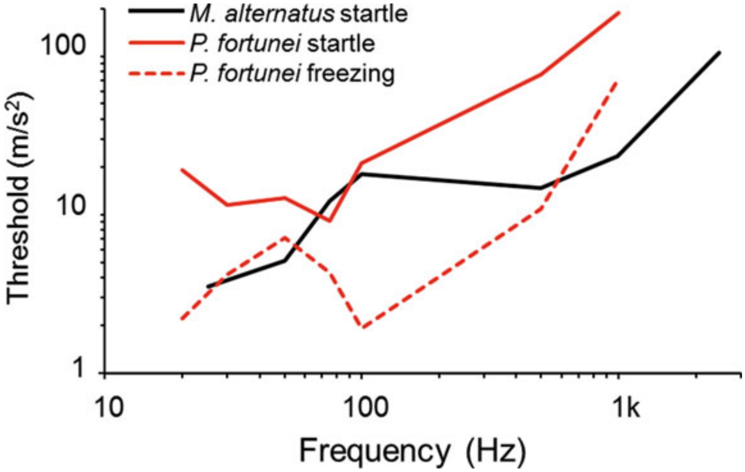


Fig. 20.4 Thresholds of behavioral responses to vibrations in *Monochamus alternatus* and *Paraglenea fortunei*: startle responses during quiescence (solid lines) in both species and freezing response during walking in *P. fortunei* (dashed line). Reproduced from Takanashi et al. (2019)

Fig. 20.5 *Moechotypa diphysis* exhibiting tonic immobility after dropping from an oak log in response to vibrations. Courtesy of Shuji Fukui

dysfunctional FCOs (Takanashi et al. 2016). *Monochamus alternatus* individuals with altered FCOs on all legs did not freeze in response to 100 Hz and 1 kHz while walking, whereas intact and sham-operated beetles froze. These findings indicate that the FCO is responsible for detecting low-frequency vibrations. Thus, the sense organ involved in the freezing behavior has been identified in a coleopteran species.

In *M. diphysis*, we investigated startle response thresholds to vibrations of 100 and 120 Hz. We observed a startle response in individual adults during a period of quiescence on a steel plate attached to a vibration exciter with variable accelerations, as previously reported in Tsubaki et al. (2014). Mean response thresholds were 0.86 m/s^2 at 100 Hz and 0.80 m/s^2 at 120 Hz ($n = 10$); thus, *M. diphysis* is more sensitive to vibrations than *M. alternatus* and *P. fortunei*. This finding is in agreement with casual observations in the field that even small-amplitude vibrations that do not evoke any detectable reactions in the two species, *M. alternatus* and *P. fortunei*, can induce drop-off behaviors in *M. diphysis* (Furukawa and Nobuchi 1996; Tsubaki et al. 2014; Takanashi et al. 2019). Immediately after dropping to the ground, *M. diphysis* often displays tonic immobility (thanotosis) with femoro-tibial joints in extended positions at angles of various degrees (Fig. 20.5).

20.3.2 Behavioral Manipulation with Vibrations

Low-frequency vibrations can be used to manipulate *M. alternatus*, *P. fortunei*, and *M. diphysis* to produce startle, freeze, and walk responses. Furthermore, low-frequency vibrations are predicted to disrupt feeding, oviposition, and other behaviors because freezing entails the sudden cessation of any ongoing behavior. A new procedure for pest management that uses vibrations to control behaviors of longicorn beetles has been developed (Takanashi et al. 2019). A prototype of a weather-resistant vibration exciter was made from giant magnetostrictive material (GMM) as a new technology for generating large-amplitude vibrations. In GMM, an

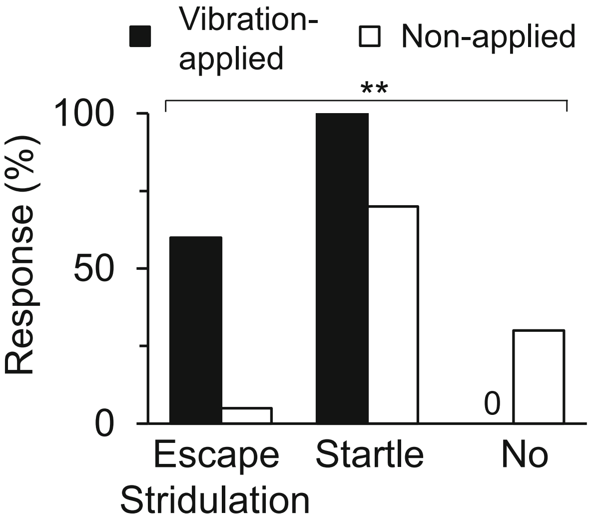


Fig. 20.6 Rates of various behavioral responses in vibration-applied and non-applied individuals of *Monochamus alternatus*. The number of responses was significantly different between the two groups according to Fisher's exact test (**$p < 0.001$, $n = 20$)

alloy of iron and rare metals, a large strain, called magnetostrain, is induced by a magnetic field (Söderberg et al. 2004).

Behavioral responses to vibrations were evaluated in *M. alternatus* individuals when they are quiescent by using a custom-made GMM vibration exciter (90 mm in length, 15 mm in diameter; Fig. 20.8). Vibrations at 100 Hz were generated for 1 to 2 s by the amplifier and function generator of the GMM vibration exciter. The vibration stimuli were applied to the femur of a prothoracic leg via a steel wire (0.6 mm in diameter, 85 mm in length; Fig. 20.2). An accelerometer with an attached data logger recorded the amplitude and other characteristics of the vibrations, as described in Takanashi et al. (2016). Amplitude at the tip of the wire was set to 5 m/s^2, which is larger than the threshold amplitude of the startle response in *M. alternatus*. In vibration-applied individuals, 60% showed escape behavior or stridulation (i.e., sound production) and 100% showed a startle response to vibrations transmitted through the wire (Fig. 20.6). In contrast, in non-applied individuals, only 5% showed escape behavior or stridulation and 70% showed a startle response to the mechanical stimulation of contacting the wire without vibrations. No response was observed in 30% of non-applied individuals. These results suggest that vibrations from the GMM vibration exciter enable behavioral manipulation of this species.

Behavioral responses of *M. diphysis* were observed to respond to vibrations produced by a different GMM vibration exciter (200 mm in length, 50 mm in diameter) (Fig. 20.7; Takanashi et al. 2019). Pulsed vibrations at 100 Hz for 1 s generated at intervals of 9 s by the amplifier and function generator of the GMM vibration exciter were applied to the bottom of a vertically oriented mushroom bed log (*Quercus crispula*; 80 mm in diameter, 740 mm in length). The accelerometer described above was used to adjust the acceleration of the vibration at the middle of the log to >1.5 m/s^2, which is larger than the thresholds of the startle response in

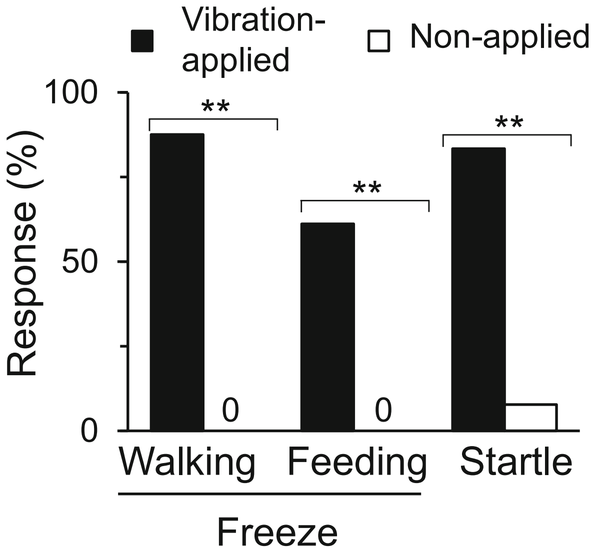


Fig. 20.7 Rates of various behavioral responses in vibration-applied and non-applied individuals of *Moechotypa diphysis*. The vibration-applied individuals showed significantly higher responses than the non-applied individuals by Fisher's exact test (**$p < 0.001$, $n = 8$–18)

M. diphysis. Overall, the vibration-applied individuals showed much higher rates of behavioral responses than the non-applied individuals (Fig. 20.7). Freeze during walk, freeze during feeding, and startle response during quiescence were induced in 61–88% of vibration-applied individuals and in 0–8% of non-applied individuals. These experiments reveal that a GMM vibration exciter can be used to manipulate behaviors of *M. alternatus* and *M. diphysis* and suggest that this procedure may be useful for behavioral disruption in pest management.

20.4 Pest Management with Vibrations

Many species belonging to both hemimetabolous and holometabolous insect orders, including Hemiptera, Coleoptera, Diptera, and Lepidoptera, exhibit sensitivity to vibrations from the larval to adult stages (Greenfield 2002; Cocroft and Rodríguez 2005; Hill 2008; Scott et al. 2010; Mazzoni et al. 2013; Takanashi et al. 2019; Kishi and Takanashi 2019b; Hofstetter et al. 2019). Since these insects are sensitive to vibrations, artificial vibrations can be applied to disrupt their various behaviors. Low-frequency vibrations at high acceleration that are above behavioral thresholds are necessary for the procedure to succeed (Takanashi et al. 2016). A GMM vibration exciter is suitable for generating vibrations to disrupt the behaviors of target pests. In addition, intermittent application of vibrations at sufficient intervals can help avoid the problem of sensory adaptation and behavioral habituation to vibrations (Fig. 20.8) (Friedel 1999; Kishi and Takanashi 2019a).

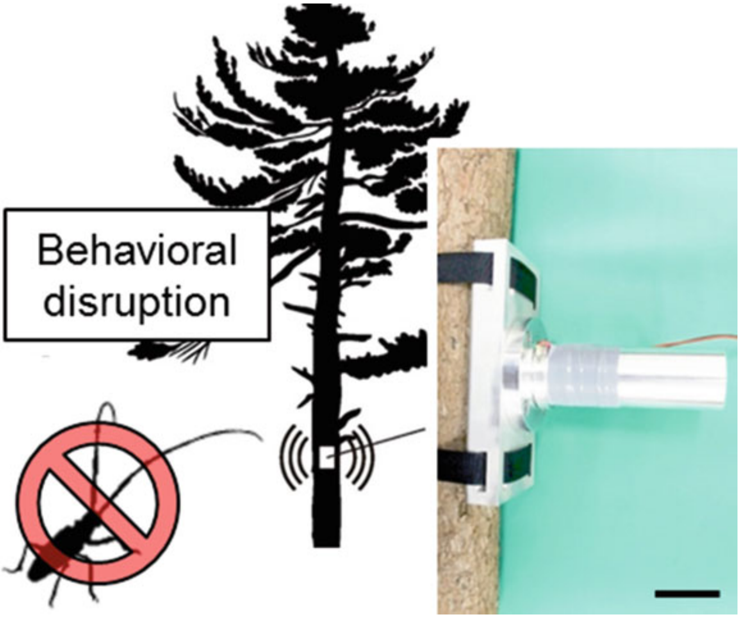


Fig. 20.8 A schematic of the vibrational pest control procedure. A vibration exciter made with giant magnetostrictive materials is attached to a tree, and it generates vibrations to disrupt behaviors of insects. Scale bar = 50 mm. Adapted from a photo of Takanashi et al. (2019)

In this study, vibrations from a GMM vibration exciter disrupted feeding and walking in *M. diphysis*, because freezing results in the cessation of ongoing behavior. Vibrations also promoted startle and escape responses in *M. alternatus* and *M. diphysis*. Disruption of residence in *M. alternatus* was demonstrated by using an exciter attached to a tree in the field (H. Sakamoto, T. Koike, N. Fukaya, T. Takanashi, in preparation). In addition, preliminary experiments have shown that the feeding of *M. alternatus* is disrupted by vibrations (T. Takanashi et al., unpublished data). The GMM vibration exciter appears to be a useful tool for vibrational management of longicorn beetle pests (Fig. 20.8). Low-frequency vibrations from the exciter are expected to be able to disrupt feeding, oviposition, and residence of longicorn beetles on host trees. It is also possible to use vibrations to enhance repellency by inducing the pests to escape from the host tree, thus causing disruption of residence.

Furthermore, a vibration exciter that uses GMM technology is able to generate vibration on various substrates (e.g., crops in greenhouses). We predict that vibrations can be applied in trees and crops damaged by various pests that exhibit vibration sensitivities. In the future, studies should explore and resolve installation of exciters and jigs for vibrational transmission, as well as ways to reduce the cost of the exciter to make it commercially practical. Vibrational pest management technologies that help to reduce the use of pesticides may become part of integrated pest management in the future (Polajnar et al. 2015; Takanashi et al. 2019). Potential negative side effects of the vibrations on plants and non-target beneficial insects must be minimized (Mitchell 1996; Polajnar et al. 2015; Nieri and Mazzoni 2018; Takanashi et al. 2019), and the most appropriate procedures need to be selected for various pests and plants. Vibrational pest management can promote integrated pest management by combining it with several existing and newly developed procedures, including physical technologies (Vincent et al. 2009; Shimoda and Honda 2013).

Acknowledgments We thank S. Fukui for providing invaluable samples of *Moechotypa diphysis*, Y. Matsui for kind advice on the GMM vibration exciter, and J. Mckenzie of the University of Canterbury for the assistance of TEM photography. This work was partly supported by MEXT/JSPS KAKENHI grants (nos. JP24120006, JP25120502 and JP18KT0042) and by Cabinet Office, Government of Japan, Cross-ministerial Strategic Innovation Promotion Program (SIP), "Technologies for creating next-generation agriculture, forestry and fisheries" (funding agency: Bio-oriented Technology Research Advancement Institution, NARO).

References

Ai H, Yoshida A, Yokohari F (2010) Vibration receptive sensilla on the wing margins of the silkworm moth *Bombyx mori*. J Insect Physiol 56:236–246

Boyan GS (1993) Another look at insect audition: the tympanic receptors as an evolutionary specializations of the chordotonal system. J Insect Physiol 39:187–200

Bräunig P (1982) Strand receptors with central cell bodies in the proximal leg joints of orthopterous insects. Cell Tissue Res 222:647–654

Breidbach O (1986) Studies on the stridulation of *Hylotrupes bajulus* (L.) (Cerambycidae, Coleoptera): Communication through support vibration--morphology and mechanics of the signal. Behav Process 12:169–186

Büschges A (1994) The physiology of sensory cells in the ventral scoloparium of the stick insect femoral chordotonal organ. J Exp Biol 189:285–292

Cocroft RB, Rodríguez R (2005) The behavioral ecology of insect vibrational communication. Bioscience 55:323–334

Endo J, Takanashi T, Mukai H, Numata H (2019) Egg-cracking vibration as a cue for stink bug siblings to synchronize hatching. Curr Biol 29:143–148

Eriksson A, Anfora G, Lucchi A, Lanzo F, Virant-Doberlet M, Mazzoni V (2012) Exploitation of insect vibrational signals reveals a new method of pest management. PLoS One 7:e32954

Field LH, Burrows M (1982) Reflex effects of the femoral chordotonal organ upon leg motor neurones of the locust. J Exp Biol 101:265–285

Field LH, Matheson T (1998) Chordotonal organs of insects. Adv Insect Physiol 27:1–228

Field LH, Pflüger HJ (1989) The femoral chordotonal organ: a bifunctional orthopteran (*Locusta migratoria*) sense organ? Comp Biochem Physiol 93A:729–743

Forestry Agency (2018) Shinrin ringyou toukei youran 2018 (in Japanese). Forestry Agency, Tokyo

Friedel T (1999) The vibrational startle response of the desert locust *Schistocerca gregaria*. J Exp Biol 202:2151–2159

Furukawa H, Nobuchi A (1996) Handbook of pathogenic fungi and pest insects in cultivated mushrooms (in Japanese). Zenrinkyou, Tokyo

Goodwyn PP, Katsumata-Wada A, Okada K (2009) Morphology and neurophysiology of tarsal vibration receptors in the water strider *Aquarius paludum* (Heteroptera: Gerridae). J Insect Physiol 55:855–861

Greenfield MD (2002) Signalers and receivers. Oxford University Press, New York

Hill PSM (2008) Vibrational communication in animals. Harvard University Press, Cambridge, MA

Hofmann T, Koch UT, Bässler U (1985) Physiology of the femoral chordotonal organ in the stick insect, *Cuniculina impigra*. J Exp Biol 114:207–223

Hofstetter RW, Aflitto N, Bedoya CL, Yturralde K, Dunn DD (2019) Vibrational behavior in bark beetles–applied aspects. In: Hill PSM, Lakes-Harlan R, Mazzoni V, Narins P, Virant-Doberlet-M, Wessels A (eds) Biotremology: studying vibrational behavior. Springer, Berlin, Heidelberg, pp 415–435

Hosomi A (1996) Effect of vibration to the infestation of *Apriona japonica* (Thomson) (Coleoptera: Cerambycidae) adults on the fig. In: Proceedings of Japan Informal Group Meeting on Human Response to Vibration held at The Hokkaido Safety and Health Service, 25–34

Kikuchi T, Cotton JA, Dalzell JJ, Hasegawa K, Kanzaki N, McVeigh P, Takanashi T, Tsai IJ, Assefa SA, Cook PJA, Otto T, Hunt M, Reid A, Sanchez-Flores A, Tsuchihara K, Yokoi T, Larsson MC, Miwa J, Maule AG, Sahashi N, Jones JT, Berriman M (2011) Genomic insights into the oof parasitism in the emerging plant pathogen *Bursaphelenchus xylophilu*s. PLoS Pathog 7:e1002219

Kishi M, Takanashi T (2019a) Tonic immobility and startle responses induced by substrate-borne vibrations in the sap beetle, *Phenolia (Lasiodites) picta* (Coleoptera: Nitidulidae). Jpn J Appl Entomol Zool 63:13–16. (In Japanese with English abstract)

Kishi M, Takanashi T (2019b) Escape behavior induced by substrate-borne vibrations in larvae of the sap beetle, *Phenolia (Lasiodites) picta* (Coleoptera: Nitidulidae). Jpn J Appl Entomol Zool 63:150–154. (In Japanese with English abstract)

Kobayashi F, Yamane A, Ikeda T (1984) The Japanese pine sawyer beetle as the vector of pine wilt disease. Annu Rev Entomol 29:115–135

Kojima W, Ishikawa Y, Takanashi T (2012a) Deceptive vibratory communication: pupae of a beetle exploit the freeze response of larvae to protect themselves. Biol Lett 8:717–720

Kojima W, Ishikawa Y, Takanashi T (2012b) Pupal vibratory signals of a group-living beetle that deter larvae: are they mimics of predator cue? Commun Integr Biol 5:262–264

Kojima W, Takanashi T, Ishikawa Y (2012c) Vibratory communication in the soil: pupal signals deter larval intrusion in a group-living beetle *Trypoxylus dichotoma*. Behav Ecol Sociobiol 66: 171–179

Kühne R (1982) Neurophysiology of the vibration sense in locusts and bushcrickets: response characteristics of single receptor units. J Insect Physiol 28:155–163

Laumann RA, Maccagnan DHB, Čokl A, Blassioli Moraes MC, Borges M (2018) Substrate borne vibrations disrupt the mating behaviors of the neotropical brown stink bug, *Euschistus heros*: implications for pest management. J Pest Sci 91:995–1004

Lujo S, Hartman E, Norton K, Pregmon E, Rohde B, Mankin RW (2016) Disrupting mating behavior of *Diaphorina citri* (Liviidae). J Econ Entomol 109:2373–2379

Matheson T (1990) Responses and locations of neurones in the locust metathoracic femoral chordotonal organ. J Comp Physiol A 166:915–927

Mazzoni V, Anfora G, Virant-Doberlet M (2013) Substrate vibrations during courtship in three *Drosophila* species. PLoS One 8:e80708

Mitchell CA (1996) Recent advances in plant response to mechanical stress: theory and application. HortScience 31:31–35

Mücke A, Lakes-Harlan R (1995) Central projections of sensory cells of the midleg of the locust, *Schistocereca gregaria*. Cell Tissue Res 280:391–400

Newland P (1991) Morphology and somatotopic organization of the central projections of afferents from tactile hairs on the hindleg of the locust. J Comp Neurol 312:493–508

Nieri R, Mazzoni V (2018) Open-field vibrational mating disruption: the effect on leafhopper pests and their predators. IOBC-WPRS Working Group, Integrated Protection in Viticulture 139:31–34

Nishino H (2003) Somatotopic mapping of chordotonal organ neurons in a primitive ensiferan, the New Zealand tree weta *Hemidenia femorata*: I. Femoral chordotonal organ. J Comp Neurol 464: 312–326

Nishino H, Field LH (2003) Somatotopic mapping of chordotonal organ neurons in a primitive ensiferan, the New Zealand weta *Hemideina femorata*: II. Complex tibial organ. J Comp Neurol 464:327–342

Nishino H, Mukai H, Takanashi T (2016) Chordotonal organs in hemipteran insects: unique peripheral structures but conserved central organization revealed by comparative neuroanatomy. Cell Tissue Res 366:549–572

Pflüger HJ, Field LH (1999) A locust chordotonal organ coding for proprioceptive and acoustic stimuli. J Comp Physiol A 184:169–183

Pflüger HJ, Bräunig P, Hustert R (1981) Distribution and specific central projections of mechanoreceptors in the thorax and proximal leg joints of locusts. Cell Tissue Res 216:79–96

Pflüger HJ, Bräunig P, Hustert R (1988) The organization of mechanosensory neuropiles in locust thoracic ganglia. Philos Trans R Soc Lond B 321:1–26

Polajnar J, Eriksson A, Lucchi A, Anfora G, Virant-Doberlet M, Mazzoni V (2015) Manipulating behaviour with substrate-borne vibrations: potential for insect pest control. Pest Manag Sci 71: 15–23

Polajnar J, Eriksson A, Virant-Doberlet M, Mazzoni V (2016) Mating disruption of a grapevine pest using mechanical vibrations: from laboratory to the field. J Pest Sci 89:909–921

Sauer AE, Stein W (1999) Sensorimotor pathways processing vibratory signals from the femoral chordotonal organ of the stick insect. J Comp Physiol A 185:21–31

Schneider W (1950) Über den Erschütterungssinn von Käfern und Fliegen. Z vergl Physiol 32:287–302

Scott JL, Kawahara AY, Skevington JH, Yen S-H, Sami A, Smith ML, Yack JE (2010) The evolutionary origins of ritualized acoustic signals in caterpillars. Nat Commm 1:4

Shelton PMJ, Stephen RO, Scott JJA, Tindall AR (1992) The apodeme complex of the femoral chordotonal organ in the metathoracic leg of the locust *Schistocerca gregaria*. J Exp Biol 163: 345–358

Shimoda M, Honda K (2013) Insect reactions to light and its applications to pest management. Appl Entomol Zool 48:413–421

Söderberg O, Sozinov A, Lindroos VK (2004) Giant magnetostrictive materials. In: Buschow KHJ, Cahn RW, Flemings MC, Ilschner B, Kramer EJ, Mahajan S, Veyssière P (eds) Encyclopedia of materials: science and technology, 2nd edn. Elsevier, Amsterdam, pp 1–3

Stein W, Sauer AE (1999) Physiology of vibration-sensitive afferents in the femoral chordotonal organ of the stick insect. J Comp Physiol A 184:253–263

Stritih Peljhan N, Rühr PT, Buh B, Strauß J (2019) Low-frequency vibration transmission and mechanosensory detection in the legs of cave crickets. Comp Biochem Physiol A 233:89–96

Takanashi T, Fukaya M, Nakamuta K, Skals N, Nishino H (2016) Substrate vibrations mediate behavioral responses via femoral chordotonal organs in a cerambycid beetle. Zool Lett 2:18

Takanashi T, Uechi N, Tatsuta H (2019) Vibrations in hemipteran and coleopteran insects: behaviors and application in pest management. Appl Entomol Zool 54:21–29

Tsubaki R, Hosoda N, Kitajima H, Takanashi T (2014) Substrate-borne vibrations induce behavioral responses of a leaf-dwelling cerambycid *Paraglenea fortunei*. Zool Sci 31:789–794

Vincent C, Weintraub P, Hallman G (2009) Physical control of insect pests. In: Resh VH, Cardé RT (eds) Encyclopedia of insects, 2nd edn. Academic Press, Cambridge, MA, pp 794–798

Wang Q (2017) Cerambycidae of the world: biology and pest management. CRC Press, Boca Raton, FL

Yazaki K, Takanashi T, Kanzaki K, Komatsu M, Levia D, Kabeya D, Tobita H, Kitao M, Ishida A (2018) Pine wilt disease causes cavitation around the resin canals and irrecoverable xylem conduit dysfunction. J Exp Bot 69:589–602

Chapter 21
Subterranean Arthropod Biotremology: Ecological and Economic Contexts

Richard Mankin

Abstract Subterranean arthropods would be ideal candidates for biotremological studies except that soil is a heterogeneous mixture of porous and solid materials with poorly characterized mechanical properties, which makes such studies difficult in situ. However, increased awareness of the impacts of subterranean arthropod herbivory on above-ground biota and the rapid development of modern electronic sensors and computer systems have encouraged efforts to conduct subterranean arthropod biotremological studies in recent years. In addition, impetus to address economic damage caused by subterranean arthropod pests has prompted multiple studies on the intra- and interspecific vibrational communication and incidental movement activities of subterranean pests. Many of these studies have been conducted on subterranean Cicadoidean, Ensiferan, Scarabaeoid, and Curculionid species that are a focus of this chapter. Studies on Lepidoptera, social insects, and Araneae also are discussed here in economic and ecological context, particularly with respect to multimodal communication.

21.1 Introduction

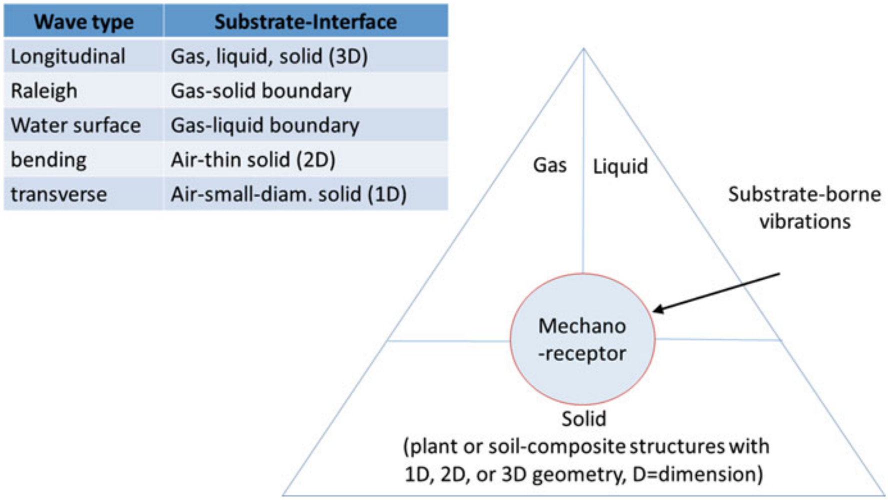
Subterranean arthropods, without opportunity to easily observe their surroundings, must rely on tactile, vibratory, or chemical senses (Funaro et al. 2018) for situational awareness, predator or conspecific detection, and communication. Above- and below-ground arthropods have a long history of eavesdropping on movements of predators or prey (Cocroft and Rodríguez 2005; Virant-Doberlet et al. 2019). However, subterranean arthropods are not often considered ideal candidates for biotremological studies.

Soil, considered one of the most complicated biomaterials on earth (Young and Crawford 2004), is a composite of porous (Sabatier et al. 1990; Lo et al. 2007) and

---

R. Mankin (✉)
Center for Medical, Agricultural, and Veterinary Entomology, Agriculture Research Service, US Department of Agriculture, Gainesville, FL, USA
e-mail: Richard.Mankin@usda.gov

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_21



Wave type	Substrate-Interface
Longitudinal	Gas, liquid, solid (3D)
Raleigh	Gas-solid boundary
Water surface	Gas-liquid boundary
bending	Air-thin solid (2D)
transverse	Air-small-diam. solid (1D)

Fig. 21.1 Effects of substrate structure and geometry on the types of substrate-borne vibrational waves to which insect mechanoreceptors are exposed during biotremological communication. See Mortimer (2017) for a review of wave speeds, distortion, and attenuation of different wave types

solid (TenCate and Remillieux 2019) materials, which complicates attempts to specify the structural and mechanical characteristics that govern signal transmission within and between the different composites (Fig. 21.1). Each type of wave in Fig. 21.1 has different wave speeds and different patterns of damping at different vibration frequencies, all of which are affected by the geometry of the structure through which the waves are passing (Michelsen 2014; Mortimer 2017; TenCate and Remillieux 2019). In addition, it is difficult to precisely measure important abiotic factors such as gas exchange and temperature that affect insect behavior and survival in soil (Villani and Wright 1990; Hagedorn et al. 2019), partly because soil macrofauna actively create channels, pores, and aggregates, and transform soil nutrients in ways that directly modify the chemical, structural, and acoustic characteristics of the soil in which they live (Brussaard et al. 1997; Veen et al. 2019). Consequently, current technologies are barely in the initial stages of developing more than a fleeting awareness of the diverse environments sustaining the subterranean biota (Mammola et al. 2019). The difficulty of precisely investigating subterranean biotremology has restricted the capability and opportunity to conduct such studies. The first subterranean insect was not even reported until 1831 (Polak 2005). The arthropod biodiversity of underground environments remains largely unexplored (Ficetola et al. 2018; Rillig et al. 2019).

For these reasons, when investigations are conducted on *Scaptocoris* burrower bugs (Čokl et al. 2006a) or *Melolontha* beetle larvae (Görres and Chesmore 2019), they typically must deal with heterogeneous substrate–mechanosensor interfaces (Akent’eva 2012; Eilers et al. 2012) that are less understood than, e.g., the interface between a stink bug subgenual organ and a plant stem (Čokl et al. 2006b; Prešern et al. 2018). Investigators must deal with information transfer processes (Michelsen 2014; Hill and Wessel 2016; Mortimer 2017) that are less well defined than processes characterized above ground, such as the transmission of information between duetting *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) in grapevines (Gordon et al. 2019).

Nevertheless, there are fundamentally important reasons to study communication and the incidental movement and feeding vibrations of arthropods in soil, given the ecological importance of subterranean herbivory (Hunter 2001; Lavelle et al. 2006; van Dam 2009; Adhikari and Hartemink 2016; Veen et al. 2019) and the evolutionary processes (Camacho et al. 1992) leading to a high diversity of known subterranean arthropod lineages (Mammola et al. 2019). Subterranean arthropods interact with the environment over a much smaller spatial scale than above-ground arthropods (Veen et al. 2019), and subterranean species sometimes are restricted to small areas such as isolated mountain valleys (van Tol et al. 2004). Population growth often is small because energy-limited and stable environments have selected for long-lived species with low metabolism and fecundity (Mammola et al. 2019). Consequently, needs for conservation efforts to protect endemic subterranean arthropods are reported often (Harvey et al. 2011), especially in view of recently reported trends of reduced biomass of flying insects in protected areas (Hallmann et al. 2017). There also are highly practical reasons to address economic damage caused by root-feeding pests (Jackson et al. 2000; Keller and Zimmermann 2006; Johnson and Murray 2008) and invasive species that directly cause damage or vector plant diseases (Fei et al. 2019).

Several examples of insect vibrational signals and eavesdropping cues in soil are considered in ecological and agro-economic contexts in this chapter. As in above-ground arthropods, stridulation (Görres and Chesmore 2019), scraping, drumming, and tapping (Hayashi et al. 2018), tremulation (Ohmura et al. 2009), and other vibratory behaviors (Funaro et al. 2018) are known to be widely employed for communication by subterranean arthropods.

21.2 Cicadoidean and Ensiferan Communication and Eavesdropping Cues

Considerable knowledge about above-ground biotremological processes has been obtained from studies in the Cicadoidea and Ensifera, where tymbals (Wessel et al. 2014) and the first stridulatory organs (Senter 2008; Strauß and Stumpner 2015) likely evolved. Tymbals are exoskeletal organs with resonant cavities and plate structures that buckle and vibrate when pulled by muscles (Wessel et al. 2014). Stridulatory organs consist of a plectrum (with a sharp large ridge) and a pars stridens (with finer parallel ribs) that move past each other while in direct contact (Senter 2008). The stridulations frequently occur as pairs when the plectrum protracts over the pars stridens, pauses, and then retracts to its original position (Mankin et al. 2009a; Görres and Chesmore 2019).

Ensiferan species are useful models for studying vibrations but more commonly are used for studies of airborne sound communication (Prešern et al. 2018). There has long been awareness of the mating calls of crickets, mole crickets, and katydids (bush crickets), several species of which produce signals with both vibrational and acoustic components (Morris et al. 1994; Gogala et al. 1996). Cicadas produce both vibrational and acoustic components during their communications, as well (Stölting et al. 2002). Bennet-Clark's (1970) investigation of the mechanism of mole cricket sound production commented that males of several mole cricket spp. had been known for centuries to produce loud mating calls that attracted females to their burrows (Howard et al. 2011; Pollack 2017). Thus, it is not surprising that two South American, *Neoscapteriscus* (Orthoptera: Gryllotalpidae) mole cricket species with loud mating calls, inadvertently introduced to Florida in the late 1800s, were among the first insects to be targeted with acoustic trapping technology after their damage to the root systems of turfgrass began causing noticeable economic damage (Ulagaraj and Walker 1973, 1975; Walker 1988). In this case, the hornlike burrows themselves were not duplicated, but loud synthetic or recorded mating call stridulations were broadcast to attract females to a swimming pool trap (Walker 1982).

Bennet-Clark (1970) reported also that some mole cricket species were very sensitive to ground vibrations; however, Hill and Shadley (1997) were first to suggest a bimodal communication mechanism in mole crickets where the airborne component of stridulation was a sexual advertisement call directed toward flying females, and the substrate-borne component influenced spacing of male burrows. Later, Hill and Shadley (2001) and Hill (2009) reported that *Gryllotalpa major* Saussure mole cricket males responded to calls played back alone through a vibration exciter without the accompanying acoustic signal. This suggests that some species of mole cricket can communicate through both acoustic and vibrational signals, as is frequently observed also in hemipterans, e.g., Schilman et al. (2001). Acoustic trapping of *Neoscapteriscus* remains an effective monitoring tool (Dillman et al. 2014; Rohde et al. 2019) but the potential for mole cricket management (Mankin and Lapointe 2003) using vibrations has not yet been investigated extensively.

21.3 Scarabaeoid Stridulatory Communication and Eavesdropping Cues

Stridulation has been reported from adults, larvae, and pupae of numerous species of soil-dwelling Scarabaeoids (Wessel 2006; Kojima et al. 2012; Barbero et al. 2012), including economically important dynastids (Bedford 1980), the near-threatened *Lucanus cervus* (L.) (Coleoptera: Lucanidae) (Harvey et al. 2011), and white grubs (Merchant et al. 2004; Wagenhoff et al. 2014) several species of which live in grassland systems where more than 70% of the invertebrate biomass is estimated to occur below ground (Seastedt and Murray 2007). Pupal vibrations are produced by rotating the abdomen and beating the pronotum against the pupal cell wall (Kojima et al. 2012). In many cases, the vibrations are powerful enough to produce audible signals (Takanashi et al. 2019) and are examples of multimodal signaling similar to those observed with mole crickets (see Sect. 21.2). In adults, stridulations often are associated with defensive (Palestrini et al. 1990) or mating behavior (Harvey et al. 2011), and in larvae and pupae, stridulations, and other vibrational cues usually are associated with defensive behaviors (Kojima et al. 2012) or avoidance of predators (Hill 2009) or cannibalism (Kočárek 2009), which occurs in *Melolontha* (Görres and Chesmore 2019). Alexander et al. (1963) described general aspects of evolutionary differentiation of stridulatory signals in Coleoptera.

It has been proposed that stridulations characterized in particular species can be monitored in field environments to acoustically identify the species presence for conservation or pest management purposes (Mankin et al. 2009a; Harvey et al. 2011; Görres and Chesmore 2019). In the absence of a stridulatory organ, subterranean insect larvae may also produce relatively stereotyped abdominal, proleg, and mandibular movements (Villani et al. 1999), which produce vibrational pulse trains (bursts) (Mankin et al. 2009b) that are useful as a means of distinguishing vibrations produced by hidden insects from background noise (Mankin et al. 2011; Jalinas et al. 2019). The types of vibrations produced depend on the friction of the interface between the insect and substrate surface. Interfaces with two dry surfaces, such as an insect integument sliding over dry soil with small asperities produce short, broadband vibration pulses similar to those produced by human footsteps on a dry surface (Ekimov and Sabatier 2006). Slip-stick friction between two wet surfaces, like wet skin on glass or brakes on a wet road (Patitsas 2010), can produce squeal vibrations with loud harmonics. Knowledge of these interfacial interactions has been used previously to help identify the species of insect that had produced the vibrations (Jalinas et al. 2019).

Other examples where the signal characteristics of incidental movements of scarabaeoids have been used to distinguish vibrations of underground larvae or adults from background noise include Mankin et al. (2000, 2007, 2009a, b), Brandhorst-Hubbard et al. (2001), Zhang et al. (2003a, b), Johnson et al. (2007), and Mankin and Moore (2010). Various instruments, including microphones, accelerometers, geophones, and piezoelectric probes, have been employed for detection (Mankin et al. 2008, 2011), as well as laser vibrometers (Zorović and Čokl 2015). A recently developed vibroacoustic device for detection of insects in trees (Potamitis et al. 2019) also provides an inexpensive method to detect subterranean arthropod vibrations, and it can be expected that even more sensitive, less expensive instrumentation will be commercialized during the next decade (e.g., Bayrakdar 2019; Rillig et al. 2019). Improved signal processing analyses have enabled better discrimination of insect-produced vibrations from background noise and nontarget arthropods (Ganchev et al. 2007; Mankin et al. 2011, 2018; Lampson et al. 2013; Korinšek et al. 2016; Hetzroni et al. 2016; Rathore et al. 2019) but there is considerable opportunity for improvements in instruments and computer analyses that automate the detection and analysis of arthropod vibrations and distinguish them from background noise. In many crops, because subterranean insect pests often occur in aggregations (Mankin et al. 2007; Inyang et al. 2019), timely use of such instrumentation can enable effective detection and targeting of infestations before above-ground damage is detected.

21.4 Curculionid and Silphid Stridulatory Communication and Eavesdropping Cues

Stridulatory organs and stridulatory behavior have been reported in adults of numerous curculionids (e.g., Mampe and Neunzig 1966; Gibson 1967; Wilson et al. 1993; Rudinsky 2009; Pureswaran et al. 2016) and silphid species (Hall et al. 2013). Curculionid stridulations may be produced by either or both sexes, depending on the species, and can be involved in mating or defense (Webb et al. 1980). Many curculionids that exhibit stridulatory behaviors are important both ecologically and economically, particularly in forests (Marini et al. 2017), and some spend part of their life cycle in the soil, including *Conotrachelus nenuphar* (Herbst), which pupates underground, mates on the ground underneath host trees, and hibernates under the orchard litter (Lafleur et al. 1987). Soil-dwelling curculionids that do not stridulate can be detected by their incidental movement and feeding sounds, including *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae), which has been the subject of tests to detect larvae in container crops (Mankin and Fisher 2002, 2007). *Otiorhynchus sulcatus* has a broad host range and reproduces by parthenogenesis (van Tol et al. 2004); consequently, a single, unmated female can rapidly infest a variety of different plants in a nursery and the larvae can cause unseen damage to the root systems. Another economically important curculionid that has not been reported to stridulate is *Diaprepes abbreviatus* (L.). Acoustic methods have been developed to detect *D. abbreviatus* larvae feeding in roots of citrus trees and distinguish their movement and feeding activity from background noise (Mankin et al. 2001; Mankin and Lapointe 2003).

As in the Ensifera (see Sect. 21.2), the communication signals of Nicrophorine burying beetles in the Silphidae have been documented for centuries (Landois 1869; Darwin 1871). Mating stridulations of the eight North American Nicrophorine species have been characterized (Hall et al. 2013) and were found to have differences that could be used over short distances to discriminate among species, but likely are involved also in competition for resources. The genus *Nicrophorus* is unusual in that both males and females take care of the brood (Smiseth and Parker 2008).

21.5 Vibrational Communication and Eavesdropping Cues in Other Arthropod Orders

Lepidopteran adults, larvae, and pupae (Chapman 1998), some of which spend a portion of their life cycles underground, have been reported to stridulate in different contexts. Stridulations of myrmecophilous butterfly larvae (Barbero et al. 2009, 2012; Casacci et al. 2019) in *Myrmica* ant nests mimic host ant stridulations (Spangler 1967) and their cuticular hydrocarbons also mimic those of their hosts, an example of multimodal mimicry. Similar mimicry has been reported by the myrmecophilous butterfly, *Spindasis lohita* (Fruhstorfer) (Lepidoptera: Lycaenidae) in *Crematogaster rogenhoferi* Mayr (Hymenoptera: Formicidae) nests (Lin et al. 2019).

The function of ant stridulations is not well understood in general (see Golden and Hill 2016), but stridulations often are produced by ants under stress and attract workers into aggregations that can provide assistance or produce nest-wide activity (Rauth and Vinson 2006; Chiu et al. 2011; Barbero et al. 2012). Ant stridulations and termite headbanging (Howse 1965; Evans et al. 2005; Inta et al. 2009; Hager and Kirchner 2013) may play somewhat similar functional roles, and they both have been observed together, e.g., when a colony of subterranean termites, *Drepanotermes* sp., was attacked by *Camponotus denticulatus* Kirby ants (Mankin and Benshemesh 2006). Termites have been shown to assess incidental vibrations produced in wood to estimate the size of a termite colony and they use resonant frequencies to assess the volume of food to which they have access (Evans et al. 2005).

Ultrasound receptor organs have evolved in many lepidopteran species in response to predation by insectivorous bats (Connor 1999) and, given the opportunity of a new communication channel, several species have employed ultrasonic signals for courtship (Spangler et al. 1984). However, because ultrasound attenuates more rapidly in soil than in air (Mankin et al. 2011), ultrasonic communication in soil is possible usually only over distances of a few centimeters; although, ultrasonic vibrations produced by termites in wood can be detected up to 2.2 m (Scheffrahn et al. 1993). Non-ultrasonic, 2000–10,000 Hz, vibrations produced by individual *Vitacea polistiformis* Harris (Lepidoptera: Sesiidae) larvae feeding on grapevine roots (Sanders et al. 2011; Inyang et al. 2019) can be detected with vibration sensors in soil over distances of about 20 cm, similar to detection distances observed in sand (Devetak 2014). Large colonies of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) in soil underneath trees can be detected with non-ultrasonic sensors over distances of 50 cm or more (Mankin et al. 2002), and can be detected over several meter distances within large hardwood trees (Osbrink and Cornelius 2013). Also, colonies of termites can extend the communication distance of headbanging in soil by responding to detected headbanging with their own signals, serving as relays to extend an alarm several meters across a large-sized colony (Hager and Kirchner 2013). Active spaces of vibrational signals, i.e., their effective detection ranges

(Mazzoni et al. 2014), are discussed by Virant-Doberlet et al. (2019) for a variety of different above-ground insects.

Subterranean arthropods are known to use vibrational cues to catch prey, including several species of antlion larva (Neuroptera: Myrmeleontidae), who wait for ants and other prey in the sand at the bottom of a funnel-shaped pit (Devetak 2014; Podlesnik et al. 2019) and grasp what falls in, or toss sand at the prey to cause a landslide that brings it within grasping distance. Araneae, some of which spin webs and identify prey by their vibrations (Parry 1965), or become prey when they seek out a predatory reduviid (Wignall and Taylor 2011), are studied above ground primarily, but many also live below ground (Lăska et al. 2011; Abrams et al. 2019) and, along with Collembola, Diptera, and Oniscidea, are among the most numerous taxa reported in soil (Lăska et al. 2011; Inyang et al. 2019). Many underground spiders stridulate during courtship (Quirici and Costa 2005) or tremulate (Carballo et al. 2017), in addition to using vibrations for prey capture.

21.6 Biotremology in Multimodal Communication

The examples (see Sects. 21.2–21.5) of vibrational communication by subterranean arthropods that also involved acoustic (mole crickets: Hill and Shadley 1997, 2001, Hill 2009; Hemiptera and Coleoptera: Takanashi et al. 2019) or chemosensory communication (butterfly larvae: Barbero et al. 2012; Casacci et al. 2019) are from several of numerous reports where multimodal communication has been identified in arthropods. Various driving forces have been proposed to explain the evolution of multimodal insect communication, including variation in localization and ranging of signal sources, “multiple message” redundancy that serves as a backup signal (Halfwerk et al. 2019), and context-dependent shifts in signal structure when one of the communication modes becomes dangerous, costly, or ineffective (Higham and Hebets 2013; Caldwell 2014). One commonality between acoustic and vibratory modes of communication is that the vibration sensors used for detection of airborne sound have evolved similarly to those used for detection of vibrations (Strauß and Stumpner 2015). It is thus not a surprise that pseudophylline katydids in neotropical rain forests have been reported to combine ultrasonic signalling in calling songs with vibrational tremulation signals, possibly to avoid bats; although, spider predation increases as a result (Morris et al. 1994). Multimodal use of pheromone and acoustic cues in pine beetles (Pureswaran et al. 2016) may be involved in species isolation between *Dendroctonus brevicomis* LeConte and *D. frontalis* Zimmerman. The extent to which multimodal signals provide additional range, redundancy, species isolation, or safety to subterranean insects has not been investigated widely, but spiders that detect insect movement and stridulation vibrations are commonly found near where subterranean insects are present (Sanders et al. 2011; Inyang et al. 2019). More often, such studies are conducted with above-ground insects. Rajamaran et al. (2018), for example, reported that female *Onomarchus uninotatus* (Serville) katydids tremulate when calling males are less than 4 m away, but perform phonotaxis when males are further than 9 m. Gordon and Uetz (2011) reported that male *Schizocosa ocreata* (Hentz) wolf spiders, which use visual and seismic signals in courtship, could use either or both modalities depending on environmental conditions.

One of the environmental conditions of interest is temperature. The effects of temperature on acoustic/vibrational/visual multimodal signals have been a topic of interest in studies of ectotherm behavior. It has been reported that temperature changes affect the timing of mating in arthropods that employ multimodal signalling (Brandt et al. 2018) but have little effect on species recognition (Greenfield and Medlock 2007).

The role of learning in vibrational and multimodal communication is largely neglected in subterranean arthropod biotremology; although, it is becoming an area of considerable research interest in studies of above-ground arthropod courtship. Several different insects and spiders have been reported to learn from acoustic, vibratory, or multimodal signals in different contexts (Dion et al. 2019). De Simone et al. (2019) reported that male *Allocosa senex* (Mello-Leitão) (Araneae: Lycosidae) wolf spiders that use multimodal chemical and vibratory communication enlarged their burrows after rejection by females. Also, burrows were shorter when constructed in fine sand, where digging is more difficult than in coarse sand, which suggests they may have learned to adapt their digging behavior to maximize reproductive success while minimizing digging effort.

21.7 Conclusions

This chapter considers twenty-first-century biotremological and related research on subterranean arthropods in relation to above-ground arthropods in ecological and economic contexts. As has been found above ground, stridulatory, scraping, drumming, tapping, and tremulation behaviors are frequently employed for courtship and defense. Vibrational eavesdropping on conspecifics, predators, and prey is widely used to enhance survival. In spider burrows, ant colonies, and termite colonies, vibrational and chemical senses frequently serve as a replacement for vision, even though the high attenuation of vibrations in soil reduces the range of detection to a few decimeters. Vibration detection methods have been developed over the last 30 years that enable the sounds of subterranean pests to be detected and discriminated from background noise, and further improvements are expected.

Present-day vibration detection instrumentation has been applied with economic benefits for early detection of crop damage by subterranean insect pests that are rarely detected in time for effective control measures using above-ground surveillance. In addition, acoustic targeting of subterranean pest aggregations enables reduction of pesticide usage in crops and forages. There is increasing concern about subterranean larvae that can injure plant root systems and predispose them to infection, as has occurred with the curculionid, *D. abbreviatus*, predisposing citrus to damage from *Phytophthora* spp. (Graham et al. 2003). Other plant diseases such as Fusarium wilt tropical race 4 may soon devastate the South American banana industry (Galvis 2019). It is not yet well investigated whether Fusarium wilt or Port Oxford Cedar root disease in North America (Fei et al. 2019) is significantly exacerbated by subterranean insects.

There also is room for expanded usage of vibration detection instrumentation in conservation research, especially because many subterranean arthropod species have limited geographic ranges. Recent alarming decreases of flying insects in protected areas (Hallmann et al. 2017) suggest that subterranean arthropod biodiversity may be in decline as well, but confirmatory studies remain to be conducted (Mammola et al. 2019). Finally, there are some areas such as the role of learning and the role of multimodal communication in subterranean arthropod courtship that has been severely neglected but beg for further consideration. Biotremological research on subterranean arthropods is likely to expand in the future as its ecological and economic relevance become more prominent and the sustainability of agriculture and forestry are threatened by increased rates of expansion of human populations and concomitant reductions in the quality and availability of water and other resources.

References

Abrams KM, Huey JA, Hillyer MJ, Humphreys WF, Didham RK, Harvey MS (2019) Too hot to handle: cenozoic aridification drives multiple independent incursions of Schizomida (Hubbardiidae) into hypogean environments. Mol Phylogenet Evol 139. https://doi.org/10.1016/j.ympev.2019.106532

Adhikari K, Hartemink AE (2016) Linking soils to ecosystem services—a global review. Geoderma 262:101–111

Akent'eva NA (2012) Multimodal sensory organs in larvae of some insect species. Entomol Rev 92: 379–389

Alexander RD, Moore TE, Woodruff RE (1963) The evolutionary differentiation of stridulatory signals in beetles (Insecta: Coleoptera). Anim Behav 11:111–115

Barbero F, Thomas JA, Bonelli S, Balletto E, Schönrogge K (2009) Queen ants make distinctive sounds that are mimicked by a butterfly social parasite. Science 323:782–785

Barbero F, Patricelli WM, Balletto E, Casacci LP, Sala M, Bonelli S (2012) *Myrmica* ants and their butterfly parasites with special focus on the acoustic communication. Psyche. https://doi.org/10.1155/2012/725237

Bayrakdar ME (2019) A smart insect pest detection technique with qualified underground wireless sensor nodes for precision agriculture. IEEE Sens J 19:10892–10897

Bedford GO (1980) Biology, ecology, and control of palm rhinoceros beetles. Annu Rev Entomol 25:309–339

Bennet-Clark HC (1970) The mechanism and efficiency of sound production in mole crickets. J Exp Biol 52:619–652

Brandhorst-Hubbard JL, Flanders KL, Mankin RW, Guertal EA, Crocker RL (2001) Mapping of soil insect infestations sampled by excavation and acoustic methods. J Econ Entomol 94:1452–1458

Brandt EE, Kelley JP, Elias DO (2018) Temperature alters multimodal signaling and mating success in an ectotherm. Behav Ecol Sociobiol 72:191

Brussaard L, Behan-Pelletier VM, Bignell DE, Brown VK, Didden W, Folgarait P, Fragoso C, Freckman DW, Gupta VVSR, Hattori T, Hawksworth DL, Klopatek C, Lavelle P, Malloch DW, Rusek J, Soderstrom B, Tiedje JM, Virginia RA (1997) Biodiversity and ecosystem functioning in soil. Ambio 26:563–570

Caldwell MS (2014) Interactions between airborne sound and substrate vibration in animal communication. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 65–92

Camacho AI, Bello E, Becerra JM, Vaticon I (1992) A natural history of the subterranean environment and its associated fauna. In: Camacho AI (ed) The natural history of biospeleology. Consejo Superior de Investigaciones Cientificas, Madrid, pp 57–95

Carballo M, Baldenegro F, Bollatti F, Peretti AV, Aisenberg A (2017) No pain, no gain: male plasticity in burrow digging according to female rejection in a sand-dwelling wolf spider. Behav Process 140:174–180

Casacci LP, Bonelli S, Balletto E, Barbero F (2019) Multimodal signaling in myrmecophilous butterflies. Front Ecol Evol 7:454

Chapman RF (1998) The insects: structure and function, 4th edn. Cambridge University Press, Cambridge

Chiu Y-K, Mankin RW, Lin C-C (2011) Context-dependent stridulatory responses of *Leptogenys kitteli* (Hymenoptera: Formicidae) to social, prey, and disturbance stimuli. Ann Entomol Soc Am 104:1012–1020

Cocroft RB, Rodríguez RL (2005) The behavioral ecology of insect vibrational communication. BioScience 55:323–334

Čokl A, Nardi C, Mauricio J, Bento S, Hirose E, Panizzi AR (2006a) Transmission of stridulatory signals of the burrower bugs, *Scaptocoris castanea* and *Scaptocoris carvalhoi* (Heteroptera: Cydnidae) through the soil and soybean. Physiol Entomol 31:371–381

Čokl A, Virant-Doberlet M, Zorović M (2006b) Sense organs involved in the vibratory communication of bugs. In: Drosopoulos S, Claridge MF (eds) Insect sounds and communication. CRC, New York, pp 45–52

Connor WE (1999) ‘Un chant d’appel amoureux’: acoustic communication in moths. J Exp Biol 202:1711–1723

Darwin C (1871) the descent of man, and selection in relation to sex. Adamant Media, London

De Simone GA, Bollatti F, Aisenberg A, Peretti AV (2019) Previous experience and substrate matter: plasticity in burrow digging in a South American wolf spider. Acta Oecologica 100: 103465

Devetak D (2014) Sand-borne vibrations in prey detection and orientation of antlions. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 319–330

Dillman AR, Cronin CJ, Tang J, Gray DA, Sternberg PW (2014) A modified mole cricket lure and description of *Scapteriscus borellii* (Orthoptera: Gryllotalpidae) range expansion and calling song in California. Environ Entomol 43:146–156

Dion E, Monteiro A, Nieberding CM (2019) The role of learning on insect and spider sexual behaviors, sexual trait evolution, and speciation. Front Ecol Evol. https://doi.org/10.3389/fevo.2018.00225

Eilers EJ, Talarico G, Hansson BS, Hilker M, Reinecke A (2012) Sensing the underground-ultrastructure and function of sensory organs in root-feeding *Melolontha melolontha* (Coleoptera: Scarabaeinae) larvae. PLoS One 7:e041357

Ekimov A, Sabatier JM (2006) Vibration and sound signatures of human footsteps in buildings. J Acoust Soc Am 120:762–768

Evans TA, Lai JC, Toledano E, McDowall L, Rakotonarivo S, Lenz M (2005) Proc Natl Acad Sci USA 102:3732–3737

Fei S, Morin RS, Oswalt CM, Liebhold AM (2019) Biomass losses resulting from insect and disease invasions in US forests. Proc Natl Acad Sci USA 116. https://doi.org/10.1073/pnas.182060116

Ficetola GF, Canedoli C, Stoch F (2018) The Racovitzan impediment and the hidden biodiversity of unexplored environments. Conserv Biol 33:214–216

Funaro CF, Böröczky K, Vargo EL, Schal C (2018) Identification of a queen and king recognition pheromone in the subterranean termite *Reticulitermes flavipes*. Proc Natl Acad Sci USA 115: 3888–3893

Galvis S (2019) Colombia confirms that dreaded fungus has hit its banana plantations. Science. https://doi.org/10.1126/science.aaz1033

Ganchev T, Potamitis I, Fakotakis N (2007) Acoustic monitoring of singing insects. In: 2007 IEEE international conference on acoustics, speech and signal processing, Honolulu, HI, pp IV-721–IV-724. https://doi.org/10.1109/ICASSP.2007.367014

Gibson LP (1967) Stridulatory mechanisms and sound production in *Conotrachelus* (Coleoptera: Curculionidae). Ann Entomol Soc Am 60:43–54

Gogala M, Popov AV, Ribarič D (1996) Bioacoustics of singing cicadas of the Western Palaearctic: *Cicadetta tibialis* (Panzer) (Cicadoidea: Tibicinidae). Acta Entomol Sloven 4:45–62

Golden TMJ, Hill PSM (2016) The evolution of stridulatory communication in ants, revisited. Insect Soc 63(2):309–319

Gordon SD, Uetz GW (2011) Multimodal communication of wolf spiders on different substrates: evidence for behavioural plasticity. Anim Behav 81:367–375

Gordon SD, Tiller B, Windmill JFC, Krugner R, Narins PM (2019) Transmission of the frequency components of the vibrational signal of the glassy-winged sharpshooter *Homalodisca vitripennis*, within and between grapevines. J Comp Physiol A 205(5):783–791

Görres C-M, Chesmore D (2019) Active sound production of scarab beetle larvae opens up new possibilities for species-specific pest monitoring in soils. Sci Rep 9:10115

Graham JH, Bright DB, McCoy CW (2003) *Phytophthora-Diaprepes* Weevil complex: *Phytophthora* spp. relationship with citrus rootstock. Plant Dis 87:85–90

Greenfield MD, Medlock C (2007) Temperature coupling as an emergent property: parallel thermal effects on male song and female response do not contribute to species recognition in an acoustic moth. Evolution 61:1590

Hagedorn F, Gavazov K, Alexander JM (2019) Above- and belowground linkages shape responses of mountain vegetation to climate change. Science 365(6458):1119–1123

Hager FA, Kirchner WH (2013) Vibrational long-distance communication in the termites *Macrotermes natalensis* and *Odontotermes* sp. J Exp Biol 216:3249–3256

Halfwerk W, Varkevisser J, Simon R, Mendoza E, Scharff C, Riebel K (2019) Toward testing for multimodal perception of mating signals. Front Ecol Evol. https://doi.org/10.3389/fevo.2019.00124

Hall CL, Mason AC, Howard DR, Padhi A, Smith RJ (2013) Description of acoustic characters and stridulatory pars stridens of *Nicrophorus* (Coleoptera: Silphidae): a comparison of eight North American species. Ann Entomol Soc Am 106:661–669

Hallmann CA, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, Stenmans W, Müller A, Sumser H, Hörren T, Goulson D, de Kroon H (2017) More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS One 12:e0185809

Harvey DJ, Hawes CJ, Gange AC, Finch P, Chesmore D, Farr I (2011) Development of non-invasive monitoring methods for larvae and adults of the stag beetle, *Lucanus cervus*. Insect Conserv Diver 4:4–14

Hayashi Y, Yoshimura J, Roff DA, Kumita T, Shimizu A (2018) Four types of vibration behaviors in a mole cricket. PLoS One 13:e0204628

Hetzroni A, Soroker V, Cohen Y (2016) Toward practical acoustic red palm weevil detection. Comput Electron Agric 124:100–106

Higham JP, Hebets EA (2013) An introduction to multimodal communication. Behav Ecol Sociobiol 67:1381–1388

Hill PSM (2009) How do animals use substrate-borne vibrations as an information source. Naturwissenschaften 96:1355–1371

Hill PSM, Shadley JR (1997) Substrate vibration as a component of a calling song. Naturwissenschaften 84:460–463

Hill PSM, Shadley JR (2001) Talking back: sending soil vibration signals to lekking prairie mole cricket males. Am Zool 41:1200–1214

Hill PSM, Wessel A (2016) PRIMER: biotremology. Curr Biol 26:R187–R191

Howard DR, Lee N, Hall CL, Mason AC (2011) Are centrally displaying males always the center of female attention? Acoustic display position and female choice in a lek mating subterranean insect. Ethology 117:119–207

Howse PE (1965) On the significance of certain oscillatory movements of termites. Insect Soc 12: 335–345

Hunter MD (2001) Out of sight, out of mind: the impacts of root-feeding insects in natural and managed systems. Agric For Entomol 3:3–9

Inta R, Evans TA, Lai JCS (2009) Effect of vibratory soldier alarm signals on the foraging behavior of subterranean termites. J Econ Entomol 102:121–126

Inyang EI, Hix RL, Tsolova V, Rohde BB, Dosunmu O, Mankin RW (2019) Subterranean acoustic activity patterns in *Vitacea polistiformis* (Lepidoptera: Sesiidae) in relation to abiotic and biotic factors. Insects 10:267. https://doi.org/10.3390/insects10090267

Jackson TA, Alves SB, Pereira RM (2000) Success in biological control of soil-dwelling insects by pathogens and nematodes. In: Gurr G, Wratten S (eds) Biological control: measures of success. Springer, Dordrecht, pp 271–296

Jalinas J, Güerri-Agulló B, Dosunmu OG, Haseeb M, Lopez-Llorca LV, Mankin RW (2019) Acoustic signal applications in detection and management of *Rhynchophorus* spp in fruit-crops and ornamental palms. Fla Entomol 102:475–479

Johnson SN, Murray PJ (2008) Root feeders: an ecosystem perspective. CABI, Cambridge, MA

Johnson SN, Crawford JW, Gregory PJ, Grinev DV, Mankin RW, Masters GJ, Murray PJ, Wall DH, Zhang X (2007) Noninvasive techniques for investigating and modelling root-feeding insects in managed and natural systems. Agric For Entomol 9:39–46

Keller S, Zimmermann G (2006) Scarabs as pests: a continuing problem. Coleopterists Soc Monogr 5:102–119

Kočárek P (2009) Sound production and chorusing behaviour in larvae of *Icosium tomentosum*. Cent Eur J Biol 4:422–426

Kojima W, Ishikawa Y, Takana T (2012) Deceptive vibratory communication: pupae of a beetle exploit the freeze response of larvae to protect themselves. Biol Lett 8:717–720

Korinšek G, Derlink M, Virant-Doberlet M, Tuma T (2016) An autonomous system of detecting and attracting leafhopper males using species- and sex-specific substrate borne vibrational signals. Comput Electron Agric 123:20–39

Lafleur G, Hill SB, Vincent C (1987) Fall migration, hibernation, site selection and associated winter mortality of plum curculio (Coleoptera: Curculionidae) in a Quebec apple orchard. J Econ Entomol 80:1152–1172

Lampson BD, Han YJ, Khalilian A, Greene J, Mankin RW, Foreman EG (2013) Automatic detection and identification of brown stink bug, *Euschistus servus*, and southern green stink bug, *Nezara viridula*, (Heteroptera: Pentatomidae) using intraspecific substrate-borne vibrational signals. Comput Electron Agric 91:154–159

Landois H (1869) Neue Beobachtungen tiber die Lauhiuberungen der Insekten. Verh Naturwiss ver Preufi Rheinl Wesif 26:67–70

Lăska V, Kopecký O, Růžička MJ, Véle A, Šarapatka B, Tuf IH (2011) Vertical distribution of spiders in soil. J Arachnol 39:393–398

Lavelle P, Decaens T, Aubert M, Barot S, Blouin M, Bureau F, Margerie P, Mora P, Rossi J-P (2006) Soil invertebrates and ecosystem services. Eur J Soil Biol 42:S3–S15

Lin Y-H, Liao Y-C, Yang C-CS, Billen J, Yang M-M, Hsu Y-F (2019) Vibrational communication between a myrmecophilous butterfly *Spindasis lohita* (Lepidoptera: Lycaenidae) and its host ant *Crematogaster rogenhoferi* (Hymenoptera: Formicidae). Sci Rep 9:18548

Lo W-C, Yeh C-L, Tsai C-T (2007) Effect of soil texture on the propagation and attenuation of acoustic wave at unsaturated conditions. J Hydrol 338:273–284

Mammola S, Cardoso P, Culver DC, Deharveng L, Ferreira RL, Fišer C, Galassi DMP, Griebler C, Halse S, Humphreys WF, Isaia M, Malard F, Martinez A, Moldovan OT, Niemiller ML, Pavlek M, Reboleira ASPS, Souza-Silva M, Teeling EC, Wynne JJ, Zagmajster M (2019) Scientists' warning on the conservation of subterranean ecosystems. BioScience 69:641–650

Mampe CD, Neunzig HH (1966) Function of the stridulating organs of *Conotrachelus nenuphar* (Coleoptera: Curculionidae). Ann Entomol Soc Am 59:614–615

Mankin RW, Benshemesh J (2006) Geophone detection of subterranean termite and ant activity. J Econ Entomol 99:244–250

Mankin RW, Fisher JR (2002) Acoustic detection of black vine weevil, *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) larval infestations in nursery containers. J Environ Hortic 20:166–170

Mankin RW, Fisher JR (2007) Acoustic detection of *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) larval infestations in nursery containers. In: Gothro P, Rosetta R, Mankin R (eds) Proceedings of the North American Root Weevil Workshop, 1–2 Nov 2001, Aurora, OR, Oregon State Univ Special Report 1065:10–15

Mankin RW, Lapointe SL (2003) Listening to the larvae: acoustic detection of *Diaprepes abbreviatus* (L.). Proc Fla State Hortic 116:305–308

Mankin RW, Moore A (2010) Acoustic detection of *Oryctes rhinoceros* (Coleoptera: Scarabaeidae: Dynastinae) and *Nasutitermes luzonicus* (Isoptera: Termitidae) in palm trees in urban Guam. J Econ Entomol 103:1135–1143

Mankin RW, Brandhorst-Hubbard J, Flanders KL, Zhang M, Crocker RL, Lapointe SL, McCoy CW, Fisher JR, Weaver DK (2000) Eavesdropping on insects hidden in soil and interior structures of plants. J Econ Entomol 93:1173–1182

Mankin RW, Lapointe SL, Franqui RA (2001) Acoustic surveying of subterranean insect populations in citrus groves. J Econ Entomol 94:853–859

Mankin RW, Osbrink WL, Oi FM, Anderson JB (2002) Acoustic detection of termite infestations in urban trees. J Econ Entomol 95:981–988

Mankin RW, Hubbard JL, Flanders KL (2007) Acoustic indicators for mapping infestation probabilities of soil invertebrates. J Econ Entomol 100:790–800

Mankin RW, Johnson SN, Grinev DV, Gregory PJ (2008) New experimental techniques for studying root herbivores. In: Johnson SN, Gregory PJ (eds) Root feeders: an ecosystem perspective. CABI, Cambridge, MA, pp 20–32

Mankin RW, Moore A, Samson PR, Chandler KJ (2009a) Acoustic characteristics of dynastid beetle stridulations. Fla Entomol 92:123–133

Mankin RW, Samson PR, Chandler KJ (2009b) Acoustic detection of Melolonthine larvae in Australian sugarcane. J Econ Entomol 102:1523–1535

Mankin RW, Hagstrum DW, Smith MT, Roda AL, Kairo MTK (2011) Perspective and promise: a century of insect acoustic detection and monitoring. Am Entomol 57:30–44

Mankin RW, Stanaland D, Haseeb M, Rohde B, Menocal O, Carrillo D (2018) Assessment of plant structural characteristics, health, and ecology using bioacoustic tools. Proc Meet Acoust 33: 010003

Marini L, Okland B, Jönsson M, Bentz B, Carroll A, Forster B, Grégoire J-C, Hurling R, Nageleisen LM, Netherer S, Ravn HP, Weed A, Schroeder M (2017) Climate drivers of bark beetle outbreak dynamics in Norway spruce forests. Ecography 40:1426–1435

Mazzoni V, Eriksson A, Anfora G, Lucchi A, Virant Doberlet M (2014) Active space and the role of amplitude in plant-borne vibrational communication. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 125–145

Merchant ME, Biles S, Mott D (2004) White grubs in Texas turfgrass. Texas A & M Agrilife Extension Bulletin E-211. https://agrilifecdn.tamu.edu/citybugstest/files/2016/07/White-grubs-in-Texas-Turfgrass-E211.pdf

Michelsen A (2014) Physical aspects of vibrational communication. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 199–213

Morris GK, Mason AC, Wall P, Belwood JJ (1994) High ultrasonic and tremulation signals in neotropical katydids (Orthoptera: Tettigoniidae). J Zool Lond 233:129–163

Mortimer B (2017) Biotremology: do physical constraints limit the propagation of vibrational information? Anim Behav 130:165–174

Ohmura W, Takanashi T, Suzuki Y (2009) Behavioral analysis of tremulation and tapping of termites (Isoptera). Sociobiology 54:269–274

Osbrink W, Cornelius M (2013) Acoustic evaluation of trees for *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) treated with imidacloprid and noviflumuron in historic Jackson Square, New Orleans, Louisiana. Sociobiology 60:77–95

Palestrini C, Pavan G, Zunino M (1990) Acoustic signals and stridulatory apparatus in *Copris incertus* Say (Coleoptera Scarabaeidae: Coprinae). Acta Zoologica Mexicana 39:1–18

Parry DA (1965) The signal generated by an insect in a spider's web. J Exp Biol 43:185–192

Patitsas AJ (2010) Squeal vibrations, glass sounds, and the stick-slip effect. Can J Phys 88:863–876

Podlesnik J, Klokočovnik V, Lorent V, Devetak D (2019) Prey detection in antlions: propagation of vibrational signals deep into the sand. Physiol Entomol 44:215–221

Polak S (2005) Importance of discovery of the first cave beetle, *Leptodirus hochenwartii* Schmidt, 1832. ENDINS 28:71–80

Pollack GS (2017) Insect bioacoustics. Acoust Today 13(2):26–34

Potamitis I, Rigakis I, Tatlas N-A, Potirakis S (2019) In-vivo vibroacoustic surveillance of trees in the context of the IoT. Sensors 19:1366

Prešern J, Polajnar J, de Groot M, Zorović M, Virant-Doberlet M (2018) On the spot: utilization of directional cues in vibrational communication of stink bug. Sci Rep 8:5418. https://doi.org/10.1038/s41598-018-23710-x

Pureswaran DS, Hofstetter RW, Sullivan BT, Potter KA (2016) The role of multimodal signals in species recognition between tree-killing bark beetles in a narrow sympatric zone. Environ Entomol 45:582–591

Quirici V, Costa FG (2005) Seismic communication during courtship in two burrowing tarantula spiders: an experimental study on *Eupalaestrus weijenberghi* and *Acanthoscurria suina*. J Arachnol 33:159–166

Rajamaran K, Nair A, Dey A, Balakrishnan R (2018) Response mode choice in a multimodally duetting paleotropical pseudophylline bushcricket. Front Ecol Evol. https://doi.org/10.3389/fevo.2018.00172

Rathore DS, Ram B, Pal BL, Malviya S (2019) Analysis of classification algorithms for insect detection using MATLAB. In: 2nd international conference on advanced computing and software engineering, 8–9 Feb 2019, Kuala Lumpur, Malaysia, pp 299–304

Rauth SJ, Vinson SB (2006) Colony wide behavioral contexts of stridulation in imported fire ants (*Solenopsis invicta* Buren). J Insect Behav 19:293–304

Rillig MC, Bonneval K, Lehmann J (2019) Sounds of soil: a new world of interactions under our feet? Soil Syst 3:45. https://doi.org/10.3390/soilsystems3030045

Rohde BB, Allen PE, Benda N, Brun A, Mankin RW, Dale AG (2019) An acoustic trap to survey and capture two *Neoscapteriscus* species. Fla Entomol 102:654–657

Rudinsky JA (2009) Chemoacoustically induced behavior of *Ips typographus* (Col.: Scolytidae). J Appl Entomol 88:537–541

Sabatier JM, Hess H, Arnott WP, Attenborough K, Römkens MJM, Grissinger EH (1990) In situ measurements of soil physical properties by acoustical techniques. Soil Sci Soc Am J 54:658–672

Sanders WR, Mankin RW, Liburd OE, Stelinski LL (2011) Acoustic detection of arthropod infestation of grape roots: scouting for grape root borer (Lepidoptera: Sesiidae). Fla Entomol 94:296–302

Scheffrahn RH, Robbins WP, Busey P, Su NY, Mueller RK (1993) Evaluation of a novel, hand-held acoustic emissions detector to monitor termites (Isoptera: Kalotermitidae, Rhinotermitidae) in wood. J Econ Entomol 86:1720–1729

Schilman PE, Lazzari CR, Manrique G (2001) Comparison of disturbance stridulations in five species of triatominae bugs. Acta Tropica 79:171–178

Seastedt TR, Murray PJ (2007) Root herbivory in grassland systems. In: Johnson SN, Murray PJ (eds) Root feeders: an ecological perspective. CABI, Cambridge, MA, pp 54–67

Senter P (2008) Voices of the past: a review of Paleozoic and Mesozoic animal sounds. Hist Biol 20:255–287

Smiseth PT, Parker HJ (2008) Is there a cost to larval begging in the burying beetle *Nicrophorus vespilloides*? Behav Ecol 19:1111–1115

Spangler HG (1967) Ant stridulations and their synchronization with abdominal movement. Science 155:1687–1689

Spangler HG, Greenfield MG, Takessian A (1984) Ultrasonic mate calling in the lesser wax moth. Physiol Entomol 9:87–95

Stölting H, Moore TE, Lakes-Harlan R (2002) Substrate vibrations during acoustic signalling in the cicada *Okaganda rimosa*. J Insect Sci 2:2

Strauß J, Stumpner A (2015) Selective forces on origin, adaptation and reduction of tympanal ears in insects. J Comp Physiol A 201:155–169

Takanashi T, Uechi N, Tatsuta H (2019) Vibration in hemipteran and coleopteran insects: behaviors and applications in pest management. Appl Entomol Zool 54:21–26

TenCate JA, Remillieux M (2019) The peculiar acoustics of rocks. Acoust Today 15(2):29–35

Ulagaraj SM, Walker TJ (1973) Phonotaxis of crickets in flight: attraction of male and female crickets to male calling songs. Science 182:1278–1279

Ulagaraj SM, Walker TJ (1975) Response of flying mole crickets to three parameters of synthetic songs broadcast outdoors. Nature 253:530–532

van Dam NM (2009) Belowground herbivory and plant defenses. Annu Rev Ecol Evol Syst 40: 373–391

van Tol RWHM, van Dijk N, Sabelis MW (2004) Host plant preference and performance of the vine weevil *Otiorhynchus sulcatus*. Agric For Entomol 6:267–278

Veen GF, Wubs ERJ, Bardgett RD, Barrios E, Bradford MA, Carvalho S, De Deyn GB, de Vries FT, Giller KE, Kleijn D, Landis DA, Rossing WAH, Schrama M, Six J, Struik PC, van Gils S, Wiskerke JSC, der Putten WH, Vet LEM (2019) Applying the above-belowground interaction concept in agriculture: spatio-temporal scales matter. Front Ecol Evol. https://doi.org/10.3389/fevo.2019.00300

Villani MG, Wright RJ (1990) Environmental influences on soil macroarthropod behavior in agricultural systems. Annu Rev Entomol 35:249–269

Villani MG, Allee LL, Diaz A, Robbins PS (1999) Adaptive strategies of edaphic arthropods. Annu Rev Entomol 44:233–256

Virant-Doberlet M, Kuhelj A, Polajnar J, Šturm R (2019) Predator-prey interactions and eavesdropping in vibrational communication networks. Front Ecol Evol. https://doi.org/10.3389/fevo.2019.00203

Wagenhoff E, Blum R, Delb H (2014) Spring phenology of cockchafers, *Melolontha* spp. (Coleoptera: Scarabaeidae) in forests of southwestern Germany: results of a 3-year survey on adult emergence, swarming flights, and oogenesis from 2009-2011. J For Sci 60:154–165

Walker TJ (1982) Sound traps for sampling mole cricket flights (Orthoptera: Gryllotalpidae: *Scapteriscus*). Fla Entomol 65:105–110

Walker TJ (1988) Acoustic traps for agriculturally important insects. Fla Entomol 71:484–492

Webb JC, Calkins CO, Carlysle T, Benner J (1980) Analysis of stress sounds produced by the plum curculio, *Conotrachelus nenuphar* (Herbst). J Ga Entomol Soc 15:419–427

Wessel A (2006) Stridulation in the Coleoptera—an overview. In: Drosopoulos S, Claridge MF (eds) Insect sounds and communication. CRC, New York, pp 397–404

Wessel A, Mühlethaler R, Hartung V, Kuštor V, Gogala M (2014) The tymbal: evolution of a complex vibration-production organ in the Tymbalia (Hemiptera excl. Sternorrhyncha). In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 395–444

Wignall AE, Taylor PW (2011) Assassin bug uses aggressive mimicry to lure spider prey. Proc R Soc Lond B 278:1427–1433

Wilson LM, Henry CS, Johnson JB, McCaffrey JP (1993) Sound production in *Phrydiuchus tau* (Coleoptera: Curculionidae). Ann Entomol Soc Am 86:621–630

Young IM, Crawford JW (2004) Interactions and self-organization in the soil-microbe complex. Science 304:1634–1637

Zhang M, Crocker RL, Mankin RW, Flanders KL, Brandhorst-Hubbard JL (2003a) Acoustic identification and measurement of activity patterns of white grubs in soil. J Econ Entomol 96: 1704–1710

Zhang M, Crocker RL, Mankin RW, Flanders KL, Brandhorst-Hubbard JL (2003b) Acoustic estimation of infestations and population densities of white grubs (Coleoptera: Scarabaeidae) in turfgrass. J Econ Entomol 96:1770–1779

Zorović M, Čokl A (2015) Laser vibrometry as a diagnostic tool for detecting wood-boring beetle larvae. J Pest Sci 88:107–112

Chapter 22
Vibrational Communication in Psyllids

Sabina Avosani, Richard W. Mankin, Thomas E. S. Sullivan, Jernej Polajnar, David Maxwell Suckling, and Valerio Mazzoni

Abstract Psyllids are small insects that can vector causal agents of serious plant diseases, such as greening in citrus and zebra chip disease in potatoes. Several invasive psyllid species are expanding their geographic range, but there are few pest management tactics available at present. Vibrational communication is a primary intra-specific communication channel within Psylloidea, being widespread among almost all the families. In psyllids, vibrational signals are used for mate location and mate choice by means of a male–female duet. Depending on the species, the first call can be emitted either by the male or the female and if a potential mate replies, the duet is eventually established. Some psyllid males produce a specific response signal when a female replies to their call, while in most other species the male uses a unique type of vibrational signal throughout the mating

---

S. Avosani (✉)
Present Address: Centre International de Hautes Études Agronomiques Mediterranéennes, CIHEAM-Bari, Valenzano (Bari), Italy

R. W. Mankin
Center for Medical, Agricultural, and Veterinary Entomology, Agriculture Research Service, US Department of Agriculture, Gainesville, FL, USA
e-mail: Richard.Mankin@usda.gov

T. E. S. Sullivan
The New Zealand Institute for Plant and Food Research Ltd, Lincoln, New Zealand
e-mail: Thomas.Sullivan@plantandfood.co.nz

J. Polajnar
Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia
e-mail: jernej.polajnar@nib.si

D. M. Suckling
The New Zealand Institute for Plant and Food Research Ltd, Lincoln, New Zealand

School of Biological Sciences, University of Auckland, Auckland, New Zealand

V. Mazzoni
Department of Sustainable Ecosystems and Bioresources, Research and Innovation Center, Fondazione Edmund Mach, San Michele all'Adige, Italy
e-mail: valerio.mazzoni@fmach.it

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_22

process. Most psyllids likely emit vibrations by means of stridulation, in which signals can be produced when the sclerotised areas of the anal vein of the wings rub against the scutellum, which bears scale-like denticles. Recent evidence suggests that novel methods of trapping or mating disruption using vibrational communication could provide solutions for monitoring and control psyllid pests. For instance, the vibrational signals of the Asian citrus psyllid, *Diaphorina citri*, and the North American tomato potato psyllid, *Bactericera cockerelli*, have been tested as monitoring and trapping tools, and similar studies have been initiated on other species.

22.1 Introduction

Psyllids are small sap-feeding insects belonging to the superfamily Psylloidea, which consists of eight hemipteran families (Aphalaridae, Calophyidae, Carsidaridae, Homotomidae, Liviidae, Phacopteronidae, Psyllidae, and Triozidae) and comprises more than 3500 species (Ouvrard 2016). They are distributed in almost all the world's climatic regions (Hodkinson 2009) and some are agricultural pests that can cause damage to the host plant, either by feeding or by transmitting pathogens including bacteria, phytoplasmas, and viruses (Munyaneza 2010; Burckhardt et al. 2014). Vector species have received great attention from researchers and pest managers because of the economic importance of the diseases that they transmit to plants (Hodkinson 1974; Munyaneza et al. 2007; Munyaneza 2010; Hall et al. 2013; Coutinho-Abreu et al. 2014). For example, the bacterium *Candidatus* Liberibacter, which is transmitted by four known psyllid species, causes important diseases in citrus and several solanaceous crops (Haapalainen 2014). A psyllid-transmitted disease known as Huanglongbing or "citrus greening disease" is associated with three species of *Ca.* Liberibacter and is considered a global threat to citrus cultivations (Gottwald et al. 2007). Another economically important disease is apple proliferation, transmitted by two *Cacopsylla* species that are controlled by insecticide treatments in Europe (Tedeschi et al. 2012).

Studies on the biology, physiology, ecology, and behaviour of these insects could provide useful information to support the development of effective control strategies. A review examined the biotic and abiotic factors affecting the life cycle in psyllids, and temperature and humidity in ecological context seemed to play major roles in psyllid evolution (Hodkinson 2009). A simple temperature-driven phenological model, based on psyllid biology and on the host plant development across a wide geographical range, demonstrates how both northern and southern limits of latitude and climate affect the insects' life cycle (MacLean 1983). The geographical range also determines whether a psyllid species can exploit different hosts or plant tissues. For instance, in the northern regions of Greenland, the willow psyllid *Cacopsylla groenlandica* (Psyllidae) develops solely on the catkins of *Salix glauca*, even in the presence of other willow species, while in southern regions the insect can complete the cycle on different plant tissues of four *Salix* species (Hodkinson 1997). In the case of psyllid vectors, similar information could be used to develop risk maps on the epidemiology and the potential outbreaks of the plant disease transmitted by the insect. Climate change should to be taken into account when modelling risk maps as well, since global warming could influence the developmental range of both pest species and pathogenic microorganisms in several ways (Ladányi and Horváth 2010). Psyllids have adapted to a range of host plants (Hodkinson 1974, 1986; Van Klinken 2000), in that most species are strictly monophagous or oligophagous and feed especially within Solanaceae, Umbelliferae, and Cruciferae (Hodkinson 2009). Host alternation during the year is common in psyllids, and adults can migrate from overwintering plants to colonise host and shelter plants (Burckhardt et al. 2014). Long-range dispersal can be a challenge for such small insects, but some migrations cover remarkable distances, as in the Asian citrus psyllid *Diaphorina citri* (Liviidae), which can move to different citrus groves even in the presence of geographical barriers such as fields and roads (Lewis-Rosenblum et al. 2015). The colonisation of a new area can lead to a shift in host range, as reported for the tomato potato psyllid *Bactericera cockerelli* (Triozidae), a major pest of potato (Knowlton and Thomas 1934). Host shifting is considered to be one of the most important drivers of speciation in psyllids (Percy et al. 2004) and depends in the first instance on the initial attractiveness of a novel host (Lapis and Borden 1993; Tsai and Liu 2000), and secondly on the ability to exploit it as a food source (Hodkinson 2009). Some psyllids accordingly inject salivary secretions to influence the physiology of the plants to enhance availability of nutrients and assist nymphal growth (Laurema 1989). Such secretions can be phytotoxic, causing necroses, galling, and malformations in the host plants (Markkula and Laurema 1971; Burckhardt et al. 2014).

Most psyllids live in dense, mixed-sex colonies (Hodkinson 2009), and research has therefore investigated the mechanisms that underlie aggregation, host location and selection. In general, psyllids reproduce sexually, but parthenogenesis has been reported in some species (Nokkala et al. 2008). Notwithstanding the available literature on the psyllid biology and ethology, there is still need for more information regarding the sexual behaviour, and particularly the sensory modalities used by these insects for mate location and selection. In fact, knowledge about the signals emitted during pair formation is an important prerequisite in order to develop successful integrated pest management strategies (Polajnar et al. 2015; Pertot et al. 2016; Takanashi et al. 2019). For this reason, besides studies on the communication of some species from Australia, e.g., Triozidae (Taylor 1985; Percy et al. 2006) and Russia, e.g., Psyllidae, Triozidae, and Aphalaridae (Tishechkin 1989, 2005, 2006, 2007), the most detailed description of psyllids' vibrational signals associated with mating has involved pest species, i.e. *Cacopsylla pyri, Cacospylla picta, D. citri,* and *B. cockerelli* (Wenninger et al. 2009; Eben et al. 2015; Oppedisano et al. 2020; Avosani et al. 2020). In this regard, since current control practices (i.e., insecticide treatments) against *B. cockerelli* did not eliminate zebra chip disease in New Zealand (Vereijssen 2020); alternative methods of control such as mating disruption and behavioural manipulation remain under investigation within this country.

22.2 Mating Communication and Pair Formation in Psyllids

Mating communication allows transfer of relevant information about the species and physiological status of the sender to the receiver (West-Eberhard 1984; Candolin 2003; Cocroft and Rodríguez 2005). Most psyllids reach sexual maturity within 24–48 h after adult eclosion and females of some species mate several times during their lifespan to continuously produce fertile eggs (Burts and Fischer 1967; Van den Berg et al. 1991). After mating, males usually search for other potential partners, while females require a longer refractory period before resuming sexual receptivity (Percy et al. 2006; Tishechkin 2007; Lubanga et al. 2014). In general, signalling systems differ between species and usually involve interactions among multiple modalities. Similarly, both vibrational and chemical communication promotes pair formation within Psylloidea, although the relative weight of the cues involved in mate finding and selection depends on the species (Wenninger et al. 2008, 2009; Lubanga et al. 2014).

22.2.1 Vibrational Communication: Mechanisms and Behaviours

Vibrational communication is considered to be the main intra-specific communication channel, being widespread among the majority of Psylloidea; it has been reported in Liviidae (Yang et al. 1986; Wenninger et al. 2009), Triozidae (Percy et al. 2006; Tishechkin 2007; Liao et al. 2016: Avosani et al. 2020), Homotomidae (Liao and Yang 2017; Liao et al. 2019), Carsidaridae (Liao et al. 2019), Aphalaridae (Tishechkin 2007; Wood et al. 2016), and Psyllidae (Eben et al. 2015; Liao and Yang 2015). Acoustic signalling in psyllids was first described by Ossiannilsson (1950) while vibrations were recorded and described in the sixties (Heslop-Harrison 1960; Campbell 1964). Much later, several studies demonstrated that vibrational signals carried along the plant surface play a key role in the mating behaviour of many species and likely constitute the primary sensory modality used to identify and locate a suitable mate in many species (i.e., Yang et al. 1986; Tishechkin 1989, 2005; Percy et al. 2006).

A proposed mechanism for signal production in psyllids is stridulation, which involves the movement of the wings in a vertical plane, along the body of the insect (Heslop-Harrison 1960; Taylor 1985; Tishechkin 2006, 2007; Eben et al. 2015; Wood et al. 2016; Oppedisano et al. 2020). In this way, vibrational signals can be produced when the sclerotised areas of the anal vein of the wings rub against the scutellum, specifically against the meso- and metathorax axillary cords, which bear scale-like denticles. The anal vein acts as a “plectrum” scraping against the surface of the axillary cord, which represents the *pars stridens* (Taylor 1985; Tishechkin 2006), as shown in Fig. 22.1. In five species of psyllids (*Macrohomotoma gladiata*

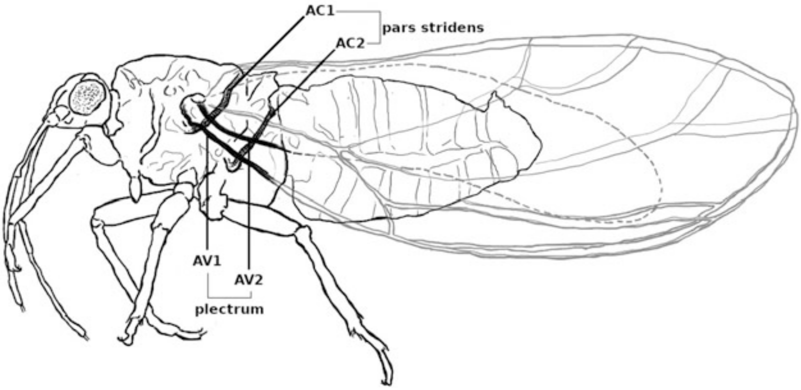


Fig. 22.1 Drawing of a male of *Cacopsylla spp*. The stridulatory organs that could be involved in the production of vibrations, as suggested by Eben et al. (2015), are coloured in black. The movement of the wings in the vertical plane enables the anal veins to act as a plectrum when they scrape against the *pars stridens,* which consists of the axillary cords. AC1: axillary cord of the mesoscutellum; AC2: axillary cord of the metascutellum; AV1: forewing anal vein; AV2: hindwing anal vein. Dashed line: hind wing (drawing courtesy of S. Avosani)

(Homotomidae), *Trioza sozanica* (Triozidae), *Mesohomotoma camphorae* (Carsidaridae), *Cacopsylla oluanpiensis* and *Cacopsylla tobirae* (Psyllidae)) vibrations are produced by means of two stridulatory mechanisms, which work together. In particular, one component involves friction between the anal area of forewings and the axillary cords on mesothorax, while the other consists in stridulation between the axillary sclerites of the forewings and the thorax (Liao et al. 2019). With this study, Liao and colleagues (2019) confirmed the crucial role of the forewings and showed for the first time that the axillary sclerites are also involved in signal production within Psylloidea. However, in the case of the five species under analysis, the hindwings are not used to emit vibrations (Liao et al. 2019), as previously hypothesised by Taylor (1985) and Tishechkin (2006). Moreover, Liao et al. (2019) demonstrated that frictional mechanisms such as the rubbing between wing–wing, leg–abdomen, or leg–wing do not play a role in signalling. Interestingly, the rapid wing vibration is hypothesised to be the source of the faint sound that was first noted in Psylloidea (Heslop-Harrison 1960). *Diaphorina citri* males and females, by contrast, use wingbeat signals for vibrational communication and have not been reported to stridulate (Wenninger et al. 2009). Indeed, signal production cannot be achieved solely via wingbeat in other psyllid species, where the stridulatory organs are found in the forewings and the thorax. As a fact, wing beat cannot be the source of substrate-borne signals when the wing beating frequency does not correspond with either the dominant or fundamental frequency of the emitted vibrational signals (Liao et al. 2019). Tishechkin (2006) described stridulatory organs in species that did not emit vibrations, while these structures were not found in some Australian species that possibly do not communicate by means of vibrations or use an alternative stridulatory organ (Percy et al. 2006).

Although stridulation appears to be a simple frictional mechanism, the resulting signals are diverse among species in terms of amplitude and temporal pattern; therefore, comparative analyses of the temporal and spectral properties of the signals could enable discrimination among different species (Tishechkin 2005; Liao and Yang 2015). In fact, the denticulate surface of the axillary cords slightly differs between psyllid families and even between morphologically identical/similar species, and these differences influence the characteristics of stridulatory signals, therefore supporting species discrimination and determining taxonomic positions (Tishechkin 2006; Liao et al. 2019). A similar relatedness between the morphology of stridulatory organs, vibrations/sound characteristics, and taxonomic relationships has been proposed and discussed for other insect species. In the genus *Typhlocharis* (Coleoptera: Carabidae), for instance, the presence of the *pars stridens* is potentially a useful character that can elucidate the taxonomic relationship between species (Zaballos and Perez-Gonzalez 2011). In velvet ants, on the contrary, the link between the structure of stridulatory organs and call patterns seems to be weak, even when characteristics of stridulatory organs are species-specific (Polidori et al. 2013). Signals generally differ substantially between sympatric species to avoid potential interference during intra-specific communication, as a form of precopulatory reproductive barrier (Claridge 1985, 1990; Coyne 2007; Tishechkin 2008). Sympatric psyllids could nonetheless produce vibrations with similar spectral and temporal patterns, if they complete their life cycle on different host plants (Tishechkin 2005, 2007; Percy et al. 2006). Signal characteristics are indeed a reliable tool that can support morphological analyses for taxonomy (Claridge 1985, 1990; Tishechkin 2014) as vibrational characters can reveal phylogenetic relationships between psyllids species, explaining if two or more species have either recently diverged or are allopatric (Percy et al. 2006). The use of vibrational features as phylogenetic characters is still under debate (Percy et al. 2006), and before using vibrational data in phylogenetic studies, it is necessary to take into account the types of selection (i.e. environmental, sexual, etc.) that operated during the process of speciation (Claridge 1990; Percy et al. 2006). At any rate, while the mere organisation and shape of sound/vibration organs cannot resolve phylogenetic problems, a multidisciplinary approach that integrates molecular data with both morphological and developmental studies could be a more effective strategy (Jost and Shaw 2006).

Psyllid signals mostly consist of short pulses, which can be arranged in longer songs (Tishechkin 2007; Eben et al. 2015). These pulses can have different spectral characteristics and be grouped in pulse trains or syllables, which in turn can be arranged to produce more complex signals used to achieve mating (Tishechkin 2005, 2007; Percy et al. 2006). As an example, a detail of the male calling signal of *B. cockerelli*, which consists of a series of pulse trains, is provided in Fig. 22.2 (Avosani et al. 2020).

As in other hemipteran insects (Virant-Doberlet and Čokl 2004), vibrational signals are species- and gender-specific and play a crucial role during pair formation within Psylloidea (Percy et al. 2006; Tishechkin 2006). In this regard, male and female tightly synchronise their signals to identify and finally locate the most suitable mate (Tishechkin 2005, 2006, 2007; Liao and Yang 2015). In most species,

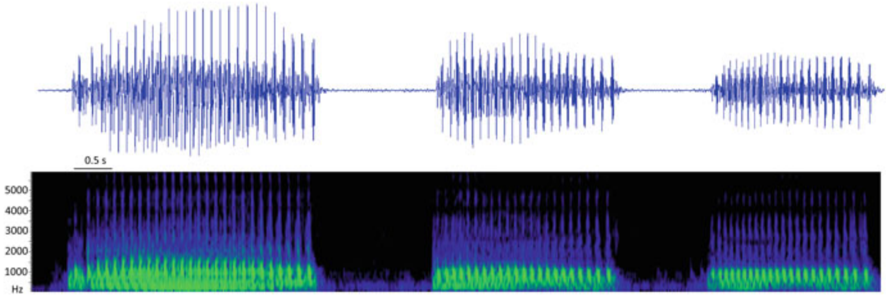


Fig. 22.2 Oscillogram and spectrogram of a section of the first part of a calling signal emitted by a *Bactericera cockerelli* male (FFT size = 256, overlap = 75%). Similarly to other psyllid species such as *Cacopsylla pyri* (Eben et al. 2015), the male call is composed of pulses arranged in pulse trains (Avosani et al. 2020). The figure shows three successive pulse trains (courtesy of S. Avosani)

the male produces the first call, to which a sexually-mature female may reply, and if so, a duet is established (Percy et al. 2006; Tishechkin 2007). In some cases, females spontaneously produce vibrational signals before or in absence of the male, as in *D. citri* and *C. pyri* (Wenninger et al. 2009; Eben et al. 2015; Mankin et al. 2020). Contrary to other psyllid species, pair formation in *C. picta* mainly started with the emission of a female calling signal, which was followed by the male signal and, potentially, the duet. Even so, the female calling activity could have been a consequence of an increased sexual motivation since the females were tested near the end of their reproductive period, as stated by the authors of the study (Oppedisano et al. 2020). In general, when the duet is established, the male starts to search for the female, who remains stationary on the plant (Percy et al. 2006; Tishechkin 2007; Liao et al. 2016; Lujo et al. 2016; Liao and Yang 2017; Oppedisano et al. 2020). Mating is eventually achieved when the male reaches the replying partner on the plant (Wenninger et al. 2009; Liao et al. 2016; Liao and Yang 2017; Oppedisano et al. 2020).

Depending on the species, the male and female signals can differ greatly in their structure, with male signals being generally longer and more complex than those of females (Percy et al. 2006; Lubanga et al. 2016; Oppedisano et al. 2020). In three *Cacopsylla* species, by contrast, the female reply has a longer duration compared to the male call, probably to provide more directional information to the searching mate (Liao and Yang 2015). In a very recent review, Liao and colleagues analysed and classified the vibrational communication of 107 species within 47 genera, providing evidence that, notwithstanding many similarities, the psyllid repertoire of vibrational signals and mating strategies are rather variegated (Liao et al. 2022). Males of *Macrohomotoma gladiata* and *Macrohomotoma robusta* (Homotomidae), for example, produce a specific response signal whenever a female replies to their call, while in other species the male uses a unique type of vibrational signal to both call and duet with the potential mate (Liao and Yang 2017). In both *Macrohomotoma* species, the male response signals resemble the female reply in

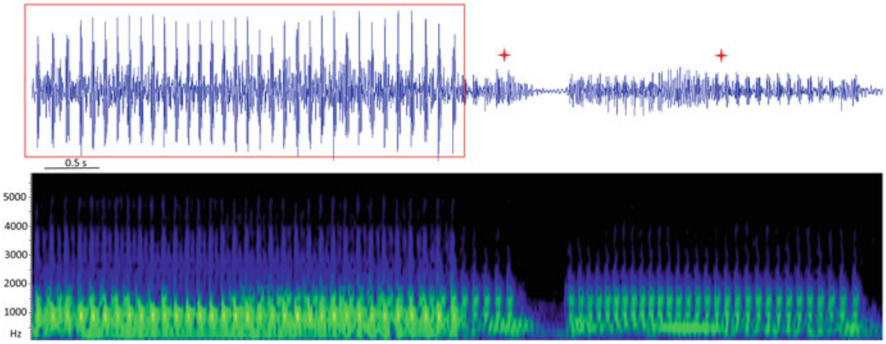


Fig. 22.3 Oscillogram and spectrogram of a section of a male–female duet in *Bactericera cockerelli* (FFT size = 256, overlap = 75%). Similarly to other psyllid species (Percy et al. 2006), the female response partially overlaps the last section of the male signal (red rectangle) and then continues after the male ceases to call (red diamonds) (Avosani et al. 2020)

the temporal structure, probably as a strategy to deceive rival males while duetting (Liao and Yang 2017). In this way, an eavesdropping male could potentially not be able to discriminate between the male and the female and consequently not reach the duetting female before the signalling male (Liao and Yang 2017). In some stonefly species, similarly to the two *Macrohomotoma* psyllids, the male signal mimics the female response during the duet (Boumans and Johnsen 2015). At any rate, the exchange of signals within the duet can be more or less synchronised in psyllids, and the reply of the female can either partially overlap or follow the male call (Eben et al. 2015; Liao and Yang 2015; Liao et al. 2019; Mankin et al. 2020; Avosani et al. 2020; Liao et al. 2022). An example of partial overlap between the male and female signal is shown in Fig. 22.3. Since the female remains stationary throughout the duet, the overlap of the male and female signals could possibly decrease the risk of eavesdropping by predators, such that "one signal" is perceived as coming from two different positions (De Groot et al. 2012). As in many other hemipterans, the male searching behaviour is characterised by the alternation of walking and calling (Polajnar et al. 2014). Male calls elicit female replies, which likely give directional information on the female's location to the male and, in this way, mediate the directional choices of the male (Percy et al. 2006; Wenninger et al. 2009). The process of pair formation in insects as small as psyllids can be very complicated in that the distance between legs is likely too short to perceive and correctly process directional information (Virant-Doberlet and Čokl 2004), but it has been demonstrated that even small insects can acquire directional information using vibrational signals (Mazzoni et al. 2014). For instance, in *Aacanthocnema dobsoni* (Triozidae), searching efficiency was experimentally confirmed on one-dimensional surfaces (i.e. plant branchlets), where males located the replying females within 30 s of duetting (Lubanga et al. 2016). Besides evaluating the calls to assess the quality of the male, psyllid females probably use other cues and accordingly choose to stop signalling before being located (Liao and Yang 2015, 2017). As an example, the responsiveness of females of *Anoeconeossa bundoorensis* (Aphalaridae) was not influenced by the parameters of male calls, which varied significantly between individuals. Since the differences in the male calls were associated with the size and the age of the insects, it is possible that females evaluated other male features to assess the quality of the potential partner (Lubanga et al. 2016). In this regard, when the male reaches the female, signal modalities other than vibrations, such as odours, could be involved and be decisive in mate preference (Wenninger et al. 2009), provided that the use of multiple cues in mate choice is common within insects (Candolin 2003).

Psyllids usually form dense populations, and this situation could be advantageous for males, which could intercept and copulate with a female without the support of vibrational signals (Tishechkin 2007). Besides occasionally achieving mating without signalling (Avosani et al. 2020), *B.cockerelli* males also try to mount other males (Avosani and Sullivan, personal observations). Similar same-sexual behaviours occur in at least 110 species among insects and arachnids and may be a result of high sexual responsiveness (Scharf and Martin 2013). Although fascinating, alternative reasons that proposed an adaptive significance for male–male interactions have been mainly rejected (Scharf and Martin 2013). Probably, a copulation attempt towards a rival does not provide any direct advantage to the mounting male, while the cost of losing the opportunity of mating with a nearby female is greater than the cost of approaching a male (Scharf and Martin 2013).

Male competitive behaviours have been described in many insect species such as delphacid planthoppers (Ichikawa 1982), leafhoppers (Mazzoni et al. 2009), and sharpshooters (Nieri et al. 2017), and may involve the production of vibrational signals aimed at decreasing the mating success of a rival male. For instance, in the leafhopper *Scaphoideus titanus,* a specific male rivalry signal is used to disrupt an ongoing duet (Mazzoni et al. 2009). Although rivalry signals have not yet been reported in psyllids, there is evidence that males interact by exchanging signals and future studies on potential rival interactions are worth being conducted (Tishechkin 2007). Male choruses have been accordingly described for some species belonging to the family Triozidae, in which chorusing may be either a cooperative strategy to increase the active space of the male calls or simply a random sum of vibrational signals emitted in unison (Tishechkin 2007; Greenfield 2015). Males of the Australian psyllid *Anoeconeossa unicornuta* (Aphalaridae) also perform choruses, regardless of the presence of a female on the plant. In particular, the male call may trigger the emission of signals from surrounding males, creating a prolonged and continuous chorus, whose role is still unknown (Liao et al. 2022).

Specific courtship vibrations are common among hemipterans such as leafhoppers (Mazzoni et al. 2009) and stinkbugs (Virant-Doberlet and Čokl 2004) to elicit the acceptance of a partner (usually the female) at short-range and differ from the signals used in the first phases of the pair formation process. In *S. titanus*, for example, pair formation is a dynamic process, whose stages are associated with different male signals, while the repertoire of the female is limited to a response signal. In this way, signals are optimised for reliability in the first stages of the mating process (identification and localisation), while the more energy-demanding signals aimed at evoking the acceptance of the female are used in proximity of the latter (Mazzoni et al. 2009). Comparable short-range courtship signals have not been reported within Psylloidea, since the male usually uses the same vibrational signal throughout the pair formation process (Tishechkin 2007). Although the repertoire of vibrational signals within Psylloidea is commonly limited to the male call and the female response, in some species signals are produced also during copulation (Tishechkin 2007). In *Colposcenia aliena* (Aphalaridae), the male produces vibratory signals, which consist of a sequence of pulses, each composed of shorter high-amplitude and longer low-amplitude fragments (Tishechkin 2007). Similarly, long signals with a varying structure are produced by the male of *Calliardia anabasidis* (Aphalaridae) once his genitalia are in contact with the female (Tishechkin 2007). Similar "copulatory" signals seem rather common among the Aphalaridae family, considering that also the male of *A. unicornuta* emits a long buzzing signal while copulating (Liao et al. 2022).

Indeed, many questions remain on the role of vibrational communication in the sexual behaviour within Psylloidea. Indeed, it seems to be important but not always necessary, since mating could occur in the absence of vibratory signals, especially in situations of high population densities (Tishechkin 2006, 2007; Liao and Yang 2017).

22.2.2 Chemical and Other Communication Modalities

Chemical cues are used by many phytophagous insects to find suitable hosts for reproduction, especially in specialist species where a correct decision during host selection is crucial for survival (Dicke 2000). Odors leading the insect to the host can derive from the plant or from conspecifics (Dicke 2000), and some psyllid species accordingly use olfaction for host finding (Moran and Brown 1973; Lapis and Borden 1993). In *D. citri*, plant volatiles play a crucial role in host selection (Patt et al. 2011; Patt and Setamou 2010; Robbins et al. 2012). Citrus trees infected by the bacterium *Candidatus* Liberibacter asiaticus released more methyl-salicylate than uninfected plants and, for this reason, were more attractive to *D. citri* (Mann et al. 2012; Martini et al. 2014). Similarly, the bacterium *Ca.* Liberibacter solanacearum, which is transmitted by *B. cockerelli*, caused an upregulation of volatiles in capsicum plants (Mas et al. 2014). The bacterium also altered the hemolymph pH and oxygen tension in the insect's body (Molki et al. 2019), but the effects on other aspects of the physiology and behaviour are not known. It would be interesting to assess whether the infection could change the attractiveness of *B. cockerelli* females and males to each others. At any rate, attractant compounds emitted by psyllids have been reported previously in the species *Cacopsylla chinensis* (Psyllidae) (Wan et al. 2013), *Cacopsylla bidens* (Psyllidae) (Soroker et al. 2004), *Cacopsylla pyricola* (Psyllidae) (Guédot et al. 2009a,b), *B. cockerelli* (Triozidae) (Guédot et al. 2010), and *D. citri* (Liviidae) (Wenninger et al. 2008).

While semiochemicals used for long-range communication have not been yet described (Lubanga et al. 2014), odors are important at short distances, considering that female-specific cuticular hydrocarbons may attract nearby males and probably enhance the efficacy of vibrational communication (Guédot et al. 2009b; Mann et al. 2013). Indeed, the role of odours depends on the species, considering that *D. citri* males increase their calling rate in absence of female odorants (Wenninger et al. 2009). Psyllid mating behaviour also relies on visual cues, as in the case of *D. citri*, where mated females can use visual cues such as male colour to assess the quality of the potential partner (Stockton et al. 2017). Besides mate choice, vision seem to be important in orientation during mate searching, as the presence of light is an important factor affecting the mating frequency of species such as *C. pyricola* and *D. citri* (Krysan 1990; Wenninger and Hall 2007). Furthermore, geotaxis and phototaxis support the male during mate finding (Yasuda et al. 2005; Wenninger et al. 2009).

22.3 Vibrational Control of Psyllid Pests

Acoustic devices can detect insect presence or absence in the field and therefore monitor the activity of potential pest populations (Mankin et al. 2011). Moreover, several studies have shown that vibrational signals can be used to manipulate the behaviour of hemipteran pests (Polajnar et al. 2015; Takanashi et al. 2019). Species-specific vibrations can accordingly attract an insect target into traps (Hartman et al. 2017; Mazzoni et al. 2017) or interfere with the communication of mating pairs (Polajnar and Čokl 2008; Čokl and Millar 2009; Eriksson et al. 2012, Lujo et al. 2016; Gordon et al. 2017; Laumann et al. 2018; Nieri and Mazzoni 2019). Many factors such as the behaviour and the size of the insect affect the efficiency of these methods, provided that broadband signals emitted by large insects can be easily discriminated from low-frequency background noise, while smaller insects can be detected when very close to the sensor or when they reach high densities and their signal active space is consequently enlarged (Mankin et al. 2011). Giving that psyllids live in dense colonies and can damage economically important crops (Munyaneza 2010), there is an increasing interest in characterising their vibrational communication and developing monitoring and behavioural manipulation control strategies by means of mechanical stimuli.

The first encouraging attempts on Psylloidea involve *D. citri*, which, as mentioned above, transmits a devastating disease to citrus trees (Bové 2006). The vibrational signals of *D. citri* were used to create a monitoring and trapping device, which is currently under implementation (Mankin et al. 2013). In particular, the female reply was transmitted to citrus plants to direct males towards the signal source, exploiting the psyllid mating duet (see Sect. 22.2.1) (Wenninger et al. 2009). Therefore, the researchers developed a device consisting of a micro-controller platform operating a piezoelectric buzzer, which mimicked the female by simulating her response after the detection of a male call on the plant (Mankin et al. 2013, 2015;

Hartman et al. 2017). The buzzer elicited behavioural responses in both males and females (Mankin et al. 2013) and successfully led males towards the signal source, reducing their mating success (Hartman et al. 2017). In fact, tested males moved preferentially towards the device than towards females and the number of matings was significantly lower in the stimulated group than in the control (Lujo et al. 2016). The signals produced by the buzzer were longer and louder than those of the female psyllids, suggesting that higher amplitude signals were more effective at disrupting mating (Polajnar et al. 2015). Even if the device competed with the females, it rarely masked their signals; thus, the authors hypothetised that a continuous application of the playback could result in a more effective disruption of mating by covering the female replies. On the other hand, since the mating duet is properly mimicked when the playback is triggered after the male call, the authors also pointed out that the continuous transmission could be disadvantageous in terms of mating disruption (Lujo et al. 2016). Besides determining what signal features and timing could underlie the most effective control strategy, an economic and convenient application method must be developed before the vibrational device could integrate the farmers' toolbox. In the case of *D. citri*, since the device needs to be applied to multiple trees in large citrus groves, studies are ongoing in order to provide a future efficient field method.

In this regard, the technology to detect and transmit insect signals is rapidly improving, enabling acoustic and vibrational stimuli to become reliable tools for pest monitoring and control (Mankin et al. 2011). Small shakers are currently applied (at 50 m intervals) to the wires that support grapevines in vineyards and used to successfully disrupt mating in the leafhopper *S. titanus* by means of species-specific interference signals (Polajnar et al. 2015; Mazzoni et al. 2019). Cosidering that a similar approach is less feasible for the control of *D. citri* on citrus groves; other strategies have been proposed and should be tested in the future (Lujo et al. 2016). The vibrations could be, for instance, induced in the host plant by means of sounds transmitted aerially by audio-speakers, as it has been tested for the control of the hemipteran pest *Amrasca devastans* and *Nilaparvata lugens* (Saxena and Kumar 1980). Sound sweeps of 90 dB have been similarly tested on *D. citri,* and provided that the preliminary results are encouraging (Lujo et al. 2016), a reliable device may be available for farmers in the near future. Nonetheless, the use of sounds in field conditions has several disadvantages, such as costs due to broadcasting over large areas, which are relatively higher than those of pesticides (Mankin and Hagstrum 2012). Moreover, the effective range of airborne signals could be reduced by frequency-dependent attenuation over distance, which is often difficult to predict in natural habitats (Nelson 2003).

Mass trapping could support both monitoring and pest control, especially if employed before the psyllid populations reach high densities within the crop, but, again, there is need of implementation of the devices to ensure economic viability and efficiency. In addition, given that *D. citri*, *B. cockerelli,* and other species also use semiochemicals for mate finding (Wenninger et al. 2008, Guédot et al. 2010, 2013), plant volatiles and/or conspecific odours could increase the attractiveness of the transmitted vibrational signals and support the development of traps, as demonstrated in the case of a stinkbug pest (Polajnar et al. 2019).

To conclude, vibrational communication plays a crucial role in the biology and ethology of psyllids, whose signal repertoire may be more or less complex, depending on the species. For these reasons, the signals emitted by these small insects may be used by researchers not only to support phylogenetic and taxonomic studies, but also to develop sustainable strategies for the control of pest species. In this regard, we are confident when affirming that mechanical stimuli are reliable tools that will integrate other pest management strategies to control and monitor psyllid populations in the field.

Acknowledgments We would like to thank Dr Rachele Nieri for reviewing the manuscript and for her precious suggestions.

References

Avosani S, Sullivan TES, Ciolli M, Mazzoni V, Suckling DM (2020) Vibrational communication and evidence of vibrational behavioural manipulation of the tomato potato psyllid *Bactericera cockerelli*. Entomol General 40:351–363

Boumans L, Johnsen A (2015) Stonefly duets: vibrational sexual mimicry can explain complex patterns. J Ethol 33(2):87–107

Bové JM (2006) Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. J Plant Pathol 88:7–37

Burckhardt D, Ouvrard D, Queiroz D, Percy D (2014) Psyllid host-plants (Hemiptera: Psylloidea): resolving a semantic problem. Fla Entomol 97:242–246

Burts EC, Fischer WR (1967) Mating behaviour, egg production and egg fertility in pear psylla. J Econ Entomol 60(5):1297–1300

Campbell KG (1964) Sound production in Psyllidae (Hemiptera). J Entomol Soc Aust (NSW) 1:3–4

Candolin U (2003) The use of multiple cues in mate choice. Biol Rev 78:575–595

Claridge MF (1985) Acoustic behavior of leafhoppers and planthoppers: species problems and speciation. In: Nault LR, Rodriguez JG (eds) The leafhoppers and planthoppers. Wiley, New York, pp 103–125

Claridge MF (1990) Acoustic recognition signals: barriers to hybridization in Homoptera Auchenorrhyncha. Can J Zool 68(8):1741–1746

Cocroft RB, Rodríguez RL (2005) The behavioural ecology of insect vibrational communication. BioScience 55:323–334

Čokl AA, Millar JG (2009) Manipulation of insect signaling for monitoring and control of pest insects. In: Ishaaya I, Horowitz AR (eds) Biorational control of arthropod pests: application and resistance management. Springer, Dordrecht, pp 279–316

Coutinho-Abreu I, McInally S, Forster L, Luck R, Ray A (2014) Odor coding in a disease-transmitting herbivorous insect, the Asian citrus psyllid. Chem Sens 39:539–549

Coyne JA (2007) Sympatric speciation. Curr Biol 17(18):787–788

De Groot M, Derlink M, Pavlovčič P, Prešern J, Čokl A, Virant-Doberlet M (2012) Duetting behaviour in the leafhopper *Aphrodes makarovi* (Hemiptera: Cicadellidae). J Insect Behav 25: 419–440

Dicke M (2000) Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. Biochem Syst Ecol 28(7):601–617

Eben A, Mühlethaler R, Gross J, Hoch H (2015) First evidence of acoustic communication in the pear psyllid *Cacopsylla pyri* L. (Hemiptera: Psyllidae). J Pest Sci 88(1):87–95

Eriksson A, Anfora G, Lucchi A, Lanzo F, Virant-Doberlet M, Mazzoni V (2012) Exploitation of insect vibrational signals reveals a new method of pest management. PLoS One 7(3):e32954

Gordon SD, Sandoval N, Mazzoni V, Krugner R (2017) Mating interference of glassy-winged sharpshooters, *Homalodisca vitripennis*. Entomol Exp Appl 164(1):27–34

Gottwald T, Da Graca J, Bassanezi R (2007) Citrus huanglongbing: the pathogen and its impact. Plant Health Progress 8:31

Greenfield MD (2015) Signal interactions and interference in insect choruses: singing and listening in the social environment. J Comp Physiol A 201:143–154

Guédot C, Horton DR, Landolt PJ (2009a) Attraction of male winterform pear psylla to female-produced volatiles and to female extracts and evidence of male-male repellency. Entomol Exp Appl 130:191–197

Guédot C, Millar JG, Horton DR, Landolt PJ (2009b) Identification of a sex attractant pheromone for male winterform pear psylla, *Cacopsylla pyricola*. J Chem Ecol 35(12):1437–1447

Guédot C, Horton DR, Landolt PJ (2010) Sex attraction in *Bactericera cockerelli* (Hemiptera: Triozidae). Environ Entomol 39(4):1302–1308

Guédot C, Horton DR, Landolt PJ, Munyaneza JE (2013) Effect of mating on sex attraction in *Bactericera cockerelli* with evidence of refractoriness. Entomol Exp Appl 149:27–35

Haapalainen M (2014) Biology and epidemics of *Candidatus* Liberibacter species, psyllid-transmitted plant-pathogenic bacteria. Ann Appl Biol 165:172–198

Hall DG, Richardson ML, Ammar ED, Halbert SE (2013) Asian citrus psyllid, *Diaphorina citri*, vector of citrus huanglongbing disease. Entomol Exp Appl 146(2):207–223

Hartman E, Rohde B, Lujo S, Dixon M, McNeill S, Mankin RW (2017) Behavioural responses of male *Diaphorina citri* (Hemiptera: Liviidae) to mating communication signals from vibration traps in citrus (Sapindales: Rutaceae) trees. Fla Entomol 100:767–771

Heslop-Harrison G (1960) Sound production in the Homoptera with special reference to sound producing mechanisms in the Psyllidae. Ann Mag Nat Hist 3:633–640

Hodkinson ID (1974) The biology of the Psylloidea (Homoptera): a review. Bull Entomol Res 64: 325–338

Hodkinson ID (1986) The psyllids (Homoptera: Psylloidea) of the Oriental Zoogeographical Region: an annotated check-list. J Nat Hist 20:299–357

Hodkinson ID (1997) Progressive restriction of host plant exploitation along a climatic gradient: the willow psyllid *Cacopsylla groenlandica* in Greenland. Ecol Entomol 22(1):47–54

Hodkinson ID (2009) Life cycle variation and adaptation in jumping plant lice (Insecta: Hemiptera: Psylloidea): a global synthesis. J Nat Hist 43:65–179

Ichikawa T (1982) Density-related changes in male-male competitive behavior in the rice brown planthopper, *Nilaparvata lugens* (Stal) (Homoptera: Delphacidae). Appl Entomol Zool 17(4): 439–452

Jost MC, Shaw KL (2006) Phylogeny of Ensifera (Hexapoda: Orthoptera) using three ribosomal loci, with implications for the evolution of acoustic communication. Mol Phylogenet Evol 38(2):510–530

Knowlton GF, Thomas WL (1934) Host plants of the potato psyllid. J Econ Entomol 27:547

Krysan JL (1990) Laboratory study of mating behaviour as related to diapause in overwintering *Cacopsylla pyricola* (Homoptera: Psyllidae). Environ Entomol 19:551–557

Ladányi M, Horváth L (2010) A review of the potential climate change impact on insect populations—general and agricultural aspects. Appl Ecol Env Res 8(2):143–152

Lapis EB, Borden JH (1993) Olfactory discrimination by *Heteropsylla cubana* (Homoptera: Psyllidae) between susceptible and resistant species of Leucaena (Leguminosae). J Chem Ecol 19:83–90

Laumann RA, Maccagnan DHB, Čokl A, Blassioli-Moraes MC, Borges M (2018) Substrate-borne vibrations disrupt the mating behaviors of the neotropical brown stink bug, *Euschistus heros*: implications for pest management. J Pest Sci 91(3):995–1004

Laurema S (1989) Free amino-acids in the psyllid *Trioza apicalis* Först (Homopt., Triozidae) and in carrot leaves. Ann Agric Fenn 28:113–120

Lewis-Rosenblum H, Martini X, Tiwari S, Stelinski LL (2015) Seasonal movement patterns and long-range dispersal of Asian citrus psyllid in Florida citrus. J Econ Entomol 108(1):3–10

Liao YC, Yang MM (2015) Acoustic communication of three closely related psyllid species: a case study in clarifying allied species using substrate-borne signals (Hemiptera: Psyllidae: *Cacopsylla*). Ann Entomol Soc Am 108:902–911

Liao YC, Yang MM (2017) First evidence of vibrational communication in Homotomidae (Psylloidea) and comparison of substrate-borne signals of two allied species of the genus *Macrohomotoma* Kuwayama. J Insect Behav 30:567–581

Liao YC, Huang SS, Yang MM (2016) Substrate-borne signals, specific recognition, and plant effects on the acoustics of two allied species of Trioza, with the description of a new species (Psylloidea: Triozidae). Ann Entomol Soc Am 109:906–917

Liao YC, Wu ZZ, Yang MM (2019) Vibrational behaviour of psyllids (Hemiptera: Psylloidea): functional morphology and mechanisms. bioRxiv 593533

Liao Y-C, Percy DM, Yang M-M (2022) Biotremology: vibrational communication of Psylloidea. Arthropod Struct Dev 66:101138

Lubanga UK, Guédot C, Percy D, Steinbauer M (2014) Semiochemical and vibrational cues and signals mediating mate finding and courtship in Psylloidea (Hemiptera): a synthesis. Insects 5: 577

Lubanga UK, Peters RA, Steinbauer MJ (2016) Substrate-borne vibrations of male psyllids vary with body size and age but females are indifferent. Anim Behav 120:173–182

Lujo S, Hartman E, Norton K, Pregmon EA, Rohde BB, Mankin RW (2016) Disrupting mating behaviour of *Diaphorina citri* (Liviidae). J Econ Entomol 109:2373–2379

MacLean SF (1983) Life Cycles and the distribution of Psyllids (Homoptera) in Arctic and Subarctic Alaska. Oikos 40:445–451

Mankin R, Hagstrum DW (2012) Acoustic monitoring of insects. In: Hagstrum DW, Phillips TW, Cuperus G (eds) Stored product protection. K-State Research and Extension Kansas State University, Manhattan, KS, pp 263–279

Mankin RW, Hagstrum DW, Smith MT, Roda AL, Kairo MTK (2011) Perspective and promise: a century of insect acoustic detection and monitoring. Am Entomol 57:30–44

Mankin RW, Rohde BB, McNeill SA, Paris TM, Zagvazdina NI, Greenfeder S (2013) *Diaphorina citri* (Hemiptera: Liviidae) responses to microcontroller-buzzer communication signals of potential use in vibration traps. Fla Entomol 96:1546–1555

Mankin RW, Rohde B, McNeill S (2015) Vibrational duetting mimics to trap and disrupt mating of the devastating Asian citrus psyllid insect pest. Proc Meet Acoust 25:010006

Mankin RW, Patel R, Grugnale M, Jetter E (2020) Effects of *Diaphorina citri* population density on daily timing of vibrational communication calls: potential benefits in finding forage. Insects 11: 82

Mann RS, Ali JG, Hermann SL, Tiwari S, Pelz-Stelinski KS, Alborn HT, Stelinski LL (2012) Induced release of a plant-defense volatile 'deceptively' attracts insect vectors to plants infected with a bacterial pathogen. PLoS Pathog 8:e1002610

Mann RS, Rouseff RL, Smoot J, Rao N, Meyer WL, Lapointe SL, Robbins PS, Cha D, Linn CE, Webster FX, Tiwari S, Stelinski LL (2013) Chemical and behavioural analysis of the cuticular hydrocarbons from Asian citrus psyllid, *Diaphorina citri*. Insect Sci 20:367–378

Markkula M, Laurema S (1971) Phytotoxaemia caused by *Trioza apicalis* Först. (Horn., Triozidae) on carrot. Ann Agric Fenn 10:181–184

Martini X, Kuhns E, Hoyte A, Stelinski L (2014) Plant volatiles and density-dependent conspecific female odors are used by Asian citrus psyllid to evaluate host suitability on a spatial scale. Arthropod Plant Interact 8:453–460

Mas F, Vereijssen J, Suckling DM (2014) Influence of the pathogen *Candidatus* Liberibacter solanacearum on tomato host plant volatiles and psyllid vector settlement. J Chem Ecol 40: 1197–1202

Mazzoni V, Presern J, Andrea L, Virant-Doberlet M (2009) Reproductive strategy of the Nearctic leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae). Bull Entomol Res 99:401–413

Mazzoni V, Eriksson A, Anfora G, Andrea L, Virant-Doberlet M (2014) Active space and role of amplitude in plant-borne vibrational communication. In: Cocroft R, Gogala M, Hill P, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 125–145

Mazzoni V, Polajnar J, Baldini M, Stacconi MVR, Anfora G, Guidetti R, Maistrello L (2017) Use of substrate-borne vibrational signals to attract the brown marmorated stink bug, *Halyomorpha halys*. J Pest Sci 90:1219–1229

Mazzoni V et al (2019) Mating disruption by vibrational signals: state of the field and perspectives. In: Hill P, Lakes-Harlan R, Mazzoni V, Narins P, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational behavior, Animal signals and communication, vol 6. Springer, Cham. https://doi.org/10.1007/978-3-030-22293-2_17

Molki B, Ha PT, Cohen AL, Crowder DW, Gang DR, Omsland A, Brown JK, Beyenal H (2019) The infection of its insect vector by bacterial plant pathogen *Candidatus* Liberibacter solanacearum is associated with altered vector physiology. Enzyme Microb Technol 129: 109358

Moran VC, Brown RP (1973) The antennae, host plant chemoreception and probing activity of the citrus psylla, *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae). J Entomol Soc S Afr 36(2): 191–202

Munyaneza JE (2010) Psyllids as vectors of emerging bacterial diseases of annual crops. Southwest Entomol 35:471–477

Munyaneza JE, Crosslin JM, Upton JE (2007) Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with “zebra chip”, a new potato disease in southwestern United States and Mexico. J Econ Entomol 100(3):656–663

Nelson BS (2003) Reliability of sound attenuation in Florida scrub habitat and behavioral implications. J Acoust Soc Am 113(5):2901–2911

Nieri R, Mazzoni V (2019) Vibrational mating disruption of *Empoasca vitis* by natural or artificial disturbance noises. Pest Manag Sci 75(4):1065–1073

Nieri R, Mazzoni V, Gordon SD, Krugner R (2017) Mating behavior and vibrational mimicry in the glassy-winged sharpshooter, *Homalodisca vitripennis*. J Pest Sci 90(3):887–899

Nokkala S, Maryanska-Nadachowska A, Kuznetsova VG (2008) First evidence of polyploidy in Psylloidea (Homoptera, Sternorrhyncha): a parthenogenetic population of *Cacopsylla myrtilli* (W. Wagner, 1947) from northeast Finland is apomictic and triploid. Genetica 133:201–205

Oppedisano T, Polajnar J, Kostanjšek R, De Cristofaro A, Ioriatti C, Virant-Doberlet M, Mazzoni V (2020) Substrate-borne vibrational communication in the vector of Apple Proliferation Disease *Cacopsylla picta* (Hemiptera: Psyllidae). J Econ Entomol 113(2):596–603

Ossiannilsson F (1950) Sound-production in Psyllids (Hem Hom.). Opuscula Entomologica 15:202

Ouvrard D (2016) Psyl’list—The world psylloidea database. Available at: http://www.hemiptera-databases.com/psyllist

Patt JM, Setamou M (2010) Responses of the Asian citrus psyllid to volatiles emitted by the flushing shoots of its rutaceous host plants. Environ Entomol 39(2):618–624

Patt JM, Meikle WG, Mafra-Neto A, Sétamou M, Mangan R, Yang C, Malik N, Adamczyk JJ (2011) Multimodal cues drive host-plant assessment in Asian citrus psyllid (*Diaphorina citri*). Environ Entomol 40(6):1494–1502

Percy DM, Page RDM, Cronk QCB (2004) Plant-insect interactions: double-dating associated insect and plant lineages reveals asynchronous radiations. Syst Biol 53:120–127

Percy DM, Taylor GS, Kennedy M (2006) Psyllid communication: acoustic diversity, mate recognition and selection. Invertebr Syst 20:431–445

Pertot I, Caffi T, Rossi V, Mugnai L, Hoffmann C, Grando MS, Gary C, Lafond D, Duso C, Thiery D, Mazzoni V, Anfora G (2016) A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. Crop Prot 97:70–84

Polajnar J, Čokl A (2008) The effect of vibratory disturbance on sexual behaviour of the southern green stink bug *Nezara viridula* (Heteroptera, Pentatomidae). Cent Eur J Biol 3:189–197

Polajnar J, Eriksson A, Stacconi MVR, Lucchi A, Anfora G, Virant-Doberlet M, Mazzoni V (2014) The process of pair formation mediated by substrate-borne vibrations in a small insect. Behav Process 107:68–78

Polajnar J, Eriksson A, Lucchi A, Anfora G, Virant-Doberlet M, Mazzoni V (2015) Manipulating behaviour with substrate-borne vibrations—potential for insect pest control. Pest Manag Sci 71: 15–23

Polajnar J, Maistrello L, Ibrahim A, Mazzoni V (2019) Can vibrational playback improve control of an invasive stink bug. In: Hill P, Lakes-Harlan R, Mazzoni V, Narins P, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational behavior, Animal signals and communication, vol 6. Springer, Cham. https://doi.org/10.1007/978-3-030-22293-2_1

Polidori C, Pavan G, Ruffato G, Asís JD, Tormos J (2013) Common features and species-specific differences in stridulatory organs and stridulation patterns of velvet ants (Hymenoptera: Mutillidae). Zool Anz 252(4):457–468

Robbins PS, Alessandro RT, Stelinski LL, Lapointe SL (2012) Volatile profiles of young leaves of Rutaceae spp. varying in susceptibility to the Asian citrus psyllid (Hemiptera: Psyllidae). Fla Entomol 95(3):774–776

Saxena KN, Kumar H (1980) Interruption of acoustic communication and mating in a leafhopper and a planthopper by aerial sound vibrations picked up by plants. Experientia 36:933–936

Scharf I, Martin OY (2013) Same-sex sexual behavior in insects and arachnids: prevalence, causes, and consequences. Behav Ecol Sociobiol 67(11):1719–1730

Soroker V, Talebaev S, Harari AR, Wesley SD (2004) The role of chemical cues in host and mate location in the pear psylla *Cacopsylla bidens* (Homoptera : Psyllidae). J Insect Behav 17:613–626

Stockton DG, Pescitelli LE, Martini X, Stelinski LL (2017) Female mate preference in an invasive phytopathogen vector: how learning may influence mate choice and fecundity in *Diaphorina citri*. Entomol Exp Appl 164:16–26

Takanashi T, Uechi N, Tatsuta H (2019) Vibrations in hemipteran and coleopteran insects: behaviours and application in pest management. Appl Entomol Zool 54:21–29

Taylor KL (1985) A possible stridulatory organ in some Psylloidea (Homoptera). Aust J Entomol 24:77–80

Tedeschi R, Baldessari M, Mazzoni V, Trona F, Angeli G (2012) Population dynamics of *Cacopsylla melanoneura* (Hemiptera: Psyllidae) in northeast Italy and its role in the apple proliferation epidemiology in apple orchards. J Econ Entomol 105:322–328

Tishechkin DY (1989) Acoustic signalization of psyllids (Homoptera: Psyllinae) of the Moscow Province. Mosc Univ Biol Sci Bull 44:20–24

Tishechkin DY (2005) Vibratory communication in Psylloidea (Homoptera). In: Drosopoulos S, Claridge MF (eds) Insect sounds and communication: physiology, behaviour, ecology and evolution. Taylor and Francis, Boca Raton, pp 357–363

Tishechkin DY (2006) On the structure of stridulatory organs in jumping plant lice (Homoptera: Psyllinea). Russ Entomol J 15:335–340

Tishechkin DY (2007) New data on vibratory communication in jumping plant lice of the families Aphalaridae and Triozidae (Homoptera, Psyllinea). Entomol Rev 87:394–400

Tishechkin DY (2008) On the similarity of temporal pattern of vibrational calling signals in different species of Fulgoroidea (Homoptera: Auchenorrhyncha). Russ Entomol J 17(4): 349–357

Tishechkin DY (2014) The use of bioacoustic characters for distinguishing between cryptic species in insects: potentials, restrictions, and prospects. Entomol Rev 94(3):289–309

Tsai JH, Liu YH (2000) Biology of *Diaphorina citri* (Homoptera: Psyllidae) on four host plants. J Econ Entomol 93:1721–1725

Van den Berg MA, Deacon VE, Thomas CD (1991) Ecology of the citrus psylla, *Trioza erytreae* (Hemiptera: Triozidae). 3. Mating, fertility and oviposition. Phytophylactica 23:195–200

Van Klinken RD (2000) Host-specificity constrains evolutionary host change in the psyllid *Prosopidopsylla flava*. Ecol Entomol 25:413–422

Vereijssen J (2020) Ecology and management of *Bactericera cockerelli* and *Candidatus* Liberibacter solanacearum in New Zealand. J Integr Agric 19:333–337

Virant-Doberlet M, Čokl A (2004) Vibrational communication in insects. Neotrop Entomol 33: 121–134

Wan J, Zhou L, Zhang Q, Xu H (2013) Extraction and GC-MS identification of sex pheromone components from winterform adults *Cacopsylla chinensis* (Yang et Li). J China Agric Univ 18(2):79–85

Wenninger EJ, Hall DG (2007) Daily timing of mating and age at reproductive maturity in *Diaphorina citri* (Hemiptera: Psyllidae). Fla Entomol 90:715–722

Wenninger EJ, Stelinski LL, Hall DG (2008) Behavioural evidence for a female-produced sex attractant in *Diaphorina citri*. Entomol Exp Appl 128:450–459

Wenninger EJ, Hall DG, Mankin RW (2009) Vibrational communication between the sexes in *Diaphorina citri* (Hemiptera: Psyllidae). Ann Entomol Soc Am 102:547–555

West-Eberhard MJ (1984) Sexual selection, competitive communication and species specific signals in insects. In: Insect communication: 12th symposium of the Royal Entomological Society of London. Academic, London

Wood R, Peters R, Taylor G, Steinbauer M (2016) Characteristics of the signals of male *Anoeconeossa bundoorensis* Taylor and Burckhardt (Hemiptera: Aphalaridae) associated with female responsiveness. J Insect Behav 29:1–14

Yang MM, Yang CT, Chao JT (1986) Reproductive isolation and taxonomy of two Taiwanese *Paurocephala* species (Homoptera: Psylloidea). Special Publication Series, Taiwan Museum, pp 177–203

Yasuda K, Kawamura F, Oishi T (2005) Location and preference of adult Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae) on Chinese box orange jasmine, *Murraya exotica* L. and flat lemon, *Citrus depressa*. Jpn J Appl Entomol Zool 49:146–149

Zaballos JP, Perez-Gonzalez S (2011) A new species of *Typhlocharis* Dieck, 1869 (Coleoptera: Carabidae: Anillini) from south Spain, with notes on the phylogenetic value of sexually related characters and the presence of stridulatory organ (pars stridens) in the genus. Zootaxa 2786(1): 42–50

Chapter 23
Potential of Biotremology for Monitoring and Control of Stink Bugs

Raul Alberto Laumann, Andrej Čokl, Maria Carolina Blassioli Moraes, Aline Moreira Dias, and Miguel Borges

Abstract Pentatomidae is one of the largest families among Heteroptera. Because of their general phytophagous habit and wide host plant spectrum, many species of stink bugs are serious agricultural pests. Behavioral manipulation of stink bugs could offer a new strategy for stink bug management based in biorational tools. Reproductive behavior of stink bugs includes communication involving interchange of chemical, mechanical, and visual signals. The knowledge of signal nature and structure, how they are transmitted by the specific channel, the reception and physiological processes involved, and how the signals of different modalities interact could help to find steps of the reproductive behavior that could be manipulated by interference with the insect's communication. Application of biotremology for stink bugs management considers two potential strategies. In one, signals could be used alone or in combination with chemical signals (pheromones) as baits to be incorporated in traps for population monitoring. Vibratory signals could be used also in indirect monitoring by the development of specific sensors to sample the signals in the field. In the other strategy, artificial or natural signals could be used to interfere in the reproductive behavior, thus disrupting communication. In this chapter, a general overview of stink bug communication is presented. Recent results of applied biotremology for stink bug monitoring and control are described and their potential for effective use is discussed. The possibility of interactions between biotremology and biological control of stink bugs is also outlined.

R. A. Laumann (✉) · M. C. Blassioli Moraes · M. Borges
Semiochemicals Laboratory, Embrapa Genetic Resources and Biotechnology, Brasilia, DF, Brazil
e-mail: raul.laumann@embrapa.br; carolina.blassioli@embrapa.br; miguel.borges@embrapa.br

A. Čokl
Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia
e-mail: Andrej.Cokl@nib.si

A. M. Dias
Zoology Post-Graduation Programme, Institute of Biology, University of Brasilia, Brasília, DF, Brazil

P. S. M. Hill et al. (eds.), *Biotremology: Physiology, Ecology, and Evolution*, Animal Signals and Communication 8, https://doi.org/10.1007/978-3-030-97419-0_23

23.1 Introduction: General Overview of Stink Bug Reproductive Behavior and Communication

23.1.1 Reproductive Behavior

Pentatomidae, with approximately 4900 species described in 10 subfamilies, is one of the largest families among Heteroptera (Rider et al. 2018). Most members of Pentatomidae are phytophagous with the sole exception of Asopinae, which feed primarily as predators of other insects and alternatively on plants (Bueno and van Lenteren 2012).

Stink bugs mainly feed on fruits and immature seeds. Most of the species are polyphagous, feeding preferentially on Leguminosae, Solanaceae, and Brassicaceae. Associated or less-preferred host plants could include species of Arecaceae, Asteraceae, Malvaceae, Poaceae, Rosaceae, Rubiaceae, and Rutaceae (Panizzi and Silva 2012; Panizzi and Lucini 2017).

Some cues originating from plants, for example, plant volatiles, could be related to host plant selection/discrimination by stink bugs (Panizzi and Lucini 2017). However, due to the wide host plant spectrum of many species, plants do not provide insects sufficient information for reliable localization of reproductive partners. Reproductive behavior of stink bugs is mediated instead by the complex pattern of communication involving interchange of chemical, mechanical, and visual signals used in different phases of the reproductive process.

Different aspects of stink bugs' reproductive behavior have been recently reviewed by Čokl et al. (2017a, 2019a), including behavioral steps, communication channels, signal characteristics, and physiology of reception, transduction, and transmission of signals. Here we provide a general overview for readers not familiar with stink bugs' biology and behavior.

The general pattern of reproductive behavior in the green stink bug, *Nezara viridula* Linnaeus 1758 (Borges et al. 1987), and several (but not all) stink bug species starts at long range with the emission of a pheromone by males that aggregates conspecifics (Nearctic species) or acts as a sexual pheromone in Neotropical species (Borges and Blassioli-Moraes, 2017; Weber et al. 2018). The male pheromone triggers production of female substrate-borne signals (calling signals) on the plant (at medium range), and these initiate communication by mechanical signals. Long sequences of female calling signals, transmitted through the plant, provide information on signaller identity and location (Čokl et al. 2017a). Males respond to the calling females with male calling song and directional searching. However, in some species males produce the calling song signals spontaneously, triggering female vibratory responses, or respond to female calling by the courtship song (Čokl et al. 2017a). The calling phase of reproductive behavior is followed by the courtship phase, which is characterized by dueting with courtship song vibratory signals, antennation, and butting at close range (Čokl et al. 2019a). Short-range communication is more complex because it includes communication with signals of different modalities, which precede copulation (Čokl et al. 2017a).

23.1.2 Chemical Communication

Communication using chemical signals (semiochemicals) is crucial in the life history of most living organisms. In insects, chemical communication is probably the primary signal used in long-range communication to attract conspecifics to the same habitat. In addition, chemical information is also used in interactions with host plants and heterospecifics.

Chemical signals have several advantages for communication: they are transmitted through the air, can be carried in the dark, can be perceived from distances ranging from short to long, up to 100 m from the source emission point, and/or transmitted after contact (Price 1997; Bradbury and Vehrencamp 2011). Another advantage is that the molecules are unstable and, when they come into contact with UV light, oxygen, and humidity of the air, they can undergo quick reactions and degradation. Therefore, these signals last long enough only to be perceived by a target organism, reducing the chance of being used as persistent cues by natural enemies or others eavesdroppers (Borges and Blassioli-Moraes 2017).

Semiochemicals are divided into two main classes: (1) pheromones, which are the chemical signals used between individuals of the same species, and (2) allelochemicals, which are the chemical signals used between individuals of different species. Pheromones and allelochemicals are sub-classified depending on their function in communication. The sex pheromone is used to attract a male or female for reproduction; the aggregation pheromone is used to aggregate individuals; the alarm pheromone is used to alert individuals to a dangerous situation; and the trail pheromone is used to guide individuals of the same species, for example, to a food source. The allelochemicals are sub-classified as allomones, kairomones, and synomones, depending on the effect on the organisms; that is, whether the interaction has a beneficial or deleterious effect on the organism. The egg parasitoid, *Telenomus podisi* Ashmead 1893 (Hymenoptera: Scelionidae), uses the sex pheromone of the Neotropical brown stink bug, *Euschistus heros* Fabricius 1798, as a kairomone to locate its preferred host (Borges et al. 1998; Silva et al. 2006); therefore, kairomones are chemicals that benefit the receiver, but not the emitter. Other examples of kairomones are the footprints released by stink bugs when they are walking on the surface of the plant. These footprints are linear and methyl branched hydrocarbons with long chains, and due to the low volatility of these compounds, they remain on the surface of the leaves. Egg parasitoids (Platygastridae) can follow these footprints to locate their host (Borges et al. 2003; Colazza et al. 2007). When soybean plants are subjected to feeding by *E. heros* they enhance volatile production. These volatiles are called herbivory-induced plant volatiles (HIPVs), and they attract the natural enemy of *E. heros* (Michereff et al. 2011). In contrast, allomones are allelochemicals that benefit the emitter, but do not benefit the receiver. The defensive compounds released by stink bugs protect them against several predators (Eliyahu et al. 2012).

23.1.2.1 Stink Bugs' Semiochemistry

Two reviews have recently been published about the semiochemistry of stink bug pheromones. Borges and Blassioli-Moraes (2017) describe in detail the role of semiochemicals in the communication process and behavior modulation, and Weber et al. (2018) examine the chemical structure of semiochemicals, their synthesis, and the role of these molecules in the communication of stink bugs. The reader is therefore directed to these thorough reviews in order to obtain more details about the chemistry of these pheromones.

Stink bugs are well known for the strong odor that they release when disturbed, caused by chemicals that are defensive compounds. In nymphs, the defensive compounds are produced and stored in the dorsal abdominal glands, while in adults, they are produced in the metathoracic glands and stored in a large reservoir (Pavis 1987; Aldrich 1988, 1995; Blassioli-Moraes et al. 2008). Defensive compounds have been identified in several species of Pentatomidae, but their functional roles have been tested in bioassays in only a few species. The chemistry of defensive compounds is composed basically of short chain (*E*)-2-alkenals, 4-oxo-(*E*)-2-alkenals (C_6, C_8, C_{10}), and linear hydrocarbons, mainly C_{11} to C_{13}. The production of these compounds changes with nymphal stage. The adults produce the same defensive compounds as the nymphs, but with fewer 4-oxo-(*E*)-2-alkenals (C_6, C_8, C_{10}), and higher levels of hydrocarbons, mainly C_{11} to C_{13}. Borges and Aldrich (1992) showed that 4-oxo-(*E*)-2-decenals are produced more profusely by first-stage nymphs of *N. viridula*, *E. heros,* and *Thyanta perditor* Fabricius 1794, and with age the level of these compounds drops; therefore, the authors propose that these compounds signal stink bugs to aggregate. However, there are very few studies that try to understand the intraspecific function of these compounds, and no study evaluates whether these chemicals could affect other signals produced by stink bugs, like vibrational signals. Most studies conducted with the defensive compounds report their function as allomones (Weber et al. 2018).

The sex pheromone of stink bugs has been identified only in 45 species, in all cases the male is the sex pheromone producer. The laboratory behavior assays conducted in several studies showed that the females respond to the conspecific sex pheromone, and to synthetic solutions containing the sex or aggregation pheromone (Weber et al. 2018; Borges and Blassioli-Moraes 2017). Behavioral reproductive studies showed that males release the sex or aggregation pheromone and females select the male for mating (Borges et al. 1987; Borges 1995; Blassioli-Moraes et al. 2014; Laumann et al. 2016). Males begin to produce the sex or aggregation pheromone when they reach sexual maturity, which in several species happened after 6–8 days in the adult stage. The chemistry of the sex and aggregation pheromones shows wide diversity in chemical structure, and they are quite complex molecules due to the presence of two or more chiral centers. However, all pheromones identified in stink bugs are fatty acids or terpene derivatives with a chain from 10 to 18 carbons. From the 45 species studied, 25 produce sesquiterpenes as sex or aggregation pheromones and 20 produce methyl esters.

Redundancy, i.e., the same molecules with the same function in different species, is common in stink bug pheromones. Specificity of the sex pheromone is provided by the presence of chiral centers in the molecules or multiple components in the blend. For example, three Neotropical stink bugs, which are rice pests, produce the compound 1,10-bisaboladien-3-ol as a sex pheromone. The rice stink bug, *Tibraca limbativentris* Stål 1860, produces two isomers of this molecule as a sex pheromone, namely isomers (3*S*,6*S*,7*R*)-1,10-bisaboladien-3-ol and (3*R*,6*S*,7*R*)-1,10-bisaboladien-3-ol (Borges et al. 2006; Blassioli-Moraes et al. 2019). However, *Oebalus poecilus* Voet 1778 (Oliveira et al. 2013) produces only one isomer, (3*S*,6*S*,7*R*)-1,10-bisaboladien-3-ol, and *G. spinosa* produces two of these isomers (but the absolute configuration has not yet been determined) (Borges and Blassioli-Moraes 2017). The two Nearctic stink bugs, *Halyomorpha halys* Stål 1855 and *Murgantia histrionica* Hahn, 1834, also share the same molecule as the sex pheromone, which is the compound 10,11-epoxy-1-bisabolen-3-ol, but with different absolute configuration. *M. histrionica* produces the isomers 3*S*,6*S*,7*R*,10*S* and 3*S*,6*S*,7*R*,10*R*; whereas *H. halys* produces the isomers 3*S*,6*S*,7*R*,10*S* and 3*R*,6*S*,7*R*,10*S*. Different male species of *Nezara* sp. and *Chinavia* sp. have a blend of *cis*-(Z)-bisabolene epoxide and *trans*-(Z)-bisabolene epoxide as a sex pheromone, and the specificity is guaranteed by different ratios of these two components in the sex pheromone blend. There are few examples of redundancy in chemical communication of stink bugs, probably due to the few species in which the sex pheromone has been identified so far. Signal redundancy in chemical communication is quite common in Lepidoptera (Mori 2007; Blassioli-Moraes et al. 2016), and also in the chemical interaction between insects and plants (Magalhães et al. 2018).

Females of *E. heros* and *Edessa meditabunda* Fabricius 1794 are attracted to synthetic mixtures of their pheromones with components that are not naturally produced by conspecific males; whereas *Thyanta perditor* and other stink bugs respond only to the isomer produced naturally. Females of *Chinavia ubica* Rolston 1983 and *Chinavia impicticornis* Stål 1872 in olfactometer bioassays only responded to synthetic solutions containing the components with the same relative configuration naturally produced by males (Blassioli-Moraes et al. 2012). The response of females to racemic mixtures minimizes the costs to produce pheromones that are to be applied for control of these pests in field conditions.

Stink bugs, in general, produce the sex pheromone during all of the photophase period, but some species showed higher levels of pheromone production during the late afternoon and in the first hours of the night. This was shown in *M. histrionica* (Zahn et al. 2008), *E. meditabunda* (Zarbin et al. 2012), *Chlorochroa uhleri* Stål 1872, and *Chlorochroa ligata* Say 1832 (Ho and Millar 2001). In general, all behavioral bioassays with Neotropical stink bugs were conducted during the late afternoon or during the night, indicating that this is the period of reproductive activity of the insects (Borges and Blassioli-Moraes 2017).

23.1.3 Vibratory Communication

Stink bugs, like many other insects, communicate on the plant by the substrate-borne component of the mechanical signals they produce as the most efficient exchange of information during calling, courting, rivalry, and other phases of mating behavior (Cocroft and Rodriguez 2005).

Stink bugs produce vibratory signals by vibration of the abdomen, tremulation of the whole body, buzzing with lifted wings, and by percussion with front legs or abdomen on the substrate. The airborne component of vibratory signals can be used for communication in the acoustic near field or by partners that stand at a short distance on mechanically isolated substrates. During courtship, nearby mates exchange information by vibratory and contact chemical signals, by vision and touch (Čokl et al. 2017a).

The main repertoire of stink bug species and sex-specific substrate-borne communication signals is produced by vibration of the abdomen. Frequency and temporal parameters of vibratory signals, identified and described in more than 37 different stink bug species, show several group characteristics (Čokl et al. 2017a). Calling, courtship, rival, and copulatory songs differ between sexes and species predominantly by duration, repetition time, amplitude modulation (AM) and the pattern of combined pulses and/or pulse trains of the song structure.

The amplitude of signals, measured at the body of stink bugs signaling on the plant (expressed in velocity units), ranges around and below 1 mm/s. The main emitted energy ranges above 60 and below 200 Hz with the dominant spectral peak in the majority of them between 80 and 100 Hz (Čokl et al. 2017a).

The stink bug sensory system that developed to detect substrate-borne vibratory communication signals with high sensitivity includes leg subgenual and joint chordotonal organs, together with non-grouped campaniform sensilla (Čokl 1983; Michel et al. 1983). Mechanoreceptors located in antennae detect high-amplitude and low-frequency vibrations (Jeram 1996; Jeram and Pabst 1996), trichobothria are highly sensitive to air particle movement in the acoustic near field (Schaefer 1975; Šolinc 2017) and Nishino et al. (2016) described mechanoreceptors in the stink bug body that might be at least indirectly involved in different processes of vibrational communication.

Recent investigations of stink bugs' reproductive behavior on a plant are focused on the less understood role of low sex- and species-specific high-amplitude vibratory signals, produced by body tremulation and vibration of lifted wings (buzzing) (Žunič et al. 2008; Kavčič et al. 2013; Laumann et al. 2013). Investigations of buzzing signals produced by vibration of lifted wings by three Neotropical stink bugs species *Chinavia impicticornis*, *C. ubica* and *Euschistus heros* reveal species' non-specific temporal parameters, characterized by irregular repetition rate and highly variable duration and have high amplitude than those of abdominal vibratory signals. Buzzing signals are emitted in the early phase of mating communication, both females and males emit signals spontaneously when alone on a plant or in presence of others individuals, including heteroespecifics. The emission of buzzing signals precede the emission of signals produced by abdomen vibrations. Buzzing signals are efficiently transmitted along and between neighboring plants. The characteristics of signals and the behavioral context in which there are emitted suggest an advertise function but the specific reactions of receptors to buzzing signals emitted on the same or other plants demand further studies (Čokl et al. 2021a). Body tremulations were recorded and observed in interactions including physical contacts between conspecific and/or heterospecific individuals, suggesting they have an aggression function. Behavioral context and underlying mechanisms of these signals demand further comparative studies. The airborne component of high-amplitude body or wing vibrations enables non-specific recognition of stink bug presence between mechanically isolated substrates at a distance of only a few centimeters (Žunič et al. 2008; Kavčič et al. 2013). Furthermore, we may speculate that air particle movement in the acoustic near field, induced by often observed tremulations during positioning of mates before copulation, informs them about the position and orientation of the body by stimulating highly sensitive trichobothria on the stink bug abdomen. The role of buzzing, tremulatory, and percussion signals is under-investigated and is currently the focus of research on mechanisms that direct stink bug reproductive behavior.

Plants represent the non-inert type of vibratory signal transmission medium (Cremer et al. 1973), changing the amplitude, frequency, and temporal characteristics of signals. Michelsen et al. (1982) confirmed that insects communicate through plants by bending waves that propagate with frequency-dependent velocity and overall low and non-linear amplitude decay. The amplitude of stem-transmitted vibratory signals varies with peaks of minimal and maximal values, regularly repeated over distances, decreasing with increasing signal frequency (Čokl et al. 2007). Michelsen et al. (1982) attributed amplitude differences at nodes and antinodes to vibratory signal transmission occurring in standing wave conditions, and Polajnar et al. (2012) demonstrated that regular amplitude variation with distance is caused by the plant's resonance.

Amplitude, frequency, and temporal characteristics of vibratory signals change during transmission through the plant according to its mechanical properties and the distance between the sender and receiver. Signal frequency components above 400 Hz are highly attenuated, as shown, for example, in stridulatory signals produced under soil by burrowing bug species *Scaptocoris carvalhoi* Becker 1967 and *Scaptocoris castanea* Perty 1830 (Cydnidae) (Čokl et al. 2006). The frequency-dependent propagation velocity of plant-transmitted vibratory signals changes the delay between more rapidly propagated higher-frequency and the slower lower-frequency spectral components of FM signals. The time delay between high- and low-frequency components of FM signals increases with increasing distance and may give information about the distance to the source. This hypothesis needs to be experimentally confirmed. Vibratory signal amplitude varies with the distance, regularly and in phase with the amplitude of its dominant frequency peak, every 20 cm for 100 Hz vibration and half of it for its first harmonic peak. Consequently the ratio of spectral peak amplitudes changes with distance, and at certain positions on the plant the amplitude of the first harmonic peak becomes dominant (Čokl et al. 2007; for a complete revision of this subject see Čokl et al. 2021b).

Plant resonance and reflections at the phase border increase duration of transmitted vibratory signals. Increased duration of fast repeated pulse-train pulses may become critical for song recognition when they fuse and change the pattern of signal amplitude modulation (AM). *N. viridula* males, for example, differentiated female signals composed of pulses or pulse trains when these were reproduced on a non-resonant substrate, but not on the plant (Miklas et al. 2001).

23.2 Reproductive Behavior in a Multimodal Communication System

Multimodal communication systems include signals transmitted by different channels carrying different information components that are detected by different receptors, in contrast with unimodal (component unique) or multicomponent (more than one component) signals, which use a single transmission channel and are detected by a single or multiple specialized receptors (Partan and Marler 2005; Higham and Hebets 2013).

In general, when management methods based on the communication process are developed, unimodal or multicomponent signals are studied and tested. However, a focus on the interaction between signals of different modalities could help to develop more efficient tools of monitoring or control by increasing the responses of target insects, developing more specific tools and reducing the risks of resistance.

The investigation of sexual communication in stink bugs has followed the general pattern of research with studies focusing principally on unimodal signals, especially pheromones (Borges and Blassioli-Moraes 2017; Weber et al. 2018) or vibratory signals (Čokl et al. 2017a). Various aspects of multisensory communication recently have been reviewed and described (Čokl et al. 2019a). Here, we give a general overview and describe in detail some new knowledge and aspects that need to be considered in pest management.

When describing reproductive behavior, Borges et al. (1987) suggested that at short range, during the courtship phase, stink bugs use signals of different modalities. Later, in a series of works with different species of stink bugs, it was confirmed that courtship in stink bugs involves substrate-borne vibratory, short-range chemical, visual, and tactile signals (Zahn et al. 2008; Silva et al. 2012; Blassioli-Moraes et al. 2013; Laumann et al. 2011, 2016; Zgonik and Čokl 2014).

In contrast, the volume of information related to multimodal communication, or interaction between signals of different modalities at long/medium range, has been less investigated. One of the examples of signal interactions is the modulation of pheromone production in *Nezara viridula* males by female calling songs (Miklas et al. 2003a). The authors measured the pheromone production by males stimulated by the female calling song of their own or an alien population, male rivalry song, and a 100 Hz artificial signal. The proportion of males stimulated with calling songs of their own or of the other population that released pheromones increased in relation to control (no vibrations or other treatments). In addition, the quantity of pheromone emission in the treatment with female calling songs in the post-stimulation period was higher than in the pre-stimulation period, but it was only observed for the calling songs of their own population. Stimulation with male rivalry songs or artificial signals did not produce any effect on males' pheromone emissions (Miklas et al. 2003a).

In a sequence of experiments to study the role of signals of different modalities in triggering vibrational communication in *N. viridula*, Zgonik and Čokl (2014) demonstrated that chemical stimuli stimulate females to start the production of calling songs. When stimulated by live males, with filter papers impregnated with a male's odors or with filter papers impregnated with synthetic pheromone, a higher proportion of females started to produce calling songs (82.8%, 61.9%, and 59.1%, respectively) in relation to non-stimulated females ($<10\%$). These results clearly show modulation of female song production by chemical signals from males (Zgonik and Čokl 2014).

Studying this phenomenon in *E. heros*, similar tendencies were found. When stimulated with synthetic male pheromones applied to filter papers, females tested on plants increased their rate of responses, and 78% started to sing in comparison with 58% of the females without stimulus. In addition, females in the presence of pheromone reduced the time to start to sing (latency time) and sang for a longer time (response time). Another interesting fact observed was that the female stimulated with male pheromone increased the number of buzzing signals produced. It was confirmed that the emission of pheromone by males, measured in ng/insect, when stimulated by *E. heros* female calling song increases threefold in relation to the emissions of non-stimulated males (Aline Dias, Raúl Laumann, Maria Carolina Blassioli-Moraes, Miguel Borges, unpublished data).

Cuticular hydrocarbons present in the footprints of insects left on plants represent other chemical signals that could interact with signals of other modalities. Because epicuticle waxes of plants absorb the footprints (Colazza et al. 2009), they could act as long-lasting signals, informing receivers about the presence of conspecifics in a specific area and inducing production of signals of other modalities. Some evidence of this hypothesis was presented by Zgonik and Čokl (2014), who interpret spontaneous singing, observed when insects are on plants, as a response to footprints. In a series of experiments, they tested how footprints of *Euschistus heros* could interfere in the vibratory communication of males and females. Results show that male footprints trigger females to produce vibratory calling signals. Males sang spontaneously in the three situations evaluated, with no significant differences between the treatments (Fig. 23.1). Males also showed a lower latency time in the presence of male footprints compared to treatment or female footprints, and this could represent a competitive interaction. When couples were tested in the presence of female or male footprints (or on surfaces without footprints) the presence of female footprints induced a significantly higher proportion of couples to mate (Dias et al. 2018). Preliminary chemical analysis for identification of the compounds in the footprints

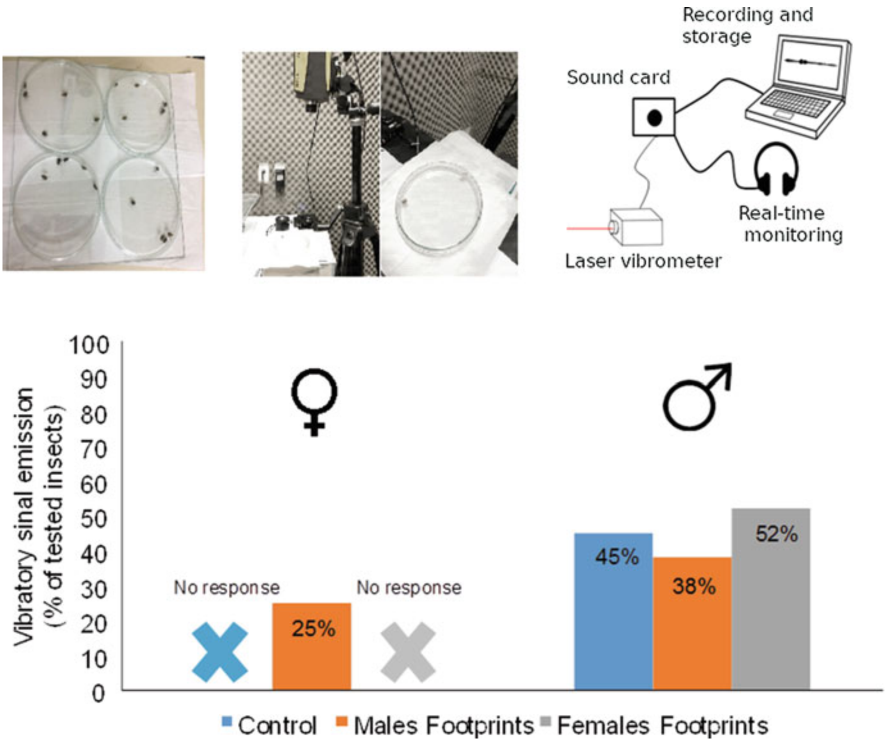


Fig. 23.1 *Top*: General setup to test the effect of stink bugs footprints in vibratory communication of *E. heros*. *Upper left*: setup to obtain arenas contaminated with male or female footprints, *upper center*: general overview of experimental design arena with male or female footprints where a male or a female were placed and system to recording the vibratory signals emission using a laser vibrometer (Polytec PDV 100), *upper right*: general setup to recording vibratory signals emission. *Below*: Percentage of *E. heros* females and males that emit vibratory signals in presence of *E. heros* males footprints, *E. heros* females footprints and Control

showed a qualitative difference in the cuticular hydrocarbons between males and females, and these differences could be the basis of recognition and discrimination by insects and could induce the specific behaviors observed (Dias et al. 2018).

23.3 Reproductive Behavior, Environment, and Complex Networks

Species success depends crucially on efficient reproduction, determined by communication adapted to different environmental conditions. Insects develop mechanisms that increase signal-to-noise ratio at the sender and receiver site. The first and most important step demands incorporation of information into signal parameters that are less influenced by mechanical properties of the transmission medium and by the impacts of environmental noise. At the receiver site, insects developed a complex multimodal sensory system that with high sensitivity detects and processes information, extracted with high selectivity from specific signal parameters. The communication distance depends on efficient tuning of signal production and sensory mechanisms with characteristics of the transmission medium. A holistic approach is needed to understand communication as a complex of mechanisms underlying information exchange at different levels from species biology, ecology, behavior, and feeding habits to the mechanisms that control signal production, detection, and processing in different transmission media and various environmental conditions.

23.3.1 Physical Limits of Stink Bug Communication

Stink bug communication starts in the field with chemical signals transmitted through the air (Borges and Blassioli-Moraes 2017; Weber et al. 2018). The communication distance and directionality of the flow of the small lipophilic molecules through the air (Weissburg 2000) depend on direction and flow of the wind, temperature, humidity, and UV radiation, which differ in the open air or within dense plant growth. Data on different environmental conditions need to use complex mathematical models to estimate the effective communication distance with chemical signals that are transmitted through the air (Laumann et al. 2017; Tillman and Cottrell 2017).

Communication with signals of different modalities faces different constraints caused by the plants. Different architecture and dimensions of plants prevent distant visual contact. Temperature and humidity gradients above soil create differently oriented local air currents around the plant and decrease the value of volatile chemical signals to mediate information on directionality to the source. Footprints left by several stink bugs on the plant lead the searching mate to multiple points on the plant. Plants that serve as low-pass filters strongly attenuate the higher-frequency airborne component of vibratory signals (sound) and for physical reasons, insects of small body size cannot produce low-frequency sound of relevantly high amplitude (Markl 1983; Bennet-Clark 1998).

Plant-borne signals are subjected to the impact of plants as a non-inert transmission medium (see Sect. 23.1.3). Wind represents the main source of abiotic noise that disturbs insect plant-borne vibrational communication. The basic frequency of wind-vibrated leaves ranges below 15 Hz (Casas et al. 1998; Barth 2002). Independently of flow direction, low-speed wind induces vibration of a leaf, with velocity reaching 60 mm/s and increasing at high speed to maximal values of 100 mm/s (Casas et al. 1998).

Vibrations induced by accidental mechanical collisions of leaves, their fluttering in the wind, falling water drops, or insects landing on the leaf are characterized by an initial phase of short duration, high frequency, and high amplitude, followed by a low-frequency phase whose velocity decays according to the plant's resonant properties (Casas et al. 1998).

Signaling with vibratory signals of most energy produced around 100 Hz has many advantages. Communication distance is enlarged by low attenuation during transmission through the plant, and the band-pass frequency characteristic of the communication channel decreases the impact of low-frequency environmental noise like wind, falling water drops, insects landing on the leaf, or their collisions (Čokl et al. 2021b).

23.3.2 Interference and Rivalry

The principal repertory of stink bug calling and courtship vibratory signals has been reviewed recently (Čokl et al. 2017a, 2019a). More attention is currently addressed to interference during conspecific and heterospecific interactions and in conflicts expressed by rivalry songs.

Interference as a consequence of overlapped vibrations of similar spectral characteristics represents another disadvantage of stink bug communication within a narrow frequency range. The AM pattern of similar-frequency, overlapped vibrations changes to sequences of readily repeated, fused, short pulses, whose duration and amplitude decrease with increasing frequency difference between masking vibrations. To avoid interference, the dueting bugs tend to alternate with signals produced within silent intervals between the mate's signal productions, and to minimize its effects by increasing the frequency difference between exchanged signals. Readily observed overlapping of calling song signals has been described in *E. heros* (Čokl et al. 2015). Female calling signals and male vibratory responses overlap because the latency of responses is shorter than female triggering signals and because the male signal is longer than the interval between two consecutive female calling song signals. The interference completely changes the AM pattern and the temporal characteristics of overlapped regions. To minimize and avoid the effect of interference, dueting bugs change their signal frequency and FM pattern to increase the difference between them, and males adjust the latency and duration of their responses to duration of intervals between female calls.

In imbalanced sex conditions, mates compete for access to copulation by rivalry that differs between males and females. Short-term male rivalry is in most cases triggered by female calling and courtship vibratory signals. Most stink bug males enter rivalry after dueting with a calling female with calling or courtship song signals. Rivalry has been described as shorter alternation with readily repeated pulses that inhibits female calling and terminates by silencing one of the rival males. The winning male responds to the female, which starts to call the male again. Such a pattern of male rivalry has been described in species like *C. impicticornis, C. ligata* and *Chlorochroa sayi* Stål 1872*, Dichelops Melacanthus* Dallas 1851*, E. heros, M. histrionica, N. viridula, Piezodorus guildinii* Westwood 1837, and *T. perditor* (Čokl et al. 2017a). Rapidly repeated male rival song pulses of duration around and below one second are produced at the dominant frequency, varying among species between 96 and 149 Hz. Their spectral characteristics differ from male calling and courtship song signals by extensive frequency modulation (FM). Females stop calling during male rivalry until one male is silenced and the winning one proceeds with calling or courting.

Female rivalry develops from simultaneous calling of several females, lasts longer and includes newly described female rival song signals of different types. Rivalry between females was first described only recently in *C. impicticornis*, *C. ubica,* and *E. heros* (Čokl et al. 2017b) and *N. viridula* (Čokl et al. 2019b). The pheromone attracts several conspecific females to land on the same plant and triggers their simultaneous production of the calling song. The pattern of female rivalry is more complex than in males and runs through different successive phases with signals of various temporal and amplitude modulation (AM) pattern characteristics. Within the same song sequence, females show high plasticity of different signal parameters, expressed as changing their temporal and frequency characteristics to avoid overlapping and interference. Rivalry terminates in the silencing of one female at different levels of alternation, with various combinations of pulses and different types of pulse trains. Females discriminate their signals by changing their temporal characteristics at the calling song level and by increasing or decreasing the dominant frequency of alternated trains of different types.

During female rivalry, males detect signals coming from different directions on a plant as a compound vibration with various temporal, frequency, and AM pattern characteristics that altogether decreases their recognition and information value regarding directionality. Consequently, males gradually stop responding and searching for rival females. De Groot et al. (2010) demonstrated in *N. viridula* that combined female playback signals inhibit male emission of vibratory responses during searching behavior. The authors have shown that stimulation with conspecific female calling song signals from two different directions increased male responsiveness when repeated simultaneously with the repetition rate characteristic of natural female signals. Studies of *N. viridula* female rivalry (Čokl et al. 2019b) have shown that during dueting with calling song, females never signal at the same repetition rate but change their duration and repetition time to avoid overlapping. Female dueting decreased and inhibited male responsiveness measured on the plant. De Groot et al. (2011) investigated the ability of *N. viridula* males to differentiate compound signals produced by several females from the same direction. Males decreased their responsiveness because they detected the overlapped signals as a compound vibration with altered temporal parameters.

23.3.3 Recognition and Directionality

In stink bugs pheromones of more or less species-specific ratios of different components do not completely isolate species, and cross-attraction has been described among various species (Borges and Blassioli-Moraes 2017; Weber et al. 2018).

Final (but not always perfect) stink bug species isolation is achieved by multimodal communication on a plant during the calling and courtship phase of mating behavior. Recognition has been studied, usually on the non-resonant substrate or on the plant, by comparing the levels of stereotyped responses to playback with natural signals and to synthesized vibratory signals of different temporal and frequency characteristics. The authors usually correlated stereotyped male responses like directionality of movement and production of the vibratory signals to playback stimuli of varied characteristics. Investigations of both processes at the courtship level are lacking.

Žunič et al. (2011) recorded male *N. viridula* calling and courtship songs as responses to playback signals in a broader range of their duration, repetition time, and dominant frequency characteristics measured for natural signals on the non-resonant substrate. Decrease of duration and inter-pulse interval below the best values, around 0.7 and 1.9 s, respectively, sharply inhibits male response. On the other hand, the authors recorded responses to signals of duration and interval values reaching 10 s. Responses were recorded in the broad frequency range of playback signals between 70 and 250 Hz; although, the dominant frequency of the naturally emitted signals ranged around 107 Hz. These results confirm the hypothesis that the sensory system is adapted to extract the relevant information on sex and species identity from signals whose characteristics change during transmission through the plant.

The decisive role of temporal parameters for stink bug female calling recognition and species isolation has been confirmed in *C. impicticornis* and *C. ubica* species, which are often found together on a plant, exhibiting similar reproductive behavior (Laumann et al. 2016). The authors described species isolation at the very early stage of reproductive behavior on a plant despite the initial heterospecific communication contact. The presence of a conspecific or heterospecific male on a plant triggers females of both species to produce female calling song, but males responded regularly only to calls of the conspecific individual. Silveira et al. (2019) recently confirmed that *C. impicticornis* and *C. ubica* males recognize the conspecific female calling song by the difference in song structure and in specific temporal characteristics.

Statistically significant differences in the *N. viridula* female calling song were described in populations investigated at the same place and in the same season in 1999 (Čokl et al. 2000) and 1 year later (Žunič et al. 2011). A species' characteristic broad response range to various temporal and frequency parameters enables communication between mates from Brazil and Slovenia (Virant-Doberlet et al. 2000) or between those from France and Guadeloupe (Miklas et al. 2003b). Vibratory signal temporal parameter differences between *N. viridula* populations from Australia and Slovenia (Ryan et al. 1996) did not prevent communication and copulation between females from Slovenia and males from Australia but, on the other hand, Australian females did not recognize Slovene male vibrational emissions and rejected copulation with them (Jeraj and Walter 1998).

Vibrational directionality of stink bugs, demonstrated first in *N. viridula* (Ota and Čokl 1991; Čokl et al. 1999) occurs by the same basic principle as described in the scorpion *Paruroctonus mesaensis* Stahnke 1957 (Brownell and Farley 1979) or the wandering spider *Cupiennius salei* Keyserling 1877 (Hergenröder and Barth 1983). Vibrational directionality is mediated by detection and processing of amplitude, phase, and/or time delay between signals coming from different directions by receptors situated in and on spatially positioned legs across the surface. Stritih et al. (2000) measured above-threshold amplitude differences of signals at the crossing between plant stem and side branch. The amplitude differences do not always favor direction of movement to the source, because the frequency-dependent amplitude variation with distance may create higher signal amplitude at the side contralateral to the calling female (Čokl et al. 2007). Brownell and Farley (1979) determined a 0.2 ms delay to be a threshold for the *P. mesaensis* directionality reaction. At about 2 cm of distance from between the legs of *N. viridula*, spread over a plant crossing (Ota and Čokl 1991), one can expect a delay of the signal arrival above 0.25 ms at propagation velocities of plant-recorded bending waves (Michelsen et al. 1982). Čokl et al. (1985) proved in *Locusta migratoria* Linnaeus 1758 that certain bimodal higher order ventral cord neurons provide directionality information by detecting one to three ms of delay in the signal arrival on different legs. The role of phase differences expressed on a crossing between low-frequency signals arriving from different directions needs to be experimentally confirmed. Prešern et al. (2018) recently confirmed in *N. viridula* that vibratory signal delay is the most reliable information for directionality to the source, with the suggested behavioral threshold between 0.3 and 0.5 ms. In certain ventral cord vibratory interneurons, described previously by Zorović et al. (2008), the authors recorded changed activity at 0.5 ms delays.

23.4 Potential of Biotremology for Monitoring and Control of Stink Bugs

Many species of stink bugs, principally those of Pentatominae and Edessinae, are serious pests of crops such as legumes, grasses, grains, vegetables and fruit, and nut trees (Panizzi et al. 2000). Their impact on agriculture has been increased by some invasive species, and they have strongly affected crops on different continents (Panizzi 2015; McPherson 2018).

The management of stink bugs relies almost entirely on the use of insecticide application, and in some places, such as Brazil, these applications have been increasing steadily in recent years (Panizzi 2013). The rational management of stink bugs needs to be based on efficient and precise monitoring systems (Borges et al. 2011a, b).

Some authors have reviewed the potential of biotremology and vibratory communication of insects to be applied in pest management (Mankin et al. 2011; Mankin 2012; Polajnar et al. 2015; Laumann et al. 2017; Takanashi et al. 2019). From these reviews, it is possible to conclude that vibratory signals of insects could be used in both monitoring and control strategies. The first of these strategies was the most used until now, especially for insects that live in cryptic conditions in plants, in wood, on soil, in stored products and in buildings. The incidental vibrations created by locomotion, feeding, or signals related to social or sexual interactions have been used to detect these insects (Laumann et al. 2017, and references therein).

However, traps with vibratory signals as bait are not yet in commercial use. An autonomous system that uses signals from the Asian citrus psyllid, *Diaphorina citri* Kuwayama 1908 (Hemiptera: Psyllidae), was developed. This system uses a microcontroller piezoelectric device that produces mimicked vibrations and monitors the insect responses (Mankin et al. 2013). A similar system was developed to detect and attract a species of leafhopper from the genus *Aphrodes* Curtis 1833 (Hemiptera: Cicadellidae) (Korinšek et al. 2016).

The application of biotremology to control insects is even less developed. One strategy explored the possibility of causing physiological disruption by substrate-borne vibrations that could cause direct mortality, or interfere with the neuroendocrine process with negative effects on the duration of development and subsequent gains in weight and size. This strategy has been tested principally in insects among stored products (Adamo and Baker 2011; Kight and Swaddle 2011; Polajnar et al. 2015). The other option includes interference with naturally produced or artificially synthetized signals in the communication of insects (Mankin 2012; Eriksson et al. 2012; Polajnar et al. 2015; Laumann et al. 2017).

23.4.1 Application of Biotremology for Monitoring Stink Bugs

The development of efficient systems for monitoring stink bugs is currently decisive for the management both of key pests and invasive species. The techniques most used for monitoring stink bugs, namely the sweep net or beat sheet sampling, have been efficient in estimating stink bugs' community composition and population densities (Todd and Herzog 1980). However, and especially in grain cultures such as soybean, corn, and beans, the actual production systems, which include expansion of cultivated areas, increased plant population, and use of taller plant cultivars, have themselves reduced the efficiency of this monitoring technique and discouraged their use (Laumann et al. 2017). For these reasons, in Brazil and other countries, control of stink bugs usually proceeds using insecticide application based on fixed dates or the phenological stage of the crops, without specific information related to population densities (Sosa-Gomez and Silva 2010).

Monitoring systems based on pheromone traps have been shown to have great potential in Neotropical (Borges et al. 2011a, b; Laumann et al. 2011; Silva et al. 2014) and Nearctic (Tillman and Cottrell 2017) stink bugs. Pheromone traps are efficient in the early detection of field invasion by stink bugs, monitoring spatial and temporal distribution and the dispersal of stink bugs in agricultural landscapes

(Tillman and Cottrell 2017). These traps could also be a very useful tool for monitoring the introduction and dispersion of invasive species (Weber et al. 2018).

However, for population density estimations, field observations have reported that in many situations trap captures did not show a significant positive relationship between the insects captured in pheromone traps and population levels estimated by sweep net or beat sheet sampling (Cullen and Zalom 2000; Borges et al. 2011a; Tillman and Cottrell 2017). In these studies, it was observed that in many cases insects are attracted to pheromone-baited traps but they are not trapped (Millar et al. 2002; Cullen and Zalom 2005; Borges et al. 2011a). Additionally, traps baited with sex pheromone capture mainly females (Borges et al. 1998; Cullen and Zalom 2005), so the inclusion of signals that specifically attract males (such as females' calling vibratory songs) could improve the efficiency of these traps (Millar et al. 2002; Laumann et al. 2017; Mazzoni et al. 2017).

Reproducing the female vibrational signal (FS2) of the brown marmorated stink bug, *H. halys*, Mazzoni et al. (2017) showed that males are significantly attracted to source points (the point where an electromagnetic vibrator or mini-shaker reproduces the stimulation signals) on plants, cardboard arenas, and a prototype of an acoustic trap. Males attracted to source points continue to search for many minutes. Despite these interesting results, the authors draw attention to the fact that 30–50% of males tested were not responsive to vibrational stimulation. This could be influenced by the physiological condition of insects, as in the experiments the exact age of insects was not established, and by the quality of signal used (Mazzoni et al. 2017). Males of the Neotropical brown stink bug, *E. heros*, are significantly attracted to the arms of two-choice arenas, the "Y" type, where the female calling song (FS1) is broadcast with mini-shakers. Interestingly, when females were tested they also significantly chose the vibrated arm (Fig. 23.2). In these arenas, an amplitude gradient was created using a cardboard substrate (vibrated arm and common arm) and the control was the opposite non-vibrated arm (Aline Dias, Raúl Laumann, Maria Carolina Blassioli Moraes and Miguel Borges, unpublished data) (Fig. 23.2). Experiments in acrylic cages with eight soybean plants and a trap (model adapted from Borges et al. 1998) placed in the middle of the cage were performed. In these cages, 30 males or females were released and the captures in vibrated (treatment) or non-vibrated (control) traps (placed individually in the cages) were monitored during periods of 24–48 h. (Aline Dias, Raúl Laumann, Maria Carolina Blassioli Moraes and Miguel Borges, unpublished data) (Fig. 23.2). In cages with vibrated traps males were captured in higher numbers than in cages with non-vibrated traps. Attraction of males to plants in contact with traps was also higher than to plants in contact with non-vibrated traps (Fig. 23.2). No effect of vibrated traps was observed in cages where females were released (Fig. 23.2). Finally, a field test in small soybean plots (20 × 20 m), where vibrated traps were compared with non-vibrated traps, showed similar results (Laumann et al. 2017). These results show the potential of vibratory signals as baits to be incorporated into traps to improve the efficiency of stink bug monitoring.

Indirect monitoring of stink bugs using vibratory signals could be achieved by development of specific sensors to sample the signals and computational procedures

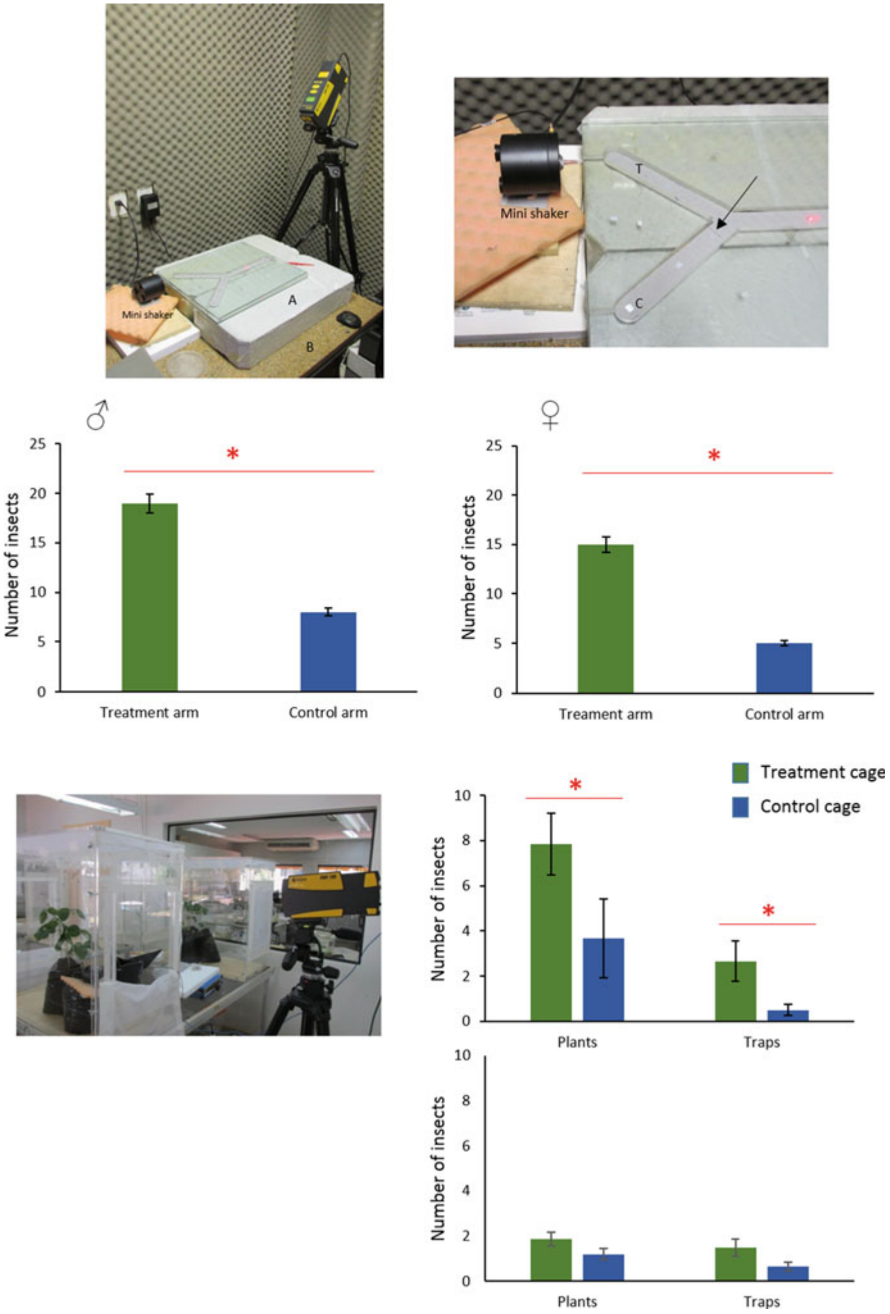


Fig. 23.2 *Upper left*: general set up for bioassays in Y arenas. Arena consist of an acrylic plate with a Y-shaped cavity (arms 12 cm common arm 18 cm). On the bottom of this cavity, cardboard with the Y format was adjusted and the upper surface was covered with a glass plate. One arm was vibrated using a small electrodynamic exiting (mini-shaker Blue & Kjaer type 4810) and the other arm was non-vibrated interrupting physical contact by a small cut (~0.5 mm). *Upper right*: detail of

for discrimination of signals from environmental noise and for species identification. Some of these systems have been tested in different insects, for example, use of linear prediction cepstral coefficient (LPCC) feature vectors and a multilayer perceptron (MLP) classifier to identify vibrations of *Aphrodes* spp. (Korinšek et al. 2016). A Gaussian mixture model (GMM) and a probabilistic neural network (PNN) allowed the identification and classification of the airborne sounds of cicadas as well as vibratory signals produced by the red palm weevil *Rhynchophorus ferrugineus* Olivier 1790 (Coleoptera: Curculionidae) (Pinhas et al. 2008).

For stink bugs, procedures based on GMM and PNN were used to discriminate vibratory signals from insect incidental sounds (ex. locomotion) and to identify two different species, the Nearctic brown stink bug, *Euschistus servus* Say 1832 and the green stink bug, *N. viridula* (Lampson et al. 2013). The signals from individuals of the two species were recorded using a piezoelectric accelerometer. Signal characteristics such as the dominant frequency, pulse duration, and 1st through 6th order linear frequency cepstral coefficients were used for the identification and classification of signals, thus discriminating signals of each species from incidental sounds with an accuracy between 70 and 90% (Lampson et al. 2013).

23.4.2 Application of Biotremology for Interference in Reproductive Behavior of Stink Bugs

The most utilized technique of interference in insect reproductive behavior is a mating disruption technique using pheromones or other semiochemicals. This technique involves permeating the crop environment with pheromones to disrupt chemical communication to prevent mating and reproduction of insects, reducing the population growth (Witzgall et al. 2010). Mating disruption using vibratory signals has been proposed recently and some experimental examples show the potential of this approach.

Probably the first attempt to use vibratory mating disruption was the work of Saxena and Kumar (1980) that showed the potential of airborne signals picked up by

Fig. 23.2 (continued) the arena. Arrow indicates the area where the connection between arms was interrupted. For bioassays, one insect (male or female) was released in the bottom of the common arm and their choice (vibrated or non-vibrated arm) was recorded. *Medium*: Response of males and females to female calling song (FS1) played back in the arenas bioassays. Asterisks indicate significant differences between choices for the treatment (vibrate arm) and control (non-vibrated arm) (χ^2 test $P < 0.05$). *Lower left*: general setup for the experiments in cage with vibrated or non-vibrated traps and soybean plants**.** Thirty males or females were released in each cage and captures in traps quantified during 8 h. *Lower right*: Number of males or females inside traps or in the plants in contact with the trap after 48 *h* of been released in the cages. Asterisks indicate significant differences in number of insects between cages with vibrated traps (treatment) and cages with non-vibrated traps (control) (GLM Analyses $P < 0.05$)

plants to interrupt the sexual behavior of the cotton leafhopper, *Amrasca devastans* Dist. (Hemiptera: Cicadellidae), and the rice brown planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). For the vineyard pest, the leafhopper *Scaphoideus titanus* Ball 1932 (Hemiptera: Cicadellidae), the strategy was to use the natural disturbance signals emitted during male rivalry (Mazzoni et al. 2009a, b). The playback of these disturbance signals reduces male calling, interrupts male–female duets, and reduces the number of copulations (Mazzoni et al. 2009b). In semi-field and field tests, the reproduction of this signal was made using electromagnetic shakers attached to the supporting metal wire where the grapevine tree stems are tied, and the number of females that remained virgins was recorded. More than 80% of females placed on vibrated plants remained virgin compared to 20% under control conditions (non-vibrated plants) (Eriksson et al. 2012). In this particular system, the disruptive signal has an intensity threshold of approximately 0.015 mm/s and the disruptive effect can be achieved up to a 10 m distance. In these conditions, 90% of tested females remained virgins after 24 h (Polajnar et al. 2016).

In another approach, mating disruption in *D. citri* was achieved with signals that mimic female signals (Mankin et al. 2013). These signals compete with those of the wild females or disrupt male–female communication. The smart system described for monitoring *D. citri* (see Sect. 23.4) was tested as a mating disruption tool. Synthetic female responses were played back with a microcontroller piezo buzzer platform after detecting a male calling. The results show a clear reduction in the rates and proportion of matings in plants where the systems were placed (Lujo et al. 2016; Mankin et al. 2016).

Other recent studies show that disturbance signals or pure tone noise could be sufficient to interfere with mating communication, avoiding the pair formation process in Cicadellidae such as the green leafhopper, *Empoasca vitis* Göthe 1875 (Nieri and Mazzoni 2019), and in the glassy-winged sharpshooter, *Homalodisca vitripenis* Germar 1821 (Gordon et al. 2017; Krugner and Gordon 2018). Because stink bugs, like many other insects, communicate in complex vibrational communication networks (Virant-Doberlet et al. 2014, 2019; Čokl et al. 2017c) there are many interference/disruptive interactions mediated by vibratory signals (see Sect. 23.3) that could be explored to identify signals/noise that interfere in communication.

Until now, the only work exploring interference in stink bugs' vibratory communication and their reproductive success by background noise was conducted with *E. heros*. Artificial pure tone vibrations between 20 and 200 Hz were played back as background noise using soybean plants as substrate (Fig. 23.3). Three different experiments were conducted, with *E. heros* female–male pairs placed on soybean plants, to test the impact of background noise in (1) vibratory signal emissions and communication, (2) reproductive behavior, and (3) reproductive success in long-term experiments (24 h). The results showed that pure tone vibrations of 75–200 Hz stimulate females to spontaneously emit calling signals and inhibited the signaling and searching of males, which thus reduces copulation of tested pairs (94.2–100% of reduction in relation to control pairs) (Fig. 23.3). Nevertheless, in long-term experiments the effects were reduced, with mating frequency falling by only 24.7% in plants with background noise in relation to plants without noise (Laumann et al.

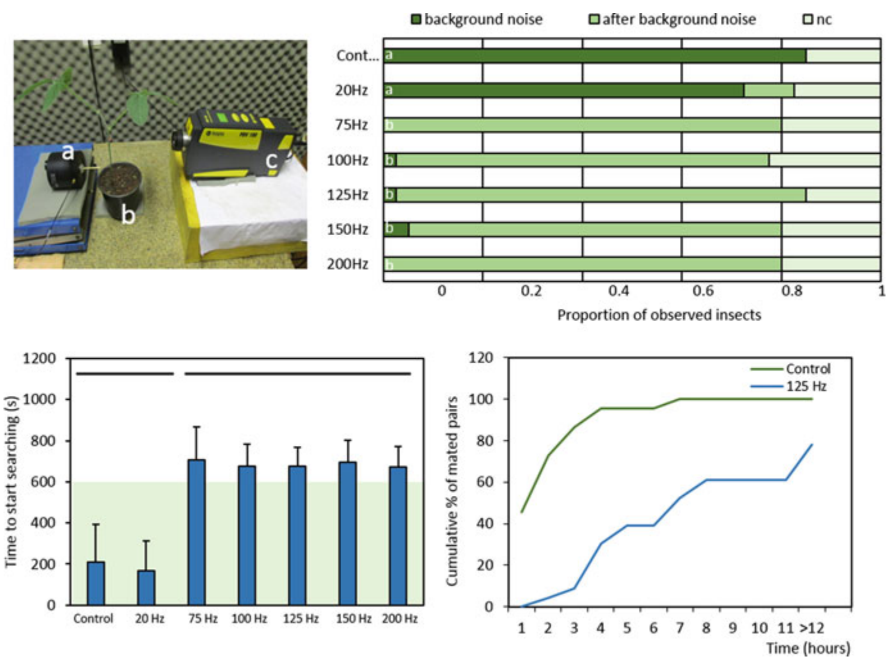


Fig. 23.3 *Upper left*: General set up for the experiments with interference noise. Soybean plants (center) with two opposite trifoliate leaves. Electrodynamic exiting (mini-shaker Blue & Kjaer type 4810, at left) for playback the pure tone background noise and laser vibrometer to monitoring signal emission by insects. For the experiments one virgin female and one virgin male were placed in the opposite leaves and the behavior and signals emission monitoring during 20 min, during the first 10 min background noise were played back and in the followed 10 min background noise was interrupted. *Upper right*: Proportion of observed pairs that mated during 10 min of pure tone frequencies emission (dark green bars, background noise), during 10 min after the interruption of emission of pure tone frequencies (green bars, after background noise) or that do not copulate (light green bars, nc). Letters inside bars at left indicate differences in proportion of pair that mate during the experimental phase when background noise were reproduced in relation to pairs in control plants (Z test $P < 0.05$ significance level was corrected by one-step Bonferroni procedure). *Lower left*: Male search latency (time to start searching) during pure tone frequencies emission lines shows standard deviation. Green area of the graph represents phase of the experiment when the background noise was played back. Black lines above bars group together treatments with non significant differences in latency (Kruskal–Wallis and Dunn tests $P < 0.005$). Lower right: Cumulative % of copulations of observed mates of mates (male and female) placed in different plants that where maintained in physical contact. Control: plants isolated of vibrations, 125 Hz: plants vibrated with continuous pure tone vibrations of 125 Hz (modified from Laumann et al. 2018)

2018) (Fig. 23.3). Authors hypothesize that the observed effect could be the result of disruption of vibrational directionality of male movement to calling females. The reduction in the efficiency of background noise to disrupt mating in long-term experiments may be a result of habituation or attraction of females to male pheromones. These results show that different aspects of behavior need to be considered for an efficient application of biotremology in insect mating disruption (Laumann et al. 2018).

Similar results were observed in *N. viridula*, in response to 100-Hz vibratory noise (Polajnar and Čokl 2008), or to simultaneous playback of female calling signals of conspecific and alien species (de Groot et al. 2010). Interestingly, white noise appeared to amplify, by stochastic resonance, *N. viridula* females' signals and enabled communication even when insect signals were at sub-threshold levels (Spezia et al. 2008), suggesting that the disruptive effects on male orientation observed in *E. heros* and *N. viridula* may be efficient in the frequency range characteristic of stink bug communication signals.

The effects of interference of rivalry signals in vibratory communication, and in disrupting mating, were also evaluated in the brown stink bug, *E. heros*, *Chinavia ubica and C. impicticornis* (Dias et al. 2021). Experiments were performed using plants vibrated with playbacks of female rivalry signals, and as control, plants with no playback of vibrational signals were used. Tests were performed with couples (male and female, $N = 20$) placed on different leaves, on vibrated and non-vibrated plants. The behavior was monitored by direct observation, and the vibrational signals were registered from the stem with a portable digital laser vibrometer. Female rival signals showed a mating disrupting effect on conspecific pairs for the three stink bug species. When insects were placed on plants where female rivalry vibratory signals of conespecifics were played-back it was observed a significant reduction of calling and courtship vibratory signals emitted, this influences the duet formation and further development of different phases of mating behavior. As a consequence, the probability of copulation in pairs on control plants (without) vibrations increased 29.41 times in *E. heros*, 4.6 times in *C. ubica* and 1.71 times in *C. impicticornis* (Dias et al. 2021)

23.4.3 Biotremology and Biological Control

The potential of use of vibratory signals in combination with natural enemies for pest management was proposed based on the fact that predators and parasitoids use substrate vibration in both prey/host search and localization (Cocroft and Rodriguez 2005). Scorpions (Brownell and van Hemmen 2001), spiders (Barth 2002; Virant-Doberlet et al. 2011), and many parasitoids (Meyhöfer et al. 1997; Meyhöfer and Casas 1999; Broad and Quicke 2000; Vilhelmsen et al. 2001) use incidental vibrations of prey/hosts when foraging. In addition, some Orussidae (Vilhelmsen et al. 2001) and Ichneumonidae (Wäckers et al. 1998; Broad and Quicke 2000) parasitoids could find their cryptic host by echolocation.

The egg parasitoid of stink bugs, *T. podisi*, uses the vibratory signals of the brown stink bug, *E. heros*, as a cue when foraging for a host. This response is highly selective regarding sex (female signals) and species (the brown stink bug against other alternative hosts) (Laumann et al. 2007, 2011). This parasitoid is the main natural enemy of the brown stink bug, which is the principal soybean pest in Brazil, and the use of the wasp in biological control programs is currently being researched, extensively. Behavioral manipulation with semiochemicals for recruitment in crops as a strategy to increase the conservative biological control of this pest shows potential (Vieira et al. 2013, 2014; Michereff et al. 2015). The possibility of using multimodal cues (chemical and mechanical) could increase the efficiency of parasitoid recruitment and consequently the impact on stink bug populations.

Another interesting possibility for joint biotremology and biological control could be their application to the manipulation of predatory stink bugs' (subfamily Asopinae) behavior. Asopinae are predators, principally, of lepidopteran caterpillars and have been extensively considered in biological control programs (De Clercq et al. 2014; Pires et al. 2015). Vibratory communication in Asopinae is characterized by a complex repertoire of tremulatory and drumming signals (Laumann et al. 2013; Žunič and Čokl 2017). Laumann et al. (2017) consider that the use of vibratory signals for manipulation of Asopinae behavior could be based on two strategies. One way is to recruit predators using conspecific vibratory signals, in combination with semiochemicals, in a similar way as described above for parasitoids. Vibratory cues from prey could also be used in this strategy. It was demonstrated that *Podisus maculiventris* Say 1832 can locate prey using the vibratory signals produced by chewing, as prey feed on plants (Pfannenstiel et al. 1995). The second possibility is to disrupt herbivores by transmitting predatory stink bug signals to plants.

23.5 Concluding Remarks

The application of biotremology in IPM as a tool for biorational stink bug management offers a potential strategy that it is beginning to be exploited. For current technological development and considering different crop systems such as fruit, grain crops, and horticulture, the strategy that could probably be implemented most immediately is the use of vibratory signals for monitoring populations. Incorporation of vibratory signals in traps does not require disruptive technological development. However, technical aspects, some of which are related to the biology and ecology of stink bugs, need to be considered. For example, it is known that directionality in stink bug males' responses to female calling songs is related to differences in arrival time at receptors located in different legs (Čokl et al. 1999), which is estimated to involve a delay of 0.5 ms (Prešern et al. 2018). This fact, more than amplitude gradient of signal transmission, needs to be considered in the design of traps (Prešern et al. 2018). Therefore, trap format, materials, and mechanism of vibration must be carefully selected and designed, in order to build more efficient traps. Another relevant point is the use of signals that maximize the attractiveness of insects. It was considered for the synthesis of optimal attrative signals to be incorporated in monitoring traps for of the brown marmorated stink bug. *H. halis* (Caorsi et al. 2021)

The interaction of chemical and vibratory signals (see Sect. 23.2) could also help to develop additional tools for monitoring. For example, the fact that female calling songs stimulate males to release pheromones could help to avoid the use of synthetic pheromone bait, because the males inside the traps could release natural pheromones. The opposite could be considered for chemical signals, such as pheromones or footprints, which stimulate the emission of female calling songs. This strategy could reduce the trap cost, by the use of a single bait, maintaining the effect of signals of two modalities (Laumann et al. 2017).

Indirect monitoring by identification of stink bug signals in the field is also a possible strategy. Some automated systems, including sensors to detect the vibrations and software to discriminate them from environmental noise and establish species and sex identity, have been developed (Laumann et al. 2017 and references therein). These systems present the opportunity of developing automatized monitoring systems that can be very useful in reducing the time and cost of monitoring, especially for crops that grow over large areas.

The use of stink bug signals in a mating disruption strategy appears to be technically more complicated for application in the field. However, in perennial crops such as fruit and nuts, the transmission of vibrations along tree surfaces may be more feasible than in other crops such as grain crops. Besides this technical problem, another potential problem is the failure of the strategy due to a specific type of resistance that, in this case, could arise when the insect becomes habituated to the disruption signals played back and treats them as environmental noise. Resistance is nowadays the principal shortcoming in any pest control technique, and the possibility of a pest evolving resistant populations needs to be considered for any control measure.

Habituation to noise is a well-documented response in insects (Mankin et al. 2011). As was described in Sect. 23.3.2, stink bugs have been shown to develop several mechanisms to either increase the signal-to-noise ratio or to avoid interference (Čokl et al. 2015; Polajnar et al. 2016), and this could explain the reduction of the effect of pure tone background noise to interrupt the communication of the brown stink bug (see Sect. 23.4.2). However, some strategies have been proposed to reduce habituation, including emission of disruptive signals using non-continuous and random programs, the emission of disruptive signals with irregular variations in pulse duration and changes in the position of the signal source, or development of devices that simulate motion (Agee 1969). Finally, the use of disrupting signals emitted in intraspecific interactions, as rivalry signals of stink bugs (see Sect. 23.4.2), presents the possibility of using natural disrupting signals with a lower probability of insect habituation than with synthetic signals or environmental noise (Polajnar et al. 2015).

The knowledge of stink bugs' vibratory communication, related to the repertoire and other aspects of reproductive behavior, has so far only been described in ~40 species of stink bugs (<0.8% of the described species). Continuous research will lead to a greater volume of information that may contribute to a better understanding of the mechanisms regulating the communication and reproductive behavior of stink bugs. This knowledge is the basis on which to build biorational pest control strategies using the application of biotremology.

Acknowledgments We thank the National Council for Scientific and Technological Development (CNPq), the Brazilian Corporation of Agricultural Research (EMBRAPA), the Research Support Foundation of the Federal District (FAP-DF), and the Slovenian Research Agency for financial support of our research. We are grateful to Profa. Susan Casement for the idiomatic revision of the manuscript.

References

Adamo SA, Baker JL (2011) Conserved features of chronic stress across phyla: the effects of long-term stress on behavior and the concentration of the neurohormone octopamine in the cricket, *Gryllus texensis*. Horm Behav 60:478–483

Agee HR (1969) Response of bollworm moths to pulsed ultrasound while resting, feeding, courting, mating, and ovipositing. Ann Entomol Soc Am 62:1122–1128

Aldrich JR (1988) Chemical ecology of the Heteroptera. Annu Rev Entomol 33:211–238

Aldrich JR (1995) Chemical communication in the true bugs and parasitoid exploitation. In: Cardé RT, Bell WJ (eds) Chemical ecology of insects II. Chapman & Hall, New York, pp 318–363

Barth FG (2002) A spider's world: senses and behavior. Springer, Berlin

Bennet-Clark HC (1998) Size and scale effects as constraints in insect sound communication. Philos Trans R Soc Lond B 353:407–419

Blassioli-Moraes MC, Laumann RA, Borges M (2008) The chemical volatiles (semiochemicals) produced by neotropical stink bugs (Hemiptera: Pentatomidae). Neotrop Entomol 37:489–505

Blassioli-Moraes MC, Laumann RA, Oliveira MW, Woodcock CM, Mayon P, Hooper A, Pickett JA, Birkett MA, Borges M (2012) Sex pheromone communication in two sympatric Neotropical stink bug species *Chinavia ubica* and *Chinavia impicticornis*. J Chem Ecol 38:836–845

Blassioli-Moraes MC, Borges M, Laumann RA (2013) Chemical ecology of insect parasitoids. In: Wajnberg E, Colazza S (eds) The application of chemical cues in arthropod pest management for arable crops. Wiley, New York, pp 225–244

Blassioli-Moraes MC, Magalhães DM, Čokl A, Laumann RA, Da Silva JP, Silva CCA, Borges M (2014) Vibrational communication and mating behaviour of *Dichelops melacanthus* (Hemiptera: Pentatomidae) recorded from the loudspeaker membrane and plants. Physiol Entomol 39:1–11

Blassioli-Moraes MC, Borges M, Viana AR, Laumann RA, Miranda JE, Magalhães DM, Birkett MA (2016) Identification and field evaluation of the sex pheromone of a Brazilian population of *Spodoptera cosmioides*. Pesq Agropec Bras 51:545–554

Blassioli-Moraes MC, Khrimian A, Michereff MFF, Magalhães DM, Hickel E, de Freitas TFS, Barrigossi JAF, Laumann RA, Silva AT, Guggilapu SD, Silva CC, Sant'Ana J, Borges M (2019) Male-produced sex pheromone of *Tibraca limbativentris* revisited: absolute configurations of zingiberenol stereoisomers and their influence on chemotaxis behaviour of conspecific females. J Chem Ecol. https://doi.org/10.1007/s10886-019-01125-w

Borges M (1995) Attractant compounds of the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae). An Soc Entomol Bras 24:215–225

Borges M, Aldrich JR (1992) Instar-specific defensive secretions of stink bugs (Heteroptera: Pentatomidae). Experientia 48:893–896

Borges M, Blassioli-Moraes MC (2017) The semiochemistry of Pentatomidae in stinkbugs. In: Čokl A, Borges M (eds) Stink Bugs: biorational control based on communication processes. CRC, Taylor & Francis, Boca Raton, pp 95–124

Borges M, Jepson PC, Howse PE (1987) Long-range mate location and close range courtship behavior of the green stink bug, *Nezara viridula* and its mediation by sex pheromones. Entomol Exp Appl 44:205–212

Borges M, Schmidt FVG, Sujii ER, Medeiros MA, Mori K, Zarbin PHG, Ferreira JTB (1998) Field responses of stink bugs to the natural and synthetic pheromone of the Neotropical brown stink bug, *Euschistus heros* (Heteroptera: Pentatomidae). Physiol Entomol 23:202–207

Borges M, Colazza S, Ramirez-Lucas P, Chauhan KL, Kramer M, Moraes MCB, Aldrich JR (2003) Kairomonal effect of walking traces from *Euschistus heros* (Heteroptera: Pentatomidae) on two strains of *Telenomus podisi* (Hymenoptera: Scelionidae). Physiol Entomol 28:349–355

Borges M, Birket M, Aldrich JR, Oliver JE, Chiba M, Murata Y, Laumann RA, Barrigossi JA, Pickett JA, Blassioli-Moraes MC (2006) Sex attractant pheromone from the rice stalk stink bug, *Tibraca limbativentris* Stal. J Chem Ecol 32:2749–2761

Borges M, Blassioli-Moraes MC, Peixoto MF, Pires CSS, Sujii ER, Laumann RA (2011a) Monitoring the neotropical brown stink bug *Euschistus heros* (F.) (Hemiptera: Pentatomidae) with pheromone-baited traps in soybean fields. J Appl Entomol 135:68–80

Borges M, Moraes MCB, Laumann RA, Pareja M, Silva CC, Michereff MF, Paula DP (2011b) Chemical ecology studies in soybean crop in Brazil and their application to pest management. In: Tzi-Bun NG (ed) Soybean—biochemistry, chemistry and physiology, 1st edn. Intech Open Access, Rijeka, Croatia, pp 31–66

Bradbury JW, Vehrencamp SL (2011) Principles of animal communication, 2nd edn. Sinauer, Sunderland

Broad GR, Quicke DLJ (2000) The adaptive significance of host location by vibrational sounding in parasitoid wasps. Proc R Soc Lond B 267:2103–2109

Brownell P, Farley RD (1979) Orientations to vibrations in sand by the nocturnal scorpions *Paruroctonus mesaensis*: mechanisms of target localisation. J Comp Physiol A 131:31–38

Brownell PH, van Hemmen JL (2001) Vibration sensitivity and a computational theory for prey-localizing behavior in sand scorpions. Am Zool 41:1229–1240

Bueno VHP, van Lenteren JC (2012) Predatory bug (Heteroptera). In: Panizzi AR, Parra JRP (eds) Insect bioecology and nutrition for integrated pest management. CRC, Boca Raton, pp 539–569

Caorsi V, Cornara D, Wells KE, Moser D, Berardo A, Miselli R, Torriani M, Pugno NM, Tasin M, Maistrello L, Mazzoni V (2021) Design of ideal vibrational signals for stinkbug male attraction through vibrotaxis experiments. Pest Manag Sci 77(12):5498–5508. https://doi.org/10.1002/ps.6590

Casas J, Bacher S, Tautz J, Meyhöfer R, Pierre D (1998) Leaf vibrations and air movements in a leaf miner–parasitoid system. Biol Control 11:147–153

Cocroft RB, Rodriguez RL (2005) The behavioral ecology of insect vibrational communication. BioScience 55:323–334

Čokl A (1983) Functional properties of vibroreceptors in the legs of *Nezara viridula* (L.) (Heteroptera, Pentatomidae). J Comp Physiol 150:261–269

Čokl A, Oto C, Kalmring K (1985) The processing of directional vibratory signals in the ventral cord of *Locusta migratoria*. J Comp Physiol A 156:45–52

Čokl A, Virant-Doberlet M, McDowell A (1999) Vibrational directionality in the southern green stink bug, *Nezara viridula* (L,), is mediated by female song. Anim Behav 58:1277–1283

Čokl A, Virant-Doberlet M, Stritih N (2000) The structure and function of songs emitted by the southern green stink bugs from Brazil, Florida, Italy and Slovenia. Physiol Entomol 25:196–205

Čokl A, Nardi C, Bento JMS, Hirose E, Panizzi AR (2006) Transmission of stridulatory signals of the burrower bugs, *Scaptocoris castanea* and *Scaptocoris carvalhoi* (Heteroptera: Cydnidae) through the soil and soybean. Physiol Entomol 31:371–381

Čokl A, Zorović M, Millar JG (2007) Vibrational communication along plants by the stink bugs *Nezara viridula* and *Murgantia histrionica*. Behav Process 75:40–54

Čokl A, Laumann RA, Žunič AK, Blassioli-Moraes MC, Virant-Doberlet M, Borges M (2015) Interference of overlapping insect vibratory communication signals: an *Euschistus heros* model. PLoS One June 22:1–16

Čokl A, Laumann RA, Stritih N (2017a) Substrate-borne vibratory communication. In: Čokl A, Borges M (eds) Stink bugs: biorational control based on communication processes. CRC, Taylor & Francis, Boca Raton, pp 125–164

Čokl A, Moreira Dias A, Blassioli-Moraes MC, Borges M, Laumann RA (2017b) Rivalry between stink bug females in a vibrational communication network. J Insect Behav 30:741–758

Čokl A, Žunič Kosi A, Virant-Doberlet M (2017c) Stink bug communication network and environment. In: Čokl A, Borges M (eds) Stink bugs: biorational control based on communication processes. CRC, Taylor & Francis, Boca Raton, pp 165–179

Čokl A, Blassioli-Moraes MC, Laumann RA, Žunič A, Borges M (2019a) Stinkbugs: multisensory communication with chemical and vibratory signals transmitted through different media. In: Hill PSM, Lakes-Harlan R, Mazzoni V, Narins PM, Virant-Doberlet M, Wessel A (eds) Biotremology: studying vibrational communication. Springer, Cham, pp 91–122

Čokl A, Žunič A, Laumann RA, Virant-Doberlet M (2019b) Female competition for availability of males in insects: the *Nezara viridula* (Linnaeus, 1758) model. Insect Sci. Available from: https://onlinelibrary.wiley.com/doi/epdf/10.1111/1744-7917.12692

Čokl A, Kosi AŽ, Moraes MCB, Borges M, Laumann RA (2021a) Stink bug inter-plant communication with signals produced by vibration of lifted wings. J Insect Behav 34(4):194–210. https://doi.org/10.1007/s10905-021-09780-2

Čokl A, Žunič-Kosi A, Stritih-Peljhan N, Blassioli-Moraes MC, Laumann RA, Borges M (2021b) Stink bug communication and signal detection in a plant environment. Insects 12(12):1058. https://doi.org/10.3390/insects12121058

Colazza S, Aquila G, De Pasquale C, Peri E, Millar JG (2007) The egg parasitoid *Trissolcus basalis* uses *n*-nonadecane, a cuticular hydrocarbon from stink bug host *Nezara viridula*, to discriminate between female and male hosts. J Chem Ecol 33:1405–1420

Colazza S, Lo Bue M, Lo Giudice D, Peri E (2009) The response of *Trissolcus basalis* to footprint contact kairomones from *Nezara viridula* females is mediated by leaf epicuticular waxes. Naturwissenschaften 96:975–981

Cremer L, Heckl M, Ungar EE (1973) Structure-borne sound, structural vibrations and sound radiation at audio frequencies. Springer, Berlin

Cullen EM, Zalom FG (2000) Phenology-based field monitoring for consperse stink bug (Hemiptera: Pentatomidae) in processing tomatoes. Environ Entomol 29:560–567

Cullen EM, Zalom FG (2005) Relationship between *Euschistus conspersus* (Hem., Pentatomidade) pheromone trap catch and canopy samples in processing tomatoes. J Appl Entomol 129:505–514

De Clercq P, Coudron T, Riddick EW (2014) Production of heteropteran predators. In: Morales-Ramos JA, Rojas MG, Shapiro-Ilan DI (eds) Mass production of beneficial organisms: invertebrates and entomopathogens. Academic, London, pp 57–100

De Groot M, Čokl A, Virant-Doberlet M (2010) Effects of heterospecific and conspecific vibrational signal overlap and signal-to-noise ratio on male responsiveness in *Nezara viridula* (L.). J Exp Biol 213:3213–3222

De Groot M, Čokl A, Virant-Doberlet M (2011) Species identity cues: possibilities for errors during vibrational communication on plant stems. Behav Ecol 22:1209–1217

Dias AM, Borges M, Moraes MCB, Čokl A, Laumann RA (2018) Chemical footprints influence on vibratory communication and mating behaviour of the Neotropical brown stink bug, *Euschistus heros* (F) (Hemiptera: Pentatomidae). In: Abstract book, 34th annual meeting of the International Society of Chemical Ecology, p 241. Available from: https://www.chemecol.org/programs/2018%20abstracts.pdf

Dias AM, Borges M, Moraes MCB, Lorran M, Coelho F, Čokl A, Laumann RA (2021) Inhibitory copulation effect of vibrational rival female signals of three stink bug species as a tool for mating disruption. Insects 12(2):177. https://doi.org/10.3390/insects12020177

Eliyahu D, Ceballos RA, Saeidi V, Becerra JX (2012) Synergy versus potency in the defensive secretions from nymphs of two Pentatomorphan families (Hemiptera: Coreidae and Pentatomidae). J Chem Ecol 38:1358–1365

Eriksson A, Anfora G, Lucchi A, Lanzo F, Virant-Doberlet M, Mazzoni V (2012) Exploitation of insect vibrational signals reveals a new method of pest management. PLoS One 7:1–5

Gordon SD, Sandoval N, Mazzoni V, Krugner R (2017) Mating interference of glassy-winged sharpshooters, *Homalodisca vitripennis*. Entomol Exp Appl 164:27–34

Hergenröder R, Barth FG (1983) Vibratory signals and spider behavior: how do sensory input from the eight legs interact in orientation? J Comp Physiol A 152:361–371

Higham JP, Hebets EA (2013) An introduction to multimodal communication. Behav Ecol Sociobiol 67:1381–1388

Ho HY, Millar G (2001) Identification and synthesis of male-produced sex pheromone components of the stink bugs *Chlorochroa ligata* and *Chlorochroa uhleri*. J Chem Ecol 27:2067–2095

Jeraj M, Walter GH (1998) Vibrational communication in *Nezara viridula*: response of Slovenian and Australian bugs to one another. Behav Process 44:51–58

Jeram S (1996) Structure and function of Johnston's organ in the bug species *Nezara viridula* (L.). Dissertation, University of Ljubljana, Slovenia

Jeram S, Pabst AM (1996) Johnston's organ and central organ in *Nezara viridula* (L.) (Heteroptera, Pentatomidae). Tissue Cell 28:227–235

Kavčič A, Čokl A, Laumann RA, Moraes MCB, Borges M (2013) Tremulatory and abdomen vibration signals enable communication through air in the stink bug *Euschistus heros*. PLoS One 8:1–10

Kight CR, Swaddle JP (2011) How and why environmental noise impacts animals: an integrative, mechanistic review. Ecol Lett 14:1052–1061

Korinšek G, Derlink M, Virant-Doberlet M, Tuma T (2016) An autonomous system of detecting and attracting leafhopper males using species- and sex-specific substrate borne vibrational signals. Comput Electron Agric 23:29–39

Krugner R, Gordon SD (2018) Mating disruption of *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) by playback of vibrational signals in vineyard trellis. Pest Manag Sci 74:2013–2019

Lampson BD, Han YJ, Khalilian A, Greene J, Mankin RW, Eg F (2013) Automatic detection and identification of brown stink bug, *Euschistus servus*, and southern green stink bug, *Nezara viridula*, (Heteroptera: Pentatomidae) using intraspecific substrate-borne vibrational signals. Comput Electron Agric 91:154–159

Laumann RL, Blassioli-Moraes MC, Čokl A, Borges M (2007) Eavesdropping on sexual vibratory signals of stink bugs (Hemiptera: Pentatomidae) by the egg parasitoid *Telenomus podisi*. Anim Behav 73:637–649

Laumann RA, Moraes MCB, Khrimian A, Borges M (2011) Field capture of *Thyanta perditor* with pheromone-baited traps. Pesq Agropec Bras 46:113–119

Laumann RA, Kavčič A, Moraes MCB, Borges M, Čokl A (2013) Reproductive behaviour and vibratory communication of the Neotropical predatory stink bug *Podisus nigrispinus*. Physiol Entomol 378:71–80

Laumann RA, Čokl A, Blassioli-Moraes MC, Borges M (2016) Vibratory communication and its relevance to reproductive isolation in two sympatric species (Hemiptera: Pentatomidae: Pentatominae). J Insect Behav 29:643–665

Laumann RA, Bottura Maccagnan DH, Čokl A (2017) Use of vibratory signals for stink bug monitoring and control. In: Čokl A, Borges M (eds) Stink bugs: biorational control based on communication processes. CRC, Taylor & Francis, Boca Raton, pp 226–245

Laumann RA, Maccagnan DHB, Čokl A, Blassioli-Moraes MC, Borges M (2018) Substrate-borne vibrations disrupt the mating behaviors of the neotropical brown stink bug, *Euschistus heros*: implications for pest management. J Pest Sci 91:995–1004

Lujo S, Hartman E, Norton K, Pregmon EA, Rohde BB, Mankin RW (2016) Disrupting mating behaviour of *Diaphorina citri* (Liviidae). J Econ Entomol 109:2373–2379

Magalhães DM, Borges M, Laumann RA, Woodcock CM, Withall DM, Pickett JA, Birkett MA, Blassioli-Moraes MC (2018) Identification of volatile compounds involved in host location by *Anthonomus grandis* (Coleoptera: Curculionidae). Front Ecol Evol 6:1–12

Mankin RW (2012) Applications of acoustics in insect pest management. CAB Rev 7:1–7

Mankin RW, Hagstrum DW, Smith MT, Roda AL, Kairo MTK (2011) Perspective and promise: a century of insect acoustic detection and monitoring. Am Entomol 57:30–44

Mankin RW, Rohde BB, Mcneill SA, Paris TM, Zagvazdina NI, Greenfeder S (2013) *Diaphorina citri* (Hemiptera: Liviidae) responses to microcontroller-buzzer communication signals of potential use in vibration traps. Fla Entomol 96:1546–1555

Mankin RW, Rohde BB, McNeill SA (2016) Vibrational duetting mimics to trap and disrupt mating of the devastating Asian citrus psyllid insect pest. Proc Meet Acoust 25:010006. Available from https://asa.scitation.org/doi/abs/10.1121/2.0000185

Markl H (1983) Vibrational communication. In: Huber F, Markl H (eds) Neuroethology and behavioural physiology. Springer, Berlin, pp 332–353

Mazzoni V, Prešern J, Lucchi A, Virant-Doberlet M (2009a) Reproductive strategy of the Nearctic leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae). Bull Entomol Res 99:401–413

Mazzoni V, Lucchi A, Čokl A, Prešern J, Virant-Doberlet M (2009b) Disruption of the reproductive behavior of *Scaphoideus titanus* by playback of vibrational signals. Entomol Exp Appl 133: 174–185

Mazzoni V, Polajnar J, Baldini M, Rossi Stacconi MV, Anfora G, Guidetti R, Maistrello L (2017) Use of substrate-borne vibrational signals to attract the brown marmorated stink bug, *Halyomorpha halys*. J Pest Sci 90:1219–1229

McPherson JE (2018) Invasive stink bugs and related species (Pentatomoidea): biology, higher systematics, semiochemistry and management. CRC, Taylor & Francis, Boca Raton

Meyhöfer R, Casas J (1999) Vibratory stimuli in host location by parasitic wasps. J Insect Physiol 45:967–971

Meyhöfer R, Casas J, Orn S (1997) Vibration-mediated interactions in a host–parasitoid system. Proc R Soc Lond B 264:261–266

Michel K, Amon T, Čokl A (1983) The morphology of the leg scolopidial organs in *Nezara viridula* (L.) (Heteroptera, Pentatomidae). Rev Can Biol Exp 42:130–150

Michelsen A, Fink F, Gogala M, Traue D (1982) Plants as transmission channels for insect vibrational songs. Behav Ecol Sociobiol 11:269–281

Michereff MFF, Laumann RA, Borges M, Michereff-Filho M, Diniz IR, Neto ALF, Blassioli-Moraes MC (2011) Volatiles mediating a plant-herbivore-natural enemy interaction in resistant and susceptible soybean cultivars. J Chem Ecol 37:73–285

Michereff MMF, Michereff Filho M, Blassioli-Moraes MC, Laumann RA, Diniz IR, Borges M (2015) Effect of resistant and susceptible soybean cultivars on the attraction of egg parasitoids under field conditions. J Appl Entomol 139:207–216

Miklas N, Stritih N, Čokl A, Virant-Doberlet M, Renou M (2001) The influence of substrate on male responsiveness to the female calling song in *Nezara viridula*. J Insect Behav 14:313–332

Miklas N, Lasnier T, Renou M (2003a) Male bugs modulate pheromone emission in response to vibratory signals of the conspecifics. J Chem Ecol 29:561–574

Miklas N, Čokl A, Renou M, Virant-Doberlet M (2003b) Variability of vibratory signals and mate choice selectivity in the southern green stink bug. Behav Process 61:131–142

Millar JG, McBrien HL, Ho HY, Rice RE, Cullen E, Zalom FG, Uokl A (2002) Pentatomid bug pheromones in IPM: possible applications and limitations. IOBC Wprs Bull 25:241–250

Mori K (2007) Significance of chirality in pheromone science. Bioorgan Med Chem 15:7505–7523

Nieri R, Mazzoni V (2019) Vibrational mating disruption of *Empoasca vitis* by natural or artificial disturbance noises. Pest Manag Sci 75:879–1198

Nishino H, Mukai H, Takanashi T (2016) Chordotonal organs in hemipteran insects: unique peripheral structures but conserved central organization revealed by comparative neuroanatomy. Cell Tissue Res 366:549–572

Oliveira MWM, Borges M, Andrade CKZ, Laumann RA, Barrigossi JAF, Blassioli-Moraes MC (2013) Zingiberenol, (1*S*,4*R*,1′*S*)-4-(1′,5′-dimethylhex-4′-enyl)-1methylcyclohex-2-en-1-ol, identified as the sex pheromone produced by males of the rice stink bug *Oebalus poecilus* (Heteroptera: Pentatomidae). J Agric Food Chem 61:7777–7785

Ota D, Čokl A (1991) Male location in the southern green stink bug *Nezara viridula* (Heteroptera: Pentatomidae) mediated through substrate-borne signals on ivy. J Insect Behav 4:441–447

Panizzi AR (2013) History and contemporary perspectives of the integrated pest management of soybean in Brazil. Neotrop Entomol 42:119–127

Panizzi AR (2015) Growing problems with stink bugs (Hemiptera : Heteroptera : Pentatomidae): species invasive to US and potential Neotropical invaders. Am Entomol 6:223–233

Panizzi AR, Lucini T (2017) Host plant-stink bug relationships. In: Čokl A, Borges M (eds) Stink bugs: biorational control based on communication processes. CRC, Taylor & Francis, Boca Raton, pp 31–85

Panizzi AR, Silva FAC (2012) Seed-sucking bugs (Heteroptera). In: Panizzi AR, Parra JRP (eds) Insect bioecology and nutrition for integrated pest management. CRC, Boca Raton, pp 295–324

Panizzi AR, McPherson JE, James DG, Javahery M, McPherson RM (2000) Stink bugs (Pentatomidae). In: Schaefer CW, Panizzi AR (eds) Heteroptera of economic importance. CRC, Boca Raton, pp 421–474

Partan SR, Marler P (2005) Issues in the classification of multisensory communication signals. Am Nat 166:231–245

Pavis C (1987) Les secretions exocrines des hétéroptères (allomones et pheromones). Une miseau point bibliographique. Agronomie EDP Sci 7:547–561

Pfannenstiel RS, Hunt RE, Yeargan KV (1995) Orientation of a hemipteran predator to vibrations produced by feeding caterpillars. J Insect Behav 8:1–9

Pinhas J, Soroker V, Hetzroni A, Mizrach A, Teicher M, Goldberger J (2008) Automatic acoustic detection of the red palm weevil. Comput Electron Agric 63:131–139

Pires EM, Soares MA, Martins R, Zanuncio JC, Sergio MP, Antonio AOM (2015) Seven decades of studies with Asopinae predators in Brazil (1933-2014). Biosci J 31:1530–1549

Polajnar J, Čokl A (2008) The effect of vibratory disturbance on sexual behaviour of the southern green stink bug *Nezara viridula* (Heteroptera, Pentatomidae). Cent Eur J Biol 3:189–197

Polajnar J, Svenšek D, Čokl A (2012) Resonance in herbaceous plant stems as a factor in vibrational communication of pentatomide bugs (Heteroptera: Pentatomidae). J R Soc Interface 9:1898–1907

Polajnar J, Eriksson A, Lucchi A, Anfora G, Virant-Doberlet M, Mazzoni V (2015) Manipulating behaviour with substrate-borne vibrations—potential for insect pest control. Pest Manag Sci 71: 15–23

Polajnar J, Eriksson A, Lucchi A, Virant-Doberlet M, Mazzoni V (2016) Mating disruption of a grapevine pest using mechanical vibrations: from laboratory to the field. J Pest Sci 89:909–921

Prešern J, Polajnar J, de Groot M, Zorović M, Virant-Doberlet M (2018) On the spot: utilization of directional cues in vibrational communication of stink bug. Sci Rep 8:5418. https://doi.org/10.1038/s41598-018-23710-x

Price PW (1997) Insect ecology. Wiley, New York

Rider DA, Schwertner CF, Vilímová J, Rédei D, Kment P, Thomas DB (2018) Higher systematics of the Pentatomoidea. In: McPherson JE (ed) Invasive stink bugs and related species (Pentatomoidea): biology, higher systematics, semiochemistry, and management. CRC, Taylor & Francis, Boca Raton, pp 25–201

Ryan MA, Čokl A, Walter GH (1996) Differences in vibratory sound communication between a Slovenian and Australian populations of *Nezara viridula*. Behav Process 36:183–193

Saxena KN, Kumar H (1980) Interruption of acoustic communication and mating in a leafhopper and a planthopper by aerial sound vibrations picked up by plants. Experientia 36:933–936

Schaefer C (1975) Heteropteran trichobothria (Hemiptera: Heteroptera). Int J Insect Morphol 4: 193–238

Silva CC, Moraes MCB, Laumann RA, Borges M (2006) Sensory response of the egg parasitoid *Telenomus podisi* to stimuli from the bug *Euschistus heros*. Pesq Agropec Bras 41:1093–1098

Silva CCA, Laumann RA, Ferreira JBC, Blassioli-Moraes MC, Borges M, Čokl A (2012) Reproductive biology, mating behavior, and vibratory communication of the brown-winged stink bug, *Edessa meditabunda* (Fabr.) (Heteroptera: Pentatomidae). Psyche 2012:1–9

Silva VP, Pereira MJB, Vivan LM, Blassioli-Moraes MC, Laumann RA, Borges M (2014) Monitoramento do percevejo marrom *Euschistus heros* (Hemiptera: Pentatomidae) por feromônio sexual em lavoura de soja. Pesq Agropec Bras 49:844–852

Silveira S, Dias AM, Gomes Lagoa AC, Blassioli-Moraes MC, Borges M, Čokl A, Laumann RA (2019) Specificity of male responses to female vibratory signals in two *Chinavia* species (Hemiptera: Pentatomidae) is based on signal structure and narrow temporal parameters. Anim Behav Cogn 6:1–12

Šolinc G (2017) Influence of temporal and spatial properties of airflow on the response of filiform sensilla of the bug *Pyrrhocoris apterus*. Thesis, University of Ljubljana, Slovenia

Sosa-Gomez DR, Silva JJ (2010) Neotropical brown stink bug (*Euschistus heros*) resistance to methamidophos in Paraná, Brazil. Pesq Aropec Bras 45:767–769

Spezia S, Curcio L, Fiasconaro A, Pizzolato N, Valenti D, Spagnolo B, Lo Bue P, Peri E, Colazza S (2008) Evidence of stochastic resonance in the mating behavior of *Nezara viridula* (L.). Eur Phys J B 65:453–458

Stritih N, Virant-Doberlet M, Čokl A (2000) Green stink bug *Nezara viridula* detects differences in amplitude between courtship song vibrations at stem and petiolus. Pflügers Arch 439(Suppl): R190–R192. https://doi.org/10.1007/s004240000141

Takanashi T, Uechi N, Tatsuta H (2019) Vibrations in hemipteran and coleopteran insects: behaviors and application in pest management. Appl Entomol Zool 54:21–29

Tillman FG, Cottrell TE (2017) Use of pheromones for monitoring phytophagous stink bug (Heteroptera: Pentatomidae). In: Čokl A, Borges M (eds) Stink bugs: biorational control based on communication processes. CRC, Taylor and Francis, Boca Raton, pp 210–225

Todd JW, Herzog DC (1980) Sampling phytophagous Pentatomidae on soybean. In: Kogan M, Herzog DC (eds) Sampling methods in soybean entomology. Springer, New York, pp 438–478

Vieira CR, Blassioli-Moraes MC, Borges M, Er S, Laumann RA (2013) cis-Jasmone indirect action on egg parasitoids (Hymenoptera: Scelionidae) and its application in biological control of soybean stink bugs (Hemiptera: Pentatomidae). Biol Control 64:75–82

Vieira CR, Blassioli-Moraes MC, Borges M, Soares CSS, Sujii ER, Laumann RA (2014) Field evaluation of (E)-2-hexenal efficacy for behavioral manipulation of egg parasitoids in soybean. BioControl 59:525–537

Vilhelmsen L, Isidoro N, Romani R, Basibuyuk HH, Quicke LJ (2001) Host location and oviposition in a basal group of parasitic wasps: the subgenual organ, ovipositor apparatus and associated structures in the Orussidae (Hymenoptera, Insecta). Zoomorphology 121:63–84

Virant-Doberlet M, Čokl A, Stritih N (2000) Vibratory songs of hybrids from Brazilian and Slovenian populations of the green stink bug *Nezara viridula*. Eur J Physiol 439:196–198

Virant-Doberlet M, King RA, Polajnar J, Symondson WOC (2011) Molecular diagnostics reveal spiders that exploit prey vibrational signals used in sexual communication. Mol Ecol 20:2204–2216

Virant-Doberlet M, Mazzoni V, de Groot M, Polajnar J, Lucchi A, Symondson WOC, Čokl A (2014) Vibrational communication networks: eavesdropping and biotic noise. In: Cocroft R, Gogala M, Hill PSM, Wessel A (eds) Studying vibrational communication. Springer, Berlin, pp 93–123

Virant-Doberlet M, Kuhelj A, Polajnar J, Šturm R (2019) Predator-prey interactions and eavesdropping in vibrational communication networks. Front Ecol Evol. https://doi.org/10.3389/fevo.2019.00203

Wäckers FL, Mitter E, Dorn S (1998) Vibrational sounding by the pupal parasitoid *Pimpla (Coccygomimus) turionellae*: an additional solution to reliability-detectability problem. Biol Control 11:141–146

Weber D, Khrimian A, Blassioli-Moraes MC, Millar JG (2018) The semiochemistry of Pentatomoidea. In: McPherson JE (ed) Invasive stink bugs and related species (Pentatomoidea): biology, higher systematics, semiochemistry, and management. CRC, Taylor & Francis, Boca Raton, pp 677–725

Weissburg MJ (2000) The fluid dynamical context of chemosensory behavior. Biol Bull 198:188–202

Witzgall P, Kirsch P, Cork A (2010) Sex pheromones and their impact on pest Management. J Chem Ecol 36:80–100

Zahn DK, Moreira JA, Millar JG (2008) Identification, synthesis, and bioassay of a male-specific aggregation pheromone from the harlequin bug, *Murgantia histrionica*. J Chem Ecol 34:238–251

Zarbin PHG, Fávaro CF, Vidal DM, Rodrigues MACM (2012) Male-produced sex pheromone of the stink bug *Edessa meditabunda*. J Chem Ecol 38:825–835

Zgonik V, Čokl A (2014) The role of signals of different modalities in initiating vibratory communication in *Nezara viridula*. Cent Eur J Biol 9:200–211

Zorović M, Prešern J, Čokl A (2008) Morphology and physiology of vibratory interneurons in the thoracic ganglia of the southern green stinkbug *Nezara viridula* (L.). J Comp Neurol 508:365–381

Žunič A, Čokl A (2017) Predatory stink bugs (Asopinae) and the role of substrate-borne vibrational signals in intra- and interspecific interactions. In: Čokl A, Borges M (eds) Stink bugs: biorational control based on communication processes. CRC, Taylor & Francis, Boca Raton, pp 59–77

Žunič A, Čokl A, Virant-Doberlet M, Millar JG (2008) Communication with signals produced by abdominal vibration, tremulation, and percussion in *Podisus maculiventris* (Heteroptera: Pentatomidae). Ann Entomol Soc Am 101:1169–1178

Žunič A, Virant-Doberlet M, Čokl A (2011) Species recognition during substrate-borne communication in *Nezara viridula* (L.) (Pentatomidae: Heteroptera). J Insect Behav 24:468–487