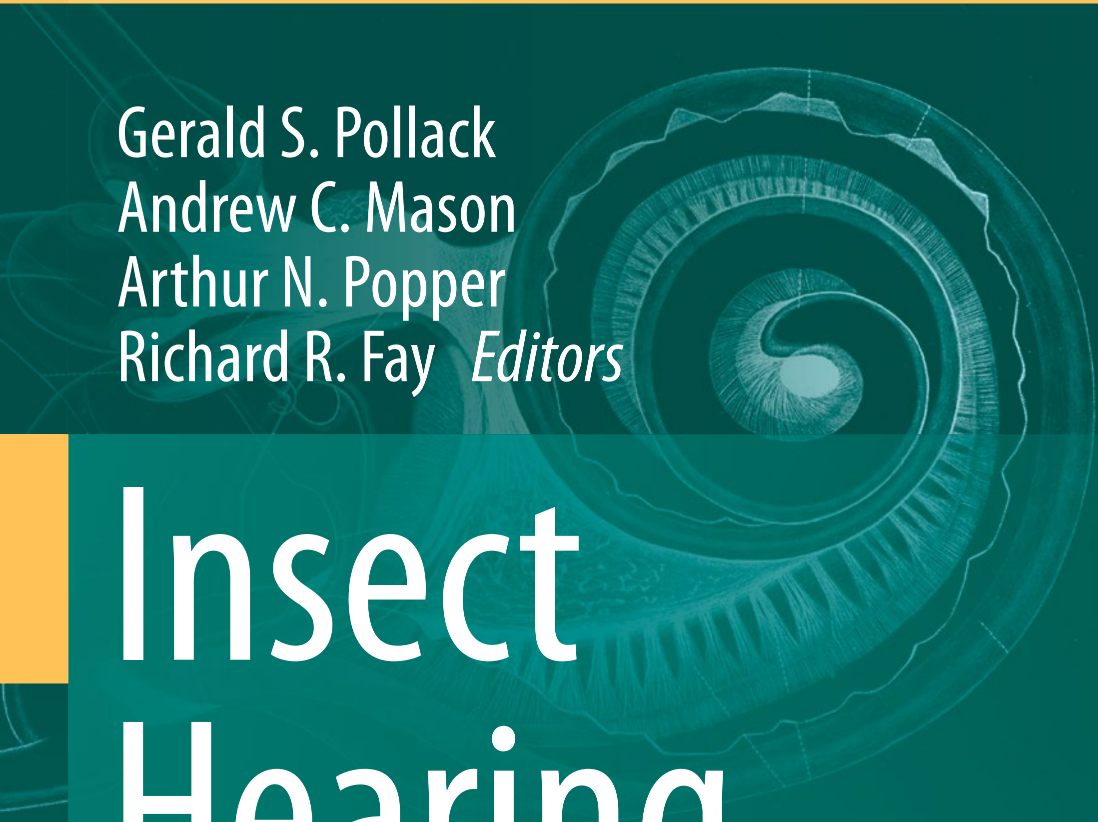
Springer Handbook of Auditory Research



Gerald S. Pollack
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Insect Hearing

Springer

Springer Handbook of Auditory Research

Volume 55

Series editors

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Insect Hearing

With 53 Illustrations

Springer

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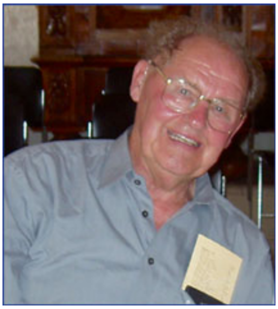

We dedicate this volume to two outstanding scientists and mentors. Franz Huber (top) pioneered the study not only of insect acoustic communication but also of the broader discipline of neuroethology. Ron Hoy's (bottom) laboratory surely holds the record for discovery of the most "new" insect auditory systems and consistently

extended the known boundaries
of invertebrate sensory perception.
Their work greatly influenced the
development of concepts such as command
neurons, central pattern generators,
and sensory pattern recognition. All of the
editors greatly value the contributions and
collegiality of Drs. Huber and Hoy.
Both of the senior editors profited from
research stays with the Huber group in
Seewiesen, and their time as postdoctoral
fellows in the Hoy lab at Cornell launched
their careers. Like dozens of other scientists,
we owe an enormous debt of gratitude
to these exceptional individuals.

Series Preface

The following preface is the one that we published in Volume 1 of the *Springer Handbook of Auditory Research* back in 1992. As anyone reading the original preface, or the many users of the series, will note, we have far exceeded our original expectation of eight volumes. Indeed, with books published to date and those in the pipeline, we are now set for over 60 volumes in *SHAR*, and we are still open to new and exciting ideas for additional books.

We are very proud that there seems to be consensus, at least among our friends and colleagues, that *SHAR* has become an important and influential part of the auditory literature. While we have worked hard to develop and maintain the quality and value of *SHAR*, the real value of the books is very much because of the numerous authors who have given their time to write outstanding chapters and to our many coeditors who have provided the intellectual leadership to the individual volumes. We have worked with a remarkable and wonderful group of people, many of whom have become great personal friends to both of us. We also continue to work with a spectacular group of editors at Springer. Indeed, several of our past editors have moved on in the publishing world to become senior executives. To our delight, this includes the current president of Springer USA, Dr. William Curtis.

But the truth is that the series would and could not be possible without the support of our families, and we want to take this opportunity to dedicate all of the *SHAR* books, past and future, to them. Our wives, Catherine Fay and Helen Popper, and our children, Michelle Popper Levit, Melissa Popper Levinsohn, Christian Fay, and Amanda Fay, have been immensely patient as we developed and worked on this series. We thank them and state, without doubt, that this series could not have happened without them. We also dedicate the future of *SHAR* to our next generation of (potential) auditory researchers—our grandchildren—Ethan and Sophie Levinsohn; Emma Levit; and Nathaniel, Evan, and Stella Fay.

Preface 1992

The *Springer Handbook of Auditory Research* presents a series of comprehensive and synthetic reviews of the fundamental topics in modern auditory research. The volumes are aimed at all individuals with interests in hearing research including advanced graduate students, postdoctoral researchers, and clinical investigators. The volumes are intended to introduce new investigators to important aspects of hearing science and to help established investigators to better understand the fundamental theories and data in fields of hearing that they may not normally follow closely.

Each volume presents a particular topic comprehensively, and each serves as a synthetic overview and guide to the literature. As such, the chapters present neither exhaustive data reviews nor original research that has not yet appeared in peer-reviewed journals. The volumes focus on topics that have developed a solid data and conceptual foundation rather than on those for which a literature is only beginning to develop. New research areas will be covered on a timely basis in the series as they begin to mature.

Each volume in the series consists of a few substantial chapters on a particular topic. In some cases, the topics will be ones of traditional interest for which there is a substantial body of data and theory, such as auditory neuroanatomy (Vol. 1) and neurophysiology (Vol. 2). Other volumes in the series deal with topics that have begun to mature more recently, such as development, plasticity, and computational models of neural processing. In many cases, the series editors are joined by a coeditor having special expertise in the topic of the volume.

Richard R. Fay, Woods Hole, MA, USA
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Volume Preface

The vast majority of ears on the planet are found on insects. Although these differ substantially in structure from the ears of vertebrates, they have evolved to serve the same functions, namely, detection and localization of predators and prey and communication with conspecifics. Auditory systems, whether insect or vertebrate, must perform a number of basic tasks: capturing mechanical stimuli and transducing these into neural activity, representing the timing and frequency of sound signals, distinguishing between behaviorally relevant signals and other sounds, and localizing sound sources. Studying how these are accomplished in insects offers a valuable comparative view that helps to reveal general principles of auditory function.

In Chapter 1, Mason and Pollack provide an overview of the entire volume and set the context for insect hearing. Next, in Chapter 2 Greenfield discusses the evolutionary origins and diversification of insect hearing. In Chapter 3, Balakrishnan reviews the behavioral ecology of insect hearing, with a focus on signaling strategies in long-range communication.

Hearing and acoustic behavior in the context of predator avoidance and defenses against echolocating bats are the subjects of Chapter 4, by Pollack. In Chapter 5, Yack provides an overview of substrate vibration senses and communication—a modality closely related to hearing. Both modalities are built on a common mechanoreceptor type, and substrate vibration sense functions analogously to hearing but under very different physical constraints. Chapter 6, by Windmill and Jackson, considers the mechanics of insect hearing and reviews the diverse mechanisms by which acoustic energy is converted to movement of specialized structures in insect ears. The cellular mechanisms for transduction of acoustic energy to electrical activity in sensory neurons are reviewed in Chapter 7 by Eberl, Kamikouchi, and Albert.

Next, in Chapter 8 Hedwig and Stumpner discuss the central auditory pathway and neurophysiological mechanisms underlying auditory behavior. In Chapter 9, Ronacher examines auditory processing in terms of information coding, feature detection, and computational algorithms. Finally, Chapter 10, by Kamikouchi and Ishikawa, provides an overview of auditory research in *Drosophila,* serving as a “case study” of a model system in which current research is addressing questions at all levels from the molecular and biophysical basis of transduction to circuit-level organization and behavior.

This volume, as most others in *SHAR*, benefits from earlier books and chapters in the series. In this case, an earlier volume on *Comparative Hearing: Insects* (Vol. 10, edited by Hoy, Popper, and Fay in 1998) provides a great deal of related material as well as a historical context for many of the amazing advances that have been made since then in insect hearing. Insect hearing has also been considered in chapters in several other volumes including a discussion on plasticity by Reinhard Lakes-Harlan in *Plasticity of the Auditory System* (Vol. 23, edited by Parks, Rubel, Fay, and Popper in 2004), insect directional hearing by Daniel Robert in *Sound Source Localization* (Vol. 25, edited by Popper and Fay in 2005), active processes by Martin C. Göpfert and Daniel Robert in *Active Processes and Otoacoustic Emissions* (Vol. 30, edited by Manley, Fay, and Popper in 2008), and transduction by Ryan G. Kavlie and Joerg T. Albert in *Insights from Comparative Hearing Research* (Vol. 49, edited by Köppl, Manley, Popper, and Fay in 2014). Finally, several chapters on hearing by Hoy and Michelsen and Larsen are found in *Perspectives in Auditory Neuroscience* (Vol. 50, edited by Popper and Fay in 2014).

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Chapter 1
Introduction to Insect Acoustics

Andrew C. Mason and Gerald S. Pollack

Abstract Insects are the most diverse taxonomic group of animals, and this is reflected in the number and variety of anatomically and evolutionarily distinct ears to be found within this group. In general terms, all auditory systems must accomplish the same basic tasks of detecting, discriminating, and localizing sound sources. Insects, therefore, present many examples of alternative mechanisms for fundamental auditory processes, and research on insect auditory systems addresses all aspects of hearing and acoustic behavior. This research is also highly integrative. Insects provide important models for studies of biophysical and molecular mechanisms of auditory transduction. The relative simplicity of insect nervous systems (compared with those of vertebrates) allows for auditory mechanisms to be studied at the level of explicitly resolved neural circuits and identifiable neurons, and these can be linked to specific behavioral functions.

Keywords Acoustic communication • Active hearing • Auditory mechanics • Auditory processing • *Drosophila* hearing • Evolution • Insect hearing • Mechanoreception • Mechanotransduction • Predator detection • Scolopidia

1.1 Hearing

Hearing, or auditory perception, refers to the detection of mechanical waves (or vibrations) propagating through a surrounding medium. These vibrations induce movement of specialized body structures (ears) and in turn activate sensory neurons to generate an auditory percept. Hearing is widespread among two disparate animal taxa: vertebrates and insects. A major goal of auditory research in general is to understand the mechanistic basis of human sound perception: as a model for the neural basis of sensory experience more generally and as a basis for maintaining normal auditory function or correcting dysfunction. In this volume, we focus on

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comparative studies of hearing in insects, with the goal of providing an overview of current research on all aspects of hearing in the most diverse and numerous animal taxon. Coverage includes the origins, evolution, and adaptive function of hearing in insects; mechanical and molecular mechanisms of auditory transduction; and organization and computational function of the auditory pathway.

All auditory systems must accomplish the same basic tasks. Sound is a source of information that can guide behavior. In a few taxa, self-generated sound or vibration is used as a mechanism for "sounding" to acquire information about the environment (Surlykke et al. 2014). In general, however, the information derived from sound refers to an external sound source. This may be a predator (to be avoided), a conspecific (to be approached), or another source that might signal the presence of a resource (such as a prey item or a host). The function of hearing, therefore, is to detect, recognize, and localize relevant sound sources, possibly among numerous irrelevant ones. This applies to insects as well as vertebrates. Whereas vertebrate hearing has a common ancestry (single evolutionary origin; Manley 2012), hearing has evolved independently many times among insects (at least 24 by current estimates; see Greenfield, Chapter 2). Thus insects are a useful group for comparative studies aimed at identifying common functional principles of hearing and auditory processing. In addition, the relative simplicity of insect nervous systems, and their "economical" design, often make these questions much more experimentally tractable than in vertebrates. Research on insect auditory systems has uncovered a variety of novel and elegant solutions to classic problems in hearing and neuroscience, including selective attention (Pollack 1988), reafference (Poulet and Hedwig. 2002), categorical perception (Wyttenbach et al. 1996), and sound localization (Robert et al. 1996). Insect hearing research also makes more directly practical contributions, as their elegant solutions to some of these sensory tasks frequently inspire novel strategies for technology development (Robert et al. 2010).

1.2 Hearing in Insects

1.2.1 Sound Pressure and Tympanal Ears

The chapters in this volume represent a relatively inclusive view of "hearing." The narrowest definition of the term (by analogy with human hearing) refers only to detection of the pressure component of airborne waves by means of a tympanal organ (Hoy and Robert 1996). Insects, largely as a consequence of their small size, operate at a wider range of spatial scales than most vertebrates. It is true that the insects that are most obviously recognizable as having an auditory system are those that communicate using acoustic signals over long distances. Examples of these include the Ensifera (crickets and kaytdids) and cicadas, and in these groups hearing is mediated by tympanal organs detecting sound pressure—unambiguously fitting the narrowest definition of hearing. The obvious acoustic signals, easily detectable to the human ear, produced by many of these species (usually males advertising to or courting females; see Balakrishnan, Chapter 3) have resulted in a long history of research on their hearing (see Pumphrey 1940). In addition to their role in intraspecific communication, the tympanal ears in many insects function in the detection of predators (mainly echolocating bats; see Pollack, Chapter 4).

1.2.2 Near-Field Hearing and Particle Displacement Ears

For many insect species, however, behaviors mediated by sound occur within the acoustic near field (see Windmill and Jackson, Chapter 6 and Kamikouchi and Ishikawa, Chapter 10), and the relevant cues are derived from the particle displacement component of sound. Examples of these include fruit flies (Drosophilidae) and mosquitoes (Culicidae), and among these groups near-field sound is detected with nontympanal ears, usually Johnston's organ, a mechanosensory structure located on the second segment of the antennae. In some species, such as fruit flies, this sensory system mediates an acoustic communication system broadly similar to those based on long-distance calls (i.e., males signaling to court females) despite the short-range nature of the signals. Others, such as mosquitoes, do not produce specialized acoustic signals, but hearing functions to allow males to detect the low-frequency tones produced by female wingbeats when they are in flight. A closely related sensory modality is fluid flow sensing, which is mediated by mechanosensory hairs activated by air particle movement over a wide range of frequencies (Bathellier et al. 2012). The hairs are often abundant and widely distributed over the insect body, but in many species they are also concentrated in specialized organs, the cerci, with functions that parallel those of the auditory system in some respects (Jacobs et al. 2008; Casas and Dangles 2010). Fluid flow senses are, nevertheless, considered a distinct modality from near-field hearing and are not discussed in this volume.

1.2.3 Sound and Vibration

Mechanical waves that function as signals or cues may also propagate in solid substrates. Sensitivity to such substrate-borne signals is ubiquitous in insects, and many species have well-developed communication systems using substrate vibration signals. There is a close relationship between sound and substrate vibration in some insect taxa. In the Ensifera, there is a close anatomical relationship between auditory and vibratory sensory structures (Strauss and Lakes-Harlan 2009). Among the true bugs (Hemiptera), cicadas make extensive and obvious use of acoustic signals that they detect using tympanal ears, whereas other closely related species communicate via signals propagated through solid (plant) substrates (Cokl and Virant-Doberlet 2003). The relationship between substrate vibration sense and tympanal hearing of airborne sound is a major question in the evolution of hearing (see Greenfield, Chapter 2).

Most reviews of insect hearing begin with a statement of the scope of material to be covered that specifically indicates the categories of “hearing” that will be included. This volume includes the full spectrum, with an admitted emphasis on airborne sound detected by tympanal ears (Hoy and Robert 1996)—this being the most extensively studied area and representing the most common and clearly acoustically active insect species. Nevertheless, the striking diversity of insect auditory systems is unified by the common underlying structure, at the cellular level, of the scolopidia comprising the sensory organs themselves (Field and Matheson 1998). In addition, a growing body of research (largely on the nontympanal ear of the fruit fly) has demonstrated a broader homology at the molecular level that encompasses both insects and vertebrates (Senthilan et al. 2012). Likewise, increasingly fine-grained analyses of the mechanics of insect typmanal organs have identified other commonalities with vertebrate systems, including traveling waves in the locust eardrum (Windmill et al. 2005), cochlea-like mechanics in katydid ears (Montealegre-Z et al. 2012; Udayashankar et al. 2012), and active amplification in a number of insect ears (Mhatre 2014). All of this highlights the value of the broadest comparative approach. There is a continuity of structure and function across the full spectrum of insect auditory mechanoreception that justifies this inclusiveness. Where this volume is slightly more conservative, on the other hand, is taxonomic scope. Unlike the previous SHAR volume on insect hearing (Hoy et al. 1998), we do not include spiders, another major arthropod group that makes extensive use of sensory information derived from substrate-borne vibrations. A number of recent reviews have covered vibrational communication in spiders (Elias and Mason 2014). Likewise, there is little mention of underwater hearing (Sueur et al. 2011).

The contents are broadly organized around three areas. Chapters 2–5 review topics on the origins and functions of hearing in insects. Chapters 6 and 7 cover mechanisms of acoustic transduction at the mechanical and cellular levels, respectively. The remaining chapters (Chapters 8–10) focus on the neural basis of hearing and auditory behavior.

1.3 Origins and Functions of Hearing

1.3.1 Evolution of Acoustic Communication in Insects

This striking diversity of ears has, at times, seemed to defy logical explanation, as (Oldroyd 1962, p. 63) observed:

> There is little rhyme or reason about which insects have tympanal organs, and whereabouts on the body they are placed. The short-horned grasshoppers have them on the first segment of the abdomen, but the long-horned grasshoppers and crickets have them on the fore-legs. Many bugs and moths have them in the thorax, but cicadas and some moths have them in the abdomen; no butterflies have them at all.

Based on current evidence, hearing (i.e., a sensitivity to airborne sound) has arisen independently at least 24 times among different insect taxa, and this list is now known to include also butterflies (Lucas et al. 2014). Studies of the origins and evolution of hearing provide an important foundation for comparative analyses, and current evolutionary and phylogenetic analyses have provided insight on origins and patterns of insect auditory diversity. In Chapter 2, Greenfield reviews current research on the evolution of hearing and communication in insects. A long-standing issue has been the question of whether insect hearing evolved in the context of intraspecific communication or predator detection. The numerous independent origins of insect hearing would suggest that there need not be a single answer to this question.

Nevertheless, within a number of large insect taxa, there are alternative models for the evolution of hearing. Among groups such as the Ensifera, where hearing is reliably associated with sound production and acoustic communication, the question remains whether hearing arose first and communication later and what evolutionary processes (e.g., sensory bias, sexual selection) resulted in the elaboration and diversification of these systems. Among insect lineages with auditory systems devoted to the detection of predators (mostly echolocation signals of bats; see Pollack, Chapter 4), the nature of selection for the evolution of hearing is clear—a set of acoustic cues highly correlated with a danger of predation makes possession of an auditory system highly advantageous. These broad generalizations are complicated by the fact that in many groups where the origin of hearing appears to have been predator detection, hearing has secondarily evolved to function in intraspecific communication. The opposite pattern is also common, in which acoustically mediated predator avoidance has evolved as an elaboration of auditory systems whose primary function is communication. Finally, the anatomical diversity of insect ears must be understood in light of the relationship between auditory organs and the other mechanoreceptive systems from which they are derived (proprioception, tactile, substrate vibration). Thus, tracing the evolutionary origins and diversification of insect hearing is a complex task but one that is central to answering the broad comparative questions.

1.3.2 Behavioral Ecology of Insect Acoustic Communication

In addition to the evolutionary origins and diversification of hearing and auditory systems in insects, much research has focused on the current functions of hearing. These fall broadly into the two categories considered in Chapter 2: intraspecific communication and predator detection. The behavioral ecology of insect hearing is reviewed in Chapter 3 by Balakrishnan. The focus here is mainly on long-distance acoustic communication, taking an integrative approach that considers production, transmission, and detection of acoustic signals. Variation in the costs and benefits of signal production and processing for both partners in a communicative exchange leads to a diversity of behavioral strategies (and signal structures) that is, to some extent, independent of the taxonomic diversity of the auditory systems involved. Behavioral studies therefore add another layer of complexity to the study of insect hearing. In addition to addressing important questions in animal behavior and evolution, clear understanding of behavioral function, and therefore the information content of signals, is vital to the broader comparative study of hearing.

In many acoustic insects, sound production serves primarily in pair formation, with males producing advertisement calls and (usually) females recognizing and localizing these signals. In some species females produce a specific response to male calls, and it is males that localize. Both sound production and movement toward a sound source may be costly because of the energetic expenditure and increased predation risk that these conspicuous activities entail. The partitioning of these costs among the sexes and across different phases of communication and mating can have a strong influence on signaling strategies and adaptations of the auditory system. In addition to mate identification, insect acoustic signals also function in aggressive contests and aggregation. In each of these contexts, individual variation in signal characteristics may carry relevant information for a receiver. Thus behavioral studies may identify multiple levels of discrimination of signal parameters that must be mediated by the auditory system—species-specific cues that identify the source as a conspecific and individual-level variation in signal parameters that correlate with some aspects of phenotypic quality. Finally, all of this information exchange relies on signal propagation through the environment. Filtering and distortion of signals due to environmental effects, and the presence of noise from other sources (anthropogenic noise is recognized as an increasingly important factor in many systems), impose further constraints on signalers and receivers.

1.3.3 Hearing for Defense

Just as for intraspecific communication, a clear understanding of the behavioral function, the relevant acoustic parameters, and the costs and benefits of alternative behavioral strategies are essential to comparative studies of the diverse insect auditory systems (in fact, the majority) that function in predator avoidance. Hearing that functions in this defensive context is reviewed in Chapter 4 by Pollack. The major difference between hearing in predator defense and in intraspecific communication is the absence of mutual interest between sender and receiver. In some contexts, there may be conflicting interests between sender and receiver in intraspecific communication. For example, signalers may benefit from exaggerating indicators of quality or competitive ability, whereas receivers will benefit from accurate information. Nevertheless, signals are by definition adapted to the function of transferring information (Bradbury and Vehrencamp 2011) and intraspecific communication will always entail some form of sender–receiver coevolution (Balakrishnan, Chapter 3). Acoustic detection of predators, on the other hand, is more purely an evolutionary arms race (Conner 2013). Acoustic predator detection in insects largely refers to the detection and avoidance of the ultrasonic echolocation calls of bats. Behavioral responses tend to be more uniform in this context—avoidance is always the adaptive response. Nevertheless, a range of avoidance tactics may be available (including eluding detection by the predator, evasive maneuvers, or defensive behaviors that may include sound production) and selection of the appropriate action is mediated by auditory analysis of the relevant acoustic cues. In addition, selection on receivers will favor rapid responses that require reliable detection and discrimination of broad classes of signals (to identify danger) but not necessarily finer, individual-level variation among examples of signals (as may often be the case for communication). A further layer of complexity arises from the fact that many species that have evolved hearing in the context of predator detection have secondarily co-opted these systems for intraspecific acoustic communication. The reverse is also true, with auditory systems whose primary function is intraspecific communication being adapted to an antipredator function. Predator avoidance hearing, therefore, provides a wide variety of examples of auditory processing with different "weighting" on the same basic components of auditory function (detection, recognition/discrimination, and localization).

1.3.4 Vibrational Signaling

It has long been recognized that substrate-borne vibration is an important source of sensory information to many animals, perhaps especially insects, and that there is a close relationship between the sensory organs that detect vibration and airborne sound in some of the most extensively studied acoustic insects (Ensifera). Nevertheless, vibration sense has tended to be less studied than hearing. At least in part, this has been due to the technical challenges of measuring and controlling vibrational stimuli. Recent decades have witnessed something of an explosion of research in substrate vibration senses in diverse animal taxa, including insects. Chapter 5 by Yack covers this important topic. Substrate vibration senses are used in many of the same behavioral contexts as airborne sound. A very different set of physical constraints, however, govern waveform structure and propagation in solids and, consequently, the detailed structure and mechanics of the associated sensory organs. These factors, in turn, affect behavioral adaptations to exploit sensory information from vibration as well as the underlying neural mechanisms. This chapter provides an overview of all these aspects of the rapidly advancing field of vibrational "hearing" in insects.

1.4 Mechanisms of Acoustic Transduction

1.4.1 Mechanical Specializations of Insect Ears

All auditory systems must work within the common constraints of physical acoustics. Some of these constraints are particularly severe for insects because of their generally small size, and the most striking aspect of the diversity of insect auditory systems is the variety of mechanisms they have evolved to convert the energy of propagating waveforms into movement of scolopidia in the auditory sensory organ. The mechanical aspects of audition in insects are reviewed in Chapter 6 by Windmill and Jackson. Because the scope of this volume includes hearing of both near- and far-field sound, two fundamentally distinct mechanisms of acoustic transduction are considered (displacement and pressure detectors, respectively). Displacement receptors are essentially fine hairs (or antennal segments), jointed at the base, which oscillate with the bulk movement of air molecules in a sound field (Robert et al. 2010). These are typically localized to the antennae and in some groups (particularly Diptera—the true flies) are associated with the complex Johnston's organ. There is no clear analogue for this transduction mechanism in vertebrate hearing. Nevertheless, beyond this initial transduction step, near-field auditory systems accomplish the same processing tasks as all auditory systems do and represent some important model systems (see Chapter 10 by Kamikouchi and Ishikawa). A thorough understanding of the mechanics of transduction in these systems is therefore of great interest.

Auditory systems functioning in the acoustic far field typically include a tympanal membrane (eardrum) as the primary input of acoustic energy. The insect body plan, a rigid exoskeleton richly supplied with mechanical extero- and proprioceptors, combined with a respiratory system consisting of a distributed network of air-filled chambers, has made for a wealth of evolutionary "opportunities" for the development of audition. This principle is clearly represented in the phylogenetic and anatomical diversity of tympanal ears among insects (Yack and Fullard 1993). The mechanics of auditory transduction represents the initial step in extracting information from relevant parameters of the sound field. Insect ears, like those of vertebrates, require mechanisms of impedance matching between the sensory structures and the medium of sound propagation to allow efficient capture of acoustic energy. Frequency analysis and directionality are important categories of information that rely on auditory mechanics. Active mechanics is emerging as a more common feature than previously appreciated across diverse auditory systems in insects.

1.4.2 Auditory Transduction

It is at the level of mechanoelectrical transduction—the conversion of mechanical energy to electrical signals at the level of primary auditory receptors—that some of the clearest commonalities among auditory systems spanning insects and vertebrates can be recognized. This topic is covered in Chapter 7 by Eberl, Kamikouchi, and Albert. At the cellular level, the tympanal membrane (or antennal flagellum) is coupled to the basic sensory units of insect hearing, scolopidia. Each of these consists of a group of cells comprising a sensory neuron with dendrite inserted in a specialized scolopale cell and a surrounding group of glial and support cells (Yack 2004). Mechanical displacement of scolopidia induces a deformation of the sensory dendrite that activates the neuron via mechanically sensitive ion channels.

The physiological properties of receptor neurons have been examined in insect auditory systems representing a range of complexity—from the relatively simple ears of some moths, comprising only a few sensory neurons, to the complex antennal ears of flies, with hundreds of sensory cells. These studies have demonstrated considerable variation and specialization in primary auditory responses. Not surprisingly, different ears may be specialized for different stimulus parameters (e.g., frequency, directionality, or temporal resolution) that may correspond with specialization of the mechanical transduction apparatus (see Windmill and Jackson, Chapter 6). But individual receptors within a single ear may also show distinct tuning and response properties, suggesting a complex interplay between the mechanical and molecular (neural) stages of auditory transduction.

Physiological studies have provided some resolution of the electrical events of transduction at the level of the multicellular structure of scolopidia. Much current research is now focused on the molecular basis of auditory transduction, and the Johnston's organ of *Drosophila* has emerged as an important model system for these studies. A number of genes affecting mechanosensory function have been identified that are homologous across insects and vertebrates. Some are implicated in specific types of hearing disorders in humans (Senthilan et al. 2012). The wealth of genetic techniques available for *Drosophila* makes this a very active and rapidly progressing area of study.

There is considerable evidence that mechanoelectrical transduction must ultimately rely on mechanically gated ion channels, that is, force generated by the mechanical transduction apparatus acting directly on the neuronal membrane to alter channel configuration. Although such a channel has not been specifically identified, molecular and genetic studies have uncovered a number of membrane channels that contribute to auditory sensitivity in *Drosophila*, and these are distributed among the multiple cell types within the scolopidia. This diversity of molecular elements and developmental mechanisms in *Drosophila* auditory transduction reflects the complexity of auditory responses observed at the level of receptor physiology. Similarly, a number of molecular elements contributing to active auditory mechanics have been identified. Unraveling these individual elements and identifying their specific contributions (Riabinina et al. 2011) to develop an explicit model of the process of mechanoelectrical transduction is an active area of research.

1.5 Neural Basis of Hearing and Auditory Behavior

1.5.1 Central Neural Processing of Sound Signals in Insects

Hearing mediates a number of important behaviors in insects (see Balakrishnan, Chapter 3 and Pollack, Chapter 4). How are these behavioral tasks accomplished by the nervous system? As mentioned previously (Sect. 1.1), the essential processing tasks of the auditory system are universal. Relevant sounds must be detected, classified (discriminated), and assigned to source locations. Nevertheless, these tasks apply to a rich variety of auditory stimuli in a wide range of behavioral contexts, and current research continues to emphasize common underlying principles of auditory function that make insects informative models. Chapter 8, by Hedwig and Stumpner, covers neural mechanisms of auditory processing in insects. Following the initial steps of mechanical and electrophysiological transduction of acoustic energy (Windmill and Jackson, Chapter 6 and Eberl, Kamikouchi, and Albert, Chapter 7), auditory information is represented in the spiking output of a population of sensory neurons. All of the information available to subsequent processing stages must be encoded at this stage. The spectral content, amplitude, and temporal patterning of acoustic stimuli all may carry information relevant for behavioral decisions, and these parameters are represented in the identities and activity patterns of auditory afferents. Sound amplitude, or intensity, is typically represented in the activity levels (spike number or rate) of auditory afferents, whereas sound frequency is usually represented in differential activation among a population of auditory sensory afferents (Mason and Faure 2004). There is, however, considerable variation across species in the design and complexity of ears and therefore in their capacities to encode and represent variation in sound parameters. Some insects, such as notodontid moths, have ears with only a single sensory neuron (Surlykke 1984), whereas others, such as mosquitoes, have auditory organs comprising thousands of receptor neurons (Field and Matheson 1998). In all cases, information encoded at the periphery is transformed as it progresses through the auditory pathway, usually with a significant reduction in the number of neurons involved in processing at higher levels in the auditory pathway. Temporal patterning of amplitude modulation is an important parameter in the classification of sound sources in many systems, and temporal processing—from the temporal resolution of the auditory periphery to the filtering of species-specific song patterns in the brain—has been an important area of research. Another important function of hearing is localization of sound sources, and extensive work has focused on neural mechanisms to extract directional information from differences in binaural responses. Auditory information must be integrated with ongoing behavior, which often in itself is a source of noise that may interfere with the ability to process auditory information. A female cricket approaching the call of a singing male by phonotactic walking must contend with the effects of her own walking movements on her ears, which are located on her front legs (Schildberger et al. 1988). A singing male cricket must have an analog to the stapedius reflex to avoid being deafened by his own sound production (Poulet and Hedwig 2006). Furthermore, these issues may be complicated by the fact that insect acoustic signals are often highly stereotyped and sustained for long periods with high redundancy, with the inevitable consequence of adaptation in auditory responses. Receivers must have mechanisms to selectively track individual sources, often among many competing simultaneous sources (Schul and Sheridan 2006). Thus insects provide a number of important examples of fundamental mechanisms of auditory processing (tonotopy, selective attention, temporal filtering, feature detection, sensorimotor integration) that have been analyzed at the level of individual neurons in explicitly mapped neuronal circuits.

1.5.2 Information Processing in the Auditory Pathway of Insects

Understanding the nature of higher level processes in the nervous system is an active area of neuroscience, and insect hearing makes important contributions here too. A practical understanding of processes such as feature detection, temporal filtering, pattern recognition, and decision making requires that the specific details of neuronal connectivity and response properties be translated into a formal description of the underlying algorithms that are implemented by neural circuitry. But this must be built from a thorough understanding of the information content of acoustic signals and its behavioral significance as well as a detailed analysis of how this information is encoded at different levels of the auditory pathway. The topic of computational models of insect auditory processing is covered by Ronacher in Chapter 9. A large number of insect taxa (especially among the grasshoppers, crickets, and katydids) communicate using acoustic signals that are highly stereotyped and sustained over long time periods. There is a huge diversity of temporal structure and spectral content among different species. Because these groups are some of the most numerous and noticeable (to humans) acoustic insects, they have been the subjects of a long history of research into the behavioral and neurophysiological aspects of acoustic communication. Thus, there is sufficient diversity of models to inform a broadly comparative approach as well as sufficiently detailed behavioral and neurophysiological data to test theoretical models for general principles of neural computation. Chapter 9 examines information coding at multiple levels in the auditory pathway, the significance of inherent variability in neural responses for the robustness of sensory coding, and modeling approaches for feature detection in auditory responses that correspond with behavioral preferences.

1.5.3 Hearing in *Drosophila*

Other chapters in this volume have reviewed research on a variety of systems and emphasized the importance of comparative studies and the diversity of insect hearing. Chapter 10 by Kamikouchi and Ishikawa reviews research on hearing in fruit flies (*Drosophila* sp.) and presents an overview of research in a single, well-studied system. Acoustic communication in *Drosophila* has been described since the 1960s (Ewing and Bennet-Clark 1968), and contemporary studies of hearing in fruit flies range from the molecular basis of auditory transduction to the development and function of central circuitry. The availability of extensive genetic tools makes *Drosophila* an important model system in integrative auditory neuroscience (Kittelmann and Goepfert 2014). Fruit fly males produce complex acoustic courtship signals that elicit responses in females as well as other males, and these responses can be manipulated in behavioral and genetic experiments (Coen et al. 2014). Fruit fly courtship signals function in the acoustic near field. Thus, unlike the majority of taxa included in this volume, the auditory system detects particle displacement via a nontympanal ear (Windmill and Jackson, Chapter 6). As mentioned previously (Sect. 1.2.3), however, the sensory mechanisms at the cellular and genetic levels are common across all types of insect ears. Genetic tools now available for *Drosophila* allow for detailed analyses of the development and neuroanatomy of the auditory system, from primary receptors to central circuits as well as the manipulation of specific elements within these circuits. Mechanical transduction by the complex antennal ear activates auditory sensory neurons in Johnston's organ, a highly complex sensory chordotonal organ comprising hundreds of receptor neurons in fruit flies. It is now understood that active amplification plays a significant role in Johnston's organ, and research in *Drosophila* has made important contributions to our understanding of the cellular and molecular mechanisms of active processes in hearing and their role in determining auditory tuning and sensitivity (Windmill and Jackson, Chapter 6; Eberl, Kamikouchi, and Albert, Chapter 7). As in any auditory system, information from sensory inputs is processed by central neural circuits to drive appropriate behavior. Studies of the development, organization, and function of auditory circuits are emerging as an important area of research in *Drosophila* (Lai et al. 2012).

1.6 Future Directions

Early research on insect hearing included a certain amount of debate on the question of whether sensory terms, such as "hearing," necessarily implied humanlike perception and therefore whether these terms could be applied to insects (which could not be assumed to possess anything analogous to subjective perception). This issue was summarized briefly by Pumphrey (1940), who argued for an operational definition of hearing as responsiveness to sound. Versions of this debate have continued, however. These might be broadly defined as the question of what studies of insect hearing (or neuroethology in general) have to say about mammalian (or human) systems. Clearly, the common structure and function of neurons and the composition of nervous systems means that simpler nervous systems, such as those of insects and other invertebrates, can be very informative about fundamental neural mechanisms from membrane biophysics to circuits. Research on insect hearing continues to make significant contributions in a number of areas including basic neuroscience, the genetics and cell physiology of hearing disorders, and sensor and signal processing technology (Wessnitzer and Webb 2006; Akcakaya et al. 2011; Liu et al. 2013).

Likewise, no one would dispute that behavioral biology, ecology, and evolution have been greatly advanced by studies of acoustic communication in insect models. But when it comes to linking specific neural mechanisms with behavior, there is sometimes debate (or confusion) about what definitions apply in the two domains. For example, discussions of neural mechanisms that underlie decision making often assume (implicitly or explicitly) that decision mechanisms must reside in the central nervous system. This reasoning has fed back to discussions of behavioral and evolutionary questions. Understanding of the role of female choice in the evolution of signals and communication has been obscured by unnecessary disputes about whether an effect of peripheral sensory filtering could be considered as part of a mechanism for “choice” (in the evolutionary sense). From an evolutionary point of view, adaptation refers to mechanisms that sort alleles between one generation and the next. Choice, in the context of sexual selection (Darwin 1871), was defined operationally—without reference to any particular internal mechanism (or subjective experience) on the part of the “chooser.” Patterns of female behavioral responses that reliably discriminate among variants of a male call, with the effect that some male phenotypes are more likely to reproduce than others, constitute female choice in the evolutionary sense. If it is subsequently determined, for one species (or signal parameter), that the neural mechanism mediating this female “choice” behavior is a bias in the peripheral sensory system that makes some male call variants more effective stimuli than others (e.g., males calling with different carrier frequencies), and for another species (or parameter) that female “choice” behavior requires the activation of network of brain neurons sensitive to the rate of amplitude modulation in the male call, does this distinguish qualitatively distinct categories of mechanism (Parker 1983)? Both contribute consistently to a female-mediated bias in male mating success, and both are ultimately mediated by a stronger or weaker stimulation of the same sensorimotor pathway.

An (arguably) unbiased perspective on insect neuroethology sees insects as providing a wealth of natural examples of mechanisms for generating adaptive behaviors in autonomous agents with an elegant efficiency of neural (and other) resources. Wehner (1987) provided a number of beautiful examples to show that apparently complex sensory processing tasks are accomplished with elegant simplicity thanks to the organization of peripheral sensory organs as environmentally matched filters. As the field progresses, more complex sensorimotor mechanisms are understood in more explicit detail at multiple levels of analysis (behavioral, neural, algorithmic), and this understanding can be validated in “real-world” models through robotics. At the same time, the active field of biologically inspired robotics demonstrates the utility of understanding the biological systems (Pfeifer et al. 2007). This inherently operational approach to sensorimotor processing and autonomous behavior shifts the debate to questions of what insects (or robots) might have to say about cognition (Wilson and Foglia 2011; Pfeifer et al. 2014). This might be considered a question of semantics (how to define “cognition”), but it might also be worth considering whether a given definition is identifying a boundary between qualitatively distinct categories (Webb 2012) or setting an arbitrary threshold across a continuum of variation (van Swinderen 2005).

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Chapter 2
Evolution of Acoustic Communication in Insects

Michael D. Greenfield

Abstract Tympanal organs for hearing in the far field have evolved on multiple occasions among insects and are currently found in seven orders. Many, if not most, cases of insect hearing probably originated as a means for detecting and avoiding predators. In particular, sensitivity to ultrasound appears to have coevolved with echolocation signaling by insectivorous bats. However, on an overall scale, hearing is relatively rare among insects in comparison with other modalities of perception, including detection of substrate vibration. Sound signaling in insects, which typically occurs in the context of mating communication, is rarer still and is known in only five orders. Phylogenetic analyses suggest that acoustic communication in the Lepidoptera and in the suborder Caelifera (grasshoppers) of the Orthoptera originated via a "sensory bias" mechanism. Hearing was ancestral and sound signaling by males subsequently arose on multiple, independent occasions. On the other hand, acoustic communication in the Cicadidae and in the suborder Ensifera (crickets, katydids) of the Orthoptera may have originated via coevolution between female perception and male signaling. The diversity of songs among acoustic insects may reflect genetic drift and reproductive character displacement. There is little evidence, however, that insect songs are adapted to specific physical environments. In one clade of acoustic insects, the diversification of song is associated with an unusually high rate of population differentiation and speciation, which may be facilitated by a genomic co-localization of loci influencing female response/preference and male signaling. The extent to which co-localization is a general factor in speciation remains to be explored.

Keywords Coevolution • Convergence • Courtship • Ecological speciation • Fisherian mechanism • Genetic drift • Phylogenetic inference • Pleiotropy • Reinforcement • Reproductive character displacement • Sensory bias • Sexual advertisement • Sexual dimorphism • Size constraints • Ultrasound

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2.1 Introduction

Evolution of acoustic communication is one of the more intriguing topics in insect hearing, but it is also one of the more intractable. The several books devoted to this topic since the 1980s attest to a general interest (e.g., Ewing 1989; Bailey 1991; Gerhardt and Huber 2002; Drosopoulos and Claridge 2006; Hedwig 2014). Some of this attraction might be explained by curious similarities between vertebrate hearing and its counterpart among insects (e.g., Boekhoff-Falk 2005; Montealegre-Z et al. 2012). The intractable nature of the topic stems from certain attributes that are common to communication in all animals. Communication entails signal production as well as the perception of those signals, and understanding the evolution of communication demands attention to these two functions as distinct entities as well as to their inseparability (Greenfield 2014a). Like other aspects of behavior, communication seldom leaves direct fossil evidence, and one must often resort to rather indirect, phylogenetic inferences to speculate on its origin. Moreover, the insect fossil record is notoriously incomplete, and where it does exist for acoustic species, the representations of the signaling and perceptual organs are generally lopsided in favor of the former (cf. Senter 2008). Nonetheless, current paleontological (e.g., Gu et al. 2012) and phylogenetic (Regier et al. 2013; Misof et al. 2014) information allows the formulation of new overviews of the evolution of acoustic communication in insects, which are presented in this chapter.

The basic propositions for the evolution of acoustic communication are that its two components, sound production and hearing, either arose jointly or that one component preceded the other. In the context of sexual communication, which is the major focus here, these two possibilities are the Fisherian mechanism (Lande 1981; Kirkpatrick 1982), wherein mating signals and preferences coevolved at and following their origin, and the so-called "sensory bias" mechanism in which the perceptual function was ancestral (Endler and Basolo 1998; see Arnqvist 2006 for a more extensive treatment of these possibilities). In this chapter a considerable amount of evidence consistent with one component, usually perception, preceding the other is presented. The various factors that may have been selected for the origin of hearing or sound production are considered, followed by discussions of how and why sound production or hearing then appeared at some later time and how and why one or both components changed over the course of their evolutionary history.

2.2 What Is Acoustic Communication?

This seemingly straightforward question is actually fraught with diverse interpretations. Although it might appear that acoustic communication could be defined simply as the transmission, via sound waves, of intraspecific messages that are, on average, mutually beneficial to the signaler and receiver (cf. Hauser 1996; Bradbury and Vehrencamp 2011), problems arise in distinguishing sound and vibration. This distinction is critical because the perception of substrate vibration and the transmission of vibration signals are rather widespread among insects and other arthropods, but hearing and acoustic signaling are quite rare (Greenfield 2002). Thus, an understanding of how acoustic communication evolved in insects demands a clear distinction between sound and vibration that is relevant to biology.

One major difference observed among sound and vibration phenomena concerns the pattern of wave dispersion in the environment. Sound waves in air or water spread more or less omnidirectionally in all three dimensions, although some local attenuation and amplification of amplitude may result from vegetation and other barriers (Römer 1993), impedance differences within the air or water arising from thermal layering (Van Staaden and Römer 1997), or the morphology and posture of the animal that is signaling (Forrest 1982). Vibrations in the substrate, on the other hand, are typically confined to the same medium in which the signaler initially generated them, for example, surface of the ground (or water), vegetation, silk webbing, or social insect nest material. Consequently, for communication to occur, the receiver(s) as well as the signaler should remain on that specific medium, implying that vibration signals usually spread in one or two dimensions only. Moreover, the transmission of a vibration signal may be strongly attenuated when the quality of the medium is altered (Elias and Mason 2014). For example, vibration signals that are transmitted effectively on wet sand may be severely weakened on dry sand (Aicher and Tautz 1990), or the carrier frequency and amplitude of signals that an herbivorous insect transmits in stems and leaves of its host plant may be greatly modified in another plant species (McNett and Cocroft 2008). The upshot of this assessment is that sound signals in air or water have the potential to reach many more local receivers than do vibration signals. Relevant to the evolution of acoustic communication, patterns of wave dispersion may have hindered certain mechanisms of speciation from functioning among insects that send and receive sound signals (see Sect. 2.6.1). Recognizing this distinguishing feature, the current chapter focuses on the evolution of sound signals propagating in the fluid medium within which the communicating individuals are immersed and of the organs that perceive these signals. Vibration communication is discussed in detail by Yack, Chapter 5.

In addition to signal dispersion, acoustic communication is also distinguished by the use of specialized perceptual organs. In the far field, most insects use tympanal organs, chordotonal organs that are fitted with an exterior membrane, the tympanum, and an air cavity behind the membrane to detect the pressure waves of sound broadcast in air or water (Hoy and Robert 1996). Notable exceptions include pneumorid grasshoppers (Van Staaden and Römer 1998) and possibly some cockroaches (Shaw 1994), which perceive sound with internal chordotonal organs that lack external anatomical features, as well as one group of sphingid moths in which overlapping scales form a “functional tympanum” (Göpfert et al. 2002). Body size may be a constraining factor in the evolution of tympanal organs, as the membrane would have to exceed a minimum diameter or be under extreme tension to respond effectively to sound delivered at all but the very highest carrier frequencies (cf. Fletcher

1992). For example, a 0.5-mm-diameter tympanum may respond maximally to 100-kHz sound delivered above a threshold amplitude of 60 dB sound pressure level (SPL; 0 dB = 20 μPa) (Rodriguez et al. 2005). Thus, the smallest insects generally do not possess tympanal organs and lack the ability to hear far-field sound (e.g., Roces and Tautz 2001). The transmission of sound into the far field, normally considered as beginning approximately one wavelength from the source, may be similarly constrained, as the diameter of the sound radiating structure may have to exceed a minimum fraction of the wavelength of the structure's vibration frequency (cf. Fletcher 1992). For example, a 0.4-mm-diameter tymbal may resonate at 100 kHz (wavelength = 3.4 mm) and generate a sound amplitude of ≈95 dB at 1 cm (Spangler et al. 1984). Such size constraints may influence the perception and propagation of substrate vibration much less, a factor that could explain, in part, why vibration communication is relatively ubiquitous among insects, even among small to minute species, less than 10 mm in length (Cocroft and Rodriguez 2005). In the near field, however, insects generally detect the particle-velocity aspect of sound with relatively unspecialized filiform hairs or other structures on the body (Tautz and Markl 1978) and appendages (Göpfert and Robert 2000), and these organs may not be strongly limited by size (e.g., Göpfert and Robert 2001). The propagation of sound into the near field, typically low in carrier frequency and generated by simple movement of unmodified wings, may also be relatively free of size constraints. Accordingly, some very small insects, for example, *Drosophila* spp. (Bennet-Clark 1971; Hoy et al. 1988), have well-developed acoustic communication in the near field. Overall, however, near-field sound signaling is not a common form of communication among insects, and other factors, possibly low inefficiency for transmitting information, may have limited its evolution. But biologists may have also overlooked some cases of near-field sound communication, as few studies have been equipped to detect it, particularly in the field.

Respecting this chapter's restriction of sound signals to those transmitted in air or water, there are several situations where the potential for acoustic communication needs clarification. Many insect species generate airborne sound as a by-product of substrate vibration signals, and in some cases biologists can monitor this signaling activity by registering the resulting sounds (Greenfield 2002). But unless the receiving individuals are equipped with hearing organs sensitive to those sounds, any communication would be along the vibration channel only. A more complex situation arises when an individual produces a substrate vibration signal that generates a concomitant airborne sound that may then cause certain distant substrates to vibrate. If a potential receiver resting on one of these distant substrates detects and responds to the vibration, then this situation would constitute acoustic communication. The air has served as the channel for transmitting the signal, and the substrate beneath the signaler and receiver functioned only to transfer the vibration between the insect and the air. Nonetheless, this particular form of acoustic communication would be somewhat restricted, being limited by the requirement that both signaler and receiver remain on a specific substrate. Potential cases may occur in spiders, and it is presented mostly as an exercise for evaluating the nature of signals and communication.

2.3 Acoustic Communication: Who Are the Actors and What Are Their Actions For?

From a phylogenetic perspective, hearing and acoustic communication are poorly represented in the animal kingdom; see Gagliano (2013) for discussion of the possibility of acoustic communication in plants. Ears that are sensitive to sound waves in air or water are known only in vertebrates and some arthropods and cephalopods. In arthropods, they are largely restricted to several orders of insects. Recent findings show that some spiders (Gordon and Uetz 2012) and crustaceans (Hughes et al. 2014) are sensitive to airborne and waterborne sound, respectively, but it is not clear whether these taxa exhibit acoustic communication as defined in Sect. 2.2. Similarly, sensitivity to waterborne sound is reported in cephalopods (Mooney et al. 2010), but there is no indication of acoustic communication in these species.

Unlike vertebrates, in which inner ear structures are found in all classes and appear to have descended from a single evolutionary origin during the Devonian Period (approx. 400 MYA before present) or earlier (Popper et al. 1992), insect ears that perceive the pressure waves of far-field sound are known in only 8 of the 32 recognized orders (Table 2.1) but have evolved independently at least 24 times, and perhaps as many as 29 (cf. Yager 1999). The uncertainty reflects the various phylogenies that have been proposed in several groups, particularly Lepidoptera and Orthoptera, and the unresolved status of these phylogenies at present. Current information shows that pressure-sensitive ears are present in the following insect orders: Orthoptera (grasshoppers, crickets, katydids), Mantodea (mantises), Hemiptera (true bugs, plant lice, cicadas), Neuroptera (net-winged insects), Diptera (true flies), Lepidoptera (moths and butterflies), Coleoptera (beetles), and possibly Blattodea (cockroaches) (Table 2.1). However, pressure-sensitive ears are not found throughout any of these orders, and in some they are known in only a few isolated groups. For example, in the Coleoptera, hearing is reported only in certain tiger and scarab beetles (Cicindellidae, Scarabaeidae; Spangler 1988; Forrest et al. 1997). When acoustic communication is considered, the distribution of confirmed cases is restricted further: Orthoptera, Hemiptera, Lepidoptera, and possibly Blattodea for pressure waves in the far field; Diptera and Hymenoptera for near-field sound (Table 2.1). Moreover, it is only in two groups of Orthoptera and one group of Hemiptera that communication by sound is widespread and a dominant form of signaling. Acoustic communication in the other orders is rare and/or a complementary behavior that functions alongside other signaling modalities. Thus, a concentration on the evolution of acoustic communication in insects is sustained by an interest in how a behavior best known in birds, mammals, and anurans originated in very different groups of organisms and attained similar levels of development despite operating under some severe handicaps, namely small size (e.g., Sueur et al. 2011). In addition, some acoustic insects have served as focal species for the study of sexual selection (Zuk et al. 2014) and speciation (Mendelson and Shaw 2005).

In all but one case, acoustic communication in insects functions in the context of mating: sexual advertisement, courtship, or intrasexual competition, the latter

Table 2.1 Distribution of hearing of airborne or waterborne sound, and production of airborne or waterborne sound, among the 32 extant orders of insects recognized by Misof et al. (2014)

Insect orders	Hearing	Sound production
Protura (coneheads)		
Collembola (springtails)		
Diplura (two-pronged bristletails)		
Archaeognatha (jumping bristletails)		
Zygentoma (silverfish)		
Odonata (dragonflies and damselflies)		
Ephemeroptera (mayflies)		
Zoraptera (ground lice)		
Dermaptera (earwigs)		
Plecoptera (stoneflies)		
Orthoptera (grasshoppers, crickets, katydids)	Tympana in several major families; internal abdominal organs in Pneumoridae	Stridulation and wing mechanisms in several major families; various sound frequencies
Mantophasmatodea (gladiators)		
Grylloblattodea (ice crawlers)		
Embioptera (web spinners)		
Phasmatodea (stick and leaf insects)		
Mantodea (mantises)	Tympana in many groups; ultrasound sensitivity	
Blattodea (cockroaches)	Internal tibial organs in one family; possible detection of far-field sound	Expulsion of tracheal air in one family; audible sound
Isoptera (termites)		
Thysanoptera (thrips)		
Hemiptera (true bugs, plant lice, cicadas)	Tympana in two families	Tymbals in two families; audible sound
Psocodea (barklice, true lice)		
Hymenoptera (sawflies, wasps, bees, ants)	Antennal organs for near-field hearing in honeybees	Wingbeat mechanism; low-frequency sound in the near field
Raphidioptera (snakeflies)		
Megaloptera (alderflies, dobsonflies)		
Neuroptera (net-winged insects)	Tympana in one family; ultrasound sensitivity	
Strepsiptera (twisted-wing parasites)		
Coleoptera (beetles)	Tympana in several genera in two families; ultrasound sensitivity	
Trichoptera (caddisflies)		
Lepidoptera (moths and butterflies)	Tympana in three major superfamilies and several additional families; ultrasound sensitivity in most groups	Tymbals, stridulation, and wing mechanisms in isolated genera and species in various groups; ultrasound frequencies typical

(continued)

Table 2.1 (continued)

Insect orders	Hearing	Sound production
Siphonaptera (fleas)		
Mecoptera (scorpionflies)		
Diptera (true flies)	Tympana confirmed in two families, with possible tympanal hearing in a third (Tuck et al. 2009); antennal organs for near-field hearing in several families	Wingbeat mechanisms in several families; low-frequency sound in the near field

Hearing refers to the perception of far-field sound except in those cases where the perception of near-field sound is associated with detecting conspecific signals. Sound production refers to acoustic signals that function in the context of intraspecific communication

including conventional display, territorial defense, and a prelude or accompaniment to aggression (see Balakrishnan, Chapter 3). Most signals are broadcast by males, but in some cases females respond to male advertisement or courtship signals with their own song and a duet ensues (Bailey 2003). These female replies are usually less intense and transmitted over a shorter distance than the male song, and they may be produced by structures that are not homologous with the male sound-producing device (Nickle and Carlysle 1975). Mosquitoes may represent an exception, as it is the female that initially broadcasts the advertisement song and is an equal partner with the male in their dialogue (Cator et al. 2009). Unlike sound production, hearing typically functions in both sexes in acoustic insects, although some level of sexual dimorphism may be present (e.g., Robert et al. 1994). Social insects use many vibration signals in various behavioral contexts, but there is only one confirmed case in which a social signal includes airborne sound that is detected and evaluated by receivers: the forager recruitment dances in *Apis* spp. honeybees (Hunt and Richard 2013). But dance language sounds function only across very short distances (Michelsen et al. 1986; Towne and Kirchner 1989) and are accompanied by substrate vibration (Nieh and Tautz 2000) and tactile signals (Rohrseitz and Tautz 1999), as well as by odor. Consequently, the sound signals of honeybees are not treated further in this chapter. Readers desiring further information on these signals and their evolution may consult Dreller and Kirchner (1993) and Kirchner (1997).

2.4 How Did Acoustic Communication Originate in Various Insect Groups?

Because hearing is more widespread than acoustic communication in insects, one may begin by considering the origin of ears and acoustic perception. One focuses on tympanal ears because these are the organs, with the exception of the abdominal chordotonal structures in pneumorid grasshoppers, that can perceive sound broadcast over relatively long distances and thereby function in all aspects of communication. It is noted that the air cavity behind the tympanal membrane is generally derived from part of the tracheal system (Hoy and Robert 1996), and among arthropods it is insects that fly or that are descended from flying ancestors that have particularly well-developed tracheal systems. Thus, it may not be a coincidence that arthropod tympanal organs are restricted to adult pterygote insects. These are the forms that already had membranous structures that could be "borrowed" for organs sensitive to the pressure waves of far-field sound should the right selection pressure occur. But what may that selection pressure have been?

2.4.1 Ultrasound Sensitivity: Insect–Bat Coevolution

Tympanate insects include Mantodea, Orthoptera (Ensifera and Caelifera), Hemiptera (Corixidae and Cicadidae), Neuroptera (Chrysopidae; lacewings), Diptera (Sarcophagidae, Tachinidae), Coleoptera (Cicindellidae, Scarabaeidae), and Lepidoptera (8 superfamilies). It is noteworthy that in 4 of these orders (Mantodea, Neuroptera, Coleoptera, Lepidoptera), comprising no fewer than 14 independent origins, tympanal hearing is specifically sensitive to ultrasound frequencies (Hoy 1992). Independence of evolution of hearing in the several groups having ultrasound sensitivity is inferred from their ears being nonhomologous organs and the principle of parsimony. The latter assumes that a recent origin of the hearing trait in the common ancestor of a group of extant species is more likely than a more ancient origin in a broader group, followed by subsequent loss of the trait in all but one lineage. Moreover, fossil evidence, molecular analyses, and historical biogeography indicate that most cases of ultrasound hearing have evolved rather recently, primarily since the beginning of the Paleogene Period (Cenozoic Era; 65 MYA before present). This inferred date for the origins of ultrasound hearing in Neuroptera, Coleoptera, and most Lepidoptera suggests a coevolutionary response to predation by insectivorous bats, whose ultrasound echolocation signals arose at that time. Among the groups with specialized ultrasound hearing, tympanal organs appear to have evolved prior to the Cenozoic Era only in the Mantodea (Yager and Svenson 2008) and in one family of the Lepidoptera [Tineidae; clothes moths (Davis 1998); note that ultrasound sensitivity is not confirmed in this group]. In the Mantodea, it is unclear whether ultrasound sensitivity arose at the origin of hearing, possibly as an adaptation for detecting inadvertent sounds made by various predators, or later as a modification of general hearing alongside the appearance of echolocation signals in bats. In summary, nocturnal flight became too dangerous in the Paleogene Period for most insects unless they were equipped to detect, and then evade, the new hunting technique employed by aerial predators. Converting part of the tracheal system for a vital perceptual function was therefore adaptive in that changing biological community (cf. Yager 1999).

The strongest evidence supporting the coevolution of insect hearing and bat predation is found in the Lepidoptera. All but one of the 10–12 independent origins of hearing in the Lepidoptera (cf. Fig. 2.1) appear to have occurred following the

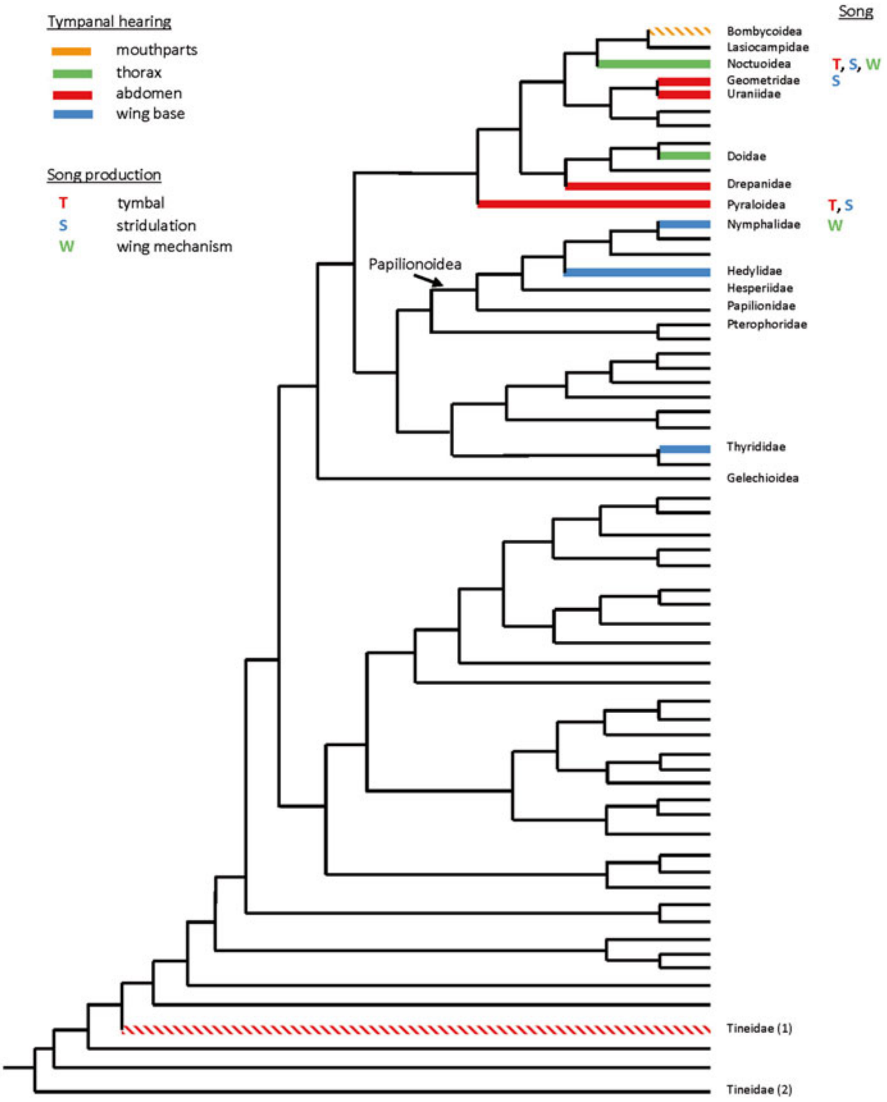

Fig. 2.1 Phylogeny of the “advanced” moths and butterflies (Lepidoptera, section Ditrisia) following maximum likelihood analyses in Regier et al. (2013), showing distribution of hearing and acoustic signaling among the superfamilies and families. In groups indicated by colored bars with diagonal shading, hearing is found only in a small percentage of genera; two independent origins of hearing are reported for the Bombycoidea (silk moths, emperor moths, and sphinx moths; Göpfert et al. 2002). In each of the four groups where acoustic signaling is indicated, song is reported in only a small percentage of species and generally involves multiple independent origins (Greenfield 2014b); there is only one confirmed report of acoustic communication in the Geometridae (geometer moths; Nakano et al. 2009)

beginning of the Paleogene Period (cf. Yack and Fullard 2000). In three of these groups with acoustic perception—Pyraloidea, Geometroidea, and Noctuoidea—ultrasound-sensitive hearing is widespread throughout a very large superfamily. Cases of hearing absence or reduction in pyraloid, geometroid, and noctuoid moths generally involve species found in regions lacking insectivorous bats (Fullard 1994) or that fly at times of the day or year when bats are not active (Fullard et al. 1997). Butterflies (Papilionoidea), which are secondarily diurnal, do not possess ultrasound hearing, but their sister group, the night-flying Hedyloidea (American butterfly moths), do (Yack and Fullard 2000). Certain thoracic structures in butterflies may be vestiges of ancestral hearing organs, and in some butterfly species these structures are adapted to hear audible sound, possibly from birds or other daytime predators (Lucas et al. 2014). Thus, a pattern of secondary loss or shift in frequency sensitivity in hearing emerges in lepidopterans that have escaped the selection pressure imposed by predatory bats. A similar process may have occurred in the Mantodea wherein most species with auditory function exhibit some level of sexual dimorphism in hearing: Female mantises, which have shorter wings, fly less, and are therefore exposed to less bat predation, generally have reduced tympanal organs (Yager 1999).

2.4.2 Sensitivity to Audible and Broadband Sound Frequencies

Hearing in the remaining groups of tympanate insects appears to be much less related to avoiding bat predation, although detection of other predators may have been a critical factor. The tympanate Caelifera (Orthoptera) and Hemiptera are largely diurnal, and their hearing is primarily sensitive to audible sound, far below the frequencies of bat echolocation signals. The tympanate Diptera are mostly nocturnal, but their hearing is also most sensitive in the audible frequency range and serves mostly as a means by which females localize their acoustic insect hosts (Lakes-Harlan and Heller 1992). In Ensifera (Orthoptera), hearing is sensitive to a broad range of frequencies, including ultrasound. Their hearing may have been the earliest to evolve among insects (prior to 250 MYA; Otte 1992), considerably before the appearance of echolocation signals in bats. However, many ensiferans are very sensitive to ultrasound sound frequencies and some exhibit specialized detection and evasion of bat echolocation signals (Faure and Hoy 2000; Schulze and Schul 2001). Paleontological evidence suggests that ultrasound sensitivity was present in katydids as early as the Paleogene Period (Rust et al. 1999).

2.4.3 Sound Signaling

The distribution of acoustic communication signals in insects differs somewhat from the distribution of hearing. Sound signaling is not only found in fewer insect orders (four as opposed to eight), but within some groups of tympanate insects,

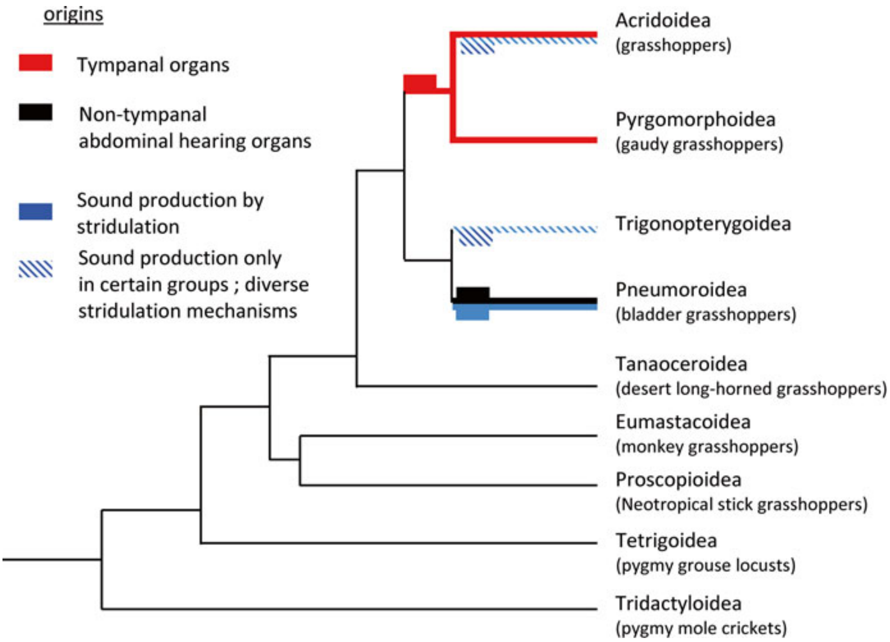

Fig. 2.2 Total evidence phylogeny of the Orthoptera, suborder Califera (short-horned grasshoppers) following maximum likelihood analysis in Song et al. (2015), showing distribution of hearing and acoustic signaling among the superfamilies (cf. Flook et al. 2000). In superfamilies indicated by colored bars with diagonal shading, acoustic signaling is found only in a portion of the group and most likely represents multiple independent origins

sound signaling occurs in only a small percentage of species. Moreover, sound signaling has evolved independently on a great many occasions in several of these groups (Greenfield 2014b).

The Lepidoptera serve as the better example of the above pattern (Fig. 2.1). Tympanal organs occur in nine superfamilies, among which they are distributed on the metathorax (two), abdomen (four), wing bases (two), and mouthparts (one). Based on current phylogenies and nonhomology of the structures, these organs represent between 10 and 12 independent origins (cf. Kristensen 2012; Regier et al. 2013). Sound signaling is known primarily in the Pyraloidea, Papilionoidea, and Noctuoidea, and within each of these three superfamilies, it is distributed in multiple, unrelated genera and species but is absent in most. These various cases of sound signaling involve nonhomologous stridulatory or percussive structures and generally represent many independent evolutionary origins.

The suborder Caelifera of the Orthoptera serves as the second example of the pattern of widespread hearing and a restricted incidence of sound signaling (Fig. 2.2). Tympanal hearing occurs only in the clade including Acridoidea (grasshoppers) and Pyrgomorphoidea (gaudy grasshoppers), although nontympanal hearing is also found in the related Pneumoroidea (bladder grasshoppers). An original

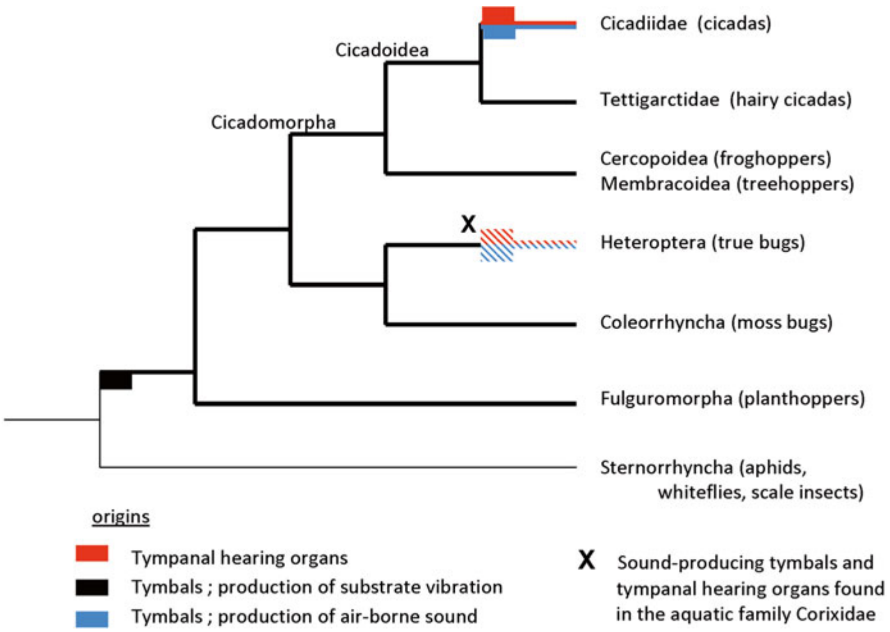

Fig. 2.3 Phylogeny of the Hemiptera (true bugs, plant lice, and cicadas) following Hoch et al. (2006) showing distribution of hearing, vibration signaling, and acoustic signaling among the major subdivisions. In subdivisions indicated by colored bars with diagonal shading, hearing as well as acoustic signaling are found only in a small portion of the group

function of hearing in the Caelifera may have been detecting the inadvertent sounds made by terrestrial predators, for example, such as mammals, reptiles, and other arthropods (Riede 1987; Flook et al. 2000). Sound signaling among Caelifera is more limited, occurring primarily in the Acridoidea, but not in all groups, and the Pneumoroidea (Flook et al. 2000). Stridulatory movements are also present in several additional families (Strauss and Lakes-Harlan 2014) where it may function in substrate or tactile vibration and/or as a visual signal. Thus, sound signaling either evolved basally in a major part of the Caelifera and was subsequently lost in many groups or, as in the Lepidoptera, evolved independently on multiple occasions. The fact that stridulation in Caelifera involves different movements and modified structures in the various groups that sing favors the latter interpretation (cf. Flook et al. 2000; Strauss and Lakes-Harlan 2014).

Acoustic communication in both the Hemiptera and the Ensifera (Orthoptera) differs from the preceding examples in that a fairly close match exists between the incidence of hearing and sound signaling. In the Hemiptera, tympanal hearing is via abdominal organs and is found only in the Corixidae (water boatmen) and the Cicadidae (cicadas; Fig. 2.3). In the latter, it is basal and occurs throughout the family, but it is not found in the sister group, the Tettigarctidae (Australian hairy cicadas; Moulds 2005; Strauss and Lakes-Harlan 2014). Most Hemiptera are sensitive to substrate vibration, though, which they detect via subgenual organs. Signaling

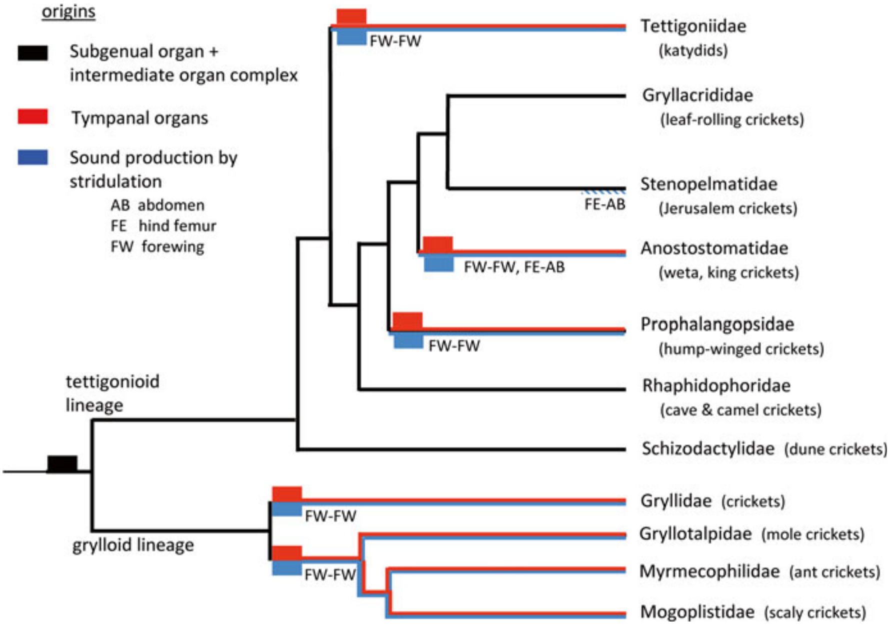

Fig. 2.4 Total evidence phylogeny of the Orthoptera, suborder Ensifera (crickets and katydids) following maximum likelihood analysis in Song et al. (2015), showing distribution of hearing and acoustic signaling among the several families (cf. Desutter-Grandcolas 2003). In groups indicated by colored bars with diagonal shading, acoustic signaling is found only in a portion of the group (and does not function in the context of intraspecific communication). The topology of families in the tettigonioid lineage is not definitive, and the number of independent origins of tympanal hearing remains uncertain. The topology of families in the grylloid lineage is more stable, but the number of independent origins of tympanal hearing in this clade is also uncertain, with one to four origins being possible based on current understanding

via specialized tymbal organs that are adapted for broadcasting waterborne or airborne sound is also found only in the Corixidae and Cicadidae. Again, in the Cicadidae, these organs are found throughout the family but are absent in the Tettigarctidae (Strauss and Lakes-Harlan 2014). Homologous tymbal organs that generate substrate vibration are much more broadly distributed in the Hemiptera, however, and the sound-producing tymbals in Cicadidae have probably evolved from these less specialized structures, which may be a basal character in all Hemiptera minus the Sternorrhyncha clade (Strauss and Lakes-Harlan 2014).

Similarly in the Ensifera, tympanal hearing occurs in several groups in both the tettigonioid and grylloid "lineages," and sound signaling is found throughout all tympanate groups in both lineages (Fig. 2.4). Tympanal organs are all located in the foretibiae, but they have probably evolved independently several times. The ancestral structure appears to be the subgenual organ/intermediate organ complex to which the tympanum and other features (e.g., crista acustica) were added in hearing species (Strauss and Lakes-Harlan 2014). Sound signaling is by means of tegminal-tegminal (forewing-forewing) stridulation in the grylloid lineage, but both tegminal-tegminal and femoroabdominal stridulation are found in the tettigonioid lineage (Strauss and Lakes-Harlan 2014). Additional, nontympanate groups in the tettigonioid lineage exhibit femoroabdominal stridulation, either to effect substrate vibration in the context of communication or as a means of startling predators. Sound signaling has been lost secondarily in some species, notably where levels of attack by phonotactic parasites are unacceptably high (e.g., Tinghitella et al. 2009). The higher level phylogeny of the Ensifera is not fully resolved (Legendre et al. 2010; cf. Song et al. 2015), and it is currently not possible to indicate relationships between some of the acoustic and nonacoustic groups with any certainty.

Acoustic communication in the Blattodea reverses the pattern described in the Lepidoptera and Caelifera. Sound production is more widespread than hearing, which is accomplished via internal chordotonal (subgenual) organs in certain species (Shaw 1994), and it has been argued that the initial and general function of sound was in startling predators. In the Diptera, sound production has been studied intensively in the Drosophilidae (fruit flies; Bennet-Clark 1971; Bennet-Clark et al. 1980), the Tephritidae (true fruit flies; Sivinski et al. 1984), and the Culicidae (mosquitoes; Cator et al. 2009; Jackson et al. 2009). These dipteran songs are relatively low in frequency (mostly <1,200 Hz) and, given that hearing in the receivers is not tympanate, effect communication primarily in the near field. There is relatively little information on sound production and hearing in related groups, which prevents evolutionary inferences at this time.

2.4.4 Origins of Acoustic Communication: Sensory Bias, Coevolution, and Motor Bias

An overall evolutionary trend that emerges from the survey presented in this section is that prior evolution of hearing in a defensive context followed by later appearance of sound signaling in some of the auditory species was likely throughout the Lepidoptera and in the Caelifera. This inferred phylogenetic sequence of events suggests the role of sensory bias in the origin of acoustic communication in these groups. As a possible scenario of the process, in an ancestral species in which males were engaging in stereotypical movement of appendages during courtship to disperse pheronomes or to create a vibration or visual display, a novel behavioral or structural modification that added sound to the display would have found an audience: hearing females. Based on the matched distributions of hearing and sound signaling in both the Hemiptera and the Ensifera, a sensory bias process appears much less likely in these two groups. There is no definitive phylogeny for the Ensifera, and the possibility that hearing and sound signaling originated at the same time via a coevolutionary process cannot be eliminated in the lineages leading to the Gryllidae and to the Tettigoniidae (cf. Desutter-Grandcolas 2003).

Acoustic communication is not a major feature of the Blattodea (cf. Nelson and Fraser 1980), but it illustrates what may be an unusual case of "motor bias." Various species were producing defensive sounds that may have involved activity and movement that males also displayed during courtship. Thus, a novel modification to the vibration-detecting subgenual organs that afforded hearing of airborne sound would have allowed improved evaluation of local males. Here, sensory modification in both males and females would be expected, as both sexes would benefit from precise evaluation of male display.

2.5 What Evolutionary Changes in Acoustic Communication Followed Its Origin?

Communication involves much more than simply detecting a stimulus and making one that can be detected. Communication implies the transfer of reliable information that reduces uncertainty about a signaler's identity, capability, and motivation. Thus, some modification of a stimulus is expected following its origin, whether via sensory bias or a coevolutionary process. Similarly, some modification in perception that would improve a receiver's evaluation is equally likely (cf. Alem et al. 2013). In acoustic communication in insects, these features would generally include identification of the signaler as a suitably compatible mate (e.g., conspecific of the opposite sex), as a potential rival (e.g., a conspecific male, for a male receiver), as a sexually mature adult, and as having a certain "quality" over and beyond merely being suitably compatible or rival. And in the case of long-range advertisement, the source of the signal would have to be localized.

Tracing the evolution of expected modifications in signals and perception would be done best with a group that currently includes a range of communication formats at different stages of development. This specification would remove the Orthoptera—both Caelifera and Ensifera—and the Cicadidae from consideration, as acoustic communication in these groups is fully fledged in most species that do sing and includes all of the elements listed previously in this section. Moreover, in the Ensifera and Cicadidae, sound signaling appears to have originated independently on very few occasions, probably only once in the Cicadidae. On the other hand, the Lepidoptera offer more possibilities for evolutionary inference because sound signaling has originated multiple times, and it exists in diverse formats ranging from simple courtship conducted at very close range to long-range advertisement.

In the nocturnal Lepidoptera, sound signaling is invariably associated at some level with bats and their ultrasound echolocation signals (Greenfield 2014b). Many species of noctuoid moths emit ultrasound signals that either jam bat echolocation systems or warn bats about the insect's chemical noxiousness (Conner 2014). In several species, these very same ultrasound signals serve as a close-range male courtship song (Simmons and Conner 1996). In other noctuoid and pyraloid moths that do not emit sound signals when interacting with bats, the males emit a very quiet call during courtship (Nakano et al. 2008, 2009) that acoustically mimics a generic bat echolocation signal (Nakano et al. 2010). These quiet calls, as well as the bat echolocation signals that they imitate, elicit an arrestment of movement in the female. In an encounter with a bat gleaning insects off surfaces by listening to their telltale noise, this arrestment response would afford a female moth some protection by silencing the sounds she inadvertently makes while moving on the substrate. But when a courting male moth is emitting the ultrasound signal, the result is that the female may remain in a receptive posture or location for a longer duration. Thus, in some cases male moths seem to have co-opted defensive signals for use in courtship, and in others they have evolved a novel courtship call that elicits a female response, increasing the likelihood that mating will take place. This latter format may be described as a male signal that "exploits" an ancestral female response exhibited in a nonsexual context, and it is consistent with male signal evolution via the sensory bias mechanism.

In both formats of acoustic courtship in moths, the male call functions only during the very final part of pair formation, most of which had been effected via a female-emitted advertisement pheromone that attracted the male. Such chemical communication is basal in Lepidoptera (Löfstedt and Kozlov 1997), and it is found in most groups except some diurnal ones, such as in the Papilionoidea (butterflies). But in several species of pyraloid and noctuoid moths, the male calls function over a much longer distance (Greenfield 2014b). Here, the male sound signals may function as an advertisement that replaces the female sex pheromone, which had been secondarily lost over evolutionary time. Such acoustic communication poses several questions. At a mechanistic level, given that the female hears both bat echolocation signaling and the male call and responds with negative phonotaxis to the former and with positive phonotaxis to the latter, how does she distinguish them? One could argue that discrimination may not be strongly selected for in moth species where the only acoustic signal is a close-range male courtship song that happens to imitate bats but where females orient over long distances to male advertisement calls, any failure to recognize males correctly could be fatal. However, this expected discrimination is problematic when one notes that moth ears, the simplest known in the animal kingdom, have only one to four peripheral neurons each (cf. Surlykke et al. 2003) and that carrier frequency, being comparable in both moth calls and bat echolocation signaling, could not be used effectively. At an evolutionary level, the major question arising is the transition from an acoustic component of courtship that plausibly appeared via a sensory bias process to a long-range male advertisement song and the concomitant loss of the female sex pheromone. What can be inferred about the evolutionary route(s) along which the various changes occurred?

Solutions to the above conundrum, as well as further questions, are forthcoming from or suggested by analyses of one acoustic moth species that has been studied intensively, *Achroia grisella* (Pyralidae: Galleriinae; lesser wax moth). Male *A. grisella* broadcast an advertisement song comprising a train of ultrasonic pulses delivered at 80–120$\cdot$s^{-1} (Spangler et al. 1984; Jang and Greenfield 1996). The song is produced while males remain stationary on the substrate and fan their wings, an activity that causes a pair of tymbals at the wing bases to resonate during each upstroke and downstroke of the wings. Each tymbal resonation yields a brief (≈100 μs) pulse of high-frequency (70–110 kHz) sound. Female *A. grisella* respond to the male song by running toward the sound source, but they arrest their movement in response to a slower (<25–55 • s^{-1}) pulse rate (Greig and Greenfield 2004), which is representative of most bat echolocation signaling during the search phase of hunting (Neuweiler 2000). Thus, the moths distinguish male song and bat echolocation signaling as expected, they do so in spite of having only three peripheral neurons per ear, and the discrimination is effected via temporal rather than spectral characters of the signals.

Nonetheless, direct evidence is limited on the evolution of the male calling song in *A. grisella* and other moths exhibiting similar pair formation. Assuming an origin via a sensory bias process, an evolutionary model must account for a radical shift in female response to an ultrasound signal from arrestment of movement, or even negative phonotaxis, to the attraction described previously in this section (see Arnqvist 2006 on the occurrence of sexual conflict in signal evolution via sensory bias). In addition, some level of signal discrimination by females and signal modification by males has probably evolved. At present, it is unknown whether the original male song was already distinguished from bat echolocation signaling by a faster pulse rate or whether the rapid rate characteristic of male song evolved later and offered females the possibility of reliable discrimination of predators and mates. Similarly, it is unknown whether females originally identified bats via their relatively slow pulse rate or whether discrimination of bat echolocation signaling and male song based on pulse rate developed once male song had evolved. In addition, male *A. grisella* emit a sex pheromone during wing fanning that currently has a weak effect on the general movement of females but not their specific orientation toward a given male. A pertinent question is whether this odor played a more critical role early during the evolution of male song by affording females a reliable opportunity to discriminate males and predators. The ability of female, and male, *A. grisella* to localize the source of male song poses yet another evolutionary question, as the defensive hearing found throughout the Pyraloidea and other moths may entail little directionality. Some resolution of these possibilities and problems could be achieved by comparative studies of acoustic species that are conducted within a phylogenetic framework.

2.6 How Do Songs Diversify?

The evolution of sound signaling and perception does not cease once acoustic communication develops as a viable means for transmitting various types of information at all stages of the mating process. Section 2.5 speculated on the evolutionary trajectory along which a simple courtship sound was eventually modified to a long-range advertisement song that indicates certain aspects of male “quality” (cf. Jia and Greenfield 1997; Jang and Greenfield 1998; Reinhold et al. 1998). But in addition to the inferred elaboration of song, lateral shifts in acoustic parameters are also expected. These latter modifications may reflect adaptation to changes in the physical and biotic environment, sexual selection favoring exaggeration of male signals, and, of course, chance, otherwise termed genetic drift (Wilkins et al. 2013). An important category of modifications are those that entail divergence between populations, which can lead to speciation. This final section reviews the circumstances under which interpopulation divergence in song may occur, which song parameters are likely to diverge, and when the end result may be speciation.

2.6.1 On the Role of Ecological Speciation

A major question in the formation of species concerns the extent to which ecological factors and chance are responsible for the initial stages of population divergence. The first possibility would occur when a single, panmictic population becomes divided in two, each part subject to a different environment that selects for a specific phenotype. When mating signals and preferences are associated with those specific phenotypes and population divergence proceeds to complete separation, "ecological speciation" is said to have taken place (Nosil 2012). Such signals and preferences are termed "magic traits" (Servedio et al. 2011) because of their dual function and consequent potential for permitting speciation. Insects associated with a particular host plant appear to be especially prone to exhibiting magic traits and undergoing ecological speciation. For example, in the Californian stick insect *Timema cristiniae* (Phasmatodea: Timematidae), two color morphs are known, one typically found on *Adenostema* shrubs and the other typically on *Ceanothus* shrubs (Nosil et al. 2002). Each form is more cryptic on its typical host shrub, and laboratory trials show that each form also prefers insects of that same form as mating partners. These mate preferences appear to be maintained indirectly by natural selection against hybrids, which are intermediate color morphs and therefore cryptic on neither host shrub.

Has ecological speciation played a role in the diversification of song among acoustic insects? A review of potential cases of this process in vibration signaling in insects provides some supporting evidence for this idea. Ultimately, these cases help illustrate why ecological speciation is much less likely to be a factor in acoustic insects. In *Enchenopa binotata* treehoppers (Hemiptera: Membracidae), several different forms are found on specific host plants that differ in the elastic properties of their branches and stems. Consequently, the vibration frequencies at which bending waves are transmitted with the least attenuation along stems differ among the plants. Male *E. binotata* transmit vibration signals along the stems of their host plants, and the peak vibration frequency observed in the signals of a population found on a given host plant corresponds with the vibration frequency that is transmitted best in the stems of that host plant (McNett and Cocroft 2008). As in *T. cristiniae* stick insects, the various *E. binotata* populations are potentially en route to speciation, with the initial stages of divergence appearing to have been facilitated by mating signals that are adapted to the specific environment of a given population (Rebar and Rodriguez 2015). However, in *E. binotata* it is unknown whether the receivers in each population are maximally tuned to these best vibration frequencies, a factor that would accelerate divergence of these incipient species (cf. Nosil 2012).

Similar correspondence has been noted between physical properties of the substrate and spectral characteristics of vibration signals in other insects and arachnids, with the general suggestion made that signalers exploit resonance to maximize transmission of their messages (e.g., Cokl et al. 2005; Polajnar et al. 2012). But processes other than ecological speciation may be responsible for diversification of vibration signals. In a survey of *Nesodyne* planthoppers in Hawaii (Hemiptera: Delphacidae), a group that has expanded via adaptive radiation, comparative analyses suggested that diversification of vibration signals arose via no fewer than three processes, ecological speciation (otherwise termed "sensory drive"), reproductive character displacement, and chance (Goodman et al. 2015). And in lacewings in the genus *Chrysoperla*, similar analyses (Henry and Wells 2004) did not reveal any correspondence between characteristics of vibration signals and mechanical properties of host plant stems and branches.

For acoustic insects, inquiry on signal diversification has yielded a markedly different picture than for vibration signaling. To date, there is no unequivocal evidence that features of the physical environment influence acoustic characters of insect song. Among birds and other vertebrates, such influences have sometimes been investigated under the rubric of the "acoustic adaptation hypothesis," which proposes that a species' sounds have been shaped by selection such that their transmission is maximized in the typical habitat (Morton 1975). Whereas some evidence in support of the acoustic adaptation hypothesis has been found for vertebrate species, particularly birds (Ryan and Brenowitz 1985; Wiley 1991), none has been revealed in comparable studies of acoustic insects (e.g., Jain and Balakrishnan 2012). Perhaps this difference reflects the shorter distances over which communication generally occurs in insects, which would impose less selection pressure favoring acoustic characters that engender maximum transmission in a habitat with a particular amount and type of clutter or other forms of interference. Some general features of insect song do appear to be adaptations for signaling in cluttered habitats, but there is little indication that they are specific characteristics of those species that frequent these environments. For example, broadband sound generally suffers less reverberation than pure-tone frequencies when transmitted through dense vegetation, a quality that would tend to preserve temporal patterns of amplitude modulation such as chirps and pulse rhythms (Römer and Lewald 1992). But broadband sound is characteristic of most cicadas, acridids, and tettigoniids, and it may simply emerge from the mechanisms of sound production in these groups. Similarly, most gryllids produce song with a rather narrow frequency band regardless of the physical habitat in which they signal (see Schmidt et al. 2013 on the adaptation of gryllid frequency bands to the biological habitat, the acoustic environment formed by other singing insects). Nonetheless, the recent analysis of a fossilized Mesozoic haglid, an extinct orthopteran group that is proposed as basal to all extant tettigonioids, indicates that these insects produced a resonant, pure-tone song via bilaterally symmetrical wings (Gu et al. 2012), a finding that may be instructive. It is inferred that such songs would have been transmitted effectively in the relatively open forests of that earlier era (cf. Römer 1993) and that asymmetrical wings and nonresonant production of songs composed of broadband frequencies—features that characterize extant tettigonioids—evolved later, possibly as the acoustic landscape changed. In an independent but parallel fashion, the ancestral condition in grylloids may have also been bilaterally symmetrical wings and pure-tone song. But asymmetric wings and broadband songs have evolved in one extant clade of eneopterine crickets (Robillard and Desutter-Grandcolas 2004). Possible selection pressures favoring such morphological and song novelty are unknown.

Among insects, the difference between acoustic adaptation and vibration adaptation, which does occur, is probably due to the strong dependence of vibration communication on the habitat. In general, the quality of a vibration signal is affected markedly by a change in substrate, whereas sound signals are affected much less by changes in the fluid medium and the general habitat, especially over short distances. Consequently, ecological speciation may be less likely to have been an important factor in the diversification of songs of acoustic insects.

2.6.2 On the Roles of Chance and Reproductive Character Displacement

If habitat is a relatively weak factor in the diversification of insect song, what accounts for the great variation in sound signals among acoustic insects? At a crude level, body size and morphology of the sound radiator may influence the carrier frequency of song, with higher frequencies generally found in smaller species (Bennet-Clark 1998). But species of a given size range often broadcast markedly different songs, and these differences usually involve temporal rather than spectral features. The best cases for analysis of song diversity in the time domain would be the several species-rich genera of nocturnal singers for which thorough phylogenetic analysis is available. Nocturnal singers are proposed because song is likely to be the major element of pair formation, as visual signaling is probably less critical. In North America two genera of Ensifera satisfy these criteria, *Gryllus* (field crickets; Gryllidae) and *Neoconocephalus* (coneheaded katydids; Tettigoniidae). Both genera exhibit a variety of intense male advertisement songs that include continuous "trills," "whines," and "rattles"; continuous songs that are interrupted with regular pauses; and discontinuous "chirps" that are repeated with a regular rhythm. The fundamental acoustic unit of these songs is the "pulse," the sound emitted during one cycle of wing movement. Thus, songs are fashioned from a continuous train of pulses, a train that is regularly interrupted by a gap representing a certain number of missing pulses, or groups of pulses (chirps) that have their own group rhythm. Pulses may also be fused into "double pulses" by more complex cycles of wing movement (Walker et al. 1973; Walker 1975). When the different temporal patterns of song exhibited in a taxon are placed in an independent phylogeny, there are few evolutionary patterns that emerge (see Snyder et al. 2009 for *Neoconocephalus*). Analysis of *Gryllus* suggested that the ancestral song was composed of intermediate-length chirps delivered at intermediate but irregular rates and that a weak evolutionary "trend" toward an increased number of pulses per chirp may have been present (Desutter-Grandcolas and Robillard 2003; see Carroll 2001 on trends in evolution). In bush crickets (Eneopterinae), a comparable analysis of the worldwide fauna also suggested an evolutionary tendency toward increased redundancy in song (Robillard and Desutter-Grandcolas 2011). But the overall picture that emerges from these various studies is one of "dynamic" evolution characterized by a high level of convergence and multiple reversals of changes in character states (Desutter-Grandcolas and Robillard 2003).

Studies on diurnal acoustic insects, in which song may represent only a small portion of a male's signaling repertoire, have added to the aforementioned impression. In *Drosophila*, where species differ in several temporal characters of male song, evolutionary trends are not apparent within the groups that have been analyzed (Hoikkala and Mazzi 2009). Similarly, analyses of gomphocerine grasshoppers, which are noted for complex, multimodal signaling (Otte 1970), have not revealed trends toward increasing complexity in song (Nattier et al. 2011). In summary, temporal song elements, being controlled by neuromuscular factors, may be particularly labile characters (cf. Fonseca et al. 2008), subject to additions, deletions, and other modifications by chance or by selection pressure for avoiding the attraction of heterospecific mates.

Genetic analyses on interspecific (Gleason and Ritchie 2004) as well as intraspecific variation in *Drosophila* (Gleason et al. 2002) and in the acoustic moth *Achroia grisella* (Limousin et al. 2012; Alem et al. 2013) generally indicate that several quantitative trait loci (QTLs) influence male song characters. These numbers can be interpreted only as minimum values. But, more importantly, some studies indicate QTLs having very major effects on song character variation (e.g., Gleason et al. 2002; Limousin et al. 2012), which suggests that an allele change at a single locus could result in a markedly different signal.

2.6.3 Does Reinforcement Occur in the Speciation of Acoustic Insects?

Whereas there is now little doubt that some amount of reproductive character displacement occurs among acoustic animal species (e.g., Höbel and Gerhardt 2003; Jang and Gerhardt 2006; cf. Walker 1974), the role of reinforcement in the divergence of sympatric populations and their eventual speciation has been less certain (Butlin 1987). The subtle distinction between these processes demands some initial clarification. In reproductive character displacement, two populations that have diverged completely in allopatry, to the point of becoming separate species, meet later in a secondary zone of contact. Owing to the disadvantages of expending time, energy, and gametes in the pursuit of heterospecific mates with which there is no possibility of fitness through producing hybrid offspring, either signals, preferences, or both evolve greater differences in the secondary sympatric zone than in the allopatric areas, where these enhanced differences are not particularly favored by selection. A related phenomenon involves selection on singing behavior in sympatry to avoid acoustic interference. For example, species that broadcast discontinuous songs may adjust their daily singing schedule such that they are not subject to interference from species broadcasting continuous songs (e.g., Greenfield 1988). In reinforcement, as strictly defined, the two populations have not separated entirely, and a hybrid zone exists between them (Liou and Price 1994). These hybrids, although viable, do have lower fitness than individuals in either allopatric population. The critical question is whether reduced hybrid viability can "reinforce" signal and preference differences while the populations still overlap in sympatry to the extent that fully separated species eventually form.

Recent models show that the process of reinforcement as described previously in this section is theoretically possible (Kelly and Noor 1996; Noor 1999; Ortiz-Barrientos et al. 2004), and various examples where sympatric speciation via reinforcement is likely to have occurred are proposed (Servedio and Noor 2003). Several acoustic insect genera exhibit well-known hybrid zones in different geographic regions of North America (e.g., Shapiro 1998; Britch et al. 2001), and it would be most appropriate to know whether reinforcement and incipient speciation are occurring in these situations or whether the hybrid zones are impeding speciation (Servedio et al. 2013). In addition, reinforcement in the past may have led to some of the fully separate species observed in these genera today.

2.6.4 On the Role of Pleiotropy in Song Evolution

When novel male mating signals evolve owing to chance or ecological factors, it does not follow that females in the population will necessarily respond to and prefer the novel signal. Biologists therefore have long sought to reconcile the stabilizing selection that generally characterizes mate recognition in animal species with the diversification of mating signals. Initial work on this problem began in the 1960s and led to formulation of the "genetic coupling hypothesis." Mating signals and references may be controlled by the same genes such that when a novel male signal appears, there is a female audience that prefers it (Alexander 1962). But various tests of genetic coupling, some conducted with acoustic insects (Hoy et al. 1977; Bauer and von Helversen 1987), failed to provide convincing support or refutation (Butlin and Ritchie 1989; Boake 1991). Instead, these breeding experiments showed how intractable the problem could be when approached via traditional methods.

The question of signal evolution in the face of stabilizing selection did not disappear, however, and recent availability of molecular genetic techniques has led to "next generation studies" using more direct approaches. These current studies ask whether signal and preference traits are influenced pleiotropically or by loci that are tightly linked physically. One of the more thorough tests of trait-preference pleiotropy, or physical linkage, has been conducted on *Laupala* crickets, a genus endemic to Hawaii that is distinguished by recent adaptive radiation and a great many species. Here, QTL studies have revealed a broad pattern of genomic "co-localization" of loci influencing male song and female preference (Shaw and Lesnick 2009; Wiley et al. 2012). But these co-localizations, particularly where they reflect actual pleiotropy, beg the question of how two seemingly disparate traits, one a motor activity and the other a perceptual and behavioral response to that activity, can be controlled by the same genetic element? In the case of *Laupala* song, the explanation may lie in a focus on timing—pulse rhythm—common to both the male song and female response and preference.

How common are cases of co-localization of signal and preference loci as reported in *Laupala* crickets, and could this phenomenon explain much of the song diversity observed among acoustic insects? At present, there is too little information to justify a definitive statement on the prevalence of co-localization. Whereas it has been found in various animal species using different signaling modalities in mating communication (Shaw et al. 2011), some studies have failed to confirm its presence (Löfstedt et al. 1989), including in acoustic insects (Limousin et al. 2012). It would therefore be safer to claim that co-localization may account for certain cases of speciation, particularly those entailing a high rate of divergence and simple differences in song that occur along a single character axis. In this regard, it is telling that population differentiation and speciation in *Laupala* are among the most rapid reported among animals (Mendelson and Shaw 2005), and song and preference differences are restricted to changes in pulse rhythm. In other words, co-localization may be the only genetic mechanism by which such high rates of diversification can be achieved. Thus, when co-localization happens to be present, speciation is likely to be the end result when allele changes appear between populations.

2.7 Summary

Tympanal organs for hearing in the far field have evolved on multiple occasions among insects and are currently found in seven orders. Many, if not most, cases of insect hearing probably originated as a means for detecting and avoiding predators. In particular, sensitivity to ultrasound appears to have coevolved with echolocation signaling by insectivorous bats. However, on an overall scale, hearing is relatively rare among insects in comparison with other modalities of perception, including detection of substrate vibration. Sound signaling in insects, which typically occurs in the context of mating communication, is rarer still and is known in only five orders. Phylogenetic analyses suggest that acoustic communication in the Lepidoptera and in the suborder Caelifera (grasshoppers) of the Orthoptera originated via a "sensory bias" mechanism. Hearing was ancestral and sound signaling by males subsequently arose on multiple, independent occasions. On the other hand, acoustic communication in the Cicadidae and in the suborder Ensifera (crickets, katydids) of the Orthoptera may have originated via coevolution between female perception and male signaling. The diversity of songs among acoustic insects may reflect genetic drift and reproductive character displacement. There is little evidence, however, that insect songs are adapted to specific physical environments. In one clade of acoustic insects, the diversification of song is associated with an unusually high rate of population differentiation and speciation, which may be facilitated by a genomic co-localization of loci influencing female response/preference and male signaling. The extent to which co-localization is a general factor in speciation remains to be explored.

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Chapter 3
Behavioral Ecology of Insect Acoustic Communication

Rohini Balakrishnan

Abstract This chapter reviews conceptual and empirical approaches to understanding insect acoustic communication using long-range acoustic signals in natural environments, primarily in the context of mate finding and mate choice. It focuses on three main issues: the role of acoustic signals in pair formation, sexual selection on acoustic signals, and strategies of acoustic signaling in noisy environments. Current hypotheses on the evolution of signaling and searching behavior in the context of mate finding are presented along with some empirical tests. The evidence for sexual selection on long-range mate attraction signals in insects is evaluated and it is concluded that the mechanisms of sexual selection on these signals remain ambiguous. Finally, sender and receiver strategies for communication in noisy environments and their relevance for dealing with anthropogenic noise are discussed.

Keywords Acoustic adaptation • Acoustic masking interference • Acoustic niche partitioning • Aggression • Bushcricket • Cicada • Courtship song • Cricket • Duetting • Female choice • Grasshopper • Katydid • Male competition • Parasitoid • Predation risk • Preference function • Sensory bias

3.1 Introduction

Insect sounds comprise a major part of natural terrestrial soundscapes today and insects were probably the first terrestrial organisms on earth to communicate using airborne sound. Fossil evidence from the Paleozoic and Mesozoic Eras suggests that crickets, katydids (bushcrickets), and cicadas were producing sounds millions of years ago that were similar to what they produce today (Senter 2008). The remarkable evolutionary persistence of acoustic signaling in these ancient insect lineages, the diversity of signals that have evolved, and the ecological challenges that such signaling systems faced and continue to face in the form of anthropogenic noise and

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land use changes make them fascinating subjects of study. Apart from crickets, katydids, and cicadas, a number of insect groups have evolved airborne sound signals and acoustic communication. A few groups of grasshoppers, including the ancient bladder grasshoppers (pneumorids), and more recent short-horned grasshoppers (acridids) (Song et al. 2015), have evolved long-distance acoustic signaling (von Helversen and von Helversen 1994; Römer et al. 2014), as have some groups of moths, notably the snout moths (pyralids) and owlet moths (noctuids) (reviewed in Greenfield 2014; Nakano et al. 2015). In all of these groups, the primary function of acoustic signals, typically produced by adult males, is to facilitate pair formation for mating. In addition, acoustic signals can form components of courtship displays or aggressive interactions at close range (Alexander 1961; Gerhardt and Huber 2002).

Crickets, katydids, cicadas, grasshoppers, and moths can hear far-field sound, typically greater than 1 kHz, using pressure or pressure-difference receivers (Gerhardt and Huber 2002; Windmill and Jackson, Chapter 6) so that the range over which communication can take place can be quite large, with bladder grasshoppers of the family Pneumoridae being able to communicate up to 450 m (van Staaden and Römer 1997). In contrast, other insect groups that have evolved acoustic signaling, such as dipterans (which includes fruit flies and mosquitos) produce low-frequency sounds with their wings that can only be sensed close to the signaler as near-field sound (Göpfert et al. 1999; Zanini et al. 2014). Such signals are thus effective only at close range. Bees also produce low-frequency sounds, especially dancing foragers, and these may provide information to nearby follower bees regarding food sources (Michelsen 2003). A number of insect groups communicate using substrate-borne vibrations in addition to, or exclusive of, airborne sound signaling (reviewed by Yack, Chapter 5). This chapter, however, focuses largely on long-range communication by insects using airborne sound.

3.2 Acoustic Signals and Pair Formation

3.2.1 Long-Range Signals and Mate Search

Mating is typically preceded by a period of courtship, often involving stereotypic displays that allow for extensive information exchange between the sexes, using a number of sensory modalities. For solitary species, however, the first problem is to locate a potential mating partner of the same species and opposite sex. In the absence of long-range signals, this would happen through random encounters. For species whose individuals occur consistently at low spatial densities, encounter probabilities by chance are likely to be low, driving the evolution of mate-search strategies. These strategies could involve movement of both sexes or one sex could remain stationary while the other searches. In a seminal paper, Hammerstein and Parker (1987) argued, using a game theoretical framework, that search behavior was likely to be frequency dependent, and the evolutionarily stable state would be one where one sex searched and the other remained stationary; either sex could end up with the searching role.

More recently, Kokko and Wong (2007) showed other equilibrium solutions, including searching by both sexes, depending on the shape of the (mate-search) cost function. When females mate repeatedly, evoking sperm competition, whether males or females do most of the searching also depends on mate encounter rates (Kokko and Wong 2007). Low probabilities of encounter and mating, as at low population densities (Cade and Cade 1992), could select for the evolution of long-range signals providing information about the sex, reproductive status, and species of the signaler. This model allows for the evolution of long-range signaling in either sex.

Most long-distance acoustic mate attraction signals are, however, produced by males (Bradbury and Vehrencamp 1998), suggesting an alternative evolutionary scenario driven by sexual selection. According to sexual selection theory, the sex that invests fewer resources in the offspring competes for the sex that invests more (the limiting sex) (Trivers 1972; Kokko and Johnstone 2002). Because females are often the limiting sex and may mate with multiple males, generating sperm competition (Parker 1970), males tend to compete for access to females. In solitary species, males that signal their presence and availability as potential mating partners using long-range signals are likely to have a selective advantage because they become more detectable and locatable by conspecific females from a distance. In this framework, long-distance acoustic signals can be thought of as typical male sexually selected traits whose evolution is driven by male competition (Darwin 1871). Acoustic signals are also energetically expensive to produce and are conspicuous to predators and parasitoids (organisms that live within a host for part of their life history and eventually kill the host) (Zuk and Kolluru 1998). This is consistent with the idea that acoustic signals are male secondary sexual traits whose evolution has been driven by competition for females. If intermale variation in these signals is indicative of differences between males that have fitness consequences for females, then female choice on these call variants is also expected to evolve.

3.2.1.1 Signaling Strategies

In species that call from defended territories or burrows, the signaling sex, typically the male, does not move from its position (Bradbury and Vehrencamp 1998). There are several reasons for this: if the male is defending and/or provides a patchy, scarce, or valuable resource, such as an optimal foraging or oviposition site, or nutrition for females (resource defense polygyny: Emlen and Oring 1977), then males would not be expected to move and the onus of search is on the female. Even if the male is not defending a resource, male–female encounter rates may be higher if the calling male acts as a stationary beacon rather than a moving acoustic target for the female to locate.

In several species of crickets, katydids, grasshoppers, and cicadas, however, calling males do move through the course of a calling night/day, walking or flying from site to site (Gwynne 1987; van Staaden and Römer 1997; Sueur 2002). In such cases, pair formation may involve only male movement or male and female movement. What factors could drive such strategies? Low density of females in the habitat is one possibility: If females are dispersed in relatively low numbers or in patchy aggregates in the habitat, then a male could increase his detectability and probability of attracting or finding females by moving from one calling site to another. The loud, low-frequency call of the male bladder grasshopper *Bullacris membracioides*, together with movement by up to 500 m before the next calling bout, may be driven by the need to find females that are at low densities or patchily distributed (van Staaden and Römer 1997; Römer et al. 2014). Another factor that could drive this behavior is pressure from acoustically orienting predators and parasitoids (Zuk and Kolluru 1998). Calling males typically face a trade-off between the chances of mate attraction and predation/parasitism (Zuk and Kolluru 1998). By moving from site to site, a calling male may decrease his chances of being preyed on or parasitized (Allen 1995). A third possibility is that male movement while calling is driven by females adopting a different strategy, by replying to the male call rather than moving (Bailey 2003).

3.2.1.2 Duetting

Duetting refers to systems in which both males and females produce sounds. In essentially all known cases of acoustic duetting in insects, the primary mate attraction signal is produced by the male, to which the female replies, typically with a predictable latency from some part of the male call (Heller 1990; Robinson 1990). Duetting systems are well described in some species of phaneropterine and ephippigerine katydids, acridid and pneumorid grasshoppers (Bailey 2003), and cicadas (Cooley 2001; Cooley and Marshall 2001). Duetting systems are diverse in terms of relative movement of the partners, ranging from systems in which females do not move and only reply to systems in which both partners move and use each other's calls to achieve pair formation (Bailey 2003).

The evolution of acoustic duetting in insects is not well understood, but possible evolutionary scenarios have been proposed (Heller 1990; Bailey 2003). Bailey (2003) assumes an ancestral non-duetting state in which only males call, with call structure, driven primarily by sexual selection, being complex and of long duration. If there is strong natural selection imposed by acoustically orienting predators or parasitoids, then male signals are expected to become short and infrequent (low duty cycle calls), making it difficult for females to detect and locate them or choose between males based on acoustic signals. This could lead to a female strategy of replying to the male call, driving the male to now use female replies to locate them (Fig. 3.1).

Although the evolution of duetting could result from the costs to signaling males, and concomitant problems for female mate search, as described in the preceding paragraph, it could also be driven primarily by the costs of female search independent of male song structure. The searching sex will have to move to find the signaling sex and incurs an energetic cost as well as an increased risk of predation. Duetting may evolve when female search costs become too high: There is evidence that female katydids may incur predation risks as high as, or even significantly higher than, calling males (Belwood and Morris 1987; Raghuram et al. 2015). This could

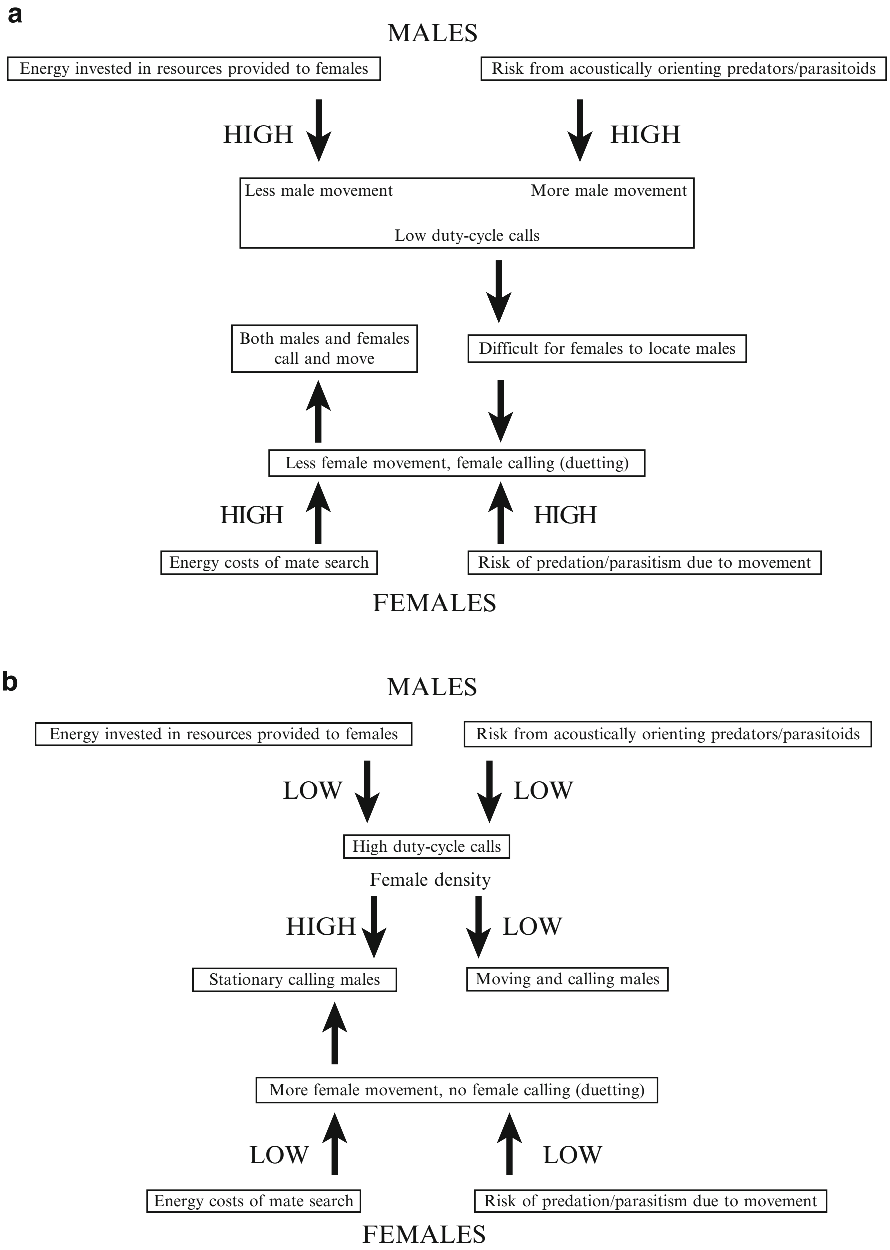

Fig. 3.1 Factors influencing the evolution of pair-forming strategies. Predicted outcomes of (**a**) high-cost and (**b**) low-cost scenarios

lead to females replying to male calls and passing on some of the risk of movement to the male in the mate search game. That this may be the case is suggested by the recent discovery of an acoustic-vibrational duet in a false leaf (pseudophylline) katydid species *Onomarchus uninotatus*, in which the male call is answered by a robust tremulation response from the female, with phonotaxis being less frequent and only attempted after a long series of tremulations (Rajaraman et al. 2015). In some cicada species, females reply to male calls with wing-flick responses, and males both call and move toward females (Gwynne 1987; Cooley 2001). In the ticktock cicada (*Cicadetta quadricincta*), males suffer higher mortality in spider webs than females (Gwynne 1987) and appear to take on the costs of both calling and movement.

Another important factor to consider in the evolution of pair-forming strategies is male investment in nutritive nuptial gifts (McCartney et al. 2012). Males of several species of the Tettigonioidea (katydids) provide a nutritious nuptial meal to the female in the form of a spermatophylax (Gwynne 2001). In some katydid species, the spermatophylax can be quite large so that male investment in a mating is high. McCartney et al. (2012) suggest that such high male investment could result in stationary males and searching females; where male investment is lower, as reflected in relatively smaller spermatophores, females may be less likely to move and use duetting instead (Fig. 3.1). Examination of several species of the katydid genus *Poecilimon*, which show a diversity of both pair formation strategies and nuptial gift size, provides correlative support to this view (McCartney et al. 2012). Because stationary males have to call for females to locate them, however, this hypothesis assumes that calling is less risky than searching, an assumption that is currently supported only by a couple of studies (Heller 1992; Raghuram et al. 2015) and needs to be examined further in comparative studies on different katydid species.

In some species, male investment in the spermatophylax has become so large that this results in a long male refractory period (non-availability as mating partners). Females now become the competing sex, competing for both matings (sperm) and the nutritious nuptial gifts (Gwynne 2001). This can result in males producing signals for low durations, lowering their investment in signaling and probably decreasing their conspicuousness to predators (Gwynne 2001). In fact, in Mormon cricket (*Anabrus simplex*) populations in which this is the case, females incur higher risks of predation than males (Gwynne 2001) in a classic sex role reversal scenario.

3.2.1.3 Alternative Strategies

When more than one pair-forming strategy occurs within one sex in a population, these are referred to as alternative strategies. Among the commonest are satellite strategies, wherein some males keep silent, remain in the vicinity of callers, and intercept females that approach the calls of these males (Cade 1975). Satellite strategies allow males to reduce the cost of pair formation, including energetic costs of calling and the risk of attracting acoustically orienting predators or parasitoids. Satellite strategies can, however, be maintained in the long run only if there are sufficient numbers of callers, and one expects an evolutionarily stable equilibrium between calling and satellite strategies (Gross 1996). Although satellite strategies have been documented, especially in field crickets (Cade 1975), there is little information on their fitness benefits and the selection pressures driving them.

The interesting situation in a Hawaiian population of the black field cricket (*Teleogryllus oceanicus*), in which the majority of males rendered mute by the "flatwing" mutation act as satellites on the few calling males (Zuk et al. 2006), may be atypical. The fact that satellite male crickets were common in populations of *T. oceanicus* that were free of parasitoid flies suggests strong alternative selective forces driving satellite behavior (Zuk et al. 1993). Satellite strategies could evolve as a result of competition among males or as a condition-dependent strategy (Gross 1996). Field cricket males can switch between calling and searching in a density-dependent manner (Cade and Cade 1992), suggesting that these alternative strategies are plastic and context dependent. In bladder grasshoppers, however, calling and non-calling strategies appear to be developmentally determined, with two morphologically distinct adult male types pursuing these alternative strategies (Römer et al. 2014). An interesting alternative strategy in tree cricket (*Oecanthus*) males is baffling (Prozesky-Schulze et al. 1975). Males sometimes cut a hole in a leaf and sing from it, using it as an acoustic amplifier and increasing call intensity.

3.2.2 Acoustic Signals and the Risk of Predation

A central feature of the hypotheses that attempt to explain acoustic pair-forming strategies is the proposed role of predation risk as a selection pressure on signal structure, signaling, and pair-forming behavior. Male katydids calling from cages placed in the field in Panamanian rain forests attracted bat predators, whereas similar cages baited with noncalling females did not (Belwood and Morris 1987). Playback of male katydid calls elicits approach by bat predators in flight cages/rooms in the neotropics (Belwood and Morris 1987), the palearctic (Jones et al. 2011), and the paleotropics (Raghuram et al. 2015). There is no doubt that calling entails predation risk, but the question is whether this constitutes a sufficiently strong selection pressure to influence call structure, calling, and searching behavior.

Belwood and Morris (1987) showed that long-duration, high duty cycle calls (produced by katydid species in neotropical forest clearings) were located much sooner by bats in flight cages than the infrequent calls of forest katydids and proposed that high predation pressure by bats may have resulted in the short, low duty cycle calls produced by forest katydid species. Although this is plausible, there are alternative explanations for low duty cycle calls, including high male investment in nuptial gifts (Gwynne 2001). Similar experiments in a paleotropical rain forest assemblage did not show an effect of call duty cycle (Raghuram et al. 2015). More importantly, most studies on the bat–katydid interaction have largely ignored non-calling females and the risks posed by searching and movement. Diet analysis of bat predators shows that female katydids are at as high, or even higher, risk of predation than acoustically signaling males, possibly because females move more than males (Belwood and Morris 1987; Raghuram et al. 2015).

3.2.3 Acoustic Signals and Parasitoids

The dramatic consequences of acoustically orienting parasitoid flies for field cricket male calling behavior can be seen in Hawaii (Zuk et al. 2006), where introduced populations of the black field cricket *Teleogryllus oceanicus* have been infested by the parasitoid fly *Ormia ochracea* (introduced from North America). A "flatwing" mutation that affects male wing stridulatory structures has spread through the population in 12–20 generations, rendering most males silent (Zuk et al. 2006). These silent males have much lower levels of parasite infestation than singing males, suggesting that the pressure from parasitic flies has been strong enough to essentially eliminate male singing behavior. This has now been seen to occur independently in populations on two different islands (Pascoal et al. 2014). The situation on the Hawaiian islands may, however, represent an extreme case, possibly driven by the lack of alternative hosts for the fly. A number of field cricket species in North America are hosts to the same parasitic fly (*O. ochracea*: Lehmann 2003) and some have high duty cycle calls, yet obviously coexist with these parasitoids, and have not lost their acoustic signals. However, parasite infestation does decrease the time spent calling (Lakes-Harlan and Lehmann 2015). The mechanisms that allow coexistence of populations of host crickets and parasitic flies are not well understood and worth examining.

Acoustically signaling katydid and cicada species are also attacked by acoustically orienting parasitoid flies. The ormiine fly *Therobia leonidei* parasitizes species of the phaneropterine katydid genus *Poecilimon* in Europe (Lakes-Harlan and Lehmann 2015). Two host species, *P. marianne* and *P. veluchianus*, produce similar calls consisting of brief, high-bandwidth pulses, differing mostly in the number of pulses per call (5–9 vs. 1). In a field study, Lehmann and Heller (1998) showed that *P. mariannae*, with the higher number of pulses/chirp was parasitized at higher levels than *P. veluchianus*, including when it was introduced into the habitat of the latter. This suggests that longer calls may make calling hosts more susceptible to acoustically orienting parasites. Whether the shorter duration of the *P. veluchianus* call is a response to parasite pressure is, however, unknown. In the case of orientation of the parasitoid fly *O. ochracea* toward the Texas field cricket (*Gryllus texensis*) calling song, Müller and Robert (2002) showed that making shorter chirps rather than long trills did not affect the ability of the fly to locate these calls. In a choice situation, however, flies oriented preferentially to calls with overall higher energy (based on a combination of call amplitude and length). The advantages of decreasing call length for male crickets and katydids are thus likely to depend on the spatial and acoustic context, underscoring the need for more studies on the ecological context of acoustic parasitism.

3.3 Acoustic Signals and Sexual Selection

Evidence for sexual selection in acoustically signaling insect species in the field comes mostly from work on field crickets. Sexual selection was inferred from the differential mating success of males differing in attributes that could affect female fitness. Cross-sectional field surveys of different field cricket species revealed that males found paired with females were likely to be older, larger, or carried lower parasite loads than unpaired males (reviewed in Zuk and Simmons 1997). Definitive evidence for sexual selection in a wild population of field crickets (*Gryllus campestris*) was provided recently by a study (Rodriguez-Munoz et al. 2010) in which the behavior of marked individuals of an entire population was tracked continuously and combined with genetic analysis to determine the fitness of individual males and females. Males had higher variance than females in lifetime reproductive success (LRS), as predicted by sexual selection theory but, interestingly, substantial numbers of females left few or no descendants, suggesting that females do not have assured fitness returns. Both males and females mated multiple times and fitness increased with number of mates for both sexes. Larger and longer lived individuals of both sexes had higher LRS.

Demonstrating sexual selection in acoustically signaling species does not necessarily mean that selection is acting on the acoustic signal. During courtship or aggression, information can be exchanged in several additional modalities, including chemosensory, tactile, visual, and vibrational, and selection could be acting on any or several of these modalities rather than, or in addition to, the acoustic signal. The demonstration of sexual selection on cuticular hydrocarbons (CHCs) in the fruit fly, *Drosophila melanogaster* (Hine et al. 2004), and the field cricket, *T. oceanicus* (Thomas and Simmons 2009; Simmons et al. 2013), both of which also produce acoustic courtship songs, are examples. In addition, postcopulatory mate choice in female insects (Eberhard 1996) may influence male LRS independent of mating success (Rodriguez-Munoz et al. 2010).

3.3.1 Sexual Selection on Close-Range Acoustic Signals

Field crickets produce both a long-range acoustic signal (calling song) and a close-range courtship song, which is often structurally different from calling song (Alexander 1961). Although courtship song was well known to be important for inducing the female to mount the male for mating (Balakrishnan and Pollack 1996), only recently has it been investigated in detail. Courtship song in the black field cricket, *T. oceanicus*, was more variable, its features more repeatable, and more expensive to produce than calling song (Hack 1998; Zuk et al. 2008; Rebar et al. 2009). Male mating success correlated positively with song duty cycle, which appears to be under directional selection (Simmons et al. 2013). Interestingly, both courtship song and CHC profile influence male attractiveness, but these were uncorrelated, suggesting that they convey different messages (Simmons et al. 2013). Courtship song may convey information about male genetic quality, whereas CHC profiles may carry information about genetic compatibility (Simmons et al. 2013).

Most studies on the courtship song of *Drosophila* have focused on its role in species recognition. Evidence for a role in sexual selection comes from both field and laboratory studies on *Drosophila montana*. Aspi and Hoikkala (1995) found that males of mating pairs caught in the field had shorter pulses and higher frequency songs compared to random unpaired males, suggesting that these song traits were preferred by females. In another field study on *D. montana*, features of male songs were seen to exhibit substantial variation, especially in frequency, which was found to be a trait preferred by females, suggesting that it was sexually selected (Ritchie et al. 2005).

3.3.2 Long-Range Acoustic Signals and Sexual Selection

Sexual selection on signals could act via male competition or female choice or be driven by sexual conflict or the need for genetic compatibility. Much of the empirical work has, however, focused on female choice, which is discussed first.

3.3.2.1 Acoustic Signals and Female Choice

Investigations of female choice in the field are rare in insects. An exception is the detailed observational study by Feaver (1983) on the black-legged katydid (*Orchelimum nigripes*). In this species, males compete for territories, and territory holders, which are often heavier and older, tend to be the callers. Females did not mate with non-calling males and, by so doing, would prefer older, heavier, territory holders. Females mated only once and were seen to spend many hours interacting with, and moving around, male territories before mating with a male. This is highly suggestive of female choice but does not address the issue of whether that choice is based on male call features.

Similarly, Kriegbaum (1989) found that females of the duetting bow-winged grasshopper (*Chorthippus biguttulus*) rarely mated in the field with males that lacked a hind leg. In this species, as in most acridid grasshoppers, sound is produced by rubbing both pairs of hind legs against the wings. When one hind leg is missing, this introduces gaps into the syllables, rendering the song unattractive (von Helversen and von Helversen 1994). There is thus correlative evidence suggesting that males with only one hind leg may be discriminated against in the wild because of their unattractive songs.

3.3.2.1.1 Preference Functions

Female choice for long-range acoustic signals has typically been investigated using playback experiments in the laboratory. These tests determine the preference function (response of females to systematic trait variation) for acoustic features (Wagner 1998; von Helversen et al. 2004) to determine preferred values of specific call features. Where a preference function is determined, this can be compared with the distribution of values of that trait in male populations to infer the pattern of selection (von Helversen and von Helversen 1994; Wagner 1998). An alternative approach gives females two simultaneously presented stimuli representing different values of a trait. These preference tests give somewhat different and complementary information to the no-choice preference functions as responses in choice scenarios, where the possibility of comparison is available, can be different (Gerhardt and Huber 2002; Dougherty and Shuker 2015).

These approaches have been used extensively on a number of acoustically communicating insects, including crickets, katydids, grasshoppers, and moths (Gerhardt and Huber 2002). A broad generalization from these studies is that call features more important for conveying information on species identity are often under stabilizing selection, whereas features likely positively correlated with energy expenditure are under directional selection (Ryan and Keddy-Hector 1992; Gerhardt and Huber 2002). Although studies on preference functions are an important first step, these are likely to be insufficient for a number of reasons detailed in the text that follows.

First, preference functions may depend on stimulus intensity [measured as sound pressure level (SPL)] (Doolan and Pollack 1985; Mhatre and Robert 2013). In the wild, receivers may hear signals at a range of SPLs, depending on instantaneous distances from senders, which will change with relative movement. Second, expression of preference functions may change with age and physiological status (Wagner 1998; Reinhold and Schielzeth 2015). Third, behavioral responses are the end result of simultaneous neural processing of a number of stimulus features, integrated to produce the final response, allowing trade-offs between multiple call features (von Helversen and von Helversen 1994; von Helversen et al. 2004). Fourth, there is individual variation in preference functions for acoustic features and in the relative weighting of different features (Jang and Greenfield 2000; von Helversen et al. 2004). If single features are under particularly strong selection, then one may expect to see a preference function that is relatively intensity invariant, whose effect is heavily weighted and relatively similar across individuals. Few studies have examined the robustness of preference functions to changes in intensity, in relation to other features, and across individuals. Finally, the relationship between the phonotaxis or duetting response and the mating preference is not always clear (Klappert and Reinhold 2003).

There have been some attempts to address these issues, typically by co-varying acoustic features and studying their interactions (Klappert and Reinhold 2003; Wagner 2011), as well as characterizing the intensity dependence of preferences (Snedden and Greenfield 1998; Nandi and Balakrishnan 2013). These studies have revealed some generalizations, including the ability of SPL to dominate over preferences for other preferred call features such as higher call rates or for leading male calls. Another approach has been to build computational models to explain preference functions and their interactions: This has been successful in correctly predicting known female responses in field crickets and grasshoppers (Ronacher et al. 2014; Ronacher, Chapter 9).

A complementary approach, aimed at determining selection on multiple acoustic features of a signal, has been to produce signal variants in which the values of each of the signal features (picked from their natural distributions) are combined randomly and to test female responses to these variants (Brooks et al. 2005).

Multiple regression-based selection analysis is then used to estimate selection gradients on call features (Lande and Arnold 1983). Such experiments have been performed on an Australian field cricket (*Teleogryllus commodus*), in the laboratory using pairwise stimulus presentation (Brooks et al. 2005) and in the field where all stimuli were played back simultaneously (Bentsen et al. 2006). In both cases, structural features of the call such as inter-pulse interval, chirp syllable number, and carrier frequency were found to be under stabilizing selection. Calling effort, as expected, was found to be under directional selection.

The field playback experiment using multiple call variants simultaneously is an attractive paradigm and lends external validity, given that the vast majority of studies describing female preferences are limited to the laboratory. These could, however, be refined to better reflect the spatial and acoustic context experienced by receivers in the field (Hirtenlehner and Römer 2014). The acoustic context can be highly variable in the wild depending on signaler densities, source SPLs, signal structure, habitat, and movement, and it is necessary to validate results obtained under laboratory conditions by testing them in realistic field scenarios. In one such attempt, Mhatre and Balakrishnan (2006) characterized the acoustic context experienced by female crickets (*Plebeiogryllus guttiventris*) in the field and incorporated it in a simulation wherein the auditory localization mechanism was modeled to produce “virtual female crickets” that performed phonotaxis when presented with multiple, simultaneously played out calling songs representing males in a dense chorus (Mhatre and Balakrishnan 2008). The same “chorus” (using speakers) was experimentally set up in the field where phonotaxis trials were carried out with real females (Mhatre and Balakrishnan 2008). The phonotaxis simulation model was able to predict outcomes at a population level. The only feature varied in this study was however call SPL. Incorporating variation in other features (Nandi and Balakrishnan 2013) may allow a predictive framework for phonotaxis outcomes (at least at the population level) that takes into account variation in individual physiology, natural call variation, and acoustic context.

To choose between males, females have to sample them. Such sampling may happen sequentially, wherein females move from one male to another, or simultaneously, if several males are audible at the same time (Murphy and Gerhardt 2002). Female tree crickets (*Oecanthus henryi*) were found to typically encounter calling males singly (only one signaler audible) and rarely in choice situations (multiple signalers audible) (Deb and Balakrishnan 2014). Despite extensive theory on optimal mate-sampling strategies (Real 1990) and some laboratory tests, it remains largely unknown whether such strategies are used in the context of female mate choice based on long-distance acoustic signals. Search costs, such as energy and time constraints, or risk of predation or parasitism should also contribute to female mating strategies and decision making (Jennions and Petrie 1997). Empirical data on these are scarce for acoustically signaling insects in natural contexts (cf. Gray and Cade 1999). The ecological and acoustic context of female mate choice on acoustic signals, and how this influences female decision making, is not well understood and requires further investigation.

3.3.2.1.2 Why Choose? Benefits of Choice and Acoustic Indicators of Male Quality

Given that there is female choice among song variants, it is important to understand why such preferences are seen. If males vary in a trait that carries benefit to females, then females may be selected to choose between them. If acoustic call features correlate with these trait values, then females should benefit from discriminating between males based on calls and approach those likely to confer higher fitness benefits. Alternatively, such "choice" may simply reflect physiological constraints imposed by phylogenetic history ("sensory biases": Ryan and Keddy-Hector 1992).

Hypotheses to explain why females choose between males fall into three major categories. Females may choose some males over others because they obtain (1) direct benefits or (2) indirect benefits or because of (3) sensory biases (Bradbury and Vehrencamp 1998). Direct benefits are those that directly increase female fitness (survival and/or reproduction) and could include nutrition (Gwynne 2001) provided directly as nuptial gifts (as in tree crickets, grigs, and katydids: Sect. 4.2.1.3) or as resource-rich territories (some grasshoppers: Greenfield 1997) or oviposition sites held by males. Larger/heavier males may provide bigger or better nuptial gifts or hold resource-rich territories (Gwynne 2001).

A number of species of katydids, true crickets, and grigs provide nuptial gifts that are consumed by females during and/or after mating (reviewed in Brown and Gwynne 1997). In some species there is evidence for direct benefits from nuptial feeding and female choice on this basis (Brown 1997a,b; Bussière et al. 2005). The links among male size, condition, nuptial gift size, and female benefit are, however, complex (Brown 2011). Nonetheless, females typically mate for shorter durations and remove the spermatophore of smaller males earlier (Deb et al. 2012).

Brown et al. (1996) showed that the carrier frequency of the male black-horned tree cricket (*Oecanthus nigricornis*) song was a reliable indicator of male size. Females chose the low-frequency songs of larger males but only when given a simultaneous choice. A similar study on a southern Indian tree cricket, *O. henryi*, also found size to be reliably indicated by male call carrier frequency (Deb et al. 2012). Females did not, however, discriminate between male calls based on frequency, even when simultaneously presented, and most females in the field could hear only one male at a time, allowing little opportunity for calls to be directly compared (Deb and Balakrishnan 2014). In addition, tree cricket carrier frequency is well-known to change with temperature (Walker 1962) and should be an unreliable signal in the field. It is therefore unlikely that tree cricket song is used by females to indicate male size. It is far more likely that females gauge male quality at close range during mating using chemosensory cues, which may include CHC profiles and/or the composition of the nuptial meal itself.

In some field cricket species, a preference for the songs of larger males has been shown and, in house crickets (*Acheta domesticus*), larger males produce more syllables per chirp (Gray 1997). In the bow-winged grasshopper (*Chorthippus biguttulus*), there is a positive correlation between some song features and male size and condition (Stange and Ronacher 2012). Direct benefits of female choice on male song have been explored in the variable field cricket *Gryllus lineaticeps* (reviewed in Wagner 2011). Female *G. lineaticeps* mating with males with higher chirp rates and durations had increased lifetime fecundity or life span, but this depended on nutritional status of both males and females (Wagner 2011). Furthermore, the preference for chirp rate itself changed depending on the number of sources (the acoustic context). These strong environmental effects underscore the difficulty of teasing apart the factors driving the evolution of female preferences on acoustic traits.

Females may also choose between males to obtain "indirect benefits," typically genetic benefits conferred by males that can increase offspring fitness (survival and/or reproduction). There is evidence of indirect benefits of choice. Female house crickets (*Acheta domesticus*) mated to more "attractive" males in the laboratory were found to have more "attractive" male offspring (Head et al. 2005), but similar experiments on the tropical house cricket (*Gryllodes sigillatus*: Ketola et al. 2007) and a bow-winged grasshopper (*Chorthippus biguttulus*) did not find such benefits, especially in the field (Klappert and Reinhold 2007). In the wax moth (*Achroia grisella*), attractive male song traits and preferences for these were found to be significantly heritable, but there was little evidence for indirect benefits in terms of egg hatchability, larval survivorship, or development time for the offspring of females mated with "attractive" males under varying environmental conditions (Jia and Greenfield 1997).

Fluctuating asymmetry (FA) has been invoked as a sensitive indicator of genetic quality (Andersson 1994). Simmons and Ritchie (1996) reported differences in the calling songs of male field crickets that reflected harp asymmetry and a preference of females for the pure-tone songs characteristic of more symmetric males. More recent studies on the mechanisms of male sound production and female phonotaxis have, however, questioned the validity of this hypothesis for field crickets (Montealegre-Z et al. 2011; Hirtenlehner et al. 2013). There is also little evidence for correlations between song traits and FA in other cricket species (Ketola et al. 2007; Deb et al. 2012) or of the effects of FA on female fitness (Brown 1997a; Ketola et al. 2007).

3.3.2.1.3 Sensory Biases

Females may prefer certain male traits due to "sensory biases," essentially because of neural circuitry that has been selected for in a context outside of mate choice and subsequently "exploited" by male signals (Ryan and Keddy-Hector 1992; Nakano et al. 2015). A possible example of this is the preference for leading calls exhibited by some female katydids, which may be a consequence of their sound localization mechanism (Greenfield 2015). The preferences exhibited for louder or longer male calls may be simply because signals with higher energy stimulate the auditory system more (Ryan and Keddy-Hector 1992). Thus, louder and longer calls are an interesting case of traits that theoretically might evoke stronger responses either because they reflect males in better condition (because these signals should be energetically more expensive to produce and may confer direct or indirect benefits on females) or because they stimulate the auditory system more (sensory bias, often referred to as "passive attraction"; Parker 1983) or both. This highlights the fact that these hypotheses for female choice need not be mutually exclusive. Dissecting which of these drives female response and its evolution to a greater or lesser extent is a difficult task.

To summarize, there is as yet little evidence to unambiguously link long-distance acoustic signal traits with female fitness benefits. The few cases where such demonstrations have been made are all in laboratory studies. The applicability of these in field conditions is unknown for several reasons: (1) the variation in male calling traits seen in the laboratory could be quite different than in the wild, given the known condition dependence of acoustic traits (Scheuber et al. 2003), especially those believed to be under directional selection; (2) the benefits of female choice may be highly context dependent, depending on factors such as nutritional status (Wagner 2011); and (3) the spatio-acoustic context in which preferences for long-distance signals are expressed are little studied and likely to be highly variable. The effects of the temporal and spatial heterogeneity of natural ecological contexts on the evolution of acoustic traits, preferences, and benefits are little explored. Given these caveats, most of the evidence so far suggests that long-range acoustic signals may be used primarily to locate a conspecific mating partner and that mate choice is more likely to be based on close-range signals, both acoustic and non-acoustic.

3.3.2.2 Acoustic Signals and Male Competition

Acoustic signals may be used in male competition for females in two major ways: either in the process of competing for resources that increase the chances of encounter with females or by acoustic rivalry in the process of attracting females (Gerhardt and Huber 2002). Signaling males may compete for and defend resources such as favorable oviposition and/or foraging sites (Greenfield 1997) that may increase both male and female fitness (resource defense polygyny). Alternatively, males may not provide resources to females but may compete among themselves primarily for favorable calling sites that increase their chances of attracting females. In both cases, contest winners are likely to have higher mating success than losers, either because females stay longer on the preferred resources (Greenfield 1997) or because more females are attracted to winners that have successfully competed for superior calling sites (Arak and Eiriksson 1992). In either case, it may then benefit losers to adopt satellite strategies, remaining as non-calling males on the territories of winners (Feaver 1983; Greenfield 1997) or as transients that remain in the vicinity of calling males and try to intercept approaching females, as in field crickets (Cade 1975; Zuk et al. 1993).

3.3.2.2.1 Acoustic Signals in Resource Defence and Aggression

Males may employ acoustic signals to deter rivals in lieu of physical aggression. For example, in the Pecos clicker grasshopper (*Ligurotettix planum*) in which males defend individual shrubs, resident and intruder males engage in antiphonal "acoustic duels," at the end of which one of the individuals (typically the intruder) leaves

(Greenfield 1997), suggesting assessment of resource-holding potential based on acoustic signals. Losers typically have lower signal rates than winners during these acoustic exchanges; some exchanges may escalate into physical aggression (Greenfield 1997). Alternatively, males may contest calling sites using acoustic signaling that can escalate into physical combat, as in the greater grig (*Cyphoderris monstrosa*; Mason 1996).

The structures of the signals used in close-range acoustic interactions can be different from the long-range advertisement signals (Alexander 1961). In many species, they differ only in call rates and intensity or in increased duration or numbers of elements, suggesting that opponents may use these acoustic signals to assess each other's fighting ability (Brown et al. 2006). The prolonged nature of some of these acoustic interactions may be a way to ensure signal honesty. In *C. monstrosa*, males that ultimately won contests were sustained singers, and song was found to be a reliable index of the ability of a male to win fights, as such males tended to win in physical fights even after they had been muted (Mason 1996).

In many field crickets, katydids, and grasshoppers, the structure of the call during aggressive interactions is different from the advertisement call (Alexander 1961). Why some taxa have evolved calls that differ in structure between advertisement and aggression, whereas others have not, is unclear. Possibly, species that use acoustic signals for assessment of fighting ability before escalation to physical aggression may rely more on features that reflect physiological condition, such as the rates, durations, and intensity of calls, whereas those that typically use aggressive acoustic signals after physical aggression may convey a different message, say of intimidation, leading to structural differences from the advertisement call. Comparative studies on the structure and function of close-range aggressive acoustic signaling may shed light on these issues.

3.3.2.2.2 Acoustic Signals and Male Aggregation

Male aggregates may be favored if they increase the number of females attracted such that there is a per capita gain for males (Gerhardt and Huber 2002; Alem et al. 2011), but empirical evidence of females being more attracted to multi-male choruses than individual calling males is slim and largely negative (Cade 1981; Guerra and Mason 2005). Alternatively, male calling aggregates may be driven by natural selection. Although acoustically orienting predators and parasitoids may find aggregates more detectable, per capita predation risk may be lower (Alem et al. 2011) owing to the dilution effect and the difficulty for predators of locating one among multiple calling individuals. Although aggregation may have benefits, it can increase male competition for females, especially if males are spaced such that their broadcast volumes overlap. If females hear multiple males simultaneously, males are in direct acoustic competition, as females will then orient toward and locate only one of the callers. Calling males may thus gain by spacing themselves such that their broadcast areas do not overlap. Indeed, there is empirical evidence that males of several calling insect species do use each other's call intensities to move apart, perhaps to avoid or minimize direct acoustic competition (reviewed in Gerhardt and Huber 2002).

On the other hand, males that have preferred traits such as higher call intensities or rates may well benefit from being near a neighbor with less preferred traits, as such males could use the increased overall broadcast volume provided by the neighbor and then "hijack" the female. Indeed, Guerra and Mason (2005) found in the katydid *Conocephalus brevipennis* that larger males and stronger singers tended to be attracted more to playbacks of conspecific male calls. The dynamics of male spacing in the field as a result of the interplay between call variation and calling site/resource availability and their consequences for male fitness are little understood and offer much scope for future investigation.

3.3.2.2.3 Acoustic Rivalry to Attract Females

In a number of species, males that can hear other callers often increase or decrease the rates or durations of their calls or both (Gerhardt and Huber 2002). Alternatively, they may change their call timings in ways that allow their call onsets to alternate with or lead those of neighboring males, leading to patterns of call alternation or synchrony (see Greenfield 2015 for an in-depth review). As females typically prefer calls with higher rates, longer durations, or leading onsets (Gerhardt and Huber 2002), this may bias their approach towards males with these traits when they can hear them simultaneously. Preferences for all of these traits can, however, be overcome by increased call intensity. In crickets and katydids, louder males also gain an advantage in direct competition because the female auditory system represents only one call pattern on each side, typically the loudest on that side (Pollack 1988; Römer and Krusch 2000).

Even if males cannot hear each other and engage in direct acoustic rivalry, there will be sexual selection on males to produce louder and longer calls, as this increases their detectability by females in space and time. Directional sexual selection on call intensity, duration, and rate can thus also be driven purely by inter-male competition for resources or direct acoustic rivalry to attract females. Distinguishing between the effects of male competition, female choice, and sensory biases therefore poses a challenge.

3.4 Acoustic Signaling in Natural Environments

3.4.1 Acoustic Masking Interference

In natural environments, acoustically signaling animals share their habitat with a number of other species, many of which may also use species-specific acoustic signals to attract conspecifics. Multiple individuals of several of these species may call together, exemplified by the loud cacophony of dawn and dusk choruses, a familiar yet little understood component of soundscapes worldwide. All of these signals are broadcast into and travel through a common transmission medium before reaching their respective receivers. This raises the question of how individuals of each species manage to communicate with conspecifics in the context of so much potential masking interference from heterospecific acoustic signals, which should impair detection, discrimination, and localization of signals (Brumm and Slabbekoorn 2005). Both senders and receivers can potentially employ a number of different strategies to decrease the probability and/or effects of acoustic masking interference (reviewed in Römer 2013; Schmidt and Balakrishnan 2015).

3.4.1.1 Sender Strategies: Acoustic Niche Partitioning

Senders could decrease the probability of acoustic overlap by "acoustic niche partitioning": moving apart in time, spectral frequency, or space, such that each species occupies a unique niche, in a manner analogous to ecological niche partitioning. Empirical studies on acoustic niche partitioning have been carried out on acoustic assemblages using the framework of ecological niche partitioning (Gotelli and Graves 1996). Typically, such studies involve an estimation of acoustic overlap between pairs of species in the assemblage, examining one dimension (trait) at a time. This is then followed by a statistical comparison of these overlap values with a null model (consisting of random combinations of the trait under study) to determine whether there is any evidence for partitioning along that dimension significantly above levels expected by chance. A second and complementary approach is to examine distributions of pairwise overlap between species in different dimensions for negative correlations. For example, species that call simultaneously (higher probability of temporal overlap) may be more likely to be selected to separate in spectral or physical space (lower probability of spectral or spatial overlap).

3.4.1.1.1 Temporal Partitioning

Temporal partitioning can be accomplished at different timescales. Species can decrease temporal overlap in their signals by (1) decreasing overlap in their breeding seasons; (2) calling at different times of the day or night (diel partitioning); (3) calling in the same diel period but in non-overlapping bouts; or (4) calling simultaneously but placing individual calls in the silent gaps between the calls of heterospecifics. Species could also evolve temporal structures that minimize signal overlap even when individuals of different species call simultaneously and pay no attention to each other (Jain et al. 2014).

Studies on cricket, katydid, and cicada assemblages have failed to find evidence of diel temporal partitioning (Sueur 2002; Diwakar and Balakrishnan 2007a; Schmidt et al. 2013). Cicadas were, however, partitioned from crickets and katydids on a diel scale in paleotropical rain forest assemblages (Diwakar and Balakrishnan 2007a; Grant 2014). Within the diel calling period, species that were more likely to call together (within 5-min time windows) were more likely to possess signal temporal structures that resulted in lower levels of overlap (Jain et al. 2014).

Masking due to temporal overlap can also occur in single-species choruses. Overlaps between the calls of individual males may obscure the species-typical temporal pattern, making it difficult for females to recognize and locate individual males, especially if they are in dense aggregates (Gerhardt and Huber 2002; Greenfield 2015). Males of some katydid species synchronize their calls, which may achieve preservation of species-typical temporal patterns, allowing an aggregate to attract females, especially since synchronized calls of aggregates can also be louder (Nityananda and Balakrishnan 2009; Hartbauer et al. 2014). If this increases per capita male fitness, then such a cooperative strategy may be selected for (Nityananda and Balakrishnan 2009). Whereas there is no evidence that the louder intensity of an aggregate is more attractive (Hartbauer et al. 2014), preservation of species-typical temporal pattern is important. Females usually prefer the calls of individual males over that of aggregates in species that do not show acoustic interactions (Guerra and Mason 2005). In the synchronizing katydid *Mecopoda elongata*, rhythm preservation appears to be important, although another factor leading to the evolution of call synchrony in this genus could be male competition to produce leading calls (Nityananda and Balakrishnan 2009; Hartbauer et al. 2014). Call alternation is another effective way to avoid overlap and appears to be used by some katydid and grasshopper species (Greenfield and Minckley 1993); from a distance, however, this would double the call rate for a listening female. It would be interesting to ask whether synchrony is more common in species with higher levels of spatial aggregation. Alternation may possibly be used more in direct inter-male rivalry because this would allow males to assess each other in the silent gaps of their singing (Greenfield 2015). Synchrony may represent either a "cooperative" outcome of a strategy in signaling aggregates that increases per capita male success or an outcome of male competition to produce leading calls that are attractive to females (Nityananda and Balakrishnan 2009; Hartbauer et al. 2014).

3.4.1.1.2 Spectral Partitioning

All of the studies on acoustic assemblages (Schmidt et al. 2013; Grant 2014; Jain et al. 2014) found overall low levels of pairwise spectral overlap between the signals of different species, but only the study by Schmidt et al. (2013) on a cricket assemblage found evidence that such low levels of overlap may be driven by selection for spectral partitioning. In a paleotropical rain forest assemblage of crickets and katydids, Jain et al. (2014) found no assemblage-wide negative correlations between spectral and temporal overlaps, suggesting that species with higher temporal overlaps do not evolve greater spectral separation. There are, however, caveats to such analyses and their interpretations. Assemblage-wide analyses can miss interactions between individual pairs of species where such evolutionary pressures may indeed operate. Species that share extensive spectral overlap may be more likely to partition their calling times (Römer 2013). In an Australian woodland shared by five calling katydid species with extensive spectral overlap, the diel calling period of one species (*Hemisaga denticulata*) did appear to be restricted and shaped by masking interference from another species (*Mygalopsis markii*) (Römer et al. 1989).

3.4.1.1.3 Spatial Partitioning

Spatial partitioning has rarely been examined in acoustic insect assemblages; the only two studies that examined this found no evidence for horizontal spatial separation between individuals of different species above levels expected by chance at either larger or smaller spatial scales (Schmidt et al. 2013; Jain et al. 2014). Vertical stratification of acoustically signaling species in an assemblage is, however, well-documented (Diwakar and Balakrishnan 2007b; Schmidt et al. 2013). The relationship between vertical stratification and levels of acoustic interference, as well as whether stratification represents a strategy to decrease acoustic masking, is unknown.

3.4.1.2 Integrating the Effects of Signaler Behavior, Signal, and Receiver Properties

The niche partitioning approach to examining acoustic interference suffers from the drawback that it investigates the different dimensions of niche separation separately and does not take into account effects of the habitat or receiver properties, both of which contribute profoundly to the amount of masking interference experienced. Estimating levels of acoustic masking interference requires all of these factors to be taken into account. Jain et al. (2014) constructed a three-dimensional acoustic active space simulation model that incorporated all of the aforementioned features and used it together with empirical data to estimate effective acoustic overlap in natural mixed-species choruses of crickets and katydids in the understorey of a rain forest. For the five species examined, the median and modal values of effective acoustic overlap (assuming receivers with frequency tuning matched to conspecific signals and typical cricket and katydid hearing thresholds) was zero. This suggests that the actual amount of heterospecific acoustic interference experienced by individual receivers in natural choruses may be quite low. This is, however, the case only if receivers are tuned to match conspecific call frequencies. If receivers are modeled as untuned, overall levels of acoustic overlap become higher but, interestingly, in pairwise comparisons between species, louder species typically experience very low levels of masking independent of call structure (Jain et al. 2014). Loud calls may thus represent a powerful strategy to reduce heterospecific acoustic masking and may be particularly useful for species with high bandwidth calls that do not have narrowly tuned receivers. It will be interesting to examine different acoustic assemblages for correlations between call bandwidth and intensity, as these appear to represent alternate, independent strategies to avoid heterospecific masking interference.

Levels of acoustic masking interference are also strongly dependent on the acoustic context, including the relative abundances of callers in heterospecific choruses (Balakrishnan et al. 2014), and are often not simply predictable from measurements of signal structure. Calls with similar carrier frequencies and bandwidths may experience vastly different interference scenarios when the relative abundances and density of mixed-species choruses change, with some species being well-buffered to changes in acoustic context and others not (Balakrishnan et al. 2014).

Signal features alone are thus likely to be inadequate proxies for levels of acoustic masking interference. The context dependence of levels of acoustic masking interference raises interesting questions about the structure and formation of multispecies acoustic choruses in the wild. It is currently unclear what drives the variation in composition and size of these choruses and whether individuals "decide" whether or not to participate depending on chorus composition and size. This is particularly important to understand from the perspective of acoustic biodiversity monitoring. If the calling behavior of individuals depends on that of other species, then the relative abundance of callers may not reflect the relative abundance of species. This would have implications for the spatial scales at which acoustic monitoring needs to be carried out to obtain reliable estimates of species relative abundances.

3.4.1.3 Receiver Strategies: Auditory Physiology

Tuning of receiver auditory systems is potentially powerful ways of overcoming heterospecific masking interference. Empirical evidence for frequency tuning matched to signal spectrum comes from physiological studies on a number of acoustic insect species (Pollack and Imaizumi 1999). Its efficacy in filtering noise in natural environments has been less investigated. Schmidt et al. (2011) investigated the frequency tuning of cricket species (Podoscirtinae and Oecanthinae) in a neotropical rain forest and found it to be narrow and well matched to conspecific signal frequencies. Its efficacy in suppressing ambient noise was investigated by making electrophysiological recordings from auditory neurons of one of these species (*Paroecanthus podogrosus*) at night in the acoustic ambience of the rain forest. There was excellent representation of conspecific call temporal pattern, as well as a high signal-to-noise ratio, indicating essentially no masking interference. This is consistent with the low levels of effective acoustic interference estimated by Jain et al. (2014) for tuned receivers in a paleotropical rain forest. The frequency filters of the neotropical rain forest crickets were more effective in tuning out ambient noise than the frequency filters of field cricket species (*Gryllus campestris* and *Gryllus bimaculatus*) in temperate regions, suggesting that such narrow tuning may be a response to the higher numbers of co-signaling cricket species in the tropics. More convincing evidence for a role for masking interference in shaping frequency tuning would, however, come from similar comparative studies on different populations of a widely distributed species occurring in different acoustic assemblages. If populations in species-rich assemblages or in assemblages with species showing high spectral overlap with the focal species were to show a sharper tuning, this would constitute stronger evidence for the evolutionary role of masking interference in shaping frequency tuning.

Apart from frequency tuning, there are a number of physiological mechanisms that could contribute to overcoming acoustic masking interference. Spatial release from masking and selective attention are two important mechanisms that allow receivers to detect and represent signal patterns in conditions of high masking noise as, for example, in the presence of multiple callers (Schmidt and Römer 2011).

Spatial release from masking refers to the fact that two sound sources can be resolved as separate if they are spatially separated. In particular, if they are on different sides of the receiver, the directionality of the ear/auditory system will allow them to be represented clearly as separate patterns (Pollack 1986). Callers in natural environments are typically spaced apart and at different distances from a given receiver. This can probably generate sufficient differences in call timing and/or intensity to allow them to be separately resolved. In addition, crickets and katydids have physiological mechanisms of selective attention, wherein only the loudest call (after filtering by the ear) on each side is represented (Pollack 1988; Römer and Krusch 2000). Because matched frequency filtering will typically make conspecific frequencies appear louder than heterospecific frequencies, this may help to filter out heterospecific "noise." That this mechanism can allow preferential representation and attention to conspecific signals in receiver auditory systems, even in the presence of spectral overlap in signals, has been shown in some elegant neurophysiological experiments on both crickets and katydids (Pollack 1988; Siegert et al. 2013).

3.4.2 The Effects of Habitat and Acoustic Adaptation

Acoustic signals are affected by the habitat through which they traverse before reaching receivers. Habitats affect sound signals in three important ways: signal attenuation, spectral filtering, and distortion of temporal pattern (reviewed in Römer 2013). All sound signals attenuate as they move away from sound sources due to spherical spreading, but obstacles such as vegetation, rocks, and the ground effect can cause further or "excess" attenuation. Excess attenuation is typically higher at ground level and in dense foliage and affects sounds of higher frequencies more than lower frequencies. High-frequency sounds therefore have smaller transmission distances and, in high bandwidth signals, this results in spectral filtering, with the high-frequency components disappearing from the spectrum at greater distances from the source (Römer 2013). Signals can also be subject to distortion of temporal patterns due to either echoes or strong amplitude modulation (AM) due to wind. In general, forest habitats should cause greater spectral filtering and reverberation, and open habitats such as grasslands should cause more AM fluctuations due to wind. These effects lead to some general predictions about the structures of acoustic signals that would be optimal in different habitats and lead to the concept of "acoustic adaptation". Signals should be selected to minimize attenuation and distortion effects in the habitats in which the signalers have evolved (Morton 1975). It predicts that long-distance acoustic signals of a given species will transmit best in native habitats.

The acoustic adaptation hypothesis (AAH) has been tested extensively in birds and frogs (Ey and Fischer 2009). There has been only one study examining the predictions of the AAH in insects at the macrohabitat level. A study on seven species of bladder grasshoppers (family Pneumoridae) in four habitats in South Africa found partial support for acoustic adaptation (Couldridge and van Staaden 2004). At the microhabitat level, the use of different calling heights by two cicada species in a vineyard was not found to correlate with advantages for signal propagation for either species (Sueur and Aubin 2003). Calling height can have a profound effect on broadcast space, and many acoustic assemblages exhibit vertical stratification of calling species (Diwakar and Balakrishnan 2007b). In an assemblage of rain forest crickets and katydids, there was, however, no evidence that vertical stratification of callers with different song structures reflected acoustic adaptation (Jain and Balakrishnan 2012). Rather, certain microhabitats such as the ground and canopy were found to be consistently poor for signal transmission regardless of signal structure. This suggests that vertical stratification of calling insect species is driven primarily by factors other than acoustic adaptation, such as avoidance of masking interference or predation.

Acoustic adaptation also makes other general predictions that have not been tested in acoustic insects; for example, acoustic assemblages in forests should on average have lower frequency tonal calls with lower repetition rates as compared to signals in open grassland habitats. The problem with examining these predictions at the assemblage level is the need to control for phylogenetic history and constraints that may result from specific sound production mechanisms. The confounding effects of body size also need to be corrected for. It may therefore be more fruitful to test the AAH on different populations of a species or on a set of closely related species occupying different habitats/microhabitats.

3.4.3 Anthropogenic Noise and Acoustic Signaling in Insects

The profound changes to the natural environment effected by humans also manifest as changes to the sonic environment. Acoustically signaling organisms have been suddenly exposed (over a period of about a hundred years) to novel anthropogenic sounds, especially in the form of technology-generated noise. One of the most pervasive is traffic noise, and its effects have been investigated in a number of terrestrial vertebrates (Barber et al. 2010), but there is a paucity of studies on invertebrates, including insects (Morley et al. 2014). Studies on the bow-winged grasshopper *Chorthippus biguttulus* (the peak frequencies of whose call lie at 6–8 kHz and could be masked by traffic noise) showed that males found in roadside habitats signaled at higher frequencies than those in quieter localities (Lampe et al. 2012). Nymphs of *C. biguttulus* exposed to road noise also produced higher call frequencies as adults, suggesting some form of adaptive developmental plasticity (Lampe et al. 2014). A recent study on tree crickets (genus *Oecanthus*; Costello and Symes 2014) of the effects of highway traffic noise on both signaling behavior by males and phonotaxis by females found no effects on male signal structure or call intensity, and females did not have a problem locating sources of male calling song even in the presence of high levels of traffic noise.

There are several possible reasons why crickets may be less affected by traffic noise. Insects that use pressure or pressure–difference receivers do not sense airborne sound below 1 kHz (as sound). Although there is spectral overlap between traffic noise and cricket call frequency, the proportion of noise energy that falls within the narrow frequency band of the call, to which receivers are typically tuned, may be quite low. Both tonal and high-frequency calls (higher than 8 kHz) of insects are thus likely to experience little masking from traffic noise. The broadband calls of many katydids and grasshoppers, with energy peaks lower than 10 kHz, may be more prone to masking interference from traffic noise. Studies on the effects of anthropogenic noise need to incorporate an examination of the physiology and ecology of the species being investigated in addition to behavioral effects (Morley et al. 2014).

A number of acoustically signaling insect species form dense, loud conspecific aggregations and have evolved powerful physiological mechanisms to deal with signaling effectively in noisy environments. These adaptations may serve them well in dealing with anthropogenic noise. Habitat fragmentation and degradation, together with uncertain seasonality posed by climate change, may be more serious causes for concern than disruption of their communication systems. Their adaptability in the face of change, however, gives grounds for optimism that these singing insects, with lineages going back to Paleozoic and Mesozoic times, will survive beyond the Anthropocene.

3.5 Summary

There are still many gaps in our understanding of insect acoustic signaling in natural environments. A comprehensive understanding of the drivers of different pair formation strategies is lacking. Most current hypotheses evoke costs of signaling, mating, and/or searching as central to the evolution of different pair-forming strategies, but there are little empirical data on such costs, especially in the field. More empirical work is needed to measure these costs (predation/parasitism and energy) in natural environments. Comparative studies on pair-forming strategies and acoustic signals need to be placed in a phylogenetic framework to obtain insights into their patterns of evolution. Mechanisms of sexual selection on long-distance acoustic signals remain a challenge. Whereas there are a large number of studies on female preference functions or multiple regression-based approaches suggesting sexual selection, it is unclear whether this is driven by female choice. The acoustic features that are typically under strong directional sexual selection, such as higher call rates, intensities, and durations could have evolved via female choice, male competition, or sensory biases. Disentangling these drivers poses a challenge. Examining benefits of choice of signal variants to females is one strategy, but this needs to be shown to be true in the wild. This is inherently difficult, not only logistically but also because there is information exchange during close-range interactions (such as courtship and aggression) as well, often based on multiple modalities. More longitudinal studies are therefore needed on the female decision-making process, especially in natural environments. Most studies on sexual selection on acoustic signals in insects have been confined to the laboratory and their external validity remains questionable. The spatial and temporal heterogeneity of the ecological context may have important influences on male and female behavior and thus on their fitness. The effects of this heterogeneity need to be explored and taken into account. Understanding of the physiological mechanisms of mate search and mate choice lags behind, and is often not integrated with, theoretical models or experimental design; a sensory ecological perspective on sexual selection will be useful. Sexual selection on acoustic signals driven by male competition has also received scant attention, even though it is an equally likely driver of signal evolution.

Regarding communication in natural environments, studies on acoustic niche partitioning have traditionally used measurements of signal features and acoustic distances without incorporating the effects of space and intensity. Whereas this is a useful first step, signal features on their own are not always good proxies for the amount of masking interference experienced. There is need to move toward models that integrate signal properties, transmission effects, and receiver physiology. In most systems, there is essentially no information on receiver positions relative to senders because females of many acoustic insects do not produce sound. This is another gap that needs to be filled to better understand masking scenarios in the field. Habitat effects on insect signals have been studied to some extent, but much more comparative work using natural signals in natural environments is needed. More studies are also needed using single or closely related species in different habitats to examine whether acoustic adaptation is an important selective force determining signal structure or signaler behavior. Phylogenetic constraints need to be explored and taken into account in comparative studies and this is not yet widespread in studies on acoustic insects. Finally, the effects of anthropogenic noise on insect signaling are just beginning to be explored. A comparative study of its effects on different types of signals would be interesting and informative, allowing identification of species or groups that may be threatened by human activities.

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Chapter 4
Hearing for Defense

Gerald S. Pollack

Abstract The appearance of echolocating bats approximately 65 million years ago presented a life-or-death challenge to nocturnally active insects, particularly those that fly at night. In response, ultrasound-sensitive ears and bat-avoidance behaviors have evolved repeatedly in insects. Avoidance responses include steering away from a distant bat, last-chance maneuvers such as diving to the ground or flying erratically to avoid capture in close encounters, and sound production to startle the bat, to warn it of the prey's distastefulness or to interfere with the bat's ability to echolocate. Some bat-avoiding insects use ultrasonic signals for intraspecific communication, presenting them with the challenge of discriminating between potential predators and mates. Evolution of the predator–prey relationship between bats and insects is ongoing, with each participant adopting new strategies to counter those of its opponent.

Keywords Acoustic startle • Bat avoidance • Echolocation • Evolutionary arms race • Negative phonotaxis • Predator–prey relations

4.1 Introduction

Most of the chapters in this volume focus on the use of acoustic signals for intraspecific communication. Yet the majority of insect auditory systems evolved in the context of predator detection (Greenfield, Chapter 2). The same characteristics of sound that favor it as a method for communication, namely its ability to be detected at a distance from its source and its capacity to encode considerable information in its spectral and temporal structure, also apply to its use for predator detection. Terrestrial predators may reveal their approach through noise produced as they walk on or through vegetation (Fullard 1988), and the vocalizations and wingbeat sounds of birds may warn of their approach (Fournier et al. 2013). The most powerful

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selection pressure favoring the evolution of predator-detecting ears, however, is exerted by bats. Of the more than 1,100 extant species of bat, approximately 70 % feed primarily on insects (Jones and Rydell 2003), which they detect and localize either by homing in on the sounds that the insects produce (e.g., mating calls) or, more commonly, by analyzing the echoes of their own ultrasonic calls that are reflected by the bodies of their flying insect prey. The appearance of these nocturnal aerial predators some 65 million years ago presented a profound challenge to night-flying insects that, until then, had the night sky essentially to themselves. Of the 24–29 independently evolved insect auditory systems known so far, at least 14 are specifically tuned to ultrasound and appear to have evolved as a direct response to bat predation (Greenfield, Chapter 2). In other cases, such as crickets and katydids, ears and mechanisms for sound production were in place and, presumably, used for intraspecific communication long before bats appeared, and ultrasound detection and avoidance were add-ons to well-established acoustic behaviors.

Predation by bats is an ongoing selection pressure that continues to shape the auditory systems of insects. Although ultrasound-sensitive ears are generally broadly tuned, the range of frequencies to which they are most sensitive is often matched to the dominant sound frequencies of the local bat community (Fullard 1998; ter Hofstede et al. 2013). Insects that evolved in or migrated to bat-free habitats tend to be less sensitive to ultrasound compared to those in bat-rich habitats, presumably because of the genetic drift that release from selection allows (Fullard 1994; Fullard et al. 2010). It is worth noting, however, that bats have also responded to the evolution of ultrasound sensitivity in insects, for example, by lowering the intensity of their echolocation calls (Goerlitz et al. 2010), by shifting their sound frequencies above or below the range to which their prey is most sensitive (Fenton et al. 1998), or by broadening the beam of their echolocation calls so as to maintain tracking of insects even as they attempt to escape (Ratcliffe et al. 2013).

In this chapter the focus is on the behavioral strategies employed by insects as countermeasures to bat predation. Although some aspects of the underlying neurobiology are discussed briefly, interested readers should consult Pollack (2015) for a more thorough review of that topic.

4.2 Flight and Sensitivity to Ultrasound

Insects are at risk of predation by aerially hawking bats only while flying. Not surprisingly, then, the ability to hear ultrasound is closely correlated with the ability to fly. In some species of mantids, females, the wings of which are reduced or absent, fly little if at all. Concomitantly, the females are less sensitive to ultrasound than males (Yager 1988). Similarly, flight in gypsy moths (*Lymantria dispar*) is limited to males and here, too, female thresholds for ultrasound are higher than those of males (Cardone and Fullard 1988). Even among moths that fly, there is a correlation across species between time spent aloft at night and sensitivity to ultrasound (ter Hofstede et al. 2008). Many species of Orthoptera (crickets, grasshoppers, katydids) are flightless and others are flight dimorphic, with some individuals, which have long wings, able to fly and others, with short or absent wings, flight incapable. Ears

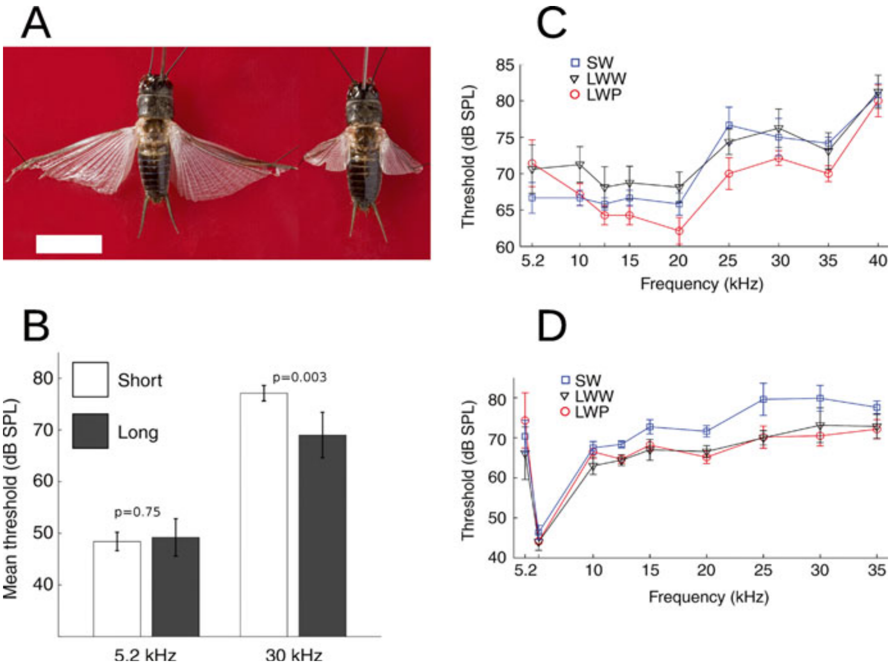

Fig. 4.1 Ultrasound sensitivity varies with flight capability. (**A**) Long-winged and short-winged *Gryllus texensis*. The front wings, which do not differ between morphs, have been removed. Scale: 1 cm. (**B**) Behavioral thresholds of long-winged and short-winged individuals for positive phonotactic steering toward a song model and negative phonotaxis away from ultrasound. (**C**) Threshold tuning curves of the AN2 neuron for long-winged individuals with functional (*pink*) flight muscles (LWP), long-winged individuals with degenerate (*white*) flight muscles (LWW) and short-winged individuals (SW). (**D**) As in (**C**), but for the ON1 neuron [(**B**)–(**D**) from Pollack and Martins (2007)]

in the flightless forms are often either absent or poorly developed (Knetsch 1939), although whether the ears of the flight-capable forms are ultrasound sensitive is not known in most cases.

Many cricket species are wing dimorphic, with long-winged, flight-capable and short-winged, flight-incapable individuals (Fig. 4.1A). Crickets use sound for intraspecific communication as well as for predator detection, and long-winged and short-winged individuals of the Texas field cricket (*Gryllus texensis*) are equally sensitive to the relatively low sound frequency that is dominant in their songs. Short-winged individuals, however, are less sensitive to ultrasound than their long-winged counterparts, as measured in both behavioral and neurophysiological experiments (Pollack and Martins 2007; Fig. 4.1B).

Even long-winged individuals may eventually lose the ability to fly because of age-related degeneration of flight muscles. An identified interneuron, AN2, that triggers negative phonotactic steering (Nolen and Hoy 1984; Marsat and Pollack 2006) is less sensitive to ultrasound in long-winged Texas field crickets with degenerate muscles than in those still able to fly (Pollack and Martins 2007; Fig. 4.1C). Because all long-winged crickets have flight-capable muscles early in adulthood, this implies that the tuning of AN2 shifts when flight ability is lost. Interestingly, tuning of another identified ultrasound-sensitive interneuron, ON1, does not differ between flight-capable and flight-incapable long-winged individuals (Fig. 4.1D). This suggests that the age-related shift in AN2 tuning is not the result of changes at the periphery (otherwise ON1 would also lose sensitivity) and points either to the synaptic connections between ultrasound-sensitive receptors and AN2 or to AN2's intrinsic electrophysiological properties as the likely sites of change in its sensitivity.

The decision to develop as either a long-winged or a short-winged adult is determined during late larval life by developmental hormones and can be manipulated experimentally by exogenous application of juvenile hormone (JH) or an analog, methoprene. Hormone treatment of larvae of the southeastern field cricket (*Gryllus rubens*) that are genetically predisposed to develop with long wings causes them to develop instead with short wings and poorly developed flight muscles (another characteristic of the short-winged form; Zera and Tiebel 1988). Short-winged-like phenotypes can also be induced by hormone treatment even in a species in which all individuals are normally long winged and flight capable (Zera et al. 1998). Hormone treatment of larvae of the Pacific field cricket (*Teleogryllus oceanicus*), a monomorphic long-winged species, results not only in underdeveloped flight muscles in adults but also in poor ultrasound sensitivity (Narbonne and Pollack 2008). Like the sensitivity difference between natural long- and short-winged crickets in dimorphic species, the loss of sensitivity to ultrasound is frequency specific; treated and control individuals are equally sensitive to the frequency used for intraspecific communication. In crickets, then, ultrasound sensitivity seems to be physiologically coupled to other flight-associated characteristics such as wing length and muscle condition, ensuring that those individuals that are able to fly have the sensory equipment required to help them avoid predation by bats.

Even among flight-capable individuals, responses to ultrasound may be evident or robust only while the insect is actively flying. Mantises (*Parasphendale agrionina*: Yager and May 1990) and tiger beetles (*Cicindela marutha*: Yager and Spangler 1997) exhibit steering responses while performing tethered flight (Sect. 4.3.1) but not when they cease flying, even while still tethered above the surface. Pacific field crickets, which respond to ultrasound stimuli with strong and consistent steering responses while flying (Sect. 4.3.1), respond only weakly and transiently while walking (Pollack et al. 1984). Parasitoid flies (*Ormia ochracea*), which locate their singing-cricket hosts through phonotaxis, orient toward both cricket songs and ultrasound stimuli while walking, but while flying they steer toward cricket songs and away from ultrasound (Rosen et al. 2009).

4.3 Defensive Strategies

Insects have evolved a number of tactics to avoid bat predation, including avoiding detection, avoiding capture once detected, warning of (or lying about) distastefulness, and interfering with the bat's ability to echolocate accurately.

4.3.1 Avoiding Detection

The ultrasound probes that bats use for echolocation attenuate rapidly with distance because of atmospheric absorption of high sound frequencies. This, together with the weak echoes returned from the small bodies of insects, limits the operating range of echolocation to only a few meters. By contrast, the sensitivity of insect auditory systems allows detection of a hunting bat at distances of tens of meters (Surlykke and Kalko 2008); thus the insect may hear the bat well before the bat detects the insect. In such cases the safest strategy for the insect may be simply to "disappear," that is, to leave the region of space being searched by the bat. That moths do just that was demonstrated by Roeder (1962), who used strobe photography to observe the responses of freely flying moths to attacking bats and to artificial ultrasound stimuli. Moths that were close to the source of ultrasound, which was consequently of high amplitude, reacted erratically by flying in loops and/or diving into the vegetation (Fig. 4.2A, B; see Sect. 4.3.2.1), but those that were distant from the sound source, and thus received low-amplitude stimuli, responded by steering systematically away from the sound source (Fig. 4.2C, D), that is, they performed negative phonotaxis. Roeder also demonstrated directed steering attempts away from the sound source during tethered flight by measuring the airflow produced by the beating wings (Roeder 1967). Negative phonotaxis to ultrasound stimuli also occurs in tethered flying crickets (Moiseff et al. 1978; Fig. 4.2E), locusts (Robert 1989), katydids (Schulze and Schul 2001), and parasitoid flies (Rosen et al. 2009). Addition of ultrasound to the normally low-frequency (2.7 kHz) mate-attraction song of southern mole crickets (*Scapteriscus borellii*) reduces the rate at which flying individuals are captured in acoustic traps, demonstrating that they, too, avoid ultrasound (Mason et al. 1998), although the kinematic details have not yet been described.

Green lacewings (*Chrysopa carnea*) respond to stimuli mimicking an approaching bat not by steering away but by folding their wings and dropping passively, a response that would bring them below the hunting bat's echolocation beam (Miller and Olesen 1979). Katydids may also exhibit a similar flight cessation response to ultrasound (Libersat and Hoy 1991; Schulze and Schul 2001).

Mantises are unique among hearing animals in having a nondirectional auditory system, with a functionally single midline ear that provides no binaural cues for determining the azimuth of a stimulus (Yager and Hoy 1986). In tethered flight, mantises respond to ultrasound with a deimatic display in which they extend their forelegs and dorsiflex the abdomen (Fig. 4.2F). This is combined with steering attempts to the left or right; the steering direction, however, is random with respect to the location of the stimulus (Yager and May 1990). In free flight, lateral steering is often accompanied by diving (Yager et al. 1990).

In contrast, tiger beetles have distinct left and right ears capable of encoding substantial binaural differences in the amplitude of an ultrasound stimulus that, in principle, could support determination of sound azimuth (Yager and Spangler 1995). Nevertheless, they, like mantises, steer randomly to one side or the other in response to ultrasound stimuli (Yager and Spangler 1997). Presumably even these

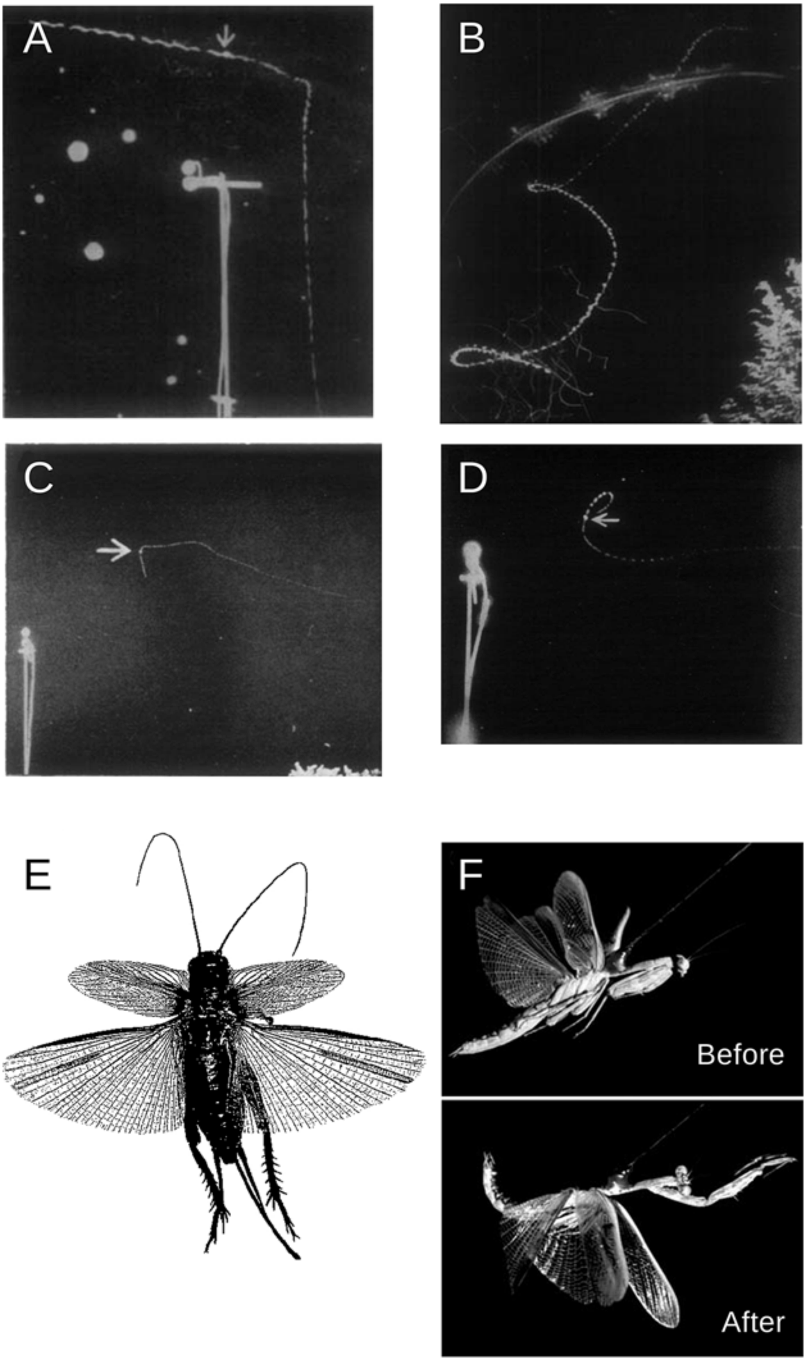

Fig. 4.2 Steering responses to ultrasound stimuli. (**A**)–(**D**) are strobe photographs showing flight paths of moths in response to artificial ultrasound stimuli (**A**, **C**, **D**) or in a close encounter with a bat (**B**). Flight paths appear as dotted lines because of the periodic stroboscopic illumination of the

randomly directed course changes suffice to remove the insect from the bat's awareness, at least some of the time.

Although, as noted in Sect. 4.2, sensitivity to ultrasound and flight ability are often linked, nonflying insects may also be at risk of predation by gleaning bats (Faure and Barclay 1994), which detect them both through echolocation and by homing in on the sounds that the insects produce (Geipel et al. 2013; Falk et al. 2015). Male wax moths (*Achroia grisella*) and katydids (*Neoconocephalus ensiger*) attract females with mate-calling songs that are rich in ultrasonic frequencies, but they fall silent when presented with bat-like ultrasound stimuli (Faure and Hoy 2000; Greenfield and Baker 2003). Female wax moths interrupt their approach to a calling male when presented with a bat-like stimulus (Greenfield and Weber 2000). The females discriminate between these two ultrasonic signals based on their differing temporal patterns. In another moth species, *Spodoptera litura*, females do not discriminate between male songs and bat calls, a situation that the males exploit by eliciting freezing responses in females, thus allowing the males to mate with the immobile females (Nakano et al. 2010).

In many moth species, stationary females attract distant males by emitting pheromones, which they disperse by fanning their wings. On presentation of bat-like stimuli, they cease wing fanning and lower their bodies close to the substrate, both of which actions presumably make them less conspicuous acoustically (Acharya and McNeil 1998).

4.3.2 Avoiding Capture Once Detected

4.3.2.1 Last-Chance Evasive Flight Maneuvers

Bats are fast and agile fliers, capable of speeds of 10 m/s or more (Hayward and Davis 1964). Although some insects can match this, speeds of <2 m/s are more typical (Dudley 2002). An insect, then, once detected, stands little chance of outflying a pursuing bat. Rather than relying on speed, many insects adopt erratic, unpredictable flight paths to elude capture. As mentioned in Sect. 4.3.1, moths that are presented with high-intensity ultrasound stimuli undergo a series of loops and dives rather than flying directly away from the stimulus (Roeder 1962). While performing tethered flight, Pacific field crickets steer away from low-intensity ultrasound stimuli but steer alternately to the left and right when stimulus intensity is high (Nolen

◄

Fig. 4.2 (continued) moth's wings. (**A**) Power dive of moth that was close to the loudspeaker (at the top of the vertical shaft) when stimulated; time of stimulation is indicated by the *arrow*. (**B**) Looping dive following close encounter with a bat, the path of which is seen above. (**C**, **D**) Two examples of directional responses away from the loudspeaker. (**E**) Steering response of a tethered cricket in response to an ultrasound stimulus presented from the left. Flexion of the legs, antennae, and abdomen to the right are diagnostic of an attempted right turn. (**F**) Deimatic display of a tethered flying mantis in response to ultrasound stimulation [(**A**)–(**D**) from Roeder (1962); (**E**) from Moiseff et al. (1978); (**F**) from Yager (2012)] and Hoy 1986a). Similarly, katydids (*Tettigonia viridissima*) that steer away from low-intensity stimuli cease flight and dive in response to high-intensity ultrasound (Schulze and Schul 2001). The diving turns of mantises, described in Sect. 4.3.1, become spiral in form when stimulus intensity is high (Yager et al. 1990).

Another auditory cue for insects about the proximity of an approaching bat is the temporal structure of the bat's echolocation calls. These occur at rates of a few per second while the bat is searching for a target (search phase), increase to tens per second once a target is acquired (approach phase), and to 100 or more per second just before the capture attempt (terminal buzz) (Griffin et al. 1960; Fenton et al. 2014). Green lacewings, on hearing a terminal buzz, often interrupt their passive drop by extending their wings, thus perturbing their otherwise predictable trajectory (Miller and Olesen 1979).

4.3.2.2 Sound Production

Tiger moths (Arctiinae), geometer moths (Geometridae), hawkmoths (Sphingidae), and tiger beetles (Cincindelidae) have independently evolved mechanisms for producing ultrasonic clicks in response to bat-like stimuli (Fig. 4.3). Tiger moths and geometer moths use specialized cuticular structures, tymbals, that generate clicks when buckled or relaxed under muscular control (Corcoran and Hristov 2014). Hawkmoths stridulate by rubbing specialized scales on their genitalia against the abdomen (Barber and Kawahara 2013). The mechanism for sound production by tiger beetles is unclear, although they do possess structures on their wings that seem well-suited for stridulation (Freitag and Lee 1972). In some of these cases, sound production has secondarily been exploited for intraspecific communication (Nakano et al. 2015; Greenfield, Chapter 2). The focus here, though, is on sound production as a defensive mechanism.

In most cases, sound production is a late component of the insect's response to echolocation calls. Like the wing-extension response of green lacewings, clicking

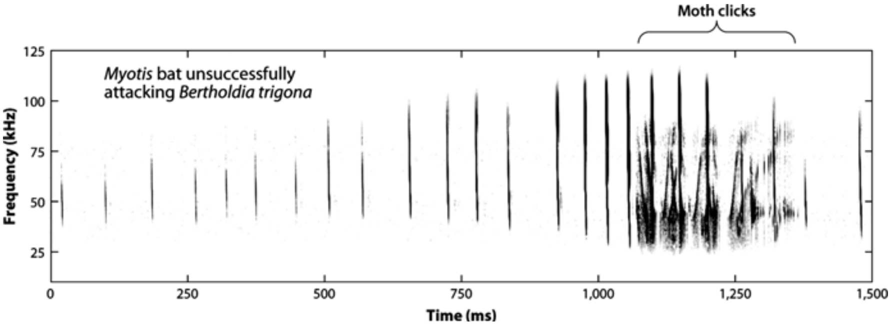

Fig. 4.3 Sound production by a moth in response to an unsuccessful bat attack. The dense sound produced by this species, *Bertholdia trigona*, thwarts attacks even by naïve bats, suggesting that it interferes with the bat's ability to echolocate accurately (From Conner and Corcoran 2012)

by the tiger moth (*Cycnia tenerea*) occurs toward the end of the bat's sequence of echolocation calls, where it appears to be triggered by the sound-pulse rates typical of approach-phase calls (Ratcliffe and Fullard 2005). The geometer moth *Eubaphe unicolor* also tends to click in the latter portion of the call sequence (Corcoran and Hristov 2014). The response latency of clicking by tethered tiger beetles is longer than that of other phonotaxis-related components of their response to ultrasound (Yager and Spangler 1997). Thus sound production, like the adoption of erratic flight paths, may be a last-chance response to avoid capture.

There are several ways in which sound production might offer protection against an attacking bat. First, it may simply startle the bat, momentarily disrupting its attack and allowing the insect to escape (Fenton and Bates 1990). Second, it might serve as an aposematic warning that the potential prey is distasteful or dangerous. Indeed, tiger moths are rejected by a number of potential predators because of their sequestration of noxious compounds from their food plants (reviewed by Dunning 1968). Third, sound production may, through Batesian mimicry, allow species that are not themselves distasteful nevertheless to dissuade bats that have learned, through their encounters with distasteful sound producers, to associate prey-produced ultrasound with unpalatability. Fourth, the prey-produced ultrasound might interfere with the bat's echolocation system, causing it to misjudge the location of the insect.

Discriminating among these alternatives is possible by observing whether/how the bat's behavior changes as a result of repeated encounters with an insect, an approach that has been pursued with studies of bat–moth interactions. Startle, unless predictive of unpalatability, should be effective only for the bat's initial capture attempts, after which the bat will have learned that the prey is harmless and palatable despite its sound production. Indeed, this is precisely what happened in laboratory tests with the big brown bat (*Eptsicus fuscus*), both when recorded moth sounds were paired with presentation of palatable prey (Fenton and Bates 1990) and when a palatable but sound-producing moth (a Batesian mimic) was offered to naïve bats (Hristov and Conner 2005). If, on the other hand, naïve bats are offered unpalatable, sound-producing prey, they should initially capture and taste them but then learn their unpalatability and avoid them. Laboratory trials demonstrated this scenario as well (Hristov and Conner 2005). Finally, if sound production interferes with the bat's ability to detect or localize the prey, then it should be equally effective against naïve and experienced bats. To date, such "jamming" has been demonstrated for only a single moth species, the tiger moth (*Bertholdia trigona*; Corcoran and Conner 2009), although field tests have demonstrated the impressive effectiveness of this defense mechanism, moths that were muted by puncturing their tymbals were more than 10 times as likely to be captured than intact individuals (Corcoran and Connor 2013).

Moth sounds fall into two general classes that seem to be well-suited for aposematic warning (or mimicking) and for jamming, respectively. Sound in the aposematic class, which includes those of known distasteful moths or their mimics, have relatively few clicks per tymbal activation and low duty cycle and are demonstrably sufficient to warn off an attacking bat. Sounds in the jamming class, which includes *B. trigona* along with many other potential but as yet untested jammers, have many clicks and high duty cycles and thus are more likely to present a stimulus at a time when it might be misinterpreted by the bat as an echo or otherwise interfere with the time-crucial processing of ultrasound in the bat's auditory system (Corcoran et al. 2011; Conner and Corcoran 2012).

4.3.3 Nonauditory Defenses

It is worth noting that hearing is not the only defense that insects have against bat predation. Indeed, nearly half of the approximately 140,000 species of moths are earless (Barber et al. 2015). Behavioral adaptations, such as avoiding flight at times and places where bats hunt, offer protection in many cases (Soutar and Fullard 2004).

Structural, rather than neurobehavioral, adaptations are another evolutionary strategy. The scales of moth wings have a honeycomb-like structure that resembles that of some man-made sound-absorbing materials. The scales enhance the absorption of sound frequencies between 40 and 60 kHz, that is, the same range most common in the echolocation calls of bats, thereby decreasing the amplitudes of echoes that the bat would receive. It is estimated that this might decrease the bat's detection range by 5–6 % (Zheng et al. 2011).

Luna moths (*Actias luna*) are large and earless. Their hindwings have long, swallowtail-like extensions that trail behind the moth and oscillate during flight. In laboratory tests, nearly half of the attacks by big brown bats were directed toward these extensions, which presumably are acoustically conspicuous, rather than toward the moth's body per se, thus leaving the vital organs of the mid-body unharmed (Barber et al. 2015). The wing extensions were often bitten off by the bat but with little impact on the moth's ability to fly. Comparison of capture rates of intact moths with those in which the wing extensions were ablated showed that the extensions afforded a level of protection, approximately 47 %, similar to that of ultrasound-sensitive ears in tympanate insects. Of course, unlike ears, the wing extensions offer protection only against one or two attacks; once they've been lost, the moth would be left completely vulnerable.

4.4 Interactions Between Defense and Reproduction

4.4.1 Attraction to a Mate Versus Repulsion by a Predator

In many cases, an insect may be en route to a potential mate when defensive action is called for. Male moths, for example, locate distant females by following their pheromone plumes. Anti-bat defensive behaviors such as negative phonotaxis or erratic loops and dives risk taking the moth far from the plume, which it might not regain, thus potentially losing a mating opportunity. Interestingly, moths are able to titrate the opposing tendencies to mate and to escape against one another, weighing the proximity of an approaching bat, as reflected by the amplitude of ultrasound, against the quality and quantity of female pheromone. In a laboratory assay, moths (*Spodoptera litteralis*) walked toward a pheromone source while being challenged with ultrasound stimuli. As mentioned in Sect. 4.3.1, walking moths freeze when presented with ultrasound. The threshold level of ultrasound required to elicit freezing was elevated by 10 to more than 40 dB in the presence of pheromone, depending on the quality and concentration of the olfactory stimulus (Skals et al. 2005).

A similar suppression of avoidance responses by mating-associated signals occurs in Pacific field crickets. The negative phonotactic response to ultrasound of females performing tethered flight is suppressed by the simultaneous presentation of a stimulus with the relatively low sound frequency that is dominant in the song that males sing to attract females (Nolen and Hoy 1986b). Response suppression in this case is ascribable to neural inhibition by low-frequency stimuli of the AN2 interneuron (Nolen and Hoy 1986b). As in the case of moths, the attractive and repulsive stimuli play off against one another quantitatively; the more intense the ultrasound, the higher the amplitude of a low-frequency signal required to suppress the avoidance response. In this case, the function of suppression may be to prevent crickets from being repelled by the high-frequency harmonics that are present in song. As a female approaches a male, these harmonics might, in the absence of low-frequency suppression, become sufficiently intense to excite the high frequency-tuned bat-avoidance circuits in the nervous system and elicit inappropriate avoidance responses (Nolen and Hoy 1986b). The neural inhibition elicited by low-frequency stimuli prevents this from happening.

4.4.2 Discriminating Mate from Predator

In contrast to the higher harmonic components of cricket song, which are present at relatively low levels and thus would be detectable only close to the source, songs of some other insects are dominated by high frequencies that could be detected by bat-sensitive insect ears at a distance. For example, males of many moth species court females with ultrasonic songs (Nakano et al. 2015), and songs of many katydids are rich in ultrasonic frequencies that overlap the frequency range that bats use for echolocation (e.g., Mason and Bailey 1998; Montealegre-Z et al. 2006). How do these insects discriminate potential mates from potential predators?

As mentioned in Sect. 4.3.1, in some moths no such discrimination occurs; the walking female's response to courtship song, freezing, is identical to her response to bat calls. Other moths, however, clearly discriminate between courtship song and bat calls. Moth ears are exceedingly simple, comprising only one to four auditory receptor neurons, and there is no indication that they are capable of frequency discrimination. Any strategy based on distinguishing between the spectra of courtship songs and bat calls is thus ruled out. Rather, the moths rely on differences in the temporal structures, or rhythms, of the signals. Male wax moths court females with a series of brief ultrasonic clicks delivered at a rate of 80–100 per second. Females perform positive phonotaxis by walking toward this signal, but they freeze if pulses are delivered at rates of less than 30 per second, which are similar to those used by bats during the search phase of their behavior. Males cease singing in response to the same, low pulse rate signal (Greenfield and Weber 2000). Reliance on a signal's temporal pattern as the basis for its identification is a common theme in insect auditory systems (see Hedwig and Stumpner, Chapter 8 and Ronacher, Chapter 9).

Unlike moth ears, katydid ears can support sophisticated frequency discrimination. The 20–40 sensory neurons in each ear are arranged tonotopically, with neighboring neurons most sensitive to different sound frequencies in a manner reminiscent of the mammalian organ of Corti (Stolting and Stumpner 1998; Montealegre-Z et al. 2012). Thus, in principle, katydids could exploit even small spectral differences between their own high-frequency calls and those of bats to discriminate between the two signals. The separation of frequency sensitivity at the periphery is, however, blurred in the central nervous system by the convergent projections of differently tuned afferents onto central neurons. One of these neurons, T1, has a large-diameter axon that promotes rapid conduction of action potentials, suggesting that it may play a role in bat-avoidance responses. Because of afferent convergence, T1 responds both to the 15-kHz frequency that is dominant in the songs of katydids in the genus *Neoconocephalus* and to the higher frequencies of bat calls. Nevertheless, when exposed to both signals simultaneously, T1 responds selectively to bat-like stimuli (Schul and Sheridan 2006; Fig. 4.4). It is able to do so because of sound frequency-specific adaptation to stimuli with high pulse rates. Frequency specificity is possible because tonotopy is expressed not only in the ear but also in the central projections of auditory afferents (Römer 1983; Stolting and Stumpner 1998). Although differently tuned afferents converge onto TN1, they do so at different places within T1's dendritic arbor. As a result local, thus frequency-specific, depression of afferent-to-T1 synapses is possible (Triblehorn and Schul 2013), allowing T1 to adapt to the high pulse rate song while remaining sensitive to lower pulse rate bat calls.

4.5 Summary

The repeated evolution of ultrasound-sensitive ears among insects attests to the strong selection pressure exerted by echolocating bats. Despite the many evolutionary routes that led to ultrasound sensitivity and bat-evasive strategies, some common themes are recognizable across taxa, such as the correlation between sensitivity to ultrasound and risk of predation and the selection of different evasive behaviors depending on the proximity of an attacking bat. This chapter has concentrated on behavior, but studies on the underlying neurobiology have also revealed many examples of convergent evolution, including large-diameter neurons that conduct action potentials rapidly, and neural mechanisms that discriminate between probable threats and innocuous ultrasonic stimuli (reviewed by Pollack 2015).

The correlation between flight and sensitivity to ultrasound begs explanation, particularly in cases where members of the same species face different exposure to

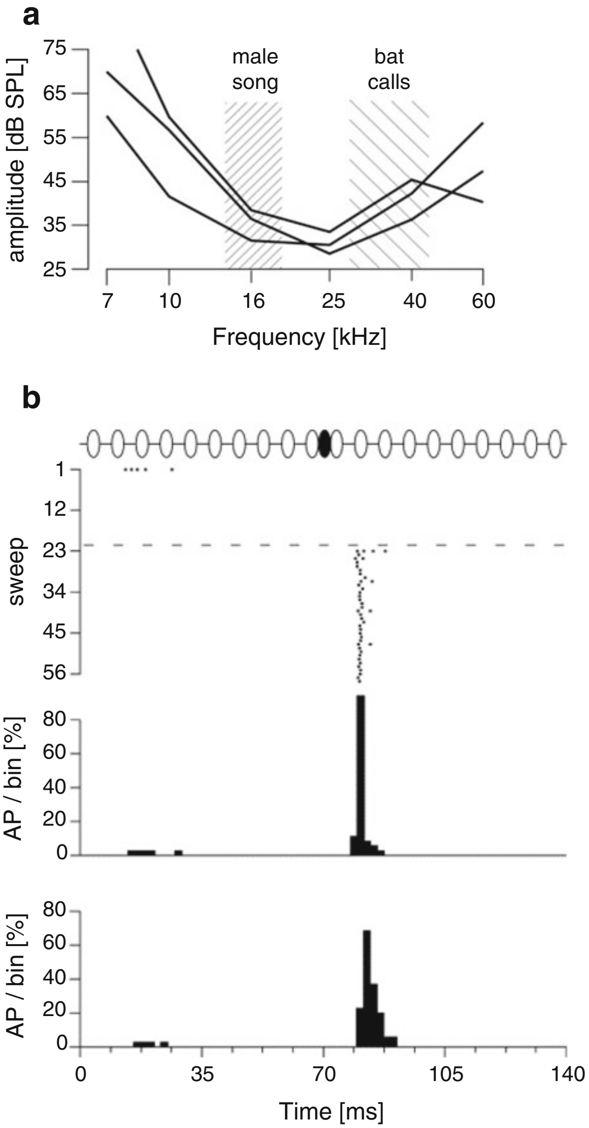

Fig. 4.4 Selective responsiveness of the katydid T1 neuron to bat-like sound in the presence of ongoing song. (**a**) Threshold tuning curves of the T1 neuron of three individuals of the katydid *Neoconocephalus retusus*. The neuron is approximately equally sensitive to the sound frequencies found in conspecific songs and in bat calls, as indicated by the *hatched vertical bars*. (**b**) *Top trace* shows stimulus pattern, with sound pulses in the model song shown in *white*, and a single bat-pulse mimic shown in *black. Second panel from top* shows raster display of TN1 responses to repeated stimulation; spikes are indicated as *dots*. The neuron responded with a few spikes on trial 1 but quickly adapted to the high pulse rate of the song model. It responded reliably to the single bat-pulse model (sweeps 23–56) despite its continued adaptation to the song stimulus. *Bottom graphs* are peristimulus-time histograms summarizing the response when both song and bat models are presented at the same amplitude, 70 dB SPL, and when the bat model is presented at 12 dB lower amplitude (From Schul and Sheridan 2006)

aerially hawking bats, whether because of differences in bat fauna across the species' range or because of developmental-, sex-, or age-related differences in flight behavior. In the former case, loss of ultrasound sensitivity in bat-poor environments may be explained by genetic drift that is permitted by the relaxation of selection pressure. Drift cannot, however, account for differences in sensitivity related to flight dimorphism, sex, or age because in these cases the flight-capable and flight-incapable individuals share the same evolutionary history. Here, the negative correlation between flight and ultrasound sensitivity suggests that the ability to hear ultrasound might be costly. Could unnecessary neural processing of ultrasound stimuli in flightless individuals impose a significant metabolic cost that would be recouped by sacrificing auditory sensitivity (Lauglin et al. 1998)? Might detection of nonthreatening ultrasound signals elicit needless startle responses that could distract individuals from activities such as foraging or mating? Examination of the relationships among an individual's condition, sexual receptivity, and ultrasound sensitivity might help to answer these questions. Further research on the cellular mechanisms linking flight and hearing would also be profitable. In crickets, JH regulates both flight-muscle condition and ultrasound sensitivity. The cellular mechanisms by which JH regulates flight-muscle development and degeneration are beginning to be understood (Zera et al. 1999; Nanoth Vellichirammal et al. 2014), but the mechanisms linking JH and poor sensitivity to ultrasound are completely unexplored.

In many insects, bat detection and evasion were the primitive functions of hearing, whereas in others bat detection was incorporated into an auditory system that had long been used for intraspecific communication, possibly by repurposing neurons and neural circuits with properties that were specialized for analyzing communication signals. Do traces of these differing evolutionary trajectories persist in the present-day bat-evasion behavior of these insects? For example, positive phonotaxis in a specific direction toward a potential mate might require more accurate sound localization than negative phonotaxis away from an approaching bat, where any maneuver that removes the insect from the bat's "search light" might suffice to avoid capture. Similarly, recognizing the songs of one's own species, and perhaps even discriminating among individuals, might require more sophisticated analysis of acoustic features than bat detection. Comparative studies of ultrasound-elicited evasive behaviors between insects in which bat detection is primitive, for example, moths, and those in which it is derived, for example, crickets or katydids, focusing on aspects such as accuracy of sound localization and analysis of acoustic features of stimuli, might offer insights into how new behavioral requirements are incorporated into existing repertoires.

The ongoing evolutionary dialog between bats and their insect prey has attracted the attention of biologists for decades, and it can be expected to continue to do so well into the future.

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Chapter 5
Vibrational Signaling

Jayne Yack

Abstract Vibrational communication is widespread in insects, yet scientists are only beginning to appreciate the importance and complexity of this communication channel. Substrate vibrations are widely available to insects living on plants, sand, soil, leaf litter, or fabricated materials such as beehives, termite mounds, or silk. Sources of vibrations important to insects may be abiotic (e.g., wind, rain) or biotic (e.g., signals or cues arising from conspecifics, predators, and even plants). This chapter focuses primarily on insects and specifically on adults that exploit plant-borne vibrations, reflecting most of the research to date. Some consideration is paid to other invertebrates such as spiders and scorpions, as well as juvenile stages such as eggs, larvae, and pupae. Topics covered include the diversity of taxa exploiting substrate-borne vibrations, the complexity of their vibratory environments, and the multitude of ways that vibrations are generated and used in social communication, finding food, avoiding predators, and monitoring the environment. Vibratory sense organs, including subgenual organs, lyriform organs, and Johnston's organs and their constituent mechanosensilla are described. The vibratory landscape of insects and other invertebrates is poorly documented for most taxa, and all lines of investigation, from "identifying the players" to understanding how complex vibratory signals are detected and processed to recognize and localize sources, are unchartered territories ripe for further investigation.

Keywords Behavior • Chordotonal organs • Communication • Insect • Mechanoreception • Scolopidia • Sensory • Subgenual organ • Substrate vibration • Vibration signals

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5.1 Introduction

Most people are familiar with insect acoustic communication signals, particularly those used for advertisement over long distances, such as the calling songs of crickets or cicadas. Insect acoustic signals have been studied for centuries, with thousands of scientific reports documenting a wide diversity of signals and their respective functions and sensory mechanisms (see Alexander 1957; Ewing 1989; Drosopoulos and Claridge 2006; Hedwig 2014). Despite this plethora of literature, the vast majority of research focuses on stimuli that are accessible to the human ear: airborne sounds transmitted as pressure waves. What many do not realize is that insects generate and tap into acoustic stimuli that are not easily detected by humans, and it has been convincingly argued that such stimuli are far more abundant and ubiquitous than airborne sounds (Cocroft and Rodríguez 2005; Hill 2008).

Acoustic signals can be broadly defined as vibrations transmitted through an elastic medium. When this occurs in a fluid (air or water), the signals are generally defined as “sound”; in solids they are generally referred to as “vibrations” or “substrate vibrations” (Windmill and Jackson, Chapter 6). There has been much discussion and debate concerning the nomenclature used to define acoustic stimuli (Hill 2014). This chapter uses the terms sound to mean airborne vibrations, near- and far-field sounds to distinguish between the displacement and pressure components, respectively, and vibrations or substrate vibrations to describe waves traveling through solids. Figure 5.1 illustrates three main types of acoustic stimuli using the example of a honeybee. If attacked, the bee can generate a buzzing or hissing sound by vibrating its wings rapidly (Rashed et al. 2009). These warning signals can be transmitted as pressure waves (far-field sounds) that are detectable by the pressure-sensitive ears of a vertebrate predator (Sen Sarma et al. 2002). Alternatively, a forager bee can communicate information about profitable food sources to a colony mate by generating oscillations of the wings and abdomen that are detected as near-field sounds by the antennae of a recruit (Kirchner 1997; Tsujiuchi et al. 2007). Recruits in turn can signal back to the forager to stop dancing and offer food samples by producing substrate-borne vibrations through the honeycomb surface (Kirchner 1997). These vibrations are detected by specialized receptors in the legs of the bee (Sandeman et al. 1996). Most research on insect acoustics has focused on communication by far-field sounds, and comparatively less is known about how insects and other arthropods use near-field sounds or solid-borne vibrations. This dearth of knowledge is partly owing to the fact that the latter types of acoustic stimuli have not been accessible to humans without the use of specialized equipment. But this is rapidly changing, at least for vibratory signals, which is the subject of this chapter.

There is an increasing awareness that animal communication through solid-borne vibrations is widespread and important. The number of published reports on vibratory communication has increased steadily over the past 20 years (Cocroft et al. 2014b). In insects and spiders, vibratory communication continues to be discovered in organisms previously thought to be “nonacoustic,” for example, some

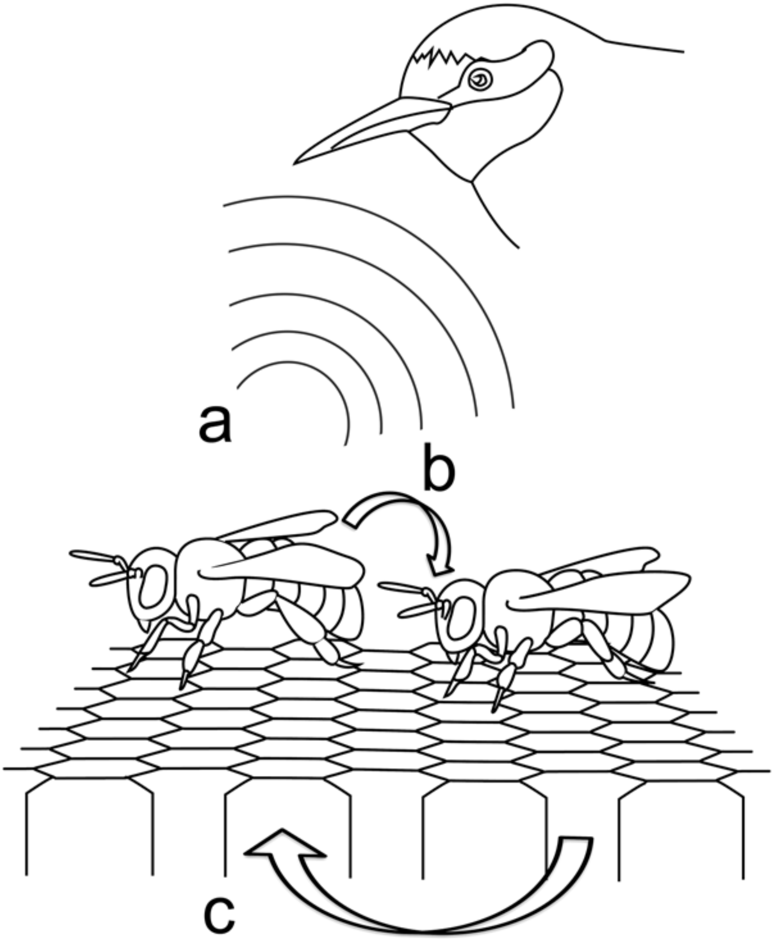

Fig. 5.1 Different types of acoustic signals produced by the honeybee. (**a**) Hissing sounds that function as antipredator signals are generated by vibrating wings and are detected by the pressure-sensitive ears of a vertebrate predator, such as a bird. (**b**) A dancing forager communicates information about a food source to a recruit through dorsoventral oscillations of its wings. These near-field sounds are detected by the recruit bee's antennal receptors. (**c**) A recruit can transmit substrate-borne signals through the wax comb to the leg receptors of a forager by pressing its thorax against the substrate and vibrating (Adapted from Kirchner 1997)

caterpillars (Scott et al. 2010), sawfly larvae (Fletcher 2007), and beetle pupae (Kojima et al. 2012). Even in those taxa already studied extensively for their use of sound communication (e.g., crickets, cicadas), vibratory communication can play an important role in their sensory ecology (Hill 2008). There are few comprehensive reviews on the subject of vibratory communication. Those with an exclusive or heavy emphasis on arthropods include Markl (1983), Virant-Doberlet and Čokl (2004), Hill (2008), and Cocroft et al. (2014a). These are highly recommended to readers wishing to explore the topic in detail. Notwithstanding the growing awareness of the vibratory sensory modality in insects, there are many unanswered questions concerning which taxa use vibrations, how they use them, the characteristics and transmission properties of signals and cues in natural habitats, and the sensory mechanisms used to detect and process vibratory stimuli.

This chapter is an introduction to the “up-and-coming” field of vibratory communication in arthropods. The focus is primarily on insects and mostly those communicating through plant tissues. However, some consideration is given to vibratory communication and reception in other arthropods such as spiders and scorpions that share similar environments and behaviors with insects. The first topic (Sect. 5.2) provides an overview of the vibratory “landscape” of an insect, including the common sources of abiotic and biotic vibratory stimuli encountered. Section 5.3 summarizes methods used to record and play back vibrations. Section 5.4 discusses the diversity of insects reported to generate and detect vibrations, and Sect. 5.5 reviews the many ways that vibrations are important to an insect’s survival. Section 5.6 reviews the main sensory organs used to detect vibrations.

The objective is to introduce readers to the literature on these topics and, importantly, to ponder unsolved problems and avenues of investigation to inspire further research.

5.2 Vibratory Landscapes

Vibrations that an arthropod might encounter in its environment are abundant and complex. There are many different vibration-generating sources, ranging from “passive” wind noise to “specialized” communication signals. Vibrations traveling through solids are far more complex than those traveling through air, and their transmission properties vary depending on the composition and geometry of the many substrates occupied by an organism in its natural habitat.

5.2.1 Types of Vibrations

Vibrations transmitted through solids have physical properties that differ from those of airborne sounds in ways that are relevant to insect communication. First, whereas airborne sounds generated by an insect can travel over long distances [e.g., >1 km in bladder grasshoppers (*Bullacris membracioides*; Van Staaden and Römer 1997)], insect-generated vibrations typically occur on a local scale, within a meter from the source. This is due to a number of factors, including the small size of an insect in relation to the size of the substrate and filtering and damping properties of the substrate (Michelsen et al. 1982; Cocroft and Rodríguez 2005). Second, airborne sound communication is not an option for many small insects because they produce high-frequency sounds that are highly attenuated in natural environments (Bennet-Clark 1998). Consequently, many small insects use solid-borne vibrations because they are the least costly for them to produce over short distances (Bennet-Clark 1998). Third, substrate vibrations are far more complex than airborne sounds. In air, one wave type (longitudinal) is propagated through a more or less uniform medium. In contrast, there are several types of vibration waves, and their transmission can be affected by substrate properties, including the type of material (e.g., herbaceous plant stems, wood, sand, rock, silk), geometry (e.g., long thin stems, flat leaves, silk strands, tree trunks), and composition and condition (e.g., heterogeneity, density, moisture content). Waves traveling through solids have been categorized based on their mode of transmission, shape, energy distribution, and motion in relation to the direction of propagation, speed, and attenuation. The main wave types used by insects include longitudinal (and quasi-longitudinal), bending, torsional, and transverse, and the type(s) that occurs in any particular scenario depends on factors such as the mechanism of signal production and the aforementioned substrate properties. This complexity has important implications for the generation and reception of vibrations and how scientists record and playback stimuli. For more detailed accounts of vibration wave types used by invertebrates, see Michelsen et al. (1982), Markl (1983), Hill (2008), and Elias and Mason (2014).

5.2.2 *Sources of Vibrations*

Vibration sources relevant to insects can be broadly categorized as abiotic or biotic, and biotic sources can be subdivided into passive cues or active signals (Fig. 5.2). A number of authors have reviewed the types of vibratory signals, cues, and noise relevant to animals (Cocroft and Rodríguez 2005; Hill 2008; Caldwell 2014; Virant-Doberlet et al. 2014). This section introduces a few of the many different vibration sources used by insects. Two main abiotic vibration sources relevant to insects are wind and rain (Barth et al. 1988; Casas et al. 1998; Cocroft and Rodríguez 2005; Virant-Doberlet et al. 2014). Wind is a major source of noise for insects communicating on plants. It induces trembling and collision in leaves and stems, resulting in a noisy vibratory environment. Wind interference, comprising vibrations typically below 200 Hz, is thought to play an important role in the selection of signal characteristics and the time of day that species living in open habitats or in the forest canopy will signal (Cocroft and Rodríguez 2005; Virant-Doberlet et al. 2014). There is also evidence that plants have evolved mechanisms, such as trembling, to enhance wind noise as a deterrent to herbivorous insects (Yamazaki 2011). Rain drops falling on plants cause intermittent and high-amplitude waveforms with most energy below 1 kHz (Fig. 5.2a) (Barth et al. 1988; Casas et al. 1998). Some insects may cue in on the vibrations to avoid activity during rainfall, while others living in geographic regions with heavy rainfall may have evolved signals with long pure tones to overcome low-frequency background interference, as suggested by Cocroft and Rodríguez (2005). Other sources of abiotic vibrational noise that may affect

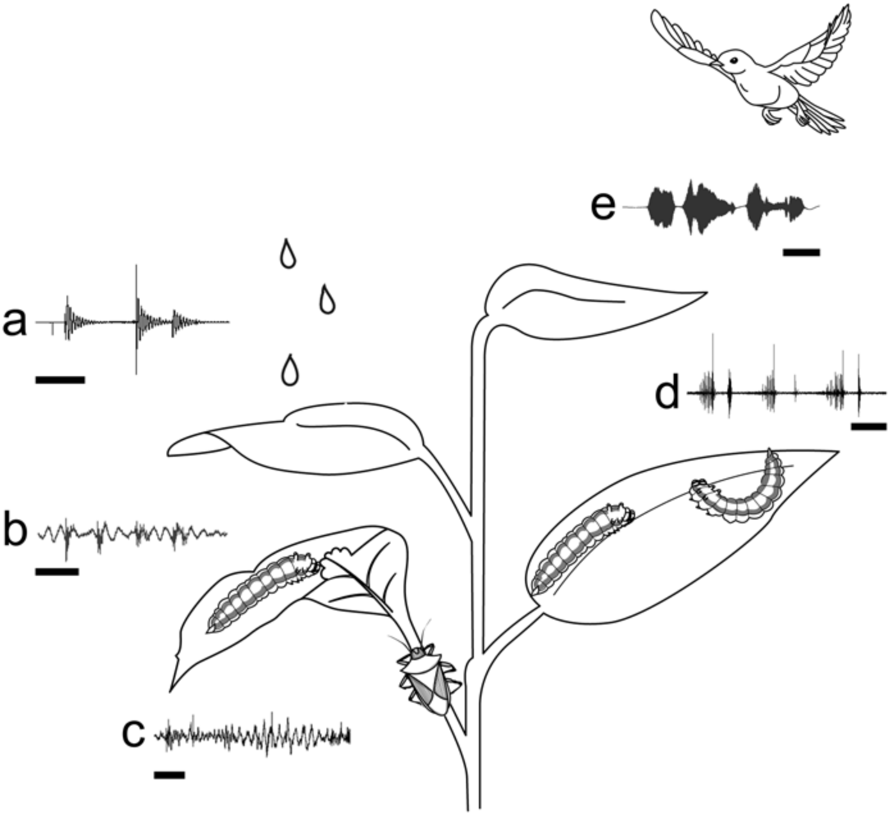

Fig. 5.2 Hypothetical "vibration-scape" of a plant-dwelling caterpillar. (**a**) Abiotic stimuli such as rain droplets may be a source of background noise or provide information about weather conditions. Various biotic sources, such as incidental vibrations generated by a caterpillar (*Drepana arcuata*) chewing (**b**) or a predator (*Podisus maculiventris*) walking (**c**), may provide useful information to a predator or prey, respectively. (**d**) Communication signals, such as those produced by resident territorial caterpillars (*D. arcuata*), inform intruding conspecifics that the leaf is occupied. (**e**) Vibrations may also be caused by airborne sounds, such as the call of a bluebird (*Sialia sialis*). All vibrations were recorded by the author in a laboratory setting on birch leaves, using a laser-Doppler vibrometer. Scale bars are 5 s (**a**), 500 ms (**b**, **c**, **d**), and 200 ms (**e**)

insects include anthropogenic noise (e.g., traffic, wind turbines) (Morley et al. 2013; Virant-Doberlet et al. 2014) and water flow (e.g., rivers, waterfalls).

Vibrations originating from biotic sources can be broadly categorized as (passive) cues and (active) signals. Although the distinction between signals and cues is not always clear, one explanation is that cues have not evolved to alter the behavior of other animals, whereas signals have evolved for that effect (Maynard Smith and Harper 2003). Vibrations from nonsignaling behaviors include those resulting from locomotion (walking, crawling, flying), feeding (chewing), or digging (Fig. 5.2b, c) (e.g., Guedes et al. 2012). There is mounting evidence that unintended receivers use such cues to escape predators or to capture prey (see Sect. 5.5.2).

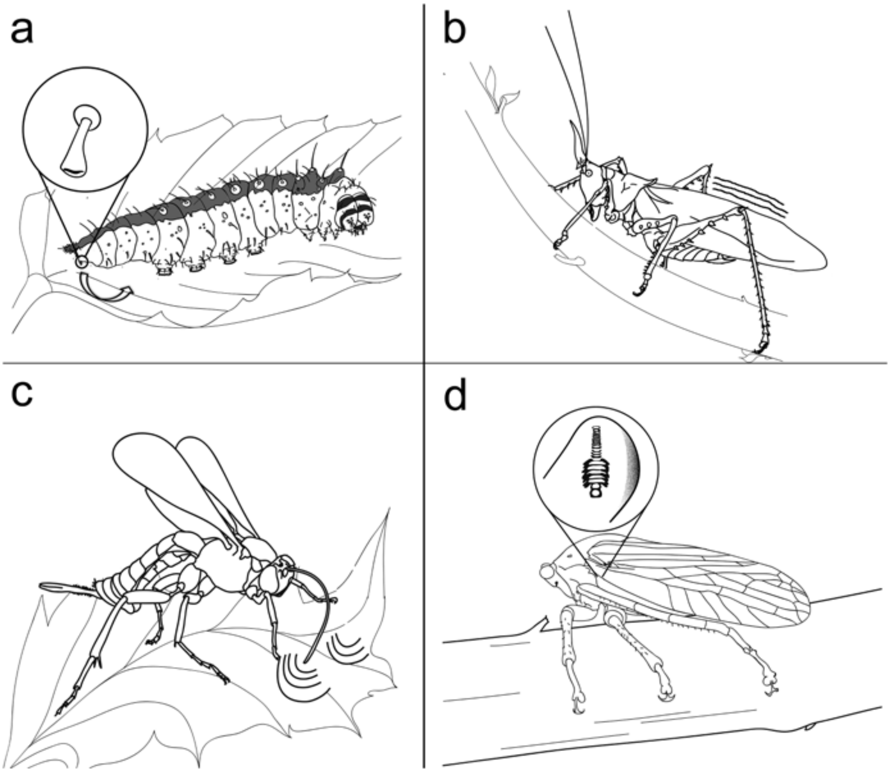

Fig. 5.3 Mechanisms for generating substrate vibration signals in insects. (**a**) Stridulation: Vibrations are generated by friction as one body part rubs against a substrate or another body part. In the caterpillar *Drepana arcuata*, bilateral anal "oars" are scraped against the leaf surface to generate vibratory signals used in territorial encounters with conspecifics. (**b**) Tremulation: Body movements such as jerking, trembling, and shaking transfer vibrations to the substrate. A male neotropical katydid, *Copiphora rhinoceros*, tremulates by bobbing up and down in a stereotyped manner during its courtship display (redrawn and adapted from Morris 1980). (**c**) Drumming: Signals are produced by striking a body part against the substrate. In this example, a parasitoid wasp (*Pimpla turionellae*) strikes its antennae against a leaf surface to echolocate (vibrational sounding) to locate a host. (**d**) Tymbal buckling: Tymbals are specialized, often ribbed, regions of exoskeleton that are popped in and out in a clicking motion by muscles attached to the inner surface of the structure. The example is a treehopper (*Aethalion reticulatum*). The tymbal inset is redrawn from Evans (1957)

In contrast to cues, signals have evolved to convey messages to intended receivers and therefore are typically conspicuous, highly redundant, and stereotyped (Johnstone 1997) (Fig. 5.2d, e). Mechanisms used to generate vibration signals in the Arthropoda are diverse, involving almost every body part imaginable. According to Hill (2008, 2014), they can be categorized into four types: stridulation, tremulation, drumming, and tymbal buckling (Fig. 5.3). Stridulation produces signals by the friction caused by one body part rubbing against another body part or against a substrate (body-substrate stridulation) (Fig. 5.3a). Mechanisms employed to generate these signals range from nonspecialized structures such as mandibles rubbing against the substrate (e.g., Ishay et al. 1974; Yack et al. 2001) to specialized file and scraper mechanisms (e.g., Uetz and Stratton 1982; Cokl and Virant-Doberlet 2003). Tremulation describes repetitive body movements such as trembling, shaking, and swaying without the insect hitting the substrate (Hill 2014; Fig. 5.3b). Such signals have narrow bandwidths compared to those produced by drumming (see later in this section). Tremulation is often used by plant-dwelling insects and spiders that signal on homogeneous substrates that permit the passage of narrowband signals (Hill 2014). Drumming, or percussion, involves some nonspecialized body part (mandibles, head, antennae, legs, abdomen) striking the substrate (Fig. 5.3c). Drumming produces broadband "noisy" signals that are most often associated with heterogeneous substrates, where the filtering characteristics of the substrate are unpredictable. With such broadband signals, temporal patterns are believed to be more important than spectral features in conveying information (Hill 2014). Tymbals are modified regions of cuticle that are buckled rhythmically by specialized muscles attached to their inner surfaces (Fig. 5.3d). Although best known for producing airborne sounds in cicadas (Cicadidae) and tiger moths (Arctiinae), they are also common in many Hemiptera that communicate primarily using plant-borne vibrations (Wessel et al. 2014). Other types of biotic signals relevant to arthropods that do not fit into the aforementioned categories include plucking a silk shelter or web (e.g., Fletcher et al. 2006; Wignall and Taylor 2011; Mortimer et al. 2014) or vibrations generated by airborne signals that are transferred and propagated as solid-borne vibrations (Fig. 5.2e; Caldwell 2014).

5.3 Vibration Recording and Playback

Over the past 50 years, scientists have developed and refined instrumentation to broaden the understanding of the extraordinary sensory capabilities of animals that communicate using ultrasonic, infrasonic, ultraviolet, infrared, geomagnetic, and chemical stimuli (Bradbury and Vehrencamp 2011). Although such instruments are portals to learning about other sensory modalities, it is crucial to be aware that each instrument imposes its own characteristics on the signal that was "intended" by the organism. Given the aforementioned complexity of substrate-borne vibrations and the heterogeneity of the substrates on which insects and other invertebrates signal, the need to take precautions for recording and playback experiments is particularly important (see Elias and Mason 2014).

A variety of instruments are available to record substrate vibrations (Cocroft and Rodríguez 2005; Elias and Mason 2014). Sensors vary in their sizes, frequency ranges, what they measure, costs, and sensitivities. Each has advantages and disadvantages, and the choice of sensor should be based on a number of factors including the size of the insect, the type of substrate, and how the recordings are to be used. Two main sensor types are used to record vibrations from insects: laser vibrometers and piezoelectric elements. Laser Doppler vibrometers (LDVs) reflect a light off the surface of a vibrating structure, providing a measure of the velocity of movement based on the Doppler shift. LDVs are ideally used in the laboratory to record vibrations from small organisms and in particular those on lightweight substrates such as herbaceous plants or spider webs. Piezoelectric elements are solid materials, usually quartz crystals or ceramic, that generate an electrical signal in response to a mechanical force. When the mass is vibrated, it produces a force that generates an electrical charge proportional to the acceleration of movement. Piezoelectric elements are often packaged as accelerometers that vary in size, bandwidth, and sensitivity. Other low-cost piezoelectric elements (e.g., guitar pickups, phonocartridges) can be adapted to monitor vibrations but are limited in that they are difficult to calibrate and couple with the substrate.

Playback devices, or actuators, also vary in their size, how they attach to the substrate, type of motion they transmit, frequency range, portability, and effects on the substrate (Cocroft and Rodríguez 2005; Elias and Mason 2014). There are three main types commonly used for invertebrate studies: mini-shakers, electromagnets, and speaker cones. Mini-shakers are acceleration actuators that use a coil and magnet to vibrate the substrate. The substrate can be coupled to the shaker using a pin or nail that is glued to the substrate or the substrate can attach directly to the shaker. Small magnets can be glued to the surface of a plant and are vibrated remotely by an electromagnet. Speaker cones are small audio speakers with their diaphragms removed to reduce the generation of airborne sounds. They are used to vibrate a lightweight substrate such as a leaf by attaching a pin to the central coil and gluing the pin to the substrate. Although playback of substrate vibrations is far more complex than playback of sounds, there are a number of solutions to common problems encountered (Cocroft et al. 2014c; Elias and Mason 2014).

5.4 Diversity of Insects Using Vibrations

Among the different forms of acoustic cues and communication signals used by insects and other arthropods, substrate vibrations are considered to be the most ancient and taxonomically widespread (Cocroft and Rodríguez 2005; Hill 2008). Cocroft and Rodríguez (2005) estimate that vibratory communication has been reported in 195,000 described insect species across 18 orders and that of all families using some form of mechanical communication, 80 % use vibrations either alone or with another mechanosensory modality. For reviews of invertebrate taxa reported to use vibrations, readers should consult Virant-Doberlet and Čokl (2004), Cocroft and Rodríguez (2005), Hill (2008), and chapters within Drosopoulos and Claridge (2006) and Cocroft et al. (2014a).

Why are substrate vibrations more commonly used by arthropods than are airborne sounds? There may be several reasons but a few in particular stand out. First, there are many sources of solid-borne vibrations that may not necessarily produce detectable airborne sounds. These include abiotic sources (e.g., wind, rain) or inadvertent body movements and activities (e.g., crawling, chewing). Such vibrations may be used as information sources even by species that have not evolved specialized communication systems. For example, some caterpillars (*Semiothisa aemulataria*) can detect the leaf vibrations induced by foraging predators (Castallanos and Barbosa 2006), and ant lion larvae (Myrmeleontidae) are extremely sensitive to the sand-borne vibrations generated by passing prey (Devetak 2014). Second, and not unrelated to the first point, is that vibration signals are typically less costly to produce than airborne sounds owing to the better impedance matching between the signaler's body and substrate compared to that of the signaler's body and air (Caldwell 2014). Very small insects, including some ants (Formicidae, Hymenoptera) and lice (Trogiidae, Psocoptera), that may be incapable of sound production can generate substrate-borne vibrations (Dumortier 1963; Kirchner 1997). Third, it may be easy to "evolve" a vibration receptor. Substrate-borne vibrations induce movements of body parts, such as legs and antennae, that are in direct contact with the substrate, and because these structures are already well "endowed" with mechanoreceptors that function to detect body movements (as proprioceptors), the evolutionary transition from proprioceptor to exteroreceptor may be relatively simple. Indeed, the borderline between the vibration sense and other forms of mechanoreception is not always clear (Kalmring 1985).

Current estimates of the number of arthropod species using vibrations are believed to be low, and researchers are still in the process of "identifying the players" (Cocroft and Rodríguez 2005; Hill 2008). Where then, should we be looking? Some taxa, including those within the Hemiptera (true bugs), Neuroptera (mayflies, ant lions, and relatives), Plecoptera (stoneflies), and Arachnida (spiders, scorpions, and relatives) have been studied in detail and are emerging as models for exploring both proximate and ultimate questions on vibratory communication. Taxa moderately represented in the literature include the Orthoptera (crickets, grasshoppers), Hymenoptera (bees, wasps, ants), Isoptera (termites), and Crustacea (crabs, lobsters, and relatives), but most others are underrepresented. Furthermore, the vast majority of reports focus on sexual behaviors of adults (Virant-Doberlet and Čokl 2004). Considering that a large portion any insect's life cycle is spent as immature, the lack of literature on the eggs, nymphs, grubs, caterpillars, maggots, and pupae that use vibratory communication is surprising. There are an increasing number of examples of juveniles using vibratory signals for a diversity of functions, including territorial behavior (e.g., Fletcher et al. 2006; Yack et al. 2014), recruitment of conspecifics or heterospecifics for foraging or defense (Cocroft and Hamel 2010), mimicry to exploit food resources (e.g., Sala et al. 2014), and parent–offspring communication (Cocroft 2001; Mukai et al. 2014). Research on acoustic communication in juveniles lags far behind that for adults and requires further investigation.

5.5 How Do Insects Use Vibrations?

Solid-borne vibrations are used by arthropods in a variety of contexts. Vibratory stimuli may be used for communication between conspecifics or heterospecifics or to gain information by monitoring abiotic events, eavesdropping on signals or cues generated by others, or through echolocation. The multitude of ways that insects and other arthropods use vibrations are discussed in Hill (2008), Virant-Doberlet et al. (2014), and chapters within Drosopoulos and Claridge (2006) and Cocroft et al. (2014a). Here, the functions are discussed under three broad categories, which are not necessarily mutually exclusive: communication signals, monitoring the environment, and obtaining food.

5.5.1 Communication Signals

Communication has been defined as the process whereby individuals exchange information using signals that have evolved for this purpose (Lindstrom and Kotiaho 2002). According to this definition, communication signals are distinct from other stimuli that may be used by an organism to gain information about its environment, which are not intended for communication. Invertebrates use vibration signals in a variety of contexts, including those involving interactions between mates, parents and offspring, heterospecifics, predators and prey, and colony members. Why use substrate-borne vibration signals over other sensory modalities? Although there are different factors that influence the evolution of one form of communication over another (Bradbury and Vehrencamp 2011), four explanations for why insects may use vibrations include the following. (1) As discussed in Sect. 5.4, vibration production can be energetically less costly than sound production, particularly for small insects signaling on plants; (2) vibrations are used in environments where chemical and sound vibration are not viable options (e.g., inside logs, soil, or termite mounds); (3) vibrations may offer a private communication channel so that a signaler avoids being exploited by predators or conspecifics that may eavesdrop on airborne or chemical signals (see Virant-Doberlet and Čokl 2004; Hill 2008 for discussion of the advantages of vibratory communication). Keeping in mind that there are a variety of ways to classify the functions and contexts of insect acoustic signals (Alexander 1967), this chapter divides the functions of vibration signals into three broad categories: reproductive behavior, predator–prey interactions, and group behavior. These categories are not intended to be mutually exclusive and are by no means comprehensive.

5.5.1.1 Reproductive Behavior

Vibratory communication signals have been implicated in a number of functions in the context of mating and reproduction, including attraction, locating a mate or rival, species recognition, courtship, competition between rivals, and pair maintenance. Signals are produced by all four previously discussed mechanisms (stridulation, drumming, tremulation, and tymbal buckling), and some species have complex signaling repertoires using multiple mechanisms [e.g., the treehopper *Ennya chrysura* (Membracidae) produces eight distinct signals (Miranda 2006)]. Other species use vibratory signals as part of a multimodal display in combination with visual or chemical signals [e.g., male jumping spiders, *Habronattus dossenus* (Salticidae), signal to females using complex visual and vibratory displays (Elias et al. 2003)]. The vast majority of studies on vibratory communication in insects and other arthropods have focused on reproductive behaviors, and reviews on the topic are included in Barth (1997), Cokl and Virant-Doberlet (2003), Virant-Doberlet and Čokl (2004), Hill (2008), and chapters within Drosopoulos and Claridge (2006) and Cocroft et al. (2014a).

5.5.1.2 Predator–Prey Interactions

Vibratory communication signals can be employed by prey to stop an attack by a predator, or by predators to facilitate prey capture (Cocroft 2001; Hill 2008; Cocroft and Hamel 2010). Antipredator signals may be directed toward the predator as aposematic or deimatic displays (Masters 1979), although there is little direct experimental evidence that vibrations alone function in these contexts. Alternatively, signals can be directed toward conspecifics or heterospecifics as alarm signals to recruit help (Cocroft and Hamel 2010). Predators may deceive prey by mimicking vibrations that attract prey. Examples of aggressive mimicry include the assassin bug (*Stenolemus bituberus*), which hunts web-building spiders by mimicking the vibrations of a struggling prey (Wignall and Taylor 2011), and the jumping spider (*Portia fibriata*), which mimics vibratory courtship signals of other species (Jackson and Wilcox 1990). Predators also locate prey by vibratory echolocation; technically, these are considered signals, as there is a sender and a receiver, although it is the same individual doing both. Echolocation is discussed further in Sect. 5.5.3.

5.5.1.3 Group Behavior

Many insects benefit from living in social groups ranging in size from two individuals to large eusocial colonies with thousands of individuals. It has been convincingly argued that vibratory communication plays an important role at all levels of group interactions and that we have just begun to explore these functions in different insect groups (see Cocroft 2001; Hrncir et al. 2006; Hill 2008; Cocroft and Hamel 2010; Hunt and Richard 2013). Reported functions of vibratory communication amid group members include recruitment to food or nesting sites (Hrncir et al. 2006), alarm signaling (Cocroft 1996; Rosengaus et al. 1999), communicating social status (Casacci et al. 2013), coordination of activities (Fletcher 2007, 2008), and parent–offspring communication for food exchange (Savoyard et al. 1998) or to stimulate egg hatching (Mukai et al. 2014).

5.5.2 Monitoring the Environment

Beyond using vibrations for communication purposes, some arthropods monitor vibration stimuli in their environments to gain information. Sources of these vibrations may be abiotic, such as wind or rain, or biotic, such as incidental cues resulting from body movements, or even communication signals that are intercepted and exploited by unintended receivers.

Vibrations caused by abiotic sources, in addition to introducing background noise (see Sect. 5.2.2), may also be information sources (Virant-Doberlet et al. 2014). Rain-induced vibrations have been reported to evoke escape responses or inhibit activity in some insects (e.g., Casas and Magal 2006; Guedes et al. 2012). Although to date there is no evidence that hatching or eclosion events are stimulated or inhibited by rain-induced vibrations, as seen in some frog embryos (e.g., Caldwell et al. 2010), this is possible for insects. Wind vibration noise has been reported to influence the activity patterns of some insects and spiders, including predators that use wind noise as a "smokescreen" to mask self-generated vibrations from their prey (Wilcox et al. 1996; Wignall et al. 2011).

Vibratory stimuli arising from biotic sources can be used by an unintended receiver. These might be incidental vibrations caused by body movements, such as chewing or walking on a plant surface. Such stimuli are used by prey or hosts to detect and avoid predators or parasitoids (e.g., Castallanos and Barbosa 2006) or, in turn, by predators and parasitoids to locate their respective prey or hosts (e.g., Pfannenstiel et al. 1995). Another intriguing possibility is that insects may acquire information from the incidental vibrations generated by plants, such as those resulting from water stress (e.g., Haack et al. 1988). The topic of plant bioacoustics is fascinating (Gagliano et al. 2012; Appel and Cocroft 2014) and is certain to reveal more ways that insects are using vibrations. Finally, insects can exploit communication signals that are intended for other recipients. Receivers may eavesdrop on the calls of conspecifics to intercept mating or to locate competitors, predators may localize prey by their calling signals, or, in turn, prey may detect potential predators (Hill 2008).

5.5.3 Vibrations for Obtaining Food

Invertebrates may use vibrations to help them obtain food in a number of ways. Predators may eavesdrop on communication signals or passive vibrations generated by prey, or colony members may recruit one another to good-quality food sources. In addition, some insects actively generate vibrations to facilitate food gathering through echolocation, buzz pollination, or even creating a "vibratome" to facilitate leaf cutting (Hill 2008). Some wasps use echolocation (or vibrational sounding) to locate a concealed host, such as a caterpillar or pupae located inside plant material or soil (Fig. 5.3c). The female wasp drums on the substrate surface using modified antennae and receives the returning vibration through subgenual organs in her legs to assess differences in the density of the substrate and thus the location of the host (Broad and Quicke 2000; Otten et al. 2001). Other insects proposed to use echolocation to assess food include termites (Evans et al. 2005) and insects living on the water surface (e.g., whirligig beetles) (Hill 2008; cf. Voise and Casas 2014). Some bees actively vibrate their flight muscles to release pollen from flower anthers, a phenomenon called buzz pollination (Hill 2008; De Luca and Vallejo-Marín 2013). Flowers and bees have formed mutualistic relationships whereby the flowers release pollen only when stimulated by a particular vibration frequency produced by the insect. In this sense, buzz pollination could be considered a form of communication between plants and insects, with plants “hearing” and responding to specific vibration signals. Finally, there is an interesting example of ants generating vibrations while feeding on leaves to facilitate leaf cutting (Tautz et al. 1995). While cutting through a leaf with their mandibles, leaf cutter ants drag their gasters on the leaf surface, causing a high-frequency vibration that stiffens the leaf to facilitate cutting, effectively functioning as a vibrotome.

5.6 Sensing Vibrations

Although most arthropods are likely capable of detecting substrate vibrations, the sensory organs have not been identified in most species. Moreover, in those species for which receptors have been identified, our understanding of how they function to detect, localize, and recognize stimuli or filter out background noise remains limited. Our knowledge of vibration receptors lags far behind that for insect tympanal ears, and there may be several reasons for this. First, unlike for tympanal ears that typically have a conspicuous tympanal membrane, there is often no distinctive external manifestation of a vibration receptor. Second, the distinction between vibration reception and other forms of mechanoreception such as touch and proprioception is not always clear (Kalmring 1985; Lakes-Harlan and Strauss 2014). Third, insects communicating with vibrations can be quite small compared to those that possess tympanal ears, making it sometimes technically challenging to conduct neurophysiological recordings to confirm vibration sensitivity. Such obstacles notwithstanding, there has been progress in understanding vibratory sensory reception in selected taxa, including those within Hemiptera, Orthoptera, Hymenoptera, Arachnida, and Crustacea (Kalmring 1985; Hill 2008; Lakes-Harlan and Strauss 2014).

5.6.1 Types of Sensilla

Like all acoustic sensory receptors in animals, those sensitive to substrate vibrations belong to a broader class of receptors called mechanoreceptors. Arthropod mechanoreception has been reviewed by several authors, including McIver (1985), Keil (1997), Barth (1997, 2004), and Field and Matheson (1998). Those specialized to detect acoustic stimuli have also been the subject of several reviews (e.g., Ewing 1989; Fullard and Yack 1993; Yager 1999; Greenfield 2002; Yack 2004; Strauss and Lakes-Harlan 2014), albeit most of these focus primarily on tympanal hearing. Mechanosensory sensilla most commonly employed as vibration receptors are hairs

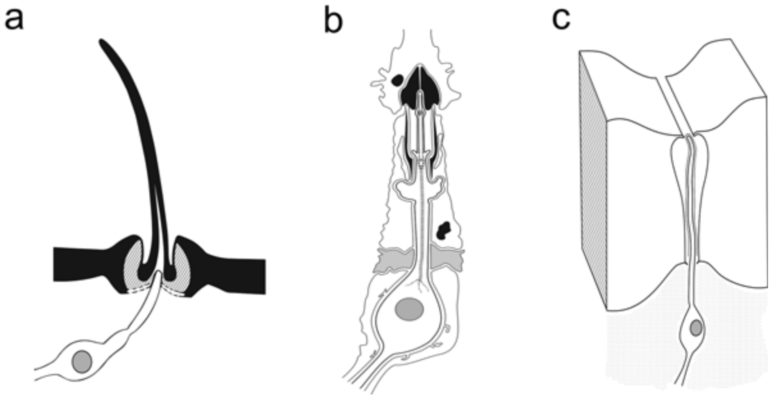

Fig. 5.4 Types of invertebrate mechanoreceptive sensilla known to function as vibration receptors. (**a**) Hair-type sensillum comprising a cuticular extension (hair shaft) innervated at its base by the dendrite of a bipolar sensory neuron. The cell responds to deflections of the hair shaft. (**b**) A scolopidium comprising a bipolar sensory neuron, a scolopale cell, and surrounding accessory cells. The dendrite tip inserts into a bullet-shaped scolopale cap produced by an attachment cell. The sensory cell is stimulated by vibrations transmitted through accessory structures. (**c**) Slit sensillum comprising a region of flexible cuticle innervated by the sensory cell dendrite. Deformation of the cuticle stimulates the sensory neuron

and bristles, scolopidia, and slit sensilla (Fig. 5.4). Other types suggested to function in vibration detection include campaniform sensilla and multipolar receptors (Lakes-Harlan and Strauss 2014).

5.6.1.1 Hairs and Bristles

Mechanosensory hairs are proposed to function as vibration receptors in insects, spiders, and scorpions (Barth 1998; Lakes-Harlan and Strauss 2014). These sensilla comprise four cell types: a hair shaft cell that secretes a hair-like cuticular projection, a socket cell, a sheath cell, and a sensory neuron (Fig. 5.4a). Hair shafts come in a variety of different forms and are variously named trichobothria (very long thin projections), hairs, bristles, and trichoid sensilla. The hair shaft typically sits in an articulating socket, into which inserts the tip of one or more sensory cell dendrites. Deflection of the hair in a particular direction deforms the dendritic tip, causing depolarization of the sensory neuron. In most cases these mechanosensory hairs are responsive to touch, but some are specialized for detecting near-field sounds, water surface vibrations, air currents, and substrate-borne vibrations (Keil 1997; Hill 2008; Lakes-Harlan and Strauss 2014). Hairs and bristles that have been implicated or confirmed to function as substrate-vibration receptors in spiders, scorpions, and insects are reviewed in Barth (1998), Hill (2008), and Lakes-Harlan and Strauss (2014).

5.6.1.2 Scolopidia

Scolopidia are internal mechanoreceptors found in the Insecta and Crustacea (spiders do not have scolopidia) (Howse 1968; Field and Matheson 1998; Yack 2004). Each scolopidium comprises one or more bipolar sensory neurons with a distal ciliated dendrite that inserts into a scolopale cap or tube (Fig. 5.4b). A scolopale cell envelopes the distal tip of the dendrite, creating an extracellular space called a lumen. Attachment and glial cells connect the sensory neuron and scolopale cell to internal anchor points. It is generally believed that deformation of the dendritic cilium leads to depolarization of the sensory cell (Mhatre 2015; Eberl, Kamikouchi, and Albert, Chapter 7). Scolopidia are extremely sensitive to vibratory stimuli. They are located throughout the body and, depending on their location and association with peripheral structures, may function as proprioceptors (detecting self-induced movements) or exteroreceptors (detecting gravitational forces, near- and far-field sounds, or substrate vibrations) (Field and Matheson 1998). Although individual scolopidia may function as vibration detectors, they are usually organized into groups called chordotonal organs that occur in the legs (subgenual organs in insects, Barth's organs in crustaceans) or antennae [Johnston's organs (JOs) in insects] (see Sect. 5.6.2).

5.6.1.3 Slit Sensilla

Slit sensilla are integumental mechanosensory sensilla that commonly occur in arachnids (Fig. 5.4c) (Barth 1997, 1998; Hill 2008). They are analogous to campaniform sensilla in insects and, similarly, function like strain gauges that detect deformation of the body wall. Each slit sensillum comprises an externally manifested elongated pit on the surface of the body wall. One or more dendrites attach to the cuticle at the base of the pit and are stimulated when the body wall is deformed. Slit sensilla occur all over the body and function mostly as proprioceptors. Some, however, are specialized to detect solid-borne vibrations and occur in organized groups such as in the lyriform organ of spiders or the basitarsal compound slit sensilla (BCSS) organ of scorpions (see Sect. 5.6.2).

5.6.2 Vibration Sensory Organs

Although there may be constituent physiological and ultrastructural properties of certain mechanosensilla that render them more sensitive to substrate vibrations, it is their associations with accessory structures and their positions and orientations within the body that play key roles in vibration sensitivity. They need to be coupled to the substrate, and therefore often occur in the distal leg regions, and are positioned such that they are sensitive to vertical displacements or acceleration caused by movements of the substrate. Vibratory sensory organs most thoroughly studied

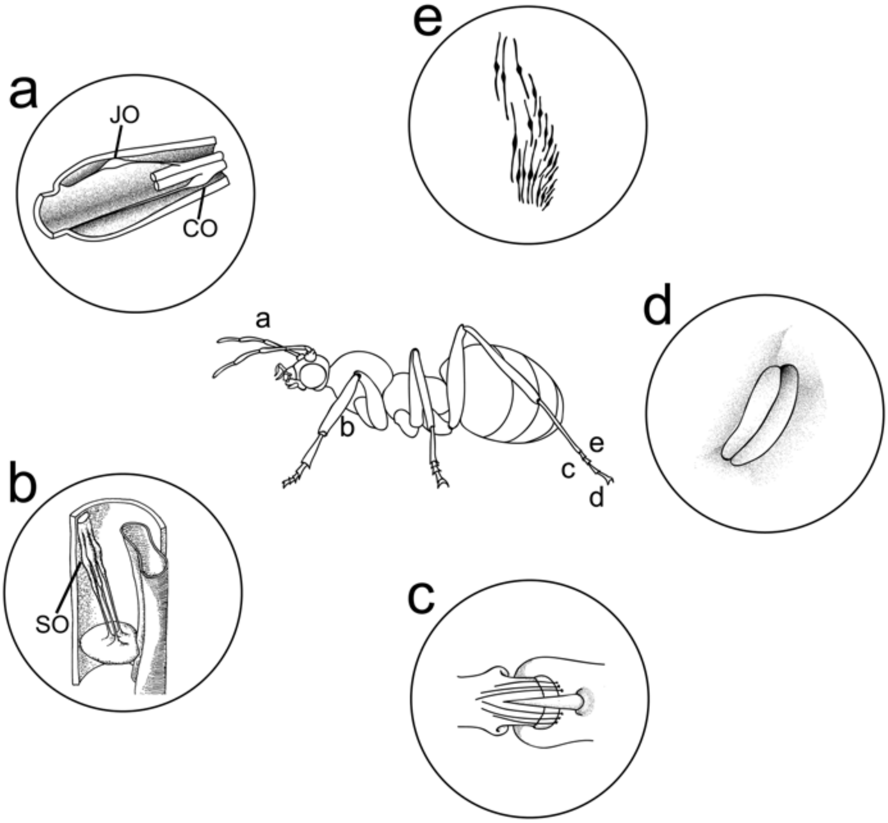

Fig. 5.5 Schematic drawing of a "generic" arthropod showing different types of vibratory sense organs and their general locations. (**a**) Antennal vibration receptors. The inset shows Johnston's organ (JO) and central organ (CO) in the third antennal segment (pedicel) of a green stink bug (*Nezara viridula*). Each sensory organ comprises several scolopidia (redrawn from Jeram and Pabst 1996). (**b**) Subgenual organs (SO) are located below the "knee" in several insects and may be developed for vibratory detection in one pair of legs or all six legs. The inset depicts the SO in the green lacewing (*Chrysoperla carnea*). Attachment cells of the scolopidia connect to a septum, and vibrations of the leg hemolymph result in stimulation of the sensory cells. (Image redrawn from Devetak and Pabst 1994). (**c**–**e**) Different vibration-sensitive cuticular sensilla on a spider leg (redrawn from Speck-Hergenröder and Barth 1988): cuticular hairs at the tarsus-metatarsus joint (**c**), a single tarsal single-slit sensillum (**d**), and the metatarsal lyriform organ (comprising several slit sensilla) (**e**)

to date in arthropods include chordotonal organs (subgenual organ, JO) and those comprising groups of slit sensilla (lyriform organ, BCSS) (Fig. 5.5). These are briefly described in Sects. 5.6.2.1–5.6.2.4. Other structures proposed to function as vibration-sensitive organs include larval antennae, prosternal organs, pleural discs, and other leg scolopidial organs such as the intermediate and femoral chordotonal organs (Saliba 1972; Meurgey and Faucheux 2006; Hill 2008; Lakes-Harlan and Strauss 2014).

5.6.2.1 Subgenual Organs

Subgenual organs (SOs) are considered to be the primary vibration receptors of insects (Field and Matheson 1998; Hill 2008; Lakes-Harlan and Strauss 2014). They are chordotonal organs located in the proximal tibia of the legs in most orders, with Diptera and Coleoptera being possible exceptions. Each SO comprises a group of scolopidia that is suspended in the hemolymph of the leg cavity but not connected to the leg joint. There is considerable morphological diversity among taxa in the shape of the organ, the number of constituent scolopidia, and the means by which they attach to the integument. Usually there is no external manifestation of the SO, but in some organisms such as parasitoid wasps that use vibrations for echolocation, the region is greatly enlarged (Broad and Quicke 2000). Although SOs can respond to a variety of mechanical stimuli, including leg movements and sounds, the best known function is vibration detection. They respond to external transient stimuli transferred through the leg from the substrate. The best studied SOs include those of selected taxa within the Neuroptera, Hemiptera, and Orthoptera (Lakes-Harlan and Strauss 2014). In green lacewings (Chrysopidae, Neuroptera), substrate vibrations stimulate the scolopidia by setting into motion the hemolymph in the leg cavity, which in turn vibrates a diaphragm to which the scolopidia are attached (Fig. 5.5b) (Devetak 1998). Localization of a vibration source may be achieved by comparing the arrival time of the signal between different legs (Virant-Doberlet et al. 2006; Lakes-Harlan and Strauss 2014).

5.6.2.2 Johnston's Organs

JOs are chordotonal organs that occur in the second antennal segment (pedicel) of pterygote insects (Field and Matheson 1998). The number, types, and arrangement of scolopidia vary between taxa. These organs have been implicated in wind detection, proprioception, and in many Diptera and Hymenoptera they are highly specialized to detect near-field sounds (Field and Matheson 1998; Eberl, Kamakouchi, and Albert, Chapter 7). In the green stinkbug (*Nezara viridula*), the JO, along with the central organ (another chordotonal organ in the pedicel), detects solid-borne vibrations (Fig. 5.5a). A male touches the branches of the fork of a plant twig with its antennae while trying to localize a female, and it is hypothesized that the antennal chordotonal organs, in conjunction with the SO, are involved in mate localization (Ota and Cokl 1991; Jeram and Pabst 1996).

5.6.2.3 Lyriform Organ

Lyriform organs are the main vibration receptors of spiders (Barth 1997; Hill 2008). They are located on the tarsi and distal ends of the metatarsi (Fig. 5.5e) and comprise parallel bundles of slit sensilla arranged such that they respond to vertical and horizontal movements of the substrate. In the wandering spider (*Cupiennius selei*), vibrations are required for detecting prey, predators, and courtship signals (Barth 1997, 1998; Hill 2008). The metatarsal lyriform organ sensilla are tuned to conspecific calls and are capable of crude frequency discrimination. The position of the legs has important implications for increasing the sensitivity and localization capabilities of lyriform organs. Spiders may take on a particular stance to enhance the transfer of vibrations to the legs, and localization of a vibration source is achieved by comparison of wave arrival times between legs.

5.6.2.4 Basitarsal Compound Slit Sensilla

In scorpions, the BCSS is considered to be homologous to the lyriform organ in spiders. The BSCC occurs on all eight legs and comprises groups of eight slit sensilla at the distal ends of the basitarsus. In the nocturnal scorpion (*Paruroctonus mesaensis*), these organs, along with tarsal sensory hairs, are used to detect and orient toward prey that cause disturbances that are propagated through sand. Scorpions position their legs in a hunting stance to optimize their ability to assess the direction of a source. Distance is assessed based on differential propagation of waves through the sand (Brownell and Farley 1979; Hill 2008).

5.7 Summary

Vibratory communication has been described as a "gold mine" for continuing research and innovation and "an exciting frontier in the study of animal behavior" (Cocroft et al. 2014b). During the past decade, as a result of increased awareness of this sensory modality and improved recording techniques, scientists have discovered that the vibratory landscape of arthropods is busy and complex. An insect can be bombarded with vibratory stimuli from multiple sources, including wind, rain, an approaching predator, or complex communication signals from a colony member, potential mate, or rival. Vibrations are used to communicate with other organisms (including plants), for orientation, to eavesdrop on potential predators or prey, or to avoid bad weather. Research to date has focused mostly on plant-dwelling insects and spiders and among those, only a select few taxa have been examined in any detail. According to Hill (2008), we are still "identifying the players" and scientists should continue to record from the natural vibratory environments of many species that have not yet been tested for vibratory sensitivities. The vibratory environments of immature insects and those residing in logs or soil are all unchartered territories ripe for exploration. The richness and complexity of this vibratory world is attributable not only to the sheer number of vibration sources but also, owing to the complexity of the communication channel, to a diversity of wave types that vary with different natural substrates. We need to understand better how waves travel in different substrates using modeling and what a receiver is experiencing by refining recording and playback methods. Finally, despite the burgeoning number of discoveries of species using vibrations and the purported importance of this sensory modality, our understanding of the sensory organs and capabilities lags behind what is known for insect hearing, vision, and chemoreception. In most taxa, vibration sensory organs have not even been identified, and there are many exciting questions to address concerning how the peripheral and central nervous systems function to process vibrations to mediate biologically relevant responses. The field is indeed a gold mine of opportunity to make new discoveries at all levels of analysis, and the potential to do so exists even in our own backyards.

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Chapter 6
Mechanical Specializations of Insect Ears

James F.C. Windmill and Joseph C. Jackson

Abstract In this chapter some of the mechanical specializations that insects have evolved to carry out acoustic sensory tasks are reviewed. Although it is easy to perceive insect hearing organs as simplistic compared to other animals, the mechanisms involved can be complex. This chapter therefore acts as an introduction to the complexities of some insect hearing systems as viewed from a mechanical perspective. The chapter provides some of the background knowledge readers require to investigate the subject in greater depth while acknowledging that this subject is an active, developing, and broad area of research. Following a brief background section on the physics of sound as applied to the insect ear, the mechanical function of several insect hearing organs is discussed in relation to the different acoustic parameters that different insect species need to evaluate, such as frequency, origin, and amplitude. A further section then follows to discuss the mechanical basis of active hearing, whereby energy is added to the hearing system to condition its acoustic response, again using available examples. Finally, the chapter concludes with a discussion on the current state-of-the-art in this active research area and makes some suggestions as to where the future may lead insect hearing mechanism researchers.

Keywords Active hearing • Antennal ears • Cicada ear • Directional hearing • Hearing mechanisms • Insect hearing systems • Locust ear • Mosquito ear • Moth ear • Tympanal ears

6.1 Introduction

The sense of hearing has evolved multiple times in the insects, fulfilling a variety of different tasks including communication (Greenfield, Chapter 2; Balakrishnan, Chapter 3) and defense (Pollack, Chapter 4). In this chapter some of the mechanical specializations that insects have evolved to carry out such acoustic sensory tasks are reviewed. Despite their perceived simplicity, the mechanics of the hearing organs of

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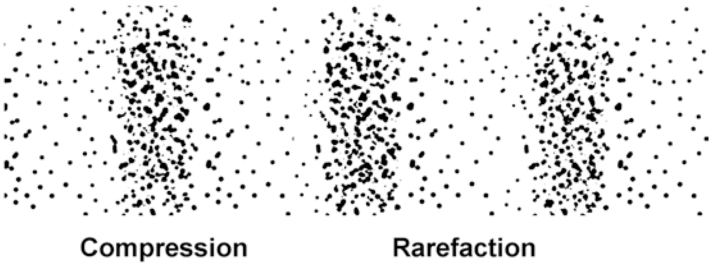

Fig. 6.1 Longitudinal sound waves traveling through a medium. These are sometimes also called compression waves, because they produce compression (the particles in the wave press closer) and rarefaction (the particles in the wave thin out). © James Windmill

insects are complex. This chapter seeks only to introduce some of these systems as seen from a mechanical perspective, providing the background knowledge required to delve further into the subject, which is an actively developing area of research. However, before considering the different mechanisms that insects use to sense sound, it is important to understand the physical forces that their hearing organs are interacting with. So the initial questions must be: What is sound? What is it that the insects are trying to sense?

6.1.1 What Is Sound?

Sound can be defined as any form of vibration through a medium, whether that is air, water, or a solid. This vibration causes the medium's particles to be displaced temporarily. The surrounding particles are thereby displaced, resulting in a wave of particle displacements that is recognized as sound. The sound waves that insects are typically considered to hear are longitudinal such that the particle displacement is parallel to the direction of travel of the wave, as shown in Figure 6.1. As sound is a wave, frequency (f), wavelength (λ), and speed (c) are used to describe it through the following equation

$$c = f\lambda$$

These are important characteristics when considering the sense of hearing in the insects. For a small animal such as an insect, the wavelengths involved in hearing can mean that evolution has led to interesting and complex mechanical solutions, some of which are described in this chapter.

6.1.2 Sound Pressure and Particle Velocity

One of the standard measures of the "loudness" of a sound is the sound pressure level (SPL). The change in pressure is caused by the compression and rarefaction of the air particles creating the sound wave. As these particles are in motion, they have their own velocity that should not be confused with the speed of the sound wave moving through the medium. The unit used is the pascal (Pa), equivalent to 1 newton per square meter. However, as the sensed change in pressure may be 10^6 (i.e., from µPa to Pa), SPL is typically quoted in decibels (dB), as the change relative to a reference sound pressure (i.e., 20 µPa). In water this reference level is normally 1 µPa; however, the acoustic impedance of water is different from that of air, which means that direct comparisons between sound in air and water are not as simple as changing the decibel reference calculation. Typically an SPL measurement is quoted for a distance from a sound source, usually 1 m, but it can be any distance chosen.

Finally, confusion is often caused by the use of sound level to mean SPL or sound intensity level. Sound intensity is a measure of the sound power per unit area, given as watts per square meter. Intensity can be considered a sound energy quantity, which is not the same as pressure (a force quantity). It is therefore incorrect to use the term intensity when discussing pressure level; rather, the amplitude of sound measured is the convention to use when talking about SPL. However, it should be noted that the normal reference levels of 20 µPa for sound pressure and 1 pW/m^2 for sound intensity mean that in decibels the sound pressure and intensity are calculated to be the same number, although they are different physical measurements.

6.1.3 Sound Attenuation

Sound attenuates as it travels through a medium. However, the extent of the attenuation depends on a number of factors. The attenuation is proportional to the distance the sound travels and also to the viscosity of the medium that the sound travels through. Other factors include whether the medium itself is traveling, for example, in air movements in windy conditions, and also the density and pressure of the medium (Bennet-Clark 1998). There is also an added dissipative effect that causes further attenuation relating to the frequency of the sound. The predominant mechanism of such attenuation is proportional to the square of frequency so at relatively low frequencies (e.g., 1 kHz), this effect is approximately 0.005 dB per meter (assuming air temperature of 20 °C and relative humidity at 50 %). As frequency increases, this attenuation factor becomes far more important such that at 100 kHz it is 3.28 dB per meter.

6.1.4 Sound over Distance: Near-Field and Far-Field Sounds

The sound field in a free field is typically divided into two regions by distance: the near field and the far field. The near field is also often subdivided into two regions, for example, the hydrodynamic and geometric near fields (Bies and Hansen 2009). However, in the context of insect hearing systems, this section discusses only the near and far fields. It is recommended that readers interested in delving deeper into this subject read Bies and Hansen (2009) or other appropriate physical acoustics books. It is also the case that the discussion of near and far fields can relate to the distance from either a source or a receiver. This chapter reviews the mechanics of receivers, the insect ears, and so the definition of near and far fields relates to the distance from the sound source that the insects are hearing.

The near field is the region immediately adjacent to the vibrating surface of the source, extending outward a distance much less than one wavelength. This region is characterized by fluid motion that is not directly associated with sound propagation and where the acoustic pressure is out of phase with the local particle velocity in the medium. The region of the sound field extending beyond the near field toward infinity is the far field, where, in the absence of reflecting surfaces, SPLs attenuate at the rate of 6 dB for each doubling of distance (not including viscous losses). The far field is characterized by satisfying the following criteria

$$r \gg \lambda/(2\pi), \quad r \gg l, \quad r \gg \pi l^2/(2\lambda)$$

where r is the distance from the source to the measurement position, λ is the wavelength of the sound, and l is the characteristic source dimension. In many cases the boundary between the near and far fields is given as approximately $\lambda/6$, where 6 is taken for 2π.

6.1.5 Introducing Insect Ear Mechanics

As mentioned in Sect. 6.1.2, sound has both pressure and particle velocity components, and therefore it is possible to preferentially detect one or the other. As both apply forces in different ways, the sensor used to detect pressure is fundamentally different from the sensor required for particle velocity detection (see Fig. 6.2). The attenuation of sound pressure with distance is less than that of particle velocity ($1/r$ vs. $1/r^2$), meaning that over long distances, pressure is more detectable.

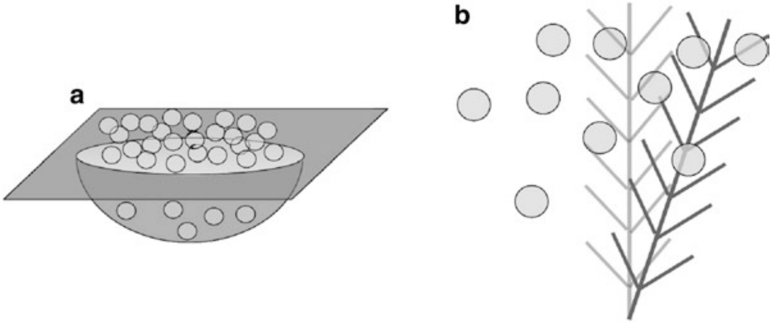

Fig. 6.2 Particles interacting with acoustic structures. (**a**) The tympanum separates regions of normal air pressure and sound-induced changes in pressure such that the mismatch in pressure creates a force that deforms the membrane. (**b**) The antenna experiences a force caused by the viscous drag of the particle motion of sound. © Joseph Jackson

As the pressure component is more pronounced at large distances, it is therefore the choice for long-range communication in land animals. To detect the pressure component, it is necessary to build a sensor that moves in response to changes in pressure. The biological solution is the tympanum (or tympanal membrane), a thin membrane of cuticle stretched over a chamber filled with air (or sometimes fluid). Tiny changes in pressure due to sound cause the tympanum to feel a force proportional to the pressure difference across the membrane, which in turn causes a deflection of the tympanum. As the pressure is oscillatory, so is the motion of the tympanum. Attached to a tympanum (not necessarily directly) is a mechanosensory system that is stretched and compressed by this tympanal motion, converting it into electrical signals in nerve cells. This solution to sound detection is used by many animals, particularly insects and in the eardrum of the vertebrate ear. In many cases, it is the only mechanical stage in sound transduction before the neurons, but in more complex systems, it can be the first part of a long chain of energy transduction. Tympanal sensors are very sensitive, typically detecting subnanometer deflections of the attachment site of the mechanosensory neurons.

Detecting the particle velocity component of sound requires a different method. A sensor must be driven by being viscously dragged by the particle motion. These sensors are either hairs or antennae and are ubiquitous in insect and arthropod taxa. A major problem with this type of sensor is that it must be external, projecting away from the body to allow efficient sampling of the fluid flow. This renders them liable to be damaged as they cannot be protected from the environment. However, these external antennae can be multifunctional, with multiple sensors giving the ability to detect sound, vibration, and wind speed and also to perform chemo- and thermoreception. They are also very sensitive so that, for example, a mosquito can detect antennal displacements of ±7 nm, corresponding to sound particle displacements of ±1 nm (Göpfert and Robert 2001).

When some of the literature on the ears of insects is examined, different insect ears are often described as near-field or far-field detectors, as well as particle velocity or pressure detectors. The interchangeable use of these terms can be confusing, so it is worth considering how they relate. First, it should be clear that when discussing near and far fields, this relates to the distance the ear is from a sound source, not the size of the ear compared to the sound wavelength. It is therefore appropriate to consider what frequency, and so wavelength, of sound different ear structures are attempting to detect. Low frequencies have long wavelengths, which also means that the near field of such sounds extends a farther distance. Within the near field, the particle velocity is high so that particle velocity sensors typically have evolved for detection in the near field of low-frequency sound sources. For example, mosquitoes and flies listen for the low-frequency (100–400 Hz) flight tones of their own species for mating. They can utilize a particle velocity ear for detecting sound in the near field (some tens of centimeters), where the particle velocity is greatest. Locusts and moths hear higher frequencies, into ultrasound (>20 kHz), where the near field is a few millimeters. To hear sounds of an approaching predatory bat, at a useful distance (as the bat flies at several meters per second), particle velocity is significantly attenuated, so the preferential hearing system to use is a pressure detection system, which is thus considered a far-field sensor. That is not to say that particle velocity in the far field is not there to be detected; it is simply that it has attenuated so much that the pressure measurement is far more appropriate.

6.1.6 Acoustic Impedance and Impedance Matching

Acoustic impedance is the ratio of complex acoustic pressure to complex acoustic volume velocity. It can therefore be considered as the complex representation of the acoustic resistance of a medium. As such, acoustic impedance can be thought of as the opposition by a medium to the flow of energy. It is conventionally given the denotation Z and measured in $\mathrm{Pa \cdot m^{-3} \cdot s}$ or in $\mathrm{Rayl \cdot m^{-2}}$, that is, 1 Rayl is 1 $\mathrm{kg \cdot s^{-1} \cdot m^{-2}}$. It is a ratio of complex numbers because, as the use of the word impedance implies, it is a combination of resistance (energy dissipation) and reactance (energy conservation). In the context of insect hearing systems, this chapter only discusses the consequences of acoustic impedance. For detailed treatments of this subject, it is recommended readers consider appropriate physical acoustics books (Fletcher 1992).

The concept of acoustic impedance is important when considering insect ears as it directly influences the mechanisms of the hearing system. For a sensor, the ear, to work most efficiently, its structure must match the impedance of the medium through which the sound travels. If the impedance is not closely matched, then the energy within the medium will not be transferred into the ear structure (causing it to move), but instead it would be reflected. A good sensor (ear) would have to be light and viscous to be susceptible to the motion of air particles or the minute pressure differences caused by sound. In fact, a structure with physical properties similar to those of the medium surrounding it would therefore be the perfect sensor, as this involves the most efficient transfer of energy from medium to ear. Taking the example of the insect tympanal membrane, this structure is extremely thin (light) and vibrates in response to changes in pressure between the external and internal sides of the membrane. Therefore the membrane vibrates most efficiently when the acoustic impedance of the sound medium is matched on each side. This impedance matching is achieved by creating an air space directly behind the membrane through a modification of the insect's internal, tubular tracheal system. There are variations and exceptions to this rule; for example, the green lacewing (*Chrysopa carnea*) is reported to have a fluid chamber backing its tympanal membrane (Miller 1970).

Finally, the specific acoustic impedance of a sound-carrying medium is dependent on the physical properties of that medium. For example, the density of water is approximately 1,000 $\mathrm{kg \cdot m^{-3}}$, whereas that of air is approximately 800 times less depending on temperature and altitude. Likewise, the speed of sound in water is approximately 1,500 $\mathrm{m \cdot s^{-1}}$—more than four times greater than that in air. Therefore, the (specific) acoustic impedance of water is approximately 3,500 times higher than that of air. This difference means that a sound of equal pressure in water to that of a sound in air is actually 3,500 times less intense than the sound in air. This is because in the air, with its lower acoustic impedance, sound has a much greater particle velocity and displacement amplitude than in water. It is this variation that leads to important differences when considering acoustics in air and underwater acoustics (see Sect. 6.1.2).

6.1.7 Pressure Difference and Pressure Gradient

Two terms that are often used to describe both microphones and tympanal insect ears are pressure difference (or sometimes pressure operated) and pressure gradient. Microphones and tympanal insect ears both work by sensing the pressure difference on either side of a thin sheet, most often called a diaphragm for microphones and a membrane for insect ears, although the terms are sometimes interchanged.

In a pressure-difference microphone, one side of the diaphragm is open to the atmosphere and is able to respond to the microscopic changes in pressure representing sound. The other side faces an enclosed volume that effectively contains a fixed "reference" air pressure. This means that the diaphragm moves in response to the difference between the pressure of the passing sound wave and the reference pressure within it. This is how an insect tympanal ear is conventionally imagined to function. However, in insects the air chamber behind the tympanal membrane is not completely enclosed, as the chamber is part of the tracheal system of air tubes used by insects to breathe. This means that the use of this nomenclature for insects is actually dependent on the frequency of the sound.

In a pressure-gradient microphone, the diaphragm is still sensitive to the difference in sound pressure on either side; however, both sides are exposed to the (same) atmosphere and therefore to the changing pressure caused by passing sound waves. In the microphone, if a sound wave arrives in the plane of the diaphragm, then there are identical pressures on both sides and so no movement of the diaphragm. There is no pressure gradient across the diaphragm and so the microphone cannot detect sounds in this orientation. If sound arrives perpendicular to the diaphragm, it will create a large pressure difference between front and rear, and it will be moved a maximum amount as a result. In insects, if the frequency of the sound arriving at the body is very low, then the wavelength is much greater than the body size of the insect. It is then possible to imagine that the tympanal membrane may undergo a pressure change on both sides, as the pressure change appears through the tracheal system. Thus, the force is exerted both externally on the tympanal membrane due to a primary sound pressure contribution from the outside and also via a secondary, attenuated contribution from the inside of the membrane. Accordingly, depending on frequency, insect tympanal ears can be described as either pressure difference or pressure gradient. However, within the literature, readers will often find these terms used interchangeably and therefore incorrectly.

6.2 The Passive Mechanics of Insect Ears

Insect auditory organs have previously been described to have four main types: trichoid sensilla, Johnston's organs, subgenual organs, and tympanal organs (Yack 2004). Subgenual organs detect substrate-borne vibrations and are discussed in Yack, Chapter 5. Of the three remaining types, the trichoid sensilla and Johnston's organ (in combination with the insect's antenna) are used to detect sound particle velocity and the tympanal organs to detect sound pressure (see Fig. 6.2). Trichoid (filiform) sensilla are hair-like cuticular projections, while the Johnston's organ sits within the pedicel of the insect's antenna. The detection of particle velocity (v_p) is achieved through drag on the filiform or antenna, as the antenna or sensilla experiences a force $F = bv_p$, where b is the viscous drag of the antenna or sensilla. This force causes a deflection of the antenna or sensilla, stimulating the sensory systems at their base. The tympanum acts to separate regions of normal air pressure P_0 and sound-induced changes in pressure P. This mismatch in pressure creates a force $F = A\Delta P$, where A is the area of the membrane. Deformation of the membrane caused by this force is detected by the sensory neuron structure attached either directly or through a secondary mechanism to the membrane.

It should also be recognized that there is a massive shift in the magnitudes that are under consideration when investigating insect ear mechanisms. The diameter of a tympanal membrane may be measured in hundreds of micrometers, if not millimeters. The thickness of that membrane will typically be measured from tens of micrometers down to less than 1 μm. However, in response to sound levels at which the ear is neurally sensitive, the tympanal membrane displacement is typically measured in nanometers, from tens of nanometers down to a fraction of a nanometer. This is a magnitude difference in meters of approximately 10 to the power 6. Anyone approaching this research field must keep this in mind, especially as figures in papers are always produced to show the data such that they are easy to view. It is easy to miss the consideration of scale.

In this section, some of the passive mechanisms by which insect ears interact with incident sounds to undertake an initial stage of information processing are reviewed. Passive refers to the fact that these interactions require no additional energy to be imparted into the hearing system; they are simply mechanical responses of the hearing structures to sound. The examples reviewed have been grouped into sound frequency analysis and sound direction analysis. This is a purely arbitrary decision made to bring some structure to the section.

6.2.1 Frequency Tuning

6.2.1.1 Moth

All moths can be described as having the "simplest" possible insect ear morphology. This is because the ears of moths have a very low number of sensory neuron structures directly attached to the tympanal membrane. They cannot therefore passively distinguish different frequencies, other than through the fact that the mechanical tuning of their ear membranes will make it preferentially sensitive to a certain frequency bandwidth, as the neuronal structures are not mechanically tuned within that bandwidth. The earliest studies of the moth ear go back to the work of Roeder in the 1950s (e.g., Roeder and Treat 1957), although many researchers have followed since.

Hearing in moths has evolved independently at least five times. In the Noctuidea, the ears are found in the metathoracic segment, with two sensory neurons directly attached to the tympanal membrane (although Notodontidae have only one neuron). In Pyraloidea, Geometroidea, and Drepanoidea, the ears have four neurons and are located in the abdomen. Finally, those moths in the Sphingidae that hear have ears on the proboscis. The principal reason for the evolution of hearing in moths is to detect the ultrasonic echolocation calls of predatory bats. Therefore, moth ear frequency sensitivity is comparable to typical bat echolocation frequencies of about 20–60 kHz.

Although the location, and to some extent morphology, of the ears found in the different hearing moth species are diverse, the conceptual understanding of the moth ear as a passive mechanical receiver is common across species (see Sect. 6.3.2 for discussion of active mechanisms in insect hearing). The general structure of the moth ear can be pictured as a tympanal membrane that is approximately circular in nature, with a single sensory neuron attachment site near the membrane's center point, as demonstrated in Figure 6.3. Thus, as a passive hearing structure, the moth ear can be thought of as a simple resonant membrane. Therefore, the frequency tuning of a moth ear should directly scale to the size of the tympanal membrane. In general this is the case, with a direct correlation between size and frequency such that larger moths are tuned to lower frequencies and smaller ones to higher frequencies.

Taking the assumption that different-sized moth ears will have very similar material properties, then the difference in tympanal membrane size also has an effect on amplitude sensitivity. A larger membrane is subject to a greater overall force compared to a smaller one when the same sound pressure is applied (assuming each membrane is the same thickness). This means that the neuron attachment site

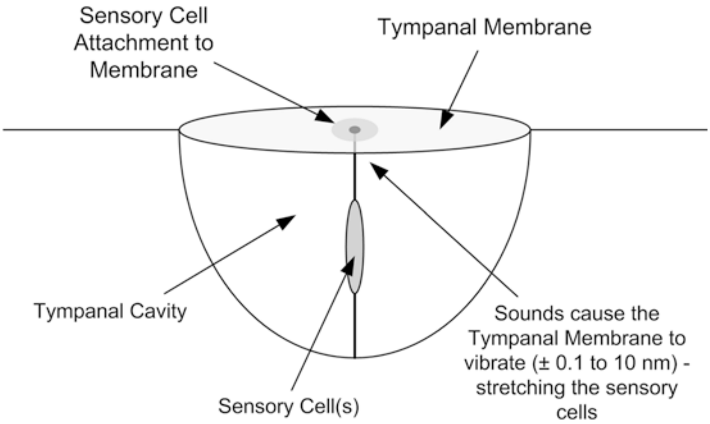

Fig. 6.3 Schematic of the structure of a moth ear as the "simplest" insect ear. © James Windmill

of the larger membrane will be deformed by a greater amount for that same sound pressure. This correlation between moth size and ear sensitivity is clearly seen when the neural sensitivity of different moths is considered (Surlykke et al. 1999). This increased sensitivity means that the larger moth, which would be more noticeable to a hunting bat, has the advantage of being able to detect the bat's echolocation calls from a greater distance than a smaller moth. Thus the apparent liability of greater size is offset by the increase in hearing sensitivity.

Of course, although it is convenient to imagine the moth ear as a simple resonant membrane, the actual morphology is more complex. For example, the noctuid tympanal ear has two membranes, the tympanal membrane and an adjacent conjunctivum membrane (also known as the counter-tympanic membrane, depending on the species). The tympanal membrane is thin, typically transparent, with a thicker area near the center where the neural attachment is sited. The second adjacent membrane is much thicker and has no neural attachment. A thicker ridge of cuticle runs between the two membranes. The mechanical response of the thinner membrane is not that of a simple membrane mode. Rather, the attachment point moves far more than the thin membrane surrounding it. Also, the two membranes deflect in antiphase at low frequencies. Then as frequency increases, the deflection of the thicker secondary membrane decreases until it appears to remain stationary. At this point, no function related to the mechanics of hearing has been put forward for the thicker secondary membrane. Currently, it is suggested that the upper membrane is simply an evolutionary remnant of the wing-hinge structure from which the tympanum evolved (Treat and Roeder 1959; Yack 2004).

Finally, as is often the case in biology, one moth ear was examined that exhibits a very wide frequency range, in contrast to that expected. The ear of the greater wax moth (*Galleria mellonella*) is sensitive to sound frequencies from 20 kHz up to 300 kHz (Moir et al. 2013). This moth listens for both bat echolocation calls and its own courtship calls. However, the upper limit of the frequency bandwidth displayed is far greater than the frequency content of either of these. Moir et al. suggested that this frequency sensitivity is due to the need for greater mechanical temporal acuity, providing the ability to separate sounds occurring at almost the same time. The response time of a mechanically resonant system such as the moth's ear to a force is inversely proportional to its bandwidth. This means that the moth's large-frequency bandwidth provides it with a much faster temporal response than a sharply tuned tympanum. The temporal acuity of the greater wax moth was estimated as about 10 μs, at least half that of the lesser wax moth (*Achroia grisella*) and six times shorter than some noctuids. This could then aid the moth in carrying out both predator avoidance and possibly courtship. The greater wax moth's ear cannot distinguish between the different frequencies of predator and potential mate. Therefore, it must use the difference in time signatures between the sounds. This has been demonstrated in the lesser wax moth, which can determine the difference between the ultrasonic clicks of its courtship calls and hunting bat calls based on the time period between pulses in each different call. Therefore, it could be that the greater wax moth has such large bandwidth sensitivity to maximize this type of capability.

6.2.1.2 Locust

The ears of different species of locusts are possibly one of the most studied insect hearing systems, from the work of Michelsen in the 1970s (Michelsen 1971) to that of many others since (e.g., Gordon and Windmill 2015). This hearing system is of great interest because as a passive mechanical system, it combines both sound reception and frequency analysis. It is also an insect that is commonly available to any researcher, as it is a ubiquitous “fresh” food for pet reptiles. The work discussed here is based primarily on research into two species, *Schistocerca gregaria* and *Locusta migratoria*.

Locusts can hear frequencies from hundreds of hertz up to greater than 30 kHz. Its ears are found on either side of the abdomen. Its tympanal membrane has several salient features. The largest area is very thin, to less than 1 μm, and hence transparent. The second smaller area of membrane is much thicker (tens of micrometers). Inside the ear sits Müller’s organ, which contains the ear’s sensory neurons (approx. 60–70), as shown in Figure 6.4. The organ connects to the membrane through three cuticular attachments that are clearly seen on the external side of the membrane.

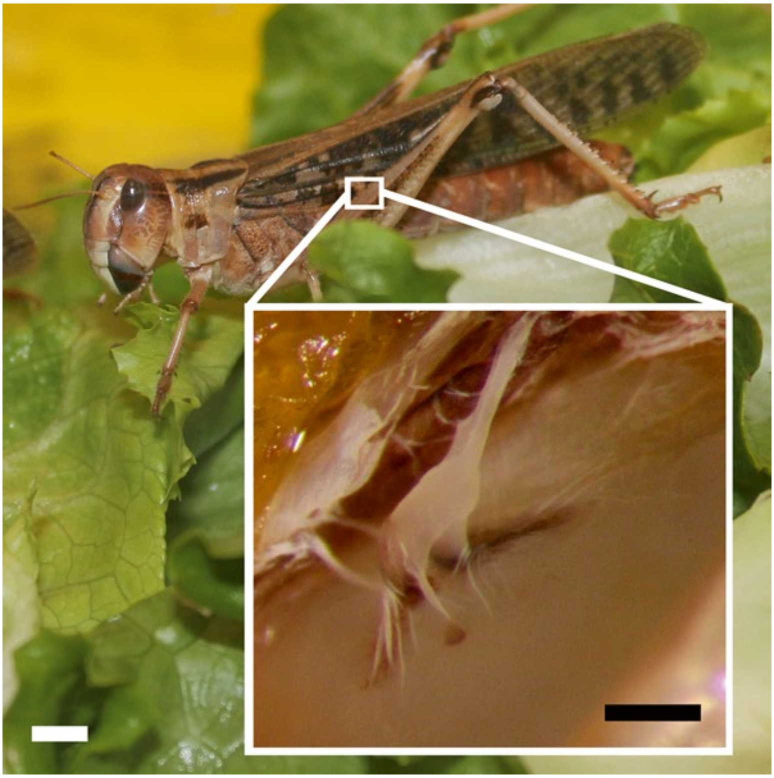

Fig. 6.4 The locust has a tympanal ear on either side of the first segment of the abdomen (white scale bar 5 mm). Inside the ear sits Müller’s organ (*inset*, black scale bar = 150 μm), which contains the ear’s sensory neurons. © Shira Gordon

Locusts are capable of distinguishing different bands of sound frequencies within an overall hearing range. This is achieved through purely passive mechanical means. When sound is incident on the locust tympanal membrane, the membrane does not resonate with the mode shapes of a membrane as would be expected. Rather, the sound energy causes the membrane to deflect such that traveling waves are generated running across the membrane (Windmill et al. 2005). These traveling waves are frequency specific such that the wave direction perceptibly alters depending on frequency. The frequency also appears to determine the eventual place on the membrane at which the traveling wave disappears. As such, the locust tympanal membrane is an example of tonotopy, whereby the frequency of the sound determines the deflections of the tympanal membrane spatially. This is analogous to the mechanism of frequency discrimination found in the mammalian ear, where frequency-specific traveling waves (von Békésy's traveling waves) occur at different positions on the basilar membrane in the cochlea to stimulate different sensory hair cells (von Békésy 1960). The locust ear distinguishes only a few frequency bands, but it does so without the need for the outer and middle ear structures in the mammalian ear. More recently, the katydid *Copiphora gorgonensis* has been found to have an ear that is an even closer analog to that of the mammals and is discussed in Sect. 6.2.1.4 (Montealegre-Z et al. 2012).

The traveling wave found on the locust tympanum initiates on the thinner membrane. It then travels across toward the thicker membrane and the connections to Müller's organ and the sensory neurons. The traveling wave always travels in the same direction, no matter what direction the sound is incident to the locust. The traveling waves caused by high-frequency sounds do not propagate onto the thicker membrane; rather they expire at a single point, which is the location of the pyriform vesicle (Fig. 6.4). Lower frequencies propagate into the thicker membrane such that below 1 kHz the whole membrane is moving, and the wavelength is such that it is easier to imagine the membrane moving with a standard mode shape.

In the mammalian basilar membrane, the existence of traveling waves has been described as depending on three criteria (Robles and Ruggero 2001). First, the displacement of the membrane should exhibit a phase lag in the direction of the wave travel. Therefore, at a given location the motion of the membrane increasingly lags the motion of its point of origin. In the locust tympanum, this increasing delay with position is found. In the locust, it is also a function of frequency as the membrane translates frequency into space. In the frequency domain, the delay is seen as a phase accumulation at high frequencies, which exceeds the high-frequency phase lag expected for a simple resonator. Second, the displacement magnitude of the membrane should have an asymmetric envelope around the point of interest where the wave is seen to compress. This is seen in the locust tympanal membrane, particularly at higher frequencies, where the leading slope of the envelope on the membrane is steeper than the trailing slope. Third, the traveling wave results from the mechanical characteristics of the membrane and, in that sense, is passive. In the locust, the traveling wave occurs in freshly dead locusts. Therefore, the motion of the locust tympanal membrane can be described as a traveling wave.

Measurements of the frequency responses of the sensory neurons in Müller's organ have shown that they correlate with the traveling wave frequencies, so that, for example, neurons running to the pyriform vesicle respond to frequencies of approximately 9 kHz and above. Other groups of neurons in Müller's organ are tuned to lower frequency bandwidths and spatially correlate with the motion of traveling waves at the same frequencies. The path of transduction of force from the traveling wave in the membrane to cause the neurons to generate electrical signals has not been adequately investigated. For example, the pyriform vesicle is clearly deflected by low-frequency sounds, as the traveling waves associated with any low-frequency sound pass through it as they cross the tympanal membrane. It is probably a reasonable conjecture to consider that the mechanical behavior of the structures of the pyriform vesicle and about the neurons associated with it relates directly to the actual response of the neurons. Or are the neurons somehow intrinsically tuned to certain frequencies? The only research to attempt to measure the motion of Müller's organ with respect to sound required sound levels far greater than the insect would usually hear, though this was with a severely dissected preparation (Stephen and Bennet-Clark 1982).

It must be noted that no two individual locusts are identical, and therefore, although all show the same characteristic traveling waves, every individual has a slightly different mechanical response. Taking this further, the desert locust (*S. gregaria*) shows an extreme phenotypic plasticity exhibited as a transgenerational accumulation of phenotypic changes driven by changes in population density. There are two extreme phenotypes, the solitarious and gregarious phases, which differ extensively in behavior, physiology, and also morphology. It has now been shown that solitarious and gregarious locusts have clear differences in their hearing, in both their tympanal and neuronal responses. The shape of the tympanal membrane is different between locust phases, with the solitarious phase having a wider membrane (on one axis of measurement). This correlated with greater displacement of the solitarious animal's tympanal membrane (when given the same sound stimulus amplitude as gregarious phase locusts). This fits with the mechanical expectations for the system, as the larger membrane has greater force applied for the same sound level, as discussed for the moth. The correlation to neuronal response was far less clear. However, this leads back to the questions of how the traveling wave on the membrane interacts with the sensory neurons in Müller's organ (Gordon et al. 2014).

Finally, understanding the mechanisms through which the locust tympanal membrane creates traveling waves is of great interest. In the quest to understand the function of the mammalian inner ear, numerous mathematical models have been generated. Resources to conduct similar work on the locust hearing system, which many might consider simpler, are rather more limited. However, progress has been made in this area. The typical engineering procedure to model and simulate a system such as a tympanal membrane is to use finite-element modeling (Reddy 2005). In practice this involves creating a two- or three-dimensional computer model of the system in question. This model is "meshed," whereby each component in the system is split into numerous blocks of smaller dimension (the elements). The computer simulation then applies physical laws to the elements in the model, working out how each element interacts with those surrounding it. The practical problem is that as the model, and resulting simulation, more closely approach the actual physical biological system, the more intensive the meshing becomes.

In the case of the locust, the tympanal membrane is not a simple flat sheet of homogeneous material. It has a specific problem in that it has a high aspect ratio; it is very thin in one dimension (thickness of micrometers) compared to the others (diameter in millimeters). This means that a very large number of elements are required for a realistic model based on the actual dimensions of the tympanal membrane. Complicating matters further are all the other related structures, including Müller's organ, the air sac(s) behind the membrane, the insect's body around the membrane, liquid-filled chambers within sections of the membrane (as shown in Malkin et al. 2014), and finally, of course, the air through which sound travels to the ear.

There are several answers to this problem. First, the power of the computing facility could be increased. However, it is very easy to approach the need for supercomputer facilities even with relatively simple insect ear models. The second approach is to move away from finite-element modeling, employing more theoretical mathematical modeling of the concepts; however, for the practical biologist (and engineer), this comes at the risk of very quickly losing the inherent physical understanding of what is being analyzed. Therefore, the third option is probably the most reasonable, making as many simplifications of the physical model of the locust tympanum as possible to be able to undertake an analysis of the system using finite-element modeling. In so doing, one hopes to walk the fine line between losing analytical rigor due to oversimplification and creating a computer model that requires far more resources and time than is feasible to run.

This latter option is the one that has been put to good effect for the locust ear (Malkin et al. 2014). This has compared experimental data with relatively simplified finite-element models to show that the locust tympanal membrane is under tension. The combination of this tension and the change in thickness across the membrane acts to generate the traveling waves seen moving across the membrane. It also makes it clear that the traveling waves, and the resulting tonotopy, are a purely mechanical effect based on the morphology and material properties of the ear. However, it is noted by Malkin et al. (2014) that their modeling relies on assuming the tympanal membrane is functioning in an isotropic linear-elastic regime, although they could certainly be anisotropic and viscoelastic. Thus, the work thus far is probably only a first approximation. This avenue of research can only grow further in the future as the ratio of price to power of computer processing continues to improve.

6.2.1.3 Cicada

Cicadas are one of the loudest groups of animals in the world. They use sound as part of the mating process, ensuring species recognition and sexual selection (Fonseca et al. 2000). Although only the male can produce sound, both sexes have hearing systems. The cicada ear combines a tympanal membrane with a sensory organ containing sensory neurons. The ear contains a surprisingly large number of sensory neurons, varying from 600 to more than 2,000 in different species. However, the calling song of cicadas tends to be centered on a single frequency that is amplitude modulated, thus typically producing continuous trains of pulses of sound. The cicada ear differs from that of the moth and locust because the sensory neuron structures do not attach directly to the tympanal membrane. In cicadas, the thin tympanal membrane exhibits a structure called the tympanal ridge, a dark area seen on the membrane, as shown in Figure 6.5. This ridge extends under the membrane through a structure known as the tympanal apodeme. This cuticular extension acts as the mechanical bridge from the membrane to inside the sensory capsule of the organ where the mechanosensory neurons attach to it.

Different cicadas display variations in the mechanical response of their tympanal ear. For example, they may have a traveling wave present on the tympanal membrane and ridge, similar to that seen in the locust, or the membrane and ridge may move with a simple drum-like motion. Sexual dimorphism has also been seen in cicada ears, whereby the male and female ears are mechanically tuned to different frequency ranges, presumably relating to selective pressures acting in different directions, linked to the different roles of each sex in sound reception and production (Sueur et al. 2010). However, in all cases, the cicada's tympanal membrane and ridge act to mechanically focus the incident sound energy to drive the activation of the sensory neurons.

Force is applied to the mechanosensory neurons through the motion of the tympanal apodeme. The apodeme appears to be a relatively thick cuticular structure and seems to act as a lever, with the external membrane and ridge at one end and the sensory neurons at the other. The apodeme appears to be stiff and does not bend, at least along the portion of the structure that has neuron connections. However, the mechanical frequency response of the apodeme is tuned about the main frequency of the cicada's calling song (Windmill et al. 2009). It thus appears to act as a passive frequency filter within the cicada ear, meaning that only energy related to the calling song reaches the sensory neurons. Also, the amplitude of the motion of the apodeme is significantly less than that of the external membrane. Thus it is also possible that one purpose of the apodeme is to reduce the amount by which the sensory neurons are stretched. It is not clear if this means, as in a simple lever system, the force applied to stretch the neurons is increased in relation to the reduction in the amplitude of motion. As such this could be some form of impedance matching, translating the motion of the thin and light tympanal membrane (which seeks to match the impedance of air) to the stretching motion of the large number of sensory cells. It should be noted, however, that experimental data on the mechanics of the cicada apodeme are so far available for only a single species (Windmill et al. 2009).

Finally, it is also not clear why the cicada has so many (hundreds) of mechanosensory neurons, especially given that the cicada calling song is an amplitude modulation of a single frequency. Electrophysiological measurements have been undertaken that show that interneurons following the sensory neurons in a cicada hearing system carry out a frequency discrimination (Fonseca et al. 2000). However, no equivalent experiments have been carried out on the sensory neurons to examine whether they are frequency tuned in some manner. As discussed in Sect. 6.3, certain insect ears are "active," such that they are motile and can add energy into the system to make ear structures move more. So, the question is open.

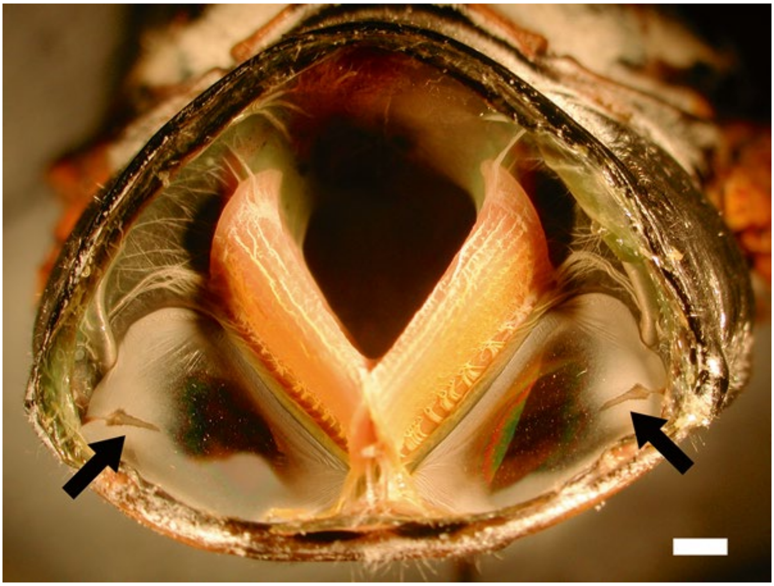

Fig. 6.5 The cicada tympanal membrane. This cutaway cross-section image shows the two tympanal membranes of a *Lyristes plebejus* (male) from an anterior view (with the pair of tymbal muscles seen as the v-shaped structure in the center). The ridge structure is clearly visible at the edge of each tympanal membrane (located by *black arrows*). Scale bar = 0.5 mm. © Jerome Sueur

Could the large number of neurons be related to frequency tuning? Or maybe, if motile, to add mechanical energy into the system? Or is there some other reason as yet to be discovered?

6.2.1.4 Katydid

Katydids (or bushcrickets) are another group of insects that use sound as part of the mating process, as males sing to attract conspecific females (Montealegre-Z and Robert 2015). The sound frequencies exploited by different species of katydids vary across a huge range from 2 to 150 kHz. Furthermore, in addition to conspecific signals, katydids are exposed to many other sounds, including ultrasound produced by bats to hunt and navigate at night, as well as the sounds produced by other nocturnal mammals that eat insects (i.e., many tropical species of katydid are nocturnal). Thus, the katydid ear has evolved within the context of communication and predator detection, meaning that many species can detect a wide range of frequencies.

The katydid ear is found in the leg of the insect, with one in each foreleg tibia, as shown in Figure 6.6. Each ear actually has a pair of tympanal membranes, one anterior and one posterior. Each tympanal membrane also has a thicker section, known as the tympanal plate. Behind the membranes is a trachea filled with air, which divides into two branches at the ear, one for each tympanal membrane. The ear is asym-

Fig. 6.6 The giant katydid *Steirodon careovirgulatum* with the ear found on the foreleg tibia (*inset*). Scale bar = 30 mm. © Fernando Montealegre-Z

metrical as the anterior branch of the trachea takes up a large part of the dorsal ear surface. In some species, the trachea plays an important role in the transmission of sound to the ear, as sound enters through a spiracle. Depending on species, there are various suggestions of different adaptations of the tracheal system in concert with the pair of tympanal membranes relating to the reception, transmission, and amplification of sounds. There is also great variation in the external morphology of the ear around the tympanal membranes, whereby in different species the tympanal membranes are either completely exposed or partially covered by cuticular structure or only one is exposed. It has been suggested that the covering structures function as sound guides to enhance directional hearing in those species. Back within the ear, the mechanosensory neurons sit in a long structure called the crista acustica. This structure sits on the dorsal wall of the anterior tracheal branch. It is surrounded by a fluid-filled cavity, described as the auditory vesicle. The sensory neurons in the crista acustica are then tonotopically organized. However, one of the most interesting morphological and mechanical points regarding the katydid is the fact that the sensory cells are not in direct contact with the tympanal membranes.

As noted previously in this section, the sensory neurons sit within a fluid-filled cavity and are not in direct contact with the tympanal membranes. Therefore, in the ear of the katydid, a mechanical transduction process is required to convert the tympanal membrane vibrations due to sound into mechanical energy in the fluid to drive the sensory cells. As discussed in Sect. 6.1, the impedances of air, cuticle, and fluid are different; therefore, the katydid ear must undertake a process of impedance conversion in a manner similar to that found in the mammalian ear. The answer is a lever system, whereby the motion of the tympanal membranes acts to drive the fluid, and then the fluid acts to drive the sensory neurons. This is important because the tympanal membranes act as simple resonant membrane structures, that is, with a central resonant frequency, whereas the crista acustica is a tonotopic system, with different sensory neurons along the crista acustica activated by different frequencies. In fact, the crista acustica displays the traveling wave motion found in the mammalian basilar membrane and the locust tympanal membrane.

Unfortunately, this is complicated somewhat by the fact that different species of katydids appear to have variations on this basic concept (Montealegre-Z et al. 2012; Palghat Udayashankar et al. 2012). In some katydids, the tympanal membrane and plate move in phase. In this case, it is suggested that pressure waves traveling in the trachea activate vibrations of the crista acustica internally. So as the tympanal membranes move with sound, the change in pressure within the trachea causes the crista acustica to move. The second case is where the tympanal membrane and plate are seen to move out of phase. For this situation, it is put forward that the tympanal plate is acting as a lever, like the ossicles in the mammalian middle ear. Here the change in air pressure causes the tympanal membranes to move, and the mechanical connection between a membrane and associated plate then acts as a lever such that the motion of the membrane is mirrored in the plate. The plate sits in contact with the fluid-filled cavity within which the crista acustica is found. The plate then couples its motion to the fluid-filled cavity, causing pressure waves within the fluid. It is then these pressure waves in the fluid that cause the sensory neurons in the crista acustica to be driven to move. In this second case, it is clear that the system is then a very close analogy to the mammalian ear. The air-driven tympanal membrane (eardrum) mechanically couples to a stiff lever system (the middle ear), which then couples to a fluid system, wherein the motion of the fluid causes the activation of the sensory neurons (the inner ear).

As mentioned previously in this section, the sensory neurons within the crista acustica are tonotopically arranged such that the sensory organ can discriminate frequencies across a range (dependent on frequency). This is seen through the formation of traveling waves through the crista acustica, with different frequencies relating to traveling waves being produced at different positions along the structure. Again this appears slightly more complex across species, relating directly to the differences between how the crista acustica appears to be driven. In the first case, where the crista acustica is driven by the change in sound pressure in the trachea, it appears that the traveling waves form whether the fluid in the cavity behind the crista acustica is present or not. In the second case, where the tympanal plate motion causes pressure waves in the fluid, it appears that removal of the fluid stops the formation of traveling waves in crista acustica, as expected. It is suggested that the traveling waves are a result of the morphology and mechanical properties of the crista acustica. This seems appropriate as the crista acustica is wide at one end, narrowing along its length. As noted previously in this section, this is again a close analog to the mammalian ear, where the basilar membrane not only fulfills the same function and displays the same vibrational motion but also has the same type of change in morphology.

Finally, it also appears that the katydid has a form of mammalian "round window," whereby the pressure change input to the fluid system is output, as a form of pressure release, because the fluid is incompressible. In some katydids, the proximal end of the fluid-filled cavity has a narrow connection with the hemolymph channel such that the pressure input relating to the sound is released into the insect's hemolymph (Montealegre-Z and Robert 2015).

6.2.2 Sound Direction

Directional hearing is usually accomplished through a mechanism that compares the sound input between two ears, relying on either interaural time or intensity differences, ITD and IID, respectively. To accomplish a timing-difference measurement, the distance sound must travel to reach each ear independently must be large enough for a detectable time delay to exist between the ears. For a small animal such as an insect, the distance between ears is generally smaller than the sound of interest's wavelength such that a time delay detection approach is not feasible.

Despite this, when the distance sound travels to reach the ears falls within half a wavelength of the incident sound, animals may be able to make a phase comparison. In this circumstance, the SPL is relatively similar at either ear, and so animals could plausibly register phase differences of the wave as it passes around the body. In addition, the air chambers in an insect's body form an internal acoustic connection between the tympanal membranes at each ear. Therefore, while a force is exerted externally on a tympanal membrane due to the external sound pressure changes, a secondary attenuated sound pressure change contribution from the incident sound is applied from the inside of the membrane via the air chamber pathway (as in a pressure-gradient receiver).

The wavelength of a sound wave in air decreases as the frequency increases. Therefore as frequency increases, there is a critical frequency beyond which the insect is unable to decipher the phase difference between the ears relating to the incident sound wave. For frequencies greater than this value, a difference in SPL must instead be detected. Theoretically, in this second case, the distance between the ears must be greater than half the wavelength of the incident sound, with this half-wavelength value corresponding approximately to the critical frequency. With higher frequencies, the insect's ears are functioning solely as pressure-difference receivers, whereby the internal sound pressure is unaffected by the change in external pressure. Increasing frequencies will result in smaller wavelengths and therefore larger sound level differences between the ears as the insect body acts as a barrier to sound propagation.

At lower frequencies, where the distance between the insect's ears fits within half a wavelength, the direction of the sound is determined by the phase of the sound wave arriving at both the external and internal (from the contralateral ear) sides of the membrane (pressure-gradient receiver); the animal's body is too small to affect the sound wave significantly as it passes and all or most of the sound diffracts easily around the body. However, for higher frequencies, sound will not be able to diffract around the animal's body and so a partial acoustic shadow is created, with a diminution of SPL on the side contralateral to the incident sound. In addition, the body creates reflections that change the sound field. When sound originates from directly in front of the animal, no difference in phase or sound level will be detected owing to the bilaterally symmetric placement of the ears. To complicate matters, sound arriving from one of the sides will create interference both from the length of the body and from the width around the body.

The accumulation of the constructive and destructive interference patterns is commonly measured by comparing the sound level within a free sound field to the altered sound level when there is an obstruction (aka body). Therefore, directional hearing is often discussed as the changes in sound level both between the ear locations and as if there was no body obstruction.

6.2.2.1 Ormia

The parasitoid fly *Ormia ochracea* is a parasite of crickets at the larval stage. As a consequence, gravid females deposit their larvae on a host cricket that is located through phonotaxis to the male cricket's mating call (Miles et al. 1995). This parasitic life cycle imposes a significant evolutionary constraint on the flies, as their body size must be limited to being less than that of their hosts. This means that the auditory organs of *O. ochracea* are separated only by a short distance such that there is less than 0.5 mm between the fly's tympanal membranes, which in turn are very small. The cricket's mating call has a relatively pure frequency tone between 4.5 kHz and 5.2 kHz (a wavelength of approximately 70 mm). This presents significant challenges for sound source localization as the ITD is 1.45 μs at a maximum azimuth of 90° and the IID in the sound pressure is extremely low. Nevertheless, despite this tiny ITD, the parasitoid fly has been shown to be able to localize a cricket song to an azimuthal accuracy of 2°. This is comparable to the directional hearing of humans, who are able to rely on much larger ITDs and IIDs given the distance between their ears. Extensive studies of the auditory system of *O. ochracea* have shown that the tiny ITD cues are amplified by means of a flexible mechanical coupling between the two sensory organs.

The ears of a parasitoid fly are unusual in that both the tympanal organs are contained within a single, undivided air-filled chamber. The auditory system is located on the front face of the thorax, with a pair of cuticular membranes serving as the tympana. The two tympanal membranes are connected through a cuticular structure referred to as the intertympanal bridge. The intertympanal bridge terminates near the center of each tympanum in a depression (the tympanal pit) that is also the attachment point of the auditory receptor neurons. It is this cuticular bridge that provides the mechanical basis for the directional sensitivity. Measurements of the tympanum using laser vibrometry have shown that the membranes vibrate with amplitude differences of approximately 12 dB and time differences on the order of 50 μs at maximum azimuth (at 6 kHz).

The coupling of the ears can be shown by manually applying a force to one membrane, resulting in the intertympanal bridge rocking about the point halfway between the pits. When stimulated acoustically, both sides of the bridge are driven by two forces of equal amplitude but slightly different phase. The overall motion of the bridge can then be decomposed into two natural modes of vibration. The first is a pure rocking mode about the center point of the bridge, while the second is a translational mode with both ends of the bridge moving in phase while the intertympanal bridge bends in the middle. The response of the hearing system at any frequency can then be described by a linear combination of these modes.

The ability of the incident sound pressures to drive each of the modes depends on the relative phase of the pressures acting on the tympana. The instantaneous sound pressure at each ear is equivalent to two sinusoidally varying point forces that are very slightly out of phase (see Fig. 6.7). The difference in these two forces provokes the rocking motion of the bridge while the sum of the forces provokes the translating mode. The instantaneous amplitude difference between the two forces then clearly depends on the wavelength of the incident sound and the natural resonance frequencies of each of the modes. In *O. ochracea* at 2 kHz, the phase delay is approximately 1°, resulting in a very low IID and a dominant translational mode. At 5 kHz, the frequency of the cricket mating call, the phase delay is larger (approx. 2.6°), which provokes a larger rocking mode supported by the proximity of this frequency range to the natural resonance of that mode at 7 kHz. In higher frequencies, the phase difference is greater, but the rocking mode is depressed by being driven above the natural frequency while the translational resonance is approached, resulting in both modes being equally strong in the system. Therefore, *O. ochracea* is critically tuned to have excellent discrimination of directional sound cues at the frequency of the cricket mating call. Hence through the addition of a mechanical link between the two tympanal membranes, the *O. ochracea* is capable of extremely fine directional hearing.

6.2.3 Other Insect Ears

Hearing in insects has evolved multiple times (Yack 2004). While the passive mechanical response of a number of insect ears has been discussed in Sects. 6.2.1 and 6.2.2 and active responses in other insects are examined in Sect. 6.3, this is by no way an exhaustive analysis of the passive mechanics of insect hearing. Current understanding depends on the resources that have been invested in the research required. Hearing in various other insects has been examined to a greater or lesser degree. A number of other insect hearing systems have been studied, two examples

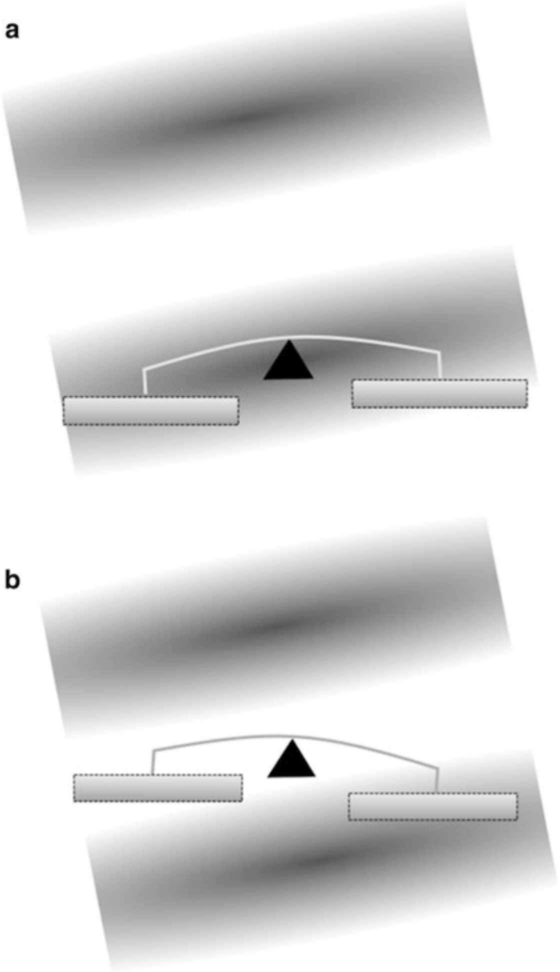

Fig. 6.7 The *Ormia* ear mechanism. The ear's two membranes are connected by an intertympanal bridge. The bridge motion has two natural modes of vibration: (1) a rocking mode about the bridge center point (triangle) and (2) a translational mode with both membranes moving in phase while the bridge bends at the center. A sound wave, depicted by shading (see Fig. 6.1), causes the system to move, with the difference in pressure between the membranes stimulating the rocking mode and the sum of the pressure on the membranes stimulating the translational mode. (**a**) At one-quarter cycle, the force from the total pressure is at a maximum while there is no pressure gradient between the membranes, reinforcing the movement of the ipsilateral membrane while repressing the movement of the contralateral membrane. (**b**) The forces from the pressure gradient and total pressure on the membrane are 90° out of phase. At three-quarters cycle, the pressure gradient is at a maximum while the total pressure is reduced to zero by the angle of the membrane to the sound wave origin. © Andrew Reid

of which, the mantis and the weta, highlight the diversity of mechanical adaptations in insect auditory systems.

The mantis hearing system is unique as it contains only a single ear and is often referred to as the cyclopean hearing system (Yager and Hoy 1986). The vast majority of mantids can hear only ultrasound frequencies (typically 30–50 kHz, but sometimes extending toward 100 kHz). The main function of the ear therefore appears to be the detection, and so avoidance, of predatory bats. This single ear of the mantis has two tympanal membranes located in a deep cuticular chamber that is found in the ventral midline of the metathorax. The teardrop-shaped tympanal membranes face each other from the walls of the groove. The mechanics of the membranes are interesting as they also utilize a traveling wave. It is also most likely that the chamber performs an acoustic function. However, research into the ear of the mantid is ongoing and is sure to reveal more about this unique ear.

As a member of the Ensiferan group, the weta is related to the katydids and crickets and so also has an ear on each foreleg tibia (Field et al. 1980). Weta produce sound by stridulation, and their hearing is typically tuned over a relatively narrow frequency range relevant to acoustic intraspecific communication. As in the other Ensiferans, the ear is composed of two tympanal membranes, one anterior and one posterior. The tympanal membranes have two distinct regions, a darkly shaded thick inner region surrounded by a thin transparent region. The inner region oscillates as a stiff plate driven by the surrounding region. As the complete tympanal membrane vibrates in a simple drum-like mode, this only provides an initial frequency discrimination based on the membrane’s drum-like response. The weta ear also has an adapted trachea, providing an air cavity behind the tympanal membranes and a crista acustica containing the sensory neurons.

The unique part of the weta ear is that the fluid in the channel that the crista acustica is sited in comprises a previously unknown form of lipid rather than the hemolymph found in katydids (Lomas et al. 2012). Furthermore, the lipid is synthesized in situ by a structure known as the olivarius, distinct from the fat body. Removal of the lipid reduces the neural auditory sensitivity of the ear. Thus the lipid channel is thought to act as a relatively solid mass preventing the crista acustica and trachea from moving dorsally. Instead, the trachea is thought to be constrained to expand laterally, stretching out the ends of the crista acustica and thus activating the sensory neurons. Clearly, questions still remain regarding this system, for example, whether the lipid’s function is purely a passive mechanical one. Therefore, research into this system continues.

6.3 The Active Mechanics of Insect Ears

While properties of insect ears that rely on structure or form to determine their acoustic function have been discussed in Sect. 6.2, there is another mechanism by which acoustic properties can be changed that does not rely, intrinsically, on ear structure. This mechanism, known as active hearing, uses metabolically dependent processes to add energy to an existing acoustic oscillator. Additional vibrational energy derived endogenously has the effect of conditioning the acoustic response of the ear for whatever purpose, typically amplification of weak signals, and increasing sensitivity.

There is a wealth of studies in the scientific literature on active hearing in the animal kingdom, the content of which cannot be reproduced in its entirety here; readers are advised to read Manley et al. (2008) and Nadrowski et al. (2011) for more detail. Instead, the general principles of how active hearing can improve audition at the periphery from a physical perspective are described, followed by examples of active hearing in the insects that act as model systems for their auditory behavior.

6.3.1 A Reduced Physical Description of the Addition of Active Properties to an Ear

A basic "ear" can be considered. Ears in nature are first and foremost passive oscillators, driven by fluctuations in fluid pressure or particle velocity in the medium through which sound propagates. As oscillators, they can be approximated as driven damped harmonic oscillators, an elementary physical model of an oscillator in which dynamic behavior is determined by three parameters (reducible to the first two): stiffness k of a linear restoring spring, linear viscous damping b of the medium in which the oscillator moves, and the mass m of the oscillator.

Oscillators of this type are linear, which in this context means that they will oscillate at the same frequency of the impinging sound field, and their response is proportional to the amplitude of the sound field. They also exhibit resonance, a preferred frequency that induces strong vibration compared with other frequencies. For insects, linear passive ears means that the ear should (1) vibrate with no distortion in response to a sound or, put another way, the ear can vibrate only at frequencies that were present in the impinging sound; (2) exhibit some resonance, whether pronounced or damped, which could be used for selectivity; and (3) respond the same way to a sound, independent of any previous sounds that may have impinged upon it. As such, this basic insect ear, when driven with a pure tone, should vibrate sinusoidally at a predictable consistent amplitude.

It is not possible here to describe the many fine details of the responses of passive oscillators to sound. Instead, the focus is to consider this basic ear already vibrating sinusoidally at a fixed amplitude as a result of a driving sinusoidal force from sound. A graph of the instantaneous displacement of this vibration oscillation versus the instantaneous velocity traces out a circle (see Fig. 6.8). In time the oscillator rotates around this circle; one cycle of this circle is one full cycle of oscillation. It is possible to imagine an active process that somehow allows the addition of energy at any point in the cycle; for example, as the oscillator traces this circular path, there is some way of "pushing" it around. Depending on how this is achieved, adding energy to different parts of the cycle has the equivalent effect of changing the effective parameters

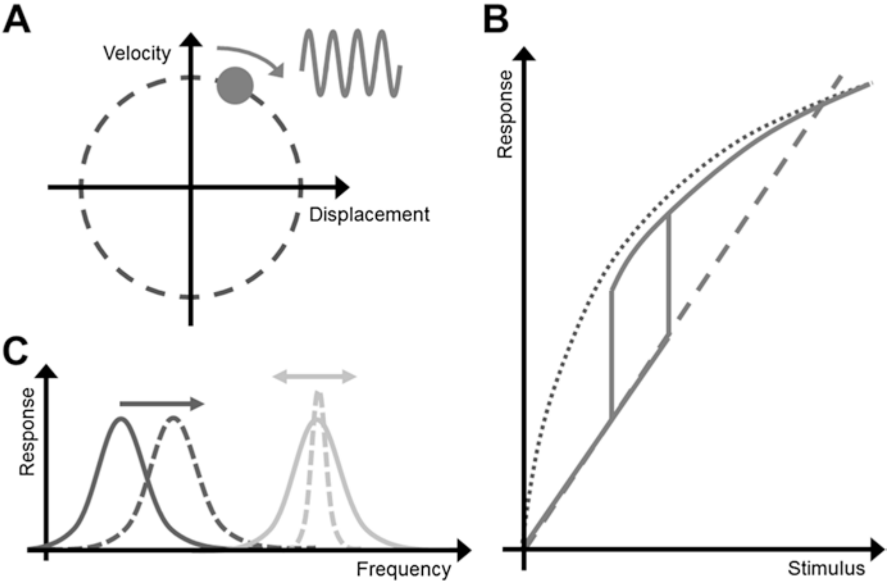

Fig. 6.8 Active insect ears. (**A**) Phase-space plot of harmonic motion. In time a sinusoidal oscillation traces out a circle. (**B**) Types of active nonlinear responses. *Gray dotted line* is a typical vertebrate hair bundle response or that of *Drosophila* or the tree cricket. *Solid line* is the mosquito nonlinearity, indicating the hysteretic response. The *dashed grey line* is a linear response. (**C**) Energy added at different points of the cycle can either shift the resonant frequency (*dark gray*) or change the effective damping and sharpen or desharpen the frequency response (*light gray*). In practice, it is some combination of each. © Joseph Jackson

of the oscillator, the stiffness and the damping components. For example, at the point of maximum velocity, the displacement is zero. If an impulsive force in the direction of motion contributed energy at this point, then it would oppose the viscous damping of the medium on the oscillator. Consequently, it would seem that the viscous damping was effectively reduced. A similar argument can be made for when the oscillator velocity is zero and the displacement is maximum; energy added at this point would affect the apparent stiffness.

This rather abstract thought experiment demonstrates how active cycle-by-cycle processes can affect the effective parameters of a simple oscillator, influencing stiffness and damping, which in turn influence the response of the oscillator; in essence, the passive oscillator has been altered and so its response to a sound stimulus will be different.

6.3.1.1 Self-Oscillation

In this basic ear, imagining an active process that can push and pull the oscillator allows predictions of how this would change the effective stiffness and effective damping of the ear. If such an active process is too strong, the effective damping becomes zero or even negative. Under these circumstances, in a basic ear, in the absence of a driving force, the active process could drive autonomous oscillation. This is possible and is considered the gold standard indicator of active hearing; understandably, as in the absence of sound, there is no external energy input into the ear, and thus it can oscillate only with an active process.

6.3.1.2 The Oscillatory Instability

That an active ear is sometimes capable of autonomous oscillation allows a generic mathematical model to be used for the dynamic behavior of this nonlinear oscillator. Such a general model derives from the normal form for a Hopf bifurcation (Pikovsky and Kurths 2003) and was successfully used to describe various phenomena in the dynamics of auditory hair bundles (Camalet et al. 2000; Eguíluz et al. 2000). This model easily explains the power law behavior of hair bundles exposed to weak acoustic stimuli. Conceptually, the predictive power of this normal form is restricted to generic properties of acoustic nonlinear sensors close to an oscillatory instability. Therefore, it cannot provide details about a particular type of sensor. However, given that such properties of active ears are generic, they can be considered important experimental phenomena that betray the presence of an active mechanism in ears.

6.3.2 Active Insect Ears

In the insects, active hearing has been found notably in mosquitoes, *Drosophila*, and a tree cricket (*Oecanthus henryi*). Self-oscillation is a characteristic property of these ears, in common with mammalian and frog saccular hair bundles (Kemp 1978; van Dijk et al. 1989). There is also evidence to suggest locusts and moths have some nonlinear active aspect to their hearing, evidenced by acoustically evoked distortion in their tympanal membranes. It seems unlikely that active hearing is restricted to these insects only. For example, little study has been made of hearing in the Nematocera, the suborder to which mosquitoes belong, despite many of this suborder having plumose antennae that could in principle be used for hearing. It is anticipated that new discoveries of active hearing in insects will occur in due course; in the meantime, what follows is a description of some well-known active hearing systems in insects.

6.3.2.1 Antennal Ears

Mosquitos and *Drosophila*, among other insects, have antennae that act as acoustic oscillators. At the base of these antennae is Johnston's organ, a chordotonal organ whose function has evolved from proprioception to acoustic sensing. This organ contains many mechanosensory units called scolopidia composed of ciliated neurons (e.g., Boo and Richards 1975). Cilia within these scolopidia are thought to be capable of generating force, thus providing an origin for energy pumping into the oscillating ear. What follows is a brief description of the two model systems for active antennal hearing in insects.

Mosquito

The male mosquito ear is a plumose antenna, at the base of which is a pestle-shaped organ called the pedicel in which lies Johnston's organ (Clements 1999). It can be considered a beam supported by a spring at the base and as such its first mode of vibration is a simple rocking motion of the antenna within the pedicel. Thus, the antenna does not bend. Johnston's organ in the mosquito consists of some 16,000 mechanosensory neurons, a very large number and probably the key to the unusual nonlinear behavior of the mosquito ear. This ear can be well approximated as a harmonic oscillator, with damping caused by the plumose antennae moving through air and stiffness dictated by the joint between flagellum and Johnston's organ. The oscillator has a primary resonance at approximately the same frequency as the female flight tone and the male ear is used to listen for a flying female.

The antenna of the elephant mosquito (*Toxorhynchites brevipalpis*) exhibits autonomous oscillation both spontaneously and elicited with microinjection of, for example, dimethyl sulfoxide (DMSO). Such oscillation is approximately sinusoidal. Experiments show that when stimulated with sound mimicking a female acoustic signal, the antenna behaves nonlinearly above a certain sound level threshold (Jackson and Robert 2006). A bistable response exists in which the antenna can oscillate with two different amplitudes. Which amplitude is "chosen" as the stable oscillation depends on whether the sound stimulus intensity was approached from a louder or a quieter sound, a phenomenon that has behavioral implications.

Antennal nonlinearity has been shown to involve significant changes in the effective damping of the antennal oscillator, with a small change in the effective stiffness. Such an effect requires energy input on a cycle-by-cycle basis, and the huge number of ciliated mechanosensory neurons is undoubtedly required to fuel this mechanical response. One potential indicator of active hearing is therefore the presence of an unusually large number of potentially motile sensors in an insect ear.

Drosophila melanogaster

Drosophila exhibit active hearing too, but with a contrasting result compared to the mosquitoes. Drosophilid antennae are of a different form to the mosquito, but the basic principles are much the same—some external paddle-like oscillator damped by air and sprung at the base of the antenna to Johnston's organ. In this animal, the chordotonal organ has a few hundred mechanosensory neurons.

The first evidence for active hearing in *Drosophila* is the ability of the antenna to self-oscillate. However, rather than an almost sinusoidal vibration as seen in the mosquito, the antenna of *Drosophila* exhibits a strong nonlinearity and the resulting self-oscillation is highly nonsinusoidal; it vibrates as if it were switching between two extreme displacements (Göpfert and Robert 2003).

The antenna exhibits an elevated mechanical response in vivo compared to a hypoxic state. When stimulated with sound, the antennae of *Drosophila* exhibit a strong change in resonant frequency. As such, the effective stiffness of the oscillator is strongly influenced by active processes, in contrast to the mosquito (Göpfert et al. 2005). Again, the suggestion is that energy is added cycle by cycle and in this case is predominantly affecting the effective stiffness.

The response to single-frequency stimuli demonstrates power law responses consistent with the generic models for active ears. As such, the ear of *Drosophila* is an excellent model system to study aspects of the evolution of active hearing in finer detail. In particular, the similarities between models for *Drosophila* hearing and vertebrate hair cells, and the amenability of experimentation on fruit flies, make the study of *Drosophila* hearing an exciting avenue for future research (e.g., Todi et al. 2008).

6.3.2.2 Tympanal Ears

Active hearing in insect tympanate ears has also been discovered, and evidence exists for active hearing in orthopterans and lepidopterans (e.g., Coro and Kössl 1998; Kössl and Boyan 1998; Windmill et al. 2006; Mhatre and Robert 2013; Mora et al. 2015). As discussed in Sect. 6.1.5, tympanal ears are drum-like sensors that detect pressure fluctuations in contrast to antennal ears, which detect fluid flow. Tympanal ears have mechanosensory organs attached directly or indirectly to the tympanal membrane. Again, the interplay between the mechanosensory attachment and the passive tympanal membrane is thought to be responsible for enabling active phenomena.

Tree Cricket

A discovery by Mhatre and Robert (2013) showed a very strong active hearing phenotype in the tree cricket (*Oecanthus henryi*). These ears are present on the foreleg of the cricket and are typically orthopteran. The remarkable aspect of this discovery is how well the ear matches the canonical properties of active hearing: strong inducible self-oscillation that is approximately sinusoidal, compressive nonlinearity with power laws consistent with a Hopf bifurcation model, and distortion that is dependent on physiological condition. Despite its very recent discovery, the tree cricket should be considered a model system for active hearing owing to it being expressed strongly in this animal.

Locust

The locust ear is a tympanal system that exhibits a vibrational tonotopy phenomenologically equivalent to the traveling wave observed in the mammalian cochlea. Furthermore, there exists evidence that the tympanal ears of locusts are also active. Unfortunately, locust hearing does not exhibit strong phenomena associated with active hearing. There is no evidence of power law responses and no evidence of self-oscillation. However, there is evidence for distortion (Kössl and Boyan 1998). At first glance, distortion without self-oscillation or power law responses would imply a nonlinear ear but not necessarily an active one. However, this distortion is shown to be metabolically dependent. Further research is needed to examine the exact nature of activity in the locust ear and what benefit the animal enjoys from it.

Noctuid Moths

Of course, nothing in nature is straightforward, and nonlinear hearing can be present in an unexpected way. The previous examples describe effects from cycle-by-cycle force feedback into an oscillating sensor, allowing changes in the effective damping and stiffness. However, there are alternative ways to change, for example, the stiffness. In the moth *Noctua pronuba*, the resonant frequency and so the stiffness of the tympanum appear to increase in response to a bat-type stimulus (Windmill et al. 2006). This phenomenon has since been observed in various moth species (e.g., Mora et al. 2015). This stiffness change is inferred from a clear change in the resonant frequency of the tympanum. However, it appears that this stiffness change is not a cycle-by-cycle feedback resulting in an effective change of stiffness; rather, it appears that it derives from a direct manipulation of the stiffness of the tympanum. Thus examples of changing stiffness, or indeed damping, are not sufficient to affirm the existence of active hearing. Nor is the presence of a power law, or distortion, which may only indicate nonlinearity. The only true proof of active hearing is self-oscillation, noisy oscillation, or metabolically dependent nonlinearity that results in amplification or distortion.

6.4 Conclusions and Future Directions

The preceding sections of this chapter have hopefully provided an inspiring, if brief, introduction into the mechanical specializations of insect ears. It is in no way a complete treatise of the subject; there are insect ears not mentioned or mentioned so briefly that those researchers who work on them will question this treatment of the subject. For example, some might ask, What happened regarding the hearing system of the cricket? Even for those insects that have been dealt with in some depth, this chapter can only act as an introduction. There is a very large body of research for aspiring researchers to work their way into.

It is clear that researchers are learning more and more about the complexities of what some would call "simple" hearing organs. The ears of different insects have evolved in various mechanical ways to carry out useful functions relating to the survival of the species through communication and defense. These hearing organs are capable of feats of sensitivity and processing similar to those found across the hearing animals. In doing so, they can mechanically process sound amplitude, frequency content, and direction, as appropriate to their ecology. This has led to many interesting variations across these themes. So, an insect might only need to pick out one frequency, but does it need to know with great accuracy where it comes from (*Ormia*) or just that the presence of that sound frequency equates to an immediate threat (moth) or a mate (possibly the same moth!)?

Through the years, the capability of researchers to utilize technology has helped generate much progress in the area. The use of a laser vibrometer to measure the nanometer-scale motion of a hearing organ is now almost ubiquitous. This is now being coupled with advances in microscopy, in particular the availability of x-ray microtomography (μCT), which allows the visualization, and measurement, of hearing organ structures and surrounding body parts in three dimensions. Thus it is now possible to see how the internal structures, air channels, and other internal organs sit in a three-dimensional space around the ear. The third area that is slowly advancing is the use of computer modeling and simulation to understand the dynamics of the mechanical function of the ear. Unfortunately, this is still limited, even with the continuous increases in computer power. At the time of writing, it is not yet possible for a 3-D μCT file to be loaded into a 3-D computer modeling suite and, after some manipulation by a researcher, a 3-D simulation of the mechanical function of the ear to be produced and directly compared with empirical measurements from a laser vibrometer. However, that day is getting closer, and researchers in the future will have access to such tools.

Unfortunately, the panacea, at least for some, described in the preceding text misses two vital points. The first one is that for any computer modeling and simulation to make sense, the material properties of the structures must be known. This is far easier said than done. Currently, mechanical models and simulations tend to take a first approximation approach, assuming the system is neatly isotropic and in a linear elastic regime. Almost certainly this is not the case, and the structures and their incorporated materials should be considered as anisotropic and viscoelastic. Furthermore, the complexity of the materials and structures exists on several scales pertinent to the mechanical function (as a millimeter-wide structure, micrometers thick, vibrates nanometers) and is always capable of providing new surprises such as the liquid cavities in the locust tympanal membrane or the lipid in the weta ear. The question for researchers in the future will continue to be how to work with all of these confounding issues such that any computer modeling and simulation provides useful additions to our knowledge.

The second point, and the end to this chapter, is to remember that these mechanical specializations found in the insect ears are inextricably linked to the evolutionary pressures acting on the different species. So although it can be very interesting for engineers, physicists, and mathematicians to look at how an ear functions, seeking understanding of the physical principles that are being utilized, it is important to ask "why?" The hearing organ performs a function relating to the ecology of the insect; therefore, a full understanding of its function is possible only by connecting how it works with why it should do so, as discussed in the other chapters in this volume.

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Chapter 7
Auditory Transduction

Daniel F. Eberl, Azusa Kamikouchi, and Joerg T. Albert

Abstract Auditory transduction, the process of converting acoustic energy into a nerve signal, couples the sound-evoked motion of an external receiver structure to the gate of a mechanosensitive ion channel. This chapter summarizes the physiological landscape of insect chordotonal auditory receptors, highlighting features that have informed the understanding of the central mechanisms and specializations of insect auditory transducers and their variation. Primarily based on combined genetic and functional experiments in the Johnston's organ of *Drosophila*, we present the current understanding of the molecular complexes associated with auditory transduction. The roles of the ciliary dendritic structures are integrated with those of the ion channels and associated complexes in the ciliary membrane. Finally, the chapter includes speculation on the foci of these mechanisms that may contribute to diverse physiological responses in insect auditory receptors.

Keywords Active mechanical amplification • Chordotonal organ • *Drosophila* • Johnston's organ • Katydid crista acustica • Locust Müller's organ • Mechanosensitive channel • Mechanotransduction • Moth ultrasonic hearing • NompC • Receptor lymph • Scolopale cell • Scolopidia • Stick insect • TRP channel

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7.1 Introduction

For a microphone, transduction is conversion of the energy from sound waves into an electrical signal. Similarly, in sensory biology, auditory transduction is the process of converting acoustic energy into a nerve signal. The acoustic energy is captured by a physical structure that resonates with the sound. This acousto-mechanical transformation of the energy allows the subsequent transduction of the resulting mechanical signal into a change in membrane potential. Among insects, there are many kinds of receiving structures, from thinned cuticular membranes or tympana that respond to pressure oscillations or gradients to antennae that oscillate in the particle displacement of near-field sound to trichoid sensilla that respond to air currents or near-field sound (Yack 2004).

With the exception of the trichoid sensilla, each of these insect auditory receptors relies on chordotonal organs, named after their mechanistic arrangement as a string or “chord” under tension. These sensory organs are also called scolopidia, based on their structural organization with a spindle-shaped scolopale cell surrounding the neuronal dendrite to enclose it in a large extracellular cavity filled with receptor lymph (Boekhoff-Falk and Eberl 2014).

This chapter surveys major designs in insect auditory organs, with a focus on ways of activating the auditory sensory neurons. Highlighted examples illustrate salient features of auditory sensory neuron physiology. A description of the molecular features of insect auditory mechanotransducers follows, with work done primarily on *Drosophila*. The chapter ends with important outstanding questions and key molecules that have yet to be identified.

7.2 Physiology of Transduction

Rather than reviewing extensively the research on physiological responses in insect auditory neurons, which has been done elsewhere (Fullard and Yack 1993; Field and Matheson 1998; Mason and Faure 2004; Nakano et al. 2015; Pollack 2015), this section summarizes selected historical vignettes that reveal salient features of chordotonal neuron physiological properties. These examples have been extensively reviewed; here they are briefly summarized in the context of how they inform the transduction mechanism.

7.2.1 Roeder’s Moth Ear Recordings

Perhaps one of the most important glimpses into the physiology of insect auditory transduction comes from the classical work of Ken Roeder on the moth auditory afferents (Roeder 1967). Many noctuid moths have a well-developed ultrasonic auditory capacity used in antipredation behavior against bats. These moths have

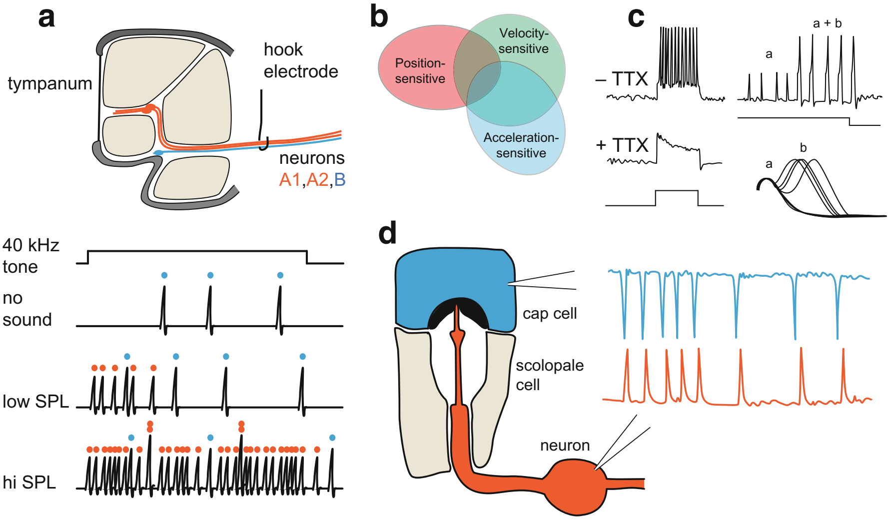

Fig. 7.1 Physiological insights into chordotonal neuron transduction. (**a**) Schematic view of Roeder's (1967) electrophysiological recordings from noctuid moth ears, which have two auditory scolopidia (A1 and A2, *orange* neurons) that innervate the ligament underlying the tympanal membrane. Using a hook electrode, he recorded activity in the auditory nerve that contains axons from these two neurons and a nonauditory neuron (B, *blue*). With no sound, only the B unit fires (*blue dots*), while a 40-kHz tone at low sound pressure level (SPL) evokes an adapting response from the A1 neuron (*orange dots*), and higher sound pressure evokes activity from both A neurons (*orange dots*), with occasional coincident firing (*double orange dots*). (**b**) Many experiments on stick insect and locust scolopidia reveal enormous variation in scolopidial response types, including position-sensitive, velocity-sensitive, and acceleration-sensitive units, as well as some combinatorial types. Not depicted are other dimensions in which these classifications can change depending on stimulus frequency or direction of movement (based on data summarized by Field and Matheson 1998). (**c**) Schematic view, based on data from Hill (1983), of intracellular recordings from locust Müller's organ auditory neuron activity in the presence of tetrodotoxin (TTX), which blocks spike formation, that reveal graded adapting receptor potentials during a 100-ms tone (*left*). Without TTX, there is no sign of adaptation. Two classes of spikes are often seen in these intracellular recordings (*right*), which Hill termed "apical" (**a**) and basal (**b**), inferred from the location of their origin in the dendrite. Apical spikes are always seen in the initial response to a tone, while basal spikes arise out of the apical spikes, evidenced by the initial shoulder on the large spikes. Superimposing a number of large and small spikes reveals a variable delay in the basal component of the large spikes. (**d**) Oldfield and Hill (1986) reported simultaneous intracellular recordings from cap cells (*blue*, also called attachment cells) and from the soma of the cognate neuron (*orange*) as schematized here from Oldfield's data. Oldfield inferred that each downward spike in the cap cell response (*blue trace*) is a negative imprint of the receptor potential, because the cap cell contacts the receptor lymph from which cations pass through mechanotransducer channels during activation. Accordingly, each cap cell negative spike is followed with a very short delay by an action potential in the neuron (*blue trace*)

metathoracic tympanal organs with two chordotonal neurons, A1 and A2 (Fig. 7.1a). Using hook electrodes to record from the tympanal nerve, Roeder took advantage of the differences in spike heights and firing patterns to identify these two units in the resulting traces, along with another nonchordotonal unit (the B cell) in the tympanic nerve IIIN1b. The B cell extends multiple dendrites into the vicinity of the articulating cuticle under the wing and reports proprioceptively on stresses imposed on the tympanic apparatus during wing movements in flight. Whereas the B cell changes its firing rate, with little adaptation, from about 5 to 300 Hz as wing position changes, the auditory chordotonal neurons respond directly to sound. These A neurons respond to sounds in the frequency range of 3 Hz to 300 kHz (Roeder 1967; Moir et al. 2013), with highest sensitivity in the middle range of about 50–70 kHz, consistent with a bat antipredation function. The two A cells show adaptation to long or continuous acoustic stimuli but no frequency discrimination (cf. Adams 1972). The A1 cell shows a lower threshold, responding to low or moderate sound levels, with A2 beginning to fire only with sounds about 25 dB louder than the A1 threshold, reflecting the behavioral urgency of a bat at close range. Minimal adaptation of the A cells occurs under stimulation with very short tone bursts that resemble a calling bat's cries. As Roeder (1967, p. 47) summarizes, "The intensity of an ultrasonic pulse is coded in the A-axon discharge as: (i) the number of A spikes per second; (ii) the activity in one or both A cells; (iii) the duration of the after-discharge; and (iv) the response time." These coding properties hold across the 40-dB suprathreshold range, above which the A cells saturate.

What can be learned about transduction from these observations? First, the number of sensory cells in the entire tympanal organ is two, so the full auditory output of the organ is represented, while at the same time, single-unit activity is distinguishable. Second, the fact that the two A cells show a different threshold reflects physiological differentiation among the sensory neurons. This could be explained by differences in the mechanisms for action potential generation, but, alternatively, it leaves open the possibility of underlying differences in the transduction mechanism itself. Third, with such high-frequency acoustic stimulation, which transforms into tympanic membrane vibrations of the same frequency, the transduction mechanism does not result in a cycle-by-cycle pattern of action potentials in the axon. Thus, a certain amount of integration takes place in, or subsequent to, the transduction events in the sensory cell. In insects with large clusters of scolopidia, the nerve response might result in tracking a higher frequency through a population mechanism.

7.2.2 *Stick Insect and Locust Femoral Chordotonal Organ Recordings*

At first glance, it may seem odd to examine femoral chordotonal organs, which mediate no known hearing function in stick insects and locusts. However, these are arguably the most extensively studied chordotonal receptors at the single-unit level. Importantly for our purposes, Field and Matheson (1998) classified 22 distinct categories of physiological responses among intracellular receptor cell recordings depending on whether they respond to position, velocity, acceleration, or a combination thereof and whether they respond in the direction of flexion, extension, or both (Fig. 7.1b). Furthermore, superimposed on these classifications is that some receptors changed categories depending on stimulation properties such as frequency (Kondoh et al. 1995). This level of receptor physiological specialization suggests that chordotonal receptors could exhibit enormous variation in transduction mechanism. It should be mentioned that there also may be mechanisms beyond the transduction apparatus that could contribute to this diversity, including mechanical and viscoelastic properties of the dendritic cap and cellular linkages to the apical and basal cuticle, subcuticular epithelium, or apodeme; the nature of intercellular adhesion junctions; the compliance of the scolopale; the ultrastructural and mechanical properties of the sensory cilium; and the composition of the receptor lymph, as well as the post-transduction physiological events in the receptor cell itself.

Because the elucidation of the molecular apparatus underlying chordotonal mechanotransduction is still in the sprouting stage, with initial insight primarily in Johnston's organ of *Drosophila* (see Sect. 7.3), it is still not clear which mechanisms could contribute to such large diversity in physiological responses. The results of single-unit analysis mentioned in this section cannot at present meaningfully enlighten the understanding of the transduction mechanisms. Importantly, this variation should strongly motivate research to discover the molecular basis of differences in transduction mechanisms.

7.2.3 Hill's Locust Müller's Organ Recordings

To approach more closely the precise events of transduction in chordotonal neurons, Hill's intracellular recordings (Hill 1983) from the locust Müller's organ are particularly revealing. On acoustic stimulation of increasing intensity, Hill observed graded potentials at the lower intensities (Fig. 7.1c). As stimulus intensity increased, evoked action potential spikes emerged, superimposed on the graded potentials, with both increasing graded potential amplitudes and increasing spike rates. This suggests a typical neuronal response from these sensory neurons. These neurons also exhibited adaptation in the spike rate during 100-ms tone stimuli. To distinguish further the receptor potentials (the transduction events) from action potentials, Hill found that tetrodotoxin (TTX) application to the preparation eliminated the action potential spikes, while the graded potentials remained (Fig. 7.1c). This allowed a more clear assessment of transduction, including verification of the graded nature of the receptor potentials and, in some of the cells, adaptation during the 100-ms tone stimuli. Although most of the recordings were from the neuron soma, Hill (1983) inferred that some electrodes penetrated the neuron in the apical dendritic regions. In these regions, the cell shows small spikes (termed apical spikes, based on inferred electrode position) that are of uniform amplitude, like an action potential, but without a baseline undershoot in the repolarizing phase, unlike a conventional action potential (Fig. 7.1c). Other records display so-called basal spikes, more like conventional action potentials in character, or a combination of apical and basal spikes. When these are combined, the basal spikes invariably arise out of the apical spikes, suggesting that when the traveling membrane depolarization reaches a certain point along the dendrite, an enhanced action potential generation mechanism engages. It is unfortunate that the electrode locations in these experiments could not be verified by an independent method. Nevertheless, if Hill's (1983) interpretations are correct, then, taken together, the immediate transduction event results in brief spontaneous discrete depolarizations or evoked graded receptor potentials, presumably in the ciliary region of the dendrite. Given sufficient summation, these potentials become small spikes in the apical dendrite, and these small spikes may in turn become full-fledged action potentials once they pass a critical region in the basal dendrite. The action potentials will propagate along the entire cell and axon to the axon terminals.

7.2.4 Oldfield's Katydid Crista Acustica Recordings

Whereas many insects lack fine frequency discrimination, instead emphasizing recognition of temporal patterning in the acoustic stimuli, other insects exhibit specialized frequency discrimination mechanisms. One such mechanism is tonotopy, which spatially separates frequencies along an anatomical gradient. Tonotopy is exemplified by the katydid crista acustica, a specialized distal segment of the subgenual organ in the prothoracic tibia (Oldfield 1982; Oldfield and Hill 1986). Depending on species, the crista acustica contains 20–50 scolopidia arranged along the dorsal surface of a tracheal tube. In some katydids, such as *Copiphora gorgonensis*, dispersive wave propagation in the acoustic vesicle adjacent to the crista acustica is initiated through a lever mechanism from the tympanal plate, allowing both amplification and tonotopic frequency separation (Montealegre-Z et al. 2012). The crista acustica responds to frequencies in the range of 4–70 kHz, and Oldfield (1982) showed in *Caedicia simplex* that the scolopidial neurons at the proximal end of the crista acustica had their lowest thresholds at low frequencies, with a gradient of sensitivities to high frequencies at the distal end. Experiments described by Oldfield and Hill (1986) on intracellular recordings in the same species have greatly informed the understanding of the transduction process. As in Hill's (1983) work in the locust, these intracellular recordings from the receptor neuron soma generate two separable event categories, which the authors interpret as large and small spikes, where the small spike can occur in the absence of a large spike, but the large spikes always initiate with a small spike embedded as a shoulder in the rising phase. These events resemble Hill's (1983) apical and basal spikes (Fig. 7.1c). Understanding of these events is greatly enhanced by their subsequent experiments recording intracellularly from the scolopidial cap cells that form the apical attachment (Fig. 7.1d). From these cells, Oldfield and Hill (1986) recorded spikes that were antiphase to those in the receptor neurons. Importantly, simultaneous recordings from the cap cell and the receptor neurons supported a temporal coupling between the cap cell activity and the smaller of the spikes in the receptor cell, these small spikes representing the receptor potential, which may or may not elicit an action potential, the larger spike. Because the cap cell membrane contacts the receptor lymph, Oldfield and Hill (1986) argue that the cap cell record reflects the ionic changes in the receptor lymph as ions flow through the opened mechanosensitive channels in the cilium, hence the reverse polarity in the spike from this cell. Consistent with this interpretation of the small spikes representing receptor potentials, hyperpolarization of the neuron soma by current injection blocked the large spikes but not the small ones, and negative current injection into the cap cell while the neuron soma was hyperpolarized evoked a burst of the small spikes in the neuron.

These experiments may represent, to date, the most direct electrophysiological access to the transduction event itself in chordotonal organs. The tight envelopment of the dendrite by the scolopale cell has presented a challenging obstacle to reliable insertion of an electrode in the dendrite. Perhaps the development of optical recording techniques, such as the genetic expression of Arclight, a gene-encoded fluorescent voltage sensor (Cao et al. 2013), in *Drosophila* will facilitate the spatial resolution of membrane voltage events associated with transduction.

7.3 Auditory Mechanotransducers

The molecular apparatuses of mechanotransduction, and more specifically the molecular identities of auditory mechanotransducer channels (aMETs), have been at the center of an ongoing controversy ever since a direct mechanical gating of aMETs was proposed (Corey and Hudspeth 1983). For the ears of both vertebrates (Furukawa and Ishii 1967; Corey and Hudspeth 1979) and insects (Albert et al. 2007), submillisecond response latencies have been reported, which are widely considered to be too short to result from an indirect, second messenger-mediated form of activation. Instead, a mechanical activation has been postulated in which the mechanical stimulus directly alters the free energy differences between the closed and open forms of the channels, thereby effectively coupling the channels' open probabilities to the mechanical stimulus (Corey and Hudspeth 1983). As a result, aMETs are directly gated by sound. A mechanical form of channel activation implies an elastic coupling of aMETs to external (i.e., extracellular) sound-receiving structures. The inherent reciprocity of this coupling, in turn, is bound to introduce distinct mechanical signatures of the gating, and adaptation, of aMETs into these external receiver structures: the receivers will be easier to move (i.e., more compliant) over that range of stimulus forces and displacements at which transducer gating occurs. Once the ion channels are all open or all closed, the receiver structure will be stiffer and thus more difficult to move. This phenomenon was named "gating compliance" and the serial elasticities that couple the receivers to the transducers are commonly referred to as "gating springs." Gating compliances have been reported for the auditory cells of both vertebrates (Howard and Hudspeth 1988) and insects (Albert et al. 2007). However, the molecular nature of the corresponding transducer channels, or their gating springs, has not yet been resolved.

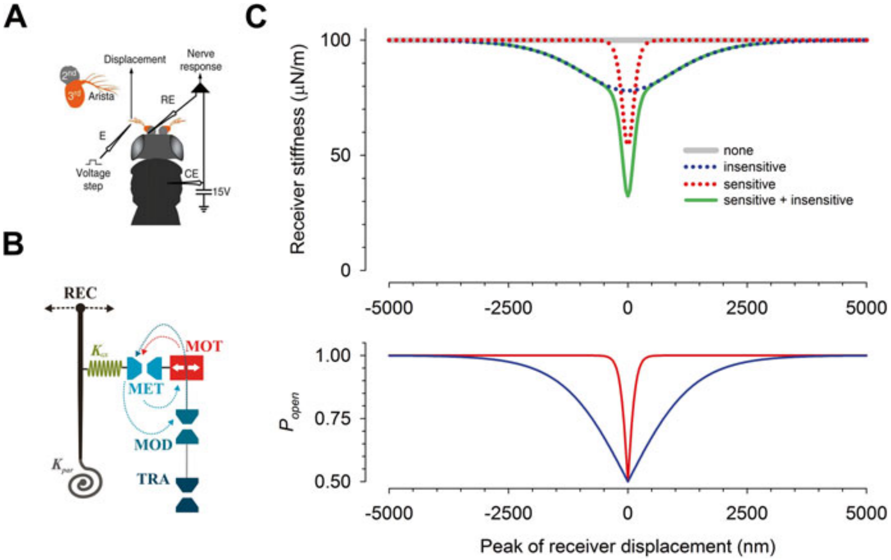

Fig. 7.2 A *Drosophila* model of auditory mechanotransduction. (**A**) Combined biomechanical and electrophysiological studies of the antennal ear of *Drosophila* have been used to dissect the mechanisms, and molecules, of auditory mechanotransduction. (**B**) The emerging view of mechanotransducer function in *Drosophila* sees external stimulus receiving structures (REC) serially coupled to mechanotransducer channels (MET) via force-transmitting elastic elements (K_{GS}). A parallel stiffness (K_{par}) summarizes all serial elasticity that does not contribute to directing forces to the MET. Motor proteins (MOT) are thought to act in series with the MET, mediating both adaptation to, and amplification of, sound-evoked signals. Downstream of transduction, further ion channels modify (MOD) the transducer signals and eventually transform them into action potentials (TRA). (**C**) Mechanotransduction in the fly's Johnston's organ (JO) has been linked to at least two independent types of MET, each of which introduces characteristic signatures into the mechanics of the antennal sound receiver. One of the two populations depends on the function of NompC and is part of a sensitive transduction pathway that contributes to sound sensation. A second population is independent of NompC and part of a less sensitive (or "insensitive") transduction pathway that contributes to the sensation of wind and gravity. The *top panel* shows the resulting dynamic receiver stiffness for four hypothetical scenarios. *Gray*: Both transducer populations are blocked, no gating whatsoever (constant stiffness); *blue*: sensitive transducers are blocked, only insensitive transducers are gated (stiffness drops over a wide range of displacements); *red*: Insensitive transducers are blocked, only sensitive transducers are gated (stiffness drops over a narrow range of displacements); *green*: both sensitive and insensitive transducers are gated (dual stiffness drop over both the narrow and the wide range). The *bottom panel* depicts the underlying open probabilities of the two transducer populations (*red*: sensitive, *blue*: insensitive)

7.3.1 *Studying aMETs in* Drosophila*: Current Insights and Ongoing Controversies*

The mechanosensory Johnston's organ (JO) of *Drosophila* resides in the second segment of the antenna (Fig. 7.2A). Over the past 10 years, the JO has been developed into a powerful model for the functional and molecular dissection of mechanosensation and specifically auditory mechanotransduction (Nadrowski et al. 2011;

Boekhoff-Falk and Eberl 2014; Albert and Göpfert 2015). Current models of fly auditory mechanotransduction have mainly focused on the specific roles of different transient receptor potential (TRP) channels expressed by the various cell types of JO (Fig. 7.2B). Belonging to the group of chordotonal organs (Field and Matheson 1998; Kavlie and Albert 2013), JO is formed by an array of approximately 200 scolopidia (see also Kamikouchi and Ishikawa, Chapter 10). Each scolopidium of JO typically comprises one to three neurons (JO-Ns) and three support cells (JO-SCs). At present, nine TRP channels have been linked to the function of the *Drosophila* JO (Zanini and Göpfert 2014), with expression reported for both JO-Ns and JO-SCs. Studies on auditory transduction proper have concentrated on three neuronal TRPs in particular: the TRPN1 channel No-mechanoreceptor-potential C (NompC) (Eberl et al. 2000), which localizes to the distal region of the mechanosensory cilium of JO-Ns and the two vanilloid transient receptor potential (TRPV) channels Nanchung (Nan) (Kim et al. 2003) and inactive (Iav) (Gong et al. 2004), which localize to the proximal part of the JO-N cilium, where a Nan/Iav dimer is deemed to form, or contribute to, a heteromultimeric channel complex (Gong et al. 2004). Mechanotransducer complexes introduce multiple nonlinearities into the response behaviors of hearing organs; this is most evident perhaps in mechanical feedback amplification, where a transducer-based process adds mechanical energy to the sound-evoked motion of a stimulus-receiving structure (Göpfert et al. 2006) (see also Sect. 7.3.2 and Windmill and Jackson, Chapter 6). Loss-of-function mutations of both NompC and Nan/Iav impair mechanically evoked responses in JO: loss of Nan/Iav abolishes compound action potential (CAP) responses in the antennal nerve completely (Kim et al. 2003; Gong et al. 2004) but increases mechanical, transducer-based amplification, whereas loss of NompC leads to a strong reduction, though not a complete loss, of CAP amplitudes (Eberl et al. 2000; Effertz et al. 2011) but virtually abolishes the mechanical, transducer-based amplification (Göpfert et al. 2006; Effertz et al. 2011). Based on the near-complete loss of transducer-based feedback amplification in the ears of *nompC-null* mutant flies and the increase in auditory amplification seen in *nan*/*iav* mutants, it was suggested that NompC might be part of true mechanotransducer channels in auditory neurons of *Drosophila*, with Nan/Iav channels playing a downstream role in mechanical gain control and action potential generation. This suggestion is consistent with the finding that the loss of NompC leads to characteristic reductions in gating compliance, which mimic the reduction in gating compliance seen after ablation of JO auditory neurons (Effertz et al. 2012). The NompC-based model of JO auditory transduction was contested, however, by a study that reported that sound-evoked giant fiber neuron (GFN) activation persists in NompC-null mutants (Lehnert et al. 2013). As the study also found that a loss of Nan/Iav, in contrast, completely abolishes the sound-evoked GFN currents, it was suggested that Nan/Iav might form the transducer channel in JO auditory neurons. Clearly, further clarification, particularly in the form of single-cell, patch-clamp recordings of JO-Ns, is required to decide whether NompC, Nan/Iav, or a third, as yet unnamed, ion channel constitutes the JO auditory transducer channel proper. Studies on *Drosophila* touch-sensitive neurons have, however, demonstrated beyond a reasonable doubt that NompC can indeed form, or contribute to, a true mechanotransducer channel (Yan et al. 2013).

7.3.2 Active Mechanical Amplification

Although fly mechanotransducer modules are still incompletely understood on the molecular level, their contributions to sensitive hearing, which in flies just as in vertebrates relies on an active process, have been analyzed in quantitative detail. A model built on the assumption that mechanically gated ion channels act in series with adaptation motor proteins could explain the response behavior of the fly's antennal ear to small stimuli, including the characteristic intensity- and frequency-dependent nonlinearities of the active process (Nadrowski et al. 2008; Fig. 7.2B, C).

The cellular basis of the active process in the antennal ear of fruit flies has been traced to JO-Ns. In fruit flies, genes that affect the function of JO-Ns, such as *beethoven* (*btv*), *touch-insensitive-larva B* (*tilB*), and *no-mechanoreceptor-potential A* (*nompA*) are necessary for active amplification (Göpfert and Robert 2003; Göpfert et al. 2005). Mutations in *btv*, *tilB*, and *nompA* cause structural defects (Eberl et al. 2000; Chung et al. 2001; Kavlie et al. 2010), which affect the mechanical properties of, or the stimulus coupling to, the JO-N dendrites. The gene *nompA* encodes an extracellular linker protein expressed in the caps, which connect the cilia of JO-Ns to the hook (Chung et al. 2001). Mutations in *nompA* disconnect JO neurons from the antennal receiver, leading to a complete loss of active amplification and elimination of the sound-evoked nerve responses (Eberl et al. 2000; Göpfert et al. 2005). Mutations in the *btv* and *tilB* genes, in turn, cause structural defects in the axonemes, which are a characteristic structure of chordotonal cilia as well as of sperm (Eberl et al. 2000). The *btv* locus encodes the intraflagellar transport (IFT) dynein heavy chain of *Drosophila*, whereas *tilB* encodes a conserved leucine-rich repeat-containing ciliary protein (Kavlie et al. 2010). Sound-evoked electrophysiological responses of JO neurons are absent in *btv*, *tilB*, and *nompA* mutants.

In mammals, the gain of the cochlear amplifier (a summary term for the hair cell-based active process) is centrally controlled through efferent pathways (Frolenkov 2006). Axons of neurons that originate in the olivocochlear complex synapse on the outer hair cells to modulate their electrical and mechanical properties, thereby providing a mechanism for cochlear gain control. In contrast, mechanical feedback amplification in *Drosophila* is not under efferent control. Silencing transmission via chemical synapses in all neurons, which also disrupts signaling from and to JO-Ns, does not affect the amplificatory gain of the antennal ear (Kamikouchi et al. 2010). Amplification in the fly ear thus seems both generated and controlled locally within JO itself.

7.3.3 Supporting Auditory Transduction: Non-Neuronal Cell Types and Ionic Homeostasis of the Extracellular Space

Next to JO-Ns, JO-SCs have been linked to distinct mechanosensory roles in the *Drosophila* JO. One type of JO-SCs, the cap cell, has been reported to specifically express the TRP channel Pyrexia, which is thought to be required for gravity sensation and gravity-related behaviors (such as the flies' negative geotaxis) (Sun et al. 2009). Another type of JO-SCs, the scolopale cell, has been shown to express specific α- (ATPα) and β- (Nrv2) subunits of the Na^+/K^+-ATPase. The knockdown of either subunit results in virtually complete deafness (Roy et al. 2013). The Na^+/K^+-ATPase of JO-Ns, in contrast, apparently uses a different β-subunit (Nrv3) (Roy et al. 2013). These findings not only highlight the multicellular nature of JO mechanotransduction, but they also stress the importance of ion homeostasis for auditory transducer function. The transducer sites in the JO-N cilia are tightly sealed against their environment by a cellular barrier formed of septate junction–linked epithelial and supporting cells (part of which are both cap and scolopale cells). The narrow cavity that is thus created around the transducers is thought to be filled with a receptor lymph, which differs from the common extracellular condition in that it is high in K^+ and low in Na^+. Electrogenic transport, such as through transmembrane ATPases in both neurons and associated supporting cells, appears to be a crucial requirement to keep the receptor lymph at the reported high positive potentials of +20 to +80 mV relative to the surrounding extracellular medium (Küppers and Thurm 1979; Kernan et al. 1994; Walker et al. 2000; Chung et al. 2001) and thereby providing a strong electrochemical driving force for currents through the transduction channels.

7.3.4 Mechanotransduction in JO: The Cilium and Mechanosensory Submodality

In *Drosophila*, there exist two classes of ciliated cells: spermatozoa and neurons of type I sense organs such as JO. The cilia of JO-Ns, which are located in the apical parts of their dendrites, are an essential component of the cells' mechanosensory organelles. It is the cilia that are widely deemed to host the mechanotransduction machinery proper. A key step in the differentiation of all eukaryotic cilia is the localization, and formation, of the basal body. Basal bodies, which designate the proximal end of the later cilium, serve as nucleation centers from which the microtubular axoneme can grow toward the distal end of the cilium. Ciliary development depends on the Rfx transcription factor (Durand et al. 2000; Laurençon et al. 2007). The ChO-specific transcriptional regulator Fd3F co-regulates chordotonal-specific ciliary genes in tandem with the pan-ciliary transcription factor Rfx (Newton et al. 2012). Several gene products have been localized to, and implicated in the function of, the basal bodies of JO-Ns; these include, for example, the coiled-coil domain proteins Chibby (Cby) (Enjolras et al. 2012), Uncoordinated (Unc) (Kernan et al. 1994; Baker et al. 2004), and Dilatory (Dila) (Ma and Jarman 2011). From the basal body, the sensory cilium is assembled through an intraflagellar transport (IFT) process that includes the anterograde kinesin II motor complex (Sarpal et al. 2003) and the IFT protein No mechanoreceptor potential B (NompB) (Han et al. 2003), as well as the retrograde dynein heavy chain 1b motor and the IFT protein Reduced mechanoreceptor potential A (RempA) (Lee et al. 2008). The fully differentiated cilium of JO-Ns is a highly compartmentalized subcellular structure specialized for the transmission, and transduction, of mechanical stimuli; a vital part of JO-N ciliogenesis is therefore the generation of JO-specific ciliary compartments. RempA is crucial for this subcompartmentalization (Lee et al. 2008), and the microtubule-associated doublecortin homolog-containing DCX-EMAP is required for the differentiation (Bechstedt et al. 2010). The Fd3F-dependent transcriptional control also includes a direct regulation of Nan and Iav, the two interdependent TRVP-channel proteins required for JO auditory function (Gong et al. 2004).

As detailed in Sect. 7.3.1, multiple ion channels have been linked to the cell-type-specific properties, and overall mechanosensory function, of JO. The sequence of events that leads from a sound-induced displacement of the dendritic cap to the generation of action potentials in the JO-N axons appears to involve a signaling chain through various TRP channels located at characteristic positions within the distal, or proximal, cilium. A *Drosophila* member of the Tubby-like protein (TULP) family, dTulp, is required for the correct TRP-channel localization (Park et al. 2013).

The transmission, transduction, and amplification of sound-induced mechanical stimuli performed by JO are biophysically and molecularly complex processes, which have been the subject of intense scientific enquiry and progress during the past decade; through the identification of some of its key developmental and homeostatic requirements, its sensory complexity is becoming better understood. The different neuronal subpopulations of JO, which vary in their respective sensitivities to oscillatory (e.g., sound) or stationary (e.g., wind/gravity) stimulation have initially been defined anatomically by their distinct target zones within the fly's brain (Kamikouchi et al. 2006). The homeodomain transcription factor Engrailed (En) is a sufficient, and most likely necessary, requirement for the subpopulation-specific axonal targeting of JO auditory neurons to the GFN (Pézier and Blagburn 2013; Pézier et al. 2014). Misexpression of En in En-negative JO-Ns causes them to form ectopic chemical and electrical synapses with the GFN; RNAi-mediated knockdown of En expression in En-positive JONs, in turn, reduces the strengths of JO-N to GFN synaptic connections. This finding is of particular interest as En is also a key regulatory factor for guidance cues that pattern retinal axon terminals in the vertebrate midbrain (Fuchs et al. 2012).

7.3.5 *Beyond the Mechanosensory Canon: Unorthodox Findings from the* Drosophila *JO*

Genetic inventories of fly (Eberl et al. 1997) and nematode (Chalfie and Sulston 1981; Chalfie and Au 1989) mechanosensory organs have provided the fundament for understanding the molecular logic of mechanosensory, and also specifically auditory, transduction. The various findings have been integrated into a canonical view that rests firmly on the interaction of three key players: ion channels, motor proteins, and cytoskeletal tethers (Chalfie 2009). A microarray-based transcriptome analysis of the *Drosophila* JO (Senthilan et al. 2012) largely confirmed the prevailing view but added a few twists to the story by reporting the expression of key components of the phototransduction cascade, including four of the fly's seven rhodopsins (Rh3, Rh4, Rh5, Rh6). Furthermore, it was shown that loss-of-function mutations of these rhodopsins greatly reduce both mechanical and electrical signatures of mechanotransducer gating in the fly's antennal ear. These findings point to specific, but as yet unknown, roles of "visual" proteins in mechanosensation and may well mark the beginning of a redefined concept of sensory specificity and its molecular requirements. The same screen, most notably, also found the pheromone-binding protein Os-C, and several ionotropic receptors, currently associated with chemosensory transduction, expressed in the *Drosophila* ear; their roles, however, have not yet been tested.

7.4 Summary

The hearing organs of insects, and the acoustic communication systems they are part of, have been the subject of intense scientific enquiry for many decades. But it was only during the last 15 years or so that we have seen substantial, and rapidly growing, progress in our understanding of the very process that brings about hearing, namely, the process of auditory transduction. Fueled by the genomic revolution and pioneered by an insect, *Drosophila melanogaster*, new ion channel families (such as TRP channels) have been identified and linked to specific roles within the auditory transduction chain. But in research as in life, the devil is in the details and the proof is in the pudding and so we are still lacking unequivocal evidence demonstrating which proteins actually form the auditory transducer channels proper. The current controversy has it that this is either NompC or a heteromeric complex built of Nan and Iav. For both possibilities there are supporting as well as contradicting data. Recent research has zoomed in on auditory transducer complexes in unprecedented molecular detail but, it seems, still not close enough to clearly resolve the component identities and their specific mechanisms. On a much more general level, and light years away from the missing final details, the molecular inventory of the *Drosophila* ear has brought to light substantial, but mechanistically as yet elusive, contributions of visual proteins, such as rhodopsins, to the process of auditory mechanotransduction. These findings shake up our concepts of sensory specificity and will clearly be hot spots of future auditory research.

Various insect ears of both the tympanal and flagellar type have been shown to display the hallmarks of active amplification (as first proposed for the human cochlea). It is currently not clear if a transducer-based feedback mechanism, such as the one reported for *Drosophila*, is the common molecular source of the observed amplification; if this turns out to be the case, it will be very interesting to see if transducer-based feedback amplifiers have evolved independently in different insect groups or rather share a common origin. Furthermore, among the JO-Ns in *Drosophila*, only two major classes have been identified, those that underlie hearing and are likely to be phasic (very fast adapting) and those that subserve wind and gravity and are likely to be more tonic (slowly adapting). This relative simplicity is an advantage for fundamental investigations of transduction mechanisms and their genetic basis, but in time one hopes to understand the complexities that contribute to the broader scolopidial physiological variation. All taken together, research on insect auditory transduction is thus a prime example to show that basic research, more than anything else, deals with the unknown unknowns. If conducted properly, it will often discover more new questions than the many answers to old ones.

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Chapter 8
Central Neural Processing of Sound Signals in Insects

Berthold Hedwig and Andreas Stumpner

Abstract The sense of hearing contributes importantly to an animal's fitness. It allows detection of predators and prey and communication with conspecifics even in the dark and over large distances. Hearing organs evolved in about 20 groups of insects. Hearing is used by moths and other insects for avoiding predatory bats; by cicada, crickets/bushcrickets, moths, and grasshoppers for intraspecific communication; and by parasitic flies to locate singing hosts. Despite the variety of these insect groups, the neural processing of sound signals faces very similar fundamental challenges related to signal detection, directional processing, frequency discrimination, pattern recognition, and coping with self-generated noise. Solutions to these problems are implemented by specific network, cellular, and synaptic properties of neural circuits. Owing to their rather simple organization, insect auditory pathways can be explored and analyzed at the level of identified neurons to reveal fundamental mechanisms of auditory processing.

Keywords Auditory pathway • Corollary discharge • Feature detection • Interneurons • Neuropil • Pattern recognition • Reciprocal inhibition • Tonotopic organization

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8.1 Introduction

The sense of hearing contributes importantly to an animal's fitness. It allows detection of predators and prey and communication with conspecifics even in the dark and over large distances. It is therefore not surprising that, driven by natural and sexual selection, tympanal hearing organs evolved in about 20 groups of insects (Yager 1999; Strauß and Lakes-Harlan 2014; see Greenfield, Chapter 2). Hearing is used by moths and other insects for avoiding predatory bats; by cicada, crickets/bushcrickets, moths, and grasshoppers for intraspecific communication; and by parasitic flies to locate singing hosts. Despite the variety of these insect groups, the neural processing of sound signals faces very similar fundamental challenges related to signal detection, directional processing, frequency discrimination, pattern recognition, and coping with self-generated noise (Pollack 1998; Stumpner and von Helversen 2001; Hennig et al. 2004; Hedwig and Pollack 2008).

The challenges of auditory processing are similar not only across different groups of insects but also between insects and hearing vertebrates. What are the neural principles and mechanisms underlying auditory processing? Acoustic signals are first coded by a population of sensory afferent neurons that carry their spike activity to the central nervous system (CNS). Central neural mechanisms refine the functional properties of the auditory pathway through specific network, cellular, and synaptic mechanisms. As an advantage due to their rather simple organization, the auditory pathways in insects can be explored and analyzed at the level of identified neurons. Here the focus is on the processing of intraspecific communication signals for mate attraction; a review of auditory predator avoidance is given in Chapter 4 by Pollack (2015).

8.2 Overview of Central Auditory Pathways

Despite the variety in insect appearance and body structure, the “Bauplan” and organization of the CNS are highly conserved. The CNS comprises a series of segmental ganglia linked by longitudinal fiber tracts. Within the ganglia, specific regions of neuropils concerned with processing of specific types of sensory information can be recognized across insects. In some cases, individually identified nerve cells are homologous between segmental ganglia or even across insect groups (Boyan 1993). Developmental and evolutionary evidence (Yager 1999; Strauß and Lakes-Harlan 2014) indicate that hearing organs derived from chordotonal organs, which are mechanosensory structures found in many regions of the body in insects. Chordotonal-derived ears have evolved in the legs of crickets and bushcrickets, the lateral body wall of grasshoppers, the prosternum (chest) of parasitoid flies, a variety of locations in moths, and elsewhere in other insects (Fullard and Yack

1993; Fig. 8.1A). Owing to the preestablished afferent projection patterns of the nonauditory precursor organs within the CNS, afferent projections of auditory neurons, which may originate at very different locations, reveal a set of common features (Fig. 8.1B). Like many chordotonal afferents, the axonal projections within the CNS do not cross the midline and stay strictly ipsilateral with respect to the auditory organ. In the body segment that carries the auditory organ, afferent projections are restricted to the corresponding ganglion (e.g., in crickets, bushcrickets) or extend over several segments (e.g., in grasshoppers, mantis, cicada, moths, flies). Like other mechanosensory afferents, auditory afferents terminate in neuropils known as the ventral association centers of the thoracic ganglia (Fig. 8.1C), where they may form a specific “auditory neuropil.” This neuropil is prominent in species with well-developed auditory pathways and may be tonotopically arranged in species with elaborate frequency processing (Römer et al. 1988; see Sect. 8.5).

The number of auditory afferents varies greatly among species; some moths employ only a single afferent and cicada, which have the most complex communication signals, use more than thousand (Yack 2004). At the thoracic level, activity is distributed to and processed by several classes of “auditory neurons”; see Boyan (1984) for a critical discussion of the term. Local neurons are contained entirely within a single segmental ganglion, ascending and descending neurons project to more anterior and more posterior ganglia, respectively, and so-called T-shaped neurons have both ascending and descending projections (Fig. 8.2). Many local neurons exhibit a bilateral functional differentiation, receiving input from afferents on one side of the ganglion and providing output via axonal projections to the contralateral neuropil, allowing for first-order binaural processing (see Sect. 8.4) in which also nonspiking interneurons may be involved (Stiedl et al. 1997). The dendrites of ascending interneurons may not be restricted to the auditory neuropil as they may receive inputs from other sensory pathways, for example, vibration. The axon can have projections on the contralateral side of the ganglion for local bilateral processing or forward activity directly toward auditory circuits in the brain. In Ensifera (i.e., crickets/bushcrickets) in which the auditory neuropil is located in the prothoracic ganglion, descending and T-shaped neurons carry auditory activity also toward the posterior thoracic ganglia, where it may be integrated into local motor circuits.

The number of ascending interneurons varies across taxa, revealing that different neuronal circuits evolved for auditory processing; there are only two in crickets (Wohlers and Huber 1982) and at least 4–5 in bushcrickets (Stumpner and Nowotny 2014), 15–20 in grasshoppers (Römer and Marquart 1984; Stumpner and Ronacher 1991), 6 in moths (Boyan and Fullard 1986), 3 in flies (Stumpner and Lakes-Harlan 1996), and 15 in cicadas (Fonseca and Correia 2007; Fonseca 2014), respectively. In some species, response properties of identified ascending neurons have been correlated with different aspects of auditory processing, including intensity tuning (see Sects. 8.3 and 8.5), directional tuning (see Sect. 8.4), and frequency tuning (see Sect. 8.5), indicating that already at the thoracic level, activity across the population of afferents is not just summed but also specifically processed before it is forwarded to the brain. The characteristic projection patterns of the ascending neurons

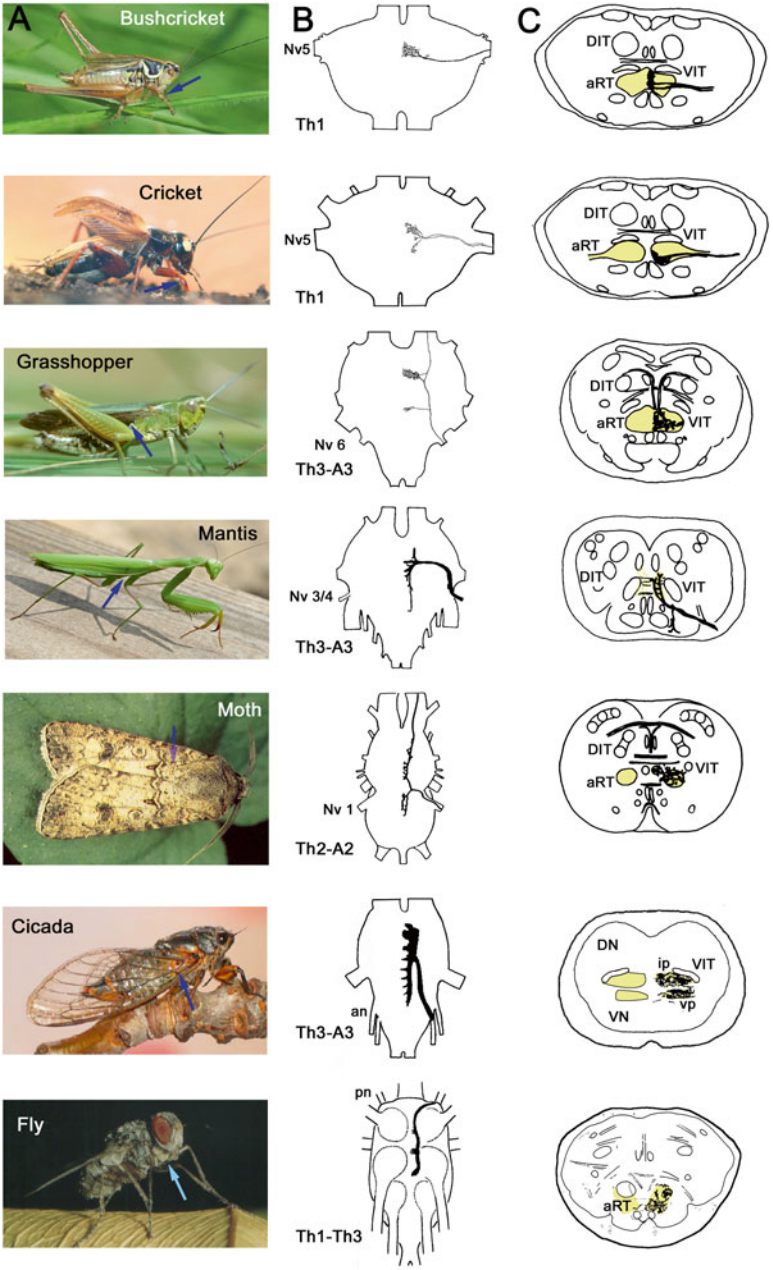

Fig. 8.1 Central auditory pathways. (**A**) Examples of insect taxa with tympanal hearing organs; position of organs indicated by *arrow*. (**B**) The central projection pattern of auditory afferents within the thoracic ganglia. (**C**) Details of the afferent axonal arborizations and auditory neuropils (marked in *yellow*) in the corresponding ganglia as indicated by transverse sections. [Image of mantis courtesy of C. Galand (www.entomart.be), image of moth courtesy of A. Surlykke, image of fly courtesy of K. G. Heller, all other images by the authors. Bushcricket afferent from Römer et al. (1988), cricket afferent after Eibl and Huber (1979), grasshopper afferent from Hedwig (1988), mantis afferent and section after Yager and Hoy (1987), moth afferent and section modified from Boyan et al. (1990), cicada afferent and section after Wohlers et al. (1979), fly afferent and

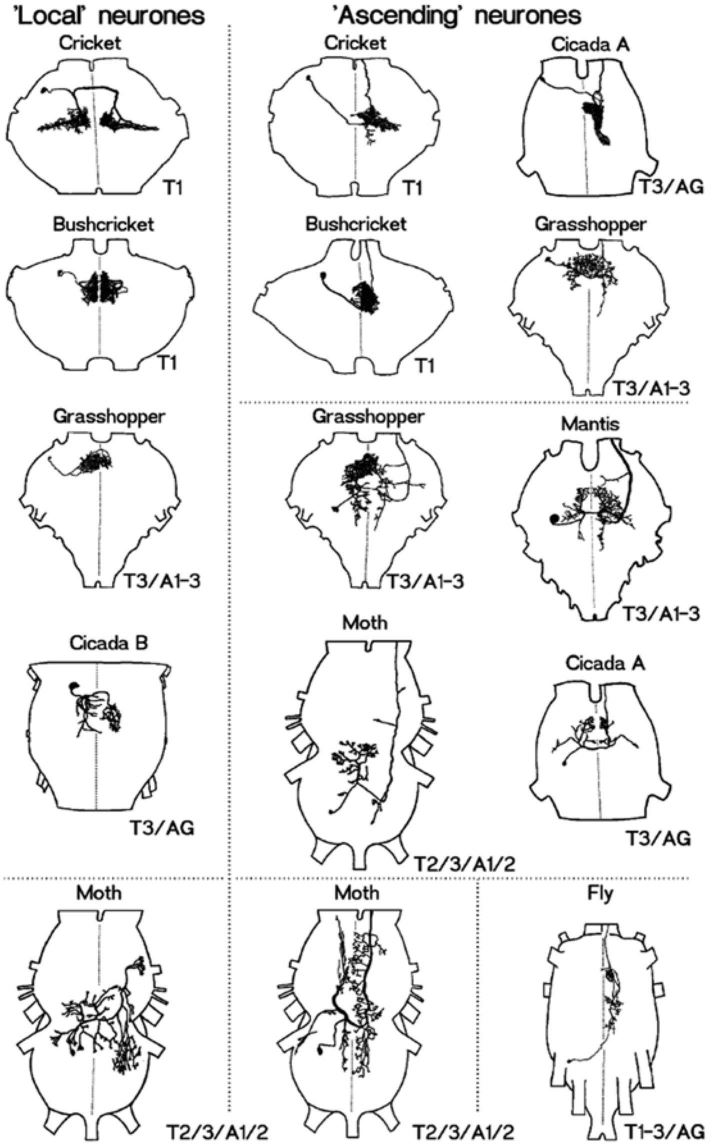

Fig. 8.2 Neuron types. Thoracic local and ascending auditory interneurons in different insects. Note the structural similarity between neurons within one class [From Stumpner and von Helversen (2001) with permission]

Fig. 8.1 (continued) section from Lakes-Harlan et al. (1999) and Stumpner et al. (2007), sections of bushcricket, cricket, and grasshopper modified from Boyan (1993).] an, auditory nerve; pn, prosternal nerve; DIT, dorsal intermediate tract; VIT, ventral intermediate tract; aRT, anterior ring tract; DN, dorsal neuropil; VN, ventral neuropil; vp and ip, ventral and intermediate projections of the sensory neurons, respectively (see Sect. 8.6), together with behavioral evidence obtained after local temperature changes of the CNS (Bauer and von Helversen 1987) and after connective-lesion experiments (Pollack and Hoy 1981; Nolen and Hoy 1984; Dawson and Fullard 1995), indicate that the brain controls acoustically mediated behavior.

8.3 Intensity Coding

8.3.1 Overview

Intensity coding is important for judging the distance of a sound source, for directional decisions and may allow differentiating between individual conspecifics when independent (e.g., spectral) information about distance is available. It is based on the activity level provided by the auditory afferents in response to a sound stimulus of a given intensity (Fig. 8.3A, B).

Auditory afferents typically have a working range of 15–25 dB or occasionally up to 40 dB (Fig. 8.3B, C) between threshold and saturation (Mason and Faure 2004). They may also show nonmonotonic intensity response functions with decreasing spike rates at high intensities (e.g., in moths; Coro and Perez 1993; Fullard et al. 1998), which may be caused by mechanical properties of the ear. Within a population of afferents, individual cells often differ in sensitivity, so that their individual dynamic ranges begin at different threshold sound levels. This so-called range fractionation increases the overall dynamic range of the system (Rheinlaender 1975; Oshinsky and Hoy 2002).

The intensity dependence of auditory afferent responses translates stimulus amplitude into specific activity levels of first-order thoracic interneurons. Inhibitory neurons in the auditory pathway, with receptor-like phaso-tonic response patterns, are the basis for subsequent processing via reciprocal or lateral inhibition to sharpen directional and frequency-specific responses. In many interneurons, this leads to nonmonotonic intensity response functions (see Sect. 8.5).

Spike rate as well as response latency of sensory neurons and interneurons depends on sound intensity, with latency decreasing and spike rate increasing with intensity (e.g., Yager and Hoy 1989; Imaizumi and Pollack 2001).

→

Fig. 8.3 (continued) of the cricket *T. oceanicus* (4.5 kHz, 500 ms). The three curves are based on recordings at three different intensities. The inset below shows the stimulus and raster plots of five exemplary responses. (**F**) Intensity response functions of neurons in different insects, all responding (phaso-)tonically to white noise stimuli or stimuli at their preferred frequency. Note, the *x*-axis does not give absolute values; curves separated for clarity. Flies: *Homotrixa alleni* and *Therobia leonidei*; mantis: *Mantis religiosa*; cicada: *Tettigetta josei*; cricket: *Gryllus bimaculatus*; grasshopper: *Chorthippus biguttulus*; bushcricket: *Neoconocephalus ensiger* [(**A**) from Boyan and Fullard (1986); (**B**) modified after Boyan and Fullard (1988); (**C**) modified after Tougaard (1998); (**D**) modified after Surlykke et al. (1988) and Sabourin et al. (2008); (**E**) modified after Benda and Hennig (2008); (**F**) modified after Schildberger (1984), Yager and Hoy (1989), Stumpner and Ronacher (1991), Stumpner and Lakes-Harlan (1996), Münch (1999), Faure and Hoy (2000), and Stumpner et al. (2007); with permission]

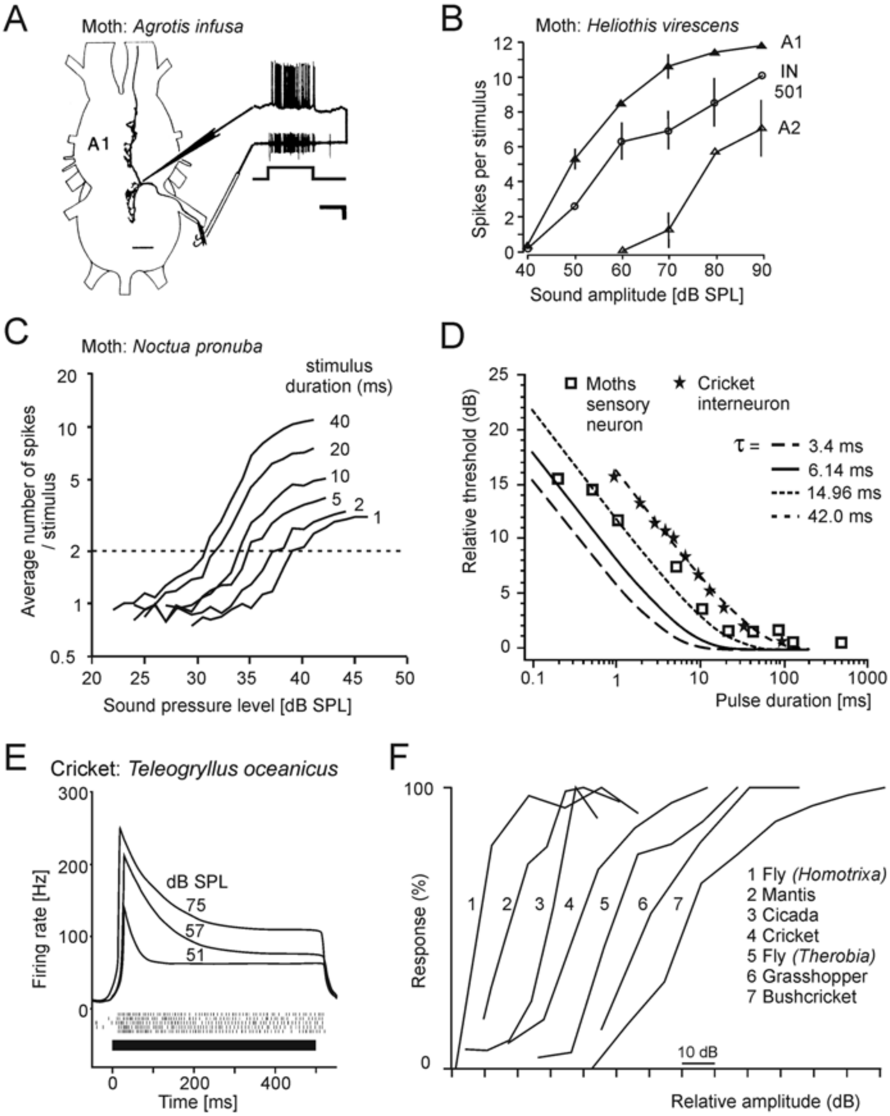

Fig. 8.3 Intensity functions. "Simple" responses and integration properties of primary sensory neurons and interneurons. (**A**) Extracellular and intracellular recording from an auditory sensory neuron (A1) in the moth *Agrotis infusa*. (**B**) Mean spiking response (±SD) of both auditory sensory neurons (A1, A2) and a tonically responding interneuron (IN 501) to 10-ms stimuli (16 kHz) of increasing sound amplitude. (**C**) Intensity response function of the A1 sensory neuron of the moth *Noctua pronuba* to stimuli of different durations. The stippled line indicates a threshold at two spikes per stimulus for calculation of data as in (**D**). (**D**) Dependence of threshold on pulse duration in sensory neurons and interneurons and graphs for various integration time constants [τ; $f(t) = -10 \log (1 - e^{-t/\tau})$] for two example insect neurons with $\tau = 14.96$ ms and 42.0 ms; data points from moth: *Agrotis segetum* sensory neuron ($n = 9$) and cricket: *Teleogryllus oceanicus* ON1 interneuron ($n = 11$). (**E**) Spike frequency adaptation in a tonically responding interneuron (AN1)

8.3.2 Temporal Integration

Insect ears integrate energy in the temporal and in the spectral domain (Tougaard 1998; Gollisch et al. 2002). As a consequence, the threshold of auditory primary sensory neurons depends on the intensity and duration of a stimulus. In a moth sensory neuron, the threshold decreases by up to 20 dB between 0.1-ms and about 30-ms pulse duration (Fig. 8.3C, D; Surlykke et al. 1988). In the simplest case, first-order local or ascending auditory interneurons show afferent-like responses with respect to stimulus integration times, and thresholds, like those of afferents, decrease with increasing stimulus duration (Fig. 8.3D) (Faure and Hoy 2000; Sabourin et al. 2008). The integration time constants of sensory neurons and interneurons range between 6 and 70 ms and clearly depend on carrier frequency, with a considerably shorter time constant at an ultrasonic compared to a sonic frequency. These values, however, are affected by additional factors such as the nonlinearity introduced through choosing a certain spiking response as threshold criterion. Using methods that avoid such nonlinearities, an energy detector in moths has been estimated to have a time constant shorter than 4 ms (Tougaard 1998).

Temporal integration also can be a means of protecting the animal from false alarms. Neural filtering against low-intensity background noise, for example, occurs at the level of single thoracic neurons in moths and crickets/bushcrickets. In an identified moth interneuron, the amplitudes and integration times of afferent-triggered excitatory postsynaptic potentials (EPSPs) are such as to cause only brief, subthreshold interneuron responses when the afferent fires below approximately 100 spikes/s, as occurs during spontaneous activity or when the insect is exposed to low-intensity ultrasound (Boyan and Fullard 1988; Fullard 1998). Only high-rate firing of the afferent, as elicited by the echolocation calls of hunting bats, can depolarize the interneuron to spiking threshold.

8.3.3 Adaptation

Responses of auditory primary sensory neurons generally copy the amplitude envelopes of pulsed sound stimuli, but they are phaso-tonic in nature. That is, for long-lasting stimuli, the initially high firing rate drops by 20–50 %, reaching a steady-state level only after about 100 ms (e.g., Fullard et al. 1998; Gollisch et al. 2002). This drop of activity to an unchanged stimulus is called adaptation and generally facilitates the detection of changes in stimulus level and helps to maintain a neuron's responsiveness. Although the rate and extent of adaptation vary according to neuron type, sound intensity, and in some cases carrier frequency, the overall effect is to emphasize pulse onsets and brief pulses in acoustic signals (Ronacher and Hennig 2004). Extremely phasic responses occur in some receptor neurons of the parasitoid fly *Ormia ochracea*, which produces only a single spike at stimulus onset regardless of stimulus duration or intensity (Oshinsky and Hoy 2002).

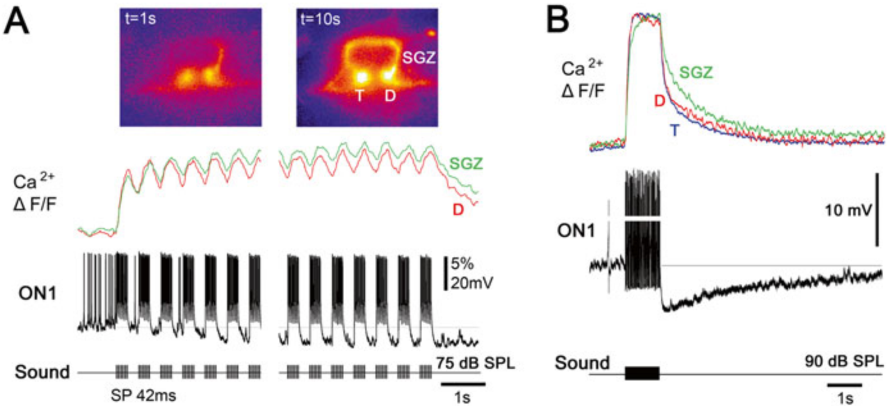

Fig. 8.4 Imaging calcium changes during signal processing. Changes in cytosolic calcium concentration as indicated by Oregon Green BAPTA-1 and intracellular recorded neuron activity during acoustic stimulation in a cricket ON1 neuron. (**A**) Repetitive stimulation with calling song causes an increase in the calcium indicator fluorescence signal that is modulated in the pattern of the sound stimulus and coupled to the spike activity. Over the course of acoustic stimulation, the membrane potential becomes more negative in line with the calcium increase; the initial background activity of the neuron is suppressed. (**B**) Calcium increase and neural activity during a 1-s acoustic stimulus. After the stimulus, the calcium signal gradually decreases in the different compartments of the neuron and the membrane potential recovers from hyperpolarisation. D, dendrites; T, axon terminals; SGZ, spike-generating zone; SP, syllable period [From Baden and Hedwig (2007) with permission]

As in receptor neurons, adaptation in tonic interneurons depends on intensity and can be fitted to linear first-order dynamics (Fig. 8.3E; Benda and Hennig 2008). Also like receptors, interneurons, in all taxa, may exhibit phaso-tonic firing patterns and saturating intensity response functions (Fig. 8.3B, F). The spike activity of these neurons copies the amplitude modulation of the stimulus (see Ronacher, Chapter 9) and forwards this activity for further processing toward the brain (see Sect. 8.6). In interneurons that integrate the inputs from many sensory neurons, the dynamic range may reach 60 dB or more (Rheinlaender 1975; Faure and Hoy 2000).

Intensity filtering provides a mechanism akin to selective attention. The cricket ON1 interneuron and thoracic interneurons in bushcrickets will respond to the louder sound only when two series of low- and high-intensity pulses are presented simultaneously (Pollack 1988; Römer and Krusch 2000). Continuous acoustic stimulation causes a gradual hyperpolarization of these interneurons, with the consequence that EPSPs triggered by low-amplitude stimuli, which are effective to elicit spiking when presented alone, remain subthreshold. The hyperpolarization of the membrane potential is coupled to an increase in the cytosolic calcium level (Sobel and Tank 1994; Baden and Hedwig 2007), which in turn is thought to activate an outward potassium current with a time constant of several seconds (Fig. 8.4A, B).

The response characteristics of interneurons depend on ambient temperature as insects are ectothermic organisms (Janiszewski and Otto 1989); robust auditory processing therefore needs to compensate for any changes in overall activity level.

8.4 Directional Processing

8.4.1 Background and Behavior

Directional processing of sounds is crucial for phonotactic orientation toward mates or prey and for predator escape responses. These behaviors pose different demands on auditory processing. During a phonotactic approach, sound intensity will increase, whereas during an escape it will decrease; also, there is only one correct direction in an auditory approach, whereas for an escape there are many, making escape responses generally less directional.

For the detection of sound direction, binaural animals rely on differences in responses of the two ears and may therefore exploit interaural time differences (ITDs) and/or interaural level differences (ILDs). These bilateral differences decline to zero as the angle of sound incidence approaches the animal's longitudinal axis. In insects, because of their small size, the bilateral time differences per se may be minute as sound takes only approximately 15 μs to travel between ears 5 mm apart. ILDs caused by sound diffraction depend on sound frequency and body size, which has a stronger effect on signals with short wavelength. As a result of sound diffraction at a cricket's body, ILDs for the calling song may be in the range of only 1–2 dB (Michelsen et al. 1994) and may not be detectable at all in parasitoid flies that localize the same signals (Robert et al. 1996). The biomechanical properties of the hearing apparatus, however, may transform and enhance the biophysical differences (Robert et al. 1996; Michelsen 1998), leading to significant bilateral differences in auditory afferent activity (see Windmill and Jackson, Chapter 6).

Different species show varying degrees of accuracy in orienting toward a sound source. Acoustically guided turning behavior of male grasshoppers is almost errorless when sound arrives from the side but becomes inaccurate in the frontal ±30° (von Helversen 1997). Bushcrickets require stimulus angles of 6–10° and 1-dB amplitude difference to turn significantly toward the more strongly stimulated side (Rheinlaender et al. 2006; Römer 2015). In contrast, parasitoid flies (Mason et al. 2001) and crickets (Schöneich and Hedwig 2010) demonstrate hyperacute directional sensitivity, especially in the frontal range of sound incidence where, in crickets, a bilateral intensity difference of only 0.4 dB is sufficient for precise orientation.

Three parameters of the bilateral afferent activity can be used to determine sound direction (Mason and Faure 2004). First, at the population level, a larger number of afferents will respond in the auditory organ driven by the louder sound (Madsen and Miller 1987; Oshinsky and Hoy 2002). Second, firing rates and spike counts of individual afferents will increase with increasing stimulus strength. Finally, response latency, which may be coded with extremely low temporal jitter (Oshinsky and Hoy 2002), decreases with increasing stimulus level. Spike rate and response latency are physiologically tightly coupled (Mörchen et al. 1978), but they can be dissociated under experimental conditions to reveal their individual impacts on the activity of directional interneurons (Rheinlaender and Mörchen 1979) and on behavior (von Helversen and Rheinlaender 1988). In behavioral tests on acridid grasshoppers, bilateral differences in stimulus level of 1.0 dB, and 0.5-ms latency differences are sufficient to allow reliable orientation to the side of the louder or earlier sound.

Directional responses at the interneuron level arise from a combination of excitation by ipsilateral acoustic stimuli and inhibition by contralateral stimuli (Gerhardt and Huber 2002; Hedwig and Pollack 2008). This processing of afferent inputs at the level of the thoracic interneurons enhances bilateral auditory contrast. The underlying neural mechanism is based on reciprocal or recurrent inhibition and is best understood in a pair of mirror-symmetrical omega-shaped interneurons (ON1; Fig. 8.5A) in the cricket prothoracic ganglion (Wohlers and Huber 1982; Wiese and Eilts-Grimm 1985). Dendrites of ON1 are restricted to one side of the ganglion, and the axonal projections overlap with the dendritic arborization of the contralateral ON1 neuron. Each neuron receives excitatory input from the afferents ipsilateral to its dendrites and monosynaptically inhibits its contralateral partner (Fig. 8.5B, C; Selverston et al. 1985). Owing to their reciprocal inhibitory connections, the ON1 neuron that is activated with a shorter latency and stronger excitation will inhibit its contralateral partner, reducing excitation and thereby also diminishing any recurrent inhibition (Fig. 8.5C). With this mechanism in place, directionality at the interneuronal level is greatly enhanced in comparison to the afferent activity (Fig. 8.5D; Boyd and Lewis 1983; Larsen et al. 1989).

As the interneuron responses become side specific, they clearly separate the left and right auditory hemispheres except in the frontal region, where the difference in activity of the left and right neurons varies linearly with stimulus direction (Fig. 8.5E). This characteristic of direction-dependent responses due to reciprocal inhibition also occurs in the ON1 of bushcrickets (Römer and Krusch 2000; Molina and Stumpner 2005). A modeling approach to the function of the inhibitory circuitry, however, implies a less significant effect of contralateral inhibition (Horseman and Huber 1994).

Bush- and tree-dwelling insects need to orient in a complex three-dimensional habitat, where orientation in elevation, as well as in azimuth, is necessary. Bushcrickets employ active scanning movements with their body that in principle could provide information about the elevation of a sound source and may require comparison of sequentially acquired auditory activity. Processing of elevation cues has recently been explored at the peripheral and central level of neuronal responses (Kostarakos et al. 2007; Römer 2015; Lakes-Harlan and Scherberich 2015).

8.4.2 *Integration of Directional Cues with Motor Responses*

Little is understood at a cellular level about how directional auditory cues are integrated into motor activity. Within the CNS, two different routes may be employed. Afferent activity could be forwarded directly to motor networks, resulting in bilaterally different reflex-like motor responses as in negative phonotaxis of flying

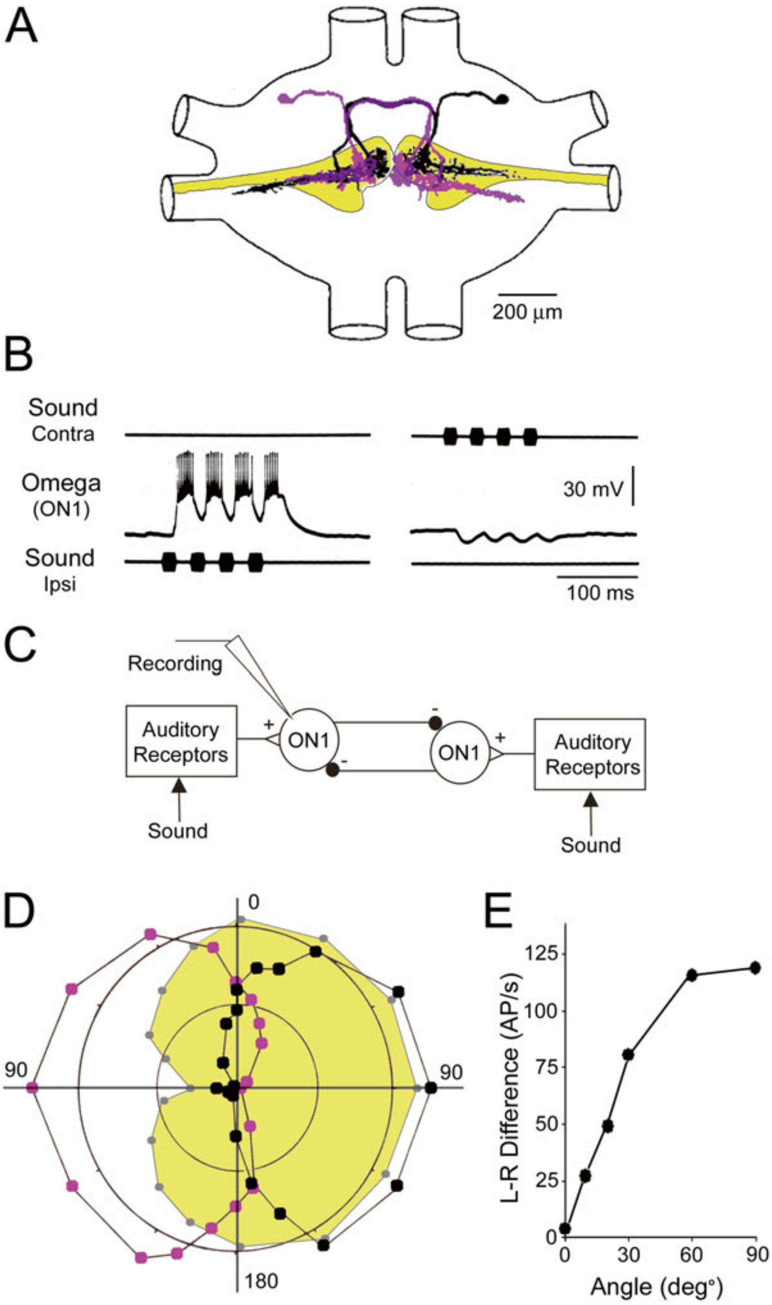

Fig. 8.5 Directional processing. Bilateral auditory contrast enhancement by reciprocal inhibition in the cricket auditory pathway. (**A**) Projection of auditory afferents (*yellow*) and of the left (*magenta*) and right (*black*) omega neurons (ON1) in the prothoracic ganglion. Dendritic and axonal arborizations of the neurons overlap with the afferent projections. (**B**) Acoustic stimulation of the ipsilateral ear elicits EPSPs and a spiking response whereas stimulation of the contralateral ear

crickets (Pollack and Hoy 1981) or as indicated by the short latency steering responses of phonotactically walking crickets (Hedwig and Poulet 2004). In the locust, interneurons receiving auditory inputs evoke flight motor and steering responses (Boyan 1984; Baader 1991), and a particular multimodal neuron, can trigger hind leg motor responses (Pearson et al. 1980). As another possibility, directional information is processed in the brain and leads to precise descending directional steering commands. Such commands have not yet been identified at the neural level.

8.5 Frequency Processing

8.5.1 Hearing Ranges and Organization of Afferents

Hearing in insects covers the sonic and ultrasonic ranges (Pollack and Imaizumi 1999). Sound frequency may be used to discriminate among predators, heterospecifics, and conspecifics but also to distinguish rivals from mating partners (von Helversen and von Helversen 1997; Pollack 2015). Frequency processing starts with a biomechanical frequency segregation in the sound-sensitive structures of the ear (see Windmill and Jackson, Chapter 6), which provides the basis for frequency tuning of auditory afferents in various types of ears (Hedwig and Pollack 2008). In moths, which have only a few primary auditory neurons, each afferent represents the full hearing range of the species (about 5 kHz to more than 100 kHz) and there is no basis for frequency discrimination in the auditory pathway (Surlykke 1984). In contrast, in the hearing organ of bushcrickets, up to 40 or more sensory neurons are each specifically tuned to a different sound frequency. Their cell bodies are arranged strictly tonotopically in the hearing organ as are their central axonal projections in the auditory neuropil (Fig. 8.6A, B; Oldfield 1983; Römer 1983). The overall hearing range, from about 2 kHz to 80 kHz or higher, is much broader than that of a single sensory neuron and provides the basis for subsequent frequency processing within the CNS (Stölting and Stumpner 1998).

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Fig. 8.5 (continued) causes only IPSPs (inhibitory postsynaptic potential). (**C**) Diagram for the reciprocal inhibition circuit in the auditory pathway. Each ON1 also inhibits contralateral ascending interneurons, which are not shown. (**D**) Directional response of the right auditory afferents (*yellow*) and of the left (*magenta*) and right (*black*) ON1 neuron. (**E**) The difference in bilateral ON1 activity of the animal, calculated from the data in (**D**), indicates in the frontal range (±30°) a linear relation to the angle of incidence with a slope of about 3 AP/s per degree. Inner and outer circles in (**D**) indicate 50 AP/s and 100 AP/s activity level for ON1; the maximum left–right response difference of the afferents corresponds to an intensity difference of 25 dB [ON1 structure and afferent projections redrawn from Wohlers and Huber (1985); intracellular recording of ON1 from Wohlers and Huber (1982) with permission; afferent activity redrawn from Boyd and Lewis (1983); ON1 activity redrawn from Wiese and Eilts-Grimm (1985)]

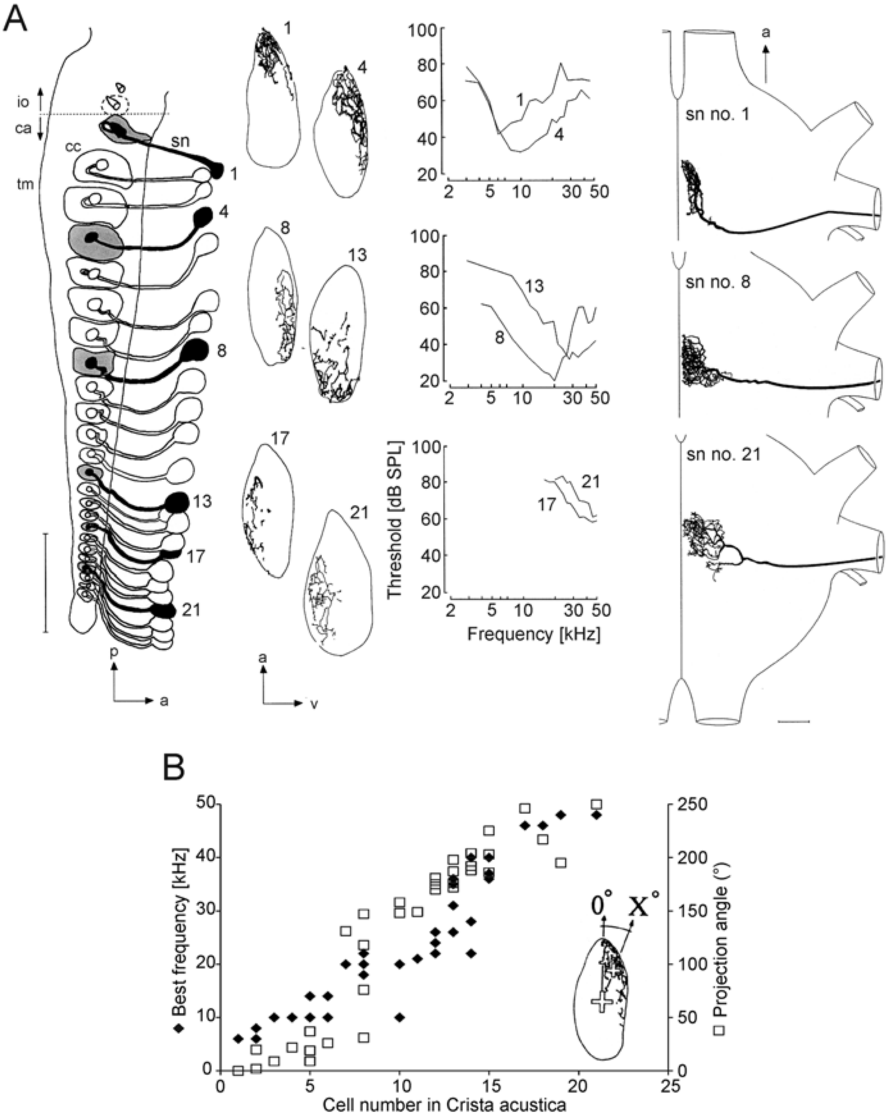

Fig. 8.6 Tonotopy. Arrangement of sensory neurons in the hearing organ (crista acustica) and projection of the sensory axons into the prothoracic ganglion of the bushcricket (*Pholidoptera griseoaptera*). (**A**) Six out of 24 cells in the crista acustica are marked (*black*) and their projection in the auditory neuropil (sagittal sections) and their frequency tuning are shown. Numbers refer to the position in the crista acustica. For three cells, the view of the prothoracic projection in the horizontal plane is given. ca, crista acustica; io, intermediate organ; tm, tectorial membrane. (**B**) Frequency of peak sensitivity ("best frequency") and projection angle ("X°") within the auditory neuropil (see inset; orientation as in **A**) for various sensory neurons recorded in different individuals of *P. griseoaptera* [Modified after Stölting and Stumpner (1998) with permission]

Grasshoppers and crickets have two hearing ranges peaking at lower sonic and ultrasonic frequencies. Even though there are three or four classes of sensory neurons in grasshoppers, their sensitivity clearly is highest either at low or at high frequencies (Halex et al. 1988; Jacobs et al. 1999). The hearing range of crickets is similarly organized to that of grasshoppers (Imaizumi and Pollack 1999). There is a categorical processing of frequencies in crickets, which show positive phonotaxis to sound signals in the sonic range and negative phonotaxis to ultrasonic signals, as in bat avoidance behavior (e.g., Wyttenbach et al. 1996). A number of further taxa, however, are not yet well studied. Cicadas can have extremely complex amplitude- and frequency-modulated calling songs (Gogala et al. 2004). Ears with more than 1,000 auditory afferents and the responses of interneurons indicate sophisticated frequency processing in the CNS (Fonseca et al. 2000; Fonseca 2014). Also in parasitoid flies, with about 50–250 sensory neurons per ear, both physiological (Stumpner et al. 2007) and behavioral (Rosen et al. 2009) results demonstrate frequency discrimination.

8.5.2 Afferent Activity Is Sharpened by Presynaptic Inhibition

In the CNS, frequency processing occurs as early as at the terminals of the afferent neurons, which are subject to frequency-specific presynaptic inhibition. Presynaptic inhibition modulates the efficiency of synaptic transmission. It is mediated by GABAergic (GABA = γ-aminobutyric acid) inputs to the afferent terminals, which cause a depolarization of membrane potential (primary afferent depolarization [PAD]) due to an increased chloride conductance. The conductance increase reduces the amplitude of the invading spikes, with the result that the release of neurotransmitter is also reduced (Watson et al. 2005). Close to the axon terminals of bushcricket afferents, PADs occur that are tightly coupled to the invading spikes (Fig. 8.7A; Baden and Hedwig 2010). These depolarizations are of central origin, although the responsible presynaptic neurons are not yet known. On acoustic stimulation, the spiking response is superimposed on a maintained PAD during which the spike amplitudes decrease. The generation of PADs is sensitive to picrotoxin, which blocks GABAergic synapses.

Ultrastructural studies (Fig. 8.7B; Hardt and Watson 1999) show input synapses to afferents from GABA-immunoreactive processes of unidentified central interneurons. In bushcrickets PADs are driven mainly, but not exclusively, by stimuli from the same side as the respective sensory neuron. This can be demonstrated by removing the ipsilateral ear, which removes all spiking activity of sensory neurons, revealing small-amplitude PADs on their terminations. These PADs then have to be of contralateral origin. Comparing the Ca^{2+} response of the afferent terminal before and after the ipsilateral ear is removed (Fig. 8.7C, D) demonstrates that tuning of the PADs in some afferents is similar to the tuning of their spike activity. In others, however, the frequency range of PADs is below or above that of the excitation, indicating that presynaptic inhibition may sharpen frequency-specific synaptic transmission of afferent activity to postsynaptic neurons.

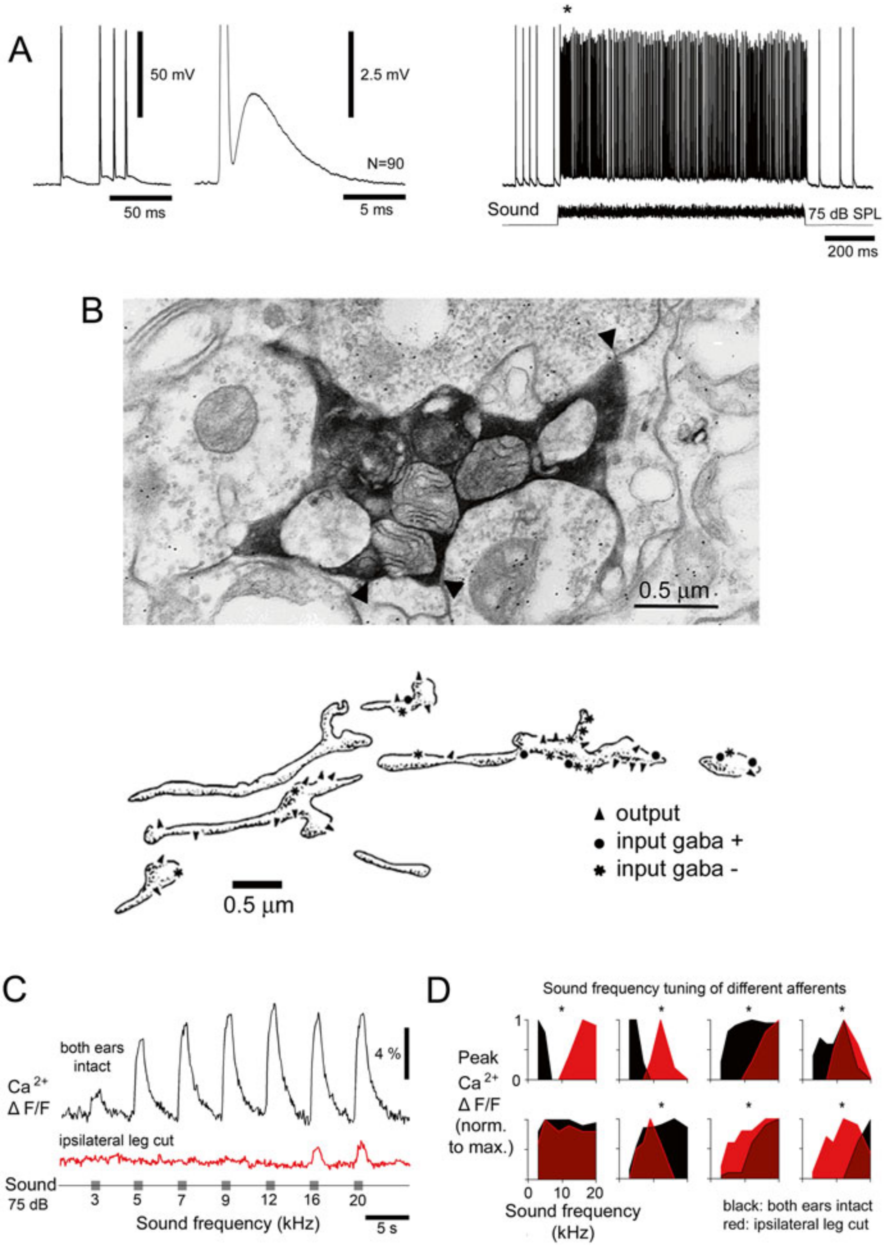

Fig. 8.7 Presynaptic inhibition. (**A**) Intracellular recording of a bushcricket (*Mecopoda elongata*) auditory afferent close to its axonal terminals in the prothoracic ganglion. *Left*: A graded primary afferent depolarization (PAD) of 2.5 mV is coupled to the end of each spike. *Right*: On maintained acoustic stimulation [white noise, 75 dB sound pressure level (SPL), 1 s] spikes ride on top of the PAD and spike amplitude is reduced, most pronounced at the stimulus onset (see asterisk). (**B**) *Top*: Ultrastructural evidence for GABA-immunoreactive processes forming synapses (*arrowheads*) on the terminals of an auditory afferent (*black*) tuned to 6 kHz in the bushcricket *Tettigonia cantans*.

8.5.3 Interneurons

While a tonotopic ordering of the central projections occurs in species with differently tuned sensory cells (grasshoppers, crickets, bushcrickets), this is less clear for interneuronal arborizations. Dendrites of some interneurons show overlap with restricted regions of the afferent projection (Römer et al. 1988); however, as interneurons also connect to other interneurons, they may branch throughout the entire neuropil, even when they receive only restricted auditory input. In crickets, correlations between low- or high-frequency tuning and anatomical characteristics of a number of mostly second-order or higher interneurons such as soma position and axonal projection in a connective have been described (Atkins and Pollack 1987).

In addition to presynaptic inhibition of afferents, frequency-specific synaptic inhibition occurs at the level of thoracic interneurons (Römer et al. 1988). Pharmacological experiments in a bushcricket revealed that this inhibition is picrotoxin sensitive; its elimination further reveals excitation by sound stimuli that initially elicited purely inhibitory responses (Fig. 8.8A–D; Stumpner 1998). Inhibitory synaptic processing therefore contributes to sharpening the frequency tuning of neurons. Among closely related species of phaneropterine bushcrickets, species-specific tuning of interneurons may be determined entirely by differences in the strength of inhibitory input (Stumpner 2002). A sharpening of frequency tuning will also occur if a neuron reaches threshold only when presynaptic firing rate is high enough to produce sufficient temporal summation and if the presynaptic firing rate depends on carrier frequency, as has been described for a bushcricket brain neuron (Ostrowski and Stumpner 2010). The Q_{10dB} value is a measure of the sharpness of tuning, that is, the higher the value, the sharper the tuning. Whereas afferents may have Q_{10dB} of up to 4, interneurons can reach a Q_{10dB} of 7. However, the Q_{10dB} values of many afferents and interneurons are in the same range of 0.5–2 (Hennig et al. 2004). The sharpness of tuning may be relevant for reducing interneuronal responses to ambient noise as compared to conspecific signals, especially in species-rich communities (Schmidt et al. 2011).

Frequency-specific inhibition also leads to complex intensity response functions of interneurons (Fig. 8.8C, F). A neuron that, at its preferred carrier frequency, receives a tonic excitation that increases with sound intensity may show an optimum-type response and a strong decrease of its activity at higher sound intensities

Fig. 8.7 (continued) *Bottom*: Reconstruction through branches of a 20-kHz afferent shows the distribution of output synapses (*arrowheads*) and input synapses from fibers that were labeled (gaba+, dots) or unlabeled (gaba–, stars) by GABA antibodies. (**C**) Cytosolic calcium change measured in the axonal terminals of an intact afferent (*black trace*) demonstrates a broad frequency tuning of the response. After the ear and spike-generating structure were removed (*red trace*), the remaining response is tuned to high frequencies only. (**D**) Different tuning of the calcium signal imaged in intact (*black*) and spike-generating zone-deprived (*red*) afferents demonstrates a different tuning of the presynaptic signal that may sharpen frequency-specific synaptic transmission. *Asterisks* indicate significant differences [(**A**), (**C**), and (**D**) from Baden and Hedwig (2010) with permission; (**B**) from Hardt and Watson (1999) with permission]

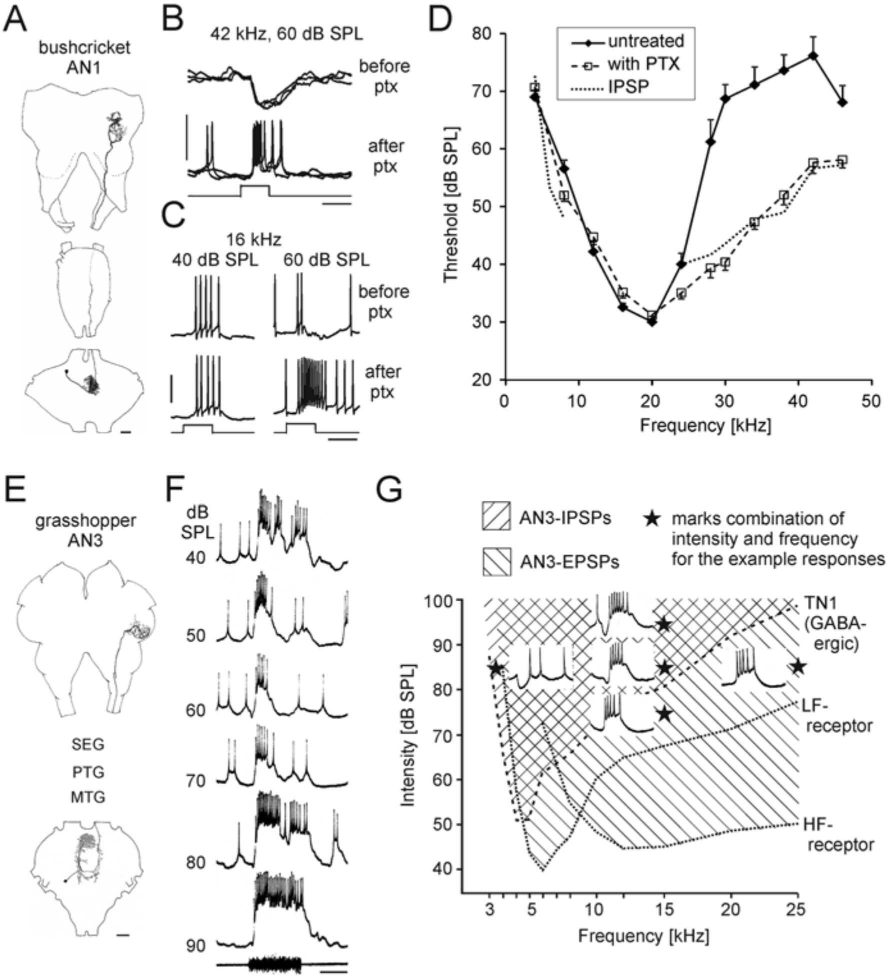

Fig. 8.8 Frequency processing. "Complex" responses to carrier frequencies and intensities in auditory interneurons. (**A**–**D**) Ascending auditory interneuron AN1 of the bushcricket *Ancistrura nigrovittata*. (**A**) Structure of the AN1 neuron in the prothoracic and subesophageal ganglion and the brain. (**B**, **C**) Responses to a 42-kHz stimulus and a 16-kHz stimulus before and after application of the chloride-channel blocker picrotoxin (ptx); bars: 50 ms, 25 mV. (**D**) Frequency tuning of AN1 before and after application of picrotoxin; means + SE, $n = 12$–13. Mean threshold for inhibition before ptx application shown as stippled line (IPSP, $n = 2$–9. [Modified from Stumpner (1997, 1998) and combined with new data]. (**E**–**G**) Ascending auditory interneuron AN3 of the grasshoppers *Chorthippus biguttulus* and *Locusta migratoria* (**G**). (**E**) Anatomy of the AN3 neuron in the metathoracic ganglion complex and the brain. MTG, mesothoracic ganglion; PTG, prothoracic ganglion; SEG, subesophageal ganglion. (**F**) Responses of AN3 to white noise stimuli of increasing intensity. (**G**) Thresholds of the presumed excitatory inputs to AN3 from low-frequency (LF) and high-frequency (HF) sensory neurons mediated via intercalated interneurons, and threshold of the GABAergic inhibition mediated via the TN1 interneuron. *Insets* show the occurrence of simple and complex responses in AN3 depending on frequency and intensity (*asterisks*) [(**E**), (**F**) from Stumpner (1988) and Stumpner and Ronacher (1991), respectively, (**G**) modified after Römer et al. (1981) with permission]

(Stumpner 1997). Such a response function is due to additional inhibitory inputs that are tuned to other frequencies and therefore become effective only at higher sound intensities (Fig. 8.8B–D). Blocking the inhibition reveals an underlying tonic excitation (Fig. 8.8B, C). The intensity-dependent responses may be even more complex in grasshoppers (Fig. 8.8E–G). When stimulated with white noise pulses of increasing intensity, the neurons show a first maximum at low intensities and then a reduced activation at intermediate intensities that is followed by a second peak of activation at high sound intensities (Fig. 8.8F, G). Such a bimodal response pattern can derive from a low- and a high-frequency evoked excitation in combination with a less sensitive inhibition at low frequencies. In grasshoppers, candidate neurons have been described that explain such a response pattern (Fig. 8.8G; Römer et al. 1981; Sokoliuk et al. 1989).

For insects with broadband communication signals, such as many bushcrickets and grasshoppers, frequency analysis may allow determination of the distance to the signaler. This is possible because in addition to the geometric spreading of acoustic energy with distance, excess attenuation occurs for higher frequencies (Römer and Lewald 1992). Therefore, the tonotopic organization of the sensory input also allows for a “coding of distance” as the activation pattern of the afferent population by a broadband signal will depend on the distance of the signaler. In the bushcricket *Mygalopsis marki*, different interneurons respond optimally to stimuli originating at different distances from the receiver (Römer 1987).

In some cases, the main frequency component of an insect's communication signals may overlap with the spectra of signals produced by predators and therefore neurons may respond to both signals. Even when the spectral content of conspecific and predator signals is different, central neurons may still respond to both due to convergence of sensory input (Ostrowski and Stumpner 2010). In these cases, differences in temporal patterns and additional context-specific sensory information need to be evaluated to allow for correct decisions (Nakano et al. 2013). When the frequency spectra do differ, the strength of synaptic input from afferents may be much stronger for ultrasound than for sonic conspecific signals (Pollack and Imaizumi 1999). Furthermore, local processing within a neuron's extended dendrites may allow segregation of signals differing in spectral content and temporal pattern (Triblehorn and Schul 2013; Prešern et al. 2015), implementing a form of auditory scene analysis or stream segregation analogous to mechanisms described for vertebrates (Moss and Surlykke 2010).

8.6 Pattern Recognition

8.6.1 Pattern Recognition: A Sequence of Feature Detection Steps

Hearing insects show typical motor responses to specific acoustic signals generated by conspecifics, predators, or prey, indicating that their auditory pathway detects and recognizes these signals as significant events within the auditory scene

(Bregman 2008). The underlying processing mechanisms are generally referred to as "feature detection" (Hoy 1978) or "pattern recognition" (von Helversen 1984; Stumpner and Ronacher 1994). Both processes are used within an operational context and their interrelationship may need to be considered. In the visual system, steps of feature detection (feature extraction) are thought to be necessary in a process underlying more complex pattern recognition (Barlow 1969). In a similar way, auditory pattern recognition may be regarded as a process involving several feature-detecting mechanisms (often called "filtering") in the frequency, the amplitude, and also the time domain, that is, when pulse intervals are crucial for communication. Different auditory patterns need to be processed and recognized, as species-specific signals that differ in carrier frequency and temporal structure are used for calling, courtship, and rivalry behavior and for response and disturbance signaling (Alexander 1962; Gerhardt and Huber 2002). For mate attraction, insects use bidirectional or unidirectional acoustic communication; so in this context either sex or (more commonly) just the females perform pattern recognition (Heller and von Helversen 1986; Robinson and Hall 2002). Otherwise, males need to employ pattern recognition for chorusing, intermale spacing, or phonotaxis to other singing males as in case of satellite males.

Frequency analysis is performed at the first level of auditory processing by the biophysical properties of the hearing organ and refined within the central auditory pathway (see Sect. 8.5). The processing of sound pulses is supported by the synchronous onset activity and the phaso-tonic responses of auditory afferents (Ronacher and Römer 1985; Nabatiyan et al. 2003) and by central mechanisms selecting the loudest signal (see Sect. 8.3). Already the activity of single auditory afferents represents fine-scale differences of intraspecific communication signals (Machens et al. 2003) and the afferent population provides the CNS with all the information available for temporal pattern recognition. However, the analysis of species-specific temporal sequences of sound pulses is not achieved in the peripheral auditory system and rather requires neural processing within the CNS. In several insect groups, ascending thoracic auditory neurons (Fig. 8.2) and their projection patterns within the brain have been characterized and local auditory brain neurons have been identified (e.g., Ostrowski and Stumpner 2010; Kostarakos and Hedwig 2012). A careful interpretation indicates that circuits for temporal auditory processing are preferentially housed in the ventral protocerebrum; however, a specific auditory brain region cannot be identified across the groups of acoustically communicating insects.

Although cicadas employ the most complex frequency- and amplitude-modulated signals, little is known about central auditory processing (Huber 1983; Fonseca 2014). In acridid grasshoppers, the amplitude modulation/temporal structure of the broadband songs appears to be crucial for pattern recognition (von Helversen and von Helversen 1983, 1987, 1998). Ascending thoracic neurons with spike activity patterns that monitor the continuity of the song pattern have been identified (Ronacher and Stumpner 1988), as well as neurons representing the basic pulse-pause unit of the song by bursting spike activity (Creutzig et al. 2009). Processing mechanisms in the brain, however, have not yet been explored.

8.6.2 Calling Song Pattern Recognition in Crickets

Neural mechanisms underlying temporal feature detection of pulse patterns are best explored in the CNS of crickets and bushcrickets. In their calling song-male crickets generate sound pulses within a very narrow carrier frequency spectrum (4–6 kHz) and combine these pulses to form complex chirp and trill patterns (Otte 1992). Conspecific females that are ready to mate approach singing males or a speaker broadcasting the calling song. In field crickets (*G. campestris*, *G. bimaculatus*, *T. oceanicus*), female phonotactic behavior is tuned to the 25–35 Hz pulse repetition rate of the male calling song as the dominant parameter for pattern recognition. It may also depend on the processing of pulse duration and chirp intervals (Doherty 1985; Hennig 2009). Elucidating the neural mechanisms underlying the response toward the species-specific pulse rate has been a major aim in behavioral neurobiology. Mechanisms such as resonant oscillations (Bush and Schul 2006), low-pass/high-pass filters (Schildberger 1984), template matching (Hoy 1978; Hennig 2003) and a delay-coincidence mechanism (Weber and Thorson 1989) have been proposed (Kostarakos and Hedwig 2012, 2015).

There is no evidence that temporal feature detection of the songs occurs at the thoracic level of the cricket CNS (Schildberger et al. 1989; Pollack 2001). However, local (ON1) and an ascending interneuron (AN1) indicate a first broad filter mechanism as they respond better and transfer more information when stimulated with sound pulses with an amplitude modulation rate below 30 Hz, which covers the range of pulse patterns for phonotactic behavior of crickets. The physiological mechanism may be due to the nature of the afferent synaptic inputs; it is not related to the reciprocal inhibition of ON1 neurons underlying directional processing (see Sect. 8.4; Marsat and Pollack 2004, 2005), which had been proposed by Wiese and Eilts-Grimm (1985). A response decrement of another ascending neuron (AN2) has been linked to phonotactic behavior (Stout et al. 2011), but details of the processing are not yet revealed.

In crickets, only one ascending auditory interneuron (AN1) forwards activity to the brain that reliably represents the temporal structure of the calling song (Wohlers and Huber 1982) (see Fig. 8.2). Its axon terminates in the ventral anterior protocerebrum. Based on intracellular recordings of local auditory brain neurons, Schildberger (1984) proposed that a combination of low-pass and high-pass neurons could constitute the feature-detecting mechanism that leads to the 30-Hz pulse-rate tuning of female phonotaxis. Detailed neuronal processing mechanisms underlying this filtering process were not revealed.

By using a cricket preparation that allows standing and phonotactic walking on a trackball (Fig. 8.9A), a group of local auditory interneurons has been identified that are closely linked to the output structures of the AN1 neuron (Kostarakos and Hedwig 2012). One identified neuron (B-LI2) simply copies the auditory stimulus pattern, whereas the spike patterns of other neurons (B-LC3 and B-LI4) exhibit a tuning that matches female phonotactic behavior (Fig. 8.9B). One particular interneuron (B-LI4) is inhibited at low and high pulse rates and spikes only at the

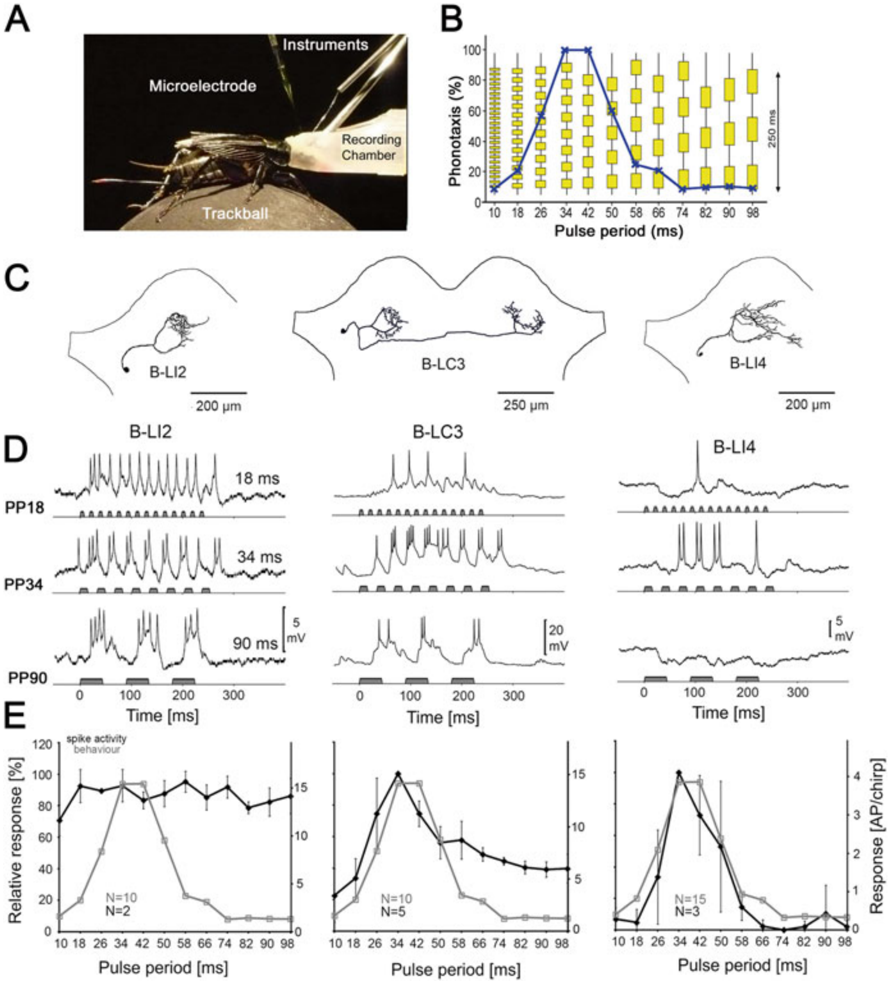

Fig. 8.9 Pattern recognition in a cricket. (**A**) Tethered female cricket (*G. bimaculatus*) positioned on a trackball for recording auditory brain neurons. (**B**) Auditory test patterns with different pulse periods and temporal tuning (*blue line*) of female phonotaxis. (**C**) Local brain neurons involved in the processing of the pulse pattern; their arborizations match the ring-like axonal arborizations of AN1 (see Kostarakos and Hedwig 2012). (**D**, **E**) Spike activity of brain neurons in response to different pulse period patterns. B-LI2 copies the sound pattern, whereas B-LC3 and B-LI4 show a tuning of their spike responses (*black line*) that matches the female phonotactic behavior (*gray line*). B-LI4 receives inhibitory and excitatory inputs and only spikes in response to the species-specific pulse period. The tuning of this neuron reveals response properties of a feature detector for the pulse period [(**C**)–(**E**) from Kostarakos and Hedwig (2012) with permission]

species-specific pulse rate. Its spiking activity closely matches the tuning of the female phonotactic behavior. The B-LI4 neuron may therefore be regarded as a feature detector for the species-specific pulse rate. Analyzing the timing of the neuronal responses reveals that some local brain neurons respond with a very long latency and only when the second sound pulse occurred (Zorović and Hedwig 2011; Kostarakos and Hedwig 2012). As the processing of any pulse rate requires at least two pulses, these responses are consistent with a coincidence detection mechanism, in which the response to the first pulse is delayed by the species-specific pulse period to coincide with the response of the second pulse (Weber and Thorson 1989; Kostarakos and Hedwig 2012). The underlying neural circuitry and the nature of the delay mechanism that explains the tuning toward the pulse periods has been described while this chapter was in press (Schöneich et al. 2015).

8.6.3 Pattern Recognition in Duetting Bushcrickets

The brain must also account for acoustic signaling behaviors that operate over longer time periods. Bushcrickets often use simple, double-pulse song patterns in the sonic and/or ultrasonic range for communication. However, in some groups with acoustically duetting mates (e.g., Phaneopterinae), male songs can be quite complex as they include both temporally patterned chirps and specific trigger pulses that are crucial to elicit the female response song (Heller and von Helversen 1986). The duetting behavior between the sexes relies not only on differences in carrier frequency but also on differences in temporal patterns. During calling, male *Ancistrura nigrovittata* (Phaneropteridae) produce chirps (pulse groups), which last about 200 ms and contain 5–9 sound pulses of 16 kHz. Chirps are produced every 800–900 ms (Fig. 8.10A) and about 350 ms after the end of a chirp a single "trigger pulse" is emitted. Females do not respond to the chirps. However, 25–30 ms after the trigger pulse, the female generates a brief ultrasonic click with her wings, which will guide the male towards her (Heller and von Helversen 1986; Dobler et al. 1994a). The female response depends strongly on the pattern of the male chirp and also on the time interval between the chirp and the trigger pulse; intervals of 250–450 ms are most efficient (Fig. 8.10B). Behavioral tests indicate that females have an expectation of the time when the trigger syllable should occur. Very motivated females respond at approximately the correct time after a chirp even if the trigger pulse is omitted (Dobler et al. 1994b). The short latency of the female's response to the male's trigger pulse does not allow for complex pattern analysis. Rather, processing and recognition of the preceding chirp signal may set an internal time window, which subsequently enables the female's short latency response to the trigger pulse. On the other hand, males will respond to female replies only if these occur within a restricted temporal "window" after the trigger syllable (Heller and von Helversen 1986).

Neural processing in the CNS of both sexes must account for these time windows, but how can this be realized? In the brains of bushcrickets, local auditory interneurons have been identified in the anterior lateral protocerebrum, with specific response properties matching the carrier frequency and timing of the communication signals (Ostrowski and Stumpner 2010, 2013). In males as well as in females, a particular neuron (LBN9) is inhibited when the bushcricket hears a male chirp at

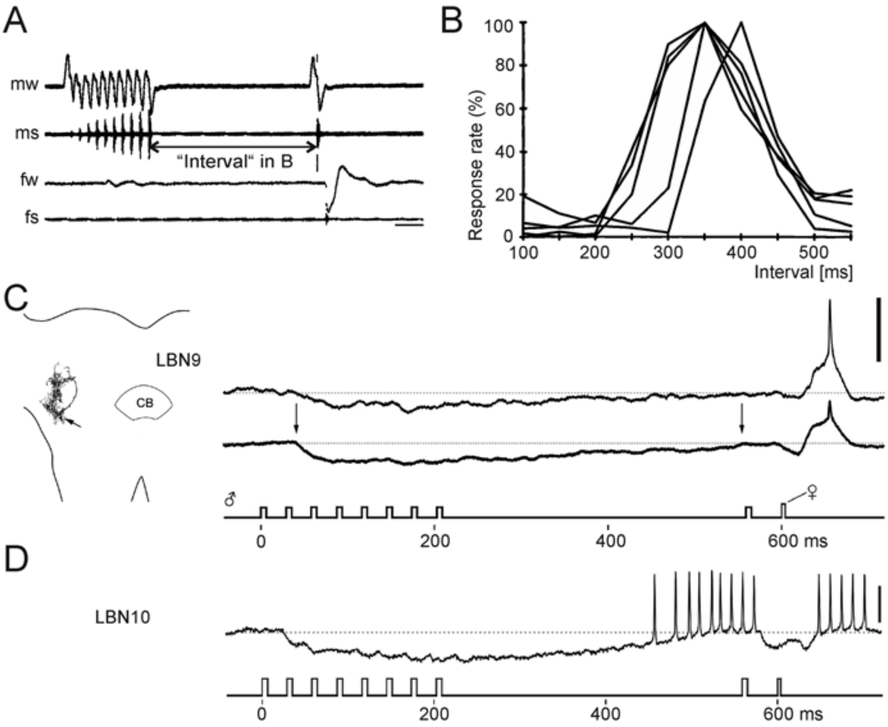

Fig. 8.10 Pattern recognition in a bushcricket. Behavioral and interneuronal responses in the bushcricket *Ancistrura nigrovittata*. (**A**) Natural duet, wing movements of male (mw) and female (fw) and sound produced (ms, fs). Upward is wing opening. Scale bar = 100 ms. The *arrow* ("Interval" in **B**) shows the interval varied in the diagram of (**B**). (**B**) Responses of females to models of the male song. Each female is represented by one line. Responses are tightly linked to the separate "trigger" pulse by the male song and occur only if the interval between trigger pulse and preceding group of pulses is between 250 and 450 ms. (**C**, **D**) Responses of brain neurons to artificial duets. (**C**) Morphology of the local brain neuron LBN9 and responses (*upper*: single response; *lower*: average of five responses) showing a long-lasting inhibition by the pulse group. (**D**) Responses of LBN10 showing a postinhibitory activation at about the time when the trigger pulse occurs in natural songs [(**A**) modified after Dobler et al. (1994b); (**B**) modified after Heller and von Helversen (1986); (**C**) from Ostrowski and Stumpner (2013); (**D**) from Ostrowski (2009); with permission]

16 kHz and is excited by a later-occurring female pulse at 24–28 kHz (Fig. 8.10C). The inhibition begins with a latency of 40 ms, builds up during the chirp, and is maintained for another 300 ms after the end of the chirp. The long duration of the inhibition thus matches the delay between the onset of the chirp and the trigger pulse. If a trigger pulse (16 kHz) is then presented, it also elicits an inhibitory response; however, a subsequent pulse at 27 kHz that, like a female response, follows the trigger pulse by 30 ms leads to excitation and spiking of the neuron. Other local brain neurons show a similar long-lasting inhibition. In one of these (LBN10; Fig. 8.10D; Ostrowski 2009), the inhibition gradually fades and is fol-

lowed by postinhibitory spiking activity. Although the functional significance of the long-lasting inhibition cannot yet be specified in detail, its time course closely matches the time window for the male trigger and the female response pulse and may be directly involved in the processing of the trigger pulses.

8.6.4 Pattern Recognition and Auditory Motor Responses

In phaneropterine bushcrickets, the very short latency female responses to the male trigger pulse indicates a functional separation of the circuitry underlying pattern recognition and that controlling the auditory motor response. The initial recognition of the male chirp seems to prime the female auditory pathway to allow for a rapid reflex-like motor response, that is, moving the wings for sound production. The single trigger pulse has no specific temporal structure and is characterized simply by its duration and frequency. Thus at this stage, a complex mechanism for pattern recognition that might involve the brain is not required, and the female motor response may be controlled by a fast local thoracic network. Such an organization would indicate a functional similarity to phonotactic steering in flying and walking crickets (Pollack and Hoy 1981; Hedwig and Poulet 2004).

8.7 Dealing with Noise due to Movement and Self-Generated Sounds

8.7.1 Effects of Motor Activity on Auditory Processing

Insect hearing organs evolved from mechanosensitive proprioceptors and they are not completely decoupled mechanically from the animal’s tracheal system and body. As a consequence, they respond not only to airborne sound but can also be activated by vibrations due to the insect’s muscle activity and movements of appendages. Such self-generated stimulation of the hearing organs causes activity of auditory afferents that is not related to acoustic stimulation. It can also lead to failure of auditory responses. Both effects pose a problem for central processing and make the coding of acoustic signals less reliable. Motor activities such as breathing, flight, passive leg movements (Hedwig 1988, 1989; Lang and Elsner 1989), or walking (Schildberger et al. 1988; Zorović and Hedwig 2011) have significant effects on the representation of auditory signals in spike patterns of afferents and interneurons. This is especially relevant when the neuronal representation of the communication signals is used for auditory orientation and pattern recognition.

Effects of motor activity on auditory processing can be resolved only in experiments that do not restrain the insect’s behavior but rather consider motor activity as the natural functional condition for auditory processing. Recordings from auditory

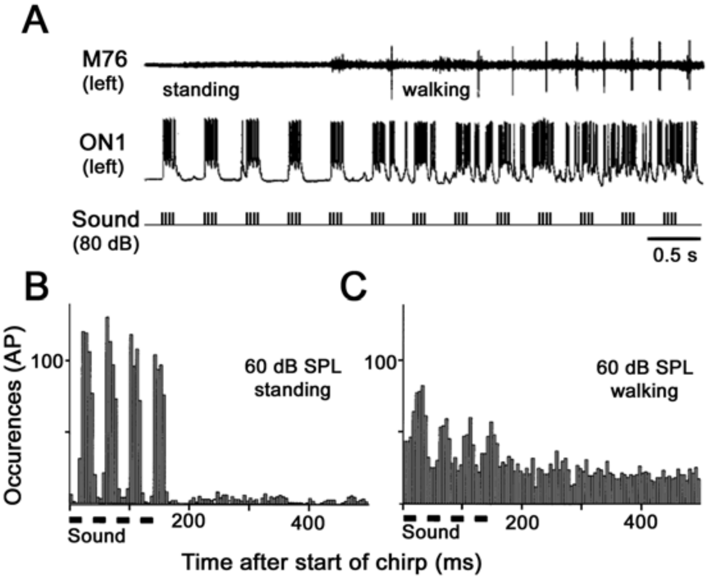

Fig. 8.11 Neural noise during walking in a cricket (**A**) Activity of a *G. campestris* ON1 interneuron in response to acoustic stimulation with calling song (80 dB SPL) in a standing and then walking cricket as indicated by the activity of a leg muscle M76. (**B**) The peri-stimulus-time-histogram (PSTH) of auditory evoked spike activity in a standing cricket gives a clear representation of the chirp pattern presented at 60 dB SPL. (**C**) Walking motor activity causes substantial background activity in the auditory pathway. The auditory response is reduced and the representation of the sound stimulus less reliable [From Schildberger et al. (1988) with permission]

neurons in the thoracic ganglia and the brain have been obtained in tethered crickets that were either standing or freely walking on a trackball system. When the insect is quietly standing and is exposed to a model of the species-specific calling song, a reliable representation of the acoustic signal occurs in the spike pattern of the thoracic ON1 neuron (Fig. 8.11A; Schildberger et al. 1988). The neural activity pattern, however, changes dramatically with the onset of walking. The walking motor activity, which will also move the hearing organ in the front leg, causes additional spikes in the interneuron and it reduces the reliability of the stimulus representation. While the insect is standing, each pulse of the sound pattern is clearly represented in the corresponding peristimulus time histogram with a response about 20 times higher than the background activity. During walking, the background spike activity increases and at the same time the response to the sound pulses decreases by about 50 % and now is only about twice as high as the background activity (Fig. 8.11B). This effect of walking on auditory processing is carried forward to the brain. Even local brain neurons show a less reliable representation of acoustic signals during walking (Zorović and Hedwig 2011). How the pattern recognition networks deal with these noisy input is not yet resolved, but once pattern recognition is activated in crickets, the system is noise tolerant and transiently responds even to nonattractive sound pulses (Poulet and Hedwig 2005).

8.7.2 Dealing with Self-Generated Sounds

All acoustically signaling species face an even more fundamental challenge: How can the signalers deal with the self-generated sound to prevent desensitization of their auditory pathway and avoid a mix-up of self-generated and external acoustic signals? A peripheral mechanism operates in cicadas, which fold their tympana during singing by contracting a detensor tympani muscle and thereby increase the auditory threshold by 20 dB (Hennig et al. 1994). In stridulating acridid grasshoppers that produce broadband signals (von Helversen 1997), the self-generated sounds and accompanying mechanical vibrations lead to an activation of the auditory afferents that also depolarizes the auditory interneurons (Hedwig and Meyer 1994). Sound stimuli presented during stridulation are masked by the self-generated afferent activity, and as a consequence interneuronal responses are strongly reduced (Hedwig 1984; Wolf and von Helversen 1986). Intracellular recordings did not provide any evidence for a central neural mechanism that modulates auditory processing and reduces interneuron responses during singing (Hedwig 1990).

The situation is different in crickets (*G. bimaculatus*), which may produce pure-tone calling songs at approximately 100 dB SPL for many hours to attract a mate, exposing their own ears to long-lasting and intense self-generated sound. There is no evidence that peripheral processes alter the sensitivity of the hearing organ (Poulet and Hedwig 2001); rather a central mechanism is employed. A central corollary discharge, that is, a signal that is generated by the motor system and forwarded to the sensory pathway, modulates self-generated auditory activity at the level of the auditory afferents and thoracic interneurons, demonstrating a concept proposed and discussed for visual pathways (von Holst and Mittelstaedt 1950; Sperry 1950). Its efficiency can be demonstrated in silently singing crickets when one of the front wings is removed to prevent sound production. At the same time, the animals are exposed to a sequence of acoustic pulses, that activate the auditory system. The auditory afferents reliably respond to the sound pulses but intracellular recordings close to the axonal terminals demonstrate that synaptic transmission is affected by a presynaptic mechanism (Fig. 8.12). The axonal arborizations close to the afferent terminals receive a PAD of 2–3 mV, which in many sensory pathways indicates presynaptic inhibition (see Sect. 8.4). The depolarization is coupled to the closing phase of the wing movements. It decreases the amplitude of the invading spikes and thereby the efficiency of synaptic transmission whenever the cricket would generate a sound pulse.

Thoracic interneurons (ON1 and AN1) respond reliably to the sound stimuli in the chirp intervals (Fig. 8.12B, C), but during the chirps, they receive a strong postsynaptic inhibition that suppresses any spike activity (Poulet and Hedwig 2002, 2003a, b, 2006). The inhibition is phase-coupled to the closing movement of the wings and it reduces spike activity to self-generated sound pulses. Presynaptic and postsynaptic inhibitions are mediated by a bilateral pair of corollary discharge interneurons that make monosynaptic connections to the afferents and the auditory interneurons (Fig. 8.12D, E). The inhibition provided by this interneuron reduces the

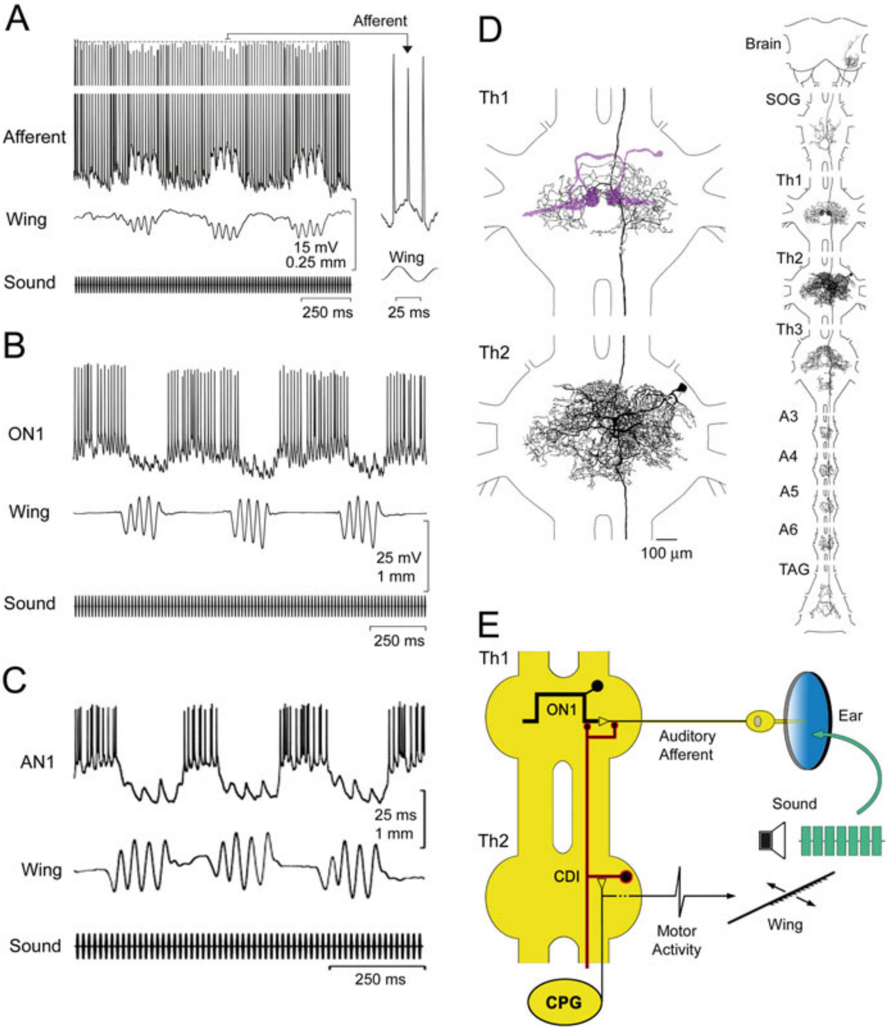

Fig. 8.12 Dealing with self-generated sound during singing. (**A**–**C**) Activity of auditory neurons in "silently" singing crickets in which one front wing is removed and the movement of the other wing is recorded (see **E**). The crickets cannot generate any sound but are exposed to a continuous sequence of sound pulses (4.5 kHz, 75 dB SPL). (**A**) An auditory afferent spikes in response to the acoustic stimuli; in phase with the wing movements the afferent receives PADs, which reduce spike height. (**B**, **C**) Auditory evoked spike activity in the ON1 and AN1 neurons occurs in the chirp intervals; the auditory response is inhibited during the singing wing movements. (**D**) Structure of the corollary discharge interneuron in the Th1 and Th2 thoracic ganglia and in the complete CNS (right). The structure of an ON1 neuron is indicated in magenta. (**E**) Proposed circuit for the underlying neuronal mechanisms. The corollary discharge interneuron is activated by the singing central pattern generator (CPG) and inhibits the auditory pathway in phase with sound production [(**A**)–(**C**) from Poulet and Hedwig (2003a,b); (**D**) from Poulet and Hedwig (2006); (**F**) from Hedwig (2006), with permission]

spike activity of auditory interneurons during singing and may serve to prevent the auditory pathway from desensitization (see Sect. 8.3). The corollary discharge interneuron has its dendrites in the mesothoracic ganglion; its axonal arborizations in the prothoracic ganglion are particularly dense and match the auditory neuropil. The interneuron may be driven directly by the singing central pattern generator housed in the abdominal ganglia, but direct evidence is so far missing.

8.8 Summary

Auditory systems evolved in a variety of insect taxa for sound processing in the context of predator avoidance and intraspecific communication. The insects' rather simple nervous systems allow analyzing neural mechanisms of auditory processing at the level of neuropils, identified neurons, and even the synaptic connections in circuits. Neurobiological studies successfully revealed principles underlying directional processing and frequency processing and, more recently, the mechanisms involved in complex corollary discharges and pattern recognition. With increasing complexity of the auditory processing task, a single-cell recording approach to characterize and manipulate the activity patterns of individual neurons may have its limits. Double intracellular recordings to reveal the flow of information within an auditory pathway are very challenging and in case of local small brain neurons, they may not be feasible at all.

Future research would greatly benefit from a molecular–genetic approach, but so far, the genome has not yet been sequenced for any acoustically communicating species using tympanic hearing organs. Such future developments may provide molecular–genetic tools to introduce calcium indicators or voltage reporters into the nervous system, generating transgenic lines with genetically encoded indicators expressed in specific subsets of neurons. Combined with intracellular recordings of identified neurons, these techniques should provide a most efficient toolbox for research. Imaging of neural assemblies in combination with electrophysiological recording of the synaptic and spike activity of identified neurons would enormously foster a detailed understanding of neural mechanisms underlying auditory processing at the level of neurons and networks. Based on such information, reliable and robust computational models could be developed to synthesize and synergistically combine the response properties of identified neurons and to test the functional properties of the modeled networks in silico. This could allow a comprehensive and detailed understanding of the way auditory circuits function and how they may have been shaped during evolution to match the processing of species-specific acoustic communication signals.

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Chapter 9
Information Processing in the Auditory Pathway of Insects

Bernhard Ronacher

Abstract The acoustic communication of grasshoppers, crickets, and katydids provides prime examples of general principles of how nervous systems represent sensory information. The processing of auditory signals in insects is innate; thus the involved neuronal modules are "hardwired." In addition, the relevant stimulus space is restricted, facilitating the investigation. A major problem of sensory processing is the trial-to-trial variability of spike trains caused by the stochastic opening and closing of ion channels. In animals that can spend only a few neurons for a given task, this unreliability of spike trains is a relevant constraint for neuronal encoding.

Signal recognition in insects depends primarily on features of the sound envelope, the pattern of amplitude modulations. The receptor neurons respond with high temporal precision and reflect the stimulus's envelope in their spike patterns. This kind of "temporal code" is later transformed to a "labeled line code" representation in which single neurons encode specific sound features. Similarly to the larger nervous systems of vertebrates, at this stage, spike rates are reduced and the presence of particular sound features can be read out from a population code. Remarkably, in insects, the sparsening and change of coding schemes occur already within a few synapses after the receptors.

Modeling studies suggest how feature detectors equipped with linear filters may explain behavioral scores found with specific stimulus variations. Remarkably, in grasshoppers and crickets, the filters found by this approach resembled Gabor filters, which allow an easy transition between behavioral preference functions found in different species.

Keywords Behavioral preference functions • Frequency discrimination • LN models • Modulation transfer functions • Peripheral filtering • Spike train variability • Temporal resolution

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9.1 Introduction

Insects must manage their lives with a rather small number of neurons. Hence, there is a strong pressure for an efficient use of computational resources. As a consequence, one observes that steps of filtering are often placed at the very periphery, thereby reducing the required processing capacities at later stages. Probably owing to constraints in the affordable number of neurons, the auditory pathway of insects is not an all-purpose processing device but rather is focused on a restricted and clearly defined set of specific tasks. The main tasks of insect hearing are not to interpret the surrounding acoustic world as such but to detect and to identify behaviorally relevant sounds. Relevant sounds belong mostly to two classes: sounds produced by friends or by foes, such as calling and courtship signals of conspecifics or sounds produced by predators (Hoy 1989; Hartbauer et al. 2010). The range of relevant stimuli and sound features of interest is therefore not too large, which greatly facilitates the investigation of how acoustic stimuli are represented within the nervous system and how stimulus representations are transformed along consecutive stages of processing.

Most progress in understanding information processing during the last decades has been made by investigating the neuronal processing of mating signals in acoustically communicating grasshoppers, crickets, and katydids, and these groups are the focus of the present chapter. The main tasks then are to recognize and localize signals of conspecifics, often under adverse conditions of high noise levels, and not to be lured by signals of related species (von Helversen and von Helversen 1994).

The auditory pathways of these insects offer several experimental advantages. (1) The recognition of calling and courtship signals are an innate capability (von Helversen and von Helversen 1975; Gottsberger and Mayer 2007). Hence the neuronal modules devoted to the processing of these sounds must be hardwired and can be observed in every individual. In addition, the signals and their correct interpretation are crucial for finding a suitable mate and therefore are under strong selection. (2) Based on their characteristic morphology, many neurons can be identified as individuals and can be recognized in every specimen. Thus we can determine synaptic connections between identified neurons and reveal processing pathways (Boyan 1992; Vogel and Ronacher 2007). These advantages make insect communication systems particularly well-suited to investigate information processing. As another advantage, as a rule the animals collaborate willingly in behavioral experiments, which allows the investigators to assess the respective relevance of general and specific sound features.

Some insects, in particular parasitoid flies, exploit the communication signals of other species to parasitize them or to prey upon them, but this aspect is not covered here (see recent reviews by Hedwig and Robert 2014; Lakes-Harlan and Lehmann 2015; for a bushcricket with particularly intriguing praying strategy see Marshall and Hill 2009).

This chapter aims to pursue the flow of information along the auditory pathway of insects, with a focus on how sound signals are encoded and represented at consecutive processing stages. In Sect. 9.2 some basic features of orthopteran sound signals are mentioned. Section 9.3 then focuses on various forms of peripheral filtering. A main function of such filtering probably is to reduce the computational load at more central stages, but, of course, peripheral filtering does reduce the versatility and leads to a restricted perception of the surrounding world. The communication signals of various insect taxa are characterized by specific patterns of amplitude modulations, and indeed it is the temporal pattern of amplitude modulations (AMs) that is decisive for signal recognition in various insects. Hence, some effort will be spent on how and with what temporal resolution AM patterns are processed at different levels of the auditory pathway (Sect. 9.4). In this context, stimulus reconstruction methods are also mentioned as a methodological and conceptional tool that advanced our understanding of neuronal information processing.

Section 9.5 deals with the variability of neuronal signals, which has profound consequences for the encoding of AM patterns and for the discrimination of similar signals. The intrinsic variability of neuronal signals is probably the main cause for a change of coding schemes that we observe when we ascend the auditory pathway. Last, in Sect. 9.6, modeling studies are presented that may help to identify more general features of neuronal computation and can provide a link between behavioral data and the processing properties observed in brain neurons.

9.2 Basic Features of Sound Signals Produced by Orthopteran Insects

Cricket songs consist of sound pulses of almost pure tones, which is the simplest form of sound. The exact peak of the dominant carrier frequency depends on species but is mostly between 3 and 6 kHz. Crickets produce these tones by moving a file located on one forewing against a scraper located at the other forewing. This results in a resonant mechanism, which allows for very loud sounds (Bennet-Clark 1999). In contrast, the communication signals of grasshoppers have a broad carrier frequency content, covering the range between approximately 2 and 50 kHz (Fig. 9.1C; see Meyer and Elsner 1996; Gerhardt and Huber 2002). Dealing with these more complex sounds, it is useful to distinguish frequencies on two time scales: (1) carrier frequencies that can encompass very high frequencies; in insect songs the carrier frequencies often extend high up in the ultrasonic range (see Fig. 9.1C; up to 100 kHz) and (2) the sound envelopes, which is the pattern of amplitude modulations of a carrier, which cover a much lower frequency range, up to some hundred hertz (Fig. 9.1A, B). This distinction is important to keep in mind in addressing the neuronal processing of frequencies.

The species-specific cues, however, lie primarily in the temporal pattern of the songs' amplitude modulations (Fig. 9.1A), and for crickets as well as grasshoppers, the temporal pattern of sound pulses is crucial for identifying a conspecific mating partner. Carrier frequencies of katydid signals are often broadband but in some species are also restricted to a narrow frequency band (Heller 1988). It has to be emphasized that in katydids as well, the species-specific pattern of amplitude modulations,

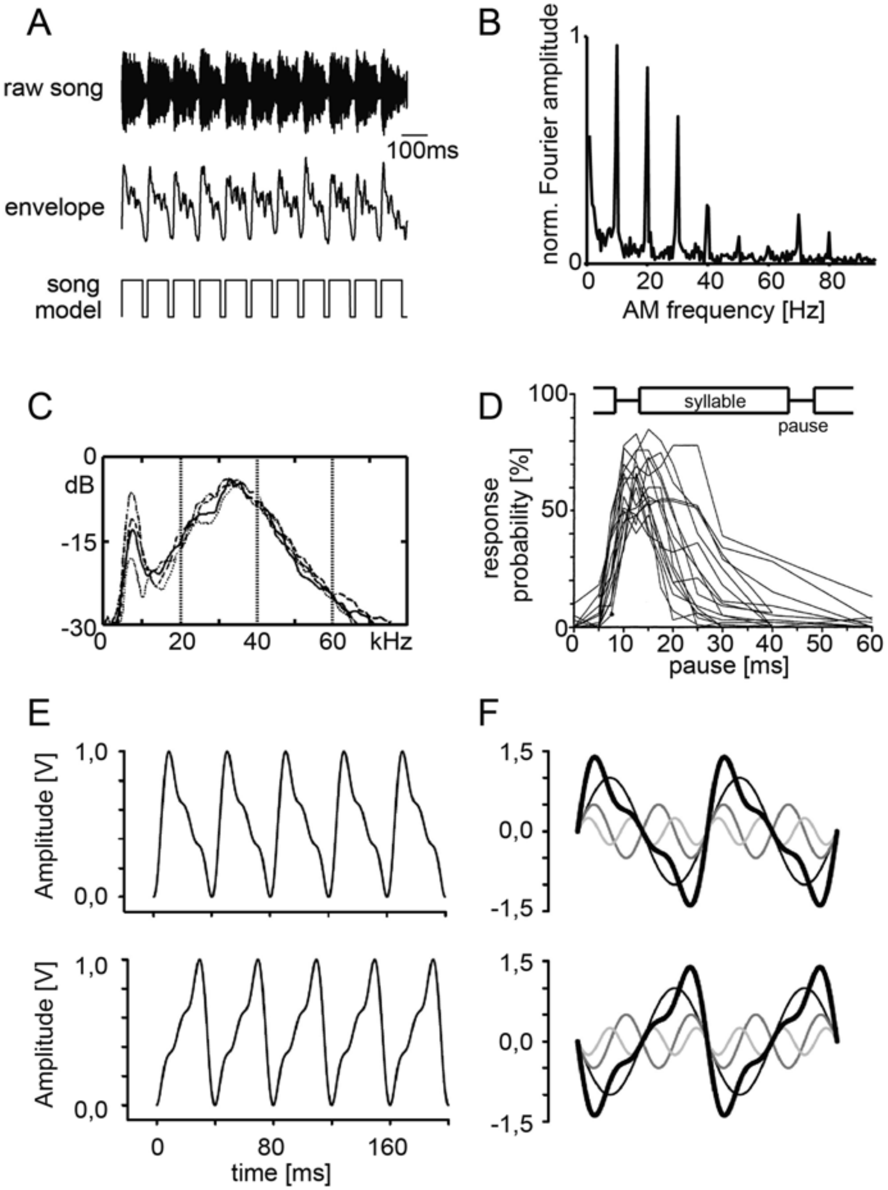

Fig. 9.1 (**A**) Section of a grasshopper song (male of *Chorthippus biguttulus*), *upper trace*; *middle*: Amplitude modulation pattern of the song (its envelope). *Lower trace*: Envelope of a song model used for behavioral tests (see **D**). (**B**) Fourier amplitude spectrum of the song envelope shown in (**A**). (**C**) Carrier frequency spectra of the songs of male *C. biguttulus*. (**D**) Female responses to rectangularly modulated broadband noise, in which the pause duration between syllables was varied; with 80-ms syllables, females preferred pauses between 10 and 20 ms. (**E, F**) Song models with a triangular envelope result from a combination of a few sinusoids (**F**). The stimulus with steep onset and slow decay was very attractive, whereas the females rejected the "inverted" signal with slow rise and steep decay (Schmidt et al. 2008) [(**A**), (**B**) from Ronacher et al. (2015); (**C**), (**D**) from von Helversen and von Helversen (1997); (**E**), (**F**) from Hennig (2009)]

or signal envelope, provides the decisive cues for species identification. With a few exceptions, the signal's carrier frequency content plays only a secondary role for species identification or sex recognition (von Helversen and von Helversen 1997; Gerhardt and Huber 2002). Therefore, in this chapter the emphasis is on the processing of sound envelopes.

9.3 Peripheral Filtering

9.3.1 Frequency Discrimination and Tuning to Species-Specific Carrier Frequencies

The capacity to discriminate between different carrier frequencies is, by and large, not well-developed in insects with tympanal hearing organs (Stumpner and von Helversen 2001; Hennig et al. 2004; cf. Fonseca 2014). Evidently, neuronal signals cannot follow sound frequencies in the kilohertz range. Carrier frequency discrimination therefore depends in the first place on a frequency-place transformation, that is, on how well different sound frequencies may stimulate different parts of the tympanum, which then can be scanned by different receptor populations (for details see Windmill and Jackson, Chapter 6).

A similar principle is realized in the mammalian cochlea, where hair cells at specific positions along the cochlea respond to particular frequencies (Montealegre-Z et al. 2012). However, compared to vertebrates, the sharpness of frequency tuning of auditory receptor neurons in insects is low (Stumpner and von Helversen 2001; Hennig et al. 2004). In katydids (Tettigoniidae), the tonotopically organized receptors could in principle provide a relatively good spectral resolution (Stumpner 1996; Hummel et al. 2011). However, at the next processing stages, in auditory interneurons, one often finds a broader tuning, indicative of a convergent input from several receptors onto these neurons (see Hennig et al. 2004; Ostrowski and Stumpner 2010). Interestingly, in an interneuron of the bushcricket (*Ancistrura nigrovittata*), the frequency tuning is sharpened by a frequency-dependent "lateral" inhibition similar to that in vertebrates (Stumpner 1998; Ostrowski and Stumpner 2010).

Many cricket species do produce signals in a narrow frequency band, pulses of almost pure tones. As a rule, these species have ears in which the lowest hearing threshold corresponds to the dominant frequency of the conspecific call (Kostarakos et al. 2009). If the receiver's ear is sharply tuned to the dominant frequency of conspecific calls, this helps to improve the signal-to-noise ratio in noisy habitats (Schmidt and Römer 2011; Schmidt et al. 2011). In addition, narrow frequency filters may contribute to the avoidance of other species that use a different carrier frequency. However, an unequivocal discrimination against different species requires a frequency analysis that is not available in crickets (see later in this section). Thus discrimination against heterospecifics would normally not be possible based on frequency cues alone; a frequency deviating from the optimal tuning would basically appear less loud, and therefore less attractive, but this disadvantage could be compensated for by increased signal energy, for example, by a nearer sender (for katydids see Deily and Schul 2006). However, if the signal frequency deviates from the optimal tuning of the receiver, this may cause localization problems, as the directionality of the hearing system depends on the frequency (Michelsen et al. 1994; Kostarakos et al. 2009).

In behavioral tests, crickets discriminate primarily between two broad frequency ranges in a kind of "categorical response" that divides the frequency axis into two parts (Hoy 1989): a broad range of frequencies below 10 kHz evokes a positive steering response. This frequency range is obviously interpreted as stemming from potential mates, whereas sound pulses with carrier frequencies above 15 kHz (up to 100 kHz) signal potential predators (bats) and evoke an avoidance response in flying crickets (Wyttenbach et al. 1996).

9.3.2 Peripheral Computations: Inhibition and Acoustic Hemispheres

In the prothoracic ganglion of crickets and katydids a pair of neurons has been identified that exhibit a characteristic shape like the Greek character omega and therefore were termed "omega neurons" (ON1). Contralateral inhibition in the ON1 neurons leads to a suppression of sounds from the opposite side, resulting in a division of the auditory world into two hemispheres (see also Hedwig and Stumpner, Chapter 8). If different sound patterns are presented from the left and right sides, the left ON1 represents only the left side pattern while the right side pattern is represented only by the right ON1 and is almost completely suppressed in the left ON1 (Römer and Krusch 2000). This inhibitory processing allows for a spatial release from masking (Schmidt and Römer 2011) and has been interpreted as a manifestation of "selective attention" (Pollack 1988). Such inhibitions also influence mate choice in katydids because they create a strong preference for signals that lead in time by suppressing the neuronal representation of follower signals (Siegert et al. 2011). The resulting "leader preference" of females has a profound impact on the formation of insect choruses (Hartbauer et al. 2005; Greenfield 2015).

9.4 Neuronal Processing of AM Patterns

Focusing on mating signals with broadband carriers, as produced by many grasshoppers and katydids, one observes that the spectral content of these signals often largely overlaps between species (Heller 1988; Meyer and Elsner 1996). In addition, owing to the dampening of high frequencies during sound propagation in the air, a signal's frequency composition arriving at the receiver depends on the distance of the sender (Römer and Lewald 1992). Therefore, a spectral analysis of the carrier frequency content, even if feasible, would not necessarily prevent attraction of heterospecific partners. As mentioned in Sect. 9.2, in the majority of insects, the most relevant species-specific features of communication signals reside in the temporal pattern of amplitude modulations and the sound envelopes, and the processing of these patterns is crucial to identify signals of conspecifics.

9.4.1 Processing of AM Patterns in the Time Domain or Frequency Domain?

Many insects produce songs that are composed of regular repetitions of highly stereotyped subunits. The rhythmic pattern of song elements manifests itself in the Fourier spectrum of the song envelope as a pattern of a few prominent modulation frequencies (Fig. 9.1A, B). This has stimulated the idea that AM patterns could be analyzed exclusively in the frequency domain, ignoring the phase information (Michelsen 1985; von Helversen and von Helversen 1998). [Figure 9.1F demonstrates how a different phase composition may yield differing song envelopes in spite of having the identical frequency composition; for a detailed description of Fourier spectra see the excellent introduction to fundamentals of hearing by Yost (2000) or Gerhardt and Huber (2002).] Such a processing in the frequency domain could be realized by a bank of filters devoted to specific modulation frequencies and performing an analysis of the song envelope's amplitude spectrum (as proposed for vertebrate hearing; see Joris et al. 2004). According to this concept, a behavioral response would be triggered if the relevant filter combination is sufficiently stimulated by the signal's AM frequency content. Indeed, several experiments have shown that crickets and grasshoppers accept song signals with randomized or shuffled patterns as conspecific, which seemed to support processing in the frequency domain (Pollack and Hoy 1979; von Helversen and von Helversen 1998; Schmidt et al. 2008).

A crucial paradigm to test this idea is to present reversed or inverted versions of an attractive signal, a procedure by which only the phase information of its Fourier components is affected but not its amplitude spectrum (compare Fig. 9.1E, F). Such behavioral experiments have been performed in grasshoppers and crickets and strongly suggest that the processing of communication signals takes place in the time domain. Grasshopper females, for example, respond well to a song model with the AM depicted in the upper part of Figure 9.1E, whereas they completely reject the reversed pattern (lower trace in Fig. 9.1E), although both stimuli are composed of the identical AM frequencies (Fig. 9.1F), only with different phases (Schmidt et al. 2008). These experiments demonstrate that the phase information cannot be ignored and thus the signals are processed in the temporal domain (von Helversen and von Helversen 1998; Hennig 2009). We should, however, be cautious to generalize these results too widely: across species, different processing options may be realized.

9.4.2 Processing of AM Patterns: Limits of Temporal Resolution

If communication signals contain high AM frequencies of some hundred hertz it is necessary to ask to what degree such modulation frequencies may convey relevant information. Can they be resolved by auditory neurons, has the receiver auditory system a reasonable temporal resolution, or do high modulation frequencies exist only as a mere by-product of sound production?

The call of the katydid (*Neoconocephalus robustus*, robust conehead) consists of a train of uniform pulses with a high pulse rate of 200 Hz, which is well copied by the ascending interneuron AN1 (Triblehorn and Schul 2009). A related species, *N. triops* (broadhead conehead), produces trains of paired pulses, the pairs being separated by a somewhat longer interval. In this species, the homologous AN1 neuron responds only with a single spike to each pulse pair, that is, with half the rate of *N. robustus*. The neuron's responses in the two species correspond nicely to the behavioral preference functions (Triblehorn and Schul 2009; see also Schul et al. 2014). Another katydid, *Tettigonia viridissima* (great green bushcricket), does also produce calls with double pulses, in which pulse pairs are separated by longer intervals. Behavioral tests in this species indicate that the 14-ms interval separating the pulses of the pair is not resolved by the pattern recognition system. A single pulse of the same duration as the pulse pair has the same attractiveness (Schul 1998). However, this melting of the pulse pair into a single auditory event occurs most probably at later processing stages, not at the level of receptors, which have a higher temporal resolution (Schul 1997).

9.4.3 Modulation Transfer Functions

Modulation transfer functions (MTFs) are a widely used paradigm to assess the temporal resolution of auditory neurons (Joris et al. 2004). The principle of these experiments is to sinusoidally modulate the amplitude of a carrier and to record the neuron's responses while the modulation frequency is systematically varied (Fig. 9.2A). Picrotoxin eliminates frequency selectivity of an auditory interneuron in a bushcricket. Two types of MTF can be discerned: rate and temporal modulation transfer functions (rMTFs and tMTFs, respectively). In the former, it is observed if a neuron responds particularly well or poorly at certain AM frequencies (the examples in Fig. 9.2B show all-pass, low-pass, and band-stop properties). For the temporal MTF, it is necessary to determine how well the spikes are locked to the AM envelope, that is, their tendency to occur at consistent points within the cycle of AM (phase locking is here expressed as vector strength; for details of the procedure see Viemeister and Plack 1993; Prinz and Ronacher 2002). Often one finds tMTFs with low-pass or band-pass properties (see Fig. 9.2C). As a convenient measure of the upper frequency limits, the corner frequency of the tMTF is used, which gives a direct measure up to which frequency a neuron can still follow the AM pattern (see vertical lines in Fig. 9.2C).

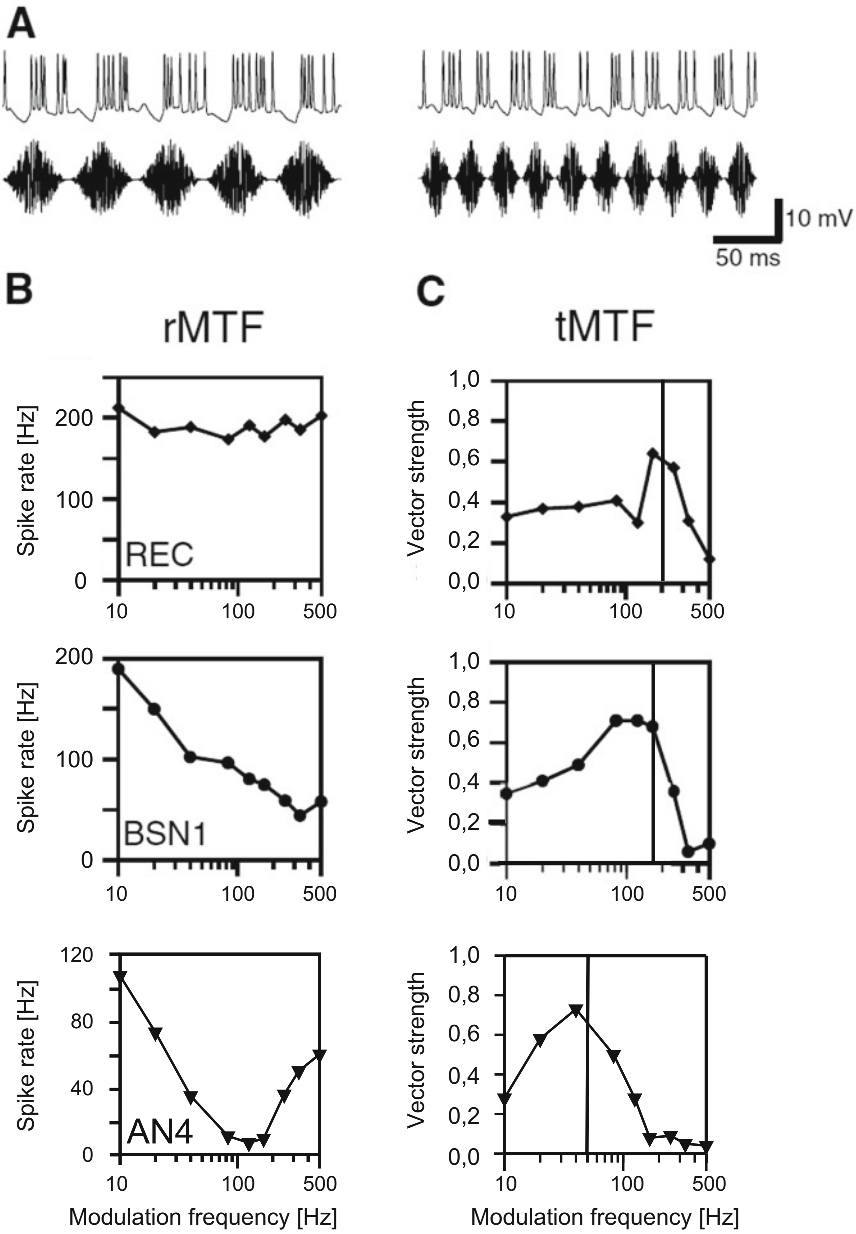

Fig. 9.2 Examples of modulation transfer functions (MTFs) of single neurons. (**A**) Spike responses to two modulation frequencies (20 and 40 Hz). (**B**) Rate modulation transfer functions (rMTFs) for neurons at different processing stages (receptor, local neuron BSN1, ascending neuron AN4). Examples show all-pass, low-pass, and band-stop rMTF. (**C**) Band-pass temporal MTFs (tMTFs) for the same neurons. The locking of spikes to the periodicity of the stimulus envelope is quantified as vector strength. *Vertical lines* indicate the neuron's corner frequencies; for the definition of corner frequencies see Wohlgemuth and Ronacher 2007 [Combined from Ronacher et al. (2008) and Wohlgemuth et al. (2011)]

As a rule, the auditory receptors of insects show a high temporal resolution and an all-pass behavior in their rMTF. Their spike rates hardly depend on the modulation frequencies presented, whereas spikes do phase lock up to high-modulation frequencies (corner frequencies of 150 to 200 Hz; see Fig. 9.2B, C). In their tMTF, receptors exhibit low-pass or band-pass properties (Prinz and Ronacher 2002). The examples in Figure 9.2 are from grasshopper neurons (average corner frequencies of receptors: 167 Hz). Auditory receptors of noctuid moths attain similar corner frequencies as found in grasshoppers (Surlykke et al. 1988). Less is known from crickets and katydids; data from Stumpner and Molina (2006) suggest that the rMTF of katydid receptor neurons have all-pass properties (for crickets see also Wendler 1990).

Compared to auditory receptors, neurons at later stages of processing tend to have lower corner frequencies, and thus a lower temporal resolution, while they exhibit more specific responses to particular AM patterns (compare Fig. 9.2B, C, lower panels). Also in other taxa, the temporal resolution of interneurons seems to be reduced compared to receptors (for crickets, see Sabourin and Pollack 2010; for katydids: Stumpner and Molina 2006; for moths: Boyan and Miller 1991; for cicadas: Fonseca 2014). An exceptional high temporal resolution is, however, reported for interneurons of some *Neoconocephalus* species (Triblehorn and Schul 2009).

The MTF paradigm can also be applied in behavioral tests (Wendler 1990; Hennig 2009). An example of a behavioral modulation transfer function obtained with a cricket (*Gryllus bimaculatus*) is shown in Figure 9.3. Males of this species produce sound pulses that are arranged in chirps of three to five pulses, separated by a longer chirp pause (Fig. 9.3A). The females prefer song models with pulse rates around 25–30 Hz (open symbols in Fig. 9.3B). The black curve shows the phonotactic responses of females toward a 4.5-kHz carrier that was continuously amplitude modulated at frequencies between 1 and 50 Hz (see insets 1, 2 in Fig. 9.3B for the stimuli used; details of the test procedure can be found in Hennig 2009). The transfer function (filled symbols) exhibits two peaks, at 3 Hz and at 25 Hz. However, with a continuous 25-Hz sinusoid stimulus, the responses were significantly reduced compared to stimuli in which the 25-Hz AM rate was presented in chirps (open symbols). The transfer function thus indicates the presence of two neuronal filters, a chirp rate filter and a pulse rate filter, and that both filters have to be activated to induce a strong response of females (Hennig 2009).

9.4.4 Stimulus Reconstruction Methods: Taking the Viewpoint of the Central Nervous System

A central nervous system (CNS) has no other information about the animal's environment than the spike trains provided by sensory neurons. Hence it is important to take the viewpoint of the CNS and ask: How much and what kind of information is represented by the spike train of a given neuron? This question has been tackled with "stimulus reconstruction" methods that allow estimating the information content of spike trains and learn what kind of information about a stimulus' properties is lost (Rieke et al. 1997). The basic idea is to record from auditory neurons while

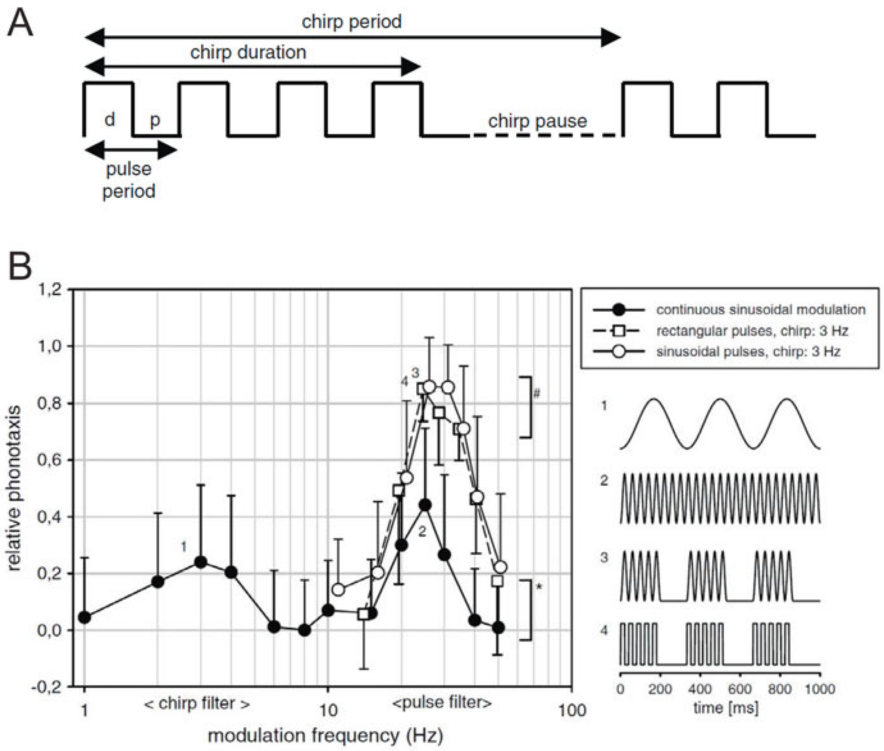

Fig. 9.3 Behavioral modulation transfer function of the cricket *Gryllus bimaculatus.* (**A**) Scheme of the song structure with different parameters are indicated. (**B**) Phonotaxis scores to a continuous sinusoidal modulation of the 4.5-kHz carrier (*filled symbols*; see insets 1 and 2 for the stimulus envelopes). *Open symbols*: phonotaxis scores obtained with patterns that combined pulse modulations with a chirp structure (see insets 3 and 4) [From Hennig (2009) with permission]

stimulating the auditory system with a stimulus that is randomly amplitude modulated (with a Gaussian amplitude distribution in a certain frequency range and a carrier that is suited to excite the neuron). Then part of the data is used to reconstruct the stimulus from the recorded spike trains in an iterative process, by replacing each spike by a filter function (see Fig. 9.4, inset in box stimulus reconstruction). The success of the procedure is then validated on the rest of the data not used to train the algorithm (see Fig. 9.4B). Using this method, it is possible to estimate the information transmitted by a neuron's spike trains and to learn what details of a stimulus' properties may not be represented by a specific neuron (Machens et al. 2001; see also Marsat and Pollack 2004). Figure 9.4B shows that the spike train of a grasshopper auditory receptor allows for an excellent reconstruction of the stimulus' amplitude modulation, provided that the modulations are not too fast (in this example <50 Hz). At higher modulation frequencies, the reconstruction success and the amount of transmitted information drop (see Machens et al. 2001). Thus, this method also yields an estimate of a neuron's upper limits of temporal resolution, similar to the tMTF paradigm.

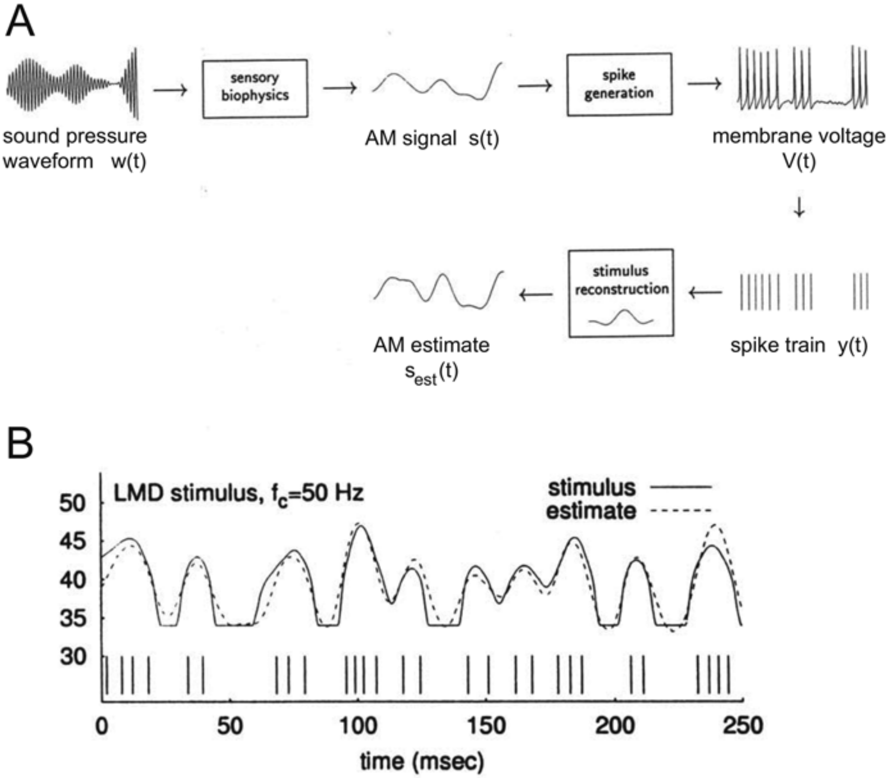

Fig. 9.4 (**A**) Principle of stimulus reconstruction. After transduction the amplitude modulation of the envelope (AM signal) elicits a spike train in the sensory neuron. Each spike is then replaced by an amplitude modulation (see stimulus reconstruction box), the exact form of which is determined in an iterative process as to yield the best prediction [$s_{est}(t)$] of the original AM signal $s(t)$. (**B**) Example of a spike train that yielded a high reconstruction success (*stippled curve*) for a random amplitude modulation (for details see Machens et al. 2001) [From Machens et al. (2001), with permission]

9.5 Variability of Neuronal Signals and Change of Coding Principles

9.5.1 "Intrinsic Noise" Induces Spike Train Variability

A fundamental problem for neuronal representations in general, and particularly for the coding of fast events, is the unreliability of neuronal signals. Even if an identical acoustic signal is repeatedly presented, the spike trains of a given neuron exhibit a certain trial-to-trial variability (see Fig. 9.5), which is due to the stochastic opening and closing of ion channels. This "intrinsic noise" sets limits to the temporal resolution (see Sect. 9.4.3) and poses a general and severe problem for neuronal encoding, in particular for animals that can spend only a small number of neurons for a given

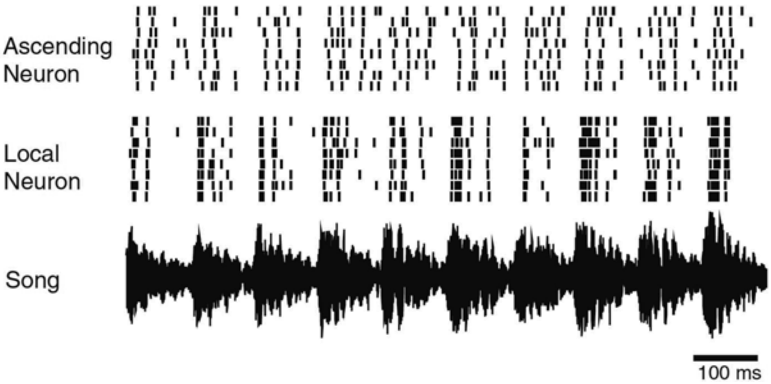

Fig. 9.5 Spiking responses of a local neuron and an ascending neuron of a grasshopper in response to a song. Spikes are shown as a "raster plot": each vertical line marks the timing of a spike, and a line represents one stimulus presentation. Note the trial-to-trial variability in spike timing, which is particularly high for the ascending neuron [From Ronacher et al. (2008), with permission]

task (Neuhofer et al. 2011). Trial-to-trial variability of neuronal responses is particularly detrimental if a receiver wants to discriminate between similar signals as is the case if females aim at selecting a high-quality partner among conspecifics (Ronacher et al. 2008; Einhäupl et al. 2011; Ronacher and Stange 2013). Note that the "intrinsic" neuronal noise operates in addition to external noise that causes a degradation of the acoustic signals while traveling from the sender to a distant receiver and thus exacerbates the ubiquitous problems of external noise (Schmidt and Römer 2011; Ronacher 2014).

In grasshoppers, the intrinsic variability of neuronal signals is lowest in auditory receptors and increases markedly at the next two stages of processing, the local thoracic neurons and the ascending neurons that transmit the information to the brain (Vogel et al. 2005). The examples in Figure 9.5 indicate that the local neuron responded more precisely, whereas the responses of the ascending neuron exhibited a particularly high trial-to-trial variability. Exploring how well different acoustic signals can be discriminated on the basis of the spike trains of single neurons, one finds that discrimination is remarkably good at the level of auditory receptors (Machens et al. 2003). At the level of ascending neurons, however, the increased neuronal variability leads to a strongly reduced discrimination performance (Wohlgemuth and Ronacher 2007).

9.5.2 *Change of Coding Schemes*

This loss of discrimination at higher processing stages was unexpected in view of rather precise behavioral responses because the ascending neurons are a bottleneck for the information available to the brain (Wohlgemuth and Ronacher 2007;

Ronacher 2014). The likely explanation for this apparent paradox is that a change of coding principles occurs already at a very early stage of processing, between the set of local neurons and the set of ascending neurons, which is only two synapses after the receptor neurons (Clemens et al. 2011). The receptors and local neurons encode the song's temporal features in the precise temporal pattern of their spiking responses. In contrast, among ascending neurons the information about song features seems to be distributed, as different ascending neurons encode different aspects of the stimulus in their spiking responses (Ronacher and Stumpner 1988; Krahe et al. 2002). Thus, the complete information about a song pattern cannot be read out from single neurons but only from a population of neurons as a *labeled line population code* (Clemens et al. 2011). Interestingly, at the level of ascending neurons the information about song features seems to be represented in spike count rather than in the exact timing of spikes (Wohlgemuth and Ronacher 2007; Creutzig et al. 2009). Thus, the information provided by the population of ascending neurons can be read out by downstream neurons simply by integrating spike count over a longer time interval. Hence, there is no need for a high temporal precision of spiking at this level—which may explain the increased variability found in ascending neurons. Remarkably, the high trial-to-trial variability observed in ascending neurons relates mainly to the timing of spikes, whereas the spike count is less variable (Neuhofer et al. 2011).

Because ascending neurons tend to respond specifically to different stimulus features it becomes evident why there is a reduced discrimination performance when focusing the analysis on the spike trains of a single neuron: If the set of acoustic stimuli does not differ in the respective feature this neuron is tuned to, the neuron's responses are, of course, not apt to allow for a discrimination.

This change of coding principles in the auditory pathway of grasshoppers is, in principle, similar to findings in the much larger nervous systems of vertebrates, but differs in that it occurs already at a very peripheral stage of processing, only few synapses after the receptor neurons (Clemens et al. 2011). Similar to reports from vertebrate brains, an increasingly sparse coding at the level of ascending neurons is observed, that is, a reduction of spike rates, and a de-correlation of responses between different neurons (Clemens et al. 2011; see also Kostarakos and Hedwig 2012).

9.6 How Many Parallel Detectors Are Needed?

A puzzling observation is that in crickets only two ascending neurons seem to be sufficient to convey the necessary information about sound signals to the brain, where the final behavioral decisions take place. In grasshoppers, in contrast, a considerable number of ascending neurons exist—on the order of 15–20 different types have been described so far (Stumpner and Ronacher 1991; Wohlgemuth and Ronacher 2007; see also Greenfield, Chapter 2). Are all of these neurons important or necessary for song recognition? How can we assess the contribution of neurons to behavioral responses? In the ideal case the stimulation of a neuron leads to a change of behavior (Schildberger and Hörner 1988). However, this type of experiments is only rarely feasible. The commonly chosen approach is to present signals of varying behavioral relevance, for example sound pulse patterns with different pulse rates (Fig. 9.3; see also Fig. 9.1D), while recording from individual neurons. If a clear correlation is observed between a neuron's response and the behavioral preference functions the scientist is satisfied and tends to assign a behavioral relevance to this neuron, or even describe it as a filter for a specific AM feature (see Ronacher and Stumpner 1988; Creutzig et al. 2010). If there is no such correlation evident, the neuron is thought to code for different or unknown features.

9.6.1 Prediction of Behavioral Responses by a Modeling Approach

A different approach has recently been undertaken, based on linear-nonlinear (LN) models applied to behavioral data, that aimed at elucidating basic principles of neuronal processing (Clemens and Ronacher 2013; see also Clemens and Hennig 2013). The principal structure of the model is depicted in Figure 9.6. The model assumes a set of parallel feature detectors, each consisting of a linear filter followed by a nonlinearity, hence the term LN model, and a step of temporal integration. After the integration, each detector yields a single number as output; these numbers are then linearly combined with certain weights to yield a prediction of the behavioral response. The experimental basis for this approach was the behavioral responses of female grasshoppers tested with a large set of different sound patterns in which several distinct features were systematically varied. Using a genetic learning algorithm, the model was trained with a part of the data and then cross-validated with the remaining data set; no a priori assumptions were made with respect to specific properties of the feature detectors or nonlinearities. This modeling approach has led to several interesting insights (Clemens and Ronacher 2013; Ronacher et al. 2015).

First, a version of the model with only two feature detectors (red and green in Fig. 9.7A) provided an excellent prediction of the behavioral data (with a high correlation between the model's feature values and behavior: $r^2 = 0.87$, Fig. 9.7F); the inclusion of a third detector yielded only a marginal improvement (Clemens and

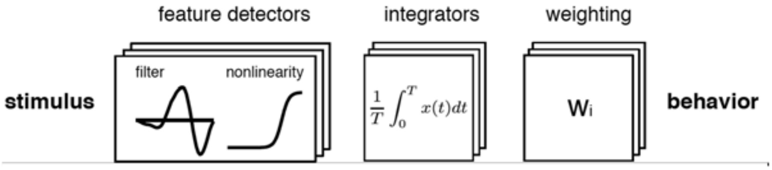

Fig. 9.6 Structure of the feature-detector model introduced by Jan Clemens. Description in the text [From Clemens and Ronacher (2013), with permission]

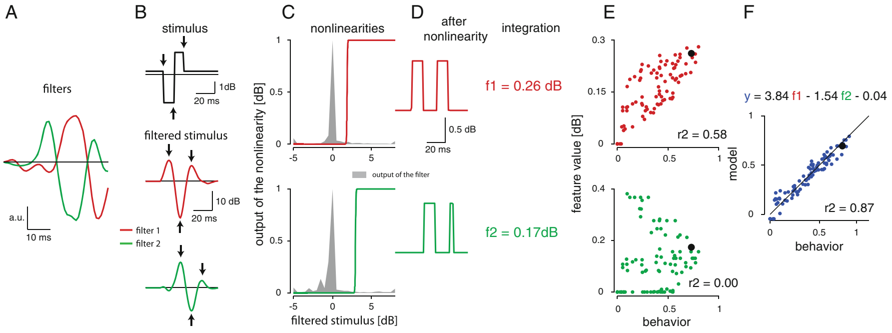

Fig. 9.7 LN feature detector model with two filters. The shape of the filters (*red* and *green*) is shown in (**A**); in (**B**), the filter response to a song model with a pause followed by an onset accentuation is demonstrated. The nonlinearities in (**C**) are very steep and let pass only positive values of the filtered stimulus above certain thresholds, leading to the picture in (**D**). These nonlinearities can be interpreted as translating a neuron's membrane depolarization into a spike rate. The integration step produces an average over time (in the example, 0.26 and 0.17, respectively). These numbers are combined with weights to yield the prediction of the behavioral response (**F**). Note that the green filter contributes to the sum with a negative value. In (**E**), the correlations of the two detectors with the behavioral data are shown separately; note that the green detector shows no correlation to the behavior at all [Modified from Clemens and Ronacher (2013), with permission]

Ronacher 2013). Note, however, that a feature detector as postulated in the model may not necessarily be implemented as a single (ascending) neuron; it may well encompass a set of neurons.

Second, the temporal integration step seemed at first glance counterintuitive because it eliminates information about the exact temporal position of sound features, in contrast to the emphasis laid on the importance of temporal patterns in Sects. 9.1 and 9.2. However, the model's filters do still respond highly specifically to the presence of certain envelope features, for example, combinations of offsets and onsets (see Fig. 9.7B). Only at the output stage, because of the integration step, it is no longer relevant at what exact position within the song this feature occurred. The temporal integration step is well in line with behavioral data, where grasshoppers responded well to shuffled songs (von Helversen and von Helversen 1998) and can indeed explain several behavioral results that remained rather enigmatic so far (Ronacher et al. 2015).

Third, a further interesting result was revealed if the correlations between behavior and each of the detectors were determined separately (Fig. 9.7E). While the output of one of the detectors exhibited a reduced but still substantial correlation with the behavior, the other detector showed no correlation to the behavior at all ($r^2 = 0.00$). Thus, this model stresses an interesting, but also disturbing, possibility that a neuron, which may contribute substantially to the overall performance, may nevertheless show no overt correlation if tested with experimental variations of behaviorally relevant parameters (for further details see Clemens and Ronacher 2013).

9.6.2 *Modeling Explains Transitions Between Behavioral Preference Functions*

The same model structure was also able to describe behavioral data obtained with two cricket species (Clemens and Hennig 2013). Although in grasshoppers and crickets, the model features were found independently, they exhibit remarkable similarities; the optimal filters found by the algorithm resemble Gabor filters, which have interesting properties. In visual systems, Gabor filters provide the best compromise between the resolution of spatial frequencies and the localization of visual patterns (Daugman 1985). If one searches for a sparse basis for natural images, the basic functions resemble Gabor filters (Olshausen and Field 1996; Bell and Sejnowski 1997). Most remarkably, Clemens and Hennig (2013) could show that a variety of different behavioral preference functions, as found in different cricket and katydid species, can be obtained with only small parameter variations in the positive and negative lobes of the filter (cf. Fig. 9.7A). The appeal of this approach is that different filter shapes can be obtained from a combination of excitatory and inhibitory inputs, with only small changes in the respective timing and the durations of synaptic potentials (see examples in Fig. 9.8). Thus, behavioral preference functions tuned to the pulse period but also tuned to pulse duration, pause duration, or duty cycle can be generated with this model (Fig. 9.8; for details see Clemens and Hennig 2013; Hennig et al. 2014). The ease with which different filter shapes and the correspondingly different preference functions can be obtained is particularly interesting in view of the evolutionary divergence of species-specific communication systems in crickets and katydids (Heller 1988; Schul et al. 2014). For example, the two closely related Australian crickets *Teleogryllus commodus* and *T. oceanicus* differ in their preference functions (period tuned in *T. oceanicus*; pulse duration tuned in *T. commodus*; Hennig 2003). The model introduced by Clemens and Hennig now suggests that a transition between the two behavioral preferences may be easily acquired by minor changes in synaptic weights and/or in the respective ion channel equipment that influence the duration of excitatory and inhibitory postsynaptic potentials (for further details see Clemens and Hennig 2013; Hennig et al. 2014).

9.6.3 *Comparison of Model Predictions and Responses of Brain Neurons*

The predictions of this modeling approach are a strong incentive to look at neuronal data obtained in crickets. As already mentioned in Sect. 9.6, in crickets only two relevant ascending neurons seem to exist, AN1 and AN2. Whereas AN2 is involved in bat escape behavior (e.g., Marsat and Pollack 2006), the AN1 is particularly relevant for processing of conspecific song patterns. AN1 copies the song pulse pattern in its spike responses, exhibiting an all-pass property for different pulse periods,

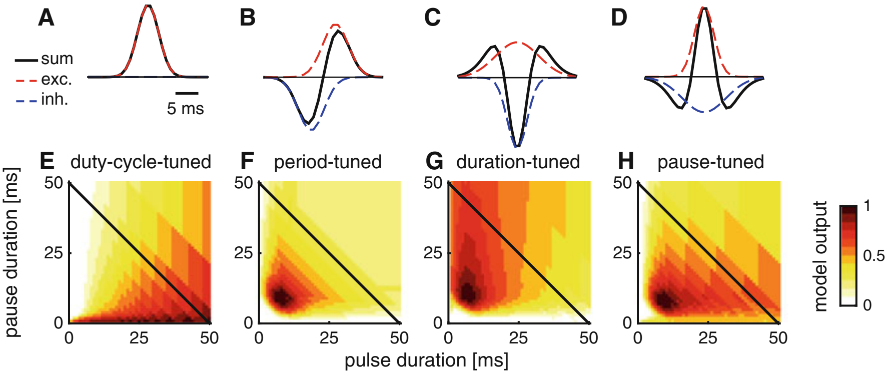

Fig. 9.8 Properties of Gabor filters can explain the profiles of various preference functions found in different species. (**A–D**) Different combinations of excitation and inhibition (*red and blue dashed lines*, respectively) give rise to different Gabor functions. No inhibition (**A**) yields a preference profile corresponding to a duty cycle tuning (**E**). Leading inhibition (**B**) yields a period tuning (**F**). Broad excitation combined with narrow inhibition (**C**) yields a Mexican-hat-like filter with pulse duration tuning (**G**). Narrow excitation paired with broad inhibition (**D**) yields pause tuning (**H**). The color code is indicated to the right of (**H**) [Modified after Hennig et al. (2014), from Ronacher et al. (2015)]

and obviously provides the basis for further computations by local brain neurons (Wohlers and Huber 1982; Kostarakos and Hedwig 2012). In *Gryllus bimaculatus,* behavioral tests revealed a filter tuned to pulse periods around 40 ms (Schildberger 1984; Hennig 2009; see Fig. 9.3). In a pioneering study, Schildberger (1984) identified brain neurons with low-pass and high-pass properties in the cricket. An AND-like combination of these two filters could then lead to the band-pass property found in behavioral tests (see Kostarakos and Hedwig 2015 for a discussion of this concept). These authors described several local neurons in the cricket brain that exhibit interesting properties (Kostarakos and Hedwig 2012, 2015; see also Hedwig and Stumpner, Chapter 8). While one of the local brain neurons (B-LI2) shows an all-pass response for pulse periods similar to AN1, other neurons, presumably representing consecutive steps of processing, show a band-pass response, with weak responses to small periods, that is, high pulse rates, and diminishing responses to large periods (see Fig. 8.9D, E in Hedwig and Stumpner, Chapter 8). The response peak of these neurons corresponds well to the behaviorally preferred periods of 34–42 ms. Interestingly, in the neuron B-LI4, with the closest correspondence to the behavioral preference function, a combination of inhibitory and excitatory postsynaptic potentials (IPSPs and EPSPs), becomes visible that shapes its preference function; only at the preferred pulse period is the neuron released from this inhibition (see Fig. 8.9 in Hedwig and Stumpner, Chapter 8, and Kostarakos and Hedwig 2012, 2015; for interactions of inhibitions and excitations in brain neurons of katydids see also Stumpner and Nowotny 2014). The observations of Kostarakos and Hedwig are well in line with the predictions of the aforementioned modeling study (Clemens and Hennig 2013; Hennig et al. 2014). In addition, the cricket neurons also exemplify the principle of sparse coding; compared to the ascending neuron AN1, in the brain neuron B-Li4 the maximum response was reduced by almost 90 % (Kostarakos and Hedwig 2015).

9.7 Summary

The acoustic communication of these insects provides prime examples of general principles of how nervous systems process and represent sensory information. At the periphery of the auditory system of grasshoppers, crickets, and katydids, the receptor neurons respond with a high temporal precision and represent the stimulus' envelope in their spike patterns, in a "temporal code." This type of representation is, however, transformed to a "place code" or "labeled line code" representation in which single neurons represent specific sound features. In these neurons, spike rates are generally reduced and the presence of a particular sound feature can be read out from the spike rate averaged over a longer time period. Remarkably, in insects, this change of coding scheme and the sparsening occur within a few synapses after the input stage, the auditory receptor neurons.

Throughout this chapter, readers will have noticed that in insects similar processing schemes are realized as in vertebrates, though based on much fewer neurons. Complex computations, which in vertebrates are performed by thousands of neurons, in insects are often concentrated within single neurons. To mention just a few general principles for which examples are also found in insects: parallel processing of information, lateral inhibition for contrast enhancement, feature extraction based on different timing of excitation and inhibition, transformation of coding from a temporal code to a place code, and sparse coding. In addition, the examples presented here demonstrate how a close combination of behavioral tests, neurophysiological studies, and computational modeling may lead to new insights and boost our understanding of neuronal information processing.

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Chapter 10
Hearing in *Drosophila*

Azusa Kamikouchi and Yuki Ishikawa

Abstract Since the first analysis of the *Drosophila* courtship song in the early 1960s, the molecular and neural mechanisms underlying acoustic communication in fruit flies have attracted the interest of many researchers studying behavioral evolution, neuroethology, sensory systems, motor pattern control, acoustic information processing, and decision making in the brain. Recent studies utilizing a wide array of genetic tools have provided novel insights into the mechanisms of acoustic communication in *Drosophila*, from genes and cells to neural circuits and behaviors. *Drosophila*, in addition to the conventional model animals such as other singing insects, mammals, and birds, thus serves as an excellent model system for analyzing the neuronal and molecular mechanisms that are essential for information processing of acoustic signals. This chapter provides an overview of our current knowledge on hearing in *Drosophila* with an introduction to their acoustic communication, the hearing organs, and cells involved in the function and development of the auditory system and the auditory neural circuits in the brain.

Keywords Acoustic communication • Active amplification • Antennal ear • Antennal mechanosensory and motor center • Auditory neural circuit • Auditory system • Courtship song • Johnston's organ • Mechanotransducer channels • Primary auditory center • Response properties

10.1 Introduction

Sound has a dual nature that includes fluctuations in pressure and oscillations of air particles. The animal kingdom has thus developed two ways of measuring sound: a pressure receiver, such as the vertebrate tympanal ear, and a movement receiver that follows the oscillation of air particles (see Windmill and Jackson, Chapter 6). Singing insects, such as crickets, katydids, and cicadas, have sophisticated tympanal ears

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capable of detecting “far-field sounds” (Yager 1999). Honeybees, mosquitoes, midges, and fruit flies detect the particle velocity component of sound by using a movement receiver at the antenna (see Ewing 1989; Kamikouchi 2013). This chapter summarizes hearing mechanisms in *Drosophila*, with a special focus on the fruit fly *D. melanogaster*. With the wealth of genetic tools currently available to visualize and manipulate particular types of cells, the hearing system of *D. melanogaster* serves as an attractive model for studying the mechanisms involved in hearing at the molecular, cellular, and neural circuit levels. This chapter reviews what and how fruit flies hear and the neural circuits from the ear to the brain. Cellular and molecular aspects of hearing are discussed by Eberl, Kamikouchi, and Albert (Chapter 7). Together, these chapters provide an overview of our current knowledge on hearing in fruit flies.

10.2 What Do *Drosophila* Hear?

The courtship of *Drosophila melanogaster* males to females involves a series of stereotyped behaviors prior to mating (Greenspan and Ferveur 2000); a male orients toward a female, chases her, taps her body with his foreleg, circles around her, licks her genitalia, and attempts to copulate. In the early 1960s, Shorey reported that the male *D. melanogaster* sends acoustic signals to the female by vibrating his wing during this complex courtship display (Fig. 10.1a; Shorey 1962). This finding was followed by the discovery that each *Drosophila* species produces a unique pattern of sounds, the courtship song, during its courtship behaviors (Fig. 10.1b; Ewing and Bennet-Clark 1968; Cowling and Burnet 1981). Since these discoveries, the courtship behavior of fruit flies has been studied by many researchers interested in exploring the cellular, molecular, and developmental bases of acoustic communication in animals.

By the early 1980s, the songs of more than 100 species of *Drosophila* and the related drosophilid genus *Zaprionus* were described (Ewing 1983). The majority of *Drosophila* species, including *D. melanogaster*, have more than one type of courtship song (Fig. 10.1b). A courting *D. melanogaster* male typically produces two types of songs, a sine song and a pulse song, alternately (Fig. 10.1c). The sine song comprises bouts of continuous oscillations at frequencies ranging between 130 and 185 Hz (Wheeler et al. 1988; Riabinina et al. 2011). The pulse song comprises a train of pulses whose dominant frequency component ranges between 150 and 200 Hz (Riabinina et al. 2011). Wing movements during *Drosophila* courtship have been interpreted as a ritualized form of flight. Indeed, the principal frequency components of flight-induced sound emissions range between approximately 145 Hz and 200 Hz in *D. melanogaster* and its sister species (Riabinina et al. 2011).

Closely related species differ in major parameters of the song, including pulse type, interpulse interval (IPI; Fig. 10.1c), and intrapulse frequency (IPF) of the pulse song, and the presence and frequency of the sine song. As for *D. melanogaster*, the time between pulses in the pulse song, the IPI, is of particular interest

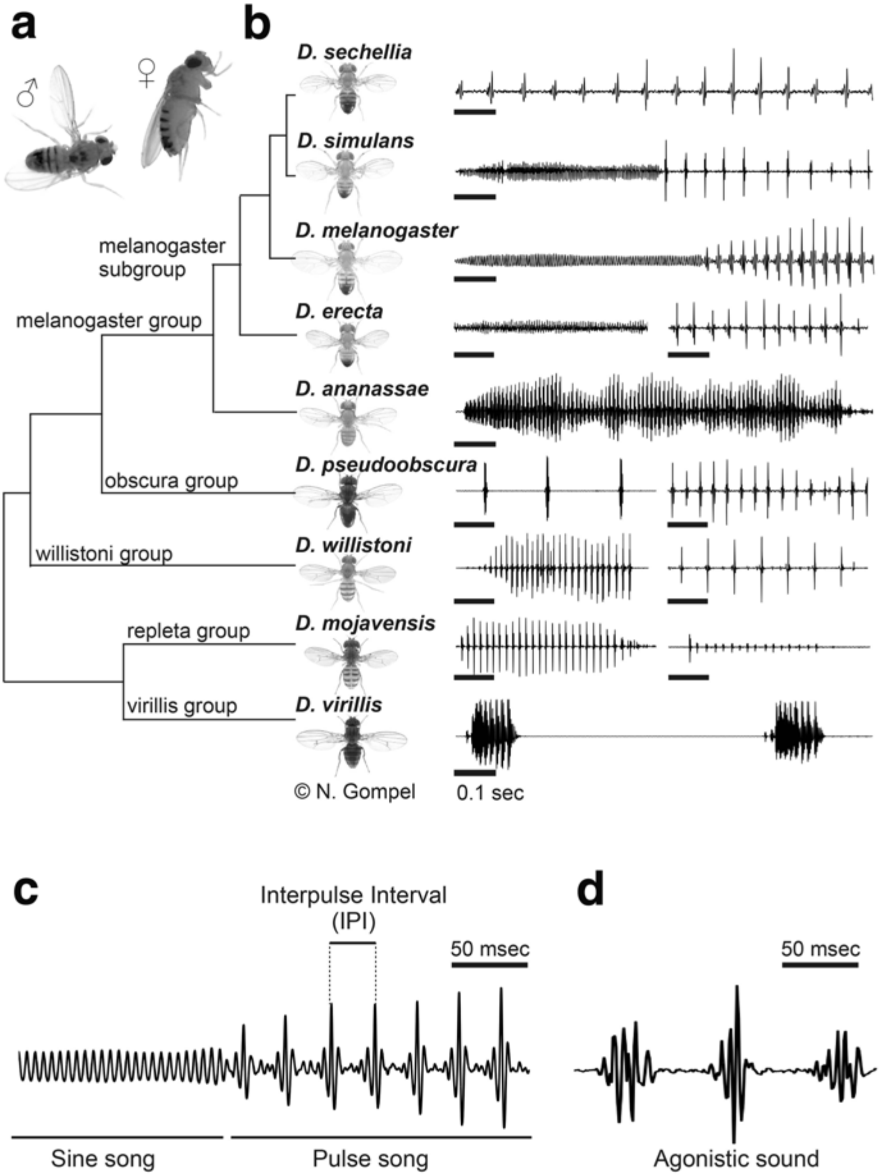


Fig. 10.1 Courtship songs of *Drosophila*. (**a**) Courtship behavior of *D. melanogaster*. The male fly vibrates his wing to generate sound, the so-called "courtship song." (**b**) Courtship songs of *Drosophila*. Fly images and phylogenetic tree are adapted from Flybase. Fly images were supplied by Dr. Nicolas Gompel. Several species have two different songs. (**c**) The sine song and the pulse song of *D. melanogaster*. (**d**) Agonistic sound of *D. melanogaster*. The sound file was provided by Dr. Thorin Jonsson

because it is a critical determinant for accelerating copulation (Bennet-Clark and Ewing 1969). Moreover, the mean IPI length differs between *D. melanogaster* and its sister species; although the actual length of the IPI fluctuates, *D. melanogaster*, *D. simulans*, *D. mauritiana*, and *D. sechellia*, have mean IPIs of 35 ms, 55 ms, 45 ms, and 85 ms, respectively (Cowling and Burnet 1981; Cobb et al. 1989). The species-specific pattern of songs, such as the IPI, is widely considered to be important for both reproductive isolation and speciation in Drosophilid flies.

It is postulated that in *D. melanogaster*, the main function of the sine song is to sexually stimulate females, whereas that of the pulse song is to trigger mating (Ewing 1989). The pulse song is produced more frequently than the sine song, but bouts of sine and pulse songs often immediately follow each other (Tauber and Eberl 2003). The wing vibrations to produce these songs generate the particle-velocity signals, whose amplitude is typically higher in the pulse song than in the sine song. While emitting the courtship song, the male stands typically within 5 mm of the female fly. At distances of 2.5 mm and 5 mm, the particle velocity levels of the natural courtship song emitted by wing vibrations of a fruit fly are estimated to be approximately 92 dB and 72 dB above 50 nm/s (i.e., 2 mm/s and 0.2 mm/s), respectively (Bennet-Clark 1971). Fruit flies detect such acoustic stimuli with the hearing organ located in the antenna, namely the “antennal ear.”

Even with intensive “singing” by a male fly, the female being courted does not always accept him as a mating partner; females sometimes emit a “rejection signal” to a courting male, which is a sound produced by wing-flicking behavior (Ewing and Bennet-Clark 1968; Paillette et al. 1991). Another example of intraspecific interactions involving the emission of sound is agonistic behavior between males, which is used to acquire or secure important resources, including food, territory, and mating partners. The rejection sound of females and agonistic signals of males both comprise a train of pulses that resembles the pulse song, but the pulse duration and IPIs are about twice as long (Fig. 10.1d; Tauber and Eberl 2003; Jonsson et al. 2011). Whether these sounds have adaptive functions, however, remains unclear.

Do fruit flies hear other types of sound such as their own flight tone and environmental noises? Because of the rapid attenuation rate of the particle velocity component of sound, their hearing system is insensitive to far-field sound. The wing beat during flight, on the other hand, produces flight tones that actuate the antennal ear of a flying fruit fly (Riabinina et al. 2011). Although it is unclear whether fruit flies use sound to control their own flight, their antennal ears are able to detect the flight tones they produce.

10.3 Behavioral Response to the Courtship Song

Females rarely accept males that do not produce the courtship song; males made mute by wing removal usually do not succeed in copulating with a target female during a 15- to 20-min observation period (Talyn and Dowse 2004; Vaughan et al. 2014). By using artificial songs to supplement the deficient courtship of wing-clipped males, the behavioral response of flies to the courtship song can be quantified experimentally. Indeed, *D. melanogaster* females mate more with wingless males when songs with species-typical parameters are played (Fig. 10.2a; Bennet-Clark and Ewing 1969; Ritchie et al. 1999). A popular method of assessing the response of flies to the courtship song is to measure their mating success under exposure to natural or artificial songs (Ritchie et al. 1999; Clyne and Miesenböck 2008). The mass-mating technique, in which tens of virgin females and wingless males are introduced into a chamber placed over a loudspeaker (Bennet-Clark and Ewing 1969), has long been used to test the effects of song parameters on mating. By using this technique, the role of the IPI in the pulse song was confirmed to be a critical parameter in enhancing mating success in *D. melanogaster*.

In such a mass-mating situation, the success rate under exposure to the courtship song reflects the sum of the responses of females and males to the song. The behavioral response of either females or males to the song can also be assessed separately by measuring the activity of flies in a unisex group. In these cases, females or males are placed in a chamber and exposed to a recorded or artificial courtship song (Fig. 10.2b). These single-sex group assays eliminate nonacoustic signals derived from the potential mating partner. Furthermore, these single-sex group assays provide useful tools for analyzing auditory effects on sexual behavior, which is independent of the interaction with mating partners. When a group of eight to nine female flies are exposed to a constant 84-dB artificial song for 1 min, for example, they slow down their movement as when accepting a courting male (Fig. 10.2b; Crossley et al. 1995).

On the other hand, male *D. melanogaster* flies in a single-sex group increase their locomotor activity and begin courting each other upon exposure to the song (Fig. 10.2b–e; von Schilcher 1976; Crossley et al. 1995). This activity increase is more obvious when the wings of the males are clipped, probably because the wingless males lack the ability to produce major rejection signals, that is, wing flicking. Such homosexual behavior between aroused males is known as “chaining behavior,” in which each male chases another male to tap its abdomen (Fig. 10.2d). Because chaining behavior is easily observed in single-sex groups of male flies on exposure to courtship song, it is a popular method of quantifying the behavioral response of males to a song (Eberl et al. 1997; Kamikouchi et al. 2009). *D. melanogaster* males maximally respond to artificial pulse songs with an IPI range between 35 and 75 ms, corresponding to the pulse song of the species (Fig. 10.2e). Songs with shorter (15 ms) and longer IPIs (95 and 105 ms) evoke the chaining response as well, but the response is lower than its maximal level (Yoon et al. 2013). In contrast, the sine song does not induce chaining behavior in males.

Once the song arouses the males to move, they continue their increased activity for at least several minutes under no-song conditions (von Schilcher 1976). This behavior of the males seems to be adaptive, as the exposure to a song implies the existence of another courting male, which indicates that there is probably a receptive female nearby. This possibility is supported by the observation in the wild that flies seem to congregate on feeding sites at certain hours of the day and to copulate there under crowded conditions (Spieth 1974).

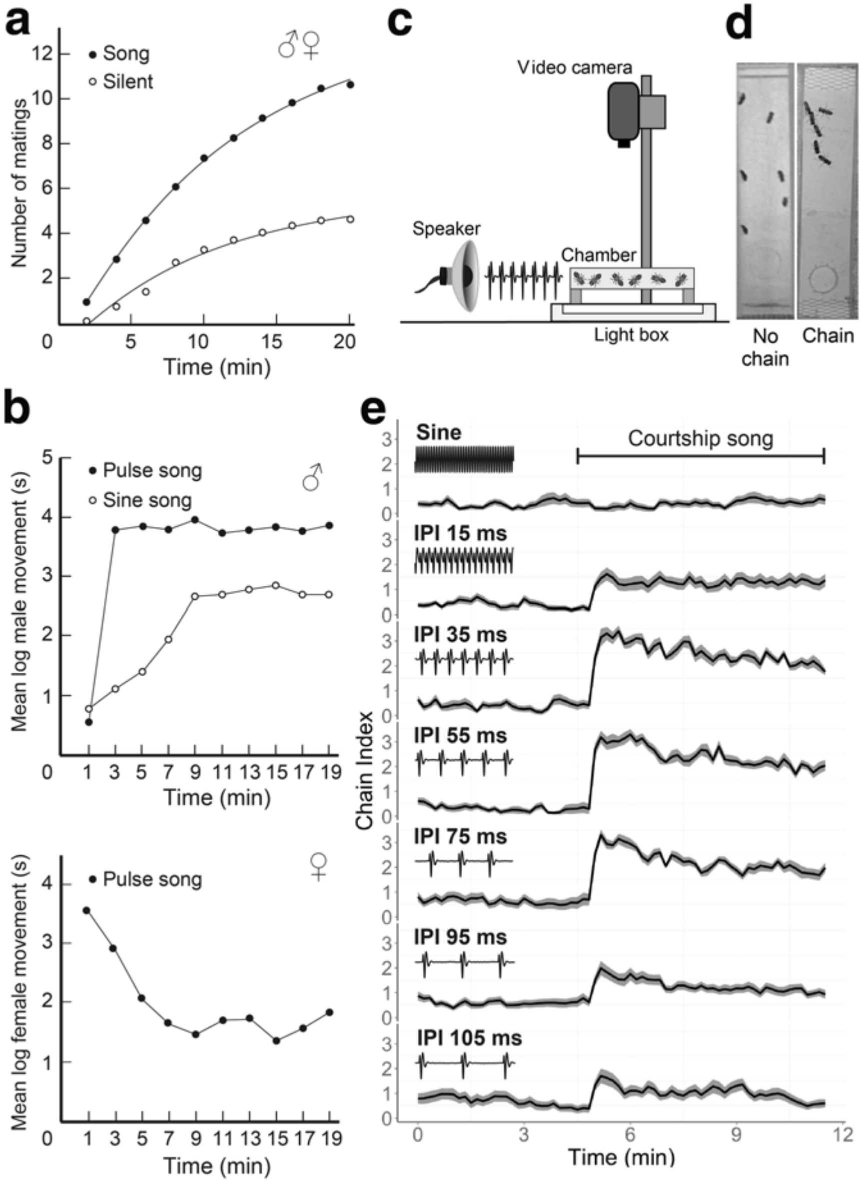


Fig. 10.2 Behavioral responses to the courtship song in *D. melanogaster*. (**a**) Stimulation with a species-specific song accelerates mating between males and females. (**b**) Exposure to the pulse song differentially affects the locomotor activity of males and females; the locomotor activity of males increases (*top*), whereas that of females decreases (*bottom*). (**c**) Experimental setup to evaluate male chaining behavior. (**d**) Chaining behavior of *D. melanogaster*. (**e**) Chaining behavior of *D. melanogaster* males is induced by the artificial pulse songs containing various interpulse intervals (IPIs) [Modified from Crossley et al. (1995); Ritchie et al. (1999); Kamikouchi et al. (2009); Yoon et al. (2013), with permission]

10.4 The Antennal Ear of Fruit Flies

The antennal ear of fruit flies comprises two functional parts: a sound receiver and an auditory sensory organ (Fig. 10.3a–c). The feathery arista at the tip of the antenna serves as the receiver, which catches the particle velocity component of sound. Laser vibrometric analysis of sound-induced vibrations revealed the mechanical coupling between the arista and the third antennal segment (a3, also referred to as the funiculus) (Göpfert and Robert 2001); the a3 and arista together represent a damped simple harmonic oscillator that vibrates back and forth when stimulated acoustically (Göpfert and Robert 2002). The a3 is flexibly articulated at its base with the second antennal segment (a2), where it interacts with Johnston's organ (JO), the auditory sensory organ of fruit flies housed in a2. Here, the proximal part of a3 forms a stalk that fits into a fossa in a2. This stalk bends laterally to form a hook that connects a3 with JO (Fig. 10.3d). Movement of the distal antenna is converted to produce oscillations of the hook, which then activates and inactivates the mechanosensory neurons in JO, namely, JO neurons. JO neurons function in an analogous manner to cochlear hair cells, though JO neurons use primary cilia instead of actin-based hair bundles as sensory organelles.

10.4.1 Johnston's Organ

Chordotonal organs are internal stretch receptors found in insects and crustaceans. JO is the largest chordotonal organ of the fruit fly, which houses approximately 480 JO neurons in *D. melanogaster* (Kamikouchi et al. 2006). JO detects the movement of the distal antenna, a3 and arista, in relation to a2 as the oscillation of the hook. In JO, repeats of the sensory unit called the scolopidium are radially organized, with their tips toward the hook (Fig. 10.3c, d; Uga and Kuwabara 1965; Kamikouchi et al. 2006). A scolopidium comprises two or three JO neurons, the scolopale cell, the cap cell, and the ligament cell (Boekhoff-Falk and Eberl 2014). Scolopale cells form a sealed space around the sensory cilia of JO neurons (Fig. 10.3c). Approximately 10 % to 15 % of JO scolopidia include three neurons, and the remainder contains two neurons (Todi et al. 2004). The cell bodies of JO neurons are distributed like a "bowl" that lies vertically with its top facing the lateral side and its bottom cut off (Fig. 10.3e; Kamikouchi et al. 2006).

10.4.2 JO Neurons

Anatomically, JO neurons are bipolar neurons. Each JO neuron has two protrusions: an axon at one side and a cilium at the other side of the cell body (Fig. 10.3c). The cilium of the JO neuron has a $9 \times 2 + 0$ axonemal ultrastructure (Todi et al. 2004), the tip of which is attached to the base of a3, the hook, via cap cells. Functionally, JO

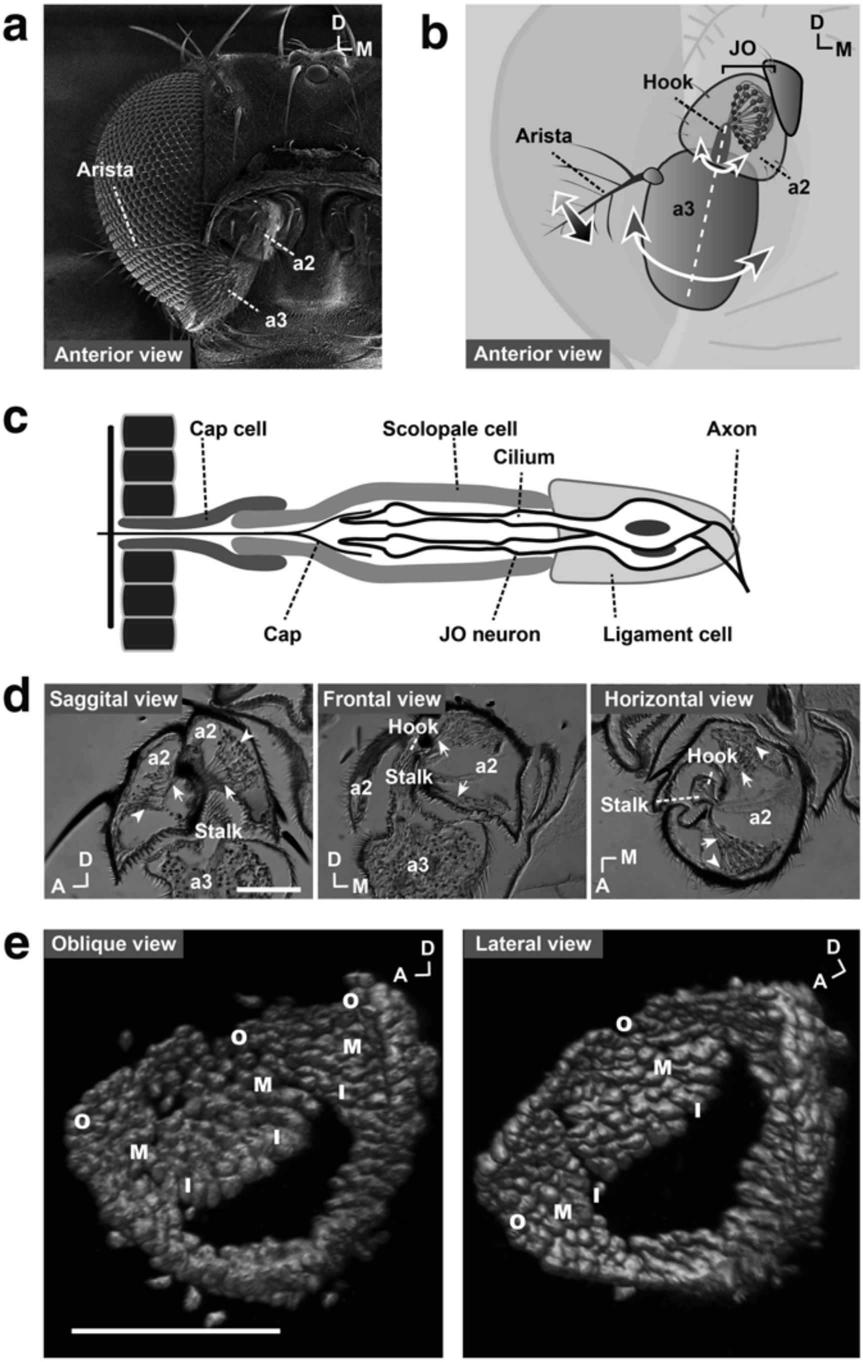


Fig. 10.3 The hearing organ. (**a**, **b**) The antennal ear of *Drosophila*. When stimulated acoustically, the arista and the third antennal segment (a3) together vibrate about the longitudinal axis of a3. The vibrations of these distal antennal parts are translated into neural activities in Johnston's organ (JO)

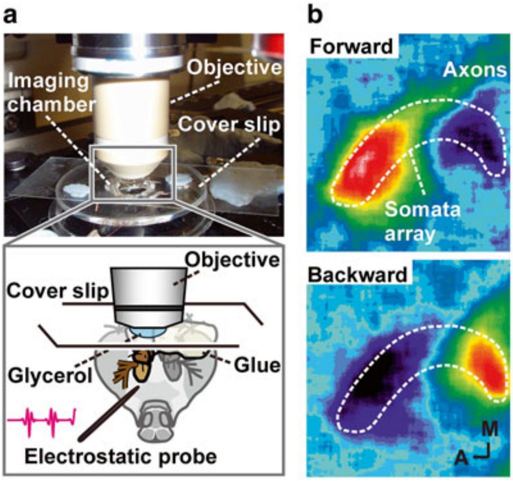


Fig. 10.4 Calcium imaging of JO neurons. (**a**) Experimental setup for calcium imaging. By using electrostatic forces generated between the stimulus electrode and a charged animal, the antennal receiver can be actuated in specific patterns. (**b**) Calcium response in Johnston's organ (JO). When the receiver was deflected statically, opposing calcium signals were detected in the anterior and posterior regions of JO. Deflecting the receiver forward evoked positive signals in the anterior region and negative signals in the posterior region [Modified from Kamikouchi et al. (2009), with permission]

neurons are stretch receptor neurons; the primary cilia of JO neurons detect the receiver's movements, such as its vibrations and static deflections. The response properties of JO neurons reflect the intrinsic and extrinsic properties of each cell. As a stretch receptor, the JO neuron is activated when the cilium is stretched and inactivated when it is compressed. Thus, tilting the antennal receiver to one direction activates one population of JO neurons and inactivates another population. Calcium imaging of JO neurons while statically deflecting the antennal receiver allows for visualization of such opposing responses (Fig. 10.4a); deflecting the receiver forward evokes positive signals in the anterior population of JO neurons and negative signals in the posterior one; backward deflection evokes the opposite signals (Kamikouchi et al. 2009; Fig. 10.4b). The existence of these two opposing neural populations explains the frequency doubling observed in the sound-evoked field potential to sinusoidal vibration stimuli recorded in the antennal nerve (Eberl et al. 2000; Albert et al. 2007).

Fig. 10.3 (continued) located within the second antennal segment (a2). (**c**) The *Drosophila* JO scolopidium. The scolopale cell wraps the sensory dendrites of JO neurons to form the scolopale space. (**d**) Sagittal, frontal, and horizontal sections through a2. *Arrowheads* and *arrows* denote the cell bodies of JO neurons and scolopidia, respectively. (**e**) Distribution of cell bodies of JO neurons. About 480 cells are organized in a "bottomless bowl" pattern in JO. This "bottomless bowl" can be divided into three concentric layers: outer (O), middle (M), and inner (I). Scale bar = 50 μm. Lines labeled A, D, and M indicate anterior, dorsal, and medial directions, respectively [Modified from Kamikouchi et al. (2006, 2009); Kamikouchi (2013); Matsuo and Kamikouchi (2013), with permission]

10.5 Active Amplification

In vertebrate ears, a process called the cochlear amplifier provides active amplification, a positive mechanical feedback system that actively increases the sound sensitivity of the ear (Hudspeth 2008). Motile hair cells in the inner ear actively enhance the tiny vibrations induced by low-level sound, thus augmenting the mechanical input experienced by the ear (Hudspeth 2014). Similar to vertebrates, hearing in the fruit fly is assisted by such active amplification (Göpfert and Robert 2003; Göpfert et al. 2005). The laser Doppler vibrometer serves as a noninvasive method for examining the antennal mechanics of fruit flies in the absence and presence of sound (Fig. 10.5a, b). Even in the absence of acoustic stimulation, the antennal receiver of the fruit fly fluctuates autonomously. This free fluctuation represents the sum of passive and active motions of the receiver, which proceed from the thermal bombardment of the receiver by surrounding air particles and mechanical feedback from JO neurons to boost the fluctuation, respectively (Göpfert and Robert 2003; Riabinina et al. 2011). In dead or transiently anesthetized flies (*D. melanogaster*), the mechanical response of the receiver remains linear and passive and is tuned to frequencies around 800 Hz, irrespective of the stimulus amplitude (Göpfert and Robert 2003). In live and nonanesthetized flies, on the other hand, active processes that enhance the vibration of the receiver alter the auditory tuning in a level-dependent manner. Here, the resonance frequency of the receiver nonlinearly shifts down in frequency toward 200 Hz when the sound is faint. Accordingly, the sensitivity around the dominant frequency component of their courtship song, which is approximately 200 Hz, increases as the stimulus intensity is decreased.

As observed in *D. melanogaster*, active amplification of the receiver's fluctuation tunes the ears of other members of the *D. melanogaster* species group. The antennal

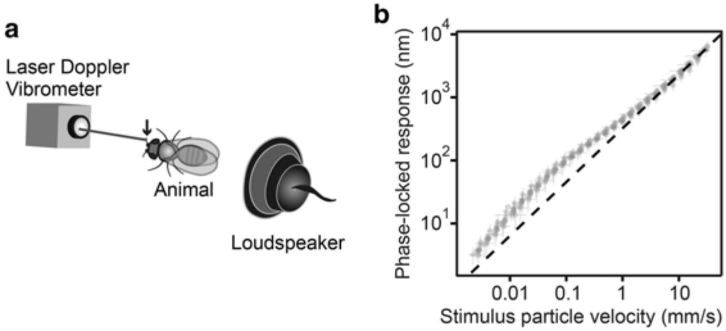


Fig. 10.5 Receiver's response to sound. (**a**) Measurement of antennal displacement with a laser Doppler vibrometer. The focus of the laser is set on the tip of the arista (*arrow*) of one antenna. Acoustic stimuli broadcast from a loudspeaker vibrate the arista together with the third antennal segment. (**b**) Nonlinear amplification of the antennal ear. The amplitude of the phase-locked response of the antennal ear (*plots*) exceeds that of the response of a passive system (*black dashed line*) when the sound is faint [Modified from Albert et al. (2006); Kamikouchi et al. (2010), with permission]

receivers of seven species of the genus *Drosophila*, *D. ananassae*, *D. erecta*, *D. mauritiana*, *D. melanogaster*, *D. simulans*, *D. teissieri*, and *D. yakuba*, are mechanically tuned to different best frequencies, each of which correlates with the high-frequency pulses of the conspecific song (Fig. 10.6; Riabinina et al. 2011). In these species, passive receivers fluctuate at the best frequencies ranging from 789 Hz in *D. melanogaster* to 991 Hz in *D. ananassae*. The active receivers, on the other hand, have best frequencies that range from 147 Hz in *D. melanogaster* to 293 Hz in *D. mauritiana*. When acoustic stimuli are large, such as when exposed to their own flight tone, the contribution of active amplification is negligible and the antennal receiver vibrates passively. The active amplification of the receiver's vibration works effectively in detecting a faint sound in the frequency range of species-specific acoustic communication.

10.5.1 Power Gain Exhibited by JO Neurons

The hallmark of active amplification is power gain in the mechanics of the receiver, which violates fundamental principles of thermodynamics (Martin et al. 2001; Nadrowski et al. 2004). JO neurons provide the mechanical energy in this amplification, which is estimated to average 19 zeptojoule (zJ) in *D. melanogaster*. This energy corresponds to 4.6 times the energy of the receiver's Brownian motion (Göpfert et al. 2005). Interestingly, this is not the maximal energy JO neurons can provide to boost the oscillations; when the physiological condition of the animal deteriorates, the antennal receiver provides excess amplification, which leads to self-sustained large-amplitude oscillations (Göpfert et al. 2005). By measuring the mechanical energy of such self-sustained oscillations of the receiver, the amount of energy the neurons are principally able to contribute is estimated to be 200 zJ. Therefore, *Drosophila* control the mechanical performance of their antennal receivers by adjusting the neural energy contribution below its maximal performance. Genes that affect the function of JO neurons, including active amplification, are discussed by Eberl, Kamikouchi, and Albert, Chapter 7.

10.6 Sensitivity of the Auditory Response

The sensitivity of the auditory system in *Drosophila* can be discussed on two distinct levels: neuronal and behavioral responses. The neural threshold to sound, measured by field potential recordings from the antennal nerve, is 5.7×10^{-2} mm s^{-1} [58 dB sound velocity level (SVL)] for a 300-Hz tone (Lehnert et al. 2013). As expected, the behavioral threshold is higher than the neural threshold. Sound-evoked behavioral thresholds have been analyzed using several behavioral assays. The courtship-related behavioral response is measured by monitoring sound-evoked chaining behavior, a display of homosexual courtship behavior in a single-sex group of

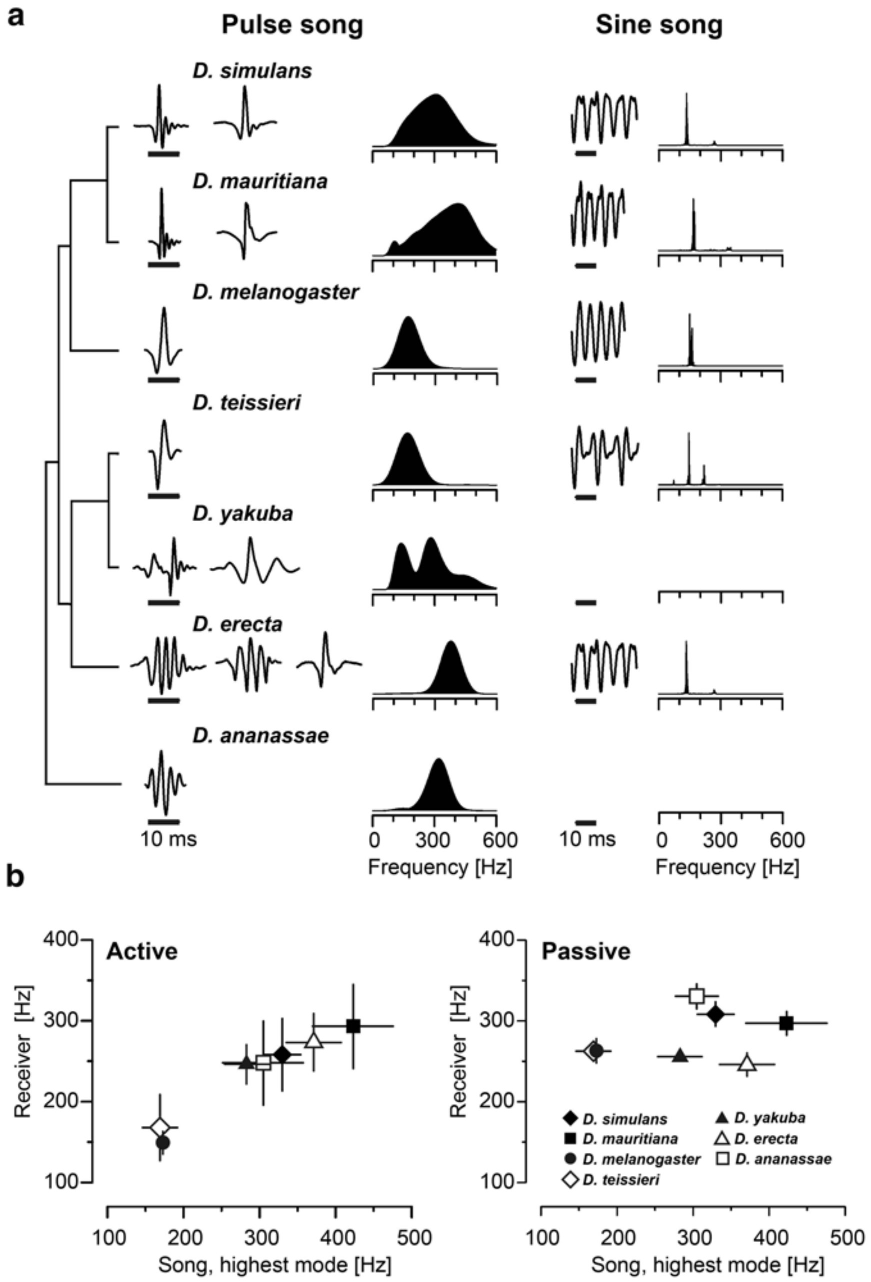


Fig. 10.6 Active tuning. (**a**) Components and spectral composition of courtship songs of *Drosophila*. (**b**) Active process tunes ears to conspecific high-frequency sound emissions [Modified from Riabinina et al. (2011), with permission]

Drosophila males (Fig. 10.2c–e; Yoon et al. 2013). Although precise measurement of the behavioral threshold of the sound-induced chaining behavior is difficult within the experimental chambers used for the chaining assay, the behavioral threshold of the chaining behavior to artificial pulse songs is estimated to be approximately 1.1 mm s^{-1} particle velocity (87 dB SVL) or less. Because the sound-evoked chaining behavior reflects not only the detection of sound but also evaluation of its quality, this threshold is probably much higher than the actual threshold of the behavioral response to sound.

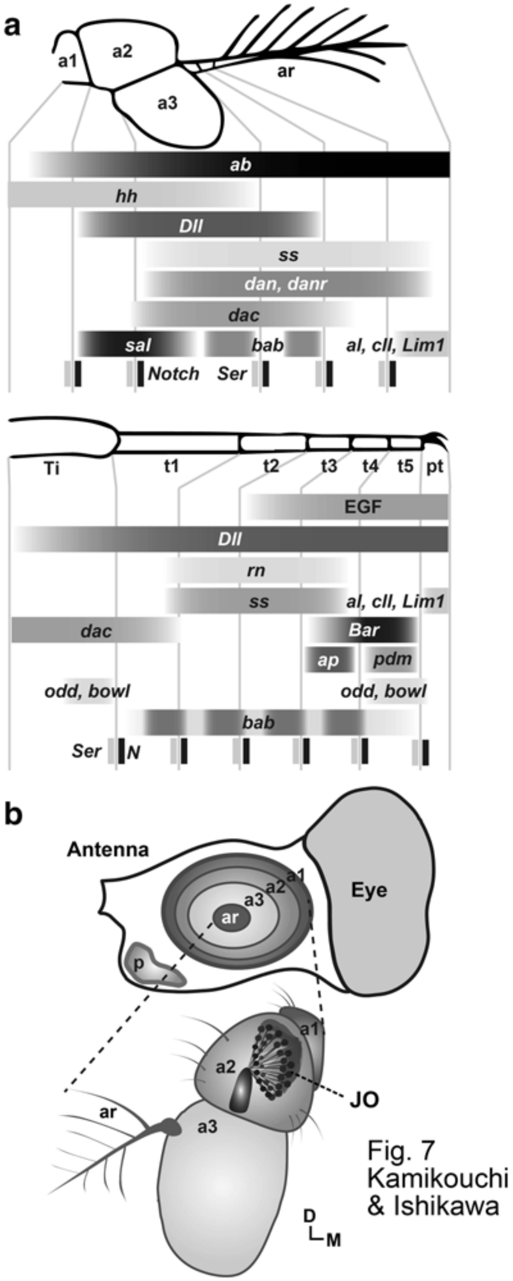
The behavioral threshold to respond to acoustic signals, as monitored by two types of behaviors (a startle behavior and tone-associated learning behavior), is indeed lower than the threshold of this chaining behavior. The threshold of the startle response to calibrated sound is measured by monitoring the sound-evoked walking behavior of a fly tethered on a spherical treadmill (Lehnert et al. 2013). For a 300-Hz tone, the behavioral threshold is as low as 1.2×10^{-1} mm s^{-1}. The threshold of the proboscis extension reflex, as an output of sound-associated conditioning, was analyzed by training the fruit fly to extend its proboscis in response to a ringing tone (Menda et al. 2011). The behavioral threshold in this learning paradigm is observed at 65 dB SVL, which corresponds to 0.9×10^{-1} mm s^{-1}. These behavioral thresholds of flies to sound are consistent with the estimated intensity range of sound that a female receives from a courting male (2 mm s^{-1} and 0.2 mm s^{-1} at a distance of 2.5 mm and 5 mm, respectively).

10.7 Development of Johnston's Organ

The insect antennae are paired appendages serially homologous to thoracic legs and wings. Consistent with this, the antenna and leg are derived from similar primordia and share most of the developmental genes that determine the proximal-distal, dorsal-ventral, and anterior-posterior axis of the appendages (Fig. 10.7a; Boekhoff-Falk and Eberl 2014). Antennae of adult *Drosophila* arise from primordia termed the antennal imaginal discs kept in the larval body (Boekhoff-Falk 2005; Jarman 2014). Cells in the disc proliferate throughout the larval stages and finally reach a size of approximately 10,000 cells before differentiation during metamorphosis (Boekhoff-Falk and Eberl 2014). This event begins in the late larval stage, and during metamorphosis in the pupa, the antennal imaginal discs are converted into three-dimensional antennae with the developing scolopidia inside (Fig. 10.7b; Jarman 2014). In the early patterning process, the genes *engrailed* (*en*) and *hedgehog* (*hh*) are expressed in presumptive posterior cells. Next, Hh induces the expression of *wingless* (*wg*) in the ventral region and *decapentaplegic* (*dpp*) in the dorsal region along the anterior-posterior compartment boundary (Diaz-Benjumea et al. 1994). During the late developmental stage, *Distal-less* (*Dll*), *homothorax* (*hth*), and the downstream genes provide the proximal-distal information of the appendage.

JO neurons and supporting cells derive from sensory organ precursors by lineage (Eberl and Boekhoff-Falk 2007). The precursors are specified by the basic helix–loop–helix proneural transcription factor Atonal (Ato), whose vertebrate homolog

Fig. 10.7 Development of the antenna. (**a**) Expression patterns of developmental genes in the antenna and leg of *D. melanogaster*. (**b**) *Top*: Eye-antennal imaginal disc of *D. melanogaster*. The maxillary palp (p), the first, second, and third antennal segments (a1, a2, and a3), and the arista (ar) derive from the larval eye-antennal imaginal disc. *Bottom*: Adult antenna. Johnston's organ (JO) is housed in the a2. Lines labeled *D* and *M* indicate dorsal and medial directions, respectively [Modified from Kamikouchi et al. (2006); Angelini et al. (2009); Bayramli and Fuss (2012), with permission]



AtoH1 (also known as Math1) is necessary for the development of hair cells in vertebrate ears and the lateral line system (Jarman et al. 1993; Bermingham et al. 1999). These genes are functionally conserved; *Drosophila ato* can fully rescue AtoH1-null mutant mice (Wang et al. 2002), whereas mouse *AtoH1* can partially rescue *ato*-mutant flies (Ben-Arie et al. 2000). Loss of *Ato* function in flies results in the failure of chordotonal organs, which include JO, to differentiate. In the developing JO, *ato* is coexpressed with several other specification genes that encode transcription factors, such as *homothorax* (*hth*), *spalt-major* (*salm*), *spalt-related* (*salr*), and *cut* (*ct*) (Dong et al. 2002; Boekhoff-Falk 2005). The products of these genes may cooperate together to regulate JO differentiation. Additional information on the development of JO can be found in Boekhoff-Falk (2005), Kernan (2007), Boekhoff-Falk and Eberl (2014), and Jarman (2014).

10.8 Auditory Neural Circuits

For studies in *D. melanogaster*, sophisticated genetic tools are available for investigating the organization of neural circuits in the brain in the context of anatomy, function, and behavior. Thousands of individual stocks of fly strains that express transcriptional activators such as *GAL4*, *LexA*, and *QF* in subsets of cell populations allow for visualization and manipulation of specific cell populations. Most of these stocks are available from several stock centers, such as the Bloomington Stock Center (http://flystocks.bio.indiana.edu), Drosophila Genetic Resource Center (https://kyotofly.kit.jp/cgi-bin/stocks/index.cgi), and Vienna Drosophila Resource Center (http://stockcenter.vdrc.at/control/main). Accordingly, *D. melanogaster* is a suitable model animal for exploring the neural mechanisms of hearing at the cellular and circuit levels (Simpson 2009; Kazama 2014). A series of *GAL4* strains, each of which labels all or smaller subsets of JO neurons and higher order auditory neurons, have been used to investigate the organization of the auditory neural circuit (e.g., Kamikouchi et al. 2006; Matsuo et al. 2014; Matsuo et al. 2016).

10.8.1 The Anatomy of JO Neuron Subgroups

GAL4 strains that label JO neurons have been used to trace the axonal projections of JO neurons (Fig. 10.8a–c; Kamikouchi et al. 2006). The axons of JO neurons stem from the basal side of the cell bodies, run along the outer surface of the somata array, and gradually assemble together (Fig. 10.8b). This axon bundle of JO neurons, together with axons of the olfactory sensory neurons and the other sensory neurons located in the antenna, forms the antennal nerve and innervates the brain. On entering the brain, the axonal projection of JO neurons separates from those of the olfactory sensory neurons innervating the antennal lobe; the axon bundle of JO neurons runs along the lateral side of the antennal lobe and spreads broadly in the

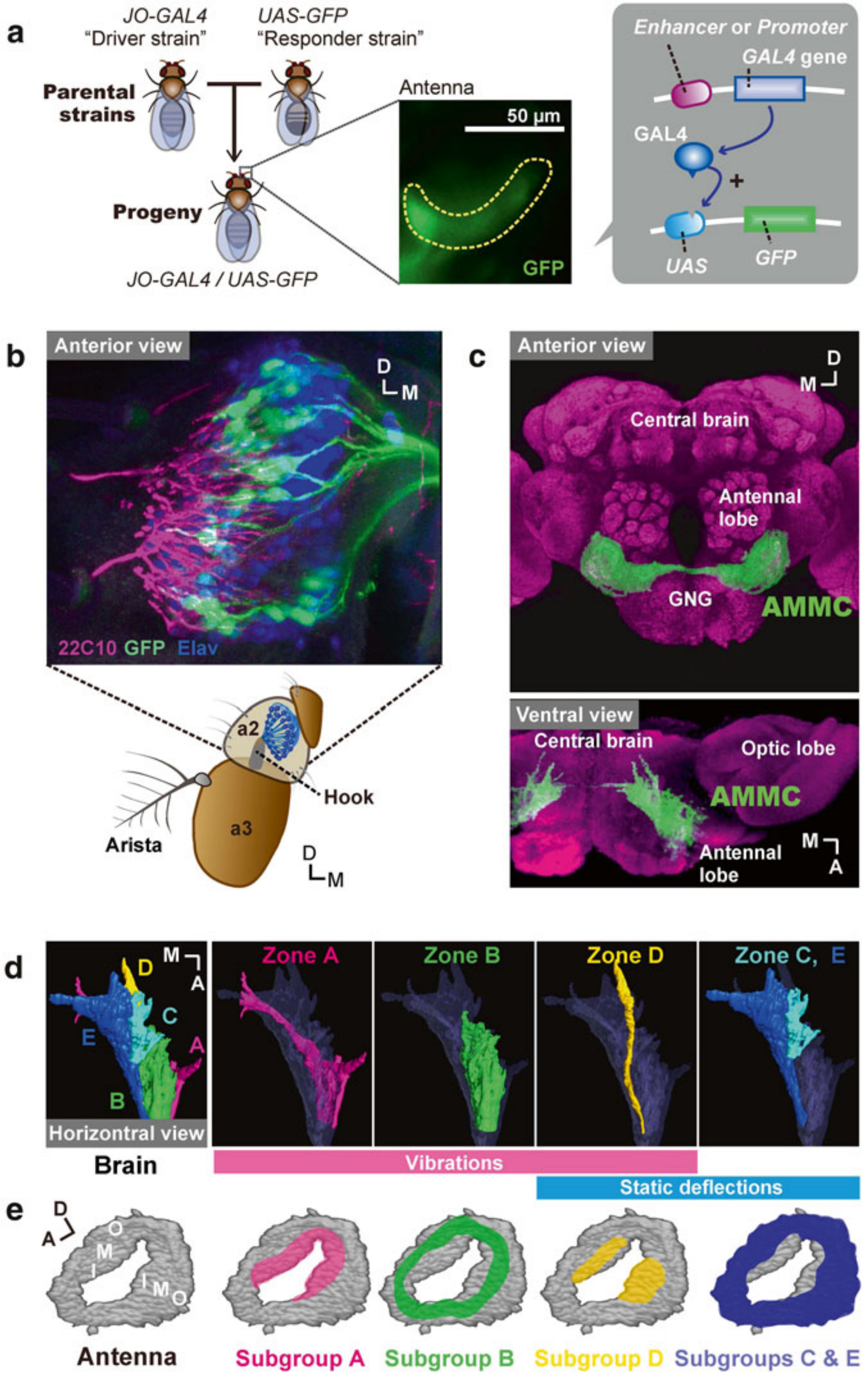


Fig. 10.8 JO neurons and their subgroups. (**a**) The binary *GAL4*/*UAS* expression system. In transgenic progeny obtained from a genetic cross of a *GAL4* strain fly and a *UAS-GFP* strain fly, the GFP reporter is expressed in a specific cell population defined by the parental *GAL4* strain

antennal mechanosensory and motor center (AMMC), paired neuropils located at the posterior-ventral side of the antennal lobes (Fig. 10.8c). A subset of axons further innervates two other neuropils: the wedge (WED, previously called the inferior ventrolateral protocerebrum) and gnathal ganglia (GNG, previously called the subesophageal ganglion).

The *Drosophila* primary center for JO neurons has a zonal structure; the overall projection area of JO neurons comprises five major branches of bundles, which divide the primary center for JO neurons into five zones (Kamikouchi et al. 2006). From the lateral to the medial side of the brain, the zones are referred to as zones A, B, C, D, and E (Fig. 10.8d). Zones B, C, D, and E are confined to the AMMC, whereas zone A extends over the AMMC, WED, and GNG. Approximately 90 % of JO neurons exclusively project to one of the five zones, whereas the remaining neurons innervate two to three zones. JO neurons that innervate a single zone are named according to the target zone; thus, neurons that innervate zones A, B, C, D, and E are referred to as subgroups A, B, C, D, and E, respectively (Fig. 10.8e). All of these subgroups have output sites in their target zone (Fig. 10.9a).

The cell bodies of each subgroup occupy distinct areas within JO in a partially overlapping manner (Fig. 10.8e; Kamikouchi et al. 2006). The somata array of JO neurons, a "bowl" with its bottom cut off (Fig. 10.3e), can be divided into three concentric layers: inner, middle, and outer layers, each of which is occupied by distinct sets of neuronal subgroups. Cell bodies of subgroups A and D JO neurons occupy the inner layer (Fig. 10.8e). Subgroup A neurons fill the large area of the inner layer, mainly the dorsal-posterior side of the array. On the other hand, subgroup D neurons form two clusters at the dorsal and ventral sides of the inner array. Subgroup B neurons are distributed like a concentric ring, mostly located in the middle layer. Subgroups C and E neurons occupy the middle and outer layers, also forming a broad ring.

10.8.2 Response Properties of JO Neuron Subgroups

Two types of antennal movement, vibrations and static deflections, can stretch and compress the cilia of JO neurons. These antennal movements are induced by distinct mechanosensory stimuli; acoustic stimuli induce antennal vibrations, whereas gravity and wind deflect the antenna in one direction statically. In an extreme case,

Fig. 10.8 (continued) (Brand and Perrimon 1993). *JO-GAL4* is a driver strain that expresses GAL4 in Johnston's organ (JO) neurons. The *dotted yellow line* indicates the array of cell bodies of JO neurons. (**b**) Anterior view of the second antennal segment. The sensory cilia (*magenta*), a subset of JO neurons (*green*), and neuronal cell bodies (*blue*) are labeled with 22C10, anti-GFP, and anti-Elav antibodies, respectively. (**c**) The axonal projection of JO neurons in the brain (*green*). The broad area of the antennal mechanosensory and motor center (AMMC) is innervated by JO neurons. GNG, gnathal ganglia. (**d**) Five zones in the primary center for JO neurons in the brain. (**e**) Distribution of cell bodies of each subgroup of JO neurons in the antenna. The "bottomless bowl" distribution of cell bodies can be divided into three concentric layers: outer (O), middle (M), and inner (I). Each layer comprises a subset of JO neuronal subgroups [Modified from Kamikouchi et al. (2006, 2009); Kamikouchi (2013); Kamikouchi and Fiala (2013); Matsuo and Kamikouchi (2013); Matsuo et al. (2014), with permission]

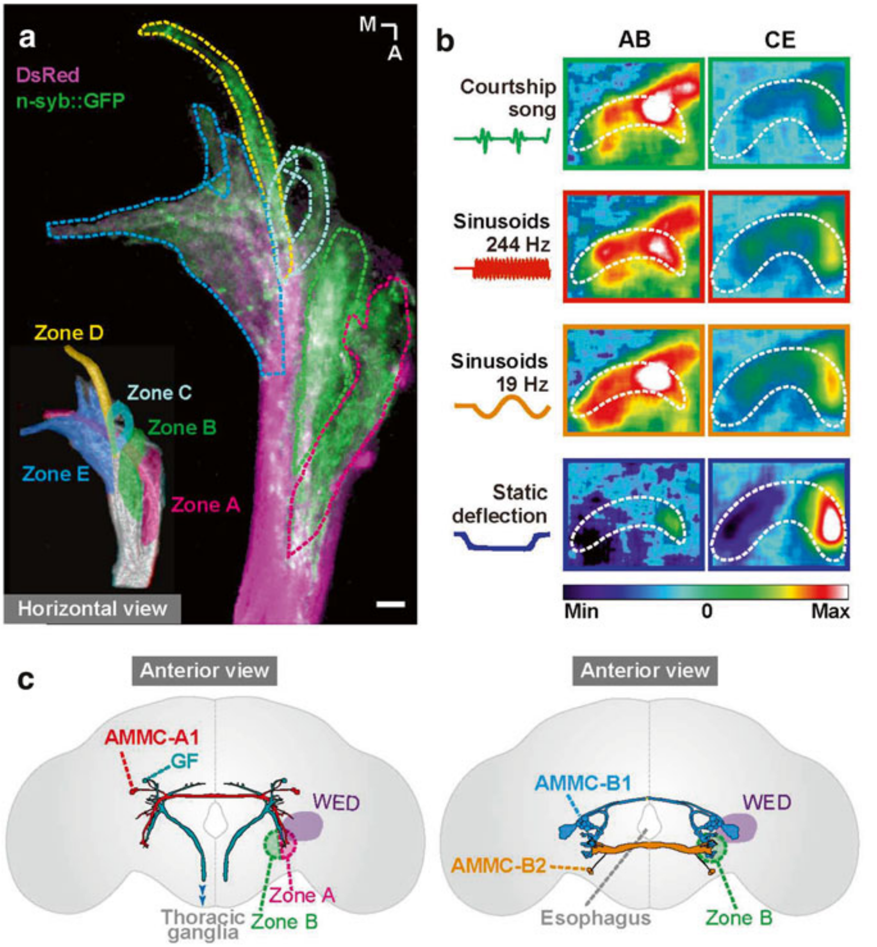


Fig. 10.9 Subgroups of JO neurons and their downstream neurons. (**a**) Output sites of JO neurons. Signals of neuronal-synaptobrevin::green fluorescent protein (n-syb::GFP) (*green*), a presynaptic reporter for chemical synapses, in JO neurons axons distribute in five target zones. Axons of JO neurons are labeled with DsRed (*magenta*). Lines labeled *A* and *M* indicate anterior and medial directions, respectively. (**b**) Response properties of subgroup A and B (AB) and subgroup C and E (CE) JO neurons to various types of antennal movements. A and B neurons respond to vibrations, whereas C and E neurons respond to static deflections. (**c**) Four types of secondary auditory neurons. *Left*: The AMMC-A1 neurons and giant fiber (GF) neurons connect zones A and B in the AMMC to the wedge (WED). *Right*: The AMMC-B1 neurons connect zone B in the AMMC to the WED. The AMMC-B2 neurons connect zone B of both hemispheres [Modified from Kamikouchi et al. (2006); Kamikouchi et al. (2009), with permission]

these three types of stimuli arrive simultaneously. How are such complex patterns of antennal movement encoded by JO neurons? Intrinsic and extrinsic properties of JO neurons are important for encoding such multimodal stimuli. As described in Sect. 10.8.1, the cell bodies of each JO neuronal subgroup occupy distinct areas in the somata array of JO neurons (Fig. 10.8e). The position of the cell bodies specifies the direction, and possibly the strength, of tension imposed on the cilia upon the movement of the hook and thus defines the activity pattern of the JO neurons.

The response properties of JO neurons are defined not only by their extrinsic properties, such as cell body location, but also by their intrinsic properties. Although single-cell electrophysiological recording is a useful method for analyzing the properties of neurons in general, it is not feasible for JO neurons because JO neurons are embedded in an anatomically complex antennal organ whose integrity is critical to their proper function. Therefore, calcium-imaging techniques are a popular tool for probing the response patterns of specific JO neuron subgroups. Because JO neuron cell bodies are located directly beneath the cuticle, the activities of these neurons can be probed noninvasively through the cuticle by expressing a genetically encoded fluorescent protein sensor, such as the GCaMP and Cameleon series, in a cell-type-specific manner (Figs. 10.4b and 10.9b; e.g., Kamikouchi et al. 2009). It is also possible to visualize the calcium response in the axons of JO neurons innervating the brain; by removing a small part of the cuticle that covers the brain, the response can be probed with a fluorescent protein sensor expressed in the axon bundle of specific subgroups (Yorozu et al. 2009; Matsuo et al. 2014). These experiments have identified intrinsic properties of JO neuronal subgroups.

Subgroups A and B JO neurons are vibration-sensitive neurons that are tuned to high (>100 Hz) and low (<100 Hz) frequency vibrations, respectively (Fig. 10.9b; Kamikouchi et al. 2009; Yorozu et al. 2009). Ablation of these subgroups abolishes the nonlinear amplification of the receiver, indicating their major contribution to active amplification (Effertz et al. 2011). The mechanotransducer channels housed by subgroups A and B JO neurons are extremely sensitive to the movement of the receiver; the transduction complex in these subgroups is gated by antennal rotations as small as 5×10^{-4} radians, which corresponds to a 74-nm displacement of the arista (Effertz et al. 2011; Lehnert et al. 2013). On the other hand, subgroups C and E neurons are more responsive to static deflections of the antennal receiver, such as those induced by wind and gravity stimuli, rather than to vibrations induced by acoustic stimuli (Fig. 10.9b; Kamikouchi et al. 2009; Yorozu et al. 2009). Each of these deflection-sensitive subgroups responds specifically to the antennal tilt in either a forward or backward direction; pushing the antenna backward (posterior deflection) activates the subgroup E neurons whereas pulling it forward (anterior deflection) activates subgroup C neurons (Yorozu et al. 2009). The directional sensitivity of these deflection-sensitive subgroups can be used to encode the direction of wind flow by comparing the activity pattern of subgroups C and E neurons in the left and right hemispheres of the brain (Yorozu et al. 2009). When compared with the subgroups that are selectively tuned either to vibrations or to static deflections, subgroup D JO neurons are unique in that they respond to both vibrations and static deflections; they prefer vibrations of approximately 200 Hz and static deflection in a forward direction (Matsuo et al. 2014). Such subgroup-selective intrinsic properties together with the location of the cell body define the response properties of each JO neuron, which in turn can encode the pattern of antennal movement.

As described in Sect. 10.8.1, the primary center for JO neurons in *Drosophila* has a zonal structure. The five anatomically defined projection zones of JO neurons constitute three functionally distinct groups: (1) primary vibration center (zones A and B), (2) primary deflection center (zones C and E), and (3) primary vibration and deflection center (zone D) (Fig. 10.8d). The primary vibration center receives acoustic information, whereas the deflection centers receive information on gravity and wind. Each zone in the primary vibration (auditory) center is tuned to a specific range of sound frequency; zone A for high frequency, zone B for low frequency, and zone D for middle-range frequency.

10.8.3 Higher Order Auditory Neurons in the Brain

A series of *GAL4* strains that label neurons innervating auditory AMMC zones has been used to identify and analyze the higher order auditory neurons in the fly brain. Four types of brain interneurons associated with the auditory AMMC zones were identified by screening 3939 *GAL4* enhancer-trap strains (Kamikouchi et al. 2009).

AMMC-A1 neurons, also called giant commissural interneurons (two cells/hemisphere), and giant fibers (GFs; one cell/hemisphere) connect the auditory AMMC zones (zones A and B) and the WED (Fig. 10.9c). These neurons are electrically coupled via gap junctions to form the GF system. AMMC-B1 neurons (approx. 10 cells/hemisphere) connect the AMMC zone B and the WED (Fig. 10.9c). These neurons are functionally subdivided into two types, B1a and B1b (Lai et al. 2012); AMMC-B1a is narrowly tuned for sensing low-frequency sound, at approximately 100 Hz, whereas AMMC-B1b is more broadly tuned. AMMC-B2 (two cells/hemisphere) neurons are bilateral neurons that connect the AMMC zone B of both hemispheres. These four types of neurons produce graded potential (non-spiking) responses to sound (Tootoonian et al. 2012).

As described in Sect. 10.8.3, the GF and the AMMC-A1 neurons form the GF system. The GF system of fruit flies mediates a short latency jump-flight response through the activation of motor neurons in the thorax (Allen et al. 2006; von Reyn et al. 2014). The GF is a pair of descending neurons activated by either visual looming stimuli or sound (Tootoonian et al. 2012; von Reyn et al. 2014).

A neural tracing technique utilizing photoactivatable green fluorescent protein (GFP) revealed another type of AMMC neuron (Lai et al. 2012). This type, the AMMC-A2 neuron, relays auditory information from the AMMC zone A to the WED. AMMC-A2 neurons have broadly tuned sensitivity to all the frequencies tested, ranging from 100 to 700 Hz.

AMMC zone D is another primary auditory center in the fly brain. Two types of interneurons were identified to innervate zone D (Matsuo et al. 2014). AMMC LNs are local neurons that distribute only in the ipsilateral auditory AMMC zones B and D. The AMMC D1 neuron is a descending neuron that connects AMMC zones B, C, D, and E and the thoracicoabdominal ganglion. The response properties of these neurons are not yet known.

10.9 Summary

For selection of the appropriate mating partner, *Drosophila* has developed a sophisticated system that produces and receives acoustic signals. In any communication system, the property of the receiver reflects the sensory signals emitted by a communication partner, and the acoustic communication of *Drosophila* is no exception. Indeed, drosophilid flies actively tune their antennal receivers to a species-specific courtship song when the sound is faint.

The sensory cells in the ear comprise five discrete subgroups (A, B, C, D, and E JO neurons) with each subgroup projecting to a different zone of the AMMC in the brain. Three subgroups of vibration-sensitive neurons (auditory subgroups A, B, and D) have different and characteristic best frequencies. The mechanism that determines the best frequency in each subgroup remains undetermined. Studies of the neural bases of acoustic information processing in the brain have led to the identification of several types of higher order auditory neurons. The overall organization of auditory neural circuits in the brain is now under extensive analysis, which will lead to a better understanding of how flies interpret the meaning of the sound by discriminating important acoustic signals from noise.

Although acoustic communication of *Drosophila* was discovered more than 50 years ago and has served as a long-standing model for studying the neural bases of communication, we still do not understand how the fly evaluates the species-specific courtship song in the brain. Anatomic and functional analyses of auditory neural circuits in the brain, as reviewed in this chapter, are important steps toward answering this question. The wide array of available genetic tools, not only to visualize but also to manipulate specific types of neurons, will facilitate dissection of the fundamental aspects of auditory sensory processing and subsequent evaluation in the brain. Moreover, a variety of courtship songs between sister species of *Drosophila* provides a unique opportunity to map and compare neurons with a crucial role in the song recognition system in each species. Given the genetic and experimental accessibility, the hearing in *Drosophila*, which includes the sensory organ, neural circuits, and behavior, clearly constitutes an attractive model system for a wide range of studies of behavioral evolution, neuroethology, sensory systems, motor pattern control, acoustic information processing, and decision making in the brain.

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