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## Directional Hearing in Insects

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### 1. Introduction

In insects, like in most other auditory animals, the presence of two bilateral auditory receivers in the sound field and their relative position on the animal's body constitute elemental initial conditions in the process of directional hearing. The problem faced by insects is intimately related to their size and the physics of sound propagation; for a vast and complex array of reasons embedded in their phylogenetic histories insects are small compared to other auditory animals, and also compared to the wavelength of most biologically relevant sounds. The ears of insects can be set so close together that the conventional cues for directional hearing become, also by human standard, barely detectable. With an interaural distance as small as the diameter of a dot on an "i," for instance, the maximum time difference is in the order of  $1 \mu\text{s}$ , a time scale admittedly delicate to handle by any nervous system. Similarly, the amplitude difference in sound pressure between the two ears can be immeasurably small. The constraint of size may thus cause severe difficulties to the processing of directional sound information. Constraints, in the course of evolutionary adaptation, however, also constitute multiple necessities that are the source of a multitude of innovations.

It is becoming increasingly apparent that, no matter how anatomically simple or how minute the auditory organs of insects may be, their sense of hearing is an act of sensation requiring great accuracy (Robert and Göpfert 2002). As astutely pointed out by Hudspeth (1997), hearing may be the most sensitive of the senses in terms of levels of detectable energy. Quantitatively, mechanoreceptor cells can detect mechanical displacements in the subnanometer range, involving energy levels close to thermal noise, or some  $4 \times 10^{-21}$  Joules (De Vries 1948; Bialek 1987; Hudspeth 1997). Some insect ears—like those of mosquitoes—may operate at similarly low levels (Göpfert and Robert 2001). In addition, audition is also designed to monitor acoustical events often more dynamic and transient than the spiking activity of neurons (for insects, see Pollack 1998; Schiolten et al 1981). Much work has been committed to the question of what are, for insects, the adequate cues—the physical quantities—that betray

the direction and/or the location of a sound source, and how do insects go about to detect them. And, crucially, can these cues be converted, and if so how, into information that coherently represents the acoustical geometry of the outside world? Witness the chapters in this volume, the question of directional hearing has a long history, the problems are admittedly complex and the vast literature to date may only herald the promising depths of future research.

Probably only sifting near the surface of a rich pool of innovations, this chapter presents the mechanisms responsible for directional hearing in insects, and attempts to advance some ideas on how to explore this pool further. This chapter intends to present the constraints imposed on insects and explain the structures and functions known to operate in the process of directional hearing in insects. At times, some subjects will not be treated with the length and depth they deserve; this is not to occlude the concepts with a barrage of data. At those moments, recommendation will be made to consult recent reviews and key original articles to gather complementary insight. Insect hearing has been the subject of several recent reviews (Yager 1999). Of particular relevance is Volume 10 in the *Springer Handbook of Auditory Research* entitled *Comparative Hearing: Insects* edited by Hoy, Popper, and Fay (Hoy et al. 1998), that presents an authoritative overview. A collection of articles published as multi-author topical journal issue (see Robert and Göpfert 2004) addresses, among varied aspects, the latest research on insect auditory anatomy (Yack 2004), neurobiology (Hennig et al 2004), and psychoacoustics (Wytenbach and Farris 2004).

## 2. What about Insect Ears?

### 2.1 Two Basic Types But Numerous Variations

The ears of insects can be categorized into two basic types, the tympanal ears and the flagellar ears. Both types, nearly always occurring as a bilateral pair, are highly sensitive to airborne vibrations and in their own way fulfill the functions of hearing organs. Figure 2.1 provides a very brief account of the diversity of insect auditory organs. Perhaps among the better known, the tympanal ear of the locust is among the “largest” of its type found in insects, yet, its tympanum spans only about 1 to 2 mm (Fig. 2.1A).

Tympanal ears can be found virtually anywhere on the general insect body plan, on the mouthparts (hawk moths), the tibia (field and bushcrickets), the abdomen (locusts and moths), the anterior thorax (parasitoid flies), the wing base (butterflies), the ventral thorax (mantisses), and the base of the neck (beetles) (reviews: Yack and Fullard 1993; Hoy and Robert 1996; Yack 2004). The basic bauplan of a tympanal ear consists in a thin cuticular membrane backed with an air-filled cavity with a mechanosensory chordotonal organ directly or indirectly in mechanical contact with the tympanum (Robert and Hoy 1998). The morphology of the tympanum, the associated air sacs, and the mechanosensory organ display a diversity that is allegedly bewildering and perhaps

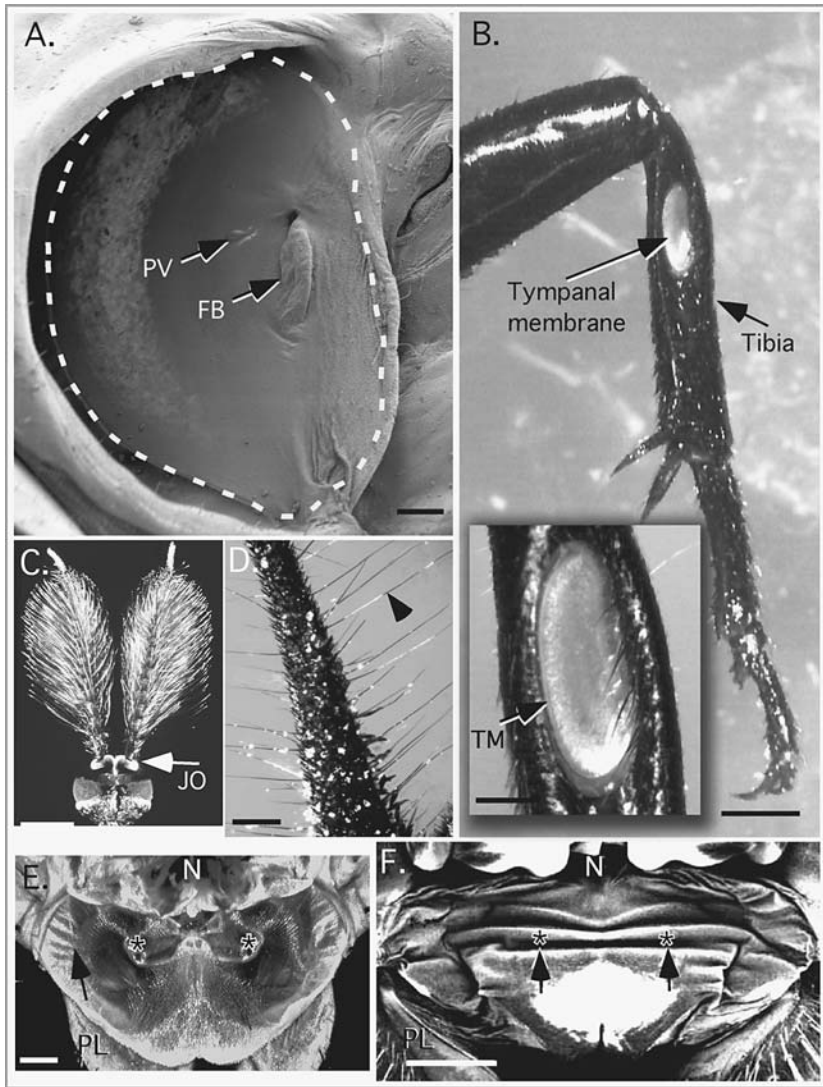


FIGURE 2.1. External auditory anatomy of a locust, a cricket, a mosquito, the cercal system of the cricket, and two parasitoid flies. (A) The tympanal ear of the locust. PV is the pyriform vesicle; FB the folded body to which high and low-frequency receptors respectively attach. The tympanal membrane is delineated by the *white stippled line*. Scale bar = 200  $\mu$ m. (B) Posterior tympanum on the tibia of the first pair of legs of the cricket *Gryllus bimaculatus*. Scale bar = 1 mm (*inset*: 250  $\mu$ m). (C) Light scanning micrograph of the antennae of the male mosquito *Toxorhynchitis brevipalpis*. The ball-like structures at the base of the antenna are the mechanoreceptive organs. Scale bar = 500  $\mu$ m. (D) Filiform hairs on the cercus of the field cricket *G. bimaculatus*. Scale bar = 200  $\mu$ m. (E) Tympanal ears of a tachinid fly (*Ormia*). *Arrow*, tympanal membrane; *arrowhead*, insertion point of the mechanoreceptive organ; N, neck; PL, prothoracic leg. Scale bar = 200  $\mu$ m. (Light scanning micrograph by D. Huber). (F) Tympanal ears of a sarcophagid fly (*Emblemasoma*). *Arrows* show insertion points of mechanoreceptive organs on the tympanal fold. Scale bar = 200  $\mu$ m.

unique to insects (Yack 2004). A partial and logically ultimate explanation for that variation is that tympanal ears have evolved independently perhaps as many as 19 times, and in at least 7 out of the approximately 25 orders of insects (Fullard and Yack 1993; Hoy and Robert 1996; Yager 1999). In view of the diversity of insects and their morphological adaptability, this figure may simply reflect the lack of research in the remaining “atympanate” orders. Notably, the absence of tympanal ears in the well-studied hymenoptera (ants, bees, and wasps), and odonata (dragonflies and damselflies) remains puzzling, and hence may deserve renewed attention. As tympanal hearing is only one of the two known methods for audition, it may be tempting to speculate somewhat, and suggest the possibility that notoriously atympanate insects (mostly the very speciose beetles and flies, but also little known insect orders) may be endowed with a yet unfamiliar sense of hearing, possibly based on the flagellar type, or some variation of it (Fig. 2.1).

## *2.2 The Directionality of Particle Velocity Receivers*

The anatomical basis of nontympanal hearing has been known for quite some time and takes the form of antennae (in mosquitoes, Johnston 1855), filiform hairs borne on the body wall (caterpillars, Tautz 1977), or the terminal cerci (crickets, Gnatzy and Tautz 1980) (Fig. 2.1). This type of auditory receiver is said to be inherently directional. This is in part because it responds to the particle velocity component of the sound field, which is a physical attribute that is vectorial (as opposed to the pressure component which is a scalar quantity). For one part, directional information is thus contained by the bulk oscillations of the air particles that take place in the direction of sound propagation, in the far field. Another component of directionality relates to the anatomical arrangement of hairlike structures and antenna. Indeed, the mobility of the sound receptor may not be isotropic. An asymmetry may then confer some directionality to the system oscillating in the sound field. In effect, as shown in the caterpillar of noctuid moths, some filiform hairs display a distinct inherent directional response, and some do not (Tautz 1977). Since the particle velocity portion of acoustic energy dominates near the sound source, these organs have also been called near-field detectors. This is not to say, however, that they will detect only sound in the near field of a sound source (from one sixth to one wavelength away from it). If endowed with enough sensitivity, particle velocity receivers may well detect sound in the far field, where particles also oscillate, albeit with much smaller magnitude. Because sound fields are usually strongly divergent close to small sound sources, such as a female mosquito, bilateral particle velocity receivers (the antennae of a male mosquito) may experience vastly different vector fields depending on their distance from the sound source and the orientation of their auditory organs' axis of best sensitivity. This alone may affect the directionality extractable by two bilaterally symmetric antennal detectors. In effect, the direction of a particle velocity vectors in a sound field near the source depends on the type of source (monopole, dipole). As a result, at

any point in space, the velocity field may or may not directly point to the sound source. The problems associated with the processing of such vectorial acoustic information have been little investigated in insects, but may well be of similar nature to those encountered by fish (Edds-Walton et al 1999). The capacity of flagellar auditory organs to detect sound directionally (mosquitoes, Belton 1974) has received only little attention recently. The physical basis of their directionality, in terms of their viscous interaction with a vectorial sound field, their own—sometimes variable—asymmetry and the coding of primary mechanoreceptors, remains unknown to date.

### 3. The Effects of Size on Directional Cues

The main acoustic cues used for directional hearing are interaural differences in intensity (IID) and in time (ITD). In addition, variations in the spectral composition of incident sounds can provide directional information about the source of sound (Middlebrooks and Green 1991; Wightman and Kistler 1997). Requiring significant diffractive effects to take place, this possibility is quite unlikely for the smaller (grasshoppers, flies), but is not excluded for larger auditory insects (locusts and bushcrickets). Another, nontrivial requirement is, of course, some sensory capacity for frequency analysis. The coding of frequency by auditory receptors and interneurons has been well documented in a variety of insects, in particular in grasshoppers and crickets (for review see Pollack 1998). Although some capacity for frequency discrimination has been demonstrated for field crickets (behavior, Wyttenbach et al 1996; neural coding, Pollack and Imai-zumi 1999), directional sound detection based on spectral variation, as known from vertebrates, has received little consideration. Such a mechanism would possibly necessitate quite a fine resolution in the frequency analysis and the sequential comparison between sounds of varying frequency composition, a processing feat that has not been demonstrated in insects.

Amplitude and time domain cues, IID and ITD, are mainly determined by the spatial separation between the ears and their position relative to the sound source. In insects, the interaural distance can vary from 1 cm (locusts, bushcrickets) to only a few millimeters (crickets, grasshoppers, moths, cicadas), or a mere 500  $\mu\text{m}$  (parasitoid flies). Consequently, interaural differences in the time of arrival of a sound wave (ITD) can easily vary from 30  $\mu\text{s}$  to 1.5  $\mu\text{s}$ . Interaural distances in insects are also often considerably smaller than the wavelength of the relevant sound, a fact that bears consequences for the other main cue (IID). Acoustical theory states that diffraction between an object of size  $r$  and a sound wave of wavelength  $l$  becomes significant when the ratio  $r:l$  exceeds 0.1 (Morse and Ingard 1968). Experiments exploring this theoretical prediction have been rarely rigorously conducted in insects; but when they have (moths, Payne et al 1966; locusts, Robert 1989; locusts and grasshoppers Michelsen and Rohrseitz 1995; flies, Robert et al. 1999), they showed diffractive effects that, as sound frequency increases, result in growing sound pressure variations and

IIDs. A systematic quantification of acoustic diffraction in a free field, using probe microphones commensurate with the task of measuring the microacoustics around the body of an insect has yet to be undertaken. Thus far, it appears that the main limiting problem has been the excessive size of probe microphones. The possible use of diffraction-related frequency cues may deserve some renewed attention in light of recent psychoacoustical evidence suggesting that the cricket may detect the direction of incident waves in the elevational plane (Wyttenbach and Hoy 1997).

Another important consequence of small body size is the reduced amount or absence of dense tissue between the auditory organs. Tympanal ears are always associated with large air sacs that generate some acoustical transparency across the body. Even for a large insect such as the locust, diffraction has limited effects (see Fig. 1 in Michelsen and Rohrseitz 1995). When ears are close together, little space is left for sound absorbing tissue to acoustically isolate the ears from each other. In locusts, some of the interindividual variation measured in the interaural transmission was attributed to differences in the amount of fat tissue between the ears (Miller 1977). In parasitoid flies, the bilateral ears even share a common air sac (Robert et al. 1996). More complete descriptions and quantifications of the biomechanics of sound propagation and the generation of cues for directional hearing in insects can be found in earlier reviews (Michelsen 1992, 1996, 1998, Robert and Hoy 1998).

## 4. Directional Receivers in Insects

Research in insect audition has uncovered a rich diversity of structures and functions that serve the purpose of directional sound detection. Taking advantage of the amenability of insects to a variety of experimental work—biomechanical, behavioral, anatomical or neurophysiological—the study of insect audition has fostered the discovery and the intimate understanding of alternative, original, mechanisms for directional hearing, such as pressure difference receivers and mechanically coupled receivers.

### 4.1 *Pressure Receivers*

Tympanal ears operating as pure pressure receivers are found on insects that are relatively large compared to the wavelength of the sound frequencies of behavioral relevance (either the courtship and mating songs or the high-frequency echolocation cries of bats). These ears are deemed to be pressure receivers because sound pressure is thought to act only on one face of the tympanal membrane, usually the external one (yet, the internal one in bushcrickets) (Fig. 2.2A) (see Michelsen 1998). In such situation, the insect's body is large enough to generate diffractive effects, resulting in overpressures and underpressures at the location of, respectively, the ear nearer and further from the sound source. Interaural pressure differences (or IIDs) are thus generated that constitute suf-

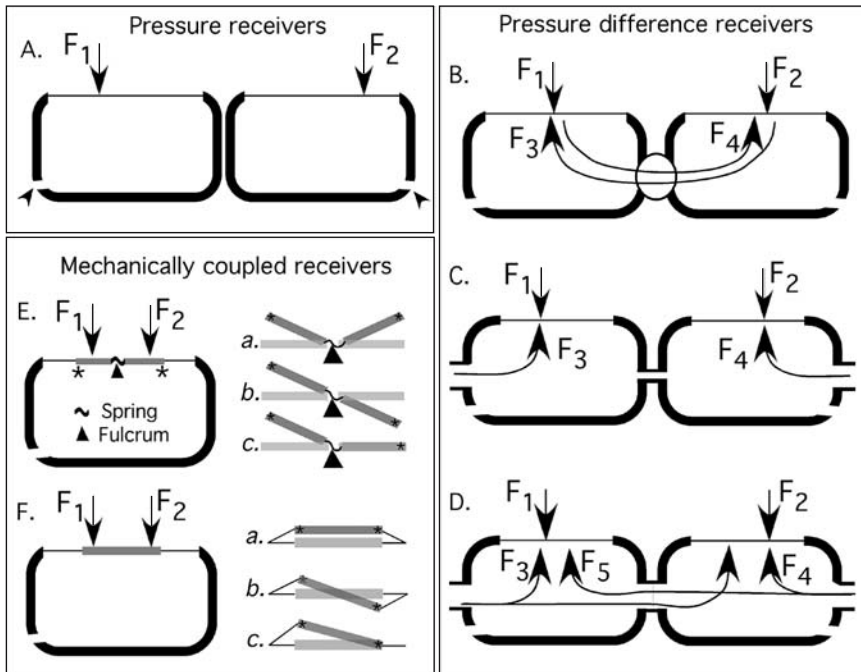


FIGURE 2.2. Directional receivers in insects. The ears are symbolized by a pair of boxes each with one thin wall representing the tympanal membrane and one small opening for static pressure equalizing (*arrowhead*). For simplicity, the action of pressure on the tympanal membrane is reduced to a point force  $F$ . (A) Pure pressure receivers. Forces act on the external side of the tympanal membranes. The two ears are acoustically independent. (B–D) Pressure difference receivers. For all cases, the forces act on both sides of the tympanal membranes. Internal sound pathways are shown by *long arrows*. (B) In locusts and grasshoppers. (C) In bushcrickets. (D) In field crickets. (E) Mechanically coupled pressure receivers in tachinid flies, the seesaw model. Forces act on the external side of each tympanum, generating asymmetrical deflections of the intertympanal bridge. (F) Mechanically coupled pressure receivers in sarcophagid flies. The deflection mode diagrams illustrate the asymmetrical mechanical behavior for both fly families. Responses are shown for three frequencies; *a*, bending at 4 kHz; *b*, rocking at 7 kHz; *c*, combination of the above at 15 kHz. Asterisks show the points of attachment of the mechanoreceptors to the tympanal system. See text for further explanations.

ficient cues for directional hearing. In a large noctuid moth, IIDs of some 20 to 40 dB were measured as a result of the substantial diffraction of sound with frequencies similar to those used by echolocating bats (30 to 60 kHz) (Payne et al. 1966). Such large IIDs are most practical for directional hearing, but they are not a prerequisite for it, nor do they indicate that the animal in question uses directional information to evade its aerial predators. Small IIDs can convey adequate information for localization or lateralization in insects. For some small

grasshoppers, IIDs as small as 1 to 2 dB have been shown to be sufficient to elicit reliable orientation toward attractive calling song (von Helversen and Rheinlander 1988; see Hennig et al. 2004). Forcibly, as the frequency of sound decreases, diffraction-related cues vanish, a constraint that has often been invoked for the tendency of insects to design calling and mating calls with high frequencies (to the inevitable cost of transmissibility) (Michelsen 1996; Bennet-Clark 1998). It is worth noting that quantitative measurements of intra-aural (i.e., behind the tympanum) pressure variation, with reference to the external pressure variation, of insect ears have proven to be very difficult and have thus far not been undertaken in noninvasive conditions. Hence, the notion of pure pressure receiver seems to rely on the recognition of only one acoustic input into the ear (the tympanum), and the absence of evidence for an alternative input.

#### *4.2 Pressure Difference Receivers*

Pressure difference receivers are distinctly more complicated, and more variable in their anatomy and modes of operation (Fig. 2.2B–D) than pressure receivers. Pressure difference receivers are typically found in insects with body sizes only a fraction of the wavelength of interest. As illustrated schematically in Figure 2.2, pressure difference receivers can take one of three forms. By definition, their mode of operation relies on the action of sound pressure on both sides of a tympanal membrane. Requiring more than one input per ear, such a mechanism was proposed a while ago as a solution to the problem of directional hearing by small animals, and in particular, among arthropods, by grasshoppers and locusts (Autrum 1940). The principle relies on ears being each endowed with two or more acoustic inputs. These supplementary acoustic inputs are adapted to conduct pressure waves to the internal side of the tympanum (Fig. 2.2B). The internal sound pressure, owing to its travel in a tracheal tube or across air sacs, undergoes different degrees of attenuation or amplification and some phase shift as a result of alterations in propagation velocity. In such a system, the force driving the tympanal membrane is the difference between the external and internal pressures (or notionally, forces) (Fig. 2.2B,  $F_1$  and  $F_3$ ). Notably, because the pressures involved are periodic, a force still acts on the tympanal membrane when internal and external pressures happen to be equal (no attenuation or amplification through internal travel) but have a phase difference. Of course, in such a system, a combination of both phase shift and amplitude difference is likely to take place, and to affect the ipsilateral and contralateral ears differentially. In theory, well-adjusted phase shifts and amplification factors could lead to constructive and destructive interference at the tympanal membranes that may greatly enhance the contrast between the two ears.

The first type of pressure difference receiver relies on the acoustic coupling between the two ears achieved by acoustically conductive tissue, or air sacs, situated between the tympanal cavities (Fig. 2.2B). For this particular anatomy, the pressure acting on the internal side of the tympanal membrane travels from



the contralateral side of the animal. This anatomical arrangement has been identified in the locust (Miller 1977). Because internal sound conduction has been shown to decrease with increasing frequency, the locust ear is deemed to operate as a pressure receiver for high frequencies and a pressure difference receiver for low frequencies. Two valuable studies have investigated in greater detail the mechanisms at work for directional hearing in large (*Schistocerca gregaria*) and small (*Chorthippus biguttulus*) grasshoppers (Michelsen and Rohrseitz 1995; Schul et al 1999). The premise of the first study was that the biomechanical mechanisms employed by large and small grasshoppers, should be scalable, owing to size and differences in the frequency range used for communication. A model was proposed that could predict directionality as a function of auditory inputs and internal interaural transmission gains and delays. Measurements failed to satisfy a simple scaling law, and it was concluded that directionality cues were poor at 5 kHz for the small grasshopper. The model would not apply as such to the smaller of the two auditory systems. This was attributed to an insufficient transmission delay in the internal interaural passage of sound. Yet, *C. biguttulus* can reliably orient to 5 kHz sound in the context of phonotactic experiments (von Helversen and Rheinlander 1988; Pollack 1998). In an effort to test the general validity of the model, Schul et al. (1999), using behavioral, acoustical, and electrophysiological methods, determined the contribution of the internal pathway responsible for the bilateral transfer of sound pressure. The acoustical measurements of Schul et al. yield transmission delays that substantially differ from those of former studies for the small species, but coincide for the large species. In particular, the delay incurred by the internal interaural sound transmission is identified—and quantified—as being essential to the generation of interaural differences, bringing to agreement phenomenology and model predictions. Therefore, it seems that the proposed two-input model featuring an interaural delay line is valid for grasshoppers and their pressure difference receiver system.

Another type of pressure difference receiver can be found in bushcrickets (katydids), which have their auditory organ (two tympana per organ, associated with one mechanoreceptive organ) on the foreleg tibia (Fig. 2.2C). Katydid can be quite large compared to the frequency of their calling songs, yet, because the tympana are borne on thin legs clear from the body wall, reduced diffractive effects can be expected. As in the large desert locusts (Robert 1989), significant diffraction-related over- and underpressures occur near the body surface. Exploiting these diffractive effects, bushcrickets possess additional acoustic inputs on their thorax, some specialized spiracles and horn-shaped atria connecting to tracheal tubes that lead to the internal side of the tympanal membranes but also to the mechanoreceptive organ (Lewis 1983). Thus, unlike grasshoppers, the pressures acting on either side of the tympanum both originate from the same side of the body (compare Fig. 2.2B, C). Notably, the tracheal tube has the shape of an exponential horn and acts like one; sound transmitted through it is amplified. The result is that the internal sound pathway dominates the force field driving the tympanal membranes (Lewis 1983). This type of pressure

difference receiver has not received as much biomechanical attention, and uncertainty remains concerning the amplification process in the horn-shaped acoustic trachea, the action of the pressure on the mechanoreceptor organ, and the role of the oft-present thin tracheal connection between the tracheal horns (Fig. 2.2C).

Another, much studied example of a pressure difference receiver is the field cricket *Gryllus bimaculatus* (Michelsen et al 1994) for which body size and wavelength of relevant calling songs are, respectively, about 0.8 cm and 7 cm. Although the interaural distance is difficult to estimate in this species—the tympanal ears are situated on the highly moveable tibia (Fig. 2.1B)—it is apparent that insufficient ITDs and IIDs are available for directional detection. The anatomical arrangement found in field crickets is the most complex known to date, employing no fewer than four inputs and one internal interaural transmission channel (Fig. 2.2D). One important operational characteristic of that system is that the two more important force inputs to the anterior tympanal membrane are the external sound pressure (Fig. 2.2D,  $F_1$ ), and the internal sound pressure ( $F_3$ ) originating from the contralateral sound input (Fig. 2.2D), unlike the situation in bushcrickets for which  $F_3$  is the dominant, ipsilateral input. In field crickets, the tracheal connection between the hemilateral tracheal tubes is larger than in bushcrickets and displays a thin septum at the midline (Fig. 2.2D). This septum has been suggested to play a crucial role in enhancing the time delay in the internal interaural transmission line (Löhe and Kleindienst 1994; Michelsen and Löhe 1995). In passing, it is worth noting that in field crickets, the anatomical relationship between the tympanal membrane and the mechanosensory organ is quite complex (Yack 2004). To what degree the vibrations of the tympanal membrane translate into mechanical actuation of the sensory organ (in magnitude and phase), and what role is played by sound pressure in the tracheal system adjacent to it, remain unknown. This problem also amounts to that of the current difficulty of measuring sound pressures in small cavities.

Multiple studies have revealed the robust capacity of crickets to readily locate the source of a calling song in intact situations but also when different parts of their tracheal anatomy and identified acoustical inputs were destroyed or plugged (Schmitz et al 1983; Weber and Thorson 1989; Doherty 1991; Michelsen and Löhe 1995). Taking nothing away from the biomechanical measurements, this behavioral evidence indicates that the four input pressure difference system is sufficient, but is not, at least in certain conditions, necessary for directional hearing.

A necessary word of caution should emphasize that the drawings of Figure 2.2 are notional and by no means intend to reflect the anatomical complexity and the actual paths taken by the multiple pressure waves propagating in cavities and long tapered tubes. If granted an explanatory value, these schematic representations are meant to illustrate the operational principles of various pressure difference receivers. Variations on that theme, with intermediate forms, or entirely new forms of internal sound transmission are likely to exist in other insects. Several accounts of the biomechanics of pressure difference receivers

have been published (Michelsen et al 1994; Michelsen 1992, 1996, 1998; Schul et al 1999), and a recent review covers the neural processing in directional hearing (Hennig et al. 2004)

Arguably, ears operating as pressure difference receivers, at least in their low-frequency range of sensitivity, may well be the most common type of auditory receiver in insects. Indeed, most auditory insects are in the biometric range (one centimetre and much smaller) that could require the presence—if not justify the evolution—of a pressure difference receiver system. Both conditions of limited diffraction and interaural acoustical coupling may thus be often fulfilled. With this in mind, the presence of sound transmission inside adapted acoustic tracheae in some insects raises interesting possibilities for other types of auditory receivers. Intriguingly, could internal sound transmission elicit vibrations of the tracheal wall that, in turn, could be detected by an associated chordotonal organ? Formulated more precisely, a thinning of the tracheal wall, or air sac, accompanied by the selective absence of taenidia (ridges acting as structural buttressing) and a few scolopidial mechanoreceptive units could act as a pressure or pressure difference receiver.

### 4.3 Mechanically Coupled Pressure Receivers

For some small insects, both interaural distance and body size are simply too small to produce IIDs and ITDs of significant magnitudes. In the little tympanate parasitoid tachinid fly *Ormia ochracea*, the ears are so close together that they link up at the midline of the animal (Fig. 2.1 E, F). For this fly, the best possible ITD has been measured to amount to  $1.45 \mu\text{s}$  ( $\pm 0.49$ , SD,  $N = 10$ ) (Robert et al 1996). For such a small insect, body size to wavelength ratio of 1:35 at best precludes significant diffractive effects (Robert et al 1999). Yet, the fly can very accurately locate her host acoustically, a field cricket singing its 5-kHz calling song (Cade 1975) using tympanal auditory organs (Robert et al. 1992; Müller and Robert 2001). Biomechanical and physiological evidence has shown that these ears are directional, and have revealed the mechanism by which they achieve this directionality (Miles et al 1995; Robert et al 1996). The process is based on the mechanical coupling between two adjacent tympanal membranes, an unconventional mechanism that is so far known to occur only in flies (Robert and Hoy 1998; Robert and Göpfert 2002). The mechanism involves the coupling of the tympana by a flexible cuticular lever; this coupling has the effect of amplifying tiny acoustic cues into more substantial interaural differences that can be processed by the nervous system. In response to the host cricket song, a trill with a carrier frequency at 4.8 to 5 kHz, this coupled tympanal system undergoes asymmetrical mechanical oscillations. Using scanning laser Doppler vibrometry, it could be shown that the oscillations arise from the linear combination of two resonant modes of vibration. Rocking like the two arms of a floppy seesaw (see Fig. 2.2E), the coupling lever and the two tympanal membranes attached to it move out of phase and at different amplitudes at frequencies close to that of the cricket song (Miles et al 1995; Robert et al 1996). Re-

markably, the mechanical ITD measured between the tympanal membranes is 50 to 60  $\mu\text{s}$  and the mechanical IID is 3 to 12 dB for sounds delivered at 90° azimuth. This mechanical ITD is thus about 40 times longer than the 1.5- $\mu\text{s}$  acoustical ITD. It is as if the ears of the fly were located some 20 mm from each other (instead of the real interaural distance of 520  $\mu\text{m}$ ). Operating as a mechanical ITD and IID amplifier, this unconventional system converts small acoustical cues into larger mechanical cues. Recent evidence reveals how these mechanical cues are used for the reliable neural coding of sound direction (Mason et al 2001; Oshinsky and Hoy 2002). These studies provide precious insight into the neural mechanisms that allow the hyperacute coding of acoustic information, a subject that is presented in Section 5 hereafter.

Directional hearing by mechanical coupling between two tympanal membranes is not unique to tachinid flies; it has also been described for a fly of another dipteran family (the sarcophagidae) illustrating a remarkable case of convergent evolution (Robert et al. 1999). As a parasitoid of cicadas, the fly (*Emblemasoma* spp.) also possesses a hearing organ on its prothorax (Fig. 2.1F). The mode of operation of this auditory organ is analogous to that of the tachinid fly *O. ochracea*, but it is not identical (Robert et al. 1999).

Phenomenologically, these two auditory systems achieve asymmetrical tympanal deflections, a prerequisite for directional hearing in these systems, but not in the same way. The tachinid and sarcophagid systems present several crucial anatomical differences that determine tympanal mechanics. In the tachinid system, intertympanal coupling is achieved by the presternum, an unpaired sclerite that spans across the midline where it is anchored to the immobile probasisternum (Fig. 2.1E). The mechanoreceptive organs attach at the end of each arm of the presternum (labeled \* in Figs. 2.1E, F and 2.2E, F) Using microscanning laser Doppler vibrometry, it was shown that this sclerite acts as mechanical lever coupling the two ears. The lever consists of two beams that are joined medially by a torsional spring (marked ~) and supported by a fulcrum (a pivot point marked by a black triangle in Fig. 2.2E). Biomechanical evidence shows that such a lever system has two degrees of freedom, resulting in a rocking mode and a bending mode. At low frequencies, the presternum undergoes bending (flexion at the immobile fulcrum), whereby both arms of the lever move together (Fig. 2.2E, a) (Miles et al 1995; Robert et al 1996). The deflection shapes of this tympanal system have been measured; the end points of the lever (\*; attachment locations of mechanoreceptor organ) experience displacements of similar amplitude at frequencies below approximately 4 kHz (Robert et al 1996). As a point of comparison, at such frequencies the tympanal system of the sarcophagid fly deflects inwards and outwards with only little bending (Fig. 2.2F, a). In effect, the deep folding running across the tympanal membranes and the presternum of the sarcophagid ear (Fig. 2.1F) generates a stiffness anisotropy making the entire system prone to oscillate about the animal's midline. Deflecting as a single beam unsupported medially, both tympana move together with only slightly different displacement amplitudes. The translational mode observed for low frequencies in sarcophagids (Fig. 2.2F, a) is thus equivalent to

the bending mode described for tachinid flies (Fig. 2.2E, *a*). At intermediate frequencies (approximately 7 kHz), both tympanal systems oscillate in a rocking mode; outward displacements at one end of the presternum are accompanied by inward displacements at the other end (*b* in Figs. 2.2E, F). In this rocking mode, both tachinid tympana oscillate about the midline, but owing to the flexibility provided by the torsional spring, they do so with some phase delay and amplitude difference (Miles et al 1995; Robert et al 1996). Notably, in the sarcophagid fly the rocking mode occurs in the absence of a fulcrum anchored at the midline of the animal. For both systems, the mechanical ITDs and IIDs (differences between one side of the tympanal system and the other) increase as frequency increases. For higher frequencies (15 kHz), a combination of the two modes dominates the motion and the side contralateral to the incident sound wave experiences low displacements (*c* in Fig. 2.2E). For the sarcophagid ears the single beam formed by the tympanal fold sways about its contralateral end (*c* in Fig. 2.2F) (Robert et al 1999). Deflection shapes thus differ between the tachinid and the sarcophagid systems, yet the deflections experienced by the points of insertion of the mechanoreceptive organs are similar (compare asterisks in Fig. 2.2E, F). The single, unpaired air space backing the tympanal system of both tachinid and sarcophagid flies cannot be a priori excluded to play a role in sound transmission similar to a pressure difference system. This question has been addressed in tachinid flies where it was shown, using acoustical and direct mechanical actuation, that interaural mechanical coupling did not depend on the presence of a finite air-filled cavity (Robert et al 1998). That study concluded that the mode of operation of these ears relies on mechanical coupling only, excluding the action of a pressure difference mode.

Both auditory systems achieve asymmetrical tympanal deflections despite interaural distances of the order of 1 mm. The interaural mechanical coupling relies on a particular morphological design that provides an anisotropy in stiffness. Through functionally convergent but anatomically divergent evolutionary innovations, these two fly families have independently solved the problem of the directional detection of low-frequency sounds by tympanal membranes separated by a fraction (1:130) of the wavelength. Other tachinid flies, from other genera have been reported to use ears to detect calling songs of their hosts at higher frequencies (10 to 15 kHz) (Lakes-Harlan and Heller 1992); from morphology alone it is likely that they use mechanical coupling for directional hearing. The morphological design and mode of action of the sarcophagid tympanal membranes show, in principle, how a millimeter-size ear can be directional by virtue of one or several folds on its membranes. Again and yielding to speculation, folds and creases along a thin tracheal tube may provide in other insects the adequate substrate, if linked to mechanoreceptor neurons, for internal auditory organs with directional characteristics. For mechanically coupled pressure receivers, the exact mechanical characteristics of the tympanal membranes, such as stiffness distributions and anisotropies, tolerances for bilateral differences, and their contributions to directionality remain uninvestigated. It is also worth noting that some 43 species in 7 genera of tachinid parasitoids have been shown

to possess a wide variety of modifications of their prosternal anatomies very reminiscent of mechanically coupled hearings organs (Huber and Robert unpublished results).

## 5. Temporal Hyperacuity in Insect Directional Hearing

In some sensory modalities, such as hearing and electroreception, the time scale of events can be far shorter than the conventional millisecond-range of neural signaling. In hearing, localization tasks near the midline often involve microsecond-scale ITDs. Defined as the capacity for submillisecond coding, temporal hyperacuity has been documented for barn owls (Knudsen and Konishi 1979; Moiseff and Konishi 1981) and electric fish (Rose and Heiligenberg 1985). Essentially, the underlying neural mechanisms have been proposed to rely on the convergence of many sensory afferents onto an interneuron acting as a coincidence detector. Interneuronal spiking would be elicited only by the coherent firing of an ensemble of afferents within a narrow window of temporal coincidence. The spiking accuracy and reliability of the primary afferents is therefore crucially important in that scheme (de Ruyter van Steveninck et al 1997). Accordingly, events following each other within microseconds are most relevant to the microscale ears of fly *Ormia ochracea*. As seen in Section 4.3, in the best-case scenario (sound source  $90^\circ$  to the side of the animal) the tympanal system amplifies temporal acoustic cues (ITDs) by about 40 times, yielding mechanical ITDs of some 50 to 60  $\mu\text{s}$ . It was shown at the mechanical level that the system of intertympanal mechanical coupling could vary its response as a function of the angle of sound incidence. Naturally, when the sound source is near the midline of the animal mechanical ITDs and IIDs decrease to values smaller than 1  $\mu\text{s}$  (Robert et al. 1996). The demonstration that the flies can use temporal sound cues at the submicrosecond scale came from a series of behavioral and neurophysiological experiments by Mason et al. (2001). Flies were tethered on their pronotum and brought to walk on an air-cushioned spherical treadmill that would record the flies' locomotory activity (Fig. 2.3A). Flies could produce walking responses oriented toward the sound source, and quite unexpectedly, they could reliably do so even though the deviation from the midline was 1 to  $2^\circ$  (Fig. 2.3B). When the amount of turning was measured as a function of the angle of azimuth of the sound source, a sigmoid response curve was revealed that displayed a smooth transition near the midline (Fig. 2.3C). This distribution of turning angles is expected from a system endowed with high accuracy of localization (as opposed to lateralization) along the midline (azimuth zero). Finally, the proportion of correct responses as a function of stimulus azimuthal angle was evaluated, revealing a remarkable reliability and repeatability at angles as low as 2 to 3 degrees (Fig. 2.3D). It must now be considered that interaural cues, when calculated for an angle of  $2^\circ$  and an interaural distance of 520  $\mu\text{m}$ , amount to a mere 50 ns for acoustical ITDs, and 2  $\mu\text{s}$  for the mechanical ITD (owing to a mechanical amplification factor of 40) (Mason et al. 2001). How

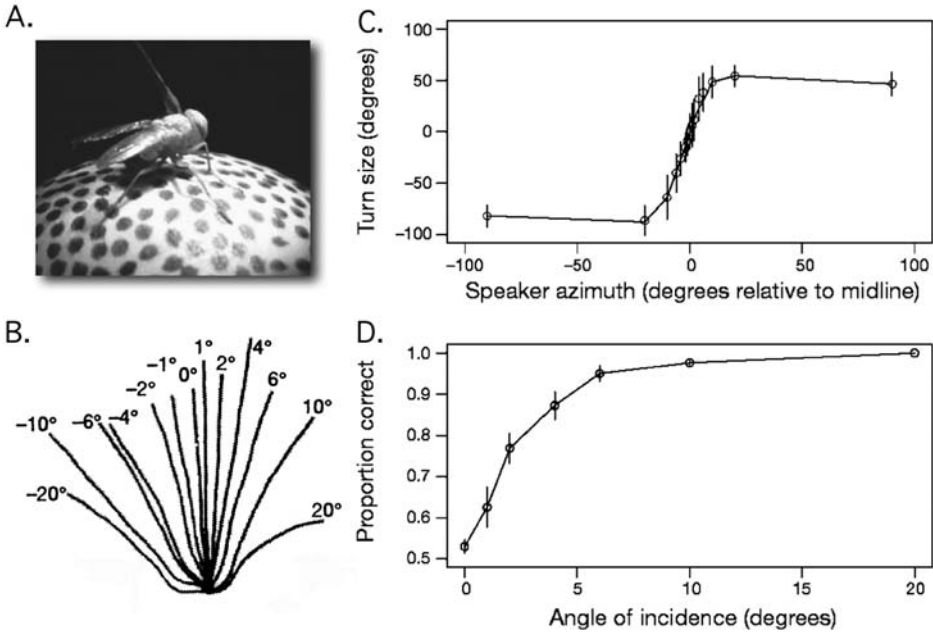


FIGURE 2.3. Phonotactic accuracy in the fly *O. ochracea* on a walking treadmill. (A) Videophotograph of the fly tethered on top of a Styrofoam ball supported by an air cushion. The locomotory activity of the fly is recorded by the resulting motion of the ball. (From Mason et al 2001, with permission.) (B) Mean paths of locomotion of one fly in response to cricket's song delivered at different angles. (C) ( $N = 7$  flies, 10 trials per fly per angle,  $\pm 1$  SD). Different azimuthal angles resulted in distinct phonotactic trajectories. (D) Proportion of correct turns as a function of azimuthal angle of incidence. A reliable oriented response occurs for angles as little as 2 to 3° ( $N = 19$  flies, 20 responses per fly per angle). (B–D modified from Mason et al. 2001; © Nature Publishing Group.)

is the observed phonotactic behavior possible in view of such small directional cues? The answer required further knowledge on the actual response characteristics of the fly's auditory primary afferent receptor neurons (Mason et al. 2001; Oshinsky and Hoy 2002).

The differences in the spike latency between left and right receptor neurons have been measured for different sound locations (Mason et al. 2001). For 90° azimuth, neural ITDs, measured as summed action potentials, amount to 150  $\mu$ s and, as the angle decreases, drop by 3.5  $\mu$ s per degree, predicting a neural ITD of 7 $\mu$ s at 2° azimuth. Hence, in view of the observed phonotactic behavior, and perhaps allowing for some degree of error in the measurements, the fly's primary afferent neurons seem capable of reliably encoding temporal events separated by a mere 10 $\mu$ s. The studies by Mason et al. and Oshinsky and Hoy together provide key evidence that such capacity is based on a remarkably fast spike time

code. First, most of the afferent neurons that were recorded were shown to respond to acoustical stimulation with a single spike (type I afferents) (Fig. 2.4A, 90 dB sound pressure level (SPL), and have very low probability of spontaneous activity (Oshinsky and Hoy 2002). Characteristically of this category of afferents, only one spike is released, irrespective of the duration of the stimulus. The latency of that single spike, measured as the time between stimulus onset and spiking, increases as stimulus amplitude decreases, resulting in a “time/intensity tradeoff” observed in numerous sensory systems (Fig. 2.4A). Such effect is useful to generate directionality. Owing to the asymmetrical mechanical deflections of the tympanal system, the primary afferents, in addition to enhanced

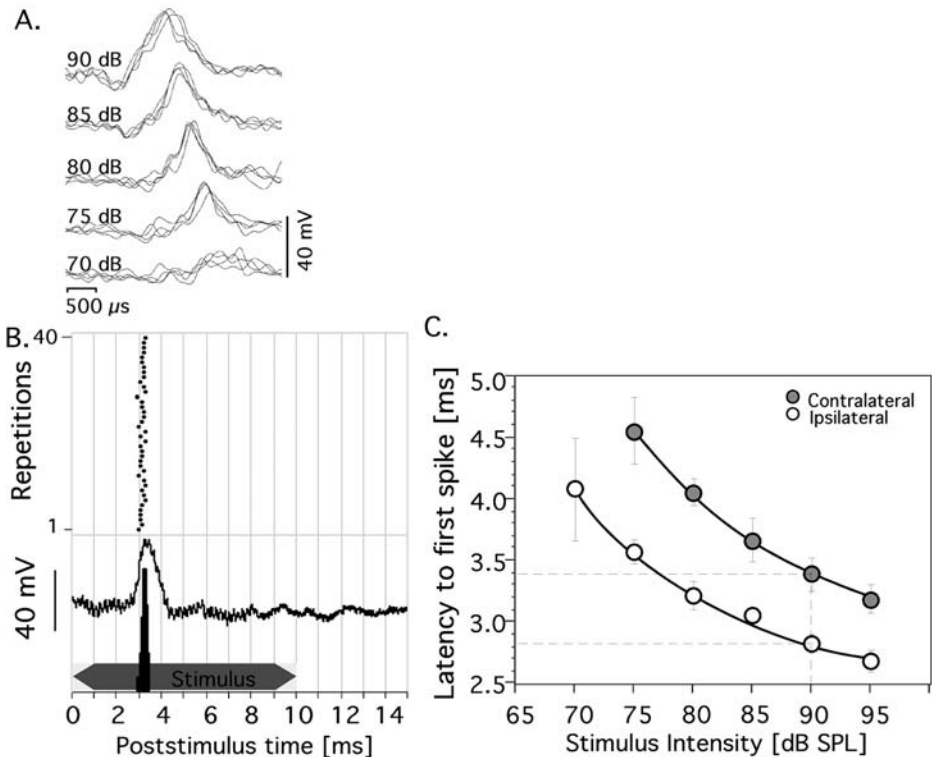


FIGURE 2.4. Temporal coding of mechanoreceptor neurons in the fly *O. ochracea*. (A) Multiple action potentials of a single neuron as a function of the amplitude of the sound stimulus in dB SPL. Low-amplitude stimuli result in a delayed action potential. (B) Response of a single receptor to a series of 40 stimulus presentations. Raster plot of the repetition, oscillogram of a single spike, and poststimulus histogram illustrate the high repeatability of the neuron’s signalling. (C) Latency of receptor spiking as a function of stimulus amplitude for ipsilateral and contralateral stimulation. For a 90-dB SPL stimulus, a receptor shows different spiking latencies, depending on whether it is ipsilateral or contralateral to the sound source. (A–C modified from Oshinsky and Hoy 2002, © 2002 by the Society for Neuroscience.)



mechanical ITDs, experience different interaural stimulus amplitudes. Yet, in detail, it is unknown whether the vibration amplitude of receptor neurons is linearly related to that of the tympanal membrane. A nonlinearity may signify a larger contrast of directionality for some range of amplitudes, generating a “foveal” zone of acuity at some stimulus amplitude. Importantly though, a difference in spiking delays is generated between the individual afferents situated in the ear ipsilateral or contralateral to the sound source (Fig. 2.4C). The response latency of single afferents from one ear was measured in response to a series of sound stimuli varying in amplitude (95 to 75 dB SPL) first broadcast ipsilaterally to that ear, and then, at the same sound pressure, from the side contralateral to that ear. This way, the difference in the latencies between ipsilateral and contralateral afferents could be estimated. For 90 dB SPL, the difference in afferent response latency was approximately 600  $\mu$ s, a delay that seemingly becomes manageable for neural processing (Fig. 2.4C).

Finally, a key observation was that the variation in spiking time (jitter) was remarkably low compared to other invertebrate sensory systems. In effect, in response to 40 successive 5-kHz tones, spiking latency was 3164  $\mu$ s with a jitter (the standard deviation of the latency distribution) of 95  $\mu$ s (Oshinsky and Hoy 2002)(Fig. 2.4B). The jitter measured for seven animals ranged from 12  $\mu$ s to 121  $\mu$ s with an average of about 70  $\mu$ s (Mason et al 2001). Thus, the uncertainty of the spike code may be about ten times larger than the temporal event it is required to code for (about 10  $\mu$ s). At this stage, this is a task that a population of primary afferents could achieve, reaching temporal hyperacuity by the coherent pooling of a large number—in the fly maybe 50 to 100—of afferent neurons (Mason et al. 2001). In this respect, the influence of stimulus amplitude on spiking jitter bears some importance.

Although it would be expected, it is unclear if and to what exact degree the spiking jitter of a receptor neuron (and hence that of the afferent population) increases as stimulus amplitude decreases (Fig. 2.4A). Such dependence could also contribute to the putative capacity for hyperacute coincidence detection of first-order binaural auditory interneurons. Critical temporal coincidence may be reached earlier and with a higher probability for the auditory channel experiencing more intense mechanical vibrations. In *O. ochracea* the primary afferents project exclusively ipsilaterally, and in the three, fused thoracic neuromeres (Oshinsky and Hoy 2002). To further evaluate the enticing possibility of coincidence detection in an insect auditory system, the neuroanatomy of the first-order interneurons, and their temporally hyperacute physiological capacity to integrate afferent signaling, need to be further studied.

## 6. Insect Psychophysics and Auditory Space Perception

### 6.1 Psychoacoustics and the Third Dimension

Until recently, studies of directional hearing in insects were mostly concerned with directional cues in the azimuthal plane. The reception of sound at the level

of the tympanal membranes, the extraction of directional cues from the sound field, and their neural coding in the elevational plane have not been given as much attention. This is perhaps a possible consequence of the tacit assumption that insect ears may not be up to the task. Compared to vertebrates and mammals in particular, insects dedicate fewer mechanoreceptor neurons (with the exception of mosquitoes) and seem to be endowed with a relatively limited capacity for signal analysis in the frequency domain (see review by Pollack 1998). In animals with bilaterally symmetrical ears, the primary cues in the elevational plane reside in the binaural (or even monaural) comparison of fine spectral characteristics of the incoming sound and their comparison over time (Middlebrooks and Green 1991, Wightman and Kistler 1997; Kulkarni and Colburn 1998). For insects, the task may be regarded as computationally demanding and hence challenging. But again, as stimulus variables and processing mechanisms may be entirely different in insects, the task is by no means impossible.

In passing, it is worth noting that acoustic events occurring at a longer temporal scale are also relevant to the sense of directional hearing in insects. For instance, crickets in simulated tethered flight show a distinct sensitivity to the precedence effect, a capacity for echo suppression that may enhance directional sound detection in some situations (Wytenbach and Hoy 1993). In crickets again, it was shown that auditory receptor neurons are liable to habituation during long bouts of simulation (8 to 10 s) in an intensity-dependent manner. This habituation process can, surprisingly, reverse the sign of the interaural difference that results from conventional auditory processing (Givois and Pollack 2000).

Some elegant experiments have shown, in effect, that Polynesian field crickets (*Teleogryllus oceanicus*) can detect and discriminate between sounds delivered at different elevational angles (Wytenbach and Hoy 1997). In that study, the minimum audible angle (MAA) was taken as a measure of spatial auditory acuity. As a standard descriptor in the field, MAA was defined as the smallest angular separation at which two sounds are perceived as coming from two distinct sources (Fay 1988). Remarkably, the experimental paradigm of choice to test discrimination in crickets was that of habituation–dishabituation. When presented with pulses of ultrasound mimicking echolocating bats, crickets initiate steering maneuvers that are part of a startle/avoidance behavior (Fig. 2.5A) (Moiseff et al 1978). This behavior is liable to habituation; the response amplitude in effect decreases with stimulus repetition (Fig. 2.5B, stimuli 1 to 5). Complying with criteria of habituation, the response decreases exponentially at a rate dependent on stimulus amplitude and repetition rate, can recover spontaneously, and with the presentation of a novel stimulus (Fig. 2.5B, stimuli T and P). Experiments required the cricket to habituate to a series of ultrasound pulses from a particular loudspeaker location, and then recorded whether the test (T) stimulus—a single ultrasonic pulse from another location, or with any other acoustical attributes—could cause dishabituation (Wytenbach et al 1996; Wytenbach and Hoy 1997). Importantly, dishabituation as such was measured as the response to a probe pulse (P) identical to the habituating pulses (Fig.

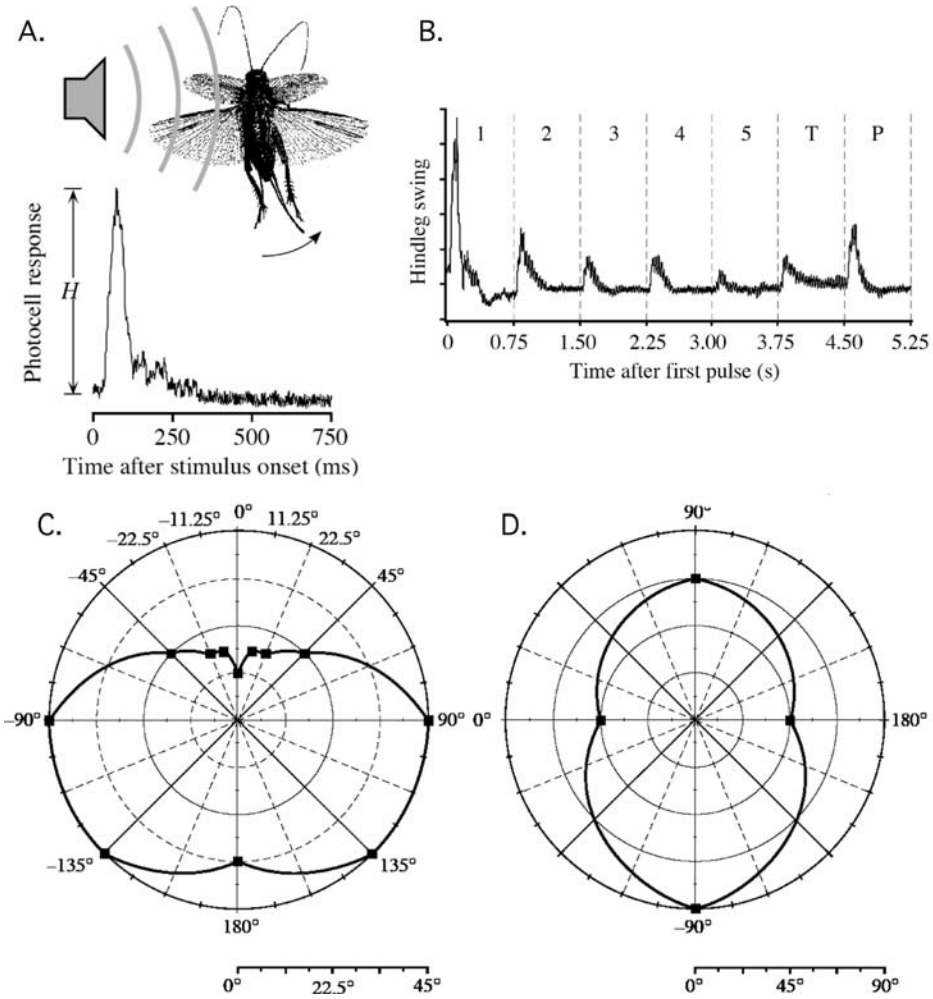


FIGURE 2.5. The spatial acuity of the cricket auditory system. (A) Behavioral response used to assess auditory acuity. The ultrasound avoidance response involves the rapid swing of the hind legs and abdomen. These are monitored by a photocell generating a voltage ( $H$ ) proportional to the amount of movement. (B) Five pulses of sound with a carrier frequency of 40 kHz were presented at regular intervals from one loudspeaker to habituate the escape response. A test pulse (T) was delivered at 40 kHz from another loudspeaker, followed by a probe pulse (P), identical to pulse 1, from the initial loudspeaker. The minimum audible angle (MAA) for a position was defined as the smallest angular separation of the loudspeaker that would evoke dishabituation. (C, D) Polar diagram displaying MAA, shown as the distance from the center of the diagram. (C) Acuity in azimuth, is best (11.25°) around 0° and worst (45°) at 90° and 135°. Data on the *left* and *right* sides of this graph are mirror images. (D) Acuity in elevation. Acuity is best (45°) in the front and rear and worst (90°). (Modified from Wyttanbach and Hoy 1997 © Company of Biologists Ltd., with permission.)

2.5B). The use of the probe stimulus establishes whether the dishabituating pulse (T) is perceived as different from the habituating pulse, although it may not elicit any behavioral response. Hence there is a need for a probe pulse to uncover the presence of dishabituation. Using such a procedure, the minimum angular separation of the acoustic stimulus source required for dishabituation to occur was interpreted as the MAA. Quantitatively, the dishabituation index was calculated by taking the difference between the response magnitudes to the probe pulse and the last habituating pulse, and by dividing this difference by the response magnitude to the first pulse in the habituating series. In the plane of azimuth, experiments with flying tethered crickets yielded MAAs of some  $11^\circ$  in front of the animal and some  $45^\circ$  to the side (Fig. 2.5C). In the elevational plane, dishabituation took place when the sound source would be displaced by  $45^\circ$  in front or rear of the animal (Fig. 2.5D). This indicates that the animal can differently perceive sound stimuli broadcast from different elevations. However, changes in the position of the sound source, or switching sound sources may introduce some experimental confounding factors, such as changes in stimulus intensity, that require appropriate controls. In the present case, changes in stimulus intensity were ineffective in the range tested (Wytenbach and Hoy 1997). In the plane of azimuth, these results concur with an earlier study, which found that a loudspeaker deviation of  $10^\circ$  in front of a tethered cricket did not elicit any behavioral response, while larger angles did (Pollack and Plourde 1982).

The habituation–dishabituation experimental paradigm is particularly useful because it allows a quantification of sensory acuity that choice experiments do not provide (Dooling and Brown 1990; Wytenbach and Hoy 1997). To date, the psychoacoustical approach in all its diversity and power has been underexploited in the study of insect sensory biology (Wytenbach and Farris 2004); it is quite likely that many insect species and other modalities are amenable to such tests.

Directional hearing in the elevation plane in field crickets makes sense in a sensory ecological context; this is also during flight at dusk and dawn that female crickets are to localize males calling from the ground. Multiple biomechanical, behavioral, and electrophysiological evidences exist that different cricket species are directionally sensitive and can perform some form of frequency analysis of incoming sound waves (Hill and Boyan 1977; Pollack and Plourde 1982; Michelsen et al. 1994). Because of the relative purity of their calling songs, field crickets may well rely on different, or unusual, stimulus variables that are perhaps related to the frequency domain and/or the multiple inputs to their auditory system, but that have thus far eluded experimental testing. Yet, the question of whether crickets, or any other insects (Pollack and Imaizumi 1999) and notably cicadas (Fonseca et al 2000), can use spectral cues for the purpose of directionality remains unanswered.

## 6.2 Directional Hearing and Range Detection

The detection of sound in the three-dimensional space is also related to the capacity to evaluate the distance of a sound source (Moore 1997). Like directional hearing, range detection may not be a necessity but it could constitute an adaptive advantage to the organisms endowed with such capacity. Would insects—or some of them—be capable of acoustic range detection?

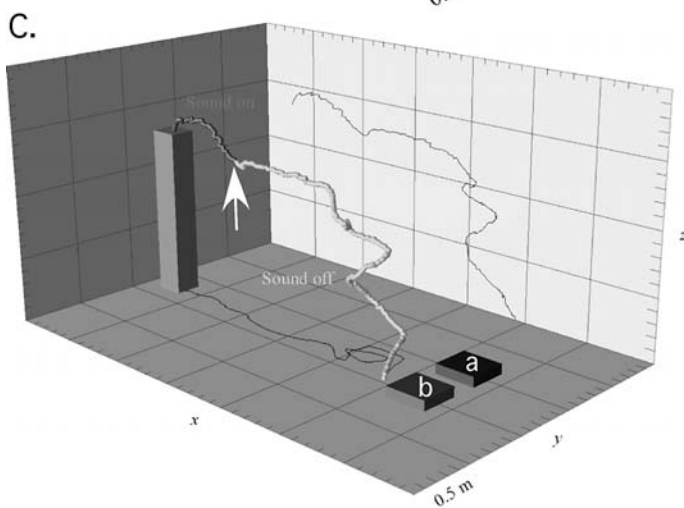
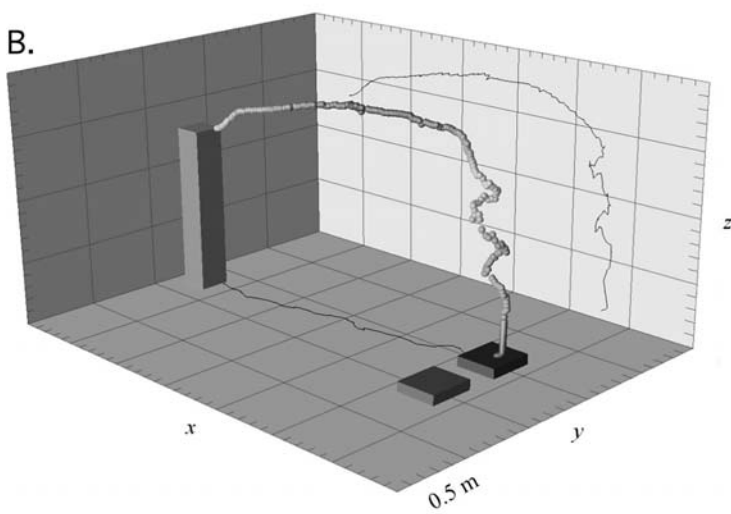
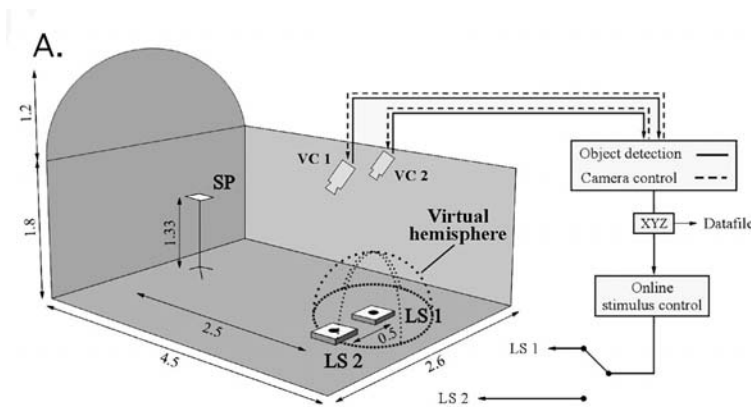
Again, attention turns to a nocturnal insect that performs phonotaxis: the tachinid fly *O. ochracea*. As a parasitoid, the female must find a suitable host for her eggs of larvae. Using her prothoracic ears, the fly finds her host in the dark, homing in on the host's calling song (Cade 1975). As the phonotactic fly flies at some height above the ground (some 1 to 2 m) (D. Robert, personal observation), and the cricket sings on the ground, the task of acoustical localization may well be a problem to solve in the three-dimensional space. The fly's task seems reminiscent of the behavior of the barn owl (Knudsen and Konishi 1979). To address the question of acoustic orientation in the dark, the three-dimensional flight trajectories of *O. ochracea* were quantified as the fly was induced to home in on the source of a cricket song placed on the ground (Müller and Robert 2001). The phonotactic flight paths were recorded in three dimensions using a stereo infrared video tracking system (Fry et al 2000). This system also allowed for controlling the delivery of sound stimuli as a function of the fly's position in space (Fig. 2.6A) (Fry et al 2004). As the phonotactic behavior is performed in the dark, it was thus possible to assess the free-flight fly's reaction to alterations in acoustic stimulation taking place at predetermined and replicable times and spaces in the flight arena. In particular, the control software of the tracking system was designed to incorporate a virtual representation of the experimental arena *in silico*. In this representation, diverse volumetric objects (such as a sphere; Fig. 2.6A) could be defined and be assigned a logical function analogous to that of a conventional light barrier. Experimental conditions could thus be programmed to change automatically and online as the animal's trajectory (its *X*, *Y*, *Z* coordinates in the virtual representation) would mathematically intersect the description of the virtual object. Local experimental conditions could thus be controlled without physically cluttering the real flight and acoustic environment in order to test the animal's reactions to novel stimuli, or the absence of them.

Tracking experiments testing the phonotactic capacity of the fly in complete darkness show that, interestingly, flies do not take the shortest path between the starting platform and the sound source. Flies do not fly a beeline to the cricket (Fig. 2.6B). Rather, trajectories comprise three distinct phases: a brief takeoff phase; a cruising phase in which course and altitude remain quite constant; and finally a terminal, landing phase. Taking place as the fly is nearer but still above the sound source, this terminal approach is characterized by a steep spiraling descent. The accuracy of the flies' phonotactic behavior is remarkable: at the end of a flight bout of about 4 m, they landed at a mean distance of 8.2 cm

(SD  $\pm$  0.6 cm,  $N = 80$  landings) from the center of the loudspeaker. One particular, and simple, experiment brought to light some unsuspected and intriguing characteristics of this fly's phonotactic capacity. As the fly was on its way to the loudspeaker, the acoustic stimulus was interrupted, thus removing the only navigational cue available. Surprisingly, the phonotactic behavior was not drastically affected, or disrupted, by the interruption of the acoustic stimulus (Fig. 2.6C). In effect, irrespective of her position in the flight room at the time of stimulus interruption, the fly initiates the descent maneuver (spiraling drop) at the appropriate time and location, not far above the loudspeaker. This results in a landing close to the now silent loudspeaker. Since other possible navigational cues are absent (visual and/or olfactory), these experiments suggest that, at the time of stimulus interruption, the fly had acquired sufficient acoustic information to navigate accurately to the sound source. It must be noted here that flies can localize the sound source without prior experience and also display no improvement (through learning) in their phonotactic abilities (Müller and Robert 2001).

Depending on their position in the flight arena, the free-flying flies respond in different ways to stimulus interruption. Most remarkably, stimulus interruption taking place whilst the fly is far away (e.g., 1.8 m) from the loudspeaker does not prevent the fly from landing close to it (Fig. 2.7). To achieve this, the fly maintains the same flight course and only initiates the spiraling descent at the appropriate time and place, landing relatively close to the loudspeaker (Fig. 2.6C). By contrast, if stimulus interruption takes place when the fly is close to target (0.6 m or less), she initiates her spiraling descent at a shorter time delay after stimulus interruption. Thus while the time of stimulus interruption is no predictor of the onset of the landing response, the fly's position relative to the loudspeaker is. Notably, these landing maneuvers are initiated at a time when sound cues are completely absent and thus seem to result from autonomous decisions. It could also be seen that, probably because of noise in the sensory and motor systems, the earlier the stimulus is interrupted, the less accurate the landing becomes (Fig. 2.7). It could thus be shown that the cessation of the acoustic stimulus, by itself, does not elicit the spiraling trajectory indicative of landing. From this it can be concluded that the fly must gather sufficient information about the spatial position of the sound source before stimulus cessation. Although it seems to rely on some form of motor memory instructed by sensory inputs, this behavior is notably different from idiotactic orientation, known of spiders and fossorial mammals, in that the fly has never been to the location of the loudspeaker before. The nature of the information gathered by the fly prior to stimulus interruption remains elusive to date.

Other experiments were conducted to test the fly's capacity to localize a sound source in midair (Fig. 2.8) (Müller and Robert, unpublished results). In a control situation, the fly was first attracted to a loudspeaker situated on the ground (ZS). In a second trial, the fly was asked to repeat the performance; as she did so, she entered the space of a virtual sphere (gray sphere; Fig. 2.8) that served as



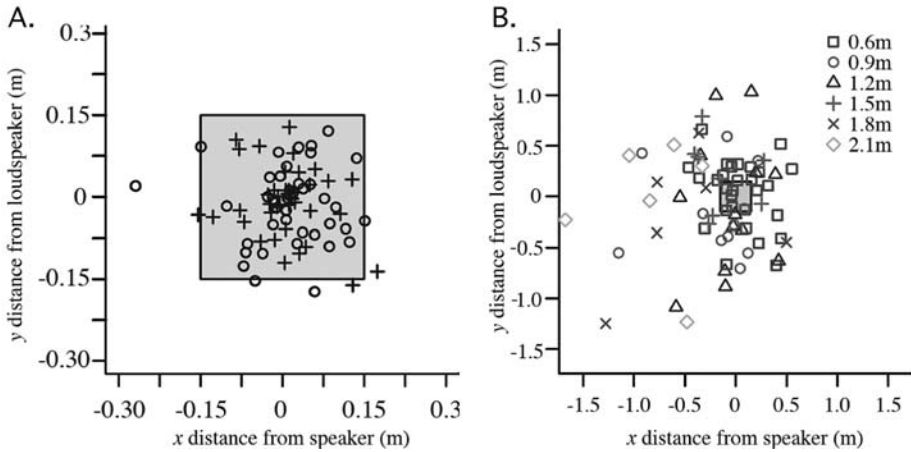


FIGURE 2.7. Landing accuracy of the fly in response of a continuous and an interrupted sound stimulus. (A) Continuous stimulus simulating a cricket song. Landings to the right (+) and left (o) loudspeaker are pooled. *Shaded area* is surface area of the loudspeaker box. After a flight approximately 3 m long, flies land within 8 cm of the center of the loudspeaker. (B) Accuracy of phonotaxis as a function of the distance of stimulus interruption. Symbols indicate the distance from the sound at which the flies were at the time of stimulus interruption. Remarkably the flies succeed at finding the sound source, even without sound cues. Accuracy decreases with increased distance. (Data modified from Müller and Robert 2001.)



FIGURE 2.6. Directional hearing as a behavior in view of three-dimensional sound localization and range finding. (A) Setup used for studying free-flight phonotaxis in insects. SP, Starting platform, VC1, 2: infrared computer-controlled pan-tilt video cameras. LS1, 2: Loudspeakers. The video signals of each camera are processed frame by frame as a stereo pair to determine the position of the fly on each frame, and used to instruct the tracking motions of both cameras. The flight path is computed in Cartesian coordinates (50 Hz sampling frequency) to yield the X, Y, Z coordinates of the animal's trajectory. This data are also used to control alterations of experimental conditions online (see Fry et al. 2004). (B) Phonotactic trajectory of the fly *O. ochracea* toward a loudspeaker broadcasting a cricket song. Under infrared darkness, the fly navigates to land on the active loudspeaker, depicted by (a) in diagram (C). (C) Experiment in which the same fly is lured toward the other loudspeaker (b) for a short time, until the sound stimulus is interrupted (arrow). In the absence of acoustic, visual or olfactory stimuli, the fly navigates to the originally active sound source.



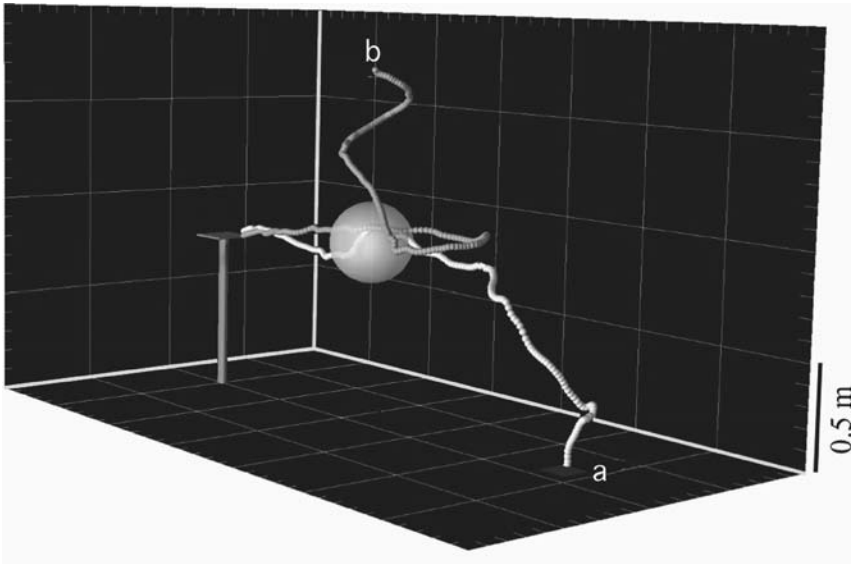


FIGURE 2.8. Orientation in the three-dimensional space in the fly *O. ochracea*. In as Fig. 2.6A and B, the fly is induced to perform a bout of phonotactically oriented flight toward a loudspeaker situated on the ground (*a*). This flight path is registered (trajectory *a*) and a virtual sphere is positioned so that it intersects that trajectory. The fly is brought back to the platform, loudspeaker *a* is activated again. The fly commences her phonotactic flight toward loudspeaker *a*, and as it enters the virtual sphere, sound is switched over to loudspeaker *b* situated directly above the sphere. Trajectory *b* indicates that the fly carries on to fly for a little while (several tens of milliseconds) before engaging in a level turn and then a spiraling ascent reaching loudspeaker *b*. Although this fly species parasitizes field crickets that sing on the ground only, the fly's auditory system is capable of finding a sound source in the three-dimensional space.

the trigger for the sound stimulus to be switched over to a target sound source (TS) straight above the sphere. The fly proved capable of pinpointing the source of sound, and of reaching it by spiraling vertically toward it.

These results seem to suggest the presence of a mechanism of acoustic detection that enables the fly to gauge both the direction (perhaps a three-dimensional vectorial representation?) and the distance of a sound source in three dimensions. Such capacity is reminiscent of the barn owl's capacity of localizing acoustic targets in both azimuth and elevation (Knudsen and Konishi 1979). Unlike the owl, the fly's ears are bilaterally symmetrical; a symmetry that was shown earlier to be a prerequisite for the mechanism used for directional hearing (Fig. 2.1E; Robert et al 1996). To date, the mechanisms supporting the proposed ability of the fly to perform three-dimensional audition and evaluate distance to target remain unknown, but certainly deserve more attention.

Recent work has shown that a conjunction of both psychophysical and sensory

ecological approaches can provide precious information, and sometimes reveal the unique and sophisticated mechanisms, and unsuspected capacities, by which insects sense their world as they rapidly pass through it (Srinivasan 1998).

## 7. Some Outstanding Issues and Prospects

Small size may well have imposed severe constraints on the mechanics of auditory receivers, but also obviously on the number of nerve cells behind them. The principles of economy employed by insects do not necessarily signify simple or crude modes of operation or reduced processing power. Rather, principles of economy can imply the implementation of alternative, possibly cunning and efficient, mechanisms that are exquisitely adapted to the task. This point has been repeatedly illustrated in insect sensory research, be it for vision, audition, olfaction, or the lesser-known modalities of thermoreception or magnetoreception. In audition, a well-known model for accurate information processing in the time domain is the Webster–Jeffress cross-correlation model for binaural processing, requiring a complex array of neurons (Jeffress 1948). It would indeed be interesting to see whether this model finds its counterpart in insect hearing, and if so, which particular form it may take at the level of interneurons. One alternative seems to involve a neuronal network that relies on the intrinsic integration properties of coincidence detector, rather than a series of delay lines temporally tuned by the differential length of transmission lines. As the question remains open, it may be useful to consider that insects have generally come up with solutions that are economical in evolutionary terms and that are more often than not, computationally undemanding but efficient.

The capacity of insects to perform some sort of auditory scene analysis, allowing them to situate themselves in space and time within their acoustic environment deserves more attention. With this respect, adapted psychophysical experimental paradigms and techniques of behavioral monitoring in unrestrained animals may be very applicable to insect systems to address testable hypotheses on the complex issues of mechanical and neural sound processing in frequency, time, and space. These studies could even be conducted in conjunction with extracellular, and intracellular electrophysiology on primary auditory afferents as well as identified interneurons. Hypotheses may address the enticing possibility that some insects can extract three-dimensional information using two symmetrical ears only. In particular, the capacity for auditory space perception—especially considering species other than *O. ochracea*—and the type of coding involved (owl-like, or else) may be particularly interesting at both fundamental and comparative levels.

Finally, one corollary and emergent outcome of insect hearing research is the extraction of operation principles for bioinspired acoustic detection technology. In the course of their evolution, insects have acquired the capacity to do small things very well. In due course, it may become a reality to see insect-inspired microsensors equip microrobots. Indeed, fly-inspired microsensors are currently

under development that seek to emulate the key characteristics of their natural analogs, such as miniaturization, accuracy, and economy of operation.

Future studies will carry on revealing the fundamental mechanisms arthropods in general, and chiefly insects, spiders, and crustaceans, employ to sense vibrations directionally, in pressure or velocity. The diversity of mechanisms used to detect sound directionally may still be much richer than presently known. Some 36 years ago, David Pye depicted insect audition (Pye 1968) in a series of verses. In a recent update to his long-lived prose, commenting on diversity, he concluded: “This list is long, the contrasts strong, and may go on for ever. And so we end with no clear trend—For Nature is so clever” (Pye 2004). Indeed, insect research contributes to enrich our knowledge of sensory systems, but also continues to impress upon us their magnificent efficiency and ever surprising diversity.

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