Chapter 4
Auditory Parasitoid Flies Exploiting Acoustic Communication of Insects

Berthold Hedwig and Daniel Robert

Abstract Hearing evolved in flies of both Dipteran families *Tachinidae* and *Sarcophagidae*, enabling the parasitic exploitation of singing orthoptera and hemiptera. Guided by acoustic communication signals, these flies identify and localise their singing target, depositing their larvae on or near the host. Larvae then develop as endoparasites, eventually killing the host. In these flies, the mechanosensory auditory organ is located on the prosternum and in most cases is less than one millimetre in size. The frontal facing tympana constitute an extreme example of adaptation in auditory micromechanics. Directional hearing relies on the mechanical coupling between the hemilateral tympana, a purely mechanical process that exploits minute interaural time differences in tympanic vibrations and enhances bilateral oscillation differences to generate a highly directional sensitivity. In tachinid fly species, the frequency response of the ears is adapted to the host communication signals. The auditory organs contain up to 250 scolopidial afferents, which are directly driven by tympanic membrane vibrations. The signals from the auditory afferents are forwarded to auditory neuropils in the three thoracic neuromeres. Further processing of intensity and directional information and also of temporal patterns involves local and also ascending auditory interneurons, which project up to the brain for final sensory-motor integration.

B. Hedwig (✉)
Department of Zoology, Cambridge, UK
e-mail: bh202@cam.ac.uk

D. Robert
School of Biological Sciences, Bristol, UK
e-mail: d.robert@bristol.ac.uk

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4.1 Introduction

Acoustic signalling rarely constitutes a private communication channel. Sound tends to radiate in all directions, propagating towards hardly predictable destinations and, sometimes, unintended receivers as predators and parasitoids. Some flies take advantage of acoustic signalling in insects to feed their larval brood. Hearing evolved among the Dipteran parasitic family Tachinidae and the flesh flies family Sarcophagidae, which are to date the only Dipteran Brachycera known to use acoustic cues to localise their host (Leonide 1969; Cade 1975; Soper et al. 1976; Fowler 1987, Barraclough and Allen 1996, Robert et al. 1998). Infestation relies on the fly planidia larvae to actively burrow their way into the host. In some species, such as *Ormia depleta*, the larvae are deposited at some distance from the host (Wineriter and Walker 1990), around the entrance of a mole cricket burrow from which the song is broadcasted. The larvae then make contact with the host by frictional encounter, or by crawling towards it. The larvae develop inside the host, a process that results in the host's death and the egress of 3–9 larvae.

There seems to be a definite advantage to find a host free of previous parasitic load; field observations of tachinids suggest that being first may be a matter of seconds. Fast and efficient host finding using acoustic cues appear to constitute a key element in the reproductive biology of Dipteran parasitoids. In populations with high infestation rates, parasitoid flies constitute significant natural selection, having a direct effect on the intraspecific communication of their hosts (Cade 1975, Walker 1993, Adamo et al. 1995a, Zuk et al. 2006).

The auditory organs of flies exhibit microscale tympanic membranes that are located at the prosternum, on the ventral prothorax at the base of the neck (Lakes-Harlan and Heller 1992, Robert et al. 1992). Insects of the order Diptera tend to be small; endoparasitic Diptera are constrained to be even smaller than their hosts, a limitation that has direct consequences for their sense of hearing. In effect, a small distance between the ears severely limits the use of interaural sound amplitude differences as directional cues. Highly acute auditory orientation, however, is possible due to an auditory system endowed with mechanically coupled tympanal membranes (Robert et al. 1994; Robert 2001; Mason et al. 2001) and appropriate neural processing at the level of auditory afferents and interneurons (Oshinsky and Hoy 2002; Stumpner et al. 2007).

Hearing research in insects has shown the diversity of miniature solutions to the problems of sound reception (Robert and Hoy 2007) and processing, (Hennig et al. 2004; Hedwig and Pollack 2008), illustrating the often unexpected routes of sensory evolution (Fullard and Yack 1993; Montealegre-Z et al. 2012). In this chapter, we review the co-evolutionary relationship between parasitoid flies and their insect hosts, focussing on the flies phonotactic behaviour (Müller and Robert 2001; Ramsauer and Robert 2000), their micro-scale hearing system (Miles et al. 1995, Robert et al. 1996) and the neural organisation of their auditory pathway (Oshinsky and Hoy 2002; Stumpner and Lakes-Harlan 1996; Stumpner et al. 2007).

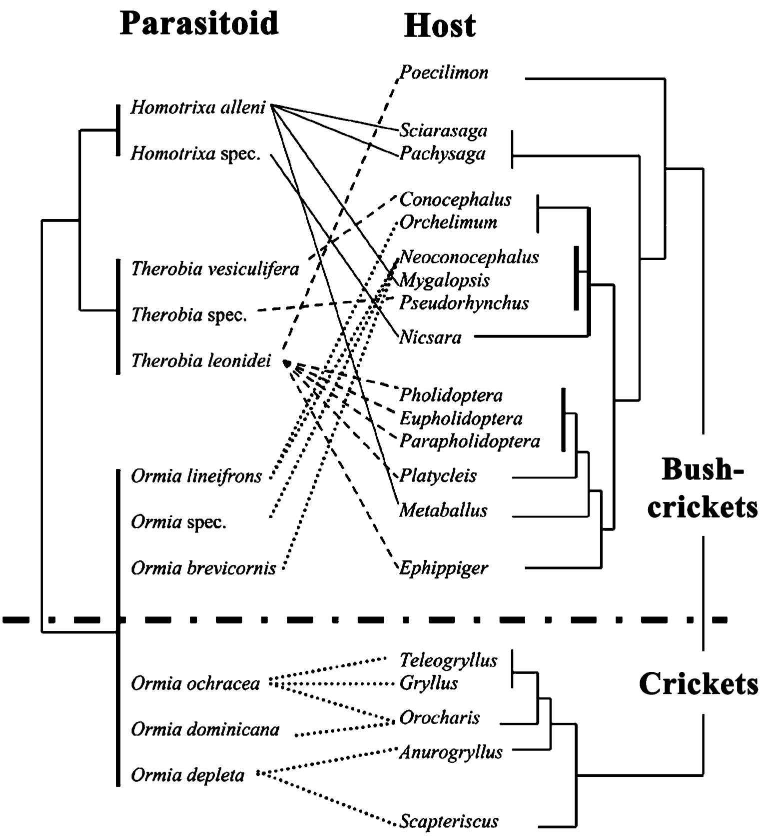

Fig. 4.1 A phylogenetic tree of parasitic Tachinid flies and their bushcricket and cricket host species for 11 Ormiini flies. Only Ormia species in North America target crickets as hosts; modified from Lehmann (2003, Fig. 4.2)–with kind permission of the author

4.2 Parasitic Hearing Flies and Hosts

Among the ~8000 species of Tachinid flies there are 67 known species in the group of parasitoid Ormiine that use acoustic cues to localise their host. Ormiini are found in tropical and subtropical regions where at least 11 species prey on various orthopteran insects. *Therobia leonide*, the only Ormiine fly in southern Europe (Léonide 1969; Lakes-Harlan and Heller 1992; Lehmann et al. 2001) and the Australian species *Homotrixa allen*, (Fig. 4.2a) (Allen et al. 1999) target bushcrickets. Further genera, such as *Phasioormia*, *Euphasiormia*, *Aulacephala*, *Mediosetiger* have been examined from museum collections and show to bear auditory organs (Huber and Robert, unpublished), however, host species are not yet known. Field collection using acoustic trapping (Wineriter and Walker 1990) has not yet been carried out across the bandwidth of Orthopteran songs. Such field prospection may uncover a greater diversity of acoustic parasitoids, and live material to examine.

In North America (Florida) gravid *Ormia lineifrons* heavily parasitises *Neoconocephalus triops* (Burk 1982); *Ormia depleta*, a species originating from South America, is attracted to the song of mole crickets of the genus *Scapteriscus* (Fowler and Kochalka 1985) and *Ormia ochracea* (Fig. 4.2b) targets singing field crickets; exhibiting regional differences in their host preferences. *O. ochracea* targets *Gryllus rubens* in Florida and in western USA it parasitises the variable field cricket *Gryllus lineaticeps* (Cade 1975). *O. ochracea* has been introduced to the Hawai'ian archipelago where it targets the Polynesian field cricket *Teleogryllus oceanicus,* another introduced species (Zuk et al. 1993). Overall, the flies seem to be opportunistic hunters with a wide range of potential hosts as auditory pattern recognition in flies is not as specific as intraspecific communication shaped by sexual selection. Lehmann (2003) speculates that bushcrickets were the hosts in the ancestral form of parasitism and that the shift to Gryllids is the derived state as these are only targeted by the North American genus *Ormia*.

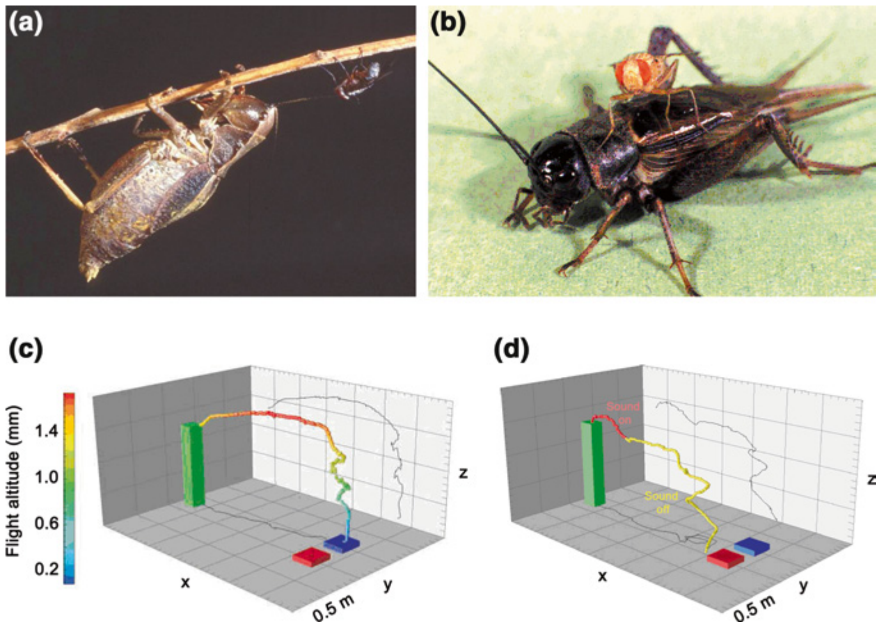

Fig. 4.2 **a** The Australian Tachinid fly *Homotrixa alleni* with its bushcricket host *Sciarasaga quadrata* in the wild. **b** The North American *Ormia ochracea* depositing larvae on her host photographed in the laboratory. **c**, **d** Phonotactic behaviour of free-flying *O. ochracea* towards a loudspeaker broadcasting a cricket song. Green block is the starting platform; red and blue are landing platforms each hosting one loudspeaker. **c** Phonotaxis to blue loudspeaker, red speaker is off. **d** Same fly attracted to red loudspeaker presenting cricket song (red trajectory) and its flight path after the song was stopped (yellow trajectory). Photo in **a** courtesy by Geoff Allen, University of Tasmania/Hobart and photo in **b** courtesy by Marie Read, Cornell University; **c** and **d** modified from Müller and Robert (2001)

Parasitoid Tachinid flies are strictly crepuscular and nocturnal with phonotaxis occurring in gravid females only. These land next to the singing host and walk towards it, dropping planidia, the mobile first instar larvae directly on the host and/or in its immediate vicinity (Cade 1975; Adamo et al. 1995b; Allen et al. 1999). In general, only singing male hosts are infested but if females respond acoustically to calling males, they can also suffer parasitism. The extension of parasitism to non-singing females arises from the flies' larviposition strategy. In *O. ochracea*, three larvae were directly deposited on the host, whilst more planidia were laid around the host, ambushing female crickets approaching the calling male. Upon contacting the host, the larvae climb on it and crawl to find soft intersegmental cuticle, through which they bore an entry point. Once inside the host, they feed on hemolymph and muscle, but keep the host alive and alert until the last stages of parasitism. Infested crickets die after 9–12 days (Walker and Wineriter 1991; Adamo et al. 1995a), upon the egress of the maggots.

Acoustically guided parasitism is also found among the *Sarcophagidae* and represents a case of a convergent evolution within the Diptera (Lakes-Harlan et al. 1999; Robert et al. 1999). In North America the sarcophagid fly *Emblemasoma sp.* and *Emblemasoma auditrix* mainly parasitises the calling males of cicada, e.g. *Okanagana rimosa* that sing during daytime (Soper et al. 1976; Farris et al. 2008). Flies first land next to the singing host and a sequence of highly specialised behaviour follows that is distinct from that generally observed in Tachinids. After visual orientation towards the host's abdomen, the fly squeezes under the cicada's wings, and proceeds to cut through the timbal organ, damaging it using their genital plate. The fly then deposits a larva within the sound-producing organ before flying away (Schniederkötter and Lakes-Harlan 2004). With a damaged sound-producing organ, male singing is less prevalent and the infected cicada deemed less likely to attract additional flies. This sophisticated parasitic strategy prevents superparasitism and therefore larval competition. Exposure to parasitoids may also be moderated by the time of day at which singing takes place. It was suggested that the different dial singing activity of three sympatric cicada species may reflect different avoidance strategies to reduce the risk of parasitism by day-active *Emblemasoma sp.* (Farris et al. 2008).

4.2.1 Host Selection and Communication Signals

Sexual selection is considered the primary force that shaped intraspecific acoustic communication signals in insects. In contrast, in parasitoid flies, auditory signal preferences with respect to sound frequency and temporal patterns have to be due to natural selection. The carrier frequency of the communication signal alone does not determine host selection (Ramsauer and Robert 2000). The processing of temporal patterns appears to be less sharply tuned as in the host species, but demonstrates some adaptation to the signal structure of the host.

In central North America (Michigan) the Sarcophagid *E. auditrix* specifically parasitises the cicada *O. rimos*a. For phonotactic approaches, *E. auditrix* favours a pulse repetition rate of 80 Hz corresponding to the chirp rate of the cicada's calling song (Köhler and Lakes-Harlan 2001; Schniederkötter and

Lakes-Harlan 2004). In more southern regions (Mississippi) *Emblemasoma sp.* is more attracted to *Tibicen pruinosa*, one of three sympatric cicada species (along with *T. chloromera*, and *Neocicada hieroglyphica*) with a pulse rate of about 1.5 Hz (Farris et al. 2008).

In the Tachinid flies *Homotrixa alleni* and *Therobia lenonidei*, host selection depends on the call structure of the host (Allen 2000; Lehmann and Heller 1998). In populations of the bushcricket *Sciarasaga quadrata* males with shorter chirps (55.4 ms versus. 64.8 ms) and higher chirp rates (112 Chirps/min versus 103 Chirps/min) were lost from the calling population due to parasitisation. In Greece, the closely related species *Poecilimon mariannae* and *Poecilimon veluchianus* generate 5–11 or just a single pulse per chirp, respectively. In experimentally mixed populations of both species *T. leonidei* infested the polysyllabic species about 3.6 times more often. However, such infestation probabilities do not necessarily reveal a preference for a specific song pattern, but rather reflect that songs with a higher pulse rate allow for a higher sampling of the host's location during the fly's approach (Lehmann und Heller 1998).

Across North America the acoustic ecology of parasitoid Ormiini may be more complex as gravid females of *O. ochracea* are attracted to different cricket songs in different regions of the US. This situation was tested by Gray et al. (2007) who exposed geographically separated *Ormia* populations in Florida, Texas, California, and Hawaii to the song models of 4 cricket species (*Gryllus rubens*, *G. texensis*, *G. lineaticeps* and *Teleogryllus oceanicus*) representing the most common host species in the study areas. These species have a carrier frequency in the range of 4–5 kHz in common but produce songs with highly different temporal patterns (Fig. 4.3). *O. ochracea* females exhibited clear local preferences and in Florida were most strongly attracted to *G. rubens* song (Walker 1993), in Texas to the song of *G. texensis*, in California to *G. lineaticeps* and to *T. oceanicus* in Hawaii. The flies' geographically indicate specific responses a host-specific adaptation of auditory processing, which may have been shaped during co-evolution of host and parasite and possible competition between sympatric fly species.

4.2.2 *Impact of Parasitic Flies on Host Communication Systems*

Cade (1975) suggested that predation by parasitic flies will act as a natural selection pressure on male reproductive behaviour. The impact of acoustic parasitoid flies on cricket populations could be closely studied on the Hawaiian archipelago where flies (*O. ochracea*) and the Polynesian field cricket (*T. oceanicus*) were recently introduced to three of the islands (Zuk et al. 2006). On the island of Kauai about 30 % of singing males were found to be infested by the flies. Notably, in the late 1990s, within about 5 years and less than 20 generations a "flat-wing" mutation spread among the cricket population. It left 90 % of the males mute, due to a reduced stridulatory apparatus with a strongly shortened and

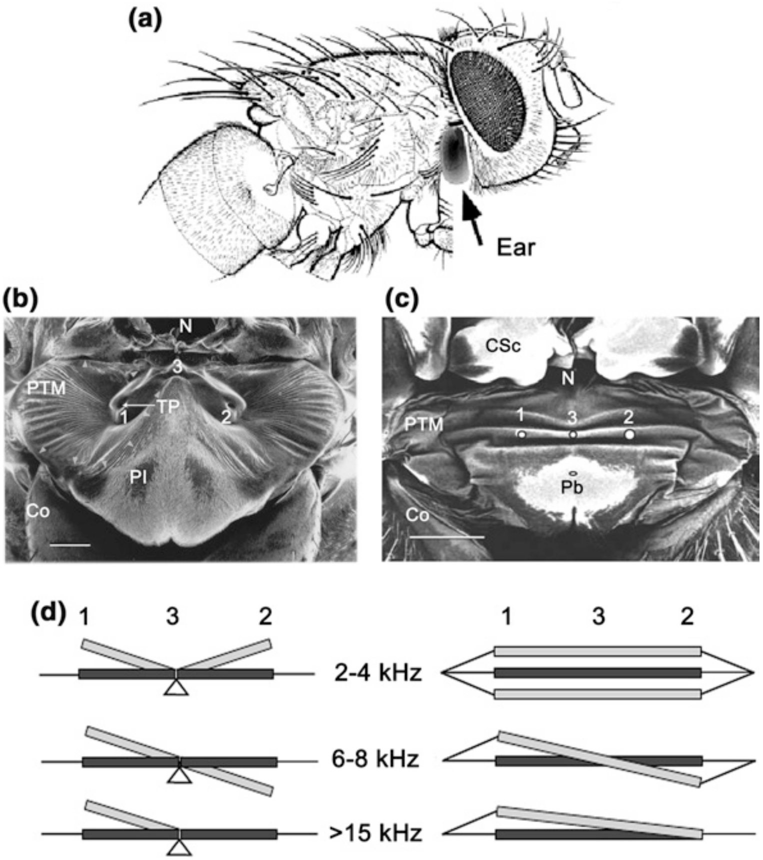

Fig. 4.3 **a** Head and thorax of a generalised Tachinid fly indicating the position of the ear at the prosternum. **b** Scanning electron micrograph of the tympanal ear of *O. ochracea* and **c** the ear of the Sarcophagid fly *Emblemasoma spp* with PTM: prosternal tympanal membrane. TP: tympanal pit of the presternum; PI: prosternal inflation; N: neck insertion; Co: prothoracic coxa and CSc: cervical sclerite; Pb: probasisternum. 1, 2 indicate attachment site of auditory afferents and 1–3 the position of laser vibrometric measurements. **d** Deflection modes of the tympanal system in *Ormia* (left) and *Emblemasoma* (right) measured at positions 1–3. The bars represent the intertympanal bridge in Ormia and the horizontal tympanal fold in Emblemasoma. Tympanic membranes lead to frequency-dependent oscillations of the attachment sites of the sensory cells, with a maximum response at 6–8 kHz. (Scale bar B: 200 µm and C: 500 µm. **a** from Yager (1999) with permission of John Wiley and Sons; **b** from Robert et al. (1994); **c**, **d** from Robert et al. (1999)

relocated file, precluding sound production. Flat-winged males cannot call and become satellite males, in the vicinity of the remaining active singers. Such mute males run a lower risk of direct fly parasitism; their rate of infestation was less than 1 %. Thus, the predation pressure of the flies established and stabilised the presence of a mutation in the cricket population that in normal populations would be detrimental (Zuk et al. 2006; Tinghitella et al. 2009).

4.3 Host Targeting Behaviour

In their search for a suitable host, the flies need to acoustically identify their target, and precisely localise its position. For small animals with a miniature brain, such a sensory task and behaviour constitutes a demanding challenge of auditory processing.

The behavioural characteristics of the phonotactic approach of *O. ochracea* were investigated using stereoscopic video tracking cameras (Müller and Robert 2001, 2002; Fry et al. 2000). Individual female flies were allowed to perform free-flight phonotaxis in a large indoor arena (Fig. 4.2c, d) and their three-dimensional flight path was tracked as they were flying in complete darkness from a starting platform to one of the loudspeakers broadcasting a cricket song from the floor. While the flies approached the sound source the delivery of the acoustic stimulus could be manipulated. The flies' trajectory was not following a straight line between start and finish. Instead they approached the sound source first maintaining a rather constant altitude (Fig. 4.2c, blue loudspeaker), following a gently meandering trajectory. At some point directly above the sound source, the flies lost altitude rapidly, entering a descending spiral that terminated on the loudspeaker. When repeated for 80 landings, the landing accuracy on the landing platform was 8.2 ± 0.6 cm (mean ± SD; N = 10 flies; Müller and Robert 2001).

When interrupting the loudspeaker broadcast at predetermined distances from the sound source this did not disrupt the phonotactic behaviour (Fig. 4.2d). The phonotactic flight was largely conserved exhibiting the typical approach pattern and final spiral dive. This behaviour is remarkable in that it implies that the flies acquired sufficient directional information as to where the loudspeaker was *before* song interruption, which then led the flies close to the sound source in darkness, even in the absence of sound. Furthermore, this result suggests the presence of a form of directional memory or at least sequential processing of acoustic signals, and its retrieval for non-idiothetic navigation.

After landing next to a host *O. ochracea* also show walking phonotaxis (Cade 1975; Walker 1993) whereby orientation and motion take place only while sound is present. The accuracy of walking phonotaxis has been tested on a treadmill and shown to be remarkably accurate, as flies localise sound sources to within 2° azimuth; revealing their acute sense of directional hearing (Mason et al. 2001).

These results highlight potential limitations of the cricket's song interruption strategy for the avoidance of acoustic parasitism. From the cricket's perspective the question arises as to what sensory capacity may be involved to warn of an approaching fly. Using the cercal system, field crickets are deemed to be capable of detecting the sounds of approaching flying or walking predators. Sensitive to the particle velocity component of a sound wave, as well as bulk motion of air (Gnatzy and Heusslein 1986; Dangles et al. 2006) the cercal system of the cricket functions as an alarm, which can trigger the interruption of singing (Dambach and Rausche 1985) and may avoid parasitism (Müller and Robert 2002).

4.4 The Ears of Parasitoid Flies

The auditory apparatus of all parasitoid flies are located on the ventral prothorax, they are characterised by a modified inflated prosternum (Fig. 4.3a; Shewell, 1987) and represent an example of convergent evolution within the Diptera (Lakes-Harlan et al. 1999; Robert et al. 1999).

4.4.1 Auditory Morphology and Comparison Between Species

In the Tachinid flies *T. leonidei* and *O. ochracea* both sexes have a balloon-like inflated prosternum filled with an air sac, however, the ears are sexually dimorphic, with the female ears larger than the male's. The ears of Ormiine flies consist of a bladder-like prosternum between the fore coxae and the head capsule (Fig. 4.3b; Robert et al. 1992, 1994). In at least two species, namely *O. ochracea* and *T. leonidei*, best auditory sensitivity is similar—but not identical—to the peak of the frequency spectrum of the host song (Lakes-Harlan and Heller 1992; Robert et al. 1992). The sensory organs proper each are enveloped in tracheal tissue and connect the sternal apophysis with the cervical membrane. The sensory organ consists of about 200 scolopidial sensory cells. The axons of the receptor cells run within a short nerve and enter the thoracic-abdominal ganglion where they form arborisations in all three thoracic neuromeres.

Hearing sarcophagids of the genus *Emblemasoma* (Robert et al. 1999, Farris et al. 2008) are parasitoids of cicadas. The bilateral tympanal membranes of the auditory organ present a series of folds spanning from one side of the prosternum to the other (Fig. 4.3c). In the sarcophagid *E. auditrix*, the bilateral tympanal membranes are median separated by sclerotised cuticular structures. The tympanal membranes extend laterally towards the coxae. Like in Ormiine flies, a single air sac fills the space behind the tympanal membranes. On each side one scolopidial organ is attached to the presternum of one tympanal membrane and the corresponding prosternal apodeme. The sense organ is located close to the apodeme and contains about 30 scolopidia, each with a sensory cell, a scolopale cell and accessory attachments cells. From the auditory organ, the auditory nerve runs caudally and the axons of the auditory afferents project into thoracico-abdominal ganglion complex. The prosternal tympanal membranes of *E. auditrix* exhibit mechanical oscillations when exposed to sound stimuli in the range of 3–30 kHz, a possible indication of its auditory sensitivity in that frequency range.

In the non-hearing atympanate Sarcophagids (e.g. *Sarcophaga bullata, Phormia regina*) a prosternal chordotonal organ in the ventral prothorax is deemed to represent the evolutionary precursor of the hearing organs (Edgecomb et al. 1995; Lakes-Harlan et al. 1999, 2007; Stölting et al. 2007). This chordotonal organ contains about 35–55 mechanosensitive afferents, which in *S. bullata* respond in a phasic-tonic way to substrate vibrations in the range of 0.2–4 kHz (Stölting et al.

2007) transmitted via the legs. It may be regarded as a pre-adaptive structure from which hearing organs independently evolved in Sarcophagids and Tachinids. In such scenario, modifications of the peripheral cuticular structures of the prosternum resulted in the sensitivity of the prosternal mechanosensory organ to shift from leg-vibrations to vibrations of the prosternal membranes. As they would evolve to be thinner, these membranes could become mechanically sensitive to sound waves, as tympanal membranes are.

Interestingly, the prosternal membranes of Tsetse files are as thin as tympanal membranes but do show some mechanical sensitivity to sound (Tuck et al. 2009). As such the prosternal region of *Glossina* might have been suggested to act as a hearing organ, as it shows some of the characteristics required. Altogether, these results may be used to hypothesise that, following this plausible evolutionary scenario, some parasitoid (or parasitic) flies employ the plesiomorphic prosternal chordotonal organ to locate their hosts using substrate vibrations.

4.4.2 Biomechanics of the Dipteran Tympanal Ears

The tympanal ears of parasitoid flies are in the majority of species less than 1 mm wide and from physical acoustics meet some limitations for processing incoming sound waves. The possible functional issues related to reduce mechanical sensitivity due to the small surface area finite thinness of tympanal membranes, mechanical stability of the entire apparatus if situated on or near moving parts, and as expected, problems with the directional detection of incident sounds. The problem of directional hearing using microscale hearing organs has been given some attention, in particular in the fly *O. ochracea* (Robert and Göpfert 2002; Robert 2005). Because the ears are set so close together on the prothorax of the flies, the acoustic information they can derive is vanishingly small, but not negligible. In Ormia, conforming to conventional acoustic cues used for directional hearing, the maximal amplitude and time differences experienced by each tympanal membrane amount to a fraction of 1 dB in amplitude and 1.45–2 μs (Robert et al. 1996), respectively—this is the best-case scenario. The time differences become even smaller when the sound source is situated near the front of the animal, yielding interaural time differences in the submicrosecond range (Robert 2001; Mason et al. 2001).

Directional hearing in tachinid flies is made possible by the capture of acoustic energy with tympanal membranes that are mechanically coupled (Robert et al. 1996; Robert 2005, 2008). Mechanical coupling is responsible for the generation of temporal and amplitude mechanical cues large enough for the directional processing of information by mechanosensory neurones in each of the auditory organs (Robert et al. 1996; Mason et al. 2001). The anatomical structure responsible for intertympanal coupling is the presternum, a sclerite that is present in most brachyceran Diptera but has developed only a particular morphology and functionality in Tachinid flies. The presternum constitutes a flexible mechanical link between the two tympanal membranes (Fig. 4.3b, c). It is also the point of attachment of the mechanosensory organs, thus presternal vibrations will determine the input to the sensory organ (Robert et al. 1992, 1996). Biomechanical studies have shown that the hemilateral branches of the presternal, the cuticle linking the tympanal pits (TP, Fig. 4.3b, c) act like a flexible see-saw, rocking back and forth in the sound field (Robert et al. 1996). At frequencies at and slightly above that of the song of the cricket host, the presternum acts as a somewhat floppy cuticular beam, resulting in a rocking motion whereby both ends of the beam do not vibrate at the same time and magnitude: The side nearer the sound source oscillates with a slight phase lead, and larger amplitude (Fig. 4.3d).

In Sarcophagid flies, intertympanal coupling has been shown to also be the basis of directional sensitivity (Robert et al. 1999). Several anatomical differences have been identified in the organisation of the tympanal membranes (Fig. 4.3). Although structurally different, the physical linkage between the two tympanal membranes was shown to generate the coupling necessary for directional hearing. The main difference is that a transversal fold on the tympanal membranes produces the stiffness necessary to the mechanical coupling, notably obviating the need for a central fulcrum point (Robert et al. 1999).

The functional properties of fly ears have prompted the development of miniature microphones, which use the physical principles discovered in flies to generate unprecedented directional sensitivity (Miles and Hoy 2006; Miles et al. 2009).

4.5 Organisation of the Auditory Pathway and Auditory Processing

The overall organisation of the central auditory pathway in flies resembles that in other insects (Stumpner and Helversen 2001; Hedwig and Pollack 2008). In all auditory flies hearing is based on scolopidial sensilla, the axons of the mechanosensitive neurons, which constitute the auditory afferents, leave the hearing organ to enter the thoracic-abdominal through a prothoracic nerve (Fig. 4.4a–c) and project to all three thoracic auditory neuropils; ascending interneurons forward auditory activity towards the brain.

4.5.1 Auditory Afferents

In Tachinid flies the total number of auditory afferents is 90–100 for *O. ochracea* (Oshinsky and Hoy 2002), about 250 for *T. lenonidei* (Lakes-Harlan et al. 2007), and in *H. alleni* there are about 200 with two groups of scolopidia considering their position and diameter (Fig. 4.5b; Stumpner et al. 2007). The number of sensory neurons is in the range of 30 for the Sarcophagid *E. auditrix* (Lakes-Harlan et al. 1999).

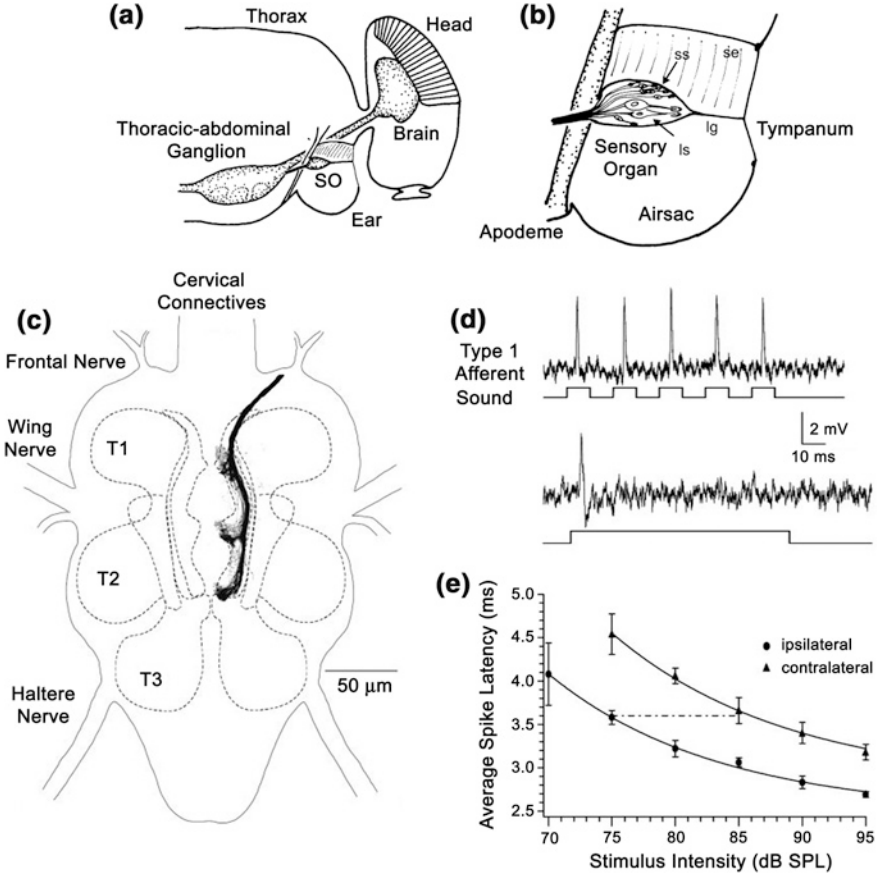

Fig. 4.4 **a** Schematic anatomy of the tympanal organ of the tachinid *H. alleni* with the location of the prosternal ear in relation to the brain and the thoracic-abdominal ganglion. SO: auditory sensory organ. **b** Details of the ear showing large (ls) and small (ss) types of scolopidia; se: septum; lg ligament. **c** The central projections of the auditory nerve in *O. ochracea* end in the ventral regions of the three thoracic neuromeres. **d** Response of a type 1 auditory afferent to synthetic cricket song with 50 pulses per second at 5 kHz, 85 dB SPL (top) and to a 100 ms pulse at 7 kHz/80 dB SPL (bottom). **e** Direction-dependent response of type 1 afferents. A latency difference occurs for identical sound intensities presented from either side of the animal. The dashed line indicates that the shift in latency due to ipsilateral versus contralateral stimulation corresponds to a 10 dB difference in stimulus intensity. **a**, **b** from Stumpner et al. (2007, Fig 4.1)-with kind permission of Springer Science+Business Media, **c**–**e** from Oshinsky and Hoy (2002) with permission of Journal of Neuroscience

The axons of the auditory afferents project from the auditory organ into the fused thoracic-abdominal ganglion complex forming a well-defined track (Figs. 4.4c, 4.5a). Axons arborise ipsilateral in all three thoracic neuromeres in the medial Ventral Association Center (mVAC). In insects in general, this is the neuropil area which receives a variety of mechanosensory inputs and which is also the projection target for auditory afferents (Stumpner and Helversen 2001; Hedwig and Pollack

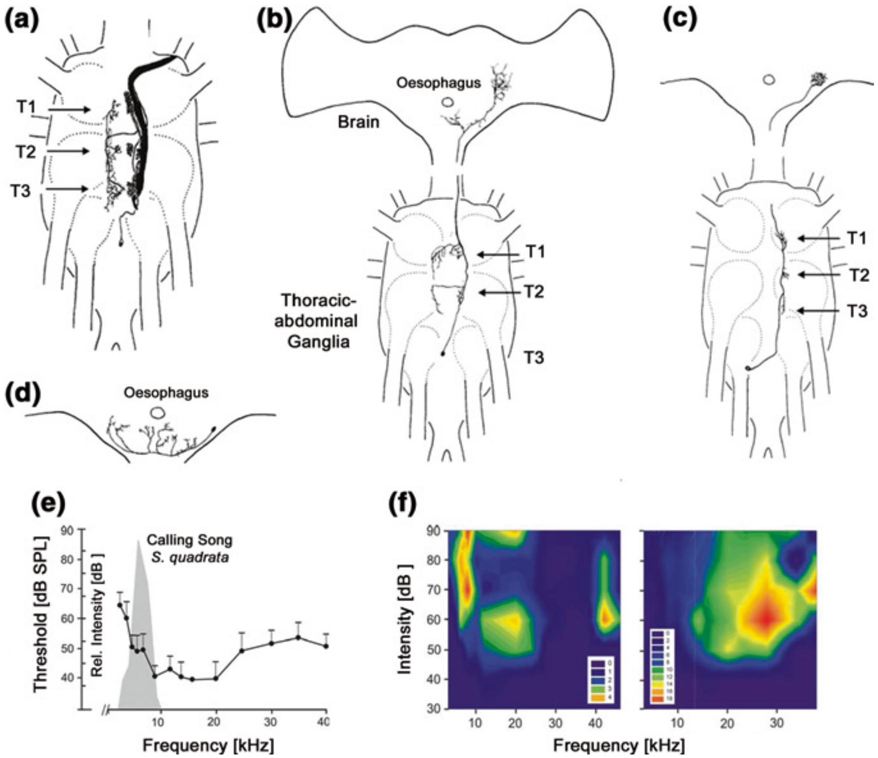

Fig. 4.5 Auditory pathway in *H. alleni*. **a** Projection pattern of auditory afferents in the thoracic neuromeres T1–T3 *right* and structure of a local bilateral auditory interneuron *left* stained via dye coupling from the sensory fibres. **b**, **c** Morphology of two ascending auditory interneurons projecting to the brain; arrows point to dendritic arborisations in the thoracic neuromeres; axonal structures are in the deutocerebrum. **d** A local bilateral auditory interneuron in the suboesophageal ganglion. **e** Frequency tuning of the ascending auditory pathway as recorded from the neck connectives plotted against the power spectrum of the host *S. quadrata left*. **f** Intensity-frequency plots, demonstrating the response properties of a low frequency interneuron and a broadly tuned interneuron. Red colours indicate high spike activity; note different scaling for both neurons. **a**–**f** modified from Stumpner et al. (2007, Figs. 4.3, Fig. 4.4b, Fig.7)-with kind permission of Springer Science+Business Media

2008). No afferents have been observed to project directly to the brain or into the abdominal neuromeres (Oshinsky and Hoy 2002; Lakes-Harlan et al. 1999).

The response properties of auditory afferents in *Ormia* appear to be adapted to processing of directional and patterned acoustic signals (Oshinsky and Hoy 2002). Although axon diameters in *Ormia* are less than 2 μm single cell recordings identified 4 types of afferents, two of which exhibit highly phasic response properties, generating only 1 or very few spikes in response to 5 kHz (80 dB SPL) sound pulses (Fig. 4.4d). Spikes as recorded in the thoracic-abdominal ganglion occur with a mean latency of 2.8 ms and moreover with an extremely low jitter of only 76 μs allowing a precise time coding of stimulus onsets, as required for acute directional processing. Upon stimulation from 90 deg ipsilateral or contralateral Type 1 afferents exhibit an intensity-dependent difference in response latency of about 0.5–1 ms (Fig. 4.4e). The shift in the latency response curves for ipsilateral and contralateral stimulation equals to a difference in sound intensity of about 10 dB, matching a corresponding difference of tympanal membrane vibrations (Robert et al. 1996).

At the population level threshold intensities spread over a wide range from 50 to 93 dB SPL with a nearly linear increase in the number of activated afferents between 68 and 88 dB SPL. Bilateral differences in the amplitude of tympanic membrane vibrations (Robert et al.1996) will therefore recruit different number of auditory afferents on both sides of the animal, supporting directional coding by means of latency differences and intensity-dependent afferent recruitment. This effect was previously reported for Orthopteran species (Römer et al. 1998), whereby latency differences may further be processed and enhanced by local auditory interneurons in the central nervous system. Coupled to the low jitter of the afferent responses is an extremely long refractory period of about 4.3 ms (Oshinsky and Hoy 2002). Thus, these auditory afferents are well suited to respond in a timely manner to the patterned structure of crickets' calling songs.

4.5.2 Structure and Response Properties of Auditory Interneurons

There is gradually increasing information on the neural basis of central auditory processing. Morphology and response properties of auditory interneurons have been analysed in the Tachinid flies *T. lenonidei* and *H. alleni* (Stumpner and Lakes-Harlan 1996; Stumpner et al. 2007). In the thoracic ganglia, dendritic arborisations of local interneurons overlap and intermingle within the axonal projections of the afferents in all three neuromeres. A bilateral arborisation pattern of some local interneurons may contribute to directional processing. At least some of the local interneurons appear to be electrically coupled to the auditory afferents, as dye—coupling during backfills of the auditory nerve also revealed the structure of the local interneurons (Fig. 4.5a–d).

Some interneurons of the ascending auditory pathway have been identified; these have a cell body in the pro, meta, or abdominal neuromere. Their dendrites overlap to different degrees with one or both sides of the auditory neuropils in the mVAC of the thoracic neuromeres. Axons of the interneurons project—in most cases contralaterally to the cell body—towards the suboesophageal ganglion and the brain. Axonal arborisations spread out in the suboesophageal ganglion (*Homotrixa*) and typically in the lateral deutocerebrum. Whereas some interneurons have wide-field projections others form a dense glomerulus-like axonal projection pattern in the brain (Fig. 4.5b, c). Overall interneurons cover a similar neuropil area in the deutocerebrum, pointing towards a specific dedicated area for auditory processing within the brain. At least one bilaterally projecting auditory neuron has been characterised in the suboesophageal ganglion; no local auditory brain neurons have been identified to date. The structure of fly auditory interneurons is highly similar in *Homotrixa* and *Therobia*, pointing towards homologous neurons involved in auditory processing in both species (Stumpner et al. 2007).

In *T. leonidei,* the ascending auditory pathway is broadly tuned to 16–40 kHz and matches the power spectrum of the bushcricket host's calling song (Stumpner and Lakes—Harlan 1996). Female thresholds were about 45 dB SPL whereas male flies were 10–20 dB less sensitive. As all interneurons in *T. leonidei* had similar tuning curves, there is no evidence for frequency discrimination.

In *H. alleni,* hearing sensitivity is similar in female and male flies. Ascending interneurons revealed an overall broad tuning (Fig. 4.5f) with a 50 dB threshold occurring between 5 and at least 40 kHz, and the lowest threshold of 40 dB SPL in the range of 10–20 kHz (Stumpner et al. 2007). Tuning of the ascending interneurons demonstrates no specific adaptation to the carrier frequency of the main host *S. quadrata*, which is at 5 kHz (Römer and Bailey 1998). However, at some interneurons give a strong response at 5 kHz when stimulated at high sound intensities (80–90 dB SPL). Interneurons differ in their frequency tuning and intensity response functions (Fig. 4.5f) indicating the possibility for frequency discrimination as a basis for frequency dependent host discrimination as *H. alleni* can also parasitize bushcricket species like *Mygalopsis pauperculus* and *Pachysaga croceopteryx* (Barraclough and Allen 1996).

In *T. leonidei* temporal processing in ascending interneurons revealed a variety of tonic, phasic and phasic-tonic response properties (Stumpner and Lakes-Harlan 1996). Shortest response latencies were only 5–10 ms and phasic spike rates as high as 500–900 AP/s. Temporal responses depended on frequency and intensity of the stimuli. As *Therobia* parasitises a range of bushcrickets with pulse rates ranging from 0.3 to 10 Hz local fly populations may be adapted to different song patterns of their hosts.

4.5.3 Sensory-Motor Integration

The neural networks for host identification and the descending networks controlling the flies' directional phonotactic approach are still unknown. It seems, however, that the neural mechanisms underlying host selection allow for an evolutionary rapid adaptation as different populations of the same species of flies may select hosts with very different acoustic signals. Earlier stages in auditory processing, including the mechanical response of the coupled ears, seem to be conducive to such flexibility. In effect, the tympanal response to sound can be directional across a large range of frequencies and easily respond to short sound transients. This part of the mechanical response therefore is not a key constraint for the specificity of host finding. Rather, higher level signal processing dealing with temporal species-specific signatures, seem to play a role.

4.6 Conclusion

Parasitoid flies show remarkable specific adaptations in their auditory host-seeking sensory equipment yet also employ opportunistic adaptation to diversify their access to their orthopteran hosts. Further studies in the physiological compatibility of parasitoid flies to their hosts and close relatives could guide further research on the process of co-evolution between singing insects and their acoustic parasitoids. To date in the context of a parasitoid life history the use of hearing has not been reported for insects other than Sarcophagid and Tachinid dipterans. Considering a broader parasitic life history, the haematophagous culicid dipteran *Uranoteania* and *Corethrella* are suggested to use their antennal hearing organ to detect and locate its frog hosts (Borkent and Belton 2006; Bernal et al. 2006).

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