Chapter 26

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Abstract

Mosquitoes generate sounds by flapping their wings in flight, which are thought to have a role in acoustic communication. Furthermore, the auditory organs of mosquitoes are the most sensitive among all arthropods reported so far. However, the function of hearing in mosquitoes is still unclear, and various debates have been raised in the scientific community. This book chapter reviews current knowledge about mosquito hearing, and is directed to the mosquito ecology community. First, we review acoustical aspects of hearing which need to be taken into account to understand the capabilities of insect sensory systems across a range of distances (near-field/far-field, active/reactive field and air particle velocity/pressure). Second, the basic mechanism of antennal hearing is explained in terms of sound-level and frequency sensitivities, interactions with the Johnston's organ and spatial hearing. Third, we review a range of theories behind the role of the acoustic interactions between male and female mosquitoes (harmonic convergence, rapid frequency modulation and species-specificity), and discuss some of the main interpretations of these behaviours. Finally, we discuss the hearing range of mosquitoes in relation to communication and sound traps.

Keywords: distortion products, harmonic convergence, mating behaviour, rapid frequency modulation, sound trap

26.1 Introduction

This book chapter is aimed mainly at the mosquito research community, and its goal is to synthesise the knowledge of the role of hearing in mosquitoes. In particular, we review key historic findings, and summarise the last two decades of research, observations and interpretations regarding the acoustic interactions within mating pairs and long-range hearing. We aim to provide enough of the basic science necessary to understand current knowledge of hearing and acoustical ecology in mosquitoes, and to catalyse new approaches to the control of diseases vectored by mosquitoes through behaviour-informed techniques and knowledge of the auditory sensory system of mosquitoes, highlighting the strengths and limitations of these approaches. Although most studies have focused on mosquito species of medical importance, we also provide examples of hearing in non-vector species.

We briefly summarise aspects of hearing that have already been reviewed elsewhere and invite the reader to refer to the following review papers concerning the physiology of hearing organs (Göpfert

and Henning, 2016), the development of sound traps and the attraction of mosquitoes to sounds (Belton, 1994) and spatial hearing (Robert, 2005; Romer, 2020). To introduce the chapters about mechanosensation, we provide a brief overview of the essential aspects of sound in relation to insect behaviour in Section 26.1.1; how sound is produced and detected by insects, how it travels through different media and how distance to the sound source plays a role in insect sensory systems.

26.1.1 Sound communication in insects

Sound production

Most insects produce sound through stridulation by rubbing one part of their body against another, such as rubbing two wings together (e.g. crickets; Rössler *et al.,* 2006), a wing against a leg (e.g. grasshoppers; Elsner, 1974), the proboscis tip against a leg (e.g. kissing bugs *Triatomine* (Manrique and Schilman, 2022), or even genitals against their abdomen (e.g. hawkmoths; Gwynne and Edwards, 1986, or water boatman; Sueur *et al.,* 2011). Insects can also use other modalities, such as drumming with their head (e.g. woodworms; Goulson *et al.,* 1994), or beating their wings (e.g. mosquitoes; Roth 1948, *Drosophila*; Bennet-Clark and Ewing, 1967, and whitefly; Kanmiya, 1996). While most of the sound production organs are also used for other functions (e.g. wings to fly), some insects have organs that are exclusively used for producing sound, e.g. the tymbals of cicadas (Boulard, 2005), and the stridulatory organs of *Triatomines* (Manrique and Schilman, 2022). According to the type of excitation, the resulting sounds range from pitch-like to broadband sounds, and a wide variety of temporal patterns.

Sound propagation

Sound is a wave that travels with step-by-step mechanical displacements of particles within a medium with elastic properties, such as air, water or soil. Solid-born sounds are often defined as 'substrate vibration' instead of sound, but they are similar mechanical phenomena in a different medium. A sound wave can travel from one to another medium, but it usually loses considerable energy at the interface between media.

Sound detection

Hearing can be broadly defined as 'the detection of a mechanical wave'. Airborne audition is present in 9 out of 30 insect orders, and most are phylogenetically independent (Göpfert and Henning, 2016). Insects can detect sounds either through tympanal membranes or antennae. Insect tympanal ears work similarly to those in vertebrate ears; a drum-like structure picks up sound pressure (i.e. the force exerted by air particles on a surface), which is transduced by similar mechanoreceptive sensilla. In contrast, antennal hearing is based on the antennal flagellum picking up the air particle-velocity component of sound through the viscous force applied by air particle friction to free-standing antennal hairs (Stokes, 1851; Fletcher, 1978). Both tympanal and antennal ears act as an interface between the air and the body of the insect. The hearing organ vibrates with the passage of a sound wave to transform the sound wave into an electrical signal (in the nervous system), which is then transmitted to the brain. Most insect transducing auditory organs, such

as the second segment of the antenna for the Johnston's organ (JO) (Johnston, 1855), are derived from stretch receptors (chordotonal organs), which can be localised in different parts of the body (Nadrowski *et al.,* 2011). When sound is picked up by an antenna, the vibration is transmitted to its base, where it compresses or stretches cells located in the JO (Boo and Richards, 1975a). The JO is present in a wide range of insects, including crickets, sand flies, mantids, midges, bees, fruit flies and mosquitoes (Göpfert and Henning, 2016).

Hearing and distance to the source

A sound-source produces two acoustic fields: a reactive field, which is associated with the local motion of the medium (i.e. the 'near-field' component) and an active field, which propagates over a greater distance (the 'far-field' component). These two fields are superimposed close to the sound source, but after a certain distance, only the active field is present. While the air pressure of a sound source usually shows an active field only, air particle velocity shows both a reactive field and an active field. This implies that the particle velocity level decreases much faster than the pressure level when moving further away from the sound source. At long-range, particle velocity and pressure attenuate at the same rate over distance (Bennet-Clark, 1998). External structures, frequently built by the insects themselves, serve to amplify the sound produced to make it travel further, such as the baffle of the tree cricket, *Oecanthidae*, on a plant leaf (Prozesky-Schule *et al.,* 1975) or the tunnel in the soil built for this purpose by the mole cricket, *Gryllotalpa* (Bennet-Clark, 1970).

26.1.2 Specificity of mosquito sound communication

The characteristic whining sound of mosquitoes, generated by the periodic beating of their two wings, is not only a by-product of their flight; female flight tones carry information, which is exploited by males to detect and locate the female. In this sense, the flight tone of the female is under natural selection pressure, and can be described as an acoustic communication signal (Laidre and Johnstone, 2013) between mosquitoes. Indeed, mosquitoes are unusual in using a pair of wings for two purposes at the same time; to fly and to communicate with each other, simultaneously. On the contrary, the fruit fly *Drosophila* communicate with each other by beating their wings, but only when they are not flying (Tauber and Eberl, 2003). 'Pound-for-pound' (i.e. per unit of weight), the sound of a mosquito wingbeat is ~3 times more intense than that of *Drosophila*, and a mosquito is more efficient by a factor of ~4 in converting mechanical power into acoustic power (Seo *et al.,* 2021), suggesting the potential importance of acoustic communication.

Mosquitoes have the most sensitive hearing organs of all arthropods reported so far (Göpfert *et al.,* 1999), and have been observed to respond to sound for a long time. Knab (1906) refers to Osten-Sacken, who wrote in 1861 that whenever the note 'A' was produced by a band, the swarm of mosquitoes hovering over his head suddenly flew downward toward his face! The mosquito hearing organ was first described by and named after Christopher Johnston (Johnston, 1855). The Johnston's organ (JO) acts as a mechano-electrical transducer, located at the base of the antennae (Figure 26.1), and houses ~15,000 sensory cells in males (Boo and Richards, 1975a), comparable to that in the human cochlea (Robles and Ruggero, 2001), versus approximately half as many in females (Boo and Richards, 1975b).
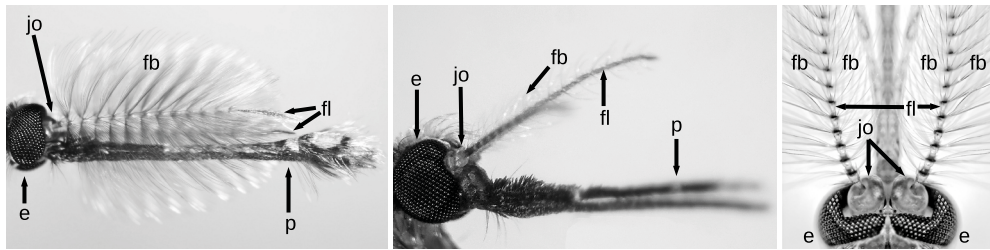
Figure 26.1. *Anopheles gambiae* male (left) and female (middle), *Culex quinquefasciatus* male (right) ©Gareth Jones. e: eyes, jo: Johnston's organ, fb: fibrillae, fl: flagellum, p: palp and proboscis. The antenna is composed of the Johnston's organ, the flagellum and the fibrillae. Note the extreme difference between the male and female antennae (not to scale).

The frequency of wing-flapping sound matches the frequency of the wingbeats (WBF), which can vary between 200-1000 times per second (Hz), depending on a range of factors, including sex (in most species females produce a lower pitch than that of males), species (Mukundarajan *et al.,* 2017) (Figure 26.2), behaviour (Pantoja-Sanchez *et al.,* 2019a), temperature (Villarreal *et al.,* 2017) and age (Costello 1974). The effect of body size on wing beat frequency is not clear; reports have varied over a range of species (Cator *et al.,* 2010; Montoya *et al.,* 2021; Pantoja-Sanchez *et al.,* 2019a; Villarreal *et al.,* 2017).

Male mosquitoes use the female flight tones to detect and locate their conspecifics in order to mate. Both sexes are sensitive to sound via the movement of their flagellum, excited by the vibration of free-standing hairs called fibrillae. The flagellum is set in the base of the JO and moves in response to sound like a joystick (Figure 26.1); males generally have more and longer fibrillae than
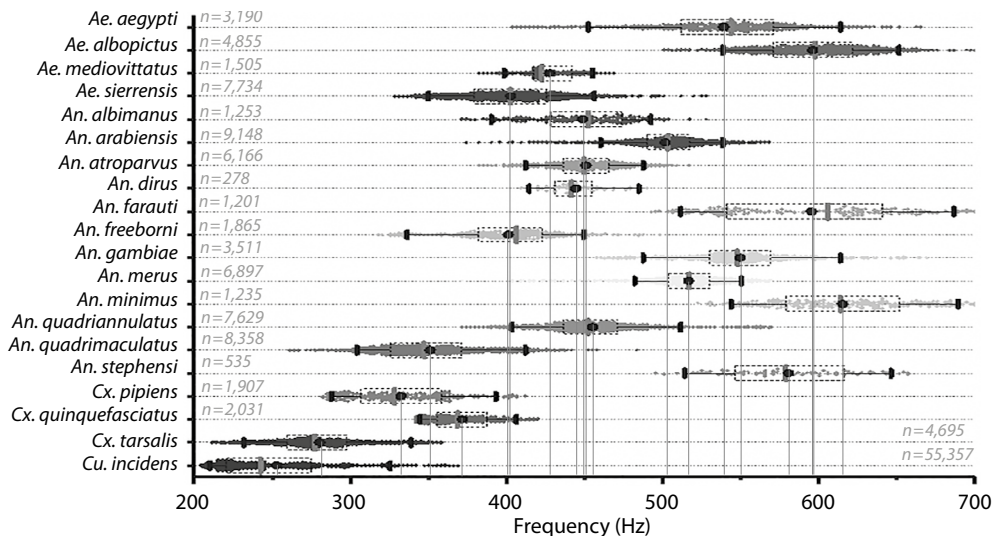

Figure 26.2. Distribution of wingbeat frequencies for female mosquitoes of 20 vector species (modified from Mukundarajan *et al.,* 2017).

females (Clements, 1999). Most studies of flight behaviour between pairs of mosquitoes have been investigated with 'tethered' mosquitoes (attached by a fine wire or hair, which enables them to flap their wings, but they are unable to control flight manoeuvres) or artificial sound sources, and therefore, little is known about the auditory interactions between males and females in free flight.

Phonotaxis (i.e. movement towards a sound source) has been found only in males. However, females of some species have been shown to respond behaviourally to the sound of males by changing their flight speed in free flight when stimulated with unnaturally high sound levels (Dou *et al.,* 2021), or by changing their WBF (Aldersley *et al.,* 2016; Montoya *et al.,* 2021; Pantoja-Sánchez *et al.,* 2019a; Warren *et al.,* 2009). Mosquito phonotactic behaviour outside a mating context has also been described for female mosquitoes, including frog-biting female mosquito *Culex territans* which are attracted to amphibian vocalisations (Bartlett-Healy *et al.,* 2008), and female *Culex tritaeniorhynchus* and *Mansonia uniformis* which are attracted to sound-baited traps (Ikeshoji, 1986; Leemingsawat *et al.,* 1988; Ogawa, 1988). Despite a smaller number of fibrillae and a lesser sensitivity to sound than males (but see Lapshin, 2013), the antennal sensitivity of females in electrophysiological studies still surpasses that of other arthropods (Göpfert and Robert, 2000).

Until 2006, the limited view of audition in mosquito behaviour was that the antennae of males are directly frequency-tuned to hear the WBF of conspecific females, in order to detect and locate them, culminating with the formation of a mating copula. Since then, researchers found that acoustic interactions occur within conspecific male-female pairs of mosquitoes (Gibson and Russell, 2006), revealing a unique mechanism of hearing, whereby a flying mosquito must 'speak' (i.e. beat its wings) to be able to hear at the same time (Warren *et al.,* 2009). The auditory mechanism involves the production of antennal 'difference tones', which enable both sexes to detect the presence of each other.

In the following section, we describe this unique hearing mechanism of mosquitoes in terms of sound-intensity sensitivity, frequency-bandwidth sensitivity, antennal distortion products and spatial hearing. In the third section, we present a range of acoustic interactions found at short range between mosquitoes, and discuss the interpretations of these findings. In the last section, we raise the question of long-range hearing under ecological conditions, and whether this could become the basis for sound traps.

26.2 Sensitivity and mechanism of mosquito antennal hearing

De Riville (1760) made what was probably one of the earliest recorded descriptions of the mating behaviour of mosquitoes. In the second half of the 19th century, many theories arose about insect hearing, without much proof (reviewed in Roth, 1948). Among them, Johnston hypothesised 'that the male should be endowed with superior acuteness of the sense of hearing appears from the fact that he must seek the female for sexual union either in the dim twilight or the dark night where nothing but her sharp humming noise can serve him as a guide' (Johnston, 1855). Forty years later, Child (1894) studied the JO (Figure 26.1) in detail and humbly named it after Johnston. Roth (1948) reported that removing the antennae of *Aedes aegypti* removes the response in male

mosquitoes to a pure tone of a tuning fork, however, the removal of the antennae is likely to have disturbed not only the hearing sensory system, but also other sensory modalities associated with flight control.

26.2.1 Sound-level sensitivity

Ecological relevance of sound levels

It was not accepted until the second part of the 20th century that mosquitoes hear air particle-velocity and not air pressure (Stokes, 1851; Fletcher, 1978). Unfortunately, particle-velocity microphones have not been developed as of yet, however it is possible to estimate particle velocity using a pressure gradient microphone (e.g. Knowles NR23158 Knowles Electronics, Itaska, IL, USA), or by using a pressure microphone at a sufficient distance from the sound source to assume equivalence between sound pressure level and particle-velocity level (Section 26.1.1). Hearing thresholds in some papers should be taken with caution (e.g. Belton, 1961; Tischner, 1953; Dou *et al.,* 2021), in which pressure levels are used to monitor the sound level that mosquitoes received, while the conditions to do so are not fulfilled (measurements were taken too close to the sound source) and without statistical evidence.

Generally, sound levels in experiments were often far higher than what a mosquito is exposed to under natural conditions, which can artificially increase the frequency band to which mosquitoes respond to under natural conditions. For example, the tuning fork experiment by Mayer (1874), with up to 103 dB sound pressure level, or the study by Roth (1948), which made the whole cage vibrate, including the substrate on which the mosquitoes were resting (108 dB sound pressure level at 6 cm). Dou *et al.* (2021) reported results on the response of male and female *Anopheles gambiae* and *Aedes aegypti* to pure tones using extremely high sound pressure levels (up to 103 dB) at a distance from the speaker where sound particle-velocity level is even higher. However, when considering naturalistic sound levels, free-flying *Anopheles coluzzii* females did not change their WBF (wingbeat frequency), their flight trajectory or their linear and angular velocities (using playback of swarming males) (Feugère *et al.,* 2021).

Hearing thresholds

Those measuring sound sensitivity in mosquitoes, and other flies, face the difficulty of monitoring how much sound energy actually reaches their antennae, because the sound-level meter is placed at a fixed-position, whereas the position of a mosquito is continuously changing during its flight. However, flight trajectories can be tracked, and the sound level of the sound stimulus can be estimated, even though the mosquitoes are at different locations, as in Feugère *et al.* (2021) and Feugère *et al.* (2022). Nonetheless, most of what is known about the auditory physiology of mosquitoes has been investigated with mosquitoes in fixed positions. In addition to measuring behavioural responses to sound levels, we can record the mechanical response of antennae, and the electrical response of the JO. For example, in *Toxorhynchites brevipalpis* male antennae, a ±2.4 nm air-particle displacement leads to a ±0.0001° deflection of the antenna. Göpfert and Robert (2000) claim that this antennal sensitivity surpasses the second most sensitive arthropod, the cricket, for

which the neuronal response threshold stands at 'only' ±0.001° of angular hair deflection. However, this publication does not state details as to how much of the vibration signal was transduced into a neural signal by the mosquito JO. Indeed, Su *et al.* (2018) measured the male threshold (defined as 10% activation of neurons) to be 10-20 nm (or ~0.001°). In terms of sound levels, the electrical response-threshold in male *Culex pipiens pipiens* JOs revealed a mean sensitivity of 32 dB SVL per JO unit (22-44 dB SVL; n=74 JO units, criterion =+2 dB above noise floor) (Lapshin and Vorontsov, 2019) and a mean of 44 dB SVL per mosquito in male *Culex quinquefasciatus* JOs (36-52 dB SVL; n=3 mosquitoes, response criterion: >10 dB above noise floor) (Warren *et al.,* 2009). In *Ae. aegypti,* male JO nerves were shown to respond to a mean of 40 dB SVL (31-50 dB SVL; n=11 males, unknown criteria over signal thresholding) (Menda *et al.,* 2019). Pennetier *et al.* (2010) measured a JO response-threshold in male *An. coluzzii* of only 10 dB SVL (particle velocity of $1.5 \pm 0.6 \times 10^{-7}$ m/s, n=2 males, criterion =1.43 recording noise floor). However, mechanical and electrical responses are not usually compared to each other using statistical tools, which means that the outcome depends on the level of background noise and signal averaging.

However, biomechanical and electrophysiology studies can only measure signals from the auditory mechanical vibration or from the nervous system, and cannot assess behaviour associated with the neural signal. What number of excited neurons are needed to be detected by a mosquito? Measures of detection are not necessarily reliable, given the complexity of defining 'detection,' so the best way to be sure whether or not a mosquito has detected a stimulus is to monitor all possible behavioural changes. In addition, to stack all the odds 'in one's favour', it is best to monitor the behavioural response to sound in a free-flying state of mating behaviour, in the context of natural physiological responses to stimuli.

Thus, Lapshin and Vorontsov (2021) measured the sensitivity of free-flying, swarming male *Aedes communis* to pure sounds in the field, and found a mean of 26 dB SVL threshold (12 °C). Under more controlled conditions, swarming *An. coluzzii* males responded to pure sounds of 26 dB SVL by increasing their WBF and their angular speed (+30 Hz, +1.6 rad/s, n=10 males); the hearing threshold of males, exposed to played-back female sound, was estimated to be at most 20 dB SVL with a 95% confidence interval (n=9 males) (Feugère *et al.,* 2022).

Effects of wingbeat flapping of a mosquito on its own hearing

The intense vibrations generated by their own wings and flight muscles during flight can have a significant effect on the detection of auditory characteristics due to the nonlinear vibrations of the antenna. Lapshin (2012) used 500 Hz sound around electrophysiological preparations to simulate the flight conditions of a male *Cx. pipiens* mosquito. Along with the flight simulation, the mosquitoes were stimulated with pure tones of different frequencies, and the responses of the auditory neurons were recorded. An improvement in sensitivity by 7 dB (2.2-fold) was observed at the main optimum frequency band (220-320 Hz) in males (Lapshin, 2012). Lapshin (2013) also showed that if non-flying female *Cx. pipiens* and *Anopheles excrucians* are stimulated during flight simulations, the acoustic sensitivity of the female is comparable to that of the male (mean response of 45 dB within the frequency range 80-120 Hz in the receptors of JOs). The underlying mechanism is presented in Section 26.2.3.

Fibrilla extension in males

Another factor enhancing sound-level sensitivity in males is the extension of fibrillae. Fibrillae are not fully extended before ~2 days after emergence, which could cause reduced attraction to sound, as measured in *Culex pipiens molestus* (Roth, 1948) and in *Ae. aegypti* (Wishart and Riordan, 1959). However, in male *Cx. p. molestus, Cx. p. pallens*, *Ae. aegypti*, *Aedes albopictus* and *Anopheles stephensi*, attraction to female flight frequencies were reported to increase until 4-5 days old (Ikeshoji, 1985). Many species of mosquito extend and collapse the fibrillae of their antennae in a circadian rhythm associated with the timing of male mating swarm activity, throughout their life cycle (Clements, 1999), such as *An. gambiae s.l.* and *An. stephensi*. The antennae of *An. gambiae s.l.* males are 4-5 times more sensitive when they are fully extended (in terms of particle-velocity) (Pennetier *et al.,* 2010). Females do not have as many fibrillae as males (Figure 26.1); similar measurements from *An. gambiae s.l.* females did not reveal diurnal shifts in the sensitivity of the flagellum, but they are 3.5 times less sensitive to males with extended fibrillae (Pennetier *et al.,* 2010). The daily erection of fibrillae was shown to be under direct nervous control in *An. stephensi* (Nijhout, 1977).

Efferent control of hearing

The involvement of the central nervous system in modulating mechanosensory organs is rarely found among arthropods. In *Cx. quinquefasciatus*, mature males keep their fibrillae extended all the time, and imaging and pharmacological studies showed that their hearing is also modulated by an efferent system. Octopamine, GABA and serotonin were identified as efferent neurotransmitters, or neuromodulators, which affect the auditory frequency tuning, mechanical amplification and sound-evoked potentials (Andrés *et al.,* 2016).

Time variance of natural sound levels

Mosquito flight sound levels induce non-linear auditory mechanisms, which could be used to enhance the female sound level, at particular distances (Jackson and Robert 2006; Lapshin, 2012). The nonlinear response of the male antennae was shown to take effect from an equivalent distance of <2 cm from the conspecific mosquito (sound particle velocity 1.2±0.2 mm/s) (Jackson and Robert, 2006).

Self-sustained oscillation

Active listening (as opposed to passive listening, i.e. in freshly dead or carbon dioxide-sedated animals), has been shown to enhance some frequency bands in mosquitoes (Göpfert and Robert, 2001; Andrés *et al.,* 2016; Su *et al.,* 2018; Figure 26.3). This is widely present in mosquitoes, as it occurs in at least three species (*Cx. quinquefasciatus*, *An. gambiae* and *Ae. aegypti*), which encompasses both the Culicinae and Anophelinae subfamilies. These species inject energy into the vibration of their flagellum ('self-sustained oscillations') when provided with dioxygen, resulting in changes in stiffness between the active and passive states of the antennae, which modifies the gain of the flagellum distortion products. It is thought that these spontaneous oscillations
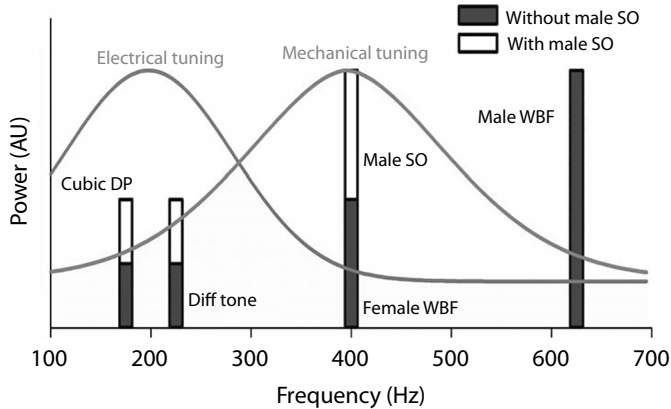
Figure 26.3. Diagrammatic representation of the mechanism of male auditory organs in the presence of the female flight tone. 'Power units' and 'scale of responses' are arbitrary. Solid lines represent Johnson's organ electrical tuning and the mechanical tuning of the flagellum for male mosquitoes. SO = self-sustained oscillation, WBF = wingbeat frequency, Diff tone = quadratic distortion product resulting in 'difference' tone between the male and the female WBFs (f2-f1), Cubic DP = cubic distortion product from the female and male WBFs (2f1-f2), (Su *et al.,* 2018).

match that of the fundamental frequency of the female flight tone, thereby, functioning as a physiological mechanism to help males detect flying females. This results in an increase in the intensity of the difference tones in the mosquito antennae. The largest increase reported is in *An. gambiae* females.

26.2.2 Frequency-range sensitivity

In the 20th century, behavioural experiments (reviewed by Belton, 1994) revealed that male mosquitoes, including *Ae. aegypti* and *Cx. pipiens*, are usually attracted to sources of pure tones with frequencies below 500 Hz, or to multi-harmonic tones provided the fundamental harmonic (≤500 Hz) is retained (Wishart and Riordan, 1959). The interpretation of these early studies was that males are attracted to female fundamental flight tones. Later, these results were enriched by electrophysiology measurements describing the precise frequency response of antennae (Göpfert *et al.,* 1999; Pennetier *et al.,* 2010), as well as more defined behavioural responses (rapid frequency modulation in free-flying mosquitoes, harmonic convergence in tethered mosquitoes; *cf* section 26.3.2 to 26.3.5) (Cator *et al.,* 2009; Gibson and Russell, 2006; Pennetier *et al.,* 2010; Simões *et al.,* 2016, 2017; Warren *et al.,* 2009).

Reports of low frequency sensitivities in behavioural experiments (<100 Hz) (Roth, 1948) were subject to caution due to the presence of speaker artefacts or cage vibrations (Wishart and Riordan, 1959). However, more recent studies report sensitivity of frequencies below 100 Hz in electrophysiology measurements, including increased sensitivity when flight tones were simulated (Warren *et al.,* 2009; Lapshin, 2013). In behavioural assays, male *Ae. diantaeus* were shown to be repelled by 140-200 Hz at 57 dB SVL (Lapshin and Vorontsov, 2018). The latter

suggests it may enable males to discriminate between flights tones of predators and of female conspecifics.

The ability of male mosquitoes to respond to frequencies above the fundamental WBF has been suggested (at least up to 2,000 Hz), based on electrophysiology measurements (Cator *et al.,* 2009), but no significant behavioural changes have been shown in response to the female second harmonic (Cator *et al.,* 2009), or any increase in response to the first two harmonics as compared to the fundamental harmonic (Feugère *et al.,* 2022). Our current understanding is that males use their own wing tone as an amplifier to hear females (Section 26.1.3); male fundamental WBFs are observed to reach up to 1000 Hz for short periods of time in swarms at ~26-28 °C (Feugère *et al.,* 2021; Garcia Castillo *et al.,* 2021; Somers *et al.,* 2022). The ability of females to hear high-pitched frog calls have been reported, however, the sound frequencies tested in experiments do not exceed ~1000 Hz (Toma *et al.,* 2019). There is no evidence that mosquitoes can hear above these frequencies. In particular, commercial 'mosquito repellents' based on ultrasounds (i.e. >20,000 Hz) have frequently been shown to be ineffective (Foster and Lutes, 1985; Jensen *et al.,* 2000; Mankin, 2012; Schreck *et al.,* 1984).

26.2.3 Antennal distortion products

Air particle velocity is picked up by friction between the air particles and the antennal fibrillae, which leads to vibrations of the flagellum, and subsequently excites the neurons of the JO within the second segment of the antennae. The JO transduces the mechanical vibration to an electric signal. In both sexes, the flagella respond over a broad frequency range from ~50 Hz to at least 2,000 Hz with a sensitive peak between 200 to 500 Hz. This has been studied in a number of species using laser interferometry to measure flagella vibrations: *Ae. aegypti* (Göpfert *et al.,* 1999; Su *et al.,* 2018), *Cx. quinquefasciatus* (Warren *et al.,* 2009; Su *et al.,* 2018), *T. brevipalpis* (Gibson and Russell, 2006) and *An. gambiae s.l.* (Pennetier *et al.,* 2010; Su *et al.,* 2018). However, the JO, which transduces the antennal vibrations, has a very narrow bandwidth, which resembles a low pass resonant filter, when measuring the compound extracellular receptor potentials using extracellular electrophysiology. Its frequency response is centred on ~300 Hz in *Cx. quinquefasciatus* and *An. coluzzii* (Warren *et al.,* 2009; Pennetier *et al.,* 2010; Simões *et al.,* 2016) and on ~400 Hz in *T. brevipalpis* (Gibson and Russell, 2006).

Many stages of sensory processing are non-linear. The only non-linearity recorded in the vibrations of the antenna (Penettier *et al.,* 2010), and indeed in the compound receptor potential of the JO (Simões *et al.,* 2016, 2018; Warren *et al.,* 2009), is quadratic distortion, which generates difference tones (f1-f2) when the antenna is excited by two tones, f1 and f2 (Figure 26.3). Cubic distortion has been recorded in the responses of JO auditory neurons, and are believed to come from the JO transduction and not from antenna vibration (Lapshin and Vorontsov, 2017). Distortions are important because these lower frequencies can be detected by the narrow-frequency bandwidth of the JO. For instance, if air particles vibrate at 400 Hz and 630 Hz, the JO will not pick up the 630 Hz because it is above its frequency tuning, however, it will pick up the variation of the 630 Hz frequency via the 630-400=230 Hz distortion, which is the 'difference' tone.

Thus, male JO nerve responses are not tuned to the female WBF but, remarkably, to the difference between the male and female WBFs. This has now been well documented in *Culex, Anopheles* and *Aedes* species (Gibson *et al.,* 2010; Simões *et al.,* 2016, 2018; Somers *et al.,* 2022; Warren *et al.,* 2009). It is this finding that enabled our understanding as to how hearing, and possibly sexual communication, occurs between male and female mosquitoes where there is sexual disparity in size, flight tones and frequency tuning of the hearing organ.

The discovery that the male JO nerve responses are tuned to the male-female 'difference' tone could explain the following:

- The differences between the WBF of the female and the JO tuning frequency in male *Ae. aegypti* (Göpfert *et al.,* 1999), *Cx. quinquefasciatus* (Simões *et al.,* 2016) and *An. gambiae s.l.* (Simões *et al.,* 2017), which are all tuned to the 'difference' tone between the WBF of the male and female, respectively.
- The apparent 'harmonic convergence' of the WBF of the male and female in the higher harmonics that they are 'not supposed to hear' (Simões *et al.,* 2016; Somers *et al.,* 2022).
- How female *Uranotaenia lowii* and *Cx. territans* mosquitoes can hear high-pitch frog calls (Bartlett-Healy *et al.,* 2008; Legett *et al.,* 2021; Toma *et al.,* 2019).
- How swarming males can avoid each other, and why males diverge their WBF in a swarm, possibly to hear each other with the low frequency difference tones (Aldersley *et al.,* 2017; Pantoja-Sanchez *et al.,* 2019a).
- How mosquitoes detect WBF of each individual over a range of temperatures. Indeed, WBF is dependent on temperature (Villarreal *et al.,* 2017), however, hearing difference-tones cancels the effect of temperature when hearing each other, as suggested by Lapshin and Vorontsov (2017).

26.2.4 Spatial hearing

Charlwood and Jones (1979) found that males from the *An. gambiae* species complex orientated towards the sound source with great accuracy; when offered five visually identical potential sources of sound 25 mm apart, they always landed within 10 mm of the actual emitter. The males ignored non-flying (wings glued together) virgin females placed in front of the emitter.

A range of mechanisms can be used to locate the source of a sound (spatial hearing), such as detecting differences in the loudness, or delay of sound, between two ears. For mosquitoes, considering that the distance between the two antennae is 100 times smaller than between two human ears, it seems improbable that they can use inter-aural time difference to locate precisely the sound sources of the audible wingbeat harmonic frequencies. However, physiological adaptations have been shown in a number of smaller animals, flies included, which increase the inter-aural differences by several orders of magnitude by the use of special organs (reviewed in Römer, 2020).

Antennal hearing is sensitive to the direction of sound, in contrast to tympanal ears, because the antennal hairs move along the air particles in parallel with the sound-propagation direction. Accordingly, Roth (1948) hypothesised that mosquitoes could locate a sound source with a single ear. This hypothesis was later rejected by showing that male mosquitoes lose their ability to

locate a sound source if one of their antennae is removed (Charlwood and Jones, 1979; Wishart *et al.,* 1962).

However, Mayer (1874) proposed and tested a mechanism consisting of a combination of triangulation and heterogeneity in the directional hearing of the particle-velocity sensing antennae. He exposed mosquitoes to the sound of a tuning fork, which elicited vigorous vibration of the antennae fibrillae, but only for particular sound directions. Indeed, the fibrillae were absolutely stationary, if they were parallel to the sound direction. Mayer then estimated the 'cone of silence' predicted from his observations experimentally, which was later estimated to be 12-20°, with an average of 15° (Wishart *et al.,* 1962). Belton (1974) made electrophysiological measurements to test this hypothesis, and validated it to be true. However, this direction-specific fibrilla vibration has to result in the same direction-specific flagellum deflection in order to elicit a consistent neuronal response, which was not measured by the above authors. The mechanisms underlying this finding have yet to be investigated. Localising a sound source this way would be possible only if the sound does not come from a broad, non-divergent source, such as a large loudspeaker, even if it is at the correct frequency and intensity, as noted previously by several authors (Belton, 1974; Wishart and Riordan, 1959). Note that the sound source of a mosquito is highly directional (Arthur *et al.,* 2014), which adds a layer of difficulty for males to monitor their distance to a female, because from the male point of view, the sound level of a female changes according to her body orientation.

In terms of physiology, Lapshin and Vorontsov (2019) used a novel form of feedback acoustic stimulation to show that sensory units with directional sensitivity throughout the 360° range of the JO are highly sensitively-tuned (mean threshold of 32±4 dB SVL) to a wide range of different frequencies within the frequency range of the JO. This finding indicates that mosquitoes could be sensitive to different frequencies throughout the direction range of their auditory system. It remains to be discovered, however, if this property of the JO is sufficient to provide the required accuracy for determining the angular coordinates of a sound source.

26.3 Acoustic interactions in mating pairs

When a male and a female are flying close enough to detect each other through sound, the male starts chasing the female. A successful mating interaction involves a high-speed chase, with the female invariably in the lead, resulting in the formation of a mating copula in flight with the two mosquitoes flying in tandem. Studying the detailed stages of mating in flight is not easy in free-flying mosquitoes because of the rapidity of the process; the range of mosquito flight speeds in mating chases varies from 0.5-1 m/s (Manoukis *et al.*, 2014). Hypotheses have been proposed to explain some of the acoustic interactions, which occur during various stages of mating, including optimisation of mate detection, female testing of male fitness, detection of conspecific cues, to avoid wasted effort or being inseminated by a non-conspecific male, easing of flight manoeuvres during in-flight formation of a copula and behavioural artefacts due to laboratory environmental conditions.

26.3.1 Early findings in harmonic convergence in paired tethered mosquitoes

Gibson and Russell (2006) described the sound interactions within male-female pairs of *T. brevipalpis*. The sound of flying pairs of 'male-male', 'female-female' and 'female-male' adults were recorded, with each mosquito tethered within a few centimetres of the other. All combinations of pairs converged their wing-flapping frequencies in unison, but the time taken to reach the first convergence was significantly shorter (on average 5 s less) when the male of the pair started to flap his wings after the female ('female-male' or 'male-male' pairs) than in the 'male-female' and 'female-female' pairs. These authors also observed, without demonstrating it by quantitative statistical analysis, that the beat frequencies of the tethered opposite-sex pairs ended up diverging after initially having converged.

Toxorhynchites brevipalpis mosquitoes are larger than most mosquito species, which facilitates handling and experimentation. This mosquito species is also unusual in that males and females are similar in size, unlike the majority of mosquito species. Thus, the convergence of wing-flapping frequencies between *T. brevipalpis* individuals is relatively natural in view of their morphological similarity. What about mosquito species that are sexually dimorphic in size, with females larger and WBF (wingbeat frequency) lower than that of males?

Cator *et al.* (2009) studied the acoustic interactions between opposite-sex pairs of tethered-flying *Ae. aegypti*. The pairs consisted of a male wing flapping at ~600 Hz and a female wing flapping at ~400 Hz, both attached by the thorax in static flight. Fourteen of the 21 pairs studied adjusted their harmonic convergence (HC) ratio to 2:3, which resulted in the third harmonic of the female coinciding with the second harmonic of the male for an average duration of 10 s. Arthur *et al.* (2014) showed that it was not due to chance. To determine what the male is sensitive to in the sound of the female, modified female-sounds were played to males. If the sound of a female with the first harmonic removed was played over a loudspeaker to a male mosquito, 39% of them converged their second harmonic to the third harmonic of the female. If only the second harmonic was played (1,200 Hz), 22% converged. The authors suggest that since a mosquito can hear these pitches, they must use the higher harmonics to adjust their fundamental frequencies.

26.3.2 Harmonic convergence: fitness testing versus mate-sound amplification?

Warren *et al.* (2009) presented a different interpretation of harmonic convergence (HC). These authors confirmed the observations of HC with tethered mosquitoes of a different species of mosquito, *Cx. quinquefasciatus,* and showed for the first time a significant effect not due to chance. Out of 20 male-female pairs, 15 pairs flew at a 2:3 HC ratio and two pairs at a 1:2 HC ratio. Same-sex pairs also converged but to a beat frequency ratio, which does not correspond to a convergence of harmonics.

While Cator *et al.* (2009) suggested that HC serves as a selection criterion for successful mating, which led to a series of papers on the correlation between HC and mating success (Cator *et al.,* 2010; Cator and Harrington 2011; Aldersley and Cator 2019; Pennetier *et al.,* 2010), Simões *et*

al. (2016) argued that HC is only an epiphenomenon of mosquito auditory functioning. Indeed, Warren *et al.* (2009) showed that HC could be interpreted as a WBF adjustment, so that the frequency difference (distortion product) falls within the bandpass range of the JO. Thus, HC indirectly leads to better hearing of the WBF of the nearby flying mate. In other words, this auditory mechanism would enable the male to detect and amplify a quiet female flight sound by using the difference of the WBF of the female and the male, respectively. Simões *et al.* (2016) confirmed this interpretation by showing that the stimuli frequencies that elicit HC without loud stimuli occurs for an HC ratio between 0.4 and 0.7, which includes the most often observed HC ratios (i.e. 1:2, 2:3 and 3:5) (Figure 26.4).

The second difference in interpretation of HC between Warren *et al.* (2009) and Cator *et al.* (2009) is that the latter measured a response in the JO to sound frequencies up to 2,000 Hz for *Aedes* mosquitoes, but only as a non-periodic response which did not preserve the all-important temporal information; which is present in the waveform of the acoustic stimulus; and which is present in the phasic electrical responses to tones at frequencies below ~500 Hz (Warren *et al.*, 2009). Incidentally, direct-current potentials associated with sensory transduction at the level of the receptors, have only been reported by one other study (Andrés *et al.,* 2016). Warren *et al.* (2009), on the contrary, found that the *Culex* JO responds only to sound up to ~500 Hz for a 10 dB signal to noise ratio (nothing measured >1 kHz). Warren *et al.* (2009) suggested that 2,000-Hz harmonics cannot be used for frequency matching because of the signal type measured by Cator *et al.* (2009): 'As in the cochlea, these sustained potentials do not preserve the phasic information necessary for auditory behaviours, such as sound localisation (Wishart *et al.,* 1962) and, indeed, frequency matching, which depends on precise phase information. Flight-tone frequency matching would be expected to require exactly this information'.
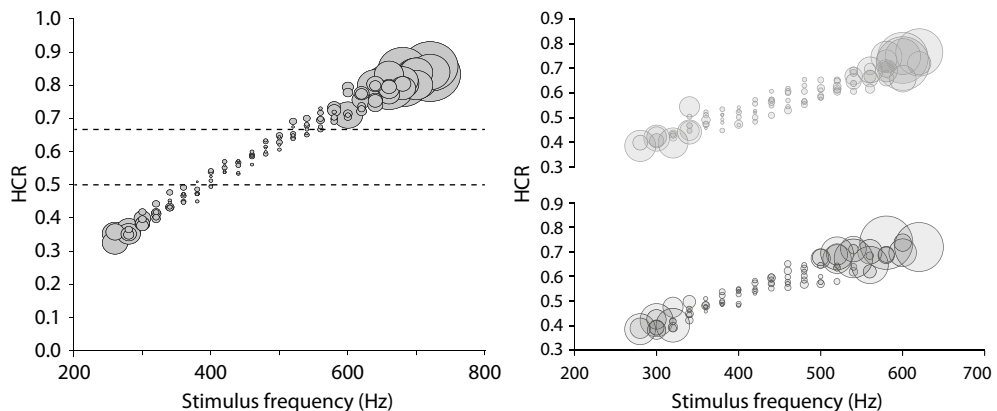
Figure 26.4. Relationship between the stimulus frequency and harmonic-convergence ratio (HCR) that elicit harmonic convergence (HC). Bubble areas are proportional to minimum stimulus intensity required to elicit HC. Dashed lines at the ratios 0.5 and 0.667 are equivalent, respectively, to 2:1 and 3:2 HCR. Left graph (*Culex quinquefasciatus*) is from Simões *et al.* (2016). Right graph (top: *Anopheles coluzzii*. Bottom: *Anopheles gambiae s.s.*) is from Simões *et al.*, 2017).

26.3.3 Methodological approaches for investigating harmonic convergence

Arthur *et al.* (2014) pointed out that the lack of spectral accuracy in the previous studies measuring harmonic convergence (HC) has prevented the degree of HC from being known, with the recent use of frequency resolution enhancement algorithms showing that convergence is generally not less than a few Hertz. The results of Garcia Castillo *et al.* (2021) with free-flying mixed-sex swarms suggest that this phenomenon would be better described as harmonic frequency difference (<50 Hz) rather than HC. Arthur *et al.* (2014) also put forward another explanation, based on a mechanical benefit of the HC for the wing flaps to form a more stable entity in mid-flight. This hypothesis is supported by a study involving free-flying males with females in flight attached to the thorax, which showed convergence only during the contact phase (Aldersley and Cator, 2019).

An important finding is that HC (or harmonic frequency difference) does not require any interaction between male and female to happen. Indeed, both Garcia Castillo *et al.* (2021) and Somers *et al.* (2022) measured HC ratio (3:2) between the male and female tones in single-sex swarms. Contrary to Garcia Castillo *et al.* (2021), the approach of Somers *et al.* (2022) is to clearly claim that the HC is an epiphenomenon of the mosquito auditory system, as proposed by Simões *et al.* (2016), which allows the male to use his own wingbeat tone as an amplifier to hear the nearby female. These authors suggest that the increase of swarming-male WBF, which leads to 3:2 HC ratio, is ruled by the male circadian rhythm independently of their behaviour. Remarkably, they re-analysed the only publicly available dataset of HC events between pairs of mosquitoes (Arthur *et al.,* 2014) and showed that the HC sequences are governed by chance, and do not happen more frequently than when both sexes are closed to each other.

In this context, if HC is an epiphenomenon, how should we interpret the correlation between HC and mating success found in many studies (e.g. Cator *et al.,* 2010; Cator and Harrington, 2011; Aldersley and Cator, 2019)? Somers *et al.* (2022) suggested that 'the finding that: (1) sons of pairs that showed more HC before mating had greater mating success; and that (2) these offspring themselves showed more HC before mating (Cator and Harrington, 2011) simply indicates that a 3:2 ratio of flight tones provides audibility and, thus, mating benefits; and (3) that flight tones are heritable traits'.

26.3.4 Rapid frequency-modulation

Roth (1948) described four behaviour patterns in response to sound in *Ae. aegypti*: (1) males start flying when sound is played back; (2) males exhibit phonotaxis; (3) a 'seizing' response (moving their body, as if they try to seize a female); and (4) a clasping response (flexing their abdomen, corresponding to the final stage of copulation when pairs make connection). During the seizing stage, Roth (1948) observed rapid frequency modulations in males, and explained that 'it is possible that the rapid vibration of the male wings after seizing the female, lessens the burden of the latter and helps to maintain the pair in flight'.

Simões *et al.* (2016; 2017) named this stereotypical acoustic behaviour 'Rapid Frequency Modulation' or 'RFM' in *Cx. quinquefasciatus* and *An. gambiae s.l.* males in response to a pure sound and defined it quantitatively by its time-frequency characteristics and by the flight path of males; it consists of a steep increase in WBF concomitant with fast phonotactic flight towards a female (or artificial sound source), followed by RFM of the wing beats, when in the immediate vicinity of a female or the female-like sound source. Male RFM behaviour involves remarkably fast changes in WBF, and can be elicited without acoustic feedback or physical presence of the female.

Aldersley *et al.* (2019) used sound recordings and high-resolution video images of *Ae. aegypti* pairs where the male was free flying and the female was tethered. These authors found RFM in 98% of the contact-making interactions. RFM started 0.4±0.2 s before male interception of the female, and generally ended before securing the tarsi and ventral alignment of the female.

Pantoja-Sanchez *et al.* (2019a) and Montoya *et al.* (2021) found RFM behaviour in free-flying pairs of *Anopheles albimanus* (Pantoja-Sanchez *et al.,* 2019a), and free-flying male and tethered female *Anopheles darlingi* (Montoya *et al.,* 2021). In *An. albimanus,* Pantoja-Sanchez *et al.* (2019a) observed, in all mating attempts, an increase in male WBF synchronised with the start of the chase, followed by an increase in female WBF. After that, both sexes exhibited RFM, however, it was not observed in all mating attempts, and the authors explained the absence of RFM was because one quarter of the couples that copulated reached the floor when making contact, which may have ended the wing flapping behaviour prematurely (Pantoja-Sanchez *et al.,* 2019a). In *An. darlingi*, Montoya *et al.* (2021) found male RFM behaviour occurred in all 40 observed mating interactions.

26.3.5 Species-specificity of wingbeat frequencies and of acoustic interactions

The question of species-specific cues in mosquito flight tones has long been contentious (Tischner, 1953). Comparison of WBF should always be performed while monitoring behaviour, since the WBF range can change substantially for a wide range of reasons (Pantoja-Sanchez *et al.,* 2019a), and between one species to another (Figure 2) (Mukundarajan *et al.,* 2017).

Whether WBF could be detected by sibling species to identify each other, such as those of the *An. gambiae s.l.*, have been debated for a while (Feugère *et al.,* 2021; Pennetier *et al.,* 2010; Simões *et al.,* 2017; Tripet *et al.,* 2004). In male swarming behaviour, Brogdon (1998) found that the WBFs of *An. gambiae* and *Anopheles arabiensis* were significantly different. Feugère *et al.* (2021) found that between *An. coluzzii* and *An. gambiae s.s.*, the latter differed slightly by ~20 Hz (860 Hz for a 70-male *An. coluzzii* swarm and 840 Hz for a 30-male *An. gambiae s.s.* swarm; 28 °C), but with a large overlap of ~50 Hz of the harmonic-peak bandwidth at -3 dB. The RFM associated with mating was found to be similar when free-flying males of both of these species were exposed to pure tones mimicking the fundamental WBF of the female (Simões *et al.,* 2017). In tethered flight, Pennetier *et al.* (2010) showed that HC occurs more consistently in same-species pairs than in mixed-species pairs of *An. coluzzii* and *An. gambiae s.s.* This intriguing finding should be further investigated using free-flying mosquitoes, or the tethered-mosquito data should be re-analysed using the methods proposed by Somers *et al.* (2022). The difference in the mean male WBF between the two

species may have slightly changed the male-female WBF ratio, thereby creating a different degree of HC between heterospecific and monospecific pairs (WBF mean±SD: *An. coluzzii* males 704±25 Hz, *An. gambiae s.s.* males 682±27 Hz, n=4 and 5, respectively; *An. coluzzii* females 467±31 Hz, *An. gambiae s.s.* females 460±26 Hz, n=6 and 5, respectively; Pennetier *et al.,* 2010).

In the wider species complex of *An. gambiae*, Charlwood and Jones (1979) conducted a series of experiments with two tethered females from different species, one being the same species of a free-flying male. The males made no discrimination between the females, and the females did not reject males, specifically (*An. gambiae s.l.* versus *An. arabiensis; Anopheles melas* versus *Anopheles merus; An. melas* versus *An. gambiae; An. arabiensis* versus *An. merus*). From the results above, mosquitoes with close-by WBF are unlikely to use acoustic cues to discriminate between interspecifics, at least it may not be a strong behavioural barrier to avoid heterospecific mating in species which are involved in on-going speciation.

26.4 Distance hearing

26.4.1 Sound sources and sound attenuation

It is assumed that the hearing distance between a male and a female is limited to a range of a few to tens of centimetres (Göpfert and Robert, 2001; Wishart and Riordan, 1959). Although their auditory organs are optimised for close-range hearing, they are not restricted to a given hearing distance (Windmill and Jackson, 2016), because they are sensitive to an intrinsic component of sound (Bennet-Clark, 1998; Menda *et al.,* 2019). Consequently, males have been shown to respond to artificially loud sound levels of played-back single female flight tones meters away from the sound source (Menda *et al.,* 2019). Thus, the debate about hearing distance should be strictly linked to sound-source power, which is expected to be linked to the resultant particle velocity around the flagellum, and the biological relevance of the sound source in the field.

The sound of a mosquito is the consequence of in-phase flapping of two wings. The particle-velocity attenuation of tethered male *T. brevipalpis* was found to be proportional to the inverse distance cubed (Jackson and Robert, 2006) in the 'near field', whereas measurements of tethered *Ae. aegypti* found that the particle-velocity amplitude attenuated slightly faster than the square of the distance, with an exponent of 2.3-2.5, which lies between that expected of near-field particle velocity from a dipole source ($1/r^3$), and that expected from a monopole source ($1/r^2$) (Arthur *et al.,* 2014). Outside the near-field, attenuation of particle velocity is expected to vary with the inverse distance squared (Bennet-Clark, 1998).

26.4.2 Long-range inter-mosquito communication

Is long-range inter-mosquito sound communication (Menda *et al.,* 2019) only possible in the laboratory, or does it also occur under natural environmental conditions? From existing results, it is reasonable to assume that to be heard at distances greater than ~10 cm from the source of mosquito sound, this sound must be more powerful than that of an individual mosquito. Mosquito species that form mating swarms can produce a relatively loud sound, easily discernible to the

human ear a few metres away, as Wesenberg-Lund (1920) observed with *Cx. pipiens* males: 'In the course of these two hours, I saw most probably about fifty times, larger and darker mosquitoes direct their way from the outside into the swarm. I could observe the females about two or three meters from the swarm; it was very interesting to see, how straight the lines were, along which these mosquitoes made their way into the swarm; the mosquitoes were always females of *Cx. pipiens*; it was as if by some magic power the insects were forced to fly directly into the swarm of dancing males. Undoubtedly, they were directed by the sound issuing from these males which I have often heard formerly and which others, standing near me, heard very distinctly'.

Indeed, some mosquito species can form relatively dense station-keeping aggregations (Manoukis *et al.,* 2009)*,* consisting of up to thousands of males (Assogba *et al.,* 2014). This raises the hypothesis that a female can be attracted from a distance to swarm sounds produced by males in established swarms. However, this hypothesis was refuted by exposing swarming *An. coluzzii* females under laboratory conditions to played-back swarming-males sound stimuli of carefully-controlled levels, and tracked distances between the speaker and the 3D positions of the mosquitoes. Females did not react to the sound by changing their WBF, their flight trajectories or flight velocities (Feugère *et al.,* 2021). To our knowledge, male swarms are the only likely candidate source of sound, which is loud enough, and fits the tuning of the mosquito organs well enough to enable inter-mosquito acoustic communication at long range. This means that *An. coluzzii* females cannot hear a male swarm before entering it. It is also unlikely that a male hears a male swarm at long range, because, although males are more sensitive to sound than females, their hearing organs are not tuned to the male WBF. Finally, as the chosen mosquito species produces large and loud swarms, Feugère *et al.* (2021) claimed that long-range interspecific acoustic communication in mosquitoes is unlikely to occur before the female mosquito enters a swarm. The differences in hearing abilities between males and females may be reduced in some species, such as *Ae. aegypti* (Dou *et al.,* 2021; Su *et al.,* 2018), and females of some species may even respond better to sound than males, such as *M. uniformis* (Ogawa, 1988), *Cx. tritaeniorhynchus* (Ikeshoji, 1986). However, the latter do not form large swarms, which could gather enough males to be audible at long range. However, this study does not eliminate the hypothesis that long-range hearing can be used for host location or for predator avoidance, providing the host/predator sound is loud enough and tuned to mosquito hearing. While there is no evidence, and no published study to our knowledge, about predator avoidance in mosquitoes in response to the sound of predators, several papers strongly suggest that frog-biting female mosquitoes use the sound of frog calls to locate them for a blood meal.

26.4.3 Female attraction to host call

Females are usually not known to exhibit phonotaxis toward the sound of the flight tone of the male. However, the following frog-biting mosquitoes have been shown to be attracted to the sound of frogs: *Cx. territans*, *Uranotaenia macfarlanei* (Bartlett-Healy *et al.,* 2008; Legett *et al.,* 2021; Toma *et al.,* 2019). Similar phonotaxis behaviours are reported in station-keeping swarming midges, which also blood feed on frogs, such as the *Corethrella* midges (Toma *et al.,* 2019). *Culex quinquefasciatus* females have recently been found to inject more energy into their antennae during the active state than any other species or sex that has been tested. This energy injection enhances

the self-oscillation amplitude of the flagella, compared to *Ae. aegypti* and *An. gambiae*, which should enable females to be more efficient at detecting sound; the authors suggest that this may help them find blood hosts (Su *et al.,* 2018), but currently, there seems that there is no behavioural evidence that female *Cx. quinquefasciatus* detect hosts by ear.

In electrophysiology, the sensitivity of female hearing at the JO neuronal level is less than that of males of *Cx. quinquefasciatus, Ae. aegypti* and *An. gambiae s.s.* (Su *et al.,* 2018), but the antennal sensitivity of female organs in electrophysiological studies still surpasses that of other arthropods (Göpfert and Robert. 2000). However, when adding the WBF of the tested sex to the opposite-sex WBF stimulus, female JO sensitivity is of the same order of magnitude to that of male (Lapshin, 2013), which suggests that the auditory environment of female mosquitoes could be more complex than that which is currently known. Possible functions for female auditory detection could include a fitness assessment of males during mosquito mating, predator avoidance, host finding and host monitoring during feeding. Behavioural tests need to be conducted.

26.4.4 Sound traps

One of the first reports of a sound trap for mosquitoes was from Maxim (1901), who managed to attract and capture male mosquitoes by broadcasting a particular note. Many others followed (reviewed by Roth, 1948; Belton, 1994), but the first thorough study of sound as an attractant bait may be that of Kahn and Offenhauser (1949). These authors used an electric screen (high-voltage) placed in front of a speaker playing-back female flight sounds of wild-caught *An. albimanus*; the numbers of male mosquitoes caught were determined by the number of electrical discharges observed on the electric screen. Ninety percent of the males caught were of the same species. The high sound levels of the played-back female flight sounds were reported to be highly unpleasant to humans, especially as the success of the method depends on the loudness of the played-back sound by attracting mosquitoes from far away. The trap was reported to be species specific and attracted *Ae. aegypti* or *An. albimanus*, as a function of the sound frequency emanating from the speaker. In comparison with a cattle trap, the sound trap of Kahn and Offenhauser (1949) was, on average, 10 times more efficient. Unfortunately, these authors did not include a 'control' trap, which would be a 'silent' playback to assess the relative efficacy of visual vs sound attraction to the trap. From their photo (Figure 4, p. 817, Kahn and Offenhauser, 1949), it seems that they put the black speaker and electrical frame on a white cloth on the ground, which may have created a strong visual attractant for swarming species.

More than a century later, the fundamental techniques for sound-attraction traps have not changed. They are based on broadcasting sound frequencies close to a WBF of a female. The frequencies of the sound trap can be set to target specific species, however, there is quite a wide range of intra-species WBFs, with inter-species frequency overlap (Figure 2; Mukundarajan *et al.,* 2017). Therefore, sound traps cannot be very specific, depending on the location.

Multi-sensory traps have been used to test multi-range attraction, e.g. long-range attractants (CO_2) combined with short-range attractants, such as sound (Ogawa 1988), or sound and swarming markers (Balestrino *et al.,* 2016; Kerdpibule *et al.,* 1989). However, so far, sound has not been

shown to be more attractive than a standard CO_2 trap (Johnson *et al.,* 2018; Pantoja-Sanchez *et al.,* 2019b). Sound traps require very loud sounds to attract mosquitoes from long range, which would create obvious disturbance in the local area.

While females are not known to exhibit phonotaxis toward the sound of males, there are several papers reporting female catching in cases in which the sound was shown to be efficient. Ogawa (1988) showed that sound (350 Hz) was necessary to catch females of *M. uniformis*, in the presence of a guinea pig cage. However, no females were caught by the sound trap in the dark, showing the need for other sensory cues to attract them. *Culex tritaeniorhynchus* females were caught with sound-only trap (and glue), but locations of traps were correlated with the location of the pigpen (Ikeshoji, 1986), or in the presence of the hamster cage and CO_2 (dry ice) (Leemingsawat *et al.,* 1988).

Attempts to catch females with sound has been shown only for a few species. Killing males cannot be very effective in reducing a mosquito population, because one male not caught can inseminate many females. Indeed, sound traps are not known to be efficient enough in the field to significantly reduce a population beyond restricted areas (Kanda *et al.,* 1990). For monitoring the presence of species, female CO_2 traps may be preferred for their efficiency, however, when male monitoring is required, sound traps may be useful for measuring the survival rate of released sterile or genetically-modified males.

26.5 Conclusions

Biomechanical and electrophysiological studies have informed many aspects of the hearing mechanisms in mosquitoes, such as sustained oscillations of the flagellum and the tuning of the Johnston's organ. Imaging and pharmacological studies have shown that mosquito auditory function is modulated by an efferent system, which is rarely found in insects. While recent free-flying behavioural experiments have measured HC between sexes, and although HC is still used as a tool to measure mating success, others showed that HC should be re-interpreted as an inter-sex WBF ratio, not governed by any interactions between the two sexes. On the contrary, several findings support the fact that HC is an epiphenomenon associated with a mechanism that optimises the audibility of a potential nearby mate, by using the wingbeat of the individual itself as an amplifier. Therefore, the idea of optimisation of hearing threshold through the increase of WBF of the male and associated antennal distortion products should be preferred to the use of HC. The RFM of males occurs only within close contact of a female after the mating chase. Its role is still unclear but does not seem necessary for copulation. It may be a way to help the copula to stabilise their combined flight trajectories.

Inter-mosquito communication is most likely restricted to short-range interactions. However, mosquitoes detect sounds over a range of distances with no limitation, provided that there is a sufficient sound level at the sound source. Thus, some frog-biting female mosquitoes exhibit phonotaxis toward host sounds, but the use of sound to detect predators remains to be investigated. Sound can be used as an attractant at long range, but in practice sound is only applied for short-range attraction, due to the ecologically-disturbing loudness that it would require. Sound-only

traps have not shown effectiveness in the field yet, but the use of sound for short-range attraction in combination of long-range attractant (CO_2) seems to achieve better results.

The hearing mechanisms of mosquitoes are probably shared with other species of the Culicomorpha infraorder, which display similar station-keeping swarming and mating behaviour, e.g. *Chironomus* (Kon, 1989; Azovsky and Fyodorova, 2003) and frog-biting midges *Corethrella appendiculata* (De Silva *et al.,* 2015).

Acknowledgements

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