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Buzzkill: Targeting the mosquito auditory system

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Buzzkill: Targeting the mosquito auditory systemMarta Andrés^{1,2}, Matthew P. Su^{1,3}, Joerg Albert^{*1,2}, Lauren J. Cator^{*4}¹Ear Institute, University College London, London, WC1X 8EE, United Kingdom, joerg.albert@ucl.ac.uk²The Francis Crick Institute, London NW1 1AT, United Kingdom³Division of Biological Science, Nagoya University, Nagoya, 464-8601, Japan⁴Grand Challenges in Ecosystems and Environment, Department of Life Sciences, Imperial College London, Ascot, SL5 7PY, United Kingdom, l.cator@imperial.ac.uk**Highlights**

- Mosquitoes, particularly males, possess a complex auditory system which underlies the attraction of males to sounds resembling female flight tones.
- Other aspects of mosquito acoustic behaviour, particularly the role of sound perception in female behaviour, are still unclear.
- Acoustic interactions between isolated male-female pairs have been investigated in detail, enabling the creation of several models of mosquito acoustic communication.
- Acoustic interactions between conspecific males and females within mating swarms have remained largely unexplored.
- There is great potential to exploit the male phonotactic response, and acoustic biology more broadly, for control but this will require enhanced collaboration between hearing physiologists, mosquito ecologists, and control experts.

Abstract

Sound plays an important role in mosquito sensory ecology. Acoustic perception and acoustically-driven behaviours therefore represent potentially effective control targets. Previous scientific efforts around acoustic-based control and surveillance have not been systematic and ambiguity around the exact role of acoustic communication in conspecific interactions remains. Here, we briefly review recent advances in mosquito auditory physiology and behavioural ecology as well as ongoing activities to incorporate sound into control and surveillance tools. We highlight areas where increased collaboration between physiologists, molecular biologists, behavioural ecologists, and control experts is needed to capitalize on this progress and realize the potential of sound-based technologies and strategies.

The WHO estimates that the wellbeing and livelihood of at least 1/3 of the human population is directly impacted by mosquito-borne diseases (1). Although progress has been made in controlling several of these diseases, signs of regression, most notably for malaria (2), have begun to appear. This distressing trend is in part due to the development of resistance to insecticide-based control strategies (3). Concurrently, other mosquito-borne diseases have emerged and expanded in response to global climatic and land-use change (4).

These threats have prompted a push to develop novel tools for vector control. Sound plays a key role in mosquito biology. Mosquito audition and acoustically driven behaviours have long been identified as promising new targets for the development of control tools (5). Here, we highlight advancements in our understanding of both the physiological mechanisms and behavioural functions of mosquito acoustic biology (Fig 1.) and how new insights in this field could be applied to controlling mosquito-borne diseases. Increased transdisciplinary collaboration in mosquito biology will enhance control.

How do mosquitoes hear?

Mosquitoes hear via a sophisticated auditory system consisting of a (i) flagellar sound receiver, (ii) the Johnston's organ (JO), which houses the auditory neurons, and (iii) auditory processing centres located in the brain. Instead of being driven by pressure changes like human ear drums, mosquito flagella are driven by friction with air particles which have themselves been set in motion by sound (the particle velocity component of sound). Mosquito flagellar ears are exquisitely sensitive. JO responses have been reported for minute displacements of the flagellar tip (6,7); saturation of auditory responses is achieved at flagellar deflection angles of less than 0.01°, making flagellar ears ~100-times more displacement-sensitive than stereociliary bundles atop the hair cells of the vertebrate ear (8).

Additionally, efferent control, which was previously believed to be unique to vertebrate ears, plays a major role in the modulation of JO function, and thereby mosquito audition. Efferent innervation releases the neurotransmitters octopamine, serotonin and GABA at various cellular locations, controlling different aspects of auditory physiology (9).

Mosquito auditory systems are also highly sexually dimorphic (10,11). The male JO, the largest chordotonal organ of any insect, contains around 16,000 neurons, which equals the number of hair cells in the human cochlea and exceeds the number of neurons in the female JO by a factor of ~ 2 (8,9). Male and female flagellae are tuned to different frequencies (~ 210 Hz for females and ~ 330 Hz for males from *Anopheles*, *Aedes* and *Culex* species), with electrophysiological responses to stimuli being far greater for males than females. Male flagellae uniquely exhibit spontaneously occurring, autonomous vibrations (12), also referred to as *self-sustained oscillations* (SOs). SOs are of almost perfect sinusoidal (i.e. mono-frequent) nature and of extremely large magnitudes, increasing the energy of flagellar oscillations by several thousand-fold (13). SOs can adjust their vibration frequencies to match that of an external pure tone. This 'entrainment' however only occurs for pure tones close to female flight tones (13), suggesting that SOs may play a role in recognition of flying females. In addition, the extent of efferent innervation also seems far greater in males than females across several species, with JO efferent innervation in female *Anopheles gambiae* almost non-existent (9,13). Taken together, these significant sexual dimorphisms likely reflect fundamental differences in the auditory behaviours of male and female mosquitoes.

What are mosquitoes listening out for?

Sound has been implicated in multiple mosquito behaviours. Males show a repeatable and highly stereotyped phonotaxis towards pure tones of between 280–600 Hz (14–16), which are in the range of frequencies produced by the beating wings of flying females. Acoustic stimuli are sufficient to induce male courtship behaviour and males will avidly respond to artificial playbacks, even attempting to copulate with speakers and tuning forks (15,17). The male phonotactic response is consistently observable and has been researched for over 100 years (18).

Male phonotactic responses are presumed to have evolved in the context of mating behaviour. Mosquitoes from several genera copulate mid-flight in aerial mating swarms [18]. There are species-specific differences in the circadian timing and magnitude of swarms as well as associated acoustic activity. In *Anopheles*, phonotactic responses appear to be restricted to the crepuscular periods in which mating takes place (5). This confined timing is also reflected in the erection of the males' antennal fibrillae, which occurs only during this period, indicating a strong circadian component to both mating and audition (5,19). Male *Culex quinquefasciatus*, whose fibrillae are permanently erect, also show a peak in phonotactic response to artificial flight tones during presumed swarming timepoints (20). Circadian components of *Aedes* spp. phonotactic responses have not yet been systematically assessed but appear to be less tightly regulated (21). In addition to responding to female flight tones, males may also respond to the flight tone of other males within the swarm. Using *Ae. aegypti* males tethered in a linear array, previous researchers found evidence that males also respond to the flight tone signals of other males. Specifically, males appeared to partition the frequency space and, when groups reached a critical threshold, transition to form clusters around common frequencies (22). Another study using free-flying *An. ablimanus* also found evidence of male-male interactions (23).

Females do not exhibit phonotactic responses. However, sound appears to also play an important role in female behaviour. When males and females move into close proximity, as in a swarm, higher harmonic components of the male and female fundamental flight tones can overlap (24–26). This phenomenon, often referred to as harmonic convergence, is not random (27) and there is consistent positive correlation between convergence and likelihood of mating success (17,28). Pairs including females which had been mated or injected with male accessory gland proteins exhibited lower rates of harmonic convergence compared to virgin females (24,29). These observations suggest that harmonic convergence may play a role in female acceptance behaviours.

It is not clear whether harmonic convergence is a by-product of the physical (e.g. aerodynamic) coordination required to form a mid-air copula or a sexually selected indicator of male quality. A recent study in which *Ae. aegypti* acoustic interactions were mapped onto behavioural interactions at a fine scale found that while convergence is positively associated with copula formation, the majority of convergence events occur after a

critical period within which female choice likely takes place (30). These results suggest that the relationship between copula formation and convergence may therefore be a consequence of this acceptance rather than a signal that females use to inform acceptance decisions. Alternatively, convergence may be a by-product of the phonotactic response being driven by distortion products created by interactions between male and female flight tones (31). The role of harmonic convergence in male-female mating interactions remains an area of ongoing investigation.

Harmonic convergence interactions were the first hint at a long suspected acoustic life for females (12). Much remains unclear regarding female hearing, particularly in terms of phonotaxis. For example, evidence from *Uranotaenia* mosquitoes and other dipteran species suggests that females may use sound outside of the mating swarm in order to identify blood hosts or to avoid predators; however no reports have found evidence of phonotaxis for human-feeding mosquito species (32–34). Whilst relatively understudied compared to males, female audition has been identified as an important research gap (6).

Targeting the mosquito auditory system to control vector populations.

Trapping. Given this rich acoustic life and the current need to identify alternative mosquito control tools, sound represents a viable control target. Traps targeting males can be used to monitor the performance and ecological impact of novel technologies based on field releases of genetically or biologically modified males. Whilst traps designed to catch females rely on chemical or physical stimuli, multiple studies have explored exploiting the male phonotactic response in acoustic lures. Sound alone, or more recently used in conjunction with chemical and visual attractants, has shown to effectively capture male *Aedes*, *Culex*, and *Mansonia* mosquitoes (34–43) (Table 1).

Mosquito lure design has not advanced at the same rate as our knowledge of the fundamental mechanisms of mosquito hearing; it has been further hampered by a lack of standardized testing methodology, e.g. regarding timing, intensity and frequency of sound stimulation, or trap placement. For example, traps have been designed under the assumption that acoustic lures should only be effective over short distances due to the near-field sensitivity of the mosquito antenna. These traps have historically used host cues to draw males in from longer distances and thus were limited to species which used host cues as part of swarm initiation. Recent discovery of longer range mosquito hearing capabilities (34) has opened up new opportunities for acoustic trap design including targeting species that do not use host cues in swarm formation, such as *Anopheles* spp. (45). Evidence for these longer-range capabilities only comes from functional tests using synthetic, loudspeaker generated sounds under lab-based settings.

The binary nature of male phonotactic responses allows for clean experimental tests of signal characteristics, including frequency and intensity, which could complement physiological assessments. Temperature and body size are known to affect sound production (46–49), but there is a paucity of clear, comparable data on the variability of male responses to female flight tones, especially under field-relevant conditions (50). The majority of behavioural work on phonotaxis has been conducted under different experimental conditions, making it difficult to compare findings across experiments and species (51). Moving forward, our improved understanding of the physiological mechanisms underlying male phonotactic responses should be combined with standardized testing protocols to improve trap design. A new generation of more dynamic sound stimulations that mimic mating acoustic interactions, or ‘swarm sounds’, could greatly improve trap efficacy. Furthermore, recent work (52) in *Culex* has highlighted the potential for using acoustic stimuli to disrupt phonotactic responses within swarms.

Insecticides. Pharmacological approaches which impair hearing and acoustic communication offer another mechanism to target the mosquito auditory system. This includes not only the auditory mechanotransducers themselves but also the various efferent components of the mosquito auditory system, which appear to be key modulators of its physiology (9). The efferent neurotransmitters octopamine and serotonin signal through G-protein coupled receptors, which have previously been recognized as a potential target for the development of next-generation insecticides (53,54). Octopamine, the invertebrate counterpart of noradrenaline, is particularly interesting as it is largely restricted to insects and present only in trace amounts in mammals (55). Octopamine receptors are therefore promising targets for highly specific insecticides with minimal off-target effects on mammals. More research is necessary to understand how these targets can be exploited for mosquito control.

Ecological and field studies can advise on how to best adapt these technologies to the field with minimal non-specific effects to other insect species.

Mass-rearing assessment. The importance of sound in mosquito mating behaviour also suggests potential roles of mosquito hearing research for the assessment and monitoring of laboratory populations. Currently, the determinants of male mating success remain unclear and this curtails our efforts to optimise mass-rearing to maximize male competitive ability. The role of female choice in male mating success and the influence of sound on female behaviours are also largely unknown. A better understanding of female hearing and controlled tests of female responses to sound in field settings are required. In particular, the ability to manipulate female hearing capabilities (which should be considerable given the size of the female JO with ~3,000-7,000 neurons depending on the mosquito species) would allow for determining the degree to which mating outcomes are dependent on female hearing and acoustic responses. Male acoustic traits identified as important for mating success can be used for high throughput monitoring of laboratory maintained lines.

Genetic Modification. Genetically disrupting mosquito hearing could be a highly effective approach to control mosquito populations due to its effect on mosquito reproduction. Employing methods such as the CRISPR-Cas system to generate knockouts for hypothesis testing and GM generation will greatly advance this effort. The molecular dissection of the vast sexual dimorphisms displayed by the ears of most mosquito species (13) will likely reveal genes exclusively involved in male audition; investigation of sex determination pathway mutants (such as *An. gambiae doublesex-female* mutants) could also help in identifying such factors (56). Recessive genes of this kind may drive the generation of novel gene drive lines which interfere with acoustic interactions during mating, thus leading to population collapse.

Conclusions

The past 100 years have seen significant advances in our understanding of both the fundamental mechanisms of mosquito audition and the application of acoustic lures under field conditions. Sound-based interventions are particularly attractive for resistant populations and should be relatively “evolution-proof” as they directly target essential components of mosquito reproductive behaviour. Though there are many ways in which sound could be utilised, the development of control tools remains restricted by our incomplete understanding of mosquito acoustic biology. Knowledge silos exist between those working on different aspects of mosquito biology and in some cases even between those working on the same aspects in different species. Increased collaboration between groups will improve current strategies and facilitate the creation of new approaches (Fig 2). Recent advances in genetic and molecular techniques which facilitate laboratory testing, as well as a renewed necessity for innovative methods of vector control, offer great opportunities to bring both lab and field researchers together. This type of collaboration will enhance our ability to exploit sound for mosquito control.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Complete physiological work up of mosquito audition reveals new auditory mechanisms. Physiological measurements of *Ae. aegypti*, *An. gambiae*, and *Cx. quinquefasciatus* indicate transduction-dependent power gain in the auditory systems of three key vector species. Includes analyses of species and sex differences in mechanotransducer function.

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In this study, the authors investigate male-male interactions in tethered *Ae. aegypti*. Results indicate that males behaviourally respond to the flight tones of other males and that group size alters both collective and individual acoustic traits. Together the data suggests that male-male acoustic interactions may be involved in swarm coherence.

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Slow-motion videography is used to document mating interactions in tethered *Ae. aegypti*. Females effectively reject males and while harmonic convergence is more frequently detected in successful mating attempts, it occurs late in the interaction and coincides with the transition to stabilized paired flight. Results suggest that female choice is a key determinant of male mating success and that harmonic convergence may be associated with success rather than a signal used by females in choice.

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Contrary to dogma, this study demonstrates that male mosquitoes will behaviourally respond to female flight tones up to 10 m. Behavioural studies are complemented by physiological recordings suggesting that the mosquito ear is able to detect females from this distance despite being a particle velocity sensor. This work opens new potential for longer range acoustic lure and disruption development.

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Figure Legends

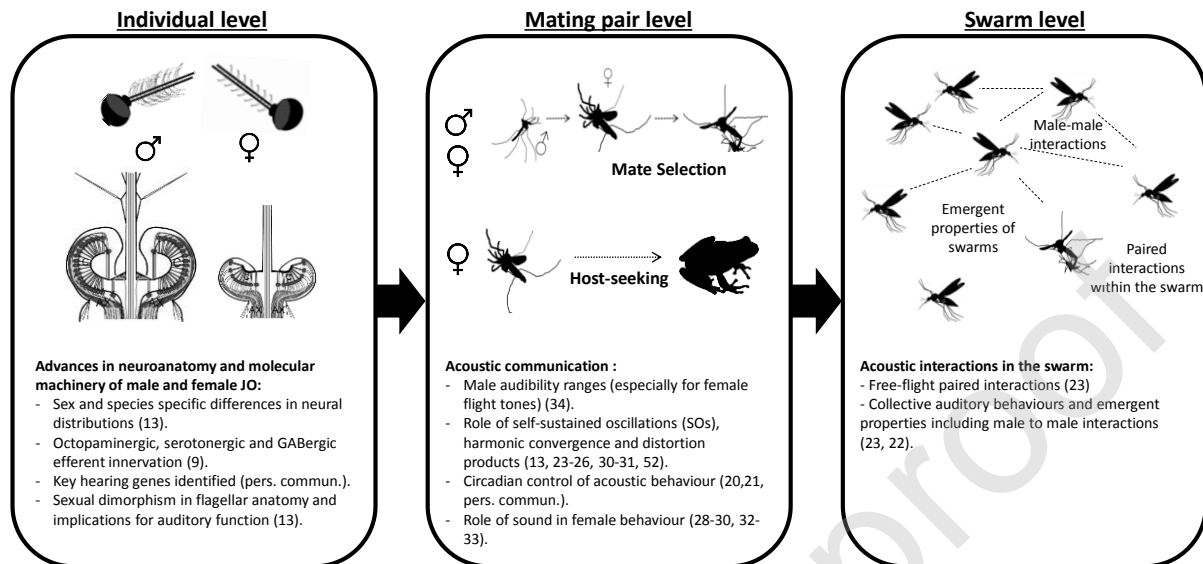


Figure 1. Advancements in understanding the role of acoustics in mosquito biology. In recent years, different studies have expanded our knowledge on mosquito acoustic biology ranging from dissection of anatomy and physiology to the acoustic properties of large swarms. Clearly, many questions remain open that should be addressed in coming years.

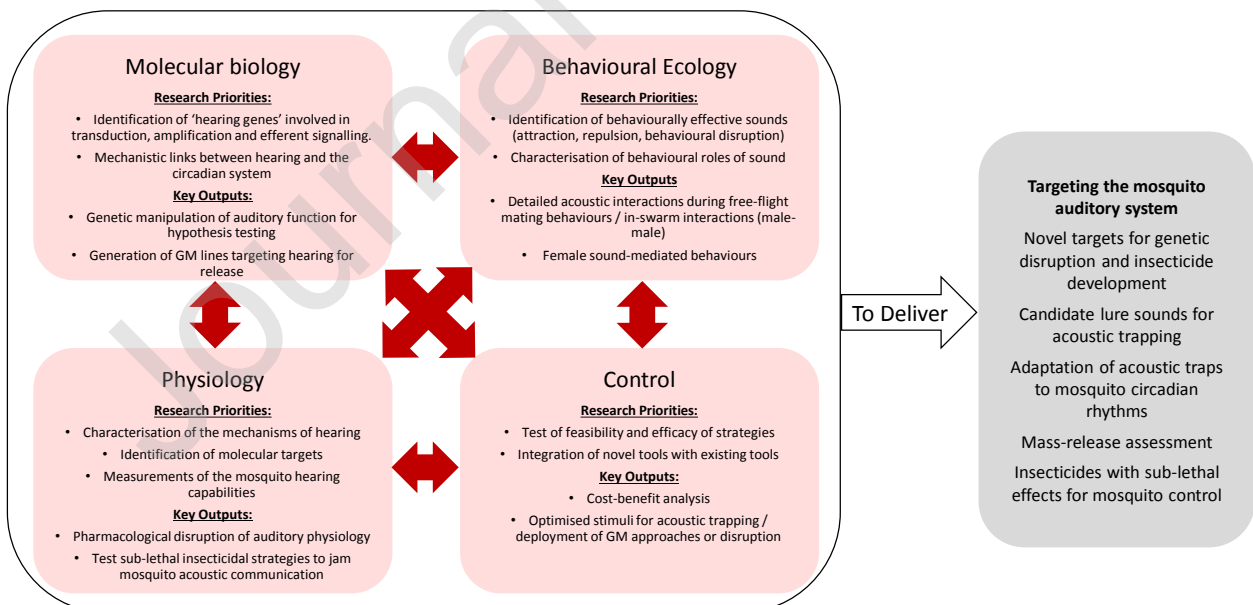


Figure 2. Opportunities for transdisciplinary research in mosquito acoustics. While there is scope for advancement in each of these field separately (highlighted in red boxes), many of the most promising avenues for advance acoustic-based control of mosquito populations will require input from multiple fields (grey box).

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Table 1: Summary of recent studies that used acoustic emissions to improve the performance of chemical or visual traps.

Species	Study	Trap	Acoustic Bait
<i>Ae. aegypti</i>	(42)	CDC Light BG-Sentinel	465 Hz pure tone played at 93 dB
	(43)	GAT	484 Hz pure tone played at 70 dB
	(35)	CDC Light	350-500 Hz Chirp played at 70-74 dB
<i>Ae. albopictus</i>	(57,58)	Custom-built	500-650 Hz Chirp played at 75 and 79 dB
<i>Ae. polynesiensis</i>	(42)	CDC Light BG-Sentinel	440 Hz pure tone played at 93 dB