Comparative aspects of hearing in vertebrates and insects with antennal ears

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Abstract

The evolution of hearing in terrestrial animals has resulted in remarkable adaptations enabling exquisitely sensitive sound detection by the ear and sophisticated sound analysis by the brain. In this review, we examine several such characteristics, using examples from insects and vertebrates. We focus on two strong and interdependent forces that have been shaping the auditory systems across taxa: the physical environment of auditory transducers on the small, subcellular, scale and the evolutionary environment within which hearing takes place, on a larger, sensory-ecological scale. We discuss briefly acoustical feature selectivity and invariance in the central auditory system, highlighting a major difference between insects and vertebrates, as well as a major similarity. By doing so within a sensory ecological framework, we aim to emphasize general principles underlying acute sensitivity to airborne sounds.

Introduction

Auditory physiology offers a distinctive perspective on the interaction between a sensory system and its environment. On the one hand, auditory systems in vertebrates and insects with sensitive hearing are capable of remarkable performances on multiple levels, including the sensory periphery where the minute energies associated with sound are converted into electrical signals, as well as higher-order brain areas, where complex natural stimuli such as human speech are processed. For example, the elastic strain caused by acoustical stimuli in the inner ear at the threshold of hearing is sub-nanometer and comparable to the distance between atoms in molecules [1]. Furthermore, thermal fluctuations in the ear's mechanotransduction apparatus are not only significant, but can be larger than the faintest audible signals, making signal detection a challenging task [2]. Ascending the sensory hierarchy, one encounters other marvels of evolution, such as

the ability of individual neurons to encode, using millisecond-long action potentials, inter-aural time differences of only about ten microseconds, and to use this information for sound-source localization [3, 4]. No engineered system has been designed yet that could understand distorted speech in a noisy and reverberating environment with multiple speakers. That our auditory system achieves this feat is testament to its remarkable performance.

On the other hand, an engineer could argue that the auditory system's performance is objectively poor even in animals with sensitive hearing: at the very first step, during the mechanoelectrical transduction in the inner ear, external sounds are distorted, or even completely suppressed, while new tones are generated by the ear itself [5]. Forward and backward masking, illusory percepts of nonexistent tones (e.g., Zwicker illusion [6]), perceptual merging of separate auditory streams, the precedence effect supressing the perception of echoes that was demonstrated in insects and vertebrates [7, 8] (and which some blind people can un-suppress), auditory hallucinations, and a frustrating inability to distinguish between distinct phonemes of a foreign language—they all indicate that evolution has not shaped the auditory system as an objective detector of acoustical reality. Instead, the auditory system, like any biological system, has evolved to help animals to find food, escape predators, and mate. The sensory ecology of each species, together with the laws of physics, are therefore the major factors controlling animal hearing.

Because sensitive hearing evolved independently multiple times in different animals, comparing how they hear is useful to understand the fundamental principles that govern auditory system's structure and function [9, 10]. In this review, we use this comparative approach to highlight several fundamental mechanisms of hearing in the peripheral and central auditory systems of insects and vertebrates, discussing similarities as well as differences in the context of the animals' sensory ecology.

Peripheral auditory system

Insects and vertebrates both transduce acoustic energy into electrical signals using highly specialized structures that, although implemented differently, follow the same biophysical principles. In both groups of animals, the need for a fast transduction of sound-evoked vibrations must be achieved through a direct coupling of the mechanical

stimulus to the mechanosensory ion channels [11]. The direct coupling assures speed but leads to nonlinearity—and therefore sound distortions—because thermodynamics imposes a nonlinear dependence of the channels' open probability on the stimulus intensity. Yet, the advantage of speed is apparently greater than the disadvantage of sounds being distorted by the ear (in some cases, as we will see below, these distortions may even be exploited as signals in their own right). Likewise, the functional advantage offered by frequency tuning, amplification of weak sounds, and (at least in vertebrates) the ear's ability to convert a million-fold range of sound intensities into a hundredfold range of mechanical or neural responses, outweighs the disadvantages of the inner-ear's instability and metabolic vulnerability (i.e., the requirement for a self-regulatory energy-expending mechanism to maintain the system's control parameters in the desired range in order to benefit functionally from the oscillatory instability) [12]. Thus, natural selection has produced ears that operate close to a bifurcation [12], which produces amplification, frequency selectivity, and a gain control, characteristics that underlie sensitive hearing in vertebrates as well as in some insects.

Insects, sound and the auditory periphery

The Encyclopedia Britannica defines sound as "*a mechanical disturbance from a state of equilibrium that propagates through an elastic material medium*". The term *sound* thus simply describes mechanical forces travelling through a (gaseous, liquid or solid) substrate, whereby the transmission of these forces occurs through series of elastic collisions between the particles within that substrate.

In animal hearing one major task of the auditory system is to secure the *audibility* of a *distinct spectrum* of such sound-associated forces, namely those of biological relevance. Sorting biologically relevant *signals* from biologically irrelevant *noise* involves the active filtering, partial amplification—or suppression—of distinct spectrotemporal features of sound. The auditory systems of different species thus display distinct degrees of *auditory specificity*, i.e., species-specific adaptations of their hearing ranges. Corresponding functional properties have been studied, and reported, for a wide range of auditory systems, including the mammalian cochlea [13], as well as insect ears [14].

In insects, where due to their small sizes evolutionary pressures for miniaturization and 'concurrent engineering' are particularly high, much of the required filtering and pre-

processing already takes place in the auditory periphery, namely on the level of the auditory transducer modules (ATMs), by which we mean (i) the auditory mechanotransducer channels (METs) proper, together with (ii) elastic components (collectively referred to as gating springs), which funnel forces to the METs, thus controlling their gating, as well as (iii) adaptation motors, acting in series with the METs and providing the forces mediating adaptation and amplification.

Insect ears can be grossly divided into two major types: *tympanal ears*, where sound-associated forces are picked up by, and exert pressure on, the area of a thin membrane (analogous to the ear drum of the mammalian ear) and *antennal ears*, which act as pendulums driven by sound-induced particle motion of the surrounding air. Whereas the pressure-sensitive tympanal ears act as far-field sensors that can operate at very high frequencies (up to 100 kHz or even higher) over long distances (tens of meters), the particle velocity sensitive antennal ears act as near-field sensors operating at lower frequencies (typically <1 kHz) over shorter distances (not expected to exceed a few centimetres). We will focus here almost exclusively on antennal ears, which, intriguingly, in many ways are similar to the cellular substrates of vertebrate hearing, i.e., the mechanosensory HCs. Considering the evolutionary distance between insects and vertebrates, it is remarkable how well hair-cell-based models of mechanotransduction [15] can capture mechanotransduction in the antennal ears of *Drosophila* [16]. We will here use the antennal ears of two Dipteran insects ('true flies'), namely Drosophilids and mosquitoes, to illustrate how the auditory periphery can play a leading role within the sensory ecology of an entire acoustic communication system. The ATMs, as we will show, confer substantial amounts of auditory specificity to the antennal ears of both mosquitoes and Drosophilid flies. Most interestingly, the auditory transducer channels of a bushcricket species (*Mecopoda elongata* L.) have recently been directly implicated in the frequency discrimination properties [17], suggesting that also in tympanal ears, METs make substantial contributions to the filtering and pre-processing of sound.

The sensory periphery: *Diptera* ('true flies')

Auditory anatomy and general principles of function

The antennal ears of *Drosophila melanogaster* are formed by two—functionally distinct—anatomical compartments: (i) the second antennal segment (A2 or *pedicellus*) houses

the (partly sound-sensitive) neurons of the Johnston's Organ (JO) and (ii) the third antennal segment (A3 or *funiculus*), together with a unilateral appendage named arista, acts as the sound receiver (Figure 1, top middle). Upon sound stimulation, A3 starts to rotate about its longitudinal axis [18] leading to the alternate stretching and compressing of two populations of JO neurons (JO-Ns) with opposite response polarity [19]. JO-Ns are part of multicellular organules called scolopidia. The scolopidia of antennal ears consist of typically 2 (occasionally 3) neurons plus 3-4 support cells [20, 21]. The *Drosophila* JO houses ~200 scolopidia (corresponding to ~500 JO-Ns), whereas the JOs of male mosquitoes can host up to ~7,500 scolopidia (corresponding to ~ 15,000 JO-Ns; ~97% of scolopidia possess two neurons) [22]. Next to containing a much larger number of neurons, the mosquito antenna differs in another important aspect from the ear of the fruit fly. The *Drosophila* antenna, as described above, forms a rotating pendulum (with only one mechanical degree of freedom), whereas the mosquito antenna conforms to the ancestral 'flagellar' condition, where the antenna's flagellum acts as an inverted pendulum that can swing in all directions within its plane of suspension (thus having two mechanical degrees of freedom). The functional anatomy of the mosquito ear is likely to reflect the major role the sense of hearing plays in mosquito mate localization [23]. No phonotaxis, or other directional auditory behaviour, has yet been reported for *Drosophila* (despite the distinct directionality of its antennal sound receiver, see ref. [24] and below).

Sensory ecology of hearing I - The antennae of Drosophila as active, mechanical pulse extractors

With regard to the passive vibrational properties of its cuticular joint (as manifest in freshly dead or CO_2-sedated animals), the *Drosophila* antennal sound receiver can be approximated as a moderately damped, simple harmonic oscillator with a linear response behaviour [25]. Mechanically coupled to the receiver, however, are directly gated ion channels situated in the membranes of JO-Ns. Essential nonlinearities, associated with the gating of MET channels, in tandem with energy input from adaptation motors, render the antennal ear as a whole both active and nonlinear [25]. Even more than that, with regard to key mechanical properties, the antennal ears are in effect dominated by the properties of their ATMs [26]. In the following we will briefly illustrate how these molecular properties are matched to, and enable, the fly ear's specific auditory tasks.

Behaviourally, hearing in flies is most closely linked to acoustic communications taking place during the mating ritual [27]. Male flies vibrate their wings to 'sing' songs to the females. In *D. melanogaster*, these songs are spectrally very simple, containing longer (typically 0.2-1s) sinusoidal, pure-tone-like, components ('sine songs') alongside shorter (~5-10 ms) pseudo-sinusoidal waveforms ('pulses'). Pulses are repeated at species-specific inter-pulse intervals (IPIs) to form trains. The IPIs are thought to represent the major signal mediating species recognition during courtship [28]. For the fly's auditory brain to be able to analyse the IPIs of the pulse trains, however, the ear must have detected these pulses in the first place. Individual pulses represent waveforms of carrier frequencies (CFs) between ~150-250 Hz [29, 30]. In its linear regime, i.e., without any contributions from mechanotransducer gating, the fly's sound receiver is tuned to best frequencies around 800-1,000 Hz. In its active state, the receiver's best frequency is shifted into the range of pulse CFs. But far more than only spectrally matching a receiver to its biologically most relevant signals, the ATMs provide active amplification, increasing the antennal displacement response to individual conspecific pulses by ~10-fold (see Figure 1). By virtue of the underlying transducer-based process, the antennae are thus able to extract pulses out of a noisy environment in an efficient and frequency-dependent way. It is one key feature of the essential nonlinearities that support hearing in both insects and vertebrates that they become relatively more prominent the smaller the forcing (and thus the fainter the sound) [31]. The dynamic range of system responses (both mechanical and electrical) is therefore heavily compressed and the ability to resolve amplitude differences sacrificed to an enhanced absolute sensitivity. In the context of a real-world pulse train this means that, by amplifying pulse-evoked receiver displacements in an intensity-dependent way, the transducer-based process levels out amplitude differences between individual pulses, effectively 'normalizing' pulse amplitudes (although it should be noted that, within certain ranges, amplitude differences can still be resolved and that this process might be further facilitated by a range fractionation between different JO-Ns). In a communication system that operates (i) with a rate code of relatively sparse events (trains typically consist of only 5-10 individual pulses) and (ii) in mechanically noisy conditions, the above system properties are likely to be adaptive. Most notably, the female ear must function during the actual mating ritual, i.e., when being chased by a singing male. Fly courtship has proven an interactive and dynamic behaviour, involving a continuous adjustment of signals [32, 33], in some species even male-female duetting [34], as well as fast movements and turns, which will

by themselves impose a considerable load of mechanical background noise on the ear. Making the job of the female's ear even more challenging, it is, by construction, acutely sensitive to variations of the angle of incidence of the sound stimulus [24]. Under these circumstances, a built-in, frequency-specific hearing aid, such as the one provided by the transducer-based process, will be of considerable value to boost the audibility of the biologically most relevant signals, i.e., individual song pulses. The widely reported intensity dependence of both frequency tuning and amplification in the *Drosophila* ear [30, 35, 36], which maximises the sensitivity and frequency match specifically for faint song components, increases the female's chance to catch every single pulse, however small. For larger pulse amplitudes, in turn, neither active amplification nor frequency optimisation are required; the mechanical properties of the (higher frequency) passive antenna will then dominate the antennal response and the (lower frequency) song pulses will simply fall into the linear part of the passive antenna's displacement response. The fly's antenna was aptly named a love song receptor almost 40 years ago [37]. Today we can add that it does this job by acting as an active, and efficient, pulse extractor. Most remarkably, the receiver's fight for audibility seems to be fought on the sender side as well, as it has been reported that *Drosophila* males continuously, and rapidly, adjust courtship song intensity to the perceived distance to the females [32].

Beyond a pioneering study [38], still very little is known about how pulse songs (or courtship songs more generally) are represented, and decoded, within the fly's auditory brain but much progress in this regard has been made over the last decades by seminal studies conducted in insects with tympanal ears, e.g., crickets. It would exceed the scope of this review to describe these advances here in detail but the interested reader is referred to the conceptual review of the topic by B. Hedwig [39].

Sensory ecology of hearing II - The antennae of mosquitoes detect, and transpose, pure tones.

Above we have discussed how the antennal ears of *Drosophila* have been shaped by, and thus betray, the specific sensory ecological needs of the flies' courtship behaviour. In this regard, the flagellar ears of mosquitoes truly sing a song of their own.

Hearing in mosquitoes is mainly about hearing another mosquito's wing beat. Males detect, locate and chase females by detecting, locating and chasing a female flight tone

[40]. Both sexes, in turn, have been reported to respond to each other's wingbeat frequencies by modulating their own [41-43], an acoustic behaviour thought to mediate male-female interactions within larger mating swarms. Just as in *Drosophila*, mosquito antennae have been reported to be active, nonlinear oscillators of exquisite nanometre-range sensitivity [23, 44-46], with the source of the observed activity and nonlinearity likely being the same as in the fly, i.e., the auditory neurons proper and their presumably active ATMs. Rather than giving a comprehensive view on acoustic communication in mosquitoes, and on the role of the transducer machinery therein, we would like to concentrate on one particular example that showcases how the very periphery of hearing may pre-process and analyse sound. For three species it has been reported that the wing-beat matching behaviour results in a convergence of, not the fundamental frequencies, but of higher harmonics [41, 43, 47]. In all three cases a match appears to be achieved around the second harmonic of the male (M2) and the third harmonic of the female (F3) wingbeat (see Figure 2). For a 'prototypical' mosquito species with a male wingbeat of ~600 Hz and a female wingbeat of ~ 400 Hz, this would result in a convergence at a frequency of ~1,200 Hz, which is commonly assumed to be considerably above the range of antennal mechanical sensitivity and thus above the mosquito hearing range. The intriguing suggestion has been made that the solution to this sensitivity dilemma, which the sensory periphery is faced with, could come from the sensory periphery itself [43, 48]. It is a key feature of active hearing organs that system's nonlinearities together with an inherent reciprocity of force transmission must lead to the generation of distortion products [5]. In case of the mosquito ear this means that even when stimulated with only two tones f_1 and f_2 (representing for example male and female flight tones), the antennal displacement response will display peaks at a set of mathematically predicted additional frequencies, i.e., distortion products. One of them, the cubic distortion product ($2f_1$-f_2), has been found to be particularly prominent in many auditory systems, including the mosquito ear [48]. As the frequency of this cubic distortion product is always lower than the primary tones' (f_1 and f_2) it may act to transpose an otherwise 'inaudible' pair of tones into an audible tone. The hypothesized mechanism has particular traction in *Culex* mosquitoes, as the female antennae in these insects are tuned to frequencies well below the male and female wingbeats—but close to the predicted cubic distortion product. Alternatively, as has been reported for *Aedes* mosquitoes [41], the antennal nerve may respond to higher frequencies of stimulation with a sustained DC-like response component, thus transposing the higher stimulus

frequency even further into the lower frequency range. Future research is needed to resolve these questions but in either case, it seems, the sensory periphery will take a centre stage role. It will be particularly intriguing to explore how the diverging sensory ecological contexts of pure-tone-dominated hearing in mosquitoes and pulse-dominated hearing in fruit flies have shaped the molecular mechanisms of their ears.

Vertebrates

Vertebrates convert sound energy into electrical signals using a single, evolutionarily conserved, type of cellular transducer. Mechano-electrical transduction (MET) occurs in hair cells, the receptor cells of the inner ear. Each hair cell has a bundle of clavate microvilli, called stereocilia, densely filled with cross-linked actin filaments and wrapped by the plasma membrane (Figure 3). Their height increases along the hair-bundle's axis of symmetry. A molecular string called a tip link connects two adjacent stereocilia of different height. It is composed of specialized cadherin molecules [49]. Its lower end (protocadherin-15) is anchored at the tip of the shorter stereocilium where the MET channels are located [50]. Its upper end (cadherin-23) is attached to the side of the taller stereocilium where myosin motors are found. Hair bundle's motion towards the taller stereocilia stretches tip links and opens MET ion channels.

The gating-spring model describes operation of this system quantitatively [15, 51]. Tension in a gating spring (tip link) opens a MET channel. The open probability (P_0) as a function of displacement (X) follows the Boltzmann relation. Channel opening produces a movement, termed the gating swing, which relaxes the gating spring. A product of the gating-spring stiffness and the gating swing defines the single-channel gating force, determines the slope of the P_0-X relation, and sets the ear's sensitivity to sound. Sensitive hearing requires a steep P_0-X relation and therefore a large gating swing [52], which is supposed to originate somehow from the MET channel's conformational rearrangement.

The *gating spring* is a key element of the gating-spring model. It represents an elastic component, or a series of elastic components, whose tension determines the MET channels' open probability. Other elastic elements, which are in parallel with the gating springs (such as stereociliary pivots), are called parallel springs; their tension does not determine the channels' open probability directly. What is a biological correlate of this model concept? Can the tip link be the gating spring? By comparing a hair

bundle's stiffness before and after disrupting tip links with a calcium chelator, the gating-spring stiffness was estimated experimentally to be between 0.4 and 4 $mN \cdot m^{-1}$ [15, 53]. Although tip links are conspicuous morphological candidates for gating springs, a crystal structure of cadherin-23 repeats indicates a value for a tip-link stiffness of 50 $mN \cdot m^{-1}$, significantly greater than the experimentally estimated values [54]. Although it was concluded based on these data that the tip link could not be the gating spring, a polymer of the size of the tip link with the Young's modulus of cadherins and with a physiological level of tension has stiffness matching the experimentally estimated values (Box 1), suggesting that tip links can be hair cells' gating springs. In order to resolve this question, it will be important to do single-molecule force-extension experiments using isolated or reconstituted tip links to test their stiffness directly as a function of applied force [55].

The gating-spring model describes mechanotransduction both in vertebrate and in insect ears [51, 56, 57]. Since insects do not have tip links, other elements of the mechanotransduction complex, e.g., the lipid bilayer and intracellular proteins in series with the transduction channels, such as the ankyrin repeat domains of the NompC channel in insects, can perform the gating-spring role as well [58-62].

Although it is possible to evoke a physiological response during signal transduction by changing the activity of only a single protein—a notable example being the detection of individual photons by the retina—collective activity of an ensemble usually provides a better signal-to-noise ratio. One hair cell may use over a hundred MET channels, located on a hair bundle's many stereocilia. Doing so, however, poses a formidable physical challenge because, unlike insects' antennae, hair cells are immersed in water, a viscous fluid. Viscous friction dissipates energy and dampens oscillations. Because sharp frequency selectivity—a fundamental property of the vertebrate auditory system—is impossible, due to fluid-structure interactions, *unless* stereocilia are grouped in a hair bundle [63] we discuss the importance of these interactions in this review (Box 2).

Some hair bundles are directly stimulated by viscous forces, and their shape reflects this fact. Alligator lizard, for example, has free-standing hair bundles with extraordinarily long and thick stereocilia [64]. In mammals, inner hair cells (IHCs) are also stimulated hydrodynamically, and their stereocilia are twice as thick as those in outer hair cells (OHCs) (500 *vs*. 250 nm in diameter). An OHC would benefit from having as many stereocilia as possible in order to maximize the force produced by the hair bundle and to have as many MET channels per cell as possible to drive the electromotility. This is

probably why its stereocilia are arranged not in a line or semi-circle as they are in IHCs but in a V- or W-shape, and they are smaller, for otherwise they wouldn't all fit in three or four rows atop a hair cell. In contrast, it would make sense for an IHC to have thick stereocilia to maximize coupling to the moving fluid. Indeed, the same number of thinner stereocilia would result in a smaller hair bundle, which would experience a smaller hydrodynamic force for the same flow, whereas a greater number of thinner stereocilia would increase the fluid coupling but also would make the hair bundle stiffer, diminishing its deflection and thus reducing the magnitude of the tip-link extension in response to the hydrodynamic force. Wider hair bundles can therefore be assumed to be more sensitive to hydrodynamic forces than narrower hair bundles of identical stiffness.

Crucially, water filling the gaps between stereocilia will allow their relative shear. Indeed, water is known to be a very good lubricant [65]. At the same time, its viscosity will suppress internally generated squeezing modes of motion and will therefore diminish sound distortions generated by the ear [66], making it a better sound detector, at least from an engineer's perspective.

Central auditory system

How sensory neurons represent (encode) natural signals is one of the classical problems in neuroscience. Using stimuli that are relevant to the animal has produced the clearest descriptions of how sensory systems work in "specialized animals", such as bats [67], electric fish [68], or barn owls [69, 70]. In each of these examples, the stimuli were both natural and simple, which was key to understanding their representations. Most of the sensory cortex in other animals and humans, however, deals with natural stimuli that are statistically complex. This complexity has slowed progress in understanding sensory representations in the brain. Advances in computational methods, however, have enabled characterization of auditory receptive fields using behaviourally relevant stimuli, both in insects and in vertebrates, revealing an interesting similarity between them. Before we address this point, however, one key difference between insect and vertebrate central auditory systems is worth noting.

In many insect species, the number of auditory neurons *decreases* from the periphery to the centre. For example, the *Drosophila* JO contains about 500 mechanosensory cells, ~75% of which (subgroups A, B and D) have been linked to auditory function [71], but the ascending mechanosensory pathways for conspecific song

detection are likely to have substantially fewer neurons [71-73]. In insect auditory systems, individual, i.e., unique, neurons can be identified, and in some species a single neuron alone can drive important behaviours, such as the bat-evasion reflex in noctuid moths [74]. Computational network models based on data from individual auditory neurons in genetically tailored *Drosophila* have been built and they have faithfully predicted key aspects of the flies' auditory behaviour [75].

In vertebrates, in contrast, the number of auditory neurons always *increases* from the periphery (cochlea) to the centre (auditory cortex) by as much as four orders of magnitude [76]. These neuroanatomical differences suggest associated functional differences: insect auditory systems are listening for sets of well-defined and species-specific sounds, whereas vertebrates, for example humans, use their auditory systems to extract a broader, and often less predictable, information about the environment. Indeed, we can hear *anything* between 20 Hz and 20 kHz, the typical frequency range of our (healthy) inner ears. The auditory system of insects might thus be thought of as a special-purpose, rigid system, adapted to detect only a handful of signals, mostly mate and predator calls. This view accords with the finding that information within the insect brain is quickly distributed into multiple parallel (and decorrelated) 'labelled lines' for the separate extraction of individual stimulus features [77]. The vertebrate auditory system, in contrast, is a general-purpose and more flexible system that is shaped by learning [78-81], affected by mood [82], focused by attention [83-85], and one that influences and is influenced by other parts of the brain, both sensory [86] and motor [87], to detect and interpret any sound that may be subjectively important at any particular moment. Our auditory system reconfigures its functional connectivity on the fly [88, 89].

This property is important because, according to the principle of efficient coding, if an animal is to extract the maximal amount of information about a stimulus whose statistics varies in time, as it usually does in natural settings, then neuronal ensembles must adjust their responses to match the varying stimulus statistics [90]. On the other hand, from the point of view of a decoder, an important property of the neuronal response is its invariance, two good examples being contrast-invariant responses to gratings in the visual cortical area V1 [91] and a time-warp-invariant processing of auditory cues [92-94]. What this means is that cortical circuits must efficiently and flexibly combine *selectivity* with *invariance* in pattern recognition.

Natural sounds, including speech, are produced by vibrating bodies and are characterized by a rapid onset and a slow decay of the envelope. The basilar membrane impulse response [95], a single hair-cell mechanical and electrical responses [96], and kernel functions representing natural sounds in efficient coding algorithms [97] all share the same asymmetrical Gabor-like shape, i.e., a sine wave multiplied by a Gaussian function. Gabor filters occur at various stages of visual and auditory processing in insects and vertebrates [98, 99]. Why are they so widespread?

One reason for a Gaussian envelope may be that a (normalized) dot product of a stimulus vector and a synaptic weight vector combined with a sigmoidal nonlinearity produces an approximately Gaussian tuning [100]. The dot product (the inner product between two vectors) would measure the similarity between a stimulus and a neuron's receptive field, and the nonlinearity comes from the action-potential generation mechanism. This is a biologically plausible way to obtain Gaussian tuning. In vision, it is well known that the Laplacian of the two-dimensional Gaussian distribution, which can be approximated by the difference of two Gaussians, e.g., with inhibition tuned more broadly than excitation, is an optimal operator to detect intensity changes in an image [101]. A second-order differential operator, it will produce filters that resemble Gabors. Crucially, the same neuronal circuit that can implement Gaussian tuning for selectivity can also implement, by using different parameter values, a MAX-like operation, which is important for invariance and one that was observed experimentally in the mammalian visual cortex [102, 103] and in the central auditory system of a songbird [104]. The predicted computational flexibility was experimentally verified in the European starling, a species of songbirds, where individual central auditory neurons could switch between the MAX-like operation for invariance and a tuning operation for selectivity depending on the stimulus and network state [104], displaying sparse responses and multidimensional selectivity [105].

A Gaussian tuning function is also useful for generalization after training on a limited data set. Specifically, radial Gaussian basis functions are smooth and interpolate well from training data to new data [106]. This property is useful for learning. Mathematically, Gabor filters have an optimal concentration both in space and time (frequency), thus introducing minimal distortions. Furthermore, Poggio et al. [107] have argued that learning invariances in the visual cortex to the group $SO_2 \times R^2$, a type of *symmetry* dealing with rigid rotation and translation, produces Gabor-like tuning. In other words, this particular *selectivity* property may be a consequence of having to deal with (and

discard) affine image transformations, which is an *invariance* property. If this argument proves correct, then it would indicate that selectivity and invariance, two pillars of natural object recognition, are not independent but are intimately connected as two parts of a whole. The similarity in central representations—across sensory modalities and animal classes—could then be linked to the conservation laws of physics, for any symmetry is associated, as Noether's theorem proves, with a conservation law.

Conclusions and future directions

Reflecting both evolutionary and functional kinship, the chordotonal organ-based ears of insects share multiple similarities with the hair cell-based ears of vertebrates. Starting with the fact that their cellular substrates, namely chordotonal neurons (ChNs) and hair cells (HCs), arise from their respective precursor cells through series of Notch- and basic helix-loop-helix (bHLH) transcription-factor-dependent, asymmetric mitoses [20, 108] the similarities extend further to the elementary process of auditory mechanotransduction, which in both HCs [109] and ChNs [110] is mediated by mechanically gated, mechanically adapting ion channels. Many features that are considered hallmarks of mammalian, or more generally vertebrate, auditory systems have been found to be phenocopied in the auditory systems of insects. Receptors in the auditory periphery of bushcrickets [111, 112], for example, are tonotopically arranged. Tonotopy is one of the fundamental organizational principles of the vertebrate inner ear, including the mammalian cochlea. As in vertebrates, the projection patterns of first-order sensory neurons in insect auditory systems can retain this tonotopic organization [113], but unlike in vertebrates, the tonotopy does not appear to extend to downstream interneurons [77]. Perhaps most strikingly of all, even the spectral decomposition of sound by way of dispersive wave propagation, which enables frequency analysis in the cochlea, has been found in an insect (*Copiphora gorgonensis* [114]). Insect ears, like their vertebrate counterparts, can produce 'phantom tones' when presented with two stimulus tones [115, 116], a phenomenon well known from the mammalian cochlea [117] and also demonstrated at the single hair-cell level [63, 118]. Even higher-order, cognitive performances, such as the categorical perception of sound frequency, have been reported in insects (*Teleogryllus oceanicus* [119]).

One of the reasons for the multiple similarities simply lies in the stunning diversity of insect hearing organs and auditory systems [120]. Based on species with tympanal ears only, it has been suggested that hearing has evolved independently at least twenty times

across the various insect orders [121]. The rich diversity of insect hearing organs, in turn, largely reflects the richness and diversity of insects themselves. Only a fleetingly small number of insect species is expected to possess a sense of hearing [122] but accounting for an expected 5.5 million species alone [123], insects have served, and will continue to serve, as a near inexhaustible treasure trove for research into hearing and acoustic communication.

A recent study may exemplify these relations and also indicate some directions that future research could take. Hearing in *Drosophila* was found to be independent of efferent control [36], and no efferent innervation had previously been reported for any other insect; but it has now been detected in the antennal ears of mosquitoes, where it modulates both the ears' frequency tuning and its amplificatory gain [124]. Efferent gain control is one of the key features of vertebrate, and specifically mammalian, hearing [125], thought to be key to the ears' exquisite auditory performance, especially in noisy environments (possibly contributing to the 'cocktail party effect'). Acoustic interactions between mosquitoes have proven more varied than previously anticipated (cf. [126, 127]), and most of them are likely to take place in larger swarms, as part of the animals' mating behaviour [128]. From an auditory perspective, such a mosquito mating swarm may well be described as the mother of all cocktail parties. It will be fascinating to see if, and if so how, efferent modulation can help to prime the mosquitoes' antennal ears for their challenging tasks. Some of the major challenges for hearing research, in turn, will be of translational nature, to translate insect studies into vertebrate research, translate findings from mosquitoes into experiments in *Drosophila*, translate studies on tympanal ears into studies on antennal ears and translate from molecular to mechanistic (and always *vice versa*).

To understand how hearing works at the most basic level—a spring pulling on an ion channel—it will be important to identify the molecular composition of both the gating spring and the MET channel. For a review of (un)likely MET-channel candidates in vertebrates, see [129]. Identifying and characterising molecular components of the mechanotransduction complex and their interaction with each other will help to understand how sounds perform work on the MET channels, i.e., the nature of the movement that transforms a sound's mechanical energy into the channel opening. This knowledge will also help to understand how channel gating contributes to sound amplification.

To understand how hearing works at the central level, i.e., at the level of algorithms and their implementation in the central auditory system, it will be important to establish more connections not only between insect and vertebrate hearing but also between insect and vertebrate *vision*. Indeed, selectivity and invariance are generic and sensory-modality-independent principles underlying natural pattern (object) recognition, but they have been mostly explored in natural and computer vision. Bringing that knowledge to bear on problems in hearing research will be a major task of future research. Finally, comparing *biological* representations of natural sounds with representations learned by *artificial* neural networks (i.e., adaptive computer programs) will help to reveal general principles of hearing in insects, vertebrates … and machines.

Acknowledgements

Work in the A.S.K. lab is funded by the BBSRC (BB/N008731/1), The Royal Society (RG140650), the Wellcome Trust (108034/Z/15/Z) and an Imperial College Network of Excellence Award. Work in the J.T.A. lab is funded by the BBSRC (BB/M008533/1 and BB/L02084X/1) and a grant from the European Research Council (H2020 - ERC-2014-CoG/648709/Clock Mechanics).

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Box 1. Can a tip-link polymer be a hair cell's gating spring?

All polymers can be divided into three classes depending on the ratio between their total length (contour length) L and the persistence length Lp that determines the distance over which the thermal forces de-correlate the tangent along the contour, i.e., oppose the tendency of the polymer to point in the same direction. If L>>Lp then a polymer is flexible: it can form loops, knots, and hairpins. Because there is only a single straight conformation of a molecule, pulling on it and straightening it decreases the entropy: stiffness of a flexible polymer has therefore a strong entropic component. If Lp>>L, a polymer is stiff. Because Lp>>L, the molecule is already straight, and therefore its stiffness is enthalpic and determined by the longitudinal compliance characterized by the elastic modulus. If L and Lp are approximately equal, however, a polymer is neither too flexible (does not form knots, for example) nor too stiff (is not a straight rod); it is *semi-flexible*. Most biological polymers, such as actin, tubulin, tropocollagen, and DNA are semi-flexible. For such polymers, *both* the entropic and the enthalpic components will contribute to the total stiffness.

To determine an order of magnitude for the stiffness of a tip link, its L, Lp, and the Young's modulus (E) of cadherins are required. The molecular-dynamics force-extension curves (Fig. S13 in Sotomayor and Schulten, 2008 [54]) give a stiffness *kappa* of C-cadherin in the presence of Ca^{2+} equal to 50 $mN \cdot m^{-1}$ (400 pN force for 8 nm extension). From which, using $E = \frac{\kappa L}{\pi r^2}$, the Young's modulus of ~ 0.37 GPa follows (Sotomayor and Schulten used L = 23 nm, and r = 1 nm). Thermal forces from the fluid will produce some bending and randomize the orientation of the polymer over the characteristic distance Lp. Unless the polymer is a homogeneous isotropic rod of a constant cross-section, the relation between E and Lp is unknown. Assuming Lp in the range of 20 to 100 nm from the EM micrographs in [49]—based on the observation that reconstituted

tip links were not straight but had several bends—one can estimate the combined stiffness of a tip link under tension using an equation originally derived by Odijk [130] in the form given in the Table 1 in [131]:

$$x = L[1 - \frac{1}{2}(\frac{k_B T}{F L_p})^{1/2} + \frac{F}{K}],$$

where x is the end-to-end distance, L is the contour length, F is force, and $K = \frac{\pi}{2} a^2 E$, where a is the radius of the tip link (4.5 nm). Adaptation motors provide several pN of pre-tension on the tip link. Taking the pre-tension of 10 pN from [132] and assuming Lp = 50 nm, one obtains the end-to-end distance of 162 nm (contour length 170 nm). The difference between the two lengths is stored in the transverse fluctuations. Then, a 1-pN increment of force elongates the polymer to 163 nm, corresponding to the stiffness of 2.7 $mN \cdot m^{-1}$. With the pre-tension of 5 pN and Lp = 50 nm, the end-to-end distance equals 159 nm (compare to 162 nm with 10 pN of pretension). As expected, less pretension corresponds to more transversal fluctuations. An additional 1 pN of force (now the total force is 6 pN) gives the end-to-end distance of 160 nm; and the stiffness is 964 $\mu N \cdot m^{-1}$. This value accords well with the experimentally estimated 0.4–4 $mN \cdot m^{-1}$ stiffness of the gating spring. Therefore, a tip link can be a hair cell's gating spring [133].

Box 2. How liquid in the inner ear has shaped the hair bundle.

A hair bundle operates at small Reynolds numbers on the order of 10^{-4}. Reynolds number is: $Re = uL\rho/\mu$, where u is the velocity, L is a linear dimension (e.g., a hair bundle's size), ρ is the density and μ is the dynamic viscosity of the fluid. Reynolds number indicates the relative importance of inertia over viscous forces for a particular type of flow. For a hair bundle, the Reynolds number much less than 1 indicates the relative importance of viscous forces.

When a solid object moves through a viscous fluid, the velocity of the fluid directly in contact with the object's surface is zero: the fluid sticks to the wettable surface (the 'no-slip' condition). This condition usually applies to hydrophilic surfaces [134]. Therefore, a gradient of fluid velocity develops in the moving object's neighbourhood. A layer of fluid is dragged along with the moving object. How far this influence spreads into the fluid, i.e., how thick is the 'boundary layer', depends on the velocity difference between the object and the fluid, on the size of the object, and on the fluid's density and viscosity (the

same factors that determine the Reynolds number). For example, a cylinder of the size of a single stereocilium oscillating at 30 Hz in water will create a boundary layer that is about 73 microns thick. Oscillating, it drags a thick layer of fluid with it and creates a gradient of fluid velocity spreading to distances much larger than its own diameter—in fact, even larger than the size of the whole hair bundle.

A hair bundle is a micrometer-sized array of cylinders that are very closely spaced: at the hair bundle's top, the ratio of the gap between two adjacent stereocilia to the diameter of a stereocilium is less than 0.1. Stereocilia are therefore completely immersed in each other's boundary layers. If fluid were forced to flow through a narrow gap between two adjacent stereocilia, a steep velocity gradient would have to develop in the fluid because of the 'no-slip' condition, and there would be a strong resistance, due to viscosity, to the flow through the gap.

The problem of flow through an infinite array of parallel cylinders at small Reynolds number was first solved by Tamada and Fujikawa (1957) [135], who found that, because of the steep velocity gradients, the drag on each cylinder in the array strongly increases compared to the drag on the same cylinder in isolation. In contrast, as they note, if an array of cylinders is finite, the fluid has an alternative to flow *around* the array rather than *through* it. In this case, very little fluid may pass between the cylinders and the drag on each of them can be much smaller that the drag on an identical isolated cylinder.

The same situation applies to a hair cell (Figure 4): by grouping stereocilia in a tightly packed hair bundle, evolution reduced the drag per stereocilium dramatically [63], making the sensitive hearing in vertebrates possible.

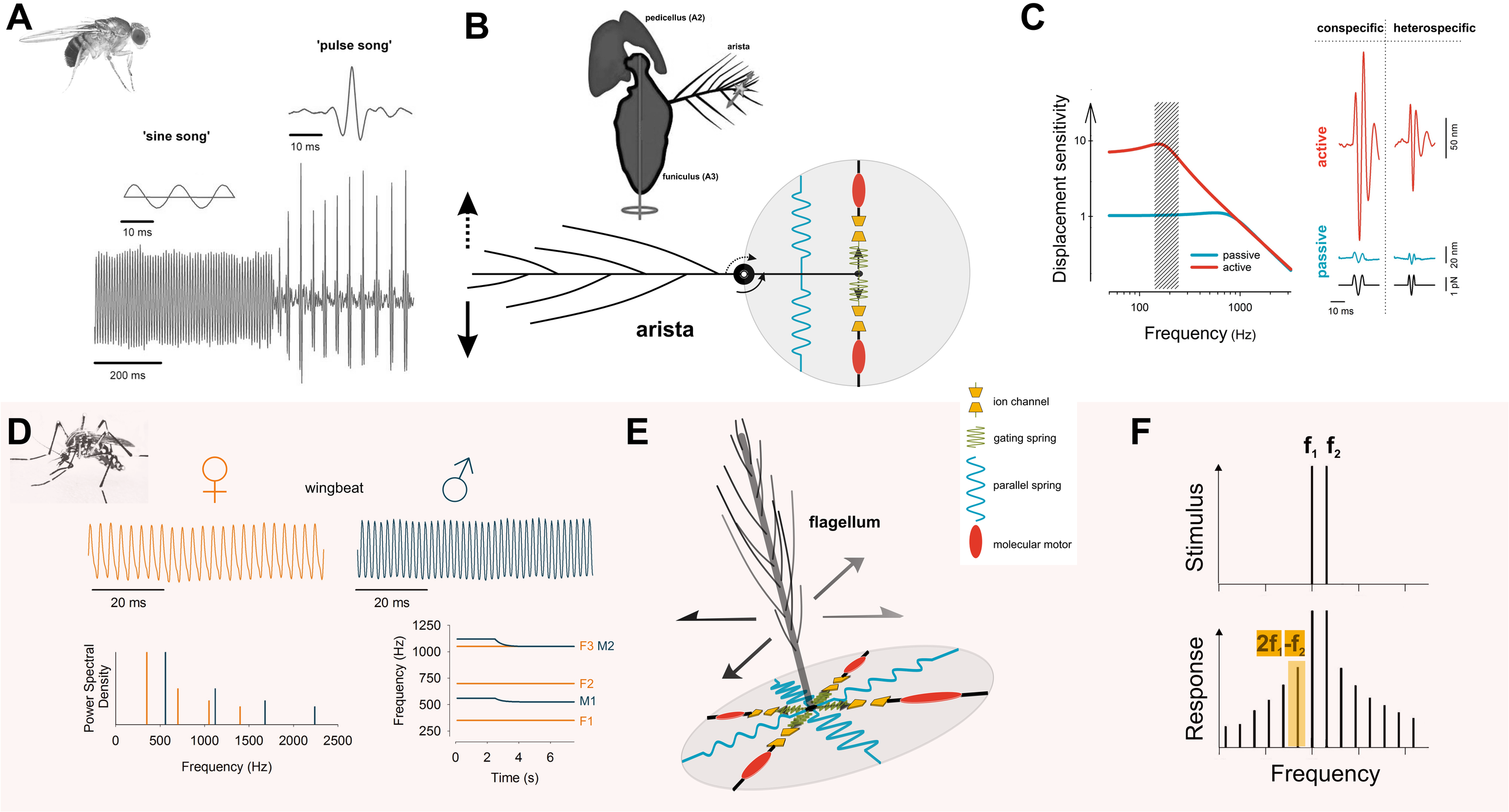
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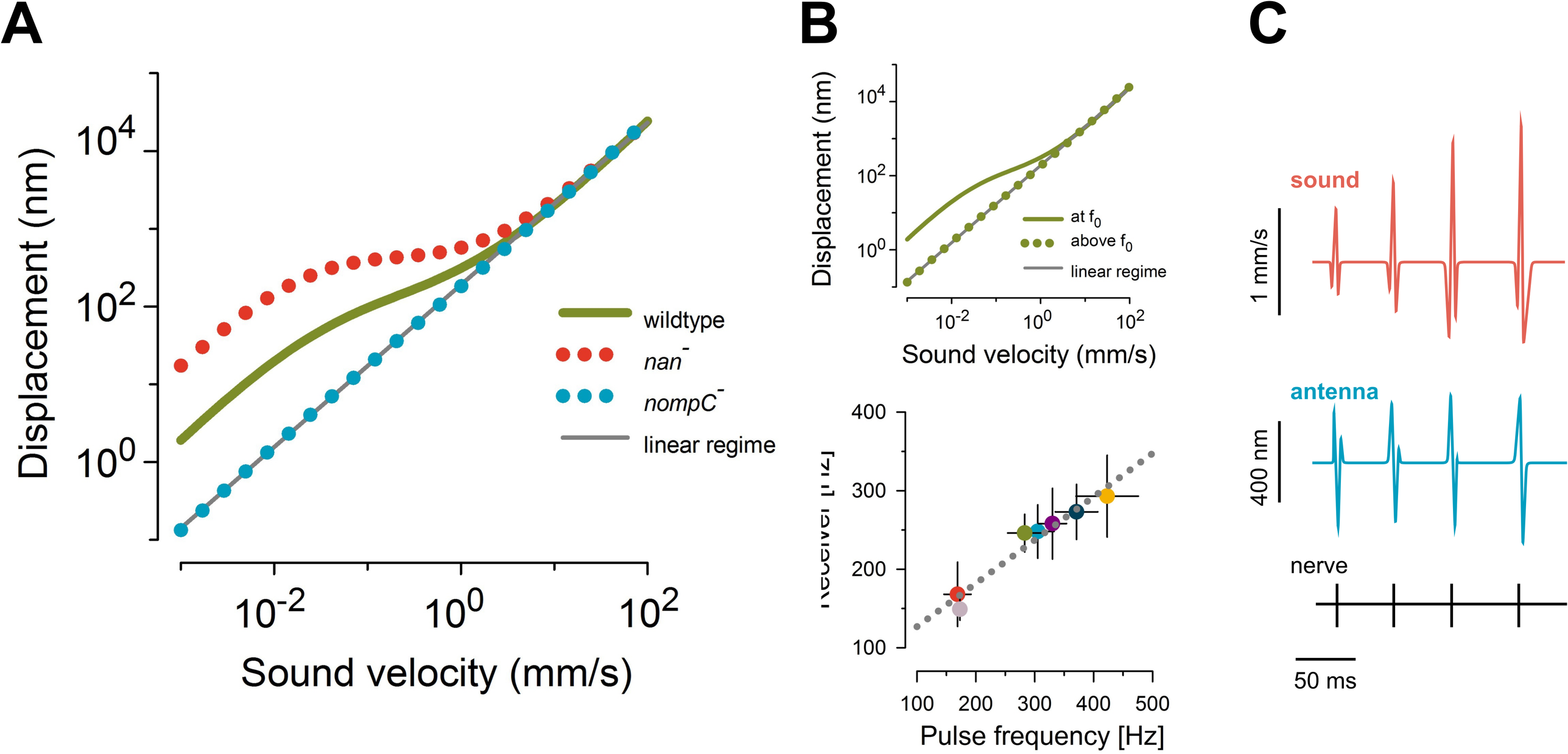
Figure 1. The sensory ecology of auditory transduction: antennal ears of Diptera. (Top: *Drosophila*; bottom: mosquitoes) From left to right: The landscape of signals used in the acoustic communication systems of fruit flies and mosquitoes is spectrally simple. It involves either pure tones (**A**, left: *Drosophila* sine songs; **D**, left: mosquito wingbeat) or minor modifications of sinusoidal oscillations (*Drosophila* pulse song, **A**, right). In some cases the emitted signal contains prominent higher-order harmonics next to its fundamental frequency (mosquito wingbeat; **D**, bottom). Sound signals in both fruit flies and mosquitoes are transduced by the mechanosensory neurons of the Johnston's Organ (JO-Ns) in the second antennal segments of their antennae (**B** and **E**). Within the membranes of JO-Ns, directly gated mechanotransducer channels are thought to associate with molecular motors to provide stimulus adaptation and amplification (overview schematics are shown in the middle panels, **B** and **E**). In *Drosophila* (**C**, left), transducer-based mechanical feedback amplification increases the antennal displacement sensitivity within the spectral range of courtship song components (hatched area) by a factor of ~10 (red) as compared to the passive system (blue). The example at the top right (**C**, right) shows the antennal deflection in response to a song pulse for an entirely passive antenna of *D. teissieri* (e.g., dead or CO_2-sedated, without contributions from active transducer modules, blue curves) and the active system (with contributions from active mechanotransducers, red curves). Note the marked increase of antennal deflection for the active state and the relative increase seen for a conspecific courtship pulse as compared to a (higher frequency) pulse of a different species (*D. yakuba*). Corresponding stimuli are shown in the bottom traces. In the ears of mosquitoes (**F**, right), a nonlinear process (most likely linked to transduction) generates distortion products. Even when stimulated with only two pure tones, f_1 and f_2 (e.g., male and female wingbeat), the antennal response will display additional tones. As these additional tones can be particularly prominent in the lower frequency range (highlighted is the cubic distortion product $2f_1$-f_2) this behaviour has been suggested as a mechanism to communicate beyond the actual auditory range [data recalculated from refs. [30], [136]].

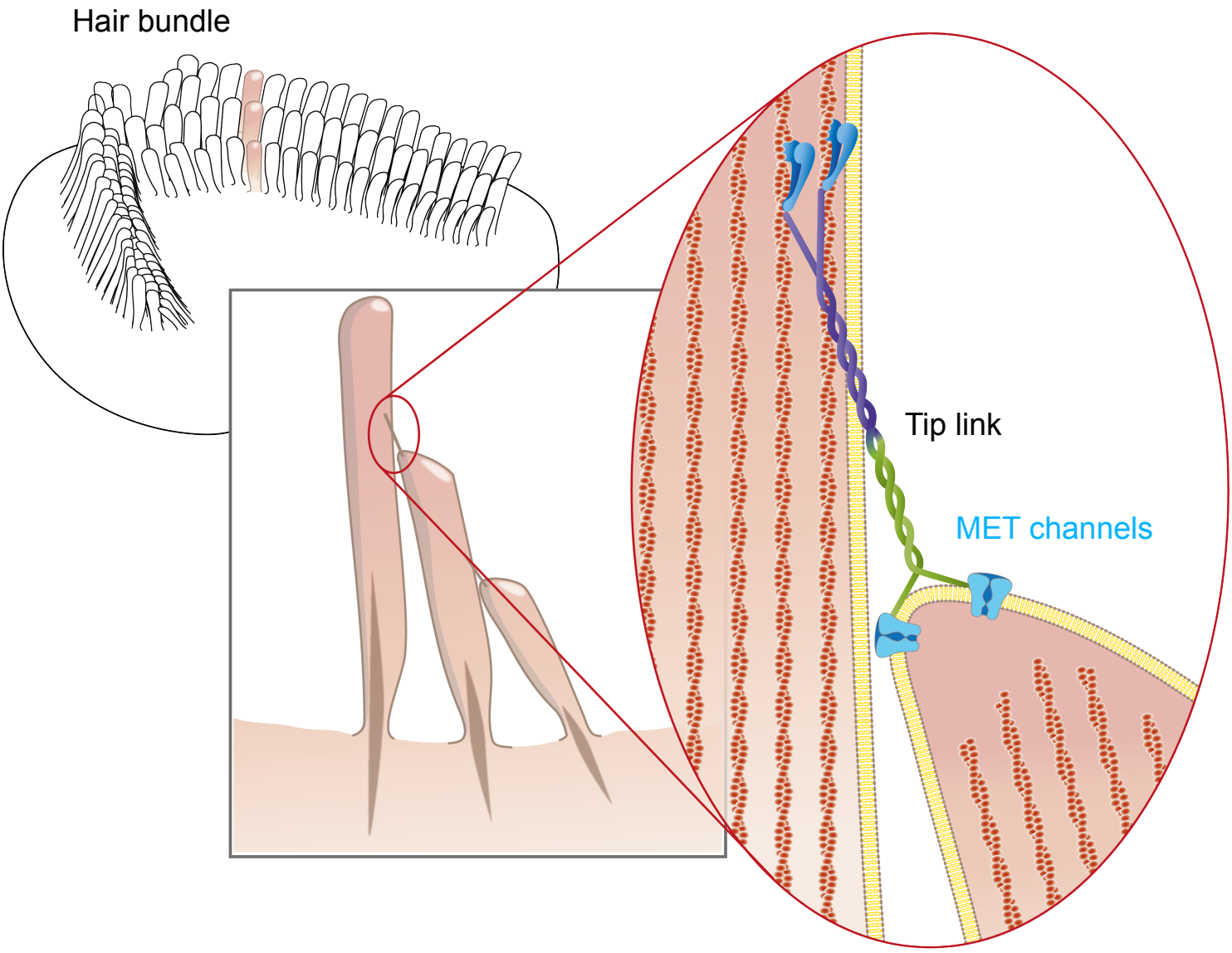
Figure 2. Transduction-dependent compressive nonlinearities reflect sensory ecological trade-offs in *Drosophila* acoustic communication. (**A**) When stimulated by pure tones at its best frequency, the wild type *Drosophila* antennal receiver displays a compressive nonlinearity, which produces relatively larger displacements for smaller stimuli (particle velocities) (green curve). This nonlinear compression originates from underlying saturating nonlinearities of mechanotransducer gating. Abolishing distinct functional components of the fly's auditory transduction chain can abolish (e.g., through loss of *nompC* function; *nompC*$^{-}$, blue) or exacerbate (e.g., through loss of *nanchung* function; nan^{-}, red) the extent of nonlinearity. The grey line depicts the linear regime of the passive antenna for comparison. (**B**, top) Transduction-related nonlinear amplification is frequency-dependent; shown is a loss of nonlinearity for stimulation ~ 2.5-times above the receiver's best frequency. (**B**, bottom) The receivers of different *Drosophila* species have been shown to display differential best frequencies, correlating with the spectral content of pulses produced by the corresponding conspecific males. (**C**) One direct result of nonlinear compression is the normalizing effect it has on response amplitude. Within the working range of the corresponding transducers, antennal deflections (middle trace) will be larger for small sound intensities (upper trace). This effect is intensity-dependent and becomes negligible for greater sound intensities, thereby reducing the effects of potential sound-amplitude differences. As stimulus amplitudes beyond the levels that saturate the transducers will not contribute to the nerve responses, this 'normalization' will be even greater at the level of the auditory nerve (bottom). As a result, a pulse train with natural amplitude variations will be converted into a train of nearly 'unitary' pulses, facilitating the robust encoding of inter-pulse intervals. Data recalculated from refs. [30], [136].

Figure 3. A schematic of a hair bundle, stereocilia, and a tip link connected to MET channels. (Left) A hair bundle of an outer hair cell (the cell body is not shown) with three rows of stereocilia. **(Middle)** A column of three stereocilia of progressively varying height. **(Right)** A zoom on the tip-link region, showing a pair of stereocilia filled with actin filaments, and a tip link composed of a cadherin-23 dimer (blue) and a protocadherin-15 dimer (green) attached to two MET channels (light blue).

Figure 4. Grouping of stereocilia in a hair bundle dramatically reduces the drag per stereocilium. Drag coefficient as a function of radius r of a cylinder displaced by a linear profile over the height on a fixed plate. The height is h = 8 μm. The drag coefficient is given for an equivalent force at the cylinder's tip. For the finite-element result (FEM) the liquid domain has an outer diameter of 62 μm and a height of 37 μm. For comparison, the analytical solutions for a rotating semi-ellipsoid (Analyt. ellipsoid) and for a cylinder (Analyt. cylinder) are shown. The latter is provided within the valid range of $r < 0.22\,h$. Experimental values of the drag coefficient for an isolated stereocilium and for an entire hair bundle without tip links are shown in black (n = 6 in each case). Note that the drag on N stereocilia in a hair bundle is only about three times greater than the drag on a single isolated stereocilium. This means that, when stereocilia are grouped in a bundle, N-3 stereocilia move as if they did not experience any viscous drag at all. With a typical N of 50–100 stereocilia, this effect decreases viscous dissipation dramatically. Adapted, with permission, from [63].


A
'pulse song'
10 ms
'sine song'
10 ms
200 ms
B
pedicellus (A2)
arista
funiculus (A3)
arista
C
conspecific
heterospecific
Displacement sensitivity
10
1
100
1000
Frequency (Hz)
passive
active
active
passive
50 nm
20 nm
1 pN
10 ms
D
wingbeat
20 ms
20 ms
Power Spectral Density
0
500
1000
1500
2000
2500
Frequency (Hz)
Frequency (Hz)
1250
1000
750
500
250
F3 M2
F2
M1
F1
0
2
4
6
Time (s)
E
flagellum
ion channel
gating spring
parallel spring
molecular motor
F
f1 f2
Stimulus
Response
2f1-f2
Frequency


A
Displacement (nm)
10^4
10^2
10^0
wildtype
nan-
nompC-
linear regime
10^{-2}
10^0
10^2
Sound velocity (mm/s)
B
Displacement (nm)
10^4
10^2
10^0
at f_0
above f_0
linear regime
10^{-2}
10^0
10^2
Sound velocity (mm/s)
400
300
200
100
100
200
300
400
500
Pulse frequency [Hz]
C
sound
1 mm/s
antenna
400 nm
nerve
50 ms


Hair bundle
Tip link
MET channels

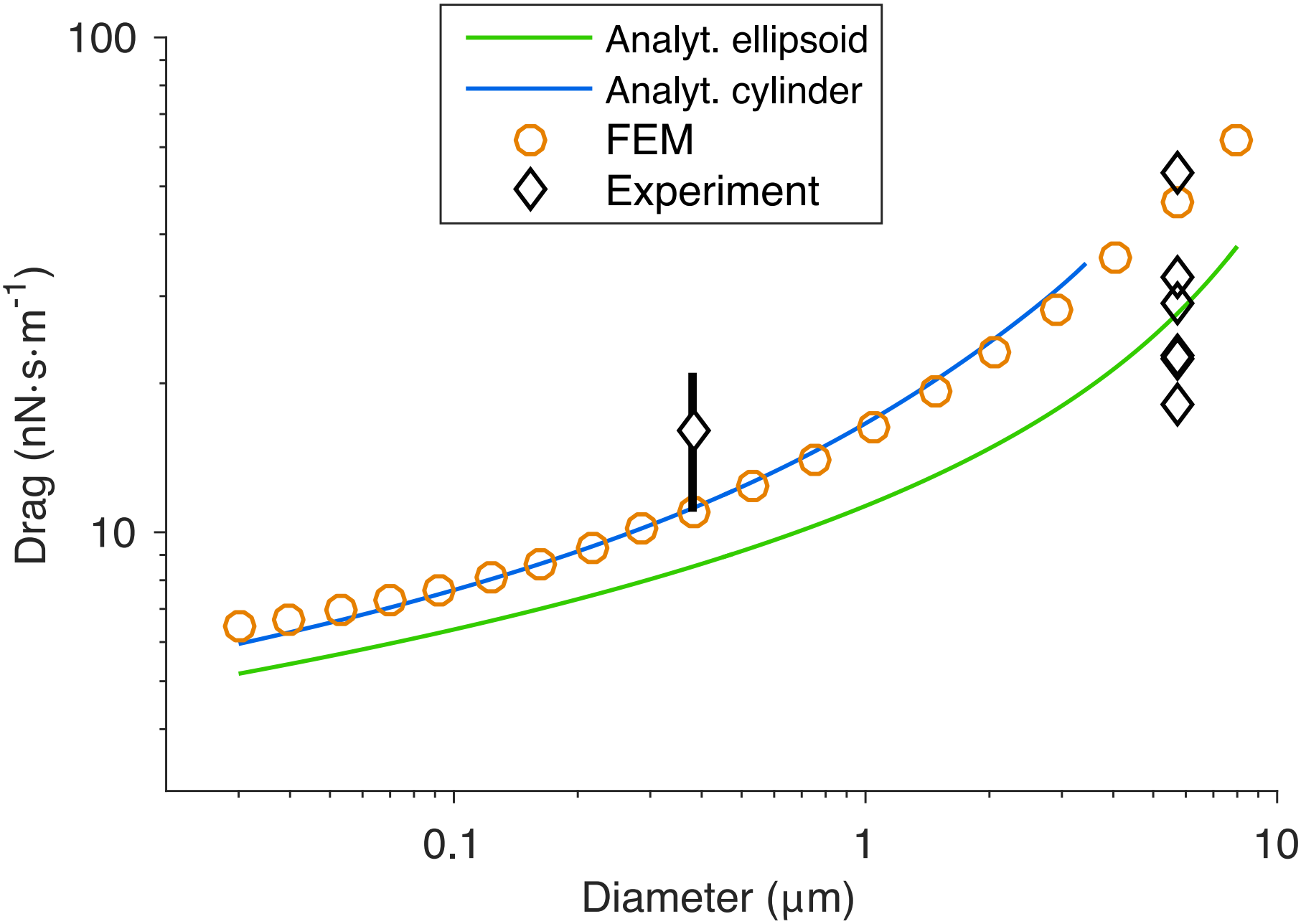

Analyt. ellipsoid
Analyt. cylinder
FEM
Experiment
100
10
Drag (nN·s·m^{-1})
0.1
1
10
Diameter (µm)

Figure 4