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Buzz pollination: Studying bee vibrations on flowers

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Summary

Approximately 6% of flowering plant species possess flowers with anthers that open through small pores or slits. Extracting pollen from this type of specialised flower is achieved most efficiently by vibrating the anthers, a behaviour that has evolved repeatedly among bees. Here I provide a brief overview of studying vibrations produced by bees and their effects on pollen release. I discuss how bee morphology and behaviour affect the mechanical properties of vibrations, and how floral traits may influence the transmission of those vibrations from the bee to the anther, thus mediating pollen release, and ultimately bee and plant fitness. I suggest that understanding the evolution of buzz pollination requires studying the biomechanics of bee vibrations and their transmission on flowers.

Keywords: Bees, behaviour, biomechanics, biotremology, buzz pollination, floral evolution, sonication, vibrations.

I. Introduction

Buzz pollination is a type of pollination in which bees use vibrations to remove and collect pollen from flowers incidentally fertilising them (Michener, 1962; Buchmann, 1983). Despite buzz pollination being known for more than a hundred years (Teppner, 2018), we are still at the early stages of understanding how floral and bee characteristics, including their biomechanical properties as well as the behaviour of bees, influence the release and collection of pollen, and ultimately plant and pollinator fitness (De Luca & Vallejo-Marín, 2013). Recently, there has been a surge of interest in the study of buzz pollination, from documenting the macroevolution of buzz-pollinated floral morphologies (Dellinger *et al.*, 2018) to characterising buzz pollination ecology and behaviour in both field (Corbet & Huang, 2014; Switzer & Combes, 2017; Mesquita-Neto *et al.*, 2018) and laboratory settings (Russell *et al.*, 2016; Whitehorn *et al.*, 2017; Arroyo-Correa *et al.*, 2018). More generally, buzz pollination provides the opportunity to investigate basic evolutionary questions including the origin and demise of complex floral adaptations (Dellinger *et al.*, 2018), the convergent evolution of flower form and function across disparate plant families (De Luca & Vallejo-Marín, 2013), evolutionary biomechanics, the evolutionary ecology of pollen rewards (Larson & Barrett, 1999), the balance between antagonistic and mutualistic interactions between plants and pollinators (Mesquita-Neto *et al.*, 2018), as well as to address fundamental questions on learning and cognition of complex motor routines in invertebrates (Russell *et al.*, 2016). The goal of this review is to summarise classic and recent work on buzz pollination, with a particular emphasis on how buzz pollination works, and to provide a framework to study the vibrational component of buzz pollination. Because the interaction between flowers and bees during buzz pollination occurs mainly in a vibrational context, studies of buzz pollination can take advantage of some of the methodological and conceptual tools developed for studying vibrations in other biological fields, namely bioacoustics and substrate-borne animal communication (biotremology) (Hill & Wessel, 2016; Mortimer, 2017).

II. Buzz pollination is an interaction between bees and flowers

Buzz pollination describes an interaction between plants with characteristic floral morphologies and a particular type of behaviour exhibited by some bees—bees are the only animals that use vibrations for pollen collection (De Luca & Vallejo-Marín, 2013), although more work is needed to determine if some flies may also collect pollen using vibrations (Buchmann *et al.*, 1977). Although bees use vibrations to collect pollen from flowers with varied morphologies (De Luca & Vallejo-Marín, 2013), typically, these flowers have poricidal anthers, which are present in roughly 6% of angiosperm species (Buchmann, 1983) across multiple different families (Vallejo-Marin *et al.*, 2010). Moreover, in addition to poricidal anthers, there are other ways to build a buzz-pollinated flower. For example, in some species of *Pedicularis*, longitudinally-dehiscent anthers are kept inside a narrow corolla tube, concealing pollen as in a poricidal anther, and bees vibrate these corolla tubes in order to rapidly extract pollen (Macior, 1968; Corbet & Huang, 2014). Thus, buzz-pollination is best thought as being associated with functional specialisation of flowers in which pollen release is restricted by modification of the stamens and, in some case, other floral parts.

Although plants with poricidal anthers are assumed to be most commonly or exclusively pollinated by vibration-producing bees (Buchmann, 1983), bees use vibrations to collect pollen from different types of flowers (De Luca & Vallejo-Marín, 2013; Russell et al., 2017). The ability to produce vibrations while visiting flowers has evolved repeatedly in the evolutionary history of bees (45 times; Cardinal *et al.*, 2018), but not all bees can use vibrations to collect pollen. Thus it is important to distinguish between the behaviour (producing vibrations on flowers or **floral vibrations**), the reliance on vibration-producing bees to set seeds (**buzz-pollinated plants**), and the pollination type or syndrome (**buzz pollination**) (for definitions see Supplementary Materials S1). Buzz pollination captures an interaction between bees and flowers representing the confluence of two related but separate phenomena: (1) The production of vibrations by bees, and (2) the effects of those vibrations on pollen release.

III. Bee vibrations

Bee species, morphology and behaviour affect floral vibrations

Bee characteristics including species identity, individual size, and behaviour influence the mechanical properties of vibrations (King, 1993; Arroyo-Correa *et al.*, 2018). Bees produce vibrations in a variety of behavioural contexts, including communication (Hrncir *et al.*, 2008), defence (De Luca *et al.*, 2014), and pollen collection (Buchmann *et al.*, 1977). Although these different vibrations are generated through the same mechanism of thoracic muscle contractions (Hrncir *et al.*, 2008), they may differ in their mechanical properties. For example, bee species differ in the frequency of floral vibrations even when visiting the same plant species (De Luca *et al.*, 2014), and the same bee species produces vibrations with different frequency and duration when visiting different plant taxa (Switzer & Combes, 2017). Yet, it remains unclear what characteristics of a bee influence the vibrations they produce during floral visitation. Some evidence suggest that bee size is positively associated with vibration amplitude (peak velocity) (De Luca *et al.*, 2013), but not with frequency (Burkart *et al.*, 2011; De Luca *et al.*, 2013; Rosi-Denadai *et al.*, 2018). Moreover, observations of bees visiting the same plant species in experimental arenas indicate that the frequency (Morgan *et al.*, 2016; Whitehorn *et al.*, 2017) and the magnitude and duration of their floral vibrations (Russell *et al.*, 2016) changes as an individual bee gains experience at manipulating flowers, although it is still unknown the extent to which this represents associative learning by the bee (Russell *et al.*, 2016; Switzer & Combes, 2016). We are still at the early stages in understanding to what extent bee characteristics, including morphology, behaviour and learning, influence the types of vibrations produced during floral visitation and more work is needed in this area.

IV. Characterising floral vibrations

Buzz pollination takes its name from the audible component, or “buzz”, that can be heard when a bee vibrates a flower (Macior, 1964). For this reason, the behaviour in which bees produce vibrations while collecting pollen from flowers is often called sonication (Cardinal *et al.*, 2018). Although, it was previously suggested that pollen release was the result of the combined effects of “acoustic turbulence” and anther vibrations (DeTar *et al.*, 1968), our current understanding suggests that sound is a by-product of the bee’s vibrations (Figure 1A) which do not contribute to pollen extraction (Buchmann, 1983; Cocroft & Rodriguez, 2005). The vibrations produced by the thoracic muscles are transmitted to the flower via direct physical contact of the bee’s body including the head (bees usually bite the anthers while vibrating, Russell *et al.*, 2016), thorax, abdomen, and to a lesser extent the legs (King, 1993) (Figure 1A). Thus, sonication (applying sound to agitate particles) may be a misleading term when studied in the context of pollen extraction, and it may be best to use “floral vibrations” or “vibrations on flowers” (See Supplementary Material S1 for a glossary). In any case, buzz pollination is a vibrational phenomenon characterised by both acoustic and substrate-borne components (Figure 1A).

Bee vibrations: acoustic and substrate-borne components

Floral vibrations are relatively complex. The vibrations produced by bees during pollen collection, can consist of a single continuous vibration lasting up to a couple of seconds or of multiple short vibrations lasting from a few tens to hundreds of milliseconds (King, 1993; King & Buchmann, 1996; De Luca & Vallejo-Marín, 2013; Arroyo-Correa *et al.*, 2018) (Figure 2). For clarity, it may be useful to define a “buzz” as an uninterrupted vibration, independently of its duration (Supplementary Materials S1). Floral vibrations contain a fundamental frequency (100-400 Hz), which often coincides with the peak or dominant frequency, and multiple harmonics of rapidly decreasing magnitude (King, 1993). The characteristics of the vibrations experienced by the anthers are also influenced by the stiffness, mass and material properties of the flower (DeTar *et al.*, 1968; Buchmann & Hurley, 1978; Arroyo-Correa *et al.*, 2018), and should change as the bee adjusts its grip onto the flower (King, 1993). The vibrations produced by bees on flowers can be characterised acoustically or as substrate-borne vibrations on the flower (Figure 1A), and both can be described with the same basic parameters of oscillatory movement (frequency, amplitude, duration) (Cocroft *et al.*, 2006). Nevertheless, the acoustic and substrate-borne vibrations do not necessarily share the same properties, particularly in their energy transfer to a substrate (Cocroft & Rodriguez, 2005; Mortimer, 2017). For instance, the frequency and duration of bee vibrations are accurately estimated either from acoustic or substrate-borne components, but the amplitude of the vibration is poorly correlated between acoustic and plant-borne components (De Luca *et al.*, 2018).

Frequency and amplitude are needed to characterise vibrations

A major challenge in current studies of buzz pollination is to achieve consistency in the terminology and methodology to characterise the vibrations produced by bees and transmitted to flowers (Supplementary Materials S1). Vibrations can be characterised by decomposing them into a sum of simple sinusoidal waves (Box 1), which can be described by their frequency (e.g., number of cycles per second, Hz) and amplitude (the magnitude of change of sinusoidal motion) (Sueur, 2018). Because amplitude can be quantified using velocity, acceleration or displacement (Sueur, 2018) it is necessary that the variable described or analysed is clearly specified, e.g., peak velocity amplitude (VA), peak acceleration amplitude (AA) or peak displacement amplitude (DA). This is also important because these different measurements of amplitude are not equally related to frequency (Figure 3; Supplementary Materials S2), and thus, the relationship between amplitude, frequency and a variable of interest (bee size or pollen release), will depend on how amplitude is expressed (Rosi-Denadai *et al.*, 2018).

In simple sinusoidal motion, peak velocity, acceleration, displacement and frequency are related to each other. Knowing the absolute magnitude of two of these variables (e.g., frequency in Hz and acceleration in ms^{-2}) allows estimating the other two (e.g., velocity and displacement) (See Supplementary Material S2). Thus a full characterisation of the vibrations observed during buzz pollination requires measuring two of these variables (King & Buchmann, 1996). Full characterisation is essential as vibrations of the same frequency may have drastically different consequences for the force with which a bee vibrates depending on the acceleration, velocity and displacement of the oscillation (Figure 3). This in turn will change the force of the substrate-borne vibrations transmitted to the flower (Buchmann & Hurley, 1978).

V. Vibrations and their effect on pollen release

We are still in the early stages of understanding the mechanism by which floral vibrations result in pollen ejection. Theoretically, the acceleration of the anther tip during vibrations should create centrifugal forces on the pollen grains causing them to be expelled out of the apical pore (King & Buchmann, 1996). In Buchmann and Hurley's (1978) model, pollen is expelled as a result of the kinetic energy transmitted from the internal walls of the vibrating anther and the pollen grains through elastic collisions. Here, pollen ejection is a function of anther geometry and the frequency and amplitude velocity of the vibrations experienced by the anther. In addition to these mechanical effects, it has been suggested that electrostatic interactions between the pollen grains and the anther walls may also play a significant role in expelling pollen from anthers (Corbet & Huang, 2014), but there is no direct evidence for this yet. Although we still lack a full model of pollen release in vibrating anthers, in principle, the accumulation of kinetic energy, centrifugal forces and, perhaps, electrostatic interactions could jointly contribute to the expulsion of pollen grains following bee vibrations.

Empirical studies suggest that the characteristics of the vibrations applied to the anthers affect pollen release (King & Buchmann, 1996). Artificial vibrations between 450-1000 Hz remove twice as much pollen than vibrations of 400 Hz (Harder & Barclay, 1994). However, within the range of frequencies produced by some bumblebees (240-405 Hz), frequency has a modest effect on pollen release (De Luca *et al.*, 2013). In contrast, peak velocity amplitude and duration have a much stronger, positive effect on pollen release during artificial vibrations (De Luca *et al.*, 2013). Intriguingly, a recent playback study found that amplitude (measured as acceleration, velocity or displacement) is positively associated with pollen release in *Solanum lycopersicum*, but the relationship of pollen release with frequency depended on whether the amplitude of the vibration was included as a covariate as velocity amplitude (positive association) or acceleration amplitude (negative association) (Rosi-Denadai *et al.*, 2018). These results indicate that pollen release is jointly influenced by multiple characteristics of the vibration, and that studying one variable "in isolation" (e.g., frequency), may not be sufficient to describe the force being transmitted to the anther and its effect on pollen release.

VI. Measuring vibrations transmitted from the bee to the anther

While estimating the frequency of bee floral buzzes is relatively straightforward (De Luca *et al.*, 2018), measuring the magnitude of the vibrations experienced by the anthers (e.g., acceleration, velocity or displacement amplitude) when a bee vibrates a flower is technically challenging. Direct measurement of the anthers' movement is complicated as the bee partially or entirely covers the anthers while vibrating the flower (Buchmann, 1983), and measuring the vibrations on the bee itself during natural pollination is practically difficult. An indirect approach consists of using accelerometers attached to another part of the flower, e.g. the pedicel (King & Buchmann, 1996) or the calyx (Arroyo-Correa *et al.*, 2018). Accelerometers can measure movement with high precision, but have the disadvantage of adding a mass to the flower (Cocroft *et al.*, 2014). In contrast, laser vibrometers can measure movement without mass loading (King & Buchmann, 2003). Measurements using either laser vibrometry or accelerometers are usually not taken on the anthers (De Luca *et al.*, 2013). This adds a variable to the measurements, namely the structural transmission path between the vibration source (the bee), the plant structure of interest (the anther) and the sensor (King, 1993; Gibson & Cocroft, 2018). The structural transmission path should be a function of the material properties of flowers and their architecture, including the characteristics of the structures connecting the anthers to the rest of the flower (DeTar *et al.*, 1968). To date, little is known about the material properties of flowers in the context of vibrations or how vibration characteristics vary over the relatively short distances between the anther and other floral parts. However, available work suggests that flowers indeed dampen vibrations even over short distances, and the amplitude (acceleration) of substrate-borne vibrations can vary significantly between different parts of the same flower (King, 1993; Arroyo-Correa *et al.*, 2018). Interestingly, even closely related plant species differ in their capacity to transmit vibrations suggesting that the material and structural properties of the flower are important in mediating the transmission of substrate-borne vibrations (Arroyo-Correa *et al.*, 2018).

VII. Conclusions

Over the last years, we have made large advances in studying the biomechanics, ecology and evolution of buzz pollination, however, two areas that are still in their infancy are: (1) experimentally testing evolutionary theories on the evolution and adaptive significance of buzz pollination, and (2) developing our understanding of the evolutionary biomechanics of buzz pollination. The first area will require, among other things, the estimation of the fitness consequence of different floral and bee characteristics, preferably under field-realistic conditions. The second will require bridging the pioneering work on buzz pollination biomechanics (DeTar *et al.*, 1968; Buchmann & Hurley, 1978; King, 1993), with a more profound understanding of bee vibrations (e.g., Hrncir *et al.*, 2008) and vibrational properties of plant structures (Arroyo-Correa *et al.*, 2018), which is a topic under rapid development in the young field of biotremology (Hill & Wessel, 2016; Mortimer, 2017; Gibson & Cocroft, 2018). By integrating the areas of animal behaviour, floral evolution and biomechanics, we have the opportunity to take buzz pollination from an initial discovery phase to a more mature, predictive field.

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Supporting Information. Note S1. Glossary of terminology commonly used in buzz pollination studies and suggestions for standardising it.

Supporting Information. Note S2. Interactive application (Shiny App) for computing and visualising sinusoidal waves from user-provided values of frequency and one additional parameter (peak velocity, peak acceleration or peak displacement).

Fig. 1 Vibrations produced by a bee visiting a buzz-pollinated flower. Upper panel: diagram of a bee vibrating the anther cone (in yellow) of a *Solanum*-like flower with poricidal anthers. Lower panel: diagram showing a single poricidal anther (in yellow) and three axes of vibration (*x*, *y*, *z*). The indirect flight muscles cause cyclical deformation of the bee's thorax, which result in vibrations (1). These vibrations are transmitted to the anther cone (4) by direct contact with the thorax, head, abdomen and to a lesser extent the legs (1–3). Vibrations are also transmitted to other parts of the flower including the petals and sepals (5). The vibrating bee also transfers energy to the surrounding air, which result in an audible component (sound; 6). Although this sound is what gives this behaviour its name (i.e. sonication or buzz pollination), the contribution of the acoustic component to the shaking of the anthers is negligible. Pollen is expelled from the anthers because of the vibrations transmitted from the bee to the anthers. In Buchmann & Hurley's (1978) biophysical model of buzz pollination, the rate of pollen release from the anther is proportional to the velocity with which the anther vibrates along either the *y*- or *z*-axes.

Fig. 2 Floral vibrations are relatively complex. The vibrations produced by a bee during pollen collection can consist of a series of multiple short vibrations (a). The characteristics of the vibrations experienced in the anthers of a flower are also affected by the stiffness, mass and material properties of the floral organs, as well as by the way in which a bee grasps the anthers. (a) Oscillogram showing instantaneous changes in amplitude (measured as acceleration, m s^{-2}) through time. (b) Frequency spectrum of the first vibration in the buzz (indicated inside the dashed lines in a). Amplitude is shown in a relative scale. The fundamental frequency in this vibration is also the dominant frequency (*c*. 370 Hz; window length = 512). (c) Spectrogram showing the magnitude of vibrations (relative amplitude, dB) across a range of frequencies (0-2.5 kHz). Lighter (yellow) areas represent frequencies of higher amplitude. The choice of analysis parameters, particularly the window length over which the FFT is calculated, determines the resolution at either temporal or frequency scales. Increased resolution at the frequency level come at a cost of decreased resolution of how the vibration changes through time. Here the window size was set to 128 samples with 75% overlap between windows. The recording shown here represents a sample of the vibrations produced by *Bombus terrestris audax* while visiting a flower of *Solanum citrullifolium* (Solanaceae) (d). The acceleration of the vibrations was measured with an accelerometer attached to the base of the flower using a metallic pin, recorded in an oscilloscope at a sampling rate of 5000 Hz (Arroyo-Correa *et al.*, 2018) and plotted in the *R* package *seewave* (Sueur, 2018).

Fig. 3 Vibrations produced by bees of different size likely differ in the magnitude of the force that can be applied to the flower. A large and a small bee can achieve, in principle, the same frequency during floral visitation (e.g. 300Hz). However, at a given frequency, the amplitude (e.g. peak displacement) of the maximum vibration that can be produced should be larger for bigger than for smaller bees (Corbet & Huang, 2014) due to their increased thorax size. For a given frequency, an increase in displacement (specifically peak displacement) will affect the magnitude of the peak acceleration and peak velocity of the vibration produced, as all these quantities are related in harmonic vibrations. Changes in both acceleration of the vibration and mass of the bee will change, in turn, the force that can be transmitted to the flower ($f = m \times a$). Lines indicate the relationship between frequency and either peak velocity (a) or peak acceleration (b) for simple sinusoidal vibrations of different peak displacements. Solid line, peak displacement, DA = 4µm; dashed line, DA = 2µm.

Box 1 Properties of vibrations

Vibrations can be described as the oscillation of a particle around an equilibrium position (Mortimer, 2017). Vibrations on a substrate have complex patterns and contain multiple frequencies simultaneously (Mortimer, 2017). These signals can be analysed by decomposing vibrations into a summation of simple sine waves, each of them with its own magnitude and frequency (Sueur, 2018). The magnitude of a vibration is affected by damping or energy loss, which is a function of the substrate properties, the medium that surrounds the substrate, and the type of vibration produced (Cocroft & Rodriguez, 2005; Mortimer, 2017). Body, boundary and bending waves form in different geometric contexts, and describe wave propagation in three, two and one dimensions, respectively. Bending waves form when rod-like structures, for example plant stems, are vibrated (Mortimer, 2017). Bending waves are a type of perpendicular waves, that is, waves where the direction of particle oscillation is perpendicular to the direction of wave propagation (Cocroft & Rodriguez, 2005). Perpendicular waves are thought to be the most important waves in substrate-borne communication (Cocroft & Rodriguez, 2005; Cocroft *et al.*, 2006; Gibson & Cocroft, 2018), and includes Rayleigh waves (Mortimer, 2017). Rayleigh waves are a mix of longitudinal and perpendicular waves produced at the boundary between media which propagate in media boundaries (Hill & Wessel, 2016). Both Rayleigh and bending waves displace particles with a shear force that is perpendicular to the plane of propagation (Hill & Wessel, 2016). The vibrational properties of plant structures is still little understood in nature (Cocroft & Rodriguez, 2005).

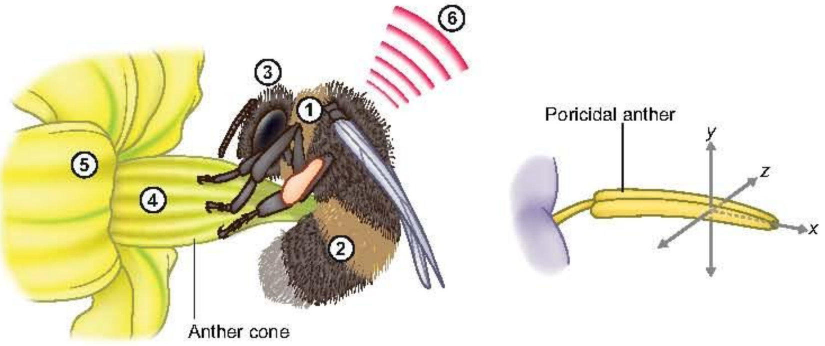

Figure 1

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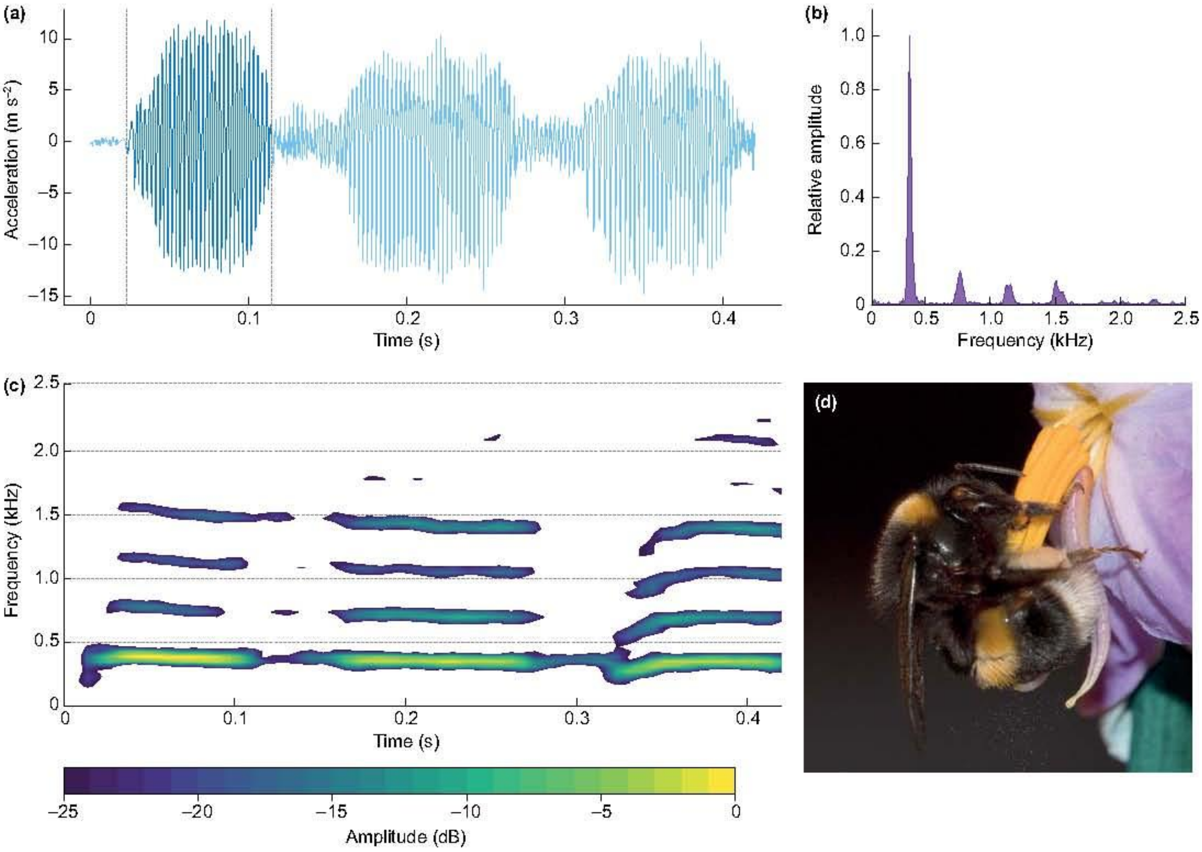

Figure 2

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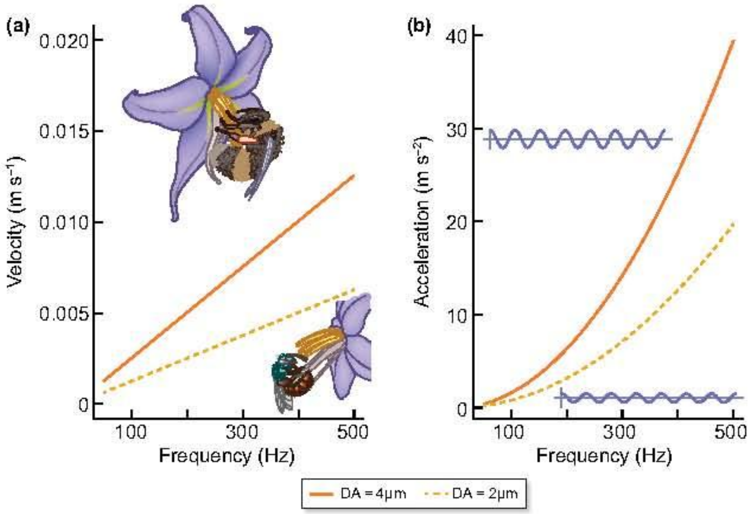

Figure 3

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