

RESEARCH ARTICLE

Floral vibrations by buzz-pollinating bees achieve higher frequency, velocity and acceleration than flight and defence vibrations

David J. Pritchard* and Mario Vallejo-Marín

ABSTRACT

Vibrations play an important role in insect behaviour. In bees, vibrations are used in a variety of contexts including communication, as a warning signal to deter predators and during pollen foraging. However, little is known about how the biomechanical properties of bee vibrations vary across multiple behaviours within a species. In this study, we compared the properties of vibrations produced by *Bombus terrestris audax* (Hymenoptera: Apidae) workers in three contexts: during flight, during defensive buzzing, and in floral vibrations produced during pollen foraging on two buzz-pollinated plants (*Solanum*, Solanaceae). Using laser vibrometry, we were able to obtain contactless measures of both the frequency and amplitude of the thoracic vibrations of bees across the three behaviours. Despite all three types of vibrations being produced by the same power flight muscles, we found clear differences in the mechanical properties of the vibrations produced in different contexts. Both floral and defensive buzzes had higher frequency and amplitude velocity, acceleration and displacement than the vibrations produced during flight. Floral vibrations had the highest frequency, amplitude velocity and acceleration of all the behaviours studied. Vibration amplitude, and in particular acceleration, of floral vibrations has been suggested as the key property for removing pollen from buzz-pollinated anthers. By increasing frequency and amplitude velocity and acceleration of their vibrations during vibratory pollen collection, foraging bees may be able to maximise pollen removal from flowers, although their foraging decisions are likely to be influenced by the presumably high cost of producing floral vibrations.

KEY WORDS: Apidae, Bee behaviour, Biomechanics, Biotremology, *Bombus*, Buzz pollination, Energetic costs, Flight, Poricidal anthers, *Solanum*

INTRODUCTION

Vibrations play an essential role in the natural behaviour of animals, particularly among invertebrates. For example, spiders and antlions use vibrations produced by prey during hunting (Mencinger-Vračko and Devetak, 2008; Guillette et al., 2009; Nakata, 2010), and larval leafminers use vibrations to detect and avoid parasitoid wasps (Djemai et al., 2001). Animal vibrations can be transmitted both through the air (sound) and through the underlying substrate (most often plant tissue) as substrate-borne vibrations (Cocroft and

Rodríguez, 2005). The substrate-borne component of vibrations can be particularly important in some contexts such as during insect communication because vibrations produced by small animals can be more efficiently transmitted through the substrate than through air (i.e. as sound) (Barth et al., 2005; Cocroft and Rodríguez, 2005; Mortimer, 2017).

Most studies of insect vibrations have focused on vibrations produced for communication or as a by-product of flight (Tercel et al., 2018; Hill et al., 2019). However, insects can use vibrations for much more than communication and locomotion. Among bees, vibrations play a particularly multifaceted role. For example, bees not only use vibrations to communicate with their nest mates (Barth et al., 2005) and as a warning or defence mechanism against potential predators (Barth et al., 2005; Hrncir et al., 2008), but also during nest construction (Rosenheim, 1987), and as a foraging tool to harvest pollen from certain flowers (Macior, 1964; Thorp, 2000; Vallejo-Marín, 2019). For example, substrate-borne vibrations are one of the ways in which some bees can rapidly dislodge and collect pollen on flowers with poricidal anthers (anthers that release pollen through small pores or slits; Buchmann, 1983). The ability to use vibrations during pollen harvesting occurs in approximately 58% of all bee (Anthophila) species including 15% of genera in all bee families (Cardinal et al., 2018), and buzz-pollination (pollination using vibrations) is associated with more than 20,000 species of flowering plants (Buchmann, 1983; De Luca and Vallejo-Marín, 2013). Despite the widespread use of vibrations across diverse behavioural contexts, including during buzz-pollination, we still know relatively little about the extent to which vibrational properties vary within the same species and across behaviours.

In bees, the same mechanism that drives the wings during flight is responsible for producing vibrations used during communication, defence and buzz-pollination. Vibrations are produced by cyclical deformations of the bee's thorax caused by the alternate contraction of dorsal longitudinal and dorsoventral power flight muscles (Hedenström, 2014). These contractions are not synchronised with nerve impulses; instead bee flight muscles are 'stretch-activated', with the stretching of one of the antagonistic pairs of muscles stimulating the contraction of the other. This cycle of stretching and contraction creates a relatively self-sustaining series of cyclical thorax contractions along longitudinal and ventral axes (Josephson et al., 2000; Dickinson, 2006), with nerve impulses mostly working to maintain this cycle or to make broad-scale changes such as an increase in power (Gordon and Dickinson, 2006).

Despite sharing a common production mechanism (thoracic power flight muscles), flight and non-flight vibrations in bees clearly have different vibrational properties. Non-flight vibrations are produced with the wings folded, effectively uncoupling power flight muscle contraction and wingbeat (King et al., 1996). For a given bee species, non-flight vibrations have higher frequencies

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than those produced during flight (King and Buchmann, 2003; Barth et al., 2005; Hrncir et al., 2008; De Luca et al., 2019), in part due to reduced drag from the wings as well as increased tension in the thoracic muscles (King et al., 1996; Hrncir et al., 2008). In contrast, non-flight vibrations produced in different contexts are superficially very similar. Both defence and floral vibrations are produced with folded wings and it is not clear to what extent non-flight thoracic vibrations have different properties from one another. Few studies have compared non-flight vibrations produced in different contexts on the same bee species. Hrncir et al. (2008) found that the frequency of vibrations produced by the tropical stingless bee, *Melipona quadrifasciata* (Apidae), during defence buzzes is approximately 60% of the frequency of vibrations used to communicate between foragers (350 vs 487 Hz, respectively). In bumblebees (*Bombus* spp.), comparison of two European species found frequency differences in non-flight vibrations, namely defence and floral buzzes. However, the direction and size of the difference in frequency between defence and floral buzzes differed between the two bumblebee species (De Luca et al., 2014). While non-flight vibrations in bees are a potentially useful system for understanding the evolution and diversification of vibratory behaviours, clearly more work is needed to characterise the exact differences between non-flight vibrations in different contexts.

Comparing the properties of vibrations produced in different behavioural contexts is technically challenging. Traditionally, substrate-borne vibrations produced by bees have been studied indirectly by recording the air-borne component of the vibration using acoustic recorders. Yet recent work indicates that although frequency components are reliably inferred from either acoustic or substrate-borne measurements, the magnitude of substrate-borne vibrations is poorly correlated with the magnitude of their acoustic component (De Luca et al., 2018). This may be because small invertebrates are poor acoustic transducers (De Luca et al., 2018), a view that is consistent with the fact that most insect communication occurs through a plant substrate, rather than through air-borne sound (Cocroft and Rodríguez, 2005). This is one reason why most of the previous work comparing the vibration properties of different bee behaviours has been focused on acoustically measured frequency differences, with relatively few studies attempting to measure both frequency and amplitude (acceleration, velocity or displacement) components (Nieh and Tautz, 2000; Hrncir et al., 2008). To get a more complete view of how vibrations differ across bee behaviours, it is necessary to capture both frequency and amplitude components (Vallejo-Marín, 2019). Vibration amplitude can be experimentally measured using vibration transducers such as accelerometers or laser vibrometers (Cocroft and Rodríguez, 2005). A full characterisation of substrate-borne vibrations is particularly important in the context of buzz-pollination because biophysical models of poricidal anthers (Buchmann and Hurley, 1978), as well as experimental tests with artificial buzzes, suggest that vibration amplitude, rather than frequency, is a key determinant of the rate of pollen ejection from flowers (De Luca et al., 2013; Rosi-Denadai et al., 2018).

In this study, we characterised for the first time, the extent to which a single species of bumblebee can modify the properties of their vibrations across multiple behaviours. Rather than just comparing flight and non-flight vibrations, we used accelerometers and laser vibrometry to directly measure the vibrational properties of buzzes produced by bumblebees [*Bombus terrestris* ssp. *audax* (Harris 1776); hereafter *B. audax*] both during flight and in two different non-flight behavioural contexts: defence and floral vibrations. In addition, we compare the floral vibrations produced by bees on two different

buzz-pollinated plant species (*Solanum rostratum* and *S. citrullifolium*, section *Androceras*, Solanaceae). Previous work has shown conflicting results on the extent to which bumblebees change the vibrations produced during floral visitation (floral vibrations), with some studies showing differences between flowers (Switzer and Combes, 2017) or with experience (Morgan et al., 2016; Switzer et al., 2019) and others showing more limited flexibility (Russell et al., 2016b). However, while other studies of bee vibrations have used non-contact methods (laser vibrometry) to look at differences in vibration properties (Conrad and Ayasse, 2015; Conrad and Ayasse, 2019), few studies to date have used these methods to examine floral vibrations directly on bees (Nunes-Silva et al., 2013). Our study addresses three specific questions: (1) what are the main differences in the vibrations produced by bumblebees across different behaviours? (2) To what extent do floral vibrations produced by the bee depend on the species of flower being visited? (3) Do the characteristics of vibrations depend on bees' morphological traits such as size?

MATERIALS AND METHODS

Study system

Bees

We used two colonies of the buff-tailed bumblebee, *Bombus terrestris audax* (Biobest, supplied by Agralan Ltd, Swindon, UK). Each colony had access to *ad libitum* 'nectar' solution (1 M sucrose solution) within the colony. Each colony was attached to a flight arena (122 cm×100 cm×37 cm), illuminated with an LED light panel (59.5 cm×59.5 cm, 48 W daylight; Opus Lighting Technology, Birmingham, UK) and maintained on a 12 h:12 h light:dark cycle. The ambient temperature was 20–23°C and relative humidity was 50–60%. In each arena, bees were also provided with a 1 mol l⁻¹ sucrose solution, *ad libitum*, from three feeders in each colony, as well as eight inflorescences (four *Solanum rostratum*, four *S. citrullifolium*) every 2 days.

Plants

We tested floral vibrations on two closely related species from the genus *Solanum* (Solanaceae). *Solanum rostratum* and *S. citrullifolium* are both nectarless species, which attract and reward pollinators solely with pollen. In common with other *Solanum* species, *S. rostratum* and *S. citrullifolium* have poricidal anthers, which requires pollinators to vibrate the anthers to release pollen. Unlike some other *Solanum* species, *S. rostratum* and *S. citrullifolium* are both heterantherous, with bees primarily focusing their attention on 'feeding anthers' presented at the centre of the flower, while a single, rarely visited 'pollinating anther' deposits pollen on the visiting bee. *Solanum* species are a classic system for the study of buzz-pollination (e.g. Buchmann and Cane, 1989; King and Buchmann, 1996), and *S. rostratum* and *S. citrullifolium* have been directly compared in a previous study that identified differences in the coupling factors of these species (Arroyo-Correa et al., 2019). Vibrations applied to *S. rostratum* show less attenuation than vibrations applied to *S. citrullifolium*, making this pair an ideal comparison for the effect of bee-produced vibrations on flowers.

Solanum rostratum and *S. citrullifolium* plants were grown from seed at the University of Stirling research glasshouses, using the method described by Vallejo-Marín et al. (2014). Seeds of *S. rostratum* were collected in Mexico (20.901°N, 100.705°W; accessions 10s77, 10s81, 10s82) and seeds of *S. citrullifolium* were obtained from self-fertilised fruits (accession 199) grown from seeds obtained from Radboud University's seed collection (accession 894750197). For daily flower provision for bees,

inflorescences were placed in water-soaked Ideal Floral Foam (Oasis Floral Products, Washington, UK) in plastic containers. For experiments, we used a single flower, cut 2–3 cm below the calyx.

Experimental methods

Recording of floral vibrations

To facilitate the recording of bee vibrations using laser vibrometry, we tagged individual bees with a small (2 mm²) piece of reflective tape placed on the dorsal part of the thorax. Bees buzzing on flowers in the flight cages were captured, placed in a freezer at –26°C for 7 min, and tagged with reflective tape using Loctite UltraControl instant adhesive (Henkel Limited, Winsford, UK). After returning to room temperature, bees resumed normal activity after approximately 7–10 min and were released back into the colony.

At least 24 h after being tagged, bees were allowed to visit flowers in the arena and a tagged bee that was actively buzzing was collected from flowers in the flight cage and released onto a single flower of either *S. rostratum* or *S. citrullifolium* in the test arena. The flower species were chosen so that each colony received the same number of flowers from each plant species. The vibrations produced by the bee were recorded simultaneously in two ways. First, we measured vibrations produced in the bee's thorax using a laser vibrometer (PDV 100, Polytec, Coventry, UK). Laser vibrometry provides a direct, contactless measure of the vibrations produced by the bee. Vibrations measured with the laser were sampled at a rate of 10,240 Hz using a low pass filter of 5 Hz, and a maximum velocity range of either 100 mm s⁻¹ (for bees 1–14) or 500 mm s⁻¹ (for bees 15–32). The laser vibrometer was placed approximately 20 cm away from the flower and aimed at the reflective tag on the bee's thorax. Second, we used an accelerometer (352C23, 0.2 g; PCB Piezotronics, Hüchelhoven, Germany) to record the vibrations transmitted from the bee to the flower (Arroyo-Correa et al., 2019). The accelerometer was attached to the calyx at the base of the flower being vibrated by the bee using a 5 mm×0.35 mm pin made from an entomological pin (Austerlitz black enameled size 0, Entomoravia, Slavkov u Brna, Czech Republic) and glued to the accelerometer with instant adhesive as described by Arroyo-Correa et al. (2019). The accelerometer and laser were set to register along the same axis of movement.

Both laser vibrometer and accelerometer data were simultaneously recorded and time-stamped using a data acquisition system (cRIO model 9040 with the C series module NI 9250; National Instruments, Newbury, UK) using a custom-made LabVIEW 2019 (National Instruments) program (see Pritchard and Vallejo-Marin, 2020, with updated version available at <https://github.com/davidjamespritchard/BuzzCatcher>). While the bee buzzed the flower, data were recorded over 2 s at a sampling rate of 10,240 Hz and saved to a file. After collecting 5–10 buzzes for each bee, the bee was caught in a 30 ml plastic container (201150; Greiner, Gloucestershire, UK), and euthanised by being placed in a –26°C freezer for 48 h. In total, we collected data for 16 bees from two colonies, eight on each flower species. For each bee we analysed an average of 6.13 buzzes ($N=98$ buzzes from 16 bees).

Recording of defence and flight vibrations

For the recording of flight and defence buzzes, bees were selected at random from the flight box. As for the flower buzzing, bees were immobilised by being placed in the freezer for 7 min. In addition to gluing a 2 mm² reflective tag to the scutum, immobile bees were also tethered to the apparatus for recording defence and flight buzzes, similar to the method used by Hrnčir et al. (2008). The neck

of the bee was held by a loop of fine nylon string threaded through a needle and attached to a syringe secured by a clamp (Fig. 1). After 7–10 min, the tethered bee had returned to regular activity levels and we continued with data collection.

To record both flight and defence buzzes, the laser vibrometer was placed above the bee and aimed at the tag on the bee's thorax. The laser beam was perpendicular to the platform on which the bee was tethered. Defence and flight vibrations measured with the laser were sampled at a rate of 10,240 Hz using a low pass filter of 5 Hz, and a maximum velocity range of 500 mm s⁻¹. To induce defence buzzes, the tethered bees were gently squeezed along the sides using featherweight forceps. To record flight buzzes, the platform underneath the tethered bee was quickly lowered, inducing the bee to start flight activity (Hrnčir et al., 2008). As before, vibration data were recorded through the cRIO data acquisition system using a custom LabVIEW program, which collected 2 s of data at a time, at a sampling rate of 10,240 Hz, with a low pass filter of 5 Hz and a velocity range of 500 mm s⁻¹. Flight and defence buzzes were recorded from 20 bees in total, with defence and flight buzzes captured from all bees. To avoid order effects, 10 of the bees had defence buzzes collected first, and 10 had flight buzzes collected first. Following recording, tethered bees were immobilised again by being placed in the freezer, removed from the tether, placed in a plastic container, and euthanised in the –26°C freezer. For each bee, we analysed an average of 5.6 flight vibrations ($N=112$ vibrations from 20 bees) and 6.8 defence buzzes ($N=136$ from 20 bees).

Bee size

Bee size was approximated using intertegular distance (ITD), the distance between the tegulae at the base of the wings (Cane, 1987). We measured ITD using a digital photograph of euthanised bees taken with a dissecting microscope (MZ6, Leica Microsystems, Milton Keynes, UK) (Fig. S1), and analysed with the Fiji distribution of ImageJ (Schindelin et al., 2012).

Data analysis

Analysis of vibrations

We used a section of each recorded vibration for analysis (Fig. 2). For floral buzzes, we selected a section of each recording that successfully captured both laser and accelerometer sensors. The sensor data (time series with voltage units) were converted from voltage to either velocity (laser) or acceleration (accelerometer) using the factory-provided conversion factors for each sensor. We zero-centred the data by subtracting the mean amplitude from each value and applied an 80–5000 Hz band-pass filter and a Hamming window (window length=512 samples), using the *fir* function in the R package *seewave* (Sueur et al., 2008). The acceleration data were converted to velocity by numerical integration using the *cumtrapz* function in the *pracma* package (<https://CRAN.R-project.org/package=pracma>), and the band-pass filter was applied again. The fundamental frequency of the analysed vibration was obtained with the *fund* function, calculated over the entire sample and setting a maximum frequency to 1000 Hz. Peak amplitude velocity for each vibration segment was calculated from the amplitude envelope calculated using the *env* function with a mean sliding window of length 2 and an overlap of 75%. All analyses were done in R version 3.6.0 (<http://www.R-project.org/>).

Transmission of bee vibrations through flowers

To quantify the extent to which the vibrations produced by bees differ from those measured in the flower itself, we calculated King's coupling factor (King, 1993). The bee's coupling factor (K_{bee}) was

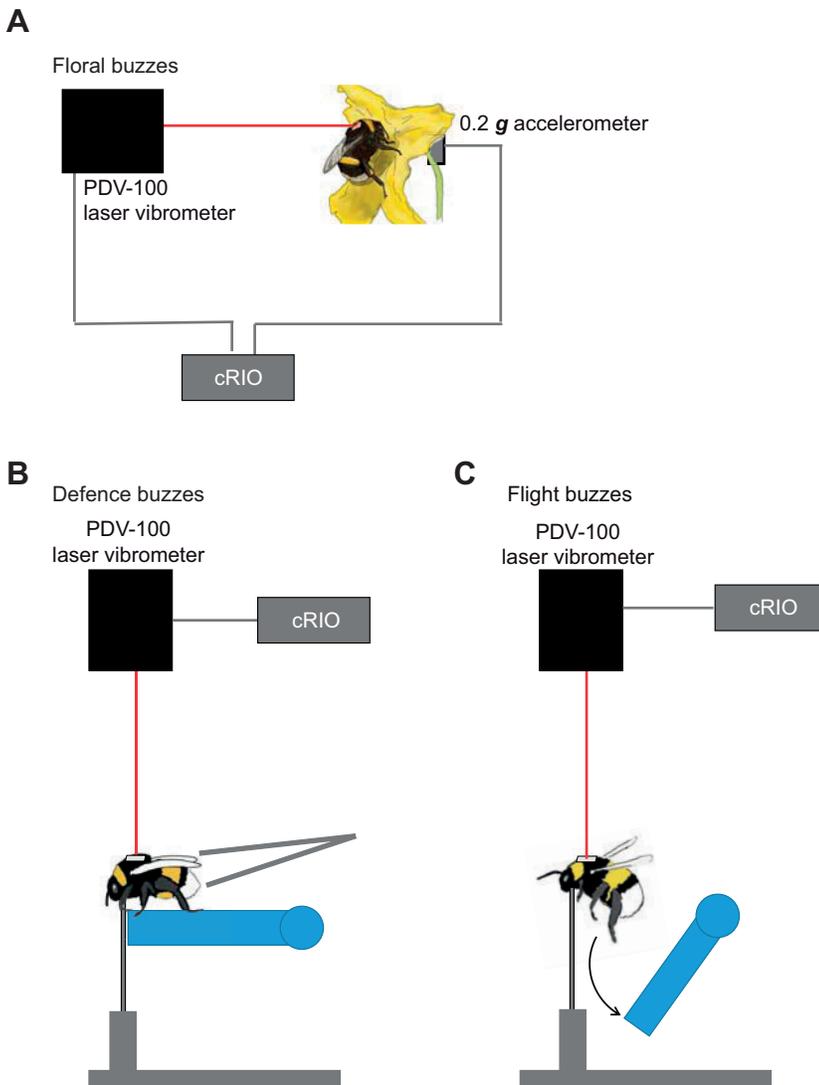


Fig. 1. Experimental set-up for measuring bee vibrations.

For floral buzzes (A), vibrations were recorded simultaneously by a PDV-100 laser vibrometer focused on a 2 mm² reflective tag on the back of the thorax of the bee, and by a 0.2 g accelerometer pinned to the calyx at the base of the flower. These measurements were sent to the compactRIO data acquisition unit (cRIO), which time-stamped the data and exported them to a file. For defence and flight buzzes (B,C), bees were tethered to a platform using a nylon wire loop fed through a blunted needle. For defence buzzes (B), bees were gently squeezed on the abdomen using featherweight tweezers. To stimulate flight (C), the platform was rapidly lowered, triggering reflexive flight. In both cases, vibrations were recorded using a PDV-100 laser vibrometer positioned above the bee and aimed at a 2 mm² area on the back of the thorax. The vibrometer then sent the data to the cRIO to be time-stamped and exported.

calculated by dividing the root mean squared (RMS) amplitude velocity of the vibration produced by the bee by the RMS amplitude velocity recorded by the accelerometer placed in the flower's calyx (Arroyo-Correa et al., 2019). We also calculated King's coupling for vibrations produced by a mechanical calibrated shaker (handheld shaker model 394C06, PCB Piezotronics). The calibrated shaker produces a vibration of constant properties (frequency=159.2 Hz, RMS amplitude velocity=9.8 mm s⁻¹) that are transmitted to a small metal plate at one end of the instrument. The metal plate of the calibrated shaker was firmly pushed against the feeding anthers of the flower, and we recorded four to five samples of 2 s each using the data acquisition system described above (see 'Analysing vibrations' section). For each flower, we selected one clean recording, converted voltage to velocity as described above, and obtained King's coupling factor for the shaker (K_{shaker}) using the ratio between expected and observed RMS velocity. Measuring both K_{bee} and K_{shaker} allowed us to compare the difference in the efficiency with which a bee and a mechanical shaker transmit vibrations to the flower.

Statistical analyses

To compare the properties of vibrations in different contexts we used linear mixed effect models using either peak velocity or

fundamental frequency as response variables, buzz type (flight/defence/floral) and ITD as explanatory variables, and bee identity as a random effect. In addition to peak velocity and frequency, which were measured directly, we also used these measures to derive the displacement amplitude (in mm) and acceleration (in mm s⁻²) of the vibration. As with velocity, we analysed the peak recordings of each of these measures with linear mixed effect models, with buzz type and ITD as explanatory variables and bee identity as a random effect. To compare the properties of floral vibrations on different *Solanum* species, we employed linear mixed effect models, using either laser-recorded peak velocity, laser-recorded fundamental frequency, accelerometer-recorded peak velocity or accelerometer-recorded fundamental frequency as response variables, flower species and ITD as explanatory variables, and bee identity as a random effect. Finally, to compare the effect of flower species and recording method on coupling factors, we used a linear mixed effect model with coupling factor as a response variable, flower species, ITD and vibration method (bee vs artificial) as explanatory variables, and bee identity as a random effect. All analyses were performed using *lme4* (Bates et al., 2015) to estimate parameters and *lmerTest* (Kuznetsova et al., 2017) to assess statistical significance.

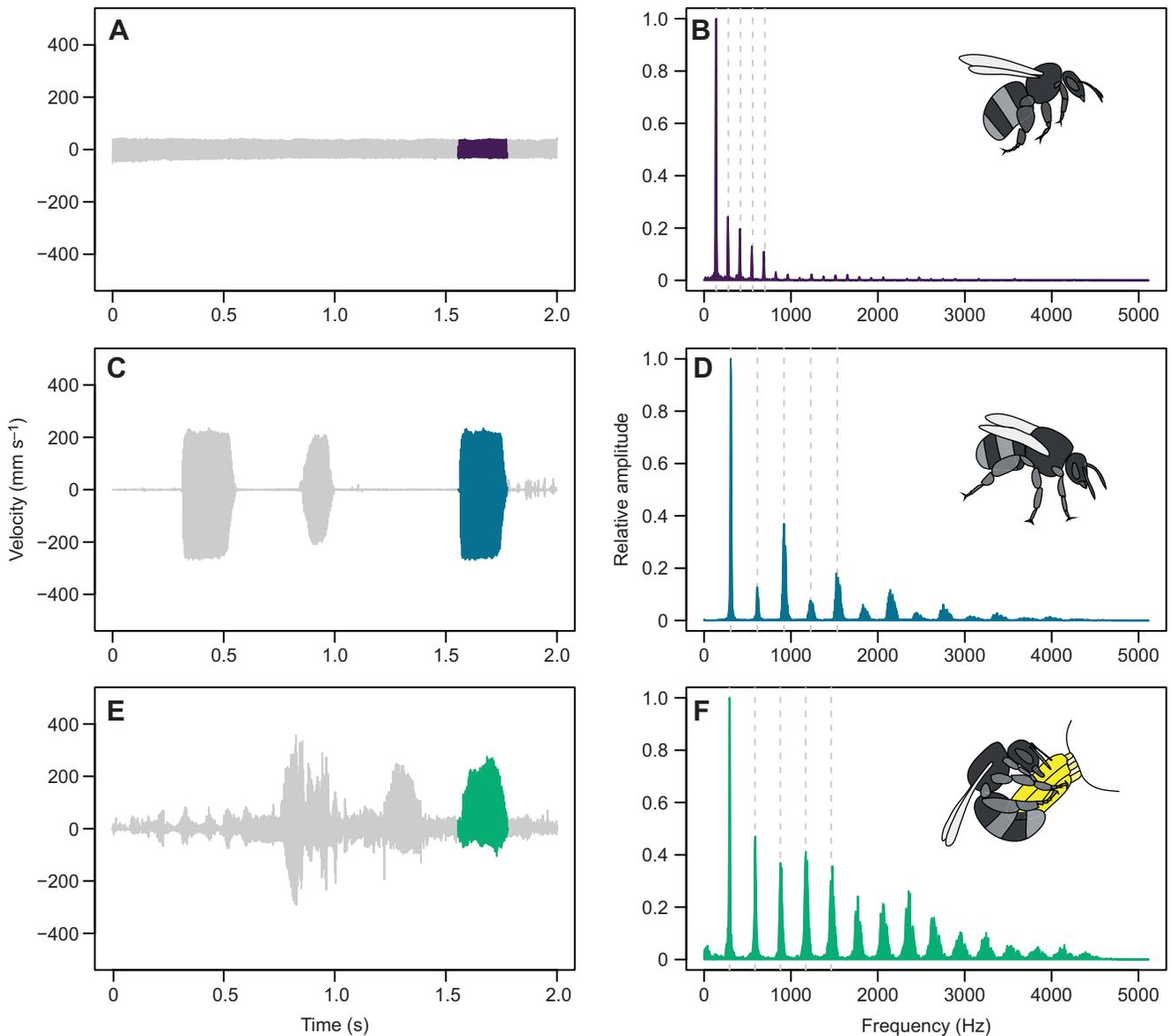


Fig. 2. Oscillograms and frequency spectra of vibrations (buzzes) produced by bumblebees (*Bombus terrestris audax*) in three different behavioural contexts: flight (A,B), defence (C,D) and buzz-pollination (E,F). (A,C,E) Buzzes in the time domain (oscillograms); (B,D,F) buzzes in the frequency domain (frequency spectra). The coloured region in the oscillogram shows the section of the buzz used to generate the corresponding frequency spectrum. The first five harmonics (multiples of the fundamental frequency) are shown as vertical dashed lines in the frequency spectra.

Ethical approval

These experiments were approved by the Animal Welfare and Ethical Review Board of the University of Stirling.

RESULTS

Comparison of buzzes produced in different behavioural contexts

The vibrations produced during flight, defence and pollen extraction differ significantly in properties including fundamental frequency and peak amplitude velocity (Table 1). The peak amplitude velocity of floral buzzes ($262.85 \pm 9.52 \text{ mm s}^{-1}$) was significantly higher than both defence ($194.85 \pm 6.12 \text{ mm s}^{-1}$) and flight buzzes ($57.29 \pm 1.28 \text{ mm s}^{-1}$; Fig. 3A; Table 1). We found no significant effect of bee size on peak amplitude velocity (Table 1). Floral buzzes also had significantly higher frequencies ($313.09 \pm 2.63 \text{ Hz}$) than both defence ($236.32 \pm 4.29 \text{ Hz}$) and flight buzzes ($136.95 \pm 1.73 \text{ Hz}$)

(Fig. 3B). We also detected an interaction between bee size and buzz type, with larger bees achieving higher frequency defence buzzes and lower frequency flower and flight buzzes than smaller bees (Table 2, Fig. S2). The differences in peak amplitude velocity across the three behaviours observed here extended to peak amplitude acceleration, with floral buzzes achieving higher accelerations ($517.77 \pm 19.40 \text{ m s}^{-2}$) than defence ($297.41 \pm 11.96 \text{ m s}^{-2}$) and flight vibrations ($49.43 \pm 1.34 \text{ m s}^{-2}$) (Fig. 3D). In contrast, the peak amplitude displacement of floral ($0.27 \pm 0.009 \text{ mm}$) and defence buzzes ($0.27 \pm 0.007 \text{ mm}$) were similar, although both were greater than the displacement amplitude of flight vibrations ($0.14 \pm 0.005 \text{ mm}$) (Fig. 3C).

Floral buzzes

Our analyses of the vibrations produced by bees while visiting flowers (floral buzzes) shows that only some of the properties of

Table 1. Analysis of *B. audax* bee size (intertegular distance) and behavioural context on the properties of thoracic vibrations measured with a laser vibrometer

Response variable	Parameter	Estimate	s.e.m.	P-value
Peak amplitude velocity (mm s ⁻¹)	Intercept (buzz type: flight)	165.71	94.16	
	Intertegular distance	-24.63	21.72	0.27
	Buzz type			<0.001
	Defence	132.68	8.54	
	Floral	207.65	14.53	
Fundamental frequency (Hz)	Intercept (buzz type: flight)	200.93	70.89	
	Intertegular distance	-14.53	16.36	0.38
	Buzz type			<0.001
	Defence	102.93	3.38	
	Floral	177.70	10.50	
	Buzz type×intertegular distance			0.002
Displacement (mm)	Intercept (Buzz type: flight)	0.24	0.11	
	Intertegular distance	-0.022	0.026	0.40
	Buzz type			<0.001
	Defence	0.11	0.011	
	Floral	0.13	0.017	
Acceleration (m s ⁻²)	Intercept (Buzz type: flight)	358.32	199.45	
	Intertegular distance	-71.09	46.01	0.13
	Buzz type			<0.001
	Defence	248.57	16.82	
	Floral	479.57	30.57	

The parameter estimates and standard errors (s.e.m.) were calculated from a linear mixed effect model with bee identity as a random factor. *P*-values for each explanatory variable were calculated using a Type III analysis of variance with Satterthwaite's estimation of degrees of freedom. Statistically significant values are in bold.

these vibrations depend on whether they are recorded on the bee or on the flower (Fig. 4). The magnitude of vibrations recorded directly on the bee had considerably higher peak velocity amplitudes (273.56±12.49 and 247.34±14.53 mm s⁻¹ for *S. rostratum* and *S. citrullifolium*, respectively) than those vibrations measured on the flower (36.61±2.30 and 19.20±1.03 mm s⁻¹ for *S. rostratum* and *S. citrullifolium*, respectively; Fig. 5A; Table 2). In contrast, the fundamental frequency of the floral vibrations was similar whether recorded directly from the bee (313.16±2.86 and 312.09±4.99 Hz for *S. rostratum* and *S. citrullifolium*, respectively) or indirectly via the accelerometer on the flower (312.70±2.92 and 313.16±4.81 Hz for *S. rostratum* and *S. citrullifolium*, respectively; Fig. 5B; Table 2). Interestingly, we observed that vibrations measured on the bee contained more harmonics (*S. citrullifolium*: 10.75±0.38; *S. rostratum*: 11.34±0.35) than those observed on vibrations measured on the flower (*S. citrullifolium*: 3.65±0.27; *S. rostratum*: 2.57±0.20) (Fig. 4).

Plant species did not significantly affect the frequency or peak amplitude velocity of floral vibrations (but see 'Transmission of vibrations through flowers' section for differences in the transmission of vibrations from bee to flower in the two *Solanum* species). Bee size (ITD) was negatively associated with fundamental frequency of floral vibrations (Fig. 5C), while bee size had no effect on their peak amplitude velocity (Table 2). We found no statistically significant interaction between bee size and plant species on either frequency or peak amplitude velocity of floral vibrations.

Transmission of vibrations through flowers

To analyse the effect of plant species on the transmission of floral vibrations through the flower, we compared King's coupling factor (*K*, the ratio of vibration magnitude produced to vibration received) for the two *Solanum* species. We found that *S. rostratum* had a significantly lower coupling factor ($K_{\text{bee}}=5.64\pm0.61$, $K_{\text{shaker}}=5.95\pm1.77$; means±s.e.) than *S. citrullifolium* ($K_{\text{bee}}=9.92\pm0.97$, $K_{\text{shaker}}=8.93\pm1.97$; Table 3; Fig. 6). Our analysis showed no difference within plant species between coupling factors calculated

from either bee floral buzzes (K_{bee}) or synthetic vibrations applied with the calibrated shaker (K_{shaker}) (Table 3), although K_{bee} is less variable than K_{shaker} (Fig. 6). We did not find an effect of bee size on coupling factor (Table 3).

DISCUSSION

Bumblebees and other buzz-pollinating bees present a unique opportunity for research on insect vibrations. In addition to producing vibrations during locomotion and as a signal to predators or conspecifics, the two forms of thoracic vibrations most commonly studied in bees and other insects, buzz-pollinating bees also use vibrations to forage. While the posture of bees during floral buzzes and defence buzzes are very similar, with both requiring the wings folded back over the body, the functions of these two buzzes are very different, making them a useful comparison for understanding how function might influence the properties of bee vibrations. In this study we directly compared these different types of vibrations within a single species of bumblebee, not only comparing flight and non-flight vibrations, but also characterising different types of non-flight vibrations. Our results show clear differences in biomechanical properties of defence and floral buzzing, as well as differences between these vibrations and those produced during flight. In addition to differences between different behaviours, we also found that the species of plant being vibrated and the size of the bee affected the properties of the floral vibrations experienced by plants.

Floral vibrations and bee size

Our results are consistent with previous work showing that plant species differ in their transmission of floral vibrations (King, 1993; Arroyo-Correa et al., 2019). Between the two studied plant species, we found that *Solanum rostratum* is better at transmitting vibrations applied on the anthers to other parts of the flower than *S. citrullifolium*, as shown by its lower coupling factor (cf. Arroyo-Correa et al., 2019). Interestingly, the coupling factor calculated using synthetic vibrations applied with a metal plate and the one calculated using vibrations applied by live bees were similar,

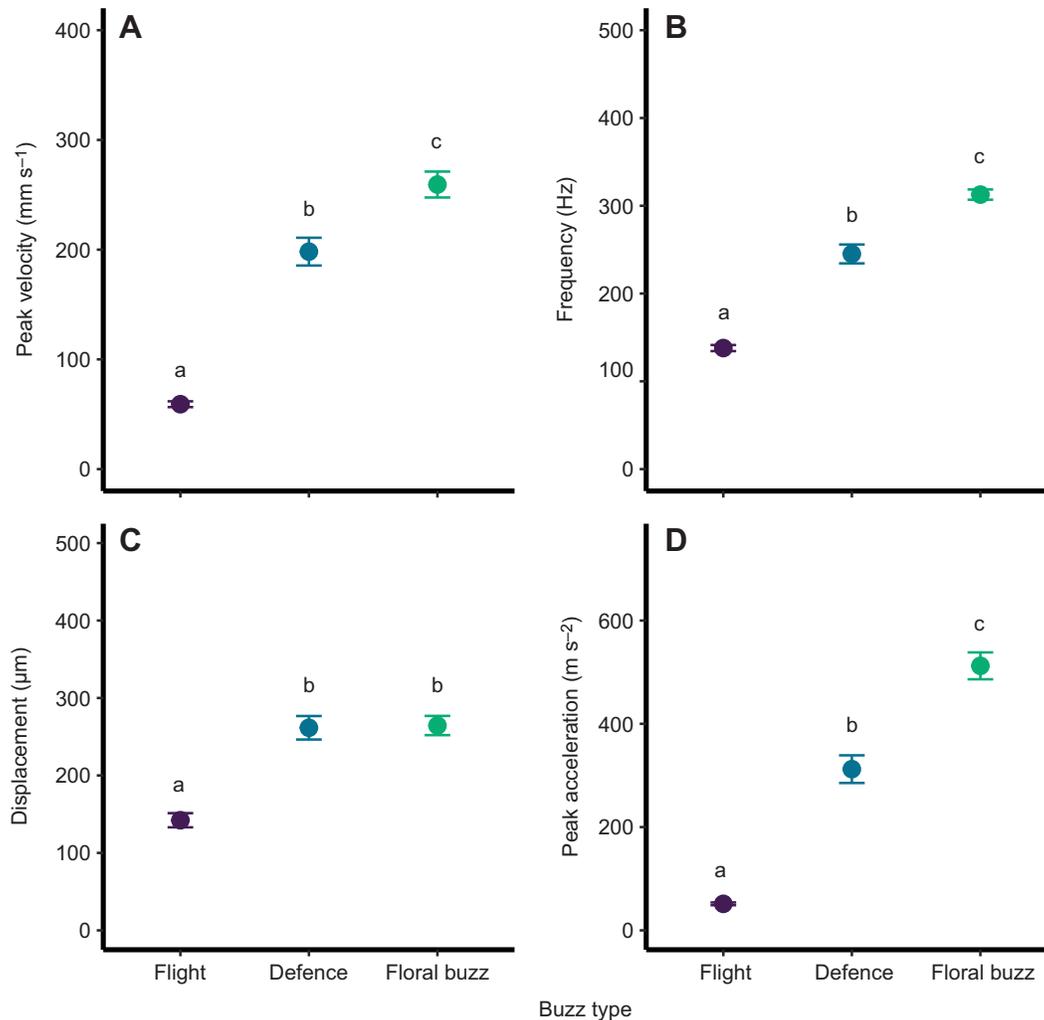


Fig. 3. Differences in the properties of vibrations (buzzes) produced in different contexts (flight, defence and floral buzzes). Vibrations differed in both peak velocity (A) and frequency (B), with floral buzzes exhibiting the highest velocity and highest frequency buzzes, and flight producing the lowest velocity and frequency vibrations. From these values we derived the magnitude of the vibrations, in terms of displacement of the thorax (C) and the acceleration (D) produced during these vibrations. Although there was no difference in the absolute magnitude of the vibrations produced during defence and floral buzzes, as the floral buzzes were faster and at higher frequency than the defence buzzes, floral buzzes showed much higher accelerations. Values are given as means \pm s.e.m.; $N=36$ bees from two colonies (16 for floral vibrations, 20 for defence and flight vibrations). Within each panel, different letters indicate statistically different mean values as assessed with a pairwise comparison of least squares means. Details of statistics are given in Table 1.

suggesting that fine floral manipulation by the bee during buzzing has little effect on the vibrations transmitted to other parts of the flower. Further analyses of the biomechanical properties of flowers are required to determine the mechanism responsible for the different coupling factors observed here and in previous studies.

We found little evidence that the magnitude of floral, flight and defence buzzes can be explained by the range of bee size variation observed within a single species of bumblebee. In contrast, bee size was negatively associated with frequency of floral and flight buzzes, but positively with defence buzzes. The frequency of flight vibrations in bees is usually negatively associated with size both within (this study) and across species (De Luca et al., 2019). For floral vibrations, the association between frequency and size seems to vary (reviewed in De Luca et al., 2019), ranging from negative, as in our study on *B. terrestris audax*, to positive (Arroyo-Correa et al., 2019), to no detectable relationship both within species (De Luca et al., 2013; Nunes-Silva et al., 2013; De Luca et al., 2014) and across multiple species (Rosi-Denadai et al., 2018; De Luca et al., 2019). Moreover, the relationship between the frequency of floral

buzzes and bee size within species may further depend on the metric of bee size used (Corbet and Huang, 2014; Switzer and Combes, 2017). Taken together, this body of work suggests that differences in size are not sufficient to explain variation in floral buzzes during buzz-pollination.

Differences among buzz types

We found that bumblebees vibrating flowers produce higher accelerations than in other behaviours, and much higher than previously thought. The floral vibrations measured in this experiment were on average 500 m s^{-2} , more than two to three times what Arroyo-Correa et al. (2019) and King (1993) calculated after measuring floral buzzing from the plant and correcting with the corresponding coupling factor. Despite this, our measurements for frequency and velocity, from which acceleration was calculated, were consistent with those found by other studies looking at flying, defence buzzing and flower buzzing bees (King, 1993; Nunes-Silva et al., 2013). Floral buzzes appear to be characterised by higher accelerations, velocities and frequencies than defence buzzes, and

Table 2. Analysis of bee size (intertegular distance), plant species and recording location on the properties of floral vibrations

Response variable	Variable	Estimate	s.e.m.	P-value
Peak amplitude velocity (mm s^{-1})	Intercept (plant: <i>S. citrullifolium</i> ; location: bee)	312.06	74.43	
	Intergular distance	-13.74	16.31	0.42
	Plant species: <i>S. rostratum</i>	22.22	12.95	0.11
	Location: flower	-233.35	9.30	<0.001
Fundamental frequency (Hz)	Intercept (plant: <i>S. citrullifolium</i> ; location: bee)	462.66	60.83	
	Intergular distance	-33.54	13.36	0.027
	Plant species: <i>S. rostratum</i>	4.40	10.12	0.67
	Location: flower	-0.20	2.07	0.92

Vibrations were recorded on *S. citrullifolium* and *S. rostratum*, both directly on the bee's thorax using a laser vibrometer and on the flower using an accelerometer. The parameter estimates and standard errors (s.e.m.) were calculated from a linear mixed effect model with bee identity as a random factor. *P*-values for each explanatory variable were calculated using a Type III analysis of variance with Satterthwaite's estimation of degrees of freedom. Statistically significant values are in bold.

both floral and defence buzzes have higher accelerations, velocities, displacement amplitude and frequencies than are produced during flight. The key question raised by our results, then, is why are the properties of floral, defence and flight vibrations so different from one another? This question can be addressed in two ways: (1) by considering how the mechanisms underlying these vibrations might

differ across behaviours; and (2) how the function of the behaviour might select for particular vibration properties.

Mechanisms of bee vibrations

All the vibrations we measured in this study were produced by contractions of the dorsal longitudinal and dorsoventral flight muscles in the thorax. The fact that these vibrations all share a common mechanism could mean that something other than the muscles might be responsible for the differences we observed. One early suggestion was whether the decoupling of the wings from the flight muscles during non-flight vibrations (defence, floral buzzes) changed the resonant properties of the thorax and led to higher frequencies. It is plausible that the deployment of the wings could lower the frequency of the vibrations; wings produce drag and inertia, which is one reason why insects with larger wings have a lower wingbeat frequency (e.g. Greenewalt, 1962; Joos et al., 1991). When insect wings are cut shorter, the frequency of flight increases (Roeder, 1951; Hrnčir et al., 2008). While wing deployment can explain the difference between flight and non-flight vibrations, it cannot explain the differences between the two non-flight vibrations (floral and defence buzzes), where the wings remained folded and the mass of the system remains unchanged.

Instead of the mechanical effect of the wings, differences between non-flight vibrations could be the result of differences in muscle activity, either in terms of increasing muscle power or by changing the stiffness and resonant properties of the thorax. Although bumblebee flight muscles are stretch activated, and so do not contract in time with motor neuron firing, studies of similar muscles in *Drosophila* show that increasing the frequency of firing increases

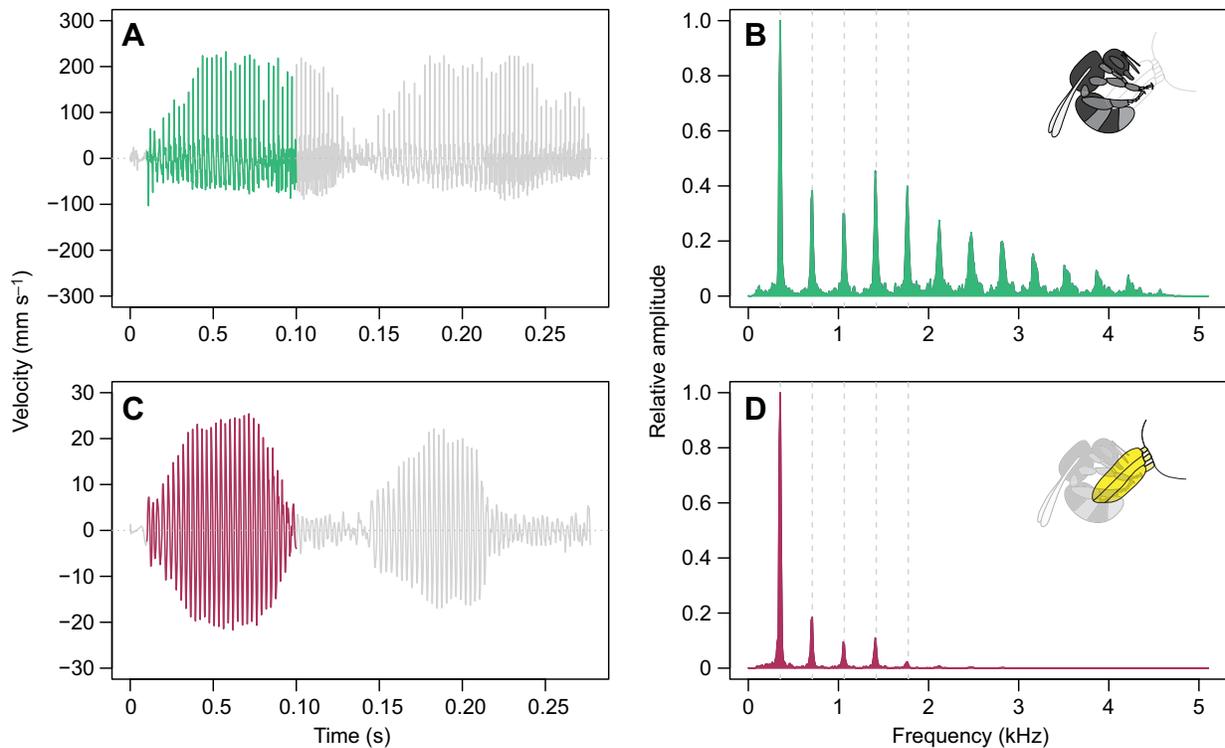


Fig. 4. Example of a floral vibration produced by *B. audax* while visiting a flower of *Solanum citrullifolium* as recorded directly from the bee and on the flower. The magnitude of the vibration, measured as velocity amplitude, is much higher when measured directly on the bee's thorax with a laser vibrometer (A), than when measured using an accelerometer attached to the base of the flower (C). In contrast, the fundamental frequency of the buzz produced during floral visitation is the same (355 Hz) when measured in the bee's thorax (B) or on the base of the flower (D). The coloured section in the oscillograms shown in panels A and C represent the section of the buzz used to calculate the frequency spectra shown in panels B and D. The dashed lines in panels B and D represent the first five harmonics of the fundamental frequency.

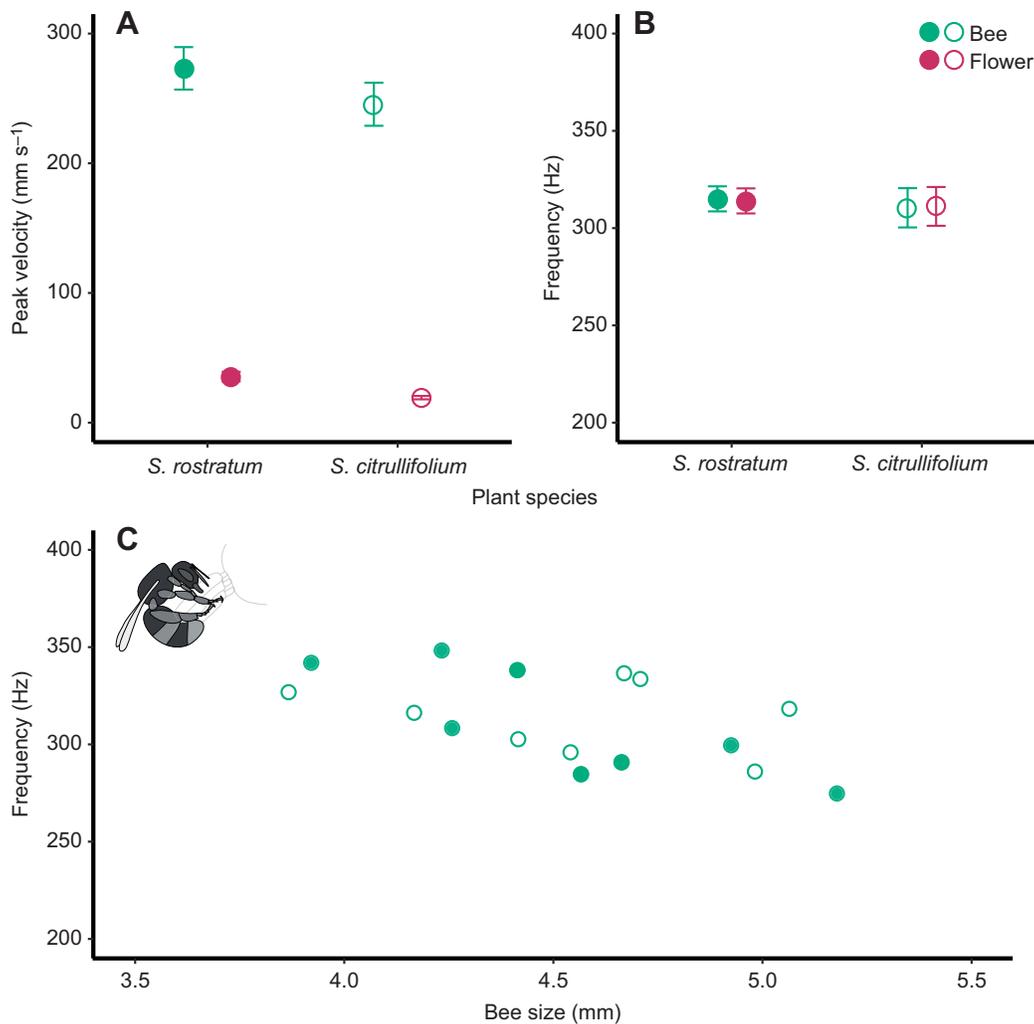


Fig. 5. Peak amplitude velocity and fundamental frequency of floral buzzes of *B. audeax* on buzz-pollinated flowers of *Solanum rostratum* and *S. citrullifolium*. (A) Peak amplitude velocity; (B) fundamental frequency; closed symbols, *S. rostratum*; open symbols, *S. citrullifolium*. Floral buzzes were recorded directly from the bee's thorax using a laser vibrometer (green symbols) or on the flower using an accelerometer attached to the calyx (magenta symbols). Vibrations recorded on the flower had significantly lower peak velocities but similar fundamental frequencies to those measured in the bee. (C) Relationship between bee size (intertegular distance) and the fundamental frequency of floral buzzes; each symbol in represents the average frequency from multiple buzzes produced by an individual bee. In panels A and B, values are means \pm s.e.m. $N=16$ bees from two colonies (eight on *S. rostratum*, eight on *S. citrullifolium*). Details of statistics are given in Table 2.

the Ca^{2+} concentration in the flight muscles, resulting in more powerful contractions (Dickinson et al., 1998; Gordon and Dickinson, 2006; Wang et al., 2011; Lehmann and Bartussek, 2017). Bees could also use other muscles to stiffen the thorax, changing its resonant properties, altering the frequency at which the cycle of stretch-activated contractions reaches equilibrium (Nachtigall and Wilson, 1967). Although these mechanisms have yet to be studied in bees, neurophysiological studies of bee flight muscles have found differences between flight and non-flight vibrations (Esch and Goller, 1991; King et al., 1996), which might also explain differences between non-flight vibrations. During flight, both the dorsoventral and dorsal longitudinal muscle sets are stimulated equally, whereas during defensive buzzes the dorsal longitudinal muscles are stimulated at twice the rate of the dorsoventral muscles (King et al., 1996). If, for example, the increased difference in activation between the flight muscle sets is responsible for the increased frequency of non-flight vibrations, then we might expect the difference in excitation between the

muscle sets to be even more extreme during floral buzzes than during defence buzzes. By comparing the mechanisms underlying floral buzzes, defence buzzes and flight in this way, we can begin to understand how bees use changes in muscular activity and associated shifts in the resonant properties of their body, to adjust the mechanical properties of their vibrations.

Function of bee vibrations

In addition to considering differences in the actions of the muscles, another approach to thinking about *why* the muscles produce vibrations with these particular properties is to consider what properties might best serve these functions. In vibratory communication, for example, the properties of the signalling environment, such as the degree of frequency filtering, determine the 'best' vibratory properties to transmit information from producer to receiver (Cocroft and Rodríguez, 2005). Similar factors could influence the 'best' properties for defence buzzes. Like the vibratory signals studied in other insect species, the function of a defence

Table 3. Effect of bee size (intertegular distance distance), flower species and vibration method on the magnitude of King's coupling factor

Response	Variable	Estimate	s.e.m.	<i>P</i> -value
Coupling factor	Intercept (flower: <i>S. citrullifolium</i> ; vibration source: shaker)	14.26	5.92	
	Intertegular distance	-0.89	1.29	0.51
	Flower species			0.002
	<i>S. rostratum</i>	-4.04	1.03	
	Vibration source			0.72
	Bee	-0.32	0.91	

Vibrations were applied to *S. citrullifolium* and *S. rostratum*, either by the bee or by pressing a calibrated shaker against the flower. The parameter estimates and standard errors (s.e.m.) were calculated from a linear mixed effect model with bee identity as a random factor. *P*-values for each explanatory variable were calculated using a Type III analysis of variance with Satterthwaite's method. Statistically significant values are in bold.

buzz is to transmit information from the producer (the bee) to a receiver (the predator). This information is effective; defence or alarm sounds produced by insects, including bumblebees, have been shown to reduce or slow down predator attacks (Masters, 1979; Moore and Hassall, 2016). The effectiveness of defence buzzes is probably affected by the properties of the vibration itself. Although in our experiment we found that defence buzzes were on average of lower frequency, peak amplitude velocity and peak amplitude acceleration than floral buzzes, these properties do not correlate with what is likely a more important property of a warning signal: volume (De Luca et al., 2018). A previous comparison of the acoustic properties of defence and floral buzzes found that defence buzzes were significantly louder than floral buzzes (De Luca et al., 2014),

and it is possible that the lower frequency or amplitude of the bee's vibrations during defence buzzing might actually increase the perceived volume of the buzz by predators. A lower frequency and velocity vibration may also be beneficial for the bee as it might be less energetically costly than the higher frequency and velocity floral buzz. Although the costs of buzzing by bees have only been measured for a handful of behaviours (Kammer and Heinrich, 1974; Heinrich, 1975), increasing the frequency and amplitude of vibrations could carry a significant cost. For instance, in the carpenter bee *Xylocopa varipuncta*, increases in the frequency and amplitude of their wingbeats when flying in less dense gases are associated with increases in their metabolic rate by over a third (Roberts et al., 2004). By using lower frequency and velocity vibrations, bumblebees might be able to perform defence buzzes for longer, increasing their effectiveness against predators.

Unlike defence buzzes, the primary function of floral buzzes is not to transmit information to receivers but to shake pollen loose from flowers. Pollen is essential for larval nutrition (Westerkamp, 1996), and bumblebees possess many specialisations to assist in pollen collection, from morphological features such as corbiculae (Thorp, 1979), to behaviour specialisations, including optimising pollen collection (Rasheed and Harder, 1997), rejecting flowers that appear empty of pollen (Buchmann and Cane, 1989; Harder, 1990), and modifying their buzzes in response to the presence or absence of pollen (Russell et al., 2016b; Switzer et al., 2019). It is possible that the properties of floral buzzes are also tuned to maximise the pollen collected from poricidal anthers. If that was the case, we would expect the properties that defined floral buzzes in this study – high frequency, velocity and acceleration – to correlate with the vibration properties that release the most pollen. Studies with artificial shakers have subjected buzz-pollinated flowers to a broad array of vibrations to determine what kinds of vibration release the most pollen (Harder

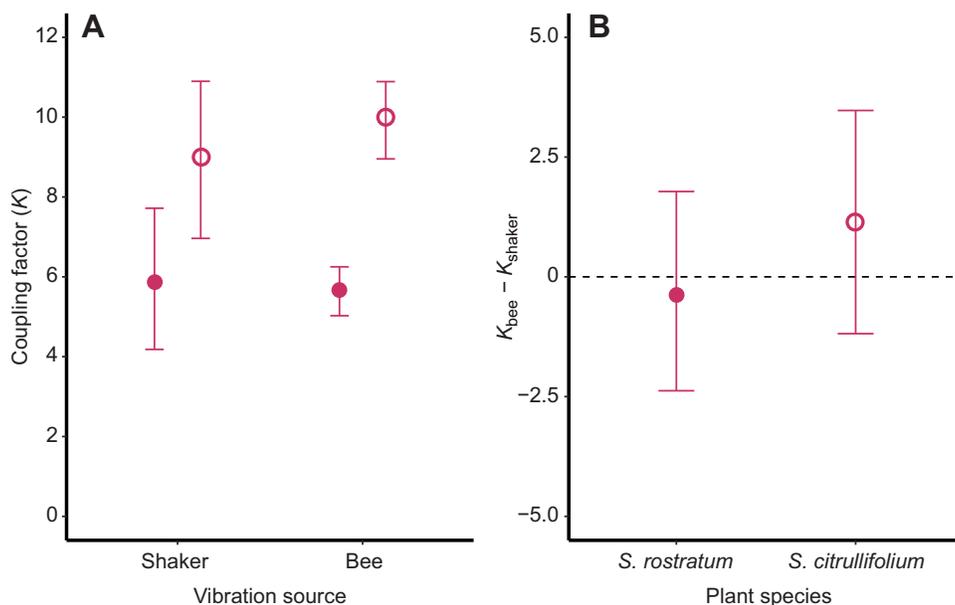


Fig. 6. Comparison of the ratio of the magnitude of the input vibration to the magnitude of the vibration measured at the sensor (King's coupling factor) on two buzz-pollinated species of *Solanum*. Coupling factors were estimated using either natural bee vibrations ('bee') or synthetic vibrations produced with a calibrated mechanical shaker ('shaker') as the input vibration. The calibrated shaker produced a vibration of fixed properties (frequency=159.2 Hz, RMS velocity=9.8 mm s⁻¹). The magnitude of the vibration produced by the bee was measured using a laser vibrometer on the bee's thorax. The vibration transmitted to the sensor on the flower was measured at the calyx using an accelerometer. Plant species consistently differ in their coupling factor with *S. rostratum* having lower values than *S. citrullifolium* (A), irrespective of whether it is calculated using bee or calibrated shaker vibrations (B). Values are given as means±s.e.m. In panel A, *N*=16 bees from two colonies (eight on *S. rostratum*, eight on *S. citrullifolium*), and 13 manual vibrations of flower (seven on *S. rostratum*, six on *S. citrullifolium*). In panel B, *N*=13 bees who had matching manual vibrations of their flower (seven on *S. rostratum*, six on *S. citrullifolium*). Details of statistics are given in Table 3.

and Barclay, 1994; De Luca et al., 2013; Rosi-Denadai et al., 2018). Although the frequency of floral buzzes appears very consistent across studies, frequency does not appear to determine how much pollen is released from anthers. Instead, as we observed, higher frequencies may result in higher velocities and accelerations, and it is these properties that most determine how much pollen an anther releases (De Luca et al., 2013; Rosi-Denadai et al., 2018). The effect of increasing the velocity or acceleration of floral buzzes on pollen release can be dramatic. De Luca et al. (2013) for example found that for a floral buzz lasting for 1 s, doubling the velocity of the buzz led to four times as much pollen being released. Rosi-Denadai et al. (2018) found a similar effect for acceleration – vibrations with a similar acceleration to the floral buzzes we recorded (500 m s^{-2}) released more than three times as much pollen as vibrations matching the flight vibrations we recorded (100 m s^{-2}), and twice as much as vibrations matching the defence buzzes (300 m s^{-2}). The accelerations we recorded from floral buzzes, therefore, are what might be expected from vibrations tuned to maximise pollen release. Producing high acceleration floral buzzes, however, is likely to have come with a cost. Although it is not clear exactly how costly these floral buzzes might be, as no-one has yet measured the metabolic cost of floral buzzing, it has been suggested that bees work to maximise the efficiency of their pollen collection (Rasheed and Harder, 1997). Their foraging decisions are therefore not just based on maximising the pollen they collect, but also based on the potential cost. If floral buzzing exerts a significant cost on bees, this cost might play an important role in their decisions about where and when to forage on buzz-pollinated flowers (Stephens, 2008).

Conclusion

Our results demonstrate clear differences between the vibrations produced by bumblebees in different contexts. In addition to the expected differences between flight and non-flight vibrations (De Luca et al., 2019), which can be partly attributed to wing deployment and different postures resulting in physical differences in drag and resonance, we also found equally sizable differences between floral and defence vibrations, in which the wings remained undeployed and posture is similar. These differences between non-flight vibrations open up larger questions about the mechanisms and evolution of insect vibrations. Currently the mechanisms that control the properties of thoracic vibrations have only been studied in a handful of contexts (Esch and Goller, 1991; King et al., 1996), with most of what we know coming from studies of flight control in *Drosophila* (Lehmann and Bartussek, 2017; Lindsay et al., 2017). The vibrations that individual bumblebees produce in different contexts exhibit stark but reliable differences in their properties, providing a model to better understand how individual insects control the properties of the vibrations they produce. By identifying homologous mechanisms as well as outlining possible constraints on how insect vibrations respond to selection, investigating the mechanisms of bumblebee vibrations can also tell us more about how these behaviours evolve. However, to understand how selection might have acted on these vibrations, it is also necessary to examine how bees use these vibrations for their particular functions. The biomechanical properties of a vibration might only be part of what makes it effective. Other behaviours can increase the effectiveness of a particular vibration by increasing the salience or memorability of a signal, such as when animals combine multiple modalities into a signal (Rowe, 1999), or by modifying the effects of the vibrations, such as when tree crickets build acoustic baffles to amplify the volume of their mating calls (Mhatre et al., 2017). During floral

buzzing, bees do not simply apply vibrations like the artificial shakers used to study pollen release. Instead, bees need to learn to handle flowers correctly, and work to get in position before starting buzzing (Macior, 1964; Laverly, 1980; Russell et al., 2016a). How bees handle flowers, where they bite anthers, and how they position themselves as they vibrate, could all influence how the high acceleration vibrations we recorded are applied to the flower and result in pollen ejection. The next step for understanding why bumblebees, and other insects, produce the vibrations they do, is to understand how other behaviours work alongside vibrations to serve their function.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: D.J.P., M.V.-M.; Methodology: D.J.P., M.V.-M.; Software: D.J.P.; Formal analysis: D.J.P., M.V.-M.; Investigation: D.J.P.; Resources: M.V.-M.; Data curation: D.J.P.; Writing - original draft: D.J.P., M.V.-M.; Writing - review & editing: D.J.P., M.V.-M.; Visualization: D.J.P., M.V.-M.; Supervision: M.V.-M.; Project administration: M.V.-M.; Funding acquisition: M.V.-M.

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Data availability

Data and code are available from Zenodo (Pritchard and Vallejo-Marín, 2020): <http://doi.org/10.5281/zenodo.3840007>.

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.220541.supplemental>

References

- Arroyo-Correa, B., Beattie, C. and Vallejo-Marín, M. (2019). Bee and floral traits affect the characteristics of the vibrations experienced by flowers during buzz pollination. *J. Exp. Biol.* **222**, jeb198176. doi:10.1242/jeb.198176
- Barth, F., Hrncir, M. and Tautz, J. (2005). Vibratory and airborne-sound signals in bee communication (Hymenoptera). In *Insect Sounds and Communication* (ed. S. Drosopoulos and M. Claridge), pp. 421-436. CRC Press.
- Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1-48. doi:10.18637/jss.v067.i01
- Buchmann, S. L. (1983). Buzz pollination in angiosperms. In *Handbook of Experimental Pollination Biology* (ed. C. E. Jones and R. J. Little), pp. 73-113. New York, NY, USA: Scientific and Academic Editions, Van Nostrand Reinhold.
- Buchmann, S. L. and Cane, J. H. (1989). Bees assess pollen returns while sonicating *Solanum* flowers. *Oecologia* **81**, 289-294. doi:10.1007/BF00377073
- Buchmann, S. L. and Hurley, J. P. (1978). A biophysical model for buzz pollination in angiosperms. *J. Theor. Biol.* **72**, 639-657. doi:10.1016/0022-5193(78)90277-1
- Cane, J. H. (1987). Estimation of bee size using intertegular span (Apoidea). *J. Kans. Entomol. Soc.* **60**, 145-147.
- Cardinal, S., Buchmann, S. L. and Russell, A. L. (2018). The evolution of floral sonication, a pollen foraging behavior used by bees (Anthophila). *Evolution* **72**, 590-600. doi:10.1111/evo.13446
- Cocroft, R. B. and Rodríguez, R. L. (2005). The behavioral ecology of insect vibrational communication. *Bioscience* **55**, 323-334. doi:10.1641/0006-3568(2005)055[0323:TBEIOV]2.0.CO;2
- Conrad, T. and Ayasse, M. (2015). The role of vibrations in population divergence in the red mason bee, *Osmia bicornis*. *Curr. Biol.* **25**, 2819-2822. doi:10.1016/j.cub.2015.08.059
- Conrad, T. and Ayasse, M. (2019). The differences in the vibrational signals between male *O. bicornis* from three countries in Europe. *J. Low Frequency Noise Vibrat. Active Control* **38**, 871-878. doi:10.1177/1461348418816263

- Corbet, S. A. and Huang, S.-Q.** (2014). Buzz pollination in eight bumblebee-pollinated *Pedicularis* species: does it involve vibration-induced triboelectric charging of pollen grains? *Ann. Bot.* **114**, 1665-1674. doi:10.1093/aob/mcu195
- De Luca, P. A. and Vallejo-Marín, M.** (2013). What's the 'buzz' about? The ecology and evolutionary significance of buzz-pollination. *Curr. Opin. Plant Biol.* **16**, 429-435. doi:10.1016/j.pbi.2013.05.002
- De Luca, P. A., Bussi re, L. F., Souto-Vilaros, D., Goulson, D., Mason, A. C. and Vallejo-Marín, M.** (2013). Variability in bumblebee pollination buzzes affects the quantity of pollen released from flowers. *Oecologia* **172**, 805-816. doi:10.1007/s00442-012-2535-1
- De Luca, P. A., Cox, D. A. and Vallejo-Marín, M.** (2014). Comparison of pollination and defensive buzzes in bumblebees indicates species-specific and context-dependent vibrations. *Naturwissenschaften* **101**, 331-338. doi:10.1007/s00114-014-1161-7
- De Luca, P. A., Giebink, N., Mason, A. C., Papaj, D. and Buchmann, S. L.** (2018). How well do acoustic recordings characterize properties of bee (*Anthophila*) floral sonication vibrations? *Bioacoustics* **29**, 1-14. doi:10.1080/09524622.2018.1511474
- De Luca, P. A., Buchmann, S., Galen, C., Mason, A. C. and Vallejo-Marín, M.** (2019). Does body size predict the buzz-pollination frequencies used by bees? *Ecol. Evol.* **9**, 4875-4887. doi:10.1002/ece3.5092
- Dickinson, M.** (2006). Insect flight. *Curr. Biol.* **16**, R309-R314. doi:10.1016/j.cub.2006.03.087
- Dickinson, M. H., Lehmann, F.-O. and Chan, W. P.** (1998). The control of mechanical power in insect flight. *Am. Zool.* **38**, 718-728. doi:10.1093/icb/38.4.718
- Djemai, I., Casas, J. and Magal, C.** (2001). Matching host reactions to parasitoid wasp vibrations. *Proc. R. Soc. Lond. B* **268**, 2403-2408. doi:10.1098/rspb.2001.1811
- Esch, H. and Goller, F.** (1991). Neural control of fibrillar muscles in bees during shivering and flight. *J. Exp. Biol.* **159**, 419-431.
- Gordon, S. and Dickinson, M. H.** (2006). Role of calcium in the regulation of mechanical power in insect flight. *Proc. Natl Acad. Sci. USA* **103**, 4311-4315. doi:10.1073/pnas.0510109103
- Greenewalt, C. H.** (1962). Dimensional relationships for flying animals. *Smithsonian Miscellaneous Collections* **144**, 1-89.
- Guillette, L. M., Hollis, K. L. and Markarian, A.** (2009). Learning in a sedentary insect predator: antlions (Neuroptera: Myrmeleontidae) anticipate a long wait. *Behav. Process.* **80**, 224-232. doi:10.1016/j.beproc.2008.12.015
- Harder, L. D.** (1990). Behavioral responses by bumble bees to variation in pollen availability. *Oecologia* **85**, 41-47. doi:10.1007/BF00317341
- Harder, L. D. and Barclay, R. M. R.** (1994). The functional significance of poricidal anthers and buzz pollination: controlled pollen removal from *Dodecatheon*. *Funct. Ecol.* **8**, 509-517. doi:10.2307/2390076
- Hedenstr m, A.** (2014). How insect flight steering muscles work. *PLoS Biol.* **12**, e1001822. doi:10.1371/journal.pbio.1001822
- Heinrich, B.** (1975). Thermoregulation in bumblebees: II. Energetics of warm-up and free flight. *J. Comp. Physiol. B* **96**, 155-166. doi:10.1007/BF00706595
- Hill, P. S. M., Lakes-Harlan, R., Mazzoni, V., Narins, P. M., Virant-Doberlet, M. and Wessel, A.** (eds). (2019). *Biotremology: Studying Vibrational Behavior*. Springer International Publishing.
- Hrncir, M., Gravel, A.-I., Schorkopf, D. L. P., Schmidt, V. M., Zucchi, R. and Barth, F. G.** (2008). Thoracic vibrations in stingless bees (*Melipona seminigra*): resonances of the thorax influence vibrations associated with flight but not those associated with sound production. *J. Exp. Biol.* **211**, 678-685. doi:10.1242/jeb.013920
- Joos, B., Young, P. A. and Casey, T. M.** (1991). Wingstroke frequency of foraging and hovering bumblebees in relation to morphology and temperature. *Physiol. Entomol.* **16**, 191-200. doi:10.1111/j.1365-3032.1991.tb00556.x
- Josephson, R. K., Malamud, J. G. and Stokes, D. R.** (2000). Asynchronous muscle: a primer. *J. Exp. Biol.* **203**, 2713-2722.
- Kammer, A. E. and Heinrich, B.** (1974). Metabolic rates related to muscle activity in bumblebees. *J. Exp. Biol.* **61**, 219-227.
- King, M. J.** (1993). Buzz foraging mechanism of bumble bees. *J. Apic. Res.* **32**, 41-49. doi:10.1080/00218839.1993.11101286
- King, M. J. and Buchmann, S. L.** (1996). Sonication dispensing of pollen from *Solanum laciniatum* flowers. *Funct. Ecol.* **10**, 449-456. doi:10.2307/2389937
- King, M. J. and Buchmann, S. L.** (2003). Floral sonication by bees: mesosomal vibration by *Bombus* and *Xylocopa*, but not *Apis* (Hymenoptera: Apidae), ejects pollen from poricidal anthers. *J. Kans. Entomol. Soc.* **76**, 295-305.
- King, M. J., Buchmann, S. L. and Spangler, H.** (1996). Activity of asynchronous flight muscle from two bee families during sonication (buzzing). *J. Exp. Biol.* **199**, 2317-2321.
- Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. B.** (2017). lmerTest Package: tests in linear mixed effects models. *J. Stat. Softw.* **82**, 1-26. doi:10.18637/jss.v082.i13
- Laverty, T. M.** (1980). The flower-visiting behaviour of bumble bees: floral complexity and learning. *Can. J. Zool.* **58**, 1324-1335. doi:10.1139/z80-184
- Lehmann, F.-O. and Bartussek, J.** (2017). Neural control and precision of flight muscle activation in *Drosophila*. *J. Comp. Physiol. A* **203**, 1-14. doi:10.1007/s00359-016-1133-9
- Lindsay, T., Sustar, A. and Dickinson, M.** (2017). The function and organization of the motor system controlling flight maneuvers in flies. *Curr. Biol.* **27**, 345-358. doi:10.1016/j.cub.2016.12.018
- Macior, L. W.** (1964). An experimental study of the floral ecology of *Dodecatheon meadia*. *Am. J. Bot.* **51**, 96-108. doi:10.1002/j.1537-2197.1964.tb06605.x
- Masters, W. M.** (1979). Insect disturbance stridulation: its defensive role. *Behav. Ecol. Sociobiol.* **5**, 187-200. doi:10.1007/BF00293305
- Mencinger-Vra ko, B. and Devetak, D.** (2008). Orientation of the pit-building antlion larva *Euroleon* (Neuroptera, Myrmeleontidae) to the direction of substrate vibrations caused by prey. *Zoology* **111**, 2-8. doi:10.1016/j.zool.2007.05.002
- Mhatre, N., Malkin, R., Deb, R., Balakrishnan, R. and Robert, D.** (2017). Tree crickets optimize the acoustics of baffles to exaggerate their mate-attraction signal. *eLife* **6**, e32763. doi:10.7554/eLife.32763
- Moore, C. D. and Hassall, C.** (2016). A bee or not a bee: an experimental test of acoustic mimicry by hoverflies. *Behav. Ecol.* **21**, arw107. doi:10.1093/beheco/arw107
- Morgan, T., Whitehorn, P., Lye, G. C. and Vallejo-Marín, M.** (2016). Floral sonication is an innate behaviour in bumblebees that can be fine-tuned with experience in manipulating flowers. *J. Insect Behav.* **29**, 233-241. doi:10.1007/s10905-016-9553-5
- Mortimer, B.** (2017). Biotremology: do physical constraints limit the propagation of vibrational information? *Anim. Behav.* **130**, 165-174. doi:10.1016/j.anbehav.2017.06.015
- Nachtigall, W. and Wilson, D. M.** (1967). Neuro-muscular control of dipteran flight. *J. Exp. Biol.* **47**, 77-97.
- Nakata, K.** (2010). Attention focusing in a sit-and-wait forager: a spider controls its prey-detection ability in different web sectors by adjusting thread tension. *Proc. R. Soc. B* **277**, 29-33. doi:10.1098/rspb.2009.1583
- Nieh, J. C. and Tautz, J.** (2000). Behaviour-locked signal analysis reveals weak 200-300 Hz comb vibrations during the honeybee waggle dance. *J. Exp. Biol.* **203**, 1573-1579.
- Nunes-Silva, P., Hrncir, M., Shipp, L., Kevan, P. and Imperatriz-Fonseca, V. L.** (2013). The behaviour of *Bombus impatiens* (Apidae Bombini) on tomato (*Lycopersicon esculentum* Mill., Solanaceae) flowers: pollination and reward perception. *J. Pollinat. Ecol.* **11**, 33-40. doi:10.26786/1920-7603(2013)3
- Pritchard, D. and Vallejo-Marín, M.** (2020). Data and code from: Floral vibrations by buzz-pollinating bees achieve higher frequency, velocity and acceleration than flight and defence vibrations [Data set]. *Zenodo*, 3840007. doi:10.5281/zenodo.3840007
- Rasheed, S. A. and Harder, L. D.** (1997). Foraging currencies for non-energetic resources: pollen collection by bumblebees. *Anim. Behav.* **54**, 911-926. doi:10.1006/anbe.1997.0487
- Roberts, S. P., Harrison, J. F. and Dudley, R.** (2004). Allometry of kinematics and energetics in carpenter bees (*Xylocopa varipuncta*) hovering in variable-density gases. *J. Exp. Biol.* **207**, 993-1004. doi:10.1242/jeb.00850
- Roeder, K. D.** (1951). Movements of the thorax and potential changes in the thoracic muscles of insects during flight. *Biol. Bull.* **100**, 95-106. doi:10.2307/1538681
- Rosenheim, J. A.** (1987). Nesting behavior and bionomics of a solitary ground-nesting wasp, *Ammophila dysmica* (Hymenoptera: Sphecidae): influence of parasite pressure. *Ann. Entomol. Soc. Am.* **80**, 739-749. doi:10.1093/aesa/80.6.739
- Rosi-Denadai, C. A., Ara jo, P. C. S., Campos, L. A. d. O., Cosme, L. and Guedes, R. N. C.** (2018). Buzz-pollination in Neotropical bees: genus-dependent frequencies and lack of optimal frequency for pollen release. *Insect Sci.* **27**, 133-142. doi:10.1111/1744-7917.12602
- Rowe, C.** (1999). Receiver psychology and the evolution of multicomponent signals. *Anim. Behav.* **58**, 921-931. doi:10.1006/anbe.1999.1242
- Russell, A. L., Golden, R. E., Leonard, A. S. and Papaj, D. R.** (2016a). Bees learn preferences for plant species that offer only pollen as a reward. *Behav. Ecol.* **27**, 731-740. doi:10.1093/beheco/arv213
- Russell, A. L., Leonard, A. S., Gillette, H. D. and Papaj, D. R.** (2016b). Concealed floral rewards and the role of experience in floral sonication by bees. *Anim. Behav.* **120**, 83-91. doi:10.1016/j.anbehav.2016.07.024
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B. et al.** (2012). Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9**, 676-682. doi:10.1038/nmeth.2019
- Stephens, D. W.** (2008). Decision ecology: foraging and the ecology of animal decision making. *Cognit. Affective Behav. Neurosci.* **8**, 475-484. doi:10.3758/CABN.8.4.475
- Sueur, J., Aubin, T. and Simonis, C.** (2008). Seewave: a free modular tool for sound analysis and synthesis. *Bioacoustics* **18**, 213-226. doi:10.1080/09524622.2008.9753600
- Switzer, C. M. and Combes, S. A.** (2017). Bumblebee sonication behavior changes with plant species and environmental conditions. *Apidologie* **48**, 223-233. doi:10.1007/s13592-016-0467-1
- Switzer, C. M., Russell, A. L., Papaj, D. R., Combes, S. A. and Hopkins, R.** (2019). Sonicating bees demonstrate flexible pollen extraction without instrumental learning. *Curr. Zool.* **65**, 425-436. doi:10.1093/cz/zoz013

- Tercel, M. P. T. G., Veronesi, F. and Pope, T. W.** (2018). Phylogenetic clustering of wingbeat frequency and flight-associated morphometrics across insect orders. *Physiol. Entomol.* **43**, 149-157. doi:10.1111/phen.12240
- Thorp, R. W.** (1979). Structural, behavioral, and physiological adaptations of bees (Apoidea) for collecting pollen. *Ann. Mo. Bot. Gard.* **66**, 788. doi:10.2307/2398919
- Thorp, R. W.** (2000). The collection of pollen by bees. In *Pollen and Pollination* (ed. A. Dafni, M. Hesse and E. Pacini, E.), pp. 211–223. Vienna: Springer.
- Vallejo-Marín, M.** (2019). Buzz pollination: studying bee vibrations on flowers. *New Phytol.* **224**, 1068-1074. doi:10.1111/nph.15666
- Vallejo-Marín, M., Walker, C., Friston-Reilly, P., Solís-Montero, L. and Igic, B.** (2014). Recurrent modification of floral morphology in heterantherous *Solanum* reveals a parallel shift in reproductive strategy. *Phil. Trans. R. Soc. B* **369**, 20130256. doi:10.1098/rstb.2013.0256
- Wang, Q., Zhao, C. and Swank, D. M.** (2011). Calcium and stretch activation modulate power generation in *Drosophila* flight muscle. *Biophys. J.* **101**, 2207-2213. doi:10.1016/j.bpj.2011.09.034
- Westerkamp, C.** (1996). Pollen in bee-flower relations some considerations on melittophily. *Botanica Acta* **109**, 325-332. doi:10.1111/j.1438-8677.1996.tb00580.x