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

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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 ants 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 Rodriguez, 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 Rodriguez, 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...
than those produced during flight (King and Buchmann, 2003; Barth et al., 2005; Hnricir 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; Hnricir 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. Hnricir et al. (2008) found that the frequency of vibrations produced by the tropical stingless bee, Melipona quadridiscata (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 airborne 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; Hnricir 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 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, Hückelhoven, 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, 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**

**Analysing 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

\[
K_{bee} = \frac{K_s}{\frac{K_f + K_s}{2}}
\]

where \(K_s\) is the coupling factor of the flower and \(K_f\) is the coupling factor of the bee. The coupling factor of the flower \(K_f\) was calculated using the method described by Arroyo-Correa et al. (2019). The bee’s coupling factor \(K_{bee}\) was calculated using the coupling factor of the flower \(K_f\) and the coupling factor of the bee’s thorax \(K_{thorax}\) using the formula:

\[
K_{bee} = \frac{K_{thorax} \cdot K_f}{K_{thorax} + K_f}
\]
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 (\(K_{\text{shaker}}\)) using the ratio between expected and observed RMS velocity. Measuring both \(K_{\text{bee}}\) and \(K_{\text{shaker}}\) 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. 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.
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±9.52 mm s\(^{-1}\)) was significantly higher than both defence (194.85±6.12 mm s\(^{-1}\)) and flight buzzes (57.29±1.28 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±2.63 Hz) than both defence (236.32±4.29 Hz) and flight buzzes (136.95±1.73 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±19.40 m s\(^{-2}\)) than defence (297.41±11.96 m s\(^{-2}\)) and flight vibrations (49.43±1.34 m s\(^{-2}\)) (Fig. 3D). In contrast, the peak amplitude displacement of floral (0.27±0.009 mm) and defence buzzes (0.27±0.007 mm) were similar, although both were greater than the displacement amplitude of flight vibrations (0.14±0.005 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

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.
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 ($K_{bee}$=5.64±0.61, $K_{shaker}$=5.95±1.77; means±s.e.) than $S$. citrullifolium ($K_{bee}$=9.92±0.97, $K_{shaker}$=8.93±1.97; 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.
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

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**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 ± 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.
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 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...
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

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

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...
and 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; Laverty, 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**


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**Data availability**


**Supplementary information**

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

**References**


