

Comparison of pollination and defensive buzzes in bumblebees indicates species-specific and context-dependent vibrations

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Abstract Bees produce vibrations in many contexts, including for defense and while foraging. Buzz pollination is a unique foraging behavior in which bees vibrate the anthers of flowers to eject pollen which is then collected and used as food. The relationships between buzzing properties and pollen release are well understood, but it is less clear to what extent buzzing vibrations vary among species, even though such information is crucial to understanding the functional relationships between bees and buzz-pollinated plants. Our goals in this study were (1) to examine whether pollination buzzes differ from those produced during defense, (2) to evaluate the similarity of buzzes between different species of bumblebees (*Bombus* spp.), and (3) to determine if body size affects the expression of buzzing properties. We found that relative peak amplitude, peak frequency, and duration were significantly different between species, but only relative peak amplitude differed between pollination and defensive buzzes. There were significant interactions between species and buzz type for peak frequency and duration, revealing that species differed in their patterns of expression in these buzz properties depending on the context. The only parameter affected by body size was duration, with larger bees producing shorter buzzes. Our findings suggest that although pollination and defensive buzzes differ in some properties, variability in buzz structure also exhibits a marked species-specific component. Species differences in pollination buzzes may have important implications for foraging preferences in bumblebees,

especially if bees select flowers best matched to release pollen for their specific buzzing characteristics.

Keywords Body size · *Bombus* · Buzz pollination · Defensive buzzes · Pollen foraging · Sonication

Introduction

Bees (Hymenoptera: Apoidea) produce vibrations in a variety of contexts including thermoregulation, communication, defense, and foraging (Heinrich 1974; Buchmann 1983; Hmcir et al. 2006). Vibrations are generated from contractions of the thoracic flight muscles and are transmitted outwards through the head, legs, and body of the bee (King 1993). Vibration-assisted foraging (buzz pollination or sonication) is used by bees across several different families (Andrenidae, Apidae, Colletidae, and Halictidae) to extract pollen from anthers, which is then collected and used as a food source (Buchmann 1983). Thousands of plant species across more than 60 families, particularly those in which pollen release is restricted to small openings or pores in the anthers' tips, are buzz-pollinated by bees (Buchmann 1983; De Luca and Vallejo-Marín 2013).

Decades of research on buzz pollination have resulted in a good understanding of the functional relationship of different buzzing properties and pollen release. For example, amplitude is an important buzz property influencing the amount of pollen released from anthers; greater amplitudes are positively correlated with increased pollen ejection loads (Buchmann and Hurley 1978; King 1993; Harder and Barclay 1994; King and Buchmann 1995; King and Buchmann 1996; De Luca et al. 2013). Our understanding of the relative importance of interspecific variability in buzzing vibrations on pollen release is less clear, despite bee-induced vibrations often being invoked as a selective force influencing the evolution of buzz-

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pollinated floral traits (Knudsen and Olesen 1993; Larson and Barrett 1999; Luo et al. 2008; Vallejo-Marín et al. 2010). Accordingly, determining the magnitude of variation in vibrational properties among bee species is important in order to understand its potential consequences on the ecology and evolution of buzz pollination.

The morphology of sonicating bees has been shown to affect certain characteristics of the vibrations they produce. For example, body mass is strongly correlated with the amplitude of buzzes in the buff-tailed bumblebee, *Bombus terrestris* (De Luca et al. 2013; but see Nunes-Silva et al. 2013). Given that pollen release is a positive function of vibration amplitude (Harder and Barclay 1994; De Luca et al. 2013), larger bees may be more efficient at removing pollen per unit of time spent foraging than smaller-bodied bees. Variation in body size can be extremely large both within and among bee species (Michener 2000), yet few studies have attempted to correlate buzzing properties with size within and among species. Quantifying these relationships is therefore necessary in order to gain a better understanding of the factors influencing variation in foraging behavior among species.

Studies of buzz pollination have used recording devices such as laser vibrometry (King and Buchmann 2003; De Luca et al. 2013), accelerometers (King 1993; King and Buchmann 1996), and audio microphones (Harder and Barclay 1994; Arceo-Gómez et al. 2011) to obtain samples of vibrations produced by bees. Audio recording devices are sufficient to capture the main features of buzz pollination vibrations and have technical and operational advantages over accelerometers and laser vibrometers (see Supplementary Material in De Luca and Vallejo-Marín 2013). However, one disadvantage is that when making recordings in the field effects of background noise from sources such as wind may interfere with the quality of recordings. For this reason, some studies have taken advantage of the fact that bees produce vibrations in contexts unrelated to foraging, for example when disturbed (i.e., defensive buzzes), and have used these vibrations as a proxy for pollination buzzes (King et al. 1996; King and Buchmann 2003; Arceo-Gómez et al. 2011). One advantage of using defensive buzzes is that bees can be captured and examined under controlled laboratory conditions where buzzes can be easily evoked, for instance by pinching a body part with forceps. Although the physiological mechanism that generates defensive and pollination vibration is the same (King et al. 1996), correspondence of specific parameters such as frequency, duration, and amplitude has not been experimentally verified. One might expect buzzes to differ though because bees may emphasize certain properties depending on the context. For example, defensive buzzes in bees are warning signals that are used to indicate aggression (Hrncir et al. 2006) and thus might be expected to be produced with a greater amplitude relative to pollination buzzes, as is seen in other types of acoustic warning displays (Evans 1997). As a

result, using defensive buzzes as a proxy for true pollination buzzes could affect our interpretations of the influence variability in buzzing properties have on specific aspects of buzz pollination, such as the removal of pollen from anthers. Clearly, more empirical data are needed to adequately address this issue.

Our aims in this study were to determine the similarity of vibrations among different species of bees and to examine the extent to which vibrations generated from buzz pollination differed from those produced in a defensive context. We used bumblebees as these organisms are well known for their use of buzz pollination vibrations (Macior 1964; Macior 1974; Buchmann 1983). We recorded pollination buzzes from freely foraging bumblebees and obtained defensive buzzes from wild-caught bees we examined in the laboratory. We predicted that bumblebee species would differ in vibrational properties such as peak amplitude as a result of inter-specific variation in the body size of workers, and that pollination buzzes would vary from defensive buzzes as a result of the different functions they perform.

Methods

Study organisms and data collection

Bumblebees exhibit considerable variation in body size within and across species (Goulson 2010) and thus are ideal for evaluating relationships between buzz properties and body size. Within colonies, workers and queens are often of markedly different size, and even workers within a colony can vary in size depending on their specific duties (Pouvreau 1989). For example, thorax widths of workers of *B. terrestris* range from 2.3 to 6.9 mm, while the mean thorax width for gynes (fertile female bees) is 7.9 mm (Goulson et al. 2002). Within the UK, there are over 20 species of bumblebees, although only a few of these tend to be commonly encountered (e.g., *Bombus terrestris*, *Bombus lucorum*, *Bombus hortorum*, *Bombus pascuorum*, *Bombus lapidarius*, and *Bombus pratorum*, as well as other species of cuckoo bumblebees; Goulson 2010). Among these species, *B. pratorum* and *B. pascuorum* are relatively smaller in size, while queens of *B. terrestris* are the largest.

Data collection took place in an experimental garden setting at the University of Stirling in central Scotland from June to September 2011, where we conducted two experiments. First, we collected worker specimens from the field and provoked them to produce defensive buzzes in the laboratory. We obtained defensive buzzes from *B. hortorum*, *B. lapidarius*, *B. pascuorum*, *B. pratorum*, *B. terrestris*, and *B. lucorum*. As in previous studies (e.g., Lye et al. 2012), workers of *B. terrestris* and *B. lucorum* were treated as a single taxon in the analysis, given that these species are morphologically

similar and extremely difficult to be reliably identified in the field. Second, we recorded pollination buzzes from freely foraging bees in an experimental array of the buzz-pollinated herb *Solanum rostratum* (Solanaceae). Pollination buzzes were recorded from *B. pascuorum* and *B. terrestris*/*B. lucorum*, as these species were the most abundant taxa that visited the array.

Experiment 1—defensive buzzes

We captured bumblebees throughout the day as they foraged on flowers using a butterfly net. We kept individuals singly in 20-ml plastic storage containers before transporting them to a laboratory with an ambient temperature of 22 °C. To minimize stress on captured bees, we limited our collecting to 15-min periods before transporting bees to the lab. We used a Zoom H4 digital recorder (Zoom Corporation, Tokyo) to make recordings (24 bit/96 kHz sampling rate). We placed the recorder on a tripod in a fixed position, with the head of the microphone set horizontally at a distance of 25 mm from a cylindrical bee marking tube (10×4 cm) which was held in position with a stand and grip. One end of the tube contained a mesh screen that faced the microphone with the other end open, allowing a bee to be inserted. Once a bee was inside, we used a sponge stopper to lightly press the bee and restrain it against the mesh to provoke defensive buzzing. Each individual was recorded for a minimum of 30 s during which a series of buzzes was usually produced. Following recording, we put the bee back into the storage container and then placed it in a refrigerating cabinet set at 2 °C for approximately 30 min to immobilize the bee to enable handling. We then removed the bee from the refrigerator and placed it in a Petri dish where it could be identified to species, after which its thorax width was measured to the nearest 0.1 mm using a set of digital calipers. Thorax width represents an accurate proxy for body size in bumblebees (Peat et al. 2005; Goulson 2010). Following this, we marked each individual with a unique code on the underside of the thorax using a non-toxic water-based marker and then released it back into the area it was caught. Marking each bee ensured that they were not used more than once.

Experiment 2—pollination buzzes

We constructed an outdoor plant array measuring 10×3 m that contained 30 potted *S. rostratum* plants set evenly apart. *S. rostratum* has often been used as a model of buzz pollination in experimental settings (Jesson et al. 2003; Vallejo-Marín et al. 2009). Experimental plants measured approximately 0.5–1 m in height, and each contained multiple inflorescences that flowered over a period of several days. The array was left for 1 week to enable naturally occurring bumblebees to become aware of the *S. rostratum* plants and regularly utilize them for pollen collection. We recorded

pollination buzzes from foraging bumblebees throughout the day with the same digital recorder used to record defensive buzzes, and we ensured that microphone settings were identical to those used in experiment 1. However, since foraging bees were not restrained, we held the recorder by hand near a worker as it foraged, keeping the microphone directed towards the dorsal surface of the bee's pronotum at all times. We utilized the same 25-mm distance between the bee and the microphone that we used in the previous experiment by affixing a plastic guide that measured 25 mm in length to the tip of the recorder so that as a bee foraged we could maintain consistency in the distance between the bee and microphone head. After recording buzzes from a bee, we captured it with a butterfly net, immobilized, measured, marked, and then released it as described in experiment 1.

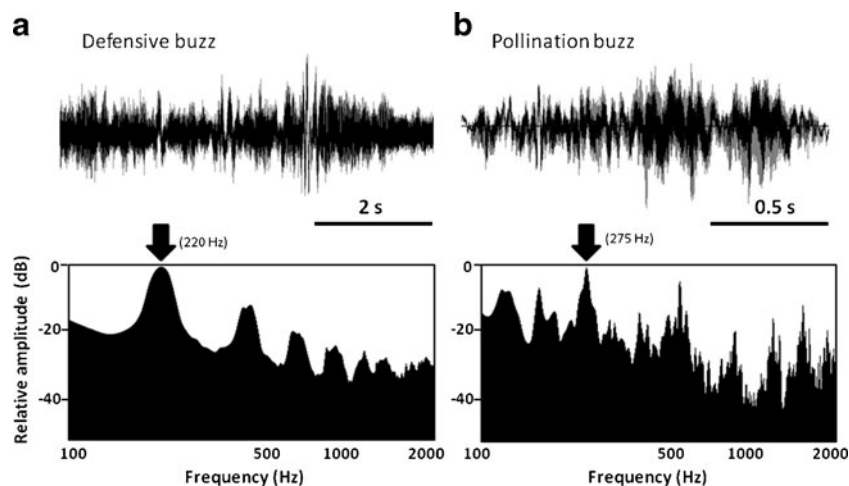
Acoustic analysis of buzzes

We analyzed temporal and spectral features of buzzing vibrations using Audacity v.1.3.11 (<http://audacity.sourceforge.net/>). We made peak frequency measurements using the “Spectrum” function (Hamming window, FFT size=8,192 Hz). For each buzz, we measured the duration (expressed as seconds, s), peak frequency (expressed as Hertz, Hz), and relative peak amplitude (expressed as decibels, dB) (Fig. 1). Relative peak amplitude measurements ranged between −26 dB (maximum) and −61 dB (minimum). To make among-buzz comparisons of this parameter more intuitive, we subsequently set 0 dB to represent the maximum peak amplitude and adjusted values accordingly relative to this reference point. Most bees would produce several buzzes, and in these cases, we measured each individual buzz and used them all in the statistical analyses.

Statistical analysis

We examined the effect of species, buzz type (defensive vs. pollination), and size (thorax width) on three characteristics of bee-produced vibrations, using mixed-effects models (R package “lme4”; Bates et al. 2013) conducted in R v.3.0.1 (R Core Development Team 2010). Only species for which it was possible to collect both pollination and defensive buzzes were analyzed with mixed-effects models, and so *B. hortorum*, *B. lapidarius*, and *B. pratorum* were not included in the statistical analysis. We carried out separate analyses for each characteristic describing a bee's vibrations: relative peak amplitude, peak frequency, and duration (De Luca et al. 2013). Mixed-effects models were chosen to account for the non-independence of multiple buzzes produced by a given individual bee. Therefore, all models initially included bee identity as a random effect; species, buzz type, and its interaction as fixed effects; and bee size as a covariate. For each model, we tested for statistical significance of main effects and interactions using likelihood ratio tests with the R package “car”

Fig. 1 Examples of **a** defensive and **b** pollination buzzes from *B. terrestris*/*B. lucorum* workers. Note the difference in time scale bars. *Y*-axis denotes amplitude. Lower panels show frequency spectrograms of each waveform. Black arrows point to the peak frequency, with the value provided in parentheses



(Fox and Weisberg 2010). We dropped non-significant effects from the final model following the principle of marginality (i.e., if an interaction was significant, we retained the variables involved in it). In order to statistically compare the expected values for different combinations of parameters (e.g., pollination buzzes in *B. terrestris*/*B. lucorum* vs. defensive buzzes in *B. pascuorum*), we calculated least squares means and 95 % confidence intervals for the final models using the package “lsmeans” (Russell 2013). Post hoc comparisons between least squares means were done using “Tukey” tests with *P* values adjusted for multiple comparisons.

Results

We recorded a total of 1,289 buzzes from female workers of five taxa: *B. hortorum*, *B. lapidarius*, *B. pascuorum*, *B. pratorum*, and *B. terrestris/lucorum*. Of these, 1,209 were defensive buzzes from 105 bees (327 from 26 bees of *B. hortorum*, 47 from 7 bees of *B. lapidarius*, 403 from 25 bees of *B. pascuorum*, 42 from 5 bees of *B. pratorum*, and 390 from 42 bees of *B. terrestris/lucorum*), and 80 were pollination buzzes from 19 bees (42 from 12 bees of *B. pascuorum* and 38 from 7 bees of *B. terrestris/lucorum*). The number of buzzes per bee ranged from 1 to 40 (mean=18.3) for defensive buzzes and 1 to 11 (mean=5.5) for pollination buzzes. Descriptive statistics (mean \pm SE) of buzzes are provided in Table 1.

Effect of species, buzz type, and body size on buzz characteristics

All three buzzing parameters were significantly influenced by species, buzz type, or their interaction (Table 2, Fig. 2). Relative peak amplitude was significantly higher in *B. terrestris/lucorum* than in *B. pascuorum* for both defensive and pollination buzzes (Tables 2 and 3). Defensive buzzes

had, on average, significantly greater amplitude values than pollination buzzes (Tables 1 and 2, Fig. 2), although the pollination buzzes of *B. terrestris*/*B. lucorum* are not statistically lower than defensive buzzes of *B. pascuorum* (Tukey test: $P > 0.05$; Table 3). In contrast, peak frequency showed a significant species*buzz type interaction (Table 2), suggesting that the effect of buzz type on frequency varied between species. Post hoc tests failed to identify significant differences between pairwise comparisons of species*buzz type combinations (Table 3), although pollination buzzes of *B. terrestris*/*B. lucorum* were higher in peak frequency than for defensive buzzes in this species, while the pattern was reversed for *B. pascuorum* (Tables 1 and 3, Fig. 2). Mean values for buzz duration (Tables 1 and 3) suggest that, overall, defensive buzzes are longer than pollination buzzes. However, we found a significant species*buzz type interaction, but in this case, a post hoc test revealed that defensive buzzes of *B. terrestris*/*B. lucorum* were significantly longer than all other species*buzz type combinations (Table 3).

We found a significant negative relationship between thorax width and duration, with larger bees producing shorter buzzes (Table 2); however, there was no effect of thorax width on either relative peak amplitude (coefficient=0.50 \pm 1.1, $P=0.64$) or peak frequency (coefficient=13.71 \pm 10.76, $P=0.21$).

Discussion

Our study reveals important but variable effects of species, buzz type, and body size on buzz structure in bumblebees. The presence of significant interactions between species and buzz type for peak frequency and duration further indicates that variability between pollination and defensive buzzes do not follow similar trends across species. As a result, using defensive buzzes as a proxy for pollination buzzes is problematic and should be avoided because there are no consistent patterns across species that justify using one buzz type as a substitute

Table 1 Inter-specific variability (mean \pm SE) in thorax width and defensive and pollination buzz properties for five *Bombus* taxa

Species	Thorax width (mm)	Defensive			Pollination		
		Relative peak amplitude (dB)	Frequency (Hz)	Duration (s)	Relative peak amplitude (dB)	Frequency (Hz)	Duration (s)
<i>B. hortorum</i>	5.06 \pm 0.11	-24.7 \pm 0.4	205 \pm 3	2.99 \pm 0.29	–	–	–
<i>B. lapidarius</i>	4.29 \pm 0.13	-28.4 \pm 1.5	200 \pm 10	7.12 \pm 1.33	–	–	–
<i>B. pascuorum</i>	4.26 \pm 0.12	-29.8 \pm 0.4	254 \pm 3	2.28 \pm 0.20	-38.1 \pm 0.7	214 \pm 8	1.07 \pm 0.11
<i>B. pratorum</i>	4.28 \pm 0.34	-34.3 \pm 1.8	151 \pm 10	4.33 \pm 0.64	–	–	–
<i>B. terrestris/B. lucorum</i>	5.34 \pm 0.13	-25.2 \pm 0.4	248 \pm 4	4.16 \pm 0.33	-30.6 \pm 1.9	289 \pm 12	0.74 \pm 0.07

No pollination buzzes were obtained from *B. hortorum*, *B. lapidarius*, or *B. pratorum*. $N = 1,289$ buzzes in total; sample sizes per species and buzz type are provided in the text

for another. Our finding that defensive buzzes had greater peak amplitudes than pollination buzzes was expected. Like other kinds of warning signals used to indicate aggression (Guilford and Dawkins 1991; Evans 1997), these buzzes are produced with more power than pollination vibrations. This was likely caused by increased physiological exertion by a bee as it attempted to escape the marking cage.

Although buzzes differed between *B. pascuorum* and *B. terrestris/B. lucorum*, with only two species directly compared, we recognize that more data from additional species needs to be collected in order to more thoroughly evaluate species' differences in buzz pollination vibrations. Yet, the current results suggest that buzzing vibrations do indeed exhibit marked species-specific characteristics. Such variability may help explain species-level patterns of foraging behavior in bumblebees. For example, King (1993) examined differences in buzzing behavior between workers of *B. hortorum* and *B. terrestris* on comfrey (*Symphytum officinale*) and found that *B. hortorum* workers used a different peak frequency, generated higher peak amplitudes, and physically manipulated flowers differently while sonicating. The author concluded that

B. hortorum was a more efficient species at vibrating comfrey anthers due to its characteristic buzzing behavior and thus was likely able to collect more pollen per flower visited. Also, when given a choice of flower species, *B. terrestris* workers tended to avoid comfrey, which the author suggested occurred because of their lower buzzing effectiveness on that plant species. In our study, we only used *S. rostratum* flowers to obtain pollination buzzes, and thus, the variability we observed between *B. pascuorum* and *B. terrestris/lucorum* could not have been due to floral effects, but rather to inherent differences between species in their buzzing behavior, similar to the findings of King (1993). Taken together, the results of these studies suggest that since bee species differ in the production of buzzes on one type of flower, it is perhaps likely that bees can also modify pollination buzzes for different floral morphologies. The correspondence between intra-specific variability in buzzing properties and floral morphology is at present poorly quantified (Harder and Barclay 1994; King and Buchmann 1996; De Luca et al. 2013), but such knowledge is crucial to better understand the factors that shape foraging relationships between buzz-pollinating bumblebees and their plant hosts.

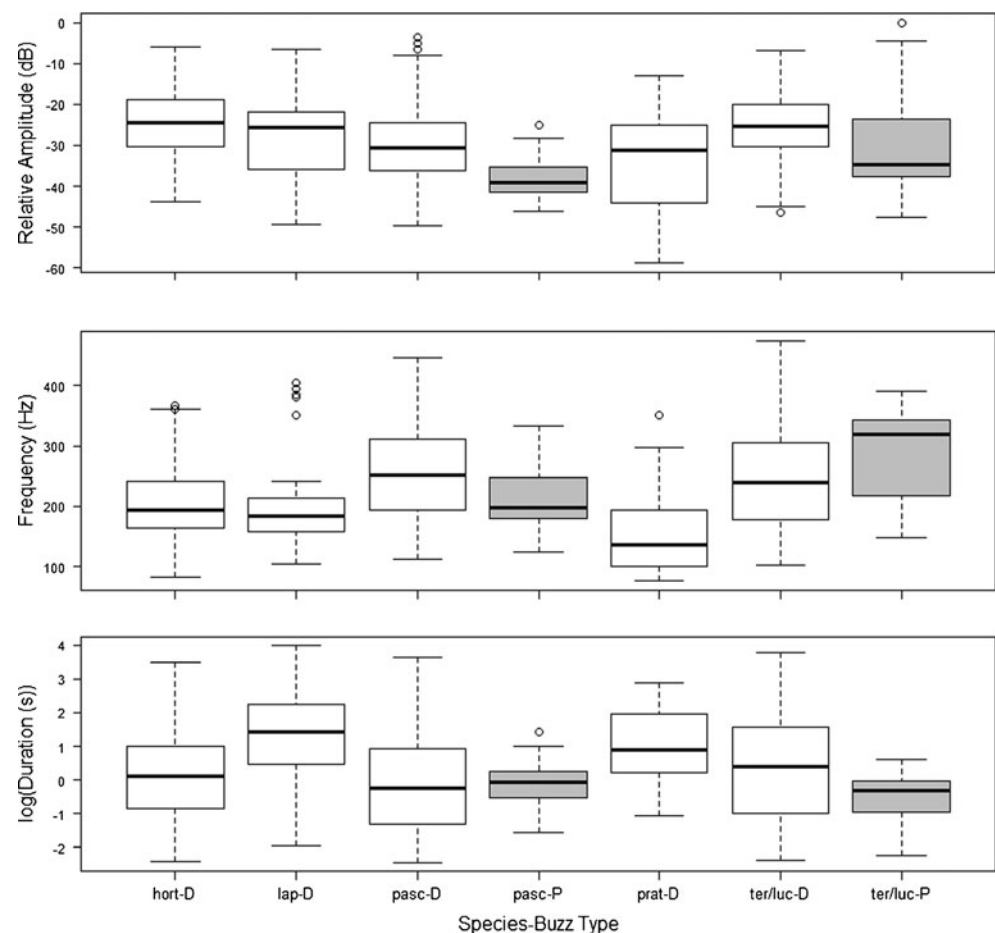
Table 2 Effect of species (*B. pascuorum* vs. *B. terrestris/B. lucorum*), buzz type (defensive vs. pollination), and body size (thorax width) on three characteristics of bee-produced vibrations

Model coefficient	Relative peak amplitude (dB)	SE	<i>P</i>	Frequency (Hz)	SE	<i>P</i>	Duration (ln(s))	SE	<i>P</i>
Species (<i>B. pascuorum</i>)	-29.09	1.07	<0.001	239.26	10.85	>0.50	2.49	0.72	<0.001
Species (<i>B. terrestris/B. lucorum</i>)	-23.62	0.88		229.86	8.62		3.55	0.88	
Buzz type	-7.91	1.65	<0.001	-25.75	20.59	0.21	-0.24	0.34	0.48
Species*buzz type	–	–	–	76.40	31.25	0.014	-1.55	0.55	<0.01
Thorax width	–	–	–	–	–	–	-0.54	0.17	<0.01

The table shows the model coefficients of separate analyses carried out for each response variable (relative peak amplitude in decibels, frequency in Hertz, and duration in seconds (natural log-transformed)) using mixed-effects models. All models included bee identity as a random effect. Final models were achieved by backward elimination of non-significant marginal terms as assessed in likelihood ratio tests of nested models. The *P* values presented here were calculated for each variable in the final model using likelihood ratio tests, with significant values indicated in italics. The interaction coefficient is given for the response of a pollination buzz by *B. terrestris/B. lucorum*. Note that *B. lapidarius*, *B. hortorum*, and *B. pratorum* were not included in the analysis as no pollination buzzes were available for these species

SE standard error

Fig. 2 Box plots depicting variation in buzzing parameters categorized by species and buzz type. *Top panel*—relative peak amplitude, *middle panel*—peak frequency, *bottom panel*—duration. The *horizontal line*, *rectangles*, and *top and bottom whiskers* show the median, interquartile range (IQR), and 1.5*IQR, respectively. *Open circles* indicate outliers. Legend: *hort B. hortorum*, *lap B. lapidarius*, *pasc B. pascuorum*, *prat B. pratorum*, *ter/luc B. terrestris/B. lucorum*, *D* defensive (white), *P* pollination (gray) buzzes



Body size had a significant effect on duration, with larger bees producing shorter buzzes. This finding has not been reported previously in studies of buzz pollination and therefore adds much needed empirical data to our knowledge of the influence of morphology on buzzing properties. The production of buzzes during foraging likely entails significant physiological costs for workers (Peat et al. 2005; De Luca et al. 2013), and thus, there may be a trade-off between size and the ability to produce long-duration vibrations if larger bees cannot sustain buzzing for prolonged periods. Duration is

positively correlated with the amount of pollen ejected from anthers in *S. rostratum* (De Luca et al. 2013), and therefore, smaller workers may still be effective at pollen collection given that they can sustain buzzing for longer periods than larger workers, at least in the *Bombus* species examined here. The lack of a significant relationship between relative peak amplitude and thorax width was unexpected, given that a previous study found a strong correlation between mass and peak amplitude for *B. terrestris* (De Luca et al. 2013). Perhaps mass is a better determinant of peak amplitude than body size

Table 3 Modeled estimates of mean effects (least squares means), 95 % confidence intervals (CI), and post hoc Tukey tests for mean differences in relative peak amplitude, peak frequency, and duration of buzzes obtained from linear mixed-effects analyses of buzz characteristics (Table 2)

Taxon*buzz type	Relative peak amplitude (dB)	95 % CI	Frequency (Hz)	95 % CI	Duration (s)	95 % CI
<i>B. pascuorum</i> *defensive	-29.09 A	-31.2 to -26.96	239 A	218–261	0.98 A	0.68–1.42
<i>B. terrestris/lucorum</i> *defensive	-23.61 B	-25.36 to -21.87	230 A	213–247	2.83 B	2.06–3.87
<i>B. pascuorum</i> *pollination	-37.00 C	-40.08 to -33.92	213 A	179–248	0.78 A	0.43–1.38
<i>B. terrestris/lucorum</i> *pollination	-31.53 A	-34.83 to -28.22	280 A	237–324	0.47 A	0.23–0.96

Within each buzz parameter, letters indicate statistically significant differences, adjusted for multiple comparisons. Duration is presented as back-transformed values

since amplitude is the result of the force applied to anthers, which depends directly on the mass of the vibrating bee (King 1993; De Luca et al. 2013). However, Nunes-Silva et al. (2013) failed to find a significant correlation between thorax weight and both frequency and amplitude in *Bombus impatiens* (Nunes-Silva et al. 2013). Regardless, there were significant differences in peak amplitude between species, with larger-bodied *B. terrestris*/*B. lucorum* producing greater-amplitude pollination buzzes than *B. pascuorum* (Fig. 2). Given that larger-amplitude buzzes release significantly more pollen in many plant species (King 1993; Harder and Barclay 1994; De Luca et al. 2013), different-sized bee species that vary in the peak amplitude of their buzzes may actively choose to forage on buzz-pollinated flowers that best match their species-specific amplitude characteristics to optimize pollen collection. Bumblebees exhibit a remarkable capacity to adjust foraging preferences based on experience, and workers will often concentrate on flowers that provide the best pollen rewards, even if other floral types are more abundant in the vicinity (Goulson 2010). Furthermore, the fact that naïve bumblebees develop buzz pollination behavior over the course of several days of repeated foraging (Laverty 1980; King 1993) suggests individual workers undergo a period of learning, maybe through trial and error, of not only how to perform buzzing but also how to focus on flowers that release the most pollen for their foraging effort (Cane and Buchmann 1989; Harder 1990; Goulson et al. 2002). Species variability in buzzing properties such as peak amplitude and duration may thus constitute a significant factor contributing to species-specific foraging preferences on buzz-pollinated flowers (Corbet et al. 1988; King 1993; King and Buchmann 1995; Kawai and Kudo 2009) and may also influence functional relationships between buzzing vibrations, floral morphology, and pollen release (Knudsen and Olesen 1993; Dulberger et al. 1994; Luo et al. 2008; Fidalgo and Kleinert 2009; De Luca et al. 2013).

One potential source of variation between pollination and defensive buzzes may have resulted from the fact that pollination buzzes were obtained from unrestrained foraging bees, while defensive buzzes were obtained from bees that were caught and examined in the laboratory. The act of capturing a bee could have introduced some stress that may have affected its subsequent buzzing behavior, but we attempted to minimize this effect by eliciting defensive buzzes within 15 min of a bee being captured. Additionally, in future studies it may be useful to obtain pollination and defensive buzzes from the same bees, in order to account for individual-level effects on variation between these different kinds of vibrations.

To conclude, the results of our study provide new evidence that the kinds of vibrations used in buzz pollination are not necessarily equivalent to vibrations given in a defensive context. Furthermore, differences in the properties of pollination buzzes among species suggest that such vibrations have an inherent species-specific component and are also influenced

by body size differences among individual bees. Whether buzz-pollinating bees attempt to match species-specific buzzing characteristics to particular floral morphologies to maximize pollen collection is currently unknown, but given our findings that demonstrate species-level differences in buzzing properties, it remains a distinct possibility.

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Ethical standards The experiments performed here comply with the current laws of the University of Stirling and the UK.

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