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Original article

Buzz-pollination in Neotropical bees: Genus-dependent frequencies and lack of optimal frequency for pollen release

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Abstract Over 50 genera of bees release pollen from flower anthers using thoracic vibrations, a phenomenon known as buzz-pollination. The efficiency of this process is directly affected by the mechanical properties of the buzzes, namely the duration, amplitude and frequency. Nonetheless, although the effects of the former two properties are well described, the role of buzz frequency on pollen release remains unclear. Furthermore, nearly all of the existing studies describing vibrational properties of natural buzz-pollination are limited to bumblebees (*Bombus*) and carpenter bees (*Xylocopa*) constraining our current understanding of this behavior and its evolution. Therefore, we attempted to minimize this shortcoming by testing whether flower anthers exhibit optimal frequency for pollen release and whether bees tune their buzzes to match these (optimal) frequencies. If true, certain frequencies will trigger more pollen release and lighter bees will reach buzz frequencies closer to this optimum to compensate their smaller buzz amplitudes. Two strategies were used to test these hypotheses: i) the use of (artificial) vibrational playbacks in a broad range of buzz frequencies and amplitudes to assess pollen release by tomato plants (*Solanum lycopersicum* L.), and ii) the recording of natural buzzes of Neotropical bees visiting tomato plants during pollination. The playback experiment indicates that although buzz frequency does affect pollen release, no optimal frequency exists for that. In addition, the recorded results of natural buzz-pollination reveal that buzz frequencies vary with bee genera and are not correlated with body size. Therefore, neither bees nor plants are tuned to optimal pollen release frequencies. Bee frequency of buzz-pollination is a likely consequence of the insect flight machinery adapted to reach higher accelerations, while flower plant response to buzz-pollination is the likely result of its pollen granular properties.

Key words: buzz amplitude; buzz frequency; floral sonication; pollination; stingless bees; vibration

Introduction

Buzz-pollination, also known as floral sonication (e.g., King & Buchman, 2003), refers to the process of pollen release from flower anther by means of thoracic vibrations by plant pollinators. This process of pollination is frequent and reported in over 50 genera of pollinators, including several species of bumblebees and stingless bees, but not honey bees (De Luca & Vallejo-Marín, 2013). Through buzz-pollination bees are able to access valuable pollen resources from over 20,000 dicot and monocot plant species from 72 families whose flowers exhibit poricidal anthers (Buchmann & Hurley, 1978; Buchmann, 1985; Proença, 1992; Arceo-Gómez et al., 2011), as well from flowers with longitudinal dehiscent anthers (Bushmann, 1985; Proença, 1992).

Buzz-pollination is the prevailing mechanism by which bees manage to release pollen from the narrow apical pore at the plant anthers, even though there are exceptions (e.g. King & Buchmann, 1995), including some bees that are able to access pollen inserting their tongue or destroying the anthers (Thorp & Estes, 1975; Renner, 1983). Therefore, many crop and wild plant species rely on buzz-pollinating bees to achieve effective pollen release and dispersal, seed formation, and fruit production (Morandin et al., 2001; Gemmil-Herren & Ochieng, 2008; Kawai & Kudo, 2009; Nunes-Silva et al., 2010). This is particularly frequent in the Neotropical region (Bezerra & Machado, 2003; Carvalho & Oliveira, 2003; Fracasso & Sazima, 2004; Nunes-Silva et al., 2010; Vinícius-Silva et al., 2017).

3

The efficiency of buzz-pollination in releasing pollen is directly affected by the buzz mechanical properties, namely duration, amplitude and frequency. Buzz frequency can be defined as the rate at which the thorax completes successive vibrational cycles (Hrncir *et al.*, 2008), which tend to be the same in other body parts such as the head (King & Buchmann, 2003). Buzz amplitude is the intensity of such vibrations, which can vary between body parts (King & Buchmann, 2003). The bumblebee *Bombus terrestris* (L.) for instance exhibits buzz duration ranging from 0.5 to 3.0 s and positively correlates with the total amount of pollen ejected (De Luca *et al.*, 2013). This relationship explains why bees increase buzz duration and number of buzzes when they perceive that flowers still have pollen available (Buchmann & Cane, 1989; Harder, 1990; Shelly *et al.*, 2000; Nunes-Silva *et al.*, 2013). Likewise, the California carpenter bee (*Xylocopa californica* Cresson) produces buzz amplitude from 0.08 to 0.15 mm peak-to-peak in the thorax and more than twice this value in the head (King & Buchmann, 2003), which also correlates to pollen ejection (Buchmann & Hurley, 1978; Harder & Barclay, 1994; De Luca *et al.*, 2013). Nonetheless, the amplitude values seem to be a mechanical consequence imposed by the pollinator body size rather than a decision based on pollen availability (De Luca *et al.*, 2013). This seems so because longer and more intense vibrations lead to higher pollen release (De Luca *et al.*, 2013), probably because of an increase in the grain kinetic energy when repeatedly hitting the inner faces of anther walls (Buchmann & Hurley, 1978). However, in contrast with the well-described effects of buzz duration and amplitude on pollen release, the effect of buzz frequency on pollen release remains unclear.

The fundamental buzz frequencies in buzz-pollination typically range from 100 to 400 Hz (Burkart *et al.*, 2011; De Luca & Vallejo-Marín, 2013). Although within narrow ranges such frequencies may not be distinguishable for pollen release (De Luca *et al.*,

2013), within broad ranges some frequencies may be more important due to the stamen-exhibited resonance (Harder & Barclay, 1994; King & Buchmann, 1995; De Luca & Vallejo-Marín, 2013), and the particulate mechanical properties of the pollen grains (King & Buchmann, 1996; Harada *et al.*, 2002). Furthermore, bees can assess the amount of pollen released per flower and change their buzzing performance accordingly, particularly its duration (Buchmann & Cane, 1989; Harder, 1990; Shelly *et al.*, 2000; Nunes-Silva *et al.*, 2013), contrasting with earlier suggestion (Hodges & Miller, 1981). These arguments led to the perception that the frequency variation observed in natural buzzes is a consequence of the bees attempting to reach more effective frequency vibrations (e.g., Harder & Barclay, 1994; Arceo-Gómez *et al.*, 2011; Burkart *et al.*, 2011; Morgan *et al.*, 2016; Switzer *et al.*, 2016).

An earlier biomechanical model of buzz-pollination predicts that velocity is the main parameter out of amplitude and frequency to explain the amount of pollen released from vibrating anthers, as velocity, acceleration and displacement are all proxies of amplitude (Buchmann & Hurley, 1978). However, subsequent findings showed that frequency actually interferes with pollen release and both variables are positively correlated at a given vibrational velocity (Harder & Barclay, 1994). The importance of buzz frequency for pollen release was further emphasized later, but this relationship is affected by buzz amplitude and how it is expressed (King & Buchmann, 1995, 1996). In fact, buzz amplitude has been independently expressed as either acceleration (e.g., King & Buchmann, 1995, 1996), displacement (e.g., Harder & Barclay, 1994; King & Buchmann, 1995), or velocity (e.g., De Luca *et al.*, 2013), and some studies do not even mention amplitude values (e.g., Buchmann *et al.*, 1977; Corbet *et al.*, 1988; Arceo-Gómez *et al.*, 2011). Regardless, some evidences suggest an optimal buzz frequency for pollen release at 500 Hz (Buchmann *et al.*, 1977; Corbet *et al.*, 1988; Harder & Barclay,

1994), but broader ranges of amplitude and frequencies have yet to be tested against pollen release besides of considering whether alternate proxies for buzz amplitude affect this relationship.

Another limitation of the available studies on buzz-pollination is the restricted focus on bumblebees (*Bombus*) and carpenter bees (*Xylocopa*) (Buchmann & Cane, 1989; Harder & Barclay, 1994; King & Buchmann, 2003; De Luca *et al.*, 2013; Nunes-Silva *et al.*, 2013; Morgan *et al.*, 2016). Some information is available about other bee genera (e.g., Burkart *et al.*, 2011), but larger samples sizes and species diversity are necessary to allow taxa comparisons limiting our understanding of how evolution shaped this behavior.

We tested whether flower anthers respond to specific frequencies optimizing pollen release and whether buzz-pollination bees tune their buzzes to reach these frequencies. If true, pointed frequencies would trigger higher pollen release regardless of the vibration amplitude and smaller bees would focus their buzz towards frequencies closer to the optimal for pollen release attempting to compensate for their small buzz amplitudes. Therefore, we aimed at answering three questions: 1) how do frequency and amplitude affect pollen release in a broad range of these variables encompassing their natural range of occurrence?; 2) do buzz frequencies differ within and among Neotropical bee species?; and 3) does bee body size correlate with buzz frequency? We used two strategies to address these issues: i) the use of (artificial) vibrational playbacks in a broad range of buzz frequencies and amplitudes to assess pollen release by tomato plants (*Solanum lycopersicum* L.), and ii) the recording of natural buzzes of Neotropical bees visiting tomato plants during pollination.

Material and methods

Playback experiment of pollen artificial release

Greenhouse-grown tomato plants (*Solanum lycopersicum* L. ac. BGH 7488) were used to collect flowers for the pollen release experiment. The individual flower plants were labelled at the 1st day of anthesis and allowed to age until the 4th day for use because pollen availability is age-dependent (Harder & Barclay, 1994), and abundant at this later age in tomato flowers. The flowers used were healthy and at the peak of their pollen release under the conditions of our study. Upon reaching this age, the flowers were cut under water at their pedicel maintaining the anthers dry, they were placed in 1 mL microcentrifuge tubes, and were subsequently transported to laboratory within a styrofoam box. No more than 15 flowers were used at a time to allow their use within two hours. Only visually healthy flowers with fully formed anthers were used without exhibiting any symptoms of dehydration or abortion. The flowers were always collected during daytime, between 9:00 am and 4:00 pm.

Pollen release was assessed by submitting individual flowers to artificial sinusoidal waves with 40 different frequency and amplitude combinations for a fixed length of time (2 s). Six replicates (i.e., flowers) were used for each signal combination. A total of 10 frequencies were tested: 100, 200, 300, 400, 500, 600, 700, 800, 1000 and 1600 Hz. Regarding the amplitudes, they varied with the applied frequency as follows: for 100 Hz the tested amplitudes were 31.25, 62.5, 125 and 187.5 m/s^2; for 200 Hz the amplitudes were 62.5, 125, 187.5 and 250 m/s^2; and for all the other frequencies the amplitudes tested were 125, 250, 375 and 500.0 m/s^2. Signals were produced, amplified and emitted by a wave generator (WW5062, Tabor Electronics Ltd., Israel), a power amplifier (Type 2718, Brüel & Kjaer, Denmark) and a mini-shaker (Type 4810, Brüel & Kjaer, Denmark).

The experiment of pollen release was performed following a completely random design with frequency and amplitude calibrations of the mini-shaker performed before each trial. This was achieved by pointing a laser vibrometer (PVD-100, Polytec Inc., Germany) perpendicularly at the mini-shaker's vibratory plate and assessing its output signal parameters in an oscilloscope (TDS2012C, Tektronix, USA). During calibration the mini-shaker amplitude was readjusted by shifting the power amplifier settings until it matched that calculated for the trial. The mini-shaker was positioned at a tripod on top of a vibration isolating table (63-500 Series Micro-g, TMC, USA). A metallic hook was attached to the mini-shaker vibratory plate, which was used to receive the flower to be used on the trial. The flower was suspended by the pedicel with its anthers pointing down deep inside a conical pollen collector without touching it and horizontal vibration was applied to the stamen. The collected pollen subjected to the desired frequency and amplitude combination for 2 s was subsequently weighted in an electronic balance (XS3DU, Mettler Toledo, USA) to determine the amount of pollen released.

Field recordings of natural pollination buzzes

The field recording of pollination buzzes was carried out using tomato plants (*S. lycopersicum* ac. BGH 7488) grown at the experimental fields of the Federal University of Viçosa (Viçosa, MG, Brazil). The visiting pollinators were observed as they approached the plants and a professional hand recorder (SongMeter SM2, Wildlife Acoustics*,* USA) was used to record the sounds during buzz-pollination. Although the flower age was not determined, the pollinators always visited healthy-looking flowers apparently at the peak of their pollen release. After taking off the bees were captured with the aid of an entomological net and placed inside glass vials with ethyl acetate to allow subsequent identification and measurement of body parameters. Each recorded buzz was divided in three sections of similar duration and the fundamental frequency of

8

each section was measured using Avisoft-SASLab Lite (Avisoft Bioacoustics, Germany); the average frequency of each buzz was the mean of the three frequencies. All bees were weighted in a precision balance (AG 200, Gehaka, Brazil) and photographed under stereo-microscope (Stemi 2000, Zeiss, Germany) to quantify their inter-tegular distance using the software Image-Pro Plus (Media Cybernetics, Inc. Rockville, EUA). Inter-tegular distance is commonly used as proxy of body size (Cane, 1987).

A second experiment was also performed, but using tomato plants of a variety with larger fruit size (*S. lycopersicum* ac. TPX-4460) and wrapping the flower buds with meshed sacks at the start of their development. The flowers remained wrapped until anthesis, when the sacks were removed exposing the flowers to buzz-pollination. After a pollinator visitation to the flower and buzz recording, performed as previously described for the prior experiment, the flower was again wrapped in meshed sack and labelled with date and pollinating visitor information. The pollinator was also captured, as previously described, aiming identification and measurements. After fruit development, the total number of seeds was recorded and associated with the respective visiting pollinator.

Statistical analyses

The relationship between the amount of pollen released, and the frequency and amplitude obtained from the playback experiment of pollen release was subjected to regression analysis using the curve-fitting procedure of TableCurve 3D v4.0 (Systat, San Jose, CA, USA), where amplitude and frequency were the independent variables and the amount of pollen released was the dependent variable. The significant regression models ($P < 0.05$) were tested from the simplest (linear and quadratic) to more complex (peak and asymptotic) models basing the model selection on parsimony, high F-values (and mean squares), and a steep increase in R^2 with model complexity. Residual distribution

was also checked for each analysis to validate parametric assumptions. Three regression analyses were modeled, each one with amplitude values expressed either as acceleration, displacement or velocity (the latter two exhibited as Fig. S1).

The results obtained with the field recordings of natural pollination buzzes were subjected to analyses of variance for each individual parameter followed by Tukey's HSD test ($P < 0.05$), when appropriate (PROC GLM, SAS Institute, Cary, NC, USA). The differences in buzz frequency during the three stages of the buzzes (i.e., at the start, middle, and end of buzz) were considered in two-way analyses of variance (species × buzz stage), while the remaining traits were assessed by regular analyses of variance. The relationship between buzz frequency during pollination and pollinator body parameters and seed production was tested using Pearson's correlation analyses ($P < 0.05$) (PROC CORR, SAS Institute).

Results

Playback experiment of pollen artificial release

Three-dimensional regression analysis testing the effect of buzz amplitude and frequency on pollen release provided significant results ($R^2 = 0.33$; $F_{2,237} = 57.61$, $P < 0.001$) (Fig. 1). Regardless of the proxy used for relative amplitude, if acceleration (Fig. 1), displacement or velocity (Fig. S1), amplitude is positively associated with pollen release – the higher the amplitude, the higher is the pollen release. The relationship between buzz frequency and pollen release is also significant, but varies with the proxy used for expressing relative amplitude (Figs. 1 and S1). Buzz frequency exhibits a negative relationship with pollen release when amplitude is expressed as acceleration (Fig. 1), but reverted to a positive one when displacement and velocity are used as proxy of buzz amplitude (Fig. S1).

Field recordings of natural pollination buzzes

The 1st field experiment of buzz-pollination in tomato plants (producing fruits of smaller size) allowed the recognition of 49 pollination events from 15 species of buzz-pollinators, among which the stingless bee *Melipona bicolor* Lepeletier was the most frequent visitor followed by the bee *Exomalopsis analis* Spinola and the bumblebee *Bombus pauloensis* Friese, with eight recorded individual visitors of the former species and six for the latter two. Regardless of the species, the buzz frequencies did not vary significantly among the three different phases of buzzing (i.e., beginning, middle, and end) ($F_{2,44} = 0.37$, $P = 0.69$), but the differences in buzz frequency among species were significant ($F_{14,34} = 24.32$, $P < 0.001$). The bee species within the same genus exhibited similar buzz frequency with member of the large-size bee genus *Centris* and the smaller stingless bees of the genus *Melipona* exhibiting the higher buzz frequencies (Fig. 2A). Individual body mass and inter-tegular distance also varied significantly among the buzz-pollinators ($F_{14,34} = 8.28$, $P < 0.001$ and $F_{14,34} = 47.40$, $P < 0.001$ respectively) (Fig. 2B and 2C). The correlation between buzz frequency and body mass was not significant (r = 0.16, $P = 0.66$, $n = 49$), nor was the correlation between buzz frequency and inter-tegular distance (r = 0.06, $P = 0.27$, $n = 49$).

In the 2nd field experiment of buzz-pollination, in which the plants used produce larger tomatoes from similar-size flowers, and seed production was also recorded, only 14 pollination events from four pollinator species were recorded. The bumblebee *Bombus morio* (Swederus) was the main visitor accounting for six of the buzz-pollination events recorded. Again, as in the 1st field experiment of buzz-pollination described above, the buzz frequencies did not vary significantly among the buzzing phases ($F_{2,11} = 0.17$, $P = 0.84$), but the differences in buzz frequency among species were

significantly different ($F_{3,11}$ = 14.79, P < 0.001) (Fig. 3A). The body mass of the visiting buzz-pollinator species also differed significantly among species ($F_{3,11}$ = 34,19, P < 0.001) (Fig. 3B), as did inter-tegular distance ($F_{3,11}$ = 389/70, P < 0.001) (Fig. 3C), but not the tomato seed production ($F_{3,11}$ = 1.19, P = 0.37) (Fig. 3D). Although individual body mass and inter-tegular distance were significantly and positively correlated with buzz-frequency on this experiment (n = 14, r = 0.67, P = 0.001 and r = 82, P < 0.001, respectively), none of these proxies for bee size (i.e., body mass and inter-tegular distance) were significantly correlated with tomato seed production (n = 14, r = - 0.12, P = 0.69).

Discussion

The role of buzz frequency among pollinators is a knowledge gap that currently exists in the rather frequent process of buzz-pollination, which we addressed here testing whether flower anthers respond to specific frequencies optimizing pollen release and whether buzz-pollination bees tune their buzzes to reach these frequencies. A (artificial) playback experiment of pollen release and field recordings of natural pollination buzzes of tomato flowers were the approaches used in the study.

The playback experiment of pollen release under a range of artificially-generated frequency and amplitude combinations indicated that both vibration characteristics, amplitude and frequency, do play a role in pollen release by tomato flowers. Although the assessments were performed on flowers at the peak of pollen release, the (reduced) pollen release expected on younger, older or moist flowers should remain consistent within the range of buzz frequencies and amplitudes assessed retaining the same trend observed in our study. Regardless of the amount of pollen available for release, the tomato flowers are efficiently pollinated through buzz-pollination receiving visit of a

range of pollinator species (e.g., Santos *et al.*, 2014; Silva-Neto *et al.,* 2017; Vinícius-Silva *et al.*, 2017). Our playback results confirmed the efficient pollen release by tomato flowers when subjected to vibration at a range of amplitude and frequencies. The role of buzz amplitude for buzz pollination is broadly recognized (Buchmann & Hurley, 1978; Harder & Barclay, 1994; De Luca *et al.*, 2013), but the role of buzz frequency in this process remains unclear (Harder & Barclay, 1994; King & Buchmann, 1996; De Luca & Vallejo-Marín, 2013; De Luca *et al.*, 2013). Our findings reinforce the notion of the importance of buzz amplitude for pollen release, the higher amplitude the higher the release, expressed by any of its common proxies (i.e., acceleration, displacement, and velocity).

Buzz frequency also provided significant effect on pollen release by tomato flowers, in addition to amplitude. Such effect was not expected within narrow frequency ranges (De Luca *et al.*, 2013), but optimal frequency leading to optimal pollen release was suspected based on the resonance exhibited by flower stamens, which would favor such release (Harder & Barclay, 1994; King & Buchmann, 1995; De Luca & Vallejo-Marín, 2013). Nonetheless, no such apparent peak on buzz frequency leading to optimal pollen release was detected in our playback experiment. Although we used a range of buzz frequencies spanning from 100 to 1600 Hz at a range of amplitudes (31.25 to 500.00 mm/s^2 of acceleration), encompassing the frequency of 500 Hz suggested as likely optimal by a few previous studies (Buchmann *et al.,* 1977; Corbet *et al.,* 1988; Harder & Barclay, 1994), pollen release exhibited significant decrease with frequency increase when acceleration was considered as the measure of amplitude; the opposite trend between buzz frequency and pollen release took place when amplitude was expressed as either displacement or velocity.

The observed results of pollen release with buzz frequency, although challenging earlier predictions based on pointed amplitude determinations and limited range of frequencies tested, is consistent with other granular systems subjected to vibration, a process known as granular fluidization (Chlenov & Mikhailov, 1965; Tai & Hsiau, 2004). This process is characterized by the granular solid matter assuming motion properties of fluids when subjected to either vertical (Chlenov & Mikhailov, 1965; Harada *et al.,* 2002; Kim *et al.*, 2002; Tai & Hsiau, 2004), or horizontal vibrations (Medved *et al.*, 2000; Medved, 2002). Previous experiments with grains retained in cylinders with small side opening and pollen piles placed on vibrating tables (King & Buchmann, 1996; Harada *et al.*, 2002), lay further credence on the role the pollen granular properties mediating its release rather than any potential anther resonance. It is worth noting though that fluidized granular systems may still exhibit response peaks at discreet vibration frequencies depending on the grain properties and characteristics of the applied vibration (Harada *et al.*, 2002), but those would be outside the range of conditions tested in our playback study of pollen release.

The field recordings of natural pollination buzzes at tomato flowers allowed us to test whether buzz-pollinating bees tune their buzzes to reach optimal frequencies for pollen release by verifying if such frequencies vary with pollinator species and their respective body size. Nonetheless, no optimal frequency for pollen release was detected in our playback study, as discussed above, and therefore the potential tuning of buzz frequency by bees does not take place. Furthermore, although buzz frequency varied among species of buzz-pollinator, the frequencies do not vary with time for each individual, nor within species, and not even among species within genera (Fig. S2). The lack of frequency variation and its independence from weight and pollination efficiency has been previously reported within individual species (Hrncir *et al.*, 2008; Nunes-Silva

et al., 2013), but the lack of correlation or independence between different bee species with varying sizes and buzz frequency leading to high pollen release (and seed formation) came as a surprise. This was so based on an earlier suggestion by Burkart *et al.* (2011). However, the difference between our findings and the expectation based on Burkart et al. (2011) may be due to relatively small species diversity recorded in our study and particularly the fact that Burkart's expectation was built on a trendline without statistical testing and without data points representing small pollinator species.

Buzzing frequency is not related to bee size and our earlier expectation that smaller bees would compensate their small buzz amplitude by reaching optimal buzz frequency leading to higher pollen release was flawed. These small species of buzz-pollinators probably use alternative mechanisms to compensate their small buzz amplitude (limited by their size). One of these potential mechanisms is the length of the buzz-pollinating activity in each visited flower since by spending more time on each flower and/or performing longer buzzes will allow the small bee species to increase pollen release (De Luca *et al.*, 2013). However, bee size is still relevant as bee allotropic relationships remain potentially important in adjusting their harvesting and carrying capacity with the amount of pollen released (Ramalho *et al.*, 1994; Muller *et al.*, 2006).

Bee size and buzz frequency did not impact seed production in buzz-pollinated flowers. A potential reason for that is the high efficiency of buzz-pollination leading to high pollination rates in tomato flowers requiring little and short amount of vibrations. In other words, there is no need to reach an optimal pollen release for efficient tomato buzz-pollination. A study with the bumblebee *Bombus impatiens* Cresson seems to lay credence to this notion, as a single visit by an individual of this species seems as efficient as several visits and forager size did not correlate with either tomato fruit weight nor seed production (Nunes-Silva *et al.*, 2013). Furthermore, as the stigma of tomato flowers

are introse to the cone of the anther, enough pollen can be released from the anthers and stoked on the stigmas after short visit of even small buzz-pollination bees. Such peculiarity also reinforces and explains the lack of correlation between bee size and buzz frequency.

The variation of buzz frequency among buzz-pollinating bees seems unrelated to pollen release, unlike earlier suspected (Burkart *et al.*, 2011; Morgan *et al.*, 2016; Switzer *et al.*, 2016). No optimal tuning of buzz frequency for pollen release was detected in our study. Nevertheless, the buzz-frequency may well be determined by the limitations on their vibrating apparatus relative to their output amplitude. In this regard, frequency variations detected across differing bee activities, i.e. flight, pollination and communication, may be due to the differences in the associated biomechanics leading to significantly higher accelerations with little changes in displacement (King *et al.,* 1996; King & Buchmann, 2003; Hrncir *et al.*, 2008; Burkart *et al.*, 2011). Thus, the range of natural buzz frequencies and amplitudes, and their respective associations, are likely limited by the morphological and physiological traits related to their flight apparatus. If true, bees may be benefiting from buzzing frequencies that return considerably larger output accelerations. These two peculiarities would explain the diverging relationship between buzz frequency and the different measures of buzz amplitude, as detected in our study.

In conclusion, we detected that buzz frequency and particularly buzz amplitude significantly affect pollen release, although this relationship depends on how amplitude is expressed, whether as acceleration, displacement, or velocity. We also detected that no optimal buzz frequency exist for pollen release and bee size is not related to buzz frequency, with low variation in the latter within genera and no significant change in the course of a buzz. The bee buzz frequency seems rather a mechanical consequence of the

flight apparatus adapted to reach large accelerations, while pollen release by buzz-pollinated flower plants seems to result from the granular biomechanical characteristics of the pollen. Both aspects require future attention.

Disclosure

The authors report no conflict of interest.

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Figure 1. Effect of artificially generated buzz frequency and amplitude on pollen release. The artificial buzzes generated had 2.0 s duration. The mesh plot indicate the amount of pollen released (μg) as predicted by the model shown on the figure, where z is pollen released (μg), x is frequency (Hz) and y is acceleration (m/s^2). The regression models, in which amplitude is expressed as either velocity or displacement, are available as the Figure S1.
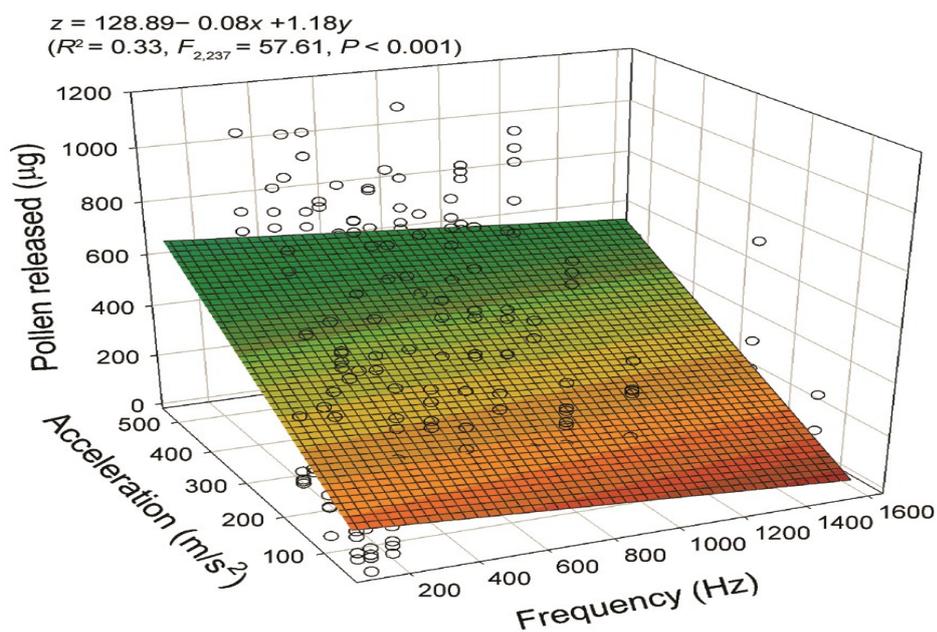
Figure 2. Field recordings (mean ± SE) of buzz frequency (A), body mass (B) and inter-tegular distance (C) of bee species visiting flowers of tomato plants of small fruit size for buzz-pollination. Bars with same letters do not differ significantly by Tukey's HSD test ($P < 0.05$).
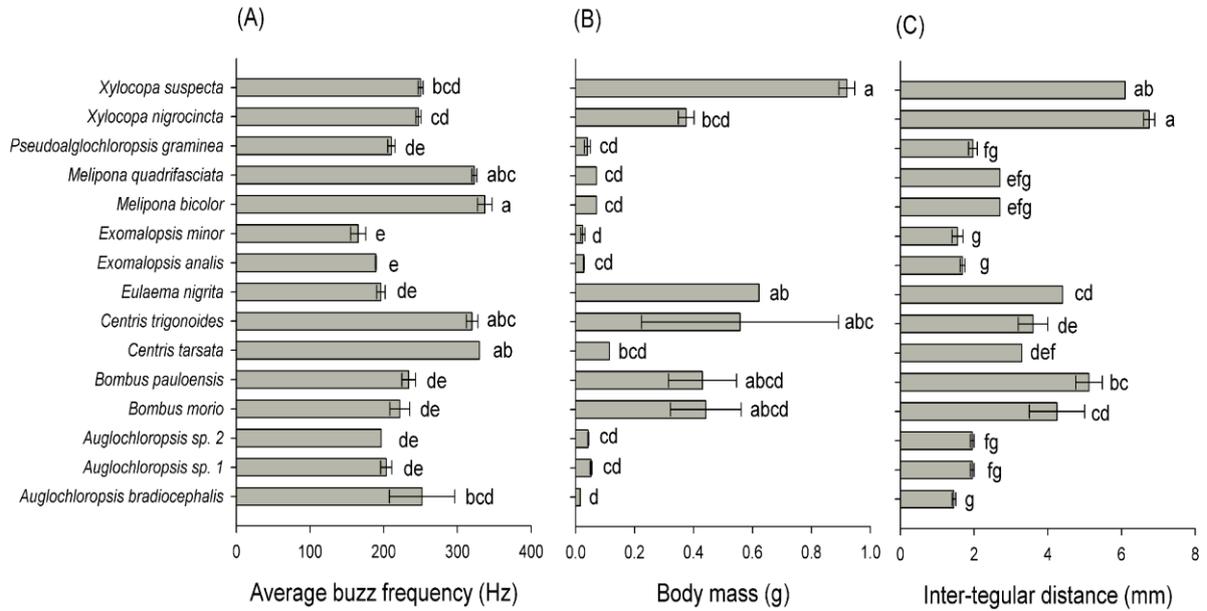
Figure 3. Field recordings (mean ± SE) of buzz frequency (A), body mass (B) and inter-tegular distance (C) of bee species visiting flowers of tomato plants of large fruit size for buzz-pollination, and the number of seeds (D) found within fruits pollinated by each species. Bars with same letters do not differ significantly by Tukey's HSD test ($P < 0.05$).