

Variability in bumblebee pollination buzzes affects the quantity of pollen released from flowers

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Abstract Buzz-pollination is a plant strategy that promotes gamete transfer by requiring a pollinator, typically bees (Hymenoptera: Apoidea), to vibrate a flower's anthers in order to extract pollen. Although buzz-pollination is widespread in angiosperms with over 20,000 species using it, little is known about the functional connection between natural variation in buzzing vibrations and the amount of pollen that can be extracted from anthers. We characterized variability in the vibrations produced by *Bombus terrestris* bumblebees while collecting pollen from *Solanum rostratum* (Solanaceae), a buzz-pollinated plant. We found substantial variation in several buzzing properties both within and among workers from a single colony. As expected, some of this variation was predicted by the physical attributes of individual bumblebees: heavier workers produced buzzes of greater amplitude. We then constructed artificial "pollination buzzes" that varied in three parameters (peak frequency, peak amplitude, and duration), and stimulated *S. rostratum* flowers with these synthetic buzzes to quantify

the relationship between buzz properties and pollen removal. We found that greater amplitude and longer duration buzzes ejected substantially more pollen, while frequency had no directional effect and only a weak quadratic effect on the amount of pollen removed. These findings suggest that foraging bumblebees may improve pollen collection by increasing the duration or amplitude of their buzzes. Moreover, given that amplitude is positively correlated with mass, preferential foraging by heavier workers is likely to result in the largest pollen yields per bee, and this could have significant consequences for the success of a colony foraging on buzz-pollinated flowers.

Keywords *Bombus terrestris* · *Solanum rostratum* · Pollinator mass · Poricidal anthers · Buzz-pollination

Introduction

In many flowering plants, pollen serves a dual function as both the carrier of male gametes and as a reward to attract pollinators. Because pollen consumed by pollinators is not available for reproduction, plants have evolved multiple strategies for regulating pollen release to ensure that a sufficient quantity is available for reproduction. Approximately 20,000 species of plants release their pollen exclusively via small pores in the anther tip (Buchmann 1983). In species with this type of poricidal anther, pollinators—typically bees (Hymenoptera: Apoidea)—generate vibrations with their indirect flight muscles to remove pollen from the anther (Macior 1964; Buchmann 1983; Endress 1994). The vibrations transmitted from the pollinator's body to the flower shake the pollen grains inside the anthers, causing them to be forcefully ejected through the terminal pores and onto the body of the bee (Buchmann

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and Hurley 1978). This behavior is known as buzz-pollination due to the characteristic sound produced as the bee vibrates its body (Macior 1974; Buchmann 1983). Buzz-pollination has been reported in 65 plant families and nearly 400 genera (Buchmann and Hurley 1978; Buchmann 1983).

The ability to extract pollen from plants using vibrations is almost entirely restricted to bees—besides bees, only one species of hover fly (Diptera: Syrphidae) is known to vibrate poricidal anthers to extract pollen (Buchmann 1983). Within the Apoidea, the ability to buzz-pollinate is widespread and likely exhibited by thousands of species (Buchmann and Hurley 1978; Thorp 2000). The family Apidae includes many species of buzz-pollinators, most notably bumblebees, *Bombus* spp. The behaviors exhibited by buzz-pollinating bumblebees are complex. During a typical visit to a buzz-pollinated plant, the bee curls the ventral side of its body around the anthers, while grabbing their bases with its mandibles (Buchmann and Hurley 1978). The bee then folds its wings and disables the indirect flight mechanism to prevent wing beating, and produces one or more series of vibrations (also called sonications or buzzes) by rapidly contracting the flight muscles (King et al. 1996). These buzzes are often alternated with pauses of varying duration in which the bee can collect pollen from its body via grooming (Macior 1964; Cane and Buchmann 1989). A typical pollination buzz lasts 0.1–8 or more seconds, and may be comprised of a series of pulses of varying duration (Buchmann 1983; King 1993). In *Bombus* spp. buzzes are produced at a peak frequency of about 300 Hz, although inter-species variation ranges from about 270–330 Hz (Corbet et al. 1988; King 1993; Harder and Barclay 1994). A key component of the vibrations is the energy transmitted to the flower's anthers, which affects the force with which pollen is ejected from the terminal pores (Buchmann and Hurley 1978), and presumably the amount of pollen that can be discharged. Buchmann and Hurley (1978) modeled the process of pollen ejection during buzz-pollination in terms of the force of impact between pollen grains and anther walls. They suggested that variation in buzzing parameters such as duration, amplitude, and frequency may lead to significant differences in the magnitude of pollen ejection, but such variation has not previously been studied systematically. In addition, buzzing properties may be influenced by physical characteristics of the pollinator (King 1993; King and Buchmann 2003). For example, in many species of insects that use vibrations to communicate, there is often a positive relationship between mass and amplitude (Cocroft et al. 2000; Cocroft and De Luca 2006), but whether heavier bumblebees generate buzz-pollination vibrations of greater amplitude by virtue of having more mass is currently unknown.

Pollen removal from anthers is a key aspect of both plant reproduction and bee foraging ecology (Buchmann 1983; Goulson 2010). Because bees use pollen as the only source of protein for their young, workers are expected to maximize pollen collection for their developing larvae and thus ensure the success of the colony. Accordingly, empirical data suggest that pollen-collecting bumblebees behave in a way that maximizes collection efficiency (protein collected/energy costs) (Rasheed and Harder 1997a, b). Optimal removal of pollen from each flower should thus be a key component of collection efficiency, as flying between flowers is very energy consuming (Rasheed and Harder 1997a) and may increase mortality risks. In addition to the fitness consequences for bees, pollen removal rates may also mediate patterns of reproductive success in plants. It is often the case in animal-pollinated plants that pollen removal per visit is negatively correlated with the probability of dispersal per pollen grain (Larson and Barrett 1999). This diminishing return arises for a variety of reasons, including a positive relationship between pollen removal and pollinator grooming, layering of pollen on the pollinator's body, and the pollinator's limited ability to carry pollen (Harder and Barclay 1994). As a consequence, plants may increase the probability of dispersal per pollen grain by restricting the amount of pollen removed during a single visit (i.e. dispensing mechanisms; Harder and Thomson 1989). For example, in buzz-pollinated *Dodecatheon* (Primulaceae), the amount of pollen ejected from anthers was found to be greatest at vibration frequencies above the natural range produced by foraging bumblebees (Harder and Barclay 1994). Although this is consistent with the hypothesis that the anthers of *Dodecatheon* function as dispensing mechanisms, the artificial buzzes that were used to vibrate the anthers in that study were applied with amplitudes that greatly exceeded those which bumblebees are naturally capable of producing. Consequently, more data are needed—especially within the natural range of variation exhibited by bumblebees—to clarify the effects of different buzz properties on pollen ejection dynamics.

In this study, we characterized natural variation in three vibrational buzzing properties (amplitude, duration, and frequency) of buff-tailed bumblebees (*Bombus terrestris*) when visiting flowers of the buzz-pollinated plant *Solanum rostratum* (Solanaceae) using laser vibrometry. We then used these data to build synthetic pollination buzzes to quantify the effect of natural variation in these parameters on pollen removal from intact anthers of *S. rostratum*. We predicted that: (1) heavier bees will generate buzz vibrations with greater amplitude as a consequence of having more body mass, (2) the amount of pollen released will increase with greater amplitude and longer duration buzzes as a result of anthers being stimulated with more force, or

for a longer period of time, respectively, and (3) if anthers function as dispensing mechanisms as suggested by Harder and Barclay (1994), then buzz properties such as frequency should have a limited effect on the magnitude of pollen removal. Our experimental methodology allowed us to independently manipulate separate buzz characteristics, thus controlling for natural correlations between these characters and allowing a powerful assessment of the relative importance of each parameter on pollen extraction. We then used response-surface analysis (Draper and John 1988) to visualize how different buzz parameter combinations interacted and affected the magnitude of pollen removal.

Materials and methods

Study system

One of the best-known plant groups requiring buzz-pollination is the genus *Solanum* (Solanaceae), which contains approximately 1,500 species with a worldwide distribution, as well as several species with agricultural importance such as tomatoes (*Solanum lycopersicon*), potatoes (*S. tuberosum*), and aubergines (*S. melongena*) (Bohs and Olmstead 1999; Knapp et al. 2004). As is common in other buzz-pollinated groups (Vogel 1978), *Solanum* lacks floral nectaries and offers pollen as the only reward to attract pollinators (Anderson and Symon 1988; Connolly and Anderson 2003). The flowers of *Solanum* have five anthers that produce numerous small pollen grains (usually between 10 and 30 μm in diameter; Knapp et al. 1998; Lester et al. 1999), which are rich in protein (Buchmann 1986; Roulston et al. 2000), dry and with a smooth, granulate wall (Anderson and Symon 1988). The styles of *Solanum* flowers are slender and have capitate stigmas that can be exceedingly small (Whalen 1979). This *Solanum* flower morphology has arisen independently in many different families of plants and represents a striking example of evolutionary convergence (Vogel 1978; Dukas and Dafni 1990; Endress 1994).

We used flowers of *Solanum rostratum* (Solanaceae) to characterize bee vibrations and to investigate the effect of buzz-pollination on pollen ejection (Fig. 1a). *Solanum rostratum* is an annual, self-compatible herb with yellow, buzz-pollinated flowers that is widespread in North America and probably originated in Central Mexico (Whalen 1979). This species also occurs outside its native range in the United Kingdom (UK), Europe, and China, where it may become an invasive weed. Each flower produces copious amounts of small pollen grains ($\sim 1.3 \times 10^6$ grains, 19 μm in diameter; Vallejo-Marin et al. 2009), which are released from two very small pores located at the

tip of each of its five anthers (Fig. 2). Flowers of this species are heterantherous, i.e. each flower produces two sets of anthers that specialize for either feeding or fertilization functions (Vallejo-Marin et al. 2010). One set consists of four bright yellow anthers that are centrally located within the flowers. Pollinators visiting these flowers focus their attention on this set of anthers, which are thus known as feeding anthers. The second set consists of a single yellow to brown-colored anther that is larger in size than the feeding anthers and deflected to either the right or left side of the flower, opposite to the location to the style. This larger anther contributes disproportionately to fertilization (Vallejo-Marin et al. 2009) and is therefore known as the pollinating anther. Each plant produces right- and left-handed—enantistylous—flowers in equal proportions (Jesson et al. 2003). There is no apparent difference in the fertility of pollen produced by the two types of anthers or the two types of flowers in *S. rostratum* (Bowers 1975).

We characterized pollination buzzes during visits to *S. rostratum* using a commercial colony of buff-tailed bumblebees, *Bombus terrestris* (Fig. 1b). This species is common and widespread in Europe and has been introduced to New Zealand, Tasmania, Chile, and Japan (Goulson 2010). Relative to other bumblebee species, it is a generalist forager visiting a broad variety of flower types, and it readily incorporates novel plant species into its diet (Goulson et al. 2002b, 2005; Lye et al. 2010). Individuals of *B. terrestris* visit other naturally occurring *Solanum* in the UK, including *S. dulcamara* (D. Souto-Vilaros and M. Vallejo-Marin, unpublished data). Although *B. terrestris* does not naturally occur in the native range of *S. rostratum*, this bumblebee species provides an excellent candidate to investigate buzz-pollination in a controlled experimental setting. In Europe, it is reared commercially to provide pollination services for a wide variety of crops and vegetables including tomatoes (Goulson 2010). During numerous field observations and previous experiments using captive colonies of *B. terrestris* foraging on *S. rostratum*, we have not observed any obvious behavioral differences during visitations to *S. rostratum* flowers compared to other medium-sized bees normally seen in its natural habitat (Vallejo-Marin et al. 2009; S. Anderson and M. Vallejo-Marin, unpublished data).

Bumblebee maintenance and training

We conducted the experiment from July to August 2010. We obtained a colony of *B. terrestris* from a commercial provider (Koppert Biological Systems, Suffolk, UK). The colony was composed of a single queen, approximately 60 adult workers, and an unknown number of developing larvae. It was housed in a cardboard box (40 \times 40 \times 30 cm) provisioned with nesting material and



Fig. 1 **a** Flowers of *Solanum rostratum* (Solanaceae). The *top* arrow indicates the four feeding anthers that the bee grasps during buzz-pollination. The *bottom* arrow indicates the single pollinating anther that deposits pollen on the bee's abdomen. **b** Worker of *Bombus*

terrestris (Apidae) buzz-pollinating *S. rostratum* inside a flight cage. The large mass visible on the bee's leg is made of *S. rostratum* pollen that the bee will transport back to the hive

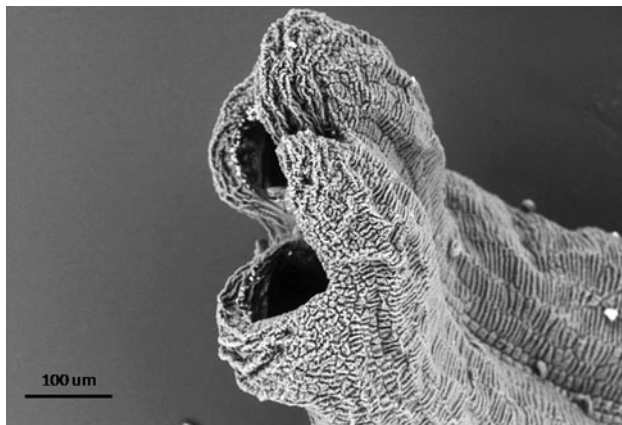


Fig. 2 Scanning electron photograph showing the terminal pores of the pollinating anther of *Solanum rostratum* (Zeiss, EVOMA15; 15 kV, WD = 6.0 mm, 39 Pa)

ad libitum nectar. Worker bees in commercially-grown colonies are not given an opportunity to forage for pollen prior to delivery; therefore, we provided them time to develop foraging behavior before beginning the experiment (King 1993). We placed the colony in a screened enclosure (4 × 3 × 2 m) located under a shaded tent (12 × 4 × 3 m) at the greenhouse facilities of the University of Stirling. We placed 2 potted *S. rostratum* plants (each about 1 m tall) approximately 1 m from the nest box. Each plant was in bloom and contained 15–20 open flowers at any given time. After positioning the plants, we opened the entrance to the nest to allow worker bees to forage. Workers from the experimental colony readily began to buzz-pollinate after discovering the flowers. We left the door to the nest open during the day (0900–1800 hours) and let bees forage for 1 week before starting recordings.

Recording buzz-pollination vibrations

We made recordings during the day, when temperatures inside the enclosure ranged from 10 to 18 °C. We recorded

the buzz-pollination vibrations using a Polytec PDV100 (Tustin, CA, USA) portable laser Doppler vibrometer (LDV). The amplitude of a vibration may be measured by displacement, velocity, or acceleration; a vibrating object must accelerate and decelerate as it moves back and forth through each cycle of vibration and there will be concomitant oscillation of all three parameters (Hill 2008). The LDV is a non-contact method for recording surface vibrations by detection of a laser beam reflected from the surface. Vibration of the surface causes a Doppler shift in the reflected beam that is proportional to the surface velocity (Aicher et al. 1983). The LDV thus provides a real-time signal of surface velocity, and we present measurements of the peak amplitude of buzz signals in velocity units. LDV sensitivity was set to 500 mm s⁻¹ V⁻¹, and signals were low-pass filtered at 5 kHz with the laser control module prior to being input into the digital port of a Sound Devices 722 (Reedsburg, WI, USA) digital audio recorder. During recording, we removed the *S. rostratum* plants from the enclosure and placed a platform (50 cm tall) in their place that contained a Styrofoam top. We inserted three bamboo sticks (30 cm long × 0.2 cm diameter) set 10 cm apart into the Styrofoam in a triangular pattern, upon which we positioned freshly cut *S. rostratum* flowers. We selected flowers whose petals had opened the morning of the day recordings were made, and attached one each by its pedicel to a bamboo stick with Blu-Tack adhesive gum (Bostik, Staffordshire, UK). We used an individual flower for about 1 h (during which several bee visits could occur) before replacing it with a freshly cut one. To increase reflectance of the laser beam we attached a small piece (1 mm²) of reflective tape to the petal of one flower, positioning it 5 mm from the base of the anthers. The other two flowers on the platform were used to help stimulate visits to the focal flower. We positioned the laser 23 cm from the focal flower and ensured that the reflective tape and laser lens were in the same horizontal plane. We monitored laser output visually with a Tequipment S61

oscilloscope (Telequipment, London, UK) to ensure recordings were not clipped and saved them as wave files (48 kHz sampling rate, 16 bit depth). We allowed 5–10 worker bees to forage freely in the enclosure at any one time, regulating bee number by closing the nest box as required to prevent too many bees from overwhelming the flowers when making recordings. We defined a single buzz-pollination event as the time from a bee's landing on the focal flower to the time at which it flew away from the platform. If the bee performed buzzing, we captured it in a vial when it left the flower and placed the vial in a cooler with ice. When recordings were completed for that day, we killed the collected bees by placing them in a freezer. The following day, we weighed them individually on an electronic balance (Denver Instrument PI 225D; Bohemia, NY, USA) to the nearest 0.1 mg.

Measuring variability in buzz parameters

We analyzed temporal and spectral features of buzz 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). To measure the peak amplitude of buzzes, we used a custom program written in Matlab v.6.5 (The MathWorks, Natick, MA, USA), which is available upon request. For each buzz, we measured the duration (expressed as seconds, s), peak frequency (expressed as Hertz, Hz), and peak amplitude (expressed as velocity, mm s^{-1}) (Fig. 3). Some bees would buzz a flower several times during a single visit, in which case we measured each individual buzz and used the mean values for that bee in subsequent analyses. To compare both within- and among-worker variation in buzz features, we calculated coefficients of variation [$\text{CV} = (\text{mean}/\text{SD}) \times 100$] for each parameter. For within-worker variability, we first measured each individual's CVs for each parameter and then calculated a grand average across workers. We examined the relationship between mass and buzz features with standard least-squares regression in JMP v.7.0 (SAS Institute 2007). We log-transformed mass, duration, peak amplitude, and peak frequency, and square-root-transformed the number of buzzes per visit (Zar 1984), prior to conducting analyses. Unless otherwise indicated, descriptive statistics are reported as the mean \pm 1SE.

Playback experiment and analysis of pollen counts

To examine the influence of natural variation in buzz parameters on pollen ejection, we constructed synthetic stimuli for use in a playback experiment. We focused on three parameters in our stimulus design: duration, peak

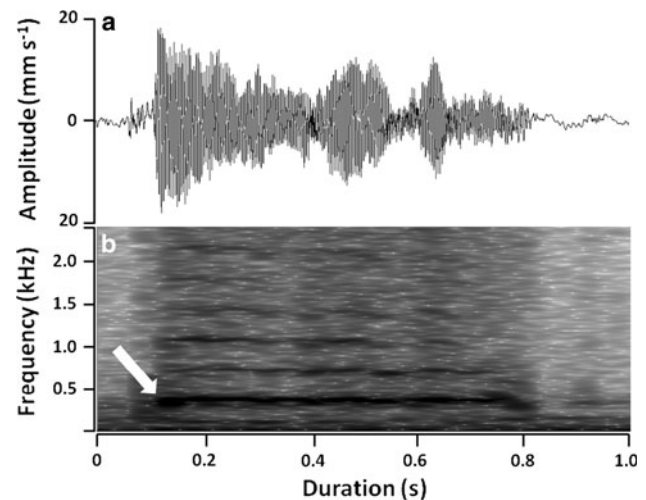


Fig. 3 A typical buzz-pollination vibration by a *Bombus terrestris* worker. **a** Amplitude waveform. **b** Spectrogram. The white arrow in the spectrogram denotes the peak frequency band, which in this example occurred at approximately 300 Hz

amplitude, and peak frequency. We varied these parameters independently and systematically at seven levels (mean log-transformed parameter value \pm 1, 2, 3 standard deviations, SDs), therefore encompassing 99 % of the naturally observed variation in parameter space (note that for peak amplitude we did not use the value corresponding to -3SD as that amplitude level (0.375 mm s^{-1}) was too low for accurate calibration with the playback transducer). Because we allowed the parameters to vary independently, we sample a greater multivariate parameter space than is actually observed in nature, but which allows us to statistically disentangle the influence of parameters that naturally covary (Brooks et al. 2005). We used Adobe Audition v.3.0 (Adobe Systems, 2007) to construct 294 stimuli ($7 \times 7 \times 6$ parameter value combinations). We shaped each stimulus with an amplitude envelope that replicated that of natural buzzes using the 'Envelope process' feature in Audition, and saved them as wave files (48 kHz sampling rate, 16 bit depth).

We conducted playbacks in August 2010 in an ambient temperature laboratory at the University of Stirling. Stimuli were broadcast from the earphone jack of a PC laptop computer and transduced with a B&K (Brüel & Kjaer, Naerum, Denmark) 2706 Power Amplifier connected to a B&K 4810 mini-shaker. We affixed a pair of BioQuip (Rancho Dominguez, CA, USA) no.4748 featherweight forceps to a metal rod (10 cm tall \times 0.2 cm diameter) that was screwed into the base of the mini-shaker. We used the forceps to hold a *S. rostratum* flower by its anthers (the flower was detached from the plant at the pedicel). We found that in pilot tests this best mimicked the manner in which a bumblebee grips a flower's

anthers with its mandibles when buzzing. Suspending the flower in this manner did not impede the ejection of pollen nor interfere with the transmission of vibrations from the forceps (Buchmann and Hurley 1978; Corbet et al. 1988). We adjusted the peak amplitudes of the stimuli at the point the forceps contacted the anthers. We calibrated stimulus amplitudes visually by monitoring signals on an oscilloscope (Telequipment S61) that was connected to the laser output, and adjusted the gain on the B&K amplifier accordingly to achieve each of the six amplitude values. Each amplitude \times frequency combination required a unique gain setting on the amplifier, thus we calibrated all stimuli at the outset so that during trials we could quickly switch between different peak amplitude values by adjusting the amplifier gain to the desired position as required. We re-checked the accuracy of the amplitudes every 4 days, but adjustments were never necessary.

We used *S. rostratum* flowers collected from three different plants that were housed in the greenhouse at the University of Stirling. Each plant had several branches (each 1–2 m in length) and contained several hundred flower buds. On the day prior to conducting a playback we enclosed 40–60 buds across the three plants in individual screen bags. This ensured that every flower used in our experiment had never been visited by an insect, therefore standardizing the pollen load across flowers. On the day we conducted a playback, we collected 15–20 freshly opened flowers in the morning (0900 hours) and placed them in a plastic container lined with moistened paper towels. We immediately transported the flowers back to the laboratory and began playbacks. After playbacks on those flowers were completed, we collected another batch of flowers and repeated the process. Consequently, no flower had been removed from a plant for longer than 170 min before it was used in a playback ($n = 23$ playback periods). We randomized the order of the stimuli during playback and followed this procedure until all 294 stimuli had been tested, using one flower per stimulus. To collect pollen we held a 1.5-ml micro-centrifuge tube that had been rinsed in 70 % ethanol so that the tips of all five anthers were slightly inserted into the tube. For each flower, we played the stimulus once, and then added 200 μ l of ethanol to the tube to suspend the pollen.

We estimated the amount of pollen ejected from a flower's anthers by counting the number of pollen grains suspended in a sub-sample of liquid extracted from the micro-centrifuge tube for each stimulus. Prior to extraction, we agitated the tube's contents using a vortexer to uniformly distribute pollen grains in the ethanol. We extracted a small amount of liquid from the tube and placed it in the well (volume = 0.9 μ l) of a

haemocytometer slide positioned under a dissecting microscope at $\times 500$ magnification. We counted the number of grains visible in the grid of the well using a hand-counter. We used the formula: [(total number of grains counted \times 200 μ l)/0.9 μ l] to estimate the total amount of pollen grains present in the tube, and rounded up or down to the nearest whole number when necessary. We repeated this process twice for each tube and used the mean value in our analyses.

We estimated the relative influence of buzz parameters on the amount of pollen ejected using standardized regression approaches similar to those used in selection analyses (Lande and Arnold 1983). Because independent variables are standardized (i.e. values are expressed in standard deviation units, SDs, with respect to the original mean across natural observations) before inclusion in the regression model, the partial regression coefficients associated with each independent variable can be easily compared regardless of differences in measurement units across traits. Prior to conducting statistical analyses we transformed pollen count values using the natural logarithm (ln) transformation.

We first removed from the dataset a small number ($n = 31$) of zero-pollen counts (see “Results”). Excluding these data had no qualitative influence on the model parameters or the overall conclusions (confirmed by examining nonparametric thin-plate splines built with and without these observations), and dramatically improved model fit as verified using visual examinations of residual distributions. Our model simultaneously estimated linear and curvilinear effects by incorporating all standardized predictors along with their squares and cross-products. This regression produces a vector of linear coefficients (one for each linear predictor) as well as a matrix of curvilinear coefficients that summarizes quadratic and correlational effects of the variables. We illustrated the partial effects of amplitude, duration, and frequency on pollen ejection by plotting the fitted effects (and 95 % confidence intervals) after fixing the other covariates to their mean values. We superimposed on the graphs the data for all playback trials, even though this includes observations where the other covariates were not at the mean value. These plots incorporate the linear and quadratic effects of each parameter, but do not illustrate any correlational (interaction) effects. Accordingly, we visualized the response surfaces for pairwise combinations of parameters using nonparametric thin-plate splines constructed using the Fields package (Furrer et al. 2010) of R software v.2.11.1 (R Core Development Team 2010). We selected smoothing parameters (lambda) for these surfaces that minimized the generalized cross-validation (GCV) scores (Schluter and Nychka 1994).

Results

Variability in buzz parameters

We recorded a total of 215 buzzes from 54 bees. Average buzz duration was 1.08 ± 0.07 s (range 0.21–3.26 s) and had a peak frequency of 324 ± 3.67 Hz (range 264–386 Hz). Average peak amplitude was 19.13 ± 1.5 mm s⁻¹ (range 1.29–84.08 mm s⁻¹). There were 3.85 ± 0.39 buzzes per flower for each floral visit (range 1–11). Mean (\pm SE) within-worker variability (CV) in buzz features was largest for duration (34.41 ± 2.49 %), followed by peak amplitude (30.16 ± 3.64 %), and then peak frequency (2.66 ± 0.25 %). Among-worker CV's followed a similar pattern, although the number of buzzes per visit was largest (74.87 %), followed by peak amplitude (57.66 %), duration (50.51 %), and peak frequency (8.28 %). Heavier bees produced buzzes of significantly greater peak amplitude than lighter bees ($r^2 = 0.15$, $P = 0.01$, $n = 40$; Fig. 4). There was no relationship between mass and buzz duration ($r^2 = 0.04$, $P = 0.2$, $n = 40$), peak frequency ($r^2 = 0.002$, $P = 0.8$, $n = 40$), or the number of buzzes per visit ($r^2 = 0.02$, $P = 0.4$, $n = 40$).

Effect of variability in buzz features on pollen ejection

Of the 31 zero-pollen counts, 14 were distributed among stimuli with the lowest peak amplitude, 14 among stimuli with the second-lowest peak amplitude, and 3 among stimuli having the mean peak amplitude. Pollen collection per stimulus averaged 49,000 grains (range 0–800,000 grains, $n = 294$ stimuli). Pollen removal increased significantly with peak amplitude and duration, with amplitude having almost four times the influence of duration over the

natural range of variation in these traits (Table 1; Fig. 5). Frequency, by contrast, had no discernible directional influence on pollen ejection (Table 1; Fig. 5). There were several statistically significant but much weaker curvilinear effects (indicated by the smaller magnitude of the coefficients for quadratic and cross-product terms): the significant negative coefficients for amplitude and duration show that the response surfaces are convex with respect to variation in these buzzing traits, i.e. that there are diminishing marginal gains for progressively higher-amplitude and longer-duration buzzes. The significant positive quadratic coefficient for frequency conversely indicates that the surface with respect to frequency is slightly concave. Finally, the significant coefficients for the cross-product of amplitude and duration indicates that these buzz properties interact in a non-additive way to determine pollen ejection.

In order to help visualize the non-additive effects of duration and amplitude on pollen removal, we built a non-parametric thin-plate spline (Fig. 6). The stronger effect of amplitude is evident in its steeper slope relative to that of duration. The interaction between these variables (as indicated by the significant cross-product term for this in Table 1), is apparent in the curvature of the contour lines. For example, the effect of duration is much stronger at higher intensities (along the top of the figure) than at lower ones (along the bottom of the figure). To illustrate the correspondence between naturally observed variation in amplitude and duration and pollen removal, we superimposed the individual buzzes recorded in the first part of our study on the pollen removal surface. Note that, since we sampled several buzzes from some bees, some individuals are represented more than once on the surface. Most of the naturally occurring buzzes are clustered in the lower left hand corner of the surface, where relatively fewer pollen grains per buzz are ejected. Many buzzes are distributed

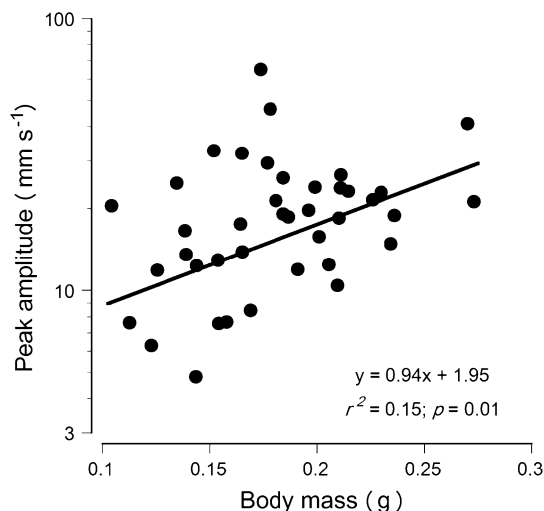


Fig. 4 Relationship between body mass and the peak amplitude of buzz vibrations, $n = 40$. Note log scale used on y-axis

Table 1 Parameter estimates for the model relating natural variation in standardized buzzing characters to natural log-transformed pollen ejection

Source	Estimate	SE	<i>t</i>	<i>P</i>
Intercept	8.21	0.19	42.98	<0.001
Amplitude	1.2	0.07	18.28	<0.001
Duration	0.31	0.05	6.7	<0.001
Frequency	-0.07	0.05	-1.39	0.16
Amplitude ²	-0.1	0.03	-2.88	0.004
Duration ²	-0.05	0.03	-1.93	0.06
Frequency ²	0.07	0.03	2.78	0.006
Amplitude × duration	0.09	0.03	3.35	<0.001
Amplitude × frequency	0.04	0.03	1.61	0.11
Duration × frequency	-0.004	0.02	-0.19	0.85

Model R^2 (adjusted) = 0.68, $F_{9,253} = 62.83$, $P < 0.001$

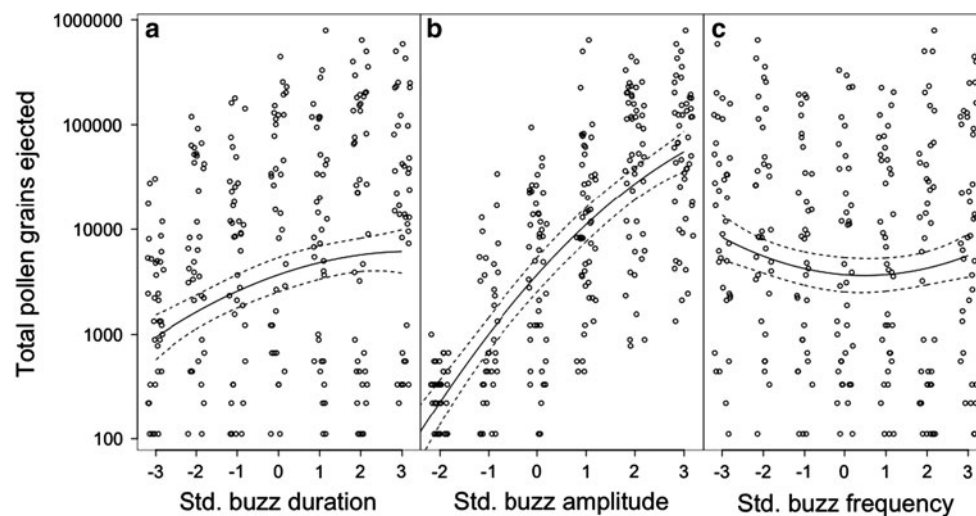


Fig. 5 Univariate plots illustrating the fitted partial effects of standardized (a) buzz duration, (b) peak amplitude, and (c) peak frequency on pollen ejection (solid line), after fixing the other covariates to the mean. Dashed lines indicate the 95 % confidence regions for these fitted effects. Open circles represent all the stimuli

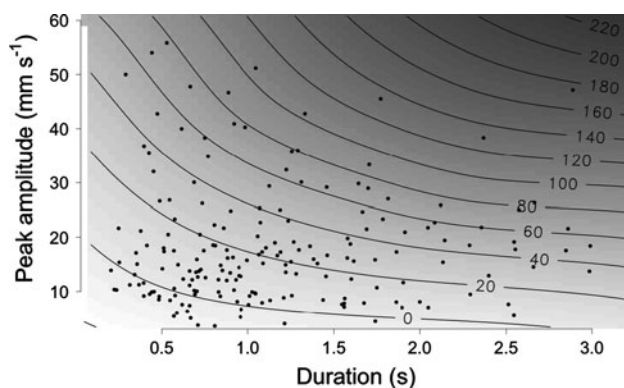


Fig. 6 The combined effects of variation in peak amplitude and duration on the amount of pollen ejected. More darkly shaded regions of the surface (top right) highlight combinations of peak amplitude and duration that result in higher levels of pollen ejection. The contour labels indicate the number of pollen grains (in thousands) represented along that contour line. Superimposed on the figure are the data points of all the natural buzzes recorded from bumblebees. Note how the majority of buzzes are clustered in a space (bottom left) that is below the combinations of peak amplitude and duration that promote the greatest pollen release

along either the duration or amplitude axis, revealing that buzzes can be long or intense, but are rarely both. Accordingly, few buzzes are located in the top right corner of the surface, where the most pollen grains are ejected.

Discussion

Our data reveal that natural variation in two buzzing features, i.e. peak amplitude and duration, greatly influences

used in the playback experiment, including those in which covariates had other than mean values. The data are plotted on the true y-coordinates, but the x-values have been jittered (randomly changed by a small positive or negative deviation) to separate overlapping observations. Note log scale used on y-axis

the magnitude of pollen release in *S. rostratum* flowers. Pollen ejection is determined principally by peak amplitude and to a lesser extent by the duration of buzz-pollination vibrations. Furthermore, pollen ejection is also moderately affected by an interaction between peak amplitude and duration such that the effect of each parameter depends to some degree on the other. The negative quadratic coefficients for peak amplitude and duration suggest a convex relationship between these buzz features and pollen removal such that higher-amplitude and longer-duration buzzes provide diminishing returns. However, given that the coefficients for these curvilinear terms are over an order of magnitude smaller than their corresponding linear effects, their contribution in shaping pollen ejection dynamics is relatively weak.

Within the natural range of buzzing frequencies used by *B. terrestris* no single value was associated with a maximum level of pollen release. In fact, plants may have no need to discriminate among individual buzz-pollinators, but would likely benefit from an optimal distribution of pollen spread among different pollinators whose buzzing vibrations naturally span a range of frequencies. The lack of a strong effect of frequency is notable though because over this frequency range (250–400 Hz) plant structures act as frequency filters, transmitting some of these frequencies exceptionally well while attenuating others (Michelsen et al. 1982; Cökl et al. 2004; Henry and Martinez-Wells 2004; McNett et al. 2006; Casas et al. 2007). This has had significant consequences for the evolution of animal vibrational communication on different types of host plants, as those frequencies that transmit well are likely to

be used exclusively in signals (Bell 1980; Morris 1980; Cocroft and Rodriguez 2005; McNett and Cocroft 2008). Although bumblebees are not using buzz vibrations to communicate, the mechanical response properties of anthers are likely to vary with frequency in a comparable manner to plant structures (e.g., stems, petioles, and leaves) used to transmit vibrations for communication. Consequently, some buzzing frequencies might be expected to propagate through anthers more efficiently than others, and thus cause pollen to be ejected from terminal pores with greater effectiveness (Harder and Barclay 1994; King and Buchmann 1996). However, at least in the range of frequencies used by *B. terrestris*, frequency had a weak influence on pollen release. This suggests an adaptation to facilitate a broad (i.e. indiscriminate) frequency filtering response in buzz-pollinated flowers, although more research is needed to test this possibility. In buzz-pollinated *Dodecatheon* flowers, the frequencies that resulted in maximal pollen removal were between 700 and 1,000 Hz, well above the frequencies that can naturally be produced by bumblebees when sonicating flowers (Harder and Barclay 1994). The authors suggested that this pattern indicates that the anthers of *Dodecatheon* function as a dispensing mechanism to restrict over-exploitation of pollen resources. Although this is certainly plausible, the relationship between frequency and pollen ejection among buzz-pollinated flower species is at present poorly quantified. Accordingly, whether ‘frequency tuning’ of anthers represents a general type of dispensing mechanism to prevent over-exploitation of pollen resources by buzz-pollinating bees remains to be more thoroughly investigated. However, even if the anthers of buzz-pollinated flowers are tuned to release more pollen at higher frequencies not used by sonicating bees, foraging workers might still partially circumvent this by adjusting buzzing behavior. For example, repeatedly buzzing a plant in a single visit is likely to remove additional pollen (Buchmann and Cane 1989; Harder 1990; Kawai and Kudo 2009), and the fact that workers in our study buzzed a flower an average of three times (and as many as 11 times) in a single visit, indicates that repeated buzzing is common during floral visits. In addition to repeated buzzing, workers may also increase pollen collection from a single flower by using longer or more intense vibrations, as these are also likely to increase the amount of pollen ejected from anthers.

Our experiment did not assess the influence of multiple buzzing bouts on the magnitude of pollen removal; however, some studies have indeed shown that repeated buzzing of a single flower enables a worker to remove additional pollen, albeit in a decelerating fashion. Pollen removal amounts for the first visit can vary from 20 to 70 % of the total available pollen in a flower’s anthers

depending on the species, with further visits removing gradually less pollen (Harder 1990; King and Buchmann 1996; Kawai and Kudo 2009). Furthermore, bumblebees will adjust their behavior when visiting previously buzzed flowers by reducing the amount of time spent buzzing (Harder 1990; Kawai and Kudo 2009), which suggests workers are able to assess the pollen load of individual flowers. Whether they also adjust the amplitude of their buzzes in response to reduced pollen availability is unknown, but given the large variability in peak amplitude both within and among workers observed in our study, it is certainly a strong possibility. Another factor that may also influence buzzing behavior is the age of a flower. Older virgin flowers release proportionally more pollen than younger ones (Harder and Barclay 1994), and thus a foraging bumblebee might be expected to increase the duration and/or amplitude of its buzzes when visiting an older flower that had not yet been visited.

Heavier bees generated buzzes with significantly greater peak amplitudes, as predicted. At the colony level, size variation among worker bumblebees is usually associated with alloethism—the division of labor based on body size. Bumblebee workers exhibit an eight-fold variation in mass within single colonies, with larger workers assuming most of the foraging duties while smaller workers tend to remain inside the nest and care for developing larvae (Heinrich 1979; Goulson et al. 2002a). Furthermore, in some species, it is the larger foragers that collect most of the pollen, while smaller foragers primarily collect nectar (Brian 1952; Free 1955; Pouvreau 1989). However, size-dependent differences in foraging specialization can also be a function of which flower types are locally available, as individual bees learn to visit flowers from which their own morphology allows them to efficiently extract rewards, whether it be pollen or nectar (Peat et al. 2005). Accordingly, the optimum size of a forager for pollen collection (and hence the size of forager which tend to specialize in pollen collection) will vary according to the local abundance and composition of different flower types. Several non-mutually exclusive hypotheses have been proposed to explain why the largest workers in a colony tend to be foragers, including having greater visual acuity, being able to conduct longer foraging trips, being better at avoiding predators, and having superior thermoregulatory abilities (Goulson 2010). Our findings offer an additional explanation: foraging by heavier bees is likely to result in larger pollen collection loads given that higher amplitude buzzes eject substantially more pollen (while lower amplitude buzzes associated with lighter bees may sometimes result in no pollen being ejected). It deserves mention, however, that since mass explains just 15 % of the variation in peak amplitude there are likely other factors (e.g., physical condition, foraging experience, or motivational state) that

affect the ability of worker bees to generate buzz vibrations of a given amplitude. Nevertheless, increased worker mass is expected to be beneficial for pollen foraging given its relationship to amplitude. Accordingly, preferential pollen collection by heavier workers should result in increased pollen yields for a colony foraging on buzz-pollinated plant species, and one likely consequence of this is improved colony survival and success as a result of a greater numbers of new workers being produced through the availability of more pollen resources (Heinrich 1979).

The distributions in parameter space of the observed buzzes are clustered in a manner that does not closely match the values for amplitude and duration that promote the greatest pollen release (Fig. 6). This could be for several reasons: (1) given that foraging is energetically expensive for bumblebees (Wolf et al. 1999; Peat and Goulson 2005), workers may be trading off increased pollen collection against energy expenditure while buzzing in order to conserve energy reserves needed for other foraging activities; (2) workers may be physiologically unable to sustain the production of buzzes that are simultaneously long and intense; (3) using forceps to mimic buzz-pollination may not have completely replicated the natural efficiency with which a foraging worker manipulates anthers for pollen extraction, and consequently, the correspondence between natural variation in values of duration and amplitude and higher amounts of pollen ejection may be closer than what is suggested in Fig. 6; future studies are planned to assess pollen loads directly from foraging workers from which buzzing vibrations have also been recorded; (4) many of the natural buzzes we recorded may represent “test-buzzes” to assess pollen availability (Buchmann and Cane 1989; King and Buchmann 1996), and so are designed to be relatively quick and inexpensive for the bee to produce; and (5) the extraction of too much pollen by producing longer or more intense buzzes may actually not be adaptive for a bee because pollen cannot be efficiently collected when too much is discharged into the air at any one time. Many of these alternatives are not mutually exclusive, and all may contribute to the variation in buzzing behavior we observed, which suggests some interesting avenues for future research. Many aspects of the interaction between bumblebees and buzz-pollinated flowers also warrant additional investigation. For example, do flowers with different floral designs have the same pollen response properties to buzz vibrations as *S. rostratum*? Do cultivated or self-pollinating species that may occasionally rely on buzz-pollination dispense pollen in a dramatically different way given their reduced reliance on pollinators? Perhaps most intriguing will be to assess the degree to which buzzing vibrations have shaped the fitness of both flowers and bumblebee colonies through their effects on pollen ejection and foraging behavior.

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References

- Aicher B, Markl H, Masters WM, Kirschenlohr HL (1983) Vibrational transmission through the walking legs of the fiddler crab, *Uca pugilator* (Brachyura, Ocypodidae) as measured by laser-Doppler vibrometry. *J Comp Physiol A* 150:483–491
- Anderson GJ, Symon D (1988) Insect foragers on *Solanum* flowers in Australia. *Ann Mo Bot Gard* 75:842–852
- Bell PD (1980) Transmission of vibrations along plant stems: implications for insect communication. *NY Entomol Soc* 88:210–216
- Bohs L, Olmstead RO (1999) *Solanum* phylogeny inferred from chloroplast DNA sequence data. In: Nee M, Symon DE, Lester RN, Jessop JP (eds) *Solanaceae IV*. Royal Botanic Gardens, Kew, pp 97–111
- Bowers KAW (1975) The pollination ecology of *Solanum rostratum* (Solanaceae). *Am J Bot* 62:633–638
- Brian AD (1952) Division of labour and foraging in *Bombus agrorum* Fabricus. *J Anim Ecol* 21:223–240
- Brooks R, Hunt J, Blows MW, Smith MJ, Bussière LF, Jennions MD (2005) Experimental evidence for multivariate stabilizing sexual selection. *Evolution* 59:871–880
- Buchmann SL (1983) Buzz pollination in angiosperms. In: Jones CE, Little RJ (eds) *Handbook of Experimental Pollination Biology*. Scientific and Academic Editions, NY, pp 73–113
- Buchmann SL (1986) Vibratile pollination in *Solanum* and *Lycopersicon*: a look at pollen chemistry. In: D’Arcy WG (ed) *Solanaceae: biology and systematics*. Columbia University Press, NY, pp 237–252
- Buchmann SL, Cane JH (1989) Bees assess pollen returns while sonicating *Solanum* flowers. *Oecologia* 81:289–294
- Buchmann SL, Hurley JP (1978) Biophysical model for buzz pollination in Angiosperms. *J Theor Biol* 72:639–657
- Cane JH, Buchmann SL (1989) Novel pollen-harvesting behavior by the bee *Protandrena mexicanorum* (Hymenoptera, Andrenidae). *J Insect Behav* 2:431–436
- Casas J, Magal C, Sueur J (2007) Dispersive and non-dispersive waves through plants: implications for arthropod vibratory communication. *Proc R Soc Lond B* 274:1087–1092
- Cocroft RB, De Luca PA (2006) Size-frequency relationships in insect vibrational signals. In: Drosopolous S, Claridge MF (eds) *Insect sounds and communication: physiology, behaviour, ecology and evolution*. CRC, Boca Raton, pp 109–120
- Cocroft RB, Rodríguez RL (2005) The behavioral ecology of insect vibrational communication. *Bioscience* 55:323–334
- Cocroft RB, Tieu TD, Hoy RR, Miles RN (2000) Directionality in the mechanical response to substrate vibration in a treehopper (Hemiptera: Membracidae: *Umberia crassicornis*). *J Comp Physiol A* 186:695–705
- Cökl A, Presern J, Virant-Doberlet M, Bagwell GJ, Millar JG (2004) Vibratory signals of the harlequin bug and their transmission through plants. *Physiol Entomol* 29:372–380

- Connolly BA, Anderson GJ (2003) Functional significance of the androecium in staminate and hermaphroditic flowers of *Solanum carolinense* (Solanaceae). *Plant Syst Evol* 240:235–243
- Corbet SA, Chapman H, Saville N (1988) Vibratory pollen collection and flower form: bumble-bees on *Actinidia*, *Symphytum*, *Borago* and *Polygonatum*. *Funct Ecol* 2:147–155
- Draper NR, John JA (1988) Response-surface designs for quantitative and qualitative variables. *Technometrics* 30:423–428
- Dukas R, Dafni A (1990) Buzz-pollination in 3 nectariferous Boraginaceae and possible evolution of buzz-pollinated flowers. *Plant Syst Evol* 169:65–68
- Endress PK (1994) Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge
- Free JB (1955) The division of labour within bumblebee colonies. *Insectes Soc* 2:195–212
- Furrer R, Nychka D, Sain S (2010) Tools for spatial data. R package version 6.3 (<http://CRAN.R-project.org/package=fields>)
- Goulson D (2010) Bumblebees: behaviour, ecology, and conservation (2nd edn). Oxford University Press, New York
- Goulson D, Peat J, Stout JC, Tucker J, Darvill B, Derwent LC, Hughes WOH (2002a) Can alloethism in workers of the bumblebee *Bombus terrestris* be explained in terms of foraging efficiency? *Anim Behav* 64:123–130
- Goulson D, Stout JC, Kells AR (2002b) Do alien bumblebees compete with native flower-visiting insects in Tasmania? *J Insect Conserv* 6:179–189
- Goulson D, Hanley ME, Darvill B, Ellis JS, Knight ME (2005) Causes of rarity in bumblebees. *Biol Conserv* 122:1–8
- Harder LD (1990) Behavioral responses by bumble bees to variation in pollen availability. *Oecologia* 85:41–47
- Harder LD, Barclay MR (1994) The functional significance of poricidal anthers and buzz pollination: controlled pollen removal from *Dodecatheon*. *Funct Ecol* 8:509–517
- Harder LD, Thomson JD (1989) Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *Am Nat* 133:323–344
- Heinrich B (1979) Bumblebee economics. Harvard University Press, Cambridge
- Henry CS, Martinez-Wells ML (2004) Adaptation or random change? The evolutionary response of songs to substrate properties in lacewings (Neuroptera: Chrysopidae: *Chrysoperla*). *Anim Behav* 68:879–895
- Hill PSM (2008) Vibrational communication in animals. Harvard University Press, Cambridge
- Jesson LK, Kang J, Wagner SL, Barrett SCH, Dengler NG (2003) The development of enantiostyly. *Am J Bot* 90:183–195
- Kawai Y, Kudo G (2009) Effectiveness of buzz pollination in *Pedicularis chamissonis*: significance of multiple visits by bumblebees. *Ecol Res* 24:215–223
- King MJ (1993) Buzz foraging mechanism in bumble bees. *J Apic Res* 32:41–49
- King MJ, Buchmann SL (1996) Sonication dispensing of pollen from *Solanum laciniatum* flowers. *Funct Ecol* 10:449–456
- King MJ, Buchmann SL (2003) Floral sonication by bees: mesosomal vibration by *Bombus* and *Xylocopa*, but not *Apis* (Hymenoptera: Apidae), ejects pollen from poricidal anthers. *J Kans Entomol Soc* 76:295–305
- King MJ, Buchmann SL, Spangler HG (1996) Activity of asynchronous flight muscle from two bee families during sonication (buzzing). *J Exp Biol* 199:2317–2321
- Knapp S, Persson V, Blackmore S (1998) Pollen morphology and functional dioecy in *Solanum* (Solanaceae). *Plant Syst Evol* 210:113–139
- Knapp S, Bohs L, Nee M, Spooner DM (2004) Solanaceae—a model for linking genomics with biodiversity. *Comp Funct Genom* 5:285–291
- Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* 37:1210–1226
- Larson BM, Barrett SCH (1999) The ecology of pollen limitation in buzz-pollinated *Rhexia virginica* (Melastomataceae). *J Ecol* 87:371–381
- Lester RN, Francisco-Ortega J, Al-Ani M (1999) Convergent evolution of heterandry (unequal stamens) in *Solanum*, proved by spermoderm SEM. In: Nee M, Symon DE, Lester RN, Jessop JP (eds) Solanaceae IV. Royal Botanical Gardens, Kew, pp 51–69
- Lye GC, Kaden JC, Park KJ, Goulson D (2010) Forage use and niche partitioning by non-native bumblebees in New Zealand: implications for the conservation of their populations of origin. *J Insect Conserv* 14:607–615
- Macior LW (1964) Experimental study of floral ecology of *Dodecatheon meadia*. *Am J Bot* 51:96–108
- Macior LW (1974) Behavioral aspects of coadaptations between flowers and insect pollinators. *Ann Mo Bot Gard* 61:760–769
- McNett GD, Cocroft RB (2008) Host shifts favor vibrational signal divergence in *Enchenopa binotata* treehoppers. *Behav Ecol* 19:650–656
- McNett GD, Miles RN, Homentcovschi D, Cocroft RB (2006) A method for two-dimensional characterization of animal vibrational signals transmitted along plant stems. *J Comp Physiol A* 192:1245–1251
- Michelsen A, Fink F, Gogala M, Traue D (1982) Plants as transmission channels for insect vibrational songs. *Behav Ecol Sociobiol* 11:269–281
- Morris GK (1980) Calling display and mating behaviour of *Copiphora rhinoceros* Pictet (Orthoptera: Tettigoniidae). *Anim Behav* 28:42–51
- Peat J, Goulson D (2005) Effects of experience and weather on foraging efficiency and pollen versus nectar collection in the bumblebee, *Bombus terrestris*. *Behav Ecol Sociobiol* 58:152–156
- Peat J, Tucker J, Goulson D (2005) Does intraspecific size variation in bumblebees allow colonies to efficiently exploit different flowers? *Ecol Entomol* 30:176–181
- Pouvreau A (1989) Contribution à l'étude du polyéthisme chez les bourdons, *Bombus* Latr. (Hymenoptera, Apidae). *Apidologie* 20:229–244
- R Core Development Team (2010) R: A language and environment for statistical computing. R foundation for statistical computing, Vienna
- Rasheed SA, Harder LD (1997a) Economic motivation for plant species preferences of pollen-collecting bumble bees. *Ecol Entomol* 22:209–219
- Rasheed SA, Harder LD (1997b) Foraging currencies for non-energetic resources: pollen collection by bumblebees. *Anim Behav* 54:911–926
- Roulston TH, Cane JH, Buchmann SL (2000) What governs protein content of pollen: pollinator preferences, pollen-pistil interactions, or phylogeny? *Ecol Monogr* 70:617–643
- SAS Institute (2007) JMP IN: the statistical discovery software: release 7.0. SAS Institute, Cary, North Carolina
- Schluter D, Nychka D (1994) Exploring fitness surfaces. *Am Nat* 143:597–616
- Thorp RW (2000) The collection of pollen by bees. *Plant Syst Evol* 222:211–223
- Vallejo-Marin M, Manson JS, Thomson JD, Barrett SCH (2009) Division of labour within flowers: heteranthery, a floral strategy to reconcile contrasting pollen fates. *J Evol Biol* 22:828–839
- Vallejo-Marin M, Da Silva EM, Sargent RD, Barrett SCH (2010) Trait correlates and functional significance of heteranthery in flowering plants. *New Phytol* 188:418–425

- Vogel S (1978) Evolutionary shifts from reward to deception in pollen flowers. In: Richards AJ (ed) The pollination of flowers by insects. Academic, London, pp 89–96
- Whalen MD (1979) Taxonomy of *Solanum* section *Androceras*. Gentes Herb 11:359–426
- Wolf TJ, Ellington CP, Begley IS (1999) Foraging costs in bumblebees: field conditions cause large individual differences. Insectes Soc 46:291–295
- Zar JH (1984) Biostatistical analysis, 2nd edn. Prentice-Hall, New Jersey