Buzz-pollination of *Hibbertia obtusifolia* by Australian native bees - a comparative study of bee buzzing behaviour in Capertee Valley, New South Wales.

Vicki Powys, December 2016

Summary:
Same-sized *Hibbertia obtusifolia* flowers were being buzz-pollinated by different-sized native bees, creating an opportunity for a direct comparison of bee buzz pitch. Popular science suggests bees may conform to a single pitch (middle C) to obtain pollen from some flowers. My study shows that buzzes spanning a full octave either side of middle C caused pollen to be released. Other aspects of bee buzzing behaviour are discussed.

Introduction

Most flowering plants have easily accessible pollen that is spread by wind and insects. However, eight percent of plants worldwide have poricidal anthers that require specialist buzz-pollination by bees. Hollow, pollen-filled anthers with a tiny opening at the top need to be shaken by buzzing (at a certain harmonic pitch) before the pollen is released. Experts are divided on the exact mechanism of this process. European honey bees cannot buzz-pollinate, but many Australian native bees can, having co-evolved with the native plants that require it. Australian native bee buzz-pollinators help maintain plant diversity in natural habitats and are also useful agricultural crop pollinators. This study compares the buzzing behaviour of six species of Australian native bee on one type of native flowering plant.

In woodland near my home, I observed six species of Australian native bee buzz-pollinating *Hibbertia obtusifolia* flowers. Flower size was a fairly uniform 20 mm diameter, but bee size ranged from 5 - 18 mm in length. Popular TV science says the exact pitch of the pollinating buzz is relevant to success in obtaining pollen; the presenter demonstrating with a musicians' tuning fork how, with some flowers, pollen will spurt forth at exactly middle C (261 hertz) (Attenborough BBC 1994; Stewart BBC 2012). Could my bees of such diverse size all conform to a single pitch? And would that pitch be middle C? Additionally, was it true that "the larger the bee, the slower the wingbeat and the lower the pitch of the flight buzz"? (Otis in Scientific American, 2005). My study investigates these beliefs, and provides data for seven Australian native bee species and the introduced European honey bee.

Study site and methods

The study was made from 21 October to 18 November, 2016, in Capertee Valley, NSW. Low-growing *Hibbertia obtusifolia* shrubs were scattered across 100 square metres of natural woodland habitat. Audio recordings were made on calm, clear mornings from about 6 - 9 a.m. Eastern Standard Time when the bees were most active. A seventh bee species was recorded on a native *Solanum* flower, and one of the *Hibbertia* bees was also recorded on a *Solanum* flower, making a useful comparison. Bees also visited flowering *Leucopogon muticus* shrubs in adjacent woodland.

Bees were recorded in the field using an Olympus LS10 recorder and directional microphone Sennheiser ME66. The faint buzzes of the three smallest bees were recorded using the built-in microphones of the LS10, held very close to the bee.

Bees were photographed and videoed using a Lumix DMC-TZ60 pocket camera. Identification to at least genus level was made from photos by bee experts Ken Walker and Megan Halcroft. Size (length) was estimated compared to flower size, or directly measured using a ruler. Buzzing behaviour was examined from slow motion playback of high-definition video footage. Pitch was measured using Praat v5.3.55 (Boersma & Weenink) linguistics software on an iMac computer. Audio clips were processed using BIAS Peak LE 5.2.1 software.

Bee buzzes have strong harmonics which are louder than the (often elusive) fundamental. I tried various audio analysis programs (Amadeus Pro, Izotope RX, Audacity, Raven Lite) however Praat was far superior in measuring the fundamental frequency of the pollinating buzz pitch. Praat has been used by other authors on bee sounds e.g. Burkart et al. (2011). I verified the Praat measurement by expanding the buzz waveform in Amadeus Pro, and measuring the number of pulses per one-tenth of a second. Pulses per second indicate the fundamental frequency. Pulses (in bees) also represent wing beats, for example 200 wing beats per second will equal 200 Hertz (Figures 1 and 2).

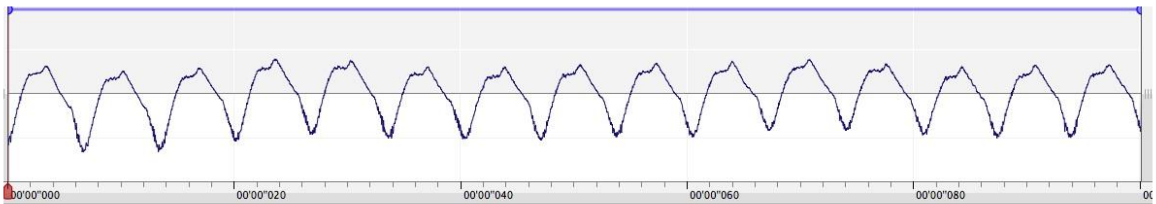

Figure 1: showing one-tenth of a second waveform in Amadeus Pro, of the flight sound of *Lasioglossum hiltacum*. There are 15 pulses or wing beats, which equals 150 wing beats per second, or 150 Hertz.

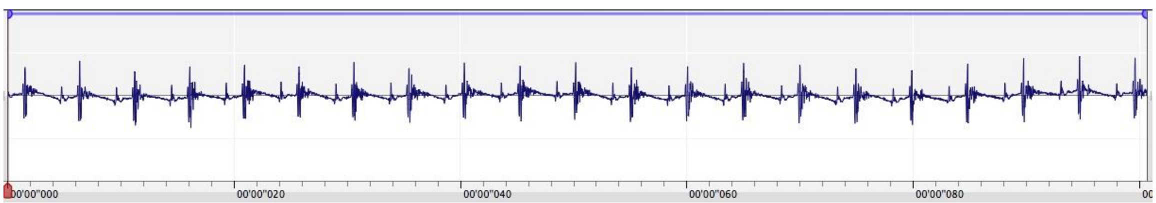

Figure 2: showing one-tenth of a second waveform of the pollination buzz of *Lasioglossum hiltacum*. There are 21 pulses or wing muscle vibrations, which equals a buzz at 210 Hertz. (Bees disengage their wings and vibrate their wing muscles, to buzz-pollinate).

From audio recordings, a total of 348 pollination buzzes, and 127 flight buzzes were analyzed, from approximately 15-20 individual bees. The duration and pitch of each buzz was measured in Praat and entered onto an Excel spreadsheet, to produce average (mean) numbers.

The number of pollination buzzes per flower could not be measured from audio clips, because some bees walked between flowers and did not fly. Pollination buzzes were counted visually from 62 video clips where a bee arrived at, buzzed then departed from any one flower.

No bees were captured nor harmed in this study.

Results

Bee species studied

Table 1 shows the eight bee species that were studied, their size, and mean pitch of both pollination and flight buzzes. "n" is the number of audio samples measured. Lower numbers for Hertz indicate a lower pitch. The list is in bee-size order from small to large. All bees were on *Hibbertia obtusifolia* except for *Lipotriches flavoviridis* which was on a *Solanum* flower.

COLOUR	COMMON NAME	SCIENTIFIC NAME	BEE LENGTH	MEAN POLLINATION BUZZ, HERTZ (n = no. of samples)	MEAN FLIGHT BUZZ, HERTZ (n= no. of samples)
red / grey		*Lasioglossum* sp	5 mm	202 (n=12)	not recorded
red / black	Reed Bee	*Exoneura* sp	5 mm	284 (n=19)	170 (n=5)
metallic green	Emerald Homalictus	*Homalictus* probably *urbanus*	5 mm	207 (n=49)	163 (n=6)
metallic striped	Nomia Bee	*Lipotriches* prob *flavoviridis*	8 mm	255 (n=29)	278 (n=2)
black / white striped		*Lasioglossum* sp	10 mm	216 (n=77)	183 (n=19)
dark red		*Lasioglossum hiltacum*	12 mm	217 (n=85)	158 (n=18)
brown / tan	European honey bee	*Apis mellifera*	16 mm	did not buzz pollinate	234 (n=6)
blue-black	Carpenter Bee	*Xylocopa aeratus*	18 mm	310 (n=75)	205 (n=77)

Table 1: Bee species, their size, mean pollination buzz & flight buzz.

Bee size

There was a big size difference between the species of bee that were studied as shown in Figure 3.

Figure 3: Four of the bees that were studied in same-sized *Hibbertia obtusifolia* flowers, showing a big difference in bee size in length and width. Clockwise from top left: red *Exoneura* species (5 mm); white-striped *Lasioglossum* species (10 mm); big carpenter bee *Xylocopa aeratus* (18 mm); red *Lasioglossum hiltacum* (12 mm).

Flowers

Hibbertia flowering lasted about a month. The yellow flowers measured about 20 mm across, with a cluster of 20-30 yellow poricidal anthers in the centre (Figure 4). The petals dropped each afternoon, and new flowers with fresh anthers opened each morning, probably encouraging the bees to an early start, with the greatest bee activity occurring between 6.30 - 9 a.m. EST when temperature ranged from 15-22 degrees Celsius. Two bee species were recorded nearby in *Solanum* species flowers that had five poricidal anthers per flower and measured about 20 mm across (Figure 4). Some bees also foraged in nearby *Leucopogon muticus* shrubs whose tiny flowers provided only nectar.

Figure 4: On left, close up of the poricidal anthers of *Hibbertia obtusifolia*. Each anther is filled with pollen and has a small opening at the top. On right, *Solanum* species flower with five poricidal anthers.

Pitch of pollination buzz

Each individual pollination buzz was measured in Praat to obtain its average pitch. A range of pitches was found for each bee species, and these are shown in Figure 5, relative to middle C. All bees were on *Hibbertia obtusifolia* flowers, except for *Lipotriches flavoviridis* which was on a *Solanum* flower. All flowers measured about 20 mm diameter. I found that:

- Bee size was not related to pollination buzz pitch (smallest and largest bees were both high pitched).
- Pitch did not need to be middle C for pollen release (all the bees were covered in pollen).
- Flower size was not related to pitch (all the flowers were the same size but bee pitches were different).
- Pitch for all bees (in musical terms) covered a full octave from E3 to E4.

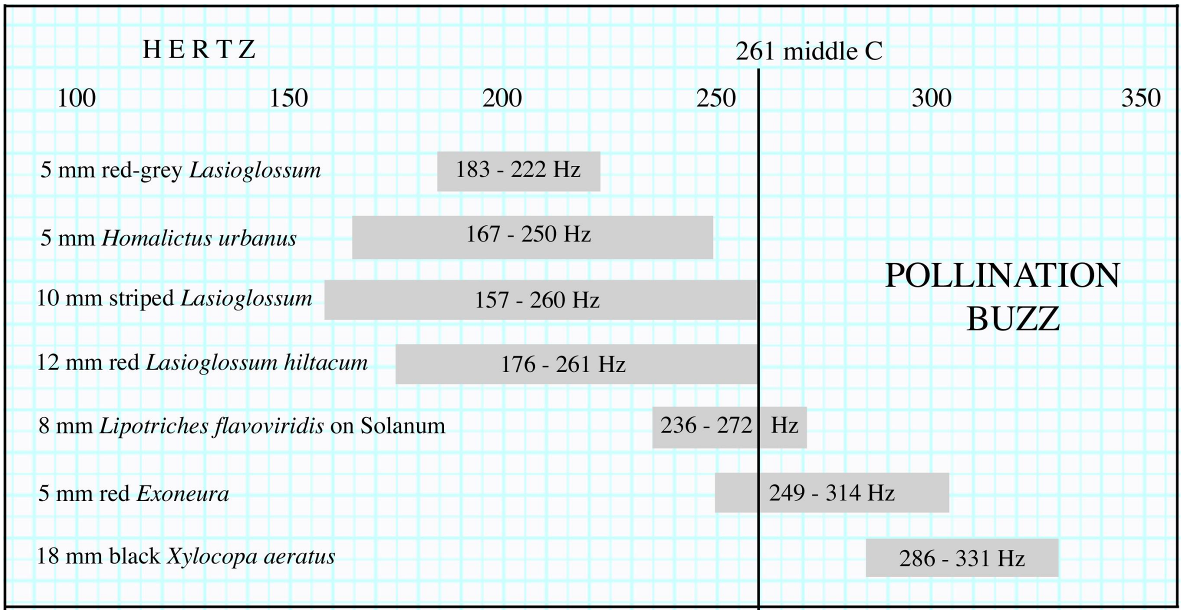

Figure 5: showing range of pitch for the pollination buzz (grey band), relative to middle C. In musical terms, the buzzes covered a full octave from E3 to E4.

When the large *Xylocopa aeratus* buzzed a *Solanum* flower, the buzz pitch was 308 Hertz (n=1), which was within the range it used for *Hibbertia* flowers.

Pitch of flight buzz

Individual flight buzzes were measured, and a range of pitches was found for each bee species (Figure 6). These were compared to pollination buzz pitch. I found that:

- Bee size was not related to the flight pitch.
- Larger bees did not always have the lowest flight pitch.
- The biggest bee had the second highest flight pitch (rather than the lowest).
- All bees but one had a flight pitch that was lower than the pollination buzz pitch.
- There was no obvious relationship between flight pitch and pollination buzz pitch, for any one species.

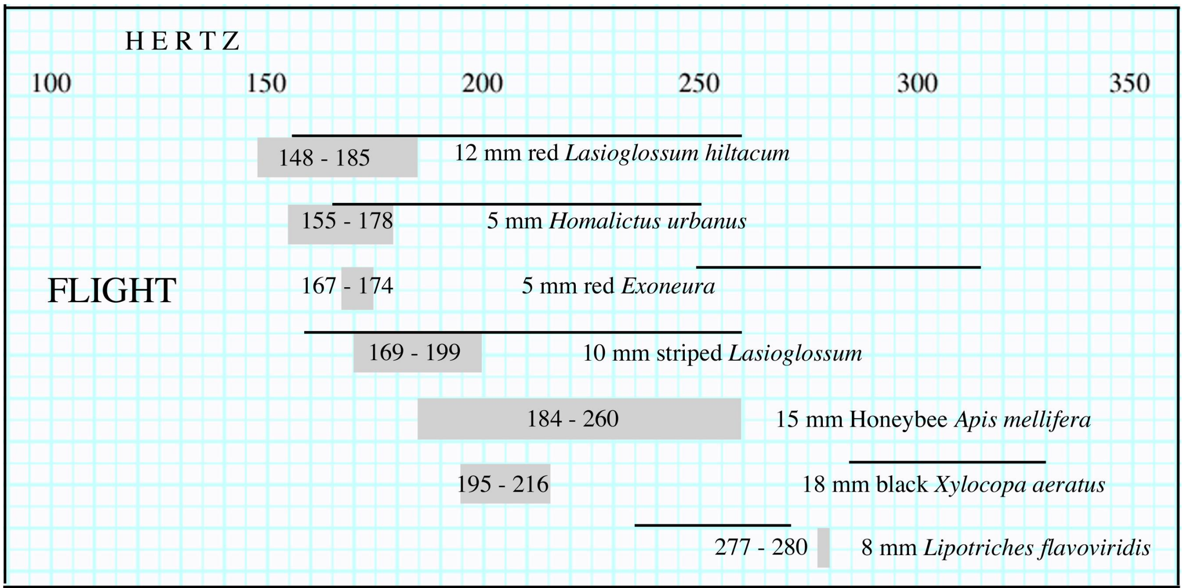

Figure 6: showing range of pitch for flight buzz (grey band), relative to the pitch of the pollination buzz (black line). Note that the honey bee did not have a pollination buzz.

Pollination buzz close-up

Single individual buzzes whilst measured in Praat to give a mean pitch, were quite variable in shape, usually with a brief high start and levelling out into a steadier tone of greater amplitude (Figure 7). Some species also ended the buzz with an upward inflection. The *Lipotriches* bee had a 3-part buzz, with two short buzzes followed by a longer buzz with rising inflection.

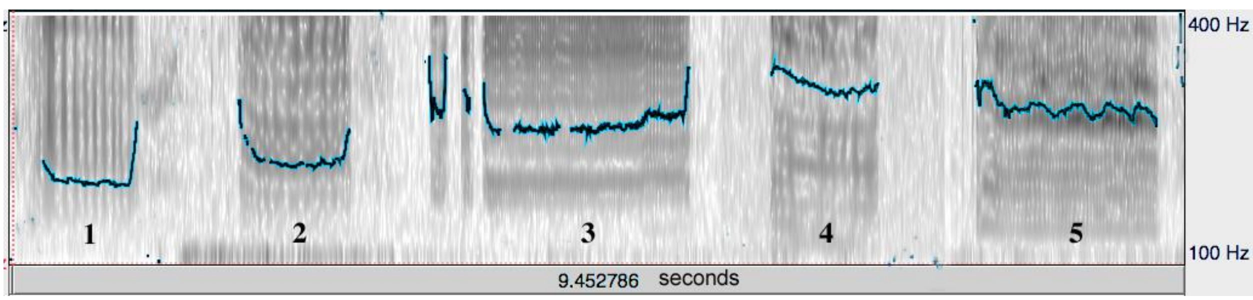

Figure 7: Praat spectrogram window comparing five examples of pollination buzzes. The curved line follows the shape of the fundamental. 1. white-striped *Lasioglossum* species; 2. red *Lasioglossum hiltacum*; 3. *Lipotriches flavoviridis* (on *Solanum* flower); 4. red *Exoneura* species; 5. *Xylocopa aeratus*.

Time spent in each flower

Did a short visit indicate easier pollen access? I looked for links between the number of buzzes per flower, the pitch of the buzz, its duration and loudness, and the size of the bee (Figure 8). I found that:

- There was a link between size of bee and time spent in a flower (smaller bees spent longer in each flower).
- A higher pitch did not always mean less time in a flower.
- Duration of the buzz was similar for all bees except one.
- Loudness may have aided success in obtaining pollen.
- Faint buzzing was still effective.

The first six bees (Figure 8) were in *Hibbertia* flowers, and as their size diminished, the number of pollination buzzes increased. (The *Lipotriches* bee in a *Solanum* flower gave many more buzzes per flower, and of a longer duration, than the other bees. This may have related to either the bee species or the flower species).

DURATION OF BUZZ	NUMBER OF BUZZES PER FLOWER	PITCH	BEE SIZE & SPECIES
0.7 SEC	1 BUZZ (n = 245)	310 HZ	18 mm *Xylocopa aeratus*
0.7 SEC	6 BUZZES (n = 4)	217 HZ	12 mm *Lasioglossum hiltacum*
0.7 SEC	7 BUZZES (n = 20)	216 HZ	10 mm striped *Lasioglossum*
0.6 SEC	9 BUZZES (n = 1)	207 HZ	5 mm *Homalictus urbanus*
0.9 SEC	9 BUZZES (n = 4)	202 HZ	5 mm red-grey *Lasioglossum*
0.7 SEC	9 BUZZES (n = 6)	284 HZ	5 mm red *Exoneura*
1.5 SEC	16 BUZZES (n = 4) IN SOLANUM	255 HZ	8 mm *Lipotriches flavoviridis*

Figure 8: Chart showing mean number of pollination buzzes per flower (n = number of flowers visited), in relation to mean pitch, bee size, and mean duration of buzz. The first six bees were in *Hibbertia* flowers, and the seventh bee in a *Solanum* flower.

The tiny 5 mm *Exoneura* bee had a high-pitched buzz averaging 284 Hertz, unlike the other 5 mm bees. This shows that a higher pitch was not the reason that bees might spend a short time in a flower, as this bee spent a long time in each flower.

Video clips confirmed that the large carpenter bee *Xylocopa aeratus* buzzed each flower only once before flying to the next flower (figure 8). All the other bees buzzed the anthers multiple times before leaving the flower. Some bees worked more quickly than others. The tiny red-grey bee was observed working in a single flower for over four minutes. All of the 5 mm bees spent time gathering together up to six anthers, before buzzing them. The white-striped *Lasioglossum* gathered ten or more anthers at a time and worked more quickly. All bees groomed themselves before flying off. The *Lipotriches* bee in the *Solanum* flower buzzed a single anther at a time and spent a long time in each flower. (See video examples in Appendix: Supplementary material).

The relative loudness of buzzes between species was measured subjectively in the field. The biggest bee was the loudest, its pollination buzz being audible from several metres away. Middle sized bees were clearly audible from 1-2 metres away. The 5 mm bees were inaudible in the field, although their hunched buzzing action was obvious. Even with the recording input volume on maximum, their buzzes were relatively faint. Yet these tiny bees were still able to obtain plenty of pollen. All of the bees studied were well-coated in pollen. Loudness combined with a high pitch and large size did seem to equate with success for the carpenter bee.

Variables for the number of pollination buzzes could include the amount of pollen available in a flower, the presence of an observer (with camera & microphone), the arrival of another bee, and the number of video samples available.

Warm-up buzzing

On cooler mornings, two medium sized bees (Table 2) were recorded giving warm-up buzzes with their wings disengaged, just before flight. The buzzes were longer, softer and much higher pitched than their pollination buzz.

BEE NAME & SIZE	WARM UP BUZZ HZ	DURATION	POLLINATION BUZZ MEAN	FLIGHT BUZZ MEAN
striped *Lasioglossum* 10 mm	330 to 380 Hz	13 sec	216 Hz	183 Hz
red *Lasioglossum hiltacum* 12 mm	300 Hz	4.9 sec	207 Hz	158 Hz

Table 2: Two examples of soft, high-pitched, warm-up buzzing from white-striped *Lasioglossum* and *Lasioglossum hiltacum.*

Other bee behaviour

Field observations and video clips showed that all the native bees folded their wings when they landed in a *Hibbertia* or *Solanum* flower, prior to buzzing (Figure 9). The bees curled their body around the anthers and clung on with their feet as they buzzed. The buzzing is said to be from the vibration of the wing muscles when the wings are disengaged. The folded wings of all bees did vibrate and for the smaller bees even the antennae vibrated. This was a good visual clue when counting the number of buzzes from movie clips. Between buzzes, the bee would comb itself downwards to transfer pollen to its back legs and belly.

Figure 9: Buzzing action of three bee species in *Hibbertia obtusifolia*, note the blur of the wings as they buzz, and for the third bee, the vibrating antennae can clearly be seen. These are screen shots from movie clips. Left to right: *Exoneura* species; red *Lasioglossum hiltacum*; white-striped *Lasioglossum* species.

All the bees that gathered pollen were assumed to be females. Female bees collect pollen, which they use as food for the larvae in their nests. Male carpenter bees could easily be identified in the field (their metallic golden colour contrasts with the blue-black females); they took nectar on nearby *Leucopogon* flowers and chased after females, but never visited the *Hibbertia* flowers. Female carpenter bees also took nectar on the *Leucopogon* flowers but did not buzz them. When the *Hibbertia* began to flower in late October, the female carpenter bees were rarely seen on the *Leucopogon*, but often on the *Hibbertia*, with perhaps 10 female carpenter bees within my 100 square metre study area. Seven bees listed here are solitary bees whose females mate once and lay their eggs in dead wood or in the ground. The introduced honey bee is a social bee, belonging to a hive with queen, drones and workers. Only once was a honey bee observed on an *Hibbertia* flower. It spent several minutes scraping at the anthers with its feet but could not obtain pollen (verified by video footage). Honey bees dominated the *Leucopogon* shrubs and fed on the nectar.

Supplementary material including pollination-buzz videos, audio clips and images are available online at my website, see Appendix.

Discussion

Pollination buzz

It was useful to have a single flower species and size, to rule out flower variables and concentrate on bee variables. Despite the predictions of popular science, an exact pitch for the pollination buzz did not occur, and pitch variation covered a full octave. All native bees were covered in pollen so the pitch they gave obviously worked for them. Amplitude could not have been a major factor, as the tiniest bees that were inaudible to the human ear, could still obtain plenty of pollen. There was no obvious sliding scale of pitch for the pollination buzz, that related to the size of the bee. For example the tiny red *Exoneura* bee (5 mm) and the largest carpenter bee (18 mm) shared a similar high pitch range. Yet two other tiny bees averaged much lower pitches. Bee structure (rather than size) may influence the pollination buzz pitch given. Attenborough (1994) and Stewart (2012) both referred to a “handsome furry” African carpenter bee that buzzed at exactly middle C to obtain pollen from pink gentian flowers *Orphium frutescens*. My carpenter bee *Xylocopa aeratus* gave a significantly higher pitched buzz than middle C, for two different types of flower.

Flight buzz

The largest bee did not have the lowest pitch of flight buzz, in my study. Almost the opposite was true – the largest 18 mm carpenter bee had the second highest pitch of flight buzz, a medium sized bee had the lowest pitch, and a 5 mm bee had the second lowest pitch. There was no pattern of bee size relative to pitch. Again, bee structure rather than size may be the determining factor.

Correlation of pollination and flight buzzes

A study in Brazil by Burkart et al. (2011) found that larger bees e.g. carpenter bees *Xylocopa frontalis* and *Xylocopa suspecta* had a pollination buzz that was twice the frequency of the flight buzz. Burkart also quoted an earlier finding by King & Buchmann (2003) that said “because of the changing resonance properties of the thorax with decoupled wings, the sound of buzzing usually has an about two times higher frequency than the sound of flight”. This rule apparently applied to a number of bee species. In my study (see Figures 1, 2 & 6 and Table 1) I could find no similar correlation between pollination and flight buzzing sounds. My largest bee had a mean pollination buzz of 310 Hz and a flight buzz of 205 Hz, a difference of about one-third.

Type of plant

Do plants of a certain size and shape require a made-to measure frequency? Other authors have looked at this question, and some have suggested that they do (e.g. Corbet & Huang 2014). My study indicates that many different frequencies worked successfully for one species and size of flower.

Other behaviour

Switzer et al. (2016) in a study in South Australia, found that Australian native blue-banded bees *Amegilla murrayensis* engaged in “head banging” to obtain pollen from the anthers of cherry tomatoes *Solanum lycopersicum*. A careful study of my video clips showed that all my bees held the anthers with their legs, but possibly not with their mandibles, nor did they appear to hit the anthers with their heads. Some bees were more tolerant of my presence than others. The large carpenter bee occasionally buzzed me by zig-zagging around me, then flying further away.

Measuring pitch

A study made in China by Corbet & Huang (2014) on bumblebees *Bombus friseanus* and *Bombus festivus*, measured the pollination buzz pitch in Raven Lite by expanding the waveform using one-tenth of a second samples. While this method was useful in my study to obtain an approximate pitch, it did not represent the true mean of a buzz. Figure 7 shows the very variable shape of each buzz and the pitch would vary depending on which tiny part of it was measured. I found it was better to get an average pitch (in Praat) and if necessary verify ballpark figures for the fundamental frequency via the waveform. Corbet & Huang also used wingbeat frequency (flight pitch) as an index of body size of bumblebees, but this method did not work in my study where I found that for Australian native bees there was no obvious relationship between flight pitch and bee size (Figure 6). Otis (2005) also found a relationship between flight buzz pitch and bee size – "The larger the bee, the slower the wingbeat and the lower the pitch of the resulting buzz", inferring that smaller bees had a higher wingbeat frequency and a higher pitched flight buzz. Otis may have observed this with bumblebees but his remark was a general one. It certainly did not apply to my Australian bees. So, a note of caution – what applies to studies in bumblebees, does not necessarily apply to Australian native bees.

Recording methods and difficulties

At the time of my study, small cicadas were starting to hatch and their persistent calls needed to be avoided. By December, the roaring noise of larger cicadas made bee recording impossible. Early morning was the quietest time, before the cicadas began calling. I could not record if it was windy. Birdsong was generally higher pitched than bee buzzes and could be filtered out, either directly in Praat or via various other audio processing applications. Lower-pitched noise from motorbikes, vehicles and jet planes overlapped with the bee sounds and could not be filtered out, thus rendering any affected clips unusable.

The ME66 directional mic has its cardioid capsule situated 14 cm from the tip of the mic, which distance proved to be a handicap when recording the faint sounds of the tiniest bees. Built-in electret mics on the LS10 recorder, even though less sensitive, proved more successful because the element could be placed much closer to the bee. For all other bees, the directional mic worked well and was useful in suppressing surrounding noise such as insects and birdsong. I also tried an electret capsule on a stick but there were problems with handling noise.

The smallest bees tolerated a microphone to within half a cm; medium bees were louder so it was not necessary to be as close and nor would they have tolerated it. I recorded the (louder but more wary) big carpenter bee at 15 cm minimum distance. For all bees I used a high sensitivity setting and near maximum recording volume (7 out of 10). Low cut was switched off. For recording and taking videos I sat on a folding stool and used elbows on knees as a tripod for the camera. Other researchers have used various recording techniques and have found that relatively simple equipment is adequate, some examples are:

- Burkhart et al. 2011: Smartphone WAV recordings processed using Audacity & Praat.
- Switzer et al. 2016: Tascam recorder with shotgun mic, processed with Audacity and analyzed using "R" and "seewave".
- Corbet & Huang 2014: recorder not stated, digital recordings processed using RavenLite.
- Duncan 2003: Sony cassette walkman with lapel microphone clipped to a stick.
- De Luca et al. 2014: Zoom H4 digital recorder, built in mics, processed using Audacity.

There is definitely room for amateur citizen scientists, through careful observations and audio recordings, to make a contribution to our knowledge on wildlife sounds including bee buzzing behaviour. At the same time, the participant is brought closer to the wonders and joys of nature.

Appendix: Supplementary material

Short videos of all the bees in this study (buzz-pollinating), also audio clips, waveforms and photos, and a short demonstration video on using Praat to measure a bee buzz, can be found at my website http://caperteebirder.com/index.php?p=1_42_Bees

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