Arthropod-Plant Interactions
DOI 10.1007/s11829-015-9407-7

ORIGINAL PAPER

Shakers and head bangers: differences in sonication behavior between Australian *Amegilla murrayensis* (blue-banded bees) and North American *Bombus impatiens* (bumblebees)

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Received: 2 November 2015 / Accepted: 20 November 2015

Abstract Many bees collect pollen by grasping the anthers of a flower and vibrating their flight muscles at high frequencies—a behavior termed sonication, or buzz-pollination. Here we compare buzz-pollination on *Solanum lycopersicum* (cherry tomatoes) by two bees that fill similar niches on different continents—in Australia, *Amegilla murrayensis* (blue-banded bee), and in North America, *Bombus impatiens* (bumblebee). We collected audio recordings of buzz-pollination and quantified the frequency and length of buzzes, as well as the total time spent per flower. We found that *A. murrayensis* buzzes at significantly higher frequencies (~350 Hz) than *B. impatiens* (~240 Hz) and flaps its wings at higher frequencies during flight. There was no difference in the length of a single buzz, but *A. murrayensis* spent less time on each flower, as *B. impatiens* buzzed the flower several times before departing, whereas *A. murrayensis* typically buzzed the flower only once. High-speed videos of *A. murrayensis* during buzz-pollination revealed that its physical interaction with the flower differs markedly from the mechanism described for *Bombus* and other bees previously examined. Rather than grasping the anther cone with its mandibles and shaking, *A. murrayensis* taps the anther cone with its head at the high buzzing frequencies generated by its flight muscles. This unique behavior, combined with its higher buzzing frequency and reduced flower visit duration, suggests that *A. murrayensis* may be able to extract pollen more quickly than *B. impatiens*, and points to the need for further studies directly comparing the pollination effectiveness of these species.

Keywords Sonication · *Solanum* · Vibration · Pollination · Native bees

Introduction

Over 200,000 plant species depend on insects for pollination (Buchmann 1983). Pollinating insects often consume both nectar and pollen, and they transfer pollen grains among plants as they travel from flower to flower, an essential step in the reproduction of many plants. Understanding the physical interactions between plants and insect pollinators (primarily bees) can provide insight into the requirements and evolution of these critical plant–pollinator relationships.

Although considered a mutualistic relationship, the interaction between plants and pollinating insects is not entirely without conflict. The conflict arises because the ideal behavior of the pollinator is different from the pollinator's perspective versus the plant's perspective (Gegear and Laverty 2001). Bees attempt to expend the least possible energy for the greatest reward; bumblebees forage for

Handling Editor: Heikki Hokkanen.

Electronic supplementary material The online version of this article (doi:10.1007/s11829-015-9407-7) contains supplementary material, which is available to authorized users.

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pollen in a manner that increases their probability of maximizing their net energy intake (Zimmerman 1982). Plants, on the other hand, would benefit most if pollinators moved sequentially among flower of the same species—a strategy that an optimally foraging pollinator would rarely use (Gegear and Laverty 2001). One evolutionary "strategy" for increasing a plant's reproductive success is to dispense only a little pollen at a time, ensuring that its flowers are visited multiple times and that pollinators must visit multiple flowers to obtain sufficient pollen (Harder and Thomson 1989). Plants may also benefit from "messy" bees that cannot clean all the pollen off their bodies, since this excess pollen is not consumed by the pollinator, and is more likely to be transferred to different flowers that the bee visits subsequently; outcrossing plants require a pollinator that accumulates pollen on its body where it has a high chance of fertilizing a conspecific plant ovule (Gegear and Laverty 2001).

These evolutionary strategies are particularly evident in the approximately 20,000 insect-pollinated plants (~8 % of angiosperms) that have evolved poricidal anthers (Buchmann 1983)—anthers with only small pores through which pollen is released. Poricidal anthers restrict direct access to pollen (De Luca and Vallejo-Marín 2013; Harder and Thomson 1989), helping to limit the amount of pollen that bees can collect during a visit and depositing pollen in locations on the bees' bodies that are poorly groomed. Throughout areas with temperate climates, *Bombus* spp. (bumblebees) play a vital role in pollinating plants with poricidal anthers, as they are capable of performing sonication, or buzz-pollination, to release pollen that is largely inaccessible to insects that do not perform this behavior (e.g., honeybees) (King and Buchmann 2003). In warmer areas, species belonging to other taxa, e.g., *Xylocopa* (Hogendoorn et al. 2000) and *Amegilla* (Hogendoorn et al. 2006), perform buzz-pollination.

Buzz-pollination has been well described in *Bombus* spp.: The bee lands on a flower, curls her abdomen around the anther tips while grasping the anthers with her mandibles, and then uses her flight muscles to vibrate her body without flapping the wings (King et al. 2006). These vibrations are transmitted through the head and body to the flower, and pollen is released from the pores onto the bee's body (De Luca and Vallejo-Marín 2013; Harder and Barclay 1994; King and Buchmann 2003). Due to the bee's position on the anther during sonication, pollen is deposited onto her ventral body surface, and although she collects some of the pollen grains, several locations on the ventral body surface are poorly groomed (Buchmann 1983; Michener et al. 1978), which facilitates the transfer of pollen to other flowers. Both species of bees groom the pollen from their bodies and place it onto specialized carrying structures (Michener et al. 1978). *Bombus* has basket-like corbiculae, while *Amegilla* has brush-like scopae for holding pollen on the hind legs (Michener 2000). *Bombus* moistens the pollen with nectar before packing it into the corbicula (Michener 2000; Michener et al. 1978). *Amegilla* packs relatively dry pollen among the hairs of the scopa (Anderson and Symon 1988).

The asynchronous flight muscles that drive the wings form part of a resonant system, whose vibration frequency depends on the mass it is driving (i.e., the mass of the wings) (Josephson et al. 2000). Thus, when the wings are disengaged during sonication, the vibration frequency of the flight muscles is higher than the bees' flapping frequency during flight (King et al. 1996).

Although much of the previous work on buzz-pollination has focused on *Bombus* spp. (Asada and Ono 1996; Buchmann and Hurley 1978; De Luca et al. 2013; Harder 1990; King 1993; King and Buchmann 2003; Morandin et al. 2001), many other bee genera perform buzz-pollination, including *Protandrena*: Andrenidae (Cane and Buchmann 1989), *Megachile*: Megachilidae (Neff and Simpson 1988), *Augochloropsis*: Halictidae (Thorp and Estes 1975), *Xylocopa*: Apidae (Hogendoorn et al. 2000; King and Buchmann 2003), *Nomia*: Apidae (Anderson and Symon 1988), and *Amegilla*: Apidae (Hogendoorn et al. 2006). Information on the mechanics of buzz-pollination in these genera is far more limited, and comparative studies of buzz-pollination mechanisms among different groups of bees are scarce.

Buzz-pollination is known to be critical for many endangered plants, such as *Dianella longifolia* in Australia (listed on the Advisory List of Rare or Threatened Plants in Victoria in 2014 and the Northern Territory Threatened Species list), which can reproduce only through buzz-pollination. In addition, the economic value of buzz-pollination is very high, as it contributes to increased yields in crops ranging from tomatoes (Asada and Ono 1996; Hogendoorn et al. 2006) to blueberries (Javorek et al. 2002) and cranberries (MacKenzie 1994).

In mainland Australia, *Bombus* spp. are not present, and multiple native bees perform buzz-pollination. The potential introduction of *Bombus* spp. to the Australian mainland for tomato pollination (Hogendoorn et al. 2006) is being debated intensively, as *Bombus* spp. have been commercialized in other parts of the world, and their effectiveness at pollinating crops in greenhouses is well established (King 1993). Native Australian bees, like *Amegilla* spp., have not been commercialized to the same degree, but research suggests that they also present a viable method of pollinating tomatoes in greenhouses (Bell et al. 2006; Hogendoorn et al. 2006). However, few studies have compared the mechanisms by which native Australian bees and *Bombus* spp. extract pollen via sonication, and buzz-pollination by *Amegilla* spp. has not been quantified.

Here we compare buzz-pollination on *Solanum lycopersicum* (cherry tomatoes) by two bees that fill similar niches on different continents—in Australia, *Amegilla murrayensis* (blue-banded bee), and in North America, *Bombus impatiens* (common Eastern bumblebee).

To determine whether these species pollinating the same flower perform buzz-pollination in the same way, we collected audio recordings of buzz-pollination and quantified the frequency and length of individual buzzes, as well as the total time spent on a single flower (which may encompass multiple buzzes). We also recorded bees during flight, to compare sonication frequency to flight frequency. Finally, we filmed *A. murrayensis* during buzz-pollination using high-speed video, to compare its physical interaction with the flower to the well-described sonication behavior of *B. impatiens*.

Materials and methods

Study species and locations

We collected audio recordings of pollination buzzes by *Bombus impatiens* (bumblebees) on *Solanum lycopersicum* "Sweet 100" (cherry tomatoes) and by *A. murrayensis* (blue-banded bees) on *S. lycopersicum* "*Heirloom Roma Cherry*" and *S. lycopersicum* "*Tommy Toe*" (cherry tomatoes) (Fig. 1). Although the varieties of cherry tomatoes (*S. lycopersicum*) used were different, the flowers are very similar in size and morphology, and thus, we do not expect that the tomato variety significantly affected buzz-pollination characteristics (Online Resource 1, Tables VII and IX). Recordings of *B. impatiens* were collected in a community garden in Carlisle, Massachusetts, USA (42°52′N; 71°32′W), and those of *A. murrayensis* at the Adelaide Botanic Garden, Adelaide, Australia (34°92′S; 138°61′E). In Australia, *S. lycopersicum* "*Tommy Toe*" plants growing in the garden were supplemented with potted tomato plants (*S. lycopersicum* "*Heirloom Roma Cherry*") to provide additional flowers. For the potted plants, we recorded if the flower had been previously visited.

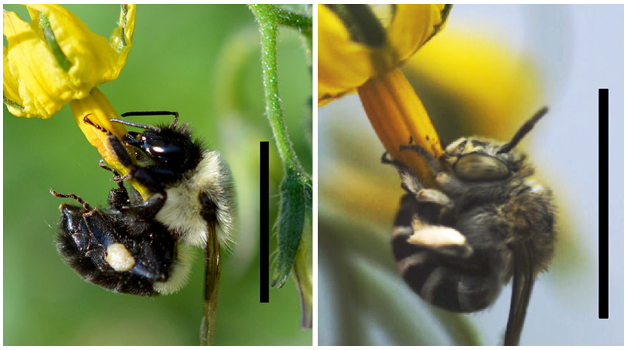

Fig. 1 Photographs of bees used in this study. *Bombus impatiens* workers (*left*) are typically larger than *Amegilla murrayensis* (*right*; Online Resource 1, Table I). Both bees are shown on *Solanum lycopersicum* flowers. *Black bars* indicate approx. 1 cm. Bumblebee photograph credit: Tim Stanley/Native Beeology

Audio recordings and analysis

We collected audio recordings with a shotgun microphone (SGM-1X, Azden, Tokyo, Japan) attached to a digital recorder (DR-100mkII, Tascam, Montebello, California), held within 3 cm of the bees' bodies. We attempted to position the microphone pointed at each bee's thorax, approximately orthogonal to the bee's frontal plane. We were not able to maintain that position for all recordings; however, we have no evidence that recording from different angles affects the analysis of sonication frequency or duration. We recorded bees while landing, buzzing flowers, and flying away, to analyze audio characteristics of both flight and buzz-pollination.

Because some bees perform multiple buzzes on a single flower with pauses in-between, we recorded the time of landing and takeoff to calculate the total visit duration. This was considered a suitable estimate of the time spent on a single blossom, since these bees generally did not crawl between tomato flowers. When audio recordings did not span the entire length of a flower visit, we excluded them from the analysis of visit duration.

After collecting audio recordings of landing, buzz-pollination, and takeoff flight, we captured bees with a net and noted the time, temperature, and relative humidity. To ensure independent samples, we either marked bees after the first capture (and excluded recaptured bees from the analysis) or collected the bees and pinned them as specimens. We measured intertegular (IT) span with digital calipers on bees that were released and with ImageJ (http://imagej.nih.gov/ij/) on photographs of pinned specimens to obtain the average size of each species (Online Resource 1, Table I). When bees performed multiple buzzes while visiting a single flower, the frequency and duration of these buzzes were averaged for statistical analysis.

We played recordings in Audacity (http://audacity.sourceforge.net/) and identified the start and end of each buzz aurally and visually to determine the buzz length. We defined buzzes that had breaks of less than about 0.1 s to be single buzzes. Figure 2a shows an oscillogram from a series of buzzes by *B. impatiens*, with a single buzz expanded in Fig. 2b. We calculated buzz frequencies in R (R Core Team 2012), using the "seewave" (Sueur et al. 2008) and "signal" (Signal Developers 2013) packages. We first filtered recordings to remove low-frequency noise and then calculated fundamental frequencies within sliding

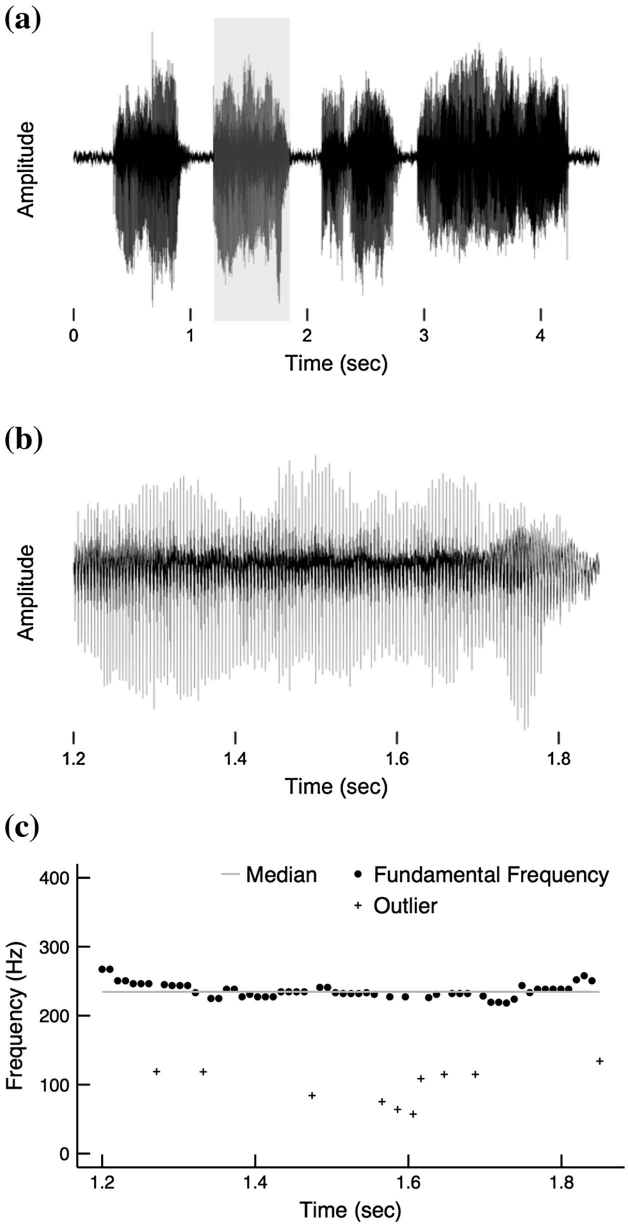

Fig. 2 Audio recordings of buzz-pollination. **a** Oscillogram showing four pollination buzzes by *Bombus impatiens* (bumblebee) on a flower of *Solanum lycopersicum "Sweet* 100" (cherry tomato). Shaded region indicates a single buzz. **b** Expanded oscillogram of the single buzz shaded in **a**. **c** Fundamental frequency calculated over the course of the buzz shown in **b**. *Dots* represents the fundamental frequencies calculated from overlapping windows of 2048 data points. Frequency measurements that were identified as outliers and removed are indicated by a *plus symbol*

windows of 2048 points, with 80 % overlap. Recordings typically contained small number points that were clearly outliers (Fig. 2c)—single data points at frequencies more than one standard deviation beyond the median fundamental frequency. These outliers were most likely artifacts caused by using a relatively small sliding window and/or collecting recordings in noisy, outdoor environments. We removed outliers and then calculated the median of the trimmed distribution to determine the frequency of each buzz (Fig. 2c).

We calculated wing beat (flight) frequency using the same method as for buzz frequency—identifying flights aurally and visually in Audacity, and then using R to calculate fundamental frequency (Online Resource 1, Table II). For one recording of a pollination buzz by *B. impatiens* and four recordings of flight by *A. murrayensis*, we were unable to obtain an accurate frequency using seewave, so we analyzed these recordings manually, by performing a fast Fourier transform (FFT) with the spectrum function in Audacity, using a Hanning window of 2048 points. We then listened to the recording and matched the sound with one of the peaks from the FFT spectrum.

Video recordings

We collected videos of *A. murrayensis* performing buzz-pollination on *S. lycopersicum* (cherry tomato) flowers in the Adelaide Botanic Garden, using a high-speed camera (TS3, Fastec Imaging, San Diego, California) recording at 2000 fps. We recorded a total of nine videos, four of which are known to be of unique individuals, because we were able to capture these bees after filming.

Statistical tests

All statistical tests were performed in R (R Core Team 2012). We used multiple linear regression to compare flight frequency, average buzz-pollination frequency, average buzz length, and visit duration between *A. murrayensis* and *B. impatiens*; this method allowed us to compare the two species of bees while accounting for environmental variables: temperature, time of day, and relative humidity. To fit the assumptions of linear regression, we squared buzz-pollination frequency, square-root-transformed flight frequency, and log-transformed buzz length and visit duration. We also used paired *t* tests to compare flight versus pollination buzz frequency for individuals within each species.

We used multiple linear regression to compare buzz-pollination characteristics for *A. murrayensis* on different tomato varieties and on virgin versus nonvirgin flowers (Online Resource 1, Tables VII–IX).

We adjusted significance level using Bonferroni correction, to account for performing multiple comparisons with the same individuals. Because we performed four multiple regressions and one *t* test, we adjusted our significance level to 0.05 divided by 5, or 0.01. We did not adjust the significance level to include the four covariates in each of the multiple regressions, because the only variable of interest was the bee species. This correction is overly conservative, but using a less-conservative adjustment would not have changed our conclusions. Graphs were made with ggplot2 (Wickham 2009).

Results

None of the buzz-pollination or flight characteristics quantified were associated with environmental variables (temperature, relative humidity, time of day). We found that buzz frequency was significantly higher for *Amegilla* than for *Bombus* ($t_{(70)} = 8.452$, p value $\ll 0.001$; Fig. 3a; Online Resource 1, Table III), and wing beat frequency was also higher for *Amegilla* than for *Bombus* ($t_{(71)} = 13.372$, p value $\ll 0.001$; Fig. 3a; Online Resource 1, Table IV). Within each bee species, the wing beat frequency during flight was significantly lower than the buzz-pollination frequency (*Amegilla* $t_{(21)} = 24.67$, p value $\ll 0.001$; *Bombus* $t_{(52)} = 16.59$, p value $\ll 0.001$).

There was no significant difference between the two bee species in the length of an individual pollination buzz (Fig. 4b; $t_{(70)} = 1.124$, p value >0.2; Online Resource 1, Table V), but *B. impatiens* spent more time on a single flower than *A. murrayensis* (Fig. 3c; $t_{(53)} = 3.974$, p value <0.005; Online Resource 1, Table VI). We found no significant differences in buzz characteristics of *A. murrayensis* when pollinating the two different varieties of *S. lycopersicum* or when pollinating unvisited versus previously visited flowers (Online Resource 1, Tables VII–IX).

The high-speed videos revealed that *A. murrayensis* differs markedly from *B. impatiens* (and many other buzz-pollinating bees described thus far) in how it physically interacts with the flower during buzz-pollination. While *B. impatiens* and other bees grasp the flower's anthers with their mandibles as well as their legs, *A. murrayensis* does not. All videos we collected showed *A. murrayensis* grabbing the anther with only its legs and repeatedly tapping the anther with its head at the high buzzing frequency that is likely generated by its flight muscles (Fig. 4, Online

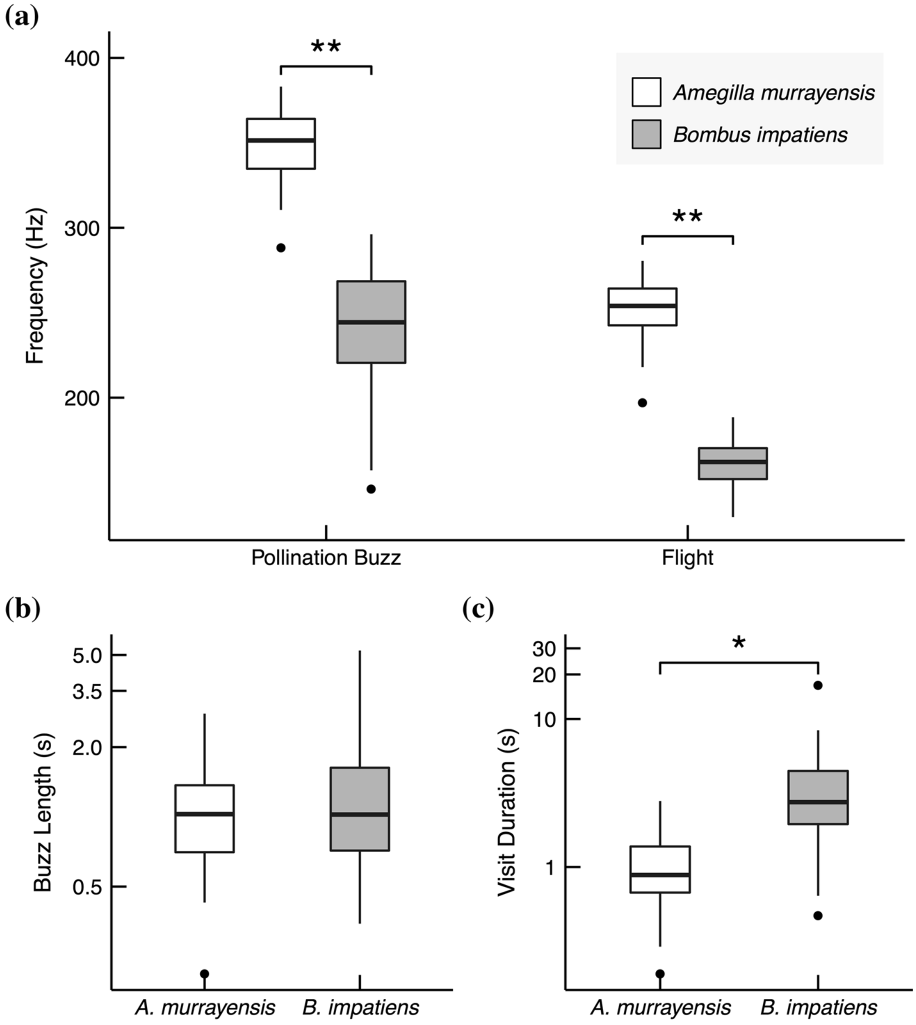

Fig. 3 Buzz-pollination and flight characteristics of *A. murrayensis* and *B. impatiens*. **a** Pollination buzz frequency of *A. murrayensis* (*white*, $n = 22$) versus *B. impatiens* (*gray*, $n = 53$), and flight (wing beat) frequency of *A. murrayensis* ($n = 23$) versus *B. impatiens* ($n = 53$). **b** Average buzz length of *A. murrayensis* ($n = 22$) versus *B. impatiens* ($n = 53$). **c** Flower visit duration of *A. murrayensis* ($n = 22$) versus *B. impatiens* ($n = 36$). *Double asterisks* and *single asterisks* indicate a significant difference at $p < 0.0001$ and 0.0005, respectively

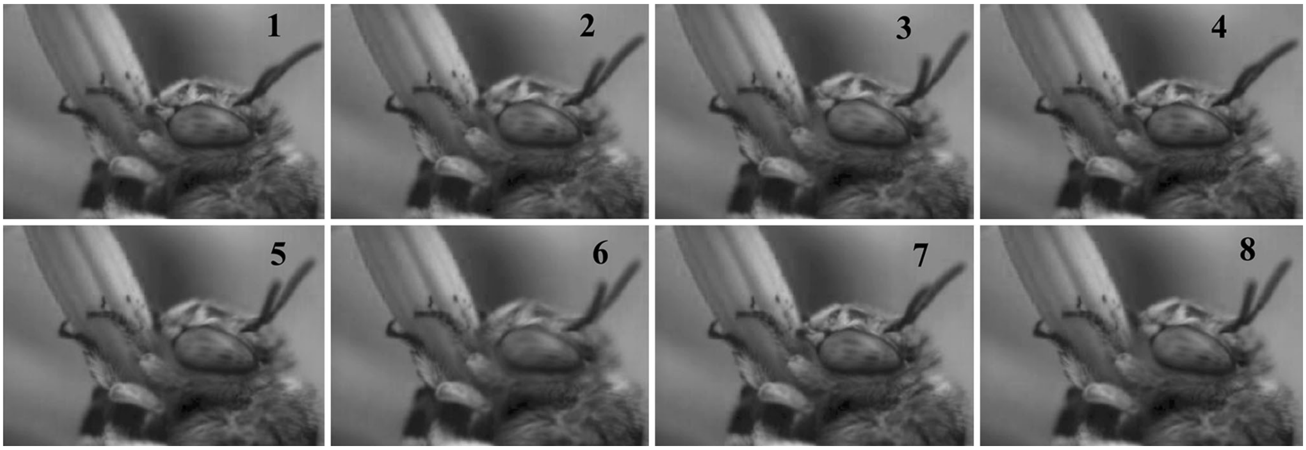

Fig. 4 Image sequence of *Amegilla murrayensis* during buzz-pollination. Rather than grasping the anther firmly with its mandibles like other buzz-pollinating bees, *A. murrayensis* taps its head against the anther of a *Solanum lycopersicum* (cherry tomato) flower at the high buzzing frequency generated by its flight muscles (approximately 350 Hz; Online Resource 1, Table II). The interval between images (from Online Resource 2) is 1/1000 of a second. The *dark marks* on the anthers were made with ink, to help visualize the movement of the anther

Resource 2). Research indicates that the flight muscles are used during sonication in *Bombus occidentalis* (King et al. 1996), and our high-speed videos show that the mesosoma of *A. murrayensis* is deforming with each tap of the head (Online Resource 2)—similar to the way that bumblebees' mesosomas deform, while they buzz (Online Resource 3). In particular, the videos for both bumblebees and blue-banded bees show the first segment of the mesosoma, called the pronotum, moving at the same frequency as the head. The mesopleuron can also be seen oscillating during buzz-pollination. In two recordings, we saw a bee briefly grasp the anther with its mandibles, but it quickly switched to the head-tapping behavior.

We noticed that *A. murrayensis* left brown marks on the anther cone—these "bee kisses" are interpreted by commercial tomato growers as a sign that bees have visited the flowers (Buchmann and Nabhan 1996). *A. murrayensis* may be damaging the anthers with impact forces, but the resulting "bee kisses" are similar to those left by *B. impatiens*.

Discussion

We found that *Amegilla murrayensis* (blue-banded bees) buzz cherry tomato flowers at significantly higher frequencies (~350 Hz) than *B. impatiens* (~240 Hz; Online Resource 1, Table II), while accounting for environmental variables. The flight (wing beat) frequencies of both species are lower than their buzz-pollination frequencies. This is likely due to the properties of asynchronous muscles, which are part of a resonant system (Josephson 2006). When the mass of the wings is reduced in this system, the wing beat frequency increases (Roberts and Cartar 2015). Likewise, when the mass on the wings is increased or simply moved further from axis of rotation, the frequency of the resonant system should decrease. The lower frequency during flapping flight is likely because the wings are extended during flapping, but held close to the body during buzz-pollination. *A. murrayensis*' flight frequency is significantly higher than that of *B. impatiens*, which is not surprising due to its smaller body size (Burkart et al. 2011). Previous studies have suggested that the amount of pollen released from poricidal anthers increases with buzz frequency (Harder and Barclay 1994) or with buzz frequency and displacement (De Luca et al. 2013; King and Buchmann 1996); thus, the higher buzzing frequency of *A. murrayensis* may be more effective at releasing pollen from the anthers.

Despite the large difference in buzzing frequency between species, the average length of a single buzz was the same in *A. murrayensis* and *B. impatiens*, with both bees buzzing in bouts lasting approximately 1 s—similar to the duration required to eject most of the pollen from *Solanum laciniatum* flowers (King and Buchmann 1996). However, *B. impatiens* spent significantly more time on a single flower (approximately 3.7 s), as compared to *A. murrayensis*, which departed after ~1 s. This difference is due to the fact that *B. impatiens* typically buzzed an individual flower several times (often gathering and cleaning pollen from its body in-between buzzes) before departing, whereas *A. murrayensis* typically buzzed a flower once and then flew away to clean pollen from its body. Occasionally, *A. murrayensis* returned to the same flower after cleaning, but most often it moved onto a new flower.

The fact that *A. murrayensis* spent significantly less time on each flower, typically buzzing the flower only once, combined with its higher (and possibly more effective) buzzing frequency, suggests that *A. murrayensis* may be able to extract pollen from flowers more quickly than *B. impatiens*. An alternative explanation for the difference between bee species in the amount of time spent on each flower is the possibility that tomatoes in Australia could provide different amounts of pollen during buzzing than tomatoes in the USA (due to potential differences in tomato varieties, local environment, visitation rates by local bees, etc.). However, because we recorded *A. murrayensis* performing only a single buzz on both virgin and previously buzzed flowers—which are known to release less pollen (King and Buchmann 1996)—we do not believe that *A. murrayensis* is adjusting the number of buzzes it performs based on the pollen reward. Whether or not *A. murrayensis* is in fact obtaining more pollen than *B. impatiens* with a single buzz, the behavior of often moving onto the next flower after performing only one buzz appears to be typical for this species, at least when foraging from cherry tomato plants.

We also found that *A. murrayensis* interacts with the flower in a unique way during buzz-pollination. Rather than grasping the anthers firmly with its mandibles and shaking (as described for *Bombus* and many other bee genera previously studied (Buchmann 1983; Buchmann and Hurley 1978; Crobet and Huang 2014; Jesson and Barrett 2005; King 1993; Online Resource 3), *A. murrayensis* taps the anthers with its head repeatedly, at the high frequencies most likely generated by its flight muscles. This "head-banging" behavior may be intentional, or it may be a side effect of the bees being unable to grasp the anthers firmly enough with their mandibles while sonicating, possibly due to their small size or insufficient grip strength—although other, smaller bee species, such as *Lipotriches* (Halictidae), have been recorded grasping anthers firmly with their mandibles during buzz-pollination (Online Resource 4), and we observed *A. murrayensis* grasping onto leaves with its mandibles while grooming its body.

The mechanical features of a buzz that have been proposed to determine how much pollen is dislodged include the length of a buzz and the maximum acceleration (often called amplitude) (De Luca et al. 2013) or velocity of the buzz (Corbet and Huang 2014). Acceleration and velocity both increase with buzz frequency and with displacement (Corbet and Huang 2014; De Luca et al. 2013). Impact forces, which occur when two objects collide, cause sharp changes in velocity, which in turn produce large spikes in acceleration. Thus, the head tapping observed in *A. murrayensis*, in which the head collides and then disengages with the anther repeatedly at high frequencies, may produce higher accelerations than grasping the anthers firmly and shaking. High accelerations produced by collisions with the anther could lead to higher pollen release rates or could help break up clumps of wet pollen. These hypotheses could be tested in future studies by manipulating flowers with mechanical shakers that either grasp firmly or collide repeatedly with the anthers, and quantifying the amount of pollen released.

If *A. murrayensis* is capable of removing more pollen with a single buzz (due to its higher buzzing frequency and/or head-tapping behavior), this could be detrimental from the plant's perspective, since a single forager is removing a large portion of its pollen. On the other hand, some researchers have suggested that high pollen removal from poricidal anthers is associated with higher pollen deposition onto stigmas (Harder 1990; Harder and Thomson 1989), although this is not always the case for plants with nonporicidal anthers (Wilson and Thomson 1991). In addition, if *A. murrayensis* routinely spends less time on a single flower, then it may move onto another flower more rapidly than *B. impatiens*, which could lead to higher pollination rates.

The relative effectiveness of *A. murrayensis* and *B. impatiens* as pollinators may also be affected by other aspects of their behavior—for instance, how well each species grooms the pollen from its body (decreasing the probability of transferring pollen between flowers), and whether the behavior of one species brings its body closer to the stigma (increasing the chances of depositing pollen). A definitive answer to the question of pollination effectiveness will ultimately require controlled experiments comparing the yield of tomato plants buzz-pollinated by *A. murrayensis* versus *B. impatiens*.

Although further work is required to make any claims about the pollination effectiveness of these different bees, our work shows that at least one native Australian bee—*Amegilla murrayensis*—differs significantly from *Bombus* spp. in several aspects of buzz-pollination, including its buzzing frequency and the amount of time spent per flower. Furthermore, our observation that *A. murrayensis* interacts with flowers in a unique way during buzz-pollination—by "head-butting" rather than "shaking" the anthers—leads to further questions about the behavioral, mechanistic, and evolutionary roots of this method of buzz-pollination.

Acknowledgments The authors thank Christopher De Ieso and Remko Leijs for help in collecting data in the Adelaide Botanic Garden, as well as the staff of the Adelaide Botanic Garden, notably Carolyn Sawtell and Robert Hatcher, for allowing us to conduct research with their plants. We would also like to thank Robin Hopkins for helpful suggestions on the manuscript. This project was funded by a Putnam Expedition Grant to C.S. from the Harvard Museum of Comparative Zoology and by the National Science Foundation (CAREER IOS-1253677) to S.C.

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