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RESEARCH ARTICLE

Resource profitability, but not caffeine, affects individual and collective foraging in the stingless bee *Plebeia droryana*

Tianfei Peng[1,‡,*], Francisca H. I. D. Segers[2,‡], Fabio Nascimento[3] and Christoph Grüter[1]

ABSTRACT

Plants and pollinators form beneficial relationships, with plants offering resources in return for pollination services. Some plants, however, add compounds to nectar to manipulate pollinators. Caffeine is a secondary plant metabolite found in some nectars that affects foraging in pollinators. In honeybees, caffeine increases foraging and recruitment to mediocre food sources, which might benefit the plant, but potentially harms the colonies. For the largest group of social bees, the stingless bees, the effect of caffeine on foraging behaviour has not been tested yet, despite their importance for tropical ecosystems. More generally, recruitment and foraging dynamics are not well understood in most species. We examined whether caffeine affects the foraging behaviour of the stingless bee *Plebeia droryana*, which frequently visits plants that produce caffeinated nectar and pollen. We trained bees to food sources containing field-realistic concentrations of sugar and caffeine. Caffeine did not cause *P. droryana* to increase foraging frequency and persistence. We observed *P. droryana* recruiting to food sources; however, this behaviour was also not affected by caffeine. Instead we found that higher sugar concentrations caused bees to increase foraging effort. Thus, unlike in other pollinators, foraging behaviour in this stingless bee is not affected by caffeine. As the Brazilian *P. droryana* population that we tested has been exposed to coffee over evolutionary time periods, our results raise the possibility that it may have evolved a tolerance towards this central nervous system stimulant. Alternatively, stingless bees may show physiological responses to caffeine that differ from those of other bee groups.

KEY WORDS: Pollination, Recruitment, Behaviour

INTRODUCTION

Plants attract pollinators by providing resources, mainly nectar and pollen. In turn, they receive visits that facilitate plant reproduction through the transfer of pollen by the pollinators (Burkle et al., 2013; Mitchell et al., 2009). As pollinators can use pollen and nectar either for themselves or to feed their offspring, this relationship between plants and pollinators usually benefits both parties. However, sometimes pollinators or plants cheat. For example, some plants attract pollinators by imitating floral signals or mating signals while not offering rewards (Schiestl, 2005; Bohman et al., 2016; Oelschlägel et al., 2015). Also, nectar-robbing bees make holes in flowers to extract nectar while providing little or no pollination service (Irwin et al., 2001; Inouye, 1980; Leadbeater and Chittka, 2008).

In nature, secondary metabolites are produced by plants as pharmacologically active toxins whose main function is to reduce leaf damage by herbivores (Bennett and Wallsgrove, 1994). Recent research has shown that secondary metabolites like caffeine (e.g. from the genera *Coffea*, *Citrus* and *Tilia*) or nicotine are added by some plants to the nectar they secrete (Kretschmar and Baumann, 1999; Wright et al., 2013; Thorburn et al., 2015; Heil, 2011). The effects of secondary metabolites on pollinators are complex and context dependent. For example, in bumble bees (*Bombus impatiens*), nicotine decreases parasite load under varying temperature conditions, while at constant temperature, it has the opposite effect (Thorburn et al., 2015). Several studies on the European honeybee (*Apis mellifera*) show that the presence of caffeine in nectar alters honeybee foraging behaviour: it increases the amount of nectar the bees drink, improves learning performance and increases recruitment and persistence to the nectar sources (Couvillon et al., 2015; Singaravelan et al., 2005; Wright et al., 2013). Similar effects of caffeine on foraging have been found in bumblebees (Thomson et al., 2015). These studies suggest that field-realistic concentrations of caffeine enhance the reward perception of temperate honeybee and bumblebee foragers. In other words, the addition of caffeine to nectar seems to have a similar effect to an increase in sugar content on bee foraging behaviour.

It has been hypothesized that plants releasing caffeine into nectar might trick pollinators into increasing foraging rates and, therefore, pollination success without offering higher quality food (Couvillon et al., 2015). The presence of caffeine could even lead to detrimental effects on honeybee colonies as it might cause colonies to focus their foraging effort on caffeinated nectar sources containing relatively low quantities of sugar (Couvillon et al., 2015; Koch and Stevenson, 2017). In the worst case, colonies could die because they are tricked into collecting low-quality resources (Koch and Stevenson, 2017). Such effects might have ecological implications through changes in plant–pollinator interaction networks and, potentially, biodiversity.

With more than 500 described species, stingless bees (Meliponini) represent the largest group of highly eusocial bees and they play key roles as pollinators in tropical and subtropical habitats (Heard, 1999; Giannini et al., 2015). Despite their number and their importance, relatively little is known about the foraging behaviour of most species (Rasmussen and Cameron, 2010; Stangler et al., 2009; Aleixo et al., 2017; Hrncir et al., 2016). Stingless bees are known to naturally forage on flowers of species belonging to *Coffea* and *Citrus* (Heard, 1999; Ricketts, 2004; Ricketts et al., 2004). Coffee, for example, has been in Brazil for nearly 300 years and Brazil has been the largest producer of coffee in the world for the last 150 years, currently producing about a third

[1]Institute of Organismic and Molecular Evolutionary Biology, Johannes-Gutenberg University of Mainz, 55128 Mainz, Germany. [2]Department for Applied Bioinformatics, Institute of Cell Biology and Neuroscience, Goethe University, 60438 Frankfurt, Germany. [3]Departamento de Biologia da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, Ribeirão Preto, São Paulo 3900, Brazil.

‡These authors contributed equally to this work

*Author for correspondence (ptianfei@uni-mainz.de)

T.P., 0000-0003-2239-9967; F.H.I.D.S., 0000-0002-8149-3809; F.N., 0000-0002-3731-7111; C.G., 0000-0001-5553-5465

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of all the coffee consumed (Fausto, 2014; Neilson and Pritchard, 2009). However, it remains unknown how stingless bees respond to caffeine and whether the collection of caffeinated nectar could have detrimental effects on tropical pollinators.

The foraging response of stingless bees and honeybees to compounds present in nectar may differ considerably. For example, the nectar of the avocado tree (*Persea americana*) contains minerals that have been shown to repel honeybees, which are not the natural pollinators of this plant, while pollinators from the native range of the plants, among which are two stingless bee species, were much less affected by these secondary nectar compounds (Afik et al., 2014). This points to taxon-specific differences in the physiological and neural response to plant compounds. Furthermore, the long-term co-existence of plants and their pollinators may allow pollinators to adapt to their preferred plants, e.g. by evolving resistance to the effects of nectar compounds. To gain a better understanding of the potential effects of caffeinated nectar on plant–pollinator interactions in the tropics, we studied the Brazilian stingless bee *Plebeia droryana*, which was found to be the most common native bee visitor of *Coffea* and *Citrus* in the state of São Paulo, Brazil (Nogueira-Neto et al., 1959; Imperatriz-Fonseca et al., 1989).

Unlike honeybees, which use the waggle dance to recruit nestmates to profitable food sources (von Frisch, 1967), *P. droryana* is not known to recruit to particular foraging locations, but successful foragers are known to produce thoracic vibrations (buzzing sound) inside the nest, which could have the function of increasing the foraging activity of the colony (Lindauer and Kerr, 1960). After discovering a food source, some stingless bee species adjust their foraging frequency, their recruitment probability or their willingness to fight for a food source according to the quality of the resource (Johnson and Hubbell, 1974; Biesmeijer et al., 1998; Jarau, 2009; Schorkopf et al., 2016). Likewise, the decision to return the next day (persistence) depends on a combination of the bees' ability to memorize food locations and information about the food source quality (Biesmeijer and Slaa, 2004; Al Toufailia et al., 2013).

Here, we examined the effects of field-realistic concentrations of sugar and caffeine on the visitation rate and foraging persistence of *P. droryana*. Additionally, as the foraging method of *P. droryana* is not well studied, we explored whether foragers might recruit nestmates to food locations offering high-quality food.

MATERIALS AND METHODS

Study species and field site

We performed our experiments on the campus of the University of São Paulo in Ribeirão Preto, Brazil. This area has a high diversity of wild stingless bee species (Cortopassi-Laurino et al., 2009) and flowering plant species belonging to the *Coffea* and *Citrus* genera (the campus is a former coffee farm). *Plebeia droryana* (Holmberg 1903) is a common species at our field site. When experimenting with foragers of one colony, we could not exclude the possibility that individuals from other colonies, e.g. up in trees or hidden from view, would also arrive at our experimental set-up. However, by marking bees individually, we could determine that the majority of foragers usually came from one focal colony. In total, we studied 10 focal colonies at 10 locations, ensuring that locations were at least 800 m apart from each other. Five of these locations were used for part 1 and the other five for part 2. Data were collected over a period of 4 weeks in March 2018 on days with good foraging conditions.

Experimental setting

We used standard training procedures to train the foragers from the focal colony to artificial feeders: two artificial feeders containing a 50% sucrose solution were placed next to the nest entrance and on differentially coloured backgrounds (yellow versus blue) to stimulate foragers to start visiting the feeders. After a group of foragers was established on each feeder, the feeders were moved to the final location while the foragers were drinking from the solution. Bees would then learn the new location of each respective feeder when returning to their nest (von Frisch, 1967; Nieh, 2004). We used this method to set up two feeders and, in order to help trained bees to learn the location and prevent switching between feeders, we used differently coloured backgrounds on a chair (height 0.5 m) (Couvillon et al., 2015). The final location of the two feeders was 10 m away from the main colony, separated by 7 m from each other. We began training in the morning and after ∼10 foragers were trained to each of the feeders, we marked all foragers individually with acrylic paint using a combination of coloured dots. In all experiments, we used unscented sucrose solutions.

Part 1: do caffeine and sugar content affect foraging in stingless bees?

In an early study, the sugar content of the nectar of coffee flowers in Sao Paulo state was found to be about 38% (range 32.8–45%; Nogueira-Neto et al., 1959) and the caffeine concentration in the nectar of three Brazilian species of *Coffea* (*C. canephora*, *C. liberica* and *C. arabica*) increased from 0.003 to 0.253 mmol l^{-1} as the sugar concentration decreased (Wright et al., 2013; Santos and Lima, 2009; Govaerts, 2009). To test whether caffeine affects foraging, we used two sucrose solutions (30% and 40%) and two caffeine concentrations (25 ppm or ∼0.14 mmol l^{-1} and 50 ppm or ∼0.28 mmol l^{-1}). For experiment 1, colonies were tested with one feeder offering a 40% sucrose solution (control feeder) and a second feeder offering a 40% sucrose solution containing a medium dose (25 ppm) of caffeine (treatment feeder). In experiment 2, colonies were tested with one feeder offering a 30% sucrose solution (control feeder) and a second feeder offering a 30% sucrose solution containing a high dose (50 ppm) of caffeine (treatment feeder). The medium and high concentrations are found naturally in the nectar of coffee plants and we mimicked the negative correlation between sucrose and caffeine concentration (Wright et al., 2013; Couvillon et al., 2015).

During the training phase, we offered 50% sucrose solution without caffeine to attract the bees. After training (but on the same day), the feeders were cleaned with water and filled with the solutions described in the previous paragraph for the treatment phase. Over a period of 120 min, we recorded the number of bees present at each feeder at 5 min intervals, including the individually marked bees and unmarked bees. We continuously recorded how often the individually marked bees were at the feeders (foraging frequency) during the experimental period by dividing the total visit period (time between the first visit and the end of the treatment) of individually marked bees by the treatment time (120 min).

To avoid disturbing the bees while they were getting familiar with the new solutions, the first count was done 10 min after the beginning of the treatment. Observers switched position every 20 min to exclude any bias caused by the attraction of the bees to one particular observer. Treatment–background (blue or yellow) combinations were randomized for each trial. In total, we marked 170 bees individually in our experiment. Of these, 27 switched between the treatment and control feeder during the treatment period; 48% of these bees had visited one feeder for more than 90% of all visits. For data analysis, we included the bees that never switched and those that had switched but visited one feeder for >90% of all visits. We excluded the remaining bees that switched (8% of all marked bees) from the statistical analysis.

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In order to find out whether caffeine affects foraging persistence in *P. droryana*, i.e. the probability of returning to the food source the day after treatment (Couvillon et al., 2015; Al Toufailia et al., 2013), we set up the same coloured backgrounds with empty, unscented feeders at the same location in the morning of the day after treatment and observed the feeders for 150 min. During these 150 min, we recorded the time when bees landed on each feeder. Then, both individually marked bees and unmarked bees were counted. We used these data to calculate the visitation rate to the now empty feeder every 5 min. Furthermore, we also calculated the probability of the individually marked bees returning to the empty feeder. We counted only those bees that landed on the feeder. The observations of persistence were made by just one observer, who checked both feeders regularly for the presence of bees.

Part 2: does *P. droryana* show recruitment to high-quality resources?

The results of part 1 suggested that sucrose concentration affects foraging motivation. To explore this further, we tested whether *P. droryana* recruits nestmates to feeders offering high-quality food (experiment 3). We provided different concentrations of sucrose solution (30% and 40%) at the two feeders, while placing both on a yellow background. Concentration–location combinations were randomized in all trials. We counted how many bees were present at each feeder every 5 min. The counting method was the same as that described above (‘Experimental setting’).

Statistical analysis

For data analysis, we used generalized linear mixed-effect models (GLMM) and linear mixed-effects models (LME) in R version 3.4.4 (http://www.R-project.org/), as implemented in the lme4 package and nlme package (Bates et al., 2015; Zuur et al., 2009). The focal colony was used as a random effect to account for the non-independence of observations from the same colony (Zuur et al., 2009). Depending on the error distribution of the response variable, we used normal (log and square-root transformed), binomial or Poisson distribution. We used colour, caffeine treatment and duration of measurement (10–120 min or 10–150 min, depending on the experiment) as fixed effects. In part 1, we were also interested in the two-way interaction between caffeine treatment and duration of measurement because the change of foraging behaviour over time might depend on the presence of caffeine. To test for the significance of interactions, we used likelihood ratio tests (LRT). The interaction between these two fixed effects was removed from the final model if it was not significant ($P>0.05$). The final model always included all three fixed effects. To test the significance of the main effects, we used Wald tests (Zuur et al., 2009).

RESULTS

Part 1: does caffeine affect foraging in stingless bees?

When the bees were offered 40% sucrose solution at one feeder with caffeine and one feeder without caffeine (Fig. 1A), the number of bees at both feeders increased with time. However, there was no significant difference in the growth trend (i.e. the interaction between time and caffeine presence) between the two feeders (GLMM, time×treatment: LRT=0.33, $P=0.56$). We found no effect of the medium dose of caffeine or background colour on the number of bees at the feeders (treatment: $z=0.37$, $P=0.71$; time: $z=11.10$, $P<0.001$; colour: $z=1.45$, $P=0.15$). The number of foragers at the feeders did not increase over time when they were offered 30% sucrose solution (GLMM, time: $z=-0.83$, $P=0.41$; time×treatment: LRT=0.03, $P=0.87$; Fig. 1B). Also, the presence of a high dose of caffeine had no effect on the number of bees at the feeder (treatment: $z=-0.21$, $P=0.83$). However, more bees visited feeders on the

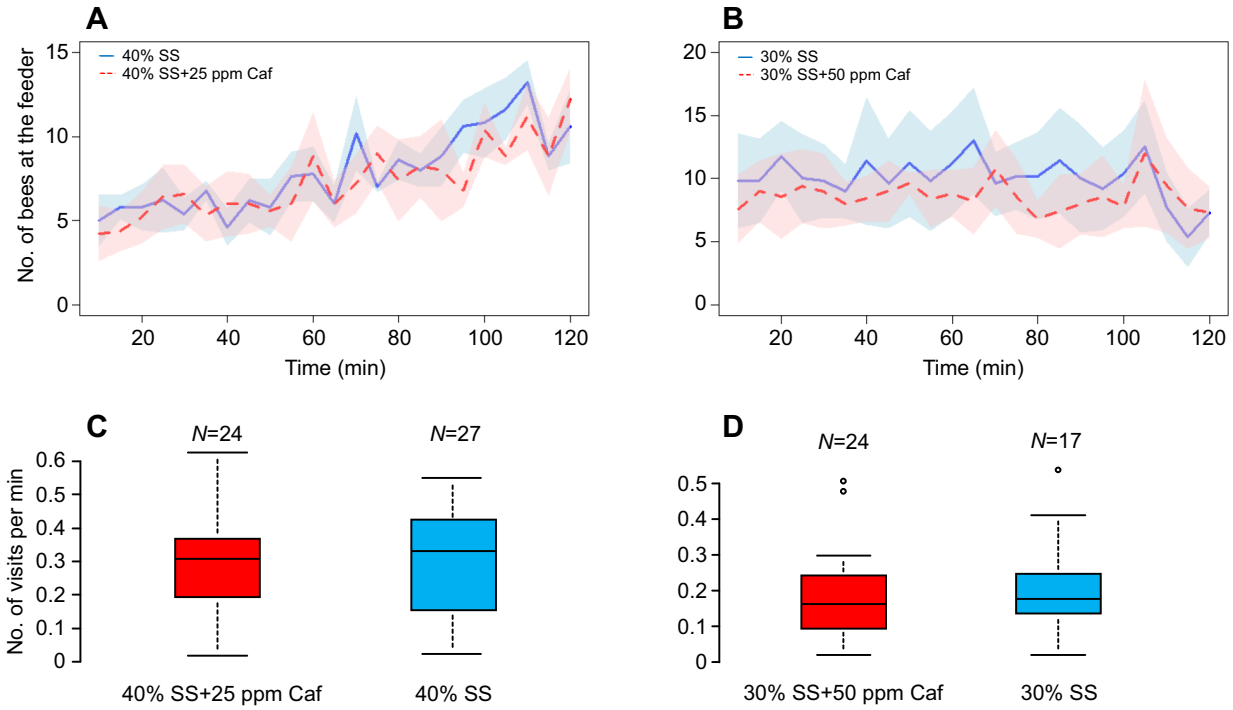

Fig. 1. Effect of caffeine on foraging. (A,B) The mean (±s.e.m., shaded area) number of bees visiting the 40% (A) and 30% (B) sucrose solution (SS) feeders, with and without a medium (25 ppm) or high (50 ppm) dose of caffeine (Caf), over a period of 120 min. (C,D) The number of visits per minute by individually marked bees to the 40% (C) and 30% (D) SS feeders with and without caffeine. The horizontal bars of the boxplots indicate the medians and the boxes delimit the first and third quartile; circles are outliers. *N* represents the number of individually marked bees.

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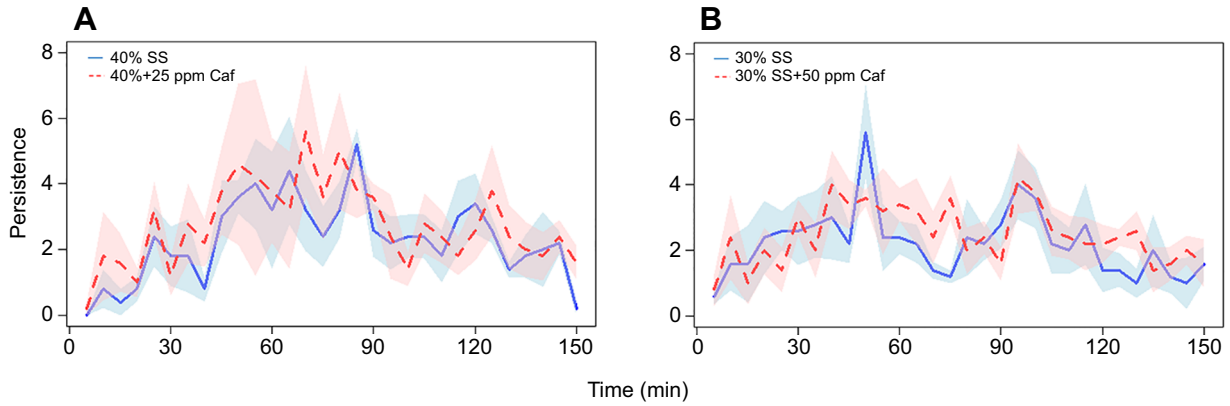

Fig. 2. Foraging persistence following treatment with caffeine. Persistence was measured as the number of bees that returned to the empty feeder the day after treatment. (A) The day after treatment with 25 ppm caffeine (Caf) dissolved in 40% sucrose solution (SS). (B) The day after treatment with 50 ppm caffeine in 30% SS. In A and B, the lines show the mean number of bees returning to the empty feeders over a period of 150 min.

yellow background versus the blue background (colour: z=10.30, P<0.001).

The foraging frequency of the individually marked bees to feeders containing 40% sucrose solution was not affected by caffeine (LME, treatment: t=−0.39, d.f.=44, P=0.70; Fig. 1C) and bees did not show a preference for a feeder based on the background colour (colour: t=−0.45, d.f.=44, P=0.66). Similarly, the number of foraging visits of individually marked bees to 30% sucrose solution did not go up or down depending on the high caffeine dose or the colour of the background (LME, treatment: t=−1.19, d.f.=33, P=0.24; colour: t=−1.54, d.f.=33, P=0.13; Fig. 1D).

The bees that were treated with caffeine did not show higher persistence compared with control bees (which only received sucrose solution without caffeine), irrespective of the dose. More specifically, in the 40% sucrose solution–medium dose caffeine treatment, the number of bees (both marked and unmarked) at a feeder was not affected by background colour during training, observation time, or whether the feeder offered caffeinated or non-caffeinated solution during training (GLMM, treatment: z=1.68, P=0.094; time: z=1.49, P=0.14; colour: z=1.33, P=0.18; time×treatment: LRT=0.87, d.f.=1, P=0.35; Fig. 2A). Likewise, in the 30% sucrose solution–high dose caffeine treatment, the presence of caffeine in the solution and observation time did not affect the number of bees (both marked and unmarked) at a feeder the next day (GLMM, treatment: z=−0.59, P=0.56; time: z=−0.28, P=0.78; time×treatment: LRT=0.95, d.f.=1, P=0.33; Fig. 2B). However, more bees (both marked and unmarked) landed on the yellow feeder (which was also the yellow feeder on the previous day) than on the blue feeder (colour: z=4.45, P<0.001).

Additionally, we examined the persistence of individually marked bees. Neither the medium dose of caffeine (binomial GLMM, treatment: z=−0.84, P=0.4; colour: z=0.13, P=0.90) nor the high dose of caffeine affected persistence (binomial GLMM, treatment: z=−1.18, P=0.24; colour: z=0.79, P=0.43). Because there was no effect of caffeine on the persistence of marked bees, we pooled the data of all bees to test whether sucrose concentration had an effect on the persistence of marked bees. However, no significant difference was found between 30% and 40% sucrose solution treatment on the persistence of the marked bees (binomial GLMM, treatment: z=0.92, P=0.36; colour: z=0.14, P=0.89).

Part 2: does *P. droryana* show recruitment to high-quality resources?

To examine whether the quality of the food source affects the number of bees at the feeders, we again offered *P. droryana* colonies two feeders, but one contained 40% sucrose solution and the other 30% sucrose solution. The background colour was kept constant (yellow). The number of stingless bees foraging at the two feeders was significantly different between the 30% sucrose solution and the 40% sucrose solution (Poisson GLMM, treatment: z=−5.86, P<0.001; Fig. 3A). Additionally, there was a significant interaction between sucrose concentration and time (time×treatment: LRT=35.40, d.f.=1, P<0.001). More specifically, the number of foragers at the feeder with 40% sucrose solution increased over time (Poisson GLMM, time: z=2.27, P=0.02), whereas the number of foragers at the 30% feeder significantly decreased over time (Poisson GLMM, time: z=−5.42, P<0.001). Focusing only on individually marked foragers, we found a significantly higher foraging frequency at the 40% sucrose solution feeder than at the 30% sucrose solution feeder; that is, 35.6% more visits per minute (LME, treatment: t=3.67, d.f.=92, P<0.001; Fig. 3B).

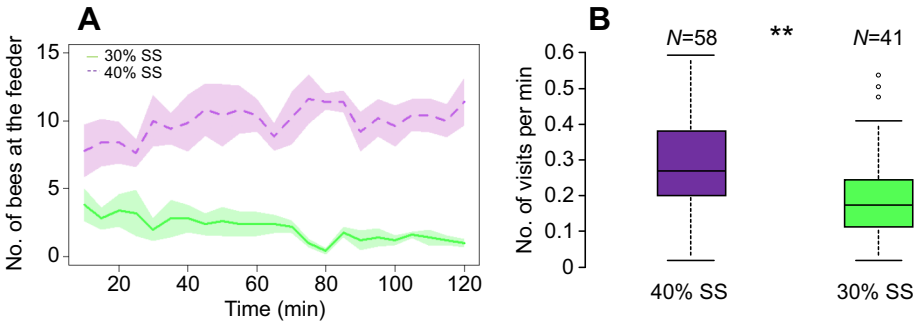

Fig. 3. Effect of resource quality on foraging. (A) The mean (±s.e.m., shaded area) number of bees visiting the 30% and 40% sucrose solution (SS) feeders over a period of 120 min. (B) The number of visits per minute made by individually marked bees to the 30% and 40% SS feeder. Circles are outliers. **P<0.01.

DISCUSSION

Our results suggest that caffeine does not affect individual and collective foraging effort in the stingless bee *P. droryana* in the range of tested concentrations in our study area. Compared with the control, sucrose solution containing caffeine did not alter the number of foragers at the food sources, the foraging frequency of individually marked bees or their persistence. We used two doses of caffeine, 25 ppm (~0.14 mmol l^{-1}) and 50 ppm (~0.28 mmol l^{-1}), which is comparable to what has been measured in the nectar of plants of the genus *Coffea* (0.003 to 0.253 mmol l^{-1}) (Wright et al., 2013). It could be argued that *P. droryana* foragers do not pay attention to the perceived energy content of solutions but prioritize other factors, such as foraging distance or the flow rate of nectar. This could explain why caffeine did not affect foraging. However, this is unlikely to explain the lack of an effect of caffeine, because *P. droryana* foragers did increase their visitation rate when we offered solutions with higher sucrose concentrations. When the concentration of sucrose solution was 40%, the number of bees at the feeders increased over time, whereas the number of foragers remained constant or decreased at 30% feeders. Additionally, the number of visits per minute was significantly higher at feeders offering 40% sucrose solution than at feeders offering 30% sucrose solution. This suggests that *P. droryana* adjusts its foraging behaviour to the perceived value of the food sources, but that caffeine does not modulate the perceived value.

Our finding that caffeine does not affect foraging effort in *P. droryana* contrasts with findings from European honeybees: caffeine intake caused changes in honeybee learning performance, foraging effort, recruitment behaviour and persistence (Wright et al., 2013; Couvillon et al., 2015). It has been suggested that plants might add caffeine to nectar to stimulate bee visitations resulting in pollination, while offering a smaller energetic reward than perceived by the bees, thus cheating in the plant–pollinator mutualism. In the tropics, stingless bees have long been recognized for their important role in pollination of *Coffea* (Heard, 1999; Ricketts, 2004; Nogueira-Neto et al., 1959). One explanation for the absence of an effect of caffeine on the foraging effort of *P. droryana* could be that this population has evolved a tolerance towards the effects of caffeine. *Plebeia droryana* has a long history of exposure to caffeinated nectar and pollen in the state of São Paulo and, thus, had many generations to adapt to caffeine in the study area.

An alternative explanation is that the effects of caffeine and other secondary plant compounds could vary among bee groups as a result physiological and neural differences among different bee taxa (e.g. Afik et al., 2014). In *A. mellifera*, caffeine functions as an adenosine receptor antagonist and affects the mushroom body neurons involved in olfactory learning and memory. The interaction of caffeine and adenosine receptors could lead to increased activation of Kenyon cells in projection neurons (Chittka and Peng, 2013; Wright et al., 2013). Ultimately, caffeine affects long-term memory by blocking adenosine receptors. However, in *P. droryana*, caffeine might be broken down before it reaches the brain. Previous work has shown that caffeine is degraded in the gut of the coffee berry borer (*Hypothenemus hampei*) and that this activity was eliminated by experimental inactivation of the gut microbiota (Ceja-Navarro et al., 2015). Studies on *Drosophila* (Bhaskara et al., 2006; Willoughby et al., 2006) and honeybees (Kucharski and Maleszka, 2005) show that caffeine regulates the genes of the cytochrome P450 family (CYP proteins), which are involved in the detoxification metabolism, by increasing their expression. However, information about the absorption, tissue distribution and metabolites of caffeine in invertebrates is still sparse and more research is needed to understand the caffeine transport mechanism in stingless bees. Caffeine may have different effects in different insect taxa as a result of potential differences in physiology. Similarly, octopamine and dopamine (which also affect reward signalling in bees) can affect different behaviours in different ant species (Kamhi and Traniello, 2013).

In honeybees, the profitability of food sources increased the persistence of foragers to an unrewarding feeding location (Al Toufailia et al., 2013). In our study, the persistence of *P. droryana* foragers was not affected by the concentration of the sucrose solution and only 23% of the marked bees returned to the empty feeder the day after treatment with 40% sucrose solution. This suggests that *P. droryana* shows much lower day-to-day persistence than *A. mellifera*, possibly because they forage on food sources that are more ephemeral.

Honeybees famously use the waggle dance to communicate the location of a profitable resource to their nestmates (von Frisch, 1967). Among the over 500 different stingless bee species (Rasmussen and Cameron, 2010), different methods to recruit nestmates to resources have been found (reviewed in Nieh, 2004; Hrncir, 2009; Jarau, 2009). Stingless bees can be recruited to specific locations by other foragers; alternatively, they can be induced to search for food sources in the environment in a spatially unspecific way. For example, returned foragers of some species vigorously run through the nest, thereby jostling their nestmates (Hrncir, 2009). Additionally, the foragers produce thoracic vibrations (sounds). Both behaviours are considered a potential mechanism to improve the recruitment abilities of nestmates (Lindauer and Kerr, 1960). A different strategy is used by species that recruit to specific food source locations using chemical compounds (Jarau, 2009; Leonhardt, 2017; Nieh, 2004). Our finding that the number of foragers at a 30% sucrose feeder decreased over time but increased at a nearby feeder that offered 40% solution could be explained by a number of processes. For instance, the different food qualities could lead to different rates of abandoning a food source. Also, discovery of a high-quality food source often stimulates the foraging activity of a colony (Schorkopf et al., 2016), which, in combination with local enhancement (the visual attraction of a food source that is occupied by other foragers; Slaa et al., 2003) could explain why forager numbers decreased at the 30% feeder, but increased at the 40% feeder. Furthermore, the findings could also be explained by foragers switching from the 30% to the 40% feeder unnoticed by the observers during the experiment. Alternatively, *P. droryana* foragers might be able to recruit nestmates to food sources. For example, it could be that the bees deposit pheromones to advertise a high-quality food source. In *Trigona recursa*, for instance, a feeder baited with pheromones attracted nestmates (Jarau et al., 2004a,b) and colonies recruited more bees to food sources of higher quality (Schmidt et al., 2006). We did not observe *P. droryana* foragers to deposit odour trails near the food source; however, *P. droryana* is a very small bee (~3 mm long) and marking behaviour might be difficult to observe. Alternatively, bees might also be attracted by footprints left on and near the feeder by foragers (Hrncir et al., 2004; Jarau et al., 2004b; Jarau, 2009). Footprint chemicals are often not very volatile, which means that other bees would have to be relatively close to perceive them (within 1 m in the much larger *Melipona seminigra*; Hrncir et al., 2004). Lindauer and Kerr (1960) found no evidence for site-specific recruitment in *P. droryana*, but their experiments should be repeated with a more representative sample size. Thus, a next step will be to further test whether *P. droryana* recruits to particular food sources and, if this is indeed the case, to elucidate the underlying mechanism.

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Competing interests
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Author contributions
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References

Afik, O., Delaplane, K. S., Shafir, S., Moo-Valle, H. and Quezada-Euán, J. J. G. (2014). Nectar minerals as regulators of flower visitation in stingless bees and nectar hoarding wasps. *J. Chem. Ecol.* **40**, 476-483. doi:10.1007/s10886-014-0455-8

Aleixo, K. P., Menezes, C., Fonseca, V. L. I. and da Silva, C. I. (2017). Seasonal availability of floral resources and ambient temperature shape stingless bee foraging behavior (*Scaptotrigona* aff. *depilis*). *Apidologie* **48**, 117-127. doi:10.1007/s13592-016-0456-4

Al Toufailia, H., Grüter, C. and Ratnieks, F. L. W. (2013). Persistence to unrewarding feeding locations by honeybee foragers (*Apis mellifera*): the effects of experience, resource profitability and season. *Ethology* **119**, 1096-1106. doi:10.1111/eth.12170

Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1. doi:10.18637/jss.v067.i01

Bennett, R. N. and Wallsgrove, R. M. (1994). Secondary metabolites in plant defence mechanisms. *New. Phytol.* **127**, 617-633. doi:10.1111/j.1469-8137.1994.tb02968.x

Bhaskara, S., Dean, E. D., Lam, V. and Ganguly, R. (2006). Induction of two cytochrome P450 genes, *Cyp6a2* and *Cyp6a8*, of *Drosophila melanogaster* by caffeine in adult flies and in cell culture. *Gene* **377**, 56-64. doi:10.1016/j.gene.2006.02.032

Biesmeijer, J. C. and Slaa, E. J. (2004). Information flow and organization of stingless bee foraging. *Apidologie* **35**, 143-157. doi:10.1051/apido:2004003

Biesmeijer, J. C., van Nieuwstadt, M. G. L., Lukács, S. and Sommeijer, M. J. (1998). The role of internal and external information in foraging decisions of *Melipona* workers (Hymenoptera: Meliponinae). *Behav. Ecol. Sociobiol.* **42**, 107-116. doi:10.1007/s002650050418

Bohman, B., Flematti, G. R., Barrow, R. A., Pichersky, E. and Peakall, R. (2016). Pollination by sexual deception—it takes chemistry to work. *Curr. Opin. Plant. Biol.* **32**, 37-46. doi:10.1016/j.pbi.2016.06.004

Burkle, L. A., Marlin, J. C. and Knight, T. M. (2013). Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science* **339**, 1611-1615. doi:10.1126/science.1232728

Ceja-Navarro, J. A., Vega, F. E., Karaoz, U., Hao, Z., Jenkins, S., Lim, H. C., Kosina, P., Infante, F., Northen, T. R. and Brodie, E. L. (2015). Gut microbiota mediate caffeine detoxification in the primary insect pest of coffee. *Nat. Commun.* **6**, 7618. doi:10.1038/ncomms8618

Chittka, L. and Peng, F. (2013). Caffeine boosts bees' memories. *Science* **339**, 1157-1159. doi:10.1126/science.1234411

Cortopassi-Laurino, M., Alves, D. A. E. and Imperatriz-Fonseca, V. L. (2009). Árvores neotropicais, recursos importantes para a nidificação de abelhas sem ferrão (Apidae, Meliponini). *Mens. Doce.* **100**, 21-28.

Couvillon, M. J., Al Toufailia, H., Butterfield, T. M., Schrell, F., Ratnieks, F. L. W. and Schürch, R. (2015). Caffeinated forage tricks honeybees into increasing foraging and recruitment behaviors. *Curr. Biol.* **25**, 2815-2818. doi:10.1016/j.cub.2015.08.052

Fausto, B. (2014). *A Concise History of Brazil*. Cambridge, UK: Cambridge University Press.

Giannini, T. C., Boff, S., Cordeiro, G. D., Cartolano, E. A., Veiga, A. K., Imperatriz-Fonseca, V. L. and Saraiva, A. M. (2015). Crop pollinators in Brazil: a review of reported interactions. *Apidologie* **46**, 209-223. doi:10.1007/s13592-014-0316-z

Govaerts, R. (2009). *World Checklist of Selected Plant Families*. London, UK: The Board of Trustees of the Royal Botanic Gardens, Kew.

Heard, T. A. (1999). The role of stingless bees in crop pollination. *Annu. Rev. Entomol.* **44**, 183-206. doi:10.1146/annurev.ento.44.1.183

Heil, M. (2011). Nectar: generation, regulation and ecological functions. *Trends. Plant. Sci.* **16**, 191-200. doi:10.1016/j.tplants.2011.01.003

Hrncir, M. (2009). Mobilizing the foraging force: mechanical signals in stingless bee recruitment. In *Food Exploitation by Social Insects: Ecological, Behavioural, and Theoretical Approaches* (ed. S. Jarau and M. Hrncir), pp. 199-221. Boca Raton, FL: CRC Press.

Hrncir, M., Jarau, S., Zucchi, R. and Barth, F. G. (2004). On the origin and properties of scent marks deposited at the food source by a stingless bee, *Melipona seminigra*. *Apidologie* **35**, 3-13. doi:10.1051/apido:2003069

Hrncir, M., Jarau, S. and Barth, F. G. (2016). Stingless bees (Meliponini): senses and behavior. *J. Comp. Physiol. A* **202**, 597-601. doi:10.1007/s00359-016-1117-9

Imperatriz-Fonseca, V. L., Kleinert-Giovannini, A. and Ramalho, M. (1989). Pollen harvest by eusocial bees in a non-natural community in Brazil. *J. Trop. Ecol.* **5**, 239-242. doi:10.1017/S0266467400003539

Inouye, D. W. (1980). The terminology of floral larceny. *Ecology* **61**, 1251-1253. doi:10.2307/1936841

Irwin, R. E., Brody, A. K. and Waser, N. M. (2001). The impact of floral larceny on individuals, populations, and communities. *Oecologia* **129**, 161-168. doi:10.1007/s004420100739

Jarau, S. (2009). Chemical communication during food exploitation in stingless bees. In *Food Exploitation by Social Insects: Ecological, Behavioural, and Theoretical Approaches* (ed. S. Jarau and M. Hrncir), pp. 223-249. Boca Raton, FL: CRC Press.

Jarau, S., Hrncir, M., Ayasse, M., Schulz, C., Francke, W., Zucchi, R. and Barth, F. G. (2004a). A stingless bee (Melipona seminigra) marks food sources with a pheromone from its claw retractor tendons. *J. Chem. Ecol.* **30**, 793-804. doi:10.1023/B:JOEC.0000028432.29759.ed

Jarau, S., Hrncir, M., Zucchi, R. and Barth, F. G. (2004b). A stingless bee uses labial gland secretions for scent trail communication (*Trigona recursa* Smith 1863). *J. Comp. Physiol. A* **190**, 233-239. doi:10.1007/s00359-003-0489-9

Johnson, L. K. and Hubbell, S. P. (1974). Aggression and competition among stingless bees: field studies. *Ecology* **55**, 120-127. doi:10.2307/1934624

Kamhi, J. F. and Traniello, J. F. A. (2013). Biogenic amines and collective organization in a superorganism: neuromodulation of social behavior in ants. *Brain. Behav. Evol.* **82**, 220-236. doi:10.1159/000356091

Koch, H. and Stevenson, P. C. (2017). Do linden trees kill bees? Reviewing the causes of bee deaths on silver linden (*Tilia tomentosa*). *Biol. Lett.* **13**, 20170484. doi:10.1098/rsbl.2017.0484

Kretschmar, J. A. and Baumann, T. W. (1999). Caffeine in Citrus flowers. *Phytochemistry* **52**, 19-23. doi:10.1016/S0031-9422(99)00119-3

Kucharski, R. and Maleszka, R. (2005). Microarray and real-time PCR analyses of gene expression in the honeybee brain following caffeine treatment. *J. Mol. Neurosci.* **27**, 269-276. doi:10.1385/JMN:27:3:269

Leadbeater, E. and Chittka, L. (2008). Social transmission of nectar-robbing behaviour in bumble-bees. *Proc. R. Soc. London. B* **275**, 1669-1674. doi:10.1098/rspb.2008.0270

Leonhardt, S. D. (2017). Chemical ecology of stingless bees. *J. Chem. Ecol.* **43**, 385-402. doi:10.1007/s10886-017-0837-9

Lindauer, M. and Kerr, W. E. (1960). Communication between the workers of stingless bees. *Bee World* **41**, 29-41. doi:10.1080/0005772X.1960.11095309

Mitchell, R. J., Irwin, R. E., Flanagan, R. J. and Karron, J. D. (2009). Ecology and evolution of plant–pollinator interactions. *Ann. Botany.* **103**, 1355-1363. doi:10.1093/aob/mcp122

Neilson, J. and Pritchard, B. (2009). *Value Chain Struggles: Institutions and Governance in the Plantation Districts of South India*. West Sussex, UK: Wiley-Blackwell.

Nieh, J. C. (2004). Recruitment communication in stingless bees (Hymenoptera, Apidae, Meliponini). *Apidologie* **35**, 159-182. doi:10.1051/apido:2004007

Nogueira-Neto, P., Carvalho, A. and Antunes Filho, H. (1959). Efeito da exclusão dos insetos polinizadores na produção do café Bourbon. *Bragantia* **18**, 441-468. doi:10.1590/S0006-87051959000100029

Oelschlägel, B., Nuss, M., von Tschirnhaus, M., Pätzold, C., Neinhuis, C., Dötterl, S. and Wanke, S. (2015). The betrayed thief–the extraordinary strategy of Aristolochia rotunda to deceive its pollinators. *New. Phytol.* **206**, 342-351. doi:10.1111/nph.13210

Rasmussen, C. and Cameron, S. A. (2010). Global stingless bee phylogeny supports ancient divergence, vicariance, and long distance dispersal. *Biol. J. Linnean. Soc.* **99**, 206-232. doi:10.1111/j.1095-8312.2009.01341.x

Ricketts, T. H. (2004). Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conserv. Biol.* **18**, 1262-1271. doi:10.1111/j.1523-1739.2004.00227.x

Ricketts, T. H., Daily, G. C., Ehrlich, P. R. and Michener, C. D. (2004). Economic value of tropical forest to coffee production. *Proc. Natl. Acad. Sci. USA* **101**, 12579-12582. doi:10.1073/pnas.0405147101

Santos, R. M. and Lima, D. R. (2009). *An Unashamed Defense of Coffee: 101 Reasons to Drink Coffee without Guilt*. Pittsburgh, USA: Xlibris.

Schiestl, F. P. (2005). On the success of a swindle: pollination by deception in orchids. *Naturwissenschaften* **92**, 255-264. doi:10.1007/s00114-005-0636-y

Schmidt, V. M., Schorkopf, D. L. P., Hrncir, M., Zucchi, R. and Barth, F. G. (2006). Collective foraging in a stingless bee: dependence on food profitability and sequence of discovery. *Anim. Behav.* **72**, 1309-1317. doi:10.1016/j.anbehav.2006.03.023

Schorkopf, D. L. P., de Sá Filho, G. F., Maia-Silva, C., Schorkopf, M., Hrncir, M. and Barth, F. G. (2016). Nectar profitability, not empty honey stores, stimulate recruitment and foraging in *Melipona scutellaris* (Apidae, Meliponini). *J. Comp. Physiol. A* **202**, 709-722. doi:10.1007/s00359-016-1102-3

Singaravelan, N., Nee'man, G., Inbar, M. and Izhaki, I. (2005). Feeding responses of free-flying honeybees to secondary compounds mimicking floral nectars. *J. Chem. Ecol.* **31**, 2791-2804. doi:10.1007/s10886-005-8394-z

Slaa, E. J., Wassenberg, J. and Biesmeijer, J. C. (2003). The use of field–based social information in eusocial foragers: local enhancement among nestmates and heterospecifics in stingless bees. *Ecol. Entomol.* **28**, 369-379. doi:10.1046/j.1365-2311.2003.00512.x

Stangler, E. S., Jarau, S., Hrncir, M., Zucchi, R. and Ayasse, M. (2009). Identification of trail pheromone compounds from the labial glands of the stingless bee *Geotrigona mombuca*. *Chemoecology* **19**, 13-19. doi:10.1007/s00049-009-0003-0

Thomson, J. D., Draguleasa, M. A. and Tan, M. G. (2015). Flowers with caffeinated nectar receive more pollination. *Arthropod. Plant. Interact.* **9**, 1-7. doi:10.1007/s11829-014-9350-z

Thorburn, L. P., Adler, L. S., Irwin, R. E. and Palmer-Young, E. C. (2015). Variable effects of nicotine, anabasine, and their interactions on parasitized bumble bees. *F1000Res* **4**, 800. doi:10.12688/f1000research.6870.2

Von Frisch, K. (1967). *The Dance Language and Orientation Of Bees*. Cambridge, MA: Harvard University.

Willoughby, L., Chung, H., Lumb, C., Robin, C., Batterham, P. and Daborn, P. J. (2006). A comparison of *Drosophila melanogaster* detoxification gene induction responses for six insecticides, caffeine and phenobarbital. *Insect. Biochem. Molec.* **36**, 934-942. doi:10.1016/j.ibmb.2006.09.004

Wright, G. A., Baker, D. D., Palmer, M. J., Stabler, D., Mustard, J. A., Power, E. F., Borland, A. M. and Stevenson, P. C. (2013). Caffeine in floral nectar enhances a pollinator's memory of reward. *Science* **339**, 1202-1204. doi:10.1126/science.1228806

Zuur, A. F., Ieno, E. N., Walker, N. and Saveliev, A. and Smith, G. (2009). *Mixed Effects Models and Extensions in Ecology with R. Statistics for Biology and Health*. New York, NY: Springer.