Foragers of the stingless bee *Plebeia droryana* inform nestmates about the direction, but not the distance to food sources

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Abstract. 1. The tropical stingless bees have evolved intricate communication systems to recruit nestmates to food locations. Some species are able to accurately communicate the location of food, whereas others simply announce the presence of food in the environment.

2. *Plebeia droryana* is a tiny Neotropical stingless bee that, until recently, was thought to use a solitary foraging strategy, that is without the use of a recruitment communication system. However, recent research has indicated that *P. droryana* might be able to recruit nestmates to specific food source locations.

3. We tested this by studying whether foragers can guide nestmates in the direction and the distance of artificial feeders placed in the vicinity of the colony. We trained bees to a scented sucrose solution feeder at 10 m and placed different feeders either in different directions (experiment 1) or in different distances (experiment 2). We found that *P. droryana* directs newcomers in the right direction, but distance information does not seem to be communicated.

4. Moreover, we then tested whether newcomers use chemical and visual cues originating from nestmates foraging at the food source, but found no evidence for the use of these social cues provided by conspecifics.

5. The potential mechanism that *P. droryana* may use to orient recruits toward the food source, however, remains unknown and requires further study.

Key words. local enhancement, recruitment, stingless bees.

Introduction

Social insects have evolved a remarkable diversity of communication mechanisms to guide nestmates to food locations (Wilson, 1971; Hölldobler & Wilson, 1990; Jarau & Hrmčíř, 2009). These communication mechanisms allow colonies to allocate workers to food sources that are too large to be exploited by an individual and, thereby, collect more food for the colony. Communication mechanisms can be divided into extranidal and intranidal mechanisms. The waggle dance in honeybees is a striking intranidal communication mechanism that has been studied extensively (von Frisch, 1967; Gould, 1975; Dyer, 2002; l’Anson Price et al., 2019). A dancing bee indicates the distance and direction of food sources to its followers (von Frisch, 1967; Dyer, 2002; Couvillon, 2012). At the same time, followers gain information about the odour of the food source, for example during trophallaxis (Gil & De Marco, 2005; Farina et al., 2005; Farina & Grüter, 2009). Extranidal communication mechanisms include the laying of pheromone trails (in many ants) or tandem running (Hölldobler & Wilson, 1990; Franklin, 2014; Czaczkes et al., 2015).

Stingless bees (Apidae, Meliponini) are a large group of eusocial hymenopterans that live in diverse tropical and subtropical habitats (Roubik, 1989). Several hundred species exist that show
a great diversity in lifestyle and ecology. While most genera play a crucial role as pollinators, a few species have evolved carnivorous or robbing lifestyles (Camargo & Roubik, 1991; Barth et al., 2008; Grüter et al., 2016). Food source communication mechanisms in stingless bees are diverse and seem to be species-specific, ranging from simply motivating nestmates to leave the nest and search for food to the precise communication of the food source location by using pheromone trails. Some species are more efficient at recruiting nestmates to food sources than honeybees (Lindauer & Kerr, 1960; Aguilar et al., 2005; Barth et al., 2008).

Intraneural recruitment communication is well-known in stingless bees. Successful foragers of many species perform “zigzag” or “jostling” runs inside the nest (Lindauer & Kerr, 1958, 1960; Hrncir et al., 2000), which usually takes place close to the nest entrance (Nieh, 1998; Hrncir, 2009). The jostling runs appear to play an important role in activating potential foragers (Hrncir et al., 2000). For example in Melipona seminigra, inactive foragers significantly increased their own jostling activity after they were jostled by a recruiting bee (Hrncir, 2009). During the jostling runs and during trophalaxis, buzzing sounds are generated with thoracic muscles (Kerr et al., 1963; Esch et al., 1965; Nieh & Roubik, 1998; Aguilar & Briceño, 2002; Nieh et al., 2003b), which may stimulate the vibrated receivers to initiate foraging (Hrncir et al., 2006, 2008; Hrncir & Barth, 2014; Krausa et al., 2017). At the same time, the information of quality and odour of a food source is potentially shared inside the colony by performing trophallaxis (Nieh et al., 2000; Aguilar et al., 2005; Jarau, 2009; Krausa et al., 2017).

Extranidal recruitment communication can involve foragers laying a scent trail when leaving a food source, as found in Cephalotrigona, Scaptotrigona, and Trigona (Lindauer & Kerr, 1958, 1960; Nieh et al., 2003a, 2004; Jarau, 2009) or the deposition of a scent beacon near the food source, which can attract other foragers (Nieh, 1998; Hrncir et al., 2004; Jarau et al., 2004; Alavez-Rosas et al., 2017). Furthermore, for some species, it has been suggested that visual tracking of guiding flights performed by recruiting foragers from nest to food source explains location-specific recruitment (Lindauer & Kerr, 1960; Aguilar et al., 2005). Recruitment success could also be the result of a combination of these mechanisms (Barth et al., 2008). However, most stingless bees do not seem to use pheromone trails to recruit nestmates, which may be due to the relatively small colony size of many species (Nieh, 2004) as the number of workers in small colonies is not sufficient to sustain the volatile pheromone trails (Beekman & Dussutour, 2009). In addition, for larger colonies, it could be easier to dominate a food source, which makes using pheromone trails potentially more beneficial (Aguilar et al., 2005). Thus, strong competition might favour recruitment communication in species with large colonies but select against recruitment in species with the small colony or body sizes (Johnson & Hubbell, 1974). Rapid and accurate communication could also be important for patchily distributed high-quality food sources, such as carrion (Noll, 1997).

Some species are able to recruit to specific locations without the use of pheromone trails. For example Partamona orizabaensis foragers can communicate the location of food sources to their nestmates but the mechanism is still a mystery (Flaig et al., 2016). Furthermore, there are species where foragers seem to only use information about the direction of food sources, without using accurate distance information (Jarau et al., 2000; Nieh et al., 2000; Nieh, 2004; Aguilar et al., 2005). More generally, recruitment communication remains poorly understood in most stingless bees. This, in turn, has hampered our understanding of how complex recruitment communication systems have evolved in social bees and how foraging strategies are related to the lifestyle of different species.

In Plebeia droyana, a small (~3 mm long) species commonly found in South America, foragers have been shown to produce buzzing sounds to alert nestmates about the presence of a food source, but Lindauer and Kerr (1958, 1960) found no evidence for specific location communication in this species. Peng et al. (2019), on the other hand, found that the number of P. droyana foragers steadily increased over time at a high-quality food source, suggesting that P. droyana foragers might provide nestmates with specific location information. The two studies differed in both the number of colonies observed and the foraging distance tested. While Lindauer and Kerr (1958, 1960) used only a single colony and a relatively large foraging distance for such a small bee, Peng et al. (2019) studied five colonies at a nearby food source (10 m). Since food source distance affects recruitment probability (Nieh et al., 2003a, 2004; Stangler et al., 2009), it is possible that foragers were not motivated to recruit in Lindauer and Kerr (1958, 1960). Here, we studied if P. droyana foragers can potentially provide direction and distance information to nestmates. Since foragers of some species are attracted by visual and chemical cues of conspecifics at food sources, so-called local enhancement (Slaa et al., 2003; Slaa & Hughes, 2009), we also explored if the presence of nestmates or their footprints at food sources affects P. droyana forager allocation.

Materials and methods

Study species and field site

We performed all experiments on the campus of the University of São Paulo in Ribeirão Preto, Brazil. This area has many different stingless bee species (Cortopassi-Laurino et al., 2009), and Plebeia droyana is among the most common ones. Wild colonies nest in tree cavities or in cavities in the walls of buildings. We used eight wild colonies for our experiments. Wild colonies were at least 100 m from each other. To prevent bees from other colonies to visit our feeders, we closed all the visible colony entrances within a 10 m radius around the focal colonies. Data were collected in February and March 2019 on days with good foraging conditions.

Experimental procedures

Foragers from the tested colonies were trained to artificial feeders by standard training procedures (see von Frisch, 1967; Peng et al., 2019, 2020). First, we placed one artificial feeder with a 50% sucrose solution next to the nest entrance to attract...
foragers to start collecting the sucrose solution. After a group of foragers was established, the feeder was moved to the final location 10 m from the tested colony while the foragers were drinking sucrose solution. Foragers would learn the new feeder location when returning to their nest. The proximity of the feeder compared to Lindauer and Kerr (1958, 1960) increased the chance that foragers would recruit, which was important to test whether this species has the potential to recruit. In all experiments, the feeder was located on a chair (height 0.5 m) with a yellow background, and the sucrose solution contained a scent, like eucalyptus, lavender, orange, or mint. Each colony was tested with only one odour (5 μl essential oil per 100 ml sucrose solution; Primavera Life GmbH, Oy-Mittelberg, Germany), which aids in the formation of spatial memories in bees (Menzel, 1999). We used the same scent in the training and testing phases in a given trial.

Experiment 1: Do P. droryana foragers provide direction information?

To test site-specific recruitment communication in P. droryana, we assessed direction (Experiment 1) and distance information (Experiment 2). For experiment 1, we began training in the morning and after ~10 foragers were trained to the feeder, we marked 10 foragers individually with acrylic paint on the thorax. The position of this training feeder was either on the left-hand side (four trials) or right-hand side (four trials) of the entrance of a tested colony (Fig. 1a). During the following 20 min, we allowed only these marked bees to visit the feeder by catching all the unmarked bees using an aspirator and the marked foragers continued to visit the feeder for 20 min. According to Peng et al. (2019), foragers perform 5–6 foraging trips on average during 20 min to a high-quality food source at this distance. This allowed us to make sure that only foragers from the focal colony were collecting food from our feeder. Second, the foragers had sufficient time to deposit chemical compounds on the feeder (therefore called FChem) and chair if they did.

After this 20-min phase, the training feeder FChem was replaced by a new, clean feeder at the training location (FTraloc). This new feeder was placed on an identical chair and yellow background. The original feeder FChem, the chair it was placed on, and the yellow background were moved to a different location that was also 10 m from the nest (Fig. 1b). In addition, we introduced a third feeder FNew, which was placed on an identical chair with yellow background 10 m from the nest, but in a different direction (Fig. 1b). During the following testing phase, all three feeders offered a 50% scented sucrose solution. In half of the trials, FChem was placed between FTraloc and FNew whereas in the other half of the trials, FNew was placed between the other two feeders (Fig. 1b). During the testing phase, the marked bees continued to collect food at the training location, now from feeder FTraloc.

During the testing period of 120 min, we caught all unmarked bees that landed on the three feeders, while allowing the marked foragers to collect food at FTraloc. If a marked bee landed on one of the other two feeders, we also caught them while on the empty feeder. However, this happened very rarely. Eight trials were carried out, one with each colony.

Experiment 2: Do P. droryana foragers provide distance information and do newcomers use local enhancement?

In order to find out whether nestmates acquire distance information and whether the local enhancement is used by newcomers, we performed a second experiment. We set up one feeder 10 m from the tested colony as described above (Fig. 2a). Ten individually marked foragers were again allowed to visit this feeder (called FTral0m) during 20 min, while unmarked bees that landed on the feeder were caught. Then, two new feeders on identical chairs and backgrounds were placed halfway between the training feeder and the nest (5 m from the nest). The two new feeders were separated by 1 m from each other. All three feeders were placed on a Petri dish (radius of 7 cm). One of the Petri dishes placed under one of the new feeders (henceforth called FBees5m) contained seven individually marked live foragers from the same colony to provide visual cues to approaching foragers (Fig. 2b). Before the foragers were transferred to the Petri dish, we chilled them on ice for 2 min to immobilise them. After carefully moving them to the Petri dish, we tightly sealed the Petri dish with tape to prevent chemical compounds from leaving the Petri dish. The other two Petri dishes did not contain bees. During the following 120 min testing phase, we captured and counted all newcomers landing on each feeder. Marked bees were only allowed to visit feeder FTral0m, otherwise, we caught them. The location of feeders FBees5m and FEmp5m (the new feeder on an empty Petri dish) was randomised in each trial (left or right from the direction between the nest and the original training feeder FTral0m).
Statistical analysis

In experiment 1, we expected that if trained foragers provided direction information in the form of a pheromone trail, visual cues, or guidance flights, most newcomers should arrive at F_{TraLoc} (the direction of this feeder was called Dir_{TraLoc}). If foragers left chemical cues on the feeder or chair (a chemical “beacon”), then newcomers should land more frequently on feeder F_{Chem} than F_{New}. If foragers did not recruit nestmates to a food source location, then the number of newcomers should be equal on all three feeders. Thus, we first used the proportion of unmarked bees at each feeder (F_{Chem}, F_{TraLoc}, and F_{New}) as a response variable to test if the foragers show a preference for a feeder and whether they could use chemical marks to choose a feeder. We also compared the effect of the spatial distribution of feeders (Dir_{Mid}: feeder direction was closest to Dir_{TraLoc}, Dir_{Far}: feeder direction was most distant to Dir_{TraLoc} and Dir_{TraLoc}) and the included colony as a random effect. However, these models failed, most likely due to the limited sample size of our experiments. Thus, in experiment 1, we performed separate tests for the two predictors.

In experiment 2, we expected that if marked foragers provided distance information, then more newcomers would arrive at F_{Tra10m}. If newcomers use local enhancement, then they should prefer F_{Bee5m} and F_{Tra10m} over F_{Emp5m}. If bees used neither distance information nor visual cues, then they should arrive in equal numbers at all three feeders. We used the proportion of unmarked bees at each feeder (F_{Tra10m}, F_{Bee5m}, and F_{Emp5m}) as a response variable.

All statistical tests were performed in R version 3.4.4 (http://www.R-project.org/). In experiments 1 and 2, we used paired sample t-test to test specific predictions about distance and direction communication. P-values were adjusted with the sequential Bonferroni correction if we used the same data for multiple comparisons (Sokal & Rohlf, 1995).

Results

Experiment 1: Do P. droryana foragers provide direction information?

Most unmarked bees arrived at feeder F_{TraLoc}, which was exploited by the marked bees during the testing phase. On average 50.53% ± 4.62% of all recruits arrived at this feeder, while 19.19% ± 2.51% arrived at the training feeder F_{Chem} and 30.28% ± 4.38% at the feeder F_{New} (Fig. 3a, Table S1). The proportion of newcomers at F_{Chem} was significantly lower than at F_{TraLoc} (paired t-test: t = −5.22, df = 7, P-adjusted = 0.0037), whereas there was no difference between the feeders with and without putative scent marks (F_{Chem} vs. F_{New} t-test paired: t = 2.04, df = 7, P-adjusted = 1.00; F_{TraLoc} vs. F_{New} t-test paired: t = −2.34, df = 7, P-adjusted = 0.10). While we were not able to formally test for an interaction between feeder type and feeder direction, Fig. S1a,b indicate that feeder type had similar effects, irrespective of feeder direction.

As mentioned, most unmarked bees (50.53% ± 4.62%) arrived at the direction of F_{TraLoc} (direction Dir_{TraLoc} in Fig. 1b), whereas 30.57% ± 3.56% landed on the feeder closest to F_{TraLoc} (direction Dir_{Mid} in Fig. 1b). Only 18.90% ± 3.44% of newcomers
Plebeia droryana use to orient recruits toward the food source

Fig. 3. Newcomer arrival at different feeders (a) or directions (b). Different letters indicate significant differences ($P < 0.05$) between the percentages of newcomers at the feeders or directions. The different shapes of dots represent individual trials according to their directions (a) or feeders (b). [Colour figure can be viewed at wileyonlinelibrary.com].

Fig. 4. The percentages of $P. droryana$ newcomers that arrived at different feeders. Black dots at the feeder $F_{Bee5m}$ represent imprisoned bees. The boxplots indicate the medians, 25% and 75% quartiles. Coloured dots at the box represent the proportion of each colony. Black dots represent individual datapoints. [Colour figure can be viewed at wileyonlinelibrary.com].

Our results suggest that $P. droryana$ foragers transmit information about the direction of food sources to nestmates, but not about its distance. In addition, neither the visual presence of nestmates nor chemical marks per se affected the number of newcomers. The finding that foragers provide directional information to nestmates is consistent with the findings of a recent study (Peng et al., 2019), but contrasts with earlier observations that have suggested that this species does not recruit to food sources (Lindauer & Kerr, 1958, 1960). We used a shorter training distance compared to these earlier studies (10 m vs. 150 m), which is likely to affect both the efficiency and the likelihood of recruitment in $P. droryana$. The proximity of the food source in our study made it more probable that foragers would recruit and, thus, reveal whether this species has the potential to recruit nestmates.

Chemical cues left at the food source do not seem to explain the recruitment pattern we found because newcomers did not land more on the feeder that was visited by marked bees during the training phase ($F_{Chem}$) compared to a new feeder at the same distance from the colony ($F_{New}$) that was not visited by marked bees earlier. This is unexpected as one way to attract nestmates is to mark the food sources using chemical compounds (Jarau, 2009). For example Melipona panamica foragers deposit scent marks that function as an olfactory beacon for themselves and for nestmates (Nieh, 1998).
same has been found in *Trigona corvina* and *Nannotrigona testaceicornis*, where newcomer bees are attracted to feeders that foragers previously visited compared to clean feeders (Schmidt et al., 2005; Boegert et al., 2006). One reason could be that the scent marks left on food sources have a short attractive range, which the *P. droryana* newcomers might not have entered. In *Melipona semiiniigra*, scent marks were perceived and attractive at a distance of about 1 m and their effect lasted about 2 h (Hrncir et al., 2004). In *Scaptotrigona aff. depilis*, this attraction range was up to 20 m (Schmidt et al., 2003). Our results indicate that *P. droryana* foragers either did not leave significant amounts of scent marks at the feeding place or that this information does not have a strong effect on recruits at the distances between food sources used in our experiment.

Alternatively, *P. droryana* foragers might use pheromone trails to lead recruits from the nest to a food resource, similar to what has been found in several species of stingless bees (Lindauer & Kerr 1958, 1960; Nieh et al., 2003a, 2004; Jarau et al., 2010; Reichle et al., 2011). The feeder that was furthest away from the feeder visited by the marked bees attracted some, but fewer bees. This suggests that recruits are sent out in the approximate, rather than the precise direction. One possible explanation is that *P. droryana* foragers deposit pheromone trails in a winding pattern when returning to the nest, similar to *Scaptotrigona postica* (Lindauer & Kerr 1958, 1960). In addition, *P. droryana* foragers do not always fly in a direct line towards the nest, but frequently perform lateral movements (Collevatti et al., 2000). This would allow recruits to discover food sources in a certain corridor, rather than a specific point in space. Even in honeybees, waggle runs vary considerably in their angle (Towne & Gould, 1988; Al Toufailia et al., 2013; Beekman et al., 2015) and flight vectors of recruits show substantial scatter (Riley et al., 2005). If the recruitment mechanism in *P. droryana* is of a similar stochastic nature, we would expect that the number of newcomers at other food sources decreases the more the route to these food sources deviates from the route between the nest and the advertised food source. Scent marking behaviour was never observed, which casts doubt over the use of pheromone trails by *P. droryana* foragers. On the other hand, *P. droryana* foragers are very small (ca. 3 mm body length), which makes it difficult for human observers to follow their flight paths and observe them landing on vegetation to deposit pheromone when they return to the nest. An alternative hypothesis is the following of guiding flights by recruiting foragers. Lindauer and Kerr (1958) suggested that *Scaptotrigona postica* recruits might use visual guidance or follow an “aerial odour tunnel” created by the recruiting forager during flight as additional information to locate a food source. These guidance flights were also observed in *Trigona corvina* (Aguilar et al., 2005). It is important to note that there might not be one single recruitment mechanism, but that *P. droryana* foragers might combine several mechanisms to recruit nestmates.

Another argument against pheromone trails is our finding that newcomers did not predominantly arrive at the food source at the correct distance. Approximately equal numbers of newcomers arrived at the three feeders in experiment 2. This suggests that the recruitment method of *Plebeia droryana* is similar to *Plebeia tica* and *Tetragonula carbonaria*, which communicate the direction but not the distance of food sources (Nieh et al., 2000; Aguilar et al., 2005). We cannot rule out that experienced foragers provide information about the distance to recruits, but that recruits do not use this information if they encounter a food source on the way to the advertised food source. Because we used identical odours for all three feeders, *P. droryana* newcomers might have searched for the food source using a combination of odour-based searching with other yet to be discovered recruitment mechanisms after leaving the nest (Aguilar et al., 2005).

Local enhancement has been shown to affect foraging decisions in honeybees, bumblebees, wasps, and stingless bees (Goulson et al., 2001; Leadbeater & Chittka, 2005; Kawaguchi et al., 2007). However, the effect of the visual presence of other individuals at food sources varies among species (Slaa & Hughes, 2009). In *Trigona amalthea*, for example local enhancement depends on the foraging experience of bees (Slaa et al., 2003). In our study, we did not find that newcomers were more attracted to the feeder that had conspecifics in its vicinity (Fig. 4), which suggests that local enhancement does not explain why more newcomers landed on the feeder *P. TraLoc* in experiment 1. It is also possible that local enhancement exists in this species, but requires the demonstrator bee to be immobile and in the immediate proximity of the food source.

**Conclusions**

We found that significantly more newcomers arrived in the direction of an exploited food source, whereas distance information does not seem to be transmitted during recruitment. The recruitment mechanism underlying this arrival pattern is still unclear. Future research should explore in more detail how foraging distance affects the efficiency and likelihood of recruitment. The recruitment method used by *P. droryana* seems to be less accurate than in some mass-recruiting species but is probably still more efficient than a random search by alarmed nestmates. Recruitment communication is similar to *P. tica*, which raises the possibility that the communication mechanism is conserved in this genus. However, this requires further research since *Plebeia* represents a large and probably non-monophyletic genus (~40 species) (Rasmussen & Cameron, 2010). Studying communication strategies in a diverse range of species is needed to gain better insights into the behavioural evolution of Meliponini and how this depends on ecological factors. Future research should also assess nest-based behaviours to find clues as to the nature of the mechanism of recruitment in *P. droryana*.

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TFP and CG conceived and designed the study, TFP, JP, JEB conducted the experiments, TFP and CG analysed the data, TFP wrote the original draft, all authors contributed to reviewing and editing the manuscript.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon request.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. The number of foragers and average of proportion at each feeder or location in experiments 1 and 2. The value represents mean ± SE.

Fig. S1. Newcomer arrival at different feeders (a, b) or directions (c, d). The different shapes of dots represent individual trials according to their directions (a) DirMid and DirTraLoc, (b) DirFar and DirTraLoc or feeders, (c) FChem and FTraLoc, (d) FNew and FTraLoc.

References


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