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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Eavesdropping, Foraging and Dominance in Keystone
Neotropical Pollinators: Stingless Bees

A dissertation submitted in partial satisfaction of the requirements for the
degree Doctor of Philosophy

in

Biology

by

Elinor Mermey Lichtenberg

Committee in Charge:

Professor James Nieh, Chair
Professor Tim Gentner
Professor David Holway
Professor Josh Kohn
Professor Jim Moore
Professor Peter Nonacs

2011

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Chair

University of California, San Diego

2011

EPIGRAPH

Oh what a glorious thing to be,
A healthy grown up busy busy bee,

...

Where is thy sting?

“The Bee Song,” Kenneth Blain

Be subtle! Be subtle! and use your spies for every kind of business

“The Art of War,” Sun Tzu

Is it the beauty of the flower,
Its honeyed sweets or fragrant power,
That first attracts the bee?

...

And all he wins, and all he gives,
Are wages but of hire!

”The Bee,” C. B. Langston

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Chapter 1, in full, is a reprint of the material as it appears in Lichtenberg, Elinor M., Michael Hrncir, and James C. Nieh. 2009. A scientific note: Foragers deposit attractive scent marks in a stingless bee that does not communicate food location

Apidologie 40: 1-2. The dissertation author was the primary investigator and author of this paper.

Chapter 2, in full, is a reprint of the material as it appears in Lichtenberg, E. M., V. L. Imperatriz-Fonseca, and J. C. Nieh. 2010. Behavioral suites mediate group-level foraging dynamics in communities of tropical stingless bees. *Insectes Sociaux* 57: 105-113. The dissertation author was the primary investigator and author of this paper.

Chapter 3, in full, is a reprint of the material as it appears in Lichtenberg, Elinor M., Michael Hrnir, Izabel C. Turattu, and James C. Nieh. 2011. Olfactory eavesdropping between two competing stingless bee species. *Behavioral Ecology and Sociobiology* 65: 763-774. The dissertation author was the primary investigator and author of this paper.

Chapter 4 is being prepared for submission of publication as Lichtenberg, Elinor M., Josh Graff Zivin, Michael Hrnir, and James C. Nieh. Value-based decision making via remote assessment of heterospecific pheromones. The dissertation author was the primary investigator and author of this paper.

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FIELDS OF STUDY

Major Field: Behavioral Ecology

Studies in Behavioral Ecology
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ABSTRACT OF THE DISSERTATION

Eavesdropping, Foraging and Dominance in Keystone Neotropical
Pollinators: Stingless Bees

by

Elinor Mermey Lichtenberg

Doctor of Philosophy in Biology

University of California, San Diego, 2011

Professor James Nieh, Chair

Animals constantly make decisions as they carry out basic functioning. Assessment of available options is facilitated by information obtained by directly sampling the environment (personal information) or by monitoring others' behavior (social information). Social information use appears widespread, and has important ecological and evolutionary effects. This may be particularly true for eavesdropping, exploitation of evolved signals that are aimed at other individuals. This dissertation addresses the roles of two types of social information by foraging stingless bees (Hymenoptera: Apidae, Meliponini): conspecific "footprints" and heterospecific

recruitment pheromones. Stingless bees are a large group of highly eusocial bees that are major tropical pollinators. Across species, they exhibit high diversity in colony sizes, foraging strategies, resource recruitment mechanisms, body sizes, and aggressiveness.

In Chapter 1, I show that *Melipona quadrifasciata* responds to social information from nestmates, showing attraction to previously visited locations. Chapter 2 finds that group-foraging species, which typically have large colonies, dominate individual resources and reduce feeding opportunities for solitary-foraging species. I then use the dominance relationships determined in Chapter 2 to study eavesdropping between a dominant-subordinate species pair: *Trigona hyalinata* and *Trigona spinipes*. In Chapter 3 I show that recruitment pheromone chemistry for the two species is distinct but overlapping, and that eavesdropping responses do not match the predicted pattern. Rather than show attraction to subordinate's pheromone, *T. hyalinata* avoided full-strength pheromone. *Trigona spinipes* was expected to avoid *T. hyalinata* pheromone, but instead showed little response. Chapter 4 expands on this work, investigating whether perceived costs determine eavesdropping responses. Consistent with this, *T. hyalinata* exhibited a concentration-dependent response to *T. spinipes* pheromone. Economic modeling confirmed that decision-making based on perceived takeover costs matches empirically-determined eavesdropping responses. This model highlights the role of dominance in predicting a species' optimal eavesdropping behavior, and degree of sensitivity to energetic constraints.

I provide the first detailed assessment of intra-guild social information use by a non-vertebrate, significantly improving understanding of eavesdropping within a trophic level. This work highlights that options' costs can drive decision-making even for animals with relatively simple brains. I also demonstrate the utility of economic decision analysis models for behavioral ecology.

Introduction

Gathering information enables animals to respond appropriately to their environment (Dall and Johnstone 2002) when faced with decisions such as where to forage, what to forage on, where to breed or with whom to mate. In addition to obtaining information through personal experience (personal information), individuals can collect social information by monitoring others' interactions with the environment (Danchin et al. 2004). Animals that consume patchily distributed food, such as mobile prey, flowering plants or carrion, improve search efficiency and ultimately fitness by using social information. When this information comes from evolved signals, unintended receivers using it exhibit "interceptive eavesdropping" (Peake 2005). Recent reviews highlight the importance of incorporating such social information into behavioral (Ydenberg 2010) and ecological (Schmidt et al. 2010) theory. Effects of eavesdropping may be particularly strong for foragers because resultant increases in foraging efficiency can cascade through food webs (Kean et al. 2003). While eavesdropping between trophic levels (e.g. predators and parasites detecting prey or hosts, prey noticing predators) is well studied (Peake 2005), little attention has been given to intra- or interspecific eavesdropping within a trophic level. Adaptive eavesdropping should yield "eavesdropping responses" to intercepted signals that maximize fitness in the face of multiple, sometimes conflicting, factors (Seppänen et al. 2007).

Within a trophic level, eavesdroppers must weigh benefits of improved search efficiency against costs of sharing or attempting to access the food source (Hall and Kramer 2008). Signals from competitors suggest that at least one individual is already at the advertised resource. Eavesdropping on competitors thus imposes opportunity costs, if the eavesdropper cannot gain access to the food, or conflict costs if the eavesdropper

attempts to displace the feeding signaler (Evans et al. 2009). This has been partially addressed by social learning theory, which outlines trade-offs between use of personal and social information for several group-foraging vertebrate systems (Kendal et al. 2009). Further research is required to fully understand the indirect effects of eavesdropped signals on the eavesdropper's perceived value of a food item and its responses to information about that item. Incorporating exploitation of competitors' food recruitment signals into empirical and theoretical work will provide insight into how diverse behavioral and ecological factors are translated into fitness terms (Hamilton 2010).

Social insects are an ideal system for studying intra-guild eavesdropping. Species often compete for food with sympatric relatives (Hubbell and Johnson 1977; Hölldobler and Wilson 1990) and exhibit clear dominant-subordinate relationships (e.g. Fellers 1987; Lichtenberg et al. 2010). They combine excellent associative learning (Dukas 2008) with powerful olfactory detection (Greenfield 2002) for successful foraging, thus possess sensory and cognitive preadaptations for eavesdropping. Ants and some stingless bees recruit nestmates by depositing attractive pheromones at a high-quality food source or as a trail between food and nest (Hölldobler and Wilson 1990; Nieh 2004). Both intra- (Boogert et al. 2006; Jarau 2009) and interspecific (Nieh et al. 2004) eavesdropping is exhibited by stingless bees. Heterospecific trail following by ants, which heavily guard trails, is rare and limited mainly to parabiogenic 'garden ant' species pairs (reviewed in Slaa and Hughes 2009; Menzel et al. 2010).

Stingless bees are eusocial corbiculate bees closely related to honey and bumble bees. They are responsible for a significant proportion of pollination in both agricultural and natural areas throughout the tropics (Heard 1999). The over 400 species occur in

mixed-species assemblages, have broad floral diets, and exhibit a wide range of foraging-related behaviors and life history traits. While some species are solitary foragers, others can recruit hundreds of nestmates to a rich resource (Jarau et al. 2003). Recruitment and food marking mechanisms include excitatory movements inside the nest, sound pulses, environmental scents, odor trails and odor marks at food sources (Nieh 2004). These marks include cues deposited as foragers walk on flowers ("footprints"; Schmidt et al. 2005) and pheromone signals. Seasonally limited food availability and significant diet overlap among species can lead to high levels of intra- and interspecific competition for food (Hubbell and Johnson 1977), and temporal and spatial resource partitioning (Willmer and Corbet 1981; Biesmeijer et al. 1999). Competitive interactions sometimes involve aggressive behaviors such as biting, which can lead to loss of body parts or death for attacker or recipient (Johnson and Hubbell 1974).

This thesis presents studies I conducted to determine how stingless bees assess both conspecific and heterospecific social information. Chapter 1 describes an experiment testing whether *Melipona quadrifasciata*, a species that does not use recruitment pheromones, is attracted to food sources previously visited by nestmates. Chapter 2 determines the dominance relationship between six sympatric stingless bee species, and assesses which species traits structure this relationship. In Chapter 3 I determine the chemical composition and glandular source of *Trigona hyalinata* recruitment pheromone, and test heterospecific eavesdropping on recruitment pheromones by this species and the subordinate *T. spinipes*. Chapter 4 investigates the effects of conflict costs on *T. hyalinata* eavesdropping decisions.

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Chapter 1

A scientific note: Foragers deposit attractive scent marks in a stingless bee that does not communicate food location

A scientific note: Foragers deposit attractive scent marks in a stingless bee that does not communicate food location*

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Melipona quadrifasciata / stingless bee / scent mark / foraging / olfactory information

Scent marking of profitable food sources improves foraging efficiency and helps social bees meet colony demands for unpredictable floral resources (Giurfa and Núñez, 1992). Sophisticated marking has evolved in the stingless bees (Apidae, Meliponini); several genera recruit nestmates to profitable food sources with scent signals (reviewed in Nieh, 2004). Scent cues such as “footprints” (Wilms and Eltz, 2008) can also facilitate navigation and orientation to rewarding parts of food patches. Scent marks may improve colonies’ competitive ability, especially in the tropics, where social bee abundance and diversity are high. We assessed scent mark use by the stingless bee *Melipona quadrifasciata*. This species poorly communicates to nestmates the location of rich food sources (Jarau et al., 2000), thus could benefit from within-patch orientation information provided by attractive odor marks. However, its ability to deposit and use field-based information is not known.

We studied four *Melipona quadrifasciata anthidioides* colonies at the Universidade de São Paulo, Ribeirão Preto in July 2007, and July–August 2008, using feeder choice tests (see, for example, Hrncir et al., 2004). To begin a trial, we allowed foragers trained to feed on 1.5 M sucrose solution to recruit 15 nestmates not previously used in the experiment. The 15 bees freely fed at a “visited” feeder for 10 min, during which we counted the number of visits. Next, we aspirated all bees and replaced the original apparatus with the visited and clean feeders on clean tripods, separated by 30 cm.

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We then released the 15 bees at the nest entrance, and recorded the number of bees landing on each feeder for 30 minutes, controlling for local enhancement by only counting bees landing alone. Data were analyzed with R v.2.6.0. To meet parametric assumptions, we applied Anscombe’s arcsine transformation to the proportion of bees landing on the visited feeder.

Foragers visited between 49 and 140 times, always more than the 40 visits required to attract *M. seminigra* foragers (Hrncir et al., 2004). *M. quadrifasciata* foragers did not use mandibular secretions as scent marks; unlike Kerr (1994) we never observed bees rubbing mouthparts on feeders. Neither preference for the visited feeder (ANOVA: $F_{3,10} = 0.53$, $P = 0.67$) nor number of visits ($F_{3,10} = 0.74$, $P = 0.55$) varied by colony. Foragers showed a preference for the previously visited feeder ($t_{13} = 6.70$, $P < 0.0001$), choosing it over the clean one 65.1% of the time (range 47.1%–76.9%). Preference for the visited feeder did not increase with the number of forager visits (linear regression: $F_{1,12} = 1.53$, $P = 0.24$, $r^2 = 0.11$).

Experienced *M. quadrifasciata* foragers deposit and orient to scent marks with similar frequency as other *Melipona* (Aguilar and Sommeijer, 2001; Hrncir et al., 2004). Given the same preference by newly-recruited *M. panamica* (Nieh, 2004), it is likely that both experienced and newly arriving *M. quadrifasciata* foragers orient towards nestmates’ scent marks within a patch. Use of scent marks to identify rewarding food sources is likely ancestral to the corbiculate bees; bumblebees and honeybees also show attraction to *ad libitum* feeders (reviewed in Saleh and Chittka, 2006). *Melipona*

quadrifasciata foragers may use context to interpret scent marks, with bees learning to associate marks with an *ad libitum* or a depleting resource. Bumblebees appear to use “footprints” in this manner (Saleh and Chittka, 2006), avoiding slowly-replenishing food sources on which recent visitors have walked. Footprints secreted from the claw retractor tendon are the source of attractive marks in *M. seminigra* (Jarau et al., 2004), thus may be used by our study species. Further work is required to identify the source and properties of *M. quadrifasciata* scent marks.

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Note scientifique sur le dépôt par les butineuses d’abeilles sans aiguillon de marques odorantes attractives qui ne donnent pas d’indication sur la source de nourriture.

Eine wissenschaftliche Notiz: Sammlerinnen einer Stachellosen Biene ohne Futterquellenkommunikation hinterlassen attraktive Duftmarken.

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Chapter 2

Behavioral suites mediate group-level foraging dynamics in communities of tropical stingless bees

Behavioral suites mediate group-level foraging dynamics in communities of tropical stingless bees

E. M. Lichtenberg · V. L. Imperatriz-Fonseca · J. C. Nieh

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Abstract Competition for floral resources is a key force shaping pollinator communities, particularly among social bees. The ability of social bees to recruit nestmates for group foraging is hypothesized to be a major factor in their ability to dominate rich resources such as mass-flowering trees. We tested the role of group foraging in attaining dominance by stingless bees, eusocial tropical pollinators that exhibit high diversity in foraging strategies. We provide the first experimental evidence that meliponine group foraging strategies, large colony sizes and aggressive behavior form a suite of traits that enable colonies to improve dominance of rich resources. Using a diverse assemblage of Brazilian stingless bee species and an array of artificial “flowers” that provided a sucrose reward, we compared species’ dominance and visitation under unrestricted foraging conditions and with experimental removal of group-foraging species. Dominance does not vary with individual body size, but rather with foraging group size.

Species that recruit larger numbers of nestmates (*Scaptotrigona* aff. *depilis*, *Trigona hyalinata*, *Trigona spinipes*) dominated both numerically (high local abundance) and behaviorally (controlling feeders). Removal of group-foraging species increased feeding opportunities for solitary foragers (*Frieseomelitta varia*, *Melipona quadrifasciata* and *Nannotrigona testaceicornis*). *Trigona hyalinata* always dominated under unrestricted conditions. When this species was removed, *T. spinipes* or *S.* aff. *depilis* controlled feeders and limited visitation by solitary-foraging species. Because bee foraging patterns determine plant pollination success, understanding the forces that shape these patterns is crucial to ensuring pollination of both crops and natural areas in the face of current pollinator declines.

Keywords Aggression · Dominance · Group foraging · Species removal · Superorganism

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Introduction

The availability of rich resources such as mass-flowering trees is important in shaping foraging behavior of tropical pollinators (Roubik, 1989; Wilms et al., 1996). Such resources attract a high diversity of visitors (Heithaus, 1979), and can be fiercely contested (Roubik, 1980; Nagamitsu and Inoue, 1997). Foraging shifts resulting from competitive interactions (e.g. Inouye, 1978) may alter pollination dynamics (Roubik and Villanueva-Gutiérrez, 2009). For social insects, intense inter- and intraspecific competition (Johnson and Hubbell, 1974; Hölldobler and Wilson, 1990; Dornhaus and Chittka, 2004) should favor strategies such as cooperative group foraging that improve foraging efficiency and resource defense. Group foragers are those who forage in the same location as nestmates.

They often use information provided by group members to locate food sources.

When animals compete for food, larger species tend to dominate (Schoener, 1983; Eccard and Ylönen, 2003) in both direct (e.g. interference competition) and indirect (e.g. exploitative competition) contests. For social animals, however, foraging in groups can improve yield through shared food location information (Clark and Mangel, 1984), increased hunting success (Bednarz, 1988), retrieval of larger food items (Traniello and Beshers, 1991), control of food (Holway and Case, 2001) or more efficient harvesting (Fernández-Juricic et al., 2004). Group foraging may be particularly important for highly social insects whose colonies act as “superorganisms” (Wilson, 1990), reproductive units whose parts, individuals, must work together to permit colony survival and reproduction. Thus, superorganism size (group size) may be more relevant than individual size for determining the outcome of dominance interactions.

Stingless bees (Hymenoptera, Apidae, Meliponini) provide a good system for studying the ecological importance of group foraging. All stingless bees are eusocial, but some species forage as individuals while others tend to forage in large groups (Johnson, 1983). These groups typically form through location-specific recruitment via odor trails or potentially referential vibrations (Nieh, 2004). Foraging strategies are likely constrained by colony sizes, which range from approx. 100 (van Veen et al., 1997) to at least 20,000 workers (Roubik, 1983). Stingless bee within-habitat diversity can range up to 62 species (Roubik, 1989) with considerable diet overlap (e.g. Wilms and Wiechers, 1997; Eltz et al., 2001). Limited food availability (Hubbell and Johnson, 1977; Eltz et al., 2002) can thus lead to high levels of both intra- and interspecific competition (Hubbell and Johnson, 1977; Nagamitsu and Inoue, 1997; Slaa, 2003).

Despite these bees’ important role as tropical pollinators (Heard, 1999), the ecological importance of stingless bee foraging strategies remains poorly understood. Several researchers have hypothesized that group foraging improves dominance for stingless bees (Johnson and Hubbell, 1975; Roubik, 1980; Nagamitsu and Inoue, 1997; Slaa, 2003). However, there are few tests of these hypotheses and no studies directly manipulate dominance by altering experimental conditions (e.g. species removal). In addition, most studies of stingless bee foraging behavior have focused primarily on aggression, comparing resource control of “aggressive” versus “unaggressive” species (Biesmeijer and Slaa, 2004 and sources therein). While aggression is a commonly proposed mechanism of interference competition (Reitz and Trumble, 2002), other traits also permit species to control or efficiently exploit a resource. For example, the stingless bee *Partamona*

orizabaensis (formerly *P. aff. cupira*, Pedro and Camargo, 2003) is “non-aggressive” (Biesmeijer and Slaa, 2004) yet in large groups can maintain control of a resource despite attack by *Trigona silvestriana* (Howard, 1985).

We experimentally altered an assemblage of stingless bees foraging at an array of feeders to investigate stingless bee dominance and foraging on a resource accessible to multiple species. We measured behavioral dominance, numerical dominance, displacement success and aggression of six Brazilian species. We tested three hypotheses: (H1) group foragers are dominant, as is found for other social insects; (H2) body size correlates with dominance (Johnson and Hubbell, 1974); and (H3) removal of group-foraging species increases feeding opportunities for remaining species. Finally, we examined the role of aggression in resource dominance.

Methods

Study site and species, and feeder array

This study was carried out at the Fazenda Aretuzina, a ranch in the state of São Paulo, Brazil, during July of 2006. This area is home to at least 12 native stingless bee species (P. Nogueira-Neto, pers. comm.). Colonies of several species were also kept in hives at the Fazenda.

We selected six species that span a broad range of foraging strategies, colony sizes, body sizes and aggression levels (based on similarity with congeners described by Biesmeijer and Slaa, 2004). These species also show overlap in plant species utilization (Table S1 in Electronic Supplementary Material): *Frieseomelitta varia* (Lepelletier, 1836), *Melipona quadrifasciata* (Lepelletier, 1836), *Nannotrigona testaceicornis* (Lepelletier, 1836), *Scaptotrigona aff. depilis*, *Trigona hyalinata* (Lepelletier, 1836), and *Trigona spinipes* (Fabricius, 1793). *Trigona* species were from wild colonies, each estimated to be 200–400 m from the feeder array and in opposite directions (Fig. S1 in Electronic Supplementary Material). The other four species occupied nest boxes dispersed in a meliponary occupying approximately 1 ha, at a density similar to that found under natural conditions (Antonini and Martins, 2003). We trained one colony of each species (von Frisch, 1967) to an artificial feeder array approximately 50 m from the center of the meliponary. Table 1 lists characteristics of the study species. Head widths were measured for 38–40 individuals (two to four colonies) of each species using a Leica M16 microscope with Leica camera attachment (model DFC500). Colony size estimates are based on reliable published data. We used descriptions of bees foraging on natural food sources to characterize foraging strategies, based on a functional definition that considers numbers of

Table 1 Characteristics of bee species studied

Species	Head width (mm)	Average colony size (# workers)	Recruitment mechanism	Group forager?	Estimated flight range (m)
<i>F. varia</i>	2.33 ± 0.06	875 (Tóth et al., 2004; Nunes et al., 2008)	SA?	No (Jarau et al., 2003)	705
<i>M. quadrifasciata</i>	3.89 ± 0.11	715 (Roubik, 1980; Wille and Michener, 1973; Tóth et al., 2004)	SM (Lichtenberg et al., 2009), 3-DS?	No (Kerr, 1994)	2000 (Kerr, 1996)
<i>N. testaceicornis</i>	1.90 ± 0.03	1,750 (Lindauer and Kerr, 1960; Jarau et al., 2003)	SM (Schmidt et al., 2005)	No (Jarau et al., 2003)	468
<i>S. aff. depilis</i>	2.69 ± 0.04	6,000 (Ramalho, 1990; Jarau et al., 2003)	OT (Schmidt et al., 2003)	Yes (Jarau et al., 2003)	903
<i>T. hyalinata</i>	2.81 ± 0.04	15,000 (D. W. Roubik, pers. comm.)	OT (Nieh et al., 2003)	Yes (Roubik, 1980)	969
<i>T. spinipes</i>	2.79 ± 0.05	5,500 (Wille and Michener, 1973)	OT (Lindauer and Kerr, 1960)	Yes (Cortopassi-Laurino and Ramalho, 1988)	840 (Kerr, 1959, <i>T. spinipes</i> = <i>T. ruficrus</i>)

Recruitment mechanisms are: three-dimensional communication of food location without the use of an odor trail (3-DS), odor trail deposited along visually prominent landmarks between the food source and the nest (OT), sounds and agitation inside the nest that do not indicate food source location (SA), and scent marking of the food source (SM). Flight ranges with no citation were estimated from van Nieuwstadt and Ruano Iraheta (1996)

nestmates visiting the same food source rather than on recruitment. Species whose colonies can forage in large groups at the same spatial location were categorized as group foraging. Those whose workers forage as solitary individuals at different spatial patches are solitary foraging. Many group-foraging species will not permit non-nestmate conspecifics to forage in close proximity (Johnson and Hubbell, 1974; pers. obs.), thus large groups of these species are generally foragers from one colony.

Feeders consisted of yellow 1.5 mL Eppendorf tubes from which four capillary tubes protruded by 1–2 mm (Figs. 1, S2). Each tube rested in a white nylon washer upon which bees stood when feeding and interacting.



Fig. 1 Artificial “flower” used in this experiment, with two feeding *S. aff. depilis* foragers

Sixteen feeders were suspended from a 15 m × 15 m grid, and were spaced every 5 m. This created a resource that was easily exploitable by all study species, despite differences in tongue length and body size. We filled feeders with 2.5 M unscented sucrose solution during training and 1.5 M unscented sucrose solution during experimentation, providing sucrose ad libitum.

Data collection

We monitored the feeder array in 5-min periods, observing from 0900 to 1146 (morning trials) or 1300 to 1546 (afternoon trials). Stingless bees show activity peaks at different times of day (Roubik, 1989). Observation during both morning and afternoon thus allowed us to study interactions over a broad time span. We began observation after sunrise because, during austral winter, chilly early morning temperatures delay foraging activity of many stingless bee species (Hilário et al., 2000). During non-removal trials (see below), each of four observers rotated among four feeders, moving sequentially down a row and then returning to the beginning of that same row. Movement between feeders occurred during 1-min pauses between observation periods.

To assess interspecific effects on foraging, we used aspirators to remove group-foraging species from feeders. We removed (1) *T. hyalinata*, (2) *T. hyalinata* and *T. spinipes*, (3) *T. hyalinata* and *S. aff. depilis* or (4) *T. hyalinata*, *T. spinipes* and *S. aff. depilis*. In all trials, we removed *T. hyalinata* because this species dominated the entire feeder array whenever it was present. For each removal combination and for the non-removal treatment,

we conducted one morning and one afternoon trial. Aspirated bees were released away from the feeder array at the end of each trial. Because removal requires constant attention to feeders, we used four feeders (one per observer) during removal trials. Observers did not move during the removal trials, but continued to implement the 1-min pause between 5-min observation periods. Observer row assignments and feeder positions within each row were randomly assigned. Non-removal and removal trials were interspersed with each other, and with several days during which data were not collected, across 10 days.

Each observer recorded the species visiting the focal feeder and all interspecific interactions. Feeders were also videotaped during observation periods, and bee interactions were verified from the video. We did not individually mark all bees because doing so would have disrupted recruits and altered results. Thus, we recorded the maximum number of bees simultaneously feeding during each period for each species rather than the total number of visits.

For each interspecific interaction that occurred during an observation period, we recorded (1) species identity and number of individuals, (2) interaction initiator, (3) interaction outcome and (4) intensity of aggression. Displacement was considered aggressive when one individual directed movement toward another bee that could cause injury (e.g. spreading mandibles or biting), or that potentially increased the aggressor's apparent size (e.g. wing or leg spreading). We defined non-aggressive displacement as the rapid departure of a bee when another bee arrived but showed no evident aggression. An individual won an interaction if her opponent moved away immediately after the encounter.

Data analysis

We calculated three measures of dominance and one index of aggression for each species. (1) *Behavioral dominance* indicates a colony's ability to control a resource. We determined the number of turnovers in favor of each species, a turnover being defined as a change in the species makes up at least 50% of individuals at a feeder. Behavioral dominance was weighted to adjust for the number of

trials each species was present at the array. (2) We use *numerical dominance* to indicate local abundance at the array. For each trial, we determined the largest number of bees visiting the array during a single observation period. Behavioral and numerical dominance were calculated separately for non-removal and removal trials. (3) For each species, we calculated displacement success—the ability to win fights—as the proportion of displacement interactions (aggressive and non-aggressive) won during non-removal trials. This measure is comparable to “dominance” of species where contests occur between individuals rather than groups (e.g. Dingemanse and de Goede, 2004; White et al., 2007). (4) *Attack probability* is the number of aggressive displacement interactions that each species initiated as a proportion of the total number of such interactions in which it was involved (Catlett, 1961). To more accurately represent species aggression, attack probability includes interactions from all trials. Due to the non-parametric nature of several indices and the fact that we compare species rather than individuals, we are sometimes limited to describing the effects of removal rather than using statistical tests. Analyses were conducted in R v. 2.8.1 (R Development Core Team, 2008).

Results

Species dominance patterns

Table 2 shows dominance values and ranks in non-removal trials for each species. *Trigona hyalinata* was clearly the dominant species, both behaviorally and numerically. All feeders were completely controlled by *Trigona hyalinata* at the end of each non-removal trial. *Scaptotrigona* aff. *depilis* and *T. spinipes* occasionally behaviorally dominated individual feeders before expulsion by *T. hyalinata*, but the remaining three species never did. *Scaptotrigona* aff. *depilis* was relatively abundant at the feeder array, maintaining on average a maximum of 11 bees/trial. The remaining species averaged between 0.5 and 3 bees/trial.

As predicted by H1, group-foraging species, which had larger colonies (Table 1), ranked above solitary-foraging

Table 2 Dominance and aggressive index values (and ranks) calculated for each species

Species	Behavioral dominance	Numerical dominance	Displacement success	Attack probability
<i>F. varia</i>	0.00 (5)	2.50 (4)	0.300 (4)	0.26 (5)
<i>M. quadrifasciata</i>	0.00 (5)	2.00 (5)	0.000 (5.5)	0.06 (6)
<i>N. testaceicornis</i>	0.00 (5)	0.50 (6)	0.000 (5.5)	0.30 (4)
<i>S. aff. depilis</i>	1.00 (2)	11.00 (2)	0.303 (3)	0.80 (3)
<i>T. hyalinata</i>	11.50 (1)	22.50 (1)	0.737 (1)	0.86 (2)
<i>T. spinipes</i>	0.50 (3)	3.00 (3)	0.332 (2)	0.89 (1)

Behavioral and numerical dominance values are for non-removal trials

species in all three dominance measures (Fig. 2a). Because stingless bee nest sizes are better known than foraging behavior, we also determined the relationship between dominance and colony size (Fig. 2b). Species with larger colonies were behaviorally dominant ($r = 0.94$, $N = 6$, $P = 0.005$) but only marginally more abundant at the feeder array ($r = 0.83$, $N = 6$, $P = 0.06$). Contrary to H2, body size did not correlate with either behavioral (Fig. 2c;

$r = 0.33$, $N = 6$, $P = 0.52$) or numerical (Fig. 2c; $r = 0.37$, $N = 6$, $P = 0.50$) dominance. We found no relationship between colony size and body size ($r = 0.03$, $N = 6$, $P = 1$). Probability of winning fights correlated with colony size ($r = 0.84$, $N = 6$, $P = 0.04$), but not with body size ($r = 0.29$, $N = 6$, $P = 0.58$). Thus, group-foraging species with large colonies (H1), but not species with larger worker body size (H2), are dominant.

Effects of species removal

Removal of group-foraging species increased feeding opportunities for the remaining species, supporting H3. All species except *N. testaceicornis* increased behavioral dominance during removal trials (Fig. 3a), yielding a more even spread of turnovers across non-removed species. The per-feeder turnover rate, however, was relatively constant across trials, averaging 0.91 turnovers/feeder without removal and 0.73 turnovers/feeder during removal trials. For all species, numerical dominance increased almost threefold with exclusion of group foragers (Fig. 3b; quasi-Poisson regression: $\chi^2_1 = 7.51$, $P = 0.006$, $e^b = 2.72$). Removing one or two group-foraging species resulted in dominance by a remaining group forager.

Solitary-foraging species are unlikely to show major increases in numerical dominance. Thus, for each treatment we also determined the number of observation periods during which each species fed. This provides a robust measure of species visitation and resource consumption, facilitating comparisons among species with different foraging strategies. All species except *N. testaceicornis* increased visitation in the absence of group foragers (*F. varia*: $\chi^2_4 = 37.01$, $P < 0.0001$; *M. quadrifasciata*: $\chi^2_4 = 28.82$, $P < 0.0001$; *N. testaceicornis*: $\chi^2_4 = 8.76$, $P = 0.07$; *S. aff. depilis*: $\chi^2_2 = 15.03$, $P = 0.0005$; *T. spinipes*: $\chi^2_2 = 17.78$, $P = 0.0001$). Solitary-foraging species benefited most from complete removal of group foragers, and occasionally were able to increase visitation even in the presence of one group-foraging species (Fig. 3c).

Aggression

All species showed some degree of aggression. We observed 499 interspecific displacements of which 59% involved aggression, 94% were one-on-one and 77% were initiated by group-foraging species. Group-foraging species were significantly more aggressive than solitary-foraging species (Table 2; Scheffé's test for proportions, $S = 9.46$, $P < 0.0005$; Zar, 1999). The majority of attacks (75%) were directed toward *M. quadrifasciata* (Table S2). Most interactions involved low levels of aggression, with prolonged

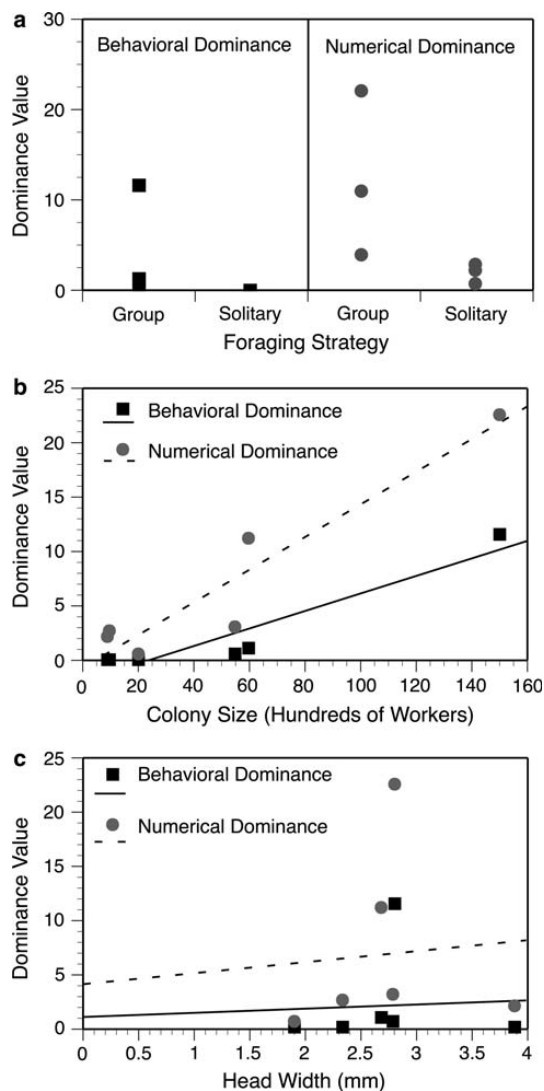


Fig. 2 a Group foragers show higher behavioral and numerical dominance than do solitary foragers. b Dominance increases with colony size. Lines were fit using least squares. c Dominance does not reflect body size. See text for statistics

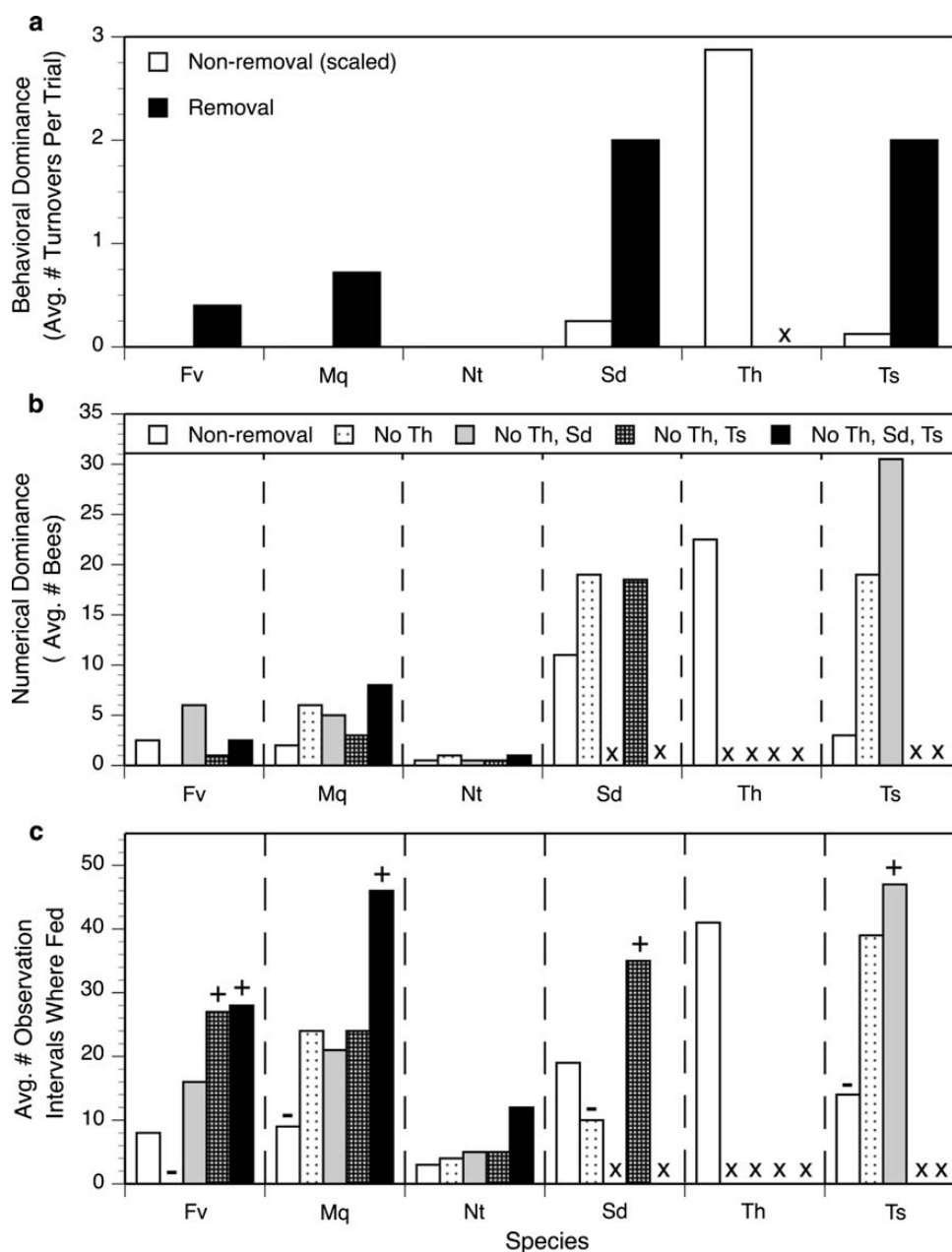


Fig. 3 *Fv*, *Friesomelitta varia*; *Mq*, *Melipona quadrifasciata*; *Nt* *Nannotrigona testaceicornis*; *Sd*, *Scaptotrigona* aff. *depilis*; *Th*, *Trigona hyalinata*; *Ts*, *Trigona spinipes*. **a** Behavioral dominance increases with removal of group-foraging species. Non-removal values are scaled by $\frac{1}{4}$ to account for the different numbers of feeders observed

in removal and non-removal trials. **b** Numerical dominance increases with removal of group-foraging species. A “x” indicates that the species was removed during that treatment. **c** Feeder visitation increases with removal of group-foraging species. “+” and “-” indicate standardized residuals >2 or <-2 , respectively. See text for statistics

fighters occurring only 13 times. Correlations between aggression and dominance were weak at the species level (behavioral dominance: $r = 0.76$, $N = 6$, $P = 0.08$;

numerical dominance: $r = 0.66$, $N = 6$, $P = 0.18$), but stronger at the individual level (displacement success: $r = 0.89$, $N = 5$, $P = 0.03$).

Discussion

We show that stingless bee species that form larger colonies and forage in large groups are able to dominate resources, altering the foraging patterns of displaced bees. Our results strongly suggest that, for highly social superorganisms, group size can have the same ecological role as body size does for non-social species. First, group-foraging species were more likely to control a resource and win individual fights than solitary-foraging species (H1). Second, worker body size did not relate to dominance (H2). Third, experimental removal of group foragers increased feeding opportunities for remaining species (H3). Numerical dominance, behavioral dominance and visitation of all species increased during removal trials. The small increases in dominance of solitary foragers after removal of group foragers enabled these colonies to feed for significantly longer. They thus likely collected more of the resource in the absence of group foragers. These experimental results are consistent with observed patterns of bee floral visitation in a Malaysian dipterocarp forest, where non-aggressive species showed increased visitation in the absence of an aggressive, dominant species (Nagamitsu and Inoue, 1997). Group-foraging species showed larger dominance increases with removal than did solitary-foraging species. However, the success of group-foraging species was not due solely to greater abundance. Feeders were often defended by a single *Scaptotrigona* or *Trigona* forager. Group forager abundance typically increased only after other species were chased away. Aggression facilitated species turnover and the subsequent increase in aggressor abundance. Our results suggest that group foraging is part of a suite of traits that evolved in several stingless bee genera as a mechanism promoting successful foraging in the face of intense competition, which can occur during times of several floral shortage (Roubik, 1989). These traits include large colonies, rapid location-specific recruitment via odor trails and aggression at food sources.

Stingless bees have likely evolved multiple strategies to improve competitive success during dearth seasons. Forming a large, aggressive group at the resource (“thug-gery”) is one strategy. Pronounced mandibular teeth, such as those characteristic of *Trigona* species (Schwarz, 1948), likely improve fighting ability of “thug” species. Some large *Melipona* species may use an alternative strategy (“tenacity”) by continuing to feed despite being the recipients of aggression. We found a high proportion of attacks directed at *M. quadrifasciata*, mainly due to this species remaining at the feeders while being bitten or returning to feeders immediately after being displaced. Very small (2–3 mm long) species likely remain competitive through a third strategy, insinuation (Johnson, 1983).

Insinuator fly away when threatened by dominant species but quickly return to nearby flowers and continue to feed.

Natural context

Aggressive and non-aggressive displacement also occurs on natural food sources. Abundance scans at a *Dombeya wallichii* tree at the Universidade de São Paulo, Ribeirão Preto revealed that *T. hyalinata* was numerically dominant, comprising 80% of bees counted (supplemental Table S3). This high abundance is somewhat surprising given the presence of over 30 honey bee colonies <50 m away. *Trigona hyalinata* bit and aggressively removed other species from flowers (0.08 displacement interactions per observer-minute versus 0.52 at the feeder array). *Trigona pallens* and *Tetragona clavipes* are also known to exhibit low to medium intensity aggression at flowers (Roubik, 1980; pers. obs.), and *Trigona cilipes* low intensity aggression (Roubik, 1980). *Trigona spinipes* (Kerr, 1959), *T. corvina* and *T. silvestriana* (Johnson and Hubbell, 1974) will fight, sometimes to the death, at flowers.

Sugar-providing feeder arrays such as those typically used for bee dominance and aggression studies are somewhat unrealistic in that they are much smaller than mass-flowering trees, important food sources for stingless bees (Endress, 1994; Ramalho, 2004). Feeder arrays may elicit more intense interactions. However, they remain useful because they permit detailed data collection of species identity and behavior.

Body size

Our results do not support H2. Body size was not a major determinant of dominance. In our study, dominant species were medium-sized. However, unlike previous research (Johnson and Hubbell, 1974), we included the *Melipona* genus, whose species have a large and robust body form (Michener, 2007) but do not forage in large groups and are non-aggressive at food sources (Biesmeijer and Slaa, 2004). Dominance studies have typically overlooked *Melipona*, although this genus is commonly found in bee–plant interaction studies (Biesmeijer and Slaa, 2006).

Aggression

Our analyses suggest that aggression can mediate dominance but should not substitute as a measure of dominance. Rather, dominance should be interpreted as the suppression of one species by another (Keddy, 2001). This may arise from aggressive interactions, unequal resource exploitation efficiency or avoidance of a food source on which the dominant species is feeding. Analysis of data from Nagamitsu and Inoue (1997) also supports using ecologically relevant

measures rather than aggression in assessing dominance. In their study, the most aggressively dominant species showed an average decrease in visitation in the presence of other species (supplemental Table S4).

Dominance and group foraging

Changes in dominance reported here and in other studies (Johnson and Hubbell, 1974; Nagamitsu and Inoue, 1997; Eltz et al., 2002; Slaa, 2003) suggest that food competition helps structure stingless bee communities. Feeding opportunities at individual resources increased with removal of group-foraging species. Group foragers typically gained control through aggression, suggesting they excel in interference competition. Solitary foragers exhibited behavioral flexibility, increasing visitation and marginally increasing local abundance in the absence of group foragers. This contradicts the prediction that foraging patterns of less aggressive species reflect floral preferences and will not be altered by removal of aggressive species (Johnson and Hubbell, 1975). Just as individuals may benefit competitively from larger body size in solitary bees (Bosch and Vicens, 2006), social bees may increase dominance with larger superorganism sizes: larger colonies whose workers forage in groups.

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Supplementary online material: figures and tables

Lichtenberg et al., Behavioral suites mediate group-level foraging dynamics in communities of tropical stingless bees

Figure S1: Map of the Fazenda Aretuzina, including locations of colonies used and the feeder array. Nests are shown in brown, roads in black, water in blue, buildings in red and the area where the feeder array was set up in green. The location of the *T. hyalinata* nest was determined through traplining from the directions that bees flew when returning to the nest from different locations.

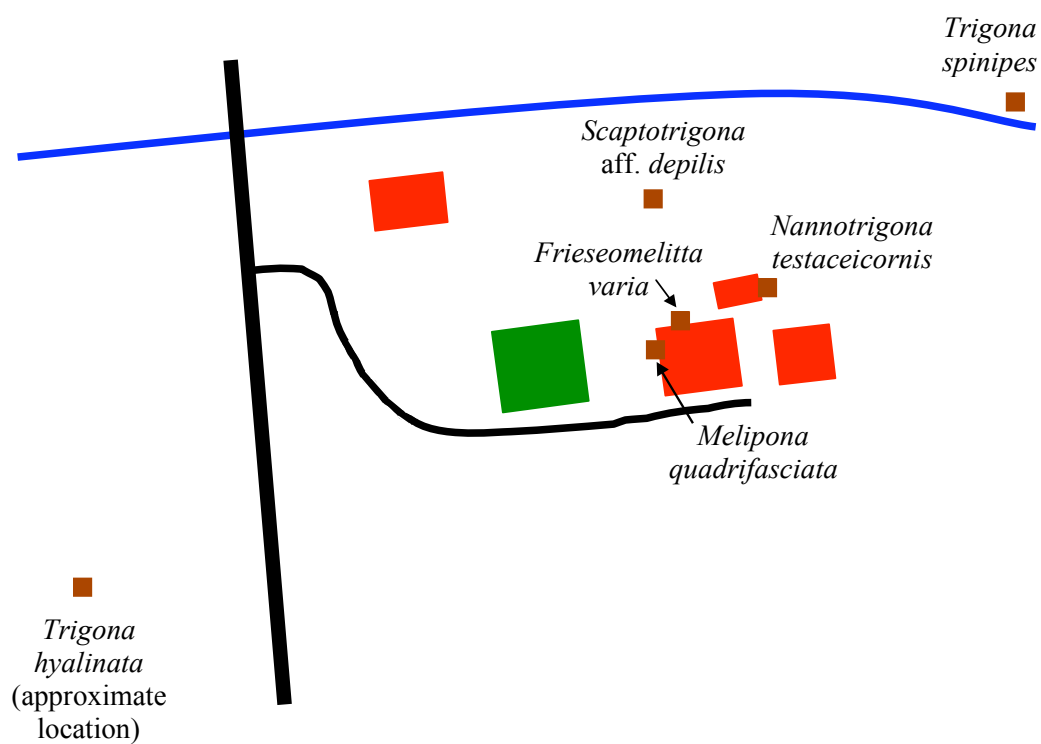


Figure S2: Close-up of the feeders used in this study showing the capillary tubes through which bees fed. A) shows *Melipona quadrifasciata* and b) *Frieseomelitta varia*.

a)



b)



Table S1: Summary of resource overlap between study species, out of 88 plant species used by more than one of the bee species. Due to the limited number of such studies which have been published, this table likely under-represents resource overlap. (Sources: Imperatriz-Fonseca et al.; Carvalho and Bego, 1996; Wilms et al., 1996; Carvalho and Bego, 1997; Viana et al., 1997; Mateus, 1998; Andena et al., 2005; Hrncir, pers. comm.; unpublished data; Menezes et al., 2007).

Species pair	Number of plant species known to be shared
<i>F. varia</i> – <i>M. quadrifasciata</i>	3
<i>F. varia</i> – <i>N. testaceicornis</i>	16
<i>F. varia</i> – <i>S. aff. depilis</i>	4
<i>F. varia</i> – <i>T. hyalinata</i>	7
<i>F. varia</i> – <i>T. spinipes</i>	21
<i>M. quadrifasciata</i> – <i>N. testaceicornis</i>	5
<i>M. quadrifasciata</i> – <i>S. aff. depilis</i>	5
<i>M. quadrifasciata</i> – <i>T. hyalinata</i>	7
<i>M. quadrifasciata</i> – <i>T. spinipes</i>	21
<i>N. testaceicornis</i> – <i>S. aff. depilis</i>	7
<i>N. testaceicornis</i> – <i>T. hyalinata</i>	11
<i>N. testaceicornis</i> – <i>T. spinipes</i>	38
<i>S. aff. depilis</i> – <i>T. hyalinata</i>	8
<i>S. aff. depilis</i> – <i>T. spinipes</i>	12
<i>T. hyalinata</i> – <i>T. spinipes</i>	31

Table S2: Frequency (and percentage of total) with which each species initiated aggressive interactions against other species.

		Recipient					
		Fv	Mq	Nt	Sd	Th	Ts
Initiator	<i>F. varia</i> (Fv)	--	2 (40%)	3 (60%)	0	0	0
	<i>M. quadrifasciata</i> (Mq)	3 (21%)	--	0 (0%)	4 (29%)	1 (7%)	6 (43%)
	<i>N. testaceicornis</i> (Nt)	3 (100%)	0 (0%)	--	0 (0%)	0 (0%)	0 (0%)
	<i>S. aff. depilis</i> (Sd)	1 (0.7%)	138 (95%)	1 (0.7%)	--	2 (1%)	4 (3%)
	<i>T. hyalinata</i> (Th)	0 (0%)	1 (4%)	1 (4%)	20 (83%)	--	2 (8%)
	<i>T. spinipes</i> (Ts)	7 (7%)	82 (78%)	2 (2%)	13 (12%)	1 (1%)	--

Table S3: Species abundance and aggression at a *Dombeya wallichii* tree located at the Universidade de São Paulo – Ribeirão Preto.

Species	Numerical (and percent) abundance	# aggressive interactions initiated
<i>Apis mellifera</i> (Africanized)	22 (10%)	0
<i>Frieseomelitta varia</i>	2 (1%)	0
<i>Nannotrigona testaceicornis</i>	1 (0.5%)	0
<i>Partamona</i> & <i>Scaptotrigona</i> spp.	11 (5%)	0
<i>Plebeia droryana</i>	2 (1%)	0
<i>Trigona hyalinata</i>	177 (80%)	10
<i>Trigona spinipes</i>	2 (1%)	0

Table S4: Aggressive dominance rankings given by Nagamitsu and Inoue (1997, Table 7) and average change in visitation in the presence of other species, calculated from Table 4. In parentheses are species binomials as proposed in a recent catalog of Indo-Malayan and Australasian stingless bees (Rasmussen, 2008).

Bee species	Aggressive dominance	Avg. change in visitation/min
<i>Trigona fimbriata</i> (<i>Homotrigona fimbriata</i>)	1	-0.05
<i>Trigona apicalis</i> (<i>Tetrigona apicalis</i>)	2	0.67
<i>Trigona melina</i> (<i>Tetragonula melina</i>)	2	0.25
<i>Apis koschevnikovi</i>	3	-5.13
<i>Trigona ventralis</i> (<i>Lepidotrigona ventralis</i>)	4	-1.11
<i>Trigona laeviceps</i> (<i>Tetragonula laeviceps</i>)	5	-2.65
<i>Trigona melanocephala</i> (<i>Tetragonula melanocephala</i>)	5	-1.36

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Chapter 3

Olfactory eavesdropping between two competing stingless bee species

Olfactory eavesdropping between two competing stingless bee species

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Abstract Foragers can improve search efficiency, and ultimately fitness, by using social information: cues and signals produced by other animals that indicate food location or quality. Social information use has been well studied in predator–prey systems, but its functioning within a trophic level remains poorly understood. Eavesdropping, use of signals by unintended recipients, is of particular interest because eavesdroppers may exert selective pressure on signaling systems. We provide the most complete study to date of eavesdropping between two

competing social insect species by determining the glandular source and composition of a recruitment pheromone, and by examining reciprocal heterospecific responses to this signal. We tested eavesdropping between *Trigona hyalinata* and *Trigona spinipes*, two stingless bee species that compete for floral resources, exhibit a clear dominance hierarchy and recruit nestmates to high-quality food sources via pheromone trails. Gas chromatography–mass spectrometry of *T. hyalinata* recruitment pheromone revealed six carboxylic esters, the most common of which is octyl octanoate, the major component of *T. spinipes* recruitment pheromone. We demonstrate heterospecific detection of recruitment pheromones, which can influence heterospecific and conspecific scout orientation. Unexpectedly, the dominant *T. hyalinata* avoided *T. spinipes* pheromone in preference tests, while the subordinate *T. spinipes* showed neither attraction to nor avoidance of *T. hyalinata* pheromone. We suggest that stingless bees may seek to avoid conflict through their eavesdropping behavior, incorporating expected costs associated with a choice into the decision-making process.

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Keywords Social information · Interceptive eavesdropping · Decision making · Dominance · Foraging · Cephalic labial glands

Abbreviations

GC-MS Gas chromatography–mass spectrometry
LGE Labial gland extract
w/w Weight/weight

Introduction

Animals at multiple trophic levels actively search for patchily distributed food such as mobile prey, flowering

or fruiting trees, or carrion. Such consumers can improve search efficiency, and ultimately fitness, by using information provided by the food itself or other organisms in the vicinity (Giraldeau 1997; Dornhaus and Chittka 2004). Use of social information (*sensu* Danchin et al. 2004) by foragers appears to be widespread. Information can come from signals (features or behaviors that have evolved to alter the behavior of the receiver in a specific way) or cues, which did not evolve because of such effects (Maynard Smith and Harper 2003). When signals provide such information, unintended receivers that use it are exhibiting “interceptive eavesdropping.” Because signals evolve through selection for information flow, they are vulnerable to selective pressures exerted by eavesdroppers (Peake 2005). Evolutionary and ecological effects of eavesdropping may be particularly strong and diverse in the context of foraging because resultant increases in food discovery efficiency cascade through food webs (Kean et al. 2003). Many examples show predators and prey benefiting from social information to locate prey and avoid predators, respectively (e.g., sources in Stowe et al. 1995; Peake 2005; Seppänen et al. 2007; Valone 2007). However, social information can also improve search efficiency within a trophic level. In this latter context, heterospecific eavesdropping (on signals) and “spying” (using social information provided by cues; Wisenden and Stacey 2005) can affect community structure. Such strategies can (1) increase the frequency of interaction among competitors (Seppänen et al. 2007) or (2) drive the formation and maintenance of foraging groups (Goodale et al. 2010) that provide benefits (e.g., protection) that overcome costs of food sharing (Stevens and Gilby 2004).

Despite the ecological implications of eavesdropping, little is known about how dominant and subordinate species competing for food use social information. To date, only a handful of studies have investigated interceptive eavesdropping on food location or quality signals by heterospecifics. Exploitation of heterospecific food location cues has also received some attention, primarily with social insects. Experiments suggest that subordinate species can avoid competitors (e.g., Pimm et al. 1985; Fletcher 2008; Evans et al. 2009; Slaa and Hughes 2009) or depleted resources (e.g., Nakashima et al. 2002; Yokoi et al. 2007) by using heterospecific visitation signals and cues. This latter phenomenon may be quite sophisticated; bumble bees can learn to be attracted or repelled by social information depending on their past experience with the food source, use it to determine when flowers replenish their nectar, and increase rejection of visited flowers when the visitor was an aggressive species. Avoidance of depleted resources through detection of chemical or visual cues likely saves bees time (Goulson 2009), thereby increasing foraging efficiency. Dominant species, on the other hand, are

sometimes attracted to food cues and signals of heterospecific competitors (reviewed in Slaa and Hughes 2009; Goodale et al. 2010). This diversity of responses suggests the rules governing eavesdropping and spying within a trophic level are more complex than in predator–prey situations and require more sophisticated decision making (Coolen et al. 2003). To understand these rules, and the effects of social information on communities and signaling systems, we must further investigate within-trophic-level eavesdropping and spying across a range of species.

Highly social insects are an excellent system for studying eavesdropping and spying by competitors. Species often compete for food with sympatric relatives (Hubbell and Johnson 1977; Hölldobler and Wilson 1990; Eltz et al. 2002) and exhibit clear dominant–subordinate relationships (e.g., Fellers 1987; Lichtenberg et al. 2010). Social insects combine excellent associative learning (Dukas 2008) with powerful olfactory detection (Greenfield 2002) for successful foraging. Using social information may thus provide fitness benefits through improved search efficiency. Both ants and several genera of eusocial stingless bees (Apidae, Meliponini) recruit nestmates by depositing attractive pheromones at a high-quality food source or as a trail between food and nest (Hölldobler and Wilson 1990; Nieh 2004). Because recruitment pheromones are signals present in the public domain, they are susceptible to eavesdropping. Stingless bee trails may be particularly at risk because the trails are much less heavily guarded than are ant foraging trails (EML, personal observation). Where ant eavesdropping has been observed, it is typically between parabolic “garden ant” species that share nests in epiphytes and thus are highly likely to encounter each others’ trails (Slaa and Hughes 2009).

Research shows that both intra- (Boogert et al. 2006; Jarau 2009) and interspecific (Nieh et al. 2004a) eavesdropping occurs in stingless bees. Coupled with patterns of response to the visual presence of heterospecifics on flowers (Slaa et al. 2003) and anecdotal observations (Kerr et al. 1963; Johnson and Hubbell 1975; Johnson 1983), these studies suggest that stingless bees actively avoid food sources of decreased resource quality or to which they will have limited access. In particular, stingless bees appear to avoid resources occupied by dominant species, thereby steering clear of conflict (the dominance motivation hypothesis). Dominant species may also benefit by following subordinates’ pheromone trails, using this social information to discover high-quality food sources that they can take over. Because stingless bees serve as major pollinators of tropical plants (Endress 1994), eavesdropping interactions between sympatric colonies may significantly affect bees’ foraging patterns and, ultimately, plant gene flow.

Here, we tested olfactory eavesdropping between two trail-laying stingless bee species that have a clear domi-

nance relationship. *Trigona hyalinata* and *Trigona spinipes* overlap in distribution (Camargo and Pedro 2007), exhibit similar floral utilization (Lichtenberg et al. 2010; unpublished data), and likely compete for resources. Both species use odor trails to recruit large numbers of nestmates to rich resources such as mass-flowering trees and sucrose feeders (Nieh et al. 2003; Nieh et al. 2004b). *Trigona hyalinata* foragers easily displace *T. spinipes* from food sources (Lichtenberg et al. 2010), both by arriving at a food source en masse and by attacking individual *T. spinipes* foragers (see Supplemental movies). In *T. spinipes* and all other trail-laying stingless bee species studied to date, recruitment pheromones come from the cephalic labial glands (Jarau 2009). An eavesdropper must both detect the target pheromone and distinguish it from its own. To show that eavesdropping is possible between these two species, we determined the chemical composition and attractiveness to nestmates of *T. hyalinata* labial gland secretions. The pheromone of *T. spinipes* is already known to have one main component: octyl octanoate (Schorkopf et al. 2007). We then tested eavesdropping between these species with preference tests. Under the dominance motivation hypothesis, we predicted that the subordinate *T. spinipes* (Lichtenberg et al. 2010) would avoid *T. hyalinata* recruitment pheromone, while the dominant *T. hyalinata* would be attracted to *T. spinipes* pheromone.

Materials and methods

Study site and colonies

We conducted research at the Universidade de São Paulo, Ribeirão Preto, in southeastern Brazil. This site is in the cerrado ecoregion and provides suitable stingless bee habitat in an otherwise urban and agricultural landscape. Multiple colonies of both *T. hyalinata* and *T. spinipes* inhabit the campus, although only a few colonies were accessible for experimentation because both species tend to nest close to the crowns of trees. We tested eavesdropping between two heterospecific colony pairs that were 100 m (2008 field season) and 500 m (2009) apart, and thus within flight range of each other (Kerr 1959). Only these four colonies could be paired with heterospecific colonies within flight range and were sufficiently low to the ground (<10 m) to allow us to train foragers to feeders.

Bees were trained to visit a training feeder located 10–15 m from their nest (exact distance depended on topography) and providing 15% weight/weight (*w/w*) sucrose solution (0.46 M). One *T. hyalinata* colony was less motivated to visit the feeder and thus was fed 30% *w/w* (0.99 M) sucrose solution. Stingless bees are known to collect nectar ranging from 5% to 67% *w/w* sugar,

with an average nectar quality of 41% (Roubik et al. 1995). We used the relatively weak 15% sucrose solution, which limits overly intense recruitment, because each colony was visiting feeders for at least a month and we did not want nests to become filled with stored honey. To train bees, we placed cotton saturated with sucrose solution at the nest entrance. When necessary, we used a pole and climbed a ladder or the tree to do this. Once bees began feeding on the sucrose solution, we gradually moved the cotton away from the nest entrance and to the final location, ensuring bees followed each move. At the training location, we transferred bees from cotton to feeders. Each feeder consisted of a small inverted jar on a grooved plastic plate (von Frisch 1967). This design provides a constant supply of sucrose solution. Feeders were supported on plastic horizontal surfaces attached to the top of tripods approximately 1 m high. Before each trial, we let bees recruit to their training feeder until there were sufficient foragers (typically 50–200) for meaningful data collection (see below).

Recruitment pheromone chemical analysis

We determined the chemical composition of *T. hyalinata* recruitment pheromone via gas chromatography–mass spectrometry (GC-MS). We dissected labial glands from five foragers from the nest used in 2009 under a stereo microscope, carefully separating the glands from all other tissues. All glands were combined in 300 μ L pure hexane (Labsynth) and dissolved at room temperature for 24 h before being stored in a freezer. To prevent contamination from alarm pheromone or other substances on the bees' cuticles, we rinsed bees five times in hexane before dissection.

We carried out the GC-MS with a Shimadzu QP-2010 GCMS system. The GC was equipped with two different columns: first a DB-5MS (30 m \times 0.25 mm, J&W Scientific, Folsom, CA, USA) and then a more polar DB-WAX column (30 m \times 0.25 mm, J&W Scientific) for better separation of similar-weight compounds. Helium was used as a carrier gas (constant linear flow rate 40.0 cm/s with the DB-5MS column, 39.7 cm/s with the DB-WAX column). With the DB-5MS column, temperature was held at 50°C for 5 min, then increased by 5°/min to 300°C, where it was held for the final 5 min. With the DB-WAX column, temperature started at 50°C, then increased by 3°/min to 240°C, where it was held for the final 5 min. We made preliminary compound identifications using the Wiley mass spectral library (McLafferty 2000), then confirmed all identifications with synthetic standards. Analytical standards' sources were: Sigma-Aldrich Corp. (St. Louis, MO, USA), hexyl octanoate and octyl octanoate; CTC Organics (Atlanta, GA, USA), octyl decanoate; Jocelyn Millar (Riverside, CA, USA), octyl hexanoate; Stefan Jarau (Ulm, Germany), decyl hexanoate and hexyl decanoate.

Preference tests

We assessed bees' responses to con- and heterospecific recruitment pheromones via preference tests, where individuals chose between two feeders each bearing different odors. Before the start of a trial we covered the training feeder, which encouraged bees to search for alternate food sources. Thus, participating bees functioned as scouts, individuals using internal information (knowledge of food types, sensory input, etc.) to locate food sources previously unknown to them (Biesmeijer and de Vries 2001). Preference tests were conducted 20–25 m from the nest and 15–20 m from the training feeder (see Fig. 1). Because bees scout independently and the potential search area increases with distance from the training feeder, new feeders farther from the training feeder will be found more slowly. Thus, we chose distances at which bees would arrive singly, yet frequently enough to gather meaningful data (at least ten decisions) during a trial. We chose a 15-min trial duration, within the 20-min retention time found for *T. spinipes* recruitment pheromone (Nieh et al. 2004b). Training feeders were set out when bees became active in the morning (c. 0900 hours) and trials continued through mid-day. Because the effects of low humidity on recruitment pheromone and bee scouting behavior are unknown, we conducted trials only when the relative humidity was above 50%. Only one colony was tested each day, participating in one to four trials of the various types described below. Solvent control and the treatment trials were interspersed.

At the testing location, we set up two feeders with 40 cm between their centers. This distance was short enough that arriving bees could smell both feeders and allowed simultaneous observation by a single observer. Recruitment pheromones have an active space of approximately 20 m under calm conditions (D. Schorkopf, personal communication). Preliminary trials showed 40 cm was far enough that bees would distinguish between the two feeders and treat them as separate odor sources. Bees arriving at the test location made a choice by landing on one of the two testing

feeders. To avoid any potentially confounding effect of food presence, these feeders were empty. We immediately removed bees once they made a choice to eliminate visual local enhancement: orientation of foragers to the visual presence of other bees (Slaa et al. 2003). These bees were marked with enamel paint, released, and not counted in subsequent trials to avoid pseudoreplication.

Pheromones

Recruitment pheromone sources were (1) fresh pheromone and (2) labial gland extract (LGE). To collect fresh pheromone, we trained one forager of the marking species to visit a feeder with 30% or 45% (1.5 M) sucrose solution (using the higher concentration when recent recruitment levels were low) approximately 10–20 m further from the nest than the training feeder. This feeder bore strips of paper upon which bees readily deposited pheromone by rubbing their mouthparts on the paper's edge while running along it. An odor mark was defined as one such rubbing event. Once the trained bee was observed odor marking and a group of recruits arrived at the feeder (indicating strong recruitment, and corresponding to the "pulses" in Nieh et al. 2004b), we harvested pheromone. We cut off the three to five strips of paper upon which bees had spent the most time odor marking, yielding seven to ten recent marks. The papers were then quickly transported (approximately 2 min in 2008, 10 min in 2009) to the other species' testing location. In 2009, when the two nests were farther apart, we stored the marked papers in a clean glass vial in a cooled container during transport to slow volatilization. These strips were placed in a slit on the platform supporting one of the testing feeders. Clean paper was placed in a slit on the other feeder.

Use of LGE facilitates the application of precise, reliable pheromone doses and yields the same orientation behaviors as naturally deposited pheromone in the three *Trigona* species previously tested (Jarau 2009). We prepared LGE in a manner similar to the sample prepared for chemical analysis, except that each bee's glands were separately dissolved in 100 μL of hexane. For LGE trials, we attached a small strip of filter paper (20 \times 5 mm) to each testing feeder. In solvent control trials testing the effect of hexane, one randomly selected strip remained dry while the other bore 10 μL of hexane. For the treatments described below, we applied 10 μL of LGE to one of the filter paper strips. Ten microliters equaled 0.1 bee equivalents, a concentration to which closely related species respond (Jarau et al. 2004). One bee equivalent is the full labial gland content from one bee. In intraspecific preference tests, we used LGE harvested from the colony being tested. Each replicate trial used LGE from a different individual. We verified that bees' responses to LGE matched behavior exhibited



Fig. 1 General layout of feeders during preference tests. Layout and exact locations used depended on topography, avoiding dense vegetation, steep slopes, roads, buildings, etc. Distances were: nest to training feeder 10–15 m, training feeder to testing feeders 15–20 m, nest to testing feeders 20–25 m, between the *center* of each testing feeder 40 cm

towards fresh pheromone. All equipment was cleaned with lab detergent or ethanol between trials.

Recruitment pheromone glandular source—intraspecific preference tests

T. spinipes produces recruitment pheromone in the cephalic labial glands and will follow an artificial trail made from labial gland extract (Schorkopf et al. 2007). In intraspecific preference tests, we determined whether *T. hyalinata* cephalic labial gland secretions are attractive to *T. hyalinata*. We compared bees' responses to LGE from nestmates to responses in solvent control trials, repeating treatment and control five times per colony. We also tested *T. spinipes* in order to have a common methodology for comparing bees' responses to nestmate recruitment pheromone with the eavesdropping responses described below. The two colonies of each species used in the eavesdropping experiment were also used for this one.

Interspecific eavesdropping

We used preference tests to determine heterospecific eavesdropping responses between *T. hyalinata* and *T. spinipes* under two conditions: (1) In the one-pheromone treatment, bees chose between a dry strip of paper and paper with 10 μ L of heterospecific LGE (five trials per colony) or between a dry strip of paper and freshly deposited heterospecific pheromone (two to three trials per colony). To test the generality of eavesdropping responses, we conducted two additional one-pheromone trials per colony using LGE from bees captured from a different location (approximately 500–1,000 m away). These latter bees were likely from a different colony, and may or may not have been encountered by test colony foragers before experimentation. (2) In the

two-pheromone treatment, bees chose between nestmate LGE and heterospecific LGE (five trials per colony).

Data analysis

For each trial, we determined the proportion of bees landing on the feeder with hexane (solvent control trials), the feeder with nestmate LGE (intraspecific preference tests) or the feeder with heterospecific LGE (eavesdropping trials). All proportions were transformed using Anscombe's arcsine transformation (Zar 1999) to meet parametric assumptions. We assessed responses to nestmate pheromone and eavesdropping with two-factor ANOVAs, including colony as a fixed effect. For each species, we conducted five separate analyses, corresponding to separate questions (see Table 1). (A1) Did the average proportion of bees preferring the feeder with nestmate LGE in intraspecific preference tests differ from the proportion that preferred the hexane feeder in solvent control trials? This tested whether the labial glands are a source of recruitment pheromone. (A2) In the one-pheromone treatment, did bees show the same eavesdropping response to heterospecific LGE and fresh heterospecific pheromone? Due to the small number of trials with fresh pheromone, we used the non-parametric Mann–Whitney *U* test and untransformed proportions for this analysis. (A3) Did bees respond the same to heterospecific LGE from a nearby and a more distant heterospecific colony? This analysis employed Kruskal–Wallis tests and untransformed proportions due to the small number of trials with the distant colonies. (A4) Was there a difference in the average proportion of bees preferring hexane in solvent control trials, heterospecific LGE in the one-pheromone eavesdropping treatment or heterospecific LGE in the two-pheromone treatment? This tested heterospecific eavesdropping responses and was followed by a Tukey HSD test to determine pairwise significant differences

Table 1 Behavioral questions addressed in this study

Question	Treatments compared
(A1) Recruitment pheromone glandular source	Solvent control Nestmate LGE
(A2) Eavesdropping on heterospecific labial gland extract (LGE) vs. natural odor marks	One-pheromone treatment Fresh odor marks
(A3) Response to labial gland extracts from different heterospecific colonies	One-pheromone treatment, nearby colony One-pheromone treatment, distant colony
(A4) Eavesdropping on heterospecific recruitment pheromone	Solvent control One-pheromone treatment Two-pheromone treatment
(A5) Response to own recruitment pheromone in the presence or absence of heterospecific pheromone	Nestmate LGE Two-pheromone treatment (proportion in opposite direction of A4 analysis)

See text for treatment descriptions

if the overall test was significant. (A5) Did the average proportion of bees landing on the feeder with nestmate LGE differ between intraspecific preference tests and the two-pheromone eavesdropping treatment? This tested whether the presence of heterospecific LGE affects bees' preference for their own recruitment pheromone. Interaction terms were removed from all analyses because they were not statistically significant. Analyses were conducted in R v. 2.9.2. Table 2 shows the number of trials conducted for each treatment and the numbers of bees participating in each trial.

Results

Recruitment pheromone chemical analysis

T. hyalinata labial gland extract contained six major components, all carboxylic esters (Fig. 2, Table 3). Octyl octanoate, the major component of *T. spinipes* recruitment pheromone (Schorkopf et al. 2007), was the most abundant component of *T. hyalinata* recruitment pheromone.

Recruitment pheromone glandular source—intraspecific preference tests (question A1)

T. hyalinata LGE was highly attractive to conspecifics, strongly suggesting it is the source gland of recruitment

pheromone for this species. Compared to their responses to hexane in solvent control trials, foragers of each species chose a feeder with nestmate LGE significantly more often than a paired feeder with no odor (A1; Fig. 3; Table 4). Preferences did not differ between conspecific colonies (Table 4). The *T. spinipes* nest we used in 2008 fell from its host tree and died before we completed our experiment, so we were only able to conduct three solvent control trials with this colony.

Interspecific eavesdropping (questions A2–A4)

For both species, response to heterospecific recruitment pheromone did not vary with pheromone source (A2, fresh pheromone vs. LGE: *T. hyalinata*, 29% vs. 34%, $U_{17}=34$, $p=0.39$; *T. spinipes*, 52% vs. 51%, $U_{19}=39$, $p=0.67$), the identity of the colony donating the LGE (A3, nearby vs. far: *T. hyalinata*, 37% vs. 29%, $K_3=3.84$, $p=0.28$; *T. spinipes*, 51% vs. 53%, $K_3=0.62$, $p=0.89$), or the colony being tested (ANOVA results in Table 4). Compared to solvent control trials, *T. hyalinata* strongly avoided *T. spinipes* recruitment pheromone (A4; Fig. 4; Table 4) in both one- and two-pheromone treatments. When choosing between conspecific and heterospecific LGE, the same proportion of *T. hyalinata* foragers preferred their own pheromone as in intraspecific preference tests (A5; Fig. 5; Table 4). *Trigona spinipes* foragers' response to *T. hyalinata* pheromone was the same as their response to hexane (A4; Fig. 4; Table 4). Although *T. spinipes* foragers appeared to choose the feeder with *T. hyalinata* LGE less frequently in the two-pheromone treatment than in the one-pheromone treatment, this difference was not statistically significant ($p=0.06$). However, the presence of *T. hyalinata* pheromone significantly reduced *T. spinipes* preference for their own LGE (A5; Fig. 5; Table 4).

Table 2 Sample sizes and bee participation for each of the treatments, separated by species

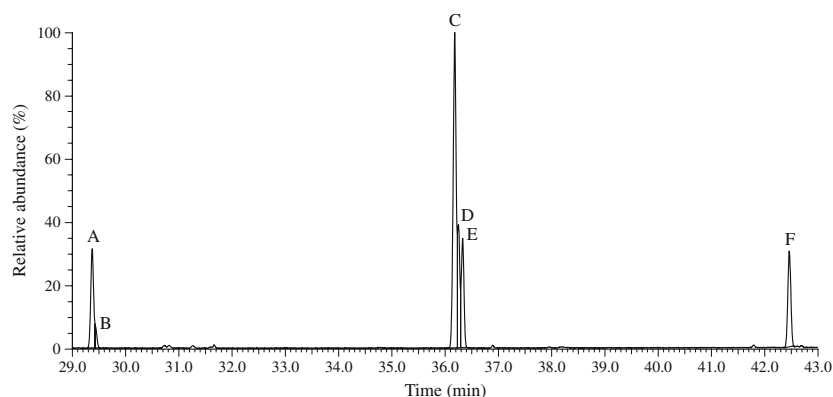
Treatment	Number of trials conducted	Mean number of bees choosing a feeder (min, max)
<i>Trigona hyalinata</i>		
Solvent control	10	20.6 (10, 38)
Nestmate LGE	11	21.2 (13, 28)
One-pheromone treatment		
Nearby colony	9	18.4 (11, 26)
Distant colony	4	15.3 (12, 18)
Fresh odor marks	4	14.8 (5, 24)
Two-pheromone treatment	11	18.4 (11, 32)
<i>Trigona spinipes</i>		
Solvent control	8	20.6 (10, 49)
Nestmate LGE	10	19.7 (11, 34)
One-pheromone treatment		
Nearby colony	10	17.2 (10, 29)
Distant colony	5	14.4 (10, 19)
Fresh odor marks	6	17.7 (12, 30)
Two-pheromone treatment	10	17.5 (12, 25)

The fresh odor marks trial with only five bees was included because death of the marking colony prevented us from repeating that trial

Discussion

Recent evidence suggests that social information use by foragers is widespread, but our understanding of how animals use such information remains limited. Most examples of interceptive eavesdropping and “spying” occur between trophic levels or emphasize copying behavior (reviewed in Dall et al. 2005; Peake 2005; Seppänen et al. 2007; Valone 2007; Goodale et al. 2010). Our results provide the most complete example to date of interceptive eavesdropping by competing social insects: we determined the composition and probable glandular source of the chemical signal, and examined reciprocal heterospecific responses to this signal in preference tests with multiple colonies. We show heterospecific avoidance eavesdropping by a stingless bee: *T. hyalinata* avoids the recruitment

Fig. 2 Gas chromatograph of *T. hyalinata* labial gland secretions (DB-WAX column). Each letter corresponds to one peak (component). Names and details of the chemical components are given in Table 2



pheromone of *T. spinipes*. Among pollinating social bees, this within-trophic-level social information can help foragers avoid unprofitable resources or conflict (e.g., Stout et al. 1998; Nieh et al. 2004a).

Chemical analysis of *T. hyalinata* and *T. spinipes* LGE demonstrates that the pheromones should be (1) detectable by both species because both contain octyl octanoate in relatively high concentrations and (2) differentiable because *T. spinipes* recruitment pheromone consists of one major component while *T. hyalinata* has six (Fig. 2). One of these, hexyl octanoate, is reported for the first time as a component of stingless bee recruitment pheromones. Unlike the pattern reported for other social insects (Slaa and Hughes 2009), eavesdropping responses did not depend on relative dominance of the eavesdropping and signaling species. In our study, the dominant species, *T. hyalinata*, avoided the recruitment pheromone of the subordinate species. *Trigona spinipes* showed no attraction to or avoidance of the dominant species' pheromone. Under the dominance motivation hypothesis, if eavesdropping decisions were based solely on relative dominance, we would have seen attraction by *T. hyalinata* foragers and avoidance by *T. spinipes* foragers to heterospecific recruitment pheromone.

Recruitment pheromones

Four trail-laying stingless bee species (in three genera), in addition to our study species, are attracted to labial gland

secretions (Jarau 2009; Stangler et al. 2009). These results, taken with the identical chemistry of *T. spinipes* LGE and odor marks (Schorkopf et al. 2007), indicate that stingless bees' recruitment pheromones are secreted by the labial glands. Our finding that *T. hyalinata* foragers are strongly attracted to nestmate LGE, and chemical similarity with congeners' LGEs, strongly suggests that recruitment pheromone comes from the labial glands in this species as well.

Trigona hyalinata recruitment pheromone composition is consistent with recruitment pheromones of congeners (Jarau 2009) and other odor-marking stingless bees (Stangler et al. 2009), which contain carboxylic and terpene esters. Octyl hexanoate, octyl octanoate, hexyl decanoate, octyl decanoate, and decyl hexanoate are shared with other species (Jarau 2009; Jarau et al. 2010), while hexyl octanoate is reported for the first time as components of stingless bee recruitment pheromones. Behavior of other *Trigona* species suggests that foragers require the entire blend of chemicals to exhibit natural trail-following behavior (Jarau 2009). Interestingly, the esters found in *T. hyalinata* recruitment pheromone are also found in other glandular extracts thought to have an attractive function. These include the Dufour's gland in *Andrena* (Fernandes et al. 1981; Hefetz 1987), *Dufourea* (Wheeler et al. 1985), and *Svastra* (Duffield et al. 1984) bee species, and mandibular and preputial glands that likely produce sex pheromones in several *Myrmecocystus* ant species (Lloyd et al. 1989) and the Brandt's vole (Zhang et al. 2007), respectively.

Table 3 Major compounds in *Trigona hyalinata* labial gland secretions, determined by GC-MS and comparison with analytical standards

Retention times are for the DB-WAX column

Peak	Compound	Retention time (min)	Relative abundance (%)
A	hexyl octanoate	29.374	13.06
B	octyl hexanoate	29.425	1.92
C	octyl octanoate	36.180	45.46
D	hexyl decanoate	36.249	12.64
E	decyl hexanoate	36.329	13.33
F	octyl decanoate	42.460	13.59

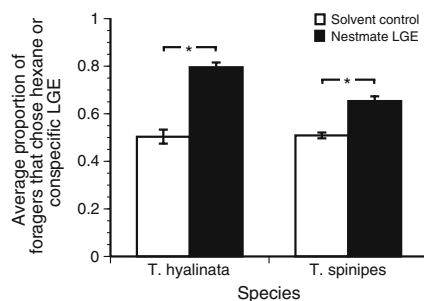


Fig. 3 Responses of *T. hyalinata* and *T. spinipes* foragers to labial gland extract (LGE) from nestmates in intraspecific preference tests, compared to responses to hexane in solvent control trials (question A1). Bars show the average proportion of foragers preferring the given odor (\pm SE). Asterisks indicate statistically significant differences ($\alpha=0.05$)

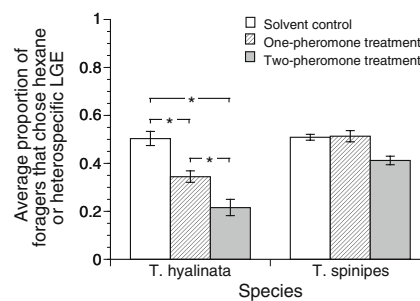


Fig. 4 Interspecific eavesdropping analysis (question A4), comparing preferences for hexane in solvent control trials with heterospecific LGE in the absence (“One-pheromone treatment”) and presence (“Two-pheromone treatment”) of nestmate LGE. Bars show the average proportion of foragers preferring the given odor (\pm SE). Asterisks indicate statistically significant differences ($\alpha=0.05$)

In intraspecific preference tests, *T. spinipes* showed weaker preference (65%) than did *T. hyalinata* (80%). *Trigona spinipes* preferences were also weaker than in other preference experiments (73%, Nieh et al. 2004b; 90%, Schorkopf et al. 2007), while *T. hyalinata* shows greater consistency across studies (81%, Nieh et al. 2003). Three major differences in life history traits between these species could be related to this, although the last two seem less likely. First, *T. spinipes* appear to be highly generalist in their floral visitation, visiting 51% of 562 plant species for

which we have collated stingless bee resource use data. *Trigona hyalinata*, however, were found on only 5% of the plants and seem to specialize on dense floral patches: trees and shrubs (unpublished data). The relationship between floral preference patterns and reliance on social information is not clear and bears further investigation. Second, at the species level *T. hyalinata* are more dominant and aggressive than *T. spinipes* (Lichtenberg et al. 2010). Third, *T. hyalinata* colonies have almost three times the number of workers as *T. spinipes* colonies have (D.W. Roubik,

Table 4 ANOVA results for recruitment pheromone glandular source and heterospecific eavesdropping questions asked in the “Data analysis” subsection of the “Materials and methods” section, separated by responding species

Research question	ANOVA output		
Effect	<i>F</i>	<i>p</i>	<i>df</i>
<i>Trigona hyalinata</i>			
(A1) Recruitment pheromone glandular source			
Trial type	67.28	<0.0001	1, 18
Colony	1.03	0.32	1, 18
(A4) Eavesdropping on heterospecific recruitment pheromone			
Trial type	20.01	<0.0001	2, 30
Colony	1.66	0.21	1, 30
(A5) Response to own recruitment pheromone in the presence or absence of heterospecific pheromone			
Trial type	0.003	0.96	1, 19
Colony	2.62	0.12	1, 19
<i>Trigona spinipes</i>			
(A1) Recruitment pheromone glandular source			
Trial type	37.23	<0.0001	1, 15
Colony	1.54	0.23	1, 15
(A4) Eavesdropping on heterospecific recruitment pheromone			
Trial type	3.19	0.06	2, 29
Colony	0.08	0.78	1, 29
(A5) Response to own recruitment pheromone in the presence or absence of heterospecific pheromone			
Trial type	12.57	0.002	1, 17
Colony	0.004	0.95	1, 17

All interactions were not significant, and were removed from analyses. All analyses in this table used LGE. Statistics for questions A2 and A3 are given in the text

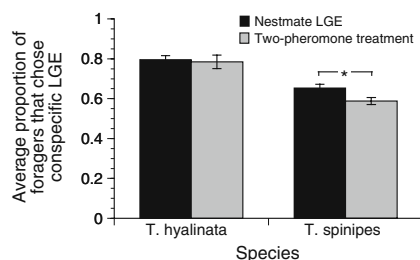


Fig. 5 Responses to nestmate LGE in the absence (“Nestmate LGE”) and presence (“Two-pheromone treatment”) of heterospecific LGE (question A5). Bars show the average proportion of foragers preferring the given odor (\pm SE). Asterisks indicate statistically significant differences ($\alpha=0.05$)

personal communication; Wille and Michener 1973). This is likely related to the dominant status of *T. hyalinata*; species with larger colonies tend to be more dominant (Lichtenberg et al. 2010). It is unlikely that species’ dominance affects degree of reliance on information provided by nestmates, since subordinate *Melipona* species show strong attraction to nestmates’ chemical “footprints” (e.g., 91%; Nieh 1998).

Most previous research on stingless bee recruitment pheromones utilized artificial trails rather than presenting odors at a food source (the feeder), as we did. This raises the possibility that pheromones deposited at the food source differ from those used in odor trails. However, no evidence indicates that stingless bees have two separate recruitment pheromones. Our protocol used the same pheromone-producing gland as artificial trail studies. In one case, chemically analyzed odor marks were collected from the feeder (Schorkopf et al. 2007). In addition, *T. spinipes* and *T. hyalinata* create polarized odor trails, depositing the majority of their pheromone within 1 m of the feeder (Nieh et al. 2004b). Because the trail is an extension of odor marks at the food source, it is reasonable to assume that pheromone deposited on the feeder is the same as trail pheromone, and that the bees obtain the same meaning from our experimental setup and odor trails.

The pheromone concentration that we used, 0.1 bee equivalents, elicited a natural response in a congener, *Trigona recurva* (Jarau et al. 2004), and in our experiments. Eavesdropping responses of *T. hyalinata* and *T. spinipes* were the same whether the treatment was fresh pheromone that had elicited strong natural recruitment or LGE (*T. hyalinata*, 29% vs. 34% of bees choosing the feeder with pheromone; *T. spinipes*, 52% vs. 51%). Each species also showed a highly significant ($p<0.0001$) preference for nestmate recruitment pheromone at 0.1 bee equivalents (Fig. 3; *T. hyalinata*, 80% choosing the feeder with pheromone; *T. spinipes*, 65%). The strength of these preferences was similar to those shown by each species in

preference tests that employed odor trails (Nieh et al. 2003; Nieh et al. 2004b). Greater or lesser amounts of LGE may elicit different eavesdropping responses than those reported here. Predatory ants eavesdropping on fig volatiles exhibit such a dose-dependent response, showing greater attraction to larger quantities of figs (Ranganathan and Borges 2009). However, our results show that 0.1 bee equivalents are sufficient to elicit both conspecific and heterospecific responses that are the same as responses to natural-deposited pheromone at approximately the same concentration.

Interspecific eavesdropping

The limited amount of natural habitat near the laboratory and *Trigona* preferences for nesting high in trees limited the number of colonies that we were able to work with. Despite this, we feel that our results reflect species-typical behaviors. Results were highly consistent across replicate colonies (Table 4), and each colony showed the same response to pheromone from two different heterospecific colonies (A3). This consistency across colonies and months also indicates that a species’ eavesdropping behavior does not vary much, if at all, with the current food needs of the colony.

Trigona spinipes foragers clearly could detect the presence of heterospecific pheromone. Preference for nestmate pheromone decreased significantly, albeit slightly (Fig. 5; going from 65% to 59% choosing the *T. spinipes* LGE; $p=0.002$), when bees chose between conspecific and heterospecific pheromones. Thus, *T. spinipes* does recognize *T. hyalinata* pheromone as different. Despite this detection ability, *T. spinipes* foragers exhibited a behavioral lack of choosiness in eavesdropping tests, showing no preference between feeders with no odor and *T. hyalinata* pheromone (Fig. 4). A similar failure to use social information has been found for three-spined sticklebacks, although the related nine-spined sticklebacks use similar social information (Coolen et al. 2003). *Trigona spinipes* are attracted to footprint cues of the subordinate *Melipona rufiventris* at certain locations (Nieh et al. 2004a), suggesting they have a species- and context-specific response to social information, and heterospecific signals and cues do not always alter their movements. Bumble bees also facultatively use social information, exhibiting visual local enhancement only when approaching unfamiliar flower types (Kawaguchi et al. 2007). Alternately, *T. spinipes* may ignore *T. hyalinata* pheromones when they cannot also see foragers on the marked food source. *Apis mellifera* ignore olfactory information when sufficient visual information is available (Giurfa et al. 1994).

Contrary to our expectation, *T. hyalinata* foragers showed strong avoidance of the subordinate species’ recruitment pheromone (Fig. 4). This result was surprising,

given previous patterns of social information use by social insects (Slaa and Hughes 2009) and the highly dominant behavior exhibited by *T. hyalinata* (Lichtenberg et al. 2010). It is unlikely that our results reflect avoidance of all non-nestmate recruitment pheromones by *T. hyalinata* foragers. Individual *T. hyalinata* foragers will fly to and attack other species at food sources (Lichtenberg et al. 2010). Given that dominant stingless bee species such as *T. hyalinata* appear to be relatively poor at discovering new food sources (the dominance-discovery trade-off, Fellers 1987; Nagamitsu and Inoue 1997), avoiding all non-nestmate odors would severely limit food intake by *T. hyalinata* colonies.

Our findings are consistent with a hypothesis of conflict avoidance through eavesdropping decisions. Attacking to gain control of an occupied resource can inflict mortality losses even for highly dominant species (Johnson and Hubbell 1974; Nieh et al. 2005). The recruitment pheromone we presented to eavesdropping *T. hyalinata* in trials with fresh odor marks and LGE corresponds to the presence of numerous subordinate foragers. In our fresh odor mark trials, we collected marks once a “pulse” (Nieh et al. 2004b) of at least 30 bees arrived at the feeder. Stingless bee trails must be actively maintained by bees interrupting their food collection, and begin to fade out after approximately 20 min without such maintenance. Thus, recruitment pheromones provide current information on both resource availability and abundance of bees already present at the resource. Under such conditions, attack may be costly for a *T. hyalinata* colony because it may require the participation of hundreds of bees, which could otherwise be recruited to non-contested food sources. *Trigona hyalinata* foragers’ decisions to not choose a resource at which social information predicts high numbers of subordinate heterospecifics may be similar to the failure of dominant *Trigona silvestriana* to drive away large numbers of subordinate bees (Johnson and Hubbell 1974). Indeed, while *T. hyalinata* can easily displace a group of foraging *T. spinipes*, they do not attempt to do so every time they encounter the subordinate species (personal observation). This behavior merits further study. One possible explanation is that social insect eavesdropping decisions include expected costs associated with each choice; research that we are currently conducting investigates this.

Different lines of evidence suggest that eavesdropping on signals and spying on cues affect the movements of social bees. First, we have observed *T. hyalinata* depositing odor marks on flowers. Second, feeders are discovered more quickly by other stingless bee species when they bear recruitment pheromone or a large quantity of footprints (Johnson 1983). Finally, interspecific interactions increase between-plant movement of honey bees (Greenleaf and Kremen 2006) and bumble bees (Kawaguchi et al. 2007),

and may do the same for eavesdropping stingless bees. Our results indicate that social information use by competitors is governed by complex rules. Potentially large ecological and evolutionary impacts make this an important area for future investigation.

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Conflict of interest The authors declare that they have no conflict of interest.

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Chapter 4

Value-based decision making via remote assessment of heterospecific pheromones

INTRODUCTION

Value maximization via decision making, in terms of fitness (or energetics as a proxy), profit, or utility, is a key theme of behavioral ecology and economics (Nicholson 2005; Birkhead and Monaghan 2010). This energetic economics view of behavior provides important insights into the rules underlying decision making by animals from cockroaches (Amé et al. 2006) to chimpanzees (Gilby and Wrangham 2007). A large body of literature supports the hypothesis that foraging-related decisions, such as whether to include a specific food type in one's diet or when to leave the current local area (or "patch") and search elsewhere for food, maximize the expected energetic payoff or minimize costs associated with each alternative (reviewed in Ydenberg 2010). Here we investigate how fight costs influence the value of a floral food source for stingless bees. We then determine how remote assessment of resource value based on heterospecific pheromones affects decisions made by individual bees.

Gathering information facilitates assessment of costs and benefits, and enables animals to respond appropriately to variable environmental conditions (Dall and Johnstone 2002). In addition to personal experience, individuals can indirectly assess the environment by monitoring others' behavior (Danchin et al. 2004). One form of indirect assessment, eavesdropping, may be particularly beneficial to decision-makers. Eavesdroppers exploit broadcast signals that have been shaped through evolution to convey specific information (Peake 2005). Foragers signal the location or quality of food sources, but may also inadvertently inform eavesdroppers about which or how many individuals are feeding. Eavesdropping also has strong potential to alter interactions within a community, and to affect evolution of communication systems (Peake 2005).

Animals that actively search for patchily distributed food (e.g. flowering trees, seeds, carrion) could improve search efficiency, and ultimately fitness, via intra-guild eavesdropping on food recruitment signals (Giraldeau 1997). Such signals attract group-mates to newly discovered resources. They are used by a variety of group-foraging organisms including birds, primates, termites, ants, and bees (Bradbury and Vehrencamp 1998). Several genera of the eusocial stingless bees recruit nestmates to rich resources, such as mass-flowering trees, via pheromone trails leading from the food back to the nest (Lindauer and Kerr 1960; Nieh 2004). Because these trails are in the public domain and there is large overlap in floral utilization among stingless bee species (Biesmeijer and Slaa 2006), species that can eavesdrop on neighbors' recruitment trails should improve fitness by gaining more information per unit search effort.

Responses of intra-guild eavesdroppers may depend on the costs associated with the advertized resource. Social learning theory predicts that individuals should copy others' choices when personally sampling the environment is costly, but refrain from doing so if copying incurs a high cost (Kendal et al. 2009). This leads us to predict that eavesdroppers will be attracted to (i.e. copy) or avoid recruitment signals depending on the energetic value of each action. Dominant species, for example, may reduce search costs by showing attraction to subordinates' pheromones, while subordinates could minimize conflict and opportunity costs by avoiding dominants' pheromones (Slaa and Hughes 2009). Such value-based use of social information is exhibited by predatory ants (Ranganathan and Borges 2009).

We previously tested eavesdropping between two trail-laying stingless bee species, and found that eavesdropping responses did not depend on relative dominance

(Lichtenberg et al. 2011). This suggests that cost assessment is based on multiple factors, not simply a species' ability to take over a food source. Here we tested the effects of one alternate criterion – perceived takeover costs – on eavesdropping decisions by the previously-tested dominant species. We first manipulated signaled takeover cost by altering concentration of the subordinate species' pheromone. Second, we developed a decision analysis model to more generally understand the role of takeover costs in decision making by stingless bees searching for new food sources. Such models facilitate analysis of behavioral responses resulting from multiple inputs, and can predict decisions of other animals faced with similar situations.

METHODS

Study site and species

Empirical work was conducted at the Universidade de São Paulo, Ribeirão Preto in southeastern Brazil. This site is in the cerrado ecoregion and provides suitable stingless bee habitat in an otherwise urban and agricultural landscape. Multiple colonies of both focal species, *Trigona hyalinata* (dominant) and *T. spinipes* (subordinate), inhabit the campus. These two species lay odor trails to recruit nestmates to rich resources, overlap in distribution (Camargo and Pedro 2007), exhibit similar floral utilization, and have a clear dominance relationship (Lichtenberg et al. 2010).

Eavesdropping experiment

We tested the eavesdropping responses of *T. hyalinata* foragers across a concentration gradient of *T. spinipes* recruitment pheromone. Each *T. hyalinata* colony was tested with pheromones from two or three *T. spinipes* colonies. Methods follow those

of Lichtenberg et al. (2011). Briefly, we assessed eavesdropping responses through preference tests, in which individuals chose between two feeders each bearing different odors. One feeder presented no odor, while the other had one of four treatments: no odor, 10 μ L of hexane, 10 μ L of labial glands dissolved in hexane (LG), or a specific number of fresh odor marks. Odor marking occurs when a bee deposits recruitment pheromone by briefly rubbing her mouthparts against a substrate (Jarau et al. 2004). Concentrations tested were: 0 (only hexane), 0.05, 0.075, 0.1 and 0.2 bee equivalents (BE); and 0 (no odor), 2, 4 and many (at least 9) odor marks. One bee equivalent is the contents of all labial glands from one bee. Each trial lasted 15 min and included the choices of at least ten bees from a single colony. For each trial, we determined the proportion of bees landing on the feeder with pheromone, hexane or (in blank control trials), an arbitrarily selected feeder. Table 4.1 shows the number of trials conducted for each treatment and the number of bees participating in each trial. Data for 0 BE, 0.1 BE, and many marks include results presented in Lichtenberg et al. (2011) as well as data from an additional *T. hyalinata* colony. Analyses were conducted in R (R Development Core Team 2011).

Recruitment

It has previously been shown that intense *T. spinipes* odor marking is soon followed by the arrival of large numbers of nestmates (Nieh et al. 2004a). To further quantify the information provided by pheromone concentration, we measured the relationship between concentration and *T. spinipes* forager abundance at the food source. One forager was trained to a location 100 m from the nest with 12.5% weight/weight (w/w) sucrose solution (equivalent to 0.375 M), a concentration low enough to avoid recruitment under prevailing natural conditions during our experiment. Once at the final

location, this was replaced with 30% w/w sucrose solution (0.99 M, a concentration that reliably elicited recruitment) and the trained bee was permitted to freely odor mark and recruit nestmates. During the following 40 min we counted the numbers of 1) odor marks placed on the feeder and 2) new recruits, in 5-min time intervals. Each newcomer was given a dot of non-toxic paint on her thorax.

This experiment was conducted five times, using two different *T. spinipes* colonies. Each repetition occurred at a different location, to avoid independent discovery of the food source by bees other than the original trained bee or recruits. For each trial we calculated the 1) cumulative number of bees and 2) total number of active odor marks at each 5-min time interval. *Trigona spinipes* recruitment pheromone has an approximately 20 min retention time (Nieh et al. 2004a), so we calculated the number of active marks as the total number of marks deposited over four time intervals (or since the start of the trial for intervals one through four). To determine the relationship between pheromone concentration and activity at the food source, we found the cross-correlation between the cumulative number of bees and the number of active odor marks in each time interval.

Model

We developed a decision analysis model of *T. hyalinata* intra-guild eavesdropping to test the role of takeover costs in eavesdropping decision making. Decision analysis models integrate uncertainties, values, and preferences to formally address the factors affecting a decision (Howard 1966) and compare the relative value of each decision option (Petitti 2000). They have provided valuable insights in a diversity of fields including medicine (Jackson et al. 2008), management (McAlpine et al. 2010), conservation (Smith 2011), and homeland security (Karvetski et al. 2011). To our

knowledge, this is the first application of decision analysis to behavioral ecology, or to decisions made by non-human animals. The facility with which this type of model handles multi-input decisions and uncertainty about exact parameter values (Petitti 2000) make it ideal for analysis of animal decision-making under natural conditions. One goal of this paper is to demonstrate the utility of decision analysis models for behavioral ecology.

We first developed a model of *T. hyalinata* eavesdropping on *T. spinipes* pheromone, and determined the effect of post-eavesdropping takeover costs on optimal decision making. This involved three key assumptions. One, stingless bee eavesdropping decisions maximize colony energetic net gain. For eusocial organisms like stingless bees, the colony is the unit of reproduction and the target of natural selection. Thus, although eavesdropping decisions are made by individual bees, the responses must reflect effects on the colony rather than the individual. Our model accomplishes this by tracking energetics of the colony as a whole. The second major assumption is that takeover of food sources with more competitors requires greater effort by eavesdroppers (Franks and Partridge 1993; Slaa 2003). Finally, we model the major cost of taking over a food source as the energy expended in recruiting enough nestmates for successful takeover. This assumes that all fight costs reflect metabolism and not physical harm, since interspecific stingless bee fights typically yield low mortality (0 to several bees, Johnson and Hubbell 1974).

The first application of our model was to ask whether these assumptions predicted the eavesdropping decision patterns that we found empirically. To do this we parameterized the model for *T. hyalinata* as the eavesdropper and *T. spinipes* as the

signaler. We ran the model across a fully-factorial set of fight duration and attraction probability (see Table 4.2) combinations, with 1000 repetitions of each combination.

Our model steps through one day (8 hrs) in 5-min intervals, and outputs the colony's net energetic gain or loss at the end of the day. The bee begins the simulated day by searching, and can either find a "contested" food source upon which non-nestmates are feeding, find an available "uncontested" food source, or fail to find food (Fig 4.1, Table 4.2). At a contested food source, the bee will show attraction to the heterospecific pheromone and fight, or will avoid the pheromone and continue to search. This corresponds to the empirically-measured eavesdropping responses. Fights last a specified duration, and represent all assessment, recruitment of nestmates, and interaction with the resident bees involved in a potential takeover event. Fight winners feed with nestmates until the end of the day, while fight losers resume searching. Bees that find an uncontested food source recruit nestmates for a specified duration, after which they feed for the rest of the day. Search, fight, and recruitment costs are based on metabolic expenditure of both active and inactive bees. Energetic benefit comes from nectar collection, which sustains current colony activity. Nectar collection above metabolic costs enables the colony to increase its honey stores and produce more brood (Roubik 1982). Parameter derivations and estimations, including additional data collection, are detailed in Appendix 4.1. The model was implemented in Python (van Rossum et al. 2010). Table 4.2 shows parameter values used for the base *T. hyalinata* analysis.

To describe the effects of fight duration and attraction probability on net benefit, we implemented quantile regression with the R package `quantreg` (Koenker 2010), and visualized regression results via the `lattice` package (Sarkar 2008). Like traditional least-

squares regression, quantile regression estimates values of a response variable conditional on one or more predictor variables. While the former summarizes means, the latter utilizes quantiles such as the median. Quantile regression does not require parametric assumptions such as normally-distributed errors with constant variance (Koenker and Hallock 2001). We chose to employ quantile regression because 1) medians minimize the influence of extreme values, 2) model data were highly skewed, and 3) linear regression residuals did not meet parametric assumptions. Because of our large sample sizes, we used the Frisch-Newton interior point method for model fitting and the Huber sandwich method for inference statistics (Koenker 2010).

From the fitted regression we calculated two descriptors of eavesdropping behavior and its consequences: the attraction-avoidance threshold and the sub-optimal attraction cost. The attraction-avoidance threshold is the fight duration at which increasing attraction probability switches from increasing to decreasing net benefit. It was calculated by setting the partial derivative of the fitted linear model (Eq. 4.1) with respect to attraction probability equal to zero and solving for fight duration. This yielded Eq. 4.2. The sub-optimal attraction cost quantifies the impact of showing attraction above the threshold. We defined attraction as showing an attraction probability of at least 0.65 (based on stingless bee odor preference experiments, e.g. Nieh 2004; Lichtenberg et al. 2009). We then compared the net benefit of runs above and below the attraction-avoidance threshold, calculating average net benefit of each portion of the parameter space via integration of the regression equation.

$$net\ benefit = b_0 + b_1 u_f + b_2 p_{attracted} + b_3 u_f p_{attracted} \quad (4.1)$$

$$threshold = \frac{-b_2}{b_3} \quad (4.2)$$

After verifying that our model captured the behavior of interest, we conducted sensitivity analyses on the probability parameters. First, we asked how altering the probability of winning a fight changes the attraction-avoidance threshold. Dominant species, by definition, should be more likely to win fights. They thus may tolerate longer fights than would a subordinate species. Second, we explored the effects of current competition level on eavesdropping decisions by altering the relative probabilities of finding a contested and uncontested food source (while holding constant the probability of finding food). In each case, we simulated 50 sets of 500 repetitions of each attraction probability-fight duration combination for each value of the winning or encountering contested food probability. Probability values tested are given in Table 4.2. We then regressed attraction-avoidance threshold and (separately) the coefficient of the interaction term on the probability of winning a fight. Attraction-avoidance threshold varied with win probability in a non-linear manner, and also exhibited heterogeneity of variance. We thus analyzed this relationship using the generalized least squares method (Zuur et al. 2009) implemented in the nlme package (Pinheiro et al. 2011), and log-transformed win probability to generate a linear relationship. We clustered standard errors, via the varIdent variance structure, based on assessment of adjacent win probabilities with similar variances (Zuur 2009; Cameron and Trivedi 2010).

Next, we tested whether our model accurately predicted eavesdropping behavior of two other species for which eavesdropping responses have been tested. Nieh et al. (2004b) showed that subordinate *Melipona rufiventris* foragers avoided *T. spinipes*

pheromone. Lichtenberg et al. (2011) found that, when subordinate, *T. spinipes* showed little response to *T. hyalinata* pheromone. We thus re-ran our model parameterized for each of these species pairs (see Table 4.2, Appendix 4.1). Experimental data led us to predict that avoidance would always benefit *Melipona* foragers, and that attraction probability would not alter net benefit for *T. spinipes*.

Finally, we explored the effects of bee traits on the energetics of eavesdropping decisions. Our parameterization method directly linked energetic costs and benefits to several life history and behavioral traits (see Appendix 4.1). This enabled us to translate the known variation in trait values across stingless bee species into variation in model parameters. Using this variation, we assessed sensitivity of the model to each energetic parameter, comparing eavesdropping behavior at the maximum and minimum values indicated by measured trait values. To facilitate comparison across different orders of magnitude and range widths, for each model parameter we computed the arc elasticities of the attraction-avoidance threshold and the sub-optimal attraction cost. Arc elasticity describes the average change in responsiveness between two points, and is calculated as the percent change in one variable with respect to the percent change in another variable.

RESULTS

Trigona hyalinata foragers exhibited a concentration-dependent eavesdropping response to both labial gland extract and fresh odor marks (Fig. 4.2; ANOVA: $F_{7,80} = 40.89, p < 0.0001$). Neither colony ($F_{2,80} = 0.94, p = 0.40$) nor pheromone source ($F_{1,80} = 2.42, p = 0.12$) affected this decision. Post-hoc testing ($\alpha = 0.05$) also permitted calibration of LG with fresh odor marks. The bees did not exhibit a preference when no

or very small quantities (0.05 BE, 2 marks) were present. They were strongly attracted to weak pheromone (0.075 BE, 4 marks), and strongly avoided strong pheromone (0.1 and 0.2 BE, many odor marks). Attraction to weak odor marks persisted for the full 15 min of a trial despite presumed pheromone volatilization (GLMM with logit link, colony and pheromone source as covariates, trial as a random effect: time coefficient = 0.0005 ± 0.04 , $z = 0.01$, $p = 0.99$).

Trigona spinipes recruitment pheromone intensity informed signal receivers of the signaling colony's level of activity at the food source. The number of foragers rose with the number of active odor marks, and continued to rise once pheromone intensity plateaued (Fig. 4.3a). Forager abundance significantly correlated ($\alpha = 0.05$) with the cumulative number of odor marks in both the current ($r = 0.94$) and previous ($r = 0.75$) 5-min time block (Fig. 4.3b).

When parameterized for *T. hyalinata* under conditions similar to those of our experiment, our model found that interspecific eavesdropping is energetically advantageous only when takeover will be quick (Fig. 4.4; Table 4.3). The attraction-avoidance threshold was at a fight duration of 13.79, which corresponds to approximately 65-70 min from resource detection to takeover. Showing sub-optimal attraction to heterospecific pheromone above the attraction-avoidance threshold cost the colony 691.39 kJ. This is equivalent to ~9.5 hours of searching. Avoiding heterospecific pheromone when attraction was optimal (sub-optimal avoidance) yielded a net benefit 747.04 kJ larger than did showing sub-optimal attraction. Thus, incorrectly showing attraction had a more negative impact on the colony than did incorrect avoidance.

Probability of winning fights had a strong, but non-linear, effect on the attraction-avoidance threshold (Fig. 4.5a; $F_{1,448} = 12,303.64$, $p < 0.0001$). The mean threshold varied from 3.60 to 16.49, which was within the empirically supported range (3-30 steps, Table 4.2) for all win probabilities. The interaction between fight duration and attraction probability was always significant. Combined, fight duration and attraction probability more strongly influenced net benefit as probability of winning increased (Fig 4.5b; linear regression: $F_{1,448} = 1,567.5$, $p < 0.0001$, $r^2 = 0.78$). Making the wrong decision was also more costly at higher probabilities of winning (Fig 4.5c; linear regression: $F_{1,447} = 4450$, $p < 0.0001$, $r^2 = 0.91$).

Model behavior was highly influenced by the probabilities of finding contested and uncontested food sources (Fig. 4.6). When the former was at least 0.045 (high competition), average attraction-avoidance threshold was greater than the empirically supported range. The optimal strategy was thus always fighting when uncontested resources were scarce. Low (< 0.01) probability of finding a contested food source yielded high variation in the threshold value. At these low probabilities, fight duration and attraction probability also did not affect net benefit. This reflects the rarity of potential for eavesdropping (marked food sources) under such conditions.

In simulations, *Melipona* foragers maximized fitness by always avoiding *T. spinipes* recruitment pheromone, regardless of fight duration (Table 4.3). Their attraction-avoidance threshold was thus essentially three, the smallest fight duration we employed.

Simulated *T. spinipes* foragers showed a milder effect of fight duration on optimal eavesdropping behavior. Net benefit was significantly affected only by the interaction between fight duration and attraction probability (Table 4.3). However, the influence of

fight duration on optimal eavesdropping behavior was weaker for *T. spinipes* than for *T. hyalinata*. The *T. spinipes* interaction term coefficient and sub-optimal attraction cost (96.98 kJ, ~4.5 h of searching) were only approximately 1/7th the *T. hyalinata* values.

The expanded sensitivity analysis suggested that the attraction-avoidance threshold is relatively insensitive to model parameters other than the probability of winning fights, and that energetics are the main determinant of the sub-optimal attraction cost (Table 4.A3, Fig. 4.7). Altering energetic or duration parameters yielded thresholds of approximately 11 to 16, a much smaller range than seen in the win probability analysis (Fig. 4.5a). Sub-optimal attraction cost, however, showed a large increase when the net energy gain while feeding was large. This energy parameter, in turn, changed the most in response to a change in recruitment intensity (Table 4.A2).

DISCUSSION

This work strongly supports the hypothesis that perceived takeover costs drive stingless bee eavesdropping decisions. *Trigona hyalinata* foragers were attracted to subordinates' recruitment pheromone only when pheromone strength correlated with few subordinates at the advertised resource. Successful takeover of weakly-attended resources should require less time and the assistance of fewer nestmates than are required at food sources marked by high pheromone concentrations (Franks and Partridge 1993; Slaa 2003). Modeling showed that this concentration-dependent eavesdropping enabled *T. hyalinata* foragers to avoid conflicts that imposed a high cost on their colony.

Our model also successfully predicted eavesdropping behavior of non-dominant stingless bee species. Simulated *Melipona* eavesdroppers' optimal strategy was avoidance

regardless of fight duration. This matches the constant avoidance *M. rufiventris* foragers showed despite weakening *T. spinipes* pheromone across 30 min trials (Nieh et al. 2004b). Model results also indicate why *T. spinipes* eavesdropping on *T. hyalinata* pheromone may not show the response expected of a subordinate species. Although simulated *T. spinipes* eavesdroppers had a short attraction-avoidance threshold consistent with frequent avoidance of the dominant's pheromone, their sub-optimal attraction cost was low. This suggests that *T. spinipes* colonies receive approximately equal payoff regardless of individual members' behavior towards a dominant species' pheromone. Value-based eavesdropping by three species that fall at opposite ends of the stingless bee phylogeny (Rasmussen and Cameron 2010) supports Pompilio and Kacelnik's predicted universality of utility-driven preference control (2010).

Sensitivity analyses showed that, in general, the dominance motivation hypothesis (dominants are attracted while subordinates avoid, Lichtenberg et al. 2011) is consistent with cost-effective eavesdropping. Our model predicts that dominant species, with a higher probability of winning fights, tolerate longer fight durations before switching to avoidance of heterospecific pheromones. Thus, experiments are more likely to establish conditions that fall to the left of the attraction-avoidance threshold. The asymptotic increase in attraction-avoidance threshold indicates that it is not necessary to win all fights in order to benefit substantially from heterospecific pheromones. In fact, a colony that wins only half its attempted takeovers will tolerate fights up to just 15 min shorter than a colony that always wins. The degree to which subordinates are affected, on the other hand, depends highly on their takeover ability. Relative dominance is species

specific (e.g. Johnson and Hubbell 1974; Lichtenberg et al. 2010), but also may depend on stage in the reproductive cycle (Johnson et al. 1987).

However, even highly dominant species such as *T. hyalinata* are constrained by the effort required for resource takeover. In fact, highly dominant species, which tend to recruit large numbers of nestmates to food sources, may be more constrained than species with little or flexible recruitment. Species that forage in large groups show wide variation in niche breadth (Biesmeijer and Slaa 2006), suggesting some mass-foraging species are willing to feed on both clumped and disperse flowers (unpublished data) for which nestmate recruitment is not necessary. Linking bee traits to model output, we see that the sub-optimal attraction cost is most sensitive to recruitment intensity. Feeding in larger groups increases the net benefit while feeding, which in turn increases the cost of making the wrong takeover decision as an eavesdropper. This constraint may potentially facilitate resource partitioning, and help explain both the high numbers of dominant stingless bee species often found in sympatry (e.g. Freitas 2001; Brosi 2009) and the overall high stingless bee diversity.

Our findings may help explain the scarcity of heterospecific trail following ants, a group well known for recruitment via odor trails. Ants walk along their trails rather than flying between pheromone-bearing landmarks, and trail strength often correlates with both food quality and activity along the trail (Hölldobler and Wilson 1990). Thus, pheromone-advertised food sources worth investigation by an eavesdropper likely require a high investment in takeover. Just as ant territory size can be constrained by economic defensibility thresholds (Hölldobler and Wilson 1990, p. 403), ant heterospecific trail

following is predicted to be limited by high attraction-avoidance thresholds and sub-optimal attraction costs.

Applying decision analysis to the behavioral ecology of intra-guild eavesdropping has shown the complex influence of perceived takeover costs on stingless bee decision making. Our empirical work has shown that predicting eavesdropping responses of competitors is not as clear-cut as with eavesdropping between trophic levels (e.g predator and prey). Modeling this decision has shown how a bee's species-specific traits, and possibly the current state of its colony, are expected to guide its responses to non-nestmate recruitment pheromones. These responses, in turn, affect plant visitation patterns and pollination success of bee-pollinated plants (Zorn-Arnold and Howe 2007). Improved understanding of intra-guild eavesdropping will thus yield insights into the fundamental link between communication networks and ecosystem services such as pollination networks.

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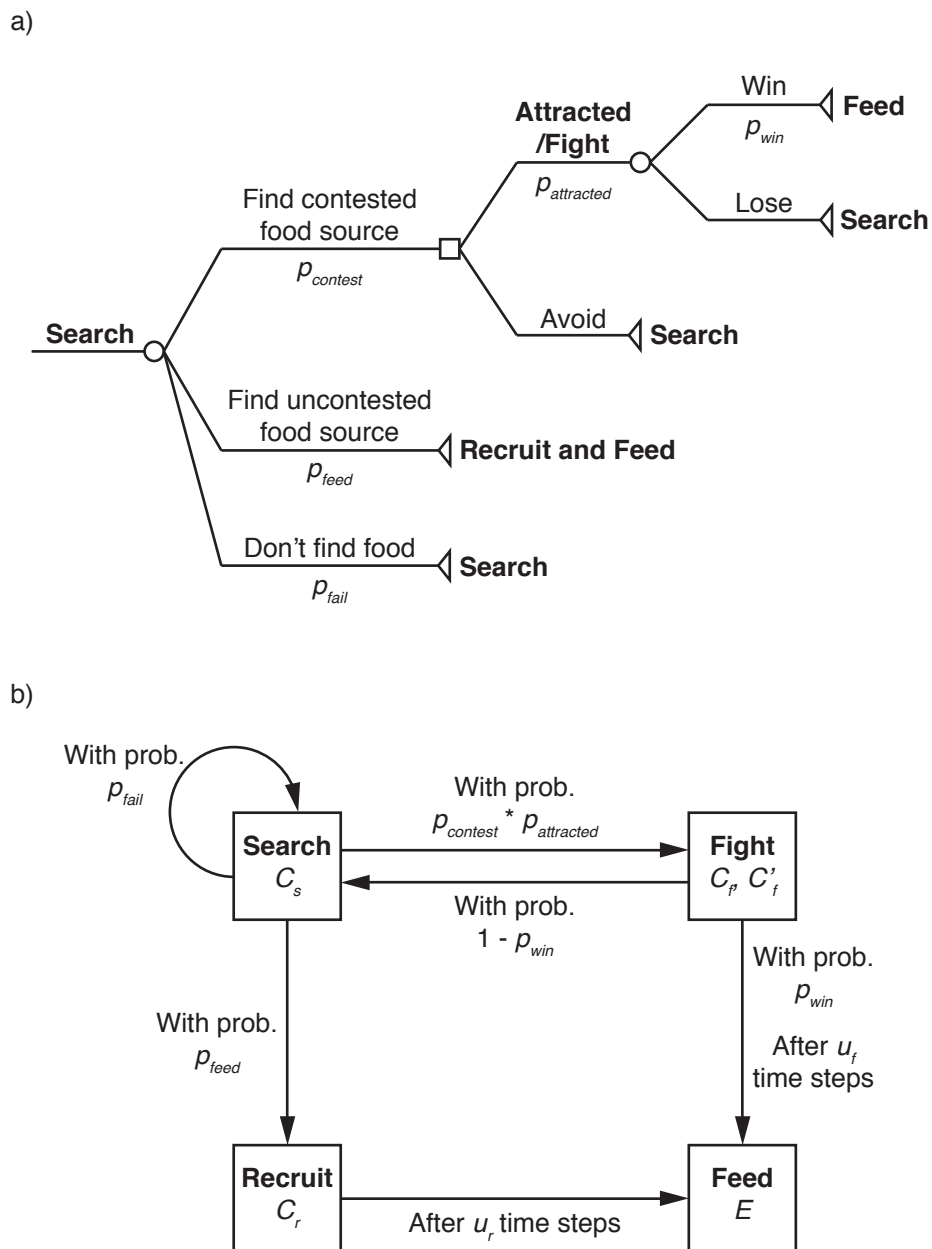


Figure 4.1 a) Decision tree and b) flow diagram for the decision analysis model of stingless bee eavesdropping. In the decision tree, circles indicate chance nodes, squares decision nodes, and triangles end nodes. Behavior states, which are shown in boxes in the flow diagram, are bolded on the decision tree. The flow diagram shows each state and the rules governing transitions between states. Symbols under behavior states are the costs and benefits associated with each state.

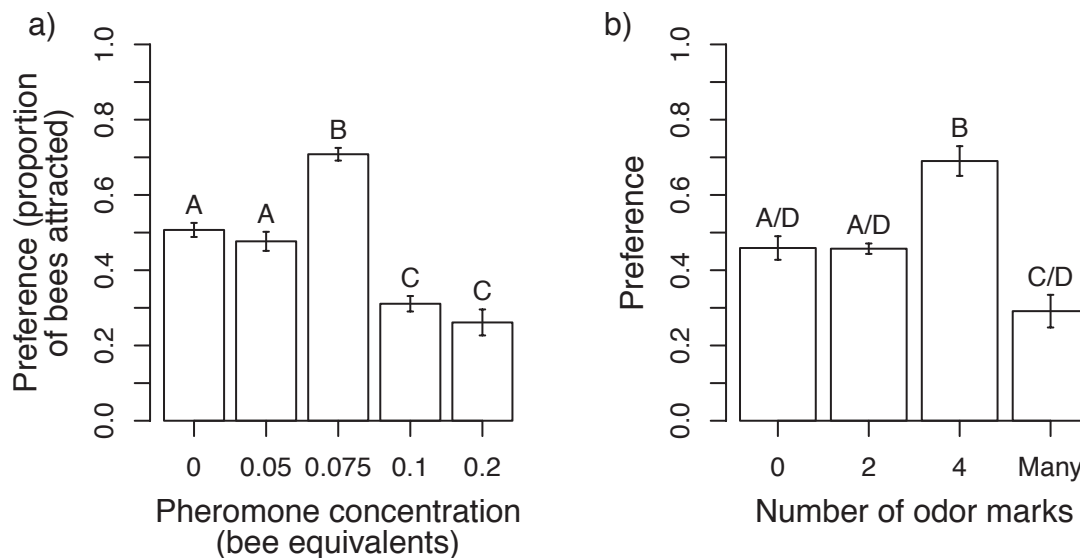


Figure 4.2 *Trigona hyalinata* foragers' eavesdropping responses across a concentration gradient of *Trigona spinipes* recruitment pheromone. Bars show the average (\pm se) preference, measured as the proportion of bees landing on the feeder with pheromone in a 15-min trial. Concentrations with different letters have significantly different means. One bee equivalent is the entire labial gland contents from one bee.

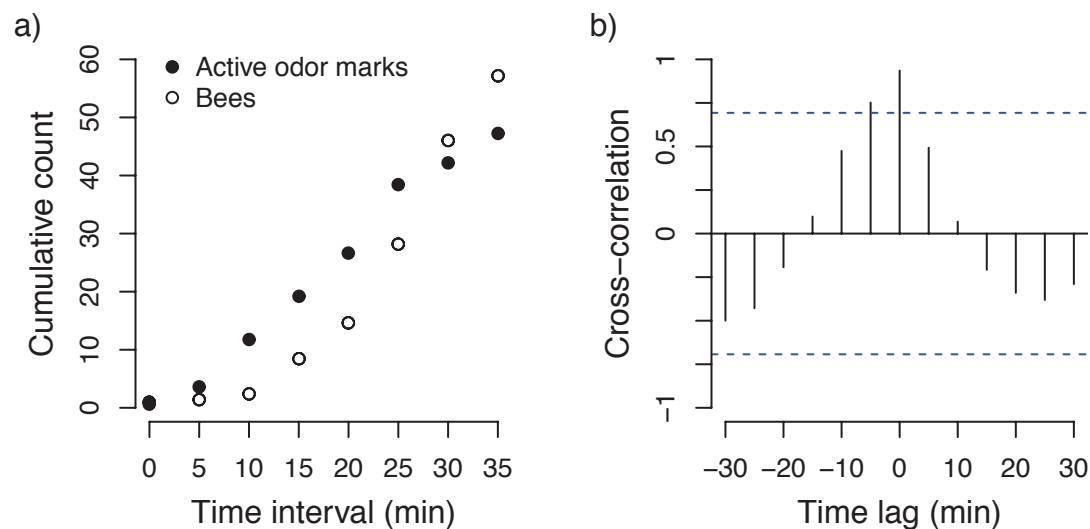


Figure 4.3 a) Build-up of *Trigona spinipes* odor marks and recruits over time, and b) the temporal cross-correlation between mark and recruit numbers. Dashed lines in b) show 95% confidence limits.

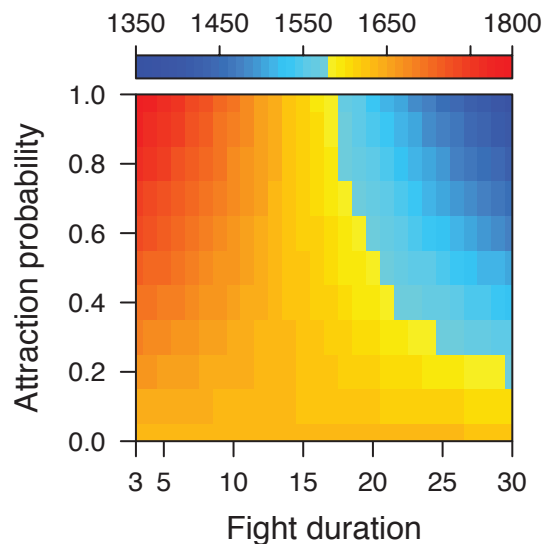


Figure 4.4 Expected net benefit (in kJ) to a *Trigona hyalinata* colony eavesdropping on *T. spinipes* recruitment pheromone, across a range of fight durations and attraction probabilities. Colors indicate the predicted model output at each combination of fight duration and attraction probability. Here, the attraction-avoidance threshold is at a fight duration of 13.79 steps, approximately where the colors shift to light orange.

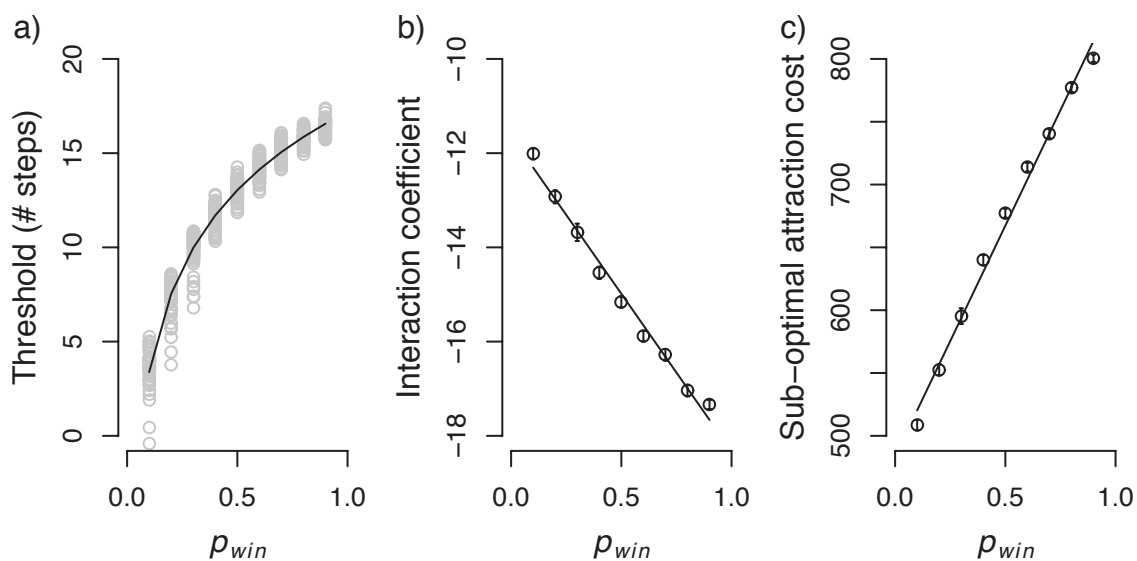


Figure 4.5 As the probability of winning fights varies: a) the attraction-avoidance threshold increases, b) the coefficient of the interaction term in the regression becomes more negative, and c) showing attraction when it would be optimal to avoid becomes increasingly costly. b and c show means \pm se. Curves are from regressions.

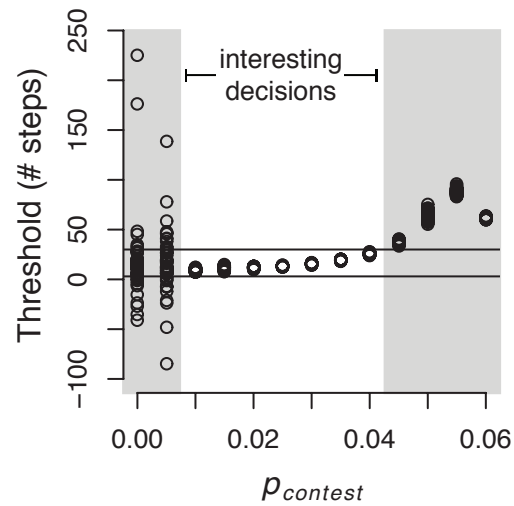


Figure 4.6 The eavesdropping model yields realistic attraction-avoidance thresholds only when the probability of finding a contested food source is between 0.01 and 0.04. Current level of competition increases with $p_{contest}$.

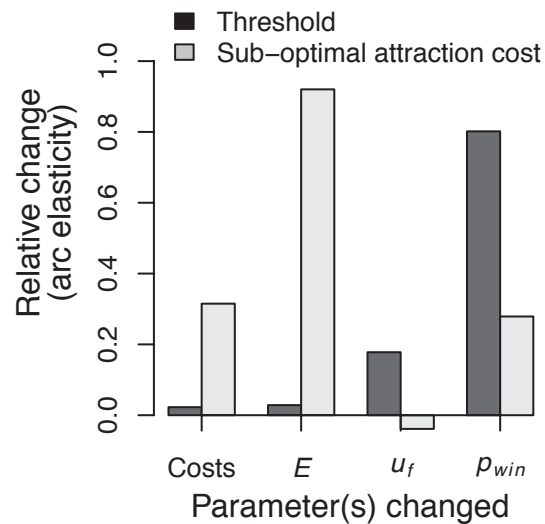


Figure 4.7 Relative effects of altering parameter values on the attraction-avoidance threshold and the sub-optimal attraction cost. Each bar shows the difference in model output between the maximum and minimum parameter values, standardized by the range of those values.

Table 4.1 Sample sizes and bee participation for each treatment.

	Treatment								
	Labial gland extract (BE)					Fresh odor marks (# marks)			
	0	0.05	0.075	0.1	0.2	0	2	4	Many
Trials conducted	16	10	14	19	10	6	6	6	4
Colonies tested	3	2	3	3	2	2	2	2	3
Mean number of choices/trial	19.00	13.60	14.21	16.53	13.20	17.83	14.50	17.67	14.75

BE – bee equivalents (see text for details)

Table 4.2 Parameter values used in the model. See text for further description of the behavior states.

Parameter	Definition	<i>Trigona hyalinata</i> values	Probability sensitivity analyses	<i>Melipona rufiventris</i> values	<i>Trigona spinipes</i> values
Energetics (kJ)					
C_s	Search cost	-6.06 kJ		-0.98 kJ	-1.78 kJ
C_f	Fight cost	-6.06 kJ		-0.98 kJ	-1.78 kJ
C'_f	Fight cost, last fight step	-6.50 kJ		-1.26 kJ	-2.21 kJ
C_r	Recruitment cost	-6.06 kJ		-0.98 kJ	-1.78 kJ
E	Net energy gain while feeding	23.79 kJ		80.28 kJ	32.29 kJ
State durations (# of 5-min intervals)					
u_r	Recruitment duration	2 steps		2 steps	2 steps
u_f	Fight duration	3 – 30 steps		3 – 30 steps	3 – 30 steps
Probabilities (in 5 min)					
$p_{contest}$	Probability of finding a contested resource	0.02	0, 0.005, 0.01, 0.01, ..., 0.05, 0.055, 0.06	0.01	0.01
p_{feed}	Probability of finding an uncontested resource	0.04	0.06, 0.055, 0.05, ..., 0.01, 0.005, 0	0.05	0.05
p_{fail}	Probability of finding no food	0.94		0.94	0.94
$p_{attracted}$	Attraction probability – probability of showing attraction to the contested food source and trying to take it over	0, 0.1, 0.2, ..., 0.9, 1		0, 0.1, 0.2, ..., 0.9, 1	0, 0.1, 0.2, ..., 0.9, 1
p_{win}	Probability of winning a fight	0.5	0.1, 0.2, ..., 0.8, 0.9	0.1	0.25

Table 4.3 Quantile regression outputs, with the model parameterized to match published eavesdropping experiments.

	Coefficient (\pm se)	t value (df=307,996)	p value
<i>Trigona hyalinata</i> base model			
(Intercept)	1632.61 (5.97)	273.57	< 0.0001
u_f	-0.1 (0.33)	-0.29	0.77
$p_{attracted}$	213.18 (9.64)	22.12	< 0.0001
$u_f \times p_{attracted}$	-15.46 (0.58)	-26.5	< 0.0001
<i>Melipona</i> model			
(Intercept)	6423.31 (10.11)	635.34	< 0.0001
u_f	-0.60 (0.52)	-1.14	0.25
$p_{attracted}$	-69.88 (18.79)	-3.72	0.0002
$u_f \times p_{attracted}$	-1.56 (1.10)	-1.42	0.15
<i>Trigona spinipes</i> model			
(Intercept)	2553.56 (4.42)	577.52	< 0.0001
u_f	0.23 (0.24)	0.98	0.33
$p_{attracted}$	9.27 (7.64)	1.21	0.23
$u_f \times p_{attracted}$	-2.32 (0.43)	-5.34	< 0.0001

APPENDIX 4.1: DETAILED DESCRIPTION OF MODEL PARAMETERS

PARAMETER DERIVATIONS

Parameters were derived from bee traits and habitat variables, from our own and published experiments. Values not known for stingless bees relied on honey bee research.

Table 4.A1 Symbols used in parameter derivations.

Symbol	Definition	Value/units
Constants		
α	Honey bee mass-specific resting metabolic rate	5.38×10^{-5} J/s/mg ¹
β	Honey bee mass-specific active metabolic rate	4.23×10^{-4} J/s/mg ¹
γ	Energetic value of 30% weight/weight (0.99 M) sucrose solution	5.68 J/ μ L ²
δ	Sucrose solution density at 20°C	1.13 J/ μ L ³
ϵ	Probability that a searching bee finds a food source	0.06 ⁴
ζ	Proportion of workers that search for food	0.025 ⁵
η	Intercept of the line predicting crop load (sucrose solution imbibed) from body size (fresh weight)	0.91 ⁴
θ	Slope of the line predicting crop load (sucrose solution imbibed) from body size (fresh weight)	0.36 ⁴
Bee traits		
w	Body size (fresh weight, empty crop)	mg
c	Size of the eavesdropping colony	# bees
R	Recruitment intensity - average # nestmates feeding at the same resource	# bees
t_v	Time in flight while collecting food	s
t_e	Time feeding (ingesting nectar)	s
t_n	Time in nest while collecting food	s
c_2	Size of the signaling colony	# workers
n_1	Nest density of the eavesdropping species	nests/ha
n_2	Nest density of the signaling species	nests/ha
Habitat variables		
d	Flowering plant density	plants/m ²
A	Area covered by a searching bee in 5 min	m ²
Intermediate calculations		
I	Average # bee visits to the food source in 5 min	--
L	Gross energy collected on one foraging trip	J
C_e	Gross metabolic costs for one foraging trip	J

Sources are: 1) Schmid-Hempel et al. 1985; 2) Kearns and Inouye 1993; Kleiber 1961 in Seeley 1994; 3) Bubník et al. 1995; 4) See following text; 5) Seeley 1995.

Energetics

Energetic parameters were based on metabolism of active (flying or recruiting) and inactive (inside the nest or ingesting food) bees, and on the energetic value of ingested nectar. All parameter values reflect five minutes of activity.

Search cost was calculated as the metabolism of the one active searching bee, plus inactive bee metabolic rate times the colony's size. We did not subtract the searching bee from colony size, since the latter is an average, and one less bee will not noticeably alter results. Written as a function of bee traits, search cost in kJ is:

$$C_s = -300 w [\alpha (c - 1) + \beta] / 1000$$

Our model divides fighting into two stages. As discussed in the text, fights take place via recruitment of sufficient nestmates to take over the discovered resource. Thus, the majority of time in the fight state is spent recruiting, and incurs the same cost as recruitment (see below). Costs are slightly elevated during the last time step in the fight state. This reflects flight of the bees to the food source and in the vicinity of the food while the subordinates leave (Lichtenberg et al. 2011). Thus more bees are active than during earlier parts of the fight:

$$C'_f = -300 w [\alpha c + R(\beta - \alpha)] / 1000$$

The final step increases costs by:

$$C'_f - C_s = -300 w (\beta - \alpha) (R - 1) / 1000$$

Recruitment covers the time period from food discovery through when recruited nestmates first feed. It involves a greater variety of behaviors than searching, but it remains largely undescribed in sufficient detail for parameterization from first principles. We thus assumed that, on average, recruitment cost equals search cost. It is likely that for

much of the recruitment process the original searching bee is flying and creating an odor trail (pers. obs.), exhibiting excitatory runs inside the nest (Nieh 2004), or inactive. Only during the last minute or two of recruitment are a large number of bees showing high activity.

Net energy gain while feeding includes gross energy collected as nectar (or sucrose solution in experiments), metabolic costs of bees inactive in the nest, and metabolic costs of foragers. Foragers are modeled as exhibiting an active metabolic rate while flying between nest and food source, but an inactive metabolic rate while standing on the food source imbibing nectar and while unloading the nectar inside the nest. Net energy gain can thus be written as:

$$E = [I(L - C_e) - 300\alpha w(c - R)] / 1000$$

To reduce the number of bee traits in the calculation, we determined the relationship between body size and crop load, the quantity of nectar a bee collects on one foraging trip. Using a database of neotropical stingless bee traits maintained by EML, we regressed crop load on body size (Fig. 4.A1; $F_{1,5} = 29.41$, $p = 0.0002$, $r^2 = 0.95$).

Substituting into the above equation and simplifying, we calculated net energy gain as:

$$E = 300 \left\| \frac{R}{t_v + t_e + t_n} \left[w \left(\gamma \theta - \beta t_v \left(1 + \frac{\delta \theta}{2} \right) - \alpha (t_e + t_n) \right) + \gamma \eta - \frac{\beta \delta \eta t_v}{2} \right] - \alpha w(c - R) \right\| / 1000$$

State durations

Bees remain in the fight and recruit states for fixed numbers of time steps.

Recruitment duration was relatively short, representing bees highly motivated to feed. We assumed that fights always last at least one time step longer than recruitment, since fights involve both recruitment and takeover. The analysis systematically varied fight duration

across an empirically-supported range (see below), using only integer values to keep model implementation reasonable.

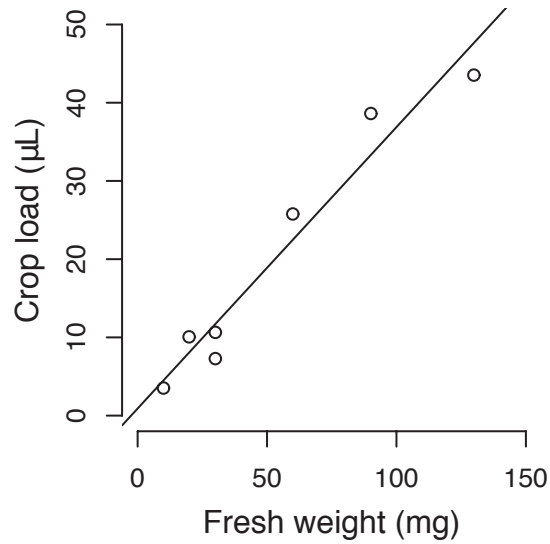


Figure 4.A1 Crop load as a function of fresh weight

Probabilities

Search-related probabilities were calculated from bee nesting traits, floral availability, and search behavior. The probability of finding a contested food source is the probability of encountering food times the probability that food is already occupied. We expanded this to:

$$p_{contest} = \frac{\epsilon^2 \zeta c_2 n_2}{dAn_1}$$

The probability of finding an uncontested food source is the probability of finding food times the probability the food is not occupied, or:

$$p_{feed} = \epsilon - \frac{\epsilon \zeta c_2 n_2}{dAn_1}$$

The probability of not finding food, then, was:

$$p_{fail} = 1 - \epsilon$$

As described in the main text, attraction probability was systematically varied between zero and one. To keep model implementation reasonable, we ran the simulation with attraction probabilities that were multiples of 0.1.

The probability of winning any given interaction depends on many factors including: species' identities, colony sizes, colony stores, current colony growth rate, current floral availability, time of year, and how many food sources a colony is already exploiting (pers. obs.). Since winning probability can thus be highly variable, we originally set it at 0.5. Deviations from this value are discussed in the Methods.

CONSTANT, DURATION, TRAIT, AND HABITAT VALUES

The probability of a searching bee finding a food source (ϵ) was originally treated as variable and calculated for both *T. hyalinata* and *T. spinipes*. For each species, we first determined the average number of bees landing in any 5 min of an eavesdropping trial, by dividing the total number of bees by three (since trials lasted 15 min). We then divided this number by the estimated number of bees present on the training feeder (see Lichtenberg et al. 2011) at the start of a trial, to estimate food encounter rates of scouting bees. Because this number was almost identical for the two species (0.0627 and 0.0605, respectively), and similar data are unpublished for other stingless bee species, we decided to treat this probability as a constant.

Although fresh weight is typically seen as an inferior measure of body size (Cane 1987), it is the standard measure used with bee metabolic values (e.g. Schmid-Hempel et

al. 1985; Wolf et al., 1989; Seeley 1994). For stingless bees it also strongly correlates with measures of sclerotized body parts, such as head width (Spearman's rank correlation: $r_s = 0.87$, $n = 16$, $p < 0.0001$). We measured fresh weight of 40 *T. hyalinata* foragers not carrying nectar or pollen using a Marte AL500 electronic balance at the Universidade de São Paulo, Ribeirão Preto in 2007. Fresh weight averaged 25 mg (± 0.6). We also measured fresh weight of five other species: *Frieseomelitta varia* (11 ± 0.3 mg), *Melipona quadrifasciata* (69 ± 1.2 mg), *Nannotrigona testaceicornis* (6 ± 0.2 mg), *Scaptotrigona* aff. *depilis* (16 ± 0.2 mg), and *T. spinipes* (20 ± 0.3 mg). Head widths for these species are reported in Lichtenberg et al. (2010). Data for ten additional species were taken from the literature (Hubbell and Johnson 1977; Roubik and Buchmann 1984; Ramalho et al. 1998; Roubik 1992; Nieh et al. 2005; Camargo and Pedro 2008; Quezada-Euán et al. 2011). Table 4.A2 shows the minimum, average, and maximum fresh weights reported across stingless bee species.

Table 4.A2 Distributions of traits that affect energetic parameters, and change in net energy gain while feeding across those ranges. Trait values are based on published and our own data. Averages were calculated when data from sufficient species were available, otherwise we show a non-extreme empirical value. Arc elasticity shows percent change in E relative to percent change in the trait.

Trait	Minimum	Average/middle	Maximum	Arc elasticity of E
w	10 mg	35.62 mg	130 mg	1.03
c, c_2	80 workers	3672.9 workers	29,855.8 workers	-3.55 (c)
R	1 recruit	154.92 recruits	1436.5 recruits	91.92
t_v	9.7 s	12.78 s	26.82 s	-5.02
t_e	7.37 s	18.46 s	25 s	-5.34
t_n	16.66 s	60.8 s	63.05 s	-1.74

As described above, we determined the linear relationship between crop load and body size to reduce the number of traits necessary for model parameterization. In 2010,

we measured the crop loads of 100 *T. hyalinata* foragers (from two colonies) collecting 30% w/w sucrose solution at a feeder located 10-15 m from the nest. Individual bees were caught as they prepared to leave the feeder (indicated by cessation of feeding and walking to the edge), and gently encouraged to regurgitate crop contents into a 20 μ L micropipette (Hirschmann[®] Laborgeräte ringcaps[®]). A small dot of enamel paint was then applied to the thorax so that no bee would be measured twice. *Trigona hyalinata* crop loads averaged 10.6 ± 0.22 μ L. Crop loads and fresh weights from seven additional species were taken from the literature (Table 4.A2; Roubik and Buchmann 1984; Hrncir et al. 2004b; Schmidt et al. 2006a; Schmidt et al. 2006b; Schmidt et al. 2008).

Species-specific colony size estimates for *T. hyalinata* and 81 other species were taken from the traits database. For species with more than one published colony size measure, we averaged all known values. Many articles report ranges of colony sizes; for these we took the midrange value. Data (summarized in Table 4.A2) come from personal communications with Meg Eckles (*Melipona panamica* colonies with 542, 346, 678, and 498 workers; *Partamona peckolti* colonies with 565 and 678 workers) and David Roubik (*T. hyalinata*, approximately 15,000 workers), and from the literature (Michener 1946; Lindauer and Kerr 1958; Lindauer and Kerr 1960; Juliani 1967; Wille and Michener 1973 and sources therein; Hubbell and Johnson 1977; Roubik 1983; Sommeijer et al. 1983; Roubik and Buchmann 1984; Sommeijer et al. 1984; Ramalho 1990; Biesmeijer 1997; Nieh and Roubik 1998; Biesmeijer et al. 1999; Cortopassi-Laurino and Nogueira-Neto 2003; Jarau et al. 2003; Nieh et al. 2003; Slaa 2003; Tóth et al. 2004 and sources therein; Aguilar et al. 2005; Hofstede and Sommeijer 2006; Cortopassi-Laurino et al. 2007; Sánchez et al. 2007; Nunes et al. 2008; Camargo and Pedro 2009).

We measured *T. hyalinata* recruitment intensity by training a single forager 25m from the nest then counting the number of recruited bees arriving over the following 3 h. As with *T. spinipes* recruitment quantification, each newcomer was marked with a small dot of non-toxic paint on her thorax, and newcomers were counted in 5-min time intervals. This was repeated three times each with two different colonies, at different locations to ensure arriving bees were recruits rather than reactivated foragers. The median number of recruits per trial was 185 (range 156-550). Because the feeding state includes build-up of bees after the initial group of recruits arrive, for *T. hyalinata* we calculated an average number of recruits present at the food source at any one 5-min time block. First, for each recruitment repetition we calculated the average number of recruits for time durations starting from the fourth step of the model (the earliest bees could feed) and successively adding one step, through the final step. We assumed that forager numbers held steady after 3 h. We then averaged these duration-specific recruit counts across trials, then across durations to obtain a final estimate of 160.70 bees feeding during any one 5-min interval. We repeated this procedure with our *T. spinipes* recruitment data, and obtained an estimated 194.38 bees feeding. Median or mean numbers of recruits were found for 14 additional species (Table 4.A2; Nieh 1998; Jarau et al. 2003; Nieh et al. 2004b; Aguilar et al. 2005; JCN unpublished data).

Lichtenberg et al. (2010) found that, for an assemblage of six stingless bee species, colony size correlated with foraging strategy (group vs. solitary). We used our database to expand on this, and found a significant correlation between colony size and recruitment intensity (Spearman's rank correlation: $r_s = 0.61$, $n = 14$, $p = 0.02$). Thus, in the traits analysis we simultaneously varied colony size and recruitment intensity to

preserve this relationship. Linear regression did not produce a good enough fit ($r^2 = 0.07$) to permit expressing recruitment intensity as a function of colony size in parameter calculations. This is likely due to the relatively small amount of available recruitment intensity data.

During *T. hyalinata* recruitment measurement, we recorded feeding time and time away from the feeder for 9-10 individual bees per repetition (59 total). We then estimated the flight distance between feeder and nest as the hypotenuse of a triangle with base 25 m and height 4 m (the approximate vertical distance between feeder and nest). Combining this distance (27.16 m) with stingless bee flight speed (4.25 m/s, Hrncir et al. 2004a), we separated time away from the nest into in-flight and in-nest components. This yielded *T. hyalinata* estimates of 23.39 ± 0.57 s feeding, 73.58 ± 1.94 s away from the nest, 12.78 s in flight, and 60.80 s in the nest. The *Melipona* eavesdropping simulation used flight (median 25.93 s) and in-nest (median 15.80 s) data collected by MH for *Melipona seminigra* foraging on 50% w/w (1.80 M) sucrose solution 50 m from the nest. Feeding times for an additional seven species, and flight and in-nest times for one other species were taken from the literature (Table 4.A2; Sommeijer et al. 1983; Roubik and Buchmann 1984; Hrncir et al. 2004b; Schmidt et al. 2006a; Schmidt et al. 2006b; Schmidt et al. 2008).

During *T. hyalinata* recruitment measurement we also noted the time at which recruits began feeding. This corresponds to transition between recruit and feed states in our model. In all trials, recruits began to feed during the second 5-min time interval. For the sensitivity analysis, we used published data (Aguilar et al. 2005) and our own experience to estimate the range of possible recruitment durations.

The minimum fight duration was always one more than the recruitment duration. The longest published takeover, between *Trigona corvina* and *Trigona silvestriana*, took 2.5 h (Johnson 1981). We thus set the maximum fight duration as 30 steps. Analyses that altered recruitment duration maintained the 28-step range of fight durations, shifting both minimum and maximum values.

Stingless bees mainly nest in tree cavities, in the ground near trees, in arboreal and or termite nests, or on branches near tree crowns (Wille and Michener 1973). Because nests in natural habitats are difficult to locate, limited nest density data are available. For *T. hyalinata* and *T. spinipes*, we estimated nest densities from nest counts on the Universidade de São Paulo, Ribeirão Preto campus and the campus' area, excluding the lake and buildings (574.61 ha, Freitas 2001). Nest densities of 26 other species were taken from Hubbell and Johnson (1977), Oliveira et al. (1995), Henriques (1997), Breed et al. (1999), Slaa (2006), and Siqueira et al. (2010). They ranged from 0.1 to 1 nest/ha.

We used bimonthly counts of the number of flowering individuals of 26 tree species in a 1 ha cerrado plot (Silberbauer-Gottsberger 2001) and the measured 224 woody plant species present in this plot (Gottsberger and Silberbauer-Gottsberger 2006) to estimate the density of flowering plants present at any given time. Flowering plant availability averaged 0.16 plants/m², ranging from 0.007 to 0.70 plants/m² across the year.

We estimated the area covered in 5 min by a searching bee by simulating bee flight in R (R Development Core Team 2011). Searching bumble (Reynolds 2009) and honey (Reynolds et al. 2007) bee flight patterns can be described by Lévy flight (with $\mu = 2$), a type of random walk where step lengths follow a probability distribution with a power-law tail. We simulated Lévy flight of bees flying for 5 min at searching flight

speed. Actively foraging honey bees fly at 6.46 m/s (Núñez 1982; Seeley 1995; Riley et al. 1996; Beekman et al. 2006), but searching bees fly at only 3.3 m/s (Andy Reynolds, pers. comm.). Thus, we assumed searching stingless bees fly at half their foraging flight speed (4.25 m/s, Hrnčir et al. 2004a), or 2.13 m/s. To determine the area covered by the simulated walk, we found its radius of gyration tensor. The eigenvectors and eigenvalues of this tensor quantify the widest and narrowest dimensions of the walk (Rudnick and Gaspari 2004). The square root of the product of the two eigenvalues is a good estimator of the arithmetic area of a random walk (Fougere and Desbois 1993). The simulation was repeated 100,000 times, and the median area calculated. Using the median allowed us to minimize the influence of flights with steps larger than is biologically realistic (~0.3% of the simulated flights).

SENSITIVITY ANALYSIS

As described in the Methods, we conducted a sensitivity analysis of energetic parameters. Table 4.A3 shows the parameter values used, and model output for each value.

Table 4.A3 Input and output values for the sensitivity analysis of energetic parameters. Regression coefficients marked with asterisks are significant ($p < 0.0001$).

Parameter(s)	Values tested		Regression coefficients		Threshold		Sub-optimal attraction cost	
	Min	Max	Min	Max	Min	Max	Min	Max
C_s, C_f, C_b, C_f'	-20/-20.5	0/-0.5	1314.98*, 0.91, 316.32*, -23.40*	1775.01*, -0.10, 153.00*, -11.84*	13.52	12.92	1081.12	530.32
E	10	130	617.30*, -0.15, 102.25*, -8, 12*	9516.37*, 1.81, 933.60*, -70.56*	12.60	13.23	365.50	3094.72
u_b, u_f	0/1, ..., 28	5/6, ..., 33	1699.63*, 0.10, 174.33*, -15.64*	1549.33*, -0.24, 228.01*, -14.28*	11.14	15.97	694.25	642.61
p_{win}	0.1	0.9	1626.14*, 1.03, 43.99, -12.02*	1658.80*, -1.04, 285.89*, -17.34*	3.60	16.49	508.52	800.49

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