doi: 10.1111/j.1420-9101.2007.01457.x

Comparative study in stingless bees (Meliponini) demonstrates that nest entrance size predicts traffic and defensivity

M. J. COUVILLON,* T. WENSELEERS,† V. L. IMPERATRIZ-FONSECA,‡ P. NOGUEIRA-NETO‡ & F. L. W. RATNIEKS*

*Laboratory of Apiculture & Social Insects, Department of Animal & Plant Sciences, University of Sheffield, Western Bank, Sheffield, UK
†Laboratory of Entomology, Zoological Institute, University of Leuven, Leuven, Belgium
‡Instituto de Biociências, Laboratório de Abelhas, Universidade de São Paulo, São Paulo, Brazil

Keywords:

forager traffic;
guarding;
nest entrance;
stingless bees.

Abstract

Stingless bees (Meliponini) construct their own species-specific nest entrance. The size of this entrance is under conflicting selective pressures. Smaller entrances are easier to defend; however, a larger entrance accommodates heavier forager traffic. Using a comparative approach with 26 species of stingless bees, we show that species with greater foraging traffic have significantly larger entrances. Such a strong correlation between relative entrance area and traffic across the different species strongly suggests a trade-off between traffic and security. Additionally, we report on a significant trend for higher forager traffic to be associated with more guards and for those guards to be more aggressive. Finally, we discuss the nest entrance of *Partamona*, known in Brazil as boca de sapo, or toad mouth, which has a wide outer entrance but a narrow inner entrance. This extraordinary design allows these bees to finesse the defensivity/traffic trade-off.

Introduction

The entrance to an insect colony is a critical location. It is where the nest, with its resources, and the outside world, with its threats, meet. The most fundamental feature of the entrance is size, and this is subject to conflicting selective pressures. To be more defensible against predators and robbers, the entrance should be smaller or even closed. But to permit foraging and to allow easy passage of forager traffic, the entrance should be larger and open. Therefore, there is a trade-off between traffic and security in nest entrance size.

Previous studies of social insect nests have indirectly demonstrated this trade-off. Honey bees (*Apis mellifera*) nest in cavities that are selected by swarms (Visscher, 2007). One nest cavity criterion that has been experimentally evaluated for swarm preference is entrance size. Swarms preferentially select medium-sized entrances, between 12.5 and 75 cm^2, over larger or smaller entrance holes (Seeley & Morse, 1978). Presumably, this intermediate size is large enough for forager traffic (populous honey bee colonies can have several hundred foragers departing each minute, Ratnieks, 1986), but small enough to defend. The ant *Temnothorax curvispinosus* will frequently modify the entrance to a new nest by reducing the size until it is small enough to be secure (Visscher, 2007). Other social insects protect against threats from predators and robbers by temporarily sealing the entrance during times when foraging will not be compromised, such as at night in bees (Roubik, 1989, 2006; Holldobler & Wilson, 1990).

Although these results are highly suggestive that there is a trade-off in entrance size, they are based on a single species. A comparative study of multiple species could provide stronger evidence. Stingless bees (Meliponini) are an ideal group for making comparisons. First, they are a species-rich group of eusocial bees with hundreds of related species (Michener, 2000). Secondly, their colonies possess immense variability in population over approximately three orders of magnitude, from *c.* 100 to 100 000 workers. Thirdly, nest predation and robbing are clearly important selective pressures. Their colonies have entrance guards to admit nestmates and deter

Correspondence: Margaret J. Couvillon, Laboratory of Apiculture & Social Insects, Department of Animal & Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK.
Tel.: +44 114 222 0149; fax: +44 114 222 0002;
e-mail: m.couvillon@sheffield.ac.uk

intruders (Suka & Inoue, 1993), and their nests often have elaborate defensive structures, including internal false nests, labyrinths and even balls of resin to roll into the entrance hole if attacked (Portugal-Araujo, 1978; Sakagami *et al.*, 1983; Wille, 1983; Melo, 1996). Finally, recent research has also provided much information on the internal phylogeny of the Meliponini (Fig. 2) (Fernandes-Salomao *et al.*, 2005; Rasmussen & Cameron, 2007).

Here, we make a comparative study of forager traffic, relative to nest entrance size and defensive behaviour, in 26 stingless bee species from São Paulo State, Brazil. Our results show that as the size of the entrance relative to the size of worker bees (entrance/bee ratio) increases, so does forager traffic. Additionally, there is a strong, significant trend for the species with higher forager traffic to have more guards and for those guards to exhibit greater aggression. Finally, we show that the extraordinary 'toad mouth' nest entrance of *Partamona*, which has a wide outer entrance but a narrow inner entrance, allows these bees to finesse the defensivity/traffic trade-off.

Methods

Study site and species

This study was conducted in São Paulo State, Brazil, using colonies at the University of São Paulo, Riberão Preto and São Paulo campuses, and especially at Fazenda Aretuzina, a farm near the town of São Simão, São Paulo State, Brazil, which is owned by Dr Paulo Nogueira-Neto and dedicated to the conservation of Brazilian wildlife and the study of stingless bees. Each location had many colonies of different stingless bee species. Most colonies studied were in hives, but all had modified the hive entrance hole with wax and propolis to construct their species-specific entrance. In addition, some wild colonies, nesting in hollow trees and on buildings, were also studied.

We studied 26 species (Table 1) of stingless bees (Meliponini). Stingless bees are a species-rich lineage (Fig. 2) of eusocial bees that are closely related to the honey bees (Apini) and bumble bees (Bombini) (Michener, 2000). Phylogenetic studies have so far not been able to resolve whether the stingless bees are the sister group of the honey bees or not. The most recent study, based on molecular data at three nuclear and one mitochondrial region, indicates that the honey bees are the sister group of the bumble bees + stingless bees (Thompson & Oldroyd, 2004).

We collected data specifically to address questions on the relationship between nest entrance size (relative to bee size) and foraging traffic, number of guards and the defensive reaction of the guards to nest disturbance. We included a species in our study only if we had data on at least three colonies, with the exception of *Trigona hyalinata*, for which only one colony was available. We included *T. hyalinata* because it is a species with high colony populations (Nieh *et al.*, 2003), and we wished to study species over as wide a range as possible. However, including or excluding *T. hyalinata* does not change any of our conclusions.

Determining ratio of entrance area to bee area

The entrance to each nest was measured using a micrometer caliper (0.1 mm gradations). Stingless bees construct their own species-specific nest entrances (Roubik, 2006), and they come in a variety of shapes including round, elliptical, slot-shaped or irregular (Fig. 1). Most of the study species had entrances that were close to round or elliptical. As we wished to measure cross-sectional area for the opening, we needed an approximate geometric shape for each entrance. For circular entrances, we measured the diameter of the circle and calculated area using $A = \pi(½D)^2$. For elliptical entrances, we measured horizontal (X) and vertical (Y) diameters and calculated area using the formula $A = \pi R_1 R_2$, where $R_1 = X/2$ and $R_2 = Y/2$. For the few that were slot/rectangular-shaped (e.g. *Friesella schrottkyi*, Fig. 1e), we calculated the area as XY. We measured the entrance of every nest used in this study to determine variability in size between and among species and calculated the average.

Samples of worker bees were taken from each species to measure the cross-sectional area of the bee. Worker bees of a particular species of stingless bee are very similar in size as they are reared in cells of defined size. However, we still wished to ensure consistency in size between and among nests. Therefore, we measured a minimum of 10 individuals per species, sampled from all study colonies. The cross-sectional area of the bee was determined by measuring the width, X, and depth, Y, of the widest part of the bee, which was usually the head, with the micrometer calipers. The heads were approximately elliptical in shape, and we determined the cross-sectional area as $A = \pi R_1 R_2$, as before.

Determining forager traffic

We video-taped 3 min of entrance activity per colony and counted the number of returning foragers per minute over the three consecutive minutes to determine the average per minute. As we wanted an upper estimate of traffic, we took data during the time of day when foraging was intense (e.g. 09:00–11:00 hours on a sunny day).

Determining guard number

Guards were identified by their unique posture and behaviour. In particular, guards tend to stand at attention at or near the hive entrance and check incoming foragers

Table 1 List of species studied arranged by genera in increasing ratio of entrance area to bee area.

Species	Entrance features	Entrance area (mm^2)	Bee area (mm^2)	Entrance area : bee area	Ave. traffic/min	Ave. no. of guards
Frieseomelitta languida	Round	5.9	2.2	2.7	4.8	2.3
Frieseomelitta varia	Round	14.0	3.2	4.4	7.1	1.6
Frieseomelitta		**10**	**2.7**	**3.6**	**5.9**	**2.0**
Melipona mandacaia	Triangular	27.3	9.3	2.9	1.8	1.0
Melipona quinquefasciata	Round	71.0	10.8	6.6	0.9	1.0
Melipona marginata	Round	16.2	5.1	3.2	3.1	1.0
Melipona scutellaris	Round, 2× entrance	88.2	13.9	6.3	8.7	1.3
Melipona bicolor	Round	26.5	11.2	2.4	2.2	1.0
Melipona quadrifasciata anthidioides	Round	56.4	12.0	4.7	3.3	1.2
Melipona rufiventris	Round	44.8	10.0	4.5	8.1	1.0
Melipona		**47.2**	**10.3**	**4.4**	**4.0**	**1.1**
Schwarziana quadripunctata	Round	14.5	2.1	6.8	17.4	3.5
Schwarziana		**14.5**	**2.1**	**6.8**	**17.4**	**3.5**
Scaura longula	Short tube	16.6	2.3	7.1	2.0	1.5
Scaura		**16.6**	**2.3**	**7.1**	**2.0**	**1.5**
Friesella schrottkyi	Slot,closed at night	11.6	1.3	8.6	7.4	3.0
Friesella		**11.6**	**1.3**	**8.6**	**7.4**	**3.0**
Tetragonisca angustula	Closed at night	29.8	2.0	15.2	10.3	11.0
Tetragonisca		**29.8**	**2.0**	**15.2**	**10.3**	**11.0**
Plebeia saiqui		9.3	2.3	4.1	5.3	1.0
Plebeia remota		11.8	2.4	4.9	5.8	1.3
Plebeia droryana	Round, 2× entrance	39.1	2.0	19.8	11.1	5.0
Plebeia pugnax		133.6	2.1	63.1	24.6	7.3
Plebeia		**48.5**	**2.2**	**23.0**	**11.7**	**3.7**
Scaptotrigona polysticta	Long tube	128.4	5.4	23.8	36.5	6.2
Scaptotrigona postica	Short tube	143.6	5.3	27.0	44.4	8.0
Scaptotrigona bipunctata	Medium tube	281.4	5.0	56.1	56.4	8.7
Scaptotrigona		**184.5**	**5.2**	**35.6**	**45.7**	**7.6**
Nannotrigona testaceicornis	Round	160.6	3.3	48.1	10.0	11.6
Nannotrigona		**160.6**	**3.3**	**48.1**	**10.0**	**11.6**
Trigona recursa	Round	649.3	3.8	170.9	40.0	8.0
Trigona spinipes	Layered entrance	2497.4	5.2	476.8	88.3	18.0
Trigona hyalinata	Layered entrance	2394.3	5.9	403.4	64.0	12.0
Trigona		**1847.0**	**5.0**	**350.3**	**64.1**	**12.7**
Tetragona clavipes	Layered entrance	3846.5	4.5	850.1	59.3	14.3
Tetragona		**3846.5**	**4.5**	**850.1**	**59.3**	**14.3**
Partamona helleri, outer entrance	Toad mouth	2083.7	5.3	392.7	26.0	4.5
Partamona helleri, inner entrance	Toad mouth	50.2	5.3	9.5	26.0	4.5

For each species, data are given on the entrance shape, including any type of special feature; entrance cross-sectional entrance area (mm^2); worker bee-cross-sectional area; ratio of these; incoming forager traffic per minute, taken as an average over 5 min foraging is intense; number of guards. The average per genus is shown in bold.

without flying off to forage themselves. In some species, one or more guards actually block the entrance, only moving back to allow forager nestmates to leave or enter. We counted the number of guards per colony at the start of each video session for every nest we included in this study and averaged these to obtain average number per species.

Statistical analyses

We determined the relationship between entrance size and traffic and between traffic and number of guards using both raw correlations, in which each species/genus was a data point, and phylogenetically independent contrasts (PICs) (Harvey & Pagel, 1991). PICs are calculated from the difference in the variables across pairs of species or higher nodes that share a common ancestor. Methods followed Wenseleers & Ratnieks (2006). Figure 2 shows the phylogeny, which was based on references 3 and 10 (Fernandes-Salomao *et al.*, 2005; Rasmussen & Cameron, 2007). Because we had clear *a priori* hypotheses, we used one-tailed tests.

To analyse the raw data, we carried out standard correlations using Minitab (Version 14). For the ratio of entrance area to bee area, we first transformed the data by taking logarithms.

Fig. 1 Nest entrances of some stingless bees in the order of increasing entrance : bee size ratio. Scale bar approximately 1 cm. (a) *Frieseomelitta varia*, (b) *Melipona marginata*, (c) *Melipona scutellaris*, (d) *Scaura longula*, (e) *Friesella schrottkyi*, (f) *Tetragonisca angustula*, (g) *Plebeia droryana*, (h) *Plebeia pugnax*, (i) *Scaptotrigona polysticta*, (j) *Scaptotrigona postica*, (k) *Trigona spinipes*, (l) *Partamona helleri* (outer entrance shown).

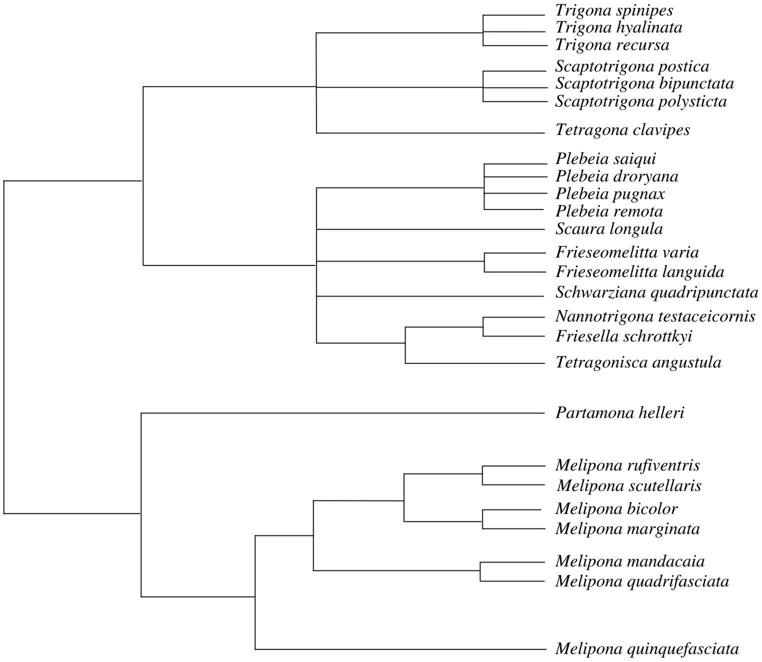

Fig. 2 Stingless bees are a species-rich lineage of eusocial bee. Phylogeny based on references 3 and 10 (Fernandes-Salomao *et al.*, 2005; Rasmussen & Cameron, 2007), where reference 2 is used for within Melipona and reference 9 is used among the genera.

Results

Table 1 shows the raw data on returning traffic and the entrance : bee area ratio. Traffic varied approximately 100-fold, from a low of 0.86 per minute in *Melipona quinquefasciata* to a high of 88 per minute in *Trigona spinipes*. The entrance : bee area ratio varied approximately 350-fold, from a low of 2.37 in *Melipona bicolor* to a high of 850 in *Tetragona clavipes*.

Table 2 groups the 26 study species into three broad categories based on the number of guards normally present at entrance and their level of aggression towards human disturbance.

Analysis at the species and genus levels

Figure 3 shows that there is, as predicted, a strong positive relationship between entrance : bee area ratio and returning forager traffic (Pearson's correlation, $r = 0.87$, $n = 25$, $P < 0.001$). This relationship was not simply a correlation between bee size and traffic (i.e. that smaller or larger bees have heavier traffic) (Pearson's correlation, $r = -0.12$, $n = 25$, $P = 0.57$) (data not shown). *Partamona helleri*, the species with the 'toad mouth' entrance (see Fig. 1L), has two data points, one each for the outer and inner entrances, and is not included in statistical analysis. We also analysed the data using the mean values for each genus. This correlation is also positive and significant (Pearson's correlation, $r = 0.88$, $n = 11$, $P < 0.001$) (data not shown).

Figure 4 shows that the relationship between the average traffic and the average number of guards is also strongly positive (Pearson's correlation, $r = 0.85$, $n = 25$, $P < 0.001$). *Partamona helleri* was excluded from the analysis. Once again, the correlation remained significant (Pearson's correlation, $r = 0.72$, $n = 11$, $P = 0.013$) when analysed at the genus level (data not shown).

Analysis of phylogenetically independent contrasts

The above conclusions of Figs 3 and 4 are also supported by analyses using PICs. Across the phylogeny with 24 contrasts, statistical significance was assessed using regression through the origin. There was a significant positive relationship between the entrance : bee area ratio and returning forager traffic ($r^2 = 0.178$, d.f. = 23, $P = 0.018$). There was also a highly significant positive relationship between average traffic and average number of guards ($r^2 = 0.494$, d.f. = 23, $P = 0.00004$).

Discussion

Our results clearly show that species or genera with greater foraging traffic have significantly larger entrances, quantified as the ratio of entrance area to bee cross-sectional area (Fig. 3, $P < 0.001$). This relationship is also supported by analysis of PICs ($P = 0.018$). This relationship has not been formally studied previously, although Roubik *et al.* (1986) incidentally commented that there was no association between nest entrance and foraging traffic. However, their comment was referring to absolute and not relative size.

Table 2 Species grouped into categories according to the number of entrance guards visible (columns) and defensivity (rows).

Few guards (1–2)	Several guards (3–5)	Many guards (6 or more)
Timid		
Plebeia remota	*Friesella schrottkyi*	*Nannotrigona testaceicornis*
Melipona mandacaia	*Scaura longula*	
Melipona quinquefasciata	*Plebeia droryana*	
Melipona bicolor	*Schwarziana quadripunctata*	
Melipona marginata	*Trigona recursa*	
Plebeia saiqui		
Melipona quadrifasciata		
Scaura longula		
Mildly defensive		
Melipona scutellaris	*Frieseomelitta languida*	*Tetragonisca angustula*
	Frieseomelitta varia	*Plebeia pugnax*
Aggressive		
Melipona rufiventris	*Partamona helleri*	*Scaptotrigona bipunctata*
		Scaptotrigona postica
		Scaptotrigona polysticta
		Tetragona clavipes
		Trigona spinipes
		Trigona hyalinata

Guard numbers are categorized as few (1–2), several (3–5) and many (>6). Defensivity is categorized as 'timid' (species with guards that retreat into the entrance when the nest is disturbed by a human); 'Mildly defensive' (species with workers that will fly out and land on a human intruder, but only when their nest is disturbed); 'Aggressive' (species that will attack a human intruder, even if the intruder merely stands by the nest; defence frequently involves biting and the recruitment of other workers to the defence).

Such a strong correlation between relative entrance area and traffic across the different species strongly suggests a trade-off between traffic and security.

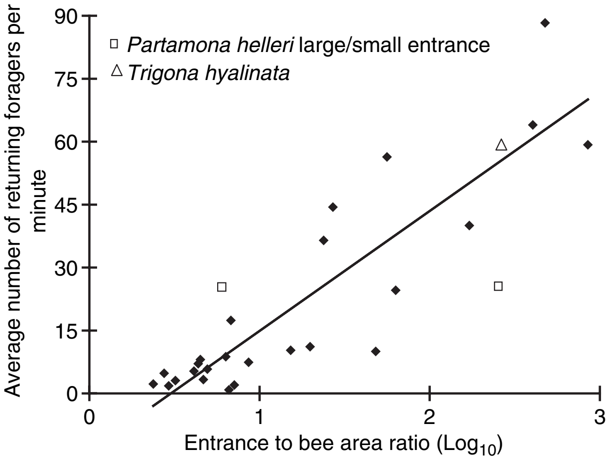

Fig. 3 Relationship between the ratio of entrance to bee area and foraging traffic. *Trigona hyalinata*, for which only one colony was studied, is shown as a triangle. *Partamona helleri* is shown as two squares, one showing the inner (small) entrance and the other the outer (large) entrance of the 'toad mouth' funnel entrance.

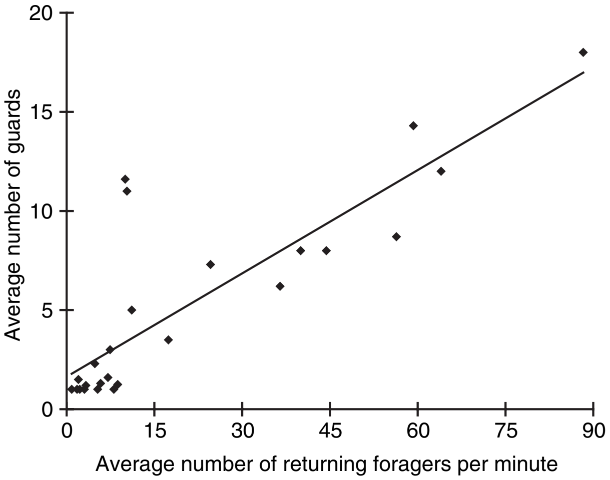

Fig. 4 Relationship between foraging traffic and average number of guards.

The existence of this trade-off and its effect on entrance design is further supported by the unusual 'double' nest entrance of *P. helleri*, which is well described by the local name 'boca de sapo', meaning toad mouth. The entrance is funnel shaped and has an outer (Fig. 1l) and inner entrances (not shown) (for detailed drawings of *Partamona* toad mouths, see Camargo & Pedro, 2003). The guards are stationed inside the funnel around the inner entrance. Usually, a small entrance constricts foraging traffic for two reasons, as the foragers must not only get through the hole, but they must also angle their approach and landing on a narrow platform – frequently we would observe foragers of different species slowing down as they approached an entrance. However, *Partamona* foragers were able to speed up in their immediate approach, crash into the toad mouth and fall into the inner hole (Camargo & Pedro, 2003), and presumably the colour of the inside surface of the funnel helps guide the foragers in, a feature that is shared with many stingless bees (Biesmeijer *et al.*, 2005) The two entrances fall considerably above and below the best fit line for the relationship between entrance size and traffic (Fig. 3). In particular, the outer entrance is more than twice as large as expected, given the traffic, and significantly so, as it is the only datum point that falls outside the 95% prediction interval. The inner entrance, which is approximately 40 times smaller than the outer entrance, is smaller than expected, given the traffic, but not significantly so, as it falls within the 95% prediction interval.

The comparison between the *P. helleri*'s outer entrance and the entrances of other species suggests that if entrance sizes were constructed purely from traffic considerations, the entrance would be considerably larger. However, in *P. helleri*, the inner entrance is actually where the guards are stationed and, as seen by the negative residual of the traffic for the larger entrance, curtails the traffic that would be capable of returning to the large entrance. The toad mouth entrance has allowed *Partamona* to finesse the trade-off between entrance size and guarding to experience the best of both worlds.

In stingless bees, the size of the nest entrance is also highly correlated with guarding behaviour, specifically with the number of guards stationed at the entrance. As the traffic increases, so too does the number of guards present at the entrance ($P < 0.001$, PICs: $P = 0.00004$) (Fig. 4). There are probably two reasons for this. First, as seen above, higher traffic is correlated with larger entrances, which need more defending. Secondly, it is presumably less costly in species with large colonies, which would have higher traffic, to invest in more guards (for example, devoting 1% of bees to guarding results in 20 vs. 2 guards in colonies of 2000 vs. 200 workers).

We also found an interesting relationship between colony defensivity strategy to disturbance and the number of guards present at the entrance. In the species with only one or two entrance guards, discretion appeared to be the better part of valour, as the guards in these species were mostly quite timid (Table 2). In these species, such as *Plebeia droryana* (Fig. 1g) and *F. schrottkyi* (Fig. 1e), the guard would retreat so that only her head or sometimes nothing was visible in the entrance hole. By contrast, in species with many guards, many workers would fly out, biting the face and head of the human intruder. At the extreme, for example, in *T. spinipes* (Fig. 1k), *T. hyalinata* (Fig. 1l) and *Scaptotrigona polysticta* (Fig. 1i), it was possible to trigger an attack simply by standing near or even below a nest higher up on a wall or in a tree. Once an attack had begun, other workers were recruited, resulting in a hasty retreat by the intruder. For mildly defensive bees such as *Tetragonisca angustula* (Fig. 1f), workers would land on the human intruder, but only if the nests were disturbed. Their response varied. *Plebeia pugnax* and *Melipona scutellaris* bit, but some species (e.g. *Frieseomelitta* spp.) in this category carried sticky resin in their pollen baskets. They would then deposit the resin on the intruder, including humans. Perhaps in the absence of strong mandibles, some bees used resin in defence. For example, when we introduced a conspecific non-nestmate into a *Frieseomelitta varia*, we observed the guard grappling with and ejecting the intruder from the platform. The intruder was not able to fly because her wings were stuck together with resin.

Most probably, the size of the colony and the level of defensivity are related. It would be maladaptive or even impossible for small colonies, with only a few hundred bees, to mount a mass defence that involved biting an intruder. For species with small colonies, timidity and retreat are a good strategy, especially as the nest is typically in a secure cavity in a tree or wall. Colonies like *T. spinipes*, who live in large nests of resin and mud, are capable of defence by overt aggression.

Our study shows that a comparative study of stingless bee nest entrances can provide convincing support for the existence of a trade-off between defence and traffic in insect societies. It would be interesting to perform a similar study within a single genus of stingless bee. The toad mouth-type entrance has evolved within *Partamona* (Camargo & Pedro, 2003). Comparative study of different *Partamona* species, both with and without the toad mouth, and also suitable outgroup species, could show how this structure altered colony defensive behaviour and, in particular, the relationship between traffic and entrance size. Unfortunately, *P. helleri* was the only *Partamona* we found in our study area. Partamona has 33 known species, and in these is great variety in entrance types and design (Camargo & Pedro, 2003).

Acknowledgments

We thank Dr Paulo Nogueira-Neto for his wonderful hospitality and helpful expertise, Claus Rasmussen for his help with the stingless bee phylogeny, Denise de Araujo Alves and Dr Vera Imperatriz-Fonseca for their friendly willingness to assist with this research, Fabio Nascimento and Ivelize Tannure-Nascimento for their friendship when we were in Brazil, and to Gisele Cabral for her help in collecting the data in Riberão Preto. MJC was funded from a Graduate Research Fellowship from the US National Science Foundation.

References

Biesmeijer, J.C., Giurfa, M., Koedam, D., Potts, S.G., Joel, D.M. & Dafni, A. 2005. Convergent evolution: floral guides, stingless bee nest entrances, and insectivorous pitchers. *Naturwissenschaften* **92**: 444–450.

Camargo, J.M.F. & Pedro, S.R.M. 2003. *Meliponini neotropicais*: o genero *Partamona* Schwarz, 1939 (Hymenoptera, Apidae, Meliponini) – bionomia e biogeografia. *Rev. Bras. Entomol.* **47**: 311–372.

Fernandes-Salomao, T.M., Rocha, R.B., Campos, L.A.O. & Araujo, E.F. 2005. The first internal transcribed spacer (ITS-1) of *Melipona* species (Hymenoptera, Apidae, Meliponini): characterization and phylogenetic analysis. *Insectes Soc.* **52**: 11–18.

Harvey, P.H. & Pagel, M.D. 1991. *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.

Holldobler, B. & Wilson, E.O. 1990. *The Ants*. Harvard University Press, Cambridge, MA.

Melo, G.A.R. 1996. Notes on the nesting biology of *Melipona capizaba* (Hymenoptera, Apidae). *J. Kans. Entomol. Soc.* **69**: 207–210.

Michener, C.D. 2000. *The Bees of the World*. Johns Hopkins University Press, Baltimore, MD.

Nieh, J.C., Contrera, F.A.L. & Nogueira-Neto, P. 2003. Pulsed mass recruitment by a stingless bee, *Trigona hyalinata*. *Proc. R. Soc. B* **270**: 2191–2196.

Portugal-Araujo, V.d. 1978. Um artefato de defensa em colonias de Meliponineos. *Acta Amazonica* **8**: 508–509.

Rasmussen, C. & Cameron, S.A. 2007. A molecular phylogeny of the Old World stingless bees (Hymenoptera: Apidae: Meliponini) and the non-monophyly of the large genus *Trigona*. *Syst. Entomol.* **32**: 26–39.

Ratnieks, F.L.W. 1986. Effect of colony population size on the efficiency nectar collection and honey production in honey bee (*Apis mellifera*) colonies. Unpublished thesis, Department of Entomology, Cornell University.

Roubik, D.W. 1989. *Ecology and Natural History of Tropical Bees*. Cambridge University Press, Cambridge.

Roubik, D.W. 2006. Stingless bee nesting biology. *Apidologie* **37**: 124–143.

Roubik, D.W., Moreno, J.E., Vergara, C. & Wittmann, D. 1986. Sporadic food competition with the African honey bee: projected impact on Neotropical social bees. *J. Trop. Ecol.* **2**: 97–111.

Sakagami, S.F., Inoue, T., Yamane, S. & Salmah, S. 1983. Nest architecture and colony composition of the Sumatran stingless bee *Trigona* (*Tetragonula*) *laeviceps*. *Kontyû* **51**: 100–111.

Seeley, T.D. & Morse, R.A. 1978. Nest site selection by the honey bee, *Apis mellifera*. *Insectes Soc.* **25**: 323–337.

Suka, T. & Inoue, T. 1993. Nestmate recognition of the stingless bee *Trigona*-(*Tetragonula*)–Minangkabau (Apidae, Meliponini). *J. Ethol.* **11**: 141–147.

Thompson, G.J. & Oldroyd, B.P. 2004. Evaluating alternative hypotheses for the origin of eusociality in corbiculate bees. *Mol. Phylogenet. Evol.* **33**: 452–456.

Visscher, P.K. 2007. Group decision making in nest-site selection among social insects. *Annu. Rev. Entomol.* **52**: 255–275.

Wenseleers, T. & Ratnieks, F.L.W. 2006. Comparative analysis of worker reproduction and policing in eusocial Hymenoptera supports relatedness theory. *Am. Nat.* **168**: 163–179.

Wille, A. 1983. Biology of the stingless bees. *Annu. Rev. Entomol.* **28**: 41–64.

Received 16 July 2007; revised 24 September 2007; accepted 25 September 2007