

## Nest Architecture and Foraging Behavior in *Bombus pullatus* (Hymenoptera: Apidae), with Comparisons to Other Tropical Bumble Bees

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**ABSTRACT:** Bumble bees (*Bombus* Latr.) are primarily a cold temperate group, but a few species live in the hot, moist conditions of tropical rainforest. We describe the external and internal characteristics of a *Bombus pullatus* Franklin nest from the tropical lowlands of Costa Rica. The nest was a large, conical mound constructed of cut vegetation on the forest floor, similar to nests of the Amazonian bumble bee, *B. transversalis* (Olivier). Maintenance of the vegetative canopy involved moving cut materials with the mandibles and a backward-directed sweeping of the legs, behaviors reported in some species of *Fervidobombus* and *Thoracobombus* and in the sister species to *B. pullatus*, *B. transversalis* and *B. atratus* Franklin. We recorded foraging activity, task specificity, and internal parasites of bees at the nest. Foragers were polylectic and peak foraging rates, particularly for pollen, were during the morning. The nest had five active entrances, and foragers tended to have a unidirectional flow through these entrances. The colony, with 414 adult workers and both worker and reproductive brood, was larger than most temperate colonies and comparable in size to nests of other tropical bumble bee species. A few workers were parasitized by the conopid fly, *Physocephala* sp.

**KEY WORDS:** *Fervidobombus*, bumblebee, tropical, nest, foraging

A robust phylogeny of the bumble bees (*Bombus* Latreille) (Kawakita *et al.*, 2004; Hines *et al.*, 2006; Cameron *et al.*, 2007) allows us to examine the evolution of traits, such as social and foraging behaviors. Yet there are large gaps in our knowledge of the behaviors associated with different bumble bee species and in our understanding of their adaptations to different environments (Sakagami, 1976). Bumble bees are most diverse throughout the cool temperate Holarctic and are one of the more cold-adapted groups of insects, with only 34 of the ~240 species occurring in the tropics (Williams, 1998). Most of these tropical bumble bee species are restricted to higher altitudes. The subgenus *Fervidobombus* Skorikov comprises the majority of the low and middle altitude bumble bee species in the Neotropical region (Williams, 1998) and includes the two species that are more abundant in the hot, moist conditions of lowland tropical rainforest: *B. transversalis* (Olivier) (Moure and Sakagami, 1962; Sakagami, 1976) and *B. pullatus* Franklin (Lievano *et al.*, 1991; Gonzalez *et al.*, 2004). While *B. pullatus* is more abundant in the tropical lowlands of Central America and northwest South America, it also occurs at higher elevations, unlike the Amazonian *B. transversalis*, which is restricted to lower altitudes (Lievano *et al.*, 1991). These two species form a monophyletic clade with *B. atratus* Franklin (Cameron and Williams, 2003; Cameron *et al.*, 2007), a species that extends into the lowland tropical rainforest but is more abundant in the highlands of northwest South America and in temperate regions of southern South America (Moure and Sakagami, 1962; Lievano *et al.*, 1991; Abrahamovich *et al.*, 2004; Gonzalez *et al.*,

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2004). Research on the nesting biology and social behavior of tropical bumble bees has focused mostly on *B. atratus* (Dias, 1960; Sakagami and Zucchi, 1965; Sakagami *et al.*, 1967; Zucchi, 1973; Lavery and Plowright, 1985; Cameron and Jost, 1998; Gonzalez *et al.*, 2004) and *B. transversalis* (Dias, 1958; Olesen, 1989; Cameron and Whitfield, 1996; Cameron *et al.*, 1999; Ramirez and Cameron, 2003; Taylor and Cameron, 2003). Our knowledge of *B. pullatus* nest architecture and biology is limited to accounts by Janzen (1971) and Chavarria (1996).

Unlike temperate bumble bee species, which form annual colonies during a 4–6 month period, *B. transversalis*, *B. atratus*, and *B. pullatus* have been collected year round and some colonies (or nests) have shown signs of perenniality (Moure and Sakagami, 1962; Janzen, 1971; Zucchi, 1973; Chavarria, 1996; Cameron and Jost, 1998; Taylor and Cameron, 2003). Tropical bumble bee colonies tend to be larger than temperate colonies, with the largest recorded colony belonging to *B. transversalis* (3056 individuals, including adults and brood, Dias, 1958) in Brazil. Other characteristics associated with the wet tropics include polygynous nests in *B. atratus* (Zucchi, 1973; Sakagami, 1976; Cameron and Jost, 1998), longer development and smaller brood size in *B. atratus* (Sakagami *et al.*, 1967; Lavery and Plowright, 1985), questionable swarming behavior in *B. atratus* (Sakagami *et al.*, 1967) and *B. pullatus* (Janzen, 1971), and extreme aggression in *B. transversalis* (Sakagami *et al.*, 1967; Olesen, 1989; Cameron *et al.*, 1999), *B. atratus* (Sakagami *et al.*, 1967; Cameron and Jost, 1998) and *B. pullatus* (Janzen, 1971; Chavarria, 1996).

Nest architecture of *B. transversalis* also appears to be modified for life in tropical lowland forests. Bumble bees usually establish nests in pre-existing cavities, either underground or beneath dead vegetation. *B. transversalis* workers cut and gather leaf litter, which they fashion into conical surface nests. Workers cut vegetation with their mandibles and move it toward the nest by sweeping it backward under the legs. Several bees doing this, sometimes in tandem, forces the cut vegetation onto the mound, where it is incorporated into the thatched nest cover. This action results in the clearing of vegetation in the vicinity of the nest and provides the bees with a dense, waterproof canopy (Cameron and Whitfield, 1996; Cameron *et al.*, 1999; Taylor and Cameron, 2003).

The first described *B. pullatus* nest was found in the leaves of a living banana tree in the lowlands of Costa Rica (Janzen, 1971). The nest was constructed of a thin vegetative mache, which sealed and closed off the nest, and had a broad entrance on one side. Chavarria (1996) found four active nests in Costa Rica located on the surface of the ground, but only described one of them. It was situated among the roots of a coffee plant and contained old, empty bee brood cells in use by fungus ants. The external architecture of this nest was not described.

Herein, we describe both the external and internal architecture of a surface nest of *Bombus pullatus* from lowland Costa Rica and compare the structure and nest construction behavior to other tropical and temperate bumble bee species. We also report on foraging activity, task specificity, and internal parasites of bees at the nest.

## Materials and Methods

### *Nest Observation*

A nest of *Bombus pullatus* was located in secondary succession rainforest once used for pasture, 1.83 km west of Estación Biológica Pitilla, Sector Pitilla, Area de

Conservación Guanacaste, Costa Rica (10.98931°N, 85.42581°W; 562 m elevation). This zone contains stretches of both primary and secondary rainforest and has a short, relatively moist dry season in March and April. We observed the colony during the wet season from July 13 to 17, 2005.

To assess foraging activity we counted the number of foragers entering and leaving the nest from each entrance for 15 minutes each hour from 10:00–16:15 on July 14, and from 07:00–16:15 on July 15. The foraging rate was calculated as the number of bees entering/min + exiting/min. During these intervals we also recorded the ambient temperature using a long-stem digital thermometer (VWRbrand®) and relative humidity using a digital hygrometer (Cooper™) in a partially shaded area similar to that surrounding the nest. Activity between sunrise (~05:30) and 07:00 and from 16:15 to sunset (~18:15) was not recorded. On July 15, we also recorded the presence of pollen on the legs of incoming foragers. To obtain data on task and entrance specificity, we individually marked (Sharpie™ paint markers, correction fluid) several bees on the outside of the nest, including those seen fanning ( $n = 2$ ), nest grooming ( $n = 1$ ), nest defending ( $n = 2$ ), and foraging ( $n = 15$ ). Nest-grooming bees were those that walked on the outer nest cover and moved nesting materials with their legs and mandibles. Nest-defending bees pursued us when we stood near the nest. Tasks and entrances used by marked bees were recorded throughout both days. By recording the time of entry and exit of marked bees we were also able to determine the length of foraging trips and time spent inside the nest between trips.

From 07:00–11:30 on July 16, bees exiting the nest were counted and collected into a large perforated plastic container. During this activity, we measured the internal nest temperature with the long-stem digital thermometer. We then cleared away vegetation around the nest structure, measured the external dimensions, and sliced through the vegetative canopy to observe the brood. Canopy thickness was measured at three points near the top and the bees remaining with the brood comb were collected and counted. Video (Canon GL-1) and digital photographs (Nikon Coolpix 5400) were taken to record numbers of cocoons, larvae, egg and larval masses, and honey and pollen pots present. All brood on the surface of the comb was counted except for approximately 12% (estimated from the portion of the circular nest obscured in photographs) located behind a sapling. This anchored portion of the canopy was left in place to keep the nest structure intact for replacement after brood observation. After 45 minutes of observing the brood comb, we returned the bees, and carefully replaced the nest canopy.

### *Statistical Analyses*

We performed Pearson-product moment correlation analyses to test if there was a significant ( $P \leq 0.05$ ) relationship between relative humidity or temperature and either the total number of foragers entering and exiting or the number of pollen foragers entering in the 15 minute observation period. Using each time period as a replicate, we used paired Student's  $t$ -tests to assess whether there was a statistically significant difference in the number of bees entering versus exiting from each entrance. All statistical analyses were performed using SAS version 9.1.3 software (SAS Institute Inc., 2002–2003). All data satisfied the assumption of normality with  $\alpha = 0.01$ .

### *Floral Visitation*

We collected pollen samples from the corbiculae of 11 returning foragers and placed each sample into a vial for identification of the number of floral morpho-species visited. These samples were processed in the lab using acetolysis (glacial acid, acetic anhydride + sulfuric acid) to remove the less-resistant organic matter, followed by alcohol dehydration. Samples were then immersed in oil for observation under a standard light microscope. We also recorded the plants that *B. pullatus* visited near the nesting area.

### *Detection of Parasites*

Prior to opening the nest we collected five foragers and three nest-defending bees directly into ethanol. We also collected a worker being killed by another worker outside the nest and another we found dead outside the nest. We examined these individuals for internal and external mites, dipteran or hymenopteran parasitoids, and ovarian development. We also mounted samples of the midgut, hindgut, and malpighian tubules onto glass slides and examined them for common internal parasites, including *Crithidia* (Euglenozoa: Kinetoplastida: Trypanosomatidae), *Nosema* (Protozoa: Microsporidia: Nosematidae), nematodes (Nematoda), and yeast (Ascomycota), using a phase contrast light microscope.

## Results

### *Nest Characteristics*

The nest consisted of a conical mound of dried vegetation on a relatively flat soil surface with dimensions 61.5 cm × 70 cm × 33.5 cm high (Fig. 1). It was constructed of thatched vegetation, mostly of small cut pieces of dried grass and cut fragments of leaves and twigs present in similar proportions to their availability around the nest. The area surrounding the nest contained a mixture of young trees, grasses, and herbs. Most of the nest was concealed and shaded by a layer of dried fallen grasses, a few live grasses, and melastomes (Melastomataceae). The nest was formed around a *Conostegia xalapensis* (Bonpl.) D. Don ex DC. (Melastomataceae) sapling, and small shoots of grasses and herbaceous plants were growing on the nest cover (Fig. 2). An area around the nest 1.25 m to the east, 0.5 m south, 0.5 m west, and 0.25 m north, was mostly cleared of vegetation, exposing a soil surface.

The nest contained five small, circular entrances, which we labeled relative to the north-facing clearing from which we observed the nest (Fig. 2): left (L), 5 cm (wide) × 2.5 (high) cm; front (F), 2.5 cm diameter; left right (LR), 2.0 cm diameter; middle right (MR), 2.5 cm diameter; and right right (RR) 2.5 cm diameter. These entrances were all on the lower portions of the nest 5–10 cm from the lower skirt of the vegetative canopy and ~3–6 cm above the ground surface. All entrances were clearly defined and flush with the outer surface of the nest (Figs. 1, 2).

The nest thatching was 5 cm thick, contained no obvious layers, and was loosely constructed, partially crumbling but remaining sheet-like when parted. No wax canopy or pillars were present. The brood filled most of the cavity beneath the nest canopy, being separated from the canopy walls by 0–5 cm (Fig. 2B, 6). The inner surface of the nest was dry and the brood was free of mold.

At the time of dissection, the colony contained 414 adult workers and appeared to be missing the queen. Among the brood were 61 small worker or male cocoons, 51



Fig. 1. Photo of the north-facing 'front' part of the nest taken near ground level, showing the nest mound, the vegetation surrounding the nest, and a cleared area in front of the nest. Two bees (dark spot in lower middle) are outside the front nest entrance.

large gyne cocoons, 147 discernable larvae, and 30 egg cells or young larval clusters (where individual larvae could not be distinguished). Older larval clusters contained from 4 to 10 individuals. When the brood is multiplied by 1.136 (to account for unobservable brood) and assuming an average of 7 individuals per egg cell or early larval cluster, the total estimated number of individuals comprising the brood was 533, amounting to ~947 live individuals in the colony. Egg cells were constructed on top of cocoons (Fig. 6A). Older larger larvae, likely queens, were incompletely covered with wax, exposed by a rather large circular opening on top of the cell (Fig. 6B).

The nest contained 22 honey pots, mostly arranged in clusters near the nest periphery. These were ovoid, less than 1.75 cm in height, constructed of dark brown wax, and with a small circular opening at the top (Fig. 6C). It is likely that these pots were constructed around clusters of old worker cocoons. The pollen pots were a similar size and shape but were open at their broadest circumference. They occurred singly, dispersed across the brood comb (Fig. 6D). There were no obvious pollen pockets, although a few pollen pots were situated laterally and underneath larval clusters, so could have functioned as a pocket in early larval development and subsequently been expanded (as noted in *B. pennsylvanicus* (DeGeer); Frison, 1930). Additional honey or pollen pots could have occurred in the underlayer of the nest. The visible empty brood cells appeared to be recently constructed and were brownish-yellow in color. Internal nest temperature prior to excavation (09:00, July 16) was 31.9°C, 5.7° warmer than the ambient external temperature at that time.

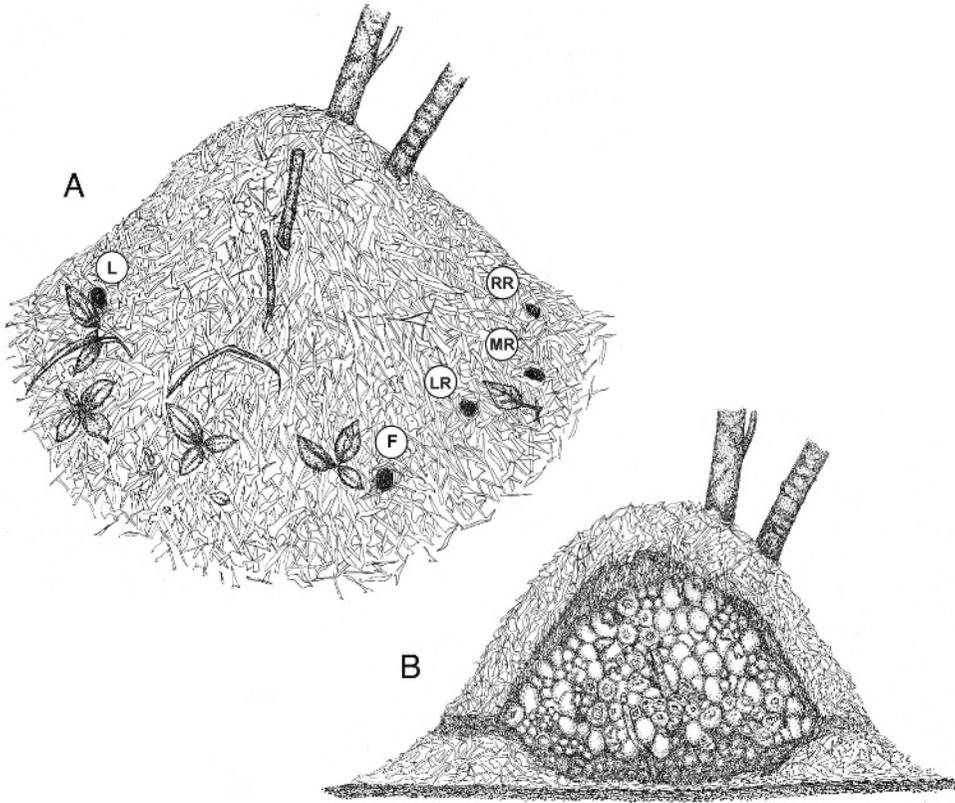


Fig. 2. A, a view from the top of the nest showing the relative locations of the nest entrances. L = left, F = front, LR = left right, MR = middle right, and RR = right right; B, a cross-section of the nest cover with the brood intact.

### *Behavioral Observations*

A few workers were observed grooming the nest by cutting up leaves and grasses present on the nest with their mandibles and moving these materials by pulling with their mandibles and front legs or using a rapid backwards-directed kicking motion of the mid and hind legs to push the materials behind them. We did not observe the movement of vegetation onto the mound. External nest groomers were seen the day after we opened the nest, but they had not increased in number. The nest canopy had been patched so that there were no exposed areas and it had a deformed conical shape. The colony was still active and contained nest groomers over a month after opening the nest (August 26, 2005).

On the afternoons of July 13 and 14, we observed bees expanding the nest canopy. This involved a bee pushing through the canopy from the inside, head-first, then turning back into the newly formed hole and kicking the vegetative material from the inside backward, thus closing the hole. This activity was repeated for ~15 minutes and resulted in a slight bulge in that part of the nest. The expansion locations from the two days were approximately 6 cm apart. Opening the nest revealed that the brood in this section projected outwards slightly more than in other areas.

The only other bees observed on the exterior of the nest were fanning along the edge of or inside an entrance, most frequently in entrance F, where the nest was most exposed to sunlight. Nest fanning occurred during most of the observation period, when temperatures ranged from 23.9–32.6°C and humidity ranged from 64–85%.

Marked workers did not exhibit strict task specificity. Nest-defending bees were seen foraging. Nest fanners were seen with pollen loads on their legs, returning from foraging, and grooming the nest for long periods of time.

During our collection of foraging data we were able to stand within 1–1.5 m of the nest to make observations. Only an occasional bee would respond to investigate our presence, but most would fly away if we remained motionless. After collecting foragers and defending bees, attempting to insert a temperature or humidity probe or cutting vegetation on the nest elicited a low buzz and up to ~20 individuals would leave the nest. These bees flew in circles within 4 meters of the nest, and did not pursue us beyond 5 or 6 meters.

### *Foraging Activity*

The foraging rate ranged from 2.6–9.5 bees/min across time periods, with the highest activity in the morning (07:00–10:00) (Fig. 3, 4). Pollen collection was highest from 07:00–09:00 and declined throughout the rest of the day, while the foraging rate for nectar foragers (i.e., those returning without pollen) remained relatively constant (Fig. 4). The percent of incoming foragers that carried pollen in a sample period ranged from 2.4 to 44.2%. There was no significant relationship between ambient temperature and number of overall foragers ( $r = -0.36$ ,  $P = 0.17$ ;  $n = 17$ ) or number of pollen foragers ( $r = -0.44$ ,  $P = 0.20$ ;  $n = 10$ ) and no significant correlation between relative humidity and overall foraging activity ( $r = 0.40$ ,  $P = 0.13$ ;  $n = 14$ ) or number of pollen foragers ( $r = 0.48$ ,  $P = 0.16$ ;  $n = 10$ ). Foragers ( $n = 7$ ) spent between 3 and 5 minutes inside the nest, with a mean of 4:05 minutes. Foraging trips ( $n = 11$ ) lasted from 30–73.5 minutes, with a mean of 51 minutes and a median of 49.5 minutes.

Some entrances were used more than others by foragers:  $L > RR > MR > F \gg LR$  (Fig. 5). The MR and RR entrances were used significantly more for entering ( $P < 0.001$ ), LR showed a trend towards more bees entering ( $P = 0.0653$ ), and L and F were used significantly more for exiting ( $P < 0.001$ ) (Fig. 5). Thus foragers did not use a single entrance for exiting and entering, but tended to have a unidirectional flow through the nest. Some marked bees were specific to a particular entrance after foraging (Table 1), but specificity in exits was not apparent. Occasionally a forager would land near one entrance, briefly investigate it, and then crawl towards and enter another entrance, sometimes by crossing over the nest cone. This provides additional evidence that foragers discriminate between nest entrances. During three occasions on one afternoon we observed a worker enter or exit by squeezing through a thin area in the upper front part of the canopy, seemingly creating its own entrance. Self-created entrances were later filled in and rethatched by nest groomers.

### *Floral Visitation*

Several *B. pullatus* individuals were seen foraging on a grassy pasture-like hilltop 1.0 km from the nest. Plants visited included: Melastomaceae–*Miconia* sp., *Monochaetum* sp.; Solanaceae–*Solanum* sp.; Fabaceae–*Mimosa* sp., *Desmodium adscendens* (Swartz) Dc.; and Rubiaceae–*Spermacoce* sp. The foragers appeared to visit nearly

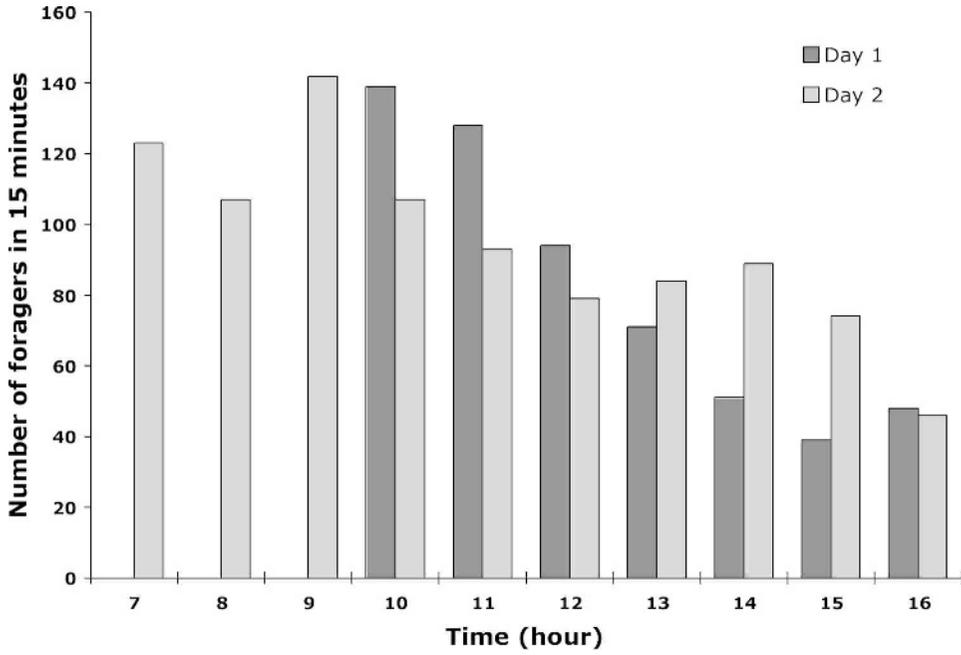


Fig. 3. The number of foragers exiting and entering the nest in a 15 minute interval at each hour across two consecutive days.

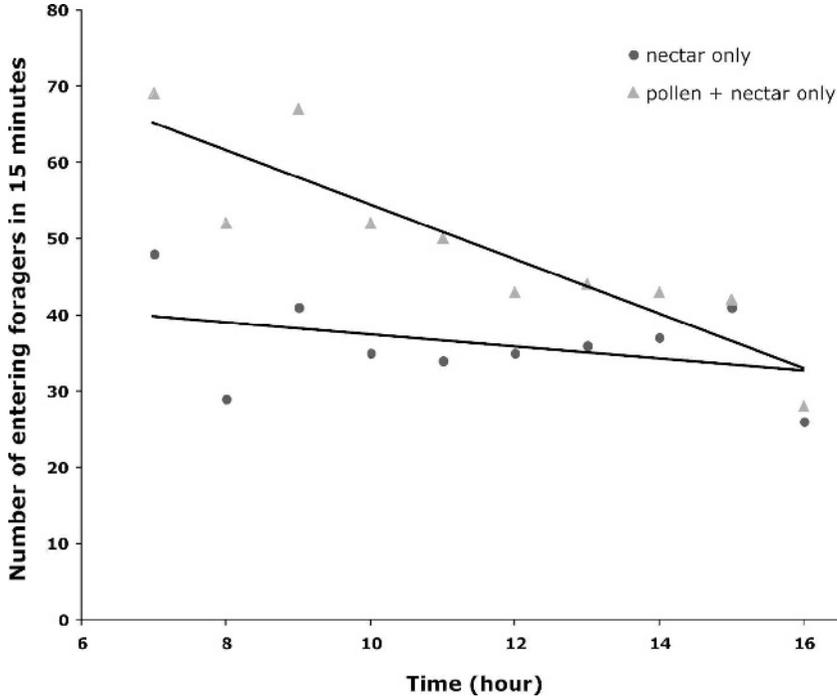


Fig. 4. The number of total entering foragers (pollen + nectar only) compared to the number of foragers collecting only nectar during a 15 minute period, partitioned by hour across Day 2.

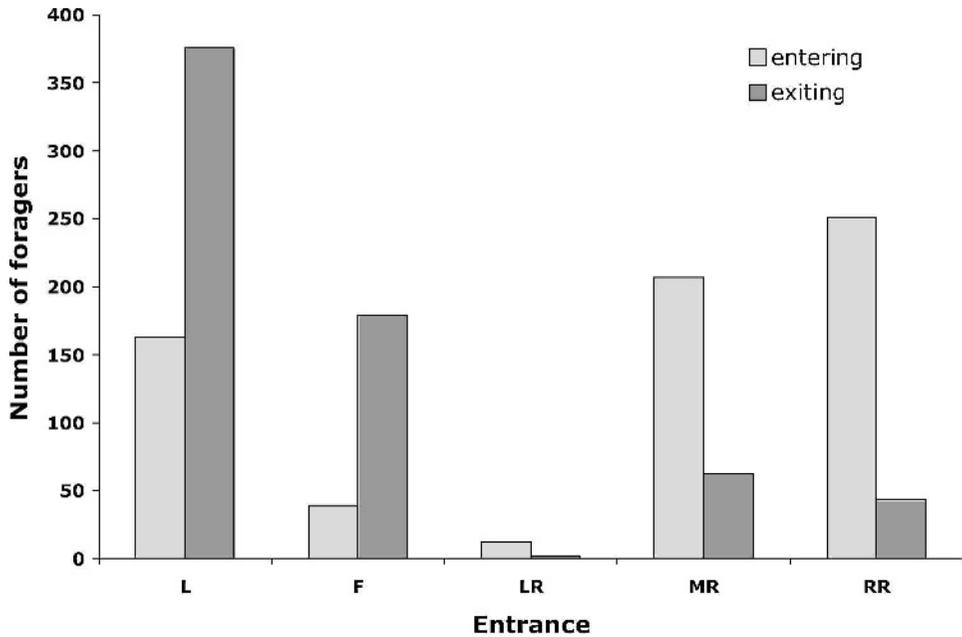


Fig. 5. The total number of foragers entering and exiting each entrance across all sampling periods. All entrances have a statistically significant difference between number entering vs. exiting (paired t-test,  $P < 0.001$ ) except LR, where  $P = 0.0653$ .

all available flowering plants in the area, including plants with tiny flowers (e.g., *Spermacoce sp.* and the melastomes surrounding the nest, with flowers  $<6$  mm). One individual was majoring on *Spermacoce sp.*

The pollen collected from 11 foragers belonged to at least six different morpho-species and included the families Asteraceae and possibly Solanaceae, Rosaceae, and Melastomaceae. Each individual bee visited one to three plant morpho-species, with most of their pollen load coming from only one or two species. Six of the 11 foragers collected pollen from only one species. The preferred plant differed among individuals.

Table 1. The number of entries and exits at each entrance by 10 marked bees.

Bee	Entering						Exiting					
	L	F	R?	LR	MR	RR	L	F	R?	LR	MR	RR
1	1					1						
2					1							
3		2				5		1				
4		1			1			1	1			
5			2			1	1					
6						3	1	2				
7			1			2	1		1			
8		1										
9	4							1				
10						1						

R? = unknown right entrance.



Fig. 6. Interior of the nest. Photo taken at a 50–70° angle from the ground surface; A, young larval or egg cells on top of cocoons; B, larval queen cells incompletely covered with wax; C, cluster of honey pots; D, pollen pot.

### *Commensals and Parasites*

No commensals were seen inside of the nest. An adult *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyiidae) was observed walking on the nest canopy with its ovipositor extended. Larvae of *Hermetia* are found in decaying vegetation. We observed *Ptecticus* sp., another stratiomyiid whose immatures develop in decomposing plant matter, flying around the nest, even flying into and temporarily disturbing a *B. pullatus* nest fanner.

The five foragers and three nest-defending workers that we collected alive had no external or internal parasites, aside from mild to moderate yeast concentrations in the gut. One of the three dissected nest-defenders had well-developed ovaries, including a fully developed egg measuring ~3.35 mm, and a second nest-defending worker and a forager had partially developed ovaries.

The worker that was killed outside the nest and the one found dead outside the nest both had their entire metasoma nearly filled and consumed by a third instar larva of *Physocephala* sp. (Diptera: Conopidae; identified using Smith, 1966). The muscle tissue of both bees was highly reduced and contained an abundance of yeast.

### Discussion

The nest architecture in *B. pullatus* showed marked similarity to nests of the tropical rainforest species, *B. transversalis*. As in *B. transversalis*, the nest consisted of a large conical mound of cut vegetation on the ground apparently constructed by

the bees. The vegetative canopy of the *B. pullatus* nest was thinner (5 cm) and more loosely constructed than is generally reported for *B. transversalis* nests (3–36 cm), which are often constructed of tightly thatched layers of cut leaves, twigs, and rootlets (Taylor and Cameron, 2003). This mound-shaped nest architecture likely protects the brood from the rain, given that well-constructed nests of both species have been dry inside and free of mold. This architecture may also provide a first defense against invading army ants (Ramirez and Cameron, 2003). All described *B. pullatus* nests (Janzen, 1971; Chavarria 1996; present study) and many of the described *B. transversalis* nests (Dias, 1958; Cameron *et al.*, 1999; Taylor and Cameron, 2003) were built around tree roots, shrubs, leaves, or saplings, which likely anchor nests and shield them from tropical rainstorms.

Some of the observed nests of the sister species to *B. transversalis* (Cameron and Williams, 2003; Cameron *et al.*, 2007), *B. atratus*, have been constructed of cut pieces of vegetation, but these nests have not been mound shaped and appeared to be modified from vegetation already covering the nest (Gonzalez *et al.*, 2004; Claus Rasmussen, pers. comm.). The *B. transversalis* nests have been more commonly observed in leaf litter substrates of primary rainforest, while the *B. atratus* nests and the *B. pullatus* nest in this study were found in more grassy substrates in secondary forest or disturbed areas.

In several *B. transversalis* nests (Cameron *et al.*, 1999; Taylor and Cameron, 2003), a *B. atratus* nest (Gonzalez *et al.*, 2004), and in the here described *B. pullatus* nest, construction and maintenance involved cutting vegetation on top of or around the nest with the mandibles and moving these pieces using the mandibles or a backwards-directed sweeping behavior of the mid and hind legs. This behavior has also been observed in some temperate species of the subgenera *Fervidobombus* and *Thoracobombus*, including *B. fervidus* (Fabricius) (*Fervidobombus*) and *B. muscorum* (Linnaeus) (*Thoracobombus* Dalla Torre), when gathering additional materials for their nests (Sladen, 1912; Plath, 1934; Free and Butler, 1959). The observed *B. pullatus* nest appeared to be in a late stage of construction, as this substrate-moving behavior was only occasionally observed and was restricted to nest repair or expansion. The development of trails or clearings in areas where materials have been collected for the nest has been documented for *B. transversalis* (Cameron and Whitfield, 1996; Cameron *et al.*, 1999; Taylor and Cameron, 2003). In *B. pullatus*, the area around the nest was mostly clear but we did not directly observe any movement of materials from this area.

Among the recorded tropical *Fervidobombus*, nests of *B. morio* (Swederus), *B. medius* Cresson, *B. bellicosus* Smith, and *B. atratus* are mostly on the surface, although a few nests of these species have been found below ground (Taylor and Cameron, 2003). *Bombus transversalis* nests have been found exclusively on the surface and *B. pullatus* nests have been surface (Chavarria, 1996; present study) or arboreal (Janzen, 1971). Surface or arboreal nests may be preferred in the tropics, as well as in arctic environments (Richards, 1973), because the moist soils make it difficult to keep the nest intact and the brood dry. Michener (1979) noted that the bee groups that are most successful in the tropics (e.g., Apidae tribes such as Euglossini, Meliponini, Apini, Xylocopini, and Centridini) either do not nest in the soil or use a thick waterproof lining on larval cells, and suggested this may be attributed to the increased risk of fungal attacks on perishable food sources and larvae in tropical soils. The increased insulation of soil cavities is also less important

in the continuously warm tropics (Janzen, 1971). The lack of wax pillars or a wax envelope in the *B. pullatus* nest is consistent with the rarity of these structures in surface nesters. Wax envelopes have been absent in most field-collected *B. atratus* (see exception in Milliron, 1971) and *B. transversalis* nests (7 of 9 nests in Taylor and Cameron, 2003).

The *B. pullatus* nest, with 414 workers and ~533 immatures, was larger than most temperate colonies, which generally contain 60–400 individuals, and was comparable in size to other tropical species (Lavery and Plowright, 1985). A survey of nine *B. transversalis* colonies yielded a maximum size of 400 adults and over 600 immatures (Taylor and Cameron, 2003), and in five highland *B. atratus* colonies, up to 80 workers and >589 immatures were recorded (Gonzalez *et al.*, 2004). The *B. pullatus* colony described by Janzen (1971) contained 343 adults and ~160 immatures and that described by Chavarria (1996) contained 500 adults.

Most of our knowledge on *Bombus* foraging and floral associations has been derived from temperate bumble bees. Like most temperate species, tropical bumble bees appear to be polylectic. Aside from our observations, polylecty has been observed in *Bombus* spp. in Brazil (Moure and Sakagami, 1962), in *B. rufipes* Lepelletier in the tropics of Southeast Asia (Kato *et al.*, 1992), and in *B. pullatus* by Janzen (1971) in Costa Rica. Polylecty may be favored in the tropics because resources are rare and sporadic and tropical bee species tend to be active throughout the year (Michener, 1979). Individuals of *B. pullatus* also sampled flowers similarly to temperate bumble bees, exhibiting majoring and minoring (Heinrich, 1976) on particular floral species. Foraging activity was highest in the morning for both *B. transversalis* (07:45 and 09:45; Cameron *et al.*, 1999) and *B. pullatus* (07:00–10:00). For *B. pullatus*, this can partly be attributed to increased pollen foraging in the morning, a trend also observed in *B. pullatus* visiting *Solanum wendlandii* Hook. f. (peaked at 08:00, Shelly *et al.* 2000) and in the Southeast Asian tropical bumble bee, *B. rufipes* (Kato *et al.*, 1992). This pattern was unrelated to humidity and temperature and may be a consequence of the morning dehiscence of anthers and their pollen availability.

Nests with two or three entrances, rather than just one, have been recorded from a few bumble bee species (e.g., Wójtowski, 1963; Ochiai and Katayama, 1982), occurring with a frequency of 13% in bumble bee nests found in Poland (Wójkowski, 1963). Dias (1958) observed three entrances in a *B. transversalis* nest, but only one was in use. Gonzalez *et al.* (2004) discovered five active entrances in a surface *B. atratus* nest. The *B. pullatus* nest observed by us also included five actively used entrances. Multiple entrances may improve nest traffic in large nests, and would be easy to create in the loose surface nests constructed by *B. atratus* and *B. pullatus*. In the *B. atratus* nest, entrances differed in their level of activity. For *B. pullatus*, not only did entrances differ in level of activity, but there was a general flow of traffic, with most bees entering in the right entrances and exiting from the left or front entrances. We also noted some forager specificity to entrances entered; e.g., one marked bee was specific to entering the less common left entrance. Entrance preference upon returning from foraging could depend on common foraging directions, nest-locating landmarks, and marking pheromone concentrations presumably deposited at entrances; the choice of exit could result from the ease of movement through the nest. Alternatively, this unidirectional flow could be attributed to a learned flow of movement and a fine spatial recognition of the nest.

The *B. pullatus* colony was relatively docile compared to *B. transversalis* colonies, where attack was expected upon approach (Olesen, 1989) and getting close to the nest usually disturbed the bees for 5–10 minutes (Cameron *et al.*, 1999). Increased aggression may help protect the bees against predation from vertebrate (Janzen, 1971) and invertebrate (Ramirez and Cameron, 2003) invaders. The tolerance of outsiders by this *B. pullatus* nest combined with the presence of five entrances and a relatively flexible thatched roof could have increased the nests susceptibility to entry by predators or parasites. The only apparent parasites, however, were conopid flies (Diptera: Conopidae), internal parasitoids which oviposit through the inter-segmental membranes of workers and males while they forage. Conopid parasitism can decrease the lifespan of a bumble bee by up to 50% and infect up to 70% of the colony (Schmid-Hempel and Durrer, 1991). Incidence of conopid parasitism among field-caught foragers commonly ranges from 10–30% in temperate Europe (Schmid-Hempel *et al.*, 1990) and has shown less prevalence in North America (Otterstatter, 2004) and at high altitudes (Korner and Schmid-Hempel, 2005). Conopids more commonly infect intermediate to large bumble bee workers (Müller *et al.*, 1996; Otterstatter, 2004), which includes the larger than average *B. pullatus*. It is intriguing that a worker containing such a parasitoid did not die directly from the parasitoid itself but rather from being killed by a fellow nest mate. This suggests that parasitized workers are recognized and removed by other members of the colony.

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