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Novel Use of Walking Trails by the Amazonian Bumble Bee, *Bombus transversalis* (Hymenoptera: Apidae)

By

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ABSTRACT During field observations of the Amazonian bumble bee, *Bombus transversalis* (Olivier), in southeastern Peru near the Rio Tambopata, we observed the use of walking trails by a subset of colony members. Workers involved in construction and maintenance of the external nest envelope follow ground trails that lead outward from the nest, gleaning and scraping root hairs and other leaf material from the substrate. Trail workers cut leaves into pieces approximately 2 cm^2 and push them behind them in the direction of the nest with their front legs. Trails radiated from the nest in opposite directions, extending 2–3 m into the forest. At least 20 workers patrolled the trails, collecting materials along them and at apparent collection sites at the ends of the trails. Observations of marked individuals indicate that workers are (at least temporarily) constant to one trail at a time.

Keywords: Bombus transversalis; Bumble bees; Trails; Pheromones.

INTRODUCTION

Ants are the preeminent ground-dwellers among social Hymenoptera. Their biomass dominates the world's tropical rain forest ecosystems, an ecological success rivaled only by the distantly related termites (Isoptera) (Wilson, 1971). Ants and termites alone among the social insects are flightless (only dispersing reproductives are winged) and both have developed walking trails as transport highways for the coordinated collection of food and other colony resources (Hölldobler & Wilson, 1990). Thus trail-use has evolved at least twice among ground-dwelling social insects, providing a striking example of evolutionary convergence in behavior. The other two groups of highly social insects, within the apid bees and vespid wasps, principally use flight for foraging. They are not known to use ground trails for locating colony resources (Wilson, 1971; Michener, 1974), although some species of stingless bees are known to follow scent marks or aerial odor trails during flight (Wille, 1983; Nieh & Roubik, 1995). Thus we were suprised in May 1995 to find the use of terrestrial trails by the Amazonian bumble bee, *Bombus (Fervidobombus) transversalis* (Olivier), whose trails resemble the ground trails of ants.

Bombus transversalis occurs only in tropical lowland rain forest habitat of the Amazon Basin. Unlike many bumble bees, which typically build their nests below ground in abandoned rodent burrows or other pre-existing cavities, *B. transversalis* constructs its nests on the surface of the soil in terra firme (never flooded) forest, on well-drained sites. Nests are fashioned from leaves, twigs and rootlets interwoven into a conical, protective nest canopy (Dias, 1958; Olesen, 1989), which bears a resemblance to some ant colonies (e.g., *Pheidole* or *Formica*) (Hölldobler & Wilson, 1990). Moreover, the colonies can become unusually large, producing several thousand offspring (Dias, 1958), whereas most *Bombus* colonies produce only a few hundred individuals. Although other species within the subgenus *Fervidobombus* nest on the soil surface and can form large colonies (Michener and LaBerge, 1954; Sakagami, 1976), only *B. transversalis* is known to construct a nest canopy of thatched leaves and twigs. Only two previous studies have described the nests of *B. transversalis*, that of Dias (1958) from the Belém region of Brasil, and that of Olesen (1989) based on a colony found in Ecuador along the Rio Napo. Those studies focused on brood counts and descriptions of nest architecture, hence details of nesting biology and foraging activity of this species are unknown or obscure.

Here we describe the characteristics of trail-making in this species, observed during a study of the nesting biology and foraging behavior of a colony located in Peru along the Rio Tambopata (12°50'S, 69°17'W), approximately 280 m above sea level. Cameron and Whitfield (1996) provided a brief initial report on trail-making behavior by this colony of *B. transversalis*. In this report we expand upon the find-

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Fig. 1. Nest canopy of *B. transversalis* near main trail at the Tambopata Preserve. A few bees can be spotted on the exterior of the canopy. This photo was taken from a vantage point to the left of and closer to the nest than that of Fig. 2.

ings presented in that paper, with new details of the nest structure and worker population, the rates and proportions of workers performing various tasks, and temporal and spatial patterns of trail construction and use.

ACKNOWLEDGMENTS

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METHODS

Two colonies were found along marked trails on the Tambopata Nature Reserve (see Pearson and Dressler, 1985 for studies of orchid bees along the same trail system). These are the first colonies of *B. transversalis* reported and observed in Peru. One colony was active and the other was inactive but had been vigorous seven months earlier (September 1994, pers. comm. T'ai Roulston) when it was first discovered. The active colony was located along "Main Trail", just past km 2.1 on the northwest side of the trail. It was situated near a stilt-rooted *Cecropia* tree on a slight rise about one m from the trail (Fig. 1). The inactive nest was located along "Swamp Trail" at approximately km 2.16. Each nest was located within approximately 100 meters of standing water. Our observations on trail use and foraging activity pertain only to the active colony.

We first observed the colony for ten days, from 20–30 May 1995, during the early part of the dry (lower water) season. Subsequently, we made periodic observations over the following months until the colony's demise during the rainy season in November 1995. We first described the nest dimensions, worker population size, and the location and length of two trails leading from the nest, apparently

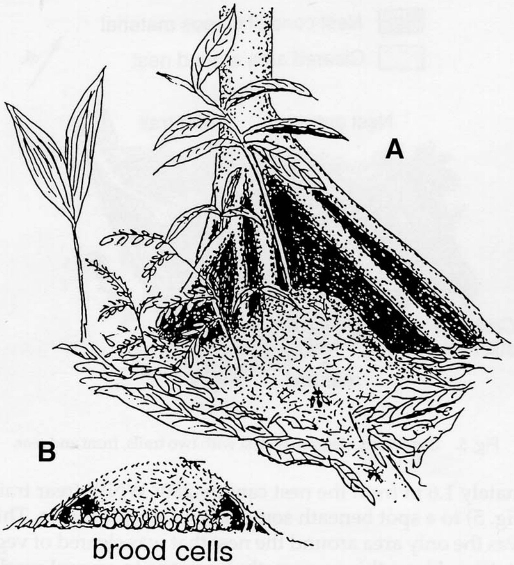

Fig. 2. Diagrams of *Bombus transversalis* nest located near *Cecropia* tree in forest site, (A) the external cone-shaped nest canopy of the colony, and (B) cross-sectional drawing of the brood comb beneath nest canopy.

Fig. 3. Photograph of the brood comb and honey pots around the perimeter.

cleared by a subset of workers (Cameron & Whitfield, 1996). Subsequently we uncovered the nest at the end of the initial 10 days of behavioral observations to determine the size and structure of the colony. All workers, including foragers, were collected alive to obtain adult and brood counts and then released back into the reassembled nest, which the workers quickly repaired.

Females were captured with aerial nets and given a distinctive color mark on the thoracic dorsum, abdomen, and/or wing using paint pens (Faber Castell). Different colors were assigned to workers engaged in (1) nest construction and trail-following at the eastern side of the colony, (2) nest construction and trail-following at the western side of the colony, and (3) colony defense (guards determined by their propensity to attack any intruder within three to four m of the nest). Foragers were easily distinguishable from other workers by their swift, direct flight in and out of the nest entrance, and they required no marking. Approximately 50 workers were marked.

During the first five days of observation, we made behavioral scans of workers outside the nest. For ten-minute periods, once each hour from sunrise to sunset, we counted all individuals active on the nest canopy, walking along trails, and flying in and out of the nest to collect food (foragers). We also marked bees that attacked us during these observations, regarding them as guard bees. Thereafter, we continued periodically to mark workers and make similar behavioral scans until November 1995, at which time the colony expired and was excavated a second time.

Species identification of the bees was confirmed by comparison with descriptions from Milliron (1973). Voucher specimens are deposited in the Arthropod Museum, University of Arkansas, Fayetteville.

RESULTS

COLONY SIZE AND NEST ARCHITECTURE

When observations began in late May 1995, the colony was in a pre-reproductive, rapid growth stage of development (see Cameron, 1989, for a description of bumble bee colony stages and behavioral castes), containing one queen and 338 workers, not including foragers away from the nest during the excavation (perhaps 10–20 workers).

The tightly woven thatching of the cone-shaped nest canopy (Fig. 2A, basal dimensions = 86.36 cm east/west, 68.58 cm north/south; 35.56 cm high; thickness of thatching = 5.1 cm) protected the brood and food-storage vessels (Fig. 2B), as well as the queen and workers engaged in nursing activities. The brood comb, in a slight depression (4–5 cm), was entirely dry and free of fungal decay or wax moths. Extremely hard, dry mud mixed with straw (5 cm thick) formed an inner band around the brood clump, lining the inner circumference of the thatched canopy at its base. The composition of this inner lining resembled a primitive clay pot. The oval-shaped comb (Fig. 3) measured 28 cm across the long axis and 20.3 cm along the short. From 26–30 wax honey pots were positioned around the perimeter of the brood comb (Fig. 3); none of these contained pollen. Some of the honey pots were unusually large

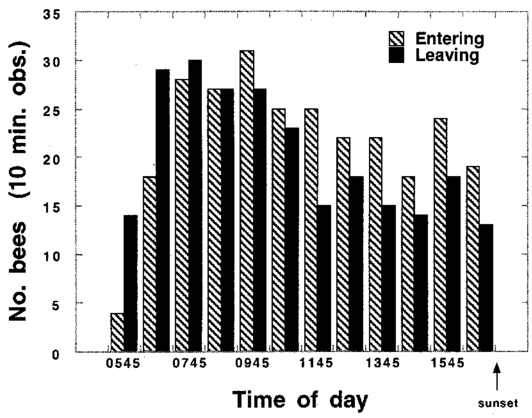

Fig. 4. Worker traffic in and out of the colony on 24 May 1995. Each bar shows traffic during a 10 min interval every hour.

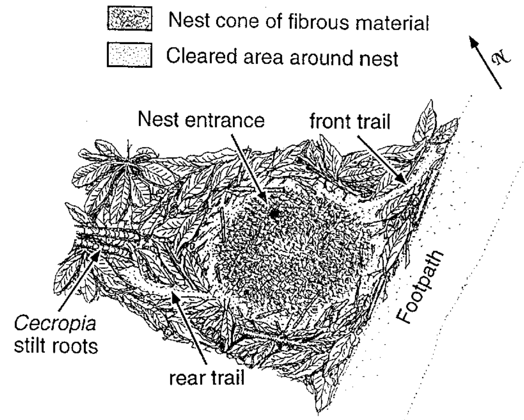

Fig. 5. Simplified diagram of nest with two trails, front and rear.

(3.5 cm tall × 1.3 cm diam.) and all were dark in color with a pebbled, coarse texture. The position, shape and texture of the honey pots suggested that they were not modified from pupal cocoons, but rather were constructed entirely of wax and perhaps sand or soil (judging from the pebbled appearance) for the sole function of storing nectar. Pollen pots, on the other hand, were modified from pupal cocoons and dispersed throughout the brood comb. New brood existed atop the older comb, consisting of the remains of empty pupal cocoons. The thickness of the entire brood clump was approximately 8.5 cm.

To minimize the amount of time the brood was exposed to the open air, we counted all brood cells on only one-half of the comb. Because egg cells, larval cells, and pupal cocoons appeared evenly distributed throughout the comb, it is reasonable to multiply this count by two to obtain an estimate of the entire brood. Thus one-half of the comb contained six egg cells, 75–80 larvae distributed among four discrete larval clumps, and 40–50 worker-sized pupal cocoons. There were no gyne cocoons, easily distinguishable from worker or male cocoons by their larger size (2×–3×).

FORAGING ACTIVITY

Specialized workers foraged for nectar and pollen throughout the nearly 12-h period of light, beginning at sunrise (0530) and continuing until sunset (1700) (Fig. 4). During peak activity (0745–0945), foragers flew in and out of the nest at an average rate of six bees per minute.

TRAIL-FOLLOWING

Females were seen following one another, often in tandem, along a cleared ground-trail that extended approximately 1.6 m from the nest canopy 280° WNW (rear trail, Fig. 5) to a spot beneath some fallen *Cecropia* leaves. This was the only area around the nest that was cleared of vegetation. Along this worn path, from one to several workers at a time were seen walking onto the trail from the nest canopy, where previously they had been engaged in canopy construction (teasing and smoothing the matted leafy outer covering of the nest with their mandibles). These workers initially drew our attention by their jabbing head movements along the soil surface and their tendency to move aside pieces of vegetation encountered in their path from the nest to the trail terminus beneath *Cecropia* leaves. At the end of the trail, workers disappeared beneath the fallen leaves, where they often remained for a period of five minutes or more, emerging from their hidden position to fly back to the nest canopy (sometimes they walked back) and begin smoothing and teasing the outer layer of nest thatching. From under the pile of dry leaves a crackling noise was audible as workers seemed to be cutting up pieces of leaves and rootlets for use in canopy construction. Because we did not wish to disturb the colony during the observations, we did not collect workers returning to the nest canopy to determine what, if anything, they carried back.

It was difficult to get closer than 1–2 m from the nest without some of the guards seeing us and attacking, necessitating protective clothing. During an attack, a large fraction of the workers on the nest canopy were disturbed, flying around in the immediate vicinity of the colony or attacking the intruder. It took five to ten minutes for the colony to return to normal after such an attack.

These preliminary observations on trail-following behavior on the west side of the nest led us to observe the

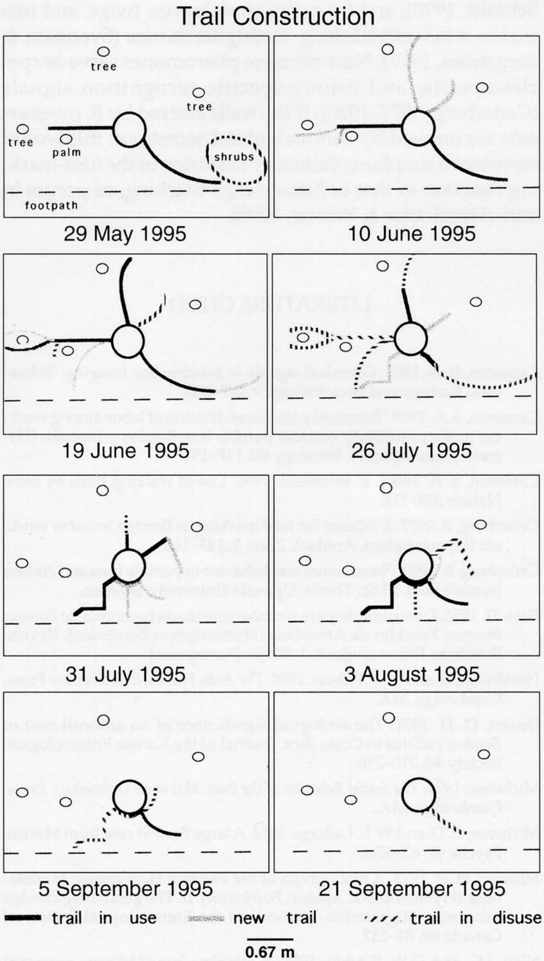

Fig. 6. Temporal patterns of *B. transversalis* trail construction and use during May–November 1995.

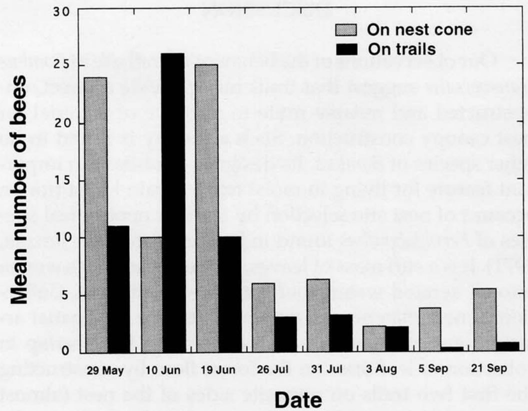

Fig. 7. Numbers of workers engaged in patrolling the nest cone, and in trail construction and use at intervals during the colony cycle.

bees on the opposite side of the nest canopy to the east (120° ENE), the side containing the single entrance to the colony (front trail, Fig. 5). Here, a second trail extended about 2 m straight out from the nest along the forest floor (Fig. 1). Like the western trail (rear trail, Fig. 5), this one was clear of vegetation and terminated underneath dense leaves. Individual workers followed this trail to the end, spent several minutes under the dense leaf material, then flew back to the canopy to begin working on the thatching. The behavior of the bees along this front trail was indistinguishable from that of the bees along the rear trail. No trails were yet seen north or south of the nest.

Trail Specificity

To determine whether workers were constant to individual trails, from 10–15 individuals were collected along each trail and marked with a distinct color (silver for the rear (W) trail, green for the front (E)). Several hours later only the silver-marked workers were seen along the rear trail and only the green along the front trail, suggesting that the two trails were used by different groups. This same distribution of marked workers was observed the following day. After two days however, several of the silver workers were seen along the front trail.

Temporal Patterns of Trail Construction and Use

During the initial 10-day observation period only two trails were visible (Fig. 6, 29 May). In subsequent weeks and months additional trails were constructed, and some trails were eventually abandoned (Fig. 6). New trails appeared to be actively maintained as outbound bees (perhaps a different group from those collecting leaves for the nest canopy) removed any fragments of leaves or twigs from the trails as they encountered them. When we dropped litter onto a trail, it was discovered and removed in five minutes or less by workers, who pushed it sideways off the trail using their mandibles, or scraped it backwards (under the body) toward the nest canopy using the forelegs.

A significant, but changing, proportion of workers was visible either on the nest canopy or on the trails (Fig. 7). Workers on the canopy appeared to be building and maintaining the elaborate thatching, using materials brought to them by the trail bees. Construction and use of the trails declined considerably in the later months of the colony cycle (Figs. 6, 7).

DISCUSSION

Our observations of the behavior of trail use in *Bombus transversalis* suggest that trails may provide a direct, unobstructed and reliable route to a source of material for nest canopy construction. Such a canopy is found in no other species of *Bombus*. Its design is doubtless an important feature for living in moist tropical rain forest (for an account of nest site selection by another neotropical species of *Fervidobombus* found in lowland forest see Janzen, 1971). It is a stiff mass of leaves, rootlets, and fibers woven into an aerated waterproof cone 10–15 cm thick. Collection of nest thatching is made efficient by the spatial arrangement of trails. The bees minimized the overlap in collection of leaf litter on the forest floor by constructing the first two trails on opposite sides of the nest (almost 180° apart), then adding subsequent trails at maximally distant positions relative to the first (90° and 45°, respectively). By maintaining the trails, which terminate in patches of dense leaf-litter, workers have a continuing supply of building materials to incorporate into the expanding canopy (Cameron and Whitfield, 1996).

An additional function of the trails may be in colony defense. The colony must protect its investment, especially the queen and new brood, from enemies that threaten to overrun the nest. Trails allow workers to monitor the vicinity of the colony, not only for vertebrate intruders but, more importantly, for predatory ants. Evidence in support of this idea was provided when we later penetrated the nest envelope to photograph the brood cells within. We collected all of the external and many of the internal bees beforehand. Within ten minutes of the disturbance, ants found their way into the colony and began stealing stored honey. We assume the ants were able to enter the colony because the normal, aggressive defense system of the guards and trail bees had been breached. The intact colony was able to sucessfully repel an attack by army ants (*Eciton*) on the morning of 19 October.

We suggest that the terrestrial trails may be considered a physical extension of the colony, analogous to the home ranges of many ants (Hölldobler and Wilson, 1990). Such home ranges are commonly marked with territorial or colony recognition pheromones (Hölldobler and Wilson, 1990). It is not yet known if pheromones are used to scent-mark trails to assist in orientation or to demarcate a colony boundary. Bumble bees apply marking pheromones in at least three contexts: (a) departing foragers deposit a pheromone from the Dufour's gland when passing between the brood cells and the nest entrance (Cederberg, 1977, 1990; Tengö et al., 1991; Oldham et al., 1994); (b) foragers scent-mark the location and vicinity of rewarding food sources (Cameron, 1981; Schmitt & Bertsch, 1990; Schmitt, 1990); and (c) males mark leaves, twigs, and tree trunks when establishing mating territories (Svensson & Bergström, 1977). Nest entrance pheromones serve as species-specific and colony-specific recognition signals (Cederberg, 1977, 1990). If the trails cleared by *B. transversalis* are marked by Dufour's gland secretions, this would represent a simple evolutionary extension of the nest-marking function to that of home-range marking, as occurs in ants (Hölldobler & Wilson, 1990).

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