

Nesting Biology, Seasonality, and Mating Behavior of *Epicharis metatarsalis* (Hymenoptera: Apidae) in Northeastern Costa Rica

RAINER THIELE^{1,2} AND BRIAN D. INOUE³

Ann. Entomol. Soc. Am. 100(4): 596–602 (2007)

ABSTRACT We investigated several aspects of the nesting biology of *Epicharis metatarsalis* Friese (Hymenoptera: Apidae) in a lowland wet-forest of northeastern Costa Rica. Five large aggregations were found 30–100 m from larger rivers at the La Selva Biological Station. The relatively short nesting season, from May to August, indicates that *E. metatarsalis* is probably univoltine. Four nests were excavated to study nest architecture, and five brood cells from three different nests were analyzed for pollen composition. Additionally, we analyzed pollen loads from 33 females returning from pollen-collecting trips, and we observed male patrolling behavior. The canopy tree *Apeiba membranacea* Spruce ex Benth (Tiliaceae) was found to be the principal pollen source, representing an average proportion of 98.5% of each pollen load and 93.4% of the larval provisions. The brood cell with the lowest proportion of *A. membranacea* pollen contained 89.3% *A. membranacea* pollen, which was 98.5% by relative volume. Pollen of *Byrsonima crispera* A. Juss. (Malpighiaceae) was the second most frequently encountered pollen with average proportions of 1.3 and 4.9% in pollen loads and larval provisions, respectively. *E. metatarsalis* can thus be considered oligolectic. The *E. metatarsalis* nesting season coincides with the relatively short flowering season of *B. crispera*, perhaps because of the importance of *B. crispera* as a source of oils rather than nectar.

KEY WORDS Apinae, bees, Centridini, oligolecty, pollination

The large centridine bee, *Epicharis metatarsalis* Friese (Hymenoptera: Apidae), has been collected in Venezuela, Panama (Friese 1900), and Costa Rica (Friese 1904, Snelling 1984) where it is most likely restricted to lowland wet forests, because no records from dry forests or higher altitudes are available (R.T., unpublished data). Like other species in the largely Neotropical tribe Centridini, which comprises the genera *Centris*, *Ptilotopus*, and *Epicharis*, *E. metatarsalis* nests in large aggregations in the ground that can consist of hundreds to thousands of nests in an area of tens to hundreds of square meters (Inouye 2000). Aspects of female *E. metatarsalis* nest location behavior have been investigated (Inouye 2000); however, little has been written about nest architecture, the provisioning of brood cells, or the behavior of males patrolling nest aggregations. Several studies on the nesting biology of *Centris* and *Epicharis* in general are available (e.g., Roubik and Michener 1980, Laroca et al. 1993, Inouye 2000), but surprisingly little is known about larval provisions for these genera (Hiller and Witt-

mann 1994, Quiroz-Garcia et al. 2001). Knowledge of the content of brood cells is important for interpreting observations of foraging bees, because it provides the most direct measure of resource use by the larvae, and it allows an evaluation of the degree of resource specialization (i.e., whether a species is oligolectic, specializing on one or very few host plants).

The majority of species in Centridini collect floral oils from flowers of Malpighiaceae and a few other plant families (Vogel 1974, Neff and Simpson 1981). Although several observations on the oil-collecting behavior of centridine bees are available (Hiller and Wittmann 1994, Vinson et al. 1997), the role of floral oils in larval provisions is poorly known. A detailed study on the nesting biology of *Epicharis dejeanii* Lepelletier (Hiller and Wittmann 1994) did not reveal oligolectic behavior (with or without correcting for pollen volume). The study of Quiroz-Garcia et al. (2001) analyzed scopal loads and larval provisions, but none of the three *Centris* species they investigated were clearly oligolectic, although *C. nitida* showed a strong preference for Malpighiaceae pollen.

In this study, we describe the nest architecture and nesting habitat of *E. metatarsalis*, and we provide a brief description of male behaviors. We also analyze

¹ Staatliches Museum für Naturkunde Karlsruhe, Erbprinzenstrasse 13, 76133 Karlsruhe, Germany.

² Corresponding author, e-mail: centris_vidua@yahoo.com.

³ Florida State University, Tallahassee, FL 32306-1100.

the pollen composition of scopal pollen loads of females returning to the nest and the composition of larval provisions. This allows us to evaluate whether *E. metatarsalis* is oligolectic and to evaluate the relevance of Malpighiaceae pollen and oil for the larval nutrition of *E. metatarsalis*.

Materials and Methods

The study was carried out at the 1,550-ha La Selva Biological Station of the Organization of Tropical Studies, located in the lowlands of northeastern Costa Rica (10° 26' N, 84° 00' W) at 37–150 m above sea level. This site is classified as tropical lowland wet forest in the Holdridge Life Zone System. Mean annual rainfall is 3,962 mm, and all months average at least 150 mm of rainfall (McDade et al. 1994). June to December, which average 350–480 mm rainfall per month, is considered the wet season. The data we present were collected during prolonged stays at La Selva from 1996 to 2000 and several shorter visits from 2001 through 2005, and result from hundreds of hours of observations.

Nesting Seasonality, Habitat, and Nest Architecture. Nesting activity was monitored at five sites on the La Selva station over 2 to 4 yr. We excavated nests at two sites, carefully removing soil in sections that allowed us to trace a nest from the surface to its terminus, including any side branches. Brood cells were collected as they were encountered and stored in vials for further analysis. Female activity and male behavior was observed for up to 6 h/d.

Male Behavior. We observed male *E. metatarsalis* behavior both at nesting aggregations and while bees were foraging in tree canopies. More detailed observations of male behavior at nesting aggregations were made in June and July 1996, 1997, and 2005. In July 2005, we counted the number of males patrolling over 1-m² quadrates and also marked individual males with fluorescent powders, so that they could be visually followed and the boundaries of the patrolled area could be assessed.

Pollen Resource Use. Samples for the study of pollen use were taken from the scopal loads of females returning to the nest and from excavated brood cells. Females returning from pollen foraging trips were caught at the nest entrance, and then they had their pollen load sampled by carefully “combing” one of the scopae with a pair of forceps (no. 5) until a pellet of ≈3–4 mm in diameter was formed. Samples were stored in 70% alcohol until acetolysis in the laboratory. Two samples were taken from the provision mass of each excavated brood cell, one sample from the bottom, or distal part, of the cell, and one sample from the middle, or central portion of the pellet. Pollen composition was similar for the two samples, so we present only pooled results.

The preparation of pollen grains was carried out following the acetolysis procedure described in Thiele (2002), except that samples were cleaned in an ultrasonic bath before and after boiling for 10 s each time. The preparation of slides and the quantitative analyses

of the samples were performed as described in Thiele (2002), except that, for each larval provision or scopal load, a total of 1,000 pollen grains was counted. Pollen volume was calculated using a volume equation for spheres ($4/3r^3\pi$), and average measurements of 10 noncollapsed, haphazardly encountered pollen grains for each pollen type. Further procedures followed the methodology of Hiller and Wittmann (1994). Pollen grains of *Byrsonima* spp. are difficult to identify due to their small size and tricolporate aperture type, which is very common among tropical Dicotyledonae (Roubik and Moreno 1991). Therefore, one subsample of each sample containing *Byrsonima*-like pollen was prepared for analysis using a scanning electron microscope (SEM). A small drop of ≈5 μl of each sample was applied on small pieces of scotch silver tape, dried at room temperature and sputtered 24 h later, following general methods for SEM.

Results

Nesting Seasonality, Habitat, and Nest Architecture. Nesting activity began between mid-April and mid-May, although in 1997, six males and one female were collected between 15 and 31 March at flowers of *Vochysia guatemalensis* D.Sm. (Vochysiaceae). Nesting activity seemed to peak during June. Very few nesting females were observed after the beginning of August. The locations of the nesting areas were 30–100 m distant from the bank of one of the two large rivers at La Selva, the Rio Sarapiquí or Rio Puerto Viejo, and separated from each other by distances ranging from 60 to 2,300 m. At all nesting sites, the shrub layer had been removed at regular intervals by human activity. Two sites (“La Guaria” and “Plagas”) were used for experimental plantations. One site (“La Flamea”) was a garden-like area and the remaining two were found in or at the edge of wide, regularly maintained trails surrounded by relatively young secondary forest. The soil at all sites was clay mixed with varying proportions of sand.

Nests at La Guaria and Plagas consisted of a vertical main tunnel with lateral tunnels of 9–12 cm branching off from the lower third of the main burrow (Fig. 1b and c). These lateral tunnels are loosely filled with soil after oviposition. A single brood cell was located at the end of each horizontal lateral tunnel. The deepest nest (Fig. 1b) was one of two excavated at Plagas. The main burrow of this nest continued 5 cm beyond the branching point of the last open lateral burrow, reaching a total depth of 120 cm. At this site, we excavated two complete nests and found a total of 21 brood cells, most of which could not be assigned to a specific nest's main burrow, because short lateral tunnels had been filled in with soil. At least five lateral tunnels were unambiguously associated with one nest. The depths of the excavated cells ranged from 62 to 115 cm. One nest at La Guaria (Fig. 1a) had a main burrow that was vertical to a depth of 52 cm, then turned away from the vertical axis at an angle of ≈100° and continued for 28 cm to a brood cell. It was difficult to categorize the slanted part as either main burrow or horizontal tun-

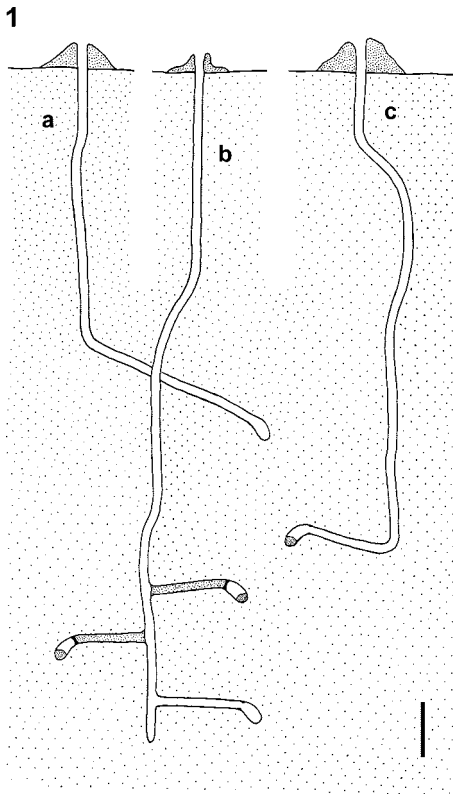


Fig. 1. Nest architecture of *E. metatarsalis* nests excavated at two different sites, with empty and partially provisioned cells. (a) Nest at Plagas. (b and c) Nests at La Guaría. Scale bar = 10 cm. See text for details.

nel. The brood cells contained larvae of different stages, and some cells were empty.

Male Behavior. Mating was observed at the nest sites and also in the top of a flowering *Dipteryx panamensis* (Pittier) Record & Mell. (Fabaceae), a canopy emergent tree. At the nest sites, males were observed throughout the nesting season flying ≈ 10 –150 cm above the leaf litter. Their flight paths mostly resembled figure eights. We observed dense clusters of males digging and fighting for emerging females on only three occasions.

Individually marked males at the “Huertos” nesting site in 2005 patrolled a range of ≈ 0.5 –2.5 m² ($n = 9$ males), pursuing females that entered these areas, and occasionally chasing males that patrolled adjacent areas. Males alternated periods of flying in circles or figure eights for 30–45 min with absences of 7–15 min, which are assumed to be foraging bouts but may also include time patrolling near inflorescences. Individually marked males patrolled nearly identical spatial locations on consecutive days.

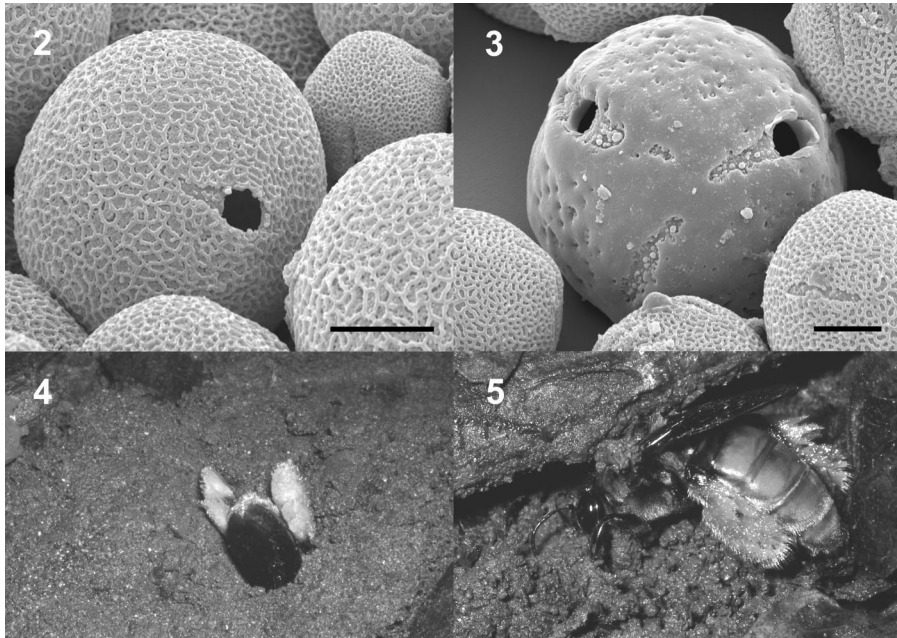
On several occasions, one of us (R.T.) was able to observe and collect males of *E. metatarsalis* flying low over the inflorescences of a 35-m-tall *D. panamensis*. Approximately 10–15 *E. metatarsalis* males occupied territories in the treetop, often flying circles and figure

eights in the same area. When a female or a group of females arrived in the treetop, males immediately pursued these females through the treetop but returned to their territories after a few seconds. Two males caught while pursuing females had protruding genitalia, which suggests that these chases were more than territorial aggression.

Pollen Resource Use. Pollen of the canopy tree *Apeiba membranacea* Spruce ex Benth (Tiliaceae) (Fig. 2) was the dominant pollen type in both samples from excavated brood cells and from scopal loads of pollen foraging bees (Fig. 4; Tables 1 and 2). The average proportion of *A. membranacea* pollen in scopal loads was 98.5% (97.7–99.2%) and in larval provisions was 93.4% (86.4–98.4%). *Byrsonima crispera* A. Juss. (Malpighiaceae) pollen (Fig. 2) averaged 1.3% (0–4.3%) of scopal loads and 4.9% (1.4–10.3%) of larval provisions. Pollen of other Malpighiaceae taxa represented from 0 to 1.4%. All other pollen types were found in low proportions. We consider the presence of pollen from species other than *A. membranacea* and Malpighiaceae taxa a by-product resulting from passive uptake during nectar foraging and not of active pollen collecting by the female.

We estimated pollen volumes for a brood cell (Table 2) that had the lowest percentage of *A. membranacea* pollen. Except for the few *Stigmaphyllon* sp. pollen grains (Fig. 3) (included in “other Malpighiaceae”), all other pollen found in this sample was smaller than that of *A. membranacea* and thus contributed very little to the total volume of pollen in the sample. Calculated per total volume, the proportion of *A. membranacea* pollen increased to 98.5%; *B. crispera* volume was only 0.6%; other Malpighiaceae was 0.3%; all other pollen represented only 0.6% of the volume. The relative proportion of *A. membranacea* by volume should be even greater in the other samples, because they contained greater proportions of *A. membranacea* pollen by counts. *Byrsonima* pollen is the dominant type of Malpighiaceae pollen collected, with only a few grains of other genera, likely because of the high local abundance and the large number of flowers per tree for both *B. crispera* and the cultivated *B. crassifolia* (R.T., unpublished data).

Close inspection of the dried provisions of two complete pellets revealed the presence of two darker layers in the light yellow pollen mass. These layers seemed to be of liquid origin, possibly floral oils, which hardened during the drying process. Of the 18 females collected at different flowers, 10 had oil loads in their scopae (Fig. 5) and their tarsal combs were covered with a thin layer of oil as well. Even the hardened scopal oil loads of pinned specimens were still shiny and transparent after 6 yr and easily recognizable as dried liquid. All 10 females with oil loads were collected at Malpighiaceae flowers: six at *B. crispera*, three at *B. crassifolia*, and one at the liana *Tetrapterys* sp. A few scattered pollen grains of another Malpighiaceae liana, *Stigmaphyllon* sp., were found in larval provisions and scopal loads. None of the females collected at nectar hosts (*D. panamensis*, *Dussia* sp. [Fabaceae], *Genipa americana* L. [Rubiaceae], *V. guatemalensis*



Figs. 2–5. (2–3) Section of brood cell samples under SEM. (2) Pollen grain of *A. membranacea* (center) and *B. crispa* (top right). (3) Large pollen grain of *Stigmaphyllon* sp. (center) among smaller pollen of *A. membranacea*. Scale bars = 10 μ m. (4–5) Females of *E. metatarsalis* returning to nest with bright yellow pollen (4) and shiny oil loads in scopae (5), respectively.

and *Vochysia ferruginea* Mart. [Vochysiaceae]) had either oil or pollen in their scopae and the tarsal combs were free of oil, indicating that bees clean themselves thoroughly between oil and nectar visits and that the presence of oil in these organs can be used as evidence for oil collecting at a given flower.

Discussion

Nesting Seasonality, Habitat, and Nest Architecture. The nesting period for *E. metatarsalis* falls within the main nesting season documented for wood-nesting centridine (i.e., *Centris analis* F. and *Centris vittata* Lepeletier) and some noncentridine bees in another study at La Selva (Thiele 2005), but it is much shorter. The shorter nesting season could be a response to flooding, because rising ground water levels during the wet season may interfere with nesting activity (see McDade et al. 1994, Thiele 2005 for precipitation data). Roubik and Michener (1980) mentioned that the nesting area of *Epicharis*

zonata F. Smith was flooded during the wet season. Although we do not have direct evidence that nests of *E. metatarsalis* were ever completely covered by the water table of nearby rivers, it is possible that at least a portion of cells were below the water table during large floods recorded at La Selva during the past 10 yr. The maximum river levels recorded at La Selva in 1998 reached the Huertos nest site and briefly covered some nest entrances.

The flowering phenology of the main pollen sources broadly overlaps with the nesting phenology of *E. metatarsalis*. The primary pollen source, *Apeiba membranacea*, was observed in peak flower from late April to early August in 1998, from May to July in 1999, and from June to August in 2000, although trees with a few scattered flowers were observed during all months except January and February. Flowering *B. crispa* were observed in June 1998 and June and July 1999. Two individuals flowered as early as late May in 1998.

Table 1. Average proportion of pollen types (percentage) in samples from scopal pollen loads of returning females across the five nesting aggregations in 1999

Nesting area	Plagas 1 19–21 VI	Plagas 2 29 VI	Huertos 21–29 VI	La Guaria 24–25 VI	La Flaminea 28 VI, 01 VII	Avg
Females sampled	5	7	6	9	6	
<i>A. membranacea</i>	98.5	98.1	98.7	99.2	97.6	98.5
<i>B. crispa</i>	1.1	1.7	0.9	0.6	2.1	1.3
Other Malphighiaceae	0.1	0	0.1	0	0	0
Other pollen	0.2	0.1	0.2	0.1	0.2	0.2

The category for other Malphighiaceae includes *Byrsonima* sp. and *Stigmaphyllon* sp.

Table 2. Average proportion (percentage) of pollen types in larval provisions

Brood cell	1	2	3	4	5	Avg
<i>A. membranacea</i>	91.7	98.0	94.0	93.9	89.3	93.4
<i>B. crispa</i>	6.2	1.8	5.1	4.8	6.5	4.9
Other Malpighiaceae	0	0	0.1	0.9	0.4	0.3
Other pollen	2.2	0.3	0.8	0.5	3.9	1.5

For each larval provision, one sample was taken from the periphery and one sample from the center of the pellet. Brood cells 1–4 were excavated at La Guaría on 15 July 1999, and brood cell 5 at Plagas 1 on 20 July 1999.

The nest architecture of *E. metatarsalis* differs from that of other *Epicharis* species studied so far (Camargo et al. 1975, Laroca et al. 1993, Raw 1992, Hiller and Wittmann 1994, Gaglianone 2005), except *E. zonata* (Roubik and Michener 1980). In both *E. metatarsalis* and *E. zonata*, the brood cells are located at the end of short lateral tunnels, which originate from the lower half of the vertical main burrow. However, the maximum depth of cells we observed for *E. metatarsalis* was markedly greater (115 cm) than for *E. zonata* (52 cm), and the preferred substrates were different (clay versus sand). The similarities in nesting habitat and architecture between *E. metatarsalis* and *E. zonata* may be a derived condition, because these species belong to the derived subgenus *Parepicharis* Moure and share several synapomorphies with species in *Triepicharis* and *Anepicharis* (Ayala 1998). More detailed nest descriptions of species in other subgenera of *Epicharis* are needed to evaluate the evolution of nest habitat and architecture in this genus.

Male Behavior. The establishment of male mating territories has been observed in several species of *Centris* (Alcock et al. 1976, Vinson et al. 1996). In *Epicharis*, only the typical male behavior of flying low over nest aggregations has been described previously (Raw 1992, Hiller and Wittmann 1994). In this study, we were able to observe male territorial behavior at flowers in the forest canopy and at nearby nesting sites. Our observations are the first to document that males patrol small territories at the nest aggregations rather than randomly flying across the entire nesting area in their search for females. We suggest there are at least three male mating strategies in the genus *Epicharis*: 1) patrolling at the nesting site, 2) patrolling flowers in the canopy, and 3) digging for females. Digging for virgin females may be a less important mating strategy for *E. metatarsalis* than for *C. pallida* (Alcock et al. 1977), because we rarely observed this behavior.

Pollen Resource. The definition of oligolecty is still debated among bee ecologists. However, host plant specialization is nevertheless an important concept in bee ecology and conservation. Some studies have used a relative proportion of 95% for the dominant pollen type as the threshold for defining oligolecty (Müller 1996, Thiele 2002), whereas other authors have pointed out that the nutritional importance of pollen cannot be measured by relative abundance alone but that it should include the calculation of pollen volume

(O'Rourke and Buchmann 1991, Silveira 1991, Hiller and Wittmann 1994). Pollen volume has rarely been considered in analyses of bee larval provisions, probably because the methodology is time-consuming. However, to classify species as oligolectes (specialists), pollen volume should be estimated for at least some samples, where the dominant pollen type is near the 95% threshold for oligolecty. This is even more important in cases where numerically dominant pollen types are relatively small.

The high proportion of *A. membranacea* pollen in our samples strongly suggests an oligolectic pollen collecting behavior for *E. metatarsalis*. Because the proportion of *A. membranacea* pollen grains in larval provisions did not always exceed a 95% threshold for oligolecty, we estimated the pollen volumes for the brood cell with the lowest relative abundance of *Apeiba* pollen (Table 2). The 98.5% relative volume of *A. membranacea* pollen in this sample clearly illustrates the significance of this species for the larval nutrition of *E. metatarsalis* and justifies categorizing *E. metatarsalis* as oligolectic. Roubik et al. (2002) found *E. metatarsalis* to be the most frequent visitor at *A. membranacea* in Panama, which is consistent with observations by one of us (R.T.) at *A. membranacea* trees close to large nest aggregations. However, because large euglossine bees like *Eulaema* and *Eufriesea* species (R.T., unpublished data) and large centridine bees (*Centris* sp. and *Ptilotopus* sp.) (D. Frame, personal communication) also have been observed collecting pollen at *Apeiba* flowers, it is possible that *E. metatarsalis* is not the only bee capable of successfully pollinating *A. membranacea*.

The collection of floral oils is common in centridine bees (Vogel 1974, Neff and Simpson 1981, Vinson et al. 1997). For most oil-collecting centridine bees, it is not known to what extent, however, these floral oils are mixed with pollen for larval nourishment or whether oil is used as construction material for cell walls and cell caps. We suggest that *E. metatarsalis* females visit Malpighiaceae flowers to collect a small quantity of floral oils and passively take up a few pollen grains of the oil host. Westrich (1989) categorizes a number of temperate zone bees as "Feuchtsammler" ("feucht"=moist, "sammeln"=collecting): bees that use nectar, possibly mixed with salivary secretions to moisten their pollen loads. In *E. metatarsalis*, Malpighiaceae oil might fulfill the same purpose as nectar for temperate zone "Feuchtsammler," i.e., increasing the stickiness of the collected pollen. The consistency of scopal pollen loads in *E. metatarsalis* is not as paste-like as described for some "Feuchtsammler" or other centridine bees (Vogel 1974; R.T. unpublished data); however, pollen loads are clearly stickier and moister than pollen from *Apeiba* anthers.

In addition to oils collected during pollen gathering, *E. metatarsalis* females bring large loads of transparent oil to the nest that are not obviously mixed with pollen. Unfortunately, we did not document at which stage of provisioning these large, probably almost pure oil loads (Fig. 5) are brought in. However, Hiller and Wittmann (1994) reported that oil was collected in

two stages during nest building and provisioning by *E. dejeanii*. In cells in which pure oil loads were brought in before pollen provisioning had started, no oil was found at the bottom of the larval food. This led these authors to conclude that pure oil loads were used for lining the inner cell. When provisioning first started, pure pollen loads were brought in, followed by loads of pollen mixed with oil. In our study, layers found in large pellets of larval provisions might have consisted of oil but also could have been nectar or of glandular origin.

At our study site, it seems that both *A. membranacea* and *B. crista* are of paramount importance for the reproduction of *E. metatarsalis*. Due to their growth or wood properties, neither species is currently being promoted in commercial wood production. Conservation of this bee species in anthropogenic environments may therefore require patches of natural forest interspersed with agricultural fields and forestry plantations. Oligolectic bees may be especially vulnerable to habitat fragmentation, because they tend to start with low levels of genetic variation (Packer et al. 2005) and have a life history of nesting in scattered aggregations.

Large-scale geographic variation in the host plant specialization of *E. metatarsalis*, and the ability of *E. metatarsalis* to facultatively use other pollen sources, should be investigated to fully evaluate the need for specific conservation measures for this species.

Acknowledgments

We thank K.-H. Hellmer for kindly helping with the SEM work and B. Rogell for collecting scopal samples and helping with the excavation at La Guaria. E. Braker and R. Marquis gave permission to excavate nests inside Plagas plots, and J. Ewel allowed us to work inside the Huertos plots. OTS course 05-03 assisted with ground-level observations of male patrolling behavior. We thank N. Underwood and two anonymous reviewers for comments. Funding for R.T. came from the Tropenökologisches Begleit-programm of the Gesellschaft für Technische Zusammenarbeit GmbH.

References Cited

Alcock, J., C. E. Jones, and S. L. Buchmann. 1976. Location before emergence of the female bee, *Centris pallida*, by its male (Hymenoptera: Anthophoridae). *J. Zool.* 179: 189–199.

Alcock, J., C. E. Jones, and S. L. Buchmann. 1977. Male mating strategies in the bee *Centris pallida* Fox (Anthophoridae: Hymenoptera). *Am. Nat.* 111: 145–155.

Ayala, R. 1998. Sistemática de los taxa supraespecíficos de las abejas de la tribu Centridini. Ph.D. dissertation, Universidad Nacional Autónoma de México, Ciudad de México, D.F.

Camargo, J.M.F., R. Zucchi, and S. F. Sakagami. 1975. Observations on the bionomics of *Epicharis* (*Epicharana*) *rustica flava* (Olivier) including notes on its parasite, *Rhathymus* sp. (Hymenoptera, Apoidea: Anthophoridae). *Stud. Entomol.* 18: 313–340.

Friese, H. 1900. Monographie der Bienengattung *Centris* (s. lat.). *Ann. K. K. Naturhist. Hofmus. (Wien)* 15: 237–350.

Friese, H. 1904. Nachtrag zur Monographie der Bienengattung *Centris*. *Ann. Mus. Natl. Hung.* 2: 90–92.

Gaglianone, M. C. 2005. Nesting biology, seasonality, and flower hosts of *Epicharis nigrita* (Friese, 1900) (Hymenoptera: Apoidea: Centridini), with a comparative analysis for the genus. *Stud. Neotrop. Fauna E* 40: 191–200.

Hiller, B., and D. Wittmann. 1994. Seasonality, nesting biology and mating behavior of the oil-collecting bee *Epicharis dejeanii* (Anthophoridae, Centridini). *Biociências* 2: 107–124.

Inouye, B. D. 2000. Use of visual and olfactory cues for individual nest hole recognition by the solitary bee *Epicharis metatarsalis* (Apoidea, Anthophorinae). *J. Insect Behav.* 13: 231–238.

Laroca, S., D. T. Reynaud dos Santos, and D. L. Schwartz Filho. 1993. Observations on the nesting biology of three Brazilian centridine bees: *Melanocentris dorsata* (Lepelletier 1841), *Ptilotopus sponso* (Smith 1854) and *Epicharitides obscura* (Friese 1899) (Hymenoptera: Anthophoridae). *Trop. Zool.* 6: 153–163.

McDade, L. A., K. S. Bawa, H. A. Hespenheide, and G. S. Hartshorn. 1994. La Selva: ecology and natural history of a Neotropical rain forest. University of Chicago Press, Chicago, IL.

Müller, A. 1996. Host-plant specialization in western palearctic anthidiine bees (Hymenoptera: Apoidea: Megachilidae). *Ecol. Monogr.* 66: 235–257.

Neff, J. L., and B. B. Simpson. 1981. Oil-collecting structures in the Anthophoridae (Hymenoptera): morphology, function, and use in systematics. *J. Kans. Entomol. Soc.* 54: 95–123.

O'Rourke, M. K., and S. L. Buchmann. 1991. Standardized analytical techniques for bee-collected pollen. *Environ. Entomol.* 20: 507–513.

Packer, L., A. Zayaed, J. C. Grixti, L. Ruz, R. E. Owen, F. Vivallo, and H. Toro. 2005. Conservation genetics of potentially endangered mutualisms: reduced levels of genetic variation in specialist versus generalist bees. *Conserv. Biol.* 19: 195–202.

Quiroz-García, D. L., E. Martínez-Hernández, R. Palacios-Chavez, and N. E. Galindo-Miranda. 2001. Nest provisions and pollen foraging in three species of solitary bees (Hymenoptera: Apoidea) from Jalisco, Mexico. *J. Kans. Entomol. Soc.* 74: 61–69.

Raw, A. 1992. Mate searching and population size of two univoltine, solitary species of the bee genus *Epicharis* (Hymenoptera) in Brazil with records of threats to nesting populations. *Entomologist* 111: 1–9.

Roubik, D. W., and C. D. Michener. 1980. The seasonal cycle and nests of *Epicharis zonata*, a bee whose cells are below the wet season water table (Hymenoptera, Anthophoridae). *Biotropica* 12: 56–60.

Roubik, D. W., and J. E. Moreno. 1991. Pollen and spores of Barro Colorado Island. *Monographs in Systematic Botany*, No. 36. Missouri Botanical Garden, St. Louis, MO.

Roubik, D. W., S. Sakai, and F. Gattesco. 2002. Canopy flowers and certainty: loose niches revisited. In Y. Basset, V. Novotny, S. E. Miller, and R. L. Kitching [eds.], *Arthropods of tropical forests. Spatiotemporal dynamics and resource use in the canopy*. Cambridge University Press, Cambridge, United Kingdom.

Silveira, F. A. 1991. Influence of pollen grain volume on the estimation of the relative importance of its source to bees. *Apidologie* 22: 495–502.

Snelling, R. R. 1984. Studies on the taxonomy and distribution of American centridine bees. *Contributions in Sci-*

- ence, Natural History Museum of Los Angeles County 347: 1–69.
- Thiele, R. 2002.** Nesting biology and seasonality of *Duckeanthidium thielei* Michener (Hymenoptera: Megachilidae), an oligolectic rainforest bee. *J. Kans. Entomol. Soc.* 75: 274–282.
- Thiele, R. 2005.** Phenology and nest site preferences of wood-nesting bees in a Neotropical lowland rainforest. *Stud. Neotrop. Fauna E* 40: 39–48.
- Vinson, S. B., G. W. Frankie, and H. J. Williams. 1996.** Chemical ecology of bees of the genus *Centris* (Hymenoptera: Apidae). *Fla. Entomol.* 79: 110–129.
- Vinson, S. B., H. J. Williams, G. W. Frankie, and G. Shrum. 1997.** Floral lipid chemistry of *Byrsonima crassifolia* (Malpighiaceae) and a use of floral lipids by *Centris* bees (Hymenoptera: Apidae). *Biotropica* 29: 76–83.
- Vogel, S. 1974.** Ölblumen und ölsammelnde Bienen, vol. 7. *Tropische Subtropische Pflanzenwelt*. 7: 1–267.
- Westrich, P. 1989.** *Die Wildbienen Baden-Württembergs*, 1st ed. Verlag Eugen Ulmer, Stuttgart, Germany.

Received 20 July 2006; accepted 20 March 2007.
