

Research article

Group nesting and individual variation in behavior and physiology in the orchid bee *Euglossa nigropilosa* Moure (Hymenoptera, Apidae)

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Received 10 December 2007; revised 2 May 2008; accepted 7 May 2008.
Published Online First 13 June 2008

Abstract. The Euglossini are a key group for studying the traits that promote or hinder highly social behavior in bees because it is the only tribe in the Apine clade without large colonies or females with distinct life histories, e.g. queens and workers. There have been few studies on behavior of orchid bee females in nests because these nests are not found easily. Taking advantage of the relatively high abundance of *Eg. nigropilosa* nests at Reserva Natural La Planada, Colombia, we examined social behavior of *Eg. nigropilosa* individuals in five nests (3 original and 2 re-used) for nine months. We report this species to have the largest colonies known for *Euglossa*, with nests reaching up to 22 individuals, and all nests containing more than one female bee from the same generation. These nests presented many traits that correspond to communal insect colonies. No generational overlap and no cooperative brood care were detected. We examined natural enemies and resource limitation as important factors for group nesting. We examined parasitoid attacks to cells in a nest with females and one without females. We also searched for nesting locations and examined nest re-use as indicators of nest site limitation. Lastly, we examined behavioral and physiological differences among females in the same nest. Such differences could be the bases for evolution of alternative life histories among group living females. We examined extent of ovary development and oviposition rates in similarly aged females in the same nest. We found large variation in reproductive effort of young females. We also examined differences in resin foraging and cell usurpation behaviors. Behavioral specialization was observed, with some individuals bringing only resin to the nest. Inside the nests, bees had territories in which they constructed and defended cells. This territoriality may be a defense against usurpation of provisioned cells by nest mates.

Keywords: Colombia, evolution of eusociality, Euglossini, nesting behavior, La Planada Natural Reserve.

Introduction

The evolution of advanced eusocial insects, characterized by the contrasting life histories of the reproductive queen and the sterile worker has intrigued evolutionary biologists for centuries (Darwin, 1859; Andersson, 1984; Page, 1997). In addition, the workers of many wasp, ant, and bee societies display a non-reproductive division of labor, in which different workers have different probabilities of performance of particular tasks (Wilson, 1971; Michener, 1974). Current understanding of social insect evolution views the unique phenotypes of social species as derived via reorganization of ancestral solitary traits (West-Eberhard, 1996; Amdam et al., 2004; Giray et al., 2005, see also Michener, 1974). Here we address the question of the origin of reproductive variation in the evolution of social organization, by detailed observations on group nesting females of the orchid bee *Euglossa nigropilosa*.

Among bees, the corbiculate apid clade, which includes bumble bees (Bombini), honey bees (Apini), stingless bees (Meliponini), and orchid bees (Euglossini), is the only group with highly eusocial behavior (Michener, 1974; Lockhart and Cameron, 2001). Euglossini is the only member of this group that presents primitive social behavior, varying from solitary to quasisocial (Dressler, 1982; Roubik and Hanson, 2004; Cameron, 2004). In consequence, Euglossini are a potentially informative group to study the evolution of social organization in insects (Garófalo, 1985; Roubik, 1989). Most studies on euglossine bees are focused on males (e.g.

Roubik and Ackerman, 1987; Eltz et al., 2003; Otero and Sandino, 2003) both because the male orchid bees display the intriguing fragrance collection behavior (e.g. Dodson et al., 1969; Eltz et al., 2003), and because the study of social behavior of Euglossini is difficult as natural nests are not easy to find and direct observations inside nests are difficult (c.f. see Soucy et al., 2003; Augusto and Garófalo, 2004).

Nests of *Euglossa nigropilosa* Moure are common at La Planada Natural Reserve (LPNR), Nariño, Colombia. LPNR is the highest location reported for a euglossini bee and is the most densely populated known for the genus *Euglossa* (Otero, 1996a, b). This species was first described from specimens collected by C.H. Dodson in Tungurahua, Ecuador, at 700–1400 m (Moure, 1965). In Colombia it is reported from Antioquia and Nariño in the Cordillera of the Andes (Bonilla-Gómez and Nates-Parra, 1992; Otero, 1996a, b). *Euglossa nigropilosa* belongs to the subgenus *Euglossa*, and previously Otero (1996b) speculated, based on nest structure, that it is communal. Communal colonies are characterized by an aggregation of more than one adult bee from the same generation with no generation overlap, no cooperative brood care and no reproductive division of labor that cohabit the same nest structure.

The cost of solitary nesting is a factor directly affected by the difficulty of nest construction which influences social organization in bees (Sakagami and Maeta, 1985; Hogendoorn and Leys, 1993; Bull and Swartz, 1996). One way to infer constraints is to quantify pollen and resin foraging in solitary and communal bees because those are behaviors linked to reproduction and nest construction (Roubik, 1989; Dunn and Richards, 2003; Paini, 2004). Solitary nesting also includes costs related to defense against natural enemies (Lin and Michener, 1972; Soucy et al., 2003). Detailed information on those factors requires direct observation of the inhabitants' behavior inside the nests (Cameron, 2004).

Taking advantage of the relatively high abundance of *Eg. nigropilosa* nests at LPNR and the opportunity to observe the behavior of the adult bees inside "natural" nests, we address the following questions: 1- What is the level of social organization in *Eg. nigropilosa* nests? We inferred the level of social organization based on traditional criteria by examining behavioral and other natural history traits. We examined overlap of generations and cooperative brood care. We also examined the average number of open cells and adult bees. A ratio close to one is expected if all individuals were reproducing (Michener, 1974; Soucy et al., 2003). 2- Is there a potential for natural enemies and resource limitation to promote group nesting in *Eg. nigropilosa*? Three important factors favoring nest cohabitation are: environmental constraints; cost of solitary nesting; and interactions between nest inhabitants. Environmental constraints include the limitation of pollen, nest materials, and nest sites (Michener, 1974; Dunn and Richards, 2003). We examined pollen foraging behavior of adult bees in the nests,

since this behavior only occurs in reproducing individuals provisioning cells for laying eggs. 3- Are there reproductive and other behavioral variation across individuals in the same nest (intranest variation)? For reproductive differences, we examined extent of ovary development, rate of oviposition, and relative age of females. For other behavioral differences, we examined cell usurpation, territoriality (cell protection), and resin foraging. Such interactions could be unequal among nest inhabitants leading to the emergence of characteristics of higher social organizations than communal. For instance, egg cannibalism could yield unequal reproduction and quasisocial organization (Garófalo, 1985), but protection against cell usurpation could counter this. Ovary development of nest mates is a useful indicator of sociality (Michener, 1974). We also analyzed behaviors to detect presence of specialization in the "non-reproductive tasks" such as resin foraging or nest repair. Presence of intranest variation in these measures can be seen as bases for mechanisms important in evolution of eusociality.

Methods

Study site

La Planada Natural Reserve is situated on the western slope of the Andes in the Department of Nariño, Colombia (77°24' W, 1°05' N). The reserve covers 3,200 ha, and has an altitudinal range from 1300–2100 m. The nests of *Eg. nigropilosa* were found at 1800 m. The mean annual precipitation is more than 4000 mm, producing high humidity with a driest period from July to August. The maximum and minimum monthly average temperatures are 24.5°C and 12° C respectively. The monthly average temperature is 19 °C and the average annual rainfall of 4087 mm (Samper, 1992, Vallejo et al., 2004).

Nests observation

Euglossa nigropilosa nests are a cluster of cells protected by a resinous envelop enclosing the entire nest area and containing adult female bees but not males (Otero, 1996b). As nests were constructed inside preformed cavities (in buildings 0.5–3 m above the ground), it was possible to remove the nest envelop to allow observations, presumably without significantly affecting bee behavior. Occasionally the bees rebuilt the envelope, in which case it was reopened.

The interiors of three *Eg. nigropilosa* nests (A, B, and E in Otero, 1996b; non of which was nest box), and the adult bee behavior and social interactions in these nests were observed during a total of 122.4 hours in ten observational periods. The eight first observational period lasted for one week each, at monthly intervals over the period April 1994, to March 1995. The ninth sampling lasted eight weeks from April 11 to June 3, 1995. An additional set of observations of the nest were performed from 16 August 1996 to 1 May 1997. Observation inside the nest was performed under a red light to minimize disturbance to the bees. Observations were recorded on audio tape.

Maps of the brood cells were drawn to record construction of new cells, provisioned cell, cell oviposition and construction of cells and envelope. The following behavior and in-nest activities were recorded: cell construction; provisioned cell; cell oviposition; envelop construction; and combat among adult bees.

Bee marking and age assessment

Each adult bee was individually marked on the thorax using a color and position code (Kearns and Inouye, 1993). White and colored liquid paper as well as Testors® paint were used (Wcislo, 1997). Because bees clean their bodies continuously, it was necessary to re-mark the individuals each week. The ages of the bees were estimated by wing damage, using a categorical scale from 1 to 4, where 1 indicates no damage and 4 the most damage (Michener, 1974). The criteria to classify wing damage were based on the proportion of the wing edge that was broken. Interpretation of wing damage data have to be interpreted with care because correlation between wing damage and bee age may not be direct and transition from wing damage category 1 to category 4 may be rapid.

Foraging trips

To determine the importance of different collecting activities by adult bees, monthly two-hour foraging observations (for one day) were done for six months from July to December 1994. Observations were mainly done during the morning when the bees foraging activity was highest and most of the collecting trips occurred. Three types of collecting trips were scored: pollen, and resin. Besides, on some trips the bee returned without any material in the corbiculae those were scored as "no loads", and they were presumably collecting nectar as energy source and/or water in hot days. Variation among nest in the frequency of different collecting trips were analyzed using a two-way ANOVA in which a factor was the type of trip, the other factor was the nest, and the response variable was the number of trips per hour. Censuses of all individuals were done for three nests (A, B and E) every month by counting the number of adults inside the nest at night. Nectar concentration of visited flowers was measured with an Atago hand Refractometer, Model ATC-1E, Brix (0–32%).

Ovary development

Thirteen bees from nests A, B, and E were dissected on 24 May 1997 to observe their ovary development under a 4x dissecting microscope and presence of sperm in the spermathecae under a 40x light microscope (Table 1). Ovarioles were classified as: developed, partially developed, and slightly developed. This classification was based on the size and appearance of the ovarioles. Developed ovarioles were the biggest with size similar to an egg; slightly developed ovarioles were the smallest. Partially developed ovarioles were intermediate in size. An Ovary Development Index (ODI) was estimated using the following formula:

$$\text{ODI} = \# \text{ developed ovarioles} \times 3 + \# \text{ partially developed} \times 2 + \# \text{ slightly developed} \times 1$$

The ODI weights the development of each ovariole and is a useful way to compare the state of the ovaries among bees. Given that *Eg. nigropilosa* has 8 ovarioles the ODI may vary from 0 to 24. The ODIs were compared among bees with no wing damage (1) vs. bees with wing damage (2–4) using an F-Test on square root transformed data. Data were approximately normally distributed.

Level of social organization

To determine the occurrence of overlapping of generations we examined the relation between adult life span and egg-to-adult period. To determine the presence of cooperative brood care we examined in-nest activities of bees in three nests as described above. To determine the presence of reproductive division of labor we examined ODI and mating status of thirteen bees in three nests. We expect all individuals to be mated and have developed ovarioles in the absence of reproductive division of labor. We also examined provisioned cells to adults ratios in five nests.

Table 1. Ovary development and fecundation of spermatheca in *Euglossa nigropilosa*. Load (Load on the corbiculae when collected entering the nest), WDO: Number of well develop ovarioles), PDO (Number of partially develop ovarioles), SDO (Number of slightly developed ovarioles), NO (Non-developed Ovarioles), E (Spermatheca). WD (wing damage after Michener 1974).

Individual	Nest	Load	WDO	PDO	SDO	NO	E	WD
CC-II 8	E	Non	4	2	2		Not observed	4
CC-II 1	E	Non	4	2	2		Mated	1
CC-II 12	E	Pollen	4	4			Not observed	1
CC-II 7	E	Resin	4	2	2		Mated	1
CC-II 5	E	Pollen	1	4	2	1	Not observed	1
CC-II 14	E	Non			2	6	Mated	1
C-I 13	B	Non	3	2	2	1	Mated	4
C-I 6	B	Pollen	2	4	2		Unmated	3
C-I 12	B	Non	1	4	3		Unmated	1
P 10	A	No apply	4	2	2		Not observed	2
P 5	A	Pollen	1	3	3	1	Not observed	3
P 7	A	Non	1	3	2	2	Mated	4
P 9	A	Pollen	2		4	2	Unmated	1

Factors influencing group nesting

One factor that may lead to group nesting is presence of opportunistic natural enemies such as parasitoids that could attack brood in unguarded nests. In a previous study, one of us (JTO), compared the percentage of cells parasitized by a parasitoid wasp in one experimental nest with no adult bees, and other active nests with adult bees present (Otero, 2001); these results, although limited were revisited in the context of group nesting in *Eg. nigropilosa* in this study. Another factor that may induce group nesting is resource limitation for which we examined building materials and nest sites as potential limitation resources. We counted re-used and newly constructed cells in nests, and resin robbing behavior. Nesting locations was used as an indicator of natural nesting sites limitation.

Mechanisms influencing social organization

We examined aggressive interactions between nest mates for protecting provisioned cells. We made observations to detect cell usurpation and egg cannibalism.

Results

Level of social organization

The three nests studied in detail had an increase in number of adult bees throughout the study period, and nest E had the biggest populations. Nests varied temporally in the number of adult female bees during the year. At the end of the study the average number of adult bees per nest was 11.9 (± 4.6 , $N = 5$). Nest E had 22 adults,

contrary to nest D that had periods without any adult bee inside (Otero, 1996a). Bees built an average of 4.2 cells (± 2.1 , $N = 13$) during the time they were observed.

The observed life span of a bee was shorter than the egg-to-adult period. The average life-span recorded for an adult female in the nest was 29.9 days (± 13.6 , $N = 13$), while the egg-to-adult period was 67 days ($N = 1$). Additionally, all of the five cells that were sealed on 11 April 1994 had live immature bees at the end of sampling, on 3rd of June, showing that the developmental period is longer than 53 days. Thus adult females of *Eg. nigropilosa* are unlikely to interact with their progeny indicating that there is no overlapping generation in *Eg. nigropilosa*.

We did not observe cooperative brood care in *Eg. nigropilosa*. None of the 45 cells recorded was constructed or worked on by more than one bee. Each bee collected resin and pollen for her own cluster of cells inside the common nest cavity. Individual clusters were adjacent to each others and comprised up to 9 cells and were better recognizable by the bee behavior. In an incidental observation, one cell with a partial pollen load, constructed by a bee that disappeared without finishing the cell, was not reused by any other bee in the same nest. Some of the resin was used a week later for cell construction.



Figure 1. Variation of ovary development index among bees with different wing damage (see text for methods description). Seven individuals of the wing damage I and six of higher damage categories were dissected.

Ovary development and fertilization

Not all adult bees reproduced equally. The most active bee constructed nine cells in 45 days; in contrast, one bee was not recorded performing reproductive activities. Most of the dissected bees had developed ovarioles (Table 1). Only one of the bees had limited ovary development, but it was young and may not have had reached the reproductive stage. The spermathecae of five of eight bees contained sperm. Most of the dissected bees (seven) were in range 1 of wing damage when captured, one had range 2, two had range 3, and three had range 4 (Table 1). One non-fertilized bee had wing damage of range 3. Bees with no wing damage, at level 1,

are thought to be younger, and they had variable ovary development, whereas older bees all had developed ovaries (Fig. 1).

Open cell to adult bee ratio

The ratio between open cells and adult female bees per nests varied (Fig. 2). The average ratio was 0.51 (± 0.33 , $N = 5$). It was significantly smaller than one ($Z=4.54$, $DF = 4$, $P=0.01$).

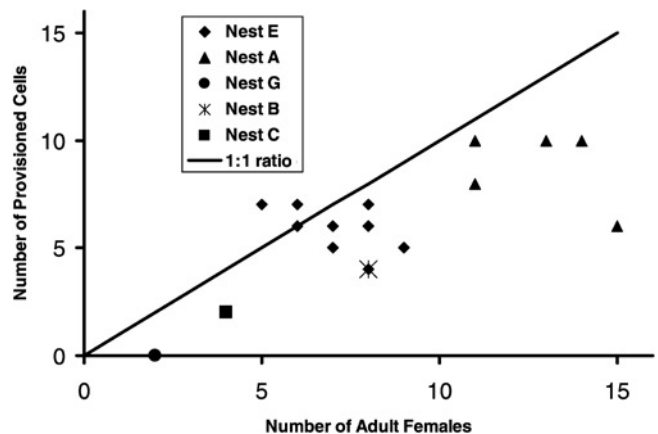


Figure 2. Number of female bees and open cells inside nest A (triangles) and nest E (diamonds) and others at the La Planada Nature Reserve between 16 August 1996 and 1 May 1997. Line represents the predicted 1:1 ratio between the number of adult females and the number of provisioned cells in communal colonies.

Foraging

Euglossa nigropilosa made trips for collecting pollen, resin and nectar. Across all nests, the most frequent trips was “no loads” (Two-way ANOVA, $F_{2,45} = 12.9$, $p < 0.0001$; Fig. 3). Pollen trips were more frequent in two of the three nests studied, but nest E had relatively more resin trips (Two-way ANOVA, $F_{4,45} = 5.9$, $p < 0.0001$; Fig. 3). All bees but one foraged for pollen and deposited in cells. The time consumed in each activity varied significantly (One way ANOVA, $F_{2,41} = 27.95$, $p < 0.0001$). The duration of pollen trips (42.0 ± 12.9 min, $N = 18$) was significantly longer than no load trips (9.3 ± 6.63 min, $N = 15$), while resin trips (18.23 ± 15.0 min, $N = 11$) were intermediate in time.

The plants used by *Eg. nigropilosa* at LPNR were located in pastures, open areas and secondary forests. Their main pollen sources at LPNR were flowers with poricidally-dehiscent anthers from two plant families: Melastomataceae and Solanaceae including *Solanum quitense* Lam., *Solanum* sp. (J. T. Otero 398, deposited at CUVC herbarium), and *Tibouchina lepidota* (Bonpl.) Baill. Pollen was extracted by buzzing. Bees used resin to construct cells, the exterior envelope, and to cover the internal cavity and holes in the nest. The resin is light

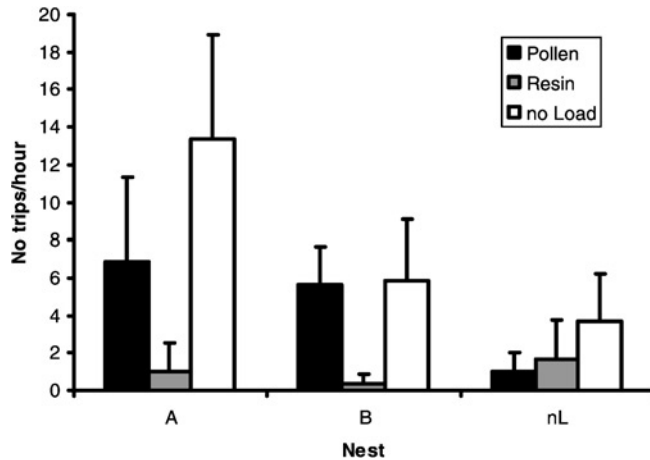


Figure 3. Number of trips with pollen, resin and “no loads” (without any material in the corbiculae, presumably nectar) during two hours monthly census of bees arriving to the three studied nests. Error bars are the standard deviations.

yellow when it arrives at the nest, but turns dark brown after oxidation and becomes brittle after desiccation. This material is collected by the bees outside the nest, presumably from flowers of *Clusia* spp., and is transported in the corbicula. When a bee arrived at the nest, the resin was deposited for storage in the bee’s territory while the bee leaves for another resin load. The main nectar sources for *Eg. nigropilosa* were two naturalized varieties of *Impatiens balsamina* L. (Balsaminaceae) with nectar concentration from 23.4% to 29.3% sucrose, and the native vine *Melothria longituba* C. Jeffrey (Cucurbitaceae) with nectar concentration 30.2% sucrose.

Although our results indicate absence of reproductive division of labor, we found some evidence of specialization for non-reproductive tasks in *Eg. nigropilosa*. Two bees were observed to specialize on tasks related to handling resin, important in cell and nest construction. One bee (CC-11) spent almost all her foraging lifetime (14 days) collecting resin and constructing the envelope. At the end of the study this bee was collected for studying ovary development. Sometimes the resin from the envelope that this bee collected was used by other bees in their cell construction. Most of the bees spent all their time constructing, supplying and protecting their own cells rather than performing labor that benefited the colony in general, such as construction of the nest envelope. Another bee (CCII-1) built nine cells in the first 45 days of its life; the last 10 days of its life, before it was collected at the end of sampling, she was less active than the previous days, remaining in its territory and leaving the nest infrequently without bringing pollen or resin for cell construction.

Factors determining social organization: natural enemies

Natural enemies of *Eg. nigropilosa* nests at LPNR were described previously. Nests were parasitized by the ectoparasitic wasp *Monodontomerus argentinus* (Torymidae) (Otero, 2001). A nest with no adults was significantly more attacked by the parasitoid *M. argentinus* than another nest that contained adult bees ($\chi^2 = 12.73$, $P = 0.0004$; Otero, 2001). The percentage of parasitized cells in an attended nest was 15% while unattended nest had 72% parasitized cells. Cells had 13.69 (9.04 SD, $n = 13$) wasps per cell. Wasps were founded at all developmental stages. *Euglossa nigropilosa* adult females appear to recognize *M. argentinus* wasps inside the nest because bees were aggressive against wasps (Otero, 2001). Bees were observed caring unidentified mites, but we did not quantify their effect on social activities.

Factors determining social organization: resource limitation

There is evidence suggesting that resin is a scarce resource for *Eg. nigropilosa*. Bees recycled resin from cells with empty cocoons in the nest. When adult bees emerged from cells, females reused them for a new brood. For cell reuse, bees introduced their head inside the cell and removed the remaining material of the cocoon with their mandibles. Afterward, the bee supplied the cell with pollen. During the observations, we recorded five cells reused in nest A and three in nest E. Some of these cells may be reused more than once. In total we observed 125 cells with brood of which 111 were new and 14 were reused.

Resin collected by a bee can be used by other bees that take it from their storage place, but bees protect their own resin and attack other bees that attempt to rob it by biting their legs. We observed five attacks by owners to bees attempting to rob resin, but most of the thefts occurred when the owner was outside the nest. Resin may be an important resource not just for *Eg. nigropilosa*, but also for other bees in the same general area. We made an unexpected observation: an unidentified species of Meliponinae entered one *Eg. nigropilosa* nest and robbed resin.

Another important scarce resource for *Eg. nigropilosa* is nesting sites. All 8 nests that we found were located in “artificial cavities” associated with human buildings. We searched 27 hours for nests in the forest and only 15 in the buildings. One caveat is that nests in human dwellings may be easier to locate.

Mechanisms of social control: territories

Cells in the nests were not apart spatially; but using the bee behavior it was possible to recognize the borders among the territories. We observed 27 territories in three

nests as indicated by 2 or more clustered cells constructed, oviposited and protected by a bee. Most agonistic interactions occur when a bee enters the territory of another bee. We have observed 127 fights between resident and trespassing bees. Nevertheless, even with observed aggressive interactions among nestmates, we found that *Eg. nigropilosa* is capable of accepting in their own nests other conspecific bees from other colonies. A bee labeled on 27 May 1994 in nest A was recorded 27 days later in nest B with a cell in construction and was supplying it with pollen.

Mechanisms of social control: cell usurpation

One attempt to usurp a provisioned cell was observed in *Eg. nigropilosa*. A bee that started a new cell after finishing the previous one left the nest. When she was outside, a second bee opened the finished cell, introduced her head inside it and remained there for approximately 30 sec, removed her head from the cell, oviposited in the cell, and capped it again. Then the usurper bee returned to her territory. As soon as the first bee arrived at the nest, she touched the usurped cell with her antennae and became excited; after walking around the cell for several seconds, the bee opened the cell, introduced her head, and oviposited and capped the cell. As an experiment, we opened one cell that was capped for a few hours without touching the interior of the cell when the owner was outside the nest. When this bee returned to the nest she performed exactly the same behavior described for the other bee.

Discussion

Level of social organization

Our principal finding is that *Eg. nigropilosa* is a communal bee: The nests studied presented many traits that correspond to communal insect colonies: 1- nests with more than one bee from the same generation, reaching up to 22 individuals, the largest colonies known for *Euglossa* spp; 2- no generational overlap; 3- no cooperative brood care; and 4- no evidence for reproductive division of labor. Nevertheless, there was variation in specialization for non-reproductive tasks. Factors such as resource recycling and defense against natural enemies may be important for group nesting.

We found no evidence of generational overlap in *Eg. nigropilosa*. Data on the longevity among *Eg. nigropilosa* females is scarce and complicated by the movement of bees between nests making difficult an accurate estimation of the average longevity for the studied species. Nevertheless, it is in the range known for other *Euglossa* spp. in nature (Dodson, 1966; Garófalo, 1985; Garófalo et al., 1998; Augusto and Garófalo, 2004) and in captivity (Ackerman and Montalvo, 1985). The 67 days The egg-to-

adult period for *Eg. nigropilosa* was only somewhat longer than in other *Euglossa* species (compare with: Dodson, 1966A; Aquino and Cuadriello, 1990; Ramírez-Arriaga et al., 1996; Eltz et al., 2003), but more data are necessary to determine whether or not this difference is significant. The relatively long egg-to-adult period of *Eg. nigropilosa* may be caused by the low temperatures at LPNR compared with other sites where *Euglossa* spp. construct nests. Higher sociality or unequal reproduction may be prevented by a lack of generational overlap due to long developmental time.

We did not observe cooperative brood care in *Eg. nigropilosa*. In *Eg. cordata* cooperative brood care is the result of cell parasitism by the dominant bee (Garófalo, 1985).

We found no evidence for reproductive division of labor, but some evidence for differential performance of non-reproductive tasks in *Eg. nigropilosa*. Reproductive division of labor with evidence of castes was reported for artificial nests cavities of *Eg. cordata*, where a dominant bee oviposited in cells constructed and provisioned by other bees (Garófalo, 1985). A similar situation was observed in nests of *Eg. townsendii* where all females reproduce but some forage and other do not (Augusto and Garófalo, 2004). The same situation was suggested for *Eg. atrovoneta* (Ramirez-Arriaga et al., 1996). In this study, some bees devoted all their time to collecting and working resin in the later part of their life, benefiting the colony. This could be a temporal component, as in *Eg. hemichlora* females start smearing resin provisions all over the walls of the nest cavity before actually starting to construct a brood cell (Eltz, pers. comm.), as reported by Garófalo (1985, 1992) for *Eg. cordata*. This change in behavior with age could be related to adult behavioral development that also underlies non-reproductive division of labor in other social Hymenoptera (Wilson, 1971; Robinson, 1992, 1999; Page, 1997; Calderón et al., 1989; Gordon, 1996).

We expected an open cell to adult bee ratio of close to one, but we found the ratio to be significantly different than one. However, even in communal systems not all bees reproduce because they may differ in reproductive quality. Alternatively, a ratio of 1 can be found in a strongly skewed system as well. Therefore the ratio of open cell to adult females should be considered only in combination with other characteristics.

Ovary development

We observed highly developed ovaries; bees that presented poor development were young (Table 1). This observation indicates that all females have reproductive potential, but this trait in itself is not exclusive of communal bees. We observed most bees to have wing damage range 1 were probably young because the first group may contain both young females with low ODI and also some reproductive females. This observation sug-

gests that ovaries mature early in the development of adult bees. Presence of spermatozoa in the spermatheca of young bees suggests that copulation is also early.

The combined evidence of ratio of provisioned cells to adult females, reproductive development in almost all individuals, foraging and cell construction and territoriality and the resulting lack of cooperative brood care all point in the same direction: *Eg. nigropilosa* have communal nests.

Factors influencing group nesting

Natural enemies

It has been postulated that natural enemies such as predators, parasites and parasitoids are important factors that facilitate the evolution of highly developed social systems in social insects (Roubik, 1989). A naïve expectation is that highly advanced insect societies should be parasite-free, but this is far from reality. Eusocial insects have an incredible diversity of parasites (Schmid-Hempel, 1998). A high incidence of parasitoids is thought to select for communal living. Evidence of this for euglossini bees was found in *Eg. nigropilosa*. An experimental nest without adults had significantly higher parasitism by the parasitoid wasp *Monodontomerus argentinus* than a control nest with adult females (Otero, 2001). *Monodontomerus* spp. are well known parasitoids of Hymenoptera. *M. argentinus* was reported to attack the solitary euglossini bee *Eufriesea nigresens* Friese in the eastern part of the Andes in Colombia (Sakagami and Strum, 1965). Hymenopteran parasitoids attack highly social insects like ants, *Polistes* wasps and honey bees. *Monodontomerus montivagus*, *M. minor* and *M. sp* are reported to attack *Bombus morrisoni*, *Polistes* sp. and *Mischocyttarus* sp. respectively (Schmid-Hempel, 1998). Furthermore, uninhabited nests of *Eg. hyacinthina* had higher incidence of natural enemies than nests with one or more females (Soucy et al., 2003). Nonetheless, the high density of closely related individuals in most large insect colonies is a perfect environment for parasites to reproduce (Schmid-Hempel, 1998). In consequence, a high incidence of parasitoids may arrest social evolution in the communal stage, especially if the parasites affect the lifespan of adult bees. The role of natural enemies such as parasitoids in the evolution of insect societies is an interesting possibility, but it must be tested more rigorously.

Resource limitation

Scarcity of nest sites and nesting materials is expected to reduce the options for independent nest foundation and may also select for communal living (Crozier, pers. comm.). The resource that momentarily limits the rate of reproduction: resin; pollen; nectar; or the availability

of nesting space are all potentially limiting. The recycling of nest building materials and cell reuse observed in *Eg. nigropilosa* are good example of inherited resources that can be recycled. We presented evidence that resin is a scarce resource for *Eg. nigropilosa* at RNLP. Of the recorded trips, resin collection represented the least frequent activity. This can be due to the methodology here used because there is the tendency of the resin-collecting trips occur in the afternoon (see Cameron and Ramirez, 2001, for *Eulaema meriana*, and Augusto and Garófalo, 2004, for *Euglossa townsendi*).

Nonetheless, when a bee finds a resin source, it collects it many times. Additionally, bees steal resin from other members of the colony. Nevertheless, our data on trip duration suggests that it is most likely pollen which is hardest to obtain. Only calculation of time budgets per cell for each resource could clarify this point.

Natural nest cavities may be infrequent in a wet and humid forest as in La Planada Natural Reserve. Under humid conditions bee nests are susceptible to fungal infection (Schmid-Hempel, 1998). All nests encountered were in human buildings that were drier than the forest sites. Reuse of cells allowed the bees to utilize the nest cavity more efficiently. The number of cells inside the nests did not increase much during nine months, suggesting that cell reuse is important; for rests in small cavities. For example, nest C had evidence of frequent cell reutilization. When there are resource limitations, the inherited resources are direct benefits for members to stay in the colony (Ragsdale, 1999), favoring the development of communal colonies.

Cell reuse is reported in other euglossini bees, *Eg. viridisima* (Aquino and Cuadriello, 1990). When euglossini bees nest in cavities, some daughters remain in the same nest; thus nests are active for several generations (Bennett, 1965; Zucchi et al., 1969a; Garófalo, 1992). This permits mothers and daughters to interact; thus generation overlap can arise. In *Eg. nigropilosa*, longevity constraints prevent generation overlap. Other euglossini bees also have long-lived nests, and some may present generation overlap, as in *Eg. hyacinthina* (Soucy et al., 2003).

Mechanisms of social control: Territories, cell usurpation and agonistic interactions

Euglossa nigropilosa can usurp provisioned cells of other bees inside the nest. Nevertheless, these attempts can be detected by the owner of the cell, who reopens the cell and presumably eats the egg and lays a new one. In contrast, in *Eg. cordata* there is a dominant bee, usually the oldest of the nest, that usurps cells and attacks other members of the nest (Garófalo, 1985). A similar situation was observed in *Eg. townsendi* (Augusto and Garófalo, 2004). Dominance was not observed in the nests of *Eg. nigropilosa* and there was no such dominance by a particular bee. *Euglossa nigropilosa* has the capability to

recognize cell usurpation, presumably based on chemical signals, but no cue, not even visual, can be excluded yet.

Agonistic interactions are an important factor determining sociability among euglossini bees. In *El. nigrita* female bees have agonistic interactions when a bee invades the territory of another female (Pereira-Martins, 1991; Zucchi et al., 1969b). In *Eg. cordata* the dominance of bees is expressed in antagonist interactions associated with egg cannibalism (Garófalo, 1985). In *Eg. nigropilosa* antagonist interactions are not associated with dominance but are related to the presence of territories in the nest.

A further evidence of aggressive interactions between neighbours in *Eg. nigropilosa* is that females had territories inside their nest. One of the most distinguishing traits of *Eg. nigropilosa* nests is the presence of territories defended by bees (Gómez, 1992; Otero, 1996). Each bee constructed her cells in a cluster and when in the nest, spent most of her time near the cell cluster. Inside the territories, bees carried out cell and envelope construction, oviposition, and defense against other bees that may enter the territory. Defense behavior was mediated by agonistic interactions among bees. Usually a bee that is in its territory attacked intruders by biting their legs. Sometimes the victim of the attack responded aggressively; agonistic behavior lasted longer than when there was no defensive response.

Mechanisms of social control: joining behavior

Joining behavior is also true for other species in Euglossini. Soucy et al. (2003) reported that females of *Eg. hyacinthina* were accepted in nests of conspecific nests. Nest aggregations of mixed species are known in the genus *Eufriesea* (González and Ospina, 2000). This tolerance is extreme in the genus *Eulaema*, which may present mixed colonies with more than one species (Roubik, 1990). This high tolerance seen among Euglossini may also be an important factor affecting evolution of social behavior in other bees of the family Apidae (Michener, 1974; Roubik, 1990).

Further studies on this species and other euglossines could add to our understanding of characteristics that favor or hamper the evolution of sociality. Specifically, one frontier could be the determination of the role of different factors on social organization, such as genetic relationships among nest inhabitants and genetic bases of differences in their behavioral phenotypes.

Acknowledgments

We thank J.C. Sandino of the Universidad Jorge Tadeo Lozano, Bogotá, Colombia, R. Ospina and G. Nates of the Universidad Nacional, Bogotá, Colombia, and C. Samper of the Smithsonian Institution for their valuable advice. At La Planada: Tata, Bello, Memo, C. Valderrama, M.F. Jaramillo and P. Moreno helped logistically, Don Segá, Ruca, Abelardo and Jaime provided company. Doña Ligia, provided food.

Hermogenes Fernandez-Marin, Beto Galindo-Cardona, B. Rivera-Marchand, D. Oskay, K. Hogendoorn, V. Grant, N. S. Flanagan and one anonymous reviewer made comments that improved the manuscript. We thank Nyeri and Inti, for their extra support. FES and the John T. and Catherine C. MacArthur Foundation supported the research. An NSF-CREST grant to TG at the Center for Applied Tropical Ecology of the University of Puerto Rico supported the preparation of the manuscript.

References

- Ackerman J.D. and Montalvo A.M. 1985. Longevity of Euglossine bees. *Biotropica* **17**: 79–81
- Aquino A. and Cuadriello J.I. 1990. Un nido de *Euglossa viridissima* Friese 1899 (Hymenoptera: Apidae: Euglossini). In: *Progr. Res. 25th Congr. Nac. Entomol. Oaxaca, México*. 117–118
- Amdam G.V., Norberg K., Fondrk M.K. and Page R.E. 2004. Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. *Proc. Nat. Acad. Sci. USA* **101**: 11350–11355
- Andersson M. 1984. The evolution of eusociality. *Annu. Rev. Ecol. Syst.* **15**: 165–189
- Augusto S.C. and Garófalo C.A. 2004. Nesting biology and social structure of *Euglossa (Euglossa) townsendi* Cockerell (Hymenoptera, Apidae, Euglossini). *Insect. Soc.* **51**: 400–409
- Bennett F.D. 1965. Notes on a nest of *Eulaema terminata* Smith (Hymenoptera, Apoidea) with a suggestion of the occurrence of a primitive social system. *Insect. Soc.* **12**: 81–92
- Bonilla-Gómez M.A. and Nates-Parra G. 1992. Abejas euglossinas de Colombia (Hymenoptera: Apidae). I. Claves ilustradas. *Caldasia* **17**: 149–172
- Bull N.J. and Schwarz M.P. 1996. The habitat saturation hypothesis and sociality in an allodapine bee: cooperative nesting is not “making the best of a bad situation”. *Behav. Ecol. Sociobiol.* **39**: 267–274
- Calderón N.W., Robinson G.E. and Page R.E. 1989. Genetic structure and division of labour in the honey-bee society. *Experientia* **45**: 765–767
- Cameron S.A. 2004. Phylogeny and biology of Neotropical orchid bees (Euglossini). *Annu. Rev. Entomol.* **49**: 377–404
- Cameron S.A. and Ramirez S. 2001. Nest architecture and nesting ecology of the orchid bee *Eulaema meriana* (Hymenoptera: Apinae: Euglossini). *J. Kansas Ent. Soc.* **74**: 142–165
- Danforth D.N., Conway L. and Shuqiong L. 2003. Phylogeny of eusocial *Lasioglossum* reveals multiple losses of eusociality within a primitively eusocial clade of bees (Hymenoptera: Halictidae). *Syst. Biol.* **52**: 23–36
- Darwin C. 1859. *The Origin of Species by Means of Natural Selection*. John Murray, London. 513 pp
- Dodson C.H. 1966. Ethology of some bees of the tribe Euglossini (Hymenoptera: Apidae). *J. Kansas Entom. Soc.* **39**: 607–629
- Dodson C.H., Dressler R.L., Hills H.G., Adams R.M. and Williams N.H. 1969. Biological active compounds in orchid fragrances. *Science* **164**: 1243–1249
- Dressler R.L. 1982. Biology of the orchid bees (Euglossini). *Annu. Rev. Ecol. Syst.* **13**: 373–394
- Dunn T. and Richards M.H. 2003. When to be social: interactions among environmental constraints, incentives, guarding, and relatedness in a facultatively social carpenter bee. *Behav. Ecol.* **14**: 417–424
- Eltz T., Roubik D.W. and Witten M.W. 2003. Fragrances, male display and behaviour of *Euglossa hemichlora*: a flight cage experiment. *Physiol. Entomol.* **28**: 251–260
- Garófalo C.A. 1985. Social structure of *Euglossa cordata* nest (Hymenoptera: Apidae: Euglossini) *Entomol. Gener.* **11**: 77–83
- Garófalo C.A. 1992. Comportamento de nidificação e estrutura de ninhos de *Euglossa cordata* (Hymenoptera: Apidae: Euglossini). *Rev. Bras. Biol.* **52**: 187–198
- Garófalo C.A., Camillo E., Augusto S.C., Jesus B.M.V. and Serrano J.C. 1998. Nest structure and communal nesting in *Euglossa (Glossura)*

- annectans* Dressler (Hymenoptera, Apidae, Euglossini). *Rev. Bras. Zool.* **15**: 589–596
- Giray T., Giovanetti M. and West-Eberhard M.J. 2005. Worker division of labor and its hormonal regulation in the primitively eusocial wasp *Polistes canadensis*. *Proc. Natl. Acad. Sci. USA* **102**: 3330–3335
- Gómez L.G. 1992. Observaciones sobre el comportamiento dentro del nido de *Euglossa* sp. (Hymenoptera: Apidae: Euglossini). *Mem. 2nd Curs. Ecol. Trop. Biol. Cons., Reserva natural La Planada – 1992*. pp 249–252
- González V.H. and Ospina R. 2000. *Eufriesea nigrescens* y *E. pretiosa* (Hymenoptera: Apidae: Euglossini): un caso de oportunismo o simbiosis? *Caldasia* **22**: 357–359
- Gordon D.M. 1996. The organization of work in social insect colonies. *Nature* **380**: 121–124
- Hamilton W.D. 1964. The genetical theory of social evolution, I and II. *J. Theor. Biol.* **6**: 1–52
- Hogendoorn K. and Leys R. 1993. The superseded female's dilemma: ultimate and proximate factors that influence guarding behaviour of the carpenter bee *Xylocopa pubescens*. *Behav. Ecol. Sociobiol.* **33**: 371–381
- Kearns C.A. and Inouye D.W. 1993. *Techniques for Pollination Biologists*. University Press of Colorado, CO. 583 pp
- Kimsey L.S. 1982. Systematics of bees of the genus *Eufriesea*. Univ. Calif. Press., Entomology 95: 1–125.
- Lin N. and Michener C.D. 1972. Evolution of sociality in insects. *Quart. Rev. Biol.* **47**: 31–159
- Lockhart P. J. and Cameron S.A. 2001. Trees of bees. *Trends Ecol. Evol.* **16**: 84–88
- Michener C.D. 1974. *The Social Behavior of the Bees*. The Belknap Press of Harvard University Press, Cambridge, MA. 404 pp
- Moure J.S. 1965. Some new species of Euglossinae bees (Hymenoptera: Apidae). *J. Kansas Entomol. Soc.* **38**: 266–277
- Otero J.T. 1996a. Aportes al conocimiento de la biología de *Euglossa nigropilosa* Moure (Apidae: Euglossini). Honors Thesis. Departamento de Biología, Universidad del Valle. 79 pp
- Otero J.T. 1996b. Biología de *Euglossa (Euglossa) nigropilosa* Moure (Hymenoptera: Apidae) I, Características de edificación en la Reserva Natural La Planada. *Bol. Ento. Univ. Valle*, **4**: 1–19
- Otero J.T. 2001. *Monodontomerus argentinus* Brethes (Hymenoptera: Torymidae): a new parasitoid of *Euglossa nigropilosa* Moure (Hymenoptera: Apidae: Euglossinae). *Pan-Pac. Entomol.* **77**: 57–60
- Otero J.T. and Sandino J.C. 2003. Capture rates of male euglossine bees across a human intervention gradient, Chocó Region, Colombia. *Biotropica* **35**: 520–529
- Page R.E. 1997. The evolution of insect societies. *Endeavor* **21**: 114–120
- Paini D.R. 2004. Nesting biology of an Australian resin bee (*Megachile* sp.: Hymenoptera: Megachilidae): a study using trap nests. *Austr. J. Entomol.* **43**: 10–15
- Packer L. 1991. The evolution of social behavior and nest architecture in sweat bees of the subgenus *Evyllaesus*: a phylogenetic approach. *Behav. Ecol. Sociobiol.* **29**: 153–160
- Pereira-Martins S.R. 1991. Biología de *Eulaema nigrita*. 2. Atividades nidais. *Papéis Avulsos de Zool. USP* **37**: 237–243
- Pereira-Martins S.R.F. and Kerr W.E. 1991. Biología de *Eulaema nigrita* 1. Construção de células, oviposição e desenvolvimento. *Papéis Avulsos do Museu de Zoologia USP* **37**: 227–235
- Ragsdale J.E. 1999. Reproductive skew theory extended: The effect of resource inheritance on social organization. *Evol. Ecol. Res.* **1**: 859–874
- Ramírez-Arriaga E., Cuadriello-Aguilar J.I. and Martínez E. 1996. Nest structure and parasite of *Euglossa atrovirens* Dressler (Apidae: Bombinae: Euglossini) at Union Juárez, Chiapas, Mexico. *J. Kansas Ent. Soc.* **69**: 144–152
- Robinson G.E. 1992. Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* **37**: 637–665
- Robinson G.E. 1999. Integrative animal behaviour and sociogenomics. *Trends Ecol. Evol.* **14**: 202–205
- Roubik D.W. and Ackerman J.D. 1987. Long-term ecology of euglossine orchid-bees in Panamá. *Oecologia* **73**: 321–333
- Roubik D.W. 1989. *Ecology and Natural History of Tropical Bees*. Cambridge University Press. New York. 514 pp
- Roubik D.W. 1990. A mixed colony of *Eulaema* (Hymenoptera: Apidae), Natural enemies and limits to sociality. *J. Kansas Entomol. Soc.* **61**: 150–157
- Roubik D.W. and Hanson P.E. 2004. *Orchid Bees of Tropical America*. Editorial INBio Costa Rica. 370 pp
- Samper C. 1992. *Natural disturbance and plant establishment in an Andean cloud forest*. Ph.D. dissertation. Harvard University, Cambridge, MA. 226 pp
- Sakagami S.F. and Maeta Y. 1985. Multifemale nests and rudimentary castes in the normally solitary bee *Ceratina japonica* (Hymenoptera: Xylocopinae). *J. Kansas Entomol. Soc.* **57**: 639–656
- Sakagami S.F. and Strum H. 1965. *Euplusia longipennis* (Friese), und ihre Merkwürdigen Brutzellen aus Kolumbien (Hymenoptera: Apidae). *Insect. Matsumurana* **28**: 83–97
- Schmid-Hempel P. 1998. *Parasites in Social Insects*. Princeton University Press. New Jersey. 409 pp
- Seeley T.D. 1995. *The Wisdom of the Hive: The Social Physiology of Honey Bee Colonies*. Harvard University Press, Cambridge, MA. 384 pp
- Soucy S.L., Giray T. and Roubik D.W. 2003. Solitary and group nesting in the orchid bee *Euglossa hyacinthine* (Hymenoptera, Apidae). *Insect. Soc.* **50**: 248–255
- Vallejo M.I., Samper C., Mendoza H. and Otero J.T. 2004. La Planada forest dynamics plot, Colombia. In: *Forest Diversity and Dynamics* (Losso E.C. and Leigh E.G., Eds). pp 517–526
- Weislo W.T. 1997. Social interactions and behavioral context in a largely solitary bee, *Lasioglossum (Dialictus) figueresi* (Hymenoptera, Halictidae). *Insect. Soc.* **44**: 199–208
- West-Eberhard M.J. 1996. Wasp societies as microcosms for the study of development and evolution. In: *Natural History and Evolution of Paper-Wasps* (Turillazzi S. and West-Eberhard M.J., Eds). Oxford University Press, Oxford. pp 290–317
- Wilson E.O. 1971. *The Insect Societies*. Harvard University Press, Cambridge, MA. 560 pp
- Zucchi R., Oliveira B.L. and Camargo J.M.L. 1969a. Notas binômicas sobre *Euglossa (Glossura) intersecta* Latr. e descrição de sua larvas e pupa (Euglossini, Apidae). *Bol. Univ. Federal do Paraná* **3**: 203–224
- Zucchi R., Sakagami S.F. and Camargo J.M.F. 1969b. Biological observations on a Neotropical parasocial bee, *Eulaema nigrita*, with a review on the biology of Euglossinae (Hymenoptera, Apidae). A comparative study. *J. Fac. Sci. Hokkaido Univ., Series VI, Zool.*, **17**: 271–380