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Research article

Nesting biology and social structure of *Euglossa* (*Euglossa*) *townsendi* Cockerell (Hymenoptera, Apidae, Euglossini)

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Summary. The nesting behavior of the euglossine bee *Euglossa townsendi* was studied on the campus of the University of São Paulo-Ribeirão Preto, SP, Brazil, from January 1994 to December 1999, using artificial nesting substrate and observation boxes. Twenty-one nests were founded during the study period, with the highest frequencies of nesting occurring during the hot, wet season. Each nest was founded by a single female and, after the completion of the last cell, she spent most of her time in the nest. The males left the nest immediately after emergence and did not return. Some females left the nest within a few days of eclosing, while others stayed in their natal nests and began to reactivate them. Reactivations were performed by a single female, by one or more females in the presence of the mother, by more than one female in the absence of the mother, and by more than one female in the presence of females that participated in the prior reactivation. According to behavior, the females were classified as forager/egg-laying and egg-laying females. The oviposition by egg-laying females was always preceded by oophagy. All of the forager/egg-laying and egg-laying females that were dissected had been inseminated. The behavior displayed by egg-laying females is characteristic of brood parasitism and fits the parental parasitism hypothesis developed as an alternative pathway by which insect sociality could have arisen. The behaviors displayed by *E. townsendi,* together with those reported for *Euglossa cordata*, show that both species have bionomic traits that resemble the conditions suggested as precursors of the origin of eusociality.

Key words: *Euglossa,* nesting behavior, brood parasitism, oophagy, social structure.

Introduction

Euglossa is the largest genus of the tribe Euglossini, with 103 described species found from northern Mexico to Paraguay and northern Argentina, and also Jamaica (Moure, 1967; Rebêlo and Moure, 1995; Engel, 1999; Michener, 2000). Although most species are considered solitary (Zucchi et al., 1969; Dressler, 1982; Kimsey, 1987; Michener, 2000; Cameron, 2004), the presence of more than one female in the nests of some species has suggested the occurrence of some kind of social organization in those nests (Dodson, 1966; Roberts and Dodson, 1967; Sakagami et al., 1967; Zucchi et al., 1969; Olesen, 1988; Eberhard, 1988; Otero, 1996). Studies on the nesting behavior of *Euglossa cordata* (Linnaeus) (Garófalo, 1985, 1987, 1992; Augusto, 1993; Augusto and Garófalo, 1994), *Euglossa atroveneta* Dressler (Ramírez-Arriaga et al., 1996), *Euglossa annectans* Dressler (Garófalo et al., 1998), and *Euglossa hyacinthina* Dressler (Soucy et al., 2003) have shown that new nests are usually established by solitary females and that the occurrence of multifemale nests is the result of nest re-use by succeeding generations. Multifemale nests may be formed by females of different generations (mother-daughter: *E. cordata*, *E. atroveneta*, *E. hyacinthina*), of the same generation (sister-sister: *E. cordata*, *E. atroveneta*, *E. hyacinthina*, *E. annectans*), and, on rare occasions, by unrelated individuals (*E. hyacinthina*) but of the same generation (*E. annectans*).

Detailed observations on the intranidal behavioral interactions of *E. cordata* females have shown that in multifemale nests the oldest female, the mother or oldest sister, becomes dominant over the others. This dominant female rarely leaves the nest, becomes the major guard bee, and oviposits in cells provisioned and oviposited in by subordinate females (daughters or youngest sisters). Oviposition by the dominant female is always preceded by oophagy and usually occurs, immediately after the subordinate female has laid and operculated the cell. According to Field (1992), the

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behavior of the dominant female is characteristic of intraspecific brood parasitism. This type of parasitism may be an alternative pathway by which insect sociality could have arisen, as suggested by Charnov (1978), Stubblefield and Charnov (1986), and Ward and Kukuk (1998). These observations, together with the occurrence of multifemale nests in other species, suggest that a more detailed knowledge of nesting behavior of *Euglossa* species could provide information on behavioral patterns that have not yet been described for the genus.

This paper presents observations on the nesting behavior of *Euglossa* (*Euglossa*) *townsendi* Cockerell, a species that, like *E. cordata*, may develop social nests in which two or more females of different or the same generation share a nest. The social organization showed by both species, *E. townsendi* and *E. cordata*, is analyzed to verify whether they fit the parental parasitism hypothesis presented by Charnov (1978) and Stubblefield and Charnov (1986) and/or the predictions in Ward and Kukuk's (1998) model.

Materials and methods

The study was conducted on the Ribeirão Preto campus of the University of São Paulo (between 21° 05′–21° 15′ S and 47° 50′–47° 55′ W), State of São Paulo, Brazil, from January 1994 to December 1997. Nests of *E. townsendi* were obtained from trap-nests according to the method of Garófalo et al. (1993). Trap-nests consisted of hollow bamboo canes which were cut so that a nodal septum closed one end of the cane. A total of 383 canes were placed horizontally in bundles of eight to eleven along two shelves in a shelter built near the laboratory. The shelves were 1.2 and 1.5 m from the ground. The canes varied in length from 8.2 to 31.0 cm and their internal diameter ranged from 0.8 to 2.5 cm, although all sizes were not equally represented. The traps were inspected three times per week with an otoscope during the study period and those occupied by *Euglossa* females were marked and left in their original places. The activity of a nest was ascertained by observing the female returning from foraging flights. Six to eight days after the females disappeared from their nests or were no longer observed performing foraging activities, the canes containing the nests were taken to the laboratory and split longitudinally into two pieces. After inspecting their structures and making measurements, the pieces of canes with the cells were placed in wooden boxes (inside dimensions: 19.8 × 5.0 × 6.0 cm) that were covered with a glass lid and had a 10-mm circular entrance hole on one side. After being transferred to the wooden boxes, the nests with a living foundress female were returned to the same places where they had been found and those whose foundresses had disappeared or were dead were left in the laboratory until the adults emerged. After the emergence of a female, the wooden boxes were fitted to laboratory wall holes; i.e., the bees were allowed to leave the boxes freely through a plastic tube connecting the boxes to the outside through the hole in the laboratory wall.

Observations of the activities of the bees in the nests were made through the glass and the activities of cell construction, provisioning, oviposition, and cell operculation were recorded for a total of 540 h. When more than two females shared a nest, they were marked with spots of color on their scutum to facilitate the identification of each individual. To verify the occurrence of oophagy and egg replacement, multifemale nests were observed for a total of 305 h. These observations were made during periods ranging from 30 minutes to 5 h after a female finished the provisioning of her cell and ovipositing in it. In addition to recorded cases, other cases of oophagy and egg replacement must have occurred when the nests were not being observed. In order to determine whether or not the females sharing a nest were inseminated, six females were removed from their nests after they had finished their reproductive activities and dissected. Each spermatheca was squashed between a slide and a coverslip, and examined under a microscope.

Statistical analyses were performed using Statistica 5.0 (StatSoft, Inc.). Throughout the text, all means are given ± SD.

Results

Nesting season, nest structure and emergence

A total of 21 nests were founded during the study period. The number of foundations per year ranged from 2 (1997) to 10 (1996) and the highest frequencies of nesting occurred during the hot, wet season (September-April).

The bamboo canes utilized by the bees ranged from 11.9 to 28.1 cm in length and from 1.1 to 2.2 cm in diameter ($n = 21$). All of the nests had a resinous wall recessed from the trap entrance in which there was a small circular hole just large enough for the female to pass. The space occupied by the nest (distance between the resinous wall and the bottom of the cane) ranged from 7.1 to 17.7 cm ($n = 21$). The nests did not show any inner wall coating; some of them had resinous particles deposited near the entrance or near the cells.

When the nests were transferred to the observation boxes, the number of completed cells in each ranged from 2 to 6 in nests whose foundresses had disappeared ($n = 4$) and from 4 to 8 in nests whose foundresses were dead ($n = 7$). In nests with live foundresses ($n = 10$), the foundresses had interrupted their field activities after building from 4 to 14 cells. After the completion of the last cell, the foundresses spent most of their time in the nest. The cells were made of dark resin, usually elliptical in shape, and 6.6–13.1 mm long ($\bar{x} = 10.7 \pm 1.4$ mm; $n = 66$) by 6.2–8.0 mm in maximum diameter ($\bar{x} = 7.1 \pm 0.5$ mm; $n = 55$). After the larva spun its cocoon, the adult female removed almost all of the resin covering the cocoon, exposing it to view, and used it for a new cell or in other activities in the nest. Therefore, the cells with prepupae and pupae usually contained only a small amount of resin in their apical end. The cells were constructed in rows with the long axes tending to be vertical or inclined. They could be found in isolated series with the cells of each series built in close contact or some cells could be isolated and other cells could be together (Fig. 1).

All emerging bees left their cells by chewing an arc around the apical end of the cell until the cap could be forced

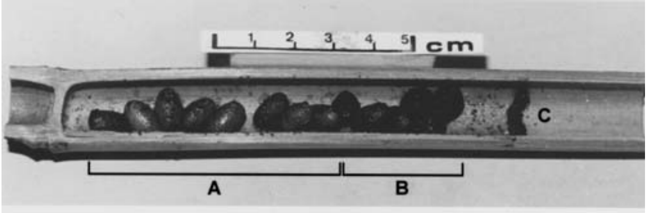

Figure 1. Bamboo cane split in half showing a fourteen-celled nest of *Euglossa townsendi*. A = oldest cells with the cocoons exposed due to removal of most of the resin by an adult female; B = newest cells built near the nest entrance; C = nest entrance.

open. Soon after emergence, both males and females would clean themselves and then leave the nest. All males and some of the females did not return to the nests, whereas some females returned and began to reactivate them.

Nest reactivation

A reactivation process (= R) is initiated when one newly emerged female remains in its natal nest and begins to work in it. Of the 73 females observed emerging, 67 (92%) returned to the nests. Only 37 (55%) of these remained in the nests and participated in reactivations. The remaining 30 females disappeared from their nests one (n = 7), two (n = 4), three (n = 11), and five (n = 8) days later because they died, started new nests, or joined existing nests. The duration of reactivation (from the beginning of female's activities until her last oviposition) (n = 19) ranged from 24 to 143 days, with longer durations in the cold, dry season. After finishing a reactivation, the females remained in the nest without performing field activities. This period of inactivity ranged from 12 to 93 days (n = 8) and was interrupted when a new reactivation was initiated. In one case only a new reactivation was initiated before the previous one had ended.

Of the 21 nests obtained, 11 were not reactivated because all of the females produced by the foundresses had dispersed from the nests (n = 4), because only males were produced (n = 1), or because all immatures were attacked by *Melittobia* sp. (Hymenoptera: Eulophidae) (n = 6). The foundresses were dead in five of these 11 nests and had disappeared in two others. In the nests with live foundresses (n = 4), the foundresses had disappeared after all individuals had emerged and left the nest. Of the 10 reactivated nests, four had live foundresses (nests 1, 2, 3 and 7) and two had dead foundresses (nests 15 and 19). The foundresses of the remaining in four nests had disappeared (nests 8, 9, 12, and 17). These nests were reactivated once (n = 4), twice (n = 5), and five times (n = 1) (Table 1).

Behavior of females during the reactivation processes

The females were classified into two groups according to their behavior: a) forager/egg-laying females (= FELF) – females that participate in the reactivation of their natal nests by constructing cells or re-using the old ones, by provisioning the cells and by ovipositing in them; and b) egg-laying females (= ELF) – females that rarely go out in the field, probably to feed, and oviposit in the cells provisioned and oviposited in by FELF; oviposition by ELF is always preceded by oophagy; the ELF can be the foundress female (= FF) when she participates in the first reactivation (= R_1) of her nest or a FELF which, after finishing the construction, provisioning, and oviposition activities, remains in the nest and oviposits in cells provisioned and oviposited in by another FELF, or a female that after emerging remains in the nest and only lays eggs. In only two cases did a female, behaving as an ELF, later perform the activities of a FELF.

Table 1. Conditions of *Euglossa townsendi* nests when transferred to the observation boxes, emergence and number of reactivations per nest

Nest no.	Examination date	Contents of the nest		Emergence			No. of dead immatures	No. of re-activations per nest
		No. of brood cells	Foundress female	☿	♂	☿/♂[2]		
1	11 Feb. 1994	5	+	3	1		1	5
2	4 Mar. 1994	13	+	4	2	6	1	1
3	4 Jan. 1995	5	+	3	2			1
4	9 Jan. 1995	6	+	0	0		6[3]	0
5	17 Apr. 1995	11	+	0	0		11[3]	0
6	17 May 1995	4	†	0	0		4[3]	0
7	23 Jun. 1995	6	+	3	1	2		2
8	23 Jun. 1995	4	+[1]	1	1	1	1	2
9	20 Dec. 1995	6	+[1]	2	1	2	1	2
10	22 Apr. 1996	8	†	0	0		8[3]	0
11	25 Jun. 1996	5	†	1	2		2	0
12	25 Jun. 1996	6	–	2	2	1	1	1
13	9 Jul. 1996	5	†	1	3		1	0
14	9 Jul. 1996	2	–	0	1		1	0
15	9 Jul. 1996	4	†	3	0		1	1
16	7 Nov. 1996	8	+	0	0		8[3]	0
17	12 Dec. 1996	6	–	3	1	1	1	2
18	6 Nov. 1996	14	+	0	0		14[3]	0
19	7 Jan. 1997	7	†	4	1	1	1	2
20	18 Mar. 1997	8	†	3	4		1	0
21	3 Apr. 1997	3	–	2	1			0

+ = live female; † = dead female; – = disappeared female; [1] = females that disappeared from the nest after they were transferred to the observation boxes; [2] individuals that emerged when the nests were not being observed and did not return to the nests; [3] immatures attacked by *Melittobia* sp.

Types of nest reactivation

Reactivation by a single female (n = 8): these reactivations were observed during the R_1 of nests whose foundresses had died (nest 19) or disappeared (nests 8, 9, 12, and 17) and during the R_2 of three nests from which the female(s) that had participated in the R_1 had disappeared (nests 8 and 17) or had been removed from the nest for dissection (nest 7). In these reactivations, the female, a FELF, behaved as a foundress, the only difference being the re-use of old cells although new ones were also constructed. In one of the eight cases observed (nest 17- R_2), the reactivation was interrupted by an attack by the cleptoparasite *Hoplostelis bilineolata* (Spinola) (Megachilidae) and the *Euglossa* female disappeared from the nest. In another case (nest 7-R_2), the female disappeared after ovipositing the fourth cell. In the six remaining cases, the females remained in their nests after finishing their reproductive activities and the number of cells laid by them ranged from 7 to 15. Four of these females disappeared from the nests before the emergence of their offspring (nests 8-R_1 and R_2, 12-R_1, and 7-R_2) and two participated in the next reactivation (= R_2) of their nests (nests 9 and 19). Nests 8, 12, and 7 ended because all progeny produced in the last reactivation process left the nest. One cell from nest 7 was parasitized by *Anthrax oedipus* Fabricius (Diptera: Bombyliidae).

Reactivation by one or more females in the presence of the mother (n = 6): matrifilial association occurred during the R_1 of nests 1, 2, 3, and 7 and during the R_2 of nests 9 and 19 in which the R_1 had been made by a single female. The number of daughters participating in these matrifilial associations was one (nest 7), two (nests 1, 2, 3, and 9), and five (nest 19). In all reactivations, the daughters behaved as FELF and the mothers as ELF. In nests 1 and 2, the mothers died after their daughters (two daughters in each case) finished their reproductive activities. In both nests, one of the daughters disappeared later, while the other one remained in the nest and either participated in the next reactivation (nest 1-R_2) or disappeared from the nest together with all individuals produced (nest 2-R_2). In nests 9 and 19, the mother disappeared before the daughters (two daughters in each nest) ended their reproductive activities. These nests ended because all of the individuals produced abandoned the nests. After the females (ELF and FELF) in nests 3 and 7 became inactive, they were collected for dissection. Nest 3 was attacked by *Melittobia* sp. and all immatures died, while nest 7 was reactivated (= R_2) by a single female.

Reactivation by more than one female in the absence of a mother (n = 1): this type of reactivation occurred during the R_1 of nest 15 whose foundress was found dead after having completed four cells. Three females ($1R_1$, $2R_1$, and $3R_1$) produced in the nest participated in the reactivation. $1R_1$ behaved as a FELF from 17 September to 5 October and then as an ELF from 6 to 11 October, ovipositing in cells that were completed by $2R_1$, which behaved as a FELF only. $1R_1$ and $2R_1$ shared the nest from 23 September to 11 October and both behaved as FELF for five days. On 7 October, a female of unknown origin (= FUO) joined the nest. During the five first days, this female behaved as an ELF and then as a FELF. After her last oviposition, the FUO remained in the nest. This female shared the nest for four days with $1R_1$ and for 15 days with $2R_1$. $3R_1$ emerged on 10 October and behaved as a FELF only. After the FUO and $3R_1$ had finished their reproductive activities, they were removed from the nest for dissection. Of the 18 brood cells built during the R_1 of this nest, six contained dead immatures that had died of unknown causes; parasites emerged from two cells, one male *Coelioxys costaricensis* Cockerell (Megachilidae) and one individual of *Anthrax oedipus oedipus* Fabricius (Diptera: Bombyliidae); four *Euglossa* females emerged from four cells; and individuals emerged from the six remaining cells when the nest was not being observed. Nest development was interrupted because all of the females left the nest.

Reactivation by more than one female in the presence of females that had participated in the previous reactivation process (n = 4): these reactivations occurred after R_1 in a single nest (nest 1) reactivated five times. These processes (R_2–R_5) had the participation of 2 (R_3), 3 (R_2 and R_5), and 5 (R_4) females that remained in the nest after emerging and worked in the presence of 1 (R_2, R_3 and R_4) and 5 (R_5) females that had participated in the previous reactivation. Of the seven females that participated in one reactivation only, six ($2R_2$, $1R_3$, $2R_4$, $1R_5$, $2R_5$, $3R_5$) behaved exclusively as FELF and one ($3R_2$) as an ELF. Of the five females that participated in two reactivation processes, three behaved as FELF and then as ELF in the same reactivation and only as ELF in the subsequent reactivation ($1R_2$, $1R_4$, $3R_4$). One female behaved as an ELF and then as a FELF in the first process and as a FELF in the second one ($4R_4$) and one female behaved as an ELF in both reactivations ($5R_4$). The only female that participated in three reactivations ($5R_3$) behaved as an ELF in all of them (Fig. 2).

Activities of the forager/egg-laying females (FELF) during nest reactivation

Construction and re-use of the cells

The number of FELF participating in each reactivation process ranged from one to four individuals and their total time of residence in the nests from six to 77 days. The FELF may build new cells or re-use the old ones during a reactivation process, utilizing the resin available in the nest or that collected in the field by herself or by another FELF if present. Resin-collecting trips (n = 35) were made from 11:00 to 18:00 h but were more frequent between 14:00 and 16:00 h (n = 16). These trips lasted from 9 to 64 minutes (x = 33.3 ± 13.3 min; n = 28). The resin loads brought into the nest were deposited on the floor of the nest near the cells or the nest entrance or on the cells themselves. The female discharged the loads on their corbiculae using their midlegs. Irrespective of the number of females participating in the reactivation process, each cell was built by a single female. Cell construction consisted of numerous trips to collect the deposited resin or the resin from the cells containing prepupae and pupae, transportation to where the new cell was being built,

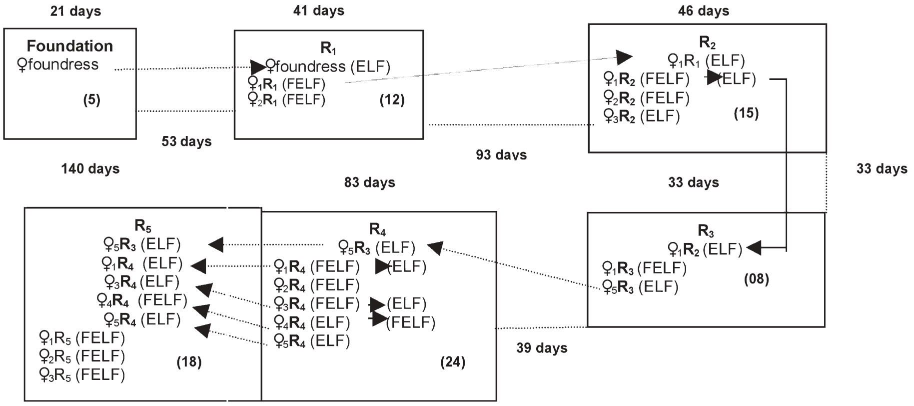

Figure 2. Schematic representation of the nest development of *Euglossa townsendi* (nest 1) with five reactivation processes. $R_1 - R_5$ = reactivation processes; ELF = egg-laying female; FELF = forager/egg-laying female; Numbers in brackets = number of brood cells built by the foundress female and during each reactivation process; Numbers above the boxes = duration (in days) of the reproductive activities of the foundress female and of each reactivation process; Numbers between the boxes = duration (in days) of the periods of inactivity between the foundation and the first reactivation process and two successive reactivation processes.

and construction of the cell. The female started provisioning when at least three-fourths of the cell had been completed. Re-use of cells occurred frequently. Cells from which the adults had emerged were cleaned, had their walls lengthened by successive additions of resin and further manipulation, and then had their inner walls lined with resin. Of the 19 reactivations observed, in only one case did the females not re-use any cell. In the 18 remaining reactivations, cell re-use ranged from 15% to 100% (x = 50 ± 26%).

Cell provisioning

Each FELF provisioned her own cell. The FELF (n = 28) began larval food-collecting trips when they were 2 to 12 days old; 68% of females began this activity when they were 2 to 6 days old. Most trips occurred between 9:00 and 12:00 h (Fig. 3) and lasted from 17.1 to 45.8 min ($\bar{x}$ = 27.9 ± 8.4 min; n = 49). When returning from the trip, the female entered the nest, walked to her cell, inspected it by introducing her head, and then introduced her abdomen into the cell and discharged the larval food. Food discharge was usually followed by rotational activity during which the female turned clockwise and/or counterclockwise, one or more times. The total time from abdomen insertion into the cell to abdomen withdrawal ranged from 14 to 52 s ($\bar{x}$ = 28.7 ± 8.7 s; n = 61) with the highest frequency (n = 37) occurring between 21 and 30 s (Fig. 4). The time spent inside the nest between provisioning trips was quite variable, ranging from 34 to 220 s ($\bar{x}$ = 80.6 ± 37.9 s; median = 72.0 s; n = 55). The duration of cell provisioning (n = 148) ranged from 1 to 6 days, with two (n = 47) and three days (n = 38) being the most frequent (Fig. 5).

Oviposition and cell operculation

The FELF (n = 28) made their first oviposition when they were five to 16 days old; in 75% of cases, the females were five to nine days old. Oviposition was always preceded by the construction of the cell collar. During this activity, the female removed portions of resin from other cells with her mandibles or collected the resin deposited on the floor of the nest and added it to the walls of the cell, increasing its height by 1–2 mm . The time spent by the female in this activity ranged from 410 to 2867 s ($\bar{x}$ = 1375.1 ± 711.78 s; n = 29). After finishing the collar, the female introduced her abdomen into the cell and oviposited. The time spent in oviposition

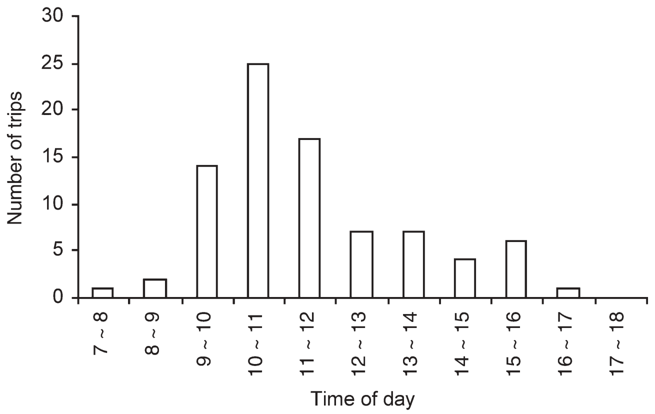

Figure 3. Larval food-collecting activity of *Euglossa townsendi* related to the time of day.

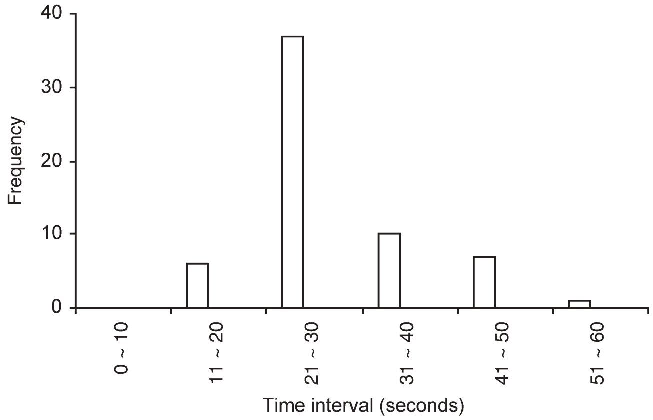

Figure 4. Time (in seconds) needed by *Euglossa townsendi* females to discharge a larval food load into the cell.

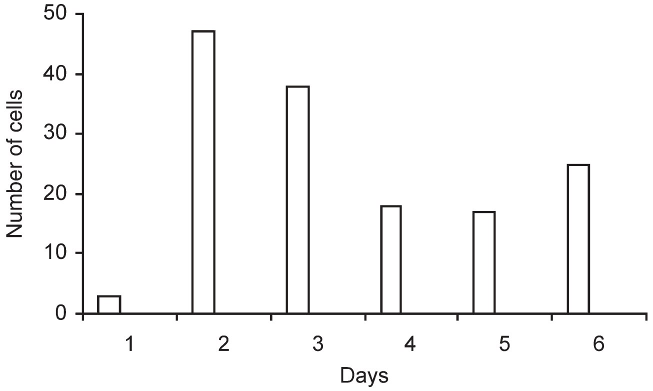

Figure 5. Time (in days) needed by *Euglossa townsendi* females to provision a cell.

ranged from 44 to 120 s ($\bar{x}$ = 67.6 ± 15.9 s; n = 33). After oviposition, the female immediately began operculation. This process was divided into two phases. In the first phase, the female, with her abdomen still in the cell, rotated her body and began to close the orifice by bending the collar with her mandibles and forelegs, while withdrawing her abdomen from the cell. The duration of this phase ranged from 63 to 208 s ($\bar{x}$ = 132.0 ± 41.4 s; n = 33). In the second phase, the female, with her body out of the cell, finished the closure by pressing the collar with her mandibles only. Then, the female left the cell many times to collect resin from adjacent cells and placed it on the apical region of the cell. The time spent in this phase ranged from 95 to 1077 s ($\bar{x}$ = 500.7 ± 273.9 s; median = 415.0; n = 33). The ovipositions (n = 50) were made mostly in the afternoon (n = 43).

The number of cells oviposited by each FELF (n = 23) ranged from one to 22 and was significantly correlated (r_s = 0.91; P < 0.05) with her period of activity (range: two to 49 days) (Fig. 6). All of the dissected FELF (n = 3) had been inseminated.

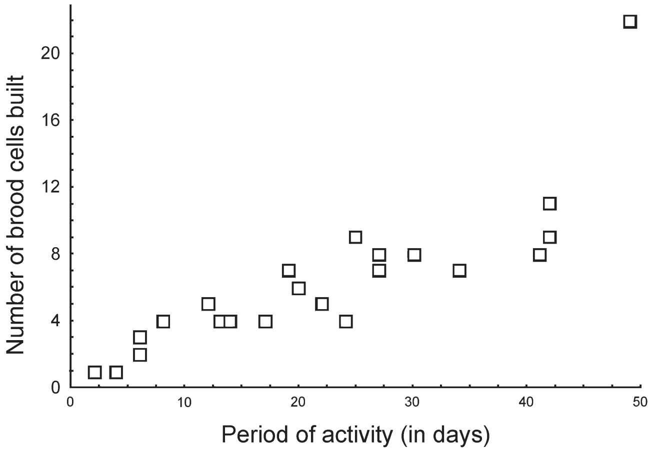

Figure 6. Relationship between the period of activity (in days) and the number of brood cells built by forager/egg-laying females of *Euglossa townsendi* (r_s = 0.91; P < 0.005).

Activities of the egg-laying females (ELF)

The number of females that behaved as ELF during a reactivation (n = 11) ranged from one (n = 6) to five (n = 1), but the maximum number of females performing such activities at the same time was four.

Such an ELF remains within the nest, her characteristic behavior is to oviposit in the cell provisioned and oviposited in by the FELF. The oviposition process by an ELF consists of a behavioral sequence of four steps: 1 – opening the cell oviposited in by a FELF; 2 – performing oophagy; 3 – ovipositing; and 4 – re-operculating the cell. Opening of the cell occurred in the presence or absence of the FELF, and the time spent by an ELF in this activity was 75 to 870 s ($\bar{x}$ = 249.1 ± 178.35 s; median = 207.5 s; n = 18). After opening the cell, the ELF puts her head inside the cell and performs oophagy, which lasts 50 to 227 s ($\bar{x}$ = 110.8 ± 46.2 s; median = 100.0 s; n = 19). Before starting oviposition, the ELF puts her head inside the cell and expands its opening by performing rotating movements. Then, she oviposits. Once oviposition is finished, the ELF immediately starts the operculation process, following the same behavioral sequence displayed by FELF.

Of the 61 egg replacements observed, 43% occurred on the same day the FELF had laid, 38% occurred one day later, and the remaining ones occurred two (11%) and three (8%) days later. The interval between oviposition by a FELF and egg replacement by an ELF on the same day ranged from 25 minutes to 4.28 h. In the reactivations where two or more ELF are present, after the first egg replacement by one ELF, the same cell may be opened again by another ELF and the egg replaced once more. This was observed on nine occasions, with the second egg replacement occurring on the same day the first ELF had laid (56% of cases) or two (33%) or four (11%) days later. On two occasions, the cells were opened again on the same day the second ELF had laid, and a new egg replacement was observed. Egg replacement was observed up to four times in one cell, and in this case the fourth process occurred four days after the third egg replacement had been observed.

The time of residence in the nests for females that behaved as ELF only (n = 4), as ELF and FELF (n = 1), and as FELF and ELF (n = 11) was significantly longer (range: 27–179 days; $\bar{x}$ = 109.7 ± 48.1 days; n = 16) than the time of residence of females that behaved as FELF only (Mann-Whitney test, Z = 3.97; P < 0.001). All of the dissected ELF (n = 3) had been inseminated.

No overt agonistic behavior was observed in any multifemale nest, and the ELF and FELF appeared to be indifferent to each other's presence.

Number of cells built during a reactivation process

Considering only those cases in which the females were alive after interrupting their reproductive activities, no difference was found between the number of brood cells built by a foundress (n = 10) and the number of cells built in one-female reused nests (n = 6) (Mann-Whitney test, Z = 1.85; P > 0.05). The total number of cells built during a reactivation process was significantly correlated ($r_s = 0.67$; P < 0.05; n = 18) with the number of FELF working on each of them (Fig. 7). However, irrespective of the number of FELF in each reactivation, the number of cells per female was not significantly different (Kruskal-Wallis test, H = 8.98; P > 0.05). In contrast, the per-capita brood production was negatively correlated ($r_s = –0.71$; P < 0.05; n = 18) (Fig. 8) with the total number of females (FELF + ELF) participating in the reactivation process.

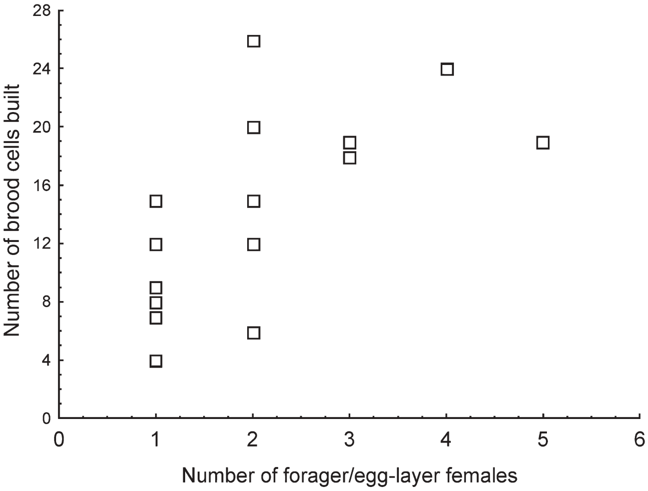

Figure 7. Relationship between the number of forager/egg-laying females and the total number of brood cells built during a reactivation process in *Euglossa townsendi* nests ($r_s = 0.67$; P < 0.05).

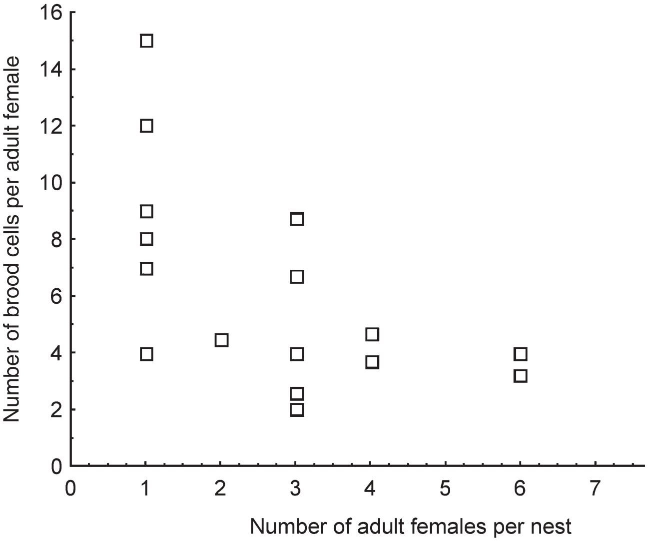

Figure 8. Relationship between the total number of adult females (forager/egg-laying + egg-laying females) participating in a reactivation process and the number of brood cells per adult female in *Euglossa townsendi* nests ($r_s = –0.71$; P < 0.05).

Egg-to-adult period

In nests active during the hot, wet season (September–April), no significant difference was found in the egg-to-adult periods between males (range: 54–74 days; $\bar{x} = 58.5 \pm 4.1$ days; n = 26) and females (range: 52–75 days; $\bar{x} = 61.9 \pm 6.8$ days; n = 35) (Mann-Whitney test, Z = –1.52; P > 0.05). A similar result was found for males (range: 64–127 days; $\bar{x} = 86.2 \pm 27.9$ days; n = 4) and females (range: 61–127; $\bar{x} = 93.5 \pm 23.8$ days; n = 14) produced in nests active during the cool, dry season (May-August) (Mann-Whitney test, Z = –0.26; P > 0.05). However, the times of development observed during each nesting period were significantly different between sexes (Mann-Whitney test, Z = –3.02; P < 0.05 for males and Z = –4.36; P < 0.05 for females).

Discussion

Nesting biology

The nesting pattern showed by *E. townsendi* was similar to those described for *E. cordata* (Garófalo, 1987) and *E. atroveneta* (Ramírez-Arriaga et al., 1996), and reflects the occurrence of a greater availability of resources during the hot, wet season.

Solitary nest foundation and nesting in pre-existing cavities, as observed in the present study, are characteristics shown by most *Euglossa* species whose nesting habits are known (Zucchi et al., 1969; Garófalo, 1985, 1992; Augusto, 1993; Garófalo et al., 1993, 1998; Ramírez-Arriaga et al., 1996).

In general, the architecture of the nests studied here was similar to that recorded by Garófalo et al. (1993) and the behavioral patterns displayed by *E. townsendi* females during cell construction, provisioning, oviposition, and operculation resembled those described by Garófalo (1985, 1987, 1992) for *E. cordata*. Only the removal of resin from cells after cocoons are spun by the larvae for the construction of a new cell and the initiation of provisioning when about three-fourths of the cell is completed are behaviors that differ from *E. cordata*.

As shown by our results and as observed by Garófalo (1987) in *E. cordata* and Ramírez-Arriaga et al. (1996) in *E. atroveneta*, the egg-to-adult period was similar for both sexes. The length of this period, however, was affected by climatic conditions, so that different values were obtained for different seasons. This was also observed in *E. cordata* (Garófalo, 1985), *E. pleosticta* Dressler, *E. truncata* Moure and Rebêlo (Garófalo et al., 1993), *Eulaema nigrita* Lepeletier (Santos and Garófalo, 1994), and the cleptoparasite *Exaerete smaragdina* (Guérin-Méneville) (Garófalo and Rozen, 2001).

In contrast to the data reported by Spessa et al. (2000) for *Amphylaeus morosus* Smith (Colletidae), the number of brood cells built by foundresses of *E. townsendi* did not differ significantly from that found in one-female reused nests.

This was also observed by Garófalo et al. (1992) in *Microthurge corumbae* Cockerell (Megachilidae) and Soucy et al. (2003) in *E. hyacinthina*. In these cases, the most important benefit of the adoption of a pre-existing nest would be the higher probability of survival for the female since she could avoid activities with a high mortality risk such as searching for a suitable nesting site and the repeated trips away from the nesting site to forage for nesting material.

In *E. townsendi*, the productivity of the nests (measured by the total number of cells built) increased with the number of females (= FELF) working in each reactivation process. These results are typical traits of colonies in which the females show independent reproductive activities, e.g., the FELF of *E. townsendi* and the females in communal associations such as those described for *Pseudagapostemon divaricatus* (Vachal) (Halictidae) (Michener,1964), *Lasioglossum hemichalceum* (Cockerell) (Halictidae) (Kukuk and Sage, 1994; Kukuk et al., 1998), *E. nigrita* (Santos and Garófalo, 1994), and *E. hyacinthina* (Soucy et al. 2003). Like *P. divaricatus*, *Ceratina japonica* Cockerell (Apidae) (Sakagami and Maeta, 1985), and *E. nigrita*, the productivity per FELF of *E. townsendi* remained constant irrespective of the number of FELF participating in the reactivation processes. On the other hand, when the FELF and the ELF were considered together, the productivity per female decreased. This decrease in productivity per female, applicable to a wide variety of social insects (Michener, 1964, 1974), is because ELF do not build cells. Thus, the more ELF in the nest, the lower the productivity per female. It is possible that a similar condition occurs in *E. hyacinthina* and would explain the lower per-capita offspring production in multifemale nests reported by Soucy et al. (2003).

Social structure and the origin of the eusociality

As observed in *E. cordata* (Garófalo, 1992) and *E. atroveneta* (Ramirez-Arriaga et al., 1996), successful nests of *E. townsendi* were founded by solitary females that, after building a number of cells, stopped foraging and remained in the nest until the brood emerged. Following the emergence of the brood, two situations could occur: all newly emerged females may leave the maternal nest or one or more than one female remains in the nest and initiates its reactivation.

Nest reactivation by one or more than one female, as observed in *E. townsendi*, has also been reported by Zucchi et al. (1969), Pereira-Martins and Kerr (1991), Santos and Garófalo (1994), and Garófalo and Rozen (2001) for *E. nigrita*, by Garófalo (1985, 1987) and Augusto (1993) for *E. cordata*, by Ramírez-Arriaga et al. (1996) for *E. atroveneta*, by Garófalo et al. (1998) for *E. annectans*, by Cameron and Ramirez (2001) for *Eulaema meriana* (Olivier), and by Soucy et al. (2003) for *E. hyacinthina*. There are, however, two significant differences in the reactivation processes shown by these species: the re-use of old cells by *Euglossa* species only and the origin of the females sharing a nest. The associated females are always of the same generation in *Eulaema* and *E. annectans* while they may also be of different generations and, on rare occasions, unrelated females in the other species and as observed in *E. townsendi*. These differences in the origin of associated females together with the behavioral patterns displayed by them result in different social structures in the nests of these species.

In the multifemale colonies of *E. townsendi*, all females are reproductively active and the activities performed by FELF and ELF are similar to those of subordinate and dominant females, respectively, in nests of *E. cordata* (Garófalo, 1985, 1987; Augusto, 1993). This task allocation shows that some females perform high-risk activities (the FELF and the subordinate females), while others do not (the ELF and the dominant female). There are, however, some important differences between these species. In *E. cordata*, task allocation is based on age, with the oldest female becoming the only dominant in the nest, and task reversal occurs only when a subordinate replaces the dominant female (Garófalo, 1985, 1987). In *E. townsendi*, other females besides the oldest female may behave as ELF, showing that age is not the only factor determining the task allocation in this species. Moreover, task reversals occur frequently, with most FELF becoming ELF later and on some occasions an ELF becoming a FELF. Thus, the presence of more than one ELF in the nest leads to the occurrence of multiple egg replacements in the same cell. In *E. cordata*, each cell is opened only once and the egg laid by the subordinate female is eaten by the dominant one, who then replaces it with her own (Garófalo, 1985, 1987; Augusto, 1993). These behaviors displayed by ELF in *E. townsendi* and by dominant females in *E. cordata* are characteristic of brood parasitism (Field, 1992) and fit the parental parasitism hypothesis put forward by Charnov (1978) and Stubblefield and Charnov (1986) as an alternative pathway by which insect sociality could have arisen. According to those authors, a mother would be under strong selection to parasitize her daughters if she had the opportunity to do so. In both *Euglossa* species such opportunity arises during nest reactivation. The mother gains a substantial selective advantage if she diverts resources from the production of grandoffspring to the production of additional offspring. The mother can do this by replacing her daughter's eggs with her own, as occurs in *E. townsendi* and *E. cordata*. Thus, by eating her daughter's eggs the mother gains high-quality nutrients which permit her to increase her longevity and fecundity in order to exploit the advantages of parasitism, as observed in *E. townsendi* and as reported by Garófalo (1985, 1987) and Augusto (1993) for *E. cordata*. Although the reproductive skew is complete in these matrifilial associations in both *Euglossa* species, to allow her daughters to oviposit and later replace their eggs with her own would also be, in these cases, a prudent selfish strategy to avoid group dispersal or lethal fighting, as predicted by the transactional models of reproductive skew (Reeve and Keller, 2001). The only significant advantage for daughters would be the acquisition of the dominant position after the death of the dominant female in *E. cordata* nests or remaining in the nest after finishing their activities as FELF and becoming an ELF, as observed in most cases in *E. townsendi* nests. Complete skew is also observed in sororal associations of *E. cor-*

data, contrary to the transactional and tug-of-war theories that predict that skews are higher when the dominants are the mothers of subordinate females than when the dominants are of the same generation as the subordinate females (Reeve and Keller, 1995; Reeve et al., 1998). On the other hand, although more detailed observation is necessary, the presence of more than one ELF in *E. townsendi* nests suggests a lower reproductive skew than that in matrifilial associations. This decrease in skew would occur because the production of offspring may be distributed among the ELF. The occurrence of more than one ELF may be associated with the absence of within-group aggression and its association with low skew seems to support the predictions by tug-of-war models.

In addition to brood parasitism when more than one ELF participates in a reactivation, the multiple egg replacements in the same cell indicate the occurrence of reproductive competition. Reproductive competition involving oophagy and egg replacement has also been observed in nests of *Xylocopa sulcatipes* Maa (Stark et al. 1990), *Xylocopa pubescens* Spinola (Hogendoorn and Velthuis, 1995; Hogendoorn, 1996), *Ceratina flavipes* Smith, *C. japonica*, and *C. okinawana* Matsumura and Uchida (Sakagami and Maeta, 1985, 1987, 1989, 1995; Maeta and Sakagami, 1995). However, in contrast to *E. townsendi*, the reproductive competition in the nests of these species occurs between the female behaving as a forager and the female behaving as a guard and, in most cases, the eggs laid by the guard were eaten and replaced by the forager. In a comparative analysis of the results obtained by those authors and those reported by Garófalo (1985) for *E. cordata*, Kukuk (1992) suggested that if the social behavior found in *E. cordata* approximates the ancestral condition for the remainder of the corbiculate bees, then the evolution of queen and worker castes could occur more readily in the Apinae where the principal reproductive is a sedentary female (the dominant female of *E. cordata*). Like in *E. cordata*, the principal reproductive in the multifemale nests of *E. townsendi* is also a sedentary female (the ELF), thus supporting the suggestion by Kukuk (1992). According to Charnov (1978), if parental parasitism is indeed the first step towards eusocial behavior, it immediately provides 'queen-like' behavior for the mother and selection would favor her giving up those duties which probably carry a considerable mortality risk. Thus, she should give up provisioning cells to remain at the nest site and parasitize her daughter cells. Interestingly, some aspects of *E. townsendi* life history are consistent with some of the predictions of the models by Ward and Kukuk (1998) about the benefits of communal nesting and the risk of brood parasitism by nest mates: 1. foundress females remain in the nests, protecting their brood after provisioning some cells; 2. some females forage, while others remain in the nest to cheat; and 3. although variable, the tendency of the fraction of females cheating increases slightly with colony size. Contrary to the prediction of the models, the number of cells provisioned by solitary females was similar to that by females sharing the nests. Although the models by Ward and Kukuk (1998) have been developed to analyze the context-dependent behavior in order to examine the transaction between solitary and communal nesting, the tactics modeled suggest a mechanism for the origin of eusociality: in a colony consisting of a mother and a group of daughters, the evolutionarily stable strategy is often for the mother to cheat and the daughters to forage but not to guard their new offspring. So, the mother would be acting as a queen and the daughters would be foraging for siblings rather than offspring – they would be the workers. The present results and those reported by Garófalo (1985, 1987) and Augusto (1993) show that both species, *E. townsendi* and *E. cordata*, have bionomical traits that resemble the conditions suggested as precursors of the origin of eusociality in Apinae.

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