

Social biology of *Augochlora* (*Augochlora*) *phoemonoe* (Hymenoptera, Halictidae) reared in laboratory nests

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Abstract Species of the subgenus *Augochlora* have been considered solitary, and their behavior was postulated as a reversal from the social condition known for its closest relatives. In this study, conclusive evidence of eusocial behavior in the wood-dwelling subgenus *Augochlora* is presented. Direct observation of behavior within artificial nests in the laboratory allowed studying the suite of behaviors that characterize social structure in the species *Augochlora* (*Augochlora*) *phoemonoe*. The following behaviors were recorded and analyzed: locomotion, feeding, construction activities within the nest, pollen collecting, guarding, oviposition, sudden retreats, antennation-tarsation, passing, and following. The last three behaviors represented interactions between nestmates. Social behavior in this temperate South American species is characterized by: a solitary nest initiation phase, followed by an eusocial phase with at least two broods; the small size of the colonies, with 1–3 first-brood females during the summer foraging period; the long-lived foundresses, alive until the end of the season; lack of morphological differentiation between dominant and subordinate individuals, evidenced by size only, but strong physiological differentiation, with a high

reproductive skew; short egg-to-adult developmental time (approximately 30 days); and delayed male production to the second or subsequent broods. Indexes of division of labor ranged between 0.32 and 0.76 for the studied nests, indicating behavioral specialization between colony members. During the eusocial phase, daughter bees had the highest frequencies of construction, pollen collection and guarding. Colony integration was mediated by high rates of social interactions, initiated by the foundress.

Keywords Augochlorini · Social behavior · Eusocial bees · Behavioral specialization · Castes

Introduction

The Augochlorini are small, usually bright green bees, exclusive to the New World. The tribe includes 25 genera (Michener 2007) and over 500 species (Moure 2007), with most diversity in tropical areas. Together with the worldwide-distributed tribe Halictini they are the only two lineages within the family Halictidae with different levels of sociality, including eusocial species (Brady et al. 2006; Michener 2007). Bees of other tribes of Halictidae are either solitary, parasitic or at most communal. The Augochlorini show diverse degrees of social behavior, from solitary to primitively eusocial, including facultative eusociality and cases of solitary behavior secondarily derived from social ancestors (Michener 1990; Danforth and Eickwort 1997; Weislo and Danforth 1997; Packer 1990; Weislo et al. 2004). Compared to the extensive literature on the social behavior of Halictini, the knowledge on the social biology of Augochlorini is scant (Packer 2006; Schwarz et al. 2007). Few species have been studied, and for many of them the information on their behavior has been inferred from nests

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in the field, based on the structure of the nests and the reproductive status of the bees at the time of nest excavation. Danforth and Eickwort (1997), in their review of social behavior in the Augochlorini, tabulated nearly 40 species for which there had been some indication of social behavior, but they considered that there was sound information for only 13 of them. Direct observation of behavior within the nests has been carried out among Augochlorini only for the solitary *Augochlora pura* in the laboratory (Stockhammer 1966), and for the facultatively eusocial *Megalopta genalis* through manipulation of natural nests and artificial observation nests in the field (Wcislo and González 2006; Smith et al. 2009; Kapheim et al. 2011; Tierney et al. 2013). The purpose of this contribution is to present detailed information on the social behavior of *Augochlora* (*Augochlora*) *phoemonoe* (Schrottky) reared in artificial nests in the laboratory.

Augochlora is one of the more diverse genera of Augochlorini, with nearly 120 named species, classified in two extant subgenera, *Oxystoglossella* and *Augochlora s. str.* (Engel 2000; Michener 2007). The two subgenera have been considered as morphologically and behaviorally distinct. Species of *Oxystoglossella* nest in the soil, and those for which social behavior is known are primitively eusocial, with castes recognized by behavior and physiology (Eickwort and Eickwort 1972). These characteristics are shared with species of the closely related genera *Augochlorella* and *Pereirapis* (Danforth and Eickwort 1997; Engel 2000), indicating that social behavior is plesiomorphic for the genus *Augochlora* as a whole.

Species of the subgenus *Augochlora* have been considered solitary (Danforth and Eickwort 1997; Wcislo and Danforth 1997; Engel 2000). They nest in decaying wood (reviewed in Dalmazzo and Roig-Alsina 2012), although one species has been reported nesting in the detritus-filled funnels of senescent bromeliads (Zillikens et al. 2001). The solitary behavior in the subgenus was postulated by Michener (1990) as a reversal to the solitary condition, associated to the lack of enemies in a newly colonized habitat, i.e., the shift from soil nesting to the use of decaying wood as a substrate. Eickwort (in Danforth and Eickwort 1997) mentioned the possibility of social behavior in the subgenus *Augochlora*, since he had dissected foraging females of the West Indian *A. magnifica* with worker-like characteristics. Recent studies of nests in the field have suggested social behavior in two species of the subgenus *Augochlora*, in the Central American *A. isthmii* (Wcislo et al. 2003) and in the South American *A. amphitrite* (Dalmazzo and Roig-Alsina 2012).

Here we present conclusive evidence of social behavior in the wood-dwelling subgenus *Augochlora*, and analyze the suite of behaviors that characterize social structure in the species *A. phoemonoe*. This species is one of the five

Augochlora present in temperate areas of southern South America (Dalmazzo and Roig-Alsina 2011). It occurs in central and northern Argentina, Uruguay, southeastern Brazil, Bolivia and Paraguay. Except for a few flower records, nothing of its biology was known to date.

Materials and methods

Laboratory settings

A flight room was installed in the facilities of the Museo Argentino de Ciencias Naturales “Bernardino Rivadavia,” Buenos Aires, Argentina, similar to those described by Batra (1964) and Stockhammer (1966). A room 2.0 × 2.5 m and 2.8 m high, with white tiles and plaster ceiling was furnished with 12 fluorescent 45 W tubes, lit nine hours a day (0900–1800 hours). The light cycle simulates the conditions of the woody environments where the species lives. Twenty artificial nests were placed on a bench 0.8 m above the floor facing a Y-shaped flight guide on the front wall. Artificial nests were constructed taking as a model those described by Michener and Brothers (1971), and modified to suit the needs of the studied species (Online Resource 1). They consisted of two glass panes 20 × 30 cm, separated 1 cm, and inserted into a wooden base. A layer of plaster of Paris in the bottom helped to keep humidity. The two panes were filled with slices of decomposing wood of *Salix sp.* The nesting substrate was collected in the field from fallen logs, where it is usual to find natural *Augochlora* nests, and sterilized in a freezer previous to use. The glass panes were covered with black plastic sheets to avoid light disturbing the nests and to facilitate observation. The room was not climate-controlled, but left to follow the day/night and seasonal fluctuations of outdoors. Fresh flowers and diluted honey were provided daily.

Seventeen females of *A. phoemonoe* were collected in the surroundings of Buenos Aires in spring (September–October) 2008 and 2009, marked on the scutum with different colors of nail polish, and introduced into the flight room. They were placed into small holes in the substrate of the artificial nest. Seven females died without any construction. Ten females started to construct their own nest, five of them continuing the small hole where they were placed, but the others selected a different place. As a result, two artificial nests had two and three foundresses respectively, but without connections between their nests. Since two females died before finishing the first cell, eight nests were successfully established, each by a single female. These nests contained the foundress female and produced at least one daughter female during the nest cycle. Daughter females were marked with a two-color code on the scutum (Online Resource 2). One color was used to indicate to which nest a

female belonged (same color as her mother) and the second one to discriminate between daughters of the same nest. Productivity per nest was assessed by the number of individuals reared throughout the season.

As an approximation to flying times of females and males in nature, collecting dates of 109 specimens (48 males, 61 females) from museum collections were analyzed. These specimens have been collected in northern Buenos Aires within a radius of 80 km around the study site by several different collectors (data pooled from Dalmazzo and Roig-Alsina 2011).

Behavioral observations

Nests were observed daily since the introduction of foundresses (September–October) until dissection of the nests at the end of the season (March). Each observation session took place between 8:00 and 20:00, totalling an average of 45 h per nest. During each observation session, each nest was observed by the same observer continuously for 20 min; the order in which the nests were observed was randomly assigned each day to avoid observation biases. Focal sampling was used to trace the behavior of individuals; the number of times (frequency counts) that each individual performed each activity or interaction from the behavioral catalogue was recorded (Online Resource 3).

The behavior of oviposition was actually observed few times (once by each of five foundresses). The total number of oviposition events in Online Resource 3 is indirectly calculated by the total number of individuals emerged from a nest. The zero oviposition frequencies for most daughter females is supported by their undeveloped ovaries. In a nest with a non-foundress female with developed ovaries the frequency of oviposition is rated as unknown (Online Resource 3).

Behavioral catalogue

Activities

Locomotion displacement of the individuals within or outside the nest, either walking or flying.

Feeding Intake of nectar or diluted honey outside the nest.

Construction Activities of tunneling, debris removal, and cell construction.

Pollen collecting Gathering of pollen from flowers and transport to the nest.

Guarding Staying at the nest entrance, often obstructing the entrance with the head or the metasomal dorsum.

Oviposition laying an egg on the food mass.

Sudden retreat A bee turns around and rapidly moves to the deepest region of the nest. This is an individual behavior with no interaction with other bees.

Interactions

Antennation-tarsation A bee approaches another bee nearly touching its head and then moves the antennae rapidly, touching the antennae and head of the second individual. In most cases the first bee also uses the foretarsi to touch the head of the second individual.

Passing A bee approaches another bee, either from the rear or from the front, and then moves past the other.

Following An individual closely approaches another one, and then turns around and moves rapidly, followed by the other bee. The movement is in any direction, either to the entrance or to the deep areas of the nest. The initiating individual begins the tandem running and dictates the direction of the movement.

Ovarian development and fertilization

The physiological condition of all females was established by fixing them in Kahle's solution at the end of the season. A few females which died in the flight room during the observation period were also fixed. Ovarian development was assessed following the classification of Michener and Wille (1961). Group A: large ovaries, well developed, usually with one or two eggs ready to lay; ovaries curved due to the pressure of the swollen posterior part. Group B: developed ovaries, but not curved, less swollen, without eggs ready to lay. Group C: undeveloped, slender ovaries. Spermathecae filled with sperm are seen as white refringent bodies, larger than empty spermathecae. Sections (10 μ thick) were made with a cryostat and stained with toluidine blue to corroborate presence of sperm.

Morphometrics

Wing and mandible wear were scored from zero (intact wings and mandibles) to three (worn mandibles and tattered wings) following Michener and Wille (1961). Four measurements were taken to assess bee size: total length (sum of lengths of head, meso and metasoma), forewing length (from wing base to apex of marginal cell), maximum width of head and length of head.

Data analysis

The significance of differences in behavioral frequencies between females were tested with non-parametric statistical tests (Mann–Whitney *U* test). Differences in morphometric

variables were tested with Chi square and Wilcoxon test. For the statistical analyses the package SPSS for Windows (v.22, SPSS Inc., Chicago, USA) was used.

Behavioral specialization

The indexes of division of labor (Gorelick et al. 2004) were used to measure the degree of specialization of individuals and the behaviors they perform. The indexes give a measure of division of labor as a function of the colony. These indexes, which derive from the index of mutual entropy (Shannon 1948; Cover and Thomas 1991), represent group attributes that allow comparisons among colonies and across species (Jeanson et al. 2005). The indexes vary between 0 and 1. To calculate $D(x/y)$ (division of tasks (Y) into individuals (X) and $D(x, y)$ (symmetric division of labor) we generated, for each nest, a matrix in which each cell showed the rate with which a specific individual was observed performing each of the behaviors listed above. The matrix was normalized, so the total of all cells added to one, and then we calculated Shannon's index H as indicated by Cover and Thomas (1991), and D indexes according to Gorelick et al. (2004).

Results

Life cycle

The eight nests established in the laboratory presented a solitary phase followed by a social phase, with one to three emerged daughter bees living with their mother.

During the solitary phase the introduced foundresses constructed a main tunnel and 1 to 4 clustered cells. These females foraged in the flight room, provisioned and laid eggs in the cells, and also guarded the nest entrances. This phase lasted an average of 30 (± 1.6) days. The developmental time of emerged females varied between 28 and 33 days ($N = 25$, median = 30) and that of emerged males varied between 27 and 30 days ($N = 23$, median = 29). The activities of the foundress and their frequencies changed drastically upon the emergence of the first daughter, marking the onset of the social phase. All emerged daughters stayed at their natal nests. Four nests had four emerged daughters, two had three, one had two, and another one a single daughter. Due to mortality of the emerged females (five bees from the four more populous nests were found dead on the floor and furniture of the flight room), no nest had more than three daughter bees during the social phase. The cells constructed and provisioned during the social phase produced a high percentage of males, which emerged in February–March. Emerged males stayed within the nest

for 12–20 h, and did not participate in any activity. At the time of dissection of the nests by the end of March, nine individuals were still at the stage of pupa, of which two were females. Nest productivity ranged from 4 to 10 individuals, with an average of 6.87 ($N = 8$). During the social phase the foundresses exited the nests only for nectar or diluted honey intake. Trophallaxis was not observed in any case. The foundresses, which were collected in spring as overwintered females, were still alive at the end of the season, so when the nests were dissected they were approximately one year old.

Social behavior

Foundress ($N = 8$) and daughter ($N = 20$) bees displayed significantly higher frequencies of locomotion than other behaviors (Wilcoxon test: $Z = -2.52$, $p = 0.01$ and $Z = -3.92$, $p < 0.001$). Daughter bees displayed significantly higher frequencies of locomotion and feeding than foundresses ($U = 20$, $p = 0.002$ and $U = 19.50$, $p = 0.002$). The frequencies of construction, pollen collecting and guarding were significantly higher for daughter bees, while foundresses displayed very low rates of these behaviors (Online Resource 3 and Table 1). Social interactions between foundress and daughters were started by the foundresses in all the nests. Statistical significant differences were found for antennation-tarsation ($U < 0.001$, $p < 0.001$), following ($U < 0.001$, $p < 0.001$), and passing ($U < 0.001$, $p < 0.001$). Daughter bees were never observed to start an interaction upon a foundress, and the rate of interactions between them was low (Online resource 3 and Table 1).

Social parameters

Foundresses presented well developed ovaries at the end of the season (Table 2). Daughter bees had slender ovaries (group C), with the exception of one individual which had developed ovaries (group B), representing 5 % of the daughter population.

Foundresses had worn wings and mandibles at the end of the season (Table 2); wings and mandibles of daughter bees were significantly less worn ($p < 0.001$, Table 2). Daughter bees with low values of wear were those last born.

The mean values of body measurements (length of body, length of forewing, maximum width of head, and length of head) were significantly larger in foundresses than in daughter bees (Wilcoxon test, $p = 0.03$, Table 3). Foundress-daughter size difference averaged 5.48 %, taking into account forewing length.

No males were produced in the first brood. The second brood consisted mostly of males (mean \pm SD = 76.2 % \pm 17.1 %, $N = 8$).

Table 1 Activities and interactions performed by foundress and daughter females observed in eight nest of *A. phoemonoe*

	Foundresses	Daughters	<i>U</i>	<i>p</i>
Locomotion				
Mean \pm SD	72.37 \pm 8.22	92.20 \pm 14.01		
Median	74	97	20	0.002
25th–75th quartiles	67.50–78.75	88.25–100.50		
Feeding				
Mean \pm SD	22.87 \pm 5.33	33.10 \pm 6.61		
Median	21	33	19.5	0.002
25th–75th quartiles	19–25.75	28–36.50		
Construction				
Mean \pm SD	3.25 \pm 1.16	31.25 \pm 6.99		
Median	3.5	33	<0.001	<0.001
25th–75th quartiles	2–4	25.25–35.50		
Pollen collecting				
Mean \pm SD	0.50 \pm 0.53	15.10 \pm 4.97		
Median	0.5	15	<0.001	<0.001
25th–75th quartiles	0–1	13–19.50		
Guarding				
Mean \pm SD	1.62 \pm 0.74	24.25 \pm 11.38		
Median	1.5	25	<0.001	<0.001
25th–75th quartiles	1–2	15.25–34.50		
Oviposition				
Mean \pm SD	4.37 \pm 1.84	0		
Median	5	0	10	<0.001
25th–75th quartiles	2.25–5.75	0		
Sudden retreat				
Mean \pm SD	14.62 \pm 4.27	1 \pm 0.64		
Median	15	1	<0.001	<0.001
25th–75th quartiles	12.50–17.75	1–1		
Antennation-tarsation				
Mean \pm SD	32.12 \pm 15.02	1.25 \pm 0.78		
Median	31	1	<0.001	<0.001
25th–75th quartiles	20–38.75	1–2		
Passing				
Mean \pm SD	18.00 \pm 6.00	0.85 \pm 0.58		
Median	16	1	<0.001	<0.001
25th–75th quartiles	13.50–21	0.25–1		
Following				
Mean \pm SD	12.50 \pm 3.74	0.65 \pm 0.67		
Median	13	1	<0.001	<0.001
25th–75th quartiles	9.50–15.75	0–1		

Median frequencies and 25th and 75th quartiles are presented for each type of activity and interaction

U: Mann–Whitney *U* test ($p \leq 0.05$) indicate statistical significant differences between foundresses and daughters

Behavioral specialization

$D(x/y)$, which measures whether a behavior is performed by a subset of all individuals or performed more equally by all

individuals within a nest, ranged between 0.32 and 0.76 for the eight nests (Fig. 1). Lower values correspond to those nests with two or three daughter bees, while the nest with the highest value (N2) had a single daughter bee. Nests with

Table 2 Physiological and morphological condition of foundress and daughter females of *A. phoemonoe* in laboratory nests

		Foundress (<i>N</i> = 8)	Daughter (<i>N</i> = 20)
Ovarian development*	A	6	0
	B	2	1
	C	0	19
Spermathecae filled with sperm*		8	0
Wings wear*	0	0	0
	1	2	9
	2	3	11
	3	3	0
Mandible wear*	0	0	0
	1	1	10
	2	3	10
	3	4	0

Bold letters and values indicate physiological and morphological categories

Number of individuals in each category is given (see text for category definitions)

Asterisks indicate significant statistical differences between foundresses and daughters (Chi square test, $p \leq 0.001$)

Table 3 Size of foundress and daughter females of *A. phoemonoe* in laboratory nests

	Foundress (<i>N</i> = 8)	Daughter (<i>N</i> = 20)
Length of body*	8.00–9.00	7.00–8.80
	(8.71 ± 0.35)	(7.82 ± 0.29)
Length of forewing*	4.60–4.85	4.00–4.75
	(4.74 ± 0.08)	(4.48 ± 0.14)
Maximum width of head*	2.40–2.90	2.00–2.60
	(3.41 ± 1.07)	(2.18 ± 0.06)
Length of head*	2.50–2.70	2.20–2.60
	(2.61 ± 0.06)	(2.37 ± 0.11)

Minimum/maximum measurements, mean, and standard deviation, given in mm

Asterisks indicate significant statistical differences between foundresses and daughters (Wilcoxon test $Z = -2.52$, $p = 0.01$)

four interacting females (N1, N3, N5, N7, N8) varied between 0.32 and 0.48, while nests with three females (N4, N6) had intermediate values. Similarly the symmetry index $D_{(x,y)}$ (0.27–0.46, Fig. 1), also takes its highest value for the nest with only two individuals. Perfect symmetry is achieved when each behavior is performed by a different individual.

Castes

Two castes are clearly differentiated in *A. phoemonoe*, according to their physiology, size and behavior. Founding females monopolize oviposition, display low rates of nest construction, guarding, and pollen collection during the social phase (Online Resource 3); they are the individuals that initiate social interactions, and are statistically larger. Daughter bees stay in the nest helping in the production of a new generation, are smaller, most of them with undeveloped ovaries, perform most tasks at the nest, and are the

subordinate individuals in social interactions. Castes are morphologically alike, except that mean size of foundresses was higher than mean size of daughter bees. Sub-castes, such as guards or foragers (Michener 1990), were not identified. No significant differences were found between daughter bees regarding the task they perform.

Discussion

The life cycle of *A. phoemonoe* is similar to that of many other halictines from temperate areas (Michener 1990; Yanega 1997; Schwarz et al. 2007). Nests were initiated in spring by solitary overwintered gynes. The first brood consisted in females, which helped produce a second brood at the end of the season. In our laboratory nests most colonies produced only males in the second brood. Studies of colonies of *Lasioglossum zephyrum* grown in the laboratory show that their productivity is affected by day

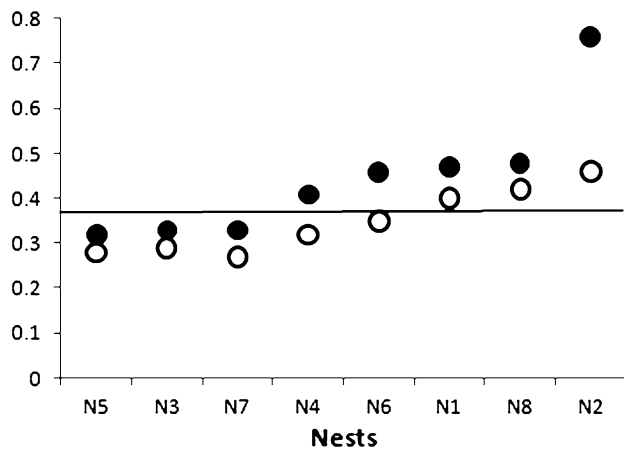


Fig. 1 Behavioral specialization in nests of *A. phoemonoe* reared in laboratory. Dots indicate division of tasks into individuals, $D(x/y)$, and open circles indicate symmetric division of labor, $D(x/y)$. The line indicates the expected value for a matrix of division of labor where a few individuals are highly specialized and the others perform the rest of the activities with similar frequency (Gorelick et al. 2004)

length and temperature (Greenberg 1982). The conditions of day length and light intensity we set in the bee room, which tried to mimic conditions in forest areas, may have been detrimental for colony growth, and the number of broods may be higher in the field than in our laboratory settings. Also, the sharp pattern of male emergence very late in the season obtained in the laboratory seems not to correlate with data from specimens collected in nature. Collecting dates of 109 museum specimens from northern Buenos Aires, (Dalmazzo and Roig-Alsina 2011) show that a few males are already flying earlier, in late spring (end of November), although they peak in mid summer (January) (Fig. 2). Nevertheless, the pattern is consistent with a delayed production of males. This protogyny is a characteristic of eusocial halictine bees (reviewed in Yanega 1997).

The egg-to-adult developmental time was similar to that of other eusocial Augochlorini with more than one seasonal brood in temperate regions (e.g., 20–31 days in *Augochlorella striata* and *A. persimilis*, Ordway 1966). Also, the developmental time was shorter than in solitary species in temperate latitudes, which takes 35–40 days in *Augochlora pura* and 37–38 days in *Agapostemon nasutus* (Stockhammer 1966; Eickwort and Eickwort 1969). In halictines with an activity period extending at least 6 months, and having a multivoltine life cycle, it is expected a short egg-to-adult developmental time (Michener 2007). The strong division of labor in *A. phoemonoe* is reflected by the high $D(x/y)$ index for the colony with just two individuals, where there is the least superposition of observed behaviors among them (Fig. 1, N2 with a foundress and a single daughter). The indexes for nests with three or four interacting females take values close to the maximum

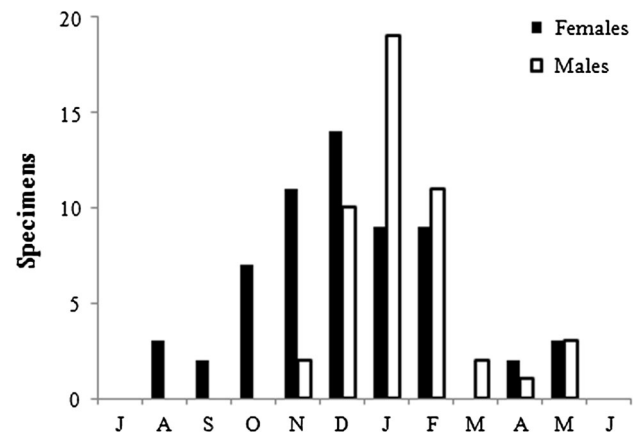


Fig. 2 Monthly records of *A. phoemonoe* bees flying in the field. Collecting dates of 109 specimens (48 males, 61 females) from northern Buenos Aires, from July to June. June and July are the coldest months. Data from museum specimens (pooled from Dalmazzo and Roig-Alsina 2011)

expected values for a matrix of division of labor where a few individuals are highly specialized and the others perform the rest of the activities with similar frequency (Gorelick et al. 2004).

The high rates of interactions between foundress and daughter bees are indicative of the communication processes that are promoting the cohesion of the colony (Michener and Brothers 1974; Breed and Gamboa 1977; Michener 1990). Antennation-tarsation, following, and passing, all interactions initiated by the foundress, would mediate integration of the colony in *A. phoemonoe*. The sudden retreat performed by foundresses, although not a part of an evident interaction with other nestmates, may have a dominant effect over the daughter bees. This behavior was observed only after the onset of the social phase, and probably has a territorial function in the domination of certain areas by the foundress, in particular the deepest areas of the nest. Such spatial segregation may enforce subordinates to perform duties according to their more peripheral locations. Similar retreats, with no obvious stimulus, have been observed in *L. zephyrum* (Michener 1990). Interactions between daughter bees were infrequent, and occurred in those nests with more individuals. Nests with three daughter bees and up to six males had the highest frequency of interactions among daughters and of daughters upon males. This may reflect a diminished dominance of the foundress when the colony grows (Michener 1990), that allows an increasing interaction between daughter bees.

Colonies of *A. phoemonoe* had a high reproductive skew. This reproductive division of labor has been postulated as the most significant parameter to characterize social behavior (Sherman et al. 1995; Lacey and Sherman 2005; Ratnieks and Wenseleers 2008). Developed ovaries and

sperm cells in the spermathecae were only found in the foundresses of *A. phoemonoe*. Most daughter bees had undeveloped ovaries and all were unfertilized. Inhibition of ovary development has been interpreted as the result of active maternal behavior upon subordinates. In the Halictini *Lasioglossum zephyrum* behaviors such as head nudging seem to mediate this inhibition (reviewed in Michener 1990). In *A. phoemonoe* antennation and tarsation would have an analogous function. However, inhibition was not absolute in the studied colonies. In a single nest, with three first-brood females, one of them had developed ovaries type B. She was unfertilized, probably because in our laboratory nests the delayed production of males to the second brood prevented females of the first brood from being fecundated. In any case, this female could have laid male eggs, and originated some of the males produced in that colony. We have no data regarding possible receptivity of first-brood females when males of the second brood emerge. If fertilized, they may become replacement reproductives, or even fly away and initiate nests of their own. More observations are needed to determine the behavioral variance probably present in this species. This study provides evidence that *A. phoemonoe* can exhibit eusocial behavior.

Species of the subgenus *Augochlora* have been considered solitary in contrast to its sister-group, the subgenus *Oxytroglossella*. This condition was inferred from extraction of nests in the field of a rather reduced number of species, and from a single detailed behavioral study in the laboratory of the solitary *A. pura* (Stockhammer 1966). Solitary behavior in the subgenus *Augochlora* was considered as derived from an eusocial ancestor (Michener 1990), since the subgenus belongs in a clade where its closest relatives are eusocial (Eickwort 1969; Danforth and Eickwort 1997; Engel 2000; Coelho 2004). All the phylogenies obtained for this clade, which besides *Augochlora* comprises the genera *Augochlorella*, *Ceratalictus* and *Pereirapis*, support an eusocial ancestor for the genus-group, although information on the social behavior of species of *Ceratalictus* is entirely lacking. Recent observation in the field of nests of *A. isthmii* (Wcislo et al. 2003) and of *A. amphitrite* (Dalmazzo and Roig-Alsina 2012) have challenged the notion that solitary nesting is the rule in the wood-dwelling *Augochlora*. Females found in nests of both species had ovaries with various degrees of development, different mandible and wing wear, and varied in size, suggesting some degree of social behavior for these species. The present study gives conclusive evidence of primitively eusocial behavior in *A. phoemonoe*. It is open to further study whether eusocial behavior is the widespread condition in the wood-dwelling *Augochlora*, or whether species of the subgenus are able to express different levels of social organization, conditioned by different environmental constraints. The related *Augochlorella striata* is known to form social or solitary nests at the high latitude limit of its

distribution (Packer 1990). Latitude and altitude are two factors that shape expression of sociality in several Halictini (Wcislo and Danforth 1997; Purcell 2011). A further plausible constraint for species of the subgenus *Augochlora* is their heterogeneous nesting substrate, highly variable in size, shape, and quality. Nesting sites from which some species have been recovered (Eickwort and Eickwort 1973; Wcislo et al. 2003; Dalmazzo and Roig-Alsina 2012), such as decomposing parts in cracks or knots of otherwise hard wood, pre-existing burrows of wood-boring insects, or narrow tree roots, impose strong limits to colony expansion. It would not be surprising to find in *Augochlora* species a social behavioral flexibility that allows them to cope with these constraints. The solitary nesting of the temperate North American *A. pura* seems to represent a true evolutionary loss of social behavior. In spite of continuous favorable conditions in the laboratory, nests reared by Stockhammer (1966) were always solitary. According to him "...evidence indicates early death of the individuals of each generation." This factor precludes any type of matrilineal association.

Conclusion

Although fieldwork is desirable to test the consistency of the observations in the laboratory nests, social behavior in *Augochlora phoemonoe* can be characterized by the following features: a solitary nest initiation phase followed by an eusocial phase with at least two broods; long-lived foundresses (alive until the end of the season), not fed by subordinates; lack of morphological differentiation between dominant and subordinate individuals, differentiated by size only; strong physiological differentiation, with a high reproductive skew; colony integration mediated by high rates of social interactions, started by the foundress; short egg-to-adult developmental time (approximately 30 days); small size of the colonies, with 1-3 first-brood females during the summer foraging period, and finally by a delayed male production to the second or subsequent broods.

The social behavior exhibited by *A. phoemonoe* is characterized by a marked division of labor within the nest. Founding females monopolize oviposition, display low frequencies of nest construction, guarding, and pollen collection, and are the dominant individuals that start social interactions. Daughter bees stay in the nest helping in the production of a new generation, perform most tasks (construction, pollen collection and guarding), and are the subordinate individuals in social interactions.

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