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Multiple Aggressions Among Nestmates Lead to Weak Dominance Hampering Primitively Eusocial Behaviour in an Orchid Bee

S Boff[1], CA Saito[2], IA Santos[1,2]

1 - Programa de Pós-Graduação em Entomologia, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo (USP), Ribeirão Preto-SP, Brazil

2 - Departamento Ecologia, Instituto de Biociências, Universidade de São Paulo (USP), São Paulo-SP, Brazil

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Corresponding author
Samuel Boff
General Biology and Bioprospecting Program, Faculty of Biology and Environmental Sciences
Federal University of Grande Dourados
Rodovia Dourados-Itahum, Km 12- Cidade Universitaria-Unidade II, Cx Postal 364
ZipCode: 79804970, Dourados, Brasil.
E-Mail: samboff@gmail.com

Abstract

Reproductive conflict expressed as aggression is common in social Hymenoptera. In eusocial species, as in honeybees, several mechanisms however alleviate the conflicts and reduce aggressive interactions. Unlike their sister group, the orchid bees do not exhibit eusocial behaviour. Instead, some of the species presents a primitively eusocial behaviour, with a single dominant female and fertile subordinate females participating on egg laying activities. In the current study we investigated the aggressive interactions of *Euglossa annectans* Dressler females through five generations of phylopatry and reuse of the natal nest. Although network analysis indicates that central individuals, those with more interactions, were more commonly the aggressors and others were more commonly the recipients, multiple attacks and several potential dominant females within the nest indicated a labile sociality. This suggests that there is an unstable social hierarchy in the species. *Euglossa annectans,* despite having overlapping generations, during which several individuals share a nest, there is no division of labour into reproductive females and interactions are often competitive. Aggressive behaviours conducted by multiple fertile females were often followed by egg, larvae or pupae replacement.

Introduction

To understand the aspects of how bee sociality evolved from solitary individuals to group living is often the aim of evolutionary biologists (Paxton et al., 2002; Schwarz et al., 2007; Kocher & Paxton 2014; Rehan et al., 2014; Andrade et al., 2016). Several insect societies rely on hierarchies to establish social organization. In eusocial insects for example the queen is the absolute reproductive dominant female while the workers (non-reproductive) are subordinated within well-defined activities organized in different castes (Michener, 1974). Despite the eusocial insects the species with plastic behaviour seem to be prone to better access social evolution. These species may offer cues of how the transition from solitary to social or vice versa evolved (Schwarz et al., 2007). Thus, studies on species whose individuals exhibit more alternative or facultative social behaviour (compared to species with predetermined caste differentiation) may help to provide more clear evidences of how advanced forms of sociality may have evolved (Dunn & Richards, 2003; Peso & Richards, 2010; Prager, 2014; May-Itzá et al., 2014; Rehan et al., 2014; Andrade et al., 2016).

The establishment of societies usually involves several behavioural traits which promote group living. The behavioural traits reflecting the interactions between reproductive conspecific females, for example, are often a key factor (Ratnieks et al., 2006; Rehan & Richards, 2013; Dolezal et al., 2014; Andrade et al., 2016). In addition to

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cooperative behaviour commonly seen between conspecifics in shared nests (Wilson, 1971; Schwarz et al., 1998; Rehan et al., 2014) there can be reproductive conflict and aggression (Breed et al., 1978; Wcislo, 1997; Rehan & Richards, 2013) which are often negatively correlated with genetic relatedness (Langer et al., 2004). Thus, aggression arises because of, or is highly correlated with, genetic relatedness, or is highly correlated with selfishness. If on one hand higher degree of altruism is found in genetically related individuals on the other hand when resources at stake is important/rare genetically unrelated societies may display equal, if not more, degree of altruism and cooperation (Andrade et al., 2016).

Reproductive conflict is wide spread in Apidae bee species (Crozier & Pamilo, 1996; Schwarz et al., 2007). Although it is minimal in eusocial species, it has been described for small carpenter bees (Rehan & Richards, 2013), bumble bees (Amsalem et al., 2009; Zanette et al., 2012), stingless bees (Peters et al., 1999; Wenseleers et al., 2003) and orchid bees (Augusto & Garófalo, 2004; 2009; 2010; Andrade-Silva & Nascimento, 2015). The latter group, the tribe Euglossini, is sister to the Apini (Cardinal & Danforth, 2011). In Apini all species are eusocial. Euglossini however do not exhibit eusocial species but are rather primitively eusocial with overlapping generation, subordination but lacks non-reproductive workers (Kocher & Paxton, 2014). In communal Euglossini species, as it was described previously (Garófalo et al. 1998; Cameron 2004; Otero et al. 2008), individuals share the same nest cavity with no interaction or alloparental care between the nestmates (da Silva et al., 2016; Dew et al., 2016). Yet primitively eusocial orchid bee species show overlapping generations, some division of labour, dominance behaviour, offspring control through oophagy (Cocom-Pech et al., 2008; Augusto & Garofalo, 2009; 2010) and may present subordinate female specialization in guarding the nest entrance (Boff et al., 2015).

In parallel to eusocial species in which only the queen lays most of the eggs, worker policing behaviour, oophagy and social contracts helps to reduce potential reproductive conflicts (Ratnieks, 1988; Ratnieks et al., 2006; Andrade et al., 2016). However, in the course of evolution some species may have selected traits in order to mitigate direct conflicts. Physiological traits (*e.g.* body size, ovary development) and social experience were potentially traits selected to avoid aggressive encounters (Wcislo, 1997; Rehan & Richards, 2010). In some species pheromones seem to play an important role controlling ovary development, making non-dominant females fail in reproduction and become loyal helpers (Dor et al. 2005). In some primitively eusocial orchid bee species, egg-laying is performed by dominant females as well as by subordinate females increasing for instance the aggression ratio between nestmates (Augusto & Garófalo, 2009; 2010).

In some bee societies, including orchid bees, totipotent individuals have plastic behaviour and can transit from one hierarchical condition to another (see Crespi & Yanega, 1995; Schwarz et al., 2007; May-Itzá et al., 2014). In the majority of species with totipotent individuals, reproductive dominance (queen-like vs worker-like females) and behavioural dominance (aggressive vs less aggressive individuals) can be predicted as it is usually the older or the bigger individual in the colony (Schwarz, 1994). Nevertheless it is not always easy to assign hierarchies for totipotent females since they can present a cryptic hierarchy (Bang & Gadagkar, 2012). Furthermore in many totipotent social Hymenoptera species, both queen-like and worker-like females are breeders (Andrade-Silva & Nascimento, 2012; Schwarz et al., 2007) and they both mate and produce offspring (sons and daughters) hence as the relatedness in the colony decreases, nestmates face more reproductive competition (Langer et al., 2004). Thus, competition and aggression between nestmates is often a mechanism used to achieve dominance on hierarchical societies (Bang & Gadagkar, 2015).

In the current study, we investigated social interactions analysing the aggression exchanged between nestmates of *Euglossa annectans* Dressler a species previously described as communal (Garófalo et al., 1998). Moreover, we established levels of attacks displayed between co-specifics and we tested if they were different among categories. Besides that, we tested if aggressive attacks towards nestmates could predict nest dominance. Finally, we tested if the number of nestmates present in the nest predicts the number of brood cells in a given generation.

Material and Methods

Nest development

The study was conducted in the Bee Laboratory at the University of São Paulo (23° 33' S, 46° 43' W), São Paulo, Brazil. The nest, previously occupied by *Tetragonisca angustula* Latreille (Apidae: Meliponini), was found in April 2010 in the garden of the Bee Laboratory with five brood cells with juxtaposed walls and only one active female of *E. annectans* called the founder female. The founder female disappeared from the nest before offspring emergences. At the end of the five brood emergences and during the sororal association of two females (parental phase) the observations ceased for a period of *ca.* 45 days, at the end of which there were 12 cells inside the nest with no female activity. At this point the nest was transferred inside the Laboratory and access to the field was offered via a short entrance/exit rubber hose tube. The nest consisted of a wooden box 20 x 30 x 8cm with a glass lid in the top which facilitated observations. Adult females present were manually removed from the nest and colour marked after placing them for two to five minutes in the fridge at 4 °C. For all generations, the nest was opened and adult females present in the nest marked only once. After marking the females, they were all delivered back to nest and their interactions were quantified.

We recorded diurnal intranidal activities along two years from September 2010 (1st generation) to March 2012 (5th generation). The total of observation covered approximately

400 hours which were not evenly distributed across days of observation. We recorded data of their nest biology and of interactions between nestmates. Daily observation time varied between 30 minutes and 6 hours. In the course of the study we recorded five generations, at least two generations per year, with overlap of generations during three generations (Fig 1). The number of operculated cells was recorded directly by mapping non-sealed and sealed cells. Sex ratio was assigned and tested through a non-parametric Chi-square test.

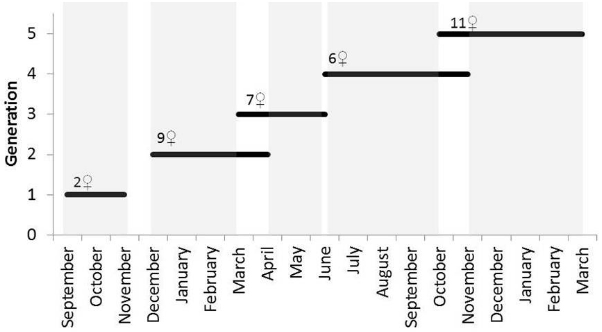

Fig 1. Nest succession of *Euglossa annectans* for two years between 2010-2012. During this time we recorded five generations. The horizontal bars show the length of time between a given generation when the first bee emerged until the period when the last bee from the same generation left the nest. The numbers over the horizontal bars followed by the symbol ♀ (female) indicate the number of active female during the generation. The light grey areas highlight the period of sororal (daughter-daughter) associations within one generation. Overlapping generations were observed among all generations.

Previous accounts of this species reported no interactions among nestmates (Garófalo et al. 1998). However as conflicts may be expected from reproductive conspecific females living together, we observed in the first generation and recorded (in the following generations) the number and duration of antagonistic interactions when body contact between nestmates was evident. The quantified aggressions were categorized according to its aggressive intensity (least aggressive "1" to most aggressive "3") as follow: 1) females push another nestmate with brief body contact; 2) female attacks another nestmate using mandibles, bites her opponent, and the opponent turns away, and 3) females attacks another nestmate using her mandibles, bites her opponent, chases her and pushes the injured female until she has left the brood cluster. We tested whether the number of quantified aggressions was different within each category using a non-parametric Chi-square test.

Aggression between distinct nestmates

During the second generation, we tracked 150 pairwise intranidal agonistic interactions (attack *vs.* injury) between seven nestmates across 14 non-consecutive days. Two females from this generation (which emerged after the marking day) were not colour marked. Thus, they did not have their interaction recorded.

We also investigated the interactions using the software Social Network Visualizer (Kalamaras, Socnet V) in order to visualize the position of the females in a central network analysis. According to the theory of central networks the most central individual is the one with the most interactions with the other nestmates (Freeman, 1978). Thus, using this approach, we were able to visualize the interactions among the females and indicate potential dominant (s) female (s), based on its centrality.

Aggression events in distinct generations

We recorded aggressive interactions observing females from four different generations (from 2nd to 5th) which were easily distinguished based on the coloured mark on their thorax (n = 20). Thus, in the 2nd and 3rd generation the observations were made on seven and eight nestmates, respectively and on two and three females, in the 4th and 5th generations, respectively. Non-marked individuals (which emerged after the marking day) did not have the aggressive interaction recorded. We present the results of aggressive interactions in terms of frequency. We also investigated the presence of potential dominant (*PD*) female(s) according to the following index of dominance: $PD=NA-NRA$, where the potential dominant (*PD*) is assigned when the difference between the number of attacks (*NA*) and the number of received attacks (*NRA*) for the same female was positive ($PD>0$). By consequence a potential subordinate female is designated when the number of received attacks (injury) from the nestmate(s) is bigger than the number of attacks she made toward her nestmate(s) ($PD<0$).

Through this approach, with bee behaviour recorded individually, we analysed if the total number of attacks was different from the total number of received attacks using a paired t-test. Out of these 20 females from four different generations, 10 attacked other females more than they were aggressed (potential dominant-*PD*, *i.e.* $PD>0$, see above explanation for potential dominant) and the other 10 females received more attacks than they initiated (potential subordinates-*PS*, *i.e.* $PD<0$). We first tested whether the number of attacks started by potential dominant and potential subordinate were different from the number of injures the same group received. Then we tested whether the number of attacks of the potential dominant females was different from the number of attacks initiated by potential subordinated females. We also tested if the number of received attacks for the potential subordinated females was higher than the number of received attacks for the potential dominant females. We used non-parametric Mann-Whitney for all paired tests.

Moreover, during our observations, we recorded number of eggs laid in a given generation and the number of females that take part in the nest reactivation in the same generation. We then tested if both variables were correlated (Pearson correlation). We also counted the frequency of egg replacement (conspecific brood parasitism) as well as the number of oophagy events carried out by the studied females. The statistical analyses were all conducted using IBM SPSS 19.

Results

Nest development

The number of eggs laid (counted by operculated cells), either in a new cell or in a reactivated one, was quite variable during the five generations. The highest number of operculations occurred during warmer season in the 5th generation followed by the 2nd generation. The lowest number of operculations occurred during the parental generation (Table 1). The number of females which engaged in reactivating the nest along five generations was positively correlated with the number of operculated cells inside the nest ($r = 0.987$, $p = 0.002$). The number of days in the nest considering females that stayed longer than 5 days was 37.6 ± 33.88 dias ($n = 34$ females). One female remained in the nest for 134 days. We did not observe the entire number of brood emergences, except for the brood from the first generation when all the individuals ($n = 12$) developed into females. However, based on the total number of cells and recorded emergences, a significant female-biased sex ratio was recorded. Even when the individuals that emerged and disappeared without been sexed and were theoretically included as males, there was still a bias to female production ($X^2 = 22.349$, df = 1, $p < 0.001$; Fig 2).

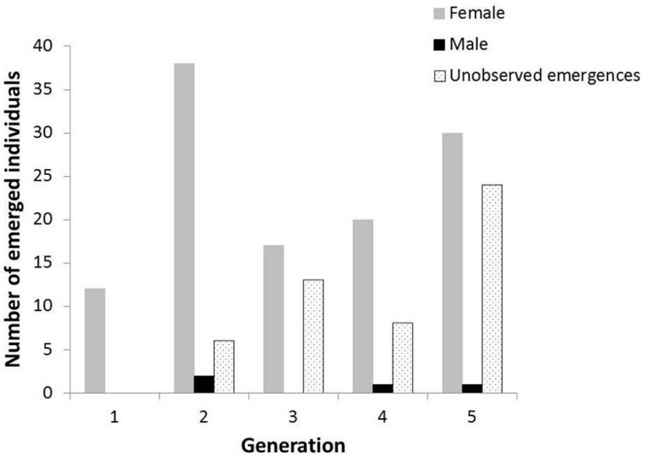

Fig 2. Number of females, males and unobserved emergences during five generations of *Euglossa annectans* based on the observed number of brood cells Except for those of the first generation, not all emerging bees were observed and sexed. The sex ratio was biased to females even when all non-sexed individuals (dotted bar) were theoretically considered as males (dotted bar + black bar = ♂ *vs.* grey bar = ♀; $X^2 = 22.349$, df = 1, $p < 0.001$).

Table 1. A summary of sororal actvities across five generations of *Euglossa annectans*.

Intranidal dynamics	Generation				
	I	II	III	IV	V
Females emerging from previous generation	3	12	38	17	20
Females that started reactivation	2	9	7	6	11
Operculated cells	12	46	30	29	55
Females emerging from current generation	12	38	17	20	30

Along the parental generation we did observe the mother leaving the nest but it did not bring any resources (eg. pollen or resin) before its complete disappearance after 30 days of nest sharing. Except for the first generation, two remaining females from previous generations were still foraging and laying eggs when the first of their brood emerged. Thus, in the beginning of the reproductive season females from previous generation ($n = 2$) and recent emergent female (s) took part in the production of a generation. The young females initiate a domestic work replacing resin inside the nest. They used to carry resin from the nest entrance to brood cells or to any other cavity in the nest or from cavities to brood cells or to the nest entrance.

Aggressive interactions

We observed attacks of different levels (from “1” to “3”, see Material and Methods) between nestmates. A female was attacked when it occupied the brood cell area (particular area where a female lays her eggs) of one of its nestmates. All females of *E. annectans* in our observation nest built brood cells with juxtaposed walls, creating an single active cellular brood cluster, similar to a comb, although with brood cells in both, vertical and inclined directions (Fig 3). In this context all females were laying eggs and operculating their cells in very close proximity to each other, leading to frequent encounters followed by aggressive behaviour.

We recorded a total of 446 aggressive interactions a long four generations. Taking into account only number of attacks towards a given female ($n = 251$), the most frequent attack registered was that belonging to category “3” ($n = 143$) followed by “2” ($n=91$). The least frequent was category “1” ($n = 17$). These results show that there were overall and pairwise significant difference between categories of attack ($X^2_{\text{overall}} = 239.086$, df = 2, $p < 0.005$; $X^2_{1\ vs.\ 2} = 50.704$, df = 1, $p < 0.005$; $X^2_{1\ vs.\ 3} = 204.313$, df = 1, $p < 0.005$; $X^2_{2\ vs.\ 3} = 79.854$, df= 1, p = <0.005). The aggression with intensity “3” had the longest mean duration (4.2s), followed by intensity “2” (2.1s) and intensity “1” (1s).

Fig 3. Nest of *Euglossa annectans* in September 2010. During this phase three females were sharing the nest (the third female is missing in the picture). The picture shows females in a matrifilial association. The mother (on the left) is observing her daughter reactivating a brood cell.

Network analysis between seven nestmates from the second generation identified the most central individual, tagged as female “1” (Fig 4). The 150 recorded interactions of aggression among these nestmates showed female “1” to be responsible for the majority of attacks (44%) towards its nestmates, followed by female “2” (19.3%). The majority of attacks that female “2” (75%) received was made by female “1”. Female “6” attacked the least (only female 1 and 7) and suffered attacks from all other nestmates (Fig 4). Female “1” was also the oldest, followed by females “2”, “3”, “4”, “5”, “6” and “7”. Only female “1” and female “7” attacked more than they were attacked (*PD* >0, see Table 2), although the latter interacted less than all other females. The other five (2-6) females (potential subordinated) were attacked more frequently than they initiated an attack (*PD* < 0, see Table 2). Across all five generations we did not observe a bee younger than this female “7” (1-3 days old) initiating a fight, but they were attacked if they walked over the brood cells of conspecific nestmates.

Although we did not track the number of eggs laid and the emergence of all females during the entire study, female “1” at this time had the highest reproductive investment since it laid more eggs (n = 6) than its nestmates.

Aggressive events from multiple generations

Out of 20 colour marked females of four generations (n = 7♀, 2nd generation; n =8 ♀, 3rd generation; n = 2♀, 4th generation; n = 3♀, 5th generation) 10 females (n = 2, 2nd generation; n = 6, 3rd generation; n=1, 4th; n = 1, 5th generation) conducted more attacks (potential dominant) towards other females compared to the number of attacks they received. The other 10 females (n = 5, 2nd generation; n=2, 3rd generation; n = 1, 4th generation; n = 2, 5th generation) were more frequently attacked (potential subordinated) compared to the number of attacks they initiated (Fig 5).

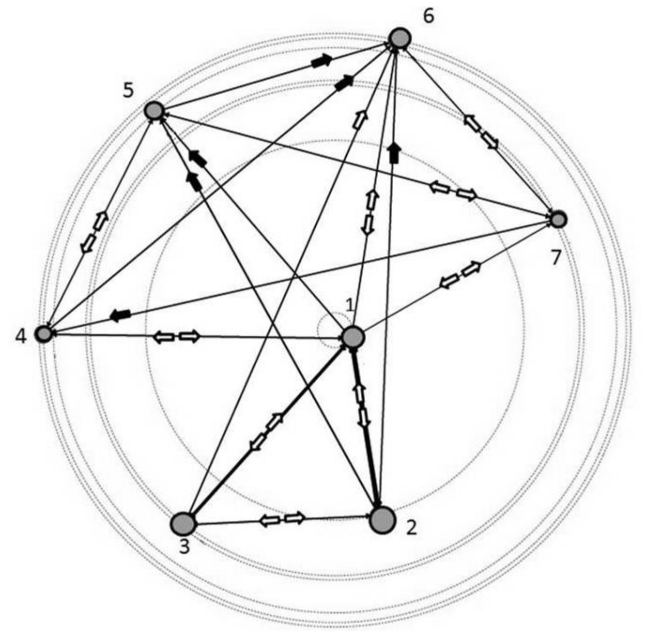

Fig 4. Results of the network analysis for seven females tracked in the second generation of *Euglossa annectans*. In the graph of the network, a link between two nodes (two females) reports the interaction. The arrows (black) departing from one node (*e.g.* female “1”) to another node (female “5”) indicate the direction of an interaction, i.e. female “1” attacking female “2”. The absence of an arrow from one node to another node or a complete absence of a link indicates absence of an attack or an interaction, respectively. If two individuals exchange mutual attacks, two arrows (white) in opposite directions are attached to the link. The thickness of the link (an interaction) indicates how many interactions between two given nestmates were observed, and the size of the node indicates how much a given bee was attacked. Thus, bigger nodes indicate a given female that received a greater number of attacks. Thus the most central bee (female “1”) was interacting more than others and also more frequently initiated an aggressive event. Every individual has a concentric circle showing the spatial place of each individual in the network.

Table 2. Agonistic interactions of seven nestmates in a sororal association of *Euglossa annectans*. *NA* = Number of attacks, *NRA* = Number of received a attacks, *PI* = Percentage of interaction, *PD* = Potential dominant.

					NA				
	Female	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***Total NRA***
NRA	***1***	-	13	3	1	0	4	3	24
	2	33	-	11	0	0	0	0	44
	3	18	7	-	0	0	0	0	25
	4	6	0	0	-	2	0	3	11
	5	5	6	0	2	-	0	7	20
	6	3	3	3	4	3	-	3	19
	7	1	0	0	0	4	2	-	7
	Total NA	66	29	17	7	9	6	16	
	PI	0.44	0.193	0.113	0.046	0.006	0.004	0.106	
	PD=NA-NRA	42	-15	-8	-4	-11	-13	9	

The mean number of attacks initiated by marked females (potential dominant + potential subordinated) was not significantly different from the mean number of injuries the same group of females received (Mann Whitney U = 190.500, $n_1 = n_2 = 20$, p = 0.799, 1 tailed). Nonetheless the number of attacks started by potential dominants was significantly higher when compared to the number of attacks started by potential subordinates (Mann Whitney U = 10.500, $n_1 = n_2 = 10$, p = 0.002, 1tailed). Albeit slightly higher, the number of received attacks for potential subordinated females was not significantly different from the number of attacks received for potential dominant females (Mann Whitney U = 33.500, $n_1 = n_2 = 10$, p = 0.218, 1 tailed). We observed that all females (either potential dominant or potential subordinated) behaved as foraging, egg laying females.

Immature offspring replacement

In the course of our observation, we recorded 60 events of two types of offspring replacement which occurred in general mainly during the wet season (55%).Twenty eight of these replacements (1st case) occurred with immatures in advanced stages of larval or pupal development stage. After having its cells opened these injured juvenile-stage bees were removed either by PD or PS from their cells before they could reach adulthood. They were left outside their cell but inside the nest, where they died. After removing the immature bee adult female often worked in the brood cell and filled it with food provision, before using it to lay her own egg inside. The frequency of larval and pupal replacement occurred mainly during the dry season (n = 18) in comparison to wet season (n = 10).

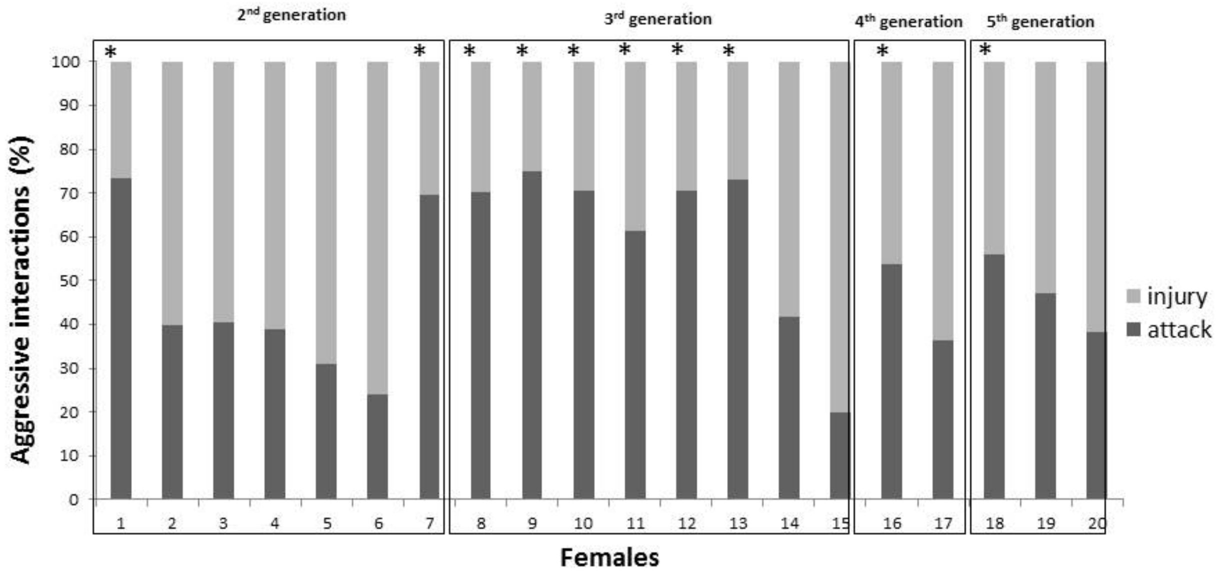

Fig 5. Agonistic interactions observed for 20 females of *Euglossa annectans* across four generations. The interactions were noted for seven females from the second generation, eight females from third generation, two females from the fourth and three females from the fifth generation. The dark grey bars show the number of attacks started by a given female (indicated from 1 to 20). The light grey bars indicate when the same female was attacked by another female. We found no significant difference when both events were compared, attack *vs* injury; paired t test (Mann Whitney U=190.500, $n_1=n_2=20$, p=0.799, 1 tailed). The symbol "*" indicates a potential dominant female and the bees with no symbol indicate a potential subordinate female (Mann Whitney U = 10.500, $n_1= n_2=10$ p = 0.002, 1tailed).

The other 32 (2nd case) offspring replacements were the result of oophagy.The majority of the observed oophagy events (60%) were made by a female which, during her lifetime, emptied the cell of another female only once. In 33% of the cases a female replaced the egg of another female twice. Only one female was observed replacing three different eggs from three different brood cells. Only in one case was an egg replacement observed up to three times in the same cell. Egg replacement was more often recorded during the wet season (n = 23) than in the dry season (n = 9). During the study the mean number of oophagies per female, considering 18 females across all 5 generations, was 1.44 (±0.61). The oophagies were performed by females from different generation as well as by females of the same generation. After oophagy females were observed bringing fresh pollen and added it to the pollen uneaten by the previous immature bees. All 60 events of replacement happened while the female that layed the initial egg was not inside the nest, suggesting that her presence in the nest is a very efficient deterrent.

Discussion

The females of the orchid bee *E. annectans* studied here are highly aggressive and the conflict between nestmates seems to be part of the intranidal female offspring control. Several females from the same and different generations were laying eggs (which developed into females) at the same time but live in a conspicuous reproductive conflict. Although we did find a clear pattern of dominance in the 2nd generation, we are aware that it was found in a reduced dataset. Considering the entire data set (four different generations) the patterns of aggressive interactions (non directional but instead with multiple directions) may suggest that dominance itself is not concentrated by only one female. The fact that the potential dominant was often attacked shows how loose her control actually was.

Unlike previous assumptions made for this orchid bee species, mentioned as communal since nestmates did not interact socially and only shared nest cavity, the females of *E. annectans* that were studied here exhibit an advanced or a transitional case of communal social organization to primitively eusocial. There were some social interactions (*e.g.* following behaviour, mutual attack, defense against intruders) between mother and daughters (matrifilial association) and also between females of the same generation (sororal association). Although we have found a clear dominance hierarchy, the presence of only one female which adressess unidirectional attacks towards her subordinates, as reported for *Euglossa fimbriata* Moure, 1968 and *Euglossa melanotricha* Moure, 1967 (Augusto & Garófalo, 2009; Andrade-Silva & Nascimento, 2015) is lacking in *E. annectans.* Instead several females attacked each other as well as multiple females replaced offspring. Those traits may broaden the complexity of more egalitarian societies since it would help to control reproductive skew.

We observed some criteria of social behaviour, according to Michener (1974): overlapping generations, aggression and egg replacement between nestmates, group nest defense (when the nest was invaded by non-identified ants and swarming individuals of stingless bees) and cooperative work between young females (1-3 days old) which replaced resin within the nest. This common behaviour trait developed by young bees seems to maximize resin use inside the nest. In contrast to primitively eusocial orchid bees there was no evidence for the presence of a single dominant female as seen for *Euglossa atroveneta* Dressler, 1978 (Ramirez-Arriaga et al., 1996), *E. fimbriata* and *Euglossa cordata* Linnaeus, 1758 (Augusto & Garófalo, 2009; 2010, respectively) and *E. melanotricha* (Andrade-Silva & Nascimento, 2012; 2015; Andrade et al., 2016) neither for the presence of a subordinated female specialized in guarding the nest entrance as reported for *Euglossa viridissima* Friese, 1899 (Boff et al., 2015).

It is known that age plays an important role in dominance and by consequence, aggression (Augusto & Garófalo, 2009; Rehan & Richards, 2013; Andrade-Silva & Nascimento, 2015). The agonistic behaviour recorded in our study was frequently started by the same, oldest female. During the tracked interactions, we observed a presumed dominance hierarchy. The observed aggressions were mainly made by only one female (oldest female) followed by a two days younger female which received 20% of all registered aggression. This hierarchy reflected the distribution of eggs and brood cells: the brood cluster of the oldest female (1) was the largest (six cells) compared to the other tracked females whose brood cluster varied from one to five cells. Brood cluster size and aggression between nestmates has been correlated in different bee societies (Batra, 1978; Kukuk, 1992; Moritz & Neumann, 2004; Augusto & Garófalo, 2010; Rehan & Richards, 2013). In the current study as all females behaved as foragers and they also layed eggs, the total costs per nest involved to produced an offspring might be higher when compared to eusocial species in which the dominant female is far less prone to be predated, since it rarely leaves the nest. Thus, the more eggs a female lays the more aggressive it may become because of its higher reproductive investment.

In the social network analysis we found that the most central position is occupied by female “1”. According to the centrality theory, more central individuals are more closely connected by interaction with all other individuals in the network (Freeman, 1978). In our analysis, centrality indicates potential dominance since female “1” interacted and aggressed more than all the other females. She also had the largest brood cluster. The frequency of attacks by female “2” was lower than the frequency of attacks by female “1”. Moreover, the former was more frequently attacked by female “1” than by any other female. Although there was no significant evidence for a single dominant female, these bees seem to be in a hierarchical tug-of-war (Langer et al., 2004), when multiple females display dominant behaviour and do not stop trying to take the dominant position. However, it may be the case that the older individuals are more prone to be aggressive towards other females as a consequence of their greater reproductive investment in the nest. Thus, oophagy or immature replacement seems to be an opportunistic behaviour either as an element of tug-of-war or parental parasitism. Although centrality seems to be a robust approach for assigning dominance it needs to be considered with caution since network interactions resulted from only one generation.

The parental parasitism hypothesis introduced by Charnov (1978) and adjusted for dominant females replacing the eggs of subordinated females (Field, 1992) proposes that daughters or younger females are submissive towards an older nestmate. Thus, unlike of what has been described for *E. cordata*, *E. fimbriata*, *E. melanotricha*, and *E. viridissima* in which the egg replacement occurs largely by one dominant female, in *E. annectans* the egg replacements were made by several females occupying the nest at the same time. This occurrence of multiple oophagies and/or the replacement of non-emerged bees better supports the tug-of-war hypothesis than the parental parasitism hypothesis. However the former hypothesis must be carefully interpreted here because the tug-of-war hypothesis originally also explains reproductive skew, an adaptative trait that describes the unequal sharing of reproduction within the group (Trubenová & Hager, 2012). Thus, the lack of a single dominant female may hampers *E. annectans* from becoming primitively eusocial.

We did not notice any female replacing its own egg, which seems to indicate that females were able to recognise their own brood cells and the eggs of their nestmates. Thus aggression between nestmates in *E. annectans* might be an adaptive response to the multiple oophagies in the nest. In the primitively eusocial orchid bee *E. fimbriata* and *E. cordata* species, only one female has been detected replacing eggs of her nestmates (Augusto & Garófalo, 2009; 2010, respectively). However in the primitively eusocial *E. viridissima* daughters were observed replacing eggs from mothers (Cocom-Pech et al., 2008). In the eusocial species *Apis mellifera* Linnaeus, female workers are responsible for oophagy of the eggs laid by other workers (policing behaviour) and are also able to eliminate some individuals, *e.g.* diploid males, but they do not replace eggs (Ratnieks 1998). In additon to egg replacement the behaviour of removing larvae/pupae from its nestmate`s cell was similar to the behaviour of *Hoplostelis bilineolata* (Spinola, 1841) (Hymenoptera, Megachilidae), in nests of *E. cordata* (Augusto & Garófalo, 1998). In *E. annectans* after the immature was dragged out from its brood cell to the nest floor it was completely ignored by any other nestmate within the nest while *H. bilineolata* places resin on the larvae of *E. cordata* (Augusto & Garófalo, 1998).

In previous studies regarding social interactions among individuals of Euglossini females, there was no evidence for attacks made by more then one female in a given nest. The exception was the communal *Euglossa nigropilosa* Moure, 1965. Although in this species multiple individuals attack each other, interactions do not seem to be associated with dominance but are related to the presence of territories in the nest (Otero et al., 2008).

Understanding the mechanisms of the major evolutionary transition from solitary to group living including their losses and benefits is one of the challenges for evolutionary biologists. The studies of species showing advanced levels of eusociality like the honeybee *A. mellifera* have contributed to knowledge about caste differentiation, division of labour and several conflicts involving workers and queens. The studies on primitively eusocial species have contributed to understand not only the transition from solitary to social but the adaptations, costs and benefits for sociality (Schwarz et al., 1998; Schwarz et al., 2007; Kocher & Paxton, 2014; Prager, 2014; Rehan et al., 2014; Andrade et al., 2016). This study shows that *E. annectans* has a unique social structure that exhibits aspects of communal and primitively eusocial societies where multiple females are prone to attack each other and multiple female are performing oophagy. This behaviour leads to a weak dominance in *E. annectans* different from *E. cordata* whose dominant females performed oophagy and replaced all the eggs of the subordinate with her own (Freiria et al., 2017). Albeit *E. annectans* is in a close phylogenetic relationship to honey bees and other socially advanced Apid species, it shows a less advanced social organization (Romiguier et al., 2015). Our find showed the importance of long term nest observations, making *E. annecatns* an important system to study female totipotency and reproductive hierarchy formation in the Apidae. Topics that motivates further exploration which may provide insights into broader patterns of social evolution in Apidae.

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