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Shared reproduction and sex ratio adjustment to clutch size in a socially polymorphic orchid bee

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Abstract

Nests of the primitively eusocial orchid bee *Euglossa viridissima* are generally founded by a solitary female but can be reactivated by female offspring, in the presence or absence of the mother. The population therefore exists of solitary and social nests that co-occur in an area. A female can stay as a subordinate helper under a dominant female or disperse to become a solitary foundress. Yet, the costs and benefits of the different social phenotypes are so far little understood. Here, we compared solitary and social nests of orchid bees. We used offspring of solitary and social nests to calculate offspring sex ratio, and applied molecular markers to calculate intranidal relatedness, infer maternity and test whether sociality may have a genetic component. We found that social nests had on average more brood than solitary nests. The overall sex ratio in the population did not differ from 1:1. However, social nests tended to produce a split sex ratio with some nests producing mainly males and others mainly female offspring. Regardless of social phenotype, the number of offspring was correlated with the sex ratio, with smaller nests having a more female-biased offspring. In social nests, not all offspring resulted from a single-mated mother, which was also the case for some solitary nests. This suggests shared reproduction in social nests and may be an indication that intraspecific parasitism and nest takeover are not uncommon. Structure analyses did not reveal different genetic background of the two social phenotypes. Our results suggest that there is no clear benefit that favours one of the two social phenotypes over the other and that the population is kept at balance in terms of sex ratio.

KEYWORDS
Euglossa viridissima, intranidal relatedness, neotropics, plastic behaviour, primitively eusocial, split sex ratio

1 | INTRODUCTION

In bees, socially polymorphic species can either live solitary or in social groups (Richards, Wettberg, & Rutgers, 2003; Wcislo, 1997). Costs and benefits for an individual living a solitary lifestyle are different from those for an individual living socially (Hamilton, 1964; Rehan, Richards, Adams, & Schwarz, 2014; Schwarz, Bull, & Hogendoorn, 1998). Theoretically, a solitary foundress has the full reproductive output of her nest (Hogendoorn & Velthuis, 1993; Reeve & Keller, 2001). She is forced, however, to leave her nest unattended while foraging for nesting material and resources, thus

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increasing the chances of nest usurpation or intra- and interspecific parasitism (Goodell, 2003). In a social organization, the dominant female might have to share the reproductive output of the nest but can otherwise reduce the risk of natural enemies predating on the offspring. A helper on the other hand might have only a small share in the direct reproduction but may benefit from indirect fitness effects through relatedness (Andrade, Miranda, Del Lama, & Nascimento, 2016), according to kinship theory (Bourke, 2011), thus increasing her inclusive fitness (Gardner, West, & Wild, 2011; Hamilton, 1964; West, 2002). By staying in the natal nest and assisting with brood care, a helper might avoid the costs of dispersal (Bonte et al., 2012; Perrin & Mazalov, 2000), the energetic costs of nest establishment, the risk of predation (Comins, Hamilton, & May, 1980; Gandon, 1999; Reader, Higginson, Barnard, & Gilbert, 2006), improve defence against parasites (Blacher et al., 2013; Hamilton, 1971; Pamminger, Foitzik, Metzler, & Pennings, 2014; Rosenheim, 1990; Wcislo, 1996) or against resource theft from conspecifics (Boff, Forfert, Paxton, Montejo, & Quezada- Euán, 2015). Furthermore, she can take over the nest when the former dominant dies or disappears. Yet, nest sharing might also imply costs such as an increase in aggression (Boff, Saito, & Alves-dos-Santos, 2017) and kin competition for local resources and reproduction. This may outweigh the benefits of kin cooperation (Boomsma & Grafen, 1991; Platt & Bever, 2009; Taylor, 1992; Wilson, Pollock, & Dugatkin, 1992).

Regarding brood production, haplodiploid reproductive females can control the sex of their progeny by either fertilizing eggs or not (Flanders, 1965; Gerber & Klostermeyer, 1970), where unfertilized (haploid) eggs will develop into males and fertilized eggs (diploid) into females. Thus, they are potentially able to adjust the sex ratio of their offspring. Factors, such as local resource availability (Fitch et al., 2019) or parasitic pressure, are hypothesized to influence offspring sex ratio (Kim, 1999; Seidelmann, Ulbrich, & Mielenz, 2010). However, the evolutionary forces that affect sex ratio (e.g. to keep the population under sexual balance) are not yet clearly understood in this system (Chapuisat & Keller, 1999; Rosenheim, Nonacs, & Mangel, 1996; Schwarz et al., 1998). In eusocial Hymenoptera, with a sterile worker caste, the optimal sex ratio (in terms of numbers of male and female offspring) for queens is 1:1 as she is equally related to her male and female offspring. This resembles the state for foundress females of solitary species. Workers of eusocial species on the other hand should bias sex ratio towards females (3:1), as they are on average more related to their sisters than to their brothers (Meunier, West, & Chapuisat, 2008; Trivers & Hare, 1976) due to relatedness asymmetries in the haplodiploid system.

Socially polymorphic species are excellent model organisms to study differences in costs and benefits between social phenotypes (Kocher & Paxton, 2014). Within the orchid bees (Apidae: Euglossini), nests of primitively eusocial *Euglossa* species are typically initiated by a solitary foundress who builds and provisions a first clutch of brood cells. She then usually stays in the nest until offspring emergence, upon which the nest can be reactivated by her with one or more of her daughters staying in a matrifilial (mother and daughter) or sororal association, that is only daughters (Andrade et al., 2016; Augusto & Garófalo, 2009, 2010). In this context, a dominant female, often the mother or the oldest female in a nest, dominates her nestmates with agonistic behaviour (Augusto & Garófalo, 2010) and claims the reproductive monopoly, for example by eating the subordinates' eggs (Cocom-Pech, May-Itzá, Medina, & Quezada-Euán, 2008; Freiria, Garófalo, & Del Lama, 2017). This behaviour may foster reproductive conflict between nestmates (Augusto & Garófalo, 2010), although monandrous systems, where a female mates with only one male, help to obtain higher relatedness among nestmates and may thus favour kin selection (Hughes, Oldroyd, Beekman, & Ratnieks, 2008).

Euglossa viridissima is a single-mated (Zimmermann, Roubik, Quezada-Euán, Paxton, & Eltz, 2009) multivoltine orchid bee species found in the Yucatán Peninsula of Mexico and in most of Central America (Burquez, 1997). This species shows a facultative social behaviour with both solitary and social nests occurring simultaneously in the same population (Cocom-Pech et al., 2008; May-Itzá, Medina, Medina, Paxton, & Quezada-Euán, 2014). Here, we studied nests of both social phenotypes and addressed the following questions that contribute to understanding social polymorphism in orchid bees. Do solitary and social nests have a different reproductive output in terms of offspring number? How does sex ratio differ between the two social phenotypes? Does clutch size have an effect on sex ratio? Does intranidal relatedness of offspring differ between solitary and social nests and how many adult females contributed to offspring production? Do individuals of the two social phenotypes have a different genetic population structure according to the type of social polymorphism?

In the light of inclusive fitness theory, we predict that social nests have a higher reproductive output than solitary nests, to make helping behaviour of subordinate females worthwhile. We also predict to find a 1:1 sex ratio in solitary nests contrasting to a female biased sex ratio in social nests. Moreover, we hypothesize that with increasing brood size, fewer female offspring is produced as a mechanism to reduce local resource competition among philopatric daughters. Lastly, we assume to find highly related offspring in solitary nests, whereas intranidal relatedness in social nests should be reduced as a consequence of shared reproduction, which should also lead to the finding of offspring genotypes coming from different mothers.

2 | MATERIALS AND METHODS

2.1 | Sampling

Fieldwork was undertaken at the Universidad Autónoma de Yucatán (87.37°W, 20.52°N), Mérida, México. Sampling occurred in 2006, 2007 (see details in Zimmermann et al., 2009) and 2014 within the same area. Sampling in 2014 was conducted by SB. Genotyping data of 2006 and 2007 of *E. viridissima* and additional information about social phenotypes of the respective nest were provided by the authors of Zimmermann et al. (2009). With these additional data, we could increase the number of social and solitary nests and improve the robustness of the analysis. All our results are based on

TABLE 1 Brood production and relatedness of *Euglossa viridissima*

2006				2007				2014				
Nest	IR	Sex ratio	Nest size	Nest	IR	Sex ratio	Nest size	Nest	IR	Sex ratio	Nest size	DM
1	0.58	0.75	7	13	0.56	2.33	10	53**	0.11	0.37	20	2
2	0.64	0.75	7	20	0.67	0.50	9	56**	0.02	0.71	10	2
3	0.63	2.33	10	21*	0.47	4.00	10	57**	0.09	1.00	6	
4	0.51	0.50	6	22	1	1.00	4	64**	0.06	3.00	12	
6*	0.82	0.10	22	23	0.81	1.4	12	65	0.14	0.66	3	2
7	/	1.00	1	24	0.54	2.33	10	69	0.56	4.00	4	1
8	1	1.00	4	25*	0.20	8.00	9	71	0.46	8.00	8	
10	0.88	1.33	14									

Note: Relatedness among offspring within nests (= intranidal relatedness IR), and number of female/male offspring for all three sampling periods. DM = diploid males.
*social nests in Zimmermann et al. (2009).
**social nests in 2014 (definition of sociality based on behavioural observations).

the original genotyping data and were thus not published in this way before.

2.2 | Molecular analysis

A total of 205 individuals (Larvae, pupae, and recent emerged offspring of both sex) from three sampling periods (*n* = 71 for 2006, *n* = 64 for 2007, *n* = 70 for 2014) were analysed. Diploid males were found in 2014 at the frequency of 7.5% (C.I. 3.1%–14.9%, calculated with http://statpages.info/confint.html), that is seven diploid males out of 93 morphologically sexed males. Brood genotypes and information on social status of nests collected in 2006 and 2007 were provided by the authors of Zimmermann et al. (2009), which we used for subsequent analyses. For individuals collected in 2014, DNA was extracted and genotyped using microsatellites (Paxton, Zobel, Steiner, & Zillikens, 2009; Souza, Cervini, Del Lama, & Paxton, 2007). Alleles were sized based on electropherograms using Fragment Profiler version 1.2 (Amersham Biosciences). For details about the molecular procedure, see Appendix S1 (A, B).

2.3 | Reproductive output

To calculate the reproductive output of solitary and social nests, all offspring (emerged adults, larvae and pupae) were counted for every nest. We tested for differences in number of offspring, see Table 1, between solitary (*n* = 15) and social (*n* = 7) nests using the Mann–Whitney *U* test.

2.4 | Sex ratio

Brood cells were opened, and pupae were sexed based on their morphology. Larvae are difficult to assign based on morphology, and hence, they were sexed by their microsatellite genotypes: larvae heterozygous at one or more polymorphic microsatellite marker (see below) were considered to be females and those homozygous for all molecular markers were classified as males. Sex ratio analyses were done for data sets including and excluding diploid males (*n* = 205 and n_{exclude} = 198, respectively). We compared the sex ratio of offspring (#female offspring/#male offspring in each nest) within social and solitary nests using a Wilcoxon signed rank test, because the two groups (females and males) were non-independent. Between social phenotypes, which are independent from each other (social vs. solitary), we used a Wilcoxon rank sum test.

2.5 | Correlating proportion of female and male offspring with clutch size

We tested for a relationship between the offspring sex ratio (females/males) with the total number of offspring using Spearman's rank correlation in both solitary (*n* = 15) and social nests (*n* = 7).

2.6 | Relatedness, maternity and genetic structure

We estimated within-nest relatedness (IR) and mean relatedness (per year) using Kingroup V1 (Konovalov, Manning, & Henshaw, 2004), using female diploid genotypes (2006: *n* = 28; 2007: *n* = 42; 2014: *n* = 23). We determined the number of potential mothers of each nest (Figure S1), assuming single mating, which is common in bees (Paxton, 2005) and supposed to be the ancestral condition in Hymenoptera (Hughes et al., 2008). This condition has been tested and verified for *Euglossa viridissima* (Zimmermann et al., 2009).

To test for the existence of genetic population structure according to the type of social polymorphism, we used the Bayesian clustering algorithm implemented in Structure 2.3.4 (Pritchard, Stephens, & Donnelly, 2000). We used three data sets to test for the occurrence of population structure: 1) all female diploid genotypes (*n* = 37), 2) two female diploid genotypes per nest (*n* = 14) and 3) all male haploid genotypes (*n* = 26). We used Structure with default

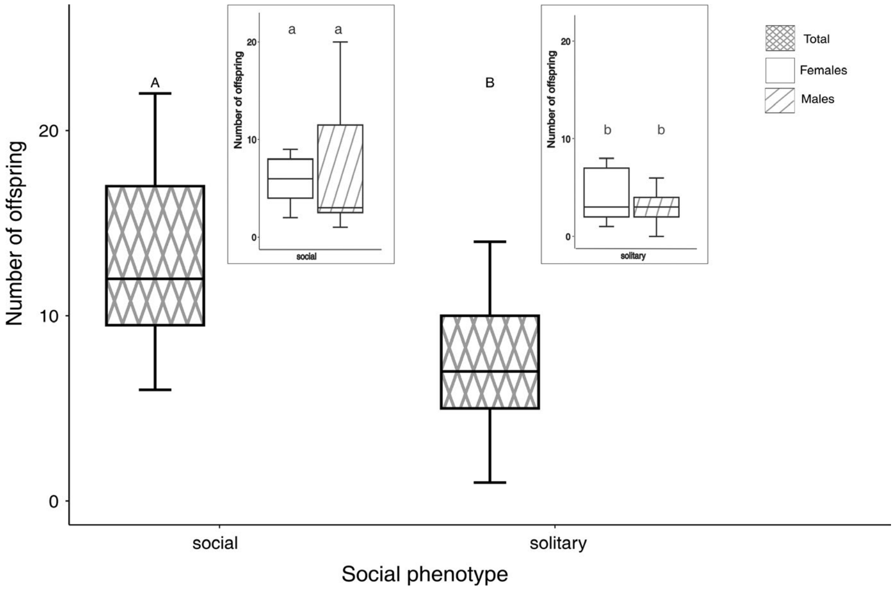

FIGURE 1 Box-Whisker plots showing the total number of offspring (crossed filled boxes) produced was higher in social then in solitary nests, and number of female (blank filled boxes) and male (diagonally filled boxes) offspring did not differ within social phenotypes. Different capital letters indicate significant differences between total number of offspring. Same lower case letters indicate no significant differences in offspring sex ratio within each social phenotype

settings except for the usage of 100,000 burn-in and 500,000 MCMC repetitions after the burn-in period. We have used the number of clusters K = 1–7 with 10 replicates each. Finally, the number of clusters explaining the best segregation of individuals according to genotypes was tested using the Web-based application Structure Harvester v0.6.94 (Earl & vonHoldt, 2012).

Statistics were conducted using SPSS v19 (IBM SPSS Statistics, 2010) and R version 1.0.153 (R Core Team, 2018).

3 | RESULTS

3.1 | Do solitary and social nests have a different reproductive output in terms of offspring number?

The median (±*SD*) number of brood produced in solitary nests was 7 ± 3.4 per nest. Seven nests were social (32%) with a median brood number of 12 ± 6. Social nests produced significantly more offspring than solitary nests (Mann–Whitney *U* test, *z* = −2.14, *p* = .015; see Figure 1).

3.2 | How does sex ratio differ between the two social phenotypes?

The number of females produced did not differ from the number of male offspring in solitary nests (Wilcoxon signed rank test: V = 72, *p* = .06) or social nests (Wilcoxon signed rank test: V = 9, *p* = .75), see Figure 1. Offspring sex ratio of solitary nests was not significantly different to that of social nests (Wilcoxon ranked sum test: W = 56.5, *p* = .80), although solitary nests had offspring with a more female biased sex ratio (Figure 2). Results did not change when including diploid males (*p* > .05).

3.3 | Does clutch size have an effect of on sex ratio?

Sex ratio and offspring number were correlated in solitary nests, although not significantly (Spearman's rank correlation: *r* = .216, *N* = 15, *p* = .439) and fairly negatively correlated in social nests, however, also not significantly (Spearman rank correlation: *r* = −.63, *N* = 7, *p* = .13). Analyses when diploid males were included gave similar results (solitary: *r* = .334, *N* = 15, *p* = .224, social: *r* = −.746, *p* = .054). Nests with a higher proportion of female offspring had a smaller clutch size (number of offspring), whereas the number of offspring was higher in nests where the proportion of female offspring was smaller. The coefficient of variation of sex ratio in social nests was higher (60%) than in solitary nests (30%). The distribution of the two social phenotypes showed that social nests tended to be located towards the extremes (Figure 3).

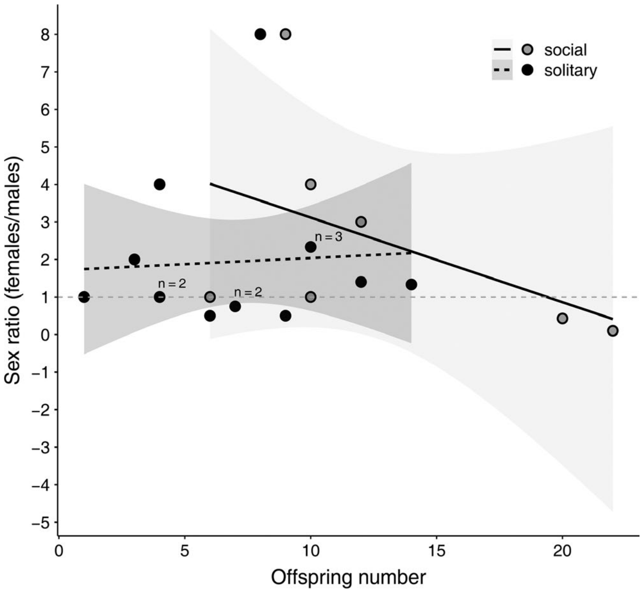

FIGURE 2 Sex ratio (#females offspring/#male offspring) in both social phenotypes. Black circles represent solitary nests, and grey circles represent social nests. For overlapping values, the number of overlaps (n) of that value was added; the lines (dashed for solitary nests, solid for social nests) indicate linear regression models with confidence interval

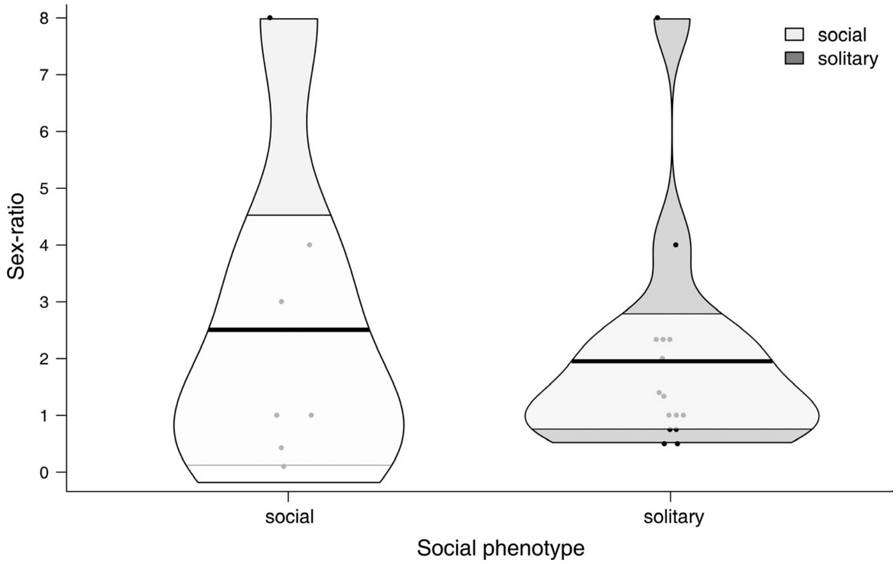

FIGURE 3 Pirate plot showing the distribution of offspring sex ratio social (light grey) and solitary (dark grey) nests. Data are shown with smoothed densities indication the distributions in each group. Central tendency (black horizontal lines) is the mean and white area around the mean represents the Bayesian 95% highest density intervals

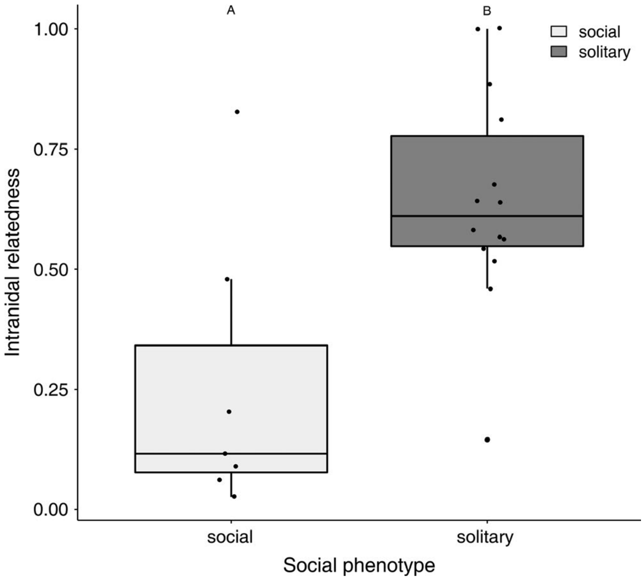

FIGURE 4 Box-Whisker plots of intranidal relatedness in both social phenotypes. Offspring of social nests (light grey boxes) were significantly less related with each other than offspring of solitary nests (dark grey boxes) Different capital letters indicate significant differences in intranidal relatedness (Mann–Whitney *U* test, $Z = -2.54$; $p = .01$)

3.4 | Does intranidal relatedness of offspring differ between solitary and social nests and how many adult females contributed to offspring production?

Offspring intranidal relatedness (IR) was continuously above 0.5 for all nests in 2006. This pattern changed in 2007 since intranidal relatedness was lower in two of the social nests (Table 1). Intranidal relatedness between nests from 2006 to 2007 differed significantly from nests of 2014 (Mann–Whitney *U* test, $Z = -3.29$, $p < .01$). When comparing the two social phenotypes across all years, we found a significant difference in the intranidal relatedness (Mann–Whitney *U* test, $Z = -2.54$; $p = .01$), where offspring of social nests was less related than in solitary nests (see Figure 4).

In 2006 and 2007, offspring from all solitary nests were assigned to only one mother that had mated with one father. The genotypes of individuals of the three social nests of these years suggest that offspring was produced by at least two different adult females. From the offspring genotypes of nests from 2014, we identified several mothers that must have produced the brood. This was the case for the social as well as for the solitary nests. From our data, it was not possible to determine the exact number of mothers; however, we could distinguish nests produced by a single mother from those produced by multiple mothers (see Figure S1a-g).

3.5 | Do individuals of the two social phenotypes have a different genetic population structure according to the type of social polymorphism?

Structure analyses did not find a significant population structure between solitary and social nests (see Appendix S1 Part C). The data set with all females showed a slight effect of differentiation but not according to social phenotype, rather according to colony of origin. The other two data sets showed no indication for population structure. This suggests that all individuals, regardless of social phenotype, come from the same population.

4 | DISCUSSION

In this study, we found that the average number of brood was higher in social nests. The overall sex ratio did not differ significantly from 1:1. Between the two social phenotypes, sex ratio did not differ significantly, but solitary nests tended to produce a more female biased brood. The number of offspring per nest was negatively correlated with sex ratio, although not significantly, showing that more female biased nests had smaller brood size. Intranidal relatedness was lower in social nests. Offspring of social nests in all years was found to be produced by several different mothers, which indicates shared reproduction. Although the majority of solitary nests were produced by one single mother, some, interestingly, contained brood that could be assigned to multiple mothers.

4.1 | Do solitary and social nests have a different reproductive output in terms of offspring number?

We found that social nests produced significantly more offspring than solitary nests, which supports Hamilton's inclusive fitness theory, where one would expect a higher reproductive output in social nests to increase the inclusive fitness of a helper and to make helping behaviour beneficial to the subordinate female (Hamilton, 1964).

4.2 | How does sex ratio differ between the two social phenotypes?

In agreement with Fisher (1930), the overall sex ratio in *E. viridissima* was not significantly different from 1:1. But we found a tendency for solitary nests to produce a higher proportion of female offspring. Foundresses might invest more in this sex to increase the chances of nest reactivation by one of her daughters. When a daughter stays in the natal nest and starts foraging and provisioning cells, the original foundress female can either assume reproductive dominance by oophagizing her daughters' eggs (Freiria et al., 2017), or by increasing her inclusive fitness by sharing reproduction with her daughters (Andrade et al., 2016). Alternatively, solitary females would invest in the sex which may be of lower cost in terms of energetic investment (Bosch & Vicens, 2005). Solitary females are forced to leave their nest unguarded while foraging, thus increasing the possibility of nest usurpation and parasitism. It might thus be beneficial to reduce foraging time by providing the cells with fewer resources. Past studies have shown that females assess the quality and quantity of available food for cell provision (Crozier & Pamilo, 1996; Rooijakkers & Sommeijer, 2009) and can decide in which sex to invest (Flanders, 1965; Gerber & Klostermeyer, 1970). It may thus be of advantage to invest in the sex which needs a reduced amount of food to develop. In terms of relatedness, the foundress female in single-mated haplodiploid species, when being solely responsible for offspring production, is equally related to her sons and daughters and might therefore allocate resources to both sexes equally (Trivers & Hare, 1976). Nevertheless, in most aculeate Hymenopteran species, females tend to be larger than males (Helms, 1994) and resource investment in both sexes could be biased. Females invest more resources into the energetically more costly sex (e.g. Rehan & Richards, 2010, but also see Mikát, Benda, & Straka, 2019). In these cases, smaller mothers tend to produce more of the sex that is energetically less costly, compared to larger ones. However, in the absence of size dimorphism between males and females, an equal offspring sex ratio in terms of resource investment might be expected. In *Euglossa viridissima*, females and males are similar regarding size and weight (Medina et al., 2016). The absence of marked size dimorphism in our study species may be a possible determinant that influences mother investment in female and male offspring and could explain the lack of significant differences in offspring sex ratio. These hypotheses need to be further explored in Euglossini. Yet, local resource competition (see below) may better explain our finding that bigger brood clutches had a lower proportion of female offspring.

4.3 | Does clutch size have an effect of on sex ratio?

Interestingly, our results indicated that in social nests, the number of offspring was strongly negatively correlated with sex ratio, though not significantly, that is nests that produced generally more brood, had a higher number of male offspring, whereas nests with smaller brood size produced a higher number of females. Local resource competition could lead reproducing females with an increased offspring number to invest more in males as they are the dispersing sex and would not compete for resources like philopatric daughters would (Chapuisat & Keller, 1999; Hamilton, 1967). Additionally, the variation in sex ratio tended to be higher in social nests than in solitary nest, thus potentially leading to a split sex ratio in which some nests produce exclusively females and others only males (Mueller, 1991). This could be an indication that reproductive females of different social status are competing about offspring production. In Andrade et al. (2016), the majority of their studied social nests had a more female-biased brood, where the subordinates generally produced a higher proportion of males, and the dominant females a higher proportion of females. Another factor that could play a role in the production of offspring sex ratio in social nests is the age of a nest, the age of egg laying females and the relatedness among the resident females (Andrade et al., 2016).

4.4 | Does intranidal relatedness of offspring differ between solitary and social nests and how many adult females contributed to offspring production?

Female offspring from social nests were less related to each other than female offspring from solitary nests. In one nest that we considered as social because two adult females were present, the intranidal relatedness was exceptionally high ($r > .8$), likely due to the fact that the only two female progenies were full sisters. In the same nest, a total of 20 male offspring were produced. From the genotypes, all brood could have been produced by only one single-mated mother. Although a single female of *E. viridissima* is capable to build more than 20 cells (AF personal observation), it is possible that the two adult females were in a matrifilial or sororal relationship, where a subordinate female produced part of the offspring. Moreover, our results indicated that offspring from some solitary nests were produced by multiple mothers and that intranidal relatedness in these nests was lower than in solitary nests where offspring could be clearly assigned to a single mother. This suggests that nest usurpation in early nest-founding stages takes place frequently in solitary nests, resulting in offspring from different mothers. During periods of limited resource availability, for example a shortage of resin to build cells, females tend to steal resources from other nests (Boff et al., 2015) or even take over entire orphaned nests if it is uninhabited (Kaitala, Smith, & Getz, 1990; SB personal observation). Alien brood is not an uncommon event and it has also been reported in other bee species, *for example Bombus hypnorum* (Paxton, Thorén, Estoup, & Tengö, 2001) and *Lasioglossum malachurum* (Soro, Ayasse, Zobel, & Paxton, 2009).

All of our studied social nests showed brood genotypes that indicate more than one reproducing mother, because all brood could not be assigned to only one single-mated female indicating that shared reproduction is frequent in social nest associations of *E. viridissima*. We observed that social nests were often shared by more than three females. One of the females was specialized in guarding the nest entrance, which might be an adaption to prevent nest usurpation and tentative robbery of nest material (Boff et al., 2015). These behaviours seem to be an adaptive response to environmental conditions when resources are scarcer (May-Itzá et al., 2014), and it might also lead to the formation of social nest associations between unrelated females, when philopatric offspring from intraspecific parasitized or usurped nests establish a social association.

4.5 | Do individuals of the two social phenotypes have a different genetic population structure according to the type of social polymorphism?

If individuals of solitary and social nests come from different gene pools, this could be an indication that the formation of social phenotype might also have a genetic component. Our structure analyses, however, did not find a pattern between individuals of solitary and social nests, indicating that all individuals arise from the same population and the expression of the social phenotype is rather a response to environmental cues than to genetic factors.

To our knowledge, this is the first study comparing solitary and social nests in this *Euglossa* species and thus contributes to the understanding of social polymorphism in bees. Social nests had a higher reproductive output in terms of offspring number, as predicted by Hamilton, 1964). Sex ratio did not differ from 1:1 in the population; however, there was a trend for higher female production in solitary nests. Even though not significant, the proportion of female to male offspring was strongly correlated with clutch size in social nests, suggesting that with increasing number of offspring, less female brood is produced. Offspring intranidal relatedness differed significantly between social and solitary nests, and reproduction among adult females in social nests is often shared. We suggest that sociality in this species seems to be an adaptive response to environmental conditions, rather than to genetic underpinning.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION

SB designed the study and collected part of data. JJQE provided samples. AF, HMGL and SB performed analysis and together with JJQE wrote the manuscript. All authors participated in interpretation of the data and reviewed and edited the manuscript.

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