



Reproductive behavior and importance of males for solitary bees of the Centridini tribe (Hymenoptera: Apidae) that nest in aggregation

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Abstract

In this study (review), we discuss the scientific literature investigating the reproductive behavior employed by Centridini males in nest aggregation and their important role in maintaining aggregation during generations. Centridini tribe is a Neotropical of oil-collecting bees, composed of only two well supported monophyletic genera of solitary bees *Epicharis* and *Centris*. These two genera have varied nesting habits, with some species forming large aggregations and pollinating from various species of angiosperms. We used Google Scholar, SciELO, and the Capes Periodical Portal with CAfe access Database to find peer-reviewed publications during May and July 2022. In addition, we use keywords directed to the topic and select only scientific articles from indexed periodicals. We found that few scientific articles are directed to studies of males in nest aggregates, and some characteristics of their reproductive behavior are described in research on nesting biology. Furthermore, with the philopatric characteristic of females for nesting, we suggest that the importance of males is underestimated and poorly understood for the maintenance of these populations. Finally, we reinforce the importance of research focused on male behavior, as well as population genetic studies to understand the real value of male dispersal in solitary bees, especially the Centridini tribe.

Keywords: *Centris*, *Epicharis*, males, neotropical, solitary bee

Introduction

Solitary bees use a wide variety of nesting substrates, digging their nests in wood or soil, constructing freestanding nest or using pre-existing cavities (Michener, 2007) ^[1]. Most of them remain with the plesiomorphic condition for the group, which is the construction of nests on the ground alone or in aggregation (Batra, 1984; Roubik, 1989; Michener, 2007) ^[2, 3, 1].

Centridini is a Neotropical tribe of oil-collecting bees, composed of only two well supported monophyletic genera of solitary bees (Moure *et al.*, 2012) ^[4]: *Epicharis* Klug, 1807 and *Centris* Fabricius, 1804. *Centris* is a more diverse genus, with about 230 species, widely distributed throughout the Americas (Michener, 2007) ^[1] while *Epicharis* currently has 35 cataloged species and has an exclusive distribution to the Neotropics (Moure *et al.*, 2012) ^[4]. Moreover, *Epicharis* dig their nests in the soil and *Centris* nesting behavior has some plasticity, composed mostly of some species that dig their nests in the soil or sometimes in termite nests and some that use pre-existing cavities (e.g., Gaglianone, 2001; Aguiar and Gaglianone, 2003; Rozen *et al.*, 2011) ^[5, 6, 7].

Most species of *Centris* and *Epicharis* are collectors of floral oils, a resource produced by species of a few plant families and used for larval feeding and nest building (see review in Alves-dos-Santos *et al.*, 2007) ^[8]. For many plants that produce floral oils, Centridini bees were considered the main pollinators (Rego and Albuquerque, 1989; Machado, 2004) ^[9, 10]. The role of Centridini in pollination is not restricted to flowers that produce floral oils, as in several cases, effective pollination has been proven during visits to the flowers of plants from different families, such as Lecythidaceae, Bignoniaceae and Leguminosae-Faboideae, which are important sources of nectar for females and males of Centridini in areas of native vegetation, while Solanaceae, Malpighiaceae, Leguminosae and Caesalpinoidea provide pollen (Teixeira and Machado, 2000, Aguiar and Gaglianone 2003) ^[11, 6].

Declines in wild bee populations have raised concern among biologists, prompting investigation into the factors contributing to the observed decreases. Stressors including pesticides, pathogens and habitat destruction have been implicated. In addition, the group's nesting biology may further raise concerns about the loss of bee species. For example, the construction of nests in aggregates and their persistence in nature (Gaglianone, 2005; Thiele and Inouye, 2007; Rocha-Filho *et al.*, 2008; Sabino *et al.*, 2017; Dec and Vivallo, 2019; Pina *et al.*, 2020) ^[12, 13, 14, 15, 16, 17] indicate that Centridini females return to their birthplace to build their nests. This probable philopatric behavior can result in a high degree of relatedness in populations, as aggregations have the potential to increase the probability of mating between family members (Paxton *et al.*, 1996; Danforth and Ballard, 2003; López-Urbe *et al.*, 2015) ^[18, 19, 20]. Inbred mating increases the phenotypic expression of deleterious recessive alleles, which can lead populations to extinction by inbreeding depression (Charlesworth and Charlesworth, 1987; Keller

and Waller, 2002) ^[21, 22]. Therefore, considering the possible costs of this nesting habit, evolutionary strategies may have emerged to minimize inbreeding matings, such as recognition between siblings and/or male dispersal to other locations.

Thus, the importance of males to the seasonal life cycle of Centridini bees may seem minor, but their role in successfully maintaining aggregate populations is underestimated. For example, within the existing bee literature, males of solitary bee are often overlooked and not included in research studies, with the thought that the caste is only important for copulation and little more. However, given the life cycle of these bees, males likely play a more critical role than their research attention currently suggests. Hence there is a need for an increase in the quantity of species studied, particularly with for male patrolling behavior, male dispersal mechanisms, mating behavior, plants visited by males, plus other biological data that may aid us comparisons within of the bees Centridini. In addition, a phylogenetic approach of these characters compared to phylogenetic studies on Centridini (e.g., Martins and Melo 2016) ^[23] can elucidate evolutionary aspects of these bees. In this study (review), we discuss the scientific literature investigating the reproductive behavior employed by Centridini males in nest aggregation and their important role in maintaining aggregation during generations.

Materials and Methods

We used Google Scholar, SciELO, and the Capes Periodical Portal with CAfe access Database to find peer-reviewed publications during May and July 2022. The search strings ('Males *Epicharis*', 'Males *Centris*', 'Males Centridini', 'Males solitary bee', 'Behavior reproductive *Centris*', 'Behavior reproductive *Epicharis*', 'Behavior reproductive Centridini', 'Behavior reproductive Solitary bees' and 'Behavior reproductive in bees agregation') were used. Of the total of all references that were identified, duplicates, conference abstracts and publications that were not relevant (i.e., did not contain males solitary bee specific data) were excluded. Peer-reviewed publications were prioritized and evaluated.

Results and Discussion

A total of 189 documents (theses, dissertations, abstracts, conferences, etc.) were found with the words used in the search engines. However, removing the duplicates, only 29 were scientific articles. Of this total, only 13 manuscripts were used in this review because it specifically presents the topic addressed (table 1).

Table 1

Term used in the search	Number of manuscript used
'Males <i>Epicharis</i> '	08
'Males <i>Centris</i> '	03
'Males Centridini'	0
'Males solitary bee'	0
'Behavior reproductive <i>Centris</i> '	02
'Behavior reproductive Centridini'	0
'Behavior reproductive Solitary bees'	0
'Behavior reproductive in bees agregation'	0

From the selected articles, we can identify that the reproductive behaviors of males in solitary bees are inserted within the articles that focused on studying the nesting biology of the species. In these studies, we found that solitary male bees do not seek out their partners randomly; exhibiting different reproductive strategies, such as: males can seek out and wrestle with other males for females in nesting areas (Alcock, Eickwort, *et al.*, 1977; Eickwort and Ginsberg, 1980; Hiller and Wittmann, 1994) ^[24, 25, 26]; carry out patrols waiting for virgin females in an emergence area (Alcock, 2013)^[27] or wait for them in flowers (Oliveira and Schlindwein, 2010) ^[30]; they can also defend territory or other attractive resources (Alcock *et al.*, 1977; Medeiros and Schlindwein, 2003) ^[24, 31] or attract females to sites without resources through pheromones (Roubik, 1989) ^[3]. In the case of territorial males, males may defend a particular group of flowering plants or not (Alcock 2013) ^[27]. Another behavior exhibited by males in areas where virgin females are emerging is to dig and enter nests in search of females even before they come to the surface (Alcock, Jones and Buchmann, 1977; Alcock, Jones and Buchmann, 2009) ^[32, 33]. In Centridini, the patrolling behaviors of males in aggregation were suggested that there are at least three mating strategies shown i) patrolling at the nesting site, ii) patrolling flowers (food resources), and iii) digging for virgin females. In aggregation, the search for females can be i) flyover near the ground and ii) flying farther from the ground, over non-flowering plants near the nesting site, may or not generate private territories (Thiele and Inouye, 2007; Pina *et al.*, 2020) ^[13, 17] To patrol the aggregate or adjacent regions males alternated periods of flying in circles, figure eights and/or zigzag.

In both *Centris* and *Epicharis*, the few studies focused on male behavior have pointed to different reproductive strategies related to male body size (Alcock *et al.*, 1977; Pina *et al.*, 2020) ^[32, 17]. The existence of alternative mating tactics and variation in the body size of males are generally reproductive strategies that minimize, for example physical combat with rivals, conferring advantages to larger males regarding access to virgin females, as observed in *Centris pallida* (Alcock *et al.*, 1977) ^[32]. However, in the study carried out by Pina *et al.* (2020) ^[17]

with *Epicharis dejeanii*, no advantage was found for any of the sizes of males in the analyzed copulations success.

In bees, cluster nesting and philopatry can provide evolutionary benefits to the population under certain conditions, decreasing, for example, high dispersal costs (Bonte, 2012) ^[34] and increasing the probability of mating encounters when population densities are low (Matthysen, 2005) ^[35]. On the other hand, it is expected that both behaviors can lead to high levels of inbreeding (Paxton *et al.*, 1996) ^[18], resulting in possible increases in the proportion of homozygotes, decreasing levels of individual genetic diversity (Hartl and Clark, 1997) ^[36] and possible reduction of the individual's fitness level (Freeland, 2005) ^[37], in addition to an inbreeding depression in the population (Allendorf *et al.*, 2012) ^[38]. Centridini aggregations often contain a high density of nests and several hundred females in one location (Thiele and Inouye, 2007; Rozen, 2016) ^[13, 7], and in some cases they can occupy a large area throughout their range. Females are supposedly philopatric and with a life cycle mostly univoltine (López-Uribe *et al.*, 2015; Dec & Vivallo, 2019) ^[20, 16]. Probably, due to the fidelity to the females' birthplaces and suitable characteristics of the soil, conducive to nesting, this aggregation of nests persists in the same place for several years in a row.

However, one factor that can reduce the long-term stability of this bee population is high levels of inbreeding. However, studies that analyze aggregated population genetic data are absent in the Tribe. On the other hand, for other solitary bee species that also nest in clusters, high levels of genetic diversity and low levels of inbreeding have been reported in the analyzed populations (Beveridge and Simmons, 2006; Lopez-Uribe *et al.*, 2015) ^[39, 20]. It is possible that male bees of the Centridini tribe are being able to disperse to more distant points within aggregations of great size in extension or even to other aggregations, which, consequently, increases the gene flow in the aggregation of nests and decreases the levels of mating between siblings, reducing the risk of inbreeding depression of the population, as suggested by the only study with *Centris* that addressed the subject (López-Uribe *et al.*, 2015) ^[20].

In bees, body size is directly related to their ability to fly (Greenleaf *et al.*, 2007; Cariveau *et al.*, 2016) ^[40, 41], consequently influencing their ability to disperse. As Centridini consists of bees considered large and fast-flying (Michener, 2007) ^[1], the plausibility of males dispersing in the Tribe is correct. Thus, it substantially increases the importance of directed studies on the behavior of males and their role in maintaining populations. Furthermore, the importance of males to the seasonal life cycle of solitary bees may seem minor, but their role in population success is underestimated. Also, as the biology and life cycle of solitary bees are distinct from social bees, it is difficult to extrapolate what is known about honey bee males to male solitary bees. For example, honey bee males mate only once, while solitary bee males have been documented to mate multiple times.

Therefore, additional work is needed to determine the degree to which males contribute to the success of the aggregate at its different stages of formation. Given the lack of research on male solitary bees, including the Centridini tribe, it is impossible to make estimates of their true contribution today. However, this information would not only provide information on the relative importance of male behavior, but would also provide clues about the impact of maladaptive sexual absences and proportions and the effects of these on the population health in aggregation.

Conclusion

Throughout this review, we evaluated the current literature on the reproductive behavior of males of solitary bees, especially of the tribe Centridini, and how they are important in maintaining the population nesting in aggregate, as well as the variables that affect the lifestyle of these bees. Our analysis compiles findings from a list of studies on the males of solitary bees and, as a result, highlights the often-overlooked ways in which males are placed in studies conducted in the aggregate. Furthermore, we present the current knowledge gaps related to these males, for example genetic studies, which offer promising areas for future research studies. Scientific projects targeting the males of both oil-collecting bees and solitary bees in general are opportune and necessary, given the aforementioned large declines in native bee populations. We hope that bee researchers will use the data highlighted here to guide future research efforts to fill in the most critical areas that lack specific work for bees that nest in clusters, as well as their participating males in this population.

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