

# Monogamy in large bee societies: a stingless paradox

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**Abstract** High genetic diversity is important for the functioning of large insect societies. Across the social Hymenoptera (ants, bees, and wasps), species with the largest colonies tend to have a high colony-level genetic diversity resulting from multiple queens (polygyny) or queens that mate with multiple males (polyandry). Here we studied the genetic structure of *Trigona spinipes*, a stingless bee species with colonies an order

of magnitude larger than those of polyandrous honeybees. Genotypes of adult workers and pupae from 43 nests distributed across three Brazilian biomes showed that *T. spinipes* colonies are usually headed by one singly mated queen. Apart from revealing a notable exception from the general incidence of high genetic diversity in large insect societies, our results reinforce previous findings suggesting the absence of polyandry in stingless bees and provide evidence against the sperm limitation hypothesis for the evolution of polyandry. Stingless bee species with large colonies, such as *T. spinipes*, thus seem promising study models to unravel alternative mechanisms to increase genetic diversity within colonies or understand the adaptive value of low genetic diversity in large insect societies.

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## Introduction

Genetic diversity within social insect colonies has proven important for the functioning of these complex societies, broadening tolerance to environmental changes (Oldroyd and Fewell 2007), improving resistance to pathogens (Schmid-Hempel 1998; Seeley and Tarpay 2007), and enhancing division of labor (Smith et al. 2008). High genetic diversity is particularly important in species with very large colonies, since such colonies have elevated risks of parasitism and more stringent requirements for efficient division of labor (Schmid-Hempel 1998; Bourke 1999; Kramer and Schaible 2013). For instance, across the social Hymenoptera (ants, bees, and wasps), species with very large colonies tend to have high intra-colonial genetic diversity (Hughes et al. 2008b; Boomsma et al. 2009; Jaffé et al. 2012).

Social Hymenoptera species with the largest known colonies exhibit either more than one queen per colony (polygyny)

or colonies headed by one queen mated with multiple males (polyandry) (Hughes et al. 2008b). Both reproductive systems increase colony genetic diversity by generating a more complex family structure. Many wood ant species (*Formica* spp.), for example, have enormous polygynous colonies, some containing thousands of queens [see Table S1 in (Hughes et al. 2008b)]. Certain army ants (*Dorylus* spp.) and leaf-cutter ants (*Atta* spp.), also have gigantic societies reaching up to millions of individuals. Yet, in these species colonies are headed by a single, multiply mated queen (Boomsma et al. 2009). Honeybees (*Apis* spp.) are among the bee species with the largest colonies, reaching tens of thousands of individuals. All honeybee species have colonies headed by one extremely polyandrous queen, which can mate with more than 50 males (Hughes et al. 2008b).

Species with large colonies headed by one singly mated queen provide notable exceptions to this general pattern and can help unravel the existence of additional mechanisms to increase genetic diversity within colonies or understand the adaptive value of low genetic diversity in large insect societies. Moreover, such species provide strong evidence against the sperm limitation hypothesis for the evolution of polyandry, which states that queen multiple mating evolved to provide queens with enough sperm to produce a large worker population (Boomsma and Ratnieks 1996; Kraus et al. 2004).

Stingless bees (Apidae: Meliponini) constitute an important challenge to understand the general importance of genetic diversity in large insect societies, with some species exhibiting colonies as large as those of polyandrous honeybees (Strassmann 2001), while the few reported polygynous species are not among those with the largest known colonies (Wenseleers and Ratnieks 2006; Alves et al. 2011). Although most analyzed species are monoandrous (see Table S4 and references therein), there is no reason to rule out the occurrence of polyandry in the group, given that polyandry has evolved independently within various tribes of other social insects [see Fig. 1 in (Hughes et al. 2008a)].

Here we studied the genetic structure of 43 wild colonies of the stingless bee *Trigona spinipes*. With colonies reaching up to 180,000 individuals, an order of magnitude larger than honeybee colonies (Michener 1974), *T. spinipes* represents an ideal model to study the general association between polygyny/polyandry and colony size (Fig. 1). Being a generalist pollinator broadly distributed across South America, *T. spinipes* is considered the ecological equivalent of *Apis mellifera* (Biesmeijer and Slaa 2006). Yet, colony genetic structure and population dynamics of this important pollinator remain unknown.

## Material and methods

Samples were collected from three Brazilian populations across three different biomes (see ESM). Adult workers from



**Fig. 1** *Trigona spinipes* nest and zoom of nest entrance (lower right). The white bar on the nest represents a 10-cm scale (photos by Fabiana C. Piocker-Hara)

32 nests were collected in Itirapina, while pupae were collected from six nests in Mossoró and five nests in São Paulo (Table S1). Samples were genotyped at five to seven polymorphic microsatellite loci: 4D, 2F, 2A, 3G, 1B, 1D (Santiago et al. unpublished), and Tang60 (Brito et al. 2009) (see ESM). In all colonies, queen genotypes were manually reconstructed along with those of their mates (Table S2). Genetic diversity measures were then computed using the genotypes of each queen and her mate (see ESM). Patriline non-detection and non-sampling errors were calculated for each colony along with matriline detection probabilities (see ESM). Finally, we performed a literature review to gather information on paternity and colony size across social bees (Table S4).

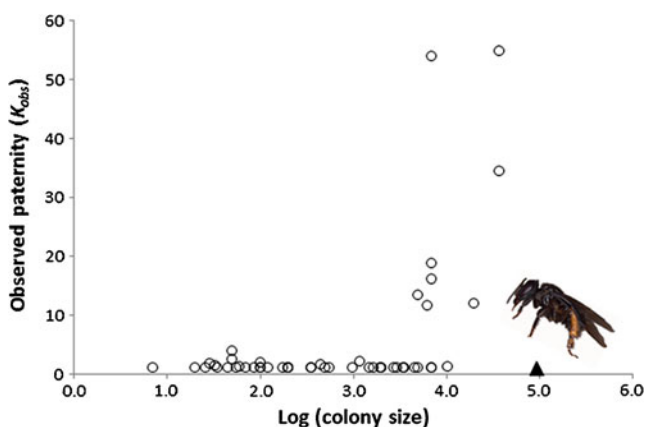
## Results

The mean number of alleles per locus was  $6.11 \pm 2.74$  with a mean expected heterozygosity ( $H_E$ ) of  $0.62 \pm 0.13$  (Table S3). No significant linkage disequilibrium between pairs of loci was detected. In all but one of the 43 study colonies, pupae and worker genotypes indicated the presence of one singly mated queen (Table S1). The exception was a colony from São Paulo, where pupae genotypes indicated the presence of two singly mated queens. Non-detection errors were very low in all colonies, ranging from  $5.09 \times 10^{-11}$  to  $9.42 \times 10^{-3}$ . The mean matriline detection probability per colony was 65 %. Non-sampling errors were low for patrilines represented in 50 % of the workers (from  $6.10 \times 10^{-5}$  to 0.13), but higher for patrilines represented in 25 and 10 % of workers (from  $1.78 \times 10^{-2}$  to 0.73; Table S1). In the Itirapina population, two loci showed significant  $F_{IS}$  estimates, but excluding these loci from the analyses did not change the main results.

## Discussion

Our results reveal the existence of a stingless bee species with very large colonies, headed by one singly mated queen, thus providing a notable exception from the general incidence of high genetic diversity in large social insect colonies (Fig. 2). The genetic markers employed were polymorphic and unlinked, and genetic diversity measures were equivalent to those of other stingless bee species (see references in Table S4). Non-detection errors were very low in all colonies, indicating that the markers employed were appropriate to detect all siring males and estimate paternity in this species. Separately, the loci employed did not allow distinguishing between mother queen-laid and daughter queen-laid workers in about a third of analyzed workers. However, the combination of all loci greatly reduced the probability of not detecting daughter queen-laid workers. Non-sampling errors, on the other hand, could have caused more important biases in our paternity estimates, as additional patriline represented in 25 % of the workers might have remained undetected in colonies with less than ten analyzed workers. Yet, no additional patrilines are likely to have remained undetected in the 13 colonies for which ten or more workers were analyzed, as this would require extreme paternity skew. By sampling pupae from different brood combs in each nest and by collecting adult workers from different nest entrances, we further reduced any potential sampling bias. As a similar genetic structure was found when analyzing adult workers and pupae samples collected from different colonies and across three different biomes, we are confident that our results reflect the biology of *T. spinipes*.

Although colony size is unlikely the main factor driving the evolution of polyandry, a significant positive association between colony size and paternity frequency has been found across all social Hymenoptera, as well as within the ants, bees,



**Fig. 2** Relationship between observed paternity and colony size across 53 species of social bees (only monogynous species included, see Table S4). White dots show paternity data reported in the literature and the filled triangle shows *Trigona spinipes* (this study)

and wasps, and independently of variation due to queen number (Jaffé et al. 2012). As polyandry is more likely to have evolved in a species like *T. spinipes* (with colonies an order of magnitude larger than those of the highly polyandrous honeybees) than in other stingless bee species with smaller colonies (Michener 1974), our results reinforce previous studies supporting the idea that polyandry did not evolve in stingless bees at all (Table S4, Fig. 2). Moreover, our results provide evidence against the sperm limitation hypothesis for the evolution of polyandry (Boomsma and Ratnieks 1996; Kraus et al. 2004), as the need for more sperm did not result in the evolution of polyandry in *T. spinipes*. For instance, *T. spinipes* queens seem able to store a similar number of sperm as *A. mellifera* queens do (Garofalo 1980), which suggests a more efficient sperm use in *T. spinipes*.

Given the importance of genetic diversity for the functioning of large insect societies, we could expect *T. spinipes* to exhibit alternative mechanisms to increase intra-colonial genetic diversity. We foresee at least three mechanisms by which this could happen: (1) frequent replacement of the old queen by her daughter (queen supersedure), who will mate with an unrelated male outside the nest and thus bring new genetic material into the colony; (2) temporal polygyny, whereby the old queen and her daughter, or a group of sister queens, temporarily co-exist in the nest mixing their worker offspring; and (3) invasion by a foreign queen, which would introduce new worker genotypes to the colony. Our data support the existence of queen invasions (one colony showed genotypes indicating the presence of two unrelated queens, Table S2), as has been described in *Melipona scutellaris* (Wenseleers et al. 2011). However, given that all remaining 42 colonies had genotypes from a single queen, queen invasions seem to be rare and monogyny seems to be the rule in *T. spinipes*, as in most other stingless bees. Moreover, colony pedigrees inferred from worker genotypes collected during a short time window do not always reflect the actual mating system (Matsuura et al. 2009), and thus we cannot make generalizations about queen supersedure or temporal polygyny. Future studies analyzing changes in colony genetic structure through time (Wenseleers et al. 2011) are necessary to reveal the incidence of temporal polygyny and queen supersedure. *T. spinipes* thus seems a promising study model to either unravel alternative mechanisms increasing colony-level genetic diversity or understand the adaptive value of low genetic diversity in large insect societies.

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