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RESEARCH ARTICLE

Colony structure, ecological correlates and nestmate recognition in the ant *Odontomachus hastatus*: a comparative study between populations with different social organisations

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Abstract
An important question in evolutionary biology is to identify the mechanisms that control the number of reproductives in social groups. Ants are appropriate models to address this question because of the variety of their social structures both within and between species, making this taxon suitable for initiating comparative studies to examine the drivers of this diversity. In this study, we developed a comparative approach between populations of the ponerine ant *Odontomachus hastatus* from Brazil and French Guiana. In Brazil, monogynous and polygynous colonies coexist in the same populations, whereas only monogynous colonies are present in French Guiana. We combined ecological, behavioural and chemical analyses to identify the factors associated with the expression of this social polymorphism. In Brazil, nest densities were higher than in French Guiana, but nesting sites were available in large quantities in both areas, indicating that habitat saturation is probably not the cause of social polymorphism. We did not detect any difference in queen size, regardless of the social structure of the colonies, suggesting that dispersal strategies may be similar between monogynous and polygynous populations. We found no influence of social structure on aggression intensity in dyadic encounters between workers. Last, we showed that the level of aggression increased with both geographical and chemical distance, but we did not find any difference in cuticular profiles between monogynous and polygynous colonies. Overall, the determinism of social polymorphism in *O. hastatus* still eludes us and calls for further field experiments coupled with genetic approaches.

Keywords Cuticular hydrocarbons · Facultative polygyny · Nest site limitation · Queen number · Recognition

Introduction

Understanding the mechanisms that shape the structure of social groups is a major goal of evolutionary biology. Social insects are valuable models to approach this question. Ants, for example, show tremendous differences in the number of reproductive queens within and between species. Monogyny (i.e. the presence of a single queen per colony) is considered the ancestral and predominant social structure (Boomsma et al. 2014). On the opposite, polygyny (i.e. the coexistence of multiple queens) is a derived trait that has evolved in many lineages (Keller 1993). Two routes are generally invoked to explain the transition to polygyny (Holldobler and Wilson 1977). The first is pleometrosis, i.e. when queens cooperate to found a colony and rear brood. The maintenance of cooperation between pleometrotic queens, though rare, can lead to the coexistence of several breeders in the same colony (primary polygyny) such as in *Neoponera* (prev. *Pachycondyla*) *villosa* (Trunzer et al. 1998). However, pleometrotic associations are usually temporary and reduced to a single laying queen (secondary monogyny) before or shortly after the emergence of the first workers. The second route is secondary polygyny which develops when established colonies adopt additional inseminated queens or as a result of colony fusion (Herbers 1993). Interestingly, the coexistence of both monogyny and polygyny within the same species, known as social polymorphism, is estimated to concern between 2%

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(Kay et al. 2022) and 15% of ant species (Boomsma et al. 2014; Boulay et al. 2014).

Habitat saturation is a common ecological cause for the transition to polygyny. One classical example is found in the ant *Temnothorax* (prev. *Leptothorax*) *longispinosus*. The addition of artificial nesting sites in the field reduces the frequency of multi-queen nests, which indicates that nest-site limitation promotes polygyny (Herbers 1986). The transition from monogyny to polygyny induces substantial changes in phenotypic traits such as morphological and behavioural differences linked to variations in dispersal strategies (Keller 1993). In socially polymorphic species, queens tend to be larger in monogynous colonies than in polygynous colonies, as they need to store energy reserves to fuel mating and dispersal flights and to invest in colony foundation (Kikuchi 2002; Rosset and Chapuisat 2007; Wolf and Seppä 2016). The social structure of colonies (monogyny or polygyny) also influences the intensity of aggression towards non-nestmates. Workers from polygynous colonies are less aggressive towards alien individuals than workers from monogynous colonies (*Solenopsis invicta*: Vander Meer and Porter 2001; *Pseudomyrmex pallidus*: Starks et al. 1998; *Pheidole pallidula*: Fournier et al. 2016; *Formica fusca*: Helanterä et al. 2011). It is not, however, a general rule as other studies have failed to confirm such patterns (*F. selysi*: Meunier et al. 2011; Rosset et al. 2007). So far, the most comprehensive studies on phenotypic traits associated with social polymorphism in ants have been conducted on a limited number of species (see: *F. selysi* and *S. invicta*, two species where queen number is under genetic control, Kay et al. 2022). To get a better picture of the correlates of social polymorphism, comparative work on a variety of ant taxa is needed, as the mechanisms underlying variations in queen numbers within species remain poorly understood (Suarez and Goodisman 2021).

Socially polymorphic species are valuable because they allow to examine the mechanisms associated with the transition from monogyny to polygyny. In this context, we investigated the behavioural, morphological, chemical and ecological correlates of social polymorphism in the ponerine ant *Odontomachus hastatus* (Fabricius). The genus *Odontomachus* contains over 70 species and has a tropical distribution (Fernandes et al. 2021). This genus has a variety of social organisation ranging from strict monogyny (e.g. *O. simillimus*: Van Walsum et al. 1998), to facultative polygyny (e.g. *O. troglodytes* (prev. *haematodes*): Colombel 1970) to strict polygyny (e.g. *O. rixosus*: Ito et al. 1996; *O. chelifer*: Azevedo-Silva et al. 2023). The species *O. hastatus* shows variations in its social structure with colonies that are facultatively polygynous in southeast Brazil (Oliveira et al. 2011) but strictly monogynous in French Guiana (Berthelot et al. 2017). This system, unique for ponerine ants, allows comparative studies between monogynous and polygynous colonies within and between populations. Here, we first characterise the habitat and demography of *O. hastatus* populations in Brazil and French Guiana. Based on the assumption that habitat saturation promotes polygyny, we expected nest sites availability to be lower in Brazil. Moreover, if polygyny is associated with a difference in the dispersal abilities of queens, we predicted that queens would be larger in monogynous than in polygynous colonies. Next, we investigated the influence of social structure (monogyny vs. polygyny) on aggression during pairwise confrontations between workers. We predicted workers from monogynous colonies to be less tolerant to non-nestmates than workers from Brazil, where polygyny might be associated with lower thresholds of acceptance. Last, we characterised the cuticular profiles of workers from Guianian and Brazilian colonies to examine the relationship between chemical distances and aggression patterns.

Materials and methods

Study area

In Brazil, fieldwork was carried out in the Cananéia estuarine lagoon complex, located on the southern coast of the state of São Paulo (Fig. 1). The area consists of three islands (Cardoso Island, Cananéia Island and Comprida Island) surrounded by channels connected to the sea (Schaeffer-Novelli et al. 1990). The predominant vegetation is Atlantic Forest, with patches of mangroves and restinga along the coast. The restinga forest has an open canopy of 5–15 m high trees growing on sandy soil, and abundant bromeliads growing both on the ground and as epiphytes (Barros et al. 1991). We studied two restinga sites about 8 km apart, one located in the Parque Estadual da Ilha do Cardoso (25°07′ S; 47°92′ W, hereafter Cardoso, abbreviated CAR) and the other in Cananéia Island (24°99′ S; 47°93′ W, abbreviated CAN). The climate is warm and humid and the average annual temperature and rainfall are 20.9 °C and 3000 mm respectively (Barros et al. 1991). Our fieldwork was conducted in November 2011 in Cardoso and in October 2012 in Cananéia.

In French Guiana, the study was conducted in the Réserve Naturelle des Nouragues, a lowland rainforest in the northern part of the Amazon rainforest. The fieldwork was carried out at two sites in January 2012: Inselberg (4°05′ N; 52°41′ W, abbreviated INS) and Saut-Pararé/COPAS (hereafter Pararé; 4°02′ N; 52°41′ W, abbreviated PAR), which are located about 6 km apart. The vegetation is typical of the vast primary forest at low altitudes, with a few inclusions of different vegetation types (palmitto-swamp forests, lianas forests and bamboo forests). The climate is humid, with a dry season (July–November) and a wet season (December–June)

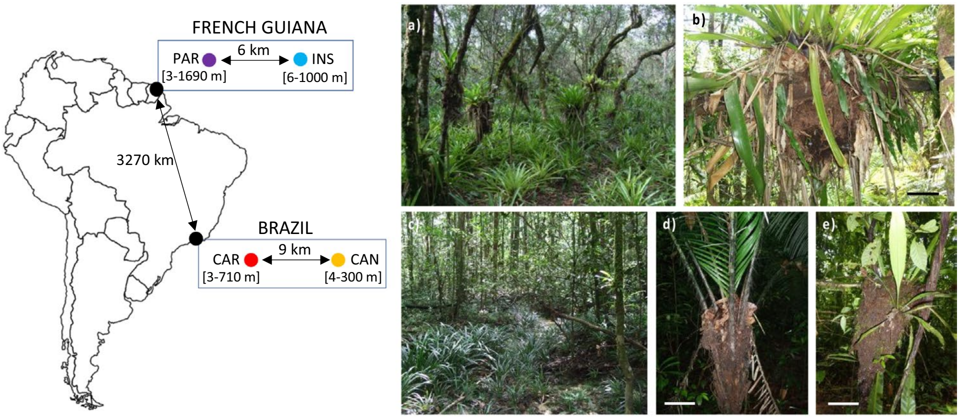

Fig. 1 Field sites in Brazil (CAR: Cardoso, CAN: Cananéia) and in French Guiana (PAR: Pararé, INS: Inselberg). Ranges of distances between colonies within each site are shown in square brackets. Photos show habitats and nests of *Odontomachus hastatus* in Brazil (**a**, **b**) and French Guiana (**c**–**e**). Scale bar: approximately 20 cm

interrupted by a short dry season in March (Sarthou and Villiers 1998). The average annual temperature and rainfall are 26.3 °C and 3000 mm respectively (Grimaldi and Riéra 2001).

Density of nests and of potential nesting sites

In Brazil, colonies of *O. hastatus* were found in the root clusters of clumped epiphytic bromeliads (*Vriesea altodaserrae*; see Oliveira et al. 2011; Camargo and Oliveira 2012). In Cardoso, we estimated the density of colonies within a 10 m distance on each side of a Y-shaped transect over a total area of about 22,000 m^2. At Cananéia, nest density was measured in a plot of 12,000 m^2 (length: 200 m, width: 60 m). At both sites, the areas sampled were representative of the habitat and all epiphytic bromeliads up to 2.5 m in height were carefully inspected for the presence of *O. hastatus*. At Cardoso, we counted the total number of epiphytic bromeliads in 4 quadrats of 100 m^2 (10×10 m) scattered along the transect. Every 10 m along the transect, we measured the nearest bromeliad and a bromeliad located 10 m on each side of the transect. At Cananéia, we counted and measured all epiphytic bromeliads in 8 plots of 100 m^2 (10×10 m). At both sites, we measured the largest diameter of the bromeliad root cluster, both for plants that were empty and those inhabited by ants (Oliveira et al. 2011).

In French Guiana, *O. hastatus* colonies were found in root clusters of epiphytic plants (*Asplenium*, *Cyclanthus*, *Philodendron*), in leaf litter accumulated at the base of palms (*Geonoma*, *Astrocaryum*, *Bactris*) or in nests built on branches or lianas (Fig. 1). At Inselberg, the number of colonies was counted within 10 m on each side of two 600 m long transects (sampled area: 24,000 m^2). At Pararé, we searched for nests within 10 m on each side of two transects of 550 m length each (sampled area: 22,000 m^2). At both sites, the areas sampled were representative of the habitat. The shapes of the nests were considered as truncated cones whose volume could be estimated by the measures of the height of the cone and its upper and lower diameters. In each sampled area, we assessed the total number of potential nesting sites by counting the number of palms with accumulated leaf litter and the number of epiphytic plants. At Inselberg, we randomly selected around 1/3 of all potential nesting sites ($N = 199$) to measure their volume and, at Pararé we measured the volume of all potential nesting sites ($N = 135$). The volume of potential nesting sites was measured by approximating their shape to a truncated cone as we did for the nests. Spatial coordinates of all inhabited nests and potential nest sites were recorded using a GPS (Garmin GPSmap 60CSx). A different method for estimating the volume of nests or potential nesting sites was used in Brazil and French Guiana to account for major differences in nest type which implies that comparisons between nest volumes in the two countries is not relevant.

Nest collection

Because *O. hastatus* has a nocturnal foraging activity (Camargo and Oliveira 2012), nests were collected during daytime to ensure the presence of all colony members. We only collected a subset of the colonies found during our survey to assess nest density. In total, we collected 49 nests of *O. hastatus*: 17 at Cardoso, 12 at Cananéia, 15 at Inselberg and 5 at Pararé. The composition of each colony (number of dealate females, winged males, winged females, workers, pupae and larvae) was determined immediately after collection. In the field, ants were kept in plastic bottles with moist tissue and were fed with ant diet (Bhatkar and Whitcomb 1970). In the lab, colonies were housed in large plastic boxes containing soil and were fed three times a week with ant diet and common green bottle flies (*Lucilia sericata*).

Head width

We measured the head width (minimum width across the eyes) of 8–10 workers from each of 11 colonies from French

Guiana, and 12 monogynous and 9 polygynous colonies from Brazil. We also measured the head width of queens (12 of colonies from French Guiana, 8 of colonies from monogynous colonies from Brazil, 2–11 queens of 7 polygynous colonies from Brazil). Head width was measured to the nearest 10^{-3} mm using a stereomicroscope Nikon SMZ1000 at a magnification of 40×. The workers and queens measured did not in all cases belong to the same colony.

Behavioural assays

We conducted dyadic encounters to assess aggression level between colonies. We considered the following types of encounters:

- Intracolonial: two workers from the same colony (controls)
- Intercolonial: two workers from different colonies
    (i) Intra-site: two workers from different colonies within the same site (Cananéia, Cardoso, Inselberg, and Pararé)
    (ii) Inter-sites: two workers from different colonies between different sites within the same geographical zone (Cananéia vs. Cardoso, Inselberg vs. Pararé)
    (iii) Inter-zones: two workers from different colonies between geographical areas (Brazil vs. French Guiana)
- Interspecific: *O. hastatus* workers were tested against workers of different, but sympatric, *Odontomachus* species. We used *O. chelifer* in Cardoso and *O. haematodus* in French Guiana. These two species share several aggressive behaviours with *O. hastatus* (mandibular strike, stinging), which allows relevant comparisons between inter- and intraspecific encounters (Raimundo et al. 2009). Intra- and inter-site dyadic encounters were conducted in the field (Cardoso, Cananéia, Inselberg, Pararé), but confrontations between workers from different areas (Brazil vs. Guiana) were conducted in Toulouse, France, in March 2012. We verified that keeping the colonies in the laboratory did not influence the outcome of the encounters by comparing the mean score of dyadic encounters performed in the field and in the lab for the same pairs of colonies (10–11 dyadic encounters for 13 pairs of colonies; data not shown). For logistical reasons, no interspecific confrontation was performed for ants collected in Cananéia.

We examined the influence of social structure on aggression by testing workers from monogynous and polygynous colonies. The social structure of all *O. hastatus* colonies used in the dyadic encounters was known, with the exception of the colonies collected in November 2012 in Cardoso, where only a sample of workers was collected from the nest surface. We used 4 monogynous colonies from Pararé, 4 monogynous colonies from Inselberg, 7 colonies (6 monogynous, 1 polygynous) from Cananéia and 11 colonies (4 monogynous, 4 polygynous, 3 unknown structures) from Cardoso. Eight to 12 replications were performed for each combination tested, except for interspecific encounters (5–11 replications). Interspecific encounters were performed as a positive control to estimate the maximum aggression score expected. A total of 1138 dyadic encounters were performed and analysed. Only a subset of the colonies collected in the field was used for the dyadic encounters.

We randomly sampled 10–15 workers from each test colony. The day before the encounters, we marked none, one or both ants to be tested the following day with a dot of coloured paint (one colour per colony) (Edding 750©) on the abdomen. In pilot experiments, we verified that the paint did not influence the outcome of the encounters. Both in the lab and in the field, the ants were kept overnight in groups of 10–15 workers in a plastic container with wet tissue and synthetic ant food. The dyadic encounters took place in a circular arena (5.5 cm diameter × 1.7 cm height). The arena was separated into two halves by a piece of cardboard inserted into slots in the arena wall. Each individual was randomly selected from the colony and carefully placed in one half of the arena. After an acclimatisation period of about 5 min, the test was started by gently sliding the cardboard. Each test lasted 10 min. We recorded up to ten confrontations simultaneously using a camera (Sony Handycam Full HD 7.1 megapixels) placed above the arenas. After each test, the arena was washed with ethanol. Each worker was used only once and the order of the dyads tested was determined randomly. Blind methods were used to analyse the video recordings, except for interspecific encounters where physical differences between species were too obvious.

The following interactions corresponding to increasing aggression were scored: ignored or weak antennation (i.e. contacts in which no ant showed interest) (score 1), moderate to vigorous antennation (score 2), backing-off (score 3), biting (score 4), mandible strike (forward lunge accompanied by rapid closure of the mandibles, score 5) and stinging (score 6). Scores of 1 and 2 were considered non-aggressive, whilst scores of 5 and 6 were considered very aggressive. The aggression score assigned to each dyad corresponded to the strongest agonistic event (1–6) observed during 10 min.

Cuticular profiles

In total, we analysed the cuticular profiles of 4 monogynous colonies from Pararé, 13 monogynous colonies from Inselberg, 14 colonies (9 monogynous, 5 polygynous) from

Cananéia and 24 colonies (20 monogynous, 4 polygynous) from Cardoso. We did not characterise the profiles of all colonies used for behavioural assays. For each colony analysed, three workers were killed and their thorax were introduced into a 1 ml glass vial and soaked in hexane (Sigma-Aldrich, HPLC quality) for 5 min. The extract was transferred to another 2 ml autosampler vial and evaporated under nitrogen stream. The dry residue was re-dissolved in 50 µl of hexane and stored at −18 °C until analysis. GC–MS analysis was performed on a mass spectrometer ISQ™QD Single Quadrupole GC–MS System (Thermo Fisher Scientific Inc., Villebon sur Yvette, France), fitted with a capillary column (Restek RTX-5MS 30 m × 0.25 mm, 0.25 µm film thickness, 5% diphenyl and 95% dimethylpolysiloxane) and a splitless injector (280 °C). Ionisation was by electron impact (70 eV, source temperature: 250 °C). Helium was the carrier gas (1.2 ml/min). The oven temperature was maintained at 70 °C after sample injection (2 µl), then programmed at 20 °C/min to 180 °C, then 5 °C/min to 320 °C and held for 10 min. Samples were automatically injected using an autosampler AS300 (Thermo Fisher Scientific Inc., Villebon sur Yvette, France). For each GC sample, peak areas were calculated by manual integration using Xcalibur 2.2 SP1 48 software and were expressed as the percentage of the total peak area.

Data analysis

All analyses were performed using R 4.2.0 (R Development Core Team 2022). We used a generalised linear mixed model (GLMM, function *glmer*) with a gamma error distribution to compare the volumes of ant nests and of potential nesting sites in French Guiana and a LMM to compare the diameters of empty and inhabited plants in Brazil. For both comparisons, data were square root-transformed and the field sites (Cardoso, Cananéia, Inselberg, and Pararé) were used as a random factor. We used linear mixed effects models (LMM) (function *lmer* in package lme4) to test for differences in colony size (after square root transformation) between monogynous colonies from French Guiana and monogynous and polygynous colonies from Brazil. Pearson correlation tests were used to assess correlations between colony size and other nest related variables (brood size, nests volumes, and workers' size). We compared head width between individuals from monogynous colonies in French Guiana and monogynous and polygynous colonies from Brazil using LMM with colony identity nested within sites as random factors. The range of variations in worker size between colonies from monogynous colonies in French Guiana and monogynous and polygynous colonies from Brazil was determined by calculating the coefficient of variation (standard deviation divided by the mean) of workers' head width for each colony. The coefficients of variation were then compared after square root transformation between monogynous from French Guiana and Brazil and polygynous populations from Brazil using an ANOVA. To compare the scores of aggression, we fitted a GLMM (function *glmer*) with a Poisson error distribution and colony identity as a random factor. *P* values were obtained with the function Anova implemented in the package *car*. We used Tukey post hoc multiple comparison-of-means tests (*glht* function in *multcomp* package) to test pairwise differences. Where necessary, we performed post hoc comparisons with the function '*glht*' with the Benjamini–Hochberg correction to adjust *P* values for multiple comparisons.

For the analysis of cuticular hydrocarbons, we only considered compounds ≥1% of the whole profile of workers from at least two colonies, which made a total of 33 compounds. The relative abundance of each compound was transformed with arcsine square root before statistical analysis. One colony from Cananéia (CAN19) showed an atypical cuticular profile in comparison to other colonies from Cardoso and was not included in subsequent analysis. Note, however, that the conclusions remained the same if this colony was included in the analysis (data not shown). We used non-metric multidimensional scaling (NMDS) plots based on a Bray–Curtis dissimilarity matrix using the *vegan* package to visualise similarities in the cuticular profiles of workers from the different sites (Guillem et al. 2016). The goodness of fit was evaluated using stress (standardised residual sum of squares) with low values (<0.05) indicating excellent data representation in reduced dimensions whilst high values (>0.2) are considered poor data representation. We complemented this approach with a similarity analysis (ANOSIM, *vegan* package) to assess the degree of separation between groups, with R values near 1 indicating strong differences between groups. We used a hierarchical cluster analysis on the Bray–Curtis dissimilarities between workers of different colonies. We used the *vegan* package to calculate the Euclidean chemical distance between colonies. Mantel permutation tests (999 permutations) were used (*vegan* package) to test the relationship between the chemical and geographical distance matrices between colonies. Pearson correlation tests were used to examine the relationship between aggression scores and geographical distances or chemical distances (measured with Bray–Curtis dissimilarities).

Results

Nest density and features

The density of nests was higher in Brazil (26.8 nests/ha at Cardoso, 10.8 nests/ha at Cananéia) than in French Guiana (7.1 nests/ha at Inselberg, 8.1 nests/ha at Pararé). Similarly, the density of potential nests sites in Brazil (1525 sites/ha at Cardoso, 717 sites/ha at Cananéia) was about 12 times higher than in French Guiana (83 sites/ha at Inselberg, 64 sites/ha at Pararé). In French Guiana, the volume of potential nesting sites (mean ± SD = 17.9 ± 17.0 l, median = 13.2 l, $N = 200$) was larger than the volume of the nests (mean ± SD = 14.3 ± 21.8 l, median = 8.4 l, $N = 91$) (GLMM (gamma): estimate = 0.08, SE = 0.02, $z = 3.85$, $P < 0.001$) (Figure S1). In Brazil, in contrast, the diameter of the root cluster of plants inhabited by *O. hastatus* (mean ± SD = 0.34 ± 0.12 m, median = 0.33 m, $N = 70$) was larger than the root cluster of empty bromeliads (mean ± SD = 0.22 ± 0.13 m, median = 0.19 m, $N = 314$) (LMM: estimate = 0.13, SE = 0.02, $z = 8.31$, $P < 0.001$). There was no difference in the diameter of the root cluster of plants inhabited by monogynous (mean ± SD = 0.31 ± 0.10 m, $N = 16$) or polygynous colonies (mean ± SD = 0.39 ± 0.17 m, $N = 11$) (LMM: estimate = 0.06, SE = 0.05, $z = 1.41$, $P = 0.17$). For queenright colonies, there was no significant relation between colony size and the volume of nests in French Guiana ($r_{13} = 0.20$, $P = 0.46$) or the diameter of the root cluster in Brazil for monogynous ($r_{14} = 0.02$, $P = 0.92$) or polygynous colonies ($r_9 = -0.07$, $P = 0.83$).

Colony demography

Queenless colonies and foundations (arbitrarily defined as queenright colonies with fewer than 5 workers) were excluded from the analysis (one nest from French Guiana containing only one queen, one nest from Brazil with one queen and 2 workers). No polygynous nests were found in French Guiana (5 out of 5 nests were monogynous at Pararé, 10/14 at Inselberg, no queens in the remaining nests collected), whilst 25% (4 out of 16) and 64% (7 out of 11) of the queenright nests were polygynous in Cardoso and Cananéia, respectively, with one queenless nest collected at each of these latter sites. Monogynous colonies from Brazil were significantly smaller (mean ± SD: 162 ± 122 workers, median = 125, range = 27–344, $N = 15$) than polygynous colonies from Brazil (mean ± SD: 368 ± 154 workers, median = 335, range = 199–626, $N = 11$) (LMM: estimate = 205.77, SE = 50.13, $z = 4.10$, $P < 0.001$) but not from colonies collected in French Guiana (mean ± SD: 225 ± 107 workers, median = 216, range = 22–436, $N = 15$) (LMM: estimate = 62.60, SE = 46.12, $z = -1.36$, $P = 0.17$) (Fig. 2). The demographics of each colony, including the number of winged females and males, are shown in Table S1.

The number of brood items (larvae and pupae) was positively correlated with colony size in French Guiana (Pearson correlation: $r_{13} = 0.85$, $P < 0.001$) (Figure S2a). In Brazil, the number of brood items was not positively correlated to colony size in monogynous (Pearson correlation: $r_{13} = 0.41$, $P = 0.13$) nor in polygynous colonies (Pearson correlation: $r_9 = 0.41$, $P = 0.21$). In Brazil, the number of dealate females ranged between 2 and 24 (mean ± SD: 7 ± 6, median = 5, $N = 11$) and was positively correlated with colony size (Pearson correlation: $r_{24} = 0.59$, $P = 0.001$) (Figure S2b).

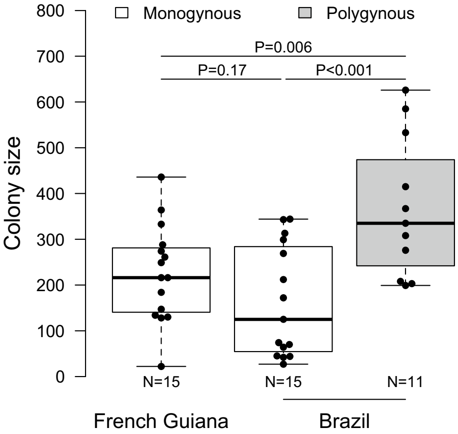

Fig. 2 Colony size (number of workers) of monogynous and polygynous colonies collected in French Guiana and Brazil. *P* values of post hoc multiple comparison-of-means Tukey tests are indicated. Each black dot represents one colony. The horizontal thick line represents the median. The box gives the interquartile range. Lower (or higher) whisker extends to the most extreme value within 1.5 interquartile ranges from the 1st (or 3rd) quartile

Head width

Workers were smaller in monogynous colonies from French Guiana (1.48 ± 0.09 mm, $N = 106$) than in monogynous colonies from Brazil (1.54 ± 0.08 mm, $N = 114$) (LMM: estimate = 0.05, SE = 0.02, $z = 2.37$, $P = 0.02$) (Fig. 3a). Workers from polygynous colonies of Brazil were larger (1.60 ± 0.07 mm, $N = 89$) than workers from monogynous colonies of Brazil (LMM: estimate = 0.06, SE = 0.02, $z = 2.57$, $P = 0.02$) and French Guiana ($z = 4.73$, $P < 0.001$) (Fig. 3a). Head width did not differ between queens from Brazil (monogynous: 1.80 ± 0.02 mm, $N = 8$; polygynous: 1.79 ± 0.03 mm, $N = 26$) and between queens from Brazil and those of French Guiana (1.76 ± 0.03 mm, $N = 12$) (Fig. 3b).

There was no correlation between colony size and average workers' head width, neither in monogynous (Pearson correlation, Brazil: $r_{10} = 0.56$, $P = 0.06$; French Guiana: $r_8 = 0.49$, $P = 0.14$) nor in polygynous colonies ($r_7 = -0.11$, $P = 0.77$) (Figure S3). The coefficients of variation of the head size of the workers were larger in colonies from French Guiana than in monogynous (ANOVA: estimate = −0.035, SE = 0.012, $z = -2.90$, $P = 0.007$ and polygynous colonies from Brazil (ANOVA: estimate = −0.039, SE = 0.013, $z = -3.01$, $P = 0.005$ (Figure S4a). The range of variation in worker head size decreased with colony size in monogynous colonies in Brazil (Pearson correlation: $r_{10} = -0.78$, $P = 0.003$),

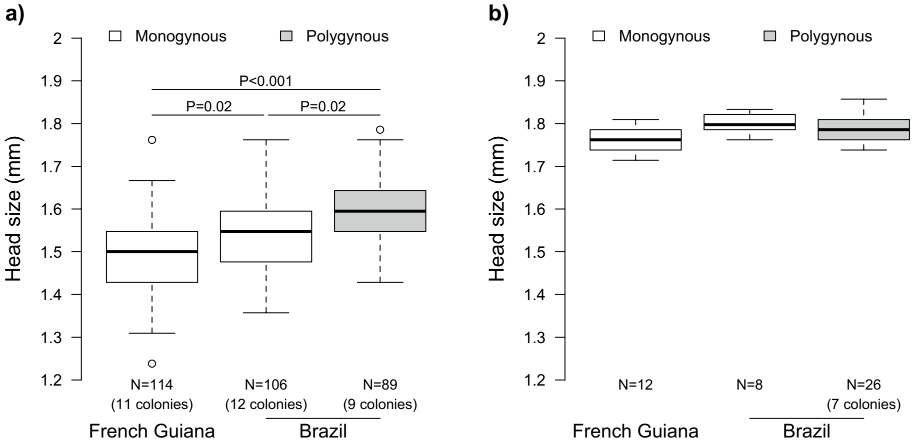

Fig. 3 Head width (mm) of workers (**a**) and queens (**b**) in monogynous and polygynous colonies from French Guiana and Brazil. *P* values of post hoc multiple comparison-of-means Tukey tests are indicated. The horizontal thick line represents the median. The box gives the interquartile range. Lower (or higher) whisker extends to the most extreme value within 1.5 interquartile ranges from the 1st (or 3rd) quartile. Circles indicate outliers

but no correlation was found for colonies in French Guiana ($r_9 = -0.01$, $P = 0.59$) and polygynous colonies in Brazil ($r_7 = 0.36$, $P = 0.35$) (Figure S4b). By grouping all colonies, regardless of their social structure, we detected no significant relationship between the coefficient of variation of worker head size and colony size ($r_{30} = -0.22$, $P = 0.22$).

Dyadic encounters

The intensity of aggression did not depend on the colonial social structure (monogyny vs. polygyny) of the interacting workers ($\text{Chi}^2 = 0.04$, $df = 2$, $P = 0.98$) (Figure S5). Interaction scores between workers collected at the same site (Cardoso or Cananéia), irrespective of the social structure of their colony of origin, were similar and reflected high tolerance. Almost all the interactions observed consisted of antennations (70% weak and 30% moderate/vigorous interactions in Cardoso and 30% weak and 70% moderate/vigorous interactions in Cananéia) (Figure S5). In subsequent analyses, we pooled the scores obtained for monogynous and polygynous colonies from each site in Brazil. For simplicity, we also aggregated the scores of all intercolonial encounters for each country, regardless of the site (for a full comparison of aggression scores across sites, see Table S2 and Figure S6). Both in Brazil and French Guiana, the score of aggression varied depending on the type of encounters (Table 1). The difference between intracolonial and intercolonial encounters was mainly due to a difference in the intensity of antennation (weak vs. vigorous antennation), with workers rarely showing the most aggressive behaviours (mandibular striking, biting) (Fig. 4). This was in stark contrast to the aggression recorded in encounters between workers of different species where the intensity of aggression was maximal (Fig. 4; Table 1). Overall, workers of *O. hastatus* from Brazilian and French Guiana populations showed similar behavioural responses to different types of encounters.

Table 1 Summary of multiple comparisons for the different categories of dyadic encounters in Brazil and French Guiana

	Estimate	SE	z	*P*
French Guiana				
Intracolonial vs. intercolonial	0.53	0.12	4.53	<0.001
Intracolonial vs. interspecific	1.49	0.11	13.46	<0.001
Intercolonial vs. interspecific	0.96	0.08	12.19	<0.001
Brazil				
Intracolonial vs. intercolonial	0.25	0.09	3.09	0.002
Intracolonial vs. interspecific	1.40	0.11	12.94	<0.001
Intercolonial vs. interspecific	1.16	0.10	11.96	<0.001

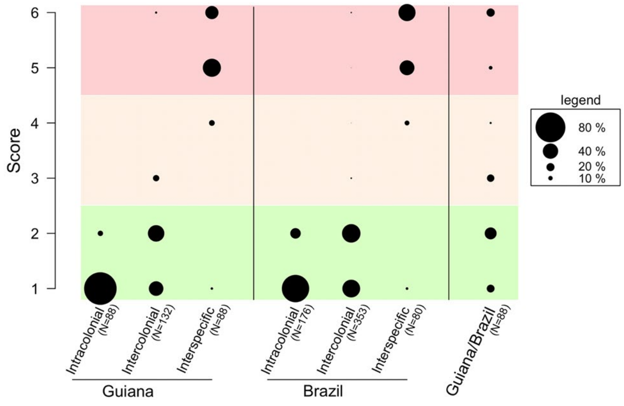

Fig. 4 Scores of aggression for dyadic encounters between workers. The size of each point is proportional to the percentage of occurrences of each score for each type of encounters. Guiana/Brazil indicates encounters between one worker from Brazil and one worker from French Guiana. The green, orange and pink background rectangles represent amicable interactions (antennations), moderate (backing-off, biting) and intense aggression (mandibular strike, sting), respectively

Interactions between workers of *O. hastatus* from colonies of French Guiana and Brazil resulted in intermediate aggression (Fig. 4).

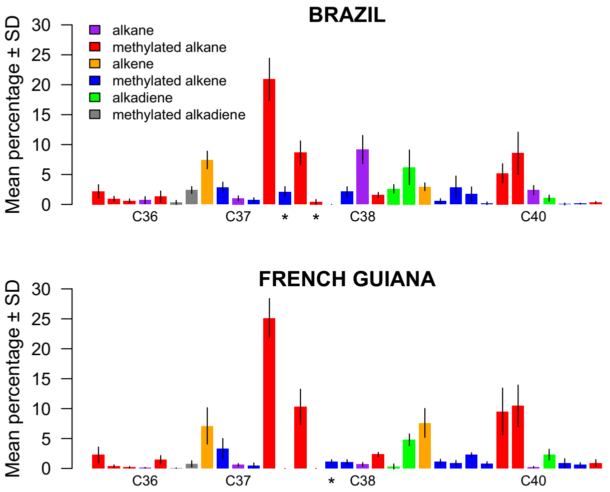

Fig. 5 Mean ± SD of the percentage of cuticular compounds for colonies from Brazil ($N_{\mathrm{total}} = 38$ of which Cardoso: $N = 24$, Cananéia: $N = 14$) and French Guiana ($N_{\mathrm{total}} = 17$ of which Pararé: $N = 4$, Inselberg: $N = 13$). Linear alkanes are indicated on the *x*-axis. The identification and relative abundance of cuticular compounds for each population and social structure are reported in Table S1. * indicate the compounds present only in the colonies from Brazil or French Guiana

Chemical analysis

The cuticular hydrocarbons of *O. hastatus* comprised a mixture of linear and methylated alkanes, methylated alkenes and alkadienes (Fig. 5). Qualitatively, we found that only three (two methyl-branched alkanes and one methyl-branched alkene) of the 33 compounds identified differed between the Brazilian and Guianese populations, representing about 3% of the total cuticle profiles. We also found that one alkadiene was present in workers from colonies at both sites in Brazil but at only one site (Inselberg) in French Guiana and, conversely, a methyl alkadiene was found on workers at both sites in French Guiana but only at one site (Cardoso) in Brazil.

Similarly, one compound found on workers from Cardoso was absent on ants from Cananéia and another compound found on workers from Inselberg was absent on individuals from Pararé (Table S1). The proportion of linear alkanes was five times larger on workers from Brazil (about 10%) than on individuals from French Guiana (about 2%).

Workers of colonies from the four populations exhibited distinct chemical profiles (ANOSIM: $R = 0.91$, $P < 0.001$) (Fig. 6). In Brazil, we detected no difference in the cuticular profiles between polygynous and monogynous colonies (Figure S7).

We next examined how aggression varied with geographic and chemical distances between pairs of colonies used for dyadic encounters (Fig. 7). Considering only the subset of colonies used for behavioural tests, chemical distances increased with geographical distances (Mantel

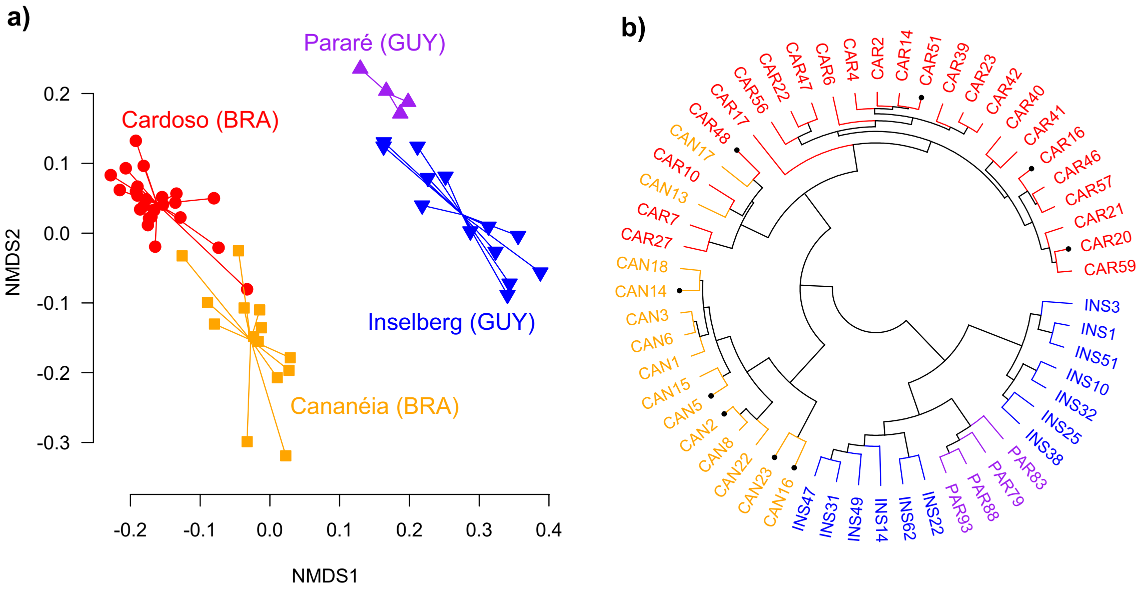

Fig. 6 **a** Non-metric multidimensional scaling ordination (NMDS) plot of the cuticular hydrocarbons of workers from monogynous and polygynous colonies of Brazil based on the Bray–Curtis dissimilarity distances. Stress = 0.06. **b** Hierarchical clustering analysis based on the Bray–Curtis dissimilarity distances of the cuticular profiles of workers, with black dots highlighting polygynous colonies

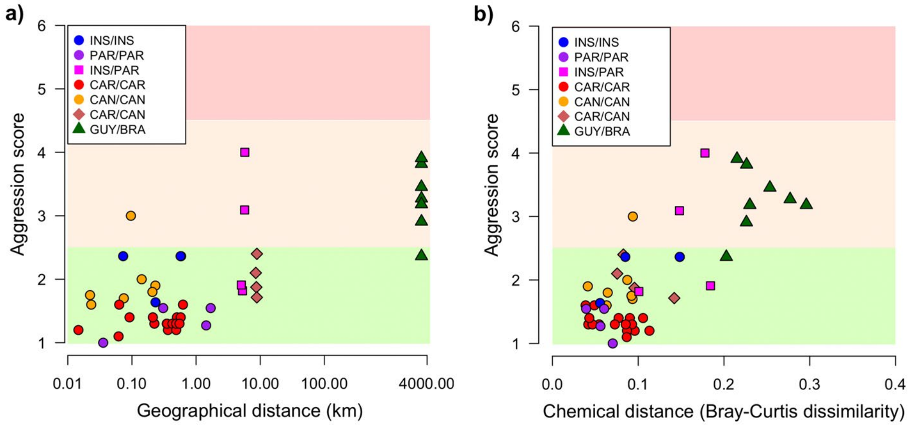

Fig. 7 Mean score of aggression as a function of **a** the geographical distance between colonies and **b** the chemical distance (assessed using Bray–Curtis dissimilarity) of the cuticular profiles of workers. Eight to ten encounters were made for each pair of colonies tested for each condition (intercolonial within sites: INS/INS, PAR/PAR, CAR/CAR, CAN/CAN; intercolonial between sites: INS/PAR, CAN/CAR and intercolonial between Brazil and French Guiana: GUY/BRA). Error bars are omitted for clarity. The green, orange and pink background rectangles represent amicable interactions (antennations), moderate (backing-off, biting) and intense aggression (mandibular strike, sting), respectively

permutation test: $r = 0.78$, $P = 0.001$) (Figure S8). Aggression increased with geographical distances between colonies whether interactions between workers from Brazil and French Guiana were taken into account (Pearson correlation: $r_{45} = 0.70$, $P < 0.001$) or not (Pearson correlation test: $r_{37} = 0.43$, $P = 0.005$) (Fig. 7a). Similarly, aggression scores and chemical distances were positively correlated (Pearson correlation: $r_{45} = 0.78$, $P < 0.001$) (Fig. 7b).

Discussion

By combining ecological, behavioural and chemical analyses, we aimed at developing a comparative approach between monogynous and polygynous populations of the ant *O. hastatus* from Brazil and French Guiana in order to identify the factors associated with the expression of social polymorphism.

Habitat saturation

Habitat saturation is a driving force frequently invoked to explain the occurrence of polygyny (Herbers 1993; Pedersen and Boomsma 1999). Given the absence of saturation of potential nesting sites in *O. hastatus*, it seems unlikely that nest-site limitation could be the cause of polygyny in Brazil. However, not all bromeliads may be suitable for nesting. Also, the finding that the root cluster of bromeliads inhabited by *O. hastatus* is larger than that of empty plants does not indicate whether colonies are preferentially founded in larger plants, whether they shape the root cluster as they grow, whether they move selectively to larger plants as colonies increase in size or whether large plants are more resistant to adverse weather conditions (rain, wind). In French Guiana, the density of *O. hastatus* colonies is three times lower than in Brazil and opportunities for nesting sites are less frequent compared to Brazil. Considering only potential nesting sites, which obviously provides a limited estimate of habitat quality, it would appear that French Guiana, which hosts only monogynous colonies, has greater habitat saturation than the sites studied in Brazil. The dominant factor in terms of habitat saturation may not be the availability of nesting sites, but rather the intensity of competition between colonies, which depends on nest density and the spatial scale of their territory (Seppä et al. 1995). The nest density in *O. hastatus* in Brazil is around 20 nests/ha, meaning that colonies are on average 20–25 m apart. Workers have been observed foraging nearly 10 m away from their colonies (Rodrigues and Oliveira 2014), which implies that the foraging range of neighbouring colonies can potentially overlap. The spatial promiscuity between mature nests can reduce the success of colony foundation by a solitary queen, due to intense scramble competition with adult colonies. On the other hand, polygynous colonies, because of their larger workforce, can mobilise a greater number of workers, who are also larger in size (as shown here), enabling them to be more successful in exploiting food sources. If we add interspecific competition to intraspecific competition, founding queens and mature colonies could, therefore, benefit from the readoption of young reproductives, a strategy that might also reduce their risk of predation.

Colony demography

No difference in colony size between monogynous nests from Brazil and French Guiana was detected, but polygynous colonies were bigger than monogynous colonies. Colony size was also correlated with the number of queens in polygynous colonies. Earlier work conducted on the same population in Brazil reported that about 80% of females in polygynous colonies have a filled spermatheca and that almost all inseminated females presented yellow bodies in their ovaries (Oliveira et al. 2011). Although dominant interactions are present in polygynous colonies, subordinate females are generally successful in laying eggs (Oliveira et al. 2011). The coexistence of multiple egg-laying queens could, therefore, explain the positive relationship between queen number and colony size. An alternative, but not exclusive, mechanism could be that the probability of recruiting additional females increases with time and therefore with colony size, as the size of a colony generally increases with its age. Assuming a constant adoption rate, the probability of switching to polygyny should thus increase with colony size.

Queen and worker size

In ants, changes in the number of queens are usually accompanied by a series of morphological and behavioural changes leading to the definition of a polygyny syndrome (Keller 1993). Typically, polygynous colonies produce small females that mate locally and re-enter natal nests or disperse by budding. This contrasts with queens produced by monogynous colonies, which are generally larger, with better dispersal abilities and found their colonies independently (Rüeppel and Heinze 1999; Peeter and Ito 2001; Wolf and Seppä 2016). In *Myrmica kotokui*, for example, queens in monogynous populations are larger than queens in polygynous populations, but no difference in head size has been detected between queens when monogynous and polygynous colonies coexist in the same populations (Kikuchi 2002). In *F. selysi*, queens are also larger in monogynous than in polygynous colonies (Meunier and Chapuisat 2009). Here, no difference in queen head size was detected regardless of the social structure of the colonies or the population origin, which could provide indirect lines of evidence that dispersal strategies do not differ between social morphs. In contrast, worker head size was influenced by both social structure and population origin with smaller head size in monogynous colonies. Since polygynous colonies are more populous, perhaps a larger workforce provides a better food supply and improved larval growth. In addition, we found no differences in worker polymorphism in Brazil between monogynous and polygynous colonies. These results contrast with the usual patterns reported across species where polygynous colonies are characterised by smaller worker size and less polymorphism than monogynous colonies (Greenberg et al. 1985; Goodisman and Ross 1996; Kikuchi 2002; Schwander et al. 2005).

Nestmate recognition

We investigated how colony social structure and distance between nests influence the behavioural responses of workers during pairwise confrontations. In Brazil, where monogyny and polygyny coexist in the same populations, we found no influence of social structure on the intensity of aggression between workers. The prediction that workers in polygynous colonies would accept non-nestmates more readily than workers in monogynous colonies was, therefore, not supported by our data. Similar behavioural patterns were observed in Brazil and French Guiana, with workers at all sites showing the same level of tolerance towards non-nestmates. It could be argued that using paired encounters in a neutral arena were not appropriate for studying nestmate recognition and that testing ants in the presence of colonial cues might have produced different results (Roulston et al. 2003; Buczkowski and Silverman 2005). However, we have previously shown that the introduction of non-nestmates into intact nests of *O. hastatus* does not elicit strong aggression with alien ants being only antennated or gently grasped by resident workers (Berthelot et al. 2017). This indicates that the behaviours observed in the present study closely reflect what is observed under more natural conditions. Workers of *O. hastatus* as well as reproductive females (see Berthelot et al. 2017) have long-chain hydrocarbons (from C35 to C45) without compounds of smaller length. Such cuticular profiles are unusual in ants, including in other species of *Odontomachus* which typically have shorter chain lengths, varying between C15 and C36 (Smith et al. 2016; de Azevedo Filho et al. 2021), even for species sharing the habitat of *O. hastatus* (e.g. *O. haematodus*). In the social parasite *Acromyrmex insinuator*, the presence of long unsaturated hydrocarbons (C43–C45) is suspected to make it easier for intruders to escape colonial recognition by host colonies (Lambardi et al. 2007). In another context, ants living in association with another ant species and displaying high interspecific tolerance also possess heavy hydrocarbons (Menzel and Schmitt 2012). The reason why long-chain hydrocarbons may promote tolerance is not yet understood, one hypothesis being that they may be difficult to detect due to their low volatility as a result of their high molecular weight (Lorenzi and d'Ettorre 2020). We can speculate that the presence of heavy compounds in *O. hastatus* contributes to amicable interactions between alien workers.

Although interactions tended to be effectively amicable, we nevertheless observed that those between workers in French Guiana and Brazil were more aggressive than those within each geographic zone, but without matching the high level of aggression observed for interspecific encounters. In *O. hastatus*, aggression increased with both the geographical and chemical distances between colonies both in Brazil and French Guiana. This trend is consistent with the dear-enemy effect, where workers show more tolerant interactions with individuals from neighbouring colonies than with ants from distant colonies (Dimarco et al. 2010). This phenomenon may be based on two mechanisms that are not mutually exclusive. First, individuals can habituate to the cues emitted by neighbours during bouts of foraging, as has been demonstrated in *Pheidole* ants (Langen et al. 2000). As indicated above, the foraging range of workers of *O. hastatus* and the distance between colonies create the conditions for frequent encounters between foragers from alien colonies. An additional explanation is that neighbours are also more likely to share recognition signals because they are potentially genetically related due to limited dispersal and/or because they live in a common environment that may have a comparable impact on the production of recognition signals.

In Brazil, we detected no difference in cuticular profiles between monogynous and polygynous colonies. The site of nest collection (Cardoso vs. Cananéia) was, however, determinant in separating the colonial cuticular signatures. It remains to be determined whether the difference in the presence/absence of hydrocarbons between sites within the same geographical area results from genetic differentiation or reflects the expression of environmental factors. An important difference in the cuticular profiles between Brazil and French Guiana concerns the relative abundance of linear alkanes, which was higher in Brazil than in French Guiana. Linear alkanes are not thought to play a role in nestmate recognition (Martin and Drijfhout 2009 but see Akino et al. 2004). Local differences in abiotic conditions such as relative humidity could explain these variations. Indeed, the composition of the cuticular blend depends on environmental conditions and linear alkanes participate in the prevention of water loss. For example, workers of the ant *Pogonomyrmex barbatus* showed an increase in the relative abundance of linear alkanes after exposure to hot and dry conditions (Wagner et al. 2001). Bioassays manipulating the colonial signature by addition of exogenous compounds should be conducted to determine which cuticular compounds contribute to nestmate recognition.

Transitions from monogyny to polygyny

Two principal mechanisms are generally invoked for the transition from monogyny to polygyny: the maintenance of cooperation between group founding queens (i.e. pleometrosis) (Holldobler and Wilson 1977) and the adoption of supernumerary females by mature colonies. Pleometrosis does not appear as a behavioural trait occurring in *O. hastatus*, since all colony foundations collected in French Guiana over several years of field work ($N > 60$ foundations) had only a single queen (Berthelot et al. 2017). This does not, however, rule out that such hypothesis could be valid for the Brazilian populations for which there is still no information about founding queens. Alternatively, polygyny could arise from the adoption of young inseminated females. However, this hypothesis does not seem to be supported since, at least for French Guiana, we showed that alien foundresses are fiercely evicted by resident workers from established colonies (Berthelot et al. 2017). Nevertheless, things may be different for the Brazilian populations for which data are lacking. Last, polygyny in Brazil could also result from the fusion of incipient colonies as suggested in other ant species (Medeiros et al. 1992; Guénard et al. 2016; Eriksson et al. 2019). In the Argentine ant *Linepithema humile*, low aggression between workers, linked to greater similarity in worker cuticular hydrocarbons, increases the chances of colony fusion (Vásquez and Silverman 2008). Here, our behavioural trials revealed amicable behaviours between non-nestmates in Brazil, suggesting that colonies could potentially merge to develop secondary polygyny. However, a similarly high degree of tolerance was found in French Guiana, and we have shown that alien mature queens, whose cuticle profiles differ markedly from those of foundresses, are readily accepted by established colonies from this population (Berthelot et al. 2017). The acceptance of mature queens but not of founding queens in colonies indicate that resident workers prioritise cues advertising fertility status over colonial cues (Moore and Liebig 2010; Berthelot et al. 2017). Despite this, polygyny does not seem to occur in French Guiana.

A large majority of colonies with more than one queen in ants generally derives from the adoption of queens in pre-existing nests or from newly mated queens remaining in their natal nest (Boulay et al. 2014). When both monogynous and polygynous colonies coexist in the same population, as here for the two sites studied in Brazil, this raises the question of why only some colonies would host supernumerary queens. One possible phenomenon that could explain the co-occurrence of monogynous and polygynous colonies is the possible existence of annual variations in environmental conditions which would imply that in some years founding queens may try to be adopted by existing colonies whilst in other, perhaps more favourable years, they would found a colony solitarily. In *Myrmica* ants for example, the temporal variation in queen numbers within colonies may be caused by cycles of queen mortality and recruitment of additional queens, possibly in response to variation in climatic conditions (Elmes and Keller 1993). A long-term field study of *O. hastatus* populations could answer this question by investigating to what extent foundation strategies, especially considering the rate of solitary foundation, depend on seasonal or annual variations in ecological parameters.

Future studies will need to characterise the genetic structure of colonies in *O. hastatus* in order to establish the relatedness between queens and workers in polygynous colonies. This will provide decisive information on the potential route to polygyny. Most work on facultative polygyny has focussed on a small number of taxa where the genetic determinism of social polymorphism is established even if the underlying mechanisms differ (Kay et al. 2022; Favreau et al. 2022). Whether social polymorphism in *O. hastatus* occurs in response to environmental constraints or genetic differences remains an open and intriguing question.

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Declarations

Conflict of interest The authors have no conflicts of interest to declare.

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