Both female castes contribute to colony emigration in the polygynous ant *Mystrium oberthueri*

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Abstract. 1. Nest emigrations are perilous for social insect colonies. Outside their nests, adults and brood are exposed to dangers. The behavioural mechanisms of emigration are thus likely to be under strong selective pressures.

2. Most studies on emigration have focused on monogynous species where survival of the queen is paramount, but emigration processes are largely unknown for species having several queens per colony.

3. In colonies of *Mystrium oberthueri* Forel, members of the morphological queen caste are as numerous as workers, although only a few of them mate and reproduce (polygyny). All queens perform intranidal tasks, such as brood care. Accordingly, we expected them to participate actively in emigration and to be less well protected.

4. Using four colonies, we studied the dynamics of 16 emigrations with a special focus on individual behavioural profile.

5. Workers were more involved than non-reproductive queens in recruitment and brood transport. Reproductive queens and young ants preferentially walked directly to the new nest without carrying brood. A chemical trail was probably used. The physiological status of individuals had more impact on their behavioural profile than their morphological caste.

6. This highly organized emigration process may underpin dependent colony foundation, as both involve the coordinated movement of nestmates.

Key words. Behaviour, caste, dependent colony foundation, emigration, ergatoid queen, *Mystrium oberthueri*, nest relocation, polygyny

Introduction

Movement in solitary animals is driven by individual decision. It is more complicated for social animals because decision and movement must be coordinated among individuals (Visscher, 2007). Strategies have evolved that facilitate the relocation of large groups, such as colonies of social insects. Such strategies are particularly important in species where colonies must move frequently. First, colonies of many ant species are indeed exposed to disturbances or changing conditions (e.g. nest destruction, predation, competition, food availability) that compel them to emigrate to new nesting sites (e.g. Wilson, 1958; Smallwood, 1982; Fukumoto & Abe, 1983; Herbers,

1986; Tsuji, 1988; Brown, 1999; Gibb & Hochuli, 2003; McGlynn *et al.*, 2004). In various species where emigration frequency was assessed, colonies moved to a new nest every few weeks (Smallwood, 1982; Kugler & del Carmen Hincapié, 1983; McGlynn *et al.*, 2004). Secondly, in species where colonies reproduce by dependent foundation, emigration is an essential part of life history: the queen and nestmate workers walk out of the mother colony to initiate a new colony (reviewed by Peeters & Molet, 2010). This contrasts with independent founding species where solitary queens fly from the mother colony to mate and found a new colony (Peeters & Molet, 2010). Dependent colony foundation resembles emigration, as it involves the coordinated movement of nestmates to a new nesting site. Emigration events could even result in colony division in some species if colonies split. Because it affects both survival and reproduction, the ability to emigrate efficiently is likely to influence colony fitness strongly.

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Compared with other social insects, ants are particularly well adapted to nest emigrations because workers do not fly and the brood is not enclosed in cells: both adults and brood can thus be carried. Nevertheless there can be a cost for colonies because adults and brood can fall victim to predators, pathogens or accidents. Thus, nest relocation is a crucial event in the life of a colony and its unfolding should be under strong selective pressures. Workers can be autonomous during emigration, i.e. walk to the new nest by themselves (Meudec, 1977; Leal & Oliveira, 1995; Brown, 1999; Franks & Sendova-Franks, 2000), but in most species the efficiency of emigration is improved by different recruitment strategies such as tandem running [*Diacamma* sp. (Fukumoto & Abe, 1983; Maschwitz *et al*., 2000; Kaur *et al*., 2012); *Pachycondyla tarsata* (Hölldobler, 1984)], adult transport [frequent in *Formica sanguinea* and *Cataglyphis iberica* (Möglich & Hölldober, 1974; Dahbi *et al*., 2008); occasional in *Basiceros manni* (Wilson & Hölldober, 1986)], or both (*Temnothorax albipennis*, Pratt *et al*., 2002). Emigrations are particularly perilous for colonies with a single queen (monogynous), and different strategies are adopted to ensure the protection of the queen on her way to the new nest. She can be transported by a worker (*T. albipennis*, Franks & Sendova-Franks, 2000), or completely surrounded by workers (*Oecophylla*, Hölldobler & Wilson, 1983). In addition, risk is reduced if the queen relocates at mid-emigration, when ant flow between the two nests peaks and both nests are equally populated, so many workers can protect her (Franks & Sendova-Franks, 2000; Avargues-Weber & Monnin, 2009). For instance, in *Diacamma indicum*, the gamergate (mated reproductive worker) generally moves to the new nest by following a tandem leader when approximately 40% of the adult ants are in the new nest (Fukumoto & Abe, 1983; Kaur *et al*., 2012). In colonies with several queens (polygynous), selective pressures on queen protection may be weaker, as the risks of all queens dying is low. Queens of the facultatively polygynous *Pachycondyla obscuricornis* have been reported to relocate alone when approximately half of the colony has moved to the new nest, regardless of queen number (Pezon *et al*., 2005). Emigration in polygynous species has been studied at the colony level (Smallwood & Culver, 1979; Stuart, 1985), but only Pezon *et al*. (2005) focused on individual behaviour. Knowledge of the dynamics of nest relocation for polygynous colonies thus remains fragmentary and, specifically, members of the queen caste have never been reported to behave as helpers during the emigration, e.g. recruiting nestmates or transporting brood and adults. Additional studies are thus needed to assess the process of emigration of polygynous colonies.

We studied colony emigration in *Mystrium oberthueri* Forel, an amblyoponine ant found in primary rainforests of Madagascar. Colonies nest mostly in damp rotten wood on the ground, including small branches and root mat, so emigrations may be relatively frequent e.g. when branches decay or dry up. *M. oberthueri* colonies contain an average of 40 adults, about half of them being ergatoid (permanently wingless) queens that are smaller than the workers (Molet *et al*., 2007a, 2009). They are numerous because they do not disperse away from their natal nest. Up to 5% of these queens are mated and reproduce. We will refer to them as 'reproductive queens', in contrast to 'non-reproductive queens', which are virgin. All queens, including reproductive ones, are specialized in brood care and intranidal tasks, whereas workers focus on foraging and nest defence (Molet *et al*., 2007b). Queens are thus multi-purpose, as found in some other species with ergatoid queens (e.g. Bolton & Marsh, 1989; Ito, 1996; Heinze *et al*., 1999).

The aim of our study was to assess the dynamics of the emigration process at three levels: whole colony, each category of adults (workers, reproductive queens, non-reproductive queens) and each individual ant. This in-depth assessment of colony emigration down to the individual level has never been attempted before. Members of the queen caste are so numerous in colonies that the value of each individual may be relatively low, making any assistance from workers unnecessary. Queens are likely to play an active role during emigration just as they do in the nest, although this role may differ from that of the workers. The relocation of queens may thus be staggered over the whole course of emigration, unlike what is found in other ant species. Finally, the reproductive status of queens may affect their behaviour: reproductive queens may be protected during emigrations while non-reproductive queens may behave more like workers.

Material and methods

Ants

Four colonies of the ant *Mystrium oberthueri* were collected in primary rainforest in Madagascar (Réserve Spéciale d'Ambatovaky, 16.82°S, 49.29°E) in February 2010. Field census indicated 24–29 workers, 15–36 ergatoid queens, as well as eggs, larvae and cocoons. In the laboratory, each colony nested in a Petri dish (10 cm diameter) filled with plaster and covered with a red film to keep the ants in the dark. A hole allowed the ants to leave or enter the dish freely. The nest was placed in a large foraging area (45 × 60 cm with a plaster substrate). Temperature was set to 25 °C and humidity to 60%. Soil particles from the field nest were added in the artificial nest to encourage ants, and they settled in only a few hours and started foraging after a few days. Fresh pieces of mealworm (*Tenebrio molitor*) were placed in the foraging area every day to feed the ants and stimulate exploration behaviours.

In order to identify each ant individually during emigrations and subsequent dissections, all ants (*c*. 220 individuals) were marked with a unique colour code consisting of paint marks (Oil-base Uni Paint Marker, Mitsubishi Pencil, Tokyo, Japan) on different body parts. We also marked eight ants that emerged in the laboratory and were less than 20 days old during emigrations (referred to as 'young' in the text). Dead queens were preserved in alcohol for dissections.

Emigrations

A second Petri dish nest, identical to the first one, was placed 20 cm away in the foraging area. Ants were free to discover

and explore this new nest for a few days before the experiment started. Emigration was then triggered by disturbing the old nest: the red film was removed, causing exposure to the light, and the dish lid was half-opened. This disturbance defined the beginning of the emigration process ($t = 0$). Emigration was then recorded using a high-definition digital video camera (Sony Handycam HDR-SR12E, Tokyo, Japan) overlooking most of the foraging box. Recording stopped when the emigration was considered completed, i.e. when less than 10% of the ants were out of the new nest (in the foraging arena or in the old nest). A total of 16 emigrations were recorded, i.e. four per colony. Ants were left undisturbed during several days between consecutive emigrations, and duration of this resting period was the same for all four colonies. We performed four replicates per colony in order to assess individual specialization across emigrations and we limited our study to four colonies so we could perform extensive individual marking and queen dissections at the end of the experiments.

Dissections

Following the final emigration, all queens ($n = 100$) from the four colonies were dissected in order to assess their reproductive status. A Leica stereomicroscope was used to check whether the spermatheca was full (indicating mating and ability to lay female eggs), whether the ovaries contained large yolky oocytes (indicating current egg-laying activity), and whether yellow bodies were present (indicating past egg-laying activity; Billen, 1982). Mated queens with active ovaries were classified as reproductive queens, while virgins were classified as non-reproductive queens.

Analysis of the videos

The movement of ants in and out of the new nest was used to quantify the dynamics of the emigration. Given that eggs and small larvae could not be visually identified or counted by eye, we considered as one 'brood item' the following: one cocoon, one large larva, one bunch of small larvae, one bunch of eggs. The time at which each individual entered or exited the new nest was noted, as well as the nature of the brood item carried. These data were used to assess demographic changes in the new nest over the course of emigration. Additional data could then be extracted, such as the time of first entry in the new nest, the time of half-relocation (when the number of ants in the new nest reached 50% of colony size) and the number of return trips or brood transports per individual and per caste.

We collected enough individual-level data to analyse them through generalised linear mixed effect modelling (GLMM). Accordingly we could assess behavioural differences between adult categories (workers, non-reproductive queens and reproductive queens) and deal with colony random effect. These individual-level data were count data (number of return trips, time of first entry, number of brood items transported) that followed a Poisson distribution. We used a log link function and the residuals were not overdispersed. When the adult category

Table 1. Demography of colonies at the time of each emigration.

Colony	Emigration no.	Workers	Non-reproductive queens	Reproductive queens	Brood items
1	1	34	22	1	11
	2	33	22	1	11
	3	33	21	1	15
	4	33	22	1	6
2	1	26	35	5	36
	2	26	37	5	31
	3	26	39	4	20
	4	28	38	5	8
3	1	30	22	1	14
	2	30	22	1	13
	3	30	21	1	16
	4	29	19	1	8
4	1	25	15	2	17
	2	25	15	2	20
	3	26	15	2	27
	4	27	16	2	5

yielded a significant effect, we calculated the inter-category percentage difference based on the reference parameter set to $b_{reference} = \exp(0) = 1$ and the estimated parameter $b_{estimated}$. The effect of emigration sequence number was also assessed to test the learning potential of ants between nest relocations. All null models were rejected in favour of the alternative complete GLMMs based on the Akaike information criterion (AIC).

Colony-level data (e.g. duration of emigration phases, percentage of workers relative to queens) were too few to be analysed through modelling (16 emigrations). In addition, these data did not follow a normal distribution. Accordingly, they were analysed using non-parametrical tests.

All statistical tests were performed using the software R, version 2.13.1 (available at http://cran.r-project.org/), packages 'car' (Fox & Weisberg, 2011) and 'lme4' (Bates *et al.*, 2011).

Results

Colony demography

Dissections at the end of the four emigration sessions revealed that each colony contained one to five reproductive queens with full spermatheca and active ovaries, whereas the remaining queens were virgin and had undeveloped ovaries. Accordingly, during the first emigration, colonies consisted of 37.7–60.5% workers, 34.9–56.5% non-reproductive queens and 1.8–7.6% reproductive queens (median for workers, 58.9%; non-reproductive queens, 39.3%; reproductive queens, 3.2%) (the demography changed slightly over the course of the experiments due to a few deaths and the emergence of new adults; Table 1).

Global dynamics of emigration

Over the course of emigration, the number of adults in the new nest generally followed a sigmoid growth over time (Figs 1 and 2). In a few cases (Fig. 1, colony 1, emigration
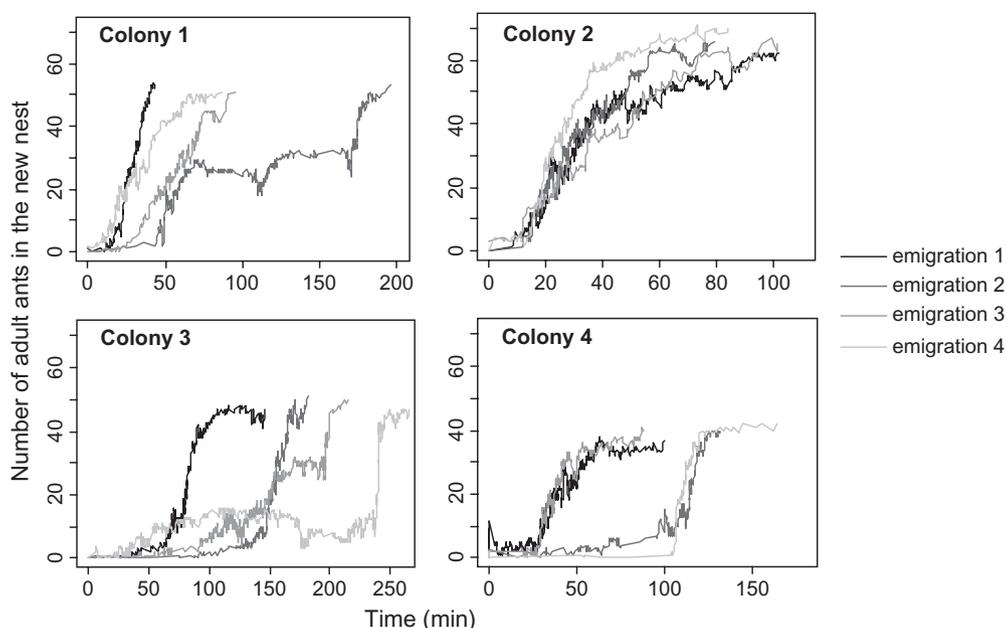
Fig. 1. Temporal dynamics of colony emigration. Each panel shows the four consecutive emigrations of a colony. Curves represent adult ants demography in the new nest.

2 and colony 3, emigrations 3 and 4), the number of adults in the new nest stagnated for a while during the emigration. This was not caused by a pause in traffic but by an equal number of ants entering and exiting the nest, which may reflect difficulties in the decision process. For statistical analyses, we set two arbitrary demographic thresholds that defined three main phases (Table 2). The initial phase lasted from the disturbance of the old nest to the point when 10% of the adults were in the new nest. Its duration ranged from 2 min 29 s to 130 min 9 s (median, 32 min 31 s) and it was characterised by a slow increase of the population in the new nest (0.7 ants min^{-1} on average). The exponential phase lasted until 80% of the adults were in the new nest. Its duration ranged from 10 min 23 s to 206 min 52 s (median, 42 min 58 s) during which a massive amount of traffic caused a steep increase in the population of the new nest (3.5 ants min^{-1}). Lastly, the final phase ended when 90% of the adults were in the new nest. It lasted from 3 min 12 s to 33 min 57 s (median, 11 min 1 s) and population increase was slower (2.6 ants min^{-1}). A percentage of 100% could never be reached because a few active individuals always explore the foraging area even outside of migration events. All adults walked to the new nest by themselves, including queens and males, and no adult transport was seen. Sequential emigration number had no effect on the duration of either the whole emigration or any of its phases ($P > 0.05$ for all linear regressions between emigration number and duration of emigration phases; Table 2).

Role of workers and queens during emigration

Over the whole emigration process, the mean number of return trips (walking to the new nest and back, all the way to the old nest or halfway, to recruit or help nestmates outside) per individual was 21% lower for non-reproductive queens than for workers [non-reproductive queens (NRQ), 0–20 return trips; workers (W), 0–22 return trips; GLMM, $b_W = 1$, $b_{NRQ} = 0.79$, $P < 10^{-6}$]. The maximum number of return trips recorded for one individual was 22 and this was achieved by a worker (mean values per colony are shown in Table 3). Furthermore, reproductive queens performed 64% fewer return trips than non-reproductive queens [reproductive queens (RQ), 0–5 return trips; non-reproductive queens, 0–20 return trips; GLMM, $b_{NRQ} = 1$, $b_{RQ} = 0.36$, $P < 10^{-6}$]. The mean values per colony are shown in Table 3 and the total number of return trips per ant across all emigrations are shown in Fig. 3. In addition, the number of return trips per individual decreased significantly over consecutive emigrations (GLMM, $b_{emigration\ number} = -0.16$, $P < 0.01$), which means that emigrations were performed more directly.

A few 'pioneer' workers and non-reproductive queens were often already present in the new nest at the beginning of the emigration. They represented up to 9.5% of colony population (zero to five individuals), and the proportion of workers relative to queens was not significantly different from the proportion in the whole colony (workers in the new nest at $t = 0$, 0–100%; workers in the whole colony, 37.7–60.5%; Wilcoxon–Mann–Whitney test with paired data, $V = 8$, $P = 0.69$). The proportion of workers entering the new nest during the initial phase was only marginally higher than the proportion of workers in the whole colony (workers entering the new nest during initial phase, 31–100%; workers in the whole colony, 37.7–60.5%; $V = 103$, $P = 0.07$). However, the mean time of first entry of workers into the new nest was significantly lower than that of non-reproductive queens (workers, 0–240 min 25 s; non-reproductive queens,
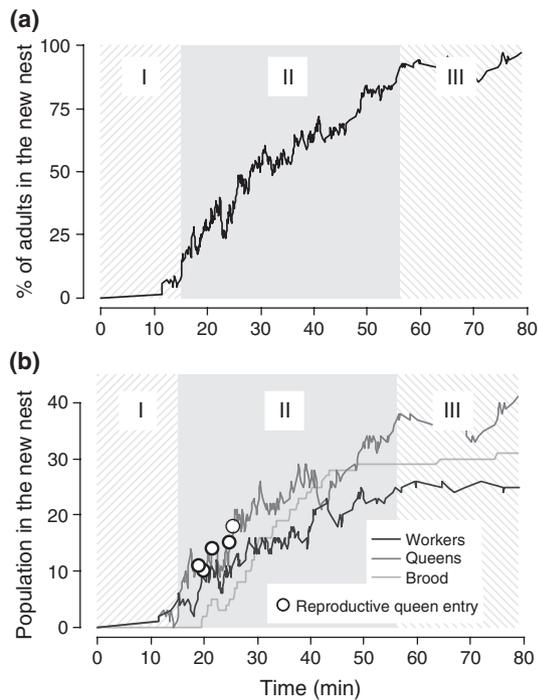
(a)

(b)

Fig. 2. Illustration of the three phases of the emigration process. (a) Total adult population in the new nest (% of total colony size). (b) Detailed worker, queen and brood populations (number of individuals). In this example (emigration 2 of colony 2), the initial phase (I) lasted 15 min 7 s, the exponential phase (II) 31 min 47 s and the final phase (III) 22 min 48 s. During the initial phase, a few ants located the nest. Then, during the exponential phase, ants moved in large numbers to the new nest, with some individuals performing numerous return trips. Reproductive queens entered the new nest during this phase. During the final phase, a few ants performed return trips to check the old nest for any remaining nestmates.

0–241 min 53 s; GLMM, $b_W = 1$, $b_{NRQ} = 1.07$, $P < 10^{-6}$). Moreover, reproductive queens entered the new nest significantly later than non-reproductive queens (GLMM, $b_{NRQ} = 1$, $b_{RQ} = 1.1$, $P < 10^{-6}$) and they were disproportionately likely to enter the new nest during the exponential phase [distribution of reproductive queen entries between the exponential phase and the two other phases (initial and final) versus distribution of all other adults entries; Pearson's χ^2 test, $\chi^2 = 9.8243$, $df = 1$, $P = 0.002$], when ant traffic between nests was maximal. Thus, workers started emigrating earlier than queens. Finally, the time of first entry of all adult ants in the new nest increased slightly with emigration number (GLMM, $b_{emigration\ number} = 1.14$, $P < 10^{-6}$).

During the final phase, the proportion of workers entering the new nest was significantly higher than the proportion of workers in the colony (workers entering the new nest during final phase, 25–96%; workers in the whole colony, 37.7–60.5%; $V = 110$, $P = 0.015$). Accordingly, workers were more involved in the last steps of the emigration than queens.

Thus, workers were more active than non-reproductive queens and they initiated and finished nest emigration, while reproductive queens performed the least number of trips

outside the nests and only when traffic between nests was maximal.

Brood transport

The brood was never transferred during the initial phase and it only started during the exponential phase. All brood items were already carried to the new nest before the end of the final phase. Accordingly, the duration of brood relocation was shorter than that of adult relocation (brood relocation, 2 min 53 s to 117 min 31 s; adult relocation, 10 min 23 s to 206 min 52 s; $V = 8$, $P < 10^{-3}$). The mean time for relocation of half the brood was not different from that for relocation of half the adults ($V = 0$, $P = 0.125$).

All castes were involved in brood transport to the new nest (Fig. 4). Median (range) percentages of transporters were as follows: 68.3% (17.2–100%) workers, 30% (0–82.7%) non-reproductive queens and 0% (0–12.5%) reproductive queens. Although 20.5% of adults were involved in transport, only 3–14% (median, 6.6%) of adults were responsible for 50% of all brood transports. The majority of transports were thus performed by a small proportion of individuals, and these were mostly workers (17.2–100%, compared with 37.7–60.5% of workers in the colony relative to queens; $V = 114$, $P = 0.018$), but these two proportions were not correlated (Spearman, $S = 427.01$, $r_S = 0.32$, $P = 0.16$). In addition, transporting workers were more implicated in terms of individual effort than transporting queens: the mean number of brood items transported by individual workers was marginally higher than that transported by queens (workers, 1–6 items; all queens, 1–6 items transported per individual; GLMM, $b_W = 1$, $b_Q = 0.8$, $P = 0.07$). The mean values per colony are shown in Table 3 and total number of transports per ant across all emigrations are shown in Fig. 4. Across the four successive emigrations, some individuals transported brood items over at least two emigrations. 33.3–87.5% were workers and 12.6–66.6% were non-reproductive queens (median, 75% workers, 25% non-reproductive queens; Fig. 5a). Reproductive queens almost never transported brood items.

Items other than brood were occasionally transported from the old nest to the new one, including 25 soil particles from the field nest (56% being transported by workers) and two pieces of mealworm (by workers only).

Passive individuals

Some individuals were passive during emigration, i.e. they neither transported brood nor performed return trips (they entered the nest once and stayed there). In each colony, 17.39–30.36% of individuals were passive over two or three emigrations (median, 25.2%; Fig. 5b), and 0–9.3% were passive across all four emigrations (median, 7.9%). Among individuals that were passive in at least two emigrations, the proportion of queens was significantly higher than the proportion of workers (queens, 32.7–52.7%; workers, 3.4–30.2%; $V = 111$, $P = 0.025$). Although a few occasionally transported brood, all reproductive queens were passive at least twice.

Table 2. Duration of the phases of each nest relocation.

Colony	Emigration no.	Initial (min)	Exponential (min)	Final (min)	Total (min)
1	1	17.1	19.3	4.3	40.7
	2	45	130.6	7.2	175.8
	3	33	54.4	7.8	90.8
	4	9.6	45.6	15.2	70.4
2	1	12.5	61.1	15.3	88.9
	2	15.1	32.8	22.8	70.7
	3	11.8	56.5	13.6	82
	4	14.7	20.5	17.4	52.6
3	1	64.6	32.6	28.1	126.3
	2	130.2	34.7	5.3	178.5
	3	92.4	107.5	3.2	203.1
	4	33.7	206.9	5.2	245.7
4	1	2.5	53.6	6.4	62.5
	2	68.1	50.7	4.6	123.3
	3	28.7	33.7	24.3	86.7
	4	106.1	10.4	14.9	131.3

Table 3. Behavioural profile of the different types of individuals during emigrations in relation to the average number of return trips performed and the average number of brood items carried to the new nest per transporter.

Colony	Emigration no.	No. of return trips			Transported items per transporter		
		W	NRQ	RQ	W	NRQ	RQ
1	1	1.09	0.86	0	3	1	NA
	2	3.39	3.41	1	1.29	1.33	NA
	3	2.51	0.76	1	1.62	1.5	NA
	4	2.24	1.91	0	1.2	NA	NA
2	1	5.92	3.09	1.8	1.7	1.61	NA
	2	3.81	2.15	0.2	2.2	1.73	NA
	3	1.85	1.23	0	2	1.2	NA
	4	1.57	1.18	0	1	1	NA
3	1	2.87	2.5	2	1.86	1	1
	2	3.2	2.86	0	1.57	1	NA
	3	6.4	4.24	1	2	1	NA
	4	7.48	5.37	0	1.14	NA	NA
4	1	4.48	4.4	5	1.7	1.2	1
	2	3.6	1.67	0.5	1.5	1.25	NA
	3	3.54	2.07	2	2	1.29	2
	4	1.15	0.25	0	2	1	NA

W, workers; NRQ, non-reproductive queens; RQ, reproductive queens; NA, no ant of this category transported brood items.

The eight young individuals of less than 20 days old were always passive.

Chemical trail

During emigrations, ants seemed to follow each other very closely and preferentially used one or two routes, suggesting that a chemical trail had been deposited on the plaster substrate. Additional evidence shows that, during their movements between the two nests, some individuals clearly rubbed the posterior end of their abdomen on the ground. Because of our video-recording design, we could not obtain quantitative data: we had a partial view over the whole foraging arena, from above, so the precise behaviour of trail-laying was often not identifiable beyond doubt.

Discussion

Dynamics of the emigration

Our observations show that colony emigration in *M. oberthueri* is a well-coordinated process. Workers, except for young ones, are the more implicated caste. They initiate the emigration and perform numerous return trips to recruit nestmates. They also carry brood items, probably lay a chemical trail, and end the emigration by performing more visits to the old nest, probably to check for any remaining
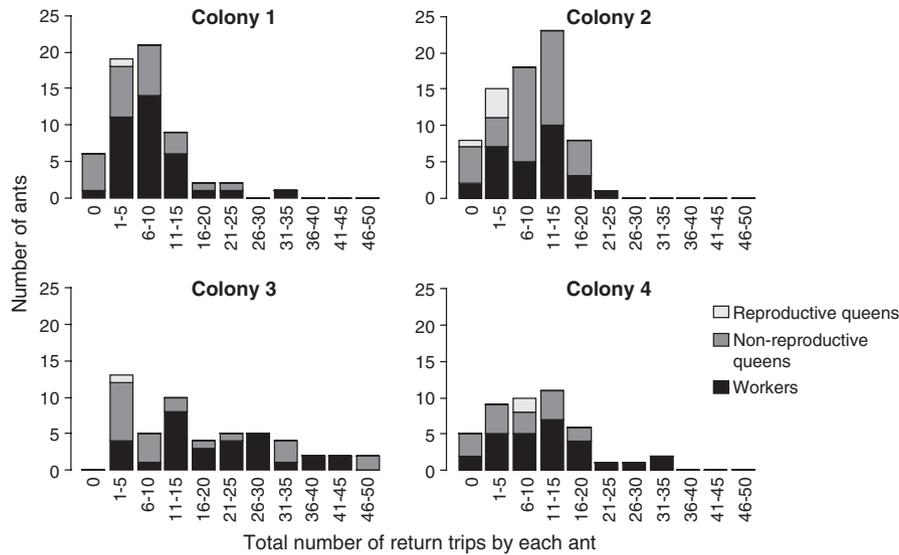
Fig. 3. Total number of return trips by each ant across all four emigrations. The highest numbers of return trips were performed by workers, except for a particularly active non-reproductive queen in colony 3 (48 return trips). Reproductive queens never performed more than eight return trips.
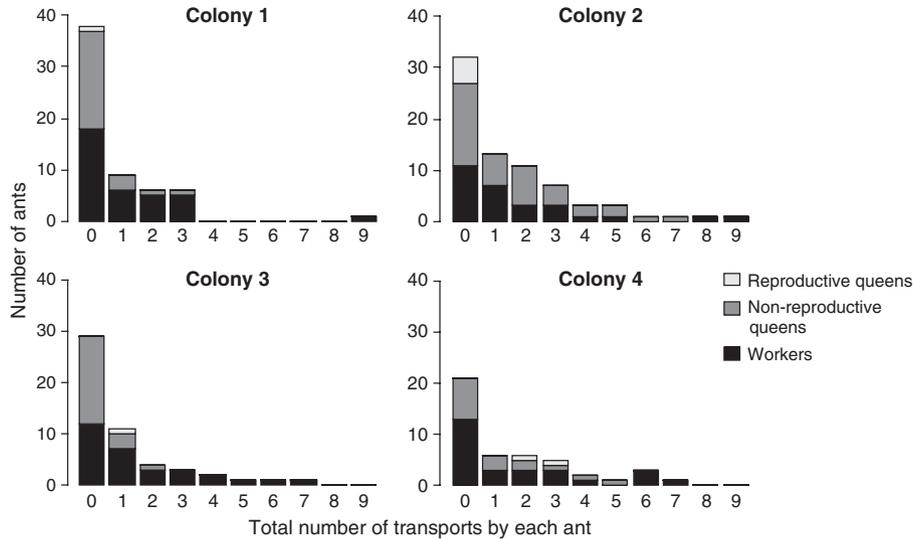

Fig. 4. Total number of brood transports by each ant across all four emigrations. Most ants did not transport any brood. The highest numbers of transports were always carried out by workers. Reproductive queens never transported brood more than three times, while a non-reproductive queen transported up to seven brood items.

nestmates or brood. The major role of older workers for recruitment is also known in other taxa such as *Apis mellifera* (Stout *et al*., 2011) and *Temnothorax* (Sendova-Franks and Franks, 1995). However, as predicted from the peculiar division of tasks between *M. oberthueri* workers and queens during sedentary phases (queens perform most of the brood care; Molet *et al*., 2007b), queens are also actively involved in the emigration process. First, just like workers, a few non-reproductive queens can be present in the new nest before emigration and thus contribute to the exploration of potential nesting sites. This contrasts with Molet *et al*. (2007b), who found that queens stayed in the nest. Secondly, queens

participate in recruitment (through return trips) and brood transport. Nevertheless, just like young workers, reproductive queens and young non-reproductive queens generally do not perform these tasks, and limit themselves to one direct trip without brood transport. Despite its peculiar caste system, *M. oberthueri* relocates its nests using a similar pattern of polyethism as monogynous species such as *Aphaenogaster senilis* (Avargues-Weber & Monnin, 2009). Individuals with experience of the outside world (older workers in *M. oberthueri*, 'outside workers' in *A. senilis*) are very active. Inexperienced individuals (non-reproductive queens and young individuals in *M. oberthueri*, 'inside workers' in
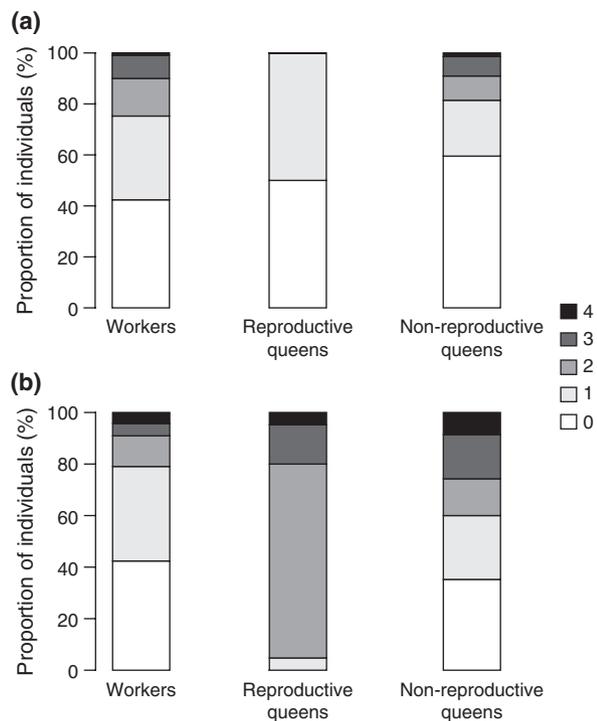
(a)

(b)

Fig. 5. Specialisation of individuals over several emigrations. The histograms show the proportion of individuals of each caste adopting the same behaviour zero, one, two, three or four times over emigrations. (a) Number of emigrations as a transporter: workers were more likely to be transporters over several emigrations, non-reproductive queens less likely, and reproductive queens only transported brood occasionally. (b) Number of emigrations as a passive individual: reproductive queens were mainly repeatedly passive individuals that neither carried brood items nor performed return trips, while almost no worker was passive three or four times.

A. senilis) are less involved. Young individuals and reproductive queens preferentially move directly to the new nest, avoiding return trips and brood transport, so they are less exposed to danger. In *M. oberthueri* colonies, the physiological status of each individual has more impact on its behavioural profile during emigration than its morphological caste.

The duration of the initial phase varies greatly. All nests being identical, nest quality cannot be incriminated. Instead, mechanisms of collective decision-making in colonies of small size could explain such stochasticity. Possible decision mechanisms include the quorum rule, as in *Temnothorax curvispinosus* and *T. albipennis* (Mallon *et al.*, 2001; Pratt, 2005; Pratt *et al.*, 2005).

Brood transport

The dynamics of adult and brood relocation are strongly linked. The brood is transported to the new nest around the middle of adult relocation. Consequently, eggs, larvae and cocoons are never left unattended and are always taken care of by many ants in both nests. Similarly, in *A. senilis*, relocations of brood and workers peak at the same time (Avargues-Weber

& Monnin, 2009). Some workers and queens were particularly prone to carrying brood, and workers were more involved overall. Division of labour among individuals is often linked to their age, with younger individuals being restricted to intranidal tasks such as brood care and older individuals switching to foraging (e.g. Tripet & Nonacs, 2004). As expected, young individuals never transported brood items, and only experienced older workers and queens transported brood items outside of the nest. Workers performed more transports than queens, so division of labour is affected by both age and caste membership, as found in worker subcastes of *Atta* leaf-cutters ants and *Lasius niger* (Wilson, 1980; Lenoir & Attaya, 1983).

Passive individuals

During each emigration, some ants from both queen and worker castes were passive, i.e. they were not involved in return trips or brood transports and they directly settled into the new nest. This was compensated by the high activity level of other nestmates. Similarly, in *T. albipennis*, the proportion of passive individuals is higher in smaller colonies because the workload of active individuals is increased (Dornhaus *et al.*, 2008). All young individuals were always passive, a probable consequence of their inexperience outside the nest and their value for the future of the colony.

Reproductive queens were almost always passive and relocated preferentially during the exponential phase, i.e. when the flow of adults between the two nests was maximal. Their behaviour is thus similar to queens of the monogynous species *A. senilis* and *T. albipennis*. This strategy allows optimal protection of these valuable individuals during the entire nest emigration, because they are always surrounded by many workers (Avargues-Weber & Monnin, 2009; Franks & Sendova-Franks, 2000). In *M. oberthueri*, the value of a reproductive queen is lower because of both polygyny and the presence of numerous potential replacement virgin queens. However, in contrast to gamergates of *Harpegnathos saltator* which mate with brothers (Peeters & Hölldobler, 1995), queens of *M. oberthueri* mate with foreign males (M. Molet, pers. obs.). Consequently, queen replacement reduces relatedness in the colony and may not be frequent.

Chemical trail

The emigration process was facilitated by the use of a chemical trail. Most individuals followed the trail, including a male present during preliminary experiments. Such a pheromone trail has been reported in the context of both foraging and nest relocation in the closely related species *Mystrium rogeri* (Hölldobler *et al.*, 1998) and in many other ant species (e.g. Leal & Oliveira, 1995; Aron *et al.*, 1989; Jeanson *et al.*, 2004). When workers of *M. rogeri* find food or a new nest, they deposit a trail pheromone secreted by the sternal gland while returning to their home nest (Hölldobler *et al.*, 1998). They do so by rubbing the ventral tip of their abdomen on the ground. This trail then guides nestmates to the distant site. We noticed *M. oberthueri* individuals performing

this abdomen-rubbing behaviour on several occasions during emigrations.

Learning potential

Emigrating to the new nest was achieved more efficiently over consecutive emigrations: ants took more time to enter the new nest at first, which might be linked to decision-making and stress, but they settled into the new nest more directly, avoiding repeated trips outside. This suggests that *M. oberthueri* ants might have a substantial learning potential. Nevertheless, our study did not focus specifically on learning, and further experiments should be carried out to assess this phenomenon.

Implications for dependent colony foundation

Colonies of *M. oberthueri* reproduce by dependent foundation (Molet *et al.*, 2009), i.e. a group of nestmates leaves the mother nest to found a new colony. This contrasts with independent foundation, where queens leave their natal nest and found a new colony alone. The process of dependent colony foundation is thus likely to resemble the process of colony emigration, except that only a fraction of the colony leaves the mother nest. Studying emigration may thus be a useful alternative to assessing the unfolding of dependent foundation, which is generally a rare and unpredictable phenomenon. Anecdotal observations of *M. oberthueri* showed that colonies in the laboratory occasionally split into two when two new nests were offered instead of one, but they always reunited after a few hours. Future studies carried out in more natural conditions, with a larger foraging area, may show that colonies that have split do not reunite again. This would be an ideal model to study the mechanisms of dependent colony founding in a polygynous ant species.

Acknowledgements

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