


## SPECIAL ISSUE ARTICLE

# The influence of colony traits on the collective behaviour of *Myrmica scabrinodis* ants

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**Abstract.** 1. Eusocial insects exhibit different kinds of collective behaviours which are the outcomes of interactions among several individuals without central control. Ant societies are ideal models to study group behaviours performed by cooperative individuals at caste or at the colony level. In addition to the ecological constraints, such as the costs of maintaining patterns of interactions, the social structure might also affect the collective behaviour in ants.

2. We tested the effect of *Myrmica scabrinodis* colony traits (number of queens, colony size, and colony age structure) on four major collective behaviours (aggression against intruders, removal of nestmate corpses, foraging, and colony relocation).

3. Our results showed that neither the number of queens nor the colony size affected the level of aggression against non-nestmates while the efficiency of corpse removal was positively correlated with both traits. The age structure of the colony influenced both the aggressiveness towards non-nestmates and the hygienic behaviours. Subcolonies containing a higher proportion of young individuals were more aggressive and less efficient in corpse removal. All studied traits affected foraging activity, as one of the most important behaviour in colony life.

4. Some of the ant collective behaviours, like foraging, are determined by many traits and their interaction, while others are mostly determined by one or a few major colony characteristics. Overall, our results suggest that individual tasks which generate collective behaviours depend on different intrinsic traits of the ant colony that make a timely and appropriate behavioural response possible in every situation.

**Key words.** Age structure, aggressive behaviour, colony size, foraging, hygienic behaviour, nest relocation, number of queens.

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## Introduction

Animal species living in groups exhibit different kinds of collective behaviours, which are the outcomes of interactions among several individuals without central control (Krause & Ruxton, 2002). Social insects such as ants, bees, and termites are ideal targets to study group behaviour. In social insects, the collective behaviour requires cooperative individuals at caste or at the colony level. In such large societies, decision-making and collective behaviours are used to be decentralised (Anderson & McShea, 2001; Hovestadt

*et al.*, 2012). Local information and simple rules guide workers that are involved in communication, or in other tasks, and the interaction among individuals results in the collective behaviour (Anderson & McShea, 2001).

Therefore, the success and efficiency of a group in task performance (e.g. foraging, nest displacement, and defence) are determined by the different characteristics of participants like, for example, individual personalities (Modlmeier *et al.*, 2014). Such differences in behaviour of group members can affect the so-called ‘ecology’ of collective behaviour that considers all the regulatory processes used to cope with biological constraints (e.g. resource availability and member interaction networks) in order to fit the environmental conditions (Gordon, 2014). In *Temnothorax longispinosus*, differences in innate aggressiveness of individuals (i.e. differences in their personality) significantly affect the colony defence ability and the nest relocation efficiency (Modlmeier *et al.*, 2014), which are collective behaviours crucial in facing ecological constraints.

In some cases, key individuals might have a great impact on the task efficiency of a group; thus, their loss could negatively influence cooperative behaviours (Keiser *et al.*, 2017). In social insects, queens represent outstanding colony members, which usually form the reproductive cast of the colony and whose presence and/or pheromones can strongly influence the behaviour of workers (Elmes, 1987; Holman *et al.*, 2010; Villalta *et al.*, 2018). For instance, queenright colonies of *Temnothorax curvispinosus* outperform colonies without queens in the majority of the collective behaviours (Keiser *et al.*, 2017). The number of queens (level of polygyny) within a colony can also influence its social defence behaviour. The colony odour which is used as a template by individuals for discriminating nestmates from strangers is fairly uniform in small monogynous colonies, but it may be fuzzy in large polygynous societies because of their greater genetic diversity (Vander Meer & Morel, 1998). A broader chemical template in polygynous colony could result in lower worker aggressiveness against intruders (Reeve, 1989; Fürst *et al.*, 2012; Csata *et al.*, 2017).

Other important factors influencing collective behaviour in ants are the colony size (Mailleux *et al.*, 2003; Dornhaus *et al.*, 2012; Leclerc & Detrain, 2018) and the experience gained by certain group members (Jeanson & Weidenmüller, 2014). In *Temnothorax albigennis*, the colony size influences some collective behaviours such as the speed with which workers discover new nest sites but does not affect other decision processes which are based on the proportion of individuals involved in distinct tasks rather than on the absolute number of colony members (Dornhaus *et al.*, 2012).

In many ant species, including *Myrmica*, age polyethism is present, meaning that young ants carry out their activities within the nest and interact exclusively with nestmates. When ageing, they begin to forage outside having the chance to meet foreign organisms and potentially undergoing their attack. Only after this ‘experience’,

individuals enhance their ability to discriminate between nestmate and strangers and develop their aggressiveness (Gordon, 2016).

Social structure and organisation in ant colonies are likely to change during the time as an effect of colony division or fusion, stochastic events or the death of key-stone individuals. Such processes occur in many ant societies leading to changes in group composition and in turn affecting the collective behaviour of colonies (Brunner & Heinze, 2009; Modlmeier *et al.*, 2014).

*Myrmica* ants have optimal characteristics for behavioural studies and specifically to investigate collective behaviours. Despite frequent changes in space and time, each *Myrmica* colony represents the fundamental unit suitable for describing the population of each species from this genus (Elmes, 1973). Distinct *Myrmica* species share very similar lifestyles (Radchenko & Elmes, 2010). The colonies are generally small and contain 200–500 workers. Nevertheless, some species, such as *Myrmica rubra*, can form larger colonies with more than 1000 individuals and the largest colonies may contain more than 2000 workers (Wardlaw & Elmes, 1996). The division of labour is correlated with worker age and physiology; older workers (foragers) have higher locomotor activity and foraging potential than younger workers, which tend to stay close to the brood (nurses; Weir, 1958). *Myrmica* colonies also contain one to many functional queens being facultative polygynous (Elmes, 1973; Elmes & Pétal, 1990; Elmes & Keller, 1993). In all *Myrmica* species, freshly mated gynes can enter diapause solitarily, form small groups with other gynes or join existing colonies (Elmes, 1982; Elmes & Pétal, 1990). In all *Myrmica* species, colony foundation occurs quite often by budding or fission of existing colonies (Bourke & Franks, 1995). In habitats occupied by stable colonies in which there is strong competition, colony fragments (gems) quickly colonise free sites, soon becoming entities separated from the colony of origin (Elmes *et al.*, 1998) or remain in contact with the mother colony-forming polycalic systems (Radchenko & Elmes, 2010). All above-mentioned characteristics of *Myrmica* ant colonies – that is number of queens, age polyethism, and colony size – represent suitable factors to investigate how social composition can influence group behaviour.

There were several studies about the influence of external factors (mostly environmental) on behavioural syndrome variability (e.g. Bengston & Dornhaus, 2014; Segev *et al.*, 2017) and its effect on colony productivity (e.g. Modlmeier & Foitzik, 2011; Modlmeier *et al.*, 2012). In this study, we examined the effect of colony traits on collective behaviours in the common European ant species *Myrmica scabrinodis*. Three colony characteristics were considered: (i) the number of queens, (ii) colony size, and (iii) colony age structure. Colony response was evaluated by examining four collective behaviours, which have important consequences for ant society fitness under natural conditions: (i) aggression against intruders, (ii) hygienic behaviour (necrophoresis), (iii) foraging, and (iv) colony relocation.

## Materials and methods

### Data collection

This study is the combined output of two initially separate studies conducted in two different laboratories. *Myrmica scabrinodis* colonies were collected in May and June 2017 at two localities: (i) in Italy at Caselette (45°6'N, 7°28'E) and in Poland near Warsaw (52°08'N, 20°43'E). Both sites are wet meadows dominated by *Molinia* spp. Ten colonies per site were collected at the minimum distance of 10 m between the nests. The average colony foraging range for *M. scabrinodis* is reported to be 2 m (Wardlaw & Elmes, 1996), so that 10 m distance ensures the sampling of separate colonies. All colonies were excavated and taken to the laboratory where the number of workers and queens was counted. In Italy, five monogynous and five polygynous colonies were collected, whereas in Poland, 10 polygynous colonies were collected.

*Myrmica scabrinodis* colonies collected in Italy were used to test the effect of queen number on collective behaviour (Set-up 1), whereas Polish colonies were used to study the effect of colony size (Set-up 2) and age composition (Set-up 3) on collective behaviour. The observations of Set-up 1 were performed at the University of Turin (Turin), whereas Set-ups 2 and 3 were carried out at the Museum and Institute of Zoology of the Polish Academy of Sciences (Warsaw).

### Experimental design

**Set-up 1 – Number of queens.** Each originally monogynous colony was divided into two subcolonies with 0 or 1 queen, whereas each originally polygynous colony was divided into three subsets with 0, 1, or 2 queens (coming from the same original colony). Characteristics like the colony composition, the number of larvae and workers were kept the same in the subcolonies to control for the effect of colony traits other than polygyny on the collective behaviour of workers (Table 1). Each subcolony received fifteen small- to medium-sized larvae and 150 workers. Ants were housed in a plastic box (19 × 16 × 7.4 cm) with interconnected chambers in their plaster bottom. Nests were watered and fed twice a week with a solution of honey water and also corpses of *Drosophila* sp. placed on a circular metallic plate (Ø 3 cm). The rim of every box was covered with paraffin to prevent workers from escaping. Behavioural assays took place between July and the first part of August 2017, at least 4 weeks after establishing subcolonies.

**Set-up 2 – Colony size.** Ten subcolonies (created by as many original colonies) that differed in worker number were established: five subcolonies with 200 individuals and five subcolonies with 50 individuals. These subcolonies did not contain queens but the other colony

characteristics were kept the same as in the previous set-up (Table 1).

**Set-up 3 – Age composition.** Ten subcolonies (all created by as many original colonies) with different worker age composition were established: five subcolonies contained 120 old and 30 young ants, while five subcolonies included 30 old and 120 young workers. The age selection was performed based on to the cuticle pigmentation, considering young workers as those with pale cuticles on thorax and yellow-brown head and gaster (group 2 according to Cammaerts-Tricot, 1974), while old workers were all foragers with very dark cuticle collected outside the nest (group 5 according to Cammaerts-Tricot, 1974). Colonies did not contain queens, but the other colony characteristics were kept constant as in the previous set-ups (Table 1).

Subcolonies were kept in plastic boxes (20 × 4.5 × 12 cm) that were connected with a plastic tube (around 11 cm) to a foraging box (11 × 8 × 4 cm). The solution of honey water and pieces of crickets were placed in the foraging arena twice a week. The rim of every box was covered with paraffin to prevent the escape of workers. Behavioural assays took place in July 2017, at least 2 weeks after establishing the different subcolonies.

### Behavioural assays

Four behavioural assays were performed to test distinct collective behaviours of ants: (i) social defensive strategies towards allospecific corpses that simulate the intrusion of foreign ants, (ii) colony reaction towards nestmates corpses, to investigate hygienic behaviours, (iii) colony foraging activity by assessing the number of workers feeding on honey baits, and (iv) the efficiency of colony relocation. The first three behaviours were tested using all set-ups, while the fourth was assessed using Set-ups 2 and 3 (colony size and age composition, respectively). Three repetitions in Set-up 1 (numbers of queens) and one replicate in Set-ups 2 and 3 per subcolony were performed for each bioassay.

**Social defensive strategy.** We used workers of *Lasius niger*, a common, similar-sized competitor of *Myrmica* ants as a proxy for 'intruder' (further on intruder). In order to eliminate behavioural variations between our stimuli and concentrate our interest on colony response, *Lasius* ants were killed before observations by freezing at –20 °C. Corpses were defrosted 10 min before the experiments and placed on round (Ø 3 cm) metal plates, 3 cm from *Myrmica* nest entrances. We used only one corpse for each subcolony.

Following the first interaction, we registered all aggressive and non-aggressive interactions repeated after every 20 s, for a total of 5 min (15 observations). All behavioural events displayed by *M. scabrinodis* workers were categorised as follows: (i) ignoring, (ii) antennation, (iii) scaring (running away after contact with the intruder corpse), (iv) biting, (v) pulling, and (vi) stinging. The

**Table 1.** Summarising table of the different experimental set-ups.

Set-ups	Subcolony composition				Treatment*
	Larvae	Queens	Workers	Worker age (old:young)	
1. Nr. of queens	15	Treatment	150	4:1	0 or 1 queen 0, 1, or 2 queens
2. Colony size	15	0	Treatment	4:1	Large Small
3. Age composition	15	0	150	Treatment	Young Old

\*Treatment subcolonies: 1. Nr. of queens: originally monogynous – with 0 or 1 queen; originally polygynous – with 0, 1, or 2 queens. 2. Colony size: large – with 200 workers; small – with 50 workers. 3. Age composition (old:young): young – worker proportion of 1:4; old – worker proportion of 4:1

latter three behaviours were considered aggressive and used later for calculating the aggression index (Martin *et al.*, 2009; Pamminger *et al.*, 2011; Maák *et al.*, 2014). If no contact was observed during the first 10–15 min, we stopped the trial and repeated later. For each trial, an aggression index was calculated as a ratio between all aggressive interactions, and the sum of all behaviours observed (following Csata *et al.*, 2017; Maák *et al.*, 2014).

*Hygienic behaviour.* To assess the hygienic behaviour of a colony, we used nestmate corpses that were killed by freezing at  $-20^{\circ}\text{C}$ . Corpses were defrosted 1 h 30 min before the experiments. This time lag ensured the appearance of corpse's signals on the surface of their cuticle (see Maák *et al.*, 2014). After that, we put five nestmate corpses on a circular metallic plate ( $\varnothing$  3 cm) at 3 cm from the nest entrance, as necrophoresis can be present inside the nest or in its close vicinity. We registered the number of corpses on the plates at the beginning and at the end of each 1-min observation and the number of workers appearing on the plate near the corpses for 1 min. The observation was repeated after every 4 min for an amount of 15 observations.

*Foraging activity.* To assess colony foraging activity, every experimental subcolony was starved for 3 days before observations. In each artificial nest, we placed a circular metallic plate ( $\varnothing$  3 cm) on which we put a drop (0.5 ml) of honey water (1:2 ratios). The metallic plate was located in the corner opposite to the nest entrance. Each observation lasted for 1 min, during which we counted the number of ants at honey-water baits. Observations were repeated every 4 min, for a total of 40 min (10 observations per subcolony).

*Colony relocation.* One day before assessing nest displacement ability, we assessed and adjusted the number and size of the larvae to be identical inside each subcolony (15 ant larvae of similar size). Inside the box, there was an original 'nest chamber' where ants used to keep their brood. The nest chamber was made by a small piece of wet sponge covered by a flowerpot saucer with a notched entrance to provide a dark place. One minute

before the observation, we placed a similar 'nest chamber' on the opposite side of the plastic box. Observations started after the old saucer and sponge were removed (to simulate nest destruction). We recorded the time of the first and the last larva was moved to the new nest. The experiments lasted for a maximum of 1 h of observation. This behaviour assay was done for Set-ups 2 and 3.

#### Statistical analyses

*Set-up 1.* In social defence bioassays, the behavioural response (recorded in the course of each 1-min observation) to the intruder was analysed with GLMM (binomial error, maximum-likelihood fit). An aggression index was calculated for each 1-min observation where the number of negative reactions was divided by the total number of behavioural responses. In the full model, the number of queens, the previous state of the colony (polygynous or monogynous), and the number of individuals around the corpses were treated as fixed factors.

In the hygienic behaviour bioassay, the number of workers that appeared around the nestmate corpses was tested using GLMM (negative binomial error distribution). In the full model, we included the number of queens, the previous state of the colony (polygynous or monogynous), and the number of corpses as fixed factors. The effect of the number of queens and the previous state of the colony (polygynous or monogynous) on the decision whether or not to remove nestmate corpses from the plate was analysed using GLMM (binomial error, maximum-likelihood fit). The corpse removal rate (hygienic behaviour assays) was analysed with the help of the Cox regression model (proportional hazard approach). In this model, the number of queens and the previous state of the colony were included as fixed factors.

The number of ants observed at honey baits, as a proxy for the foraging activity, was tested with GLMM (negative binomial error distribution). We used the same fixed factors as applied in the previous models. In all the models above, the repetitions and the original nest IDs were included as random factors.



*Set-ups 2 and 3.* The aggression index was tested using generalised linear models (GLM, binomial error distribution). Two separate models were used to analyse the two set-ups (treatments): colony size and age composition. Treatments were included as fixed factors, whereas the number of individuals interacting with the intruder was included as a covariate. The number of workers that interacted with the nestmate corpses was tested using GLMs (negative binomial error distribution). In these models, the colony size or age composition was included as fixed factor and the number of nestmate corpses as a covariate. The removal rate of nestmate corpses (necrophoresis) was tested with the help of Cox regression. In the models, the treatment (colony size or age composition) was included as fixed factor. We used GLMs (binomial error distribution) to test for the influence of colony size or colony composition on the ant's decision to transport away or not the corpses (hygienic behaviour assay) from the experimental plates. The effects of colony size or age composition on the number of ants observed at honey baits (foraging activity assay) were tested with GLMs (negative binomial error distribution).

In the colony relocation experiment, we analysed the total time spent by workers to relocate all the larvae (relocation time) in the new nest location with the help of GLMs (Gaussian error distribution). In the models, the effect of the treatment (colony size or age composition), the time needed to move the first larvae, and the interaction between the treatment and time were included as fixed factors.

Statistical analyses were performed using the package R version 3.4.2 (R Core Team 2017).

Generalised linear models were performed using the 'glm' function from the Stats and 'glm.nb' from the Mass packages (Venables & Ripley, 2002). GLMMs were

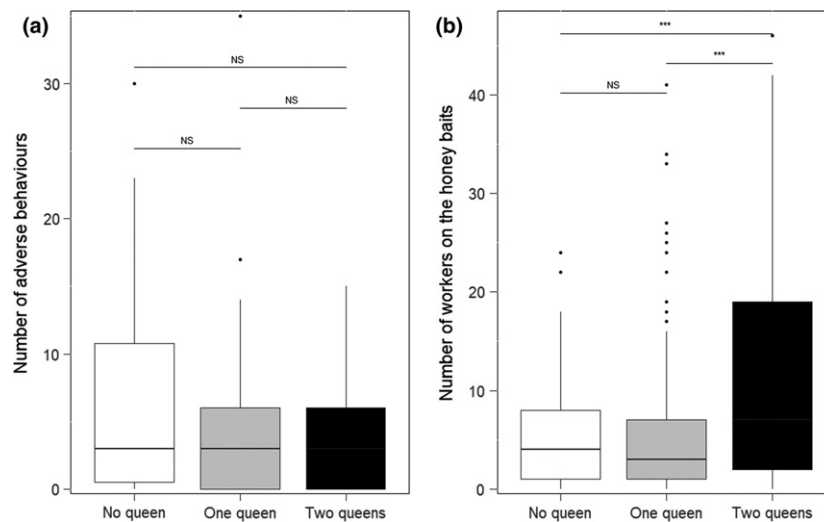
performed using the 'glmer' and 'glmer.nb' functions in the lme4 package (Bates *et al.*, 2014). The best models were selected with automated model selection with the help of *stepAIC* function in GLMs (MASS package, Venables & Ripley, 2002) and with the help of *dredge* function in GLMMs (MuMIn package, Bartoń, 2013). Cox regression analysis was done with the use of the 'coxph' function from the survival (Therneau, 2015a) and the 'coxme' function from the coxme package (Therneau, 2015b). Taking into account the experimental design and the low number of variables included in the models, the results of the full models were used. The post hoc sequential comparisons among factor levels (both GLMMs and Cox regression analysis) were carried out using the 'lsmeans' function from the lsmeans package (Russell, 2016).

## Results

### *Set-up 1 – Number of queens*

The number of queens and the original state of the colony (mono- or polygynous) did not have any significant effect on the aggression index (GLMM  $-0.5 < z < -0.1$ ,  $P > 0.77$ ; Fig. 1a). Despite the number of queens in the colony, the higher the number of *M. scabrinodis* workers found around the intruder corpse, the lower was the aggression index observed (GLMM  $z = -2.06$ ,  $P = 0.04$ ).

The number of workers that interacted with the nestmate corpses was influenced by both the original state of the colony and the number of queens placed in the subsets (Table 2). However, only the number of queens in the subsets had a significant effect on the number of removed corpses of nestmates and on the rate of corpse removal



**Fig. 1.** Difference in the number of adverse behaviours against the competitors (a) and in the number of workers on the honey baits (b) in the case of *Myrmica scabrinodis* subcolonies without, with one, or with two queens. Central measure = median, box = interquartiles, whiskers = 2 \* SD and data points are 'outliers'. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , NS = not significant.

**Table 2.** Results of the GLMM analysis of the hygienic behaviours towards nestmate corpses as a function of the number of queens and the original state of ant colonies (monogynous or polygynous). For the fixed factor ‘number of queens’, the results of the post hoc sequential comparisons among factor levels are reported.

Statistical analysis	Fixed effects	<i>z</i>	<i>P</i>
Number of workers around the corpses	Original status	<b>2.82</b>	<b>0.004</b>
	Queen 0–1	1.06	0.54
	Queen 0–2	<b>–4.23</b>	<b>&lt;0.001</b>
	Queen 1–2	<b>–5.00</b>	<b>&lt;0.001</b>
Corpse removal rate	Original status	1.01	0.31
	Queen 0–1	<b>3.25</b>	<b>0.003</b>
	Queen 0–2	–0.32	0.95
	Queen 1–2	<b>–2.88</b>	<b>0.01</b>
Number of corpses removed	Original status	1.65	0.09
	Queen 0–1	1.81	0.16
	Queen 0–2	–1.97	0.12
	Queen 1–2	<b>–3.20</b>	<b>0.004</b>

Significant values are reported in bold.

(Table 2; Fig. S1). When more than one queen was present in the subcolony, workers were less involved in hygienic behaviour.

In foraging assays, subcolonies with two queens had significantly more workers at the honey baits compared to subcolonies without queens (GLMM  $z = 4.54$ ,  $P < 0.001$ ) and with only one queen (GLMM  $z = 4.3$ ,  $P < 0.001$ ; Fig. 1b). No differences were found between subcolonies

without and with one queen (GLMM  $z = 0.36$ ,  $P = 0.93$ ; Fig. 1b). The original state of the colony did not have a significant effect (GLMM  $z = 1.23$ ,  $P = 0.22$ ) on the number of workers at honey-water baits.

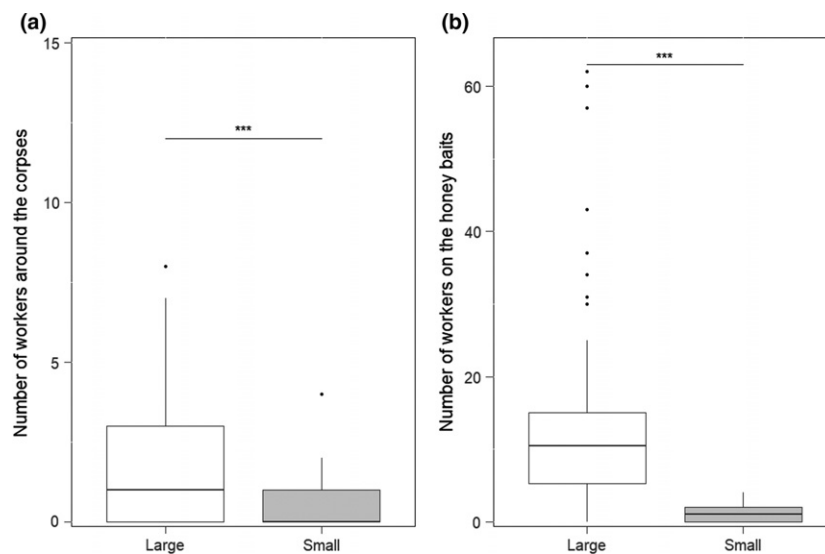
#### Set-up 2 – Colony size

In the social defence behaviour directed towards the intruder corpse, no significant differences were detected between big and small colonies in the aggression index (GLM  $z = 0.27$ ,  $P = 0.79$ ) and in the number of individuals at the metal plate (GLM  $z = 0.59$ ,  $P = 0.56$ ).

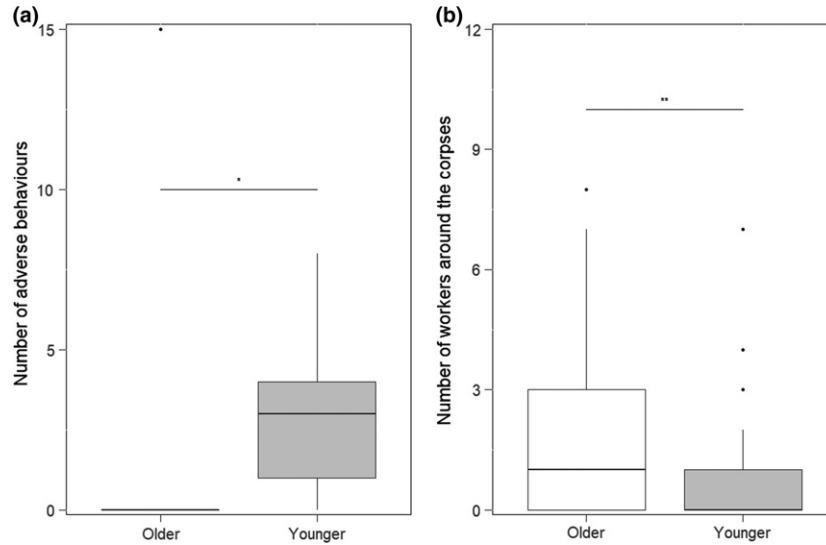
In larger subcolonies, significantly more workers were present around the nestmate corpses performing hygienic behaviour (GLM  $z = 4.61$ ,  $P < 0.001$ ; Fig. 2a). The number of nestmate corpses present on the experimental plates had no significant effect (GLM  $z = 1.65$ ,  $P = 0.07$ ). Colony size did not influence the nestmate corpse removal rate (Cox  $z = -0.57$ ,  $P = 0.57$ ; Fig. S2) or the number of corpses transported away (GLM  $z = -0.37$ ,  $P = 0.71$ ).

During the foraging assays, in large subcolonies, higher numbers of workers gathered at the honey-water baits (GLM  $z = 11.92$ ,  $P < 0.001$ ; Fig. 2b).

Large subcolonies relocated their nest in a shorter time interval than small ones (GLM  $z = -3.54$ ,  $P = 0.01$ ). The relocation time of the first larvae had a positive influence on the total relocation time (GLM  $z = 5.07$ ,  $P = 0.002$ ). However, when considering the interaction of these two variables, the total transport time was lower in smaller colonies (GLM  $z = -4.07$ ,  $P = 0.006$ ). In other words, the overall time needed for nest displacement, which includes the new nest site discovery, is lower in larger colonies, but



**Fig. 2.** The effect of colony size (4:1 – large/small) on the number of workers present around the corpses (a) and on the honey baits (b). Central measure = median, box = interquartiles, whiskers = 2 \* SD and data points are ‘outliers’. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , NS = not significant.



**Fig. 3.** The effect of colony age composition (older – 4:1 old/young ratio, younger – 1:4 old/young ratio) on the number of adverse behaviours against a competitor (a) and on the number of workers present around the corpses (b). Central measure = median, box = interquartiles, whiskers = 2 \* SD and data points are ‘outliers’. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , NS = not significant.

the time span from the first to the last larval transportation is shorter in smaller colonies.

#### Set-up 3 – Colony composition

Subcolonies with higher young worker ratio showed a higher aggression index (GLM  $z = 2.33$ ,  $P = 0.02$ ; Fig. 3a), and, as the number of individuals on the plates increased, the collective aggression behaviour was also increasing (GLM  $z = 2.18$ ,  $P = 0.03$ ).

Hygienic behaviour was performed by a larger number of workers in colonies where the majority of individuals were old (GLM  $z = -3.05$ ,  $P = 0.002$ ). The number of nestmate corpses present on the experimental plates had no significant effect on collaborative hygienic behaviour (GLM  $z = -1.79$ ,  $P = 0.1$ ). Subcolonies with higher old ant ratio showed a faster corpse removal rate (Cox  $z = -2.08$ ,  $P = 0.04$ ; Fig. S3). However, colony age structure did not have a significant effect on the number of nestmate corpses transported away (GLM  $z = -1.58$ ,  $P = 0.11$ ). In subcolonies with higher old worker ratio, more foragers were found on the honey baits ( $z = 2.55$ ,  $P = 0.01$ ; Fig. 3b). On the other hand, colonies with a larger proportion of young individuals were slightly more efficient in nest relocation (GLM  $z = 2.26$ ,  $P = 0.06$ ). However, the time of the relocation of the first larva (GLM  $z = -0.53$ ,  $P = 0.62$ ) or their interaction ( $z = -1.50$ ,  $P = 0.18$ ) was not significant.

#### Discussion

We analysed the effects of queen number, colony size, and age composition on the efficiency of the most

important collective behaviours. Our results showed that all the colony traits examined had a strong influence on nest hygienic behaviours and foraging activity, that is the tasks which have significant impacts on colony state and fitness (Carroll & Janzen, 1973; Cremer *et al.*, 2018). Other collective behaviours were affected only by more specific colony characteristics, that is social defence by colony age composition, whereas nest displacement efficiency by colony size.

#### Hygienic behaviours

The presence of waste and corpses inside the nest can lead to the establishment and spread of pathogens, for example fungi. However, by removal of corpses, the possibility of a pathogen to reproduce and/or reach maturity is interrupted, and the colony survival can be enhanced (Diez *et al.*, 2013, 2014; López-Riquelme & Fanjul-Moles, 2013; Sun & Zhou, 2013). Colonies of *Myrmica* ants are able to assess the risk level associated with the presence of potentially infectious corpses in the nest and modify their investment into hygienic behaviours accordingly (Diez *et al.*, 2015). Our results showed that this collective behaviour is finely adjusted by several and distinct colony traits. We found that when more than one queen occurred in the original colony, the number of workers involved in hygienic behaviours was higher. In general, polygynous colonies produce more workers to fulfil several distinct tasks with high efficiency (Hölldobler & Wilson, 1990; Debout *et al.*, 2007). If this was true in the original colonies, it cannot be applied to subsets where the number of workers might be too low to be split between tasks fulfilling the needs of two queens and brood, without dropping the efficiency of some more specialised tasks, as nest

hygiene. When we tested subsets with different numbers of queens, we found the existence of a trade-off in the investment of the colony in the foraging activity or the hygienic behaviour. Bazazi *et al.* (2016) showed that in subcolonies with two queens, the division of labour is targeted to cope with the elevated needs of food more than hygienic performances. When only one queen was present, the corpse removal rate was higher compared to subcolonies without a queen. This may be because a colony without the queen rapidly changes its social organisation or colony cohesion (Vander Meer *et al.*, 2008) by allocating resources differently, for example in rearing new reproductive individuals. However, reduced corpse removal ability is likely to result in an increased rate of mortality of ant workers, leading to lower colony fitness (Bot *et al.*, 2001; Diez *et al.*, 2014). Both a reduced worker lifespan and a high vulnerability to diseases have been demonstrated in queenless ant nests (Keiser *et al.*, 2017).

The health risks imposed by pathogens can be closely related to colony size. Due to a plethora of hygienic behaviours (López-Riquelme & Fanjul-Moles, 2013; Sun & Zhou, 2013; Cremer *et al.*, 2018), ants living in larger groups have usually lower mortality and decreased pathogen transmission (Hughes *et al.*, 2002; Leclerc & Detrain, 2018). We observed a stronger hygienic activity by workers in bigger colonies, which is consistent with the recent finding that larger colonies of a *Myrmica* species are faster in rejecting elements of waste infested with the spores of a generalist entomopathogenic fungus (Leclerc & Detrain, 2018). If we take into account that necrophoresis is one of the most important social prophylactic mechanisms (Renucci *et al.*, 2011; Diez *et al.*, 2013), this is not surprising. However, we found that the efficiency of corpse removal was not affected by the colony size but by its age structure. When the number of old workers is high, the corpse transport rate increased. This is in line with the results found for *Myrmica rubra*, where the workers performing hygienic behaviours were mostly foragers, that is older individuals. When nestmate corpses were present in larger numbers, a temporal specialisation on this task could be observed in these workers (Diez *et al.*, 2013).

#### *Foraging activity*

Foraging efficiency is a principal link between individual-level performance and colony-level success and in several species is mostly affected by the colony size and the social environment (Herbers & Choiniere, 1996; Dornhaus *et al.*, 2012). As expected, we measured the best foraging performance (greater recruitment to honey baits) in larger colonies of *M. scabrinodis* or with a higher proportion of old workers or with greater number of queens. Increased colony size usually reduces the proportion of workers required to forage and maintain the ant society (Hölldobler & Wilson, 1990; Herbers & Choiniere, 1996; Dornhaus *et al.*, 2012; Hovestadt *et al.*, 2012). Large colonies have a greater number of foragers or inactive workers

that can be recruited as foragers (Schafer *et al.*, 2006). Moreover, adult individuals require mainly carbohydrate diet for energy (Dussutour & Simpson, 2009), so the larger the number of workers, the larger is the amount of carbohydrates needed. On the other hand, because younger ants accomplish tasks inside the nest, having a majority of older individuals in the colony means possessing a greater number of members ready to go outside and able to fulfil riskier tasks (Moroñ *et al.*, 2008; Giraldo & Traniello, 2014). Colonies with more old foragers could have some advantage, as workers had time to acquire important information about the colony state and the position of food sources in its surroundings (Herbers & Choiniere, 1996; Giraldo & Traniello, 2014).

#### *Social defence*

In ants, the defence mechanism and aggression vary across the species and can be differently affected by colony traits. As for *Myrmica*, it seems that only the ratio between young and old individuals affects the colony aggressiveness.

Our findings are consistent with Bengston and Dornhaus (2014) who found that the behavioural syndromes were not affected by the number of queens, colony size, or the number of brood. However, they showed a link between the aggressiveness and foraging effort as some ant species exploit closer resources and avoid aggressive engagements with invaders, while others travel farther and respond with higher intraspecific aggression but invest less in the exploitation in the food sources (Bengston & Dornhaus, 2014).

The presence of the queens can influence and also modify the behaviour of workers (Vander Meer & Alonso, 2002; Chapman *et al.*, 2011; Villalta *et al.*, 2018). In many species, monogynous colonies show more aggressive reactions than polygynous ones mostly depending on the genetic distance between adverse colonies (Martin *et al.*, 2009; Fournier *et al.*, 2016). In North America, where *M. rubra* is invasive and usually has polygynous colonies, it was found that the aggression of this species increased linearly with the distance between the polygynous systems (Garnas *et al.*, 2007). However, in other species, no differences in aggression levels between polygynous or monogynous colonies were found (Stuart, 1991; Satoh & Hirota, 2005). Similarly, larger colonies showed more aggressive behaviours in some ant species (Stuart, 1991; Batchelor & Briffa, 2011; Barbieri *et al.*, 2015) but did not influence the aggression in others (Barbieri *et al.*, 2015).

In our study, the higher aggressiveness found in colonies with a higher proportion of young individuals can be the outcome of two main factors. Usually, older workers are more aggressive than younger ones (Chapman *et al.*, 2011). In some ant species, individual aggression increases with age and experience (Van Wilgenburg *et al.*, 2010; Norman *et al.*, 2014) and can also be task-dependent, as workers that perform tasks outside can meet non-nestmates or enemies and have to implement their defence



ability (Sturgis & Gordon, 2012). On the other hand, at least when polygynous and monogynous subsets were tested, we found that the number of workers (recruitment) had a significant effect on the aggression index; when recruitment rate is low, participants compensate it with higher aggressiveness (e.g. Hölldobler & Wilson, 1990). Therefore, when colonies were manipulated to test differences in age structures, the lower number of old workers reacted with enhanced pugnacity which increased the overall aggressive index, similarly as it happens when ants have to assess the quality of a fluctuating resource (Franks *et al.*, 2015). This finding suggests that in *Myrmica* ants, the defence of the colony can be regarded as decision-making process based on a quorum, where all the participants perceive the ratio of experienced nest-mates and adjust their own aggressiveness accordingly (e.g. Hölldobler & Wilson, 1990; Gordon, 2010).

#### Nest displacement efficiency

In general, if a task is performed by several individuals, the negative effects of a mistake can be minimalised (Herbers & Choiniere, 1996). This becomes even more important during nest displacement, where the goal is to keep the colony unified and to move as fast as possible to a new nest site (Pratt, 2005). Based on our results, the time of colony relocation was lower in larger colonies and it was positively affected by the shorter time at which the first larva was moved.

Having only one available new nest site, our larger colonies seemed to find it faster, which shortened their total time of transport; however, the relative transport time between the first and last larvae was lower in smaller colonies. This implies that a higher proportion of ants performed, such a transport, or the number of transports performed by an individual was higher. Similarly to our results, also in other ant species, it was found that the speed of discovery of new nesting sites was faster in larger colonies (as the result of higher number of scouts; Dornhaus & Franks, 2006; Cronin, 2012) but the time taken for nest moving did not differ between small and large colonies (Dornhaus & Franks, 2006). The lower transport time of small colonies compensated their longer time needed for the new nest site discovery and decision-making processes (Dornhaus & Franks, 2006). Smaller colonies require a larger proportion of informed individuals to make accurate decisions (Couzin *et al.*, 2005), and the relative quorum threshold that determines the beginning of the transport is usually higher (Dornhaus & Franks, 2006; Cronin, 2012). Moreover, Dornhaus and Franks (2006) pointed out that the importance of learning is higher in colonies with fewer workers, as smaller colonies performed much better after repetition of the experiment.

The collective behaviours of *Myrmica* ants are influenced by several colony traits in a different manner. The more important they are for the colony fitness, the higher is the number of traits that can modify their outcomes.

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#### Conflict of interest

The authors declare no conflict of interest. There are no disputes over the ownership of the data presented, and all authors' contributions have been attributed appropriately.

#### Author contribution

SB, MW, JC, FB, and IM conceived the experiment and designed methodology; all the authors performed the experiments and collected the data; IM and LPC performed the statistical analysis; and IM, LPC, MW, and FB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

#### Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** Estimated functions for Cox regression of time to corpse removal based on the number of queens in the case of polygynous and monogynous *Myrmica scabrinodis* colonies.

**Figure S2.** Estimated functions for Cox regression of time to corpse removal based on the difference in colony size in *Myrmica scabrinodis* sub-colonies.

**Figure S3.** Estimated functions for Cox regression of time to corpse removal based on the difference in colony structure in *Myrmica scabrinodis* sub-colonies.

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