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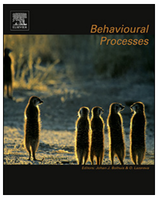

What's going on at the entrance? A characterisation of the social interface in ant nests

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ABSTRACT

Nest entrances are key locations where information about environmental opportunities and constraints are shared between foragers and inner-nest workers. However, despite its functional value, we still lack a detailed characterisation of the interface between the nest and the environment. Here, we identified the social interface in the ant *Myrmica rubra* as being the population of ants that faced the nest entrance and that received significantly more contacts from returning foragers than other nearby ants. We also spatially delineated the entrance area that hosted the social interface, a 2-centimetre radius area from the nest openings, which influences the position, orientation, and behaviour of ants. Then, we studied the impact of additional entrances on this social interface as well as on the flow of foragers. The size of the social interface increased according to the number of open entrances through the progressive reorientation of the ants toward new openings. We also observed a significant, although less than proportional, increase in the flows of ants that were progressively distributed homogeneously between all open entrances. Thus, our work highlights the flexibility of both the social interface and the flow of foragers to changes in the numbers of passageways between the nest and the environment.

1. Introduction

Interaction networks are essential components in the communication dynamics of social groups as they shape the flow of information between their members. In these networks, individuals mainly exchange information with congeners with whom they have privileged connections. In this respect, some key-individuals are essential to information sharing as they reach a wide audience through numerous connections, or as they connect subgroups between each other (Travers and Milgram, 1969; Watts and Strogatz, 1998). These key individuals are found in numerous biological systems, in humans (Danon et al., 2011; Goldenberg et al., 2009), in primates (Leca et al., 2003; Sueur and Petit, 2008), in insect societies (Jeanson, 2012; Mersch, 2016; Pinter-Wollman et al., 2017).

In ants, the interaction network of workers is not built on random connections but is segregated into different social subgroups. This fragmented network results namely from the spatial specialization of workers depending on the different tasks they perform in the colony (nurses, foragers, or nest cleaners; Boi et al., 1999; Gordon, 2010; Mersch, 2016). Each of these social subgroups nevertheless interact either directly or indirectly through key workers (transfer workers, Robson and Traniello, 1999; Tschinkel and Hanley, 2017). In this context, the nest structure and the topology of the galleries play an important role as they are known to shape the spatial distribution of the workers, the network of interactions between nestmates and hence the collective behaviours that emerge from these interactions (Buhl et al., 2004; Pinter-Wollman et al., 2017; Tschinkel, 2004). The nest architecture namely provides key locations where ants will naturally aggregate and where interactions will be facilitated (Pinter-Wollman et al., 2017).

One of these key locations is the entrance chamber, where a high rate of contacts are made between foragers and inner nest workers (Pinter-Wollman et al., 2013) and where workers exchange food and information about the surrounding environment (Gordon, 2010; Pinter-Wollman et al., 2013). In particular, the workers that stand near the entrances constitute a pool of potential recruits (de Biseau and Pasteels, 2000; Pinter-Wollman et al., 2011; Pless et al., 2015) that is known to shape the colony foraging response for a wide range of recruitment

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*Abbreviations:*C, L, L', R, R', Central, Left, Left', Right, Right', refer to the position of the 5 entrances of the nest with the Central entrance being located in the middle, Left and Right entrances being adjacent to the central entrance, and Left' and Right' entrances being placed at the far right and far left side from the central entrance (see Fig.1)

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types, from small colonies performing tandem-running recruitment (Richardson et al., 2018) to larger colonies performing group or mass recruitment (Collignon et al., 2012; de Biseau and Pasteels, 1994; Mailleux et al., 2011). In addition to their role in food exploitation, the nest entrances are also strategic places where ants can be recruited for the defence of the colony territory (Hölldobler and Lumsden, 1980; Whitehouse and Jaffe, 1996), can reject intruders (Huang, 2010), or can check sanitary risks associated to diseased nestmates or contaminated items (Leclerc and Detrain, 2016).

Thus, the entrances of a nest can be defined as a physical interface, that is characterized by the structure and repartition of the openings, but also as a social interface, that hosts workers waiting for the information brought back by the scouts and/or interacting with the flow of incoming individuals. As a physical interface, it appears as a highly flexible structure of which the location and number of openings can change depending on underground shifts in the spatial organization of workers (Hughes, 1990) or on changes in the distribution of food sources (McIver, 1991; Robinson, 2014). As a social interface, it appears as an essential relay between the outer and the inner nest workers. However, there is currently no quantitative study on its population size as well as on its flexibility in response to changes in the physical interface. Therefore, we aimed to better characterize the behaviour of the population of workers that is located near the opening of single-entrance nests housing colonies of the ant *Myrmica rubra*. We assumed that ants belonging to the social interface should be located nearby nest openings and should face nest entrances to favour encounters with incoming individuals, while such an orientation should vanish in the deeper nest. After defining the entrance area, where the proximity to a nest opening influenced the position and orientation of ants, we checked whether the ants belonging to the social interface were actually more likely to interact with incoming foragers. Finally, we investigated to which extent a higher number of entrances influenced the population size of this social interface as well as the level of information exchange through the resulting flows of foragers.

2. Material & methods

2.1. Ant colonies

Myrmica rubra is a polygynous and monomorphic ant species that is common in European temperate areas. *Myrmica rubra* is an opportunistic and omnivorous species whose workers can retrieve small preys or trigger mass recruitment toward larger food sources (personal field observations, Cammaerts-Tricot, 1974; Davidson, 1998). This species builds nests that are composed of relatively independent subunits exchanging brood and individuals and that are connected to the environment through several entrances (personal field observations). Eight colonies of *M. rubra* were excavated from earth banks in a semi-open grassland located in Stambruges (50°50′81.2″N, 3°71′33.4″E) and Udange (49°37′49.2″N 5°45′45.1″E) (Belgium) in September 2014. In the laboratory, ants were reared in artificial nests placed in foraging arenas (45 x 30 cm) with Fluon-coated walls (Whitford, UK) to prevent ants from escaping. The floor of each arena was covered with plaster and watered every day to provide humidity. Laboratory conditions were kept at 21 ± 0.4 C° and 52 ± 2% relative humidity, with a constant photoperiod of 12 h a day. Each colony had access to water, to a 0.3 M sucrose solution ad libitum, and was given *Tenebrio molitor* mealworms twice a week. Each of the eight colonies contained one queen, 280 to 400 workers and brood covering 10 to 15% of the nest area. All colonies were housed in circular nests made of a laser-cut cardboard perimeter covered with a Plexiglas ceiling. A red filter was fitted on the ceiling to darken the nest interior while still enabling the observation of workers. Internal dimensions of the circular nests were 8-cm diameter and 2-mm-high in order to obtain a mono-layer of workers, facilitating their tracking inside the nest. The nest had five entrances (10 mm wide and 5 mm long) evenly spaced (10 mm) along one half of the nest perimeter (Fig. 1). The central entrance (C, Fig. 1) was constantly open while the four other entrances (L, L’, R and R’, Fig. 1) could be closed or open by using small pieces of cardboard (Fig. 1). Before carrying out the experiments, all colonies were first moved into nests with one entrance and were allowed to acclimatize for 48 h before being tested. For all the observations presented below, three replicates were carried out on each of the eight tested colonies for a total of 24 experiments. Ants were given at least 48 h between successive replicates.

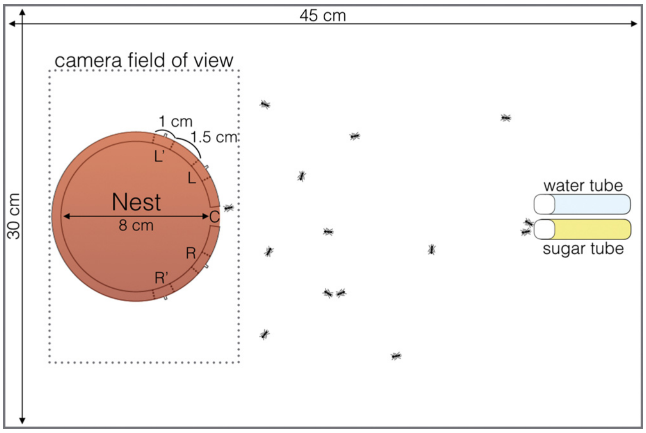

Fig. 1. Experimental arena. The arena was covered with a plaster floor. The circular nest was covered with a red filter. The central entrance (C) was always open, while the four other entrances could be closed or open using a small removable cardboard door (dotted line). L and L’ stand for the entrances located on the left side from the central door, R and R’ for the entrances located on the right side.

2.2. Locating the social interface at the nest entrance

The population of ant workers located around the nest entrance and facing the nest openings will be referred throughout this work as ‘the social interface’. Indeed, we postulated that because of their position and orientation towards nest openings, this social interface was composed of the first ants that would encounter individuals coming from the outside. To locate the social interface, we took one picture of each of the eight ant nests with webcams (Logitech C920) and we mapped out the orientation of the ants within the nest. This procedure was repeated three times per colony on three consecutive days, for a total of 24 pictures. On these pictures, we separated the ants into eight groups based on their radial distance from the entrance. These groups corresponded to concentric rings centred on the entrance and spaced by 0.5 cm (Fig. 2a). In each of these eight rings, we determined, for all the ants, their orientation to the entrance. To do so, we computed an abdomen-head vector and an abdomen-entrance vector by marking the head and abdomen positions of every ant individual. The smallest angular difference between these two vectors (ranging between -180° and 180°, 0° meaning the ants were facing the entrance) gave us the orientation of each ant individual towards the entrance (Fig. 2b).

In order to spatially define the nest entrance area, we pooled all the orientation values of ant individuals and we checked for a preferential ant orientation per 0.5 cm ring. To this aim, for each 0.5 cm ring, we tested for the uniformity of individual ant orientations by performing a Hodges-Ajne test and we computed the resulting orientation vector (Batschelet, 1981).

It is important to mention that, based on our results, we established the social interface to be located within a 2 cm radius from the nest entrance (see Results Section 3.1), a natural cut-off in which the ants were significantly more oriented toward the entrance. All our following analyses used this 2 cm criterion to spatially define the entrance area that hosted the social interface. In addition, we found that in the two

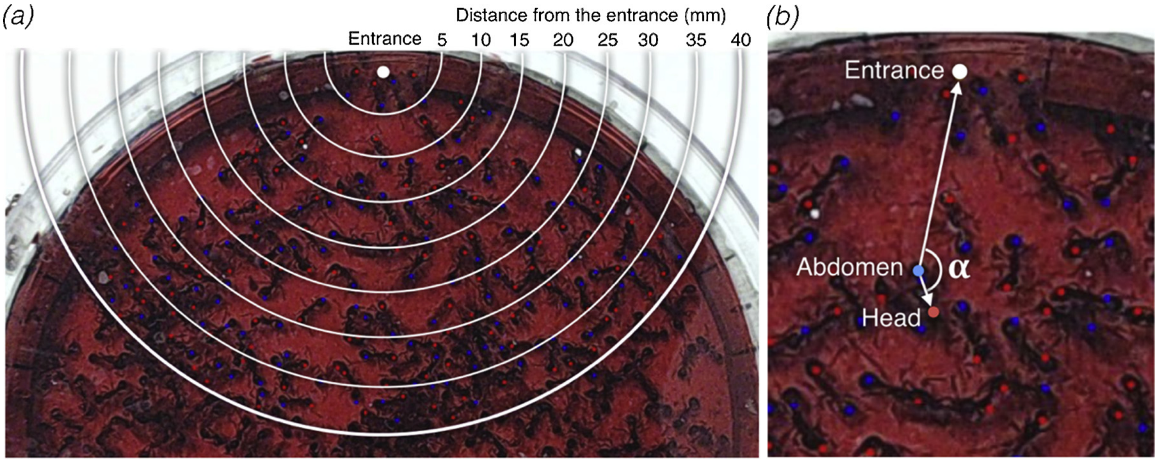

Fig. 2. Determination of the ant orientation to the entrance. (a) Each ant orientation was characterized within 4 cm from the nest entrance. This area was divided into eight rings spaced by 5 mm and centred on the entrance. (b) The orientation of each ant was determined by using two vectors: abdomen-head vector and abdomen-entrance vector. Red dots represent the head of the ant, blue dots represent the abdomen of the ant, α represents the resulting value of the ant orientation towards the nest entrance.

first centimetres, the deviation from a theoretical uniform orientation distribution occurred between -40° and 40°. This angular range was later used to discriminate oriented ants facing the entrance from non-oriented ants.

2.3. *Characterisation of the population of ants in the entrance area*

We investigated whether ants from the social interface (i.e. workers oriented toward the entrance) differed in their spatial persistence in the entrance area, as well as in their propensity to interact with incoming foragers. In one-entrance nests, we determined three groups of workers: ants that joined the entrance area by coming from the outside (entering ant), ants that were initially present in that area and that were either “oriented” toward the entrance (oriented ant), or “non-oriented” toward the entrance (non-oriented ant). For each tested colony, we measured, on video recordings made during the first replicate (15 fps, resolution 1920 × 1080 pixels, Logitech C920 webcams), the time spent in the entrance area by 15 ants: five oriented ants, five non-oriented ants and five entering ants, for a maximum duration of 5 min. We restricted the number to five ants per category as it was the minimum number of ants present in the entrance area for all video recordings. To avoid any spatial bias, we chose for each oriented ant, a corresponding non-oriented ant that was at equal distance from the entrance. We also made sure to choose individuals scattered over the whole entrance area. We calculated the survivorship curves of the time that ants spent in the entrance area. We compared the different groups (oriented ants, non-oriented ants and entering ants) by using a log-rank (Mantel-cox) test, with a Bonferroni correction for multiple comparisons.

Next, we characterized the propensity of oriented and non-oriented ants to interact with returning foragers. We measured the number and duration of the contacts that oriented and non-oriented ants received from all the entering ants. Individuals were chosen in the same way as above mentioned. These measures were taken until the focal ant left the entrance area or for a maximum duration of five min per ant (Table 1). We compared the proportion of oriented and non-oriented ants that were contacted by entering ants by using chi square tests, as well as the number and duration of these contacts by using Mann-Whitney tests.

2.4. *Impact of increasing the number of nest entrances on the social interface*

We monitored how the population size as well as the spatial organization of workers from the social interface changed for three successive configurations of nest openings: one entrance, three entrances and five entrances (Fig. 3). Ants were settled in one entrance nests (entrance C open, Fig. 1) for 48 h prior the experiments. The day of the experiment, we first recorded the spatial distribution of the ants in the one-entrance nest. We then opened the left (L) and right (R) entrances that were adjacent to the central door (Fig. 1), thereby forming a three-entrance nest. After one hour, we opened the two remaining entrances L’ and R’ (Fig. 1) in order to form a five-entrance nest. We maintained this latter configuration for 20 h in order to check for the stability of the spatial organization of workers (Fig. 3).

Table 1
Ant orientation and length of the resulting vector according to the distance from the nest entrance.

Entrance area (cm)	Ant orientation (mean ± sd, °)	Length of vector r
0–0.5	−8.05 ± 51.15	0.67
0.5–1.0	−0.29 ± 65.47	0.52
1.0–1.5	9.59 ± 81.45	0.36
1.5–2.0	10.86 ± 95.49	0.25
2.0–2.5	21.13 ± 113.81	0.14
2.5–3.0	−46.28 ± 134.09	0.064
3.0–3.5	−74.22 ± 134.08	0.064
3.5–4.0	−138.26 ± 117.77	0.12

We assessed how the sequential opening of new entrances influenced the social interface by comparing the number and the orientation of ants located in the five entrance areas between each successive nest configurations (i.e. n_{1door} vs n_{3doors}, n_{3doors} vs n_{5doors}, n_{5doors} vs $n_{5doors+20h}$). For the number of ants in the entrance areas, we used Wilcoxon signed rank tests with Bonferroni correction. In regards to the orientation of the ants, we focused our analysis on the ants located in the central entrance as well as in the two adjacent left and right entrances (L and R, Fig. 1). We paid special attention to this population of ants because they experienced all possible changes in the opening status of entrances (closed door, open door, opening of an adjacent door, 20 h stabilisation, Fig. 3). We then measured the orientation of all the ants present in the 2 cm entrance area in front of the left and right doors and compared the angular distributions across the multiple treatments. We compared how the distributions of ant orientations within each entrance changed between the successive nest configurations by using a Watson-Wheeler test (Batschelet, 1981).

Furthermore, for each nest configuration, we assessed the impact of new openings on the flow of ants by quantifying the inflows and outflows of ants passing through each open door for 10 min (Fig. 3). In order to set apart fluctuations of ant flows resulting from manipulation

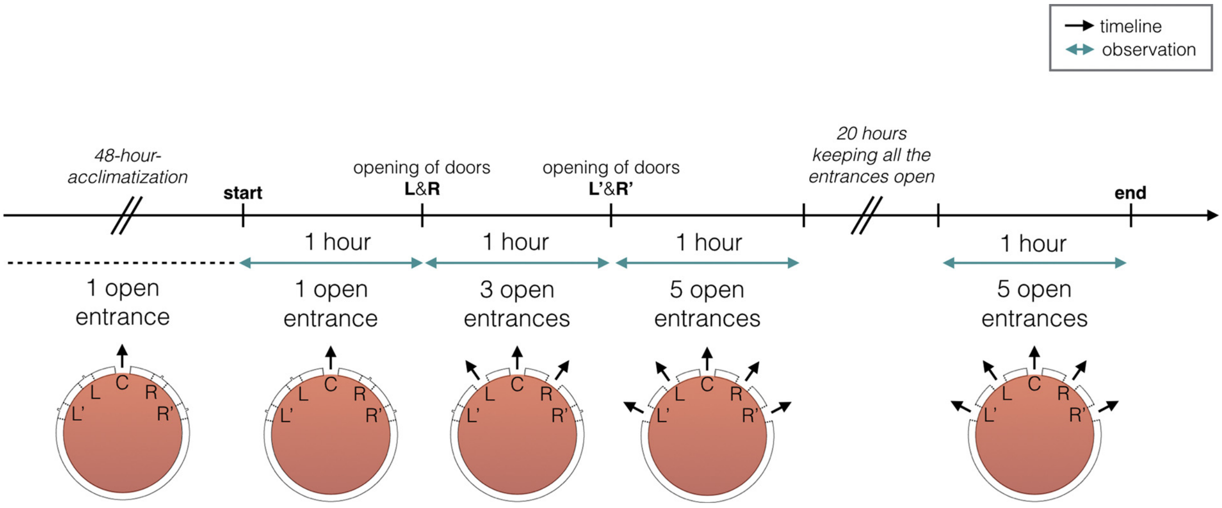

Fig. 3. Experimental procedure. The three successive configurations of open nest entrances are represented: one entrance, three entrances or five entrances. Entrances were named according to their position to the central entrance (C). L and L' stand for entrances located to the left, R and R' stand for entrances located to the right.

disturbances, this counting started 15 min after the opening of the new nest entrances. Due to a recording issue, ant flows were not measured for one experiment. As the population size (and hence the number of foragers) varied between colonies, we used flow fold-changes (instead of absolute flow values) to assess the impact of additional nest openings. We took the flows of foragers in the one-entrance configuration as the reference value and calculated the fold-change of the total flows after each opening of additional doors. We assessed the impact of sequential openings by comparing total flows between each successive nest configurations (i.e. $\text{flow}_{1\text{door}}$ vs $\text{flow}_{3\text{doors}}$, $\text{flow}_{3\text{doors}}$ vs $\text{flow}_{5\text{doors}}$, $\text{flow}_{5\text{doors}}$ vs $\text{flow}_{5\text{doors}+20\text{h}}$) using Wilcoxon signed rank tests with Bonferroni correction.

Finally, for each replicate, we computed the proportion of the total inflows and outflows of ants passing through each open door. After each additional door opening, these proportions were compared between doors using a Friedman test followed by a Dunn-Šidák test post hoc.

3. Results

3.1. Locating the social interface at the nest entrance

We postulated that the nest entrance hosted the social interface of the colony, i.e. the population of ants that were more likely to interact with incoming ants by being oriented toward the nest opening. By measuring the orientation of the ants in eight 0.5-cm-concentric rings, from the entrance to the centre of the nest, we delineated the entrance area. We found that for the five rings located between 0 and 2.5 cm from the nest entrance, as well as for the last ring between 3.5 and 4 cm, the observed orientation distributions were significantly different from a uniform distribution (Hodges-Ajne test, $p < 0.001$ for each ring between 0 and 2.5 cm and for the ring between 3.5 and 4 cm, Fig. 4). In the areas between 0 and 2 cm, the distribution was unimodal and centred around 0° (Fig. 4a), with a resulting vector indicating a preferential orientation of ants toward the entrance (see Table 1 for orientation mean ± sd and length of the resulting vector). Between 2 and 2.5 cm, the orientation distribution displayed three local maxima (at 40°, -10 and -80°, Table 1, Fig. 4b), indicating a transition between a preferential orientation toward the entrance, and random orientations. Between 2.5 and 3.5 cm, the distribution was uniform (Hodges-Ajne test, $p > 0.05$, Fig. 4b, Table 2) indicating that the ants did not show any preferential orientation. Eventually, between 3.5 and 4 cm, the distribution showed several local maxima and the resulting vector indicated a preferential orientation toward the back half of the nest, where the queen and brood were located (Table 1).

These angular distributions allowed us to delineate the area in which the nest entrance had a significant influence on the orientation of the ants. In our experimentals, this area was located within a 2 cm radius centred on the nest entrance where the majority of ants had a preferential orientation toward the entrance and thus composed the social interface. This area is referred hereafter as the nest entrance area.

3.2. Characterisation of the population of ants in the entrance area

In order to better characterise the population of ants present in the entrance area, we focused on the behaviour of three groups of individuals. We found no significant difference between the first two groups, oriented ants and non-oriented ants, regarding the duration of their stay in the entrance area (Log-rank test, oriented ants versus non-oriented ant, $\chi^2 = 0.94$, $p = 0.33$, Fig. 5). However, the staying durations of oriented and non-oriented ants were significantly longer than those of entering ants (Log-rank test, $n_{\textit{Entering ants}} = n_{\textit{Oriented ants}} = n_{\textit{Non-oriented ants}} = 40$, entering ants versus oriented ants, $\chi^2 = 5.41$, $p < 0.001$; entering ants versus non-oriented ants, $\chi^2 = 8.62$, $p < 0.01$; Fig. 5). This suggested that the ants in the entrance area were spatially specialised by standing for prolonged duration in the vicinity of nest openings, with more than a half of these ants staying longer than our five minutes of observation (57% and 62% of non-oriented ants ($n = 40$) and oriented ants ($n = 40$) respectively). Conversely, outside nest workers merely transited through the entrance area before either going deeper near the brood or exiting the nest again. Among the ants that remained in the entrance area, 48% of non-oriented ants (11 out of 23 ants) and 72% of ants facing the nest entrance (18 out of 25 ants), maintained their initial body direction.

As above mentionned, we assumed that oriented ants constituted the social interface that acted as a first line of exchange between returning foragers and the ant nest. To validate this assumption, we recorded the number of contacts that oriented and non-oriented ants each received from entering ants. The contacts between entering ants and nestmates consisted almost exclusively in antennations, with only two trophallaxis observed out of 144 contacts. Seventy-five percent of the oriented ants received contacts from the entering ants (Table 2), whereas a significantly smaller proportion of the non-oriented ants, only 45%, were contacted by the entering ants (Chi square test, $p < 0.05$, Table 2). Thus, the ants coming from the outside mainly interacted with the oriented ants, which themselves represented only 46% of the ants present in the entrance area. In addition, the number of

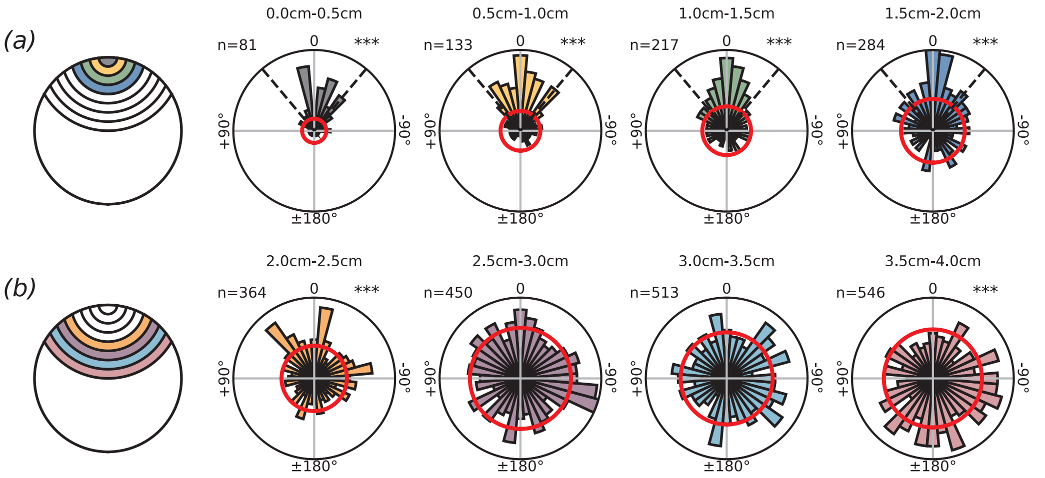

Fig. 4. Worker orientation as a function of the distance to the nest entrance. Zero indicates the direction to the nest entrance. Ant orientation was characterized from the nest entrance to the centre of the nest. Each colour correspond to a 0.5 cm concentric ring in this area. Roses represent the distributions of the experimental ant orientations for each 0.5 cm ring. For each given ring, distributions were compared to theoretical uniform distributions (red circles) of the orientations (Hodges-Ajne test, ***, p < 0.001). (a) The distributions were unimodal and pointed toward the entrance up to two centimetres, which delineated the entrance area. In this entrance area, the main deviation from the uniform distribution laid between -40° and 40° for the two first centimetres. (b) The distributions from 2 cm up to 4 cm, were homogeneous and/or did not point toward the entrance.

contacts per capita was significantly higher for the oriented ants, with a mean of 2.45 contacts per ant, compared to a mean of 1.15 contacts per non-oriented ant (Mann-Whitney test, $p < 0.01$, Table 2). Contact duration were twice as high for the oriented ants as for the non-oriented ants but this difference was not statistically significant (Table 1). Once being contacted, 23% ($n = 30$) of the oriented-ants and 11% of the non-oriented ants ($n = 18$) exited the nest to start foraging. Interestingly, among ants that left the entrance area to engage in inner nest activities 68% were non-oriented ants.

3.3. *Impact of an increasing number of nest entrances on the social interface*

In order to assess the impact of additional nest entrances on the social interface, we measured the orientation and number of ants present in the entrance area in each of the four nest configurations. Our results showed that the orientation of the ants in front of the left door and the right door were significantly influenced by the number of open entrances (Watson-Wheeler test; Left door: $W = 24.952$, $p_{\text{left}} < 0.001$, Right door: $W = 37.017$, $p_{\text{right}} < 0.001$, Fig. 6). In both left and right entrance areas, the ants reoriented themselves to face the corresponding left or right entrance (see orientation values in Table 3; Fig. 6). By contrast, the orientation of the ants in front of the central entrance was not influenced by the successive openings of additional entrances (Watson-Wheeler test; Central: $W = 5.2731$, $p_{\text{central}} > 0.05$, see orientation values in Table 3).

As regards to the total number of ants located in the five entrance areas (Fig. 7a), we observed a mean of 90 ants when only one entrance was open. This number of ants did not change significantly between successive experimental steps (Wilcoxon signed-rank tests with Bonferroni correction, critical p-value = 0.016; 1 door vs 3 doors, $n = 24$, $p = 0.031$; 3 doors vs 5 doors, $n = 24$, $p = 0.35$; 5 doors vs 5 doors + 20 h, $n = 24$, $p = 0.094$, Fig. 7b). This result suggests that there was no major mobilisation of additional ants around newly open entrances. Thus, although the ant response was not instantaneous, the colony was able to adapt the size of its social interface to the number of open nest entrances. This phenomenon occurred mostly through the progressive reorientation of the ants toward newly open entrances rather than through an increase in the number of ants located in their vicinity.

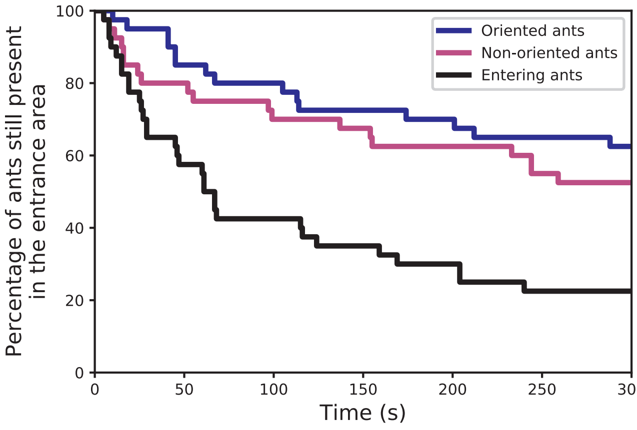

Fig. 5. Persistence of the ants in the entrance area as a function of their initial position. Ants could be either facing the entrance (oriented ants, vector between -40° to 40°), not oriented toward the entrance (non-oriented ants, vector between -180° to -40°, and 40° to 180°) or entering the nest (entering ants). For each category of initial position, five ants per colony were tested ($n = 40$ per category).

Similarly, we analysed the influence of additional open entrances on the inflows and outflows of ants. At the start of the experiment, in the one-entrance nests, we observed a large variability of ant flows between colonies. Incoming flows ranged from 7 to 74 incoming ants/10 min, with a mean of 32 ants entering the nest. This variability was observed as well for the outgoing flows, ranging from 9 to 58 ants/10 min exiting the nest, with a mean of 31 outgoing ants. Thus, for each colony, we computed the fold-changes of the incoming and outgoing flows that were elicited by the opening of new doors, taking the one-entrance-nest flow as the reference value (Fig. 8). Our results showed that both inflows and outflows significantly increased by 50% when we tripled the number of open entrances (Wilcoxon signed-rank test, 1 door vs 3

Table 2
Contacts made by entering ants towards oriented and non-oriented ants in the entrance area.

	Oriented ants	Non-oriented ants	p	Statistical test
Proportion of contacted ants	0.75 ($n = 40$)	0.45 ($n = 40$)	$\chi^2 = 6.3$, $p = 0.012$*	Chi square
Number of contacts received (mean ± sd)	2.45 ± 2.41 ($n = 40$)	1.15 ± 1.75 ($n = 40$)	$U = 1088.5$, $p = 0.004$**	Mann-Whitney
Contact time (mean ± sd, s)	13 ± 16.97 ($n = 30$)	6 ± 5.03 ($n = 18$)	$U = 329$, $p = 0.21$	Mann-Whitney

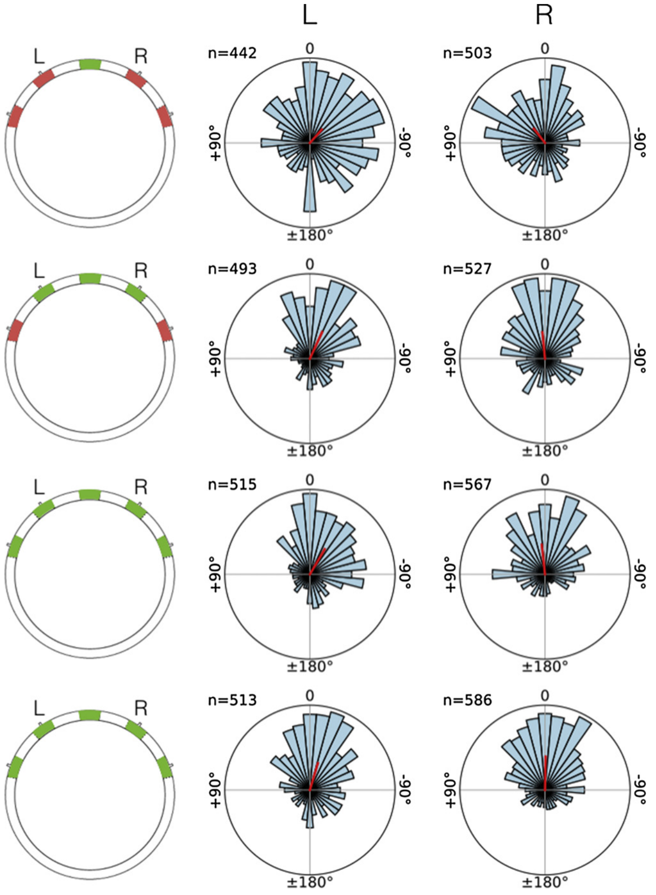

Fig. 6. Influence of the opening of additional doors on the ant orientations in the nest entrance area. On the left are presented the different nest opening configurations. Open entrances are represented in green. Closed entrances are represented in red. Rose plots represent the distribution of the ant orientations for the left (L) and right (R) entrance for the different nest opening configurations. Red lines indicate the resulting orientation vector.

doors, $n=23$, $p < 0.0001$ for both the inflow and the outflow, Fig. 8). The inflow of ants kept on increasing to almost twice the initial flow when we opened five entrances (Wilcoxon signed-rank tests with Bonferroni correction, 3 doors vs 5 doors, $n=23$, $p < 0.01$ for the inflow, Fig. 8). After 20 h, neither the outflow nor the inflow changed (Wilcoxon signed-rank test, 5 doors vs 5 doors + 20 h, $n=23$, $p > 0.05$, Fig. 8), indicating that the number of foragers was stabilized. Thus, additional entrances led to a significant increase of the flows of ants, although it was less than proportional to the number of open entrances.

Finally, we determined whether the ants preferred to use one specific entrance. Just after the opening of new entrances, the incoming and outgoing ant flows spread between all the open entrances but some entrances were temporarily favoured. For instance, in the three-entrance nests, the inflow and outflow of ants through each door did not differ significantly from each other (inflow: Friedman test, $n=23$, $\chi^2=1.66$, $p > 0.05$; outflow: Friedman test, $n=23$, $\chi^2=2.85$, $p > 0.05$). However, in the five-entrance nests, ants walked through the central door significantly more often than through the adjacent R and L doors for the incoming flow (Friedman test, $n=23$, $\chi^2=18.66$, $p < 0.001$; Dunn-Šidák test post-hoc, L versus C, $p < 0.001$; R versus C, $p < 0.001$; Fig. 9) and the outgoing flow (Friedman test, n = 23, $\chi^2=18.24$, $p < 0.001$; Dunn-Šidák test post-hoc, L versus C, $p < 0.05$; R versus C, $p < 0.05$; Fig. 9). Eventually, after 20 h, the incoming flows spread homogeneously between the five open entrances (Friedman test, $n=23$, $\chi^2=7.26$, $p > 0.05$; Fig. 9). The outgoing flows also tended to spread homogeneously as well, except for the flows through door L and R' that were significantly different (Friedman test, $n=23$, $\chi^2=13.06$, $p < 0.05$; Dunn-Šidák test post-hoc, L versus R', $p=0.034$). Overall, these results indicated that foragers dynamically reorganized themselves when additional entrances were open. Although the ants immediately used new entrances, it took several hours for the flows of ants to become similar between the different open entrances, with ants not favouring a particular nest opening.

4. Discussion

Our study showed that the orientation of the ant workers towards the nest entrance could be used as a criterion to delineate the entrance area. Based on this criterion, the entrance area extended up to 2 cm from the entrance in our *M. rubra* experimental nests. At this front location, 46% of individuals preferentially faced the nest entrance while ants located deeper in the nest were randomly oriented. These ants, that faced the nest entrance, were more engaged in contacts with incoming ants, thereby acting as a “sensory” interface with the outside environment. This work highlights the role of the entrance area as a key-location for information sharing between outside foragers and nestmates which then compose the social interface of the colony.

This social interface is expected to be of primary importance for the colony as its workers are the first to perceive recruitment signals or flows of nutrients brought back by foragers (Pinter-Wollman et al., 2013; Pless et al., 2015). Likewise, this social interface is ideally located for checking the sanitary status of incoming nestmates (Leclerc and Detrain, 2016) and for rejecting any intruder trying to enter the nest. In a few ant species, individuals of the social interface may show striking examples of morphological specialisation such as phragmosis in majors of *Cephalotes* and *Pheidole* genera of which the large and/or flattened head blocks the nest entrances against attacking invaders (Eisuke, 1993; Huang, 2010; Powell, 2008). In case of little to no worker polymorphism, as in the majority of ant species including *M. rubra* species, the specialization of workers belonging to the social interface is far less obvious. In *Myrmica rubra*, Pamminger et al. (2014) have shown that behavioural syndromes were nevertheless associated with ants staying at specific locations in the nest, in the absence of visible physical polymorphism. According to their results, the role of the entrance workers seemed to be in relation with the foraging activity, those workers being regularly observed patrolling outside the nest. Likewise, in our study, we have shown that workers belonging to the social interface remained in the nest entrance area much longer than incoming foragers and, when leaving it, seemed more prone to forage outside the

Table 3
Orientation of the ants and length of the resulting vector in the different nest entrance areas areas as a function of the nest opening configuration.

Entrance area	Central		Left		Right	
Nest configuration	Ant orientation (mean ± sd, °)	Length of resulting vector *r*	Ant orientation (mean ± sd, °)	Length of resulting vector *r*	Ant orientation (mean ± sd, °)	Length of resulting vector *r*
1 door open	−6.29 ± 71.63	0.45	−41.76 ± 99.73	0.21	38.02 ± 101.45	0.21
3 doors open	8.15 ± 71.88	0.45	−24.67 ± 82.77	0.35	5.65 ± 90.10	0.29
5 doors open	−7.05 ± 71.32	0.46	−30.46 ± 84.37	0.34	6.14 ± 84.08	0.34
5 doors open + 20 h	−8.68 ± 74.62	0.43	−16.44 ± 86.91	0.32	−1.05 ± 79.52	0.38

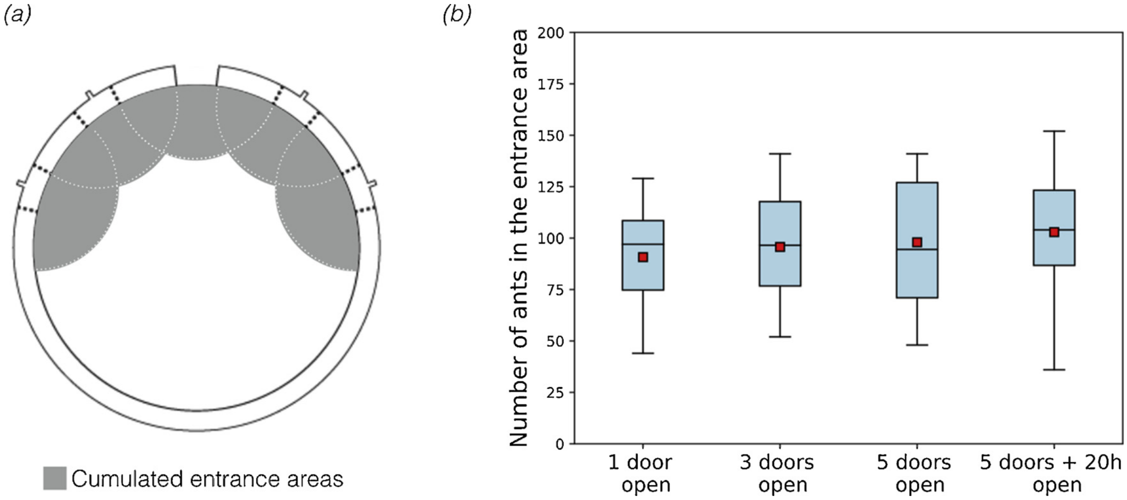

Fig. 7. Influence of the opening of additional doors on the total number of ants present in the entrance areas. (a) The cumulated entrance areas are represented in grey. White dotted lines represent each 2 cm entrance area. (b) Cumulated number of ants in the five entrance areas for the different configurations of nest openings. Boxplots give medians and quartiles, red squares indicate means ($n = 24$, Wilcoxon signed-rank tests with Bonferroni corrections).

nest than going deeper inside the nest.

The entrance area can host a significant part of the ant colony (up to one third of the *M. rubra* population in our experimental nests). As previously reported in many ant species that dig subterranean nests - e.g. *Pogonomyrmex barbatus* and *P. badius* (Gordon, 2010; Tschinkel, 2015) - the nest entrances are connected to enlarged superficial chambers that can contain more than 40% of the colony workers (Tschinkel, 2004). From a functional perspective, the aggregation of many workers in nest entrance areas can facilitate food exchanges and information transfer about environmental opportunities. It also allows a quick and efficient mobilisation of this pool of potential recruits towards new food resources favouring the emergence of collective exploitation of food sources (Beckers et al., 1992; Beekman et al., 2001; Collignon et al., 2014; de Biseau and Pasteels, 2000; Pasteels et al., 1987; Pinter-Wollman et al., 2013).

The present study also showed that the size of this social interface tended to increase with the number of nest entrances. Instead of massively mobilising additional workers to the vicinity of the newly opened gates, the ant workers that were already present in these areas simply reoriented themselves towards the new nest entrances. This confirmed the key-role of the entrance area as a hotspot for information sharing. Indeed, the reorientation of workers towards new entrances allowed them to face the incoming flows of ants and thereby to increase their interaction rates, as also shown in Pinter-Wollman et al., 2013, where interactions among ants located in the vestibule and returning foragers were clustered near the nest exit.

Several explanations could be given in order to account for the limited mobilisation of additional workers (up to 10%) towards new nest openings. First, our experimental nests consisted in a single chamber that was designed to house approximately 300 individuals. This resulted in an almost homogeneous distribution of the ants with many workers already present near the nest walls. Multi-chambers nests, with a network structure closer to natural ant nests, may show a more clear-cut change in the number of ants located in the nest entrance area. Second, the limited mobilisation of nestmates close to new entrances could indicate that each colony has to deal with other essential tasks such as queen and brood care, in which the number of engaged workers is incompressible. Third, the number of ants at the entrance area could depend on the level of risks to which the ant colony is exposed. Indeed, studies on honeybees have shown that the number

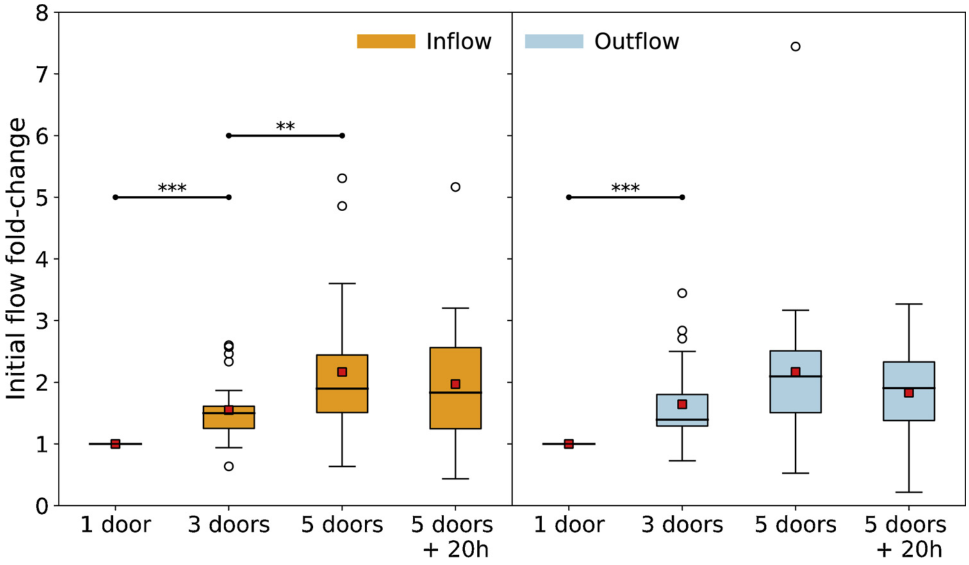

Fig. 8. Initial flow fold-change in response to door opening. The inflow was represented in orange and the outflow in blue. Presented are medians and quartiles, red squares indicate means and circles indicate outliers ($n = 23$, Wilcoxon signed-rank tests with Bonferroni correction, ** $p < 0.01$, *** $p < 0.001$).

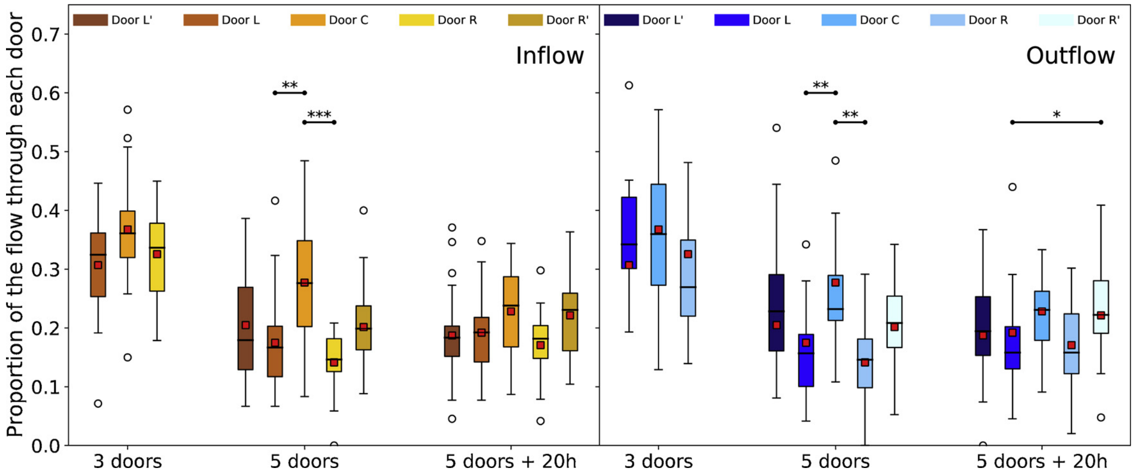

Fig. 9. Proportion of ants walking through each open door for the inflows (yellow) and the outflows (blue). Boxplots give medians and quartiles, red squares indicate means and circles indicate outliers ($n = 23$ for each entrance, Dunn-Šidák post-hoc test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

of guards rapidly increased if numerous intruders were detected at the hive entrance (Couvillon et al., 2008). In this work on *M. rubra* colonies, we observed the ants in a non-competitive context and in the absence of intruders. Future studies could interestingly test whether ant colonies that regularly face potential intruders would better adjust the population size of the social interface to the number of nest entrances, thereby prioritising an efficient control of all the gates.

Finally, our study showed that ants quickly made use of the new entrances to exit and enter the nest. Indeed, the additional passageways resulted in an increase of the inflow and outflow of ants. Thus, even in the absence of external stimuli such as the discovery of new food sources, the total ant traffic was twice as high when we opened four additional entrances. As this increase was observed in ant colonies fed ad libitum, the investment of ants in foraging activities was driven not only by the internal motivation and nutritional needs of the colony, but also by the configuration of the interface between the nest and the environment. Furthermore, although the ants were reared in a one-entrance-nest condition, their preference to use this better-known entrance compared to the more recently open ones was temporary. While the initial entrance was still favoured after the opening of four additional doors, this preference vanished the following day, on which no hierarchy appeared clearly in the level of use of the nest entrances. Such an even distribution of the ants between the different entrance areas reflects a propensity of the ants to use all the available nest entrances, which may favour the exploration and the exploitation of a wider foraging area (Deffernez et al., 1990; Robinson, 2014).

The number of nest entrances should however be limited, as too many passageways could be detrimental to the colony. First, it may compromise the physical safety of the nest, for example by facilitating access to predators or pathogens, by favouring flooding through the additional openings or by impairing the regulation of the nest homeostasis. Second, an increase in the number of nest entrances may lead to a decreased efficiency of the decision-making process at the collective level. Indeed, the entrance area is the location where information about resources are conveyed by different competing recruiters, which finally leads to the selective exploitation of the best food source (Beckers et al., 1993, 1990; Pasteels et al., 1987). In this context, further studies could investigate whether multiple entrances could disrupt this selection process of competing recruitment signals and could result in a loss of efficiency in foraging choices made at the colony level.

Declarations of interest

The authors have no competing interests.

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