

Spatial organization in a dimorphic ant: caste specificity of clustering patterns and area marking

Grégory Sempo,^a Stéphanie Depickère,^b and Claire Detrain^a

^aUnit of Social Ecology, CP 231, Université Libre de Bruxelles, Bld du Triomphe, 1050 Brussels, Belgium, and ^bINLASA, Entomología Médica, Rafael Zubietta N°1889 (Lado del Estado Mayor General), Miraflores Casilla M—10019, La Paz, Bolivia

Living in groups constitutes the root of social organization in animals. Likewise, the spatial aggregation between members of insects societies plays a crucial role in social cohesion and division of labor, namely, in polymorphic ant species. In the present paper, we show caste-specific aggregation patterns in the strictly dimorphic *Pheidole pallidula* ant species. We investigate the influence on the clustering of ants exerted by direct contacts between nest mates as well as by indirect cues through chemical marking. In a homogeneous environment deprived of chemical cues, majors show a higher aggregation level than minors and a centripetal behavior. By contrast, minors are more scattered in the experimental arena and display a centrifugal behavior. In addition, area marking laid by minors enhances their own aggregative behavior while contributing to the localization of the spontaneously aggregating majors. Such differences in aggregative patterns as well as their adaptive value have to be coupled with the mobility level and the task performance efficiency of each worker caste. Contrary to majors that are likely to aggregate, highly mobile minors, scattered inside and outside the nest colony, can detect colony needs and can carry out most of the daily tasks for which they are more efficient than majors. *Key words*: aggregation, caste, chemical marks, division of labor, social organization. [*Behav Ecol* 17:642–650 (2006)]

Aggregation occurs in many biological systems (Parrish and Hammer 1997; Parrish and Edelstein-Keshet 1999; Krause and Ruxton 2002). There is a wealth of literature dealing with cost/benefit for animal individuals to organize themselves in space and time as aggregates. Although living in groups implies costs, such as sharing of resources (Giraldeau and Caraco 2000), sexual competition (Moller and Birkhead 1993), or increased parasite burdens (Van vuren 1996), aggregation can also bring advantages by facilitating information transfer (Ward and Zahavi 1973), cooperative foraging (Goss-Custard 1977; Zwarts and Drent 1981; Creel S and Creel NM 1995), thermoregulation (Ancel et al. 1997), and protection against predators (Hamilton 1971; Bertram 1978; Treherne and Foster 1980).

In social insects, aggregation plays a key role in the organization of their complex societies. Modulation of the aggregation level, namely, through the use of recruiting signals, is at the core of collective behaviors shown by nest mates in the context of defense (for ants: Wilson 1974, 1976a, 1976c; for bees: Millor et al. 1999), foraging (for ants: MacKay WP and MacKay EE 1984; Gordon 1996; Detrain and Deneubourg 1997), nest construction (for ants: Rasse and Deneubourg 2001; for bees: Karsai and Penzes 1993), or swarming (for ants: Anderson et al. 2002; for bees: Visscher and Camazine 1999). Moreover, within the nest, spatially defined areas exist in which groups of specialized individuals are more likely to stay, leading to a spatial aggregation of individuals belonging to the same ethological caste (for ants: Wilson 1980; Franks and Sendova-Franks 1992; Sendova-Franks and Franks 1995; for bees: Trumbo et al. 1997). This clustering helps workers

collaborate into colony maintenance or brood care, facilitates the collective performance of spatially related tasks and hence increases the ergonomic efficiency of the colony by notably minimizing the travel time between successive tasks (Wilson 1976b, 1985; Seeley 1982, 1985). Although costs and benefits of aggregation are well documented, data about underlying behavioral mechanisms are sparser and have a recognized need for further advances. In this respect, aggregation patterns in insect societies can result from 2 main phenomena:

1. a sorting according to environmental heterogeneities and/or
2. an interindividual attraction.

Regarding the influence of environmental heterogeneities, many aggregation patterns are shaped by physical factors that are external to the colony, that is, temperature or humidity (for ants: Ceuster 1977; Roces and Nuñez 1989; Cole 1994; for bees: O'Donnell and Foster 2001). In addition, aggregation of social insects can result from intrinsic attraction between nest mates (for ants: Depickère et al. 2004a; for bees: Camazine et al. 2001). In ants, this interattraction is based on cues of nest mates' presence that can be either direct, such as antennal contacts (Depickère et al. 2004a, 2004b), or indirect, such as chemical marks laid actively (e.g., recruitment trail to an aggregation site) (Jeanson et al. 2004), or can occur passively on the ground (e.g., area marking by cuticular hydrocarbons) (Hölldobler and Wilson 1990).

In this paper, we will examine the aggregation patterns of *Pheidole pallidula*, an ant species with a strictly dimorphic worker caste. By comparing the aggregation dynamics of minors and majors, we will search for caste-specific clustering behaviors and we will underline the influence of direct cues, such as the presence of nest mates, on the aggregation process. Then, we will test the influence of an indirect cue consisting of chemical marks deposited by each caste on the area, on the aggregation patterns of minors and majors.

Address correspondence to G. Sempo. E-mail: gsempo@ulb.ac.be.
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METHODS

Collection and rearing of colonies

Nineteen colonies of *P. pallidula* were collected on calcareous slopes found around the village of Gonfaron (Var District, France). Colonies were composed of 1 queen, approximately 5000 workers, and brood including eggs, nymphs, and all 3 larvae instars. The worker population was composed of around 90% minors and 10% majors, a proportion close to that of natural colonies (Passera 1977).

In the laboratory, collected colonies were reared using moistened nest tubes (length, 16 cm; diameter, 1.5 cm) placed in plastic trays (length, 30 cm; width, 20 cm; height, 4 cm) coated on the walls using Fluon to prevent ants from escaping. Ants were fed with brown sugar solution (concentration: 1 M) and dead cockroaches (*Periplaneta americana*) and had a permanent access to water. Colonies were maintained in the dark at 28 ± 1 °C.

Experimental setup

We investigated the aggregation behavior of the 2 *P. pallidula* castes in 3 dimensions, 2 in space (in a circular arena) and 1 in time (for 3 h). The experimental setup was composed of a paperboard arena (thickness, 3 mm; diameter, 20 cm). This arena was laid on a transparent glass plate. In order to avoid bias in the aggregation patterns of ants, we suppressed visual landmarks around the arena by placing it into a black cylinder (30-cm height). This cylinder had a hole on its top, which was equipped with the digital camera lens (Panasonic WV-BP330). This camera allowed us to record the position of each ant during the 3 h of the experiment. Necessary illumination was provided by a black chamber bulb (15 W) placed under the arena. This setup gave us the best compromise between a low illumination and a high contrast on video recordings. Indeed, the illumination of ants from below permitted us to record their shadow.

Dynamics of aggregation

We studied the aggregation response of 3 experimental groups over the circular arena, in a randomly assigned testing order. The ground paper was free of any previous ant marks. These 3 experimental groups of ants consisted in pure groups including 50 minors (11 replicates), pure groups of 50 majors (10 replicates), or mixed groups composed of 25 individuals of each caste (10 replicates). The number of workers involved in each test was limited to 50 individuals, which was found to be the best compromise between studying aggregative behavior at relatively low ant density (0.16 ants/cm²) and keeping the size of the arena small enough to allow the identification of worker caste as well as further analysis of digitized snapshots (see next paragraph). These low-density testing conditions guaranteed that cluster formation resulted from aggregation processes based on interindividual interactions or on attraction toward chemically marked areas, instead of resulting from overcrowding. Minor and major workers used for the experiments were withdrawn from nest tubes and introduced in the center of the arena. Among the population of majors, we decided not to pick up “replete” individuals specialized in fat storage (Lachaud et al. 1992), due to the probable atypical aggregative behavior of this major subcaste.

The Cartesian coordinates of ant's thorax were determined by using digitized snapshots of the experimental setup at different times (1 min, 5 min, and every 5 min until 180 min), for a total of 37 snapshots. Software developed in our laboratory was used to generate these coordinates by locating the thorax of all individuals. These positions were used to com-

pute different indexes characterizing the aggregation of each caste in the different experimental conditions. In this respect, the arena was divided into 5 concentric fixed-width rings (2 cm), the outermost ring matching the edge of the arena. We then calculated the density of castes (number of individuals/cm²) in the different rings, taking into account the increasing area of rings toward the edge of the arena. To emphasize a preference in the spatial position of ants over the arena, we compared the observed densities with that expected from a homogeneous distribution of individuals in the arena (total number of individuals in the arena/total surface of the arena). We also determined the number and the fraction of aggregated individuals of each caste in the setup. Two ants were considered as aggregated when the distance (thorax to thorax) between 2 or more individuals was lower or equal to 7.5 mm. This distance criterion takes into account the size of individuals (minors: 1.6–2.6 mm, majors: 3.3–4.9 mm) and allows us to group all individuals belonging to the same cluster without including ants moving in the arena or belonging to another cluster. For all the clusters formed in the arena, we analyzed their relative number and size and, for mixed groups, their caste composition.

Influence of area marking on aggregation dynamics

In a second set of experiments, we observed the influence of chemical marks deposited by minors or majors on the aggregation patterns and on the choice of a clustering site by each caste. In this setup, we covered the ground of the arena (thickness, 3 mm; diameter, 20 cm) with a white paper sheet (80 g/m²) at the beginning of the experiment. Two circular walls (height, 3 mm; diameter, 6 cm) delimited 2 small areas whose centers were symmetrically positioned at 5 cm from the center of the arena. We then introduced either 25 majors or 25 minors in the small arenas and let them mark the substrate for 2 h. After this marking phase, individuals as well as small arenas were removed. In the center of the setup we then introduced 50 minors or 50 majors originating from the colony to which belonged the previously used workers. During the 2 h, we counted in 5-min intervals, the number of ants remaining inside each of the 2 circular areas. We quantified the clustering behavior of minor and major workers faced with the following binary choices:

- a site marked by minors and a site marked by majors (8 replicates),
- a site marked by majors and an unmarked control site (8 replicates).

These different experiments were performed in a randomly assigned testing order.

Statistical analyses

Data from all the experiments were tested from deviance from normality using Kolmogorov–Smirnov test. When the normality conditions were met, we carried out parametric tests, otherwise, we performed corresponding nonparametric tests. Percentage data were arcsine transformed to satisfy normality (Zar 1999). The effect of group type (pure group of minors or majors or mixed group), of distance from the setup center, and of interaction between these 2 factors was tested by using a 2-way analysis of variance (ANOVA). On each group, we examined by a 1-way ANOVA the effect of distance from the setup center on the densities/number of workers observed at the end of the experiment. These ANOVAs were followed by a Bonferroni post hoc test when required. A 1-sample *t*-test was used to examine whether observed densities significantly differ from densities expected from a homogeneous distribution of workers over the area. The expected densities would

then be 0.16 workers/cm² for pure groups and 0.08 workers/cm² for each caste in mixed groups. In addition, we tested between castes differences in their spatial position by using unpaired *t*-tests comparing the densities of minors and majors at *t* = 180 min in the central or in the peripheral ring.

Changes over time in the spatial distribution of workers were analyzed by a 2-way ANOVA for repeated measures in order to assess for main effects of time, group type, and interaction of both factors on the size of the largest cluster. Time was the within-subject factor, whereas group type was the between-subject factor. Repeated methods of contrasts were used for pairwise comparisons of all time step values against the final size of the largest cluster at *t* = 180 min (simple contrast). The dynamics of cluster formation was then considered to have reached a plateau when these pairwise comparisons were no longer statistically significant. The testing of a linear time effect was achieved by using simple linear regression from the 20th minute (end of initial dispersal phase of ants) until the end of the aggregation process, when the plateau value is reached.

Concerning the influence of area marking on aggregation, a 2-way ANOVA for repeated measures was used to assess the effect of time, marking, and their interaction on the mean number of aggregated ants.

All *P* values were 2-tailed, and means are provided with ± 1 SE. All calculations were carried out using SPSS 13.0 software (SPSS Inc.). The significance of the statistical tests was fixed to $\alpha = 0.05$. Data satisfied the assumptions of each test.

RESULTS

Caste specificity of patterns and dynamics of aggregation

The spatial distribution of ants gave us overall information on the position of aggregation sites as well as on possible inter-caste differences in thigmotactic behavior. After their introduction in the experimental setup, both castes instantly spread from the center of the arena to its periphery. Such a centrifugal behavior was, however, more pronounced among minors than majors. Indeed, in pure minor groups (11 replicates), after 3 h of testing, minors were predominantly localized close to the periphery of the arena (ring 8–10 cm: $75 \pm 5\%$ of tested minors), whereas only $2.4 \pm 0.7\%$ of them were still present in the central ring (0–2 cm). As a result, minors' density was significantly higher in the peripheral ring than in the other rings of the arena (Figure 1A; 1-way ANOVA: $F_{4,50} =$

27.8, $P < 0.0001$; Bonferroni post hoc for ring 8–10 cm vs. other rings: $P < 0.001$; for other comparisons: $P > 0.05$). Their density in this peripheral ring reached twice the value of that predicted from a homogeneous distribution (1-sample *t*-test: $t_{10} = 8.1$, $P < 0.0001$).

Regarding pure groups of majors (10 replicates), at the end of the experiment, a high fraction of individuals was restricted either at the periphery (ring 8–10 cm: $40 \pm 7\%$ of tested majors) or at the center of the arena (ring 0–2 cm: $20 \pm 7\%$ of tested majors). In terms of density, their level of presence in the peripheral ring was similar to that predicted by the homogeneous distribution (Figure 1A; 1-sample *t*-test: $t_9 = 0.50$, $P = 0.63$). This also revealed their manifest inclination to aggregate in the center of the arena (ring 0–2 cm), as their density in this area was significantly higher than those in the other rings (Figure 1A; 1-way ANOVA: $F_{4,45} = 4.5$, $P = 0.004$; Bonferroni post hoc comparisons for ring 0–2 cm vs. other rings: $P < 0.05$, for the other comparisons: $P > 0.05$) and was 4.8 times higher than predicted from a homogeneous distribution (Figure 1A; 1-sample *t*-test: $t_9 = 2.3$, $P < 0.05$).

Therefore, the density of workers varied according to their distance from the center (Figure 1A; 2-way ANOVA: distance from the center, $F_{4,95} = 47.1$, $P < 0.0001$): the high density in the peripheral ring resulting from their thigmotactic behavior (Figure 1A; Bonferroni post hoc comparisons for ring 8–10 cm vs. other rings: $P < 0.0001$, for the other comparisons: $P > 0.7$). Furthermore, spatial patterns significantly differed between pure minor and pure major groups (2-way ANOVA: caste \times distance from the center, $F_{4,95} = 11.1$, $P < 0.001$). Indeed, thigmotaxis was essentially found in minors, the density of which was higher near the edges of the arena while majors were aggregated in a more central position (Figure 1A; unpaired *t*-test for comparisons between minor and major densities in ring 0–2 cm: $t_{19} = 2.5$, $P < 0.05$, in ring 8–10 cm: $t_{19} = 4.0$, $P < 0.001$, in the other rings: $P > 0.05$).

The concurrent introduction of 25 minors and 25 majors (10 replicates) led to a dispersal of majors in the complete arena because their densities did not differ between rings (Figure 1B; 1-way ANOVA: $F_{4,45} = 2.2$, $P > 0.05$). Majors' aggregation extended to the 2 central rings and their density at the arena center (ring 0–2 cm) did not differ from the 0.08 majors/cm² predicted by a homogeneous distribution (1-sample *t*-test: $t_9 = 1.49$, $P > 0.05$). Regarding minors, in mixed group tests, there was no preference for the edge of the arena (Figure 1B; 1-way ANOVA: $F_{4,45} = 2.06$, $P > 0.05$). Indeed, in comparison with pure minor groups, they slightly

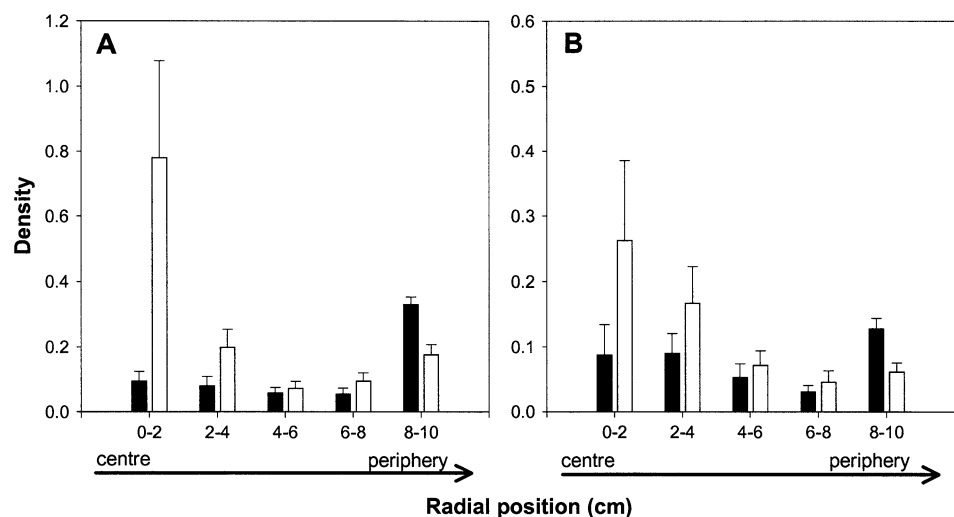


Figure 1
Spatial distribution of the ants after 180 min in the 5 concentric rings of the arena. (A) Density of minors (minors/cm², black, 11 replicates) and majors (majors/cm², white, 10 replicates) in pure colonies. (B) Density of minors (minors/cm², black, 10 replicates) and majors (majors/cm², white, 10 replicates) in mixed colonies.

avoided the periphery at the benefit of the arena center, their density in the peripheral ring did not differ from that expected from a homogeneous distribution of minors (1-sample t -test: $t_9 = 0.34$, $P = 0.74$). As a consequence, the presence of majors appeared to counteract minors' thigmotactic behavior with the arena edges and to enhance their aggregation in the center of the arena.

To summarize, minors and majors differed qualitatively and quantitatively in their spatial distribution inside the arena. In the absence of any other stimuli than the presence of individuals of their own caste, majors tended to spontaneously aggregate in the center of the arena, whereas minors were more mobile and had a preference to remain close to edges. In experimental groups incorporating both castes, minors had a more uniform spatial distribution in the arena than in pure groups, their aggregation in the center of the arena being enhanced by the presence of a majors' cluster in this spot. On the other hand, the intrinsic tendency of majors to aggregate in the center of the setup was unchanged in the presence of minors, although a slight expansion of the aggregation site (ring 0–4 cm) was observed in comparison with pure major groups.

The spatial distribution of ants relatively to the center of the arena and the fraction of aggregated individuals gave us some information about the spatial distribution of ants but none about their clustering patterns. Indeed, based on our definition of aggregation (see Methods), a high fraction of aggregated ants could result from a high number of duos and trios or from 1 large cluster composed of many workers. We therefore analyzed the cluster size distribution in order to address the aggregative behavior of castes in more detail.

For each type of group, the decrease in size of the largest cluster after 5 min (Figure 2) showed that most individuals (about 90%) left the center of the arena directly after their introduction in the setup. However, the number of ants departing from the arena center in the first 5 min differed among groups (1-way ANOVA: $F_{2,28} = 4.95$, $P < 0.01$; Bonferroni post hoc, pure major vs. pure minor: $P < 0.05$, pure major vs. mixed group: $P < 0.05$, pure minor vs. mixed group: $P > 0.05$). Indeed, during the first minute, almost all minors were dispersed, with only 6.7 ± 1.3 minors remaining in the largest cluster, whereas approximately 40% of majors (18 ± 4.7 majors) were still grouped in a central cluster (Figure 2). The lower scattering of majors was probably due to their lower mobility. In all our experiments, clustering was thus an active behavior with workers coming back to the center to aggregate instead of having a passive behavior with workers simply staying where they were experimentally introduced.

The size of the largest cluster did not differ between groups but significantly changed over time (2-way repeated measures ANOVA: group type, $F_{2,28} = 1.01$, $P > 0.05$; time, $F_{36,1008} = 14.24$, $P < 0.05$), the interaction term between these 2 factors being also significant (2-way repeated measures ANOVA: time \times group type, $F_{72,1008} = 1.54$, $P < 0.01$).

When analyzing the aggregation dynamics of each group type, we find out that the size of the largest cluster significantly changed over time (1-way repeated measure ANOVAs: pure minor group, $F_{36,360} = 5.4$, $P < 0.0001$; pure major group, $F_{36,324} = 4.7$, $P < 0.0001$; mixed group, $F_{36,324} = 7.7$, $P < 0.0001$). Indeed, after an initial dispersal of ants over the arena, they started aggregating and the size of the largest cluster reached a plateau value that was determined using simple contrasts post hoc tests ($P > 0.05$; see Methods for details). The time elapse required to reach the maximal cluster size was 45 min in groups of minors, whereas 110 min was needed for groups of majors. Mixed groups showed an intermediate time value of 90 min for the stabilization of largest cluster size. During the growing phase, the size of the largest cluster in-

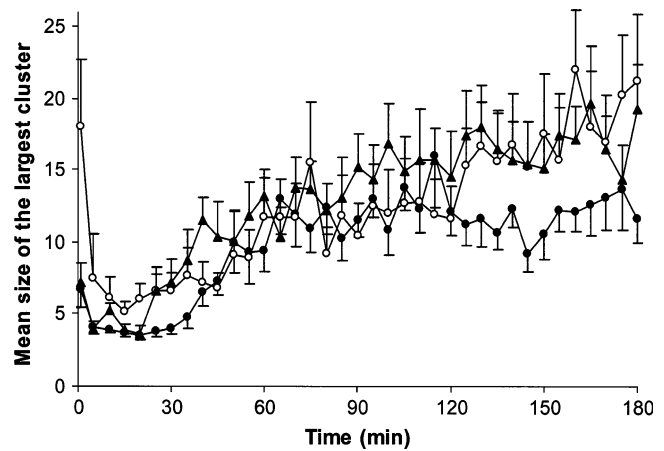


Figure 2

Change over time, along the experiment, of the mean size of the largest cluster (\pm SE) observed in pure minor (black circle, 11 replicates), pure major (white circle, 10 replicates), or mixed (black triangle, 10 replicates) groups.

creased linearly for all groups from the 20th minute until the plateau (linear regression: minor group, $20 \leq t < 45$ min, $F_{1,64} = 23.01$, $P < 0.0001$, $R^2 = 0.26$; major group, $20 \leq t < 110$ min, $F_{1,198} = 15.28$, $P < 0.0001$, $R^2 = 0.07$; mixed group, $20 \leq t < 45$ min, $F_{1,138} = 23.63$, $P < 0.0001$, $R^2 = 0.15$).

The distribution of cluster sizes at the end of the experiment showed that the proportion of aggregated ants in clusters of 5 individuals or less did not differ between the 3 groups (Figure 3; 1-way ANOVA on arcsine-transformed data: $F_{2,28} = 1.03$, $P > 0.05$). On the other hand, the fraction of the aggregated population present in very large clusters (more than 20 ants) significantly differed between groups (Figure 3; 1-way ANOVA: $F_{2,28} = 4.36$, $P < 0.05$; Bonferroni post hoc comparisons: $P < 0.05$ only between minor and major groups). Indeed, although clusters of more than 20 minors were never observed, they were found out in half of the experiments with pure major groups, these very large clusters including up to 40 majors. In mixed groups, the distribution of cluster sizes was close to that obtained in pure groups of majors: large aggregates of more than 20 ants occurred in 40% of the experiments. In these mixed groups, small clusters including up to 5 workers were composed of $49.2 \pm 5.5\%$ of minors (number of aggregates = 48), whereas clusters of more than 5 workers included $66.9 \pm 5.8\%$ of majors (number of aggregates = 13). Although not significant (1-tailed Mann–Whitney test: $U = 227$, $n = 61$, $P = 0.068$), this trend shows the propensity of majors to aggregate into larger clusters.

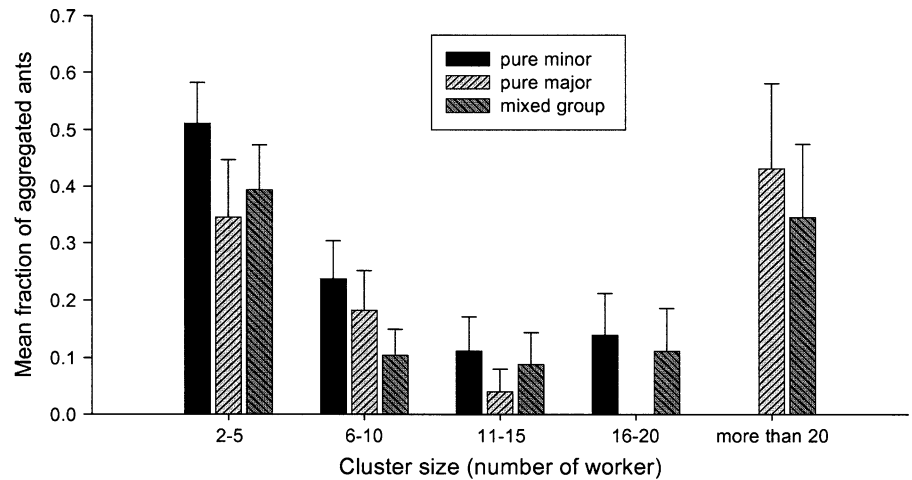
Influence of area marking on the aggregation of castes

We then addressed the potential influence of previous marking (by nest mates belonging to their own or to the other caste) on the choice of an aggregation site and its dynamics.

Concerning minors, the mean number of aggregated individuals changed over time, whereas there was no significant interaction between time and marking type (Figure 4A; 2-way repeated measures ANOVA: time, $F_{24,336} = 4.6$, $P < 0.0001$; marking type \times time, $F_{24,350} = 0.43$, $P = 0.992$). The spatial distribution of minors was significantly influenced by the type of area marking (Figure 4A; 2-way repeated measures ANOVA: marking type, $F_{1,350} = 129.73$, $P < 0.0001$). Indeed, minors preferentially stayed in the area previously marked by individuals of their own caste. However, the density of minors on the

Figure 3

Distribution of the fraction of individuals among the different cluster sizes at the end of the experiment ($t = 180$ min). Experiments were performed using pure minor colonies (11 replicates, total of aggregated ants: 318), pure major colonies (10 replicates, total of aggregated ants: 339), or mixed colonies composed of an equal number of minors and majors (10 replicates, total of aggregated ants: 345).

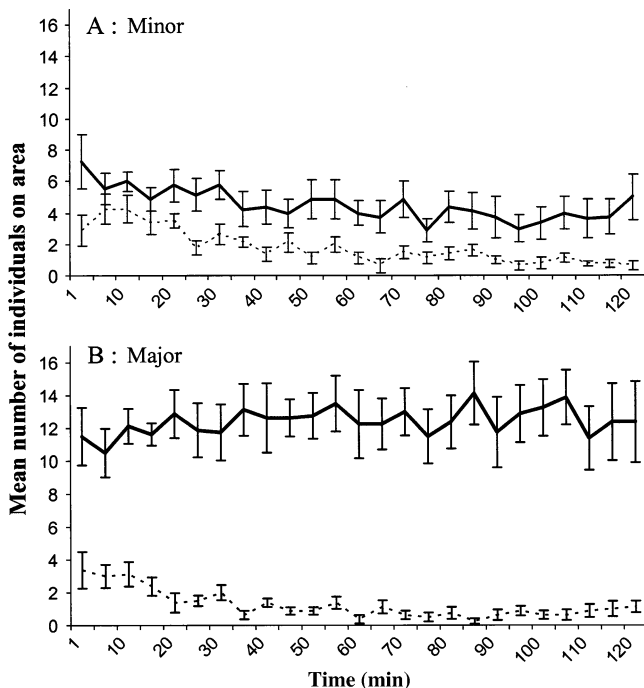


preferred marked area (0.18 ± 0.05 minors/cm² at $t = 120$ min) was very low and did not significantly differ from the expected density of a homogeneous distribution (1-sample t -test: $t_7 = 0.33$, $P > 0.05$). As usual, the aggregation level of majors was significantly higher than that of minors, the total fraction of majors in both areas ($27 \pm 14\%$ of majors at $t = 120$ min) being twice as high as that of minors ($11 \pm 8\%$ of minors) (unpaired t -test: $t_{14} = 2.856$, $P < 0.05$). Neither the time nor the interaction between time and marking type influenced the spatial distribution of majors (Figure 4B; 2-way repeated measures ANOVA: time, $F_{24,336} = 0.60$, $P > 0.05$; marking type \times time, $F_{24,336} = 1.11$, $P > 0.05$). But, interest-

ingly, majors significantly preferred to stay in the area marked by minors instead of that marked by their own caste (Figure 4B; 2-way repeated measures ANOVA: marking type, $F_{1,14} = 57.89$, $P < 0.0001$). In terms of density, the presence of majors on the area marked by minors was then 2.75-fold higher than that expected from a homogeneous distribution (0.44 ± 0.09 majors/cm²; 1-sample t -test: $t_7 = 3.18$, $P < 0.02$). This rapid and stable choice of a site (Figure 4B) shows that, for majors, chemical marks laid by minors were more efficient aggregation stimuli than their own marking.

The high efficiency of chemical cues laid by minors to induce aggregation could hide from view a weak—but nevertheless existing—influence of majors' marking on *P. pallidula* aggregative behavior. Therefore, we compared the influence of an area marked by majors versus a blank area free of any ant marks, using the same experimental procedure as described previously. The total mean number of aggregated minors significantly changed over time with no significant interaction between time and marking type (Figure 5A; 2-way repeated measures ANOVA: time, $F_{24,336} = 7.69$, $P < 0.0001$; time \times marking type, $F_{24,336} = 1.13$, $P > 0.05$). The presence of majors' area marking influenced the spatial distribution of minors (Figure 5A; marking, $F_{1,14} = 8.55$, $P < 0.05$). Indeed, after 2 h, the marked area attracted more minors than the blank, although the mean number of minors was weak and similar for both areas (Figure 5A; paired t -test: $t_{14} = 0.16$, $P = 0.88$). When majors are faced with the same choice, there was no significant effect of time nor of interaction between time and marking on their aggregation response (Figure 5B; 2-way repeated measures ANOVA: time, $F_{24,350} = 0.20$, $P = 1.00$; time \times marking type, $F_{24,336} = 0.69$, $P < 0.0001$). By contrast, there was a significant marking-type effect (Figure 5B; 2-way repeated measures ANOVA: marking, $F_{1,14} = 8.18$, $P < 0.05$). Majors were attracted by their own chemical marks, their number in this marked area being significantly higher than that in the blank at the end of the experiment (Figure 5B; paired t -test: $t_7 = 2.13$, $P = 0.04$). Thus, chemical marks deposited by majors can be perceived as a factor enhancing aggregation, but due to the weakness of this stimulus, its influence was highly variable and evidenced only for the major caste that is the most prone to aggregate.

To summarize, chemical marks deposited by minors elicited the aggregation of both minors and majors. On the other hand, chemical marks of majors had no influence on the spatial distribution of minors and a weak effect on majors which led to a diffuse majors' aggregation in the center of the arena.

**Figure 4**

Change over time of the mean number of individuals (mean \pm SE) on the area previously marked by minors (solid line) or by majors (dashed line) when (A) minors are introduced in the arena (8 replicates) and (B) majors are introduced in the arena (8 replicates).

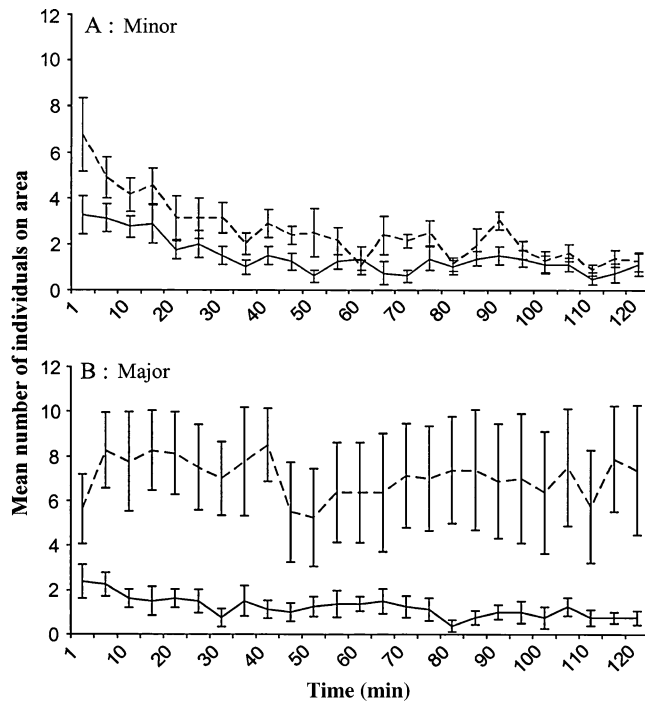


Figure 5
Change over time of the mean number of individuals (mean \pm SE) on the area previously marked by majors (dashed line) or on an unmarked area (solid line) when (A) minors are introduced in the arena (8 replicates) and (B) majors are introduced in the arena (8 replicates).

DISCUSSION

Caste-specific aggregation patterns and division of labor

One of the goals of behavioral ecology is to understand the processes regulating the distributional patterns of organisms in time and space. In insect societies, the spatiotemporal distribution of workers is extremely important in the social organization of labor. This study is a first step in the understanding of processes regulating the spatial organization of worker castes in polymorphic ant species. It emphasizes caste specificity in the aggregative behavior of *P. pallidula* minors and majors. In a homogeneous environment deprived of any pre-existing cue, the 2 worker castes differ in their localization and aggregation levels: after an initial dispersal, majors have a high propensity to aggregate into a large central cluster, whereas most minors keep on walking close to the edges of the arena. The aggregation of majors in the center of the arena could result from the initial placing of tested majors at this location. This would lead to a higher local density of area marking that, despite its weak enhancing effect on aggregative behavior, may be sufficient to nucleate and localize the cluster of majors.

The caste to which a given worker belongs has thus a determinant influence on the distribution of cluster sizes. Although only a small number of individuals (50 ants) were observed, one may expect that caste-specific aggregation behaviors would be maintained in larger groups, matching *P. pallidula* natural colonies in size. Additional tests with a much larger number of ants would be interesting to carry out and could evidence slight variations around these caste-specific aggregation schemes. For instance, the more likely presence of workers displaying very low activity level (e.g., newly hatched workers) in large populations (Aarab et al. 1990) would nucleate additional and/or larger aggregates, even in

pure groups of minors. Furthermore, changes in colony size (or workers density) that alter the rates of interactions between nest mates could influence the aggregation dynamics (e.g., by speeding it up) or the clustering patterns (e.g., emergence of several coexisting clusters instead of one majors' cluster).

A similar caste difference as that found between *P. pallidula* minors and majors was previously reported in *Leptothorax unifasciatus*, in which less active individuals occupied a central position within the nest, whereas more active ones displayed a peripheral position (Sendova-Franks and Franks 1994). Similarly, the caste specificity in *P. pallidula* aggregative behavior can be related to differences in the activity rate and mobility level of minors and majors. Indeed, *P. pallidula* minors, which represent around 90% of the population (Passera 1977), are also the most active workers as well as the most mobile both inside (Sempo and Detrain 2004) and outside the nest (Detrain and Pasteels 1991). Around 60% of minors within the nest are moving or engaged in a task performance (Sempo and Detrain 2004). On the other hand, the high aggregation of majors can be related with their low mobility and activity because a maximum of 39% of the majors were carrying out tasks or moving within the nest (Sempo and Detrain 2004).

Such intercaste differences may have ergonomic implications on the colony social organization. Both the dispersion and the high mobility of minors would allow them to find and exploit resources, to assess colony needs, and to detect risks arising from the presence of competitors and/or predators. Thus, this caste of minors appears as the most appropriate for relaying all kinds of information and for carrying out most of the colony tasks. By contrast, due to their low mobility and their high spatial localization, it is less likely for majors to encounter external or inner-nest stimuli as well as to exchange information with nest mates. Therefore, majors have a limited perception of colony needs or environmental stimuli and are less involved in colony activities, as reported for several dimorphic *Pheidole* species (Wilson 1984; Brown and Traniello 1998).

High clustering behavior of majors: functional considerations

A relevant question now arises, about the adaptive value, at the colony level, of having a major caste with a low mobility and a high propensity to aggregate that make this caste usually "disconnected" from stimuli. This specificity matches the ergonomic organization of the colony, in which the morphologically specialized caste of majors is weakly involved in daily tasks. Although majors are able to carry out almost all inner-nest tasks (Sempo and Detrain 2004), they are less efficient than minors. Regarding outside nest activities, majors are less prone to explore the foraging area (Detrain and Pasteels 1991) or to retrieve food individually (Detrain 1990) and are unable to lay a trail (Ali et al. 1988). Due to their high response thresholds, majors are selectively mobilized to forage only in case of massive recruitment to large prey or competing ants (Detrain and Pasteels 1991, 1992). Because of the high manufacturing costs of *P. pallidula* majors (higher protein requirements and longer larval development [Passera 1974]), their low involvement in external tasks could be an adaptive way to reduce their exposure to risky situations such as exploration and thus to minimize energy consumed to replace lost individuals.

Apart from their frequent involvement in a subset of tasks (Sempo and Detrain 2004), *P. pallidula* majors remain stationary and form clusters localized in sensitive nest areas, such as the entrance or the brood pile where they can either quickly

react to strong attacks (Detrain and Pasteels 1992) and to large food sources recently discovered (Detrain 1990; Detrain and Pasteels 1991) or can ensure brood protection and removal (personal observation).

In addition, replete majors involved in lipid storage (Lachaud et al. 1992) are expected to display a similar—or even higher—aggregative behavior: this could ease the food flow between nest mates with faster travel times for foragers regurgitating food and for nurses dispatching food during starvation periods.

Chemical cues as enhancers of ants' clustering

In many invertebrate and vertebrate species, the spatial localization of individuals is influenced by indirect cues of nest mates' presence (Wyatt 2003). For example, indirect cues as chemical trails and area marking are widely used during foraging activities by various animals such as gastropods (*Littorina littorea*, Edwards and Davies 2002), ants (Hölldobler and Wilson 1990), bees (Cameron 1981; Gilbert et al. 2001), Norway rats (*Rattus norvegicus*, Galef and Buckley 1996), or naked mole-rats (*Heterocephalus glaber*, Judd and Sherman 1996). These trail-laying and marking behaviors are often coupled with direct interactions between nest mates such as food sharing or tactile invitations between recruiters and recruits (von Frisch 1967; Hölldobler and Wilson 1990; Judd and Sherman 1996). In ants, apart from the spontaneous tendency for nest mates to aggregate, chemical marks can speed up clustering and can therefore facilitate the cooperation between nest mates. These enhancing marks can be deposited actively (as in recruitment trails [Hölldobler and Wilson 1990]) or passively by walking ants (e.g., footprint cuticular hydrocarbons [Yamaoka and Akino 1994; Devigne and Detrain 2002; Depickère, Fresneau, Detrain, et al. 2004]). The aggregative role of cuticular hydrocarbons is also documented in other insects (cockroaches: Rivault and Cloarec 1998; Rivault et al. 1998, and triatomine bugs: Figueiras and Lazzari 1998; Vitta et al. 2002). Our results demonstrated that, in *P. pallidula*, ground marks laid by minors—and to a far less extent those laid by majors—actually influence the cluster localization and increase the aggregation of workers. However, at this stage, we cannot make conclusions about the origin of these attractive chemical cues: they can result from exploratory trails laid by minors, the only *P. pallidula* caste to possess trail pheromone in their poison gland (Detrain et al. 1987; Ali et al. 1988), or they can be footprint cuticular hydrocarbons or other glandular secretions (e.g., Dufour gland compounds). Although preliminary chemical analysis did not detect any major difference between castes in the profile of cuticular hydrocarbons (JC de Biseau, personal communication), differences in the attractiveness of area marking could arise from quantitative differences in cuticular compounds of *P. pallidula* minors and majors (Wagner et al. 1998; Kaib et al. 2000; Bonavita-Cougourdan et al. 2001; Greene and Gordon 2003). Only elements of answers can be brought to the question why area marking laid by minors was selected as an enhancing cue for ants' clustering. Because chemical cues greatly influence the spatial and social organization of the colony, minors, which are numerically dominant and represent the most informed caste, should be ideally responsible for laying chemical compounds which act on the aggregation of nest mates. This would allow an efficient tuning of nest mates' density to actual needs. Moreover, by enhancing aggregation, area marking laid by minors may contribute to social cohesion: the higher interaction rates inside clusters could enhance the mixing of cuticular hydrocarbons as well as the setting up of the colonial odor (Gestalt colony odor) (Crozier and Dix 1979; Isingrini and Lenoir 1986; Dahbi et al. 1999).

Understanding the external and internal forces acting on individuals' aggregation is a fascinating challenge. The present study demonstrates that interattractions between individuals, either direct or indirect, determine patterns of grouping as well as their caste specificity. Aggregation patterns are also governed by the responses of ants to environmental factors. In this respect, the well-known influence of the queen or the brood pile on the spatial distribution of individuals within the nest (Franks and Sendova-Franks 1992) deserves further investigation. The range of aggregating processes is potentially wide in ants and the likely payoffs are substantial. Especially, the cues that individuals use to aggregate can be highly informative in terms of ecological as well as evolutionary issues.

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