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## Ants can sort their brood without a gaseous template

Received: 23 September 2004 / Revised: 2 August 2005 / Accepted: 4 August 2005 / Published online: 12 October 2005  
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**Abstract** A fundamental issue of collective intelligence is whether the collective pattern or process is based on environmental information that explicitly codes for it or arises through self-organization of the individuals. Sometimes, these alternatives occur together. Adaptive systems may also be capable of utilizing different types of mechanism under different conditions. Sendova-Franks et al. (Anim Behav 68:1095–1106, 2004) demonstrated evidence for a self-organization mechanism of brood sorting in the ant *Temnothorax albipennis*, where the brood are sorted in a series of bands or concentric annuli that increase in size with distance from the colony centre. The work by Cox and Blanchard (J Theor Biol 204:223–238, 2000) suggests an alternative or complementary mechanism whereby the brood pattern is specified by the template of a CO<sub>2</sub> gradient. Here, we test for a gaseous template as a necessary condition for brood sorting. Under the experimental condition, we pumped the air out of the nest continuously to prevent the accumulation of any gaseous substances. We compared the brood pattern between the experimental and control conditions according to four characteristics: mean distance from centre, mean nearest-

neighbour distance, shape and area. Under the experimental condition, the order of brood types according to the first two characteristics was the same as in the control. The area of the brood pattern was smaller, and its shape elongated under the experimental condition. As expected on the basis of these differences, mean distance from centre was greater and mean nearest-neighbour distance was smaller under the experimental condition (although not statistically significantly) and by the expected amount. We found evidence that ants avoid placing brood in the strongest airflow stream. This could explain the reduced area and elongated shape of the brood pattern under the experimental condition. We conclude that a gaseous template is not a necessary condition for brood sorting.

**Keywords** Ant · *Temnothorax* · Brood sorting · Template · Self-organization · Collective intelligence

Communicated by K. Ross

**Electronic supplementary material** Supplementary material is available in the online version of this article at <http://dx.doi.org/10.1007/s00265-005-0078-8> and is accessible for authorized users.

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### Introduction

Social insect colonies have the amazing ability to find flexible solutions to problems that are beyond the scale of an individual member's overview. Recent studies have demonstrated that such collective intelligence (Franks 1989) is exhibited in the choice of a new nest site (Dornhaus et al. 2004; Seeley and Visscher 2004), the division of labour (Sendova-Franks et al. 2002), foraging (Couzin and Franks 2003; Devigne et al. 2004), building (Franks et al. 2004), improvement of collective performance with experience (Langridge et al. 2004), worker sorting (Sendova-Franks and Van Ient 2002) and brood sorting (Sendova-Franks et al. 2004). The collective intelligence of social insects is also an inspiration in computer science (Marshall et al. 2003) and robotics (Wilson et al. 2004), where the concept of swarm intelligence is finding practical applications (Bonabeau et al. 1999) such as ant colony optimization (Dorigo and Stützle 2004).

A prime example of collective intelligence which involves the building of a structure is brood sorting in

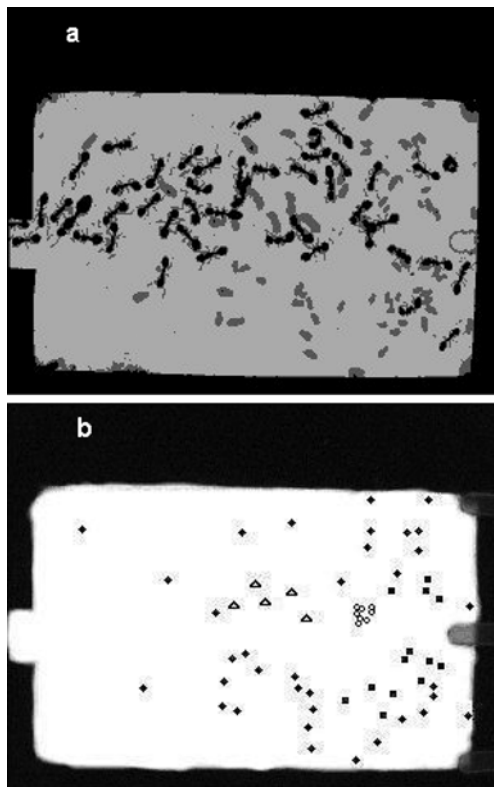
ants (Franks and Sendova-Franks 1992). *Temnothorax* ants (formerly *Leptothorax* ants; Bolton 2003) sort their brood into a series of bands or concentric annuli in order of increasing brood size. The smallest brood type, the eggs and small larvae, are near the colony centre; further outwards are the medium larvae followed by the large larvae on the periphery. The prepupae and pupae have an intermediate position usually between the medium and large larvae (Fig. 1). In addition to this order of the brood types, the pattern is characterised by the increasing space (domain of care) allocated to individual items with increasing distance from the colony centre (Franks and Sendova-Franks 1992; Fig. 1). The brood items cannot change position on their own, and any movement of the brood is a result of carrying by the adult ants. The necessity to sort the brood anew arises when a *Temnothorax* colony emigrates to a new nest site, an event that may occur frequently in the field (Partridge et al. 1997). Brood sorting continues during the everyday life of the colony, albeit at a much slower rate, as brood items change type with development and need to be moved in the appropriate position for that type in the brood pattern.

A crucial distinction, in attempting to understand the underlying mechanism of a collective intelligence phenomenon, such as brood sorting, is whether the structure is built through a process of self-organization or one of its alternatives, such as a leader, a blueprint, a recipe or a

template (Camazine et al. 2001, p. 47). A self-organization mechanism for brood sorting would involve only interactions among the individual ants, and the rules specifying these interactions would be executed using only local information without reference to the global pattern (Camazine et al. 2001, p. 8). The alternative scenario would not require interactions among the individual ants, and, in the particular case of a template, ants would use information represented in the environment as a full-size guide that specifies the whole pattern (Camazine et al. 2001, p. 49). These alternatives are not mutually exclusive. They could act in combination, as, for example, in the building of a perimeter nest wall in *Temnothorax* ants (Franks and Deneubourg 1997) or the royal chamber in termites (Bonabeau et al. 1998) where the spatial position of building is guided by a template but the building itself is a self-organized process.

Recently, Sendova-Franks et al. (2004) demonstrated evidence that brood sorting in *Temnothorax* is a self-organized process consisting of two phases. This process involves the following algorithm for individual ant behaviour. In the first phase, termed clustering, an ant is more likely to pick up a brood item of any type; the more isolated it is and more likely to put it down, the more brood there is in the immediate neighbourhood. In the second phase, termed spacing, an ant attempts to tend the brood, and, if the item does not have enough space around it to allow tending, the ant picks it up, carries it in a random direction for the duration of the giving-up time specific for its type and puts it down in the first available space. The giving-up time for carrying an item is an inverse function of the weight for its type. Therefore, larger brood items are carried for shorter periods. However, they are moved on straighter paths. Since larger brood items have bigger domains of care (Franks and Sendova-Franks 1992), i.e. they require more space to allow tending, they are picked up more often and are displaced further from the colony centre than smaller brood items.

Although the self-organization mechanism proposed by Sendova-Franks et al. (2004) could sort the brood, this in itself does not preclude the possibility that, for example, in the clustering phase, ants rely on the source of a template as a pivot for clustering and, in the spacing phase, they rely on the template itself as an indicator of the relative positions of different brood types. A candidate for such a template exists. It is gaseous substances emanating from the queen, the workers and the brood. Cox and Blanchard (2000), for example, suggested that there might be a CO<sub>2</sub> gradient inside the nest with the highest concentration in the colony centre and the lowest at the edges. Ants can sense tiny changes in CO<sub>2</sub> levels (Kleineidam et al. 2000), and it is reasonable to suppose that *Temnothorax* can treat the CO<sub>2</sub> gradient as a position-fixing template. The colony members could act as a source of CO<sub>2</sub>, which diffuses from the colony centre outwards. It could be the case that ants associate the positions of different types of brood with particular concentrations of CO<sub>2</sub> along its gradient so that the smallest items are associated with the highest concentration and the largest items are associated with the lowest



**Fig. 1** The sorted brood pattern of a *T. albipennis* colony: **a** a video frame of a colony inside the experimental nest, **b** the mapped point pattern of the brood in **a**, where each point represents the *x*- and *y*-coordinates of the item's centre; ○ eggs and small larvae, ■ medium larvae, ◆ large larvae, △ pupae

concentration. A worker would pick up a brood item if it is in an area of lower or higher concentration than the one appropriate for its type and drop it when the concentration is at the appropriate level. The two antennae would allow workers to turn in the direction of higher concentration (Couzin and Franks 2003). The same logic could apply to other gaseous substances released by the colony members. Such a template-based mechanism could also lead to the sorted brood pattern described earlier.

The aim of the present study is to find out whether a gaseous template is a necessary condition for brood sorting to occur. Our approach is to compare the brood patterns of colonies under normal conditions with their brood patterns when the air is being pumped continuously through the nest to prevent any gas from accumulating and any potential gaseous template from being established.

We compare the brood pattern under the two conditions according to four characteristics: (1) mean distance from centre, (2) mean nearest-neighbour distance, (3) shape and (4) area of the whole pattern.

When air is pumped through the nest continuously, we expect that if ants rely on the source of a gaseous template as a pivot for clustering, they would fail to cluster the brood, abandon the new nest or fail to emigrate altogether. If ants rely on the gaseous template itself to indicate the relative positions of different brood types, we expect that the brood would not be sorted according to either mean distance from centre or mean nearest-neighbour distance.

## Methods

### Experiments

#### *Procedure for brood pattern experiments*

The experiments took place in the period from 26 July to 4 September 2002 and involved five *Temnothorax albipennis* colonies collected from Dorset, England, in the spring of the same year. Each colony underwent a 48-h treatment under the experimental condition and a 48-h treatment under the control condition. The treatment of each colony under the control condition followed within 48 h of its treatment

under the experimental condition. Replications were carried out sequentially, and the five colonies were allocated to replication numbers at random (Table 1).

Both the experimental and the control condition involved an emigration to a new nest site (Sendova-Franks and Franks 1995a) within a plastic experimental arena with dimensions 22×22×5 cm and inside walls coated in Fluon. To induce each colony to emigrate, we removed the roof (the top microscope slide) of its current nest (Sendova-Franks and Franks 1995a). The current nest was of the standard type (Fig. 2a). We then placed this old nest without its roof and the removed roof (with any brood or ants clinging to it) side by side on the experimental arena next to a new intact nest for the ants to occupy. The new intact nest was of the experimental type (Fig. 2b). This manipulation marked the beginning of the 48-h treatment under the experimental condition. A similar procedure with the current and new experimental nest marked the beginning of the 48-h treatment under the control condition.

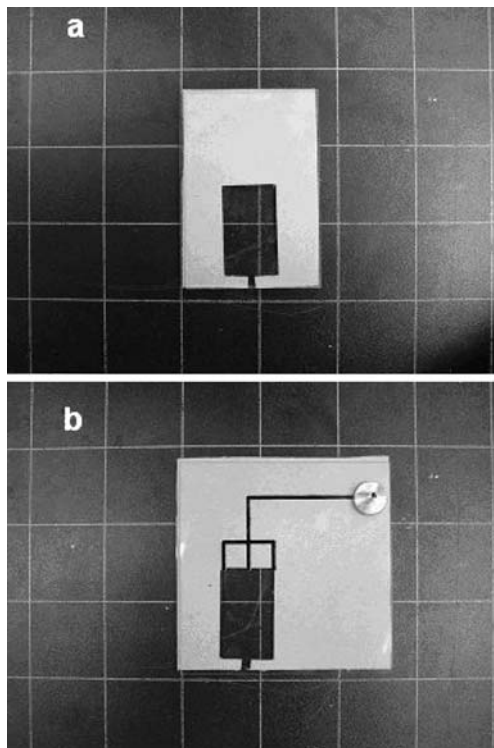
The standard nest comprised a 35×21×0.8-mm chamber and 5×4×0.8-mm entrance tunnel cut into cardboard sandwiched between two 76×51×1-mm microscope slides (Fig. 2a). The experimental nest had a chamber and entrance tunnel of the same dimensions as the standard nest, but they were cut into cardboard sandwiched between two larger microscope slides, 76×76×1 mm (Fig. 2b). Using a diamond drill bit, we scored three 1.5-mm-wide grooves into the top slide of the experimental nest (Fig. 2b). The grooves led to an aluminium connector (Fig. 2b) that joined the nest to an 8-mm-long silicon tubing attached to a pump.

Under the control condition, the pump was switched off. Under the experimental condition, the pump was switched on. The air pumped into the scored grooves, passed through the nest and exited through the entrance tunnel opposite (Fig. 2b). We measured the speed of air movement at the beginning and end of treatment under the experimental condition of each of the five colonies by placing a hot-wire anemometer at the outside opening of the nest entrance tunnel. The median of these ten-speed values was 0.25 m/s and the range was 0.23–0.28 m/s. Since the area of the outside opening of the nest entrance tunnel was 3.20 mm<sup>2</sup>, the airflow rate there was 800 mm<sup>3</sup>/s (3.20 mm<sup>2</sup>×250 mm/s). This in turn means that the air in the 604-mm<sup>3</sup> volume of the

**Table 1** The number of brood items of each type in each of the five experimental colonies under the experimental (E) and control (C) conditions

Brood type	Colony									
	16		6		9		2		15	
	E	C	E	C	E	C	E	C	E	C
Small larvae	9	13	11	21	4	7	12	10	17	31
Medium larvae	7	15	24	22	15	9	37	37	19	24
Large larvae	5	4	10	15	14	13	5	8	4	7
Prepupae	0	0	0	0	0	0	0	0	0	0
Pupae	12	6	4	5	1	0	0	0	0	0
Total	33	38	49	63	34	29	54	55	40	62

Colonies are ordered from left to right according to the experimental replication sequence



**Fig. 2** **a** A standard nest. **b** An experimental nest: the 1.5-mm-wide grooves scored into the top glass slide have been traced with *black ink* on the picture for better visibility

nest ( $588 \text{ mm}^3$  for chamber and  $16 \text{ mm}^3$  for entrance tunnel) was completely replaced approximately every 0.8 s.

At the end of the 48-h treatment, under each of the experimental and control conditions, we made a record of the brood pattern for each colony. We used a Sony XL1s DV camcorder mounted on a copy stand above the experimental arena. The camera's field of view included the nest cavity and a small part of the surrounding walls. To eliminate shadows over the brood pattern, the experimental nest was illuminated from underneath by two 9-W fluorescent lamps contained within a plastic box under the experimental arena. The lamps were cooled with a super-low-noise fan that produced minimal vibrations. We stored the digital images on the hard drive of a PC with Adobe Premier 6 via a FireWire card connection to the camera. We generated a mapped point pattern of the brood for each of the five colonies under each of the two conditions. Using Scion Image Beta 4.02 for Windows 95 to XP (<http://www.scioncorp.com>), we recorded the  $x$ - and  $y$ -coordinate of the centre and the type (see "Introduction") of each brood item in the pattern.

#### *Procedure for nest airflow pattern experiments*

To test the hypothesis that under the experimental condition (pump switched on), workers placed the brood in the part of the nest that was least disturbed by the airflow, we employed five nest designs: (1) the design used in the brood pattern experiments above (Fig. 2b) and (2) four additional

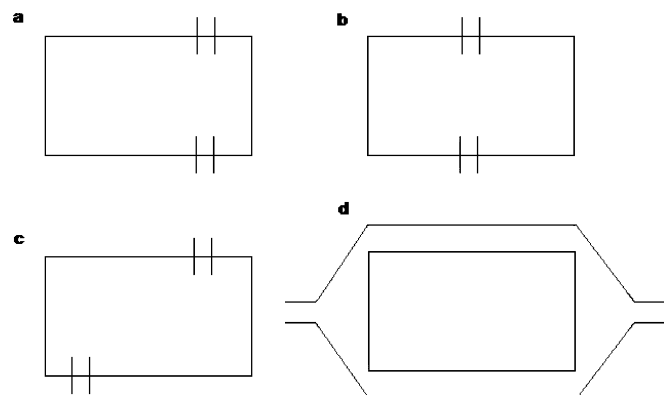
nest designs (Fig. 3). With the latter four designs, we aimed to channel the air stream through (a) one side of the nest (Fig. 3a), (b) the middle of the nest (Fig. 3b), along the diagonal of the nest (Fig. 3c) and (d) uniformly along the whole nest (Fig. 3d). First, we prompted a different colony to emigrate in each of the four additional nest designs under the experimental condition (as opposed to the control condition) described in the subsection on the brood pattern experimental procedure above. A record of the brood pattern in each of these four nest designs was taken after 48 h. Such a record was already available for the original nest design used in the brood pattern experiments.

Second, after the inhabiting colonies had emigrated back into their standard nests, we submerged the empty nests of five different designs in water and pumped red food dye through the bottom (Fig. 3a–c), right (Fig. 3d) or left (Fig. 7a) air inlet(s) using a hand-held syringe. The ensuing flow patterns were recorded using Adobe Premiere 6 and then played back in slow motion. For each nest design, we selected the frame near the beginning of this record that demonstrated where the flow was the strongest. We then superimposed this dye frame with the frame taken 48 h after the ants emigrated into the nest for each of the five designs.

#### Analysis

##### *Mean distance from centre and mean nearest-neighbour distance*

The centre of the brood pattern was defined as the mean  $x$ - and  $y$ - coordinates of the eggs and small larvae (the colony centre in the study of Sendova-Franks and Franks 1995b). The eggs and small larvae form the biological but not



**Fig. 3** Plan of the four additional nest designs for the nest airflow pattern experiments (in addition to the original nest design for the brood pattern experiments; Fig. 2b). In all cases, the *rectangle* represents the nest chamber cut into cardboard. In **a**, **b** and **c**, the *short, parallel lines* indicate the positions of the air inlet and outlet scored in the top microscope slide of the nest. The inlet is at the *bottom* and the outlet at the *top* of the page. In **d**, the *hexagonal shape* surrounding the *rectangle* of the nest chamber is scored in the top slide of the nest together with the inlet on the right side and the outlet on the left side of the page

necessarily the geometric centre of the brood pattern (e.g. in cases where bands are formed rather than concentric circles) and represent a logical anchor for describing the pattern. Three brood types, eggs and small larvae (henceforth collectively referred to as small larvae), medium larvae and large larvae were included in the analysis. Of the remaining two types, prepupae and pupae, only pupae were present in the experimental colonies and then in only half of the treatment replications (Table 1). These two brood types only occur during a short period of the year and, due to their scarcity, were not included in the analysis.

For each of the two response variables, mean distance from centre and mean nearest-neighbour distance, we had 30 observations (two levels of the factor condition  $\times$  three levels of the factor brood type  $\times$  five replicates/blocks/colonies). To correct heteroscedasticity and skewness, we applied the transformation  $X' = \sqrt{X + 0.5}$  to the response variable mean distance from centre and the transformation  $X' = \log(X + 1)$  to the response variable mean nearest-neighbour distance (Zar 1999, p. 275). We modelled each of these two response variables with a general linear model (GLM) based on a split-plot design (Cochran and Cox 1957, p. 293), where condition was the whole-block factor because each level of this factor was tested separately within a block/colony and brood type was the split-block factor because all three levels of this factor were tested simultaneously within the same block/colony. We implemented the models in Minitab under the GLM function with the five terms: (1) colony (random factor), (2) condition, (3) interaction between colony and condition, (4) brood type and (5) interaction between condition and brood type. The third term, interaction between colony and condition, represented Error A in the split-plot design model (Cochran and Cox 1957, p. 298; method checked against the numerical example in the study of Cochran and Cox 1957, pp. 299–301).

### Shape and area

From biological considerations, the shape of the brood pattern under both experimental and control conditions is best modelled by a planar region with a smooth boundary. For geometric simplicity, an elliptical disc is appropriate. However, the data did not meet the assumptions of normality and unimodality implicit in the bivariate normal ellipse model (White and Garrott 1990). We therefore estimated area by approximating the elliptical disc by a minimum convex polygon (MCP) (Kenward and Hodder 1996), with its centre calculated as the harmonic mean of all brood positions in the pattern.

We estimated the area  $A$  of brood patterns of colonies under experimental and control conditions [the number of items in each of the five pairs of brood patterns ranged from 29 to 63 (Table 1), but only small, medium and large larvae were included in the analyses] with the Ranges V software (Kenward and Hodder 1996). For robustness of the estimates, we used the areas of the 50% MCP, comprising the

50% of the data closest to the MCP centre. We also calculated the median distance of brood positions from the MCP centre, i.e. the maximum distance from the centre for the data included in the 50% MCP. This was our measure of the semi-major axis  $a$  of the ellipse approximated by the MCP. Our measure of the semi-minor axis  $b$  of the ellipse was calculated using the equation,  $A = \pi ab$ , where  $A$  is the area of the elliptical disc.

Our aim was to check whether any differences between the control and experimental conditions with regard to the observed mean distance from centre,  $\bar{D}_c$ , and the observed mean nearest-neighbour distance,  $\bar{D}_{nn}$ , could be expected simply on the basis of differences in the area and shape of the brood pattern under the two conditions. For this purpose, we calculated the expected mean values for distance from centre,  $\bar{D}'_c$ , and nearest neighbour distance,  $\bar{D}'_{nn}$ , for each of the five colonies under each of the two conditions on the basis of the observed value of the area of the brood pattern,  $A = \pi ab$ , and the measure of its shape, the ratio  $R = a/b$ . The number of brood items,  $m$ , was an additional parameter required for the calculation of  $\bar{D}'_{nn}$ . We then compared the percentage difference between  $\bar{D}_c$  for control and  $\bar{D}_c$  for experiment with the percentage difference between  $\bar{D}'_c$  for control and  $\bar{D}'_c$  for experiment. Similarly, we compared the percentage difference between  $\bar{D}_{nn}$  for control and  $\bar{D}_{nn}$  for experiment with the percentage difference between  $\bar{D}'_{nn}$  for control and  $\bar{D}'_{nn}$  for experiment.

We calculated  $\bar{D}'_c$ , expressed as a function of  $A$  and  $R$ , with the formula:

$$\bar{D}'_c(A, R) = \frac{4}{3} \sqrt{\frac{AR}{\pi^3}} E\left(\frac{\sqrt{R^2 - 1}}{R}\right)$$

(see S1 for derivation).

To calculate  $\bar{D}'_{nn}$ , we made use of the following formula for  $m$  points chosen at random in a bounded convex planar region ( $R$ ) of area ( $A$ ):

$$\bar{D}_{nn} = \frac{1}{2} \sqrt{\frac{A}{m}} + \frac{L}{m} \left( p + \frac{q}{\sqrt{m}} \right),$$

where  $L$  is the length of the boundary of  $R$  and  $p = 0.051$  and  $q = 0.042$  are constants evaluated through simulation (Donnelly 1978; Diggle 1983, p.17). This formula is valid for areas with relatively smooth boundaries (Donnelly 1978) as is the case in the present study. Expressed in terms of the parameters  $A$  and  $R$ ,  $\bar{D}'_{nn}$  is:

$$\bar{D}'_{nn}(A, R, m) = \frac{1}{2} \sqrt{\frac{A}{m}} + \frac{4}{m} \sqrt{\frac{AR}{\pi}} \left( p + \frac{q}{\sqrt{m}} \right) E\left(\frac{\sqrt{R^2 - 1}}{R}\right)$$

(see S2 for derivation).

All computations of expected values were carried out with *Maple8*.

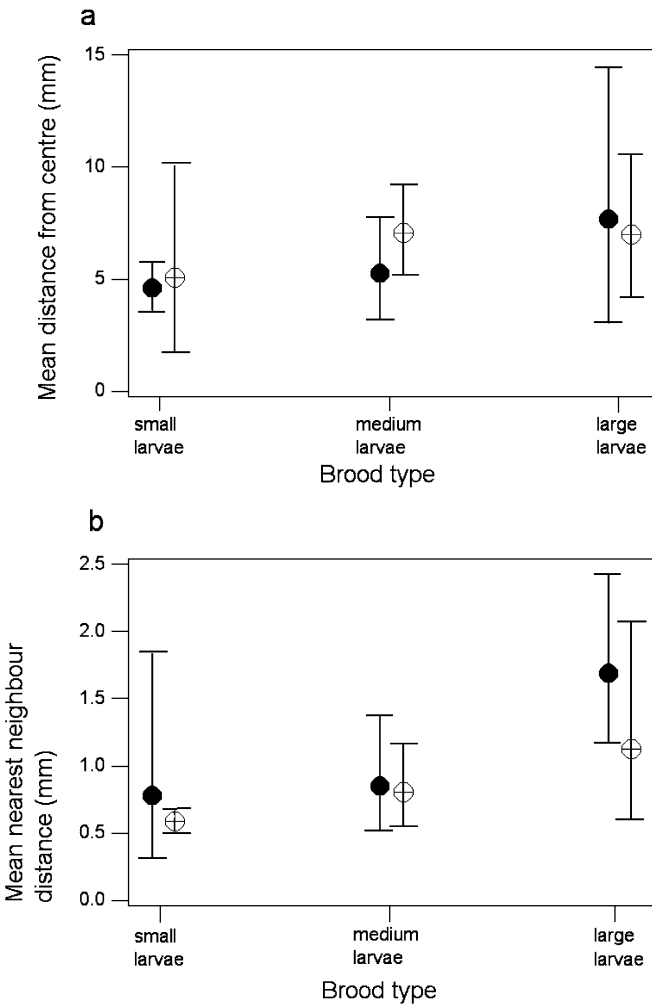
**Results**

Brood pattern experiments

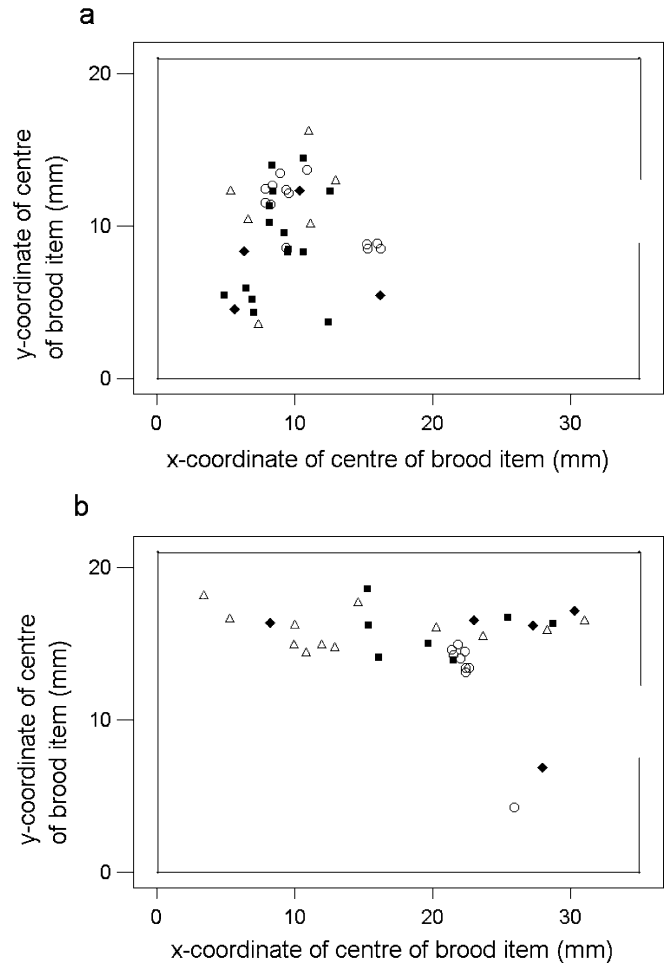
All five experimental colonies emigrated to the new nest, and none attempted to abandon it after the emigration under experimental conditions.

*Mean distance from centre and mean nearest-neighbour distance*

We found no difference between the control and experimental conditions in the relative order of the three brood types with regard to  $\bar{D}_c$  (no interaction between brood type and condition  $F_{2,16}=0.79, P=0.472$ ; Fig. 4a). Over both conditions, the brood were in the sorted order: small larvae closest to the centre and large larvae closest to the periphery (main effect of brood type  $F_{2,16}=3.37, P=0.060$ ). The sig-



**Fig. 4** Interaction plots for the two factors brood type and condition: **a** mean distance from centre (mm), mean with 95% confidence intervals; **b** mean nearest-neighbour distance (mm), mean with 95% confidence intervals; ● control, ○ (with a cross) experiment



**Fig. 5** An example of the mapped point pattern of the brood under **a** the control condition and **b** the experimental condition, both patterns are for colony 16. The black lines indicate the walls of the nest and the opening to the right represents the exit; ○ eggs and small larvae, ■ medium larvae, ◆ large larvae, △ pupae

nificant effect of brood type was entirely accounted for by the significant difference in  $\bar{D}_c$  between small and large larvae (Tukey simultaneous tests small vs large larvae,  $T=2.59, P=0.049$ ; small vs medium larvae,  $T=1.41, P=0.361$ ; medium vs large larvae,  $T=1.18, P=0.479$ ). We found no difference in  $\bar{D}_c$  between the control and experimental conditions over all brood types (no main effect of condition:  $F_{1,4}=1.04, P=0.365$ ).

We found no difference between the control and experimental conditions in the relative order of the three brood types with regard to  $\bar{D}_{nn}$  (no interaction between brood type and condition  $F_{2,16}=0.73, P=0.499$ ; Fig. 4b). Over both conditions, the spacing between items was in the correct relative order for a sorted brood pattern (Franks and Sendova-Franks 1992): small larvae closest to neighbours, large larvae furthest from neighbours (main effect of brood type  $F_{2,16}=11.99, P=0.001$ ). The significant effect of brood type was accounted for by the significant difference in  $\bar{D}_{nn}$  between small and large larvae as well as between medium and large larvae (Tukey simultaneous tests small vs large larvae,  $T=4.74, P<0.001$ ; small vs medium larvae,  $T=1.31,$

$P=0.410$ ; medium vs large larvae,  $T=3.43$ ,  $P=0.009$ ). We found no difference in  $\bar{D}_{nn}$  between the control and experimental conditions over all brood types (no main effect of condition  $F_{1,4}=2.14$ ,  $P=0.218$ ).

### Shape and area

The shape of the brood pattern under the experimental condition was elongated and approximately elliptical in comparison to the approximately circular disc shape of the brood pattern under the control condition (Fig. 5). This is confirmed by the higher value of the ratio  $R$  for the brood pattern under the experimental condition compared to the control condition in all colonies (Fig. 6a). Furthermore, the area of the brood pattern in all colonies was smaller under the experimental condition than under the control condition (Fig. 6a).

The mean value for  $\bar{D}'_c$  was 2.709 mm under experimental conditions and 2.606 mm under control conditions (Table 2). Therefore,  $\bar{D}'_c$  under experimental conditions was 3.802% greater than under control conditions. This is qualitatively the same and quantitatively comparable to the observed values where  $\bar{D}_c$  under experimental conditions (6.320 mm) was 8.908% greater than under control conditions (5.757 mm). See also Fig. 4a.

The mean value for  $\bar{D}'_{nn}$  was 0.616 mm under experimental conditions and 0.752 mm under control conditions (Table 2). Therefore,  $\bar{D}'_{nn}$  under experimental conditions

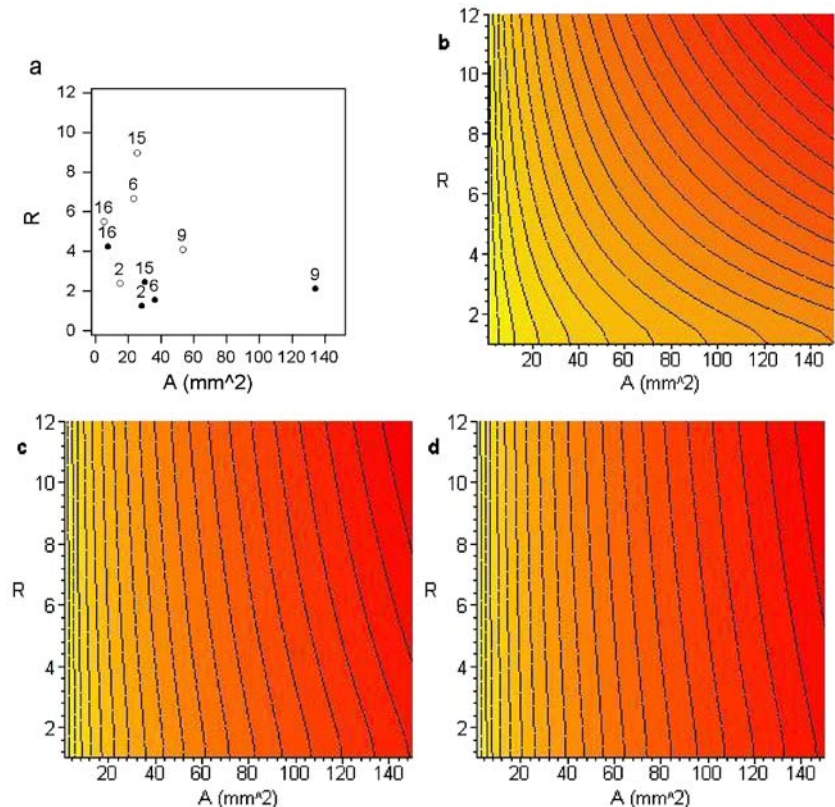
was 22.078% smaller than under control conditions. This is qualitatively the same and quantitatively comparable to the observed values where  $\bar{D}_{nn}$  under experimental conditions (0.811 mm) was 28.237% smaller than under control conditions (1.040 mm). See also Fig. 4b.

Therefore, differences in mean distance from centre and mean nearest-neighbour distance for the brood pattern under the control and experimental condition could be expected simply on the basis of differences in the area and shape of the brood pattern. Indeed, the observed differences, although not statistically significant, had the same directions and comparable magnitudes to those of the respective expected differences.

To get a better feel for the geometrical context of these results, we examined the behaviour of the functions  $\bar{D}'_c(A, R)$  and  $\bar{D}'_{nn}(A, R, m)$ , the latter for a fixed  $m$ , in a biologically relevant region of  $(A, R)$  parameter space. We considered the region of parameter space defined by  $1 \text{ mm}^2 < A < 150 \text{ mm}^2$  and  $1 < R < 12$  (Fig. 6b–d), these limits being chosen on the basis of the values for our five experimental colonies (Fig. 6a). We considered two values for the number of items,  $m=11$  and  $m=31$ , representing the two extreme values in our data (Table 2).

$\bar{D}'_c$  increases with both increasing area and increasing  $R$  ratio (Fig. 6b). As stated earlier, there was a difference between the two conditions in both area and  $R$  ratio: in all experimental colonies, the area of the brood pattern was greater and its  $R$  ratio was smaller under the control condition than under the experimental condition (Fig. 6a). Therefore, if the difference in the  $R$  ratio was higher in

**Fig. 6** **a** Relationship between area ( $\text{mm}^2$ ) and  $R = \frac{a}{b}$  for the brood patterns in the five experimental colonies; *numbers* above the points represent colony identity number,  $\bullet$  value under control condition,  $\circ$  value under experimental condition; **b** contour plot of  $\bar{D}'_c(A, R)$ , values for the expected mean distance from *centre* increase with successive contours from the *left side* to the *upper right corner*; **c** contour plot of  $\bar{D}'_{nn}(A, R, 11)$ , values for the expected mean nearest-neighbour distance increase as in **b** above; **d**  $\bar{D}'_{nn}(A, R, 31)$ , interpretation of contours as in **c** above



**Table 2** Expected mean distance from centre,  $\bar{D}'_c(A, R)$ , and expected mean nearest-neighbour distance,  $\bar{D}'_{nn}(A, R, m)$ , for the brood patterns of the five colonies under the experimental (E) and control (C) conditions (mm)

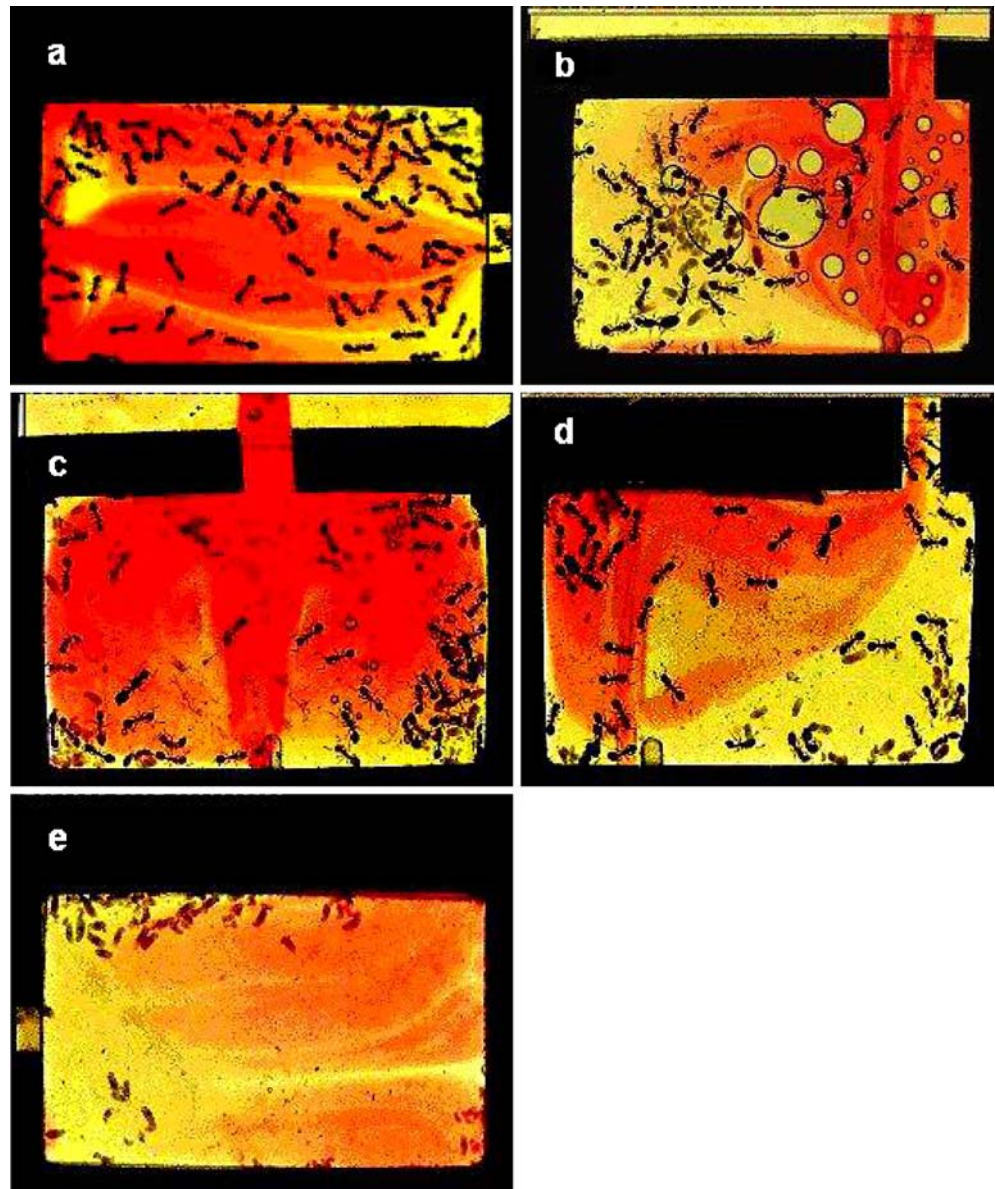
Colony	$\bar{D}'_c(A, R), E$	$\bar{D}'_c(A, R), C$	$\bar{D}'_{nn}(A, R, m), E$	$m_E$	$\bar{D}'_{nn}(A, R, m), C$	$m_C$
16	1.333	1.444	0.416	11	0.395	16
6	3.084	2.348	0.579	23	0.603	29
9	3.785	4.837	1.014	17	1.685	15
2	1.650	2.017	0.403	27	0.542	28
15	3.694	2.382	0.670	20	0.537	31
Mean	2.709	2.606	0.616		0.752	

See “Methods” for details

magnitude than the difference in area,  $\bar{D}'_c$  was greater under the experimental condition (e.g. colony 15; Fig. 6a,b). Conversely, if the difference in area was higher in magnitude than the difference in the  $R$  ratio,  $\bar{D}'_c$  was greater under the control condition (e.g. colony 9; Fig. 6a,b).

$\bar{D}'_{nn}$  increases predominantly with increasing area (Fig. 6c,d). The importance of the  $R$  ratio diminishes very quickly for values of  $m$  up to approximately 20 items, leading to only small differences in the plots for  $m=11$  and  $m=31$  (Fig. 6c,d). Hence, greater area generally means a

**Fig. 7** Photographs of nest airflow pattern experiments for the **a** original experimental nest design, **b–e** the four additional nest designs: nest design 1, see Fig. 3a; nest design 2, see Fig. 3b; nest design 3, see Fig. 3c; nest design 4, see Fig. 3d. In **a**, the airflow is directed from the left to the right, in **b**, **c** and **d**, from the bottom to the top, and in **e**, from the right to the left



greater  $\bar{D}'_{nn}$ . Since in all colonies, the area was greater under the control condition,  $\bar{D}'_{nn}$  also tended to be greater under the control condition.

Finally, we remark that qualitatively similar results to those above hold when the region is taken to be a rectangle, rather than an elliptical disc, but we do not give the details here.

### Nest airflow pattern experiments

The visualisation of the airflow pattern in relation to the brood pattern inside the original experimental nest design and the four additional nest designs (Figs. 2b, 3 and 7) supports our hypothesis that the elongated shape of the brood pattern under the experimental condition results from the ants' avoidance of placing brood items in the air stream. As expected according to this hypothesis, the ants placed their brood patterns in the areas of lowest airflow, as illustrated by the lowest concentration of dye (Fig. 7).

With the original experimental nest design, we aimed to minimise the areas in the nest with lower airflow. These areas were between the three jets and near the wall close to the exit (Fig. 7a). That is where the ants placed their brood (Figs. 5b and 7a). The first of the additional nest designs (Fig. 3a) aimed to channel the air stream through one side of the nest, leaving the main nest area with a very low rate of flow. As expected, the workers placed their brood in the main nest area where the flow rate was lowest. There was a very good overlap between the area of least flow and the area of the highest brood density in the superimposed images of the dye and the ants (Fig. 7b).

The second design (Fig. 3b) aimed to split the brood into two clusters, one on each side of the air stream where the flow rate was lower. As expected, there was a very good overlap between the position of the brood and the lowest concentration of dye (Fig. 7c). We expected the third design (Fig. 3c) to lead to a similar split of the brood into two clusters as in the second design (Fig. 7c) but with one cluster into each of the diagonally opposite NW and SE corners (Fig. 7d). Indeed, this was the case. The cluster in the NW corner was still on the left-hand side of the main jet, although the dye spread quickly both to the left and to the right. The final, fourth, additional design (Fig. 3d) aimed to create as near uniform flow along the nest as possible by placing the whole nest area inside the air stream. Even though the difference was negligible, the point of least flow was in the corners on either side near the outlet. This was where the brood were predominantly placed (Fig. 7e).

## Discussion

Our results demonstrate that a gaseous template is not a necessary requirement for brood sorting to occur in the ant *T. albipennis*. We pumped the air through the nest continuously under the experimental condition to prevent any potential gaseous template from accumulating. Never-

theless, not only did the ants emigrate without any problems but the brood were also sorted within 48 h. The small larvae were closest to the centre of the pattern. The medium larvae were further outwards followed by the large larvae on the periphery. The small larvae were closest to neighbours and the large larvae were the furthest.

The only difference between the control and experimental conditions was in the shape and area of the brood pattern. Under the experimental condition, the area was smaller and the shape was elongated compared to the circular disc under the control condition. On the basis of these differences in shape and area, the theoretical expectation was that mean distance from centre should be greater and mean nearest-neighbour distance should be smaller under the experimental condition. This was indeed the case, and the observed magnitudes of these differences (although not statistically significant) were comparable to their expected values. Therefore, although the airflow did reduce the area and elongate the shape of the brood pattern, there was no evidence that it disrupted the underlying mechanism of sorting. Consequently, even if a gradient of CO<sub>2</sub>, or any other gaseous substance produced by the colony members, exists inside the nest and even if ants use it for brood sorting under normal conditions, there was no evidence that they are not able to sort their brood in the absence of such a template.

Our result that *Temnothorax* ants could sort their brood in the absence of a template lends support to the idea that they use a self-organization mechanism such as the one proposed by Sendova-Franks et al. (2004). Such a mechanism could explain the elongation of the shape and the reduction in the area of the brood pattern we found under the experimental condition. Under the experimental condition in the present study, the brood were placed parallel to a hypothetical line drawn between the air inlet and the outlet, which suggests that the ants did not find the airflow favourable. Nevertheless, not a single one of our colonies attempted to escape the airflow by abandoning the experimental nest. Instead, each and every one of them placed their brood in the area of the nest where the airflow rate was the lowest. Our interpretation is that in the second phase of sorting (spacing) under the experimental condition, ants dropped brood items according to the giving-up time for their type as suggested by Sendova-Franks et al. (2004) but not beyond the line of the air stream. Thus, under the experimental condition, the ants effectively moved their brood predominantly along a line corresponding to the orientation of the airflow. This in turn led to the elongated shape and smaller area of the brood pattern when the air was pumped through the nest. As expected, when the ants had a smaller and elongated area in which to sort their brood, items were placed on average further from the centre and closer to one another.

Our result that ants are able to sort their brood in the absence of a template is analogous to the result from another study of sorting phenomena in ants. Backen et al. (2000) found that the presence of a potential template, such as the brood pattern or all the workers, is not a necessary condition for worker sorting in *Temnothorax* ant colonies. Sendova-

Franks and Van Ient (2002) proposed self-organization mechanisms to explain the phenomenon of worker sorting.

We cannot conclude, however, that ants do not use a template for brood sorting under any circumstances. We have only demonstrated that they do not rely on it in brood sorting associated with colony emigration. A template is not a necessary condition, but it may be a sufficient condition for brood sorting under certain circumstances. For example, when more than half of the brood are already sorted inside the nest, they themselves could act as a template for any additional items that are brought later into the nest. However, this may be difficult to distinguish experimentally from a sieving process, for example, whereby smaller items are more likely to percolate through to the centre of the pattern.

A gaseous template and a CO<sub>2</sub> one in particular (Cox and Blanchard 2000) is very likely to exist in *T. albipennis* ant nests. It would be surprising and illuminating if ants have not evolved mechanisms to take advantage of such information available to them.

Given the amazing ability of social insects to solve problems under many types of condition, it is quite possible that they can use more than one mechanism for solving a particular type of problem (Blazis 2002), and, if the conditions for one are absent, they could use an alternative. This is another yet unexplored area of collective intelligence, which could hide yet unsuspected lessons of adaptability for artificial systems such as software and robotic agents.

**Acknowledgements** We thank Antony Alexiev, Anna Dornhaus, Nigel Franks, Elizabeth Langridge, Francois-Xavier Dechaume Moncharmont and Tom Richardson for helpful suggestions and discussions. ASF, SS and CM gratefully acknowledge the EPSRC for support. All experiments carried out in this study comply with the current law in the UK.

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