REVIEW

When social behaviour is moulded in clay: on growth and form of social insect nests

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ABSTRACT

The nests built by social insects are among the most complex structures produced by animal groups. They reveal the social behaviour of a colony and as such they potentially allow comparative studies. However, for a long time, research on nest architecture was hindered by the lack of technical tools allowing the visualisation of their complex 3D structures and the quantification of their properties. Several techniques, developed over the years, now make it possible to study the organisation of these nests and how they are built. Here, we review present knowledge of the mechanisms of nest construction, and how nest structure affects the behaviour of individual insects and the organisation of activities within a colony.

KEY WORDS: Collective animal behaviour, Nest architecture, Nest building, Ants, Termites, Stigmergy, Self-organisation

Introduction

Animal groups often exhibit various forms of collective activities such as synchronisation, coordinated motion or collective decision making (reviewed in Camazine et al., 2001; Sumpter, 2010). All these collective activities depend upon information flowing across the group. Information can be shared in several different ways depending on the group considered: examples include vocal calls, and visual and chemical signals or cues.

In the case of social insects, the collective activities of a colony often result in the formation of complex physical structures such as networks of trails (Perna and Latty, 2014; Czaczkes et al., 2015), shelters (Anderson and McShea, 2001) and, most notably, nests (Hansell, 2005; Grassé, 1984). These structures are not simply the by-product of animal interactions, because they also mediate the flow of information that is required for the building of the nest itself, in a form of indirect communication known as stigmergy (Grassé, 1959; see below). Because of these properties, nest building in social insects has long been considered an example of self-organisation in nature and its understanding can potentially also inform us on other phenomena of biological self-organisation.

If we try to order animals according to their ability to build large and complex structures, we will probably find out that social insects such as ants and termites – and not our close phylogenetic relatives, the great apes – are the closest followers of talented human architects (Hansell and Ruxton, 2008).

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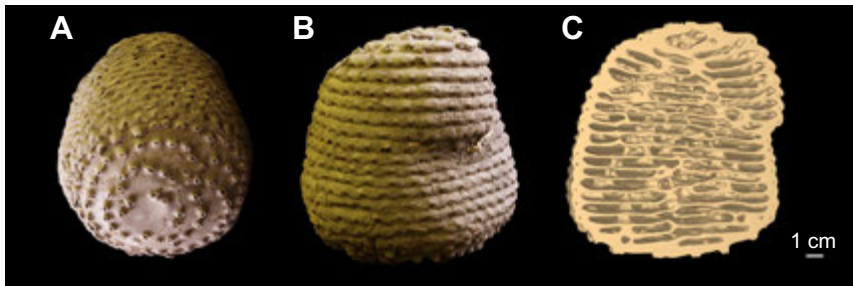
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Termites of the genus *Apicotermes* provide examples of the high level of architectural complexity achieved by termites, of which they probably represent one extreme in terms of regularity and symmetry of features (Fig. 1). These termites, which live in African Savannahs and forests, build relatively small nests that are difficult to spot, as the nest itself and all the galleries used by the termites in their movements for foraging are completely underground. Over the outer surface of these nests, there is a series of regularly spaced pores that supposedly ensure air conditioning and gas exchange with the outside environment. Inside the nests, which are about 20–40 cm high, are a succession of large chambers stabilised by pillars and connected by both direct passages and helicoidal ramps (see Fig. 1; Desneux, 1952, 1956; Schmidt, 1960).

Apicotermes nests are just one example of the complex and diverse architecture of nests built by social insect colonies. These include both hypogeous nests and above-ground structures built on trees or on the surface of soil. Building materials can also be very different, ranging from clay, to fecal pellets, to paper and wax. The forms range from a network of underground galleries (most frequent in hypogeous ant and termite nests) to complex structures alternating bubble-like chambers and corridors (Fig. 2).

In terms of size, the largest nests are those of some fungus-growing ant and termite species. For instance, in many termites of the subfamily *Macrotermitinae*, nests can attain sizes up to 7 m high and 12 m in diameter, against a size of the workers that build them of the order of 1 cm (Grassé, 1984). Excavation of nests of the leaf-cutter ant (*Atta laevigata*) revealed a system of galleries containing up to 7800 chambers and reaching a depth of 7 m below ground (Moreira et al., 2004).

In such large nests, it is unlikely that any single individual has a global view of the overall organisation of the structure as a whole. Yet, many nests clearly do have a coherent organisation at the global scale. It is precisely because of their organisation that some termite nests can be passively ventilated and cooled (Lüscher, 1955; Korb and Linsenmair, 1999, 2000; Turner, 2001; Korb, 2003; King et al., 2015; see also Turner, 2000, for a review) or dried (Schmidt et al., 2014). The organisation of communication pathways across the nests also appears to be optimised at the scale of the entire nest. For instance, Perna et al. (2008b) measured the length of all the possible paths inside nests of the termite *Cubitermes* sp., and observed that these paths were much shorter than would be expected if adjacent chambers were simply interconnected randomly. In simulations, we could reproduce optimised paths similar to those observed in real termite nests by assuming that termites initially establish a more densely connected network of chambers and galleries and they subsequently prune the connections (i.e. they close the corridors) that support less traffic.

The size of many ant and termite nests indicates that their construction requires a large number of building or digging actions; their coherent global organisation suggests that these actions must be coordinated in order to produce a coherent structure. For instance,

Fig. 1. Complex nest architecture. (A,B) Termite nest of the genus *Apicotermes*. These nests are built in underground cavities 30–60 cm deep in the soil. On their external surface, they present a series of regularly spaced pores that enable communication between the outside of the nest and a series of circular corridors moving inside the external nest wall. (C) X-ray tomography scan of the nest, allowing visualisation of the internal structure, which is composed of large, regularly spaced chambers, delimited by thin floors of clay. The different levels are supported by pillars and interconnected by ramps.

Tschinkel (2004) estimated the amount of work required for a colony of Florida harvester ants to dig their underground nest, observing that about 5000 workers (15–20 g) must excavate about 20 kg of sand in 4–5 days every time the colony moves to a new nest, something that happens at least once or twice a year (Tschinkel, 2004).

Here, we provide a review of the scientific literature that has addressed the following questions: how do insects coordinate their activities to build such elaborated nests?; and what are the strategies found by evolution to obtain structures that meet the functional needs of a colony, depending on colony size and environmental conditions? We focus our review on examples of nests built by ants and termites, either by digging or through building behaviour.

Visualising the internal structures of nests

Two major technical obstacles hinder our understanding of the organisation of these structures and the mechanisms that lead to their formation. The first is more practical, and it consists in the difficulty in actually visualising the internal structures of the nests, hidden metres underground, or inside large mounds. The second is more conceptual; it is the problem of finding synthetic and appropriate descriptors for the structures that we observe. Loosely speaking, it is the problem of finding the 'average' nest design among all those built by a given species in spite of the fact that all details are different from one nest to another.

In the case of underground nests, very detailed and beautiful reconstructions of the underground galleries have been obtained by pouring casting materials such as metal alloys or dental plaster into the nests, with a series of techniques developed over more than a century (Smith, 1898; Ettershank, 1968; Williams and Lofgren,

1988; reviewed in Tschinkel, 2010). For instance, Mikheyev and Tschinkel (2004) created casts of the nests of *Formica pallidefulva* with dental plaster that they subsequently used to record measures of the distribution of nest volume as a function of depth, or of the shape of chambers inside the nest. Casts can be used to obtain accurate reconstructions of the nests. The major drawback of this technique is that it requires destruction of the nest and the entire colony.

An alternative to the use of casts is provided by medical imaging techniques. As far back as 1956, the Belgian naturalist Desneux (1956) had the idea of using tools developed for medical imaging to visualise the internal organisation of intact termite nests. He followed this idea by taking X-ray radiographs of the *Apicotermes* nests that we mentioned above (see Fig. 1). However, these first imaging trials remained an isolated case for long time. To our knowledge, it is only in 2001 that medical imaging techniques were used again to visualise termite nests and in this case also to compare the fraction of built material and internal empty space across different species (Hervier et al., 2001).

One of the major advantages provided by medical imaging techniques is that they are non-destructive, and in principle can also be used on active nests, still inhabited by insects. In fact, Fuchs et al. (2004) used both X-ray tomography and endoscopy to observe the nest-building behaviour of the termite *Cryptotermes secundus* within soft wood. X-ray tomography could be used to detect the architecture of the nest and the position of the major chambers that could then be accessed with endoscopy to directly observe termite behaviour. Unfortunately, endoscopy did not prove as useful as hoped because the termites exhibited clear reactions to the presence of the endoscope.

The non-invasive character of X-ray tomography was fully exploited by Halley et al. (2005), who followed with repeated scans
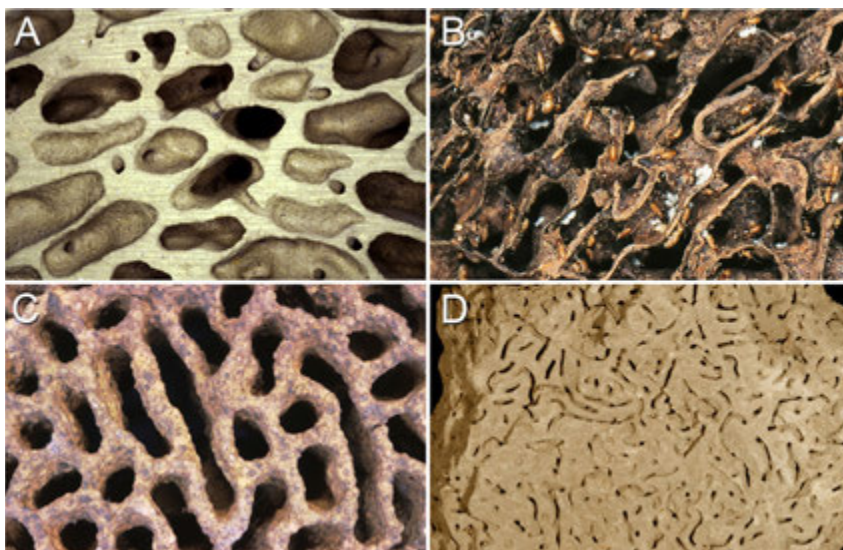

Fig. 2. Examples of various structures built by termites. (A) Section of a *Cubitermes* sp. nest showing the chambers and the galleries that connect them. (B) Arboreal structure built by *Nasutitermes* sp. (C) Fungus growing structure built by *Macrotermes* sp. (D) Pseudowall surrounding a calie of *Sphaerotermes sphaerotorax*.

the development of nests dug by Argentine ants in sand over a period of months, and by Himmi et al. (2016), who studied the 3D structure of the nest-gallery system of the termite *Incisitermes minor* in naturally infested timbers. While the resolution of medical X-ray tomography is usually limited to the millimetre scale (depending on acquisition parameters), it is also possible to achieve much better resolutions, of the order of 50 μm, by using micro-tomography. This was done, for instance, by Minter and collaborators (2012), who applied this technique to monitor the growth of nests of *Lasius flavus* ants. The repeated exposure to X-ray radiation at the levels used in these experiments did not have detectable effects on the digging behaviour of ants.

The techniques described above have made it possible to visualise, with a high level of detail, the galleries and chambers inside the nests of ants and termites. In addition, both tomography and casts allow quite accurate measurement of the internal volumes of different structures and counting of the number of characteristic nest elements, such as chambers, shafts or fungus-growing structures.

However, it is clear that many functional properties of a nest, such as the regulation of internal environmental conditions, the defence of the colony, etc., depend not only on the size of the nest and of its parts but also on how these different parts are arranged and interconnected. This 'topological' pattern of interconnections can be studied by mapping the connections and paths existing inside a nest onto a network, which can be further characterised by computing relevant topological estimators. For instance, Buhl and collaborators (2004b) mapped the network of galleries produced by ants of the species *Messor sanctus* in a thin layer of sand. Galleries produced in these experimental conditions formed a meshed network, which provided a balance between efficiency (a measure of how fast ants can reach different parts of the nest) and robustness (a measure of how well the network can keep its functionality when a fraction of connections are blocked). The meshed networks of galleries produced by *M. sanctus* were quite different from those found by Perna et al. (2008a) inside the mounds produced by termites of the genus *Cubitermes* in natural conditions. *Cubitermes* networks were 'sparse'; that is, they were traversed by a small number of paths compared with what would have been possible to realise (see Fig. 3). Perna et al. (2008a) argued that the absence of alternative paths between different parts of the nest could facilitate the defence of the nest in the event of an attack by ants, because blocking only one or a few corridors would be sufficient to isolate the attacked part of the nest. In fact, in the *Cubitermes* genus, the soldier termites have a large sclerified head that precisely fits the width of the small corridors connecting the chambers, and corridor blocking is a common defence strategy (Dejean and Fénéron, 1999). Together, these studies show how, by finding appropriate descriptors of the nest structure, it is sometimes possible to get an insight into the functional properties of the nests.

A variety of forms

The nests of ants and termites present a large variety of forms. While some have a regular arrangement in floors, as for the *Apicotermes* sp. nest of Fig. 1, the structure of most nests appears more chaotic at first sight. Fig. 2 shows some examples of the variety of structures built by termites. These range from chambers interconnected by small corridors, which are common in many nests of the Termitinae subfamily (Fig. 2A), to convoluted surfaces built with paper-like material on trees (Fig. 2B). Termites in the subfamily Macrotermitinae produce specialised structures with a high surface to volume ratio for the cultivation of fungi (Fig. 2C).
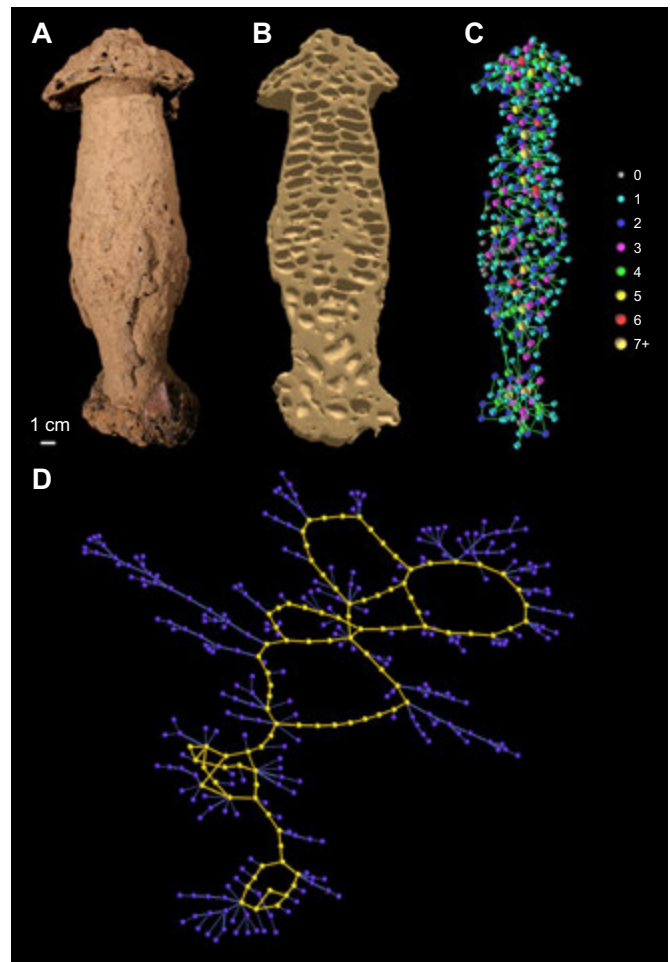

Fig. 3. Topological pattern of paths in a nest. (A) Termite nest of the genus *Cubitermes* sp. (Central African Republic). (B) Tomographic section of the same nest. (C) Representation of the chambers and galleries as a network, where each node corresponds to a chamber in the original nest and each edge to a corridor. The colour of the nodes corresponds to the number of corridors connected to that chamber. (D) A planar representation of the same network. A few long communication channels (yellow) cross the whole nest, and several groups of chambers organised in tree-like structures are connected to these channels. The number of connections in this network is rather low, considering that theoretically all the nodes that are adjacent in C could easily be connected directly with a corridor.

Other nests present underground structures, such as the numerous tiny galleries in *Sphaerotermes sphaerotorax* nests, which, as can be guessed from their structure and material composition, are probably the result of a building process (Fig. 2D). In fact, these structures appear to be made of a homogeneous mixture of clay and fine sand that confers on them smoothness and solidity – a composition that can be different from that of the coarser soil surrounding the nest (Grassé, 1984). It is surprising that such structures are found completely underground and the building process remains unclear.

Many ant nests are dug underground. While these nests also present a wide diversity of forms, they are usually composed of a small number of easily recognisable elements: vertical shafts connecting horizontal chambers (Tschinkel, 2015). The shape of the chambers themselves can be variable, but their distribution – as observed in several species – tends to be top-heavy (chambers are more frequent close to the surface of the ground) in all observed species.

With very few exceptions, underground nests have a tree-like structure; that is, they usually do not contain loops. This was

accurately measured by Monaenkova and collaborators (2015) for nests built by the fire ant under laboratory conditions and is also usually observed in nests of different ant species from the field (Délye, 1971; Lys and Leuthold, 1991). The only way in which a loop can be produced in a digging process is if the growing tip of one gallery encounters another gallery (Gautrais et al., 2014). In the absence of specialised mechanisms that allow digging ants to 'sense' the presence of another gallery through the soil, these reconnections are relatively rare, except if the density of galleries is high or if their growth is constrained. In artificial digging conditions, this happens, for instance, when the nests are constrained in 2D set-ups (Buhl et al., 2006), and it is possible that in nature loops also form more frequently when digging progresses almost in two dimensions, such as when insects dig under a stone or at the interface between soil layers with different composition. Insect behaviour during digging is also important in terms of increasing or decreasing the number of loops. For instance, Su and collaborators (2004) studied the galleries excavated by termites (*Coptotermes formosanus* and *Reticulitermes flavipes*) in a thin layer of sand and observed that in spite of being constrained to dig in two dimensions, the number of loops remained low, compared with what was produced in computer simulations of termite digging. In fact, specific digging 'rules' such as initiating new galleries at angles as large as possible from each other (Robson et al., 1995), or maintaining a digging direction oriented away from the point of initiation of the tunnel (Bardunias and Su, 2009), are mechanisms that also allow the appearance of loops to be minimised.

There are examples in which the same species, and even the same insect colony, can produce different forms of nests depending on the material or on the mechanism of nest construction (e.g. through building or through digging). For example, the black garden ant *Lasius niger* digs underground nests composed of multiple galleries characterised by an almost complete absence of loops (Sudd, 1972; Rasse and Deneubourg, 2001). However, it also re-organises the rejected material to build mounds that are filled with meshed networks of highly connected corridors (Khuong et al., 2016). Some termite species such as *Nasutitermes longipennis* build the external part of the nest in sand and clay cemented with stercoral mortar, while the chambers inside the nest are built of paper (Grassé, 1984).

The opposite is also true and similar forms are sometimes produced by phylogenetically distant groups. For instance, the structure shown in Fig. 2B is built by a termite (of the genus *Nasutitermes*), but under visual inspection, at least, it presents strong similarities with structures built by some ants, such as the nests of *Lasius fuliginosus*.

The observation that the same species of social insects can produce very different structures, and that phylogenetically distant species can produce nests with a similar overall appearance calls for more detailed studies of the nest-building behaviour of individual insects. Did different nest-building behaviours evolve several times in different taxa? Are a few behavioural modules sufficient to produce the large variety of observed nests depending on environmental parameters (such as the building material used for construction) and on the physiology of the species (including the intensity of the response to different pheromones or the evaporation rate of the pheromones themselves)?

Nest building and the coordination of individual activities

The collective construction of large spatial structures by hundreds or thousands of insects clearly depends upon the successful coordination of activities across different members of a colony. The first and most detailed explanation of the coordination mechanism at work in termites was proposed by Pierre-Paul Grassé (1959). Grassé (1959) suggested that insects do not need to share information directly (e.g. through antennal contacts or other forms of direct communication) in order to coordinate their building activities. Instead, indirect interactions of the workers with the building substrate would be sufficient. Every time a termite worker executes a building action in response to a local stimulus, such as adding or removing a piece of material from the existing nest structure, it modifies the environment, producing new stimuli. These new stimuli in turn induce new behavioural responses in the same worker, or potentially in any other worker in the colony. The stimulus itself can be one particular configuration of the building material, possibly impregnated with pheromones. Coordination is simply achieved through judiciously chosen stimulating patterns of matter to which insects are sensitive. The whole sequence of stimuli and behavioural responses leads to an almost perfect collective construction that may give the impression that the whole colony is following a well-defined plan. Grassé (1959) gave the name 'stigmergy' to this general mechanism of coordination of activities (see Theraulaz and Bonabeau, 1999, for a historical review).

Research since Grassé (1959) has confirmed that, for most tasks, insects do not need to share information directly or to recognise each other on an individual basis. However, the regulatory mechanisms are not limited to stigmergy. Two regulatory mechanisms involve responses to the characteristics of the environment inside and around the nest: (1) stigmergic stimuli, such as the configuration of the growing material, and (2) orientation and gradients of physical and chemical quantities. These gradients pre-exist in the environment and are not necessarily modified by the actions of insects. Another two regulatory mechanisms pertain to the insects themselves: (3) responses to the flow and density of nestmates, and (4) individual 'memory', which can refer both to the actual cognitive capabilities of insects and to their physical characteristics, such as the size of their body, used as a template for measuring or for producing structures with a size comparable to that of individual insects.

There are a large number of stigmergic stimuli capable of triggering or modulating the building behaviour of ants and termites. For instance, Sudd (1970a,b) observed how the digging behaviour of ants is stimulated by the presence of a pre-existing vertical tunnel. He also noticed that ants from two different species differed in their preference for digging either in continuity with the existing tunnel or at an angle from it, and he suggested that these differences might play a role in determining the different nest shapes produced by each species. While in this example ants responded only to the configuration of the digging site (the presence of a pre-existing tunnel), in general the stimulus that elicits digging behaviour can also involve the presence of pheromones released by other insects. Evidence for such digging pheromones was found by, for example, Chen and Zhang (2013) in the ant *Solenopsis invicta* and by Pielström and Roces (2013) in *Atta vollenweideri*, but not by Bruce (2016) in *Acromyrmex lundi*.

Concentrations of specific chemical or physical quantities can also modulate the digging activity of ants and termites. One such modulator of ant digging behaviour is carbon dioxide, which has an attractive effect on fire ants and elicits their digging activity (Hangartner, 1969). As carbon dioxide is produced by the metabolism of the colony, it can also act as a cue that carries information about colony size in relation to the size of the nest. Other physical quantities such as circulating dry air (e.g. Bollazzi

and Roces, 2007) can also act as a trigger and as a template for nest-building behaviour.

The recognition that nest building in social insects is typically a collective endeavour does not exclude the fact that many social insects are also capable of performing complex behaviours at the individual level. For instance, it is known that ants can rely on memory to return to the location of foraging sites (Sundström, 1993), and it seems plausible that they also rely on memory when returning to the location of a building or digging site. Digging termites can also integrate their moving direction, for instance to recover the overall direction of a gallery after encountering an obstacle (Bardunias and Su, 2009).

In general, the coordination of nest-building behaviour does not rely on a single mechanism; instead, multiple signals and cues cooperate in triggering the appropriate responses by nest-building insects. For example, Bruinsma (1979) studied the behaviour of termites *Macrotermes subhyalinus* building the royal chamber, and found evidence for the existence of at least three distinct pheromones that modulate the behaviour of worker termites. (i) The *M. subhyalinus* queen emits a pheromone that diffuses, creating a gradient around its body; the gradient of pheromone concentration then acts as a chemical template, which stimulates the workers to deposit their pellets at a certain concentration along this gradient; that is, at a certain distance from the queen. (ii) Workers impregnate the building material with a 'cement pheromone' secreted from their buccal cavity. This substance has been shown to increase the likelihood of termites conducting a variety of tasks at the location of previous labour (Petersen et al., 2015). Finally, (iii) trail pheromones deposited by termites provide long-range guidance for workers to the building site.

In a recent study on nest-building behaviour by *L. niger* ants, Khuong et al. (2016) found evidence for two distinct types of interactions of ants with the substrate. *Lasius niger* ants build above-ground nests composed of multiple pillars that are progressively expanded near their top to form a roof, on top of which the ants build subsequent layers of pillars and roofs. As in the case of *M. subhyalinus*, a cement pheromone added to the building material allows the ants to identify the active building sites. Computer simulations indicated that the evaporation of this pheromone determines the spacing of pillars and the resulting shape of the

nest (Fig. 4). In contrast, the mechanism for regulating the height of the roof did not involve pheromones or complex collective regulation. In this case, the ants deposited their pellets on top of the pillars as long as the pillar height was shorter than the length of their own body, then they started to deposit building materials on the sides of the pillars. So, the ants' own body served as a template for determining the height of the roof. There could be a functional regulation associated with these distinct mechanisms of regulation based on pheromones and on using the ant body as a template. In fact, when the environment is hot or dry, the evaporation of pheromone triggers a transition towards the production of larger shelters that are likely to be more appropriate for the protection of the colony from desiccation; the height of the roof, determined by a different form of regulation, remains constant independent of the environmental temperature. These regulations are not encoded in ant behaviour: they are a genuine product of the interplay between the construction process and the chemical properties of the building pheromone.

When the size of a nest structure is small compared with the size of insects, a single insect can accurately produce it using its own body as a template, or relying on individual-level capabilities such as proprioception and memory. Conversely, individuals in a large colony are unlikely to possess an internal representation of large-scale structures, such as the size and form of the entire nest. In some cases, the environment itself can provide a template for regulating size and providing orientation at scales larger than the size of the insects, for instance by forming gradients of temperature and humidity through the soil, or through the action of oriented physical parameters, such as gravity (Sudd, 1972) and magnetic fields.

However, it should be noted that the regulation of nest size and form can usually also be achieved in the absence of these templates, as a result of self-organisation processes whereby a large-scale and regular structure 'emerges' from multiple local interactions of insects with each other and with the substrate (Theraulaz et al., 2002).

The interplay between templates and self-organised regulation is likely to be very general in social insect nest construction, with small structures mainly determined by insects using their body as a template and large structures determined by collective interactions. For instance, in digging termites the size of galleries scales with the
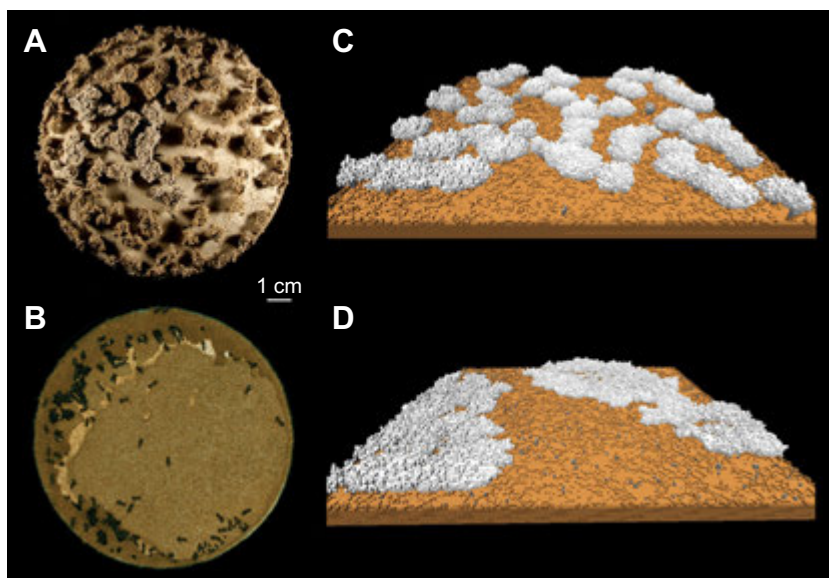

Fig. 4. Phenotypic plasticity of nest architecture in *Lasius niger*. (A,B) Under experimental conditions, ants build structures whose shape varies with environmental conditions, ranging from a large number of thin pillars and walls (A) to a small number of pillars covered with a large roof (B). (C,D) Simulations of growth dynamics in a 3D stochastic model of ant nest construction suggest that the lifetime of the building pheromone added by ants to the building material is a highly influential parameter that controls the growth and form of nest architecture: a slow rate of pheromone evaporation (C) determines the appearance of regularly spaced pillars and walls, while fast pheromone evaporation (D) results in a much smaller number of pillars.

body size of individuals (Haifig et al., 2011). Conversely, in most ant and termite species, the size of the nest is observed to closely match the size of the entire colony (e.g. Franks et al., 1992; Franks and Deneubourg, 1997; Tschinkel, 1987, 1999; Buhl et al., 2004a; Mikheyev and Tschinkel, 2004; Su and Lee, 2009). The adjustment of nest size to colony size was investigated experimentally in the ant *L. niger* by Rasse and Deneubourg (2001). These researchers measured the amount of sand extracted from soil during nest digging as a function of colony size. The digging rate decreased when the nest approached its final volume, but if additional ants were introduced into the colony, digging started again and nest size converged to a new size that matched the requirements of the larger colony. The important thing in these examples is that in order to regulate nest size with respect to the size of a colony, insects do not need to estimate directly either of these quantities. Regulation of nest size can be entirely based on insects responding to the density of neighbours that they encounter in their local perception range. A high local density of neighbours would elicit specific digging or building behaviours, which would have the overall effect of producing a match of the size of the nest with the space requirements of the entire colony (Buhl et al., 2005; Franks et al., 1992).

The size of the population of insects that live inside a nest is also directly correlated with the complexity of the nest architecture. For instance, the nests built by *Leptothorax* ants, whose colony size ranges between 50 and 500 individuals, are very simple 2D structures: a circular wall built with sand grains interrupted by a few small passages used by foragers (Franks and Deneubourg, 1997). In comparison, in large termite nests hosting millions of insects, one can identify at least half a dozen elementary components such as the royal cell, the galleries, the chambers of different size and shape, fungus garden structures and the system of tunnels and ventilation shafts that ensures the homeostasis of the nest, i.e. the regulation of temperature and oxygen levels (Korb and Linsenmair, 1999, 2000; Korb, 2011). As the nest grows, its complexity increases. In part, this increase in complexity can be explained by the fact that as the population increases, the occupied space becomes larger and the probability of finding heterogeneities and various gradients (temperature, humidity, CO_2) within the space occupied by the colony also increases. These environmental heterogeneities may greatly modulate individual behaviour and consequently the shape and structure of a nest can be modified according to the variation in environmental conditions (Wilson, 1971; Korb and Linsenmair, 1998; Frouz, 2000; Garnier et al., 2007).

Colony size and density can also directly affect the behaviour of individual insects and the possibility of their interaction with each other and with the substrate, leading to transitions in the way the nest grows that are density dependent. For instance, Toffin and collaborators (2009, 2010) observed that *L. niger* ants confined to digging their nest in a 2D substrate produced an initially circular cavity, but this cavity subsequently evolved into a ramified structure. The main parameter that determined the transition from circular to ramified shape was the density of ants at the digging front: at the beginning of excavation, crowding of ants along the perimeter of the nest resulted in nearly homogeneous digging and circular nest expansion; as the density of ants at the front of the excavation decreased, ants started to concentrate their activity at a few localised sites and the ramifications started to appear. In this case, the transition resulted from an exclusively passive regulation, as it was determined by the availability of digging sites. However, density can also more directly affect the behaviour of insects, determining behavioural shifts. This is the case, for instance, for

subterranean termites, for which traffic congestion determines the formation of queues. The time spent waiting in queue was one of the factors that determined a change of behaviour at the individual level, with a higher probability of initiating digging activity on the lateral walls of the tunnel (Bardunias and Su, 2010).

Nest shape, occupancy and the organisation of activities within a colony

One of the implications of the concept of stigmergy is that individuals do not choose the activities they perform; instead, their activities are determined by the stigmergic stimuli available around them. In other words, there would be a close relationship between how individuals occupy their nest and what activities these individuals perform.

The most extreme formulation of this hypothesis was proposed by Franks and Tofts (1994). These authors emphasised the role that the spatial distribution of ants within their nest would have on the division of labour of ant colonies, as compared with the role played by other determinants of ant behaviour, such as previous experience and physiological or developmental state.

Today, there is general agreement that the position within a nest is not the only determinant of an ant's behaviour, and that physiology and previous experience, including interactions with nestmates, play an important role in determining which activities a particular ant will perform. What remains true is that we do clearly observe a spatial segregation of ant castes inside and outside the nest. For instance, Tschinkel (1987, 2004) observed that older workers of the Florida ants *Prenolepis imparis* and *Pogonomyrmex badius* tend to move upward within a vertical test apparatus to assume positions in the upper parts of the nest, which indicates that there is at least a strong correlation between spatial position and performed activities. In other words, whether task allocation within the colony depends on interaction with nestmates or on stigmergic stimuli, these two forms of regulation remain highly correlated because individuals of different castes segregate on a spatial basis and ultimately encounter and interact with other individuals frequenting the same areas of the nest.

Recent studies have tracked the position of all individuals in ant colonies over several weeks, with the main purpose of identifying the patterns of interactions among individuals of different castes (Moreau et al., 2011; Jeanson, 2012; Mersch et al., 2013; Pinter-Wollman et al., 2013). While the nests used in these studies had a relatively simple structure, with just one or a few chambers, individual ants showed a clear spatial fidelity, which was also the main determinant of their pattern of encounters with other ants (Fig. 5).

If the size and shape of a nest affect the patterns of movement of insects and their encounter rate, it is likely that nest shape might also have an overall effect on colony-level performance on different tasks, such as foraging and social immunity. For instance, a theoretical study by Pie et al. (2004) indicates that nest shape has a possible impact on disease spreading across a colony. A recent study (Pinter-Wollman, 2015) indicates that nest shape could affect the speed at which information about a new food source spreads across the colony, determining the patterns of recruitment of foragers to the food source.

A number of techniques exist for tracking the spreading of food within ant colonies, ranging from visual identification of trophallaxis events, to the automatic tracking of radio-labelled food and to the use of fluorescent markers (Buffin et al., 2009; Sendova-Franks et al., 2010; Greenwald et al., 2015). However, the use of all these techniques is limited to simple 2D nests, and to our
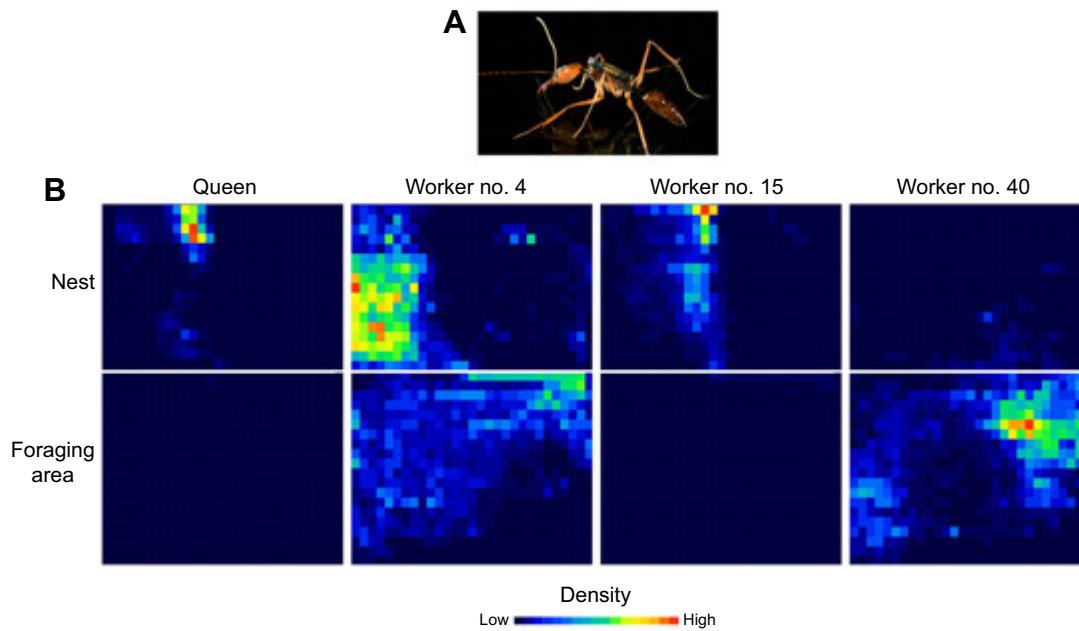
Fig. 5. Mapping the motion and interaction networks of ants within a nest with radio frequency identification (RFID) tags. (A) An *Odontomachus hastatus* ant equipped with an RFID tag; ants were tracked with RFID tags for a period of 3 weeks. (B) Spatial analysis of tracking data reveals a high level of variability in space occupation among ant workers: some ants stay mostly inside the nest within specific locations or in the foraging area, while others explore a much greater space. The differences in mobility patterns determine the potential encounters between ants and the resulting interaction network (based on data from Jeanson, 2012).

knowledge no currently developed technique allows monitoring of insect movements or food spreading inside nests built in more natural conditions. At present, the only accurate measurement of the distribution of individuals inside nests in the field can be obtained by making casts of the nests with certain specific materials, such as wax and dental plaster, that allow the corpses of insects to be retrieved at approximately the same position that they were when the cast was performed (Tschinkel, 2010). Clearly, however, these methods are completely destructive and do not allow quantification of the flow of insects or food in active nests.

Conclusions

Technical advances in our capacity to visualise and measure complex 3D structures are now making it possible to characterise and compare nest structures produced by different species. In parallel, direct observations of the behaviour of individual insects, in combination with novel tracking technologies, make it possible to characterise the movement patterns and the building behaviour of individual insects in response to local environmental stimuli.

Recent studies of nest-building behaviour have already addressed the problem of the relationship between individual-level actions and the growth of structures produced at the colony level, for some nest species and for some types of nests. Further studies are required to extend the existing approaches to a larger number of species and nest structures in a comparative way, to eventually achieve a more detailed understanding of the relationship between nest-building behaviour and the forms of the nest produced from an evolutionary perspective.

Today, we know that the number of distinct actions involved in nest building and digging at the individual level is relatively small, possibly limited to simple actions such as picking up and depositing pellets and moving in different directions in response to the configuration of the building material and to pheromones. Such individual-level actions can be remarkably similar over a diversity of species. The resulting complexity of the nest architecture emerges as the result of a self-organised process, which involves the recurring execution of these simple actions and the combination of simple regulatory mechanisms such as the density of insects or various templates.

Moreover, the insects are able to perceive and respond to a wide range of cues and signals that are present in their environment, and these signals are mainly activating or inhibiting. In turn, the behavioural responses that are triggered by these signals clearly depend on their intensity and on the context in which they are released. And, finally, the combination of stigmergic behaviours and environmental templates is able to increase the flexibility and the variety of the collective patterns that can be built by social insects. In particular, when the environmental conditions are changing, the same behavioural mechanisms lead to the construction of structures that look very different. For instance, when the size of a nest increases, the variety of signals and cues that are likely to be encountered by the insects within the nest increase substantially and this may, in part, explain why the most populous termite societies also build nests with the most complex architecture.

Studies directed at exploring the relationship between nest organisation and social organisation of the colony will also be important in the coming years because they will provide explanations of how nest-building activity and sociality may have co-evolved. Recall that the building or digging of nests is a costly endeavour, whose benefits are shared across all the individuals inhabiting the same nest, which would also explain why sociality (and in particular eusociality) and nest building behaviour frequently co-occur and have probably evolved together multiple times across unrelated taxa. Indeed, one functional consequence of building and living within a nest is a substantial change of the world in which insects are living. And if ants and termites are able to modify their environment and to control in some way the flow of matter and energy in their ecosystems, they are likely to modify their fitness. These ideas are at the core of the Niche Construction theory

and such processes might have played an important role in the evolution of social insects (Odling-Smee et al., 2003).

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