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Variation in nestmate recognition ability among polymorphic leaf-cutting ant workers

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ABSTRACT

A key feature for the success of social insects is division of labour, allowing colony members to specialize on different tasks. Nest defence is a defining task for social insects since it is crucial for colony integrity. A particularly impressive and well-known case of worker specialization in complex hymenopteran societies is found in leaf-cutting ants of the genera *Atta* and *Acromyrmex*. We hypothesized that three morphological worker castes of *Acromyrmex echinatior* differ in their likelihood to attack intruders, and show that major workers are more aggressive towards non-nestmate workers than medium and minor workers. Moreover, minors do not discriminate between nestmate and non-nestmate brood, while larger workers do. We further show that *A. echinatior* ants use cuticular chemical compounds for nestmate recognition. We took advantage of the natural variation in the cuticular compounds between colonies to investigate the proximate factors that may have led to the observed caste differences in aggression. We infer that major workers differ from medium workers in their general propensity to attack intruders (the "action component" of the nestmate recognition system), while minors seem to be less sensitive to foreign odours ("perception component"). Our results highlight the importance of proximate mechanisms underlying social insect behaviour, and encourage an appreciation of intra-colony variation when analysing colony-level traits such as nest defence.

1. Introduction

The stability of social insect colonies is maintained by an efficient recognition system, with fine-tuned communication processes allowing workers to prevent potentially dangerous non-nestmates from entering the colony (d'Ettorre and Lenoir, 2010; Sturgis and Gordon, 2012). In general, recognition systems can be partitioned into three components: production, perception, and action (Starks, 2004). The production component of nestmate recognition involves colony-specific "labels" in form of chemical odours. Social insects are thought to form a neural representation of the colony odour, called the template. When workers encounter another individual, they perceive the other worker's label and somehow estimate how different it is from their own template ("label-template differential", measured in the perception component, cf. Sherman et al., 1997). In the action component of the recognition system, workers follow behavioural decision rules. Here they decide, based on the perceived label-template differential, whether or not to attack the encountered individual. Whether the estimation of the label-template differential is reliable, and hence the error rate of intruder detection, depends on the accuracy of both the own template and the perception of the other ant's label (Sherman et al., 1997; van Zweden and d'Ettorre, 2010).

In ants, the nestmate recognition label typically consists of a blend of long-chain hydrocarbons covering the cuticle. The blend can be complex and dynamic, with many compounds varying in quantity between colonies and in quality and quantity between species (d'Ettorre and Lenoir, 2010; van Zweden and d'Ettorre, 2010; Sturgis and Gordon, 2012). Leaf-cutting ants of the genus Atta, however, emit volatile colony-specific odours (Hughes et al., 2001a) that might be used for nestmate recognition (Hernandez et al., 2002). For the species studied here, *Acromyrmex echinatior*, we present evidence that the volatiles Atta uses are not necessary for nestmate recognition, but that the ants rely on a blend of long-chain substances (Supplementary material 1).

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While recognition and discrimination stabilize groups, an important reason that groups are successful is that they allow for specialisation and division of labour. In ant colonies, queens specialise on reproduction, while workers conduct a broad spectrum of tasks ranging from foraging to brood care. Due to the diversity of tasks to be completed, large colonies profit from a further division of labour among workers, which can be based on age polyethism, task specialisation, and worker polymorphism (Robinson, 1992; Chittka and Muller, 2009). In leaf-cutting ants, for example, which live in symbiosis with a fungus, small workers (minors) mostly work inside the nest and engage in brood care and fungus farming. Large workers (majors), on the other hand, are efficient foragers carrying large leaf fragments, and they also act as guards at the nest entrance (Wilson, 1980; Hart et al., 2002). Within each morphological caste, workers may switch tasks as they age, and some workers may specialize on certain tasks (Julian and Cahan, 1999; Camargo et al., 2007; Francelino et al., 2008; Waddington and Hughes, 2010). Both worker morphology and behaviour can be, at least in part, genetically determined (Hughes et al., 2003; Waddington et al., 2010).

The worker castes can also differ in their propensity to attack intruders. Older or larger workers, or those that work outside the colony, are more likely to attack (Free, 1965; Wilson and Hölldobler, 1985; Sturgis and Gordon, 2013). In Atta leaf-cutting ants, major workers defend the colony against invertebrate intruders, but against vertebrate enemies a distinct caste of even larger soldier workers has evolved (Whitehouse and Jaffé, 1996). Minor workers, on the other hand, are particularly attracted to alarm pheromones (Hughes et al., 2001b).

In the study presented here, we tested whether morphological worker castes of the leaf-cutting ant *A. echinatior* differ in their nestmate recognition behaviour, and if so, what the proximate mechanism behind the variation is. *Acromyrmex* cultivate a symbiotic fungus and have large long-lived colonies with complex division of labour among workers, which is reflected by the presence of morphologically different worker castes varying in size (Bot and Boomsma, 1996; Dijkstra et al., 2005). A dedicated soldier caste, however, as in the related genus *Atta*, is absent. An effective nestmate recognition system is crucial in these leaf-cutting ants because colonies are constantly threatened by competitors, predators, and parasites (Schultz et al., 1998; Bekkevold et al., 1999). The entrances to *Acromyrmex* nests are typically, but not always, guarded by major workers, who are also the main foragers. Minor workers, in contrast, are mostly inside the nest itself, and typically take care of brood and fungus (although some variation may occur; VN, pers. obs.; cf. Camargo et al., 2007 for Acromyrmex subterraneus).

We tested in behavioural experiments whether individuals belonging to three different morphological worker castes differed in their propensity to attack conspecific intruders. We found that major workers are most aggressive, and minor workers least aggressive. In a second step, we investigated the proximate mechanism underlying the observed behavioural difference among morphological worker castes, which could be caused by differences in the perception or the action component of the recognition system (Starks, 2004). These two hypotheses can be tested using variation in the recognition labels between colonies. When ants from colonies with very different labels encounter each other, they experience a large label-template differential, meaning that it must be obvious to detect that the other ant is an enemy. When the labels are more similar, non-nestmates may be harder to detect (reduced stimulus concentration has a similar effect: Cini et al., 2009; Ichinose and Lenoir, 2010). If some workers were less aggressive because they followed a decision rule preventing them from ever attacking non-nestmates, we would expect them to be peaceful no matter how large the label-template differential. In contrast, if these ants were aggressive when the label-template differential is large, but not when it is small, they might simply not be sensitive enough to detect non-nestmates as intruders when the differential is small.

2. Material and methods

2.1. Study organisms

2.1.1. Collection and animal keeping

The experiments were conducted using workers from colonies of *A. echinatior* that had either been kept in our laboratory in Denmark for more than one year (Copenhagen colonies) or had been recently collected in the field (Gamboa colonies). The Copenhagen colonies had been collected between 2003 and 2008 in Gamboa, Panama, and were maintained in a climate-controlled room at the University of Copenhagen under standardized conditions of 70% relative humidity, temperature of 25 °C and a photo-period of 12:12 h L:D. Colonies were fed twice a week with fresh bramble leaves, apple and rice. All colonies were kept in Fluon (De Monchy, The Netherlands) coated plastic boxes and the fungus garden (>1 L) was placed under inverted plastic beakers and covered with flowerpots. The Gamboa colonies were collected in Gamboa in 2010, within an area of 2 km^2, and were kept there in plastic boxes, similar to Copenhagen colonies, but under naturally varying conditions of temperature (25–30 °C) and humidity (75–100%). The Gamboa colonies were fed daily with *Lagestroemia speciosa* leaves and mango fruits. When collecting the colonies, we made sure to collect the entire intact fungus garden including the queen. This procedure minimises the loss of workers that are inside the nest; it is likely, however, that we missed those ants that were foraging or working to defend the colony during collection, which may have slightly altered the colony structure. After colony collection, the ants managed to clean and rebuild the fungus garden in less than a day. We conducted the first experiments earliest two days after collection.

2.1.2. Morphological worker castes

The three worker castes used in the behavioural assays differed in their head width (majors > 2.0 mm; medium workers ca. 1.6 mm; minors < 1.2 mm), as an indicator for overall body size (Bot and Boomsma, 1996; Dijkstra et al., 2005). We only took fully pigmented workers to avoid using young "callow" workers, which might not bear any significant amount of recognition cues (Dahbi et al., 1998; Breed et al., 2004). Our design is restricted to account for worker polymorphism and may miss part of the behavioural variation within these size classes, be it due to task specialisation or temporal polyethism. However, the minor workers differed from the majors in the location they were collected from. We collected minor workers from inside their colony. Minors could be easily picked up from the surface of the fungus garden in sufficient numbers, and they reacted only little to the disturbance caused by opening the cover. In our laboratory colonies, minor workers can hardly be seen outside the fungus garden, and also in the field we never encountered minors outside the nest (PdE & VN personal observation). In contrast to minor workers, medium and major workers were collected from the foraging arena outside the nest. Since medium and major workers tend to swarm out to attack potential intruders when one lifts the bowl covering the fungus garden, it would be difficult to systematically pick only inside or only outside workers. Therefore, to simplify the experiments and reduce the potential for experimental noise, we only took medium and major workers from the foraging arena.

2.2. Experimental design and procedures

To estimate variation in aggression among the morphological worker castes, we conducted aggression tests between pairs of