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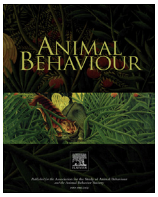

Caste-dependent brood retrieval by workers in the ant *Formica exsecta*

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ABSTRACT

The ability to distinguish friends from foe is a widespread phenomenon among social animals. In ants, recognition of intruders is important for the maintenance of colony integrity and survival. Intruders are typically adult, but the acceptance of non-nestmate brood could result in severe fitness costs, depending on the caste of the brood. Accepting non-nestmate worker brood may not carry a cost, as they should not drain resources of the adoptive colony but may instead add to the workforce. Sexual brood, however, would typically not contribute to colony performance, yet require resources, and should thus be rejected. Here, we tested whether workers of the narrow-headed ant, *Formica exsecta*, which strongly discriminate between adult nestmates and non-nestmates, also discriminate between nestmate and non-nestmate pupae. Furthermore, we investigated whether the caste of the brood (workers/sexuals) affects discrimination. We carried out analysis of surface chemicals to investigate whether the chemical distance between colonies was associated with the propensity to accept non-nestmate pupae. We show that worker pupae were retrieved irrespective of their origin, whereas nestmate sexual pupae were retrieved at a slightly higher rate than non-nestmates. Our chemical data, however, suggest that both the reproductive and the worker brood carry sufficient chemical information for discrimination, as they both express colony signatures. However, this information is acted upon only in the case of sexual brood. Our results thus suggest that workers selectively capitalize on the chemical information in agreement with fitness predictions, albeit to a lower extent than during discrimination between adult individuals.

The ability to discriminate between friend and foe plays a crucial role in the evolution and maintenance of eusocial societies. Through efficient recognition, altruistic behaviours can be directed towards related individuals and the colony and its resources protected from exploitation by unrelated intruders (d'Ettorre & Lenoir, 2011; Hepper, 1991). To this end, social insects have developed precise discrimination abilities, and most species are able to discriminate between adult nestmates and non-nestmates very accurately (van Zweden & d'Ettorre, 2010). There is, however, an essential difference between recognition and discrimination. Recognition is the internal neural process of detecting and classifying another individual as a member of the group (the perception component, Mateo, 2004). Discrimination relates to an action (e.g. acceptance, aggression), based on the information perceived during recognition (Liebert & Starks, 2004; action component, Reeve, 1989). Therefore, recognition does not necessarily imply action, which can be context dependent (Bos, Guerrieri, & d'Ettorre, 2010; Chapuisat, 2004; Downs & Ratnieks, 2000; Nehring, Evison, Santorelli, d'Ettorre, & Hughes, 2011; Sturgis & Gordon, 2012).

Even though social insects are extremely good at discriminating between adult group members and alien individuals, whether they can, or even need to, distinguish between nestmate and non-nestmate brood is less clear. Given that the immature stages of social insects are immobile, they are not potential intruders of colonies. Thus, the ability to distinguish between nestmate and non-nestmate brood could be under less stringent selection. None the less, the ability to discriminate against non-nestmate brood may be favoured in species that are targeted by social parasites. Social parasites, whether conspecific or heterospecific, enter the nest and exploit colony resources to their own benefit (Schmid-Hempel, 1995), thus severely reducing the fitness of the hosts

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(Buschinger, 2009; d'Ettorre & Heinze, 2001; Topoff, 1990). By doing so, social parasites manipulate the workers of their host in one way or another, causing host workers to care for parasite brood at the expense of the colony's own brood (Davies, Bourke, & de L Brooke, 1989; Lenoir, d'Ettorre, Errard, & Hefetz, 2001). Thus, in addition to discrimination against adult intruders, accurate discrimination against non-nestmate brood may prevent a social parasite from gaining foothold in the colony (Achenbach & Foitzik, 2009; Achenbach, Witte, & Foitzik, 2010; Johnson, Topoff, Vander Meer, & Lavine, 2005). Social parasite pressure may hone precise discrimination between species, which could also select for more precise recognition abilities within species, as has been suggested in the ant *Formica fusca* (Chernenko, Helanterä, & Sundström, 2011).

Evidence for discrimination against non-nestmate brood in social insects varies across species. Some species discriminate between nestmate and non-nestmate brood (Bonavita-Cougourdan, Clément, & Lange, 1987; Fénéron & Jaisson, 1992; Helanterä & Sundström, 2007; Klahn & Gamboa, 1983; Lenoir, 1984; Meunier, Delaplace, & Chapuisat, 2010; Pirk, Neumann, & Hepburn, 2007; Tan et al., 2009; Visscher, 1986), whereas others may readily accept brood of unfamiliar origin (Crosland, 1988; Goodloe & Topoff, 1987; Holzer, Kümmerli, Keller, & Chapuisat, 2006; Maeder, Freitag, & Cherix, 2005; Tan et al., 2009). Furthermore, some species can distinguish nestmate from non-nestmate brood, but nevertheless adopt some non-nestmate brood (Souza, Della Lucia, Errard, Richard, & Lima, 2006; Viana et al., 2001). Species-specific differences in brood discrimination may be context dependent, and thus reflect the evolutionary and ecological history of the species.

Discrimination stringency should depend on the costs and benefits of discrimination, and their fitness consequences (Reeve, 1989). Thus, worker brood from another colony may be accepted, as they could contribute to the colony workforce, once eclosed (Buschinger, Ehrhardt, & Winter, 1980; Fénéron & Jaisson, 1995; Isingrini, Lenoir, & Jaisson, 1985; Orivel, Errard, & Dejean, 1997). Hence, they would not impose a cost but would contribute to the workforce of the adoptive colony. Conversely, sexual brood would usually not contribute to colony performance, but instead require colony resources during maturation, yet provide no fitness returns to the resident colony members (but see Nehring, Boomsma, & d'Ettorre, 2012). Thus, non-nestmate sexual brood should not be accepted. Despite these relatively straightforward fitness predictions for acceptance of worker versus sexual brood, the effect of caste on the acceptance or rejection of brood remains largely unexplored (Achenbach et al., 2010).

Nestmate recognition in social insects is based on a colony-specific ‘chemical signature’, which consists of a characteristic mixture of cuticular hydrocarbons (Lenoir, Fresneau, Errard, & Hefetz, 1999). Most studies on recognition cues have been carried out on adult individuals (van Zweden & d'Ettorre, 2010), but surface chemistry has been proposed to be the most likely basis also for brood recognition (Achenbach et al., 2010; Helanterä & d'Ettorre, 2014; Souza et al., 2006; Viana et al., 2001). None the less, few studies have combined behavioural assays on brood recognition with chemical analysis (Achenbach et al., 2010; Johnson et al., 2005; Souza et al., 2006).

Here, we tested whether workers of the ant *Formica exsecta* can discriminate between nestmate and non-nestmate pupae. We also investigated whether the caste of the brood (workers/sexuals) affects discrimination. Thus, if the caste of the brood does not influence retrieval decisions, nestmate brood should be retrieved at a faster rate than non-nestmate brood, irrespective of the caste of the brood. Furthermore, non-nestmate sexual brood should be retrieved at a slower rate than nestmate sexual brood, whereas no such difference would be expected in the rate of retrieval of worker brood. Finally, we carried out chemical analysis to reveal possible differences between different castes and investigated whether the chemical distance between colonies has an effect on the rates of brood retrieval.

METHODS

Study Population

Our study population of the narrow-headed ant, *F. exsecta*, is in the Hanko peninsula of southwestern Finland. This population has been studied extensively with respect to demography, dispersal, relatedness and inbreeding (Chapuisat, Sundström, & Keller, 1997; Haag-Liautard, Vitikainen, Keller, & Sundström, 2009; Sundström, Chapuisat, & Keller, 1996; Sundström, Keller, & Chapuisat, 2003; Vitikainen, Haag-Liautard, & Sundström, 2011; Vitikainen, Haag-Liautard, & Sundström, 2015). In addition, nestmate recognition and the surface chemistry of these ants have been studied intensively (Martin & Drijfhout, 2009; Martin, Helanterä, & Drijfhout, 2008a; Martin, Shemilt, & Drijfhout, 2012; Martin, Vitikainen, Drijfhout, & Jackson, 2012; Martin, Vitikainen, Shemilt, Drijfhout, & Sundström, 2013). This species founds colonies by temporary parasitism on *Serviformica* species (Collingwood, 1979; Czechowski, Radchenko, & Czechowska, 2002; Seifert, 2007), but may itself also occasionally be targeted by social parasites, such as the mound-building wood ants of the *Formica* s.str. group. Colonies can be either monogyne (one reproductive queen) or polygyne (multiple queens). Based on previous work (Sundström et al., 1996) we chose only colonies known to be monogyne for this study.

Experimental Set-up

We conducted two sets of tests with identical set-ups, to measure the propensity of workers to retrieve nestmate versus non-nestmate worker pupae (set 1) and sexual pupae (set 2), respectively. We collected workers (ca. 300 workers each colony) and pupae from 20 colonies for each of the experiments (four of which were used in both experiments), by collecting nest material from inside the nest, and brought these to the laboratory. The collected nest fragments were housed in boxes (25 × 15 cm and 10 cm high), the walls of which were coated with Fluon and the bottoms lined with peat as nest material. The ants were kept at room temperature (ca. 21 °C), fed Bhathkar–Whitcomb diet (Bhatkar & Whitcomb, 1970), and watered daily.

Behavioural Assays

Two days after collection, 20 nurse ants were chosen, based on their proximity to the brood in the nestbox, and placed in small experimental boxes (diameter 7 cm, height 5 cm; Fig. 1a) lined with a thin layer of peat. In addition, five nestmate pupae (depending on the set-up, worker or sexual pupae) were added, as the lack of access to familiar brood may cause workers to decrease their preference for nestmate brood (Fénéron & Jaisson, 1995; Hare & Alloway, 1987; Lenoir, 1981). The ants were left to habituate overnight. The following day, each experimental nest was given the choice between 10 nestmate and 10 non-nestmate pupae (either all worker-destined [set 1] or all sexual [set 2]), with each colony assigned as the donor of non-nestmate pupae for one recipient colony. The pupae were marked with either a red or a blue marker pen (YOKEN xylene-free, low-odour permanent marker) close to the meconium, with the colours assigned randomly for each trial. At the start of the trial the pupae were placed in a circle in an arena (diameter 22 cm, height 5 cm), equidistant from the central