

Decision rules for egg recognition are related to functional roles and chemical cues in the queenless ant *Dinoponera quadriceps*

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Abstract The capacity to distinguish colony members from strangers is a key component in social life. In social insects, this extends to the brood and involves discrimination of queen eggs. Chemical substances communicate colony affiliation for both adults and brood; thus, in theory, all colony members should be able to recognize fellow nestmates. In this study, we investigate the ability of *Dinoponera quadriceps* workers to discriminate nestmate and non-nestmate eggs based on cuticular hydrocarbon composition. We analyzed whether cuticular hydrocarbons present on the eggs provide cues of discrimination. The results show that egg recognition in *D. quadriceps* is related to both age and the functional role of workers. Brood care workers were able to distinguish nestmate from non-nestmate eggs, while callow and forager workers were unable to do so.

Keywords *Dinoponera quadriceps* · Functional roles · Egg recognition

Introduction

Recognition is a fundamental feature that permits cooperative groups to obtain benefits through helping. Social insects employ a complex chemical communication system, which mediates most interactions between colony members (Hölldobler and Wilson 1990; Lenoir et al. 2001). The capacity for nestmate recognition enables individuals to protect their colonies against intruders and parasites (Beekman and Oldroyd 2008; Brandt et al. 2005). At the group level, discriminators use available information to distinguish between members and non-members (Dietemann et al. 2003; Couvillon et al. 2007).

The importance of cuticular hydrocarbons for intra-colonial communication and individual recognition in social insects is well known (Breed 1998; Vander Meer and Morel 1998; Nunes et al. 2008; Tannure-Nascimento et al. 2008). Recognition odors are determined mostly by genetic factors (Carlin and Hölldobler 1986), but environmental origin is also important for the acquisition of colonial cues (Crosland 1988; Obin and Vander Meer 1989). In ants, cuticular hydrocarbon profiles are acquired within 48 h of emergence (Errard 1994), and callow workers are more likely to be accepted into alien colonies than older workers (Stuart 1992). Indeed, the ontogeny of nestmate recognition in ants seems to be related to individual age (Lenoir et al. 1999). For example, in the primitive ant, *Ectatomma tuberculatum*, adults preferred nestmate larvae over alien conspecific brood when 2–10 weeks old, a period corresponding to nursing behavior in this species (Féron and Jaisson 1992). However, workers outside this age group were unable to discriminate larvae (Féron and Jaisson 1995).

Previous studies have shown that workers across Hymenoptera are able to recognize queen-laid from

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worker-laid eggs (e.g., honeybees, Ratnieks and Visscher 1989; ants, Endler et al. 2004; and paper wasps, Dapporto et al. 2007). In *Dinoponera quadricaps*, the cuticular hydrocarbons present on the surface of the eggs differed between those laid by gamergates and subordinate workers. Gamergates' eggs had a higher proportion of 9-hentriacontene than substitute alphas' and subordinates' eggs (Monnin and Peeters 1997). However, in *Formica fusca*, experienced colonies exposed to eggs laid by non-nestmate queens induced to acceptance of alien eggs, demonstrating that workers respond to the diversity of cues experienced in the colony (Helanterä et al. 2007).

The present study aims to investigate the ability of *D. quadricaps* workers to recognize eggs laid by nestmate and non-nestmate gamergates. We analyzed cuticular hydrocarbons present on the surface of eggs to verify quantitative differences that provide cues for egg discrimination. We then tested whether egg discrimination is related to age and to the functional roles of workers.

Materials and methods

D. quadricaps is a queenless ant with small colonies. Dominance interactions lead to a linear hierarchy, and only the alpha worker (gamergate) copulates (Monnin and Peeters 1997, 1999). In this study, four entire colonies of *D. quadricaps* were collected in São Cristóvão, Sergipe State and transferred to the laboratory. Colonies housed in plastic boxes (45×35×10 cm) with internal chambers were connected to the foraging arena by a plastic tube. All adult ants were marked with paint dots on the thorax for individual identification. Temperature and humidity conditions were maintained under natural conditions (27°C and 60% humidity, 12:12-h light/dark). Colonies were fed with small pieces of fruits and cockroaches four times per week, and water was provided ad libitum.

We investigated egg recognition using three different groups of workers. According Féron and Jaisson (1995), three distinct age-based tasks can be recognized in ponerine ants: nest maintenance, brood care, and foraging. Experimental individuals were classified as callows (1–7 days old), brood care workers (7–45 days old), and foraging workers (45+ days old).

We conducted a series of bioassays in which we recorded the behavioral response of workers to nestmate and non-nestmate eggs. We used a glass arena (16×10×7 cm) with a "T" corridor made with glass through which each experimental worker walked to access the eggs. We introduced four eggs (two from the experimental colony and two from a foreign colony) per trial in order to verify recognition. Each pair of eggs was placed on a clean filter paper at the edges of the arena and separated by 3 cm. A

single trial began when the testing worker was released into the arena and started to explore the space. For 10 min we observed whether the worker antennated, bit, collected, or ignored the eggs. A generalized linear model (GLZ) was used to verify which factors affected egg recognition. Colony and age were entered into the analysis as the independent variable and egg acceptance as the dependent variable using a binomial error structure, with a specified probit link function (Sokal and Rohlf 1995). We considered a positive response when the worker collected a nestmate egg. Negative responses were entered into the analysis when workers either ignored the eggs or collected non-nestmate eggs. All tests were performed with the program Statistica 6.0 (StatSoft Inc.).

Gas chromatography–mass spectroscopy analyses of eggs' surfaces were performed to verify differences in their chemical profiles (see [Electronic supplementary material](#)).

Results

Egg recognition was determined by colony origin and individual roles of *D. quadricaps* workers. In 135 trials, workers recognized their nestmate egg in 51 instances (37.8%), ignored eggs in 69 instances (51.1%), and made erroneous decisions in only 15 trials (11.11%). As expected, the generalized linear model showed that age had a significant effect on egg recognition ($\chi^2=75.06$; Wald statistics=46.40; $p<0.001$; $df=2$). Colonies had no influence on the results ($\chi^2=0.40$; Wald statistics=0.41; $p=0.93$; $df=3$), so discriminator workers were equally able to recognize nestmate eggs depending upon their ages.

Figure 1 shows that an average of $78\pm9.7\%$ of brood-care workers were able to recognize nestmate eggs, while only $10\pm2.2\%$ of foraging workers recognized eggs from their colonies. On three occasions (8.4%) did brood care workers collect alien eggs, while 12 foraging workers (17.2%) collected non-nestmate eggs (about 20% of them).

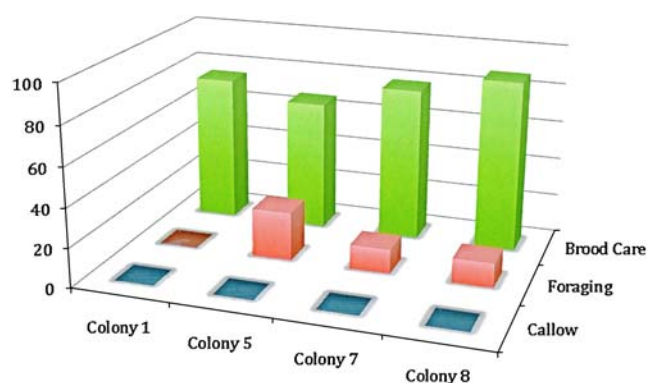


Fig. 1 Proportion of nestmate egg recognition according to the functional roles of workers

In stark contrast, callows failed to collect a single nestmate or alien egg.

Ants behaved clearly distinctly during the experiments. Nurse workers were very precise to recognize nestmate eggs when passed on them, so they stopped the arena exploring, antennated, and collected the eggs. Foragers quite often ignored or passed on both kinds of eggs without interest (52 out of 79 trials). Callow workers were always inactive or motionless in the most trials. Latency time of nestmate egg recognition varied significantly among brood care workers during the experiments: the time in which nurses collected non-nestmate eggs was significantly lower than that spent to recognize nestmate eggs (nestmate eggs, mean $\pm$ SD=76.55 $\pm$ 64.74 s, range=4–243 s; non-nestmate eggs, mean $\pm$ SD=330.33 $\pm$ 170.24 s, range=134–420 s; Mann–Whitney test, $Z=2.48$; $p<0.05$). Time spent by foragers to collect eggs were not different according their source (nestmate eggs, mean $\pm$ SD=136.75 $\pm$ 109.63 s, range=40–280 s; non-nestmate eggs, mean $\pm$ SD=154.37 $\pm$ 104.65 s, range=45–360 s; Mann–Whitney test, $Z=0.34$; $p=0.73$).

Hexane washes of the eggs revealed about 40 compounds or mixtures of compounds, 29 of which were

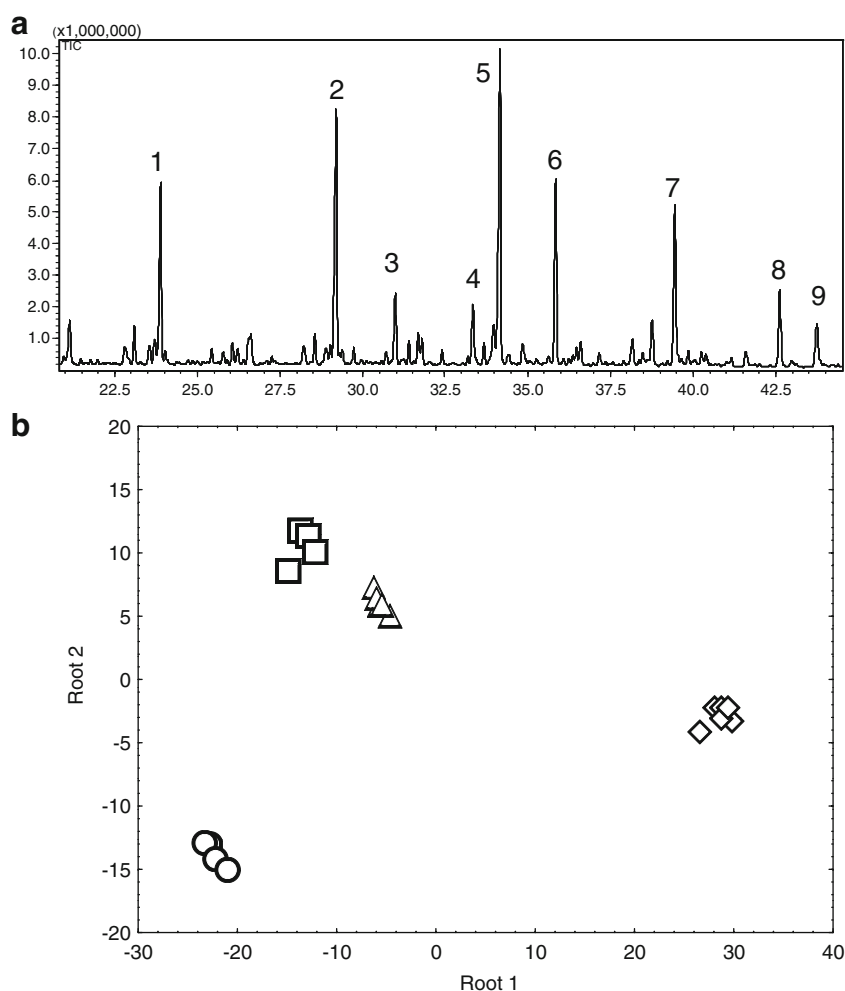
identified as hydrocarbons (see [Electronic supplementary material](#)). These were linear alkanes, alkenes, and methyl-branched alkanes (with chain lengths of between C_{23} and C_{31}). Figure 2 shows a chromatogram of extract from the surface of a typical *D. quadricaps* egg.

Discriminant analysis significantly differentiated chemical profiles of egg surfaces (Wilk's Lambda=0.00007; $F_{27,21}=98.98$; $p<0.00001$). Predicted groups were 100% scored according their colonies (Fig. 2b). Heptacosene was the most important compound that differentiated among the groups (F remove=50.40; $p<0.0001$). Other hydrocarbons that also varied were 11-, 13-, and 15-methyl nonacosane and 3-methyl heptacosane.

Discussion

The ability of ant workers to recognize seems to be related to social contexts and in-nest activities. As in other ponerine ants, egg discrimination in *D. quadricaps* varied according to workers' roles. Brood care workers were able to discriminate nestmate from non-nestmate eggs in almost

Fig. 2 **a** Chromatogram of *Dinoponera quadricaps* egg surface showing the main compounds present: 1 C_{23} , 2 C_{25} , 3 C_{26} , 4 C_{27} :2, 5 C_{27} , 6 3Me C_{27} , 7 11-, 13-, 15 Me C_{29} ; 8 C_{31} :1; 9 C_{31} . **b** Discriminant analysis comparing the major compounds from four distinct colonies. Proportion of variance explained by root 1 was 80%, while root 2 was 15%. Circles colony 1, diamonds colony 5, squares colony 7, triangles colony 8



80% of trials. Error choices and latency time of recognition reinforced these results. On the other hand, callow workers were unable to recognize nestmate eggs (or eggs at all for that matter). This same pattern was found in *E. tuberculatum* in relation to nestmate larvae and cocoons (Féneron and Jaisson 1995). Conversely, callow workers of *Cataglyphis cursor* less than a day old were able to discriminate nestmate from alien larvae (Isingrini and Lenoir 1988). In *Camponotus japonicus*, brood recognition ability is achieved as the brain matures (Hara 2003). In this same species, it has been shown that antennal sensillae respond to chemicals of non-nestmate individuals (Ozaki et al. 2005), evidencing a learning mechanism to detect colony-specific signals.

The onset of foraging activities seems to be concomitant with a reduction of egg recognition in *D. quadricaps*. In fact, foraging workers collected about 20% of the alien eggs, and the time spent with each egg collection was significantly longer in our experiments. In *E. tuberculatum*, behavioral repertoire of foraging workers differs from nurses, and brood tending is unlikely to be performed by the former (Féneron and Jaisson 1995). Another proximate explanation for the loss of egg recognition might be a consequence of the lack of chemical template updating of the eggs to the foraging workers. Foraging workers spend a long part of their time performing outside activities. This changing from inside nest tasks to external work may affect sensorial abilities, as workers begin to explore the surrounding world. In fact, the learning familiarization of brood chemical template in ants appears to require continuous presence inside the chamber (Féneron 1993). However, founding associations of *Pachycondyla* queens show memory retrieval after even 24 h of separation (Dreier et al. 2007). The mechanisms involving the update of chemical profiles in adult individuals and brood still need experimental testing to be clarified.

Our results showed that eggs differ in many compounds between distinct colonies. Eggs had a blend composed of several hydrocarbons that varied quantitatively across the colonies, including hentriacontene (gamergate's substance). It is known that ants use cuticular hydrocarbons to discriminate nestmates from non-nestmates (Bonavita-Cougourdan et al. 1987; Lahav et al. 1999; Denis et al. 2006). Colony members bear the same odor pattern because it is continuously exchanged among nestmates. According to the Gestalt theory (Crozier and Dix 1979), nestmates blend their hydrocarbons to form a common colonial signature. In this study, we showed that eggs could also reflect colony identity in ants. Cuticular hydrocarbon profiles present on the surface of eggs varied according to their colony. This variation may have been the cue used by *D. quadricaps* brood care workers to discriminate between nestmate and alien eggs.

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