Animal Behaviour 88 (2014) 113–124

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Nestmate discrimination in the social wasp *Ropalidia marginata*: chemical cues and chemosensory mechanism

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A R T I C L E I N F O

Article history:
Received 3 April 2013
Initial acceptance 10 June 2013
Final acceptance 10 October 2013
Available online 20 December 2013
MS. number: 13-00292R

Keywords:
adult
behavioural sequence analysis
bioassay
chemosensory cue
cuticular hydrocarbon
gas chromatography
nestmate discrimination
newly eclosed individual
Ropalidia marginata

Nestmate discrimination plays an important role in preserving the integrity of social insect colonies. It is known to occur in the primitively eusocial wasp *Ropalidia marginata* in which non-nestmate conspecifics are not allowed to come near a nest. However, newly eclosed females are accepted in foreign colonies, suggesting that such individuals may not express the cues that permit differentiation between nestmates and non-nestmates. As cuticular hydrocarbons (CHCs) have been implicated as chemosensory cues used in nestmate recognition in other species, we investigated, using bioassays and chemical analyses, whether CHCs can play a role in nestmate recognition in *R. marginata*. We found that individuals can be differentiated according to colony membership using their CHC profiles, suggesting a role of CHCs in nestmate discrimination. Non-nestmate CHCs of adult females received more aggression than nestmate CHCs, thereby showing that CHCs are used as cues for nestmate recognition. Contrarily, and as expected, CHCs of newly eclosed females were not discriminated against when presented to a foreign colony. Behavioural sequence analysis revealed the behavioural mechanism involved in sensing nestmate recognition cues. We also found that newly eclosed females had a different CHC profile from that of adult females, thereby providing an explanation for why young females are accepted in foreign colonies.

Communication is an integral part of all living beings, and can be found at various levels of biological organization, ranging from the molecular level (intra- and intercellular communication) to the organismal and colonial or societal levels (communication between individuals and between groups of individuals). Interindividual communication plays an important role in the lives of social animals, as they have to perform various complicated social behaviours and social decision making. This is best exemplified in social insects, in which a large number of individuals (often thousands or millions) communicate and coordinate with each other to perform the tasks necessary for their existence efficiently. Nestmate discrimination, that is, recognizing colony members (nestmates) and differentiating them from nonmembers (non-nestmates) is an example of interindividual communication that is widespread in social insects (Gadagkar, 1985; Gamboa, Reeve, Ferguson, & Wacker, 1986; Gamboa, Reeve, & Pfennig, 1986; Pfennig, Gamboa, Reeve, Reeve, & Ferguson, 1983; van Zweden & d'Ettorre, 2010). Individuals recognize and differentiate between nestmates and non-nestmates and accordingly do or do not allow them to enter their nest, thus preventing social parasitism or theft of valuable resources from the nest. This also helps maintain group adhesion, allowing individuals to show altruistic behaviours towards nestmates, while avoiding or attacking non-nestmates, thus having an implication for colony fitness (Wilson, 1971). Thereby nestmate discrimination plays a key role in preserving the integrity of social insect colonies and is important for the organization and maintenance of eusociality.

The mechanism of nestmate recognition involves one individual perceiving the chemical cues present on the body surface of another (Dapporto, Fondelli, & Turillazzi, 2006; Howard & Blomquist, 2005; Martin, Vitikainen, Helantera, & Drijfout, 2008; van Zweden, Dreier, & d'Ettorre, 2009; van Zweden & d'Ettorre, 2010). It is believed to occur by matching a label (chemical signature containing nestmate/non-nestmate cues) with a template (representation of colony odour in memory), and, depending on the similarity or difference between the label and template, a conspecific is accepted into or rejected from the colony (Gadagkar, 1985; Sturgis & Gordon, 2012; Tsutsui, 2004; van Zweden & d'Ettorre, 2010). Over the years, cuticular hydrocarbons (CHCs) have come

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0003-3472/$38.00 © 2013 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.anbehav.2013.11.017

to be recognized as chemical cues used in nestmate recognition in various social insect species (Akino, Yamamura, Wakamura, & Yamaoka, 2004; Dapporto et al., 2006; Gamboa, Grudzien, Espelie, & Bura, 1996; Howard & Blomquist, 2005; Lenoir, Fresneau, Errard, & Hefetz, 1999; Lorenzi, Sledge, Laiolo, Sturlini, & Turillazzi, 2004; Martin et al., 2008; Ozaki et al., 2005; van Zweden et al., 2009; van Zweden & d'Ettorre, 2010). The relative proportion of compounds in CHCs have been found to differ between different colonies of a species, thereby giving rise to a colony-specific blend, and there is behavioural evidence for the involvement of CHCs in nestmate discrimination (Akino et al., 2004; Foitzik, Sturm, Pusch, d'Ettorre, & Heinze, 2007; Lahav, Soroker, & Hefetz, 1999; Lorenzi, Bagnères, Clement, & Turillazzi, 1997). Thus CHCs can function as chemical cues perceived when individuals come into contact and can be used by individuals to differentiate nestmates from non-nestmates.

The study of nestmate discrimination can be broadly divided into two components: (1) chemical cues or chemosensory cues, that is, the chemicals involved in forming the signal based on which interindividual chemical communication resulting in nestmate recognition occurs, generally exemplified by CHCs present on the cuticle of individuals, and (2) chemosensory mechanisms, that is, the proximate mechanisms (behavioural and neurobiological) used to perceive the chemical cues involved in nestmate recognition, and thereby to make the decision whether or not to allow an individual into a colony. The chemosensory cues that form the nestmate signal have been considered as the 'expression component' of nestmate recognition, wherein the polymorphic phenotypic cues or labels (generally CHCs in the case of social insects) are expressed on the external body surface of individuals, and such cues can be both inherent and derived from the environment (Tsutsui, 2004). The chemosensory mechanisms used in perceiving nestmate cues fall under the purview of the 'perception component' of nestmate recognition (Mateo, 2004). Lastly, following perception of the 'nestmate' cues, depending on the similarity to or difference between the perceived cue and template, a decision is made whether or not to allow an individual into a colony. This falls under the 'action component' of nestmate recognition (Liebert & Starks, 2004). Studies that have investigated nestmate discrimination in social insects have primarily focused on the chemosensory cues (the expression component) by looking at chemical differences in CHC profiles of colonies, sometimes followed by bioassays (involving the action component) to show that CHCs are indeed the chemical cues involved in nestmate discrimination (Akino et al., 2004; Dapporto et al., 2006; Foitzik et al., 2007; Gamboa et al., 1996; Howard & Blomquist, 2005; Lahav et al., 1999; Lenoir et al., 1999; Lorenzi et al., 1997; Lorenzi et al., 2004; Martin et al., 2008; van Zweden et al., 2009; van Zweden & d'Ettorre, 2010). Studies investigating the chemosensory mechanisms that are used to perceive nestmate discrimination cues (the perception component) are relatively rare (Brandstaetter & Kleineidam, 2011; Brandstaetter, Rössler, & Kleineidam, 2011; Leonhardt, Brandstaetter, & Kleineidam, 2007; Ozaki et al., 2005). Hence there is a need to incorporate investigation into the chemosensory mechanisms in addition to looking at the chemosensory cues in order to have a more comprehensive understanding of chemical communication.

Ropalidia marginata is a primitively eusocial paper wasp found in peninsular India. These wasps build nests out of cellulose material collected from plants. Each colony has one queen that monopolizes reproduction, and the other individuals function as sterile workers that perform foraging and other tasks needed to maintain a colony. Males stay on the nest for a brief period (usually up to 7 days), after which they leave the nest for a solitary life, and thus nest members are primarily females. The colony cycle is aseasonal, that is, nests are founded and abandoned throughout the year, and there is no body size variation between individual wasps from season to season. These wasps generally do not allow any non-nestmate conspecifics to come near their nest (Venkataraman & Gadagkar, 1992; Venkataraman, Swarnalatha, Nair, & Gadagkar, 1988). Young or newly eclosed individuals, however, are often accepted into foreign colonies, suggesting that such individuals do not express the cues that permit differentiation between nestmates and non-nestmates (Arathi et al., 1997; Venkataraman & Gadagkar, 1995). As CHCs have been implicated as chemosensory cues involved in nestmate recognition in various species, we investigated whether CHCs can be used for nestmate recognition in *R. marginata*. Since non-nestmates are attacked when they come near a foreign colony, we predicted that non-nestmate CHCs should receive more aggression than nestmate CHCs. Contrarily, since newly eclosed individuals are not discriminated against when presented to a foreign colony, we predicted that there should not be any difference between the aggression received by nestmate CHCs and that received by non-nestmate CHCs, when CHCs of newly eclosed individuals are used. Hence in addition to revealing the chemical cues used in nestmate recognition in *R. marginata*, this study also allowed us to show how the age of an individual has an effect on whether the individual will or will not be discriminated against in a foreign colony.

We also unravelled, using behavioural sequence analysis, the behavioural mechanism used in perceiving nestmate discrimination cues. Behavioural sequence analysis was first developed as a technique for the social sciences to study events that are sequential in nature or to analyse any context in a temporal manner (Abbott, 1995). It has been applied in animal behaviour, often to observe interactive social behaviour, and has mostly been used to look at behaviour occurring in successive time units or at which behaviour follows which behaviour (Bakeman & Gottman, 1997; Fagen & Young, 1978; Maubourguet, Lesne, Changeux, Maskous, & Faure, 2008; Slooten, 1994). It has not yet been applied to look at chemical communication in social insects. We used behavioural sequence analysis to look at which behaviour follows which behaviour and thereby to find out the behavioural chemosensory mechanism that is used for perceiving the chemical cues used in nestmate recognition. Aggression is the key behaviour for categorizing the response of a wasp towards a stimulus containing 'nestmate' cues as acceptance or rejection. High aggression is expected with non-nestmate CHCs implying rejection, and lower aggression or no aggression is expected with nestmate CHCs implying acceptance. Hence by looking at the behaviours that precede aggression, in a stimulus-specific manner, and based on the relative frequency with which aggression follows a particular given behaviour in response to a nestmate recognition stimulus, we could find out the behaviour that is involved in perceiving nestmate cues.

METHODS

Chemical Analysis

Colony differences in CHC profiles

To investigate whether CHCs can convey information on nest membership, we analysed the CHCs of adult females (>5 days old) from six different colonies. Postemergence nests of *R. marginata* were collected from various localities in Bangalore (13°00′N, 77°32′E), India, and transplanted to the vespiary at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore. The nests were maintained in closed cages made of wood and fine mesh, and provided with food, water and building material ad libitum. All adults were uniquely colour-coded with small spots of Testors