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SHORT COMMUNICATION

An absence of aggression between non-nestmates in the bull ant *Myrmecia nigriceps*

**Ellen van Wilgenburg · Susie Dang ·
Amy-Louise Forti · Tessa J. Koumoundouros ·
Anna Ly · Mark A. Elgar**

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Abstract The ability of social insects to discriminate against non-nestmates is vital for maintaining colony integrity, and in most social insect species, individuals act aggressively towards non-nestmates that intrude into their nest. Our experimental field data revealed that intra-colony aggression in the primitive bulldog ant *Myrmecia nigriceps* is negligible; our series of bioassays revealed no significant difference in the occurrence of aggression in trials involving workers from the same, a close (less than 300 m) or a far (more than 1.5 km) nest. Further, non-nestmate intruders were able to enter the nest in 60% of our trials; a similar level was observed in trials involving nestmates. These results suggest that workers of *M. nigriceps* are either unable to recognize alien conspecifics or that the costs of ignoring workers from foreign colonies are sufficiently low to favor low levels of inter-colony aggression in this species.

Keywords Nestmate recognition · *Myrmecia* · Hymenoptera

Introduction

Social insects engage with their colony mates in profoundly cooperative activities, including the construction of nests, rearing brood, foraging, and defending their nest against enemies (Wilson 1971; Hölldobler and Wilson 1990; Bourke and Franks 1995). The latter is particularly crucial to maintaining the integrity of the colony and, thus, deriving the benefits of social living. Threats to the colony can come from both conspecifics and other species that may invade the nest and exploit the resources therein (e.g., Hölldobler and Wilson 1990; Schmidt-Hempel 1998). Thus, many social insects have highly sophisticated recognition systems that allow individuals to distinguish between nestmates and alien conspecifics. These recognition systems rely on chemical cues, typically cuticular hydrocarbons that derive from environmental or genetic sources (Lahav et al. 1999; Thomas et al. 1999; Wagner et al. 2000).

Recognition of non-nestmates is typically manifested in aggressive behavior, which has been documented in termites, ants, bees, and wasps (for reviews, see Breed and Bennett 1987; Vander Meer and Morel 1998).The intensity of aggression between non-nestmates varies depending on the context (e.g., Starks et al. 1998; van Wilgenburg et al. 2005) and genetic and geographic distance between colonies and local conditions (e.g., Beye et al. 1998; Thomas et al. 1999). Only a few species show no aggression towards non-nestmates: most notable are several introduced species of ants for which there is an apparent absence of any non-nestmate aggression (Hölldobler and Wilson 1977). The low level of intraspecific territoriality and aggression in these so-called unicolonial populations is usually attributed to a loss of genetic diversity during introduction (Tsutsui et al. 2000). Alternatively, a lack of inter-colonial aggression may stem from the potential costs associated with discrimination. Reduced levels of aggression may occur when colonies are rarely in contact, if the costs of fighting are high and the benefits of excluding intruders low (Reeve 1989).

Many investigations of inter-colonial aggression involve “more advanced” social insects, including honey bees *Apis mellifera* (Breed 1998) and ants within the sub-families

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E. van Wilgenburg (✉) · S. Dang · A.-L. Forti ·
T. J. Koumoundouros · A. Ly · M. A. Elgar
Department of Zoology, The University of Melbourne,
Victoria 3010, Australia
e-mail: ellenvw@gmail.com

Dolichoderinae (e.g., meat ants *Iridomyrmex purpureus*, Thomas et al. 1999; van Wilgenburg et al. 2006; Argentine ant *Linepithema humile* Tsutsui et al. 2003), Myrmicinae (e.g., fire ants *Solenopsis invicta*, Obin 1986; Morel et al. 1990), and Formicinae (e.g., wood ants, *Formica* sp. Beye et al. 1998; Pirk et al. 2001; carpenter ants *Camponotus* sp, Carlin and Hölldobler 1986; green tree ants *Oecophylla smaragdina*, Elgar and Allan 2006). In contrast, few studies have considered nestmate recognition in species with more primitive social systems, and there are not many accounts of inter-colonial aggression in primitive ants.

Bull ants (*Myrmecia*, Myrmeciinae) have many primitive characteristics: Colonies are small and comprise between a few dozen to a few thousand workers, there is little morphological difference between queens and workers, and workers forage individually using primarily visual and tactile cues (Haskins and Haskins 1951; Hölldobler and Wilson 1990). Importantly, their chemical communication system is relatively unsophisticated (Hölldobler and Wilson 1990). While recent ant phylogenies (Moreau et al. 2006; Brady et al. 2006) challenge the long-held view that the Myrmeciinae is one of the most ancient ant lineages, the Myrmeciinae does represent a lineage of ants that diverged relatively early. The Australian bull ant *Myrmecia nigriceps* is among the larger species of *Myrmecia*, characterized by long, straight mandibles and large eyes (Shattuck 1999). Little is known regarding the species' basic biology. A colony of *M. nigriceps* nests in the ground, with a characteristic gravel mound surrounding the nest entrance. Workers are extremely aggressive and will chase and attack any intruders including other insects, spiders, echidnas, and humans that disturb their nest (Eriksson 1985; Gilhotra and Brown 2006). We conducted field experiments to investigate the behavior of workers of *M. nigriceps* towards conspecific intruders. We sourced non-nestmate intruders from both near and far nests to investigate whether the response of workers is modulated according to the distance between nests.

Materials and methods

We conducted field experiments during April 2006 at the Long Forest Nature Conservation Reserve, Victoria, Australia. We located 35 colonies from among three sites that were located within 3 km of each other. Nests at each site (A, B, and C) were marked with flagging tape, and their positions recorded on a GPS.

Bioassay

We conducted a series of bioassays in which we recorded the behavioral response of individuals of *M. nigriceps* to an 'intruder' worker placed near the entrance hole of their nest. The intruder workers were drawn from the same (control) nest: a 'near' nest, defined as a neighboring nest from the same site, less than 300 m away and a far nest from either of the two other sites, more than 1.5 km away. We differentiated between intruders and other ants by marking intruders with a small dot of nontoxic acrylic paint on their abdomen. Individuals were first chilled in a container for up to 1 h to make them behaviorally inert before being painted, and all of the ants were acclimated to ambient temperature before being subjected to an assay.

We obtained intruder workers by collecting three worker ants from each of the 35 marked colonies by gently scratching around the nest entrance and allowing a worker to climb onto a stick. We subjected each colony to the three treatments in succession; the order of treatments was randomized for each colony. The bioassays were performed blind with respect to treatment to prevent observer bias. We noted whether the intruder was small or large, based on observer experience. Each assay lasted 5 min, and we allowed at least 10 min between assays. Thus, we conducted a total of 105 assays on 35 nests, where nests served as both donor and recipient colonies.

For each assay, we noted the following behaviors: aggression (any seizing, biting, or stinging between intruder and resident); antennation (touching with the antennae between intruder and resident); entered nest (intruder entered the nest and disappeared from view); emerged from nest (the intruder reemerged from the nest before the end of the assay); left the nest mound (the intruder left the gravel mound of the nest);and returned (the intruder returned to the nest mound before the end of the assay).

Results

After release, the intruder worker walked around the nest mound, and in roughly a third of the trials, they would encounter and antennate with a resident worker; workers were similarly likely to antennate nestmates as non-nestmates (χ^2=0.25; *df*=2; *p*=0.88). We observed aggression in only 14 of the 105 trials, and antennation preceded aggression in all but three of the trials in which aggressive behavior occurred. The frequency of aggression was not influenced by the treatment; workers were no more likely to respond aggressively to nestmates than non-nestmates (χ^2= 0.17; *df*=2; *p*=0.92, Fig. 1). The size of the intruding ant also had no significant effect on the frequency of aggression (χ^2=979; *df*=1; *p*=0.322).

Remarkably, 63% of the non-nestmate workers entered the foreign nests, and the decision of an intruder to enter the nest was not influenced by whether she was a nestmate or non-nestmate (χ^2=3.45; *df*=2; *p*=0.18). There was no significant

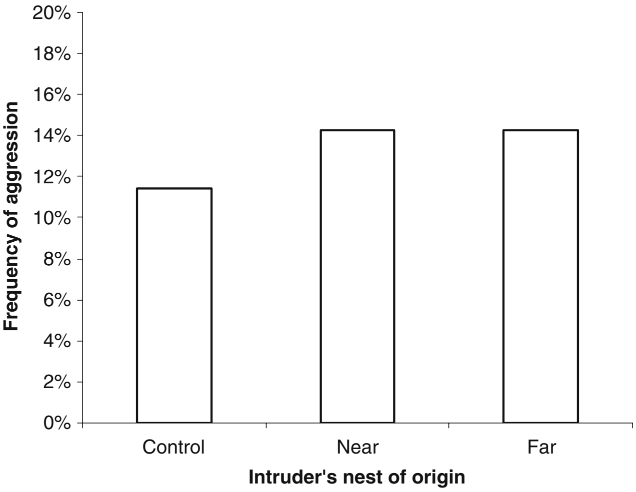

Fig. 1 Frequency of trials during which aggression was observed after the introduction of intruding ants from either the trial nest (control), near nests, and far nests

difference in the proportion of control, near, and far ants that subsequently reemerged from the nest entrance after entering it (χ^2=1.715; *df*=2; *p*=0.42) and those that left the nest mound (χ^2=0.33; *df*=2; *p*=0.85). However, of the workers that left the nest mound, significantly more control than near or far ants subsequently returned to the nest mound within the 5-min assay (χ^2=8.28; *df*=2; *p*=0.02, Fig. 2).

Discussion

Workers of the bull ant *M. nigriceps* generally respond passively to non-nestmate conspecifics that approach the nest entrance. Remarkably, intruding ants could enter and remain in the nest without attracting the kind of aggressive response that is typical of other species. In many cases, non-nestmate workers reemerged from the nest several minutes after entering it, and a few of these workers were likely to have remained for longer; the proportion of nestmates and non-nestmates that reemerged from the nest was similar, suggesting that non-nestmate workers were not necessarily attacked while inside the nest. Crosland (1989) reports low levels of intraspecific aggression in the genus *Myrmecia*, but this is the first time it has been confirmed by rigorous field experiments. While nestmate and non-nestmate workers behaved similarly around the nest mound, nestmates workers were more likely to return to the nest mound after leaving it than were their non-nestmate counterparts.

There are several reasons why workers of *M. nigriceps* fail to respond differently to nestmate and non-nestmate intruders. First, species of the primitively social genus *Myrmecia* may lack the general olfactory acuity to distinguish between individuals according to differences in their cuticular chemistry. This seems unlikely because nestmate recognition is reported in several species with less advanced forms of social organization (e.g., *Polistes* wasps, Gamboa 2004 and halicitine bees, Kukuk et al. 1977; Smith 1983). Moreover, workers of *Myrmecia gulosa* use cuticular hydrocarbons to discriminate between individuals within the colony (Dietemann et al. 2003). Second, the workers may be able to distinguish between individuals on the basis of cuticular hydrocarbons, but there was insufficient genetic or environmental variation between colonies in our sample to allow any distinction. This lack of variation is thought to explain why workers of the Argentine ant *L. humile*, in introduced populations, treat nearly all conspecifics as nestmates and form so-called uni-colonial populations (Tsutsui and Case 2001). However, colonies of *M. nigriceps* in our population show none of the characteristics that are typically associated with uni-coloniality, including colony budding, high worker numbers per nest, and high nest densities (Bourke and Franks 1995). More importantly, true uni-coloniality is often associated with introduced species, and *M. nigriceps* is native to the area.

Workers of *M. nigriceps* may be able to distinguish between nestmate and non-nestmate conspecifics, but may not respond aggressively because of the costs. For example, the lack of intra-colony interaction, the high cost of unintentionally attacking nestmates, and the low cost of accepting non-nestmate conspecifics may explain the low levels of aggression in the wood ant *Formica paralugubris* (Chapuisat et al. 2005). Workers of *M. nigriceps* may tolerate non-nestmate intruders for similar reasons. Colonies in this population are widely spaced, reducing the likelihood of interactions between members of different colonies. Indeed, the infrequent social interaction between

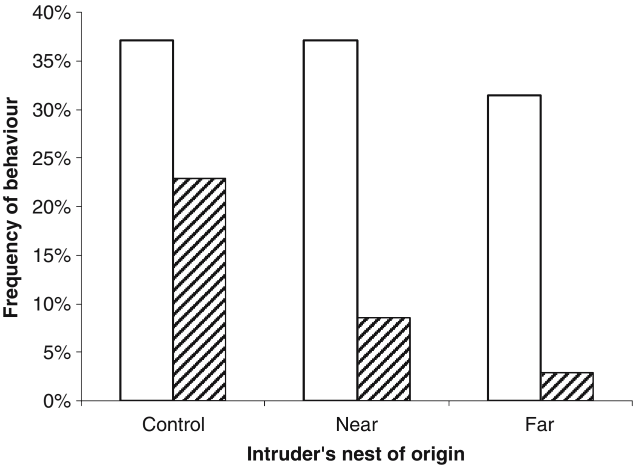

Fig. 2 The frequency of trials during which intruders left the nest (*open bars*), and returned to the nest (*hatched bars*), for intruders from control, near, or far nests

individuals of *M. nigriceps* corresponds with similar behavior reported for another primitively socially ant *Nothomyrmecia macrops* (Jaisson et al. 1992). More significantly, workers of *M. nigriceps* are extremely aggressive to heterospecific intruders, and thus, physical contests between individuals might result in death or serious injury to both parties. The loss of individual workers may be nontrivial, given the relatively small colony size of this species. Perhaps this cost of aggression also favors nonthreatening behavior on the part of foraging workers that happen to encounter the nest of an adjoining colony.

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