Host colony integration: *Megalomyrmex* guest ant parasites maintain peace with their host using weaponry

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Social parasites exploit resources of other social species, to the detriment of their host. In order to enter and integrate in a host colony, social parasites must avoid being detected as a non-nestmate. The parasites, therefore, use one or a combination of chemical strategies: (1) producing recognition cues that match host’s (mimicry), (2) acquiring recognition cues from the hosts or its nest (camouflage), (3) not producing recognition cues (insignificance) and/or (4) using substances for confusing, suppressing or appeasing the host (weaponry). In this study, we investigate the integration strategy of *Megalomyrmex symmetochus* ants into colonies of the fungus-growing ant *Sericomyrmex amabilis*. We compared the chemical odour profiles of parasitized and nonparasitized *S. amabilis* colonies with the profiles of the parasites. Additionally, we conducted behavioural assays, where we introduced a single ant, being either a nestmate, a conspecific non-nestmate or a parasite into an arena with five *S. amabilis* workers and scored the behaviour of the latter ants. The chemical analysis revealed that the social parasites have distinct odour profiles and share only one hydrocarbon with its host, have a low overall abundance of cuticular hydrocarbons and have high concentrations of venom-derived alkaloids. In behavioural experiments, we found that workers of nonparasitized colonies fight against parasite intruders, whereas workers of parasitized colonies treat introduced parasites (from their own and from another parasitized colony) similar to their conspecific nestmates. All workers (parasitized or not) show more submissive behaviour towards parasitized workers and parasites than towards nonparasitized workers. The chemical analysis of odour profiles suggests that the parasites use a chemical insignificance strategy. Furthermore, the chemical and behavioural data suggest that the parasites use weaponry to maintain an amiable association with their host ants. We discuss the biological significance of the lack of aggression in *S. amabilis* workers from parasitized colonies.

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Sophisticated mechanisms have evolved to protect social insect colonies (e.g. ants, some bees and wasps) from invasion (Breed & Bennett, 1987; Gamboa, Reeve, & Pfennig, 1986; Hölldobler & Wilson, 1990). Therefore, a successful exploitation of social insect colonies requires strategic evasion of organized defence tactics. The recognition of colony members is a fundamental component of being social, and thus allows amiable social groupings and loyalty between colony members. The nestmate recognition system of social insects is predominantly chemically based and allows individuals to discriminate between members from their own colony (nestmates) and members from a different colony (non-nestmates). In ants, nestmate recognition is based on long-chained species-specific cuticular hydrocarbons (CHCs) present on the exoskeleton (Brandstaetter, Endler, & Kleineidam, 2008; Lahav, Soroker, Hefetz, & Vander Meer, 1999; Martin & Drijfhout, 2009). CHCs are thought to have evolved primarily as protection against desiccation (Lockey, 1988), and gained secondarily a function for identification of colony membership, where neighbouring colonies of the same species have the same CHCs, which only differ in quantity (d’Ettorre & Lenoir, 2009). The odour profile of a colony is not only genetically determined, but also influenced by environmental factors, like nest
chemical parasites can produce nestmate recognition cues that match the host’s (Akino, 2008; Lenoir, 1998). When label and template are similar, the encountered ant will be recognized as a nestmate; if mismatched, the encountered ant is recognized as a non-nestmate and may be attacked (Vander Meer & Moré, 1998). Since the colony odour changes over time, the neuronal template needs to be updated as well (Vander Meer et al., 1989). Social parasites, defined as a social organism that exploits another social organism, avoid host attack by circumventing detection or through host domination (Buschinger, 2009). Social parasites overcome host detection by using one or more chemical strategies: mimicry, camouflage, insensitivity and/or weaponly (Akino, 2008; Lenoir, d’Ettorre, Errard, & Hefetz, 2001). Some parasites can produce nestmate recognition cues that match the host’s chemical profile (chemical mimicry) while others can acquire them by exposure to the nest environment or host individuals (chemical camouflage). An impressive example for these two strategies is the caterpillar of the butterfly Maculina rebelli that parasitizes Myrmica ant colonies (Akino, 2008; Nash, Ais, Maile, Jones, & Boomsma, 2008). The caterpillars produce host recognition cues and are carried by ant workers into the nest. Later they acquire additional hydrocarbons within the nest, making their CHC profile nearly identical to their host’s (Akino, Knapp, Thomas, & Elmes, 1999). Similarly, social parasite wasp CHC profiles change to match colony-specific host odour following infiltration and during host colony integration (Sledge, Dani, Cervo, Dapporto, & Turillazzi, 2001). Other parasite species lack an abundant CHC profile, when recognition cues are absent the parasites appear to be chemically ‘invisible’ to the host (i.e. chemical insensitivity). This strategy is used during host colony infiltration by the social parasitic ant Acromyrmex insinuator. The parasites avoid host colony aggression by producing fewer hydrocarbons relative to their host and bearing increased n-alkane levels (Nehring, Dani, Turillazzi, Boomsma, & d’Ettorre, 2015). Besides circumventing detection, parasites can also produce chemicals to attack or confuse the host, disrupting nestmate recognition and host defence behaviour (chemical weaponry). This strategy is used in the slave-making ant species Polyergus rufescens. The usurping queen uses secretions from its Dufour’s gland as an appeasement allomone (Mori, Grasso, Visicchio, & Le Moli, 2000) or repellent (d’Ettorre, Errard, Ibarra, Francke, & Hefetz, 2000).

The expected evolutionary response of hosts towards social parasites can be either in the form of resistance or tolerance, both adaptive solutions to parasite exploitation. Parasite resistance may involve direct host aggression towards the parasite, preventing a successful attack. It may also involve a hierarchical sequence of resistance behaviours that occur over time (Kilner & Langmore, 2011). In other circumstances, hosts use a ‘tolerance’ strategy to minimize detrimental fitness impacts of parasites, in other words, it is better to consent than to risk death (Svensson & Råberg, 2010). Tolerance behaviour would be expected in host species that lack an effective defence (e.g. toxic poison, strong mandibles) or species that do not recognize the parasite as a threat but instead as a harmless nestmate.

The social parasitic ant species Megalomyrmex symmetochus (Formicidae: Solenopidini) (Wheeler, 1925) parasitizes the fungus-growing ant Sericomyrmex amabilis (Formicidae: Attini; Attina) (Wheeler, 1925) by living within the nest and consuming the brood and fungus garden of the host (Adams et al., 2013; Adams et al., 2013; Bruner, Wcislo, & Fernández-Marin, 2014; Bruner et al., 2014; Wheeler, 1925). The interactions between the host and parasites are typically amiable. However, aggression has been observed in field and laboratory colonies when the two species are producing sexuals (Boudinot, Sumnicht, & Adams, 2013). In addition, the parasite workers chew the wings from the host female sexuals (i.e. gynes), prohibiting these individuals from dispersing. The parasite workers are armed with toxic alkaloid venom that can kill the host and the hosts’ enemies (Adams et al., 2013). In contrast, S. amabilis workers do not appear to have a toxic venom, but are capable of biting off legs and antennae from their opponents (Adams et al., 2013; Boudinot et al., 2013). Their reaction to threat is often crypsis, during which ants tuck their antennae and head under and play dead, similar to other attine host species (Adams, Jones, Longino, Weatherford, & Mueller, 2015).

The infiltration strategy (i.e. initiation of the association) and integration strategy (i.e. maintenance of the association) of M. symmetochus parasites into colonies of S. amabilis are currently unknown. In this study, we investigate the integration strategy of M. symmetochus parasites using chemical analysis and a behavioural approach. If host colonies and parasites have similar CHC profiles (i.e. mimicry or camouflage), then we predicted that workers from a parasitized colony would not react to the parasites but workers from a nonparasitized colony would react aggressively, just as they would to a conspecific non-nestmate. If the parasites’ CHCs are very low in abundance, then we predicted that the host as well as nonparasitized ants would behave as if they did not detect the parasites (i.e. chemical insensitivity). If the CHC profile of the parasite is not similar to their host’s and causes a behavioural reaction by S. amabilis workers from parasitized and nonparasitized colonies, it would suggest a weaponry strategy. We found no evidence for mimicry or camouflage. In contrast, our chemical analysis suggests that the parasites use a chemical insensitivity strategy. Furthermore, the chemical and behavioural data support the hypothesis that the parasites use weaponry to maintain an amiable association with their host ant species S. amabilis.

**METHODS**

**Study Animals**

Sericomyrmex amabilis colonies or subcolonies (referred to as colonies hereafter) were collected along Plantation Road in Gamboa (9°9′36″N, 79°44′24″W) and on Barro Colorado Island (9°9′36″N, 79°50′24″W), Republic of Panama between 2011 and 2013. Queenright and queenless laboratory colonies can be kept alive for years under humid laboratory conditions as long as they forage to feed their garden (R. M. M. Adams, personal observation). In our experiments, 16 laboratory host colonies contained M. symmetochus social parasites (referred to as parasitized colonies) and 19 S. amabilis colonies were without parasites (nonparasitized colonies).

**Creating CHC Extracts**

We randomly collected workers from parasitized and non-parasitized colonies (host and parasites, if present) from their nestboxes into filter paper-lined petri dishes, allowed them to acclimate, then froze them over night at −80 °C (colonies and species were kept in separate dishes). For each extract, we selected three workers and put them in a 2 ml vial with ca. 100 μl of pentane (cuticular wash). The vials were gently agitated for 1 min using a vortex machine with a slow rotating speed. After waiting 10 min and gently shaking the vial again, the solution was moved to a 200 μl insert and evaporated. The samples were frozen until chemically analysed. In total, we created 19 host ant extract samples from four parasitized colonies, 36 ant extracts of eight colonies. The chemical analysis was performed using gas chromatography coupled with mass spectrometry. The chemical profiles were compared using principal component analysis (PCA). The PCA analysis showed a clear separation between host and parasite colonies, indicating that the parasites use a chemical insensitivity strategy to avoid detection by the host colonies.
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