

# Chemically armed mercenary ants protect fungus-farming societies

Rachelle M. M. Adams<sup>a,b,1</sup>, Joanito Liberti<sup>a</sup>, Anders A. Illum<sup>a</sup>, Tappey H. Jones<sup>c</sup>, David R. Nash<sup>a,1</sup>, and Jacobus J. Boomsma<sup>a</sup>

<sup>a</sup>Centre for Social Evolution, Department of Biology, University of Copenhagen, DK-2100 Copenhagen, Denmark; <sup>b</sup>Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560; and <sup>c</sup>Department of Chemistry, Virginia Military Institute, Lexington, VA 24450

Edited by Bert Hölldobler, Arizona State University, Tempe, AZ, and approved August 4, 2013 (received for review June 20, 2013)

The ants are extraordinary in having evolved many lineages that exploit closely related ant societies as social parasites, but social parasitism by distantly related ants is rare. Here we document the interaction dynamics among a *Sericomyrmex* fungus-growing ant host, a permanently associated parasitic guest ant of the genus *Megalomyrmex*, and a raiding agro-predator of the genus *Gnampotogenys*. We show experimentally that the guest ants protect their host colonies against agro-predator raids using alkaloid venom that is much more potent than the biting defenses of the host ants. Relatively few guest ants are sufficient to kill raiders that invariably exterminate host nests without a cohabiting guest ant colony. We also show that the odor of guest ants discourages raider scouts from recruiting nestmates to host colonies. Our results imply that *Sericomyrmex* fungus-growers obtain a net benefit from their costly guest ants behaving as a functional soldier caste to meet lethal threats from agro-predator raiders. The fundamentally different life histories of the agro-predators and guest ants appear to facilitate their coexistence in a negative frequency-dependent manner. Because a guest ant colony is committed for life to a single host colony, the guests would harm their own interests by not defending the host that they continue to exploit. This conditional mutualism is analogous to chronic sickle cell anemia enhancing the resistance to malaria and to episodes in human history when mercenary city defenders offered either net benefits or imposed net costs, depending on the level of threat from invading armies.

evolutionary transition | symbiosis | Attini | Solenopsidini

Ant societies retain much of their coherence through chemical nestmate recognition (1, 2), which allows resident workers to differentiate between friend and foe by colony-specific chemical signatures (3). This recognition system is not infallible, however; numerous species have evolved ways to evade detection so they can exploit ant colonies through invasion, usurpation, or thievery (2). One common route to social parasitism is shown convergently by many ant genera in which social parasites are their host's closest relatives, a scenario that might have arisen through sympatric speciation (4, 5). At the other end of the spectrum are interactions with different insect orders, such as parasitic beetles and butterfly caterpillars, that drain host ant colonies of resources while remaining protected by crypsis or chemical mimicry (6).

Intermediate types of parasitic interactions involving ants that exploit other, distantly related ants are rare (7). Several species of *Megalomyrmex* (Solenopsidini) belong to this category, associating in varying capacities with fungus-growing ant hosts (Attini), a clade of the same ant subfamily (8–12) (Fig. 1). Most details about the diversity and life history adaptations of these ants have been clarified only recently (11), and much of their biology remains to be discovered (*SI Text: Study System* and *Tables S1* and *S2*). Free-living predatory *Megalomyrmex* are known to biosynthesize venom alkaloids that are used in defense (13, 14) whereas the fungus-growing ant associates seem to aggressively dispense these venoms when they attack host colonies

(15). Some of these parasites are obligate or facultative thief ants consuming brood and fungus gardens (15), whereas others are specialized agro-predators that move from one host colony to the other after usurping fungus gardens and killing or chasing away the resident ants (10). Despite the often high densities of their attine hosts, these *Megalomyrmex* social parasites remain rare, with parasitism rates of ~1.5–14% (10, 15), similar to the rates of many other social parasites (5).

Behaviorally derived lineages of *Megalomyrmex* have become guest ant parasites of the higher attine genera *Trachymyrmex* and *Sericomyrmex* (11, 16, 17) (Fig. 1A). The *Trachymyrmex* specialist, *Megalomyrmex adamsae*, appears to have remained as equally rare as the agro-predators and thief ants (11), but the *Sericomyrmex* specialist *Megalomyrmex symmetochus* (Fig. 1B) is surprisingly common, with a prevalence of >80% in some host populations (16). Newly mated guest ant queens of both species likely enter host colonies by stealth and establish themselves in the fungus garden, where their developing colony will consume host brood and fungus garden for years (11). Their presence slows host colony growth and also prevents or reduces host reproduction, because both guest ant species clip the wings of host gynes (virgin queens), but not males (11). Such mutilation reduces host reproduction and dispersal via mating flights, but likely increases guest ant fitness when these mutilated females adopt worker tasks (11, 18). Like all guest ant social parasites, *M. symmetochus* retains a fully functional worker caste (16, 19), in contrast to many social parasites that exploit the services of their phylogenetically similar host colony without the need to produce workers (5).

The maintenance of a large worker caste despite permanent cohabitation with a host colony may have several purposes, all based on some specialized role for the parasite workers. First, it

## Significance

We document the behavioral interactions among three ant species: a fungus-growing host ant, a permanently associated parasitic guest ant, and a raiding agro-predator ant. We show that the presence of guest ants becomes advantageous when host ants are attacked by raider ants, because guest ants use alkaloid venom to defend their host ant colony. Furthermore, detection of the guest ant odors is sufficient to discourage raider scouts from recruiting nestmates to host colonies. Guest ants likely have evolved this protective behavior because they also perish when their host colony dies.

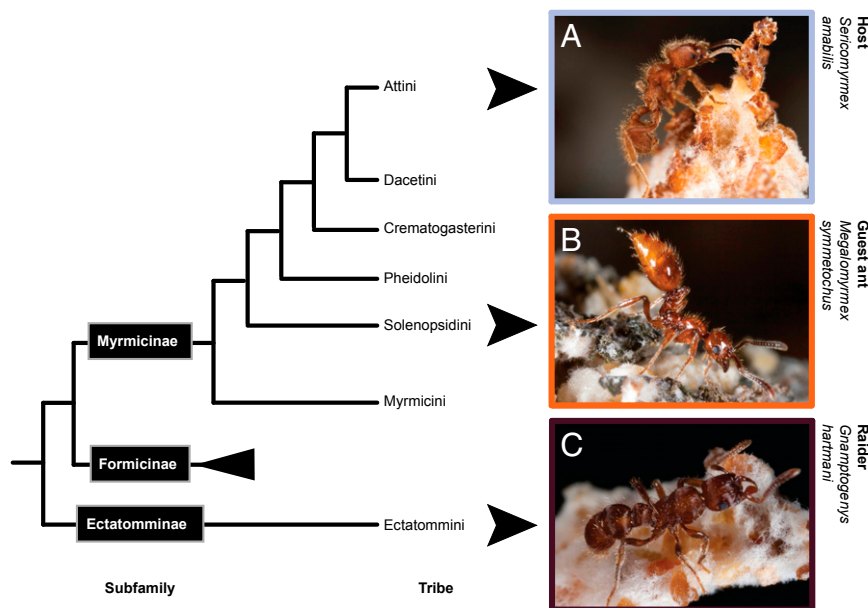
Author contributions: R.M.M.A., J.L., A.A.I., D.R.N., and J.J.B. designed research; R.M.M.A., J.L., A.A.I., and T.H.J. performed research; R.M.M.A. and D.R.N. analyzed data; and R.M.M.A., D.R.N., and J.J.B. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

<sup>1</sup>To whom correspondence may be addressed. E-mail: rmmadams@gmail.com or drnash@bio.ku.dk.

This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1311654110/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1311654110/-DCSupplemental).



**Fig. 1.** Subfamily and tribe-level tree after Brady et al. (8) indicating the different phylogenetic positions of the interacting ant species. (A) The fungus-growing ant host *S. amabilis*. (B) The guest ant social parasite *M. symmetochus* (in a stilted stance, emitting volatile alkaloids from its protruding sting). (C) The *G. hartmani* raiding agro-predator. *M. symmetochus* and *G. hartmani* have independently specialized on using *S. amabilis* fungus gardens and brood as food.

may be that the distantly related host workers are unable to feed the social parasite larvae (5) even though adult hosts and parasites are adapted to the same highly specific fungal diet. Another possibility is that the guest ant colony remains at risk of occasional attack by the host workers; such antagonism has been observed between *M. symmetochus* workers and host workers in older colonies (*SI Text, Study System*). But *Sericomyrmex* ants have only vestigial stings (20) and often feign death when attacked, and so this does not explain the large number of parasite workers. Thus, it seems reasonable to hypothesize that a large number of *Megalomyrmex* guest ant workers continue to remain essential for the survival and reproductive success of their own mother queen in ways other than merely reinforcing their dominance over *Sericomyrmex* host workers and caring for their own brood.

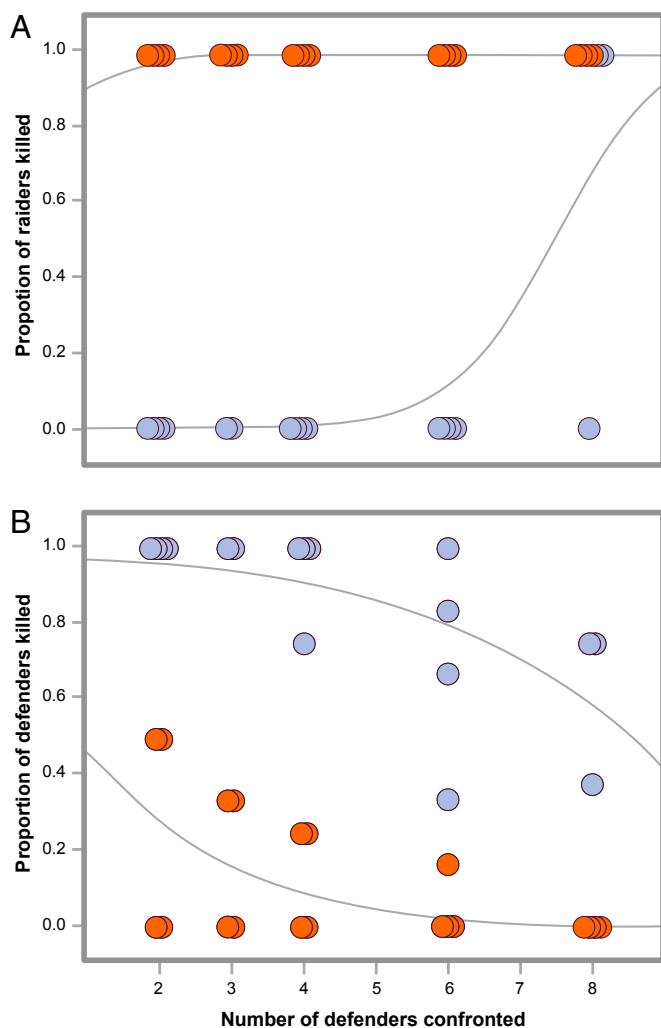
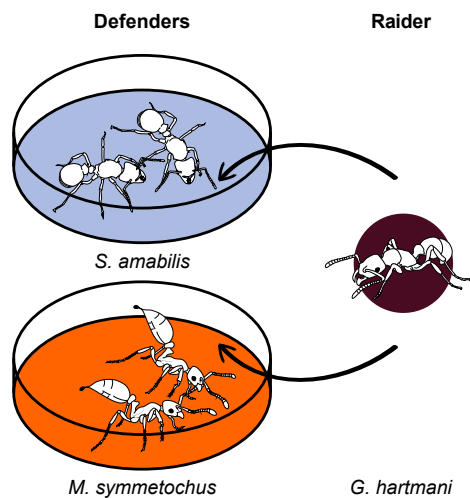
Neither founding queens nor mature colonies of *M. symmetochus* guest ants are known to move to other host colonies later in life, implying that guest ant reproductive success is completely dependent on the continued well being (albeit not the reproduction) of host colonies (11). Thus, we conjectured that *M. symmetochus* guest ants might act as defenders when host colonies are attacked by natural enemies that are sufficiently effective to pose a significant threat. Such a specialized enemy, the unrelated agro-predator ant *Gnamptogenys hartmani* (Ectatomminae: Ectatommini) (Fig. 1C and Fig. S1A), was recently seen to raid colonies of *Sericomyrmex* in Panama and to usurp their gardens and nest structures with remarkable efficiency (21). This finding suggests that the enhanced mortality risk emanating from these raids might have produced an unusual secondary mutualism between the socially parasitic guest ants and their hosts. Rather than merely reducing worker production as ant social parasites normally do, the *M. symmetochus* guest ants produce a seemingly excess number of workers (Table S2) that constantly patrol the host nest. These guest ants' potent alkaloid weaponry, which originally secured their establishment at a chronic cost to the host colony, potentially could also serve to protect the host from greater harm in the direct interest of the guest ant parasite.

We tested this idea in a series of controlled laboratory experiments aimed at quantifying the damage by *G. hartmani* agro-predator parasites and the defense efficiency of *Sericomyrmex* hosts with and without guest ants (*SI Text: Study System*). We found that hosting even a moderate number of *Megalomyrmex* guest ants provides almost complete protection against *G. hartmani* raids, because (i) guest ants are much more efficient than host ants in killing intruding *G. hartmani* workers; (ii) guest ants reduce host ant mortality inflicted by the raiding agro-predators; and (iii) scouts of *G. hartmani* preferentially recruit nestmates to *Sericomyrmex* host colonies whose odor indicates an absence of guest ants.

## Results and Discussion

After a number of staged encounters with guest ant-infected subcolonies of *S. amabilis* in the laboratory, it became clear that *G. hartmani* scouts were immediately attacked by the *M. symmetochus* guest ants, and that raids were often deterred (Movies S1 and S2). Not only were *G. hartmani* workers killed during the altercations with the *M. symmetochus* defenders, but some were also attacked later by members of their own raiding party (Fig. S1B), suggesting that *M. symmetochus* venom is both toxic and causes confusion in *G. hartmani* ants. In addition, experimentally stung *G. hartmani* ants more often avoided contact with a naïve sister compared with controls ( $2.88 \pm 0.72\%$  of time spent together vs.  $33.27 \pm 7.01\%$ ; Welch's  $t = 4.31$ ,  $df = 1$ ,  $P = 0.0073$ ) (*SI Text: Methods and Results* and Fig. S1 C and D), but when contact was made, stung ants were often attacked (four of six replicates) and sometimes killed, indicating that *M. symmetochus* venom disrupts nestmate recognition abilities of *G. hartmani*, much like other antagonistic chemicals used by ant exploiters (2, 22).

To formally investigate the efficiency of *M. symmetochus* and *S. amabilis* defenses against a single *G. hartmani* ant, we conducted a series of experiments with a varying number of opponents (two, three, four, six, or eight workers of *S. amabilis* or *M. symmetochus*) (Fig. 2). We found that *M. symmetochus* workers were much more effective than *S. amabilis* host workers at killing *G. hartmani* raiders [binomial generalized linear model (GLZ), likelihood ratio (LR)  $\chi^2 = 42.3$ ,  $P < 0.0001$ ] (Fig. 2A). Surviving *S. amabilis* hosts sometimes fled to the roof of the nest box to escape their predators, as reported previously by Dijkstra



**Fig. 2.** Defense efficiencies of host and guest ants. (A) The resulting mortality after a single *G. hartmani* agro-predator interacted with groups of two to eight *S. amabilis* host ants (blue Petri dish) or *M. symmetochus* guest ants (orange Petri dish) after 24 h. Defender category significantly affected *G. hartmani* worker mortality (binomial GLZ, LR  $\chi^2 = 42.34$ ,  $P < 0.0001$ ), with *S. amabilis* effective in killing only when greatly outnumbering *G. hartmani* (blue dots) and *M. symmetochus* significantly more effective in killing regardless of their number (orange dots). (B) The overall mortality inflicted by the *G. hartmani* worker on host or guest ant defenders differed significantly (binomial GLZ, LR  $\chi^2 = 18.84$ ,  $P < 0.0001$ ), with *S. amabilis* defenders (blue

dots) taking proportionally much higher casualties. The proportional mortality of both defenders decreased significantly with an increasing number of defenders (binomial GLZ, LR  $\chi^2 = 7.29$ ,  $P = 0.0069$ ), but this decrease did not differ between the defending species (interaction between number and species of defenders, binomial GLZ, LR  $\chi^2 = 0.0996$ ,  $P = 0.752$ ). Ant drawings courtesy of Rozlyn Haley.

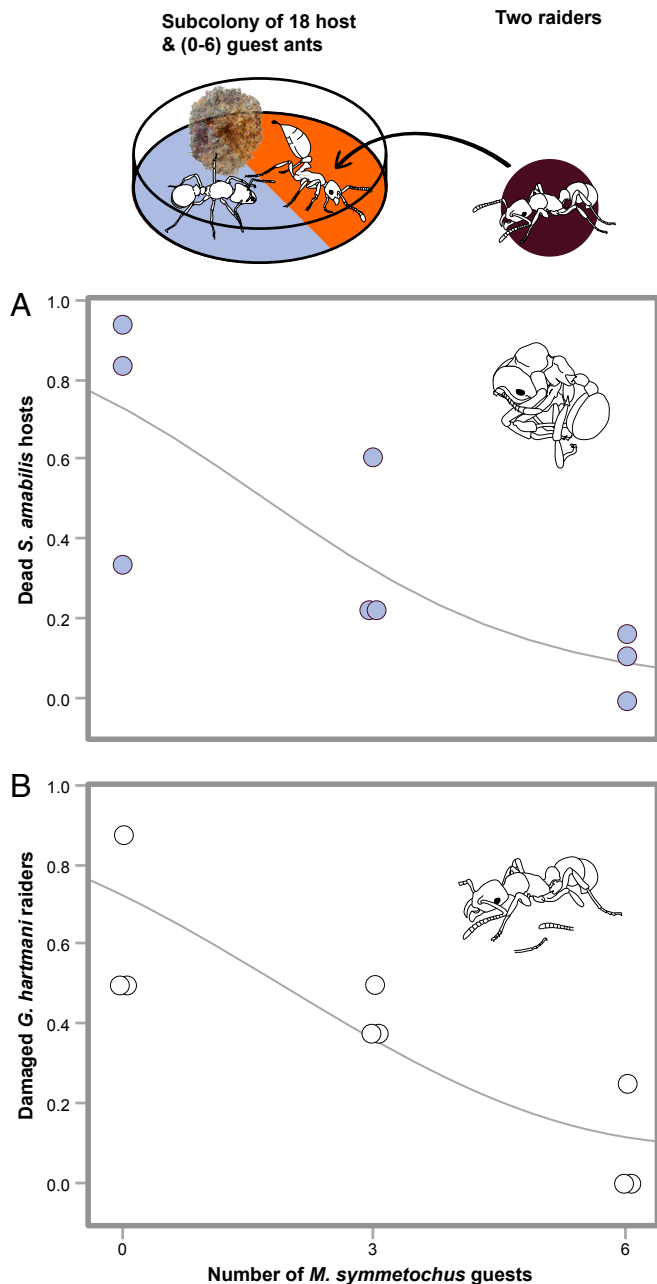
and Boomsma (21), and never killed the intruding *G. hartmani* worker in the first hour of the trial ( $n = 17$ ). However, *M. symmetochus* workers pursued, attacked, and killed the *G. hartmani* raiders in 45% of 20 trials in the first hour and in 100% of 20 trials within 24 h (Fig. 2A). Furthermore, the guest ant workers were significantly less likely than the *S. amabilis* host workers to be killed by the *G. hartmani* worker (binomial GLZ, LR  $\chi^2 = 7.29$ ,  $P = 0.007$ ) (Fig. 2B). A higher number of defenders of either species was generally associated with a reduced average casualty rate (LR  $\chi^2 = 18.85$ ,  $P < 0.0001$ ), and this reduction was similar for both species (species  $\times$  number interaction, LR  $\chi^2 = 0.0996$ ,  $P = 0.752$ ).

When attacking, *G. hartmani* workers lock on to their *S. amabilis* opponents and sting repeatedly, releasing their potent nonvolatile venom (*SI Text: Methods and Results*). These one-to-one engagements can last from approximately 20 seconds to up to 10 min, during which time the *S. amabilis* defenders may use their relatively powerful mandibles to bite off legs or antennae of the *G. hartmani* attackers (Fig. S1A) but then feign death, prompting the *G. hartmani* workers to release them. This cycle can repeat itself several times before the *S. amabilis* workers are eventually killed. In contrast, the *Megalomyrmex* defensive strategy is aggressive and uses an approximate 1:1 mix of two isomers of butylhexylpyrrolizidine alkaloids (*SI Text: Methods and Results*) dispensed from their specialized sting as an aerosol or contact venom (Fig. 1B, *SI Text: Methods and Results*, and Movie S2). To evaluate the complex interactions between all three ant species, we conducted a second experiment using larger and more natural (i.e., with fungus garden fragments) subcolonies of *S. amabilis* with and without a variable number of guest ants in which we introduced two *G. hartmani* raiders.

In this more complex interaction experiment, both introduced *G. hartmani* workers were killed in six of the nine *S. amabilis* subcolonies across the range of zero, three, or six *M. symmetochus* defenders (Fig. 3). When mortal damage was also considered (i.e., at least six of eight possible *G. hartmani* extremities lost), all trials left at least one *G. hartmani* dead or doomed after 24 h independent of the number of guest ants (binomial GLZ, LR  $\chi^2 = 0.286$ ,  $P = 0.593$ ). As in the first experiment (Fig. 2B), the *G. hartmani* raiders were very effective at killing a high proportion (70% on average) of the *S. amabilis* host workers in the absence of *M. symmetochus*, but as the number of *M. symmetochus* guest ants increased, host casualties decreased to rather low values (LR  $\chi^2 = 10.93$ ,  $P = 0.0009$ ) (Fig. 3A). This finding indicates that the numerical ratios of interacting ants used in this experiment were well balanced, such that adding defenders of each category had a noticeable effect. The external damage to *G. hartmani* workers (i.e., the proportion of the maximum  $2 \times 8$  raider legs and antennae that were missing) decreased with increasing numbers of defending guest ants (LR  $\chi^2 = 14.17$ ,  $P = 0.0002$ ) (Fig. 3B), supporting behavioral observations that *G. hartmani* workers were indeed killed by the guest ants and not by the host ants. As in the first experiment (Fig. 2B), there was little difference in guest ant mortality between treatments (LR  $\chi^2 = 0.232$ ,  $P = 0.630$ ), indicating that as few as three *M. symmetochus* workers offer adequate protection to *S. amabilis* colonies when there are two *G. hartmani* intruders. This protection was even greater when the ratio of *M. symmetochus* to *S. amabilis* was increased to 1:3 (6 to 18; Fig. 3A), a ratio close to that seen in field colonies (Table S2).

Adams et al.





**Fig. 3.** Host survival rates and raider mutilations. When threatened, *M. symmetochus* guest ants use toxic venom, whereas *Sericomyrmex* hosts mutilate intruders by removing appendages. (A) When 18 host ants were confronted with 2 intruding *Gnamptogenys* workers without (0) or with (3 or 6) *Megalomyrmex* guest ants, the proportion of *S. amabilis* deaths was decreased (binomial GLZ, LR  $\chi^2 = 10.93$ ,  $P = 0.0009$ ). (B) The same increased number of guest ants was also associated with a reduced rate of extremity damage in *G. hartmani* workers (binomial GLZ, LR  $\chi^2 = 14.18$ ,  $P = 0.0002$ ), consistent with the fact that the agro-predator raiders were no longer attacked by *S. amabilis* defenders because they were killed by guest ants rather than by physical mutilation by the host ants. Ant drawings courtesy of Rozlyn Haley.

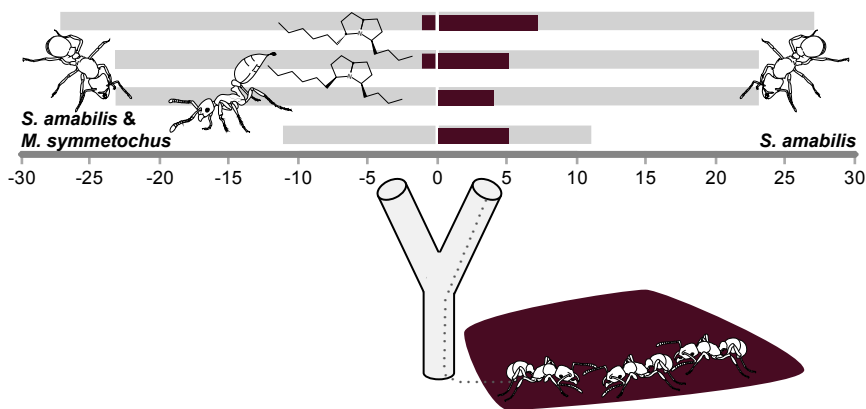
In the more realistic scenario of mixed subcolonies (Fig. 3), the guest ants remained more effective at killing *G. hartmani* raiders than their *S. amabilis* hosts, who needed much larger numbers to mount at least some resistance against one or two *G. hartmani* intruders. (Compare Fig. 24, in which eight *S. amabilis* workers were able to kill a single *G. hartmani* raider in two

out of three trials, but at the cost of 63% mortality.) However, under field conditions, scouts of *G. hartmani* normally return to their nest to recruit a column of nestmates before initiating a raid. This may easily involve 100 or more *G. hartmani* (Fig. S2), which can quickly overwhelm an *S. amabilis* colony (21). Thus, hosting and feeding an *M. symmetochus* guest ant colony likely would have substantial fitness payoffs for *S. amabilis* when the risk of a raid by *G. hartmani* is high, and these benefits would be even greater if *G. hartmani* colonies preferred to raid *S. amabilis* colonies without guest ants.

To test this possibility, we conducted a y-tube choice experiment, which showed that *G. hartmani* preferentially initiated raids on *S. amabilis* colonies without guest ants relative to control colonies with guest ants (binomial GLZ, LR  $\chi^2 = 18.12$ ,  $P < 0.0001$ ) (Fig. 4, SI Text: Methods and Results, Fig. S2, Movie S3, and Table S3). Experimental *Gnamptogenys* scouts were allowed minimal contact with resident *S. amabilis* workers through a mesh separating their colonies, and they were never observed to have physical contact with guest ants. Thus, it is likely that the volatile components of the *M. symmetochus* venom were the main factors deterring raids (Fig. 4). Fig. 1B depicts a worker projecting its sting to disperse two isomers of 3-butyl-5-hexylpyrrolizidine (Fig. S3); SI Text: Methods and Results provides information on chemical analysis. This finding suggests that direct contact with *M. symmetochus* defenders is not necessary for a *G. hartmani* scout to determine whether a *S. amabilis* colony is worth recruiting to. However, field colonies of *M. symmetochus* can have hundreds of workers that are spread out across all chambers of a host colony (Table S2). Furthermore, physical contact between a *G. hartmani* scout and an *M. symmetochus* defender is likely, given that *M. symmetochus* workers recruit nestmates from their deeper fungal cavity when an intruder is detected (Movie S2), further enhancing the prophylactic inhibition of *G. hartmani* raids.

Our results confirm that socially parasitic *M. symmetochus* guest ants can serve as protective symbionts of their *S. amabilis* hosts. This development is remarkable because, despite the parasitic (i.e., maintenance) costs, the relationship between the host and guest ants has shifted to a context-dependent mutualism in which the cost to the host is compensated for by a secondary protection benefit against a shared natural enemy. It has turned an interaction governed by negative antagonistic selection into one characterized by positive reinforcement, allowing the guest ants to become unusually common (in 73% of host nests on average; SI Text: Methods and Results and Table S2). This finding reinforces the concept that mutualistic interactions are actually driven by mutual exploitation (23), and that the outcomes can be mutualistic win-win situations under certain conditions and parasitic win-lose situations in other circumstances. This variation may be a key factor in the coevolution of such interactions, and is one of the cornerstones of the geographic mosaic theory of coevolution (24, 25).

Our results suggest that *M. symmetochus* guest ant prevalence should be positively correlated with *Gnamptogenys* agro-predator density across sites, as has been shown in other protective symbionts (26, 27). We would also expect the guest ant *M. adamsae* and its host *T. zeteki* to suffer much less from *Gnamptogenys* raids given the typically much lower host colony infection rates (~1–6%; ref. 11), but we lack the comparative data needed to test this possibility. This dynamic coevolutionary scenario would seem to be conditional on each of the partners being largely or fully dependent on the others, with attine ants rearing a peculiar food source for which the two parasitic ants compete, one (*Gnamptogenys*) as a destructive and highly virulent agro-predator and the other (*Megalomyrmex*) as a milder and chronic disease. However, this tripartite interaction likely owes its evolutionary stability to the milder parasite's alkaloid weaponry, which can control the more virulent raiders without major cost.



**Fig. 4.** A *G. hartmani* laboratory colony was given repeated choices between four pairs of *S. amabilis* host colonies with or without *M. symmetochus* guest ants (size-matched so that total number of ants, fungus garden volume, and nest box size were approximately equal). Gray bars represent the number of replicate trials for each pair, and dark-colored bars represent raids that were initiated after single *G. hartmani* scouts had inspected one or both of the maize separations with the experimental *S. amabilis* colonies. Recruited columns of raiding *G. hartmani* were preferentially directed toward *S. amabilis* colonies without guest ants (binomial GLZ, LR  $\chi^2 = 18.12$ ,  $P < 0.0001$ ). Chemical structures (Upper Left) represent the *M. symmetochus* venom compounds (5Z,8E)-3-butyl-5-hexylpyrrolizidine and (5E,8E)-3-butyl-5-hexylpyrrolizidine, detected from air samples. Ant drawings courtesy of Rozlyn Haley.

Although pyrrolizidines are not unique to *M. symmetochus* and have convergently evolved in other ant genera (28), our study illustrates that such alkaloids are detectable and functional in interactions with ants from a distant subfamily, the Ectatomminae (Fig. 1). Previously, ant alkaloids were considered general repellents used during competitive intraspecific interactions and thief ant raids (ref. 29 and references therein). Such broad functionality remains compatible with specific effects on the *Gnamptogenys* raiders as long as they remain vulnerable to these venoms.

Examining the different strategies of the two exploiters may hold the key to understanding their stable coexistence. *S. amabilis* sites without *G. hartmani* raiders would be influenced by the maintenance costs of infection with *M. symmetochus* guest ant parasites, which would tend to reduce *S. amabilis* densities and impose selection on traits that would decrease host colony susceptibility to guest ant infiltration (e.g., queen aggression, detoxifying enzymes effective against *Megalomyrmex* alkaloids). This would make the mildly virulent chronic guest ant rare while at the same time creating ideal conditions for the more virulent *G. hartmani* raiders to invade. Such population invasion would shift selection on the host to allow more frequent guest ant infiltration. Colonies with protective guest ant symbionts would then increase in the population, providing fewer attractive host colonies for *G. hartmani* to raid, which in turn would reduce the fitness of *G. hartmani*, making the agro-predators rare once again. Renewed selection against guest ant infiltrations would then be expected.

Another major factor in maintaining some form of dynamic equilibrium between the two social parasites and their shared host appears to be the life-long association of *M. symmetochus* with a single host colony, similar to the association between *M. adamsae* guest ants and their *Trachymyrmex* host colonies (11). This form of obligate perennial colony-level association tends to select for low virulence or prudent exploitation (30), implying that the cost to host colonies of maintaining guest ants might quickly shift to a net benefit when more virulent and mobile alternative parasites appear. The characteristics of the association between *Megalomyrmex* guest ants and their hosts remains fundamentally antagonistic, however (11), explaining the aggression between *S. amabilis* host workers and their *M. symmetochus* guest ants seen in both the field and the laboratory. This

effect is likely driven by a window of conflict over resource allocation, because the *S. amabilis* host can still realize some reproductive success by producing males and thus has no interest in allowing *M. symmetochus* guest ant colonies to grow much beyond the number of workers needed for optimal protection.

Overall, the dynamic interactions among the three ant species studied here resemble human military history. Many medieval cities maintained contingents of mercenary soldiers in times when mobile invasive armies posed a threat, despite their maintenance costs, which quickly became prohibitive after peace treaties were signed. Thus, both *M. symmetochus* guest ants and human mercenaries can be considered alien soldier castes that defend against larger evils as long as they are worth their keep. Another relevant analogy is the maintenance by heterozygote advantage of sickle cell anemia as a chronic human disease in areas where virulent malaria is endemic (31), that is, a chronic disease is maintained because it makes carriers resistant to a potentially lethal disease. Thus, we would expect *Sericomyrmex* populations without *Gnamptogenys* raiders to have a lower prevalence of *M. symmetochus*, because this situation would select for partial resistance against invasion by guest ants.

## Materials and Methods

**Biological Material.** Four parasitized and four nonparasitized *S. amabilis* colonies, along with a single *G. hartmani* colony, were collected in May 2009, 2010, and 2011 near Gamboa and El Llano in the Republic of Panama (Table S1). All colonies were transferred to Copenhagen, Denmark and kept in an environmentally controlled rearing room at a constant temperature of 25 °C and relative humidity of 60–70%. Ant vouchers from all colonies used in this study are deposited at the Museum of Natural History, Smithsonian Institute, Washington, DC and at the Smithsonian Tropical Research Institute, Balboa, Republic of Panama.

**Guest Ant Venom Function.** Pilot experiments were staged, introducing the *G. hartmani* colony into guest ant-infested *S. amabilis* subcolonies to establish whether guest ants exhibit defensive reactions. The observation of intracolony conflict of *Gnamptogenys* raiders prompted a more controlled study of pairwise interactions between two *G. hartmani* workers (SI Text: Methods and Results). The treatment consisted of a *Megalomyrmex* “stung” individual introduced to a naïve *G. hartmani* worker ( $n = 6$ ; Fig. S1 C and D). Avoidance or attraction behavior was scored for 1 h. Control experiments were designed similarly, except that the introduced worker was rubbed with empty soft forceps rather than with a live *Megalomyrmex* worker stinger.

The proportion of time that the workers spent in close proximity was compared using Welch's *t* test (allowing for heterogeneous variances).

**Defense Efficiency in Two-Species Interactions.** A single starved *G. hartmani* scout worker and two, three, four, six, or eight *S. amabilis* or *M. symmetochus* worker opponents (10 combinations in all) were placed in a small arena, and mortality was assessed after 24 h. The experiment was repeated using subcolonies derived from four source colonies: Mb, Mc, Md, and Me (SI Text: *Methods and Results* and Table S1). Mortality was analyzed using JMP version 9.02 (SAS Institute) to fit a generalized linear model with binomial errors. Opponent type and number of opponents were fitted as main effects, together with their interaction. Because the *G. hartmani* mortality data had quasi-complete separation, we performed Firth-adjusted maximum likelihood analysis.

**Three-Species Interactions, Survival, and Mutilation.** Subcolonies consisting of 18 *S. amabilis* workers, a fungus garden fragment ca. 1.5 cm in diameter, and zero, three, or six *M. symmetochus* workers were set up in medium-sized Petri dishes from three parasitized source colonies: Mb, Mc, and Md (SI Text: *Methods and Results* and Table S1). Two starved *G. hartmani* workers were introduced into each Petri dish, and ant mortality and damage to *G. hartmani* worker appendages were assessed after 1 h and 24 h. Mortality and damage were analyzed with JMP using generalized linear models with binomial errors, correcting for overdispersion of the data as necessary.

**Raid Preference Choice: Parasitized vs. Nonparasitized.** With the use of a bifurcating olfactometer (i.e., y-tube), a laboratory colony of *Gnamptogenys hartmani* was given the choice of recruiting nestmates to size-matched pairs of parasitized or nonparasitized *S. amabilis* colonies (Fig. S2). Colony combinations (Mb+Sb, Mc+Sc, Md+Sd, and Me+Se; Table S1) were tested 11, 24, 29, and 23 times, respectively (SI Text: *Methods and Results* and Table S3). A

mesh screen at the entrance of each *S. amabilis* colony allowed airflow and minimal (antennal) interactions between *G. hartmani* scouts and *S. amabilis* workers, and prohibited contact between *G. hartmani* scouts and *M. symmetochus* guest ants. Where raids occurred, their direction (accumulating *G. hartmani* workers on one side; Fig. S1 and Movie S3) was scored blindly from video recordings and analyzed using a generalized linear model with binomial errors to test for overall bias toward or away from parasitized nests, taking into account any differences between colony pairs. Firth-adjusted maximum likelihood estimates were used, because there were no raids on parasitized nests in two of the four pairs of colonies.

**Volatile Chemical Analyses.** All three species were extracted in methanol and chemically analyzed for volatile compounds by GC-MS following established methods (32). To determine whether the venom alkaloids were dispensed in the air by *M. symmetochus* workers, headspace analysis using a solid-phase microextraction (SPME) fiber assembly carboxen/polydimethylsiloxane (57318 SUPELCO; Sigma-Aldrich) was conducted on a sample of eight *M. symmetochus* ants from colony Me (SI Text: *Methods and Results*). For comparison, 10 ants and a small amount of fungus garden from a nonparasitized colony (RMMA100611-03; Table S1) were analyzed as well.

**ACKNOWLEDGMENTS.** We thank the staff and researchers at the Smithsonian Tropical Research Institute for help with logistics and facilities, the Autoridad Nacional del Ambiente y el Mar for permission to sample ants in Panama and export them to Denmark, Friluftslund A/S and John B. Anderson for equipment, and Rozlyn E. Haley for the ant drawings. We also thank two anonymous reviewers for comments and suggestions that improved this article. This work was funded by a Marie Curie International Incoming Fellowship [237266—evolutionAry traNsitions: Chemical Ecology of Parasitic Societies (ANCEPS), to R.M.M.A.], a Smithsonian Molecular Evolution Postdoctoral Fellowship (to R.M.M.A.), and a grant from the Danish National Research Foundation (DNRF57, to J.J.B.).

- Lenoir A, D'Ettorre P, Errard C, Hefetz A (2001) Chemical ecology and social parasitism in ants. *Annu Rev Entomol* 46:573–599.
- Akino T (2008) Chemical strategies to deal with ants: A review of mimicry, camouflage, propaganda, and phytomimesis by ants (Hymenoptera: Formicidae) and other arthropods. *Myrmecol News* 11:173–181.
- Guerrieri FJ, et al. (2009) Ants recognize foes and not friends. *Proc Biol Sci* 276(1666):2461–2468.
- Buschinger A (1986) Evolution of social parasitism in ants. *Trends Ecol Evol* 1(6):155–160.
- Buschinger A (2009) Social parasitism among ants: A review (Hymenoptera: Formicidae). *Myrmecol News* 12:219–235.
- Hölldobler B, Wilson EO (1990) *The Ants* (Belknap Press of Harvard Univ Press, Cambridge, MA), pp 471–529.
- Huang MH, Dornhaus A (2008) A meta-analysis of ant social parasitism: Host characteristics of different parasitism types and a test of Emery's rule. *Ecol Entomol* 33:589–596.
- Brady SG, Schultz TR, Fisher BL, Ward PS (2006) Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proc Natl Acad Sci USA* 103(48):18172–18177.
- Longino JT (2010) A taxonomic review of the ant genus *Megalomyrmex* Forel (Hymenoptera: Formicidae) in Central America. *Zootaxa* 2720:35–58.
- Adams RMM, Mueller UG, Schultz TR, Norden B (2000) Agro-predation: Usurpation of attine fungus gardens by *Megalomyrmex* ants. *Naturwissenschaften* 87(12):549–554.
- Adams RMM, Shah K, Antonov LD, Mueller UG (2012) Fitness consequences of nest infiltration by the mutualist-exploiter *Megalomyrmex adamsae*. *Ecol Entomol* 37:453–462.
- Brandão CRF (1990) Systematic revision of the Neotropical ant genus *Megalomyrmex* Forel (Hymenoptera: Formicidae: Myrmicinae), with the description of thirteen new species. *Arquivos de Zoologia* 31:411–481.
- Jones TH, Blum MS, Fales HM, Brandão CRF, Lattke J (1991) Chemistry of venom alkaloids in the ant genus *Megalomyrmex*. *J Chem Ecol* 17:1897–1908.
- Jones T, et al. (1999) Dialkylpyrrolidines from the ants *Megalomyrmex cyendyria* Brandão and *M. latreillei* Emery. *Caribb J Sci* 35:310–311.
- Adams RMM, Longino JT (2007) Nesting biology of the arboreal fungus-growing ant *Cyphomyrmex cornutus* and behavioral interactions with the social-parasitic ant *Megalomyrmex mondabora*. *Insectes Soc* 54:136–143.
- Wheeler WM (1925) A new guest-ant and other new Formicidae from Barro Colorado Island, Panama. *Biol Bull* 49:150–181.
- Schultz TR, Brady SG (2008) Major evolutionary transitions in ant agriculture. *Proc Natl Acad Sci USA* 105(14):5435–5440.
- Nehring V, Boomsma JJ, d'Ettorre P (2012) Wingless virgin queens assume helper roles in *Acromyrmex* leaf-cutting ants. *Curr Biol* 22(17):R671–R673.
- Martin SJ, Jenner EA, Drijfhout FP (2007) Chemical deterrent enables a socially parasitic ant to invade multiple hosts. *Proc Biol Sci* 274(1626):2717–2721.
- Hermann H, Moser J, Hunt A (1970) The hymenopterous poison apparatus, X: Morphological and behavioral changes in *Atta texana* (Hymenoptera: Formicidae). *Ann Entomol Soc Am* 63:1552–1558.
- Dijkstra MB, Boomsma JJ (2003) *Gnamptogenys hartmani* Wheeler (Ponerinae: Ectatommini): An agro-predator of *Trachymyrmex* and *Sericomyrmex* fungus-growing ants. *Naturwissenschaften* 90(12):568–571.
- Thomas JA, et al. (2002) Parasitoid secretions provoke ant warfare. *Nature* 417(6888):505–506.
- Herre EA, Knowlton N, Mueller UG, Rehner SA (1999) The evolution of mutualisms: Exploring the paths between conflict and cooperation. *Trends Ecol Evol* 14(2):49–53.
- Thompson JN (2005) *The Geographic Mosaic of Coevolution* (Univ of Chicago Press, Chicago).
- Forde SE, Thompson JN, Bohannan BJM (2004) Adaptation varies through space and time in a coevolving host-parasitoid interaction. *Nature* 431(7010):841–844.
- Jaenike J, Unckless R, Cockburn SN, Boelio LM, Perlman SJ (2010) Adaptation via symbiosis: Recent spread of a *Drosophila* defensive symbiont. *Science* 329(5988):212–215.
- Kwiatkowski M, Vorburger C (2012) Modeling the ecology of symbiont-mediated protection against parasites. *Am Nat* 179(5):595–605.
- Jones T, Blum M, Fales H, Thompson C (1980) (5Z, 8E)-3-Heptyl-5-methylpyrrolidines from a thief ant. *J Org Chem* 45:4778–4780.
- Jones TH, Blum MS, Fales HM (1982) Ant venom alkaloids from *Solenopsis* and *Monomorium* species. *Tetrahedron* 38:1949–1958.
- Frank SA (1996) Models of parasite virulence. *Q Rev Biol* 71(1):37–78.
- Allison AC (1954) Protection afforded by sickle-cell trait against subtertian malarial infection. *BMJ* 1(4857):290–294.
- Adams RMM, et al. (2012) A comparative study of exocrine gland chemistry in *Trachymyrmex* and *Sericomyrmex* fungus-growing ants. *Biochem Syst Ecol* 40:91–97.