Correspondence

Ant farmers practice proactive personal hygiene to protect their fungus crop

Citlalli Morelos-Juárez[1], Thomas N. Walker[1], Juliane F.S. Lopes[2], and William O.H. Hughes[1,*]

Agricultural systems often involve monocultures that are vulnerable to competitors and pathogens. Successful agriculture, therefore, relies on preventing the contamination of the crop by detrimental organisms or on removing such organisms. The fungus-growing ants exhibit one of the most ancient forms of agriculture, farming a clonal fungal crop that is highly susceptible to competitive and pathogenic microorganisms [1,2]. Like human farmers, the ants have a suite of reactive and prophylactic defence mechanisms, including the application of pesticides and weeding [3-5]. Here, we show that fungus-growing ants also engage in proactive self-cleaning behaviour to remove undetected microbes and prevent them from contaminating the vulnerable fungal crop. Although many social animals show reactive hygiene, the behaviour detailed here is proactive and a response to the detection of vulnerable individuals rather than the threat itself.

Humans and fungus-growing ants (Figure 1A) developed and evolved agriculture approximately 10,000 and 50 million years ago, respectively [2,6]. In both cases, the crops on a farm are frequently highly related monocultures that are vulnerable to competitors and pathogens. The ants in particular suffer from a specialist fungal weed, *Escovopsis*, which feeds on the fungal crop [1]. Both societies also include immature individuals, which are susceptible to pathogens. Humans and fungus-growing ants thus have mechanisms to protect their vulnerable crops and young from microbial threats. The ants prophylactically apply chemical pesticides that are produced by themselves and by symbiotic bacteria [3-5]. The ants also deal reactively with hostile microbes that they detect, weeding them out of their fungal crops and grooming them off their bodies [7,8]. A further level of defence can be provided if societies can prevent vulnerable crops and young coming into contact with hostile microbes in the first place. However, this can be difficult because individuals may frequently be contaminated with microbes that they do not detect at the time of contamination. This may be because they do not recognise the contamination — for example, because the microbes are present in low numbers or of a species which is not harmful to the individual itself — or because they do not sense the microbes — for example, because the microbes contaminate a body surface lacking the sensilla to detect them. Humans defend against this threat in settings such as hospitals and industrial animal production by proactive self-cleaning to remove undetected microbes before interacting with vulnerable individuals. Here, we examine whether fungus-growing ants show this level of defence.

The crucial point for contamination prevention in social insect colonies will be when an individual re-enters its nest. Prior to this, individuals may come into contact with the diversity of microbes which abound in the extranidal environment; after it, they may interact with, and potentially contaminate, their vulnerable crop and young. We observed the entrances of laboratory colonies of *Acromyrmex subterraneus molestans* fungus-growing ants and found that 90% of ants (n = 256) self-groomed immediately

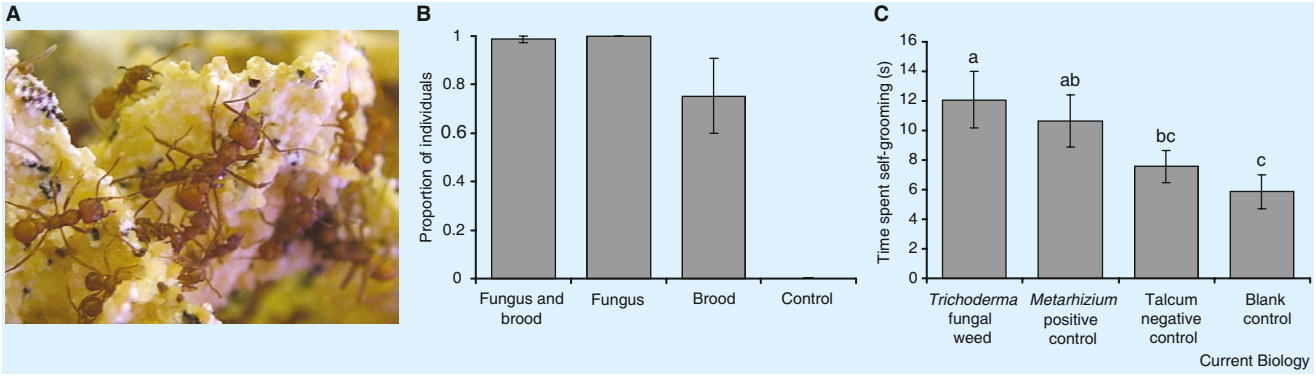

Figure 1. Proactive self-grooming in farming ants.

(A) Fungus-growing ants, such as *Acromyrmex echinatior* shown here, farm a mutualistic fungus crop which they must protect against other microbes. (B) Fungus-growing ants self-groom upon re-entering their nest. While some respond if the nest contains brood, virtually all ants engage in this proactive self-cleaning if the nest contains their fungal crop. Data presented are the mean ± s.e. proportions of *A. echinatior* workers self-grooming immediately upon entering nest chambers containing either the fungus crop, brood, both, or neither. (C) Ants spend longer cleaning themselves when they sense their fungal crop if they have been contaminated by other fungi. The mean ± s.e. lengths of time that *A. echinatior* workers spent self-grooming immediately upon entering the fungus chamber when they had been exposed to either the *Trichoderma viridae* fungus, which can outcompete their fungal crop but is not directly hazardous to the ants themselves, the *Metarhizium anisopliae* fungus entomopathogen, which served as a positive control, inert talcum powder negative control material or were left uncontaminated as a blank control. Letters above columns indicate treatments which differed significantly from one another in LSD *post hoc* pairwise comparisons.

upon entering the nest chamber. Importantly, this self-grooming took place before ants had directly contacted the fungal crop or brood, and thus before any contamination could occur. Self-grooming is effective in leaf-cutting ants at removing fungal spores from the cuticle [8], which are then gathered in the infrabuccal pocket. Material in this pocket is exposed to antibiotics produced by the ants and their bacterial mutualists before being regurgitated away from the fungus garden, so the procedure is effective at removing the threat from contaminants [9]. Ants normally self-groom as soon as they detect microbes harmful to themselves [8], so this behaviour on entering their nest would seem potentially to be directed at the removal of undetected microbes to protect other, more vulnerable individuals.

This behaviour could be stimulated by the ants sensing their fungal crop, their young or simply that they have re-entered their nest. To distinguish between these possibilities, we set-up twenty mini-nests of *Acromyrmex echinatior* fungus-growing ants with nest chambers containing either the fungal crop, brood, both or neither. We found that virtually all ants self-groomed as soon as they entered chambers containing the fungal crop either with or without brood, many also did so when entering chambers containing brood, but none did so when chambers contained neither fungal crop nor brood (Generalized Linear Model: $\chi^2 = 158.7$, df = 3, $P < 0.0001$, $n = 20$; Figure 1B). The proactive self-cleaning behaviour is, therefore, specifically stimulated by ants sensing the presence of vulnerable individuals, particularly the fungal crop, most probably via volatile chemicals. In nature, this may take place upon entry to the fungus chamber or the tunnels leading to it.

If the self-cleaning is truly a proactive mechanism to protect vulnerable crops and young, then individuals that have been contaminated by hostile microbes should self-groom for longer. To examine this, we set-up twenty mini-nests of *A. echinatior* containing fungal crop and brood, and then introduced into these ants which we had contaminated with the *Trichoderma viridae* or *Metarhizium anisopliae* fungi, talcum powder or nothing. *T. viridae* is an aggressive competitor of the fungal crop but is not directly hazardous to the ants themselves [7]. *M. anisopliae* is a virulent pathogen of the ants which served as a positive control [8,10], while talcum powder is an inert material that acted as a negative control and uncontaminated ants were a blank control. We found that treatment had a significant effect on the length of time ants spent self-grooming upon entering the nest chamber (ANOVA: $F_{3,16} = 3.5$, $P = 0.04$). Ants exposed to *Trichoderma* or *Metarhizium* self-groomed for longer than those exposed to nothing or, to a lesser extent, talcum powder (Figure 1C). While self-grooming stimulated by *Metarhizium* could be for self-defence or to protect brood, *Trichoderma* only presents a hazard to the fungal crop and not to the ants themselves. The most parsimonious explanation is that the ants are able to recognise previously undetected contamination, particularly by fungal spores, and respond by self-grooming longer to protect their fungal crop. Engagement in self-grooming is proactive and stimulated by the presence of the fungal crop, but the duration of self-grooming is then extended if ants during self-grooming detect contamination by competitive or pathogenic fungi.

Social insects are renowned for the scrupulous hygiene shown by many species to remove potentially pathogenic organisms from the body surfaces of both themselves and their nestmates. Similar self- and allo-grooming behaviours are shown by many other social animals as well. All are consistent in that the behaviour is elicited either by the detection of the pathogenic organisms or a contaminated individual. The self-cleaning behaviour shown here is different in being directed at protecting vulnerable individuals, and in being proactive rather than simply reactive to the detection of pathogens. Such proactive self-cleaning is therefore not limited to humans and may be common in other social animals which need to protect vulnerable brood or crops.

Supplemental Information

Supplemental Information includes supplemental experimental procedures and can be found with this article online at doi:10.1016/j.cub.2010.04.047

Acknowledgements

We would like to thank the Smithsonian Tropical Research Institute and E.A. Herre for providing facilities in Gamboa, the Autoridad Nacional del Ambiente (ANAM) for permission to collect and export the *A. echinatior* colonies, J.J. Boomsma for kindly allowing us to use colonies Ae216 and Ae312, IBAMA for permission to collect and export the *A. s. molestans* colonies from Brazil and the three anonymous reviewers for their constructive comments. We are also grateful to the Royal Society and the Leverhulme Foundation for funding.

References

1. Currie, C.R., Mueller, U.G., and Malloch, D. (1999). The agricultural pathology of ant fungus gardens. Proc. Natl. Acad. Sci. USA *96*, 7998–8002.
2. Schultz, T.R., and Brady, S.G. (2008). Major evolutionary transitions in ant agriculture. Proc. Natl. Acad. Sci. USA *105*, 5435–5440.
3. Fernandez-Marin, H., Zimmerman, J.K., Nash, D.R., Boomsma, J.J., and Wcislo, W.T. (2009). Reduced biological control and enhanced chemical pest management in the evolution of fungus farming in ants. Proc. R. Soc. Lond. B *276*, 2263–2269.
4. Sen, R., Ishak, H.D., Estrada, D., Dowd, S.E., Hong, E., and Mueller, U.G. (2009). Generalized antifungal activity and 454-screening of *Pseudonocardi*a and *Amycolatopsis* bacteria in nests of fungus-growing ants. Proc. Natl. Acad. Sci. USA *106*, 17805–17810.
5. Currie, C.R., Scott, J.A., Summerbell, R.C., and Malloch, D. (1999). Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. Nature *398*, 701–704.
6. Gupta, A.K. (2004). Origin of agriculture and domstication of plants and animals linked to early Holocene climate amelioration. Curr. Sci. *87*, 54–59.
7. Currie, C.R., and Stuart, A.E. (2001). Weeding and grooming of pathogens in agriculture by ants. Proc. R. Soc. Lond. B *268*, 1033-1039.
8. Hughes, W.O.H., Eilenberg, J., and Boomsma, J.J. (2002). Trade-offs in group living: transmission and disease resistance in leaf-cutting ants. Proc. R. Soc. Lond. B *269*, 1811–1819.
9. Little, A.E.F., Murakami, T., Mueller, U.G., and Currie, C.R. (2006). Defending against parasites: fungus-growing ants combine specialized behaviours and microbial symbionts to protect their fungus gardens. Biol. Lett. *2*, 12–16.
10. Hughes, W.O.H., Petersen, K., Ugelvig, L., Pedersen, D., Thomsen, L., Poulsen, M., and Boomsma, J.J. (2004). Density-dependence and within-host competition in a semelparous parasite of leaf-cutting ants. BMC Evol. Biol. *4*, 45.

[1]Institute of Integrative and Comparative Biology, University of Leeds, Leeds LS2 9JT, UK. [2]Pós-Graduação Comportamento e Biologia Animal, Instituto de Ciências Biológicas/UFJF, Campus Universitário de Martelos, 36.036-330, Juiz de Fora, MG, Brazil.
*E-mail: w.o.h.hughes@leeds.ac.uk