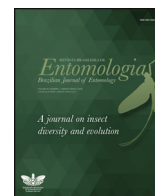




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The trade-off between the transmission of chemical cues and parasites: behavioral interactions between leaf-cutting ant workers of different age classes



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ABSTRACT

Social animals are faced with an intriguing dilemma. On the one hand, interactions between individuals are essential to exchange information and to promote cohesion, while on the other hand such interactions carry with them the risk of catching and transmitting parasites. This trade-off is particularly significant for social insects because low within-colony genetic diversity makes their colonies potentially vulnerable to parasites while frequent interactions are essential to the development of the colonial odor profile necessary for nestmate recognition. Here we investigate whether social interactions between young and old leaf-cutting ant workers show evidence of this trade-off. We find that old workers engage in more selfgrooming and mandibular scraping than young workers, both in keeping with old workers having been more exposed to parasites. In contrast, we find that young workers engaged in more allogrooming than old workers, which seems likely to have a different motivation possibly the transfer of recognition cues. Furthermore, young workers tended to engage in allogrooming with other young workers, although it was the old workers that were most active and with whom allogrooming would seem likely to optimize information or chemicals transfer. This suggests that young workers may be attempting to minimize the risk of parasite transmission during their social interactions. Although limited to behavioral data, these results hint that ant workers may be sensitive to the trade-off between the transmission of recognition cues and disease, and adjust their social interactions accordingly.

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Introduction

Interactions are fundamental to the organization of all societies. Even in simple societies, individuals share food resources or nesting sites, and exchange information to maintain group cohesion (Wilson, 1975; Kraus and Ruxton, 2002). The transmission of information becomes still more important as social complexity increases. Advanced societies, such as those of humans and social insects, are characterized by a division of labor that is underpinned by information transfer. However, social interactions also carry with them a risk of parasite transmission, which is largely dependent on the interaction rate between infected and susceptible hosts (Anderson and May, 1981; McCallum et al., 2001). Although

this effect will depend on the transmission mode of parasites (Côte and Poulin, 1995), and may be mitigated by decreased inter-group transmission (Wilson et al., 2003), parasites are nevertheless often predicted to be a particular hazard for social animals (Alexander, 1974; Freeland, 1976; Kraus and Ruxton, 2002). Social interactions may therefore involve a trade-off between the need to exchange information and the risk of parasite transmission.

This trade-off may be particularly significant for social insects. Social insect colonies are characterized by low within-colony genetic diversity, which may greatly facilitate the transmission and evolution of any parasite that enters a colony (Hamilton, 1987; Schmid-Hempel, 1998; Boomsma et al., 2005; Hughes and Boomsma, 2006; Morelos-Juárez et al., 2010). Social insects defend themselves against this threat at several levels. Workers have effective individual defenses, consisting of selfgrooming to remove parasites from their cuticle, the prophylactic production of antibiotics to make their cuticles a more hostile environment for any parasites they encounter, and an effective immune response to

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deal with any parasites that successfully infect them (Hughes et al., 2002; Poulsen et al., 2002a; Rosengaus et al., 2004; Baer et al., 2005; Rosengaus et al., 2007; Okuno et al., 2012). At a second level, social insects also engage in group-level defenses, allogrooming one another to remove parasites, possibly exchanging antibiotic secretions, and dealing hygienically with waste material (Rosengaus et al., 1998; Bot et al., 2001; Hughes et al., 2002). At a third level, social insect colonies are subdivided both architecturally, with nests often consisting of multiple chambers, and demographically, with younger individuals tending to be located at the center of nests and older individuals toward the exterior (Hölldobler and Wilson, 1990; Robinson, 1992). In addition, hazardous tasks such as waste management are often partitioned such that those individuals most exposed to the hazard have least interaction with the remainder of the colony (Hart and Ratnieks, 2001, 2002). Models suggest that the combination of this structuring with group-level and individual defense mechanisms is key to parasite transmission within social insect colonies being minimized (Naug and Camazine, 2002; Pie et al., 2004; Fefferman et al., 2007).

While parasites select for social insects to minimize their social interactions, the need for information transfer conversely requires these to be frequent to, in particular, develop the necessary cues to allow nestmate recognition. Although other pheromones may play a secondary role in some taxa (Hughes et al., 2001; Hernandez et al., 2006), the primary cues used for nestmate recognition are normally non-volatile cuticular hydrocarbons (CHCs) (Lahav et al., 1999; Howard and Blomquist, 2005; Guerrieri and D'Ettorre, 2008; Martin et al., 2008). Individuals transfer these CHCs between one another by allogrooming or trophallaxis to produce a colony-specific blend or 'gestalt' odor which allows nestmates to be distinguished from non-nestmates (Soroker et al., 1995; Boulay et al., 2000; Lenoir et al., 2001; Soroker et al., 2003) so CHCs constitutes a great source of information and when they are transferred not just the gestalt odor is obtained but so the information about individual colony origin. Colony specific odors that maintain colony insularity by rejecting any individual that carries a different odor are well known in social insects (Breed, 1983; Wilson, 1971). Individuals eclose with an individually-distinctive profile of cuticular hydrocarbons and acquire the gestalt odor rapidly over the days following eclosion (Morel et al., 1988; Dahbi et al., 1998; Kaib et al., 2000; Cuvillier-Hot et al., 2001). Individuals continue to biosynthesize cuticular hydrocarbons throughout their life, so continually need to interact with nestmates to maintain and to contribute to their gestalt colony odor formation and avoid redeveloping an individually-distinctive profile (Boulay et al., 2000; Boulay and Lenoir, 2001).

As with other group-living animals, social insect workers therefore need to balance the need for social interactions to transfer chemical cues with the risk of the same interactions transmitting parasites. Here we investigate whether some social behaviors in leaf-cutting ants show evidence of such a trade-off. Four behaviors were examined: allogrooming, selfgrooming, mandibular scraping and antennation.

Allogrooming is the principal mechanism by which CHCs are transferred between leaf-cutting ants because this species only rarely engage in trophallaxis (Moreira et al., 2006). Also it is an important mechanism of disease resistance, but carries with it the risk of disease transmission (Rosengaus et al., 1998; Hughes et al., 2002; Fefferman et al., 2007). Although allogrooming may also serve to increase immunity (Traniello et al., 2002; Ugelvig and Cremer, 2007), it seems probable that this benefit will normally be outweighed by the risk of infection. Selfgrooming both plays a role in disease defense removing fungal spores and other contaminants from the cuticle (Hughes et al., 2002) and in the development of the gestalt odor. Mandibular scraping most probably has a hygienic function in which the effective action is in one direction and

transfers dirt material that has accumulated on the mandibles from the cleaned to the cleaning part (Lopes et al., 2005). Antennation was measured as a general measure of social activity. We compared all four behaviors between young and old workers.

Young workers are most valuable to the colony because of their long expected life. Their tasks are generally limited to within, and often at the center of the nest (Robinson, 1992; Camargo et al., 2007), so they may be less exposed to parasites than older workers which engage in moving soil, waste management or foraging (Schmid-Hempel, 1998; Boomsma et al., 2005). This contrast in exposure is likely to be particularly strong for leaf-cutting ants because young workers reside exclusively within the fungus garden. The ontogenetic differences lead to several hypotheses: mandibular scraping will be more common in old workers than young workers because the former are more likely to be contaminated with soil or food debris; selfgrooming will be more common in old workers than young workers because the tasks of the former (soil moving, waste management, foraging) carry more risk of parasite exposure; allogrooming will also be more frequent for old rather than young workers for the same reason, and in addition will tend to be within age categories in order to limit transmission by dividing interaction networks (Naug and Camazine, 2002; Pie et al., 2004; Fefferman et al., 2007). In contrast, information transfer would predict similar rates of selfgrooming and allogrooming for old and young workers because both groups need to develop and maintain their CHC profile. Furthermore, information transfer would be optimized by allogrooming between age categories in order to prevent them developing distinct CHC profiles which would interfere nestmate recognition.

Material and methods

The study was conducted with two colonies of *Acromyrmex crassispinus*, collected at Jaguariaíva-PR (24°32' S; 49°57' W), and two colonies of *Acromyrmex rugosus*, collected at Botucatu-SP (22°52'20" S; 48°26'37" W), both locations being in southeast Brazil. *A. crassispinus* is found mainly in pine forests and uses monocotyledonous as fungus substrate while *A. rugosus* occurs in grassland and disturbed habitat using dicotyledonous plants as substrate (Verza et al., 2007). The two species otherwise have very similar ecology and life-history, with both nesting within the soil and reaching a similar size at maturity with one or two fungus gardens. Here, they were chosen for easy access and rearing capability of these species. Also, both species show the classic pattern of alloethism for *Acromyrmex*, with a continuous size-frequency distribution around two modes, normally termed small workers and large workers (Wetterer, 1999; Hughes et al., 2003). Small *Acromyrmex* workers reside within the fungus garden where they care for the brood and fungus. Large workers also remain within the fungus garden while young, where they serve as a substrate for the culturing of antimicrobial bacteria to protect the fungus garden (Currie et al., 1999, 2003; Poulsen et al., 2002b). Medium and large old-aged workers work outside the fungus garden and nest, foraging for vegetation, removing waste and building the nest (Camargo et al., 2007).

Here, we considered that young workers (light brown color) were 1 week old, and old workers (dark brown color) were more than 3 and 4 weeks old. This assumption is based on study from Camargo et al. (2007), where leaf-cutting ants start to participate in activities outside the nest by 3 or 4 weeks of age, and defined as "outside workers". Thus all of them should be older than 1 month. On the other hand young worker as defined as "inside workers" and all of them should be younger than 1 week.

The experimental colonies were maintained in the laboratory at $24 \pm 1^\circ\text{C}$ and $\text{RH } 80 \pm 10\%$ on a diet of *Ligustrum* sp. and *Acalypha*

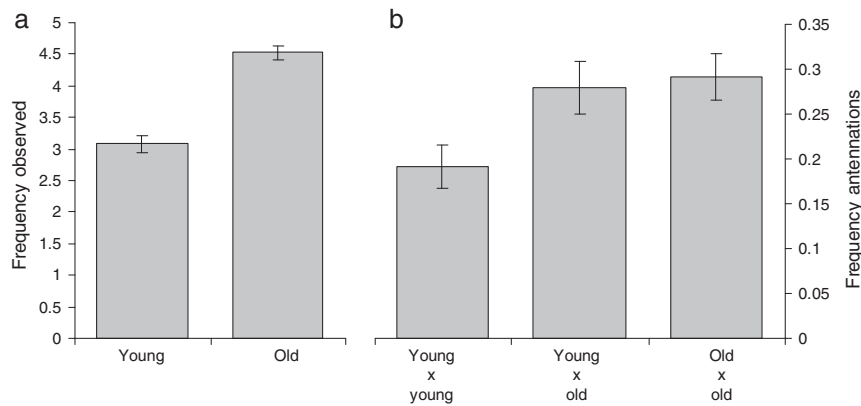


Fig. 1. Overall activity levels. Mean \pm s.e. frequencies that young and old ants were: (a) observed engaging in one of the focal behaviors and (b) observed engaged in antennation with another ant, during a 15 s observation period.

sp. The colonies each had a single 500 mL fungus garden housed in a plastic container, the bottom of which was covered with a 1-cm layer of plaster to maintain the humidity of the fungus garden. Each colony had a connection to a foraging arena, made out of glass, and a container in which the ants deposited waste. Young and old workers were identified by their light and dark cuticular coloration and captured inside the fungus garden chamber and foraging arena, respectively. The cuticle of *Acromyrmex* workers darkens progressively as they age from a light to very dark brown color (Armitage and Boomsma, 2010). While cuticular coloration only provides an approximate measure of age, it does therefore allow young and old workers to be very reliably distinguished.

For each colony, 50 large workers (1.3–1.7 cm body length in *A. crassispinus* and 1.0–1.3 cm body length in *A. rugosus*) of each age category had a different colored paint dot applied to their thoraxes to facilitate subsequent identification and were placed in a lidded glass experimental arena (square of 225 cm²). Colored pens (EddingTM, Edding International GmbH, Ahrensburg, Germany) were used to label the workers because of their excellent adherence, quick drying and good visibility (Camargo et al., 2007).

The lids of the experimental arena were demarked into a grid of 9 squares. The ants were left for 5 min to become accustomed to the arena, which was then videoed using a microcamera (Pacific-Co) for 45 min. Subsequently, the frequency of behavioral interactions between young and old workers in the arena was counted, as well, between same age groups. Four focal behaviors were recorded: (1) antennation, (2) selfgrooming, (3) mandibular scraping and (4) allogrooming. The ages (young or old) of ants involved and the direction of each behavior was also recorded. The behaviors in each

square were counted for a 15 s period, at intervals of 45 s. Grid squares were observed in a random order, with the set of nine squares making up the arena being recorded 13 times over the course of the 45 min. The dataset for each arena thus consisted of nine replicate counts (squares), each lasting 15 s, with there being 13 repeated measures over time of each set of squares. The data were $\log(x + 1)$ transformed and analyzed with repeated-measures analyses of variance followed by paired *t*-test with *p*-value adjustment by Benjamini–Yekutieli method.

Results

Overall, old workers were observed engaging in the four focal behaviors significantly more often than young workers ($F_{1,64} = 194$, $p < 0.0001$; Fig. 1a), even though the same numbers of each category were present in the arenas. The number of antennations involving old workers, either with other old workers ($p = 0.02$) or with young workers ($p = 0.041$), was also significantly higher than for antennations between young workers ($F_{1,64} = 5.36$, $p = 0.006$; Fig. 1b). Selfgrooming was observed almost twice as often by old workers as by young workers ($F_{1,64} = 56.3$, $p < 0.0001$; Fig. 2a). Mandibular scraping was a relatively infrequent behavior that consequently did not differ significantly between the age groups ($F_{1,64} = 0.528$, $p = 0.591$), although it was observed approximately twice as often between old workers than involving young workers (Fig. 2b). Finally, the frequency of allogrooming differed significantly between the age groups ($F_{3,128} = 6.7$, $p = 0.0003$). Allogrooming was most common between young workers, and was approximately equally frequent between old workers as between

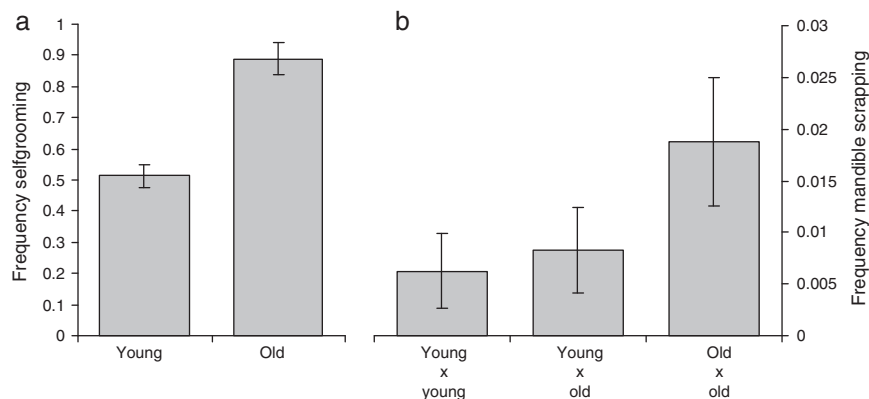


Fig. 2. Mean \pm s.e. frequencies that young and old ants were: (a) observed selfgrooming and (b) observed engaged in mandible scraping with another ant, during a 15 s observation period.

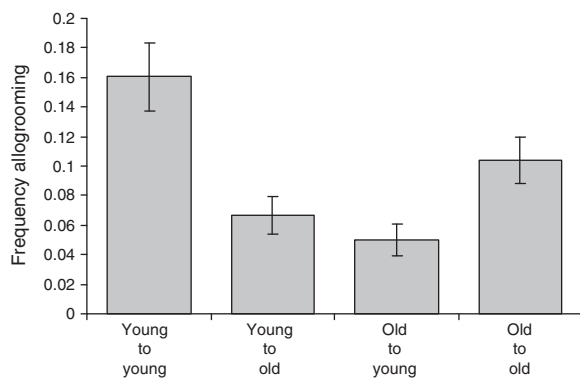


Fig. 3. Mean \pm s.e. frequencies that young and old ants were observed giving or receiving allogrooming during a 15 s observation period.

young and old workers (Fig. 3). Allogrooming between young and old workers was similarly likely to involve each age group as the giver or receiver of the interaction. Accordingly, young workers engaged in allogrooming significantly more often than old workers (young worker interactions vs. old worker interactions: $F_{1,64} = 4.43$, $p = 0.039$), in spite of being less active overall. Young workers were more likely to engage in allogrooming with other young workers than with old workers ($F_{1,64} = 4.87$, $p = 0.031$), whereas old workers were similarly likely to engage in allogrooming with the different age groups ($F_{1,64} = 0.638$, $p = 0.428$). No trophallaxis was observed between any of the ants during the experiment.

Discussion

In spite of the highly artificial experimental set-up, we nevertheless found clear evidence of an ontogenetic difference in social interactions. These results show us that *Acromyrmex* workers therefore need to balance the need for social interactions to transfer chemical cues related to nestmate recognition with the risk of the same interactions transmit parasites.

Old workers, which were in general more active, selfgroomed more often than young workers, and engaged in more mandibular scraping. However, young workers allogroomed more often than old workers, in spite of their overall lower activity. Furthermore, allogrooming by young workers was non-random, being preferentially directed at other young workers.

Selfgrooming removes fungal spores and other contaminants from the cuticle (Hughes et al., 2002), while mandibular scraping is most probably to remove material that has accumulated on the mandibles (Lopes et al., 2005). Old workers are likely to have been most exposed to parasites and other microbes because they carry out all the hazardous tasks, such as foraging, soil moving and waste management (Schmid-Hempel, 1998; Boomsma et al., 2005; Camargo et al., 2007). Selfgrooming may also be important in blending recognition cues, but this would predict similar rates both for young and old workers. It therefore seems most likely that the higher rates of selfgrooming and mandibular scraping observed in old workers are for hygienic reasons. Morelos-Juárez et al. (2010) show that leaf-cutting ants are engaged in proactive self-cleaning behavior to remove undetected microorganism and prevent them from contaminating the vulnerable fungal crop.

Explaining the higher rates of allogrooming in young workers is more problematic. Possibly the level of interaction needed by young workers to develop their gestalt profile is greater than the level needed by old workers to maintain it (Morel et al., 1988; Dahbi et al., 1998; Boulay et al., 2000; Kaib et al., 2000; Boulay and Lenoir, 2001; Cuvillier-Hot et al., 2001). Alternatively, it may be not so much that young workers have high rates of allogrooming, but rather

that old workers have reduced rates, perhaps in order to reduce the risk of them transmitting parasites to nestmates (Rosengaus et al., 1998; Hughes et al., 2002; Fefferman et al., 2007). The fact that young workers preferentially allogroomed with other young workers is intriguing. If allogrooming was governed solely by information transfer then it seems probable that allogrooming would tend to be between age categories because this would optimize the development of a single colony-level gestalt odor. Similarly, the greater activity of old workers means most allogrooming should have been with old workers if interactions were random. Finally, if allogrooming was driven solely by the requirements of the receiving individual, then this again would predict that old workers should receive most allogrooming given that their tasks are likely to involve greater exposure to parasites (Schmid-Hempel, 1998; Boomsma et al., 2005), and given that their higher rates of selfgrooming and mandibular scraping support this. Young workers therefore engaged in allogrooming not with those that would optimize information transfer, nor the most active individuals, nor those most in need of cleaning, but rather those that presented the least risk from parasites. This suggests that they are sensitive to the risk of parasite transmission and adjust their social interactions to minimize this risk. Another alternative explanation is that young workers engaged in higher levels of allogrooming within their class because of they are in a learning process of the colony odor. Also their own chemicals will be an integral part of the gestalt odor of the colony. In this study we have only investigated behavioral interactions. While previous studies have demonstrated that such interactions can transmit both information, in the form of CHCs (Soroker et al., 1995, 2003; Boulay et al., 2000; Lenoir et al., 2001), and parasites (Rosengaus et al., 1998; Schmid-Hempel, 1998; Hughes et al., 2002; Boomsma et al., 2005; Fefferman et al., 2007), we did not attempt to quantify either here, so the implications of our study must remain somewhat speculative. Nevertheless, it seems certain that the trade-off between information transfer and parasite transmission is real and may be of particular importance in social insect colonies with their low genetic diversity and high need for information transfer. The behavioral results we have presented here suggest that leaf-cutting ants may be sensitive to this trade-off.

Conflicts of interest

The authors declare no conflicts of interest.

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References

- Alexander, R.D., 1974. The evolution of social behavior. *Ann. Rev. Ecol. Syst.* 5, 324–383.
- Anderson, R.M., May, R.M., 1981. The population dynamics of microparasites and their invertebrate hosts. *Philos. Trans. R. Soc. Lond. B* 291, 451–524.
- Armitage, S.A.O., Boomsma, J.J., 2010. The effects of age and social interactions on innate immunity in a leaf-cutting ant. *J. Insect Physiol.* 56, 780–787.
- Baer, B., Krug, A., Boomsma, J.J., Hughes, W.O.H., 2005. Examination of the immune responses of males and workers of the leaf-cutting ant *Acromyrmex echinator* and the effect of infection. *Insectes Soc.* 52, 298.
- Boomsma, J.J., Schmid-Hempel, P., Hughes, W.O.H., 2005. Life histories and parasite pressure across the major groups of social insects. In: Fellowes, M.D.E., Holloway, G.J., Rolff, J. (Eds.), *Insect Evolutionary Ecology*. CABI Publishing, Wallingford, pp. 139–175.
- Bot, A.N.M., Currie, C.R., Hart, A.G., Boomsma, J.J., 2001. Waste management in leaf-cutting ants. *Ethol. Ecol. Evol.* 13, 225–237.

- Boulay, R., Lenoir, A., 2001. Social isolation of mature workers affects nestmate recognition in the ant *Camponotus fellah*. *Behav. Processes* 55, 67–73.
- Boulay, R., Hefetz, A., Soroker, V., Lenoir, A., 2000. *Camponotus fellah* colony integration: worker individuality necessitates frequent hydrocarbon exchanges. *Anim. Behav.* 59, 1127–1133.
- Breed, M.D., 1983. Nestmate recognition in honey bees. *Anim. Behav.* 31, 86–91.
- Camargo, R.S., Forti, L.C., Lopes, J.F.S., Andrade, A.P.P., Otatti, A.L.T., 2007. Age polyethism in the leaf-cutting ant *Acromyrmex subterraneus brunneus* Forel, 1911 (Hymenoptera: Formicidae). *J. Appl. Entomol.* 131, 139–145.
- Côte, I.M., Poulin, R., 1995. Parasitism and group size in social animals: a meta-analysis. *Behav. Ecol.* 6, 159–165.
- Currie, C.R., Bot, A.N.M., Boomsma, J.J., 2003. Experimental evidence of a tripartite mutualism: bacteria protect ant fungus gardens from specialized parasites. *Oikos* 101, 91–102.
- Currie, C.R., Scott, J.A., Summerbell, R.C., Malloch, D., 1999. Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. *Nature* 398, 701–704.
- Cuvillier-Hot, V., Cobb, M., Malosse, C., Peeters, C., 2001. Sex, age and ovarian activity affect cuticular hydrocarbons in *Diacamma ceylonense*, a queenless ant. *J. Insect Physiol.* 47, 485–493.
- Dahbi, A., Cerda, X., Lenoir, A., 1998. Ontogeny of colonial hydrocarbon label in callow workers of the ant *Cataglyphis iberica*. *C. R. Acad. Sci. III* 321, 395–402.
- Fefferman, N.H., Traniello, J.F.A., Rosengaus, R.B., Calleri, D.V., 2007. Disease prevention and resistance in social insects: modeling the survival consequences of immunity, hygienic behavior, and colony organization. *Behav. Ecol. Sociobiol.* 61, 565–577.
- Freeland, W.J., 1976. Pathogens and the evolution of primate sociality. *Biotropica* 8, 12–24.
- Guerrieri, F.J., D'Etorre, P., 2008. The mandible opening response: quantifying aggression elicited by chemical cues in ants. *J. Exp. Biol.* 211, 1109–1113.
- Hamilton, W.D., 1987. Kinship, recognition, disease, and intelligence: constraints of social evolution. In: Ito, Y., Brown, J.L., Kirkkawa, J. (Eds.), *Animal Societies: Theories and Facts*. Japan Scientific Societies Press, Tokyo, pp. 81–100.
- Hart, A.G., Ratnieks, F.L.W., 2001. Task partitioning, division of labour and nest compartmentalisation collectively isolate hazardous waste in the leafcutting ant *Atta cephalotes*. *Behav. Ecol. Sociobiol.* 49, 387–392.
- Hart, A.G., Ratnieks, F.L.W., 2002. Waste management in the leaf-cutting ant *Atta colombica*. *Behav. Ecol. Sociobiol.* 13, 224–231.
- Hernandez, J.V., Goitia, W., Osio, A., Cabrera, A., Lopez, H., Sainz, C., Jaffe, K., 2006. Leaf-cutter ant species (Hymenoptera: *Atta*) differ in the types of cues used to differentiate between self and others. *Anim. Behav.* 71, 945–952.
- Hölldobler, B., Wilson, E.O., 1990. *The Ants*. Belknap Press, Cambridge.
- Howard, R.W., Blomquist, G.J., 2005. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annu. Rev. Entomol.* 50, 371–393.
- Hughes, W.O.H., Boomsma, J.J., 2006. Does genetic diversity hinder parasite evolution in social insect colonies? *J. Evol. Biol.* 19, 132–143.
- Hughes, W.O.H., Howse, P.E., Goulson, D., 2001. Mandibular gland chemistry of grass-cutting ants: species, caste, and colony variation. *J. Chem. Ecol.* 27, 109–124.
- Hughes, W.O.H., Eilenberg, J., 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.
- Hughes, W.O.H., Sumner, S., Van Borm, S., Boomsma, J.J., 2003. Worker caste polymorphism has a genetic basis in *Acromyrmex* leaf-cutting ants. *Proc. Natl. Acad. Sci. U. S. A.* 100, 9394–9397.
- Kaib, M., Eisermann, B., Schoeters, E., Billen, J., Franke, S., Francke, W., 2000. Task-related variation of postpharyngeal and cuticular hydrocarbon compositions in the ant *Myrmecaria eumenoides*. *J. Comp. Physiol. A* 186, 939–948.
- Kraus, J., Ruxton, G.D., 2002. *Living in Groups*. Oxford University Press, Oxford.
- Lahav, S., Soroker, V., Hefetz, A., Vander Meer, R.K., 1999. Direct behavioral evidence for hydrocarbons as ant recognition discriminators. *Naturwissenschaften* 86, 246–249.
- Lenoir, A., Hefetz, A., Simon, T., Soroker, V., 2001. Comparative dynamics of gestalt odour formation in two ant species *Camponotus fellah* and *Aphaenogaster senilis* (Hymenoptera: Formicidae). *Physiol. Entomol.* 26, 275–283.
- Lopes, J.F.S., Hughes, W.O.H., Camargo, R.S., Forti, L.C., 2005. Larval isolation and brood care in *Acromyrmex* leaf-cutting ants. *Insectes Soc.* 52, 333.
- Martin, S.J., Vitikainen, E., Helanterä, H., Drijfhout, F.P., 2008. Chemical basis of nest-mate discrimination in the ant *Formica exsecta*. *Proc. R. Soc. Lond. B* 275, 1271–1278.
- McCallum, H., Barlow, N., Hone, J., 2001. How should pathogen transmission be modelled? *TREE* 16, 295–300.
- Moreira, D., Erthal, M., Carrera, M., Silva, C., Samuels, R., 2006. Oral trophallaxis in adult leaf-cutting ants *Acromyrmex subterraneus subterraneus* (Hymenoptera, Formicidae). *Insectes Soc.* 53, 345–348.
- Morel, L., Meer, R.K., Lavine, B.K., 1988. Ontogeny of nestmate recognition cues in the red carpenter ant (*Camponotus floridanus*). *Behav. Ecol. Sociobiol.* 22, 175–183.
- Morelos-Juárez, C., Walker, T.N., Lopes, J.F.S., Hughes, W.O.H., 2010. Ant farmers practice proactive personal hygiene to protect their fungus crop. *Curr. Biol.* 20, 553–554.
- Naug, D., Camazine, S., 2002. The role of colony organization on pathogen transmission in social insects. *J. Theor. Biol.* 215, 427–439.
- Okuno, M., Tsuji, K., Sato, H., Fujisaki, K., 2012. Plasticity of grooming behavior against entomopathogenic fungus *Metarhizium anisopliae* in the ant *Lasius japonicus*. *J. Ethol.* 30, 23–27.
- Pie, M.R., Rosengaus, R.B., Traniello, J.F.A., 2004. Nest architecture, activity pattern, worker density and the dynamics of disease transmission in social insects. *J. Theor. Biol.* 226, 45–51.
- Poulsen, M., Bot, A.N.M., Nielsen, M.G., Boomsma, J.J., 2002a. Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants. *Behav. Ecol. Sociobiol.* 52, 151–157.
- Poulsen, M., Bot, A.N.M., Currie, C.R., Boomsma, J.J., 2002b. Mutualistic bacteria and a possible trade-off between alternative defence mechanisms in *Acromyrmex* leaf-cutting ants. *Insectes Soc.* 49, 15–19.
- Robinson, G.E., 1992. Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* 37, 637–665.
- Rosengaus, R.B., Maxmen, A.B., Coates, L.E., Traniello, J.F.A., 1998. Disease resistance: a benefit of sociality in the dampwood termite *Zootermopsis angusticollis* (Isoptera: Termitidae). *Behav. Ecol. Sociobiol.* 44, 125–134.
- Rosengaus, R.B., Traniello, J.F.A., Lefebvre, M.L., Maxmen, A.B., 2004. Fungistatic activity of the sternal gland secretion of the dampwood termite *Zootermopsis angusticollis*. *Insectes Soc.* 51, 259–264.
- Rosengaus, R.B., Tara, C., Katerina, G., James, F.A.T., 2007. Inducible immune proteins in the dampwood termite *Zootermopsis angusticollis*. *Naturwissenschaften* 94, 25–33.
- Schmid-Hempel, P., 1998. *Parasites in Social Insects*. Princeton University Press, Princeton.
- Soroker, V., Vienne, C., Hefetz, A., 1995. Hydrocarbon dynamics within and between nestmates in *Cataglyphis niger* (Hymenoptera: Formicidae). *J. Chem. Ecol.* 21, 365–378.
- Soroker, V., Lucas, C., Simon, T., Fresneau, D., Durand, J.L., Hefetz, A., 2003. Hydrocarbon distribution and colony odour homogenisation in *Pachycondyla apicalis*. *Insectes Soc.* 50, 212–217.
- Traniello, J.F.A., Rosengaus, R.B., Savoie, K., 2002. The development of immunity in a social insect: evidence for the group facilitation of disease resistance. *Proc. Natl. Acad. Sci. U. S. A.* 99, 6838–6842.
- Ugelvig, L.V., Cremer, S., 2007. Social prophylaxis: group interaction promotes collective immunity in ant colonies. *Curr. Biol.* 17, 1967–1971.
- Verza, S., Forti, L.C., Lopes, J.F.S., Hughes, W.O.H., 2007. Nest architecture of the leaf-cutting ant *Acromyrmex rugosus rugosus*. *Insectes Soc.* 54, 303–309.
- Wetterer, J.K., 1999. The ecology and evolution of worker size-distribution in leaf-cutting ants (Hymenoptera: Formicidae). *Sociobiology* 34, 119–144.
- Wilson, E.O., 1971. *The Insect Societies*. Belknap Press, Cambridge.
- Wilson, E.O., 1975. *Sociobiology: The New Synthesis*. Belknap Press of Harvard University Press, Harvard.
- Wilson, K., Kneil, R., Boots, M., Koch-Osborne, J., 2003. Group living and investment in immune defence: an interspecific analysis. *J. Anim. Ecol.* 72, 133–143.