

Prudent inquilines and proactive hosts: behavioral dynamics between an ant social parasite, *Megalomyrmex symmetochus* and its fungus-growing ant host, *Sericomyrmex amabilis*

G. Bruner · W. T. Wcislo · H. Fernández-Marín

Received: 10 April 2013 / Revised: 25 November 2013 / Accepted: 27 November 2013 / Published online: 29 December 2013
© International Union for the Study of Social Insects (IUSI) 2013

Abstract Ant social parasites evolve adaptive relationships with their hosts. Theoretically, coevolution predicts strong selection to maximize fitness of the parasite that minimizes costs to its host, which potentially leads to the evolution of benign interactions. We studied the demographic and behavioral traits of the ant social parasite *Megalomyrmex symmetochus* (Solenopsidini), an agro-predator that feeds on larvae and fungal garden products of their host, *Sericomyrmex amabilis* (Attini). Based on demographic data from 15 parasitized colonies, the proportion of parasitic workers to those of the host is 1:2. Moreover, defensive prophylactic behaviors observed during infections with *Metarhizium brunneum*, a generalist entomopathogen, and *Escovopsis*, a specialized fungal garden parasite, showed that *S. amabilis* works extensively to remove and control fungal infections, in contrast to *M. symmetochus*. *M. symmetochus*, however, performed intraspecific allogrooming during infections with *Escovopsis* and *M. brunneum*, suggesting that they may recognize

fungal pathogens and indirectly limit dispersion of spores. Our results indicate that *M. symmetochus* did not have a strong role in maintaining a hygienic nest.

Keywords Amelioration · Coevolution · Symbiosis · Xenobiosis · Social parasitism

Introduction

Parasitism is a common life-history strategy, and such species play a key role in shaping life on earth (e.g., Price, 1977; Ewald, 2004). Generally, it is assumed that parasites impose an on-going cost to host fitness (Anderson and May, 1982). Some coevolutionary models predict a modulation of virulence and reciprocal adaptive dynamics that may result in a more stable relationship (Dawkins and Krebs, 1979; Ebert, 1998; Ebert and Herre, 1996), under some conditions, such as a highly host-specific parasite (Kirchner and Roy, 2002). Generalist parasites are less likely to evolve reduced virulence because they confront multiple hosts, and the different hosts confront multiple pathogens (Thomas et al., 2003; Vojvodic et al., 2012; Hodgson et al., 2004). These conditions lead to parasite–parasite competition, increasing both parasites' virulence and host-resistance mechanisms.

Social insect colonies provide exceptional opportunities to study host interactions with multiple parasites, because their colonies present a buffered niche that is exploited by numerous parasites and symbionts (Hölldobler and Wilson, 1990; Schmid-Hempel, 1995; Bruner et al., 2012), including non-social parasites as flies or hymenopteran parasitoids, or social parasites that exploit host brood care behavior and food resources (Schmid-Hempel, 1995). Generally, it is assumed that ant social parasites are costly for the host colony. It is, however, increasingly evident that different

G. Bruner · W. T. Wcislo · H. Fernández-Marín (✉)
Smithsonian Tropical Research Institute, Apartado Postal
0843-03092, Panama City, Republic of Panama
e-mail: hermozenes_f@hotmail.com;
H Fernandez@indicat.org.pa

Present Address:

G. Bruner
Department of Bacteriology, University of Wisconsin, Microbial
Sciences Building, 1550 Linden Dr., Madison, WI 53706, USA

H. Fernández-Marín
Instituto de Investigaciones Científicas y Servicios de Alta
Tecnología, PO Box 0843-01105, Edificio 219, Panamá 5, Ciudad
del Saber, Clayton, Panama City, Republic of Panama

parasitic species differ greatly in their impact on hosts (Brandt et al., 2005; Hare and Alloway, 2001; Hovestadt et al., 2012). Given that queen-tolerant ant parasites tend to be host-specific, co-exist long term with their hosts, and likely have vertical transmission, they have apparently evolved more benign relationships with their hosts (Brandt et al., 2005). Therefore, if ant social parasites have to compete against possible parasitic competitors and deal with the same potential parasites (e.g., entomopathogens), we should expect some antagonistic interactions with some parasites. Nevertheless, most experimental studies have been concerned with the cost imposed by the parasite (i.e., fitness cost), leaving aside the question of whether host-beneficial behaviors evolved.

Colonies of some attines species (including *Sericomyrmex*, *Trachymyrmex*, *Cyphomyrmex*, and *Apterostigma*) can be usurped by specialized ant social parasites, *Megalomyrmex*, which consume larvae and the fungal cultivar (Adams et al., 2000, 2012; Brandão, 2003; Wheeler, 1925). Although most *Megalomyrmex* ants are considered free-living or potentially facultative parasites, some species have been found only exclusively with colonies of attine ants (Brandão, 2003; Buschinger, 2009; Longino, 2010; Adams et al., 2012). *Sericomyrmex amabilis* (Attini), a non-leaf-cutter higher attine, is typically a host of *M. symmetochus* (Adams et al., 2012; Adams and Longino, 2007; Brandão, 2003; Longino, 2010; Wheeler, 1925), which has been thought to cohabit passively with its host, raising the hypothesis that *Megalomyrmex* may evolved behavior beneficial to its host, such as disease management (e.g., Currie and Stuart, 2001; Fernández-Marín et al., 2006, 2009; Morelos-Juárez et al., 2010). In colonies of *S. amabilis* cohabiting with *M. symmetochus*, we investigated the relation of host colony size and parasite load. We collected host nests over a relatively long time, and we were able to excavate them carefully and check that all parasitizes were collected. Moreover, we experimentally explored the proactive and hygienic behaviors between *M. symmetochus* and *S. amabilis*, during microfungal infections, and evaluated how they shape host survival.

Materials and methods

Ant collections

Fifteen colonies of *S. amabilis* containing the ant social parasite *M. symmetochus* were collected near Gamboa in central Panama ($N = 2$), and along nearby Pipeline Road (0.3 and 1 km from the park entrance, $N = 5$) and Plantation Road (2 and 2.5 km from the park entrance, $N = 8$). Those colonies were collected during the rainy seasons from 2010 to 2012, and some containing sexual individuals. Details of

the colonies are included per year: in 2010, six colonies were collected, including two in July, one in August, and two in November, one in December (with sexual males); in 2011, four colonies were collected, including one each month, July, August, October (with sexual males) and December; and in 2012 five colonies were collected, including two in April (one with sexual females and males, and another with sexual males), one in July, and two in August (Table 1).

Colonies were excavated to 30 cm depth from nest entrances using a paleontological pick. Fungal gardens and worker ants were collected using a spoon, forceps and manual aspirator. Colonies were transported to and maintained in the facilities of the laboratories at Smithsonian Tropical Research Institute, in Gamboa and kept in plastic containers (~ 25 °C and 12:12 h of light:dark). We returned to the site 1 day after collections to confirm that the colonies were collected completely. In the laboratory, we quantified the number of workers, queens and alates for each colony for both species *S. amabilis* and *M. symmetochus*. Eggs, larvae and pupae were not counted to avoid disturbing the fungal garden. Colonies were kept using moistened plaster on the floor of the containers and fed every 3 days/week with corn meal and young leaves of *Lagerstroemia speciosa* (Lythraceae) and flowers of *Ixora coccinea* (Rubiaceae).

Sericomyrmex–*Megalomyrmex* prophylactic behavior

To determine the level of performance of hygienic tasks by *S. amabilis* and *M. symmetochus*, we quantified the responses of both species by experimentally simulating a fungal garden infection using a specialized cultivar parasite, *Escovopsis* sp. and the generalist entomopathogen, *Metarhizium brunneum*. All fungi were cultured under laboratory conditions using potato dextrose agar (PDA pre-made mix, 39 g/l); *Escovopsis* had been isolated from the fungal garden of *S. amabilis* and *M. brunneum*, from dead queens of *Atta colombica* (cf. Fernández-Marín et al., 2006). Four colonies of *S. amabilis* containing *M. symmetochus* were

Table 1 Demography [mean \pm SD (range)] of colonies of *Sericomyrmex amabilis* containing the social parasite *Megalomyrmex symmetochus*

	<i>Sericomyrmex</i>	<i>Megalomyrmex</i>	<i>U</i>	<i>P</i>
Worker	791.6 \pm 639.6 (45–2,569)	385.2 \pm 171.6 (55–671)	58	0.025
Dealate queen	0.8 \pm 0.67 (0–2)	1.6 \pm 1.18 (0–5)	48	0.025
Alate male	1.73 \pm 4.25 (0–14)	22.13 \pm 50.71 (0–189)	77	0.402
Alate queen	1.73 \pm 6.71 (0–26)	10.13 \pm 33.59 (0–130)	80	0.453

P values represent the difference between ant species using Mann–Whitney *U* test; $N = 15$ colonies

used in the experiment. For each nest, we set up 12 subcolonies containing 0.3 g of fungal garden placed in a Petri dish (100 mm × 15 mm). Four subcolonies each were assigned to one of the three treatments: *Escovopsis* sp., *M. brunneum*, and control (no conidia). In the treatments, we used sterile entomological forceps to gently brush pure conidia from a piece (~3 mm³) of PDA medium over the fungal garden. For the control, we rubbed sterile forceps over the fungal garden. Then, we randomly selected ten workers of *S. amabilis* and ten workers of *M. symmetochus* from the fungal garden of the same colony and placed them in a Petri dish that contains 0.3 g of the fungus garden. We closed the Petri dish and 10 min after infection recorded the prophylactic behaviors (see definitions below) for both species using a stereoscope (0.7×, Bausch and Lomb) during 60 min. After infections, we record the behavior for 60 min. The observations were recorded as follows: each 10 min during the hour, we recorded the number of workers that were grooming the fungus garden. In continuous observations, we recorded the frequencies of ants engaged in metapleural gland (MG) grooming, cultivar planting, weeding, and allogrooming. Since our observations were not scans of individual ants, our data are summed for ants observed in the field of view of a stereomicroscope at 10×.

Behavioral definitions

We quantified the following prophylactic behaviors: (1) the number of workers cleaning the infected area; (2) MG grooming, when an ant rubbed its foreleg during the opening of the MGs and passed the foreleg to the glossa following fungal grooming of the infected area; (3) cultivar planting, when an ant placed a piece of uninfected garden over an infected area; (4) weeding, which involved removing of a piece of the infected garden; (5) intraspecific allogrooming, when an ant groomed any body part of a conspecific over infected area; and (6) interspecific allogrooming, when an ant groomed any body part of a heterospecific ant (Fernández-Marín et al., 2006, 2009, 2013; Morelos-Juárez et al., 2010).

To compare colony demography between *Sericomyrmex* and *Megalomyrmex*, we used Mann–Whitney *U* tests. The prophylactic behavioral data were transformed using automated data preparation (default settings) and analyzed using linear mixed models (SPSS v. 20) fitted to restricted maximum likelihood including the fixed effects, ant species (*Sericomyrmex* and *Megalomyrmex*) nested within colony factor, pathogen factor (*Escovopsis*, *Metarhizium* and control), the prophylactic behaviors (ant grooming, MG grooming, planting, weeding, and interspecific and intraspecific allogrooming) nested within the ant factor and colony as random factor. We also included the interactions fungus × ant nested to colony, and ant × fungus ×

prophylactic behaviors nested to colony. We did not exclude any statistically non-significant factor from the model. To compare *Sericomyrmex* and *Megalomyrmex* prophylactic behaviors within pathogen treatments, we used pairwise comparisons of the estimated marginal means. The pairwise comparisons were adjusted using Sidak corrections for multiple comparisons.

Results

The relative numbers of workers and queens of *Sericomyrmex* and *Megalomyrmex* differed significantly ($P = 0.025$, and $P = 0.025$, respectively), but there were no differences between alate males ($P = 0.402$) and alate females ($P = 0.453$) (Table 1). There was a significant correlation between number of workers of *Sericomyrmex* and *Megalomyrmex* ($r = 0.83$, $P < 0.001$) (Fig. 1), so that the *Megalomyrmex*: *Sericomyrmex* worker ratio was approximately 1:2. For the main effects, there was an overall significant difference within ant species treatments ($F_{3,576} = 3.78$, $P = 0.01$), and occurrence of prophylactic behaviors differed significantly ($F_{5,576} = 35.99$, $P < 0.0001$). There were no significant differences within pathogen treatments ($F_{2,576} = 0.253$, $P = 0.777$), nor across colonies ($F_{3,576} = 1.79$, $P = 0.147$), but there were significant interactions effects on pathogen × ants species ($F_{12,576} = 3.78$, $P < 0.0001$), and pathogens × ant species × prophylactic behaviors ($F_{110,576} = 8.70$, $P < 0.0001$).

There was an overall greater prophylactic behavioral response from *Sericomyrmex* workers than *Megalomyrmex* workers within pathogenic infections. A pairwise comparison between ant species showed that the number of workers

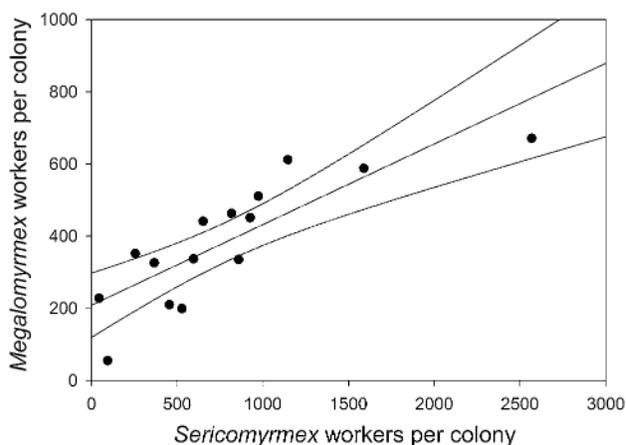


Fig. 1 Relationship between the numbers of ant workers of *Sericomyrmex amabilis* (host) and *Megalomyrmex symmetochus* (social parasite) per colony. Solid line [$y = 208.302 + (0.224 \times x)$] and dashed lines represent the main regression and 95 % confidence intervals, respectively

grooming was greater for *Sericomyrmex* than *Megalomyrmex* within pathogen infections for *Escovopsis* and *Metarhizium* ($F_{1,576} = 166.19$, $P < 0.0001$; $F_{1,576} = 118.60$, $P < 0.0001$) (Fig. 2). However, *Megalomyrmex* workers grooming was greater than *Sericomyrmex* during the control ($F_{1,576} = 67.25$, $P < 0.0001$) (Fig. 2). MG

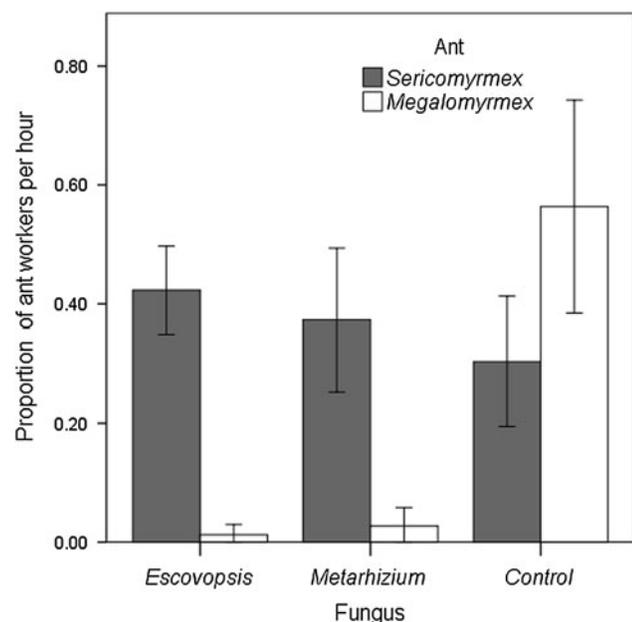


Fig. 2 Mean (\pm SD) number of workers of *Sericomyrmex amabilis* and *Megalomyrmex symmetochus* observed during fungal grooming

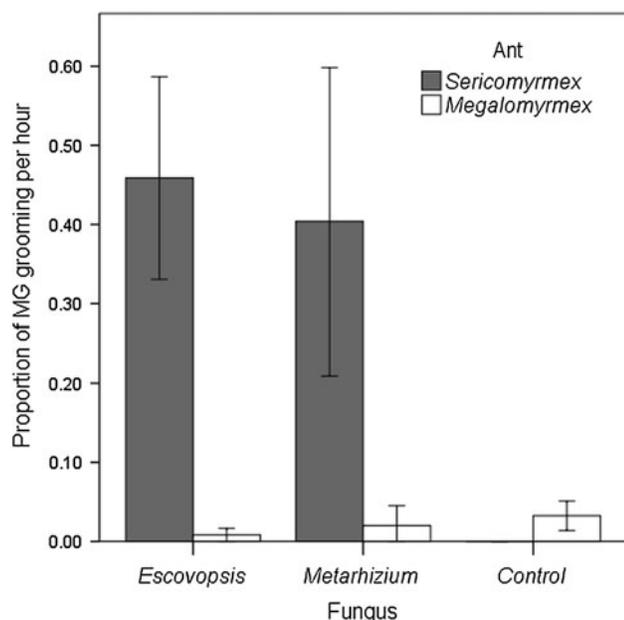


Fig. 3 Mean (\pm SD) metapleural gland (MG) grooming response of *Sericomyrmex amabilis* and *Megalomyrmex symmetochus* during treatments

grooming behavior was greater for *Sericomyrmex* within *Escovopsis* ($F_{1,576} = 201.74$, $P < 0.0001$) (Fig. 3) and *Metarhizium* infections ($F_{1,576} = 146.51$, $P < 0.0001$); there was no difference in the control ($F_{1,576} = 1.03$, $P = 0.310$). *Sericomyrmex* groomed (interspecific allogrooming) more frequently than *Megalomyrmex* during control trials ($F_{1,576} = 10.50$, $P = 0.001$) and *Escovopsis* infections ($F_{1,576} = 10.54$, $P = 0.001$) than during *Metarhizium* infections ($F_{1,576} = 0.27$, $P = 0.870$). Both ant species did not show significant differences within pathogen infections for weeding ($F_{1,576} = 0.21$, $P = 0.646$; $F_{1,576} = 0.96$, $P = 0.375$; $F_{1,576} = 0.00$, $P = 1.00$ for *Escovopsis*, *Metarhizium* and control, respectively), planting behavior ($F_{1,576} = 3.11$, $P = 0.077$; $F_{1,576} = 325$, $P = 0.072$; $F_{1,576} = 0.00$, $P = 1.00$ for *Escovopsis*, *Metarhizium* and control, respectively) and intraspecific grooming ($F_{1,576} = 1.17$, $P = 0.279$; $F_{1,576} = 0.430$, $P = 0.512$; $F_{1,576} = 3.11$, $P = 0.077$ for *Escovopsis*, *Metarhizium* and control, respectively).

Discussion

Our colony demographic data suggest that both host and parasite queens cohabit the same nest and that the production of *M. symmetochus* workers may be associated with *S. amabilis* colony size, supporting the hypothesis that *M. symmetochus* may coexist long term and could increase the number of individuals both species, as an obligatory queen-tolerant inquiline (Adams et al., 2000, 2012; Adams and Longino, 2007; Brandão, 1990, 2003). This hypothesis is consistent with the putatively iteroparous strategy of *M. symmetochus* hosted by *S. amabilis*, which enables the host to continue production of sexuals. In contrast, *Acromyrmex echinator* hosts a semelparous parasite, *Acromyrmex insinuator* (Bekkevold and Boomsma, 2000), where parasite workers increase the parasites' fitness, which reduces the production of host sexuals (Sumner et al., 2003). Regulation of worker production by *M. symmetochus* may be a stable strategy that keeps *S. amabilis* relatively healthy, allowing the parasite time to reach sexual maturity and exploit the host over a long term. Interestingly, we observed that 2 of the 15 host colonies attacked and killed all worker ants of *M. symmetochus* 2 months after being collected, which suggests that this coexistence may be perturbed by unknown factors (e.g., disease outbreaks, or laboratory conditions). These behavioral conflicts show that *S. amabilis* can defend their nests against the social parasite. Although selection of defensive mechanisms against *M. symmetochus* may be plausible in some populations, it is likely to occur during the foundation of the colony and not once

the parasite load is high, given that parasite-host conflicts are costly for both species. In addition, the abundance of *M. symmetochus* workers (Table 1) is considerably higher than many obligatory inquiline ants, which usually are not abundant (Hölldobler and Wilson, 1990). Wheeler (1925) reported no more than 75 *Megalomyrmex* workers per colony cohabited in colonies of *S. amabilis*. Why does *M. symmetochus* allocate so much energy to worker production instead of producing alates? One possible explanation is that *M. symmetochus* synchronize peak alate production with that of their hosts, which will create a larger pool of small, incipient host colonies to parasitize. Social and brood parasites life cycles are typically synchronized to seasonal patterns of host availability (Calero-Torralbo and Valera, 2008; Payne, 1973; Wcislo, 1987).

Megalomyrmex symmetochus intraspecific allogrooming was greater during infections with dry conidia of *Metarhizium* and *Escovopsis* than the control trial, suggesting that *M. symmetochus* detected infections but directed benefits to conspecifics. *M. symmetochus*, therefore, may indirectly decrease disease transmissions in the nest by reducing dispersion of fungal spores. Furthermore, *S. amabilis* workers performed interspecific grooming, suggesting that *M. symmetochus* also exploit the active prophylactic behavior of its host. Allogrooming is an important prophylactic behavior in social insects to prevent fungal parasite transmission (Walker and Hughes, 2009; Reber et al., 2011; Hughes et al., 2002; Rosengaus et al., 1998; Morelos-Juárez et al., 2010). Our survey shows for the first time that the behavioral dynamics between an ant social parasite and its host during a pathogenic fungus outbreak, however, do support the idea that social parasites *Megalomyrmex* is helping with the disease management of *Sericomyrmex* ants. The *Sericomyrmex*–*Megalomyrmex* interaction is mediated on one hand by the balanced display of proactive defensive prophylactic behaviors for suppressing possible fungal pathogens by the host *S. amabilis*, and, on the other hand, *M. symmetochus* may be a prudent ant social parasite in exploiting its host. In addition, the parasite (*Megalomyrmex*) may provide additional benefits to its host. For example, *Sericomyrmex* colonies parasitized by *M. symmetochus* better resisted invasion by the agro-predator, *Gnamptogenys harmanni*, since *M. symmetochus* help defend the host nests, including killing the *Gnamptogenys* ants (Adams et al., 2013).

Acknowledgments We thank the Autoridad Nacional del Ambiente for research and export permits, and STRI staff for logistic support. This work was supported by Re-insertion grant from SENACYT and Tupper Postdoctoral Fellowships from STRI (to HF-M), grants from International Collaboration Program at SENACYT to WTW and HF-M (09-038), and general research funds from STRI (to WTW).

References

- Adams R.M.M., Mueller U.G., Schultz T.R. and Norden B. 2000. Agro-predation: usurpation of attine fungus gardens by *Megalomyrmex* ants. *Naturwissenschaften* **87**: 549-554
- Adams R.M.M. and Longino J.T. 2007. Nesting biology of the arboreal fungus-growing ant *Cyphomyrmex cornutus* and behavioral interactions with the social-parasitic ant *Megalomyrmex mondabora*. *Insect. Soc.* **54**: 136-143
- Adams R.M.M., Shah K., Antonov L.D. and Mueller U.G. 2012. Fitness consequences of nest infiltration by the mutualist-exploiter *Megalomyrmex adamsae*. *Ecol. Entomol.* **37**: 453-462
- Adams R.M.M., Liberti J., Illum A.A., Jones T.H., Nash D.R. and Boomsma J.J. 2013. Chemically armed mercenary ants protect fungus-farming societies *Proc. Natl Acad. Sci. USA* **110**: 15503-15504
- Anderson R.M. and May R.M. 1982. Coevolution of hosts and parasites. *Parasitology* **85**: 411-426
- Bekkevold D. and Boomsma J.J. 2000. Evolutionary transition to a semelparous life history in the socially parasitic ant *Acromyrmex insinuator*. *J. Evol. Biol.* **13**: 615-623
- Brandão C.R.F. 1990. Systematic revision of the Neotropical ant genus *Megalomyrmex* Forel (Hymenoptera: Formicidae: Myrmicinae), with the description of thirteen new species. *Arq. Zool.* **31**: 1-91
- Brandão C.R.F. 2003. Further revisionary studies on the ant genus *Megalomyrmex* Forel (Hymenoptera: Formicidae: Myrmicinae: Solenopsidini). *Pap. Avul. Zool.* **43**: 145-159
- Brandt M., Foitzik S., Fischer-Blass B. and Heinze J. 2005. The coevolutionary dynamics of obligate ant social parasite systems between prudence and antagonism. *Biol. Rev.* **80**: 251-267
- Bruner G., Fernández-Marín H., Touchon J.C. and Wcislo W.T. 2012. Eggs of the blind snake, *Liotyphlops albirostris*, are incubated in a nest of the lower fungus-growing ant, *Apterostigma cf. goniodes*. *Psyche* 2012: 1-5
- Buschinger A. 2009. Social parasitism among ants: a review (Hymenoptera: Formicidae). *Myrmecol. News* **12**: 219-235
- Calero-Torralbo M.A. and Valera F. 2008. Synchronization of host-parasite cycles by means of diapause: host influence and parasite response to involuntary host shifting. *Parasitology* **135**: 1343-1352
- Currie C.R. and Stuart A.E. 2001. Weeding and grooming of pathogens in agriculture by ants. *Proc. R. Soc. London Ser. B* **268**: 1033-1039
- Dawkins R. and Krebs J.R. 1979. Arms races between and within species. *Proc. R. Soc. London Ser. B* **205**: 489-511
- Ebert D. 1998. Experimental evolution of parasites. *Science* **282**: 1432-1435
- Ebert D. and Herre E.A. 1996. The evolution of parasitic diseases. *Parasitol. Today* **12**: 96-101
- Ewald P.W. 2004. Evolution of virulence. *Infect. Dis. Clin. North Am.* **18**: 1-15
- Fernández-Marín H., Bruner G., Gomez B.E., Nash D.R., Boomsma J.J. and Wcislo W.T. 2013. Dynamic disease management in *Trachymyrmex* fungus-growing ants (Attini: Formicidae). *Am. Nat.* **181**: 571-582
- Fernández-Marín 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. London Ser. B* **276**: 2263-2269
- Fernández-Marín H., Zimmerman J.K., Rehner S.A. and Wcislo W.T. 2006. Active use of the metapleural glands by ants in controlling fungal infection. *Proc. R. Soc. London Ser. B* **273**: 1689-1695

- Hare J.F. and Alloway T.M. 2001. Prudent *Protomognathus* and despotic *Leptothorax duloticus*: differential costs of ant slavery. *Proc. Natl Acad. Sci. USA* **98**: 12093-12096
- Hodgson D.J., Hitchman R.B., Vanbergen A.J., Hails R.S., Possee R.D. and Cory J.S. 2004. Host ecology determines the relative fitness of virus genotypes in mixed-genotype nucleopolyhedrovirus infections. *J. Evol. Biol.* **17**: 1018-1025
- Hölldobler B. and Wilson E.O. 1990. *The Ants*. Harvard University Press, Cambridge, MA
- Hovestadt T., Thomas J.A., Mitesser O., Elmes G.W. and Schonrogge K. 2012. Unexpected benefit of a social parasite for a key fitness component of its ant host. *Am. Nat.* **179**: 110-123
- 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. London Ser. B* **269**: 1811-1819
- Kirchner J.W. and Roy B.A. 2002. Evolutionary implications of host-pathogen specificity: fitness consequences of pathogen virulence traits. *Evol. Ecol. Res.* **4**: 27-48
- Longino J.T. 2010. A taxonomic review of the ant genus *Megalomyrmex* Forel (Hymenoptera: Formicidae) in Central America. *Zootaxa* **58**: 35-58
- Morelos-Juárez C., Walker T.N., Lopes J.F. and Hughes W.O.H. 2010. Ant farmers practice proactive personal hygiene to protect their fungus crop. *Curr. Biol.* **20**: R553-554
- Payne R.B. 1973. The breeding season of a parasitic bird, the brown-headed cowbird in central California. *Condor* **75**: 80-99
- Price P.W. 1977. General concepts on the evolutionary biology of parasites. *Evolution* **31**: 405-420
- Reber A., Purcell J., Buechel S.D., Buri P. and Chapuisat M. 2011. The expression and impact of antifungal grooming in ants. *J. Evol. Biol.* **24**: 954-964
- Rosengaus R.B., Maxmen A.B., Coates L.E. and Traniello J.F.A. 1998. Disease resistance: a benefit of sociality in the dampwood termite *Zootermopsis angusticollis* (Isoptera: Termopsidae). *Behav. Ecol. Sociobiol.* **44**: 125-134
- Schmid-Hempel P. 1995. Parasites and social insects. *Apidologie* **26**: 245-245
- Sumner S., Nash D.R. and Boomsma J.J. 2003. The adaptive significance of inquiline parasite workers. *Proc. R. Soc. London Ser. B* **270**: 1315-1322
- Thomas M.B., Watson E.L. and Valverde-Garcia P. 2003. Mixed infections and insect-pathogen interactions. *Ecol. Lett.* **6**: 183-188
- Vojvodic S., Boomsma J.J., Eilenberg J. and Jensen A.B. 2012. Virulence of mixed fungal infections in honey bee brood. *Front. Zool.* **9**: 5
- Walker T.N. and Hughes W.O.H. 2009. Adaptive social immunity in leaf-cutting ants. *Biol. Lett.* **5**: 446-448
- Wcislo W.T. 1987. The roles of seasonality, host synchrony, and behaviour in the evolutions and distributions of nest parasites in Hymenoptera (Insecta), with special reference to bees (Apoidea). *Biol. Rev.* **62**: 415-443
- Wheeler W.M. 1925. A new guest-ant and other new Formicidae from Barro Colorado Island, Panama. *Biol. Bull.* **49**: 150-181