Ecological Entomology

Ecological Entomology (2018), DOI: 10.1111/een.12512

The farming ant *Sericomyrmex amabilis* nutritionally manages its fungal symbiont and its social parasite

JONATHAN Z. SHIK,[1,2] ANGELO CONCILIO,[3] THOMAS KAAE[4] and RACHELLE M. M. ADAMS[1,*] [1]Department of Biology, Centre for Social Evolution, University of Copenhagen, Copenhagen, Denmark, [2]Smithsonian Tropical Research Institute, Ancon, Republic of Panama, [3]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Torino, Italy and [4]Department of Biology, University of Copenhagen, Copenhagen, Denmark

Abstract. 1. When parasites exploit mutualisms involving food exchange, they can destabilise the partnership with costs to interacting partners. For instance, the ant *Sericomyrmex amabilis* farms fungal symbionts to produce food, but, in so doing, attracts parasitic *Megalomyrmex symmetochus* guest ants that infiltrate fungus-farming ant societies and live with their hosts their entire lives.

2. The present study examined whether host foraging in parasitised colonies shifts towards nutritional requirements of the parasitic guest ants as inferred from the parasite's elemental content (%C, %N, and C:N).

3. Laboratory feeding experiments with nutritionally defined diets indicated that *S. amabilis* ants harvest protein-biased substrate, and more total substrate when hosting *M. symmetochus* relative to when provisioning their fungus gardens and nestmates.

4. Field surveys further showed that parasitised colonies incur reductions in fungus garden nutritional quality and quantity, brood mass, and host worker body condition. And yet these costs appear manageable across growing seasons, as parasitised fungal cultivars appear to provide sufficient nutrition for stable populations of host ants.

5. The approach developed here shows how behavioural strategies for nutrient regulation can extend beyond the needs of the individual to entire fungus-farming systems, and implies that *S. amabilis* dynamically adjusts collective foraging strategies when parasitised to enhance long-term symbiotic stability.

Key words. Ecological stoichiometry, geometric framework, nutritional ecology, social parasite, symbiosis.

Introduction

When a bacterial pathogen invades a host, it colonises an ecosystem with a finite pool of nutrients (Smith, 2007) and competes for resources with a resident community of microbes (Chow *et al.*, 2010). The supply of nutrients can thus shift the competitive edge for or against antagonistic symbionts (Solomon *et al.*, 2003; Smith, 2007). However, the nutritional mechanisms governing such symbiotic stability often remain elusive, as the nutritional needs of symbionts (i.e. hosts, pathogens, parasites, mutualists) can be difficult to study outside their integrated symbiotic network (Chaffron & von Mering, 2007). Ant experiments overcome such methodological constraints, as their modular structure means that individuals (e.g. ants and symbionts) can be added, subtracted, and studied in isolation. In addition, ant colonies are often invaded by social parasites, other ant species that consume host resources (Buschinger, 2009). To our knowledge, no other studies have tested: (i) how host colonies adjust their foraging behaviours to manage the nutritional requirements of hosting co-evolved symbionts living in their nests; or (ii) whether such dynamic behaviours enhance long-term symbiotic stability.

The ant *Sericomyrmex amabilis* (Hymenoptera: Formicidae: Attini: Attina) farms fungal symbionts for food in Neotropical forests (Ješovnik *et al.*, 2017). Workers collectively harvest bits of decaying organic matter and plant material used as manure

Correspondence: Jonathan Shik, Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark. E-mail: jonathan.shik@gmail.com

*Current address: Evolution, Ecology and Organismal Biology, The Ohio State University, 318 West 12th Avenue, 300 Aronoff Laboratory, Columbus, Ohio 43210, U.S.A.

to nurture their co-evolved fungus in subterranean nests (Leal & Oliveira, 2000; de Fine Licht & Boomsma, 2010). *Megalomyrmex symmetochus* ants live in *S. amabilis* nests as social parasites and consume host brood and fungus garden (Adams *et al.*, 2013). Social parasites are typically closely related to their hosts (i.e. Emery's rule; Huang & Dornhaus, 2008; Rabeling *et al.*, 2014; Schär & Nash, 2014; Rabeling *et al.*, 2015) and are predicted to have similar nutritional requirements as their hosts. *Megalomyrmex symmetochus* is unique in this respect, descending from an insectivorous ant lineage (Boudinot *et al.*, 2013) and thus being distantly related to its fungivorous *S. amabilis* host (Ward *et al.*, 2014). And, as attine fungal symbionts thrive on carbohydrate-rich substrates, with moderate protein levels inhibiting fungus growth and survival (Shik *et al.*, 2016), we thus hypothesised that host ants face challenges when foraging to meet divergent nutritional demands of their co-evolved fungal cultivars and social parasites. We explored how host ants overcome these nutritional challenges by performing controlled laboratory feeding experiments within the geometric framework (GF; Simpson & Raubenheimer, 2012). We compared foraging responses of *S. amabilis* workers for two limiting macronutrients (protein and carbohydrates) in isolated groups of workers, in the presence of their fungal symbiont and also when hosting social parasites.

Body elemental stoichiometry (e.g. C:N:P) is often used to infer nutritional requirements (Sterner & Elser, 2002; Davidson, 2005; Kaspari *et al.*, 2012; Reich, 2014), and organisms are predicted to forage for foods rich in elements they need to build important biomolecules (e.g. carbon-rich carbohydrates, nitrogen-rich proteins, phosphorus-rich ATP, etc.) (Kay *et al.*, 2005; Yoshida, 2006). However, as parasitic *M. symmetochus* ants do not forage outside their host nests, they only have access to host brood and fungus. The costs of hosting *M. symmetochus* ants are therefore likely to be mediated by the match between the parasites' nutritional requirements and the nutritional content of resources available in host colonies. A mismatch between parasite resource demands and available protein-poor fungal cultivar would cause parasites to consume more protein-rich host brood to achieve a balanced diet. This would induce greater costs to host colonies because the fungus garden is typically in excess, whereas workers and reproductives are a more limiting resource (Shik *et al.*, 2014). We used stoichiometric analyses of the elemental content of the ants and their fungal food first to explore whether available foods (e.g. fungus garden and brood) represent nutritionally distinct resources, and then to visualise the longer-term nutritional costs of hosting social parasites in field-collected colonies. We further tested the prediction of higher %N content in *M. symmetochus* than in *S. amabilis*, as arthropod predators (consuming protein-rich prey) tend to have higher body %N than herbivores (consuming protein-poor vegetation) (Fagan *et al.*, 2002; Davidson, 2005).

Exploring the mechanisms governing symbiotic stability over different timescales required a multifaceted approach. To link these facets to our research aims, we measured short-term behavioural responses of host ants with nutrient feeding experiments; determined the long-term costs of hosting social parasites by comparing parasitised and non-parasitised demographic and stoichiometric measurements; and assessed whether social parasite ant nutrient requirements are matched to the resources available in host nests.

Materials and methods

Experimental colonies

We harvested *S. amabilis* colonies from Soberania National Park in May 2015 (Panama: 9.154 51°N, 79.735 83°W), excavating five non-parasitised colonies (23 garden chambers with host adult ants and brood; hereafter, subcolonies), and four parasitised colonies (eight subcolonies with *M. symmetochus* ants) (demographic data provided in Table S1). We established subcolonies in the laboratory (24 °C) in separate cotton-lined Petri dishes within larger foraging arenas and allowed them to acclimate for *c*. 7 days with *ad libitum* water and polenta.

Short-term host foraging flexibility

We used a diet-choice laboratory experiment to measure protein:carbohydrate (P:C) foraging targets of isolated *S. amabilis* worker groups ($n = 10$), non-parasitised *S. amabilis* subcolonies ($n = 13$), and parasitised *S. amabilis* subcolonies ($n = 8$). Replicates in the isolated worker group experiment were from non-parasitised colonies and contained 17–50 workers (mean ± SD, 40.1 ± 11.7), representing field variation in workers per chamber. We provided ants with paired 1:3 and 3:1 P:C agar-based diet cubes (*c*. 1 g) containing 1.3 g agar litre^{-1} and 40 g protein + carbohydrates litre^{-1}, modified from Dussutour and Simpson (2008). We replaced diets every second day and measured foraging over 9 days (isolated worker groups and non-parasitised subcolonies) or 4 ± 3 days [parasitised subcolonies: 7 days (four subcolonies) or 1 day (four subcolonies)].

We calculated daily per-worker protein and carbohydrate harvest from dietary P:C ratios, drying post-experiment diets at 60 °C for 24 h and measuring dry mass loss (to 0.1 mg) based on dry:wet mass ratio of control diet cubes (Appendix S1). We tested for parasite effects on feeding experiment results (daily per-worker total, protein, and carbohydrate diet harvest), using general linear mixed model analyses (SAS V9.3) with colony ID as a random factor and including *S. amabilis* worker number as a covariate. We used *post hoc* Tukey tests to further evaluate significant main effects.

Long-term host demographic and stoichiometric responses

We weighed field-collected subcolonies before the feeding experiment and calculated worker number (sum of live and dead) and brood mass following the experiment. Initial 'field' fungus garden mass was estimated by subtracting worker and brood mass from initial subcolony mass (Table S1). We used the mixed-model approach described for the feeding experiments to test for differences in fungus mass and elemental composition of fungus and host worker (%C, %N, and C:N) between colonies from the two treatments (parasitised versus non-parasitised). Within colonies of each treatment (parasitised

or non-parasitised), we used paired *t*-tests to compare the total mass of C and N in cohabitating fungus and host workers. We also tested for parasite effects on final brood mass using a non-parametric Wilcoxon signed-ranked test, as these data were not normally distributed.

Match between parasite nutritional requirements and available resources

Directly measuring the nutritional intake targets of *M. symmetochus* workers, as we did with isolated groups of *S. amabilis* workers, would have enabled us to test if host ant foraging shifts towards the nutritional requirements of their parasite. However, repeated attempts to perform feeding experiments with isolated groups of *M. symmetochus* were unsuccessful because the social parasites do not forage outside of the host nest. As a proxy for parasite nutritional requirements, we measured the %C and %N of *M. symmetochus* workers ($n = 8$ parasitised colonies) and compared these values with those of cohabiting *S. amabilis* workers ($n = 8$ parasitised colonies) and their fungal cultivars (samples from seven parasitised colonies; one fungal sample was lost during CN analyses).

Samples were from freshly harvested colonies (as per Shik *et al.*, 2016). Ant measurements of C and N were carried out on four oven-dried workers per colony, and fungal cultivar measurements were carried out on dried, homogenised 1-mg subsamples from actively growing parts of the fungus garden. Focusing on the subset of parasitised colonies, we examined whether *M. symmetochus* workers had higher %C and %N content than either their fungivorous *S. amabilis* host ants or their available fungal food source. We used a paired *t*-test, as this analysis included *M. symmetochus* worker samples paired with host ant or fungal cultivar samples from the same colonies.

We also explored whether host brood represents a nutritionally distinct food source for *M. symmetochus* relative to cultivated fungus, sampling *S. amabilis* larvae (four samples: two to four pooled larvae per sample) and pupae (five samples: three to four pooled pupae per sample). While low sample sizes precluded statistical analyses involving host brood C:N, these data were sufficient to visualise differences. *Sericomyrmex amabilis* brood were sampled from three colonies (TK150510, AC150510, MS150507) harvested from the same Panamanian study populations in May 2015, but analysed in June 2016, after they had been maintained for a year under laboratory conditions in Copenhagen.

Results

Short-term host foraging flexibility

When provisioning fungal gardens, *S. amabilis* ants regulated diet harvest around a nutritional target with more carbohydrates than protein (P:C ∼ 3:4; Fig. 1a). This foraging strategy included significantly more carbohydrates than when workers foraged in groups of nestmates without fungus garden ($F_{2,27} = 10.61$; $P = 0.0004$; Fig. 1a,b; Table S2). In contrast, presence of fungus garden did not influence worker protein foraging, as the significant treatment effect ($F_{2,25} = 30.35$; $P = 0.0001$; Table S2) was driven by parasite treatment effects (Tukey test, $P < 0.01$) and not differences between isolated worker groups and fungus-farming workers (workers with their gardens) (Tukey test, $P > 0.05$) (Fig. 1b). When hosting *M. symmetochus* parasites, *S. amabilis* workers harvested significantly more total diet ($F_{2,25} = 15.80$; $P = 0.0001$: Table S2), carbohydrates ($F_{2,27} = 10.61$; $P = 0.0004$; Table S2), and protein ($F_{2,25} = 30.35$; $P = 0.0001$; Table S2) per day than other symbiont treatments (Fig. 1b), and a more protein-biased nutritional target (P:C ∼ 7:4) (Fig. 1a).

Long-term host demographic and stoichiometric responses

We found significant differences between non-parasitised and parasitised colonies when comparing mass and elemental compositions. The fungus garden had around 50% less garden mass (parasitised, 2270.33 ± 800.57 mg; non-parasitised, 4147.38 ± 850.40 mg; $F_{1,19} = 25.18$; $P = 0.0001$; Fig. 2a) and around 61% less host brood mass per nest chamber (parasitised, 43.29 ± 24.22 mg; non-parasitised, 113.86 ± 52.11 mg; $Z = 44.0$, $P = 0.002$; Fig. 2b). Elemental composition also varied among symbionts depending on whether or not their colony was parasitised. First, carbon was present in significantly lower amounts in the cultivars ($F_{1,18} = 6.99$, $P = 0.017$; Table S2, Fig. 2c) and *S. amabilis* host ants ($F_{1,19} = 8.54$, $P = 0.009$; Table S2, Fig. 2d) from parasitised colonies. Second, nitrogen was lower in parasitised cultivar ($F_{1,18} = 4.54$, $P = 0.047$; Table S2, Fig. 2c), but not in parasitised host workers ($F_{1,18} = 0.01$, $P = 0.917$; Table S2, Fig. 2d).

Despite these differences, parasitised colonies appear stable for years. Even though nests with *M. symmetochus* had lower amounts of less nutritious fungal cultivars (Fig. 2c), fungal food was still abundant. The garden weighed *c.* 18.5% more than the *S. amabilis* and had greater masses of C ($t_{7,0.05} = 7.13$, $P = 0.0002$) and N ($t_{7,0.05} = 6.56$, $P = 0.0003$) than of ant tissue. Fungal crop carbon and nitrogen ratios were also similar in non-parasitised and parasitised nests (non-parasitised, C:N = 18.2 ± 2.14; parasitised, C:N = 19.7 ± 1.86; $P = 0.146$; Table S2). Finally, worker number per chamber was not lower in parasitised colonies ($F_{1,19} = 0.65$; $P = 0.4289$; Table S1), indicating stable host worker populations in parasitised colonies despite reductions in worker body %C (Fig. 2d, Table S2).

Match between parasite nutritional requirements and available resources

Elemental analyses suggested that fungal cultivars, host larvae, and host pupae represent nutritionally distinct foods (fungus C:N = 19.67 ± 1.86; host larvae C:N = 6.80 ± 0.37; host pupae C:N = 5.52 ± 0.25; Fig. 3). These foods also had different elemental ratios than *M. symmetochus* consumers (parasite C:N = 4.95 ± 0.34, $n = 8$) (Fig. 3). First, fungal cultivars had significantly lower concentrations of both N ($t_{6,0.05} = 30.85$, $P = 0.0001$) and C ($t_{6,0.05} = 5.69$, $P = 0.001$) compared with

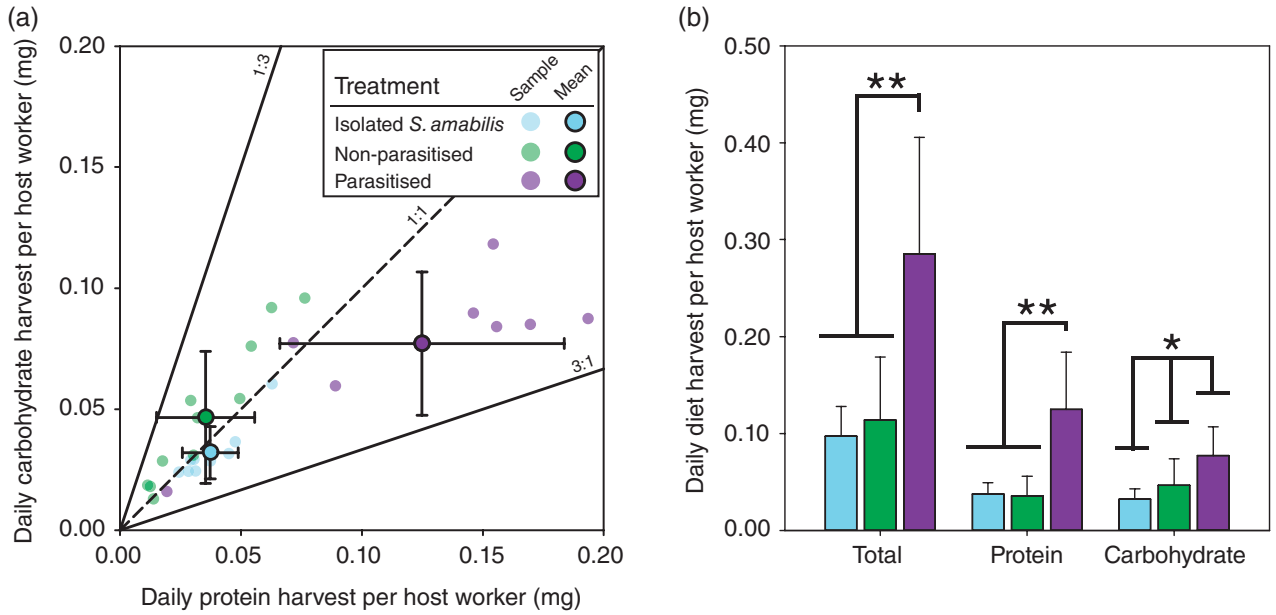

Fig. 1. Linking host foraging behaviour and symbiont status using laboratory feeding experiments with nutritionally defined protein:carbohydrate (P:C) diets. (a) Diet choice experiments indicate distinct P:C nutritional targets selected by *Sericomyrmex amabilis* host ants when provisioning isolated groups of nestmates (P:C ~ 7:6), non-parasitised colonies (P:C ~ 3:4), or parasitised colonies (P:C ~ 7:4). Solid lines indicate 'intake rails' bounding the nutritional options of the ants that were allowed to select between 1:3 and 3:1 P:C diets. The dashed line indicates a 1:1 P:C blend, the boundary between carbohydrate-biased and protein-biased foraging choices. (b) Daily diet harvest per host worker (total, protein, carbohydrate) did not differ between isolated worker groups and workers also provisioning fungus gardens, but all three measures were significantly higher in parasitised colonies. Horizontal lines indicate results of *post hoc* Tukey tests (significance levels: $*P < 0.05$, $**P < 0.01$). Data are means ± SD.

M. symmetochus ants (Fig. 3). Second, both *S. amabilis* larvae (6.95 ± 0.38 %N) and pupae (8.62 ± 0.46 %N) had qualitatively lower N content than did the social parasites (9.89 ± 0.52 %N) (Fig. 3). However, while predatory arthropods tend to have higher %N than arthropods at lower trophic levels (Fagan *et al.*, 2002), *M. symmetochus* parasites (9.89 ± 0.52 %N) and strictly fungivorous *S. amabilis* host workers (10.09 ± 0.54 %N) had statistically similar %N ($t_{7,0.05} = 0.74$, $P = 0.485$; Fig. 3). In contrast, *M. symmetochus* (48.82 ± 1.44 %C) had significantly higher %C than *S. amabilis* workers (45.78 ± 1.43 %C) ($t_{7,0.05} = -5.21$, $P = 0.001$; Fig. 3).

Discussion

We present a multifaceted approach combining behavioural laboratory experiments and demographic field observations to explore how host ants simultaneously manage the nutritional requirements of two symbiotic partners with divergent nutritional demands. We found that host *S. amabilis* workers exhibit dynamic collective foraging decisions in the presence of *M. symmetochus* social parasites. They not only increase the amounts of harvested nutrients, but also select substantially more protein than carbohydrates than do host ants in non-parasitised colonies. We also showed large performance costs of parasitism to *S. amabilis* ants (lower brood mass per chamber and lower host worker elemental content) and to fungal cultivars (lower biomass and lower elemental content of fungi per chamber). However, these costs appear manageable from the hosts' point of view, given that parasitised fungal crops still contained far more C and N than could likely be consumed by host workers, and also because these colonies exhibit stable worker populations indicative of colony survival across growing seasons.

Protein-biased foraging in parasitised colonies may reflect protein requirements in social parasites, given that *M. symmetochus* has an N-biased C:N ratio relative to its available foods, and that it exhibits nearly identical %N (9.9 %N) with a facultative thief ant and predatory relative *Megalomyrmex silvestrii* (10.0 %N, Kaspari *et al.*, 2012). However, fungivorous *S. amabilis* host ants are also composed of 10.1 %N, suggesting complex relationships between an organism's body C:N and its dietary carbohydrate:protein requirements (Wilder & Eubanks, 2010). Moreover, as predatory arthropods can be limited by a wide range of nutrients, such as lipids (Wilder *et al.*, 2013) and salt (Kaspari *et al.*, 2008), further study will be required to explain why *S. amabilis* workers harvest more protein under parasitised conditions.

We propose two potentially overlapping proximate hypotheses to explain protein-biased foraging by host ants. First, hosts may use excess protein to rear more workers and counter consumption of host brood by the parasites. The shunting of resources to colony growth has been linked to reductions in the fat content of adult workers (Tschinkel, 1993) and may help explain the reduced %C in *S. amabilis* workers from parasitised colonies. Second, the nutritional content of fungus gardens probably depends on the nutritional content of foraged substrate

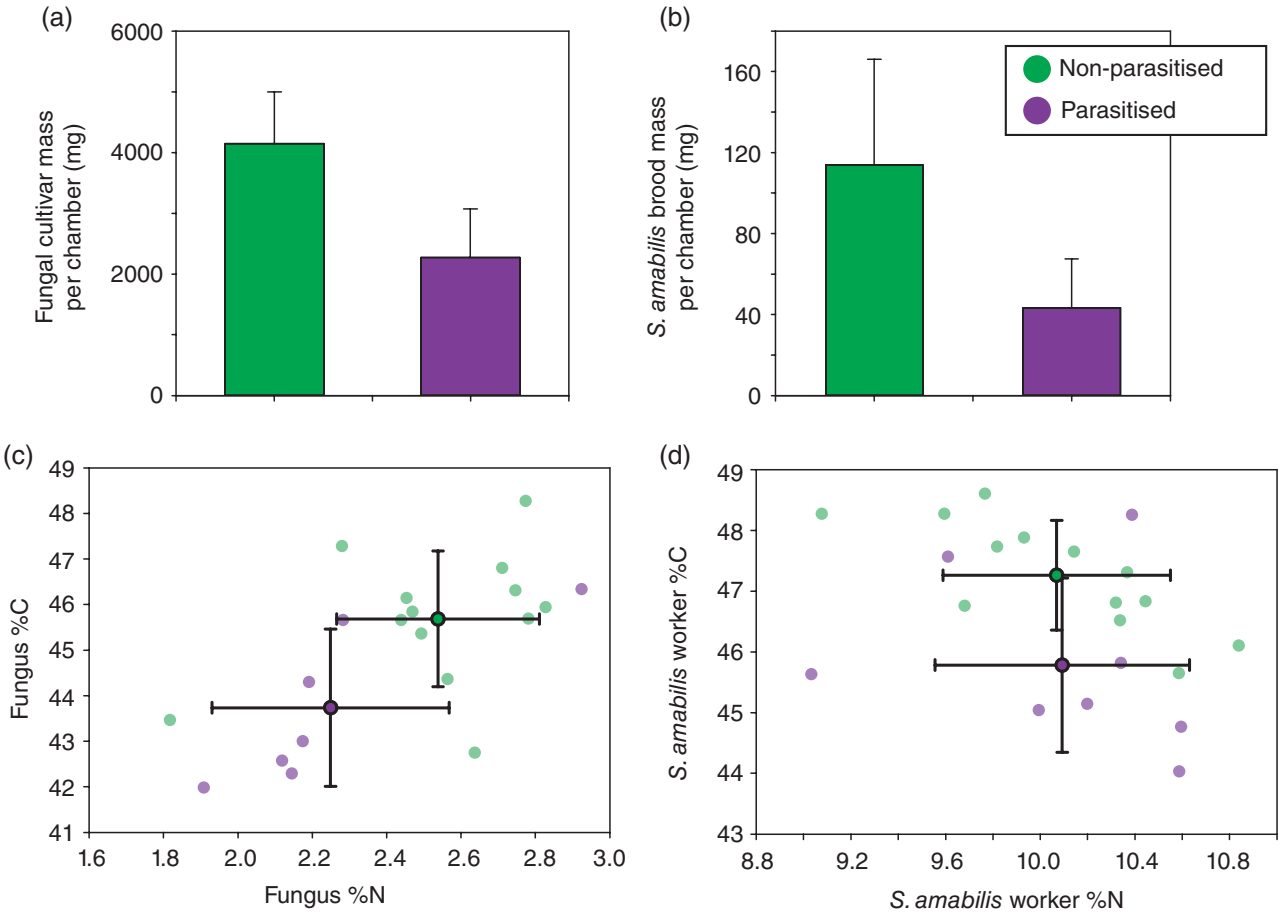

Fig. 2. Long-term performance costs of hosting *Megalomyrmex symmetochus* social parasites inferred using demographic and stoichiometric data from field-collected *Sericomyrmex amabilis* colonies. Parasitised colonies had significantly lower fungus cultivar mass (a) and brood mass per chamber (b). (c) Parasitised colonies contained fungal cultivars with significantly lower carbon and nitrogen, suggesting they were less nutritious. (d) Host workers had significantly lower %C (but not %N) in parasitised colonies. Data are means ± SD. Brood and garden masses were measured wet. Elemental %C and %N were measured from dry mass.

(Shik *et al.*, 2016). Host ants may thus forage to increase fungus protein content to encourage parasitic consumption of fungal crops rather than host brood. This would shift the direct fitness costs imposed by host brood consumption to indirect costs of fungus consumption.

Our data supporting long-term symbiotic stability corroborate previously published data indicating high rates of parasitised host colonies (*c*. 80%; Adams *et al.*, 2013; Wheeler, 1925), with most containing large numbers of host workers (*c*. 800 workers), sexual ants (Bruner *et al.*, 2014), and eight to 12 garden chambers produced over several years. While the geometric framework foraging experiments in this study represent behavioural snapshots, they provide a methodological template for nutritionally parsing the resource exchange governing long-term symbiotic stability. Especially promising applications include exploring host–parasite interactions in the context of dynamic nutritional foraging behaviours that enable colonies to respond to specific nutrient shortfalls. This is expected to occur within (Bazazi *et al.*, 2016) and across seasons (Leal & Oliveira, 2000; Mooney & Tillberg, 2005), and also in the context of a colony's demographic composition (Yang, 2006) and reproductive status (Tschinkel, 1993).

Parasites generate substantial nutritional costs for their hosts (Kilner & Langmore, 2011). Symbiotic species networks make it difficult to pinpoint parasite nutritional requirements and how they influence the performance of the symbiosis as a whole (Chaffron & von Mering, 2007). In this context, fungus-farming ant colonies allow the quantification of resource exchange among associates because the symbioses can be 'disassembled' into fungal cultivars and ants of different life stages. The nutritional requirements of each component can then be studied in isolation. Building on this foundation, future research can explore whether the symbiotic stability stems from host ants adjusting to parasite-induced costs. Moreover, as a diversity of social parasites exploit attine-farming systems (Adams *et al.*, 2000; Adams *et al.*, 2015; Rabeling *et al.*, 2015), comparative insights will be gained by studying nutritional dynamics in more or less virulent host–parasite systems. For example, the highly virulent *Megalomyrmex wettereri* usurps fungus-farming *Cyphomyrmex longiscapus* colonies, stealing host brood and

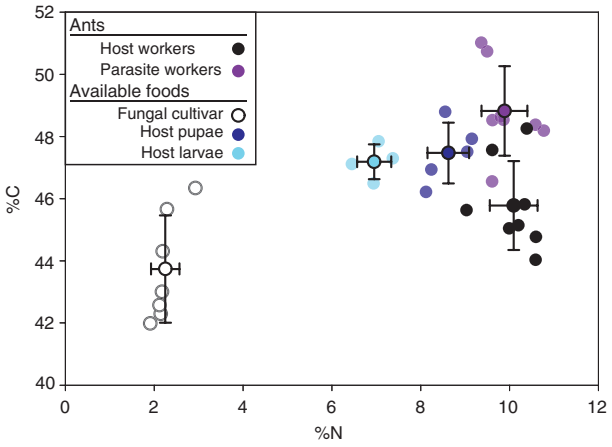

Fig. 3. Elemental composition (%C and %N) of potential foods available to non-foraging *Megalomyrmex symmetochus* social parasites inside host colonies. Fungal and adult ant tissues were collected from freshly harvested parasitised colonies, and host larvae and pupae were sampled from non-parasitised *Sericomyrmex amabilis* laboratory colonies (see Methods for details). Mean values for each tissue type are plotted with black bidirectional error bars (± SD).

fungus garden by chasing away the host workers (Adams *et al.*, 2000). In contrast, the thief ant *Megalomyrmex mondaboroides* lives beside the host, repeatedly raiding host brood and grazing on the garden of a *Cyphomyrmex costatus* (Adams *et al.*, 2015). In both systems, the parasites abruptly take resources, but in the former, the *C. longiscapus* host abandons most of its garden resource while fleeing with small bits of garden and brood. Comparing social parasite systems using our approach can link mutually beneficial partners (ants and their fungus) and mutualist-exploiting parasites and help to explain how symbioses persist when nutritional interests are split in several directions.

Acknowledgements

We thank the staff and researchers at the Smithsonian Tropical Research Institute (STRI) for logistical support, and the Autoridad Nacional del Ambiente y el Mar for permission to sample ants in Panama and export them to Denmark. Jacobus Boomsma provided insightful comments on an earlier draft. For assistance in the field, we thank participants of the 2015 Tropical Behavioural Ecology and Evolution course, organised and financially supported by the University of Copenhagen and STRI. Elemental analyses were performed in the laboratory of Ben Turner at STRI. Finally, we thank three anonymous reviewers and the Associate Editor for their detailed comments; with their scrutiny, our paper was greatly improved. JZS was supported by a Postdoctoral Fellowship from the Smithsonian Institution Competitive Grants Program, and by a Marie Curie International Incoming Fellowship (327940 INSEAME). RMMA was supported by the Ohio State University and the Center of Social Evolution, Copenhagen Demark.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12512

Appendix S1. Detailed description of methods for diet preparation, analyses of %C and %N, and isolated worker group feeding experiment.

Table S1. Demographic composition of field-collected parasitised ($n = 8$) and non-parasitised ($n = 13$) *Sericomyrmex amabilis* subcolonies. Colonies were harvested near La Seda creek along Pipeline Road in Soberenia National Park in Panama. Each colony includes discrete nest chambers connected by tunnels. As colonies distribute resources across chambers, nest chambers represented modular replicates of a colony's allocation to growth (brood) and maintenance (adult workers). Given the limited availability of parasitised colonies, we performed the feeding experiment at the subcolony level, while including colony ID as a random factor in analyses. Colony ID consists of the collector's initials, year, month, day, and subcolony number. Days indicates the duration of feeding experiments. Garden mass was calculated by subtracting ant mass (adults and brood of hosts and parasites) from the mass of the entire subcolony just after field collection. Sericomyrmex amabilis worker number was calculated from dead workers collected during the experiment [dead worker numbers (mean ± SD) from non-parasitized colonies: <1 ± 0.7, and from parasitized colonies: 0 ± 0] and surviving workers following the feeding experiment. Mass was measured from fresh material (mg).

Table S2. Results of mixed-model analyses on: (i) the laboratory diet feeding experiment (top); and (ii) elemental composition of field-collected colonies (bottom). The diet experiment analysis tested the effects of symbiont treatment (isolated host worker groups, non-parasitised colonies, parasitised colonies) on daily harvest per host worker (total, protein, carbohydrate) with initial *S. amabilis* worker number included as a covariate. We removed non-significant interactions between symbiont treatment and initial worker number from the carbohydrate harvest analysis. The elemental composition analysis tested the effects of parasite status (parasitised versus non-parasitised colonies) on the %C and %N of the fungal cultivar and host *S. amabilis* workers. In both analyses, colony ID was included as a random factor.

References

Adams, R.M.M., Mueller, U.G., Schultz, T.R. & Norden, B. (2000) Agro-predation: usurpation of attine fungus gardens by *Megalomyrmex* ants. *Naturwissenschaften*, **87**, 549–554.

Adams, R.M.M., Liberti, J., Illum, A., Jones, T.H., Nash, D.R. & Boomsma, J.J. (2013) Chemically armed mercenary ants protect fungus-farming societies. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 15752–15757.

Adams, R.M.M., Jones, T.H., Longino, J.T., Weatherford, R.G. & Mueller, U.G. (2015) Alkaloid venom weaponry of three *Megalomyrmex* thief ants and the behavioral response of *Cyphomyrmex costatus* host ants. *Journal of Chemical Ecology*, **41**, 373–385.

Bazazi, S., Arganda, S., Moreau, M., Jeanson, R. & Dussutour, A. (2016) Responses to nutritional challenges in ant colonies. *Animal Behavior*, **111**, 235–249.

Boudinot, B.E., Sumnicht, T.P. & Adams, R.M.M. (2013) Central American ants of the genus *Megalomyrmex* Forel (Hymenoptera: Formicidae): six new species and keys to workers and males. *Zootaxa*, **3732**, 1–82.

Bruner, G., Wcislo, W.T. & Fernández-Marín, H. (2014) Prudent iniquilines and proactive hosts: behavioural dynamics between an ant social parasite, *Megalomyrmex symmetochus* and its fungus-growing ant host, *Sericomyrmex amabilis*. *Insectes Sociaux*, **61**, 83–88.

Buschinger, A. (2009) Social parasitism among ants: a review (Hymenoptera: Formicidae). *Myrmecological News*, **12**, 219–235.

Chaffron, S. & von Mering, C. (2007) Termites in the woodwork. *Genome Biology*, **8**, 229.

Chow, J., Lee, M.S., Shen, Y., Khosravi, A. & Mazmanian, S.K. (2010) Host-bacterial symbiosis in health and disease. *Advances in Immunolology*, **107**, 243–274.

Davidson, D.W. (2005) Ecological stoichiometry of ants in a New World rain forest. *Oecologia*, **142**, 221–231.

Dussutour, A. & Simpson, S.J. (2008) Description of a simple synthetic diet for studying nutritional responses in ants. *Insectes Sociaux*, **55**, 329–333.

Fagan, W.F., Siemann, E., Mitter, C., Denno, R.F., Huberty, A.F., Woods, H.A. *et al.* (2002) Nitrogen in insects: implications for trophic complexity and species diversification. *The American Naturalist*, **160**, 784–802.

de Fine Licht, H.H. & Boomsma, J.J. (2010) Forage collection, substrate preparation, and diet composition in fungus-growing ants. *Ecological Entomology*, **35**, 259–269.

Huang, M.H. & Dornhaus, A. (2008) A meta-analysis of ant social parasitism: host characteristics of different parasitism types and a test of Emery's rule. *Ecological Entomology*, **33**, 589–596.

Ješovnik, A., Sosa-Calvo, J., Lloyd, M.W., Branstetter, M.G., Fern Ández, F. & Schultz, T.R. (2017) Phylogenomic species delimitation and host-symbiont coevolution in the fungus-farming ant genus *Sericomyrmex* Mayr (Hymenoptera: Formicidae): ultraconserved elements (UCEs) resolve a recent radiation. *Systematic Entomology*, **42**, 523–542.

Kaspari, M., Yanoviak, S.P. & Dudley, R. (2008) On the biogeography of salt limitation: a study of ant communities. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 17848–17851.

Kaspari, M., Donoso, D., Lucas, J.A., Zumbusch, T. & Kay, A.D. (2012) Using nutritional ecology to predict community structure: a field test in Neotropical ants. *Ecosphere*, **3**, 93.

Kay, A.D., Ashton, I.W., Gorokhova, E., Kerkhoff, A.J., Liess, A. & Litchman, E. (2005) Toward a stoichiometric framework for evolutionary biology. *Oikos*, **109**, 6–17.

Kilner, R.M. & Langmore, N.E. (2011) Cuckoos versus hosts in insects and birds: adaptations, counter-adaptations and outcomes. *Biological Reviews*, **86**, 836–852.

Leal, I.R. & Oliveira, P.S. (2000) Foraging ecology of attine ants in a Neotropical savanna: seasonal use of fungal substrate in the cerrado vegetation of Brazil. *Insectes Sociaux*, **47**, 376–382.

Mooney, K.A. & Tillberg, C.V. (2005) Temporal and spatial variation to ant omnivory in pine forests. *Ecology*, **86**, 1225–1235.

Rabeling, C., Schultz, T.R., Pierce, N.E. & Bacci, M. Jr. (2014) A social parasite evolved reproductive isolation from its fungus-growing ant host in sympatry. *Current Biology*, **24**, 2047–2052.

Rabeling, C., Schultz, T.R., Bacci, M. Jr. & Bollazzi, M. (2015) *Acromyrmex charruanus*: a new iniquiline social parasite species of leaf-cutting ants. *Insectes Sociaux*, **62**, 335–349.

Reich, P.B. (2014) The world-wide "fast-slow" plant economics spectrum: a traits manifesto. *Journal of Ecology*, **102**, 275–301.

Schär, S. & Nash, D.R. (2014) Evidence that microgynes of *Myrmica rubra* ants are social parasites that attack old host colonies. *Journal of Evolutionary Biology*, **27**, 2396–2407.

Shik, J.Z., Santos, J.C., Seal, J.N., Kay, A., Mueller, U.G. & Kaspari, M. (2014) Metabolism and the rise of fungus cultivation by ants. *American Naturalist*, **184**, 364–373.

Shik, J.Z., Gomez, E.B., Kooij, P.W., Santos, J.C., Wcislo, W.T. & Boomsma, J.J. (2016) Nutrition mediates the expression of cultivar-farmer conflict in a fungus-growing ant. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 10121–10126.

Simpson, S.J. & Raubenheimer, D. (2012) *The Nature of Nutrition: A Unifying Framework from Animal Adaptation to Human Obesity*. Princeton University Press, Princeton, New Jersey.

Smith, V.H. (2007) Host resource supplies influence the dynamics and outcome of infectious disease. *Integrative and Comparative Biology*, **47**, 310–316.

Solomon, P.S., Tan, K.C. & Oliver, R.P. (2003) The nutrient supply of pathogenic fungi: a fertile field for study. *Molecular Plant Pathology*, **4**, 203–210.

Sterner, R.W. & Elser, J.J. (2002) *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton University Press, Princeton, New Jersey.

Tschinkel, W.R. (1993) Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle. *Ecological Monographs*, **63**, 425–457.

Ward, P.S., Brady, S.G., Fisher, B.L. & Schultz, T.R. (2014) The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). *Systematic Entomology*, **40**, 61–81.

Wheeler, W.M. (1925) A new guest-ant and other new Formicidae from Barro Colorado Island, Panama. *Biological Bulletin*, **49**, 150–181.

Wilder, S.M. & Eubanks, M.D. (2010) Might nitrogen limitation promote omnivory among carnivorous arthropods? Comment. *Ecology*, **91**, 3114–3117.

Wilder, S.M., Norris, M., Lee, R.W., Raubenheimer, D. & Simpson, S.J. (2013) Arthropod food webs become increasingly lipid-limited at higher trophic levels. *Ecology Letters*, **16**, 895–902.

Yang, A.S. (2006) Seasonality, division of labor, and dynamics of colony-level nutrient storage in the ant *Pheidole morrisi*. *Insectes Sociaux*, **53**, 456–462.

Yoshida, T. (2006) Ecological stoichiometry and the shape of resource-based tradeoffs. *Oikos*, **112**, 406–411.

Accepted 2 February 2018

Associate Editor: Sophie Evison

