Ecological Entomology

Ecological Entomology (2020), DOI: 10.1111/een.13002

Dinner with the roommates: trophic niche differentiation and competition in a mutualistic ant-ant association

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Abstract. 1. The potential for competition is highest among species in close association. Despite net benefits for both parties, mutualisms can involve costs, including food competition. This might be true for the two neotropical ants *Camponotus femoratus* and *Crematogaster levior*, which share the same nest in a presumably mutualistic association (parabiosis).

2. While each nest involves one *Crematogaster* and one *Camponotus* partner, both taxa were recently found to comprise two cryptic species that show no partner preferences and seem ecologically similar. Since these cryptic species often occur in close sympatry, they might need to partition their niches to avoid competitive exclusion.

3. Here, we investigated first, is there interference competition between parabiotic *Camponotus* and *Crematogaster*, and do they prefer different food sources under competition? And second, is there trophic niche partitioning between the cryptic species of either genus?

4. Using cafeteria experiments, neutral lipid fatty acid and stable isotope analyses, we found evidence for interference competition, but also trophic niche partitioning between *Camponotus* and *Crematogaster*. Both preferred protein- and carbohydrate-rich baits, but at protein-rich baits *Ca. femoratus* displaced *Cr. levior* over time, suggesting a potential discovery-dominance trade-off between parabiotic partners. Only limited evidence was found for trophic differentiation between the cryptic species of each genus.

5. Although we cannot exclude differentiation in other niche dimensions, we argue that neutral dynamics might mediate the coexistence of cryptic species. This model system is highly suitable for further studies of the maintenance of species diversity and the role of mutualisms in promoting species coexistence.

Key words. Cryptic species, Formicidae, neutral theory, niche partitioning, nutrition, parabiosis, species coexistence mechanism, trade-offs.

Introduction

All organisms preferably occur in environments most suited for their physiological needs that is their fundamental niches (Hutchinson, 1957). However, as most resources are limited, organisms with similar ecological requirements have to compete for them resulting in realised niches different from their fundamental niches. If one species is a stronger competitor, this should lead to competitive exclusion of the other species (Gause, 1932; Hardin, 1960). One mechanism to avoid competitive exclusion is niche partitioning. It can occur in various different dimensions, like spatial, temporal, or dietary differentiation (Tanaka *et al.*, 2010; Stuble *et al.*, 2013; Houadria

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et al., 2015; Grevé *et al.*, 2019). Especially dietary (trophic) niche partitioning is considered a key mechanism of species coexistence (Rosumek *et al.*, 2018; Grevé *et al.*, 2019). Ants are among the most abundant and species-rich terrestrial arthropods especially in tropical rainforests, and high competition between locally co-occurring species can shape ant communities worldwide, often via direct behavioural interactions (Savolainen & Vepsäläinen, 1988; Hölldobler & Wilson, 1990). Therefore, the pressure for niche partitioning among ant species should be high. Nest sites or food sources are often limiting resources for ants (Blüthgen & Feldhaar, 2010). A species' food choice may hence strongly depend on its competitive abilities, since dominant species often monopolise and aggressively defend suitable food sources (Hölldobler, 1983; Dejean *et al.*, 2005). Strong competition for food sources between dominant and submissive species could result in behavioural or physiological trade-offs, for example the discovery-dominance trade-off (Fellers, 1987; Sarty *et al.*, 2006) or the thermal vulnerability-dominance trade-off (Cerdá *et al.*, 1998).

Associations between organisms can result in costs and benefits for each party, or even both at the same time (Bronstein, 2001). An important cost for associated species, especially if they are taxonomically similar, is competition. An exceptional form of association is the parabiosis, which is an intimate association between two ant species that live together in the same nest. The ants tolerate each other, but keep their brood separate (Orivel *et al.*, 1997; Menzel *et al.*, 2008). The neotropical parabiotic ants *Camponotus femoratus* and *Crematogaster levior*, are among the ecologically most dominant arboreal species, and share so-called ant gardens as their nests (Davidson, 1988). While *Camponotus* probably profits from the resource discovery abilities of *Crematogaster* and follows interspecific pheromone trails to food sources (Vantaux *et al.*, 2007; Menzel *et al.*, 2010, 2014), *Crematogaster* benefits from the nest-building abilities of *Camponotus* and its aggressive nest defence (Orivel & Dejean, 1999; Vantaux *et al.*, 2007; Youngsteadt *et al.*, 2008; Menzel & Blüthgen, 2010; Vicente *et al.*, 2014). Despite these benefits, it is likely that the two species compete with each other, the more so as they belong to the same family, which is highly unusual for interspecific associations (Menzel *et al.*, 2012).

Recent studies found that both *Ca. femoratus* and *Cr. levior* actually consist of two cryptic species each (*Ca. femoratus* PAT and PS; *Cr. levior* A and B). They differ genetically and possess different cuticular hydrocarbon profiles, but largely occur in sympatry, and can even co-occur within few metres distance (Hartke *et al.*, 2019). Interestingly, both *Ca. femoratus* PAT and PS live in association with *Cr. levior* A and B. Hence, there is no mutual specialisation or partner preference (Hartke *et al.*, 2019). However, across French Guiana, *Ca. femoratus* PS is less common in the wetter and slightly cooler East compared to the West of the country, while *Ca. femoratus* PAT occurs in all sampled areas (Hartke *et al.*, 2019). Apart from that, no ecological factors (e.g. canopy cover or presence of certain ant garden plants in their nests) were identified so far that differed between the cryptic sister species of either *Ca. femoratus* or *Cr. levior* (Hartke *et al.*, 2019). Therefore, here we investigated if there is trophic niche partitioning between them as a potential explanation for their co-occurrence.

Trophic niches are most easily assessed by observating feeding behaviour in a natural environment. However, behavioural observations of feeding choices actually represent 'temporal snap-shots' of the current food preferences or choices of best option in the presence of a competitor. In addition, the attractiveness of baits can be influenced by current nutritional needs and limitations (Kay, 2004): if an ant colony needs protein, and protein availability is limited, ants will prefer this bait (Kaspari *et al.*, 2020). This is why indirect measurements, such as analysis of fatty acids or stable isotope signatures, can be a useful complement to obtain insights into more long-term feeding differences of terrestrial arthropods (Ruess & Chamberlain, 2010; Rosumek *et al.*, 2017, 2018). Neutral lipid fatty acids (NLFAs) are stored in the fat bodies of arthropods and are a major source of energy (Stanley-Samuelson *et al.*, 1988). Among these, unsaturated fatty acids are preferable to saturated ones because they are easier to mobilise and metabolise, thus providing a better way of energy storage (Price, 2010; Guglielmo, 2018). Animals frequently incorporate dietary fatty acids into their body tissue without modifications, which is why they can be used to infer trophic transfer between consumer and diet (i.e. dietary routing) (Ruess & Chamberlain, 2010). Specific fatty acids can be used as biomarkers when they are specific to certain food sources. But also more widespread fatty acids can accumulate in a consumer, thus indicating its dietary origin (Ruess & Chamberlain, 2010; Rosumek *et al.*, 2017). Hence, fatty acid profiles can be highly useful to study trophic niche differentiation. A third powerful tool commonly used to infer the trophic position of an organism in a food web or to detect trophic niche partitioning is stable isotope analysis. ^{15}N isotopes accumulate in the food chain due to differential digestion or fractionation during metabolic processes by the consumer (Post, 2002; Heethoff & Scheu, 2016). For example predators usually have higher $\delta^{15}N$ than primary consumers, and ^{13}C isotopes can additionally inform about the carbon sources used by a consumer. In particular, C_3 and C_4 plants bear different ^{13}C signatures, which is also detectable in organisms eating the plant material or consuming nectar (McCutchan Jr *et al.*, 2003; Swap *et al.*, 2004; Blüthgen & Feldhaar, 2010).

In this study, we followed the integrative framework of Rosumek *et al.* (2018) to investigate differences in the realised trophic niche among parabiotic ants. Since *Ca. femoratus* and *Cr. levior* are almost exclusively found in parabiosis (Davidson, 1988), it is impossible to obtain isotope or fatty acid data from field nests without the parabiotic partner. Hence, we can only obtain data on the realised trophic niche, but not the fundamental niche, and try to infer the potential for competition from these data.

We combined cafeteria experiments, NLFA and stable isotope analyses to shed light on niche differences (1) between *Camponotus* and *Crematogaster* and (2) among the cryptic species of each genus. Our questions were first, is there interference competition between the mutualists and if so, which food sources do they compete for? And second, is there trophic niche partitioning between the cryptic species of *Ca. femoratus* and *Cr. levior*?

Table 1. Frequency of cryptic species and species combinations at the three sampling sites.

(A) Frequency of cryptic species

Site	*Ca. femoratus* PAT	*Ca. femoratus* PS	*Cr. levior* A	*Cr. levior* B	unknown *Ca. femoratus*	unknown *Cr. levior*
Paracou	5	9	14	1	3	2
Camp Patawa	14	0	7	7	0	0
Sinnamary	6	5	3	9	2	1

(B) Frequency of species combinations sharing a nest

Site	PAT/A	PAT/B	PS/A	PS/B	unknown *Ca. femoratus*	unknown *Cr. levior*
Paracou	4	1	9	0	2	1;0
Camp Patawa	7	7	0	0	0	0;0
Sinnamary	0	6	3	2	1	0;1

The numbers represent the 44 colonies tested in the cafeteria experiment. There were no colonies with known *Ca. femoratus* species and unknown *Cr. levior* species.

Materials and methods

Study sites and species identification

Diet experiments and sample collection took place in three different sites in French Guiana in October 2018. These were the Paracou Research Station (n = 17 parabiotic nests; 5°14.04 N, 52°54.28 W), next to the Route de Saint-Èlie near Sinnamary (n = 13 nests; 5°17.49 N, 53°14.46 W), and close to the village of Kaw next to the D6 road near Camp Patawa (n = 14 nests; 4°32.56 N, 52°09.45 W). *Crematogaster levior* A, B, and *Ca. femoratus* PAT occur at all three sites, whereas *Ca. femoratus* PS occurs only at Paracou and Sinnamary but not near Camp Patawa (Hartke *et al.*, 2019) (see Table 1b for chemotype combinations per site).

The cryptic species identity of the tested colonies was identified using cuticular hydrocarbon (CHC) extracts of five *Cr. levior* or one *Ca. femoratus* worker taken from nests prior to the experiment (Table 1) and analysed using gas-chromatography mass-spectrometry (GC–MS, see Sprenger *et al.*, 2019 for details on the method). Unfortunately, due to sample loss the identity of five *Camponotus* and three *Crematogaster* colonies is unknown (Table 1); these samples were retained in the dataset but the identity was set to NA. For the same reason, there were only 86 instead of 88 fatty acid samples.

Cafeteria experiments

We conducted cafeteria experiments by offering five different food sources on a PVC platform (16.5 cm × 14 cm with a V-shaped notch for the trunk) attached to the vegetation 1–3 m away from the parabiotic nest (N = 44). The food sources offered were 1) a protein source resembling vertebrate carcasses (sausage, i.e. chicken luncheon meat, Zwan, Almelo, The Netherlands), 2) a sugar source resembling natural sugar sources like extra floral nectaries (20% v/v sugar solution), 3) a fat source resembling plant elaiosomes (10% v/v oleic acid solution), 4) a nitrogen source resembling bird faeces (10% v/v uric acid solution), and 5) crushed plant seeds as a starch source (Sittich Perle®, Vitakraft, Bremen, Germany). The different food sources were placed as a circle on the platform in a randomised order. Pictures were taken after 15, 60, and 120 min to document the number of ants at each time point. The number of foragers at each food source was counted in a 1 cm diameter around each food source.

Statistical analysis of cafeteria experiments

All statistical tests were conducted in R v. 3.6.0. First, we analysed whether any food sources were visited more intensely by either *Camponotus* or *Crematogaster*. We did not exclude any species artificially, thus, most data points originate from bait platforms visited by both species at the same time. First, we performed a 'hotlink' analysis (Junker *et al.*, 2010; Grevé *et al.*, 2019), which compares the relative food preferences of the two genera while competing (i.e. the realised trophic niche). To exclude random encounters at a food source, we only included observations that had at least five ants at the bait and those represented at least 10% of total workers observed; here, occurrences at a bait were either scored as present or absent. Second, we tested for overall differences in food choice between genera and cryptic species with a permutational ANOVA (PERMANOVA; software PRIMER 6 v. 6.1.14 & PERMANOVA+ v. 1.0.4, Primer-E Ltd.), and whether the level of inter-colony variation of food choices differed between genera or cryptic species using PERMDISP based on Bray-Curtis dissimilarities (commands *adonis* and *betadisper* + *permutest*, R package *vegan*, Oksanen *et al.*, 2019). Here, forager numbers at each bait were used. In the PERMANOVA, we furthermore included the test site as well as the time point as fixed factors. Third, we separately analysed the numbers of either *Camponotus* or *Crematogaster* ants at the different baits, as well as the summed numbers of workers of either species. Each of these variables was used as dependent variable in a linear mixed effects model (LMM), with the fixed factors 'time point' (1–3), 'cryptic species identity', 'cryptic species identity of the partner', and 'number of workers of the partner species at the respective bait', and 'colony ID' as random factor (R package *lme4*, command *lmer*, Bates *et al.*, 2015). To avoid over-parametrisation, we allowed two-way and three-way interactions, but not higher-level interactions. For the same

reason, we determined beforehand (using Akaike's Information Criterion, AIC) whether 'site' (Patawa, Paracou, or Sinnamary) was to be included as fixed or random effect, or not at all; the respective model with the lowest AIC was then chosen for further analysis. All numbers were log+1-transformed in each model. We chose to analyse absolute numbers rather than *Camponotus*-*Crematogaster* ratios since we deemed them more informative and since at some baits, only one species was present. As the number of foragers at some baits was very low, we transformed the data to binomial variables (present or absent) for seeds and uric acid in *Camponotus* and for oleic acid in *Crematogaster*, and calculated generalised LMMs (command *glmer* with binomial error distribution) with similar fixed and random factors. In each model, non-significant interactions were removed in a stepwise fashion, until only significant interactions remained.

NLFA analysis

Before starting each cafeteria experiment, we collected two *Crematogaster* and two *Camponotus* workers (one backup sample each) that were freeze-killed and kept at −20 °C until fatty acid extraction. The extraction protocol followed the steps described in Rosumek *et al.* (2017). In brief, fatty acids were extracted from whole body individual workers by immersing them in a 2:1 chloroform-methanol (v/v) mixture for 24 h. NLFA were separated from phospholipid fatty acids (PLFA) using chloroform- and hexane-conditioned SiOH-columns (Chromabond, 1 ml/100 mg, Macherey-Nagel, Düren, Germany). The NLFAs were eluted with chloroform, while PLFAs remained in the column. We let the solvent evaporate under a gentle nitrogen stream and re-dissolved the NLFAs in a dichloromethane-methanol (2:1 v/v) solution. For quantification, we added 10 µl of nonadecanoic acid (C19:0, solved in dichloromethane-methanol 2:1 v/v, 0.2 mg ml^{-1}) as an internal standard. For analysis, the fatty acids were derivatised to fatty acid methyl esters (FAME) with 20 µl trimethylsulfonium hydroxide (TMSH; Sigma-Aldrich, Munich, Germany).

In total, we analysed 86 FAME samples with GC–MS. 2 µl of the samples were injected into the GC (7890A, Agilent Technologies, Santa Clara, California) that was equipped with a Zebron Inferno ZB5-HT capillary column (length 30 m, Ø 0.25 mm, 0.25 µm coating, Phenomenex, Aschaffenburg, Germany) in the splitless mode. As carrier gas, we used helium at a flow rate of 1.2 ml per minute. Initially, the oven had a temperature of 60 °C and heated up with 15 °C per minute until it reached 150 °C. In the following, the temperature increased with 3 °C min^{-1} up to 200 °C and then with 10 °C min^{-1} up to 320 °C. This temperature was held constant for additional 10 min. The separated FAMEs were transferred to the MS (575C, Agilent Technologies) and fragmented by an electron beam at 70 eV. We identified them via their fragmentation patterns (i.e. molecular and diagnostic ions). Resulting chromatogram peaks were integrated manually using the software MSD ChemStation (E.02.02.1431, Agilent Technologies).

We compared the fatty acid profiles between genera, cryptic species (21 *Cr. levior* A, 18 *Cr. levior* B, 24 *Ca. femoratus* PAT, 15 *Ca. femoratus* PS and 8 of unknown cryptic species identity), and sites using a PERMANOVA based on Bray-Curtis dissimilarities and tested if they differed in variance using PERMDISP (commands *adonis* and *betadisper* + *permutest*, R package *vegan*, Oksanen *et al.*, 2019). In addition, we used linear models to test for differences in fatty acid traits, that is, absolute quantity of fatty acids as well as proportions of saturated, mono-unsaturated, and poly-unsaturated fatty acids. If necessary, the values were transformed to meet the model assumptions. Finally, we used a Mantel test based on Pearson correlation to test if the fatty acid profiles of *Crematogaster* and *Camponotus* workers from the same nests were correlated.

Stable isotope analyses

In total, we analysed 72 samples from 38 parabiotic nests (22 *Cr. levior* A, 12 *Cr. levior* B, 21 *Ca. femoratus* PAT, and 12 *Ca. femoratus* PS and 5 of unknown cryptic species identity). All samples were collected before the cafeteria experiments. Each sample consisted of three ant workers without gaster, which was dried in a dry oven. We measured the isotope composition of nitrogen (N) and carbon (C) using standard gases (N_2 and CO_2) in a coupled elemental analyser – isotope ratio mass spectrometer (EA-IRMS). For the analysis, we used a Carlo Erba 1108 elemental analyser (Carlo Erba, Milano, Italy) coupled to a delta S isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) via a ConFlo III open-split interface (Thermo Fisher Scientific, Bremen, Germany) in a dual element analysis mode. The standard gases were calibrated against international standards (N_2 in air and V-PDB) using reference substances (N1 and N2 for the nitrogen isotopes; CH6, CO8, and NBS18 for carbon isotopes; standards from the International Atomic Energy Agency, Vienna, Austria).

We compared the $\delta^{15}N$ and $\delta^{13}C$ signatures (= $(R_{sample} / R_{standard} - 1) \times 1000$ [‰]; with R being the ratio of heavy to light isotopes) between genera, cryptic species and sites using linear models (LM). For the models, we each used the cryptic species nested in genus and the sampling site as fixed effects. Further on, we separately tested if the $\delta^{15}N$ and $\delta^{13}C$ signatures of *Camponotus* and *Crematogaster* of the same nest were linked to each other using LM with site as additional fixed factor.

Results

Cafeteria experiments

On average, more *Crematogaster levior* (mean ± SEM: 25.20 ± 3.34) than *Camponotus femoratus* workers (16.95 ± 1.46) visited the bait platforms (sum of individuals on all baits). Both numbers increased over time (LMM: *Camponotus*: $\chi^2_2 = 27.44$, $P < 0.001$; *Crematogaster*: $\chi^2_2 = 18.55$, $P < 0.001$; increase from 15 to 60 min, but not from 60 to 120 min in both genera). By reciprocally testing the effect of the worker numbers, we found that the number of *Camponotus* workers was negatively affected by the number of *Crematogaster* workers ($\chi^2_1 = 7.29$, $P = 0.0069$) but not the other way around ($\chi^2_1 = 0.05$, $P = 0.82$). This is most likely due to the

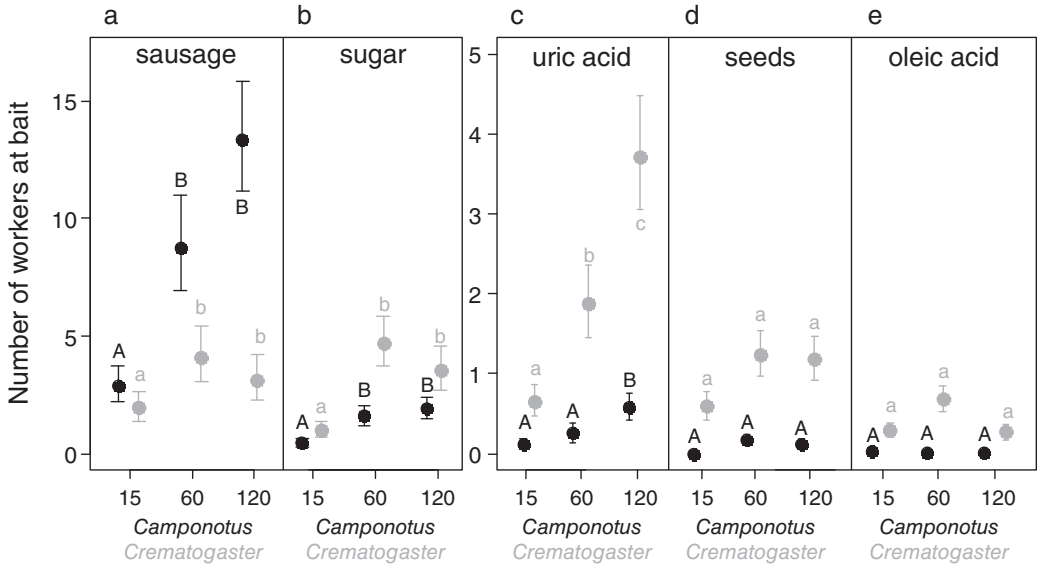

Fig. 1. Food choice and competition between *Camponotus femoratus* and *Crematogaster levior*. The plots show back-transformed means ± SEM. of the worker numbers of *Ca. femoratus* (black) and *Cr. levior* (grey) at each three time points (after 15, 60, and 120 min) for five different baits: sausage (a), sugar (b), uric acid (c), seeds (d), and oleic acid (e). Letters indicate statistical differences between time points in *Camponotus* (capital letters) and *Crematogaster* (lower letters).

Table 2. Results of the hotlink network analysis.

Genus	Sausage	Sugar	Uric acid	Seeds	Oleic acid
After 15 min					
Camponotus	**0.046**	0.86	1	1	1
Crematogaster	0.99	0.46	0.46	0.11	1
After 60 min					
Camponotus	**<0.001**	0.92	1	1	1
Crematogaster	1	0.17	**0.022**	0.14	0.53
After 120 min					
Camponotus	**<0.001**	0.85	1	1	1
Crematogaster	1	0.28	**<0.001**	0.08	1

Significant *P*-values (printed in bold) indicate that colonies used one of the baits more frequently than expected by their total number of *Crematogaster* and *Camponotus* workers at the five baits. The baits are ordered according to attractiveness (i.e. mean number of attracted foragers).

stronger effect of the test site for *Crematogaster* ($\chi^2_2 = 9.59$, $P = 0.008$; fewer *Crematogaster* workers at Camp Patawa compared to Sinnamary: post-hoc Tukey test: $t_4 = -3.09$, $P = 0.011$; Fig. S1). Worker numbers did not differ between cryptic species of either *Camponotus* or *Crematogaster* (both $P > 0.17$).

Sausage and sugar were the most attractive baits for both *Camponotus* and *Crematogaster* (Fig. 1a,b). Here, the hotlink analysis showed that the two genera were equally often foraging at sugar, after correcting for their different overall occurrences (all $P \geq 0.17$; Table 2). However, *Camponotus* workers were clearly more frequent at sausage than *Crematogaster*. This was significant for all three time points (Hotlink analysis: $P = 0.046$, $P < 0.001$ and $P < 0.001$, respectively, Table 2). *Crematogaster*, in turn, foraged more intensely than *Camponotus* at uric acid after 60 and 120 min ($P = 0.022$ and $P < 0.001$). Taken together, food choice (i.e. the number of workers foraging at the five baits) differed between the two genera (PERMANOVA: pseudo-$F_1 = 21.29$, $P = 0.001$), but not between the cryptic species within each genus (pseudo-$F_2 = 1.03$, $P = 0.40$). Overall food choice also differed between time points (PERMANOVA: pseudo-$F_2 = 7.03$, $P = 0.001$); and genus effects differed between sites (interaction genus: site: pseudo-$F_2 = 2.45$, $P = 0.003$) and between time points (interaction genus: time: pseudo-$F_2 = 2.10$, $P = 0.016$). In addition, food choice was more variable in *Crematogaster* (PERMDISP: $F_1 = 18.07$, $P = 0.001$).

At the sausage baits, the numbers of *Camponotus* negatively affected the number of *Crematogaster* workers and vice versa (LMM: effect of *Crematogaster* on *Camponotus*: $\chi^2_1 = 19.87$, $P < 0.0001$; effect of *Camponotus* on *Crematogaster*: $\chi^2_2 = 20.25$, $P < 0.0001$) and were higher after 60 and 120 min than after 15 min in both genera (effect on *Camponotus*: $\chi^2_2 = 51.74$, $P < 0.0001$; effect on *Crematogaster*: $\chi^2_2 = 21.79$, $P < 0.0001$; Fig. 1a). The effect size differed between *Camponotus* PAT and PS: after 60 min, the effect of the number of *Ca. femoratus* PS workers on the number of *Crematogaster* workers was more negative than that of PAT workers

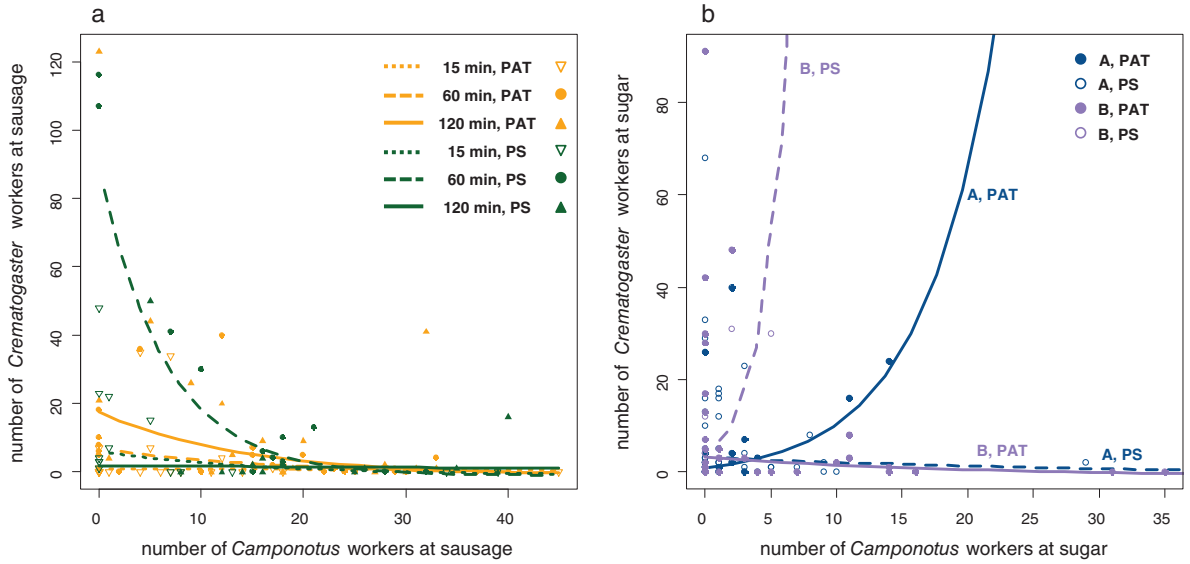

Fig. 2. Cryptic species interactions in competition at two different baits (sausage and sugar). (a) Plot of the interaction between number of *Camponotus* workers (x-axis) versus the number of *Crematogaster* workers (y-axis), the cryptic species identity of *Camponotus*, and the time point at the sausage bait. We plotted log-regression lines for each cryptic species (*Ca. femoratus* PAT: orange; PS: green) at each time point (15 min: dotted line; 60 min: dashed line; 120 min: solid line). The data points for each regression are represented by different symbols. (b) Plot of the interaction between number of *Camponotus* workers (x-axis) versus number of *Crematogaster* workers (y-axis) and cryptic species identities of *Ca. femoratus* and *Cr. levior* at the sugar bait. We plotted log-regression lines for each combination of cryptic species (*Cr. levior* A, *Ca. femoratus* PAT: blue, solid line; A, PS: blue, dashed line; B, PAT: purple, solid line; B; PS: purple, dashed line). The data points for each regression are represented by open or closed circles in the colours described before. [Colour figure can be viewed at wileyonlinelibrary.com].

(three-way interaction between number of *Camponotus* workers, *Camponotus* species, and time: $\chi^2_2 = 10.65$, $P = 0.0049$; Fig. 2a).

Contrary to the sausage baits, at sugar, the numbers of workers increased over time in parallel in both genera (*Camponotus*: $\chi^2_2 = 16.75$, $P = 0.0002$; *Crematogaster*: $\chi^2_2 = 25.96$, $P < 0.0001$; Fig. 1b). Here, the abundance of *Crematogaster* workers differed between sites (see supplement). Interestingly, worker number on sugar baits was affected by the composition of the species pair: *Camponotus* and *Crematogaster* numbers increased in parallel in pairs of either *Ca. femoratus* PAT/*Cr. levior* A (n = 33 nests) or *Ca. femoratus* PS/*Cr. levior* B (n = 6 nests), but not in the other two combinations (PS/A: n = 36; PAT/B: n = 42) (three-way interaction between numbers of either *Ca.* or *Cr.* workers, *Camponotus* species and *Crematogaster* species: effect on *Ca.*: $\chi^2_1 = 3.98$, $P = 0.046$; on *Cr.*: $\chi^2_1 = 6.88$, $P = 0.0087$; Fig. 2b).

Uric acid was generally more often visited by *Crematogaster*, whose numbers increased over time (LMM: $\chi^2_2 = 34.62$, $P < 0.0001$; Fig. 1c). Seeds and oleic acid were rarely visited by both genera, and nearly not at all by *Camponotus* (Fig. 1d,e). More detailed results can be found in the supplementary materials.

NLFA analysis

The fatty acid profiles strongly differed between *Camponotus* and *Crematogaster* (PERMANOVA: pseudo-$F_1 = 14.65$, $P = 0.001$) although their level of variation among samples was similar (PERMDISP: $F_1 = 1.15$, $P = 0.29$). The profile of *Ca. femoratus* PAT differed from PS (species nested in genus: pseudo-$F_2 = 3.76$, $P = 0.003$; PERMANOVA with *Camponotus* subset: pseudo-$F_1 = 9.49$, $P = 0.001$), while *Cr. levior* A and B did not differ (PERMANOVA with *Crematogaster* subset: pseudo-$F_1 = 0.59$, $P = 0.66$). The variability of fatty acid profiles did not differ between cryptic species (PERMDISP: *Ca. femoratus* PAT and PS: $F_1 = 3.49$, $P = 0.069$; *Cr. levior* A and B: $F_1 = 0.11$, $P = 0.75$).

As expected, *Camponotus* had higher absolute fatty acid amounts than the much smaller *Crematogaster* (LM: $F_1 = 45.97$, $P < 0.0001$; Fig. 3a). However, the cryptic species of either genus did not differ in their absolute fatty acid quantity (cryptic species identity nested in genus: $F_2 = 0.76$, $P = 0.47$; Fig. 3a). Ants from Paracou contained slightly more fat than those from Patawa ($F_2 = 4.16$, $P = 0.020$; Tukey: $t_{68} = 2.81$, $P = 0.017$).

Fatty acid composition differed between *Camponotus* and *Crematogaster*: While *Crematogaster* had more saturated fatty acids (LM: $F_1 = 18.78$, $P < 0.0001$; Fig. 3b), *Camponotus* had more mono-unsaturated ones ($F_1 = 11.98$, $P = 0.0009$; Fig. 3c). This effect was driven by *Ca. femoratus* PAT, which had more mono-unsaturated fatty acids than *Ca. femoratus* PS and the two *Crematogaster* species (cryptic species identity nested in genus: $F_2 = 4.45$, $P = 0.015$, post-hoc Tukey test PAT vs. PS: $t_{72} = 2.94$, $P = 0.023$; Fig. 3c). On the other hand, *Crematogaster* had more di-unsaturated fatty acids than *Camponotus* ($F_1 = 6.29$, $P = 0.014$; Fig. 3d), while the cryptic species within each genus did not differ ($F_2 = 0.82$, $P = 0.44$; Fig. 3d). We did

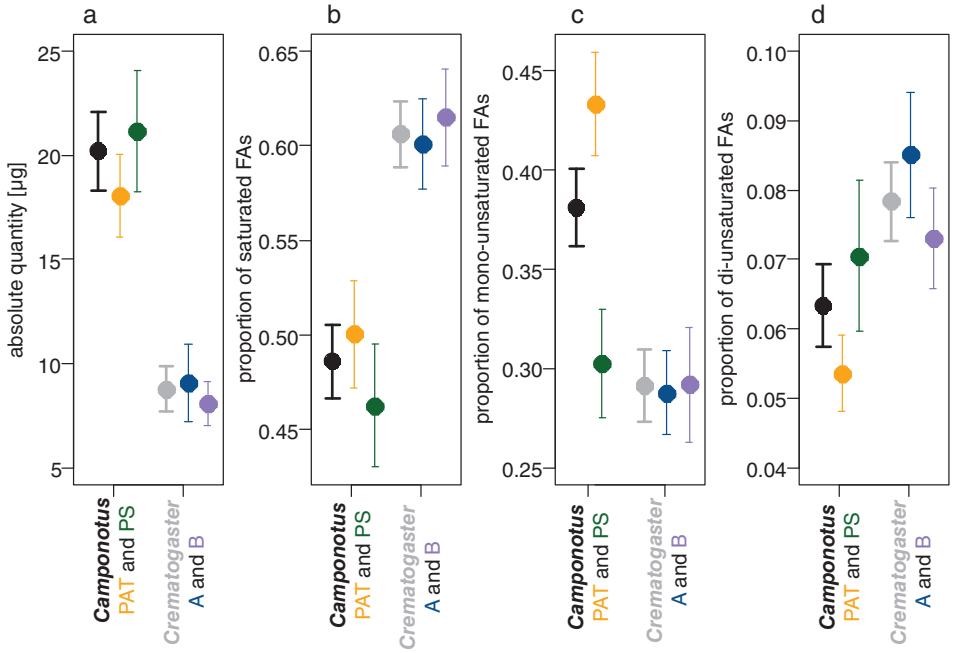

Fig. 3. Absolute quantity and composition of neutral lipid fatty acids in the cryptic species of *Ca. femoratus* and *Cr. levior*. The plots show back-transformed means ± SEM of the absolute quantity (a) or the relative proportion of structural classes of fatty acids (b–d). *Camponotus femoratus* PAT is represented in orange, PS in green, and *Cr. levior* A in blue, B in purple. The bold plots in black (*Camponotus*) and grey (*Crematogaster*) represent the averages of the two cryptic species per genus. [Colour figure can be viewed at wileyonlinelibrary.com].

not find evidence that fatty acid profiles between *Camponotus* and *Crematogaster* from the same ant garden were correlated (Mantel test: $R^2 < 0.0001$, $P = 0.43$).

Stable isotope analyses

Stable isotope signatures of nitrogen were lower in *Camponotus* than in *Crematogaster* (LM: $F_1 = 6.19$, $P = 0.016$; Fig. 4). $\delta^{15}N$ also differed between sites ($F_2 = 4.18$, $P = 0.020$), but not between cryptic species (cryptic species identity nested in genus: $F_2 = 0.05$, $P = 0.95$; Fig. 4).

The $\delta^{13}C$ signature neither differed between *Camponotus* and *Crematogaster* ($F_1 = 0.10$, $P = 0.75$) nor among sampling sites ($F_2 = 1.94$, $P = 0.15$). However, *Cr. levior* A had higher $\delta^{13}C$ signatures than B (cryptic species identity nested in genus: $F_2 = 3.27$, $P = 0.045$; A vs. B: $t = -2.30$, $P = 0.025$; Fig. 4), while there was no difference between the cryptic *Ca. femoratus* species (PAT vs. PS: $t = 0.88$, $P = 0.38$).

The $\delta^{15}N$ signatures of *Camponotus* and *Crematogaster* ants of the same ant garden co-varied (LM: $F_1 = 6.73$, $P = 0.015$), but the $\delta^{13}C$ signatures did not ($F_1 = 0.25$, $P = 0.62$). Site did not influence the covariation of either isotope signature ($\delta^{15}N$: $F_2 = 1.03$, $P = 0.37$; $\delta^{13}C$: $F_2 = 1.17$, $P = 0.32$).

Discussion

In the present study, we aimed to answer two questions: 1) Do the parabiotic species differ in their realised trophic niches and is there potential for competition for certain food sources? Costs due to the competition with the partner should reduce the net benefit from the interaction and hence the stability of the association. 2) Is there trophic niche partitioning between the cryptic species? Differences among the cryptic sister taxa may be relevant in the light of competitive exclusion when both occur in sympatry but may also matter for coevolution with the parabiotic partner, for example if one of the cryptic species shows less interference competition against the partner than the other. In the following, we will discuss how our findings help us to understand competition and feeding ecology within the parabiosis and how the cryptic species of *Ca. femoratus* and *Cr. levior* might coexist without mutual exclusion.

Competition between parabiotic ants

Our cafeteria experiments indicate that there is competition between *Camponotus* and *Crematogaster* for certain food sources although their realised food niches differed (cf. PERMANOVA results) and *Crematogaster* was more variable in its food choice (cf. PERMDISP results). *Camponotus* foraged at a higher frequency at sausage as indicated by the 'hotlink' analysis (Table 2; Fig. 1a), and their numbers negatively affected the numbers of *Crematogaster* workers, suggesting competition and displacement from this bait (Figs 1a and 2a). These findings imply that *Ca. femoratus* probably displaced *Cr. levior* from sausage baits. This goes in line with earlier experiments on protein-rich baits such as wasp larvae, termites, locusts, or crushed insects (Swain, 1980; Vantaux *et al.*, 2007).

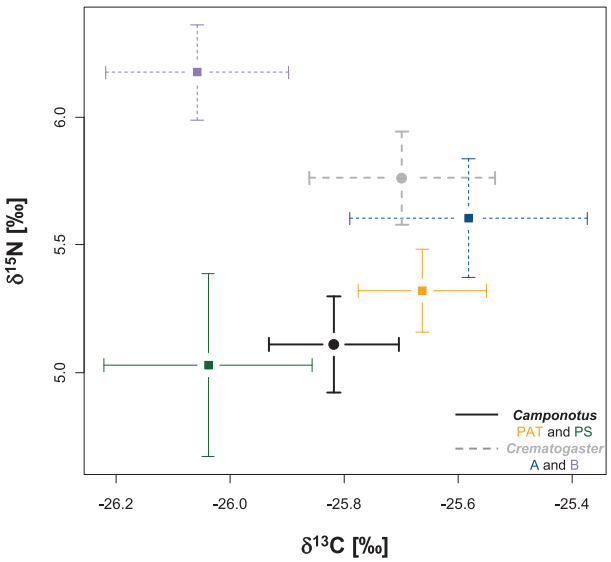

Fig. 4. Stable isotope signatures of $\delta^{15}N$ and $\delta^{13}C$ in the cryptic species of *Ca. femoratus* and *Cr. levior*. The plots with squares show means ± SEM of the $\delta^{15}N$ signature (y-axis) and the $\delta^{13}C$ signature (x-axis) of *Ca. femoratus* PAT (orange, solid line), PS (green, solid line), *Cr. levior* A (blue, dashed line), and B (purple, dashed line). The bold plots with dots in black with solid lines (*Camponotus*) and grey with dashed lines (*Crematogaster*) represent the averages of the two cryptic species per genus. [Colour figure can be viewed at wileyonlinelibrary.com].

There is evidence that *Crematogaster* can find protein-rich food sources before *Camponotus* and retrieve food pieces before getting displaced (Vantaux *et al.*, 2007; Menzel *et al.*, 2014), which is consistent with our results where the negative effect of *Camponotus* on *Crematogaster* also increased with time. This discovery-dominance trade-off among the partners could reduce competition (Fellers, 1987; Parr & Gibb, 2010), which has been found for multiple ant communities (Fellers, 1987; Sarty *et al.*, 2006), even though it seems too rare to explain local ant co-occurrence in many cases (Parr & Gibb, 2012).

At sugar baits the numbers of *Camponotus* and *Crematogaster* increased in parallel (albeit only in two cryptic species pairs: *Ca. femoratus* PAT/*Cr. levior* A or *Ca. femoratus* PS/*Cr. levior* B; Figs 1b and 2b). This lack of competition for carbohydrates confirms experiments in which *Ca. femoratus* and *Cr. levior* were found simultaneously feeding on sugar baits (Swain, 1980). Since both species forage arboreally, it seems likely that sugar sources such as trophobionts or extrafloral nectaries are less limited than prey items or other protein sources (Davidson *et al.*, 2003). Early observations of the parabiosis between *Cr. levior* and *Ca. femoratus* even reported interspecific trophallaxis (Wheeler, 1921), which we, however, never observed. Despite this seemingly peaceful relationship, Davidson (1988) found that *Camponotus* monopolised higher-quality honey baits, but did not exclude *Crematogaster* from less attractive baits.

Crematogaster workers often fed on uric acid (resembling bird faeces). This matches recent observations that *Cr. levior* (but also *Ca. femoratus*) foraged more frequently on bird faeces than expected by chance (Grevé *et al.*, 2019). Interestingly, in our study *Cr. levior* was more frequently found on uric acid than *Camponotus*. It seems possible that *Ca. femoratus* is less nitrogen-limited, both because it forages (and monopolises) protein baits such as sausage and because, in contrast to *Cr. levior*, it can also metabolise urea, for example from mammal urine, due to *Blochmannia* endosymbionts (Sauer *et al.*, 2000; Feldhaar *et al.*, 2007).

Trophic niche partitioning between mutualists

Both *Camponotus* and *Crematogaster* mostly foraged at sausage and sugar baits, like many other ant species, which mainly forage on protein and carbohydrate sources (Houadria *et al.*, 2015). After 60 min, however, *Camponotus* started displacing *Crematogaster* from the sausage bait, which then started to recruit to uric acid instead. Indeed, the fatty acid analysis indicated differences in the trophic niches between *Camponotus* and *Crematogaster* with the former having more mono-unsaturated fatty acids and the latter having more saturated and di-unsaturated fatty acids (Fig. 3). Such strong differences are unlikely to be exclusively caused by differences in fatty acid metabolism, and are probably due to different diets (Budge *et al.*, 2006). In ants, enrichment of dietary fatty acids seems to be similar among species kept on the same diet, and it significantly contributes to overall changes in the NLFA profiles over time (Rosumek *et al.*, 2017). Similarly, quantitative fatty acid composition correlates with the amount of dietary precursors fed to herring gulls (Käkelä *et al.*, 2009), and thus fatty acid profiles can be used to track different food sources (Bayes *et al.*, 2014). Beside the differences between *Camponotus* and *Crematogaster*, the consistently different proportion of unsaturated fatty acids between *Ca. femoratus* PAT and PS, therefore, suggest differences in the dietary composition.

Signatures of $\delta^{15}N$ and $\delta^{13}C$ for *Ca. femoratus* and *Cr. levior* from our study resembled those of an earlier study on community level (Davidson *et al.*, 2003). While *Camponotus* and *Crematogaster* had similar $\delta^{13}C$ signatures, $\delta^{15}N$ was lower in *Camponotus*, which agrees with earlier findings (Blüthgen *et al.*, 2003; Davidson *et al.*, 2003). Formicines (like *Camponotus*) usually forage on lower trophic levels (i.e. being less predatory and/or more engaged in trophobiotic associations) than Myrmicines (like *Crematogaster*) (Blüthgen *et al.*, 2003; Csata & Dussutour, 2019). While both *Ca. femoratus* and *Cr. levior* tend trophobionts (Davidson, 1997; Davidson *et al.*, 2003), such associations are more common in *Camponotus* and other Formicines (Davidson, 1997; Blüthgen & Feldhaar, 2010; Zhang *et al.*, 2012; Menzel *et al.*, 2014). This might explain the lower $\delta^{15}N$ in *Ca. femoratus* although this species forages on protein more than other species (this study; Grevé *et al.*, 2019). Our results coincide with previous data from a paleotropical parabiosis, where *Ca. rufifemur* had lower $\delta^{15}N$ than its parabiotic partner *Cr. modiglianii* (Menzel *et al.*, 2012).

The correlation in $\delta^{15}N$ signatures of *Camponotus* and *Crematogaster* from the same nest was independent of the site, which suggests trophic niche overlap between the mutualistic partners, potentially causing competition. Beside overlap in the

trophic niche, both *Ca. femoratus* and *Cr. levior* tend to forage during the day (whereas most other *Camponotus* species are nocturnal) (Grevé *et al.*, 2019). The additional overlap in temporal niche may additionally increase interference competition. Alternatively, it is possible that the two species forage different food items, but exchange food in the nest. However, so far we did not observe any trophallaxis or other food exchange even in lab colonies.

Niche partitioning versus neutral processes in cryptic species

In the cafeteria experiments, the realised trophic niche did not differ between the cryptic species of *Ca. femoratus* and *Cr. levior*. Nevertheless, the cryptic species of *Ca. femoratus* differed in their NLFA composition, with PS having more variable fatty acid profiles and PAT containing way more mono-unsaturated fatty acids. This suggests that PAT and PS use different food resources, for example different prey species. However, there are only few studies investigating NLFAs in ants (Rosumek *et al.*, 2018), and we cannot pinpoint the precise resources so far. In *Cr. levior*, species A had a significantly higher $\delta^{13}C$ signature compared to B, while they did not differ in their fatty acid profiles. Differences in the $\delta^{13}C$ signature between the cryptic *Cr. levior* species could be affected by differential use of plant extrafloral nectaries or trophobionts on different plants: C_3 and C_4 plants differ in ^{13}C abundance (Swap *et al.*, 2004; Blüthgen & Feldhaar, 2010), which is also reflected in sugars taken up by plant suckers. All these differences are subtle, but suggest that despite the large overlap, there is at least weak niche partitioning between the cryptic species of both genera, which may prevent competitive exclusion in the long term.

Niche partitioning should allow cryptic species to escape competitive exclusion, for example via spatial partitioning as shown in butterflies or fig wasps (Vodă *et al.*, 2015; Darwell & Cook, 2017) or trophic differentiation as shown for bats (Siemers *et al.*, 2011) or dolphins (Owen *et al.*, 2011). *Ca. femoratus* PAT and PS have different climate niches, but still often occur in sympatry (Hartke *et al.*, 2019). Here, we showed that their trophic niches also largely overlap, despite some evidence for niche partitioning. This resembles findings in a species complex of freshwater amphipods with overlapping isotopic signatures (Dionne *et al.*, 2017), and suggests that niche differences between co-occurring species can be subtle, especially among closely related species. If species entirely overlap in their niche and fulfil similar functions in an ecosystem, they are considered 'neutral species' within (but not beyond) their functional group (McPeek, 2017). Such neutral species can persist in the same community via random processes like ecological drift, or if competitive superiority is context-dependent (Leibold & McPeek, 2006; Andersen, 2008; Dionne *et al.*, 2017; Gilbert & Levine, 2017). The weak, albeit significant, differentiation of dietary niches seems not sufficient to explain their co-existence from a niche-based view. Hence, *Ca. femoratus* PAT and PS, and *Cr. levior* A and B may actually represent two pairs of 'nearly' neutral species. Competitive outcomes might be context-dependent in that they vary with colony size, but also with the identity of the parabiotic partner.

Conclusions

Despite their mutualistic relationship, *Ca. femoratus* and *Cr. levior* compete for certain food sources. Often *Cr. levior* is the first at protein sources, but gets displaced by *Ca. femoratus* over time, which suggests a discovery-dominance trade-off between the mutualistic partners (Fellers, 1987; Sarty *et al.*, 2006). Nevertheless, the realised food niches of the mutualists differ, as indicated by food choice, fatty acid profiles, and stable isotope signatures.

The cryptic species of both genera showed only subtle differences in their trophic niches and seem to feed on largely similar food sources. Since so far, climatic partitioning was only found for *Ca. femoratus* (Hartke *et al.*, 2019), it remains open at least for *Cr. levior* A and B how the cryptic species avoid competitive exclusion. Sympatric coexistence of the cryptic species may be also mediated by partitioning in yet unknown niche dimensions, but probably to a major degree by neutral processes (Hubbell, 2005; Adler *et al.*, 2007), especially since the cryptic species seem to be nearly neutral species as outlined above. Neutral processes equalise fitness differences between species (Chesson, 2000), and hence limit competitive exclusion of one species by another (Andersen, 2008). In our study system, they may include competitive outcomes depending on environmental conditions or on the parabiotic partner species, for example if one of the partner species displaces one or the other cryptic species faster from baits (for which we found evidence) or takes part in competitive encounters with neighbouring colonies. The species complexes of *Ca. femoratus* and *Cr. levior* offer the intriguing chance to further investigate how mutualistic interactions affect coexistence and the roles of niche partitioning versus neutral processes among closely related species.

Acknowledgements

We thank Aurélie Dourdain for permission to work at the Paracou Research Station and Lindon Yansen for assistance at the station. To J.-A. Cerda we are grateful for logistic support at Camp Patawa. Further on, we thank Freya Zäpernick and Nicolas Goß for their help with the experiments and fatty acid extractions and Carina Bauer and Christine Tiroch for technical assistance in isotope ratio mass spectrometry. Finally, we thank Simone Glaser and two anonymous reviewers for comments on earlier versions of this manuscript. This study was supported by the German Research Foundation (DFG) as a grant to FM (ME 3842/5-1 and ME 3842/6-1), TS (SCHM 2645/7-1) and BF (FE 1333/7-1) as well as the Feldbausch Stiftung of the Johannes Gutenberg-University Mainz to CM. The authors declare no conflict of interest. Open Access funding enabled and organized by ProjektDEAL.

Author contributions

PPS, JH, BF, TS, and FM conceived and designed the experiments. CM performed the experiments in the field and the fatty acid analysis, GG performed the stable isotope analysis. PPS, CM, and FM analysed the data. PPS and FM wrote the manuscript; other authors provided editorial advice.

Data availability statement

The datasets generated during the current study are available in the online supplement (Tables S1-S3).

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Further results from cafeteria experiment.

Fig. S1. Total number of *Crematogaster levior* workers at the baits at different sites. The plots show back-transformed means ± SEM of the number of *Cr. levior* workers at Paracou, Camp Patawa, and Sinnamary. Different letters in the plots indicate statistically different comparisons.

Table S1. Raw data of the cafeteria experiment.

Table S2. Raw data of the fatty acid analyses.

Table S3. Raw data of the stable isotope analyses.

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Accepted 4 December 2020
Associate Editor: Adam Hart