Original Article

Prudent behavior rather than chemical deception enables a parasite to exploit its ant host

Thomas Parmentier,[a,b,c] Frederik De Laender,[c] Tom Wenseleers,[b] and Dries Bonte[a]

[a]Department of Biology, Terrestrial Ecology Unit (TEREC), Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium, [b]Laboratory of Socioecology and Socioevolution, KU Leuven, Naamsestraat 59, B-3000 Leuven, Belgium, and [c]Research Unit of Environmental and Evolutionary Biology, Namur Institute of Complex Systems, and Institute of Life, Earth, and the Environment, University of Namur, Rue de Bruxelles 61, 5000 Namur, Belgium

Many parasites display complex strategies to evade host detection. The principal view is that the parasites of social insects deceive their host by means of advanced chemical adaptations such as mimicking the cuticular host recognition cues, being chemically odorless, or emitting manipulative volatiles. Apart from these chemical adaptations, parasites of social insects may also use simpler behavioral strategies to evade host detection. As yet, such a behavior has rarely been studied. Here we tested which chemical and behavioral strategies the unspecialized parasitic rove beetle *Thiasophila angulata* uses to avoid detection by its aggressive *Formica rufa* red wood ant host. Chemical comparisons of the beetle's and the host ants' cuticular hydrocarbons showed that the beetle carried an idiosyncratic cuticular profile that was clearly different from that of its host. Beetles that were isolated from their host or were placed in the nests of another *Formica* species perfectly retained their original cuticular profiles and provoked equal levels of aggression. These results suggest that the beetles do not avoid host detection through chemical deception. In contrast, the beetle adapted its behavior to avoid aggression by the ants. In the presence of ants, the beetle behaved much more prudently by hiding more frequently and engaging in less risky runs. Overall, these results highlight that for relatively unspecialized parasites, general strategies such as prudent behavior can be equally effective as more specialized deception strategies to evade host detection.

Key words: chemical mimicry, hydrocarbon, myrmecophile, red wood ant, risk avoidance, Staphylinidae.

INTRODUCTION

Many animals skillfully mask their presence to avoid adverse interactions with other animals. Although these concealment strategies have mainly been examined in predator–prey interactions (Stevens and Merilaita 2009; Mokkonen and Lindstedt 2016), it is generally assumed that they also play a crucial role in other types of interactions, such as those between hosts and parasites (Mokkonen and Lindstedt 2016). Some parasites try to stealthily exploit their host through visual deception. Egg mimicry of avian brood parasites is a classic example of visual host deception by a parasite (Stoddard and Stevens 2010). Parasites may also use acoustic trickery as demonstrated in the ant-parasitic caterpillar *Maculinea* (Barbero *et al.* 2009) and in cuckoo chicks (Davies *et al.*

1998). Parasites can even mislead the host with chemical signals (Bagnères and Lorenzi 2010), which are, for example, exploited by the parasitic beetle *Eremostibes opacus* to enter the burrows of its beetle host without being noticed (Geiselhardt *et al.* 2006). Instead of deceiving the host with visual, acoustic, or chemical tactics, parasites can also conceal their presence and avoid antagonistic interactions simply by hiding or by reducing their foraging. The crucial role of these types of prudent behavior in predator–prey interactions has been experimentally (e.g., Dill *et al.* (1997) and Jennions *et al.* (2003)) and theoretically (Ma *et al.* 2009) explored. These studies show that prudent behavior is a simple, but very efficient and widespread tactic to evade predation (Lima and Dill 1990). As yet, the importance of prudent behavior in host-parasite interactions has been largely overlooked. Only a few studies reported prudent behavior in parasites to avoid host detection and aggression (von Beeren *et al.* 2010; Koop *et al.* 2012; Nazzi and Le Conte 2016). The relative importance of prudence

Address correspondence to T. Parmentier. E-mail: thomas.parmentier@ugent.be.

compared with more advanced deception strategies has not been determined yet.

Strategies to deceive the host are particularly well-studied in the diverse community of parasites living in social insect colonies (Lenoir et al. 2001; Bagnères and Lorenzi 2010). Workers of a social insect colony share a unique chemical odor, which in most groups is based on a blend of cuticular hydrocarbons (CHCs) (van Zweden and d'Ettorre 2010). Individuals whose chemical bouquet deviates from that of the colony are expelled or attacked. By mimicking their host's chemical profile, parasitic arthropods can infiltrate into the colony without being noticed (Lenoir et al. 2001; Bagnères and Lorenzi 2010). Parasitic arthropods can synthesize the components themselves ("chemical mimicry") or acquire the colony odor passively ("chemical camouflage") from the host colony (van Zweden and d'Ettorre 2010). Alternatively, parasitic arthropods may mask their presence by suppressing their complete chemical odor profile ("chemical insignificance") or some key components ("chemical transparency") (Lenoir et al. 2001; Martin et al. 2008). Finally, parasites can manipulate the social insect host by secreting volatiles (Hölldobler and Wilson 1990; Stoeffler et al. 2007; Stoeffler et al. 2011). Although most studied parasites of social insects rely on one or more of these advanced chemical strategies, recent research has suggested that a large group of rather unspecialized parasites of social insects might employ different strategies (Parmentier et al. 2017). Unspecialized or generalist parasites are clearly recognized as intruders and provoke host aggression to a varying degree (Parmentier et al. 2016a), whereas specialized parasites actively interact with their host and are royally treated, for example, being groomed, fed, and transported. To limit host aggression, unspecialized parasites mainly avoid interactions with their host (Kistner 1979; Hölldobler and Wilson 1990). There are some indications that parasites of social insects also resort to simple behavioral strategies such as hiding to mask their presence or at least minimize the number of antagonistic interactions (von Beeren et al. 2010; Parmentier 2016a), but this has not been tested yet.

European red wood ant (Formica rufa group) nests attract a diverse community of nest-inhabiting parasites (Parmentier et al. 2014). The abundant parasitic beetle Thiasophila angulata shows poor similarity in CHC composition to its red wood ant host (Parmentier et al. 2017). Even with low levels of similarity, the beetle could trick the host by either exposing or suppressing some key recognition cues. However, the high level of elicited aggression (Parmentier et al. 2016a) suggests that the beetle does not trick the host through its cuticular profile. We therefore hypothesized that this beetle relies on prudent behavioral strategies rather than on advanced chemical deception to mask its presence in the colonies of its ant host. We tested this prediction: first by examining whether the chemical profile of the beetle changed in the presence of its host and second by analyzing the effect of the ant host on the beetle's behavior.

MATERIALS AND METHODS
Study system and sampling

The rove beetle T. angulata (Erichson, 1837) (Staphylinidae: Aleocharinae) is an obligate ant symbiont, a so-called myrmecophile, that thrives in the nests of mound-building Formica ants (Parmentier et al. 2014). The complete life cycle takes place within ant nests (Zagaja et al. 2017). The beetle parasitizes its host by preying on ant brood and pilfering prey collected by the ants (Parmentier et al. 2016b; Zagaja et al. 2017). The beetle can

be found throughout the nest, but has a strong preference for the brood chambers, which are characterized by high densities of workers and a constant supply of resources (Parmentier et al. 2016a). The myrmecophilous beetle can reach high densities (a maximum density of 45 individuals in 1 L of nest material in our study region) and was found in almost all mounds sampled in northern Belgium so far (Parmentier et al. 2015a, personal observations). It is also a common red wood ant guest in other regions in Europe (Päivinen et al. 2004; Härkönen and Sorvari 2014; Zagaja et al. 2017). The beetle looks very similar to nonant associated relatives and does not directly interact with its hosts (Freude et al. 1974). Like free-living rove beetles of the Staphylinidae subfamily Aleocharinae, they can defend themselves against enemies by secreting repellent volatiles from an abdominal gland, known as the tergal gland (Steidle and Dettner 1993). Zagaja et al. (2017) demonstrated that the tergal gland of T. angulata produced an irritant secretion mainly consisting of quinones. These compounds are very general and are widely employed by rove beetles, regardless of whether the species is associated with social insects (Steidle and Dettner 1993). These volatiles do not mask the beetle's identity, whereas more specialized myrmecophilous beetles deceive the host by the secretion of appeasing or host-mimicking volatiles (Blum et al. 1971; Stoeffler et al. 2007; Stoeffler et al. 2011).

Beetles and ant workers were captured in 5 different mounds of a colony of the red wood ant F. rufa (subgenus Formica s. str.) Linnaeus, 1761 in Boeschepe, France (50°47′48.48″N, 2°40′31.00″E) from May 2017 until March 2018. The mounds were lined along a forest edge over a distance of 100 m. The mounds were part of a large supercolony that consisted of more than 30 cooperating mounds distributed along a forest edge of ca. 500 m. The colony was headed by thousands of queens (highly polygynous). Workers, brood, and food resources were constantly exchanged between the different mounds and no aggression among the mounds was observed. Therefore, a common colony odor is expected to be shared by the different mounds of the supercolony. Beetles were separated from the nest by spreading out nest material into a large plastic tray. We collected the beetles with an aspirator and stored them in large plastic containers with nest material and host workers.

In addition, we collected workers of the ant Formica cunicularia (subgenus Serviformica, relatedness of Formica subgenera, see Goropashnaya et al. (2012)) from a colony residing in sandy soil in an urbanized region (Middelkerke, Belgium, 51°9′27.44″N, 2°44′55.37″E). This ant species is not known as a host for T. angulata, but the beetle can survive well in lab colonies of this ant for at least 20 days (Parmentier et al. 2016c).

Effect of beetle isolation or transplantation on F. rufa aggression

In this experiment, we wanted to test whether F. rufa workers would behave differently towards isolated beetles or beetles transplanted into the nests of another host species. We allocated T. angulata beetles to 3 treatment groups: 1) isolated beetles: beetles isolated from their host ants, 2) transplanted beetles: beetles transferred to the nonhost F. cunicularia, and 3) native beetles: beetles collected from their native F. rufa nest. For the isolation treatment, we distributed 15 beetles over 3 containers (3 × 5 beetles) (diameter 9 cm) filled with a 2-cm-thick layer of plaster. We coated the inner walls of the container with fluon to prevent beetles from climbing up. We added one Eppendorf tube stuffed with moist cotton and provided a cut maggot of the common green bottle fly Lucilia sericata (Meigen), which was replaced after 5 days. We carved furrows into the plaster,

which enabled the beetles to hide. The setup for the transplanted treatment was similar, but here the 3 containers also housed 40 *F. cunicularia* workers. After 10 days, we removed the beetles (15 for each treatment) from the containers and placed them individually in snap lid vials (4.5 cm diameter) with a plaster bottom and made from hard plastic. On the same day, we went to the field to collect 15 individuals of *T. angulata* in the *F. rufa* supercolony. These "native beetle" individuals were also individually housed in snap lid vials.

We scored the aggression of a *F. rufa* worker towards *T. angulata* individuals in a circular, plastic cup (7 cm diameter, 5 cm height) with a bottom layer of plaster of Paris (ca. 1 cm thick) and with the inner side coated with Fluon. A worker of the test colony was added and allowed to acclimatize for 30 min. Then a *T. angulata* rove beetle subjected to 1 of 3 treatments was added. After 30 s, 15 consecutive interactions between ant and beetle were recorded. Ant aggression was quantified as the proportion of interactions that were aggressive (biting, snapping, or opening of the mandibles) out of the total number of interactions. As the beetles were individually housed in small containers, we were able to conduct this experiment blindly with respect to treatment (isolated vs. transplanted vs. native). Aggression towards every beetle was tested in 3 runs with 3 different ant workers (15 × 3 = 45 trials assigned to each of 3 treatments). There was a time gap of at least 60 min between 2 trials with the same beetle. As previous research showed that *F. rufa* aggression depends on the size of the worker (Parmentier *et al.* 2015b), we controlled for worker size in each trial. Head width was used as a proxy for size and was measured after the aggression trials using a stereomicroscope equipped with an eyepiece graticule.

Effect of the host on the beetle's chemical profile

Here, we wanted to test whether the presence of the host affects the beetle's cuticular chemical profile. To do so, we compared the cuticular chemical profile of the 45 beetles subjected to 3 treatments (isolated, transplanted, and native beetles) explained in the previous section. Directly after the aggression experiments (see above), we transferred the beetles with clean forceps to separate 2-mL glass vials (Sigma-Aldrich). An isolation or transplantation period of 10 days is sufficiently long for myrmecophiles to acquire or lose CHCs (see, e.g., Vander Meer *et al.* (1982), Akino *et al.* (1996), and von Beeren *et al.* (2012)). We also stored 12 *F. rufa* workers and 17 *F. cunicularia* workers in 2-mL glass vials. The beetles and ants were killed by freezing and were kept in the freezer at −21 °C until solvent extraction and GCMS analysis. We extracted the cuticular compounds for 10 min in 2-mL vials capped with a PTFE septum (Sigma-Aldrich) in 30 µL of hexane (HPLC grade, Sigma-Aldrich) for a beetle and in 100-µL hexane for a worker of *F. rufa* or *F. cunicularia*. The hexane extract was transferred to another vial. The samples were left to evaporate at room temperature in a laminar fume hood and stored at −21 °C prior to analysis. Beetle samples were diluted again in 10-µL hexane, *F. rufa,* and *F. cunicularia* samples in 30-µL hexane. We injected 2 µL of each hexane extract into a Thermo GC (Trace 1300 series) coupled with a MS (ISQ series, −70 eV, electron impact ionization) and equipped with a Restek RXi-5sil MS column (20 m × 0.18 mm × 0.18 µm). We selected splitless injection and maintained an inlet temperature of 290 °C. We used the following temperature program: 1 min at 40 °C, 2 temperature ramps from 40 to 200 °C at 20 °C min⁻¹ and from 200 to 340 °C at 8 °C min⁻¹, after which the final temperature of 340 °C was held for 4 min. We used helium as a carrier gas at a flow rate of 0.9 mL min⁻¹. We ran a linear C7 to C40 alkane ladder standard (49452-U, Supelco) at 3 different concentrations (0.001,

0.01, and 0.1 µg/mL) before and directly after the samples. The order in which samples were run was randomized. The relationship between peak area and concentration was linear on a log–log scale (Parmentier *et al.* 2017) and absolute quantification (to determine the total amount produced in ng per individual) was performed using interpolation on a log–log scale, based on the peak areas of the closest eluting n-alkane of our external alkane ladders for each peak. Retention indices (Kovats indices) of all peaks were calculated using cubic spline interpolation (Messadi *et al.* 1990) using the elution times of the external alkane ladders. CHCs were identified on the basis of expected mass spectrometric fragmentation patterns and retention indices already determined for both *T. angulata* and *F. rufa* in a previous study (Parmentier *et al.* 2017). For each species, we selected the peaks that eluted between *n*-C20 and *n*-C40 and comprised on average more than 0.1% of the total peak area between *n*-C20 and *n*-C40.

Beetle behavior in absence versus presence of *F. rufa*

Here, we assessed whether the beetle adjusted its behavior when exposed to ants. For this experiment, we used different beetle individuals than for experiments 1 and 2. The beetles were also collected from the *F. rufa* supercolony and subsequently placed in separate 4.5-cm diameter snap-lid containers with a plaster bottom. A maggot was offered to each beetle for a period of 3 to 4 h and then the beetle was starved for 48 h. We subsequently introduced the beetle to an arena (9-cm diameter, plaster bottom and Fluon coated) either with or without ants (for each treatment 50 replicates with unique beetle individuals). For the treatment with ants, 40 *F. rufa* workers of the host colony were added to the arena. We carved a standardized pattern of 8 furrows into the plaster (Figure 1), which enabled the beetles to hide during the experiment. Ants could not access these hiding places. In the middle of the arena, we fixed a maggot with an insect needle (Figure 1). We recorded the beetle's behavior in 45 snap shots over a period of 90 min. The beetle and the ants were allowed to acclimatize for 30 min before the beetle's behavior was recorded. To do so, we took a set of 2 pictures every 2 min with a 5-s time interval using a Nikon D5100 camera and DigiCamControl software. We performed all tests under red light to mimic dark nest conditions. We distinguished 5 different types of beetle behavior: 1) feeding on the maggot in the center of the arena, 2) hiding in a furrow, 3) resting (no movement in the 5-s interval), 4) walking (distance of maximum 2 cm traveled in the 5-s interval), and 5) running (distance of more than 2 cm traveled in the 5-s interval). The distance traveled by the beetle in an interval of 5 s was estimated by calculating the Euclidean distance between the beetle's positions in both pictures using the software ImageJ. Arenas were reused, but we carefully rinsed the plaster bottom with hexane and ethanol after each trial to remove all leftover chemical cues.

Data analysis

Effect of beetle isolation or transplantation on *F. rufa* aggression

We ran generalized linear mixed models (GLMM) with a binomial distribution in R (version 3.4.2, package lme4) to compare the proportion of aggressive interactions towards *T. angulata* across the 3 treatment groups, whilst controlling for the size of the ant workers "Treatment" and "worker size" were included in these models as a fixed factor and covariate, respectively. As we used the same
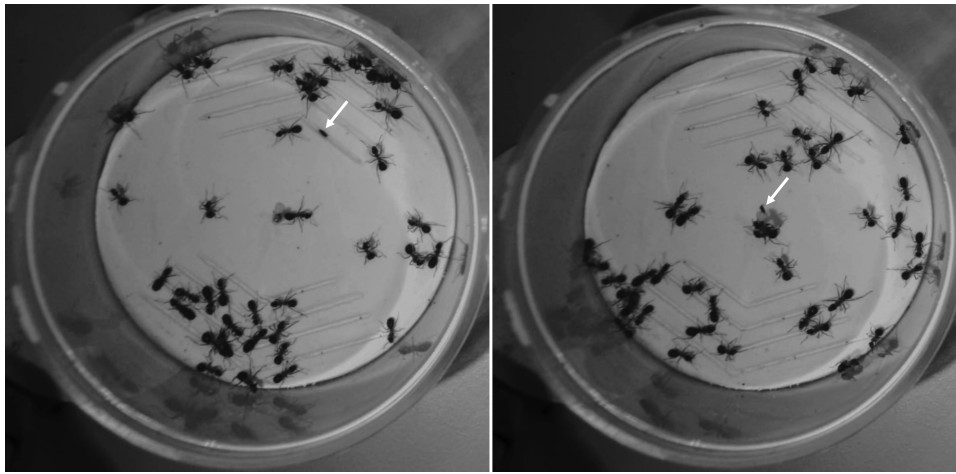
Figure 1

Arenas used in the experiment in which we compared the behavior of *T. angulata* with and without *Formica rufa* workers under red light. A standardized pattern of 8 V-shaped furrows was carved into the plaster bottom and offered hiding places for *T. angulata* (beetle indicated with arrow on left figure). A cut maggot was pinned in the center of the arena to test the feeding frequency of the beetle (feeding beetle indicated with arrow on right figure).

beetle individuals in this experiment in subsequent trials, we also included a random intercept "beetle id," We also added an observation level random factor to deal with overdispersion (Browne *et al.* 2005). Significance of model parameters was tested using type-III tests using the "Anova" function in the R-package "car."

Effect of the host on the beetle's chemical profile

We focused on CHCs in the chemical profiles as they serve as the main recognition cues in ants (van Zweden and d'Ettorre 2010). First, we compared the CHC composition among *F. rufa*, *F. cunicularia*, and the beetle *T. angulata*. We only compared the CHCs that were present in all 3 species. The mass of each hydrocarbon peak was standardized relative to the total sample mass of hydrocarbons shared by the 3 species. This generates a compositional dataset (constant sum of 1 for all peaks in each sample). The proportional CHC values are not allowed to vary independently, which violates the basic assumptions of standard statistical analyses. However, the effect of the constant sum constraint (constant sum of 1) on the covariance and correlation matrices can be effectively removed by the centered log ratio (CLR) transformation (Aitchison 1986):

$$Z_{i,j} = \ln\left[Y_{i,j} / g\left(Y_j\right)\right],$$

where Y_{ij} is the (relative) mass of peak i for individual j, $g(Y_j)$ is the geometric mean of (relative) masses of all CHC peaks for individual j, and Z_{ij} is the transformed mass of peak i for individual j. As the CLR transformed data did not follow the multinormal distribution, we applied nonmetric multidimensional scaling (nMDS, 2 dimensions) to plot the dissimilarities among the CLR-transformed profiles of the ants and beetles. The analysis was run in R (version 3.4.2) using the package "vegan." We chose for this multivariate analysis the Euclidean distance as distance index rather than the Bray–Curtis dissimilarity index, as the latter gives misleading values with the negative CLR-transformed data. We conducted a Permanova (Permutational Analysis of Variance, function "adonis" in R, 999 permutations) to test differences in the shared CHC profile among *F. rufa*, *F. cunicularia*, and *T. angulata* and using Euclidean distance as a distance measure. In this analysis, we did not account for the different treatments imposed on the beetles. Next, we wanted to test whether the CHCs of the 3 experimental groups of

beetles differed. We ran a second nMDS with 2 dimensions based on a matrix of pairwise Euclidean distances between the CLR-transformed CHC quantities (ng) of the beetles. Significance was tested by a Permanova (function "adonis" in R package vegan, 999 permutations), based on a matrix of pairwise Euclidean distances. Finally, a Kruskal–Wallis test was performed to test differences in total amount of CHCs (ng) per beetle among the 3 experimental beetle groups.

Beetle behavior in absence versus presence of *F. rufa*

The frequency of the beetles displaying each type of behavior (feeding, hiding, resting, walking, and running) was compared between the treatment with and without ants. As these data are also compositional (the frequency of all recorded behaviors per beetle sum up to 1% or 100%), we here applied a CLR transformation (Aitchison 1986) as well. Some beetles did not display 1 of 5 behaviors in the 45 snap-shot observations. As the CLR transformation cannot handle zero values, we replaced zero values in the dataset with 1/45, the lowest possible frequency of a behavior. Subsequently, we tested differences in the behavioral repertoire (Euclidean distance matrix) in the presence and absence of ants with a Permanova (function "adonis," 999 permutations). The Permanova was more robust than a multinomial regression. The latter generated unreliable *P* values as we could not control for overdispersion. Next, we separately compared the CLR transformed frequencies of each of 5 recorded behaviors in the presence and absence of ants using Wilcoxon signed-rank post hoc tests. We selected these nonparametric tests to account for non-normality in the CLR-transformed behavioral data. To reduce type I errors in these 5 post hoc tests, the threshold level of significance α was set at 0.010 (0.05/5) using the Bonferroni correction.

RESULTS

Effect of beetle isolation or transplantation on *F. rufa* aggression

F. rufa workers could not distinguish beetles living in their own nest from beetles that were isolated for 10 days or from those that were housed in a colony of another *Formica* species for 10 days. They showed a similar level of aggression towards the 3 treatment groups

(GLMM, Chisq = 1.187, $P = 0.525$). Beetles sometimes raised their abdomen, likely to secrete repellent substances. Abdomen raising might stop or lower the aggression response of an interacting worker (video in Supplementary Material 1). However, in most cases, the beetle needed swift movements to escape, when the interacting worker continued to show aggression or when other aggressive workers detected the beetle (video in Supplementary Material 1). We added worker size as an additional fixed factor in the statistical model of this experiment as we previously found that larger workers showed less aggression (Parmentier et al. 2015b). Here we did not find an effect of worker size on aggression (GLMM, Chisq = 2.065, $P = 0.151$), but this could be explained by the fact that workers were selected within a narrower size range (head width between 1.2 and 1.7 mm) than in the previous study (head width between 1.0 and 2.2 mm).

Effect of the host on the beetle's chemical profile

In line with our previous study (Parmentier et al. 2017), we found clear qualitative differences in the general cuticular profile of the beetle and its host ant (Figure 2). Only 24 of 57 (42.1%) hydrocarbons detected in F. rufa were found in T. angulata (Supplementary Table S1). There were also quantitative differences in the CHC composition. The beetle, F. rufa and F. cunicularia each formed a completely separate group in an nMDS plot (2D stress 0.09) based on the 19 CHC peaks shared by the 3 species (Figure 3a). A Permanova test confirmed that their shared CHC profiles were significantly different (Permanova—adonis, 999 permutations, $F = 69.56$, $R^2 = 0.66$, $P = 0.001$). In addition, we found no evidence that the host or a nonhost Formica species affected the parasite's idiosyncratic chemical profile. The CHC profiles ($N = 39$ peaks) of T. angulata subjected to 3 treatments (isolated, transplanted, and native) could not be separated graphically in an nMDS plot (Figure 3b, 2D stress 0.20). Consequently, no effect of treatment group on beetle CHC composition could be demonstrated (Permanova—adonis, 999 permutations, $F = 1.43$, $R^2 = 0.06$, $P = 0.112$). Finally, there is no indication that hydrocarbons are lost or gained during isolation and transplantation as the total amount of CHCs per beetle individual did not differ among the 3 experimental groups (Kruskal–Wallis, chi-squared = 1.82, $P = 0.403$).

Beetle behavior in absence versus presence of F. rufa

The behavioral repertoire of the beetles was significantly different in the presence of ants (Permanova—adonis, 999 permutations, $F = 19.12$, $R^2 = 0.16$, $P = 0.001$). They spent significantly more time hiding in the furrows ($P < 0.001$, Bonferroni-corrected $\alpha = 0.010$) and initiated fewer runs ($P < 0.001$, Bonferroni-corrected $\alpha = 0.010$) in the presence of ants compared with the treatment without ants. We did not find an effect of ant presence on the frequency of feeding ($P = 0.672$), walking ($P = 0.015$), or resting ($P = 0.077$) with a Bonferroni-corrected significance level set at $\alpha = 0.010$ (Figure 4).

DISCUSSION

Our results show that the unspecialized parasitic beetle T. angulata does not invest in advanced chemical adaptations to deceive its red wood ant host, but rather depends on prudent behavior to avoid antagonistic interactions. It is often assumed that chemical deception strategies are the rule in parasites of social insects (Lenoir et al. 2001; Nash and Boomsma 2008; Kronauer and Pierce 2011;

Parmentier et al. 2017). Here we demonstrate that behavioral strategies can be equally effective.

Species coexistence theory predicts that specialist parasites should show a high degree of host specificity, as their adaptations are targeted to host-specific traits, whereas generalist parasites use strategies that can be employed to associate with different host species (Futuyma and Moreno 1988; Sasal et al. 1999; Kneitel and Chase 2004). Specialist parasites of social insects mimic their host's bouquet of CHC, which serve as the nestmate recognition system in most social insects (Lenoir et al. 2001; Akino 2008; Parmentier et al. 2017). This enables the parasite to successfully enter the social life of the colony without being noticed and to be treated (e.g., groomed, fed, and transported) as a fellow nestmate. As CHC profiles are colony/species-specific, these parasites can only target one or a few related hosts at best (von Beeren et al. 2018). Less specialized parasites rely on chemical insignificance (nondetectable CHC concentrations) or mimic or suppress (chemical transparency) a set of key cues (Martin et al. 2008; von Beeren et al. 2018). These strategies could already reduce aggression considerably and allow for a higher potential host range. However, we confirm the results of a previous study in which we showed that T. angulata carries a distinct profile from their host with detectable concentrations of CHCs (Parmentier et al. 2017). It has been reported that parasites could acquire host CHCs through physical contact with the host (rubbing) or by eating its workers or larvae (Elgar and Allan 2004; von Beeren et al. 2011; von Beeren et al. 2018). There could be a CHC transfer from the red wood ant host to T. angulata by both mechanisms as the beetle preferentially lives in the center of the nest in chambers with high worker densities and also feeds on ant brood (Parmentier et al. 2016a). However, this study clearly demonstrated that the beetles keep their idiosyncratic chemical profile in isolation of the ant. They also did not change their chemical profile when introduced into a crowded lab nest of the ant F. cunicularia, a congeneric ant species of the red wood ant host but with a distinct profile. The amount of CHCs also did not differ between beetles captured in the nest and those that were isolated or kept in a nonhost Formica nest. The nonflexibility of the chemical profile is unusual as most other studies have reported significant transfer of CHCs from the host to the parasite (Hojo et al. 2009; von Beeren et al. 2011; von Beeren et al. 2018). Some parasites could even acquire CHCs from an alternative host to facilitate their adoption (Akino et al. 1996; Lenoir et al. 2013; Le Conte et al. 2015). The fact that ants aggressed isolated, adopted, and nonisolated beetles to a similar degree further confirms that T. angulata did not alter its chemical profile to mask its presence or to reduce aggression.

In concert with chemical mimicry of the cuticle, specialist parasites of social insects can also emit deceptive volatiles produced in glands (Hölldobler and Wilson 1990). These volatiles typically function as appeasing substances or act as host alarm pheromones (Blum et al. 1971; Kistner and Blum 1971; Hölldobler and Wilson 1990; Steidle and Dettner 1993; Stoeffler et al. 2011). Now and then, T. angulata emits volatiles from the tergal gland by raising its abdomen. The tergal gland secretions of T. angulata are mainly composed of toxic quinones and are very similar to the compounds that non-ant-associated relatives use to deter aggressors (Zagaja et al. 2017). The beetles thus make use of general defensive compounds, rather than advanced manipulating secretions to avoid ant aggression. The volatile defensive compounds could temporarily repel some host workers, but most persisted in their hostile attitude. This defensive behavior and swift movements enabled the beetle to escape briefly from direct ant aggression (video in Supplementary
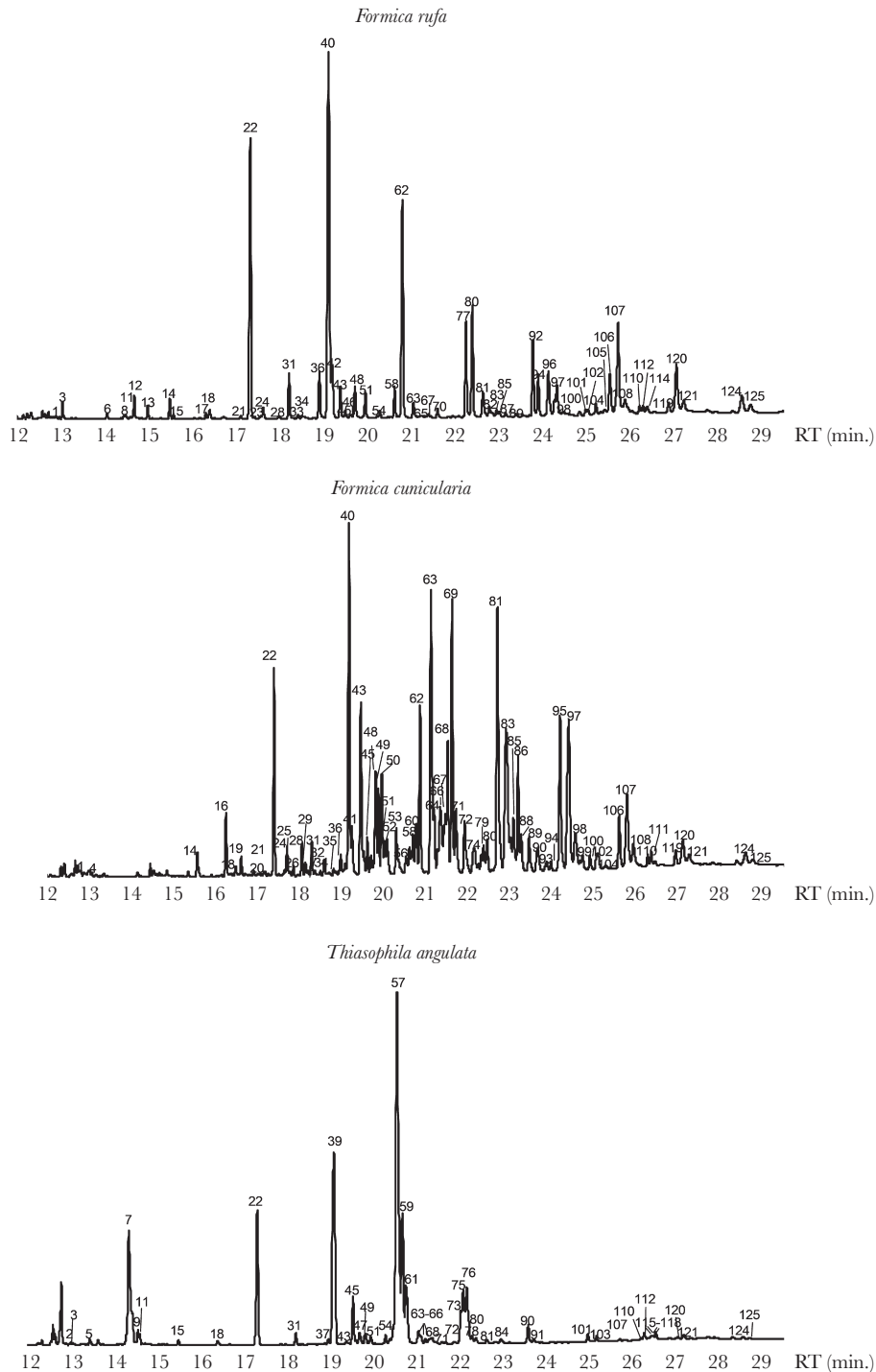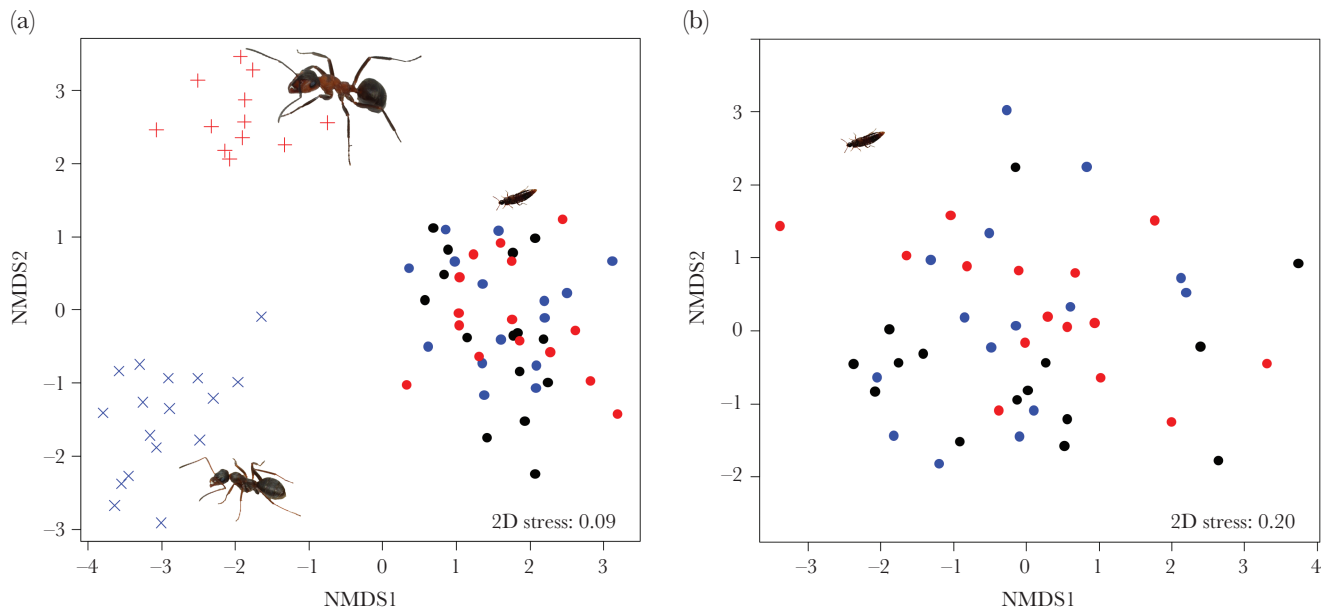
Figure 2

Representative chromatograms of *F. rufa*, *F. cunicularia*, and *T. angulata*. Peak identities can be found in Supplementary Table S1.

Material 1). Continuously resorting to these strategies is costly though, and a more durable strategy is needed.

We found that the beetle avoided persistent aggression by drastically adjusting its behavior. Well-known behavioral strategies used by many animals to avoid antagonistic encounters with their enemies are hiding and reducing their activity (Wisenden *et al.* 1997; DeWitt *et al.* 1999; Caro 2005; Hedrick and Kortet 2006). In this study, the beetles hid much more frequently and undertook less risky runs when associated with ants than when

ants were absent. This enabled the beetles to efficiently mask their presence in the nest. Red wood ant nests are characterized by a mound of organic material consisting of leaf fragments, needles, small sticks, and other organic material. The nest architecture therefore offers many possibilities for hiding from the host. Red wood ants, like many other animals, detect fast-moving organisms much more efficiently than resting or slow-moving individuals (Dorosheva *et al.* 2011; Parmentier *et al.* 2016a). Consequently, the beetle also benefits from avoiding fast runs. In contrast to highly

Figure 3

Graphical representation of the dissimilarities among the CHC profiles of the ants and beetles subjected to 3 different treatments. (a) nMDS-plot visualizing the CHC dissimilarities of *T. angulata* (o), its host ant *F. rufa* (red +), and the nonhost *F. cunicularia* (blue x). *T. angulata* was subjected to three treatments: *T. angulata* collected in *F. rufa* nests are depicted with red circles (native), beetles isolated for 10 days depicted with black circles and those transplanted into a *F. cunicularia* lab nest for 10 days with blue circles. The underlying Euclidean distance matrix is based on the CHCs ($N = 19$) shared by *F. rufa*, *F. cunicularia*, and *T. angulata*. (b) nMDS-plot visualizing CHC dissimilarities of *T. angulata* subjected to 3 treatments: *T. angulata* collected in *F. rufa* nests are depicted with red circles, beetles isolated for 10 days depicted with black circles, and those transplanted into a *F. cunicularia* lab nest for 10 days with blue circles. The underlying Euclidean distance matrix is based on the CHCs ($N = 39$) shared by the beetle individuals.
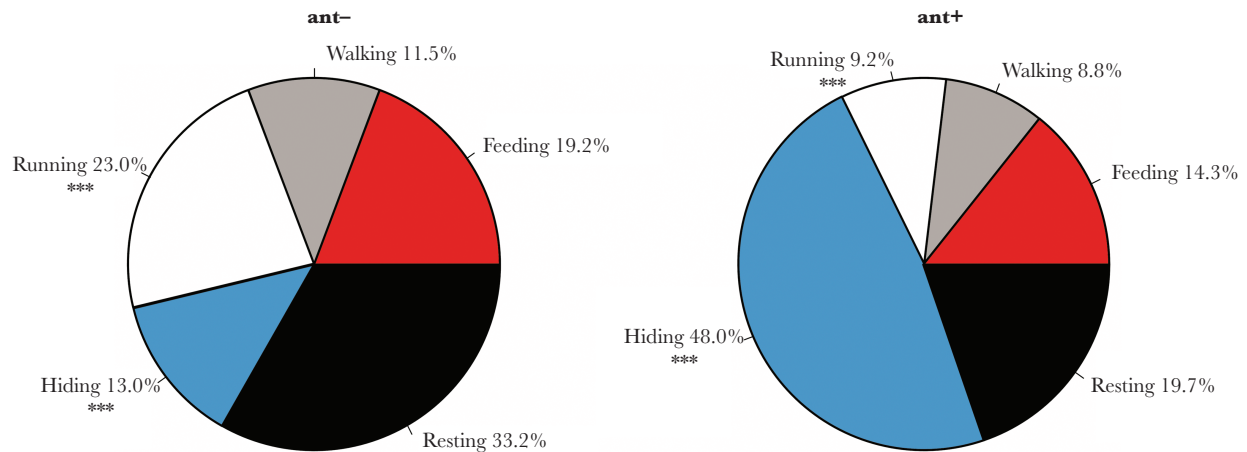
Figure 4

Behavioral repertoire of *T. angulata* with (ant+) and without (ant-) its host. The mean proportion (50 unique beetles tested in each treatment) that a behavior was recorded out of 45 snap-shot observations (every 2 min) in the presence or absence of ants is given for 5 behaviors in a pie chart. The frequencies of hiding and running were significantly different ($P < 0.001$, indicated with ***) with a Bonferroni-corrected $\alpha = 0.010$.

specialized beetles that capitalize on host-specific deception strategies, *T. angulata* can easily target multiple host species using its prudent behavior given that there are enough hiding places in the nest. *T. angulata* shows moderate host specificity. It associates with multiple mound-building *Formica* species and *Lasius* ants with arboreal nests (Parmentier *et al.* 2014). These nests are rather unusual as they all are stuffed with organic material rather than inorganic soil material. They provide many more hiding places than soil nests. It appears that the beetles are adapted to a life in an organic ant nest but show few adaptations to the host itself. Interestingly, the beetle can even survive well in lab nests with

hiding possibilities of nonhosts such as *F. cunicularia* (Parmentier *et al.* 2016c).

Species coexistence theory also predicts that generalist parasites should show wider resource use, but be less efficient in exploiting the resources shared with specialists (Futuyma and Moreno 1988; Sasal *et al.* 1999; Kneitel and Chase 2004). *T. angulata* shows little specificity in resource use as it lives as a scavenger and feeds on brood as well as dead and living prey and may feed on organic nest material as well (Parmentier *et al.* 2016b). They are probably less successful in exploiting the host than highly specialized ant parasites such as *Maculinea* caterpillars (Thomas and Elmes 1998). We

did not observe that *T. angulata* fed less on dead prey in the presence of its hosts. However, in a previous study, we showed that the beetle consumed considerably less brood when ants were present (Parmentier *et al.* 2015b). Theoretical models and empirical studies (Martin 1999; Jennions *et al.* 2003; Cooper and Frederick 2007) underline that hiding time is a trade-off between costs of not obtaining resources outside the refuge and benefits of protection against host aggression. The generalist species *T. angulata* provokes aggression and needs to balance risky foraging activities with rest periods in refuges, whereas specialized species can continuously exploit the host without being noticed (Hölldobler and Wilson 1990; von Beeren *et al.* 2018).

A growing number of spectacular examples of host deception by parasites are being discovered. These parasites typically deploy very specialized chemical, morphological, and acoustic trickery to gain access to the host's resources (Saul-Gershenz and Millar 2006; Bush *et al.* 2010; Flower 2011; Mokkonen and Lindstedt 2016). Unspecialized parasites, in contrast, tend to draw much less attention in literature and their strategies are underexplored. Here, we found an unspecialized parasite that does not deceive its host, but relies on simple prudent behavioral strategies and general defensive volatiles to exploit the host's resources. Further studies on unspecialized parasites could help us to better understand the role of behavior in the origin of parasitism and its evolution towards more specialized strategies.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/.

FUNDING

Data Accessibility Statement: Analyses reported in this article can be reproduced using the data provided by Parmentier *et al.* (2018).

Handling Editor: Luke Holman

REFERENCES

Aitchison J. 1986. The statistical analysis of compositional data. London: Chapman and Hall.

Akino T. 2008. Chemical strategies to deal with ants : a review of mimicry, camouflage, propaganda, and phytomimesis by ants (Hymenoptera : Formicidae) and other arthropods. Myrmecological News. 11:173–181.

Akino T, Mochizuki R, Morimoto M, Yamaoka R. 1996. Chemical camouflage of myrmecophilous cricket *Myrmecophilus* sp. to be integrated with several ant species. Jpn J Appl Entomol Zool. 40:39–46.

Bagnères AG, Lorenzi MC. 2010. Chemical deception/mimicry using cuticular hydrocarbons. In: Blomquist G, Bagnères A-G, editors. Insect hydrocarbons biology, biochemistry, and chemical ecology. New York: Cambridge University Press. p. 282–324.

Barbero F, Thomas JA, Bonelli S, Balletto E, Schönrogge K. 2009. Queen ants make distinctive sounds that are mimicked by a butterfly social parasite. Science. 323:782–785.

von Beeren C, Brückner A, Maruyama M, Burke G, Wieschollek J, Kronauer DJC. 2018. Chemical and behavioral integration of army

ant-associated rove beetles - a comparison between specialists and generalists. Front Zool. 15:8.

von Beeren C, Hashim R, Witte V. 2012. The social integration of a myrmecophilous spider does not depend exclusively on chemical mimicry. J Chem Ecol. 38:262–271.

von Beeren C, Maruyama M, Hashim R, Witte V. 2010. Differential host defense against multiple parasites in ants. Evol Ecol.. 25:259–276.

von Beeren C, Schulz S, Hashim R, Witte V. 2011. Acquisition of chemical recognition cues facilitates integration into ant societies. BMC Ecol. 11:30.

Blum MS, Crewe RM, Pasteels JM. 1971. Defensive secretion of *Lomechusa strumosa*, a myrmecophilous beetle. Ann Entomol Soc Am.. 64:975–976.

Browne WJ, Subramanian SV, Jones K, Goldstein H. 2005. Variance partitioning in multilevel logistics models with over-dispersion. J R Stat Soc A. 168:599–613.

Bush SE, Kim D, Reed M, Clayton DH. 2010. Evolution of cryptic coloration in ectoparasites. Am Nat. 176:529–535.

Caro TM. 2005. Antipredator defense in birds and mammals. Chicago: The University of Chicago Press.

Cooper WE, Frederick WG. 2007. Optimal time to emerge from refuge. Biol J Linn Soc. 91:375–382.

Davies NB, Kilner RM, Noble DG. 1998. Nestling cuckoos, *Cuculus canorus*, exploit hosts with begging calls that mimic a brood. Proc R Soc B Biol Sci. 265:673–678.

Dewitt TJ, Sih A, Hucko JA. 1999. Trait compensation and cospecialization in a freshwater snail: size, shape and antipredator behaviour. Anim Behav. 58:397–407.

Dill LM, Fraser AHG, Va BC. 1997. The worm re-turns: hiding behavior of a tube-dwelling marine polychaete, *Serpula vermicularis*. Behav Ecol. 8:186–193.

Dorosheva EA, Yakovlev IK, Reznikova ZI. 2011. An innate template for enemy recognition in red wood ants. Entomol Rev.. 91:274–280.

Elgar MA, Allan RA. 2004. Predatory spider mimics acquire colony-specific cuticular hydrocarbons from their ant model prey. Naturwissenschaften. 91:143–147.

Flower T. 2011. Fork-tailed drongos use deceptive mimicked alarm calls to steal food. Proc Biol Sci. 278:1548–1555.

Freude H, Harde KW, Lohse GA. 1974. Käfer Mitteleuropas. Bd 5. Staphylinidae II (Hypocyphtinae und Aleocharinae), Pselaphidae. Krefeld: Goecke et Evers Verlag.

Futuyma DJ, Moreno G. 1988. The evolution of ecological specialization. Annu Rev Ecol Syst.. 19:207–233.

Geiselhardt SF, Geiselhardt S, Peschke K. 2006. Chemical mimicry of cuticular hydrocarbons – How does *Eremostibes opacus* gain access to breeding burrows of its host *Parastizopus armaticeps* (Coleoptera, Tenebrionidae)? Chemoecology. 16:59–68.

Goropashnaya AV, Fedorov VB, Seifert B, Pamilo P. 2012. Phylogenetic relationships of Palaearctic *Formica* species (Hymenoptera, Formicidae) based on mitochondrial cytochrome B sequences. PLoS One. 7:e41697.

Härkönen SK, Sorvari J. 2014. Species richness of associates of ants in the nests of red wood ant *Formica polyctena* (Hymenoptera, Formicidae). Insect Conserv Divers. 7:485–495.

Hedrick AV, Kortet R. 2006. Hiding behaviour in two cricket populations that differ in predation pressure. Anim Behav.. 72:1111–1118.

Hojo MK, Wada-Katsumata A, Akino T, Yamaguchi S, Ozaki M, Yamaoka R. 2009. Chemical disguise as particular caste of host ants in the ant inquiline parasite *Niphanda fusca* (Lepidoptera: Lycaenidae). Proc Biol Sci. 276:551–558.

Hölldobler B, Wilson EO. 1990. The ants. Cambridge (Massachusetts): Belknap Press of Harvard University Press.

Jennions MD, Backwell PRY, Murai M, Christy JH. 2003. Hiding behaviour in fiddler crabs: how long should prey hide in response to a potential predator? Anim Behav.. 66:251–257.

Kistner DH. 1979. Social and evolutionary significance of social insect symbionts. In: Hermann HR, editor. Social insects. Vol. 1. New York: Academic press. p. 339–411.

Kistner DH, Blum MS. 1971. Alarm pheromone of *Lasius* (*Dendrolasius*) *spathepus* (Hymenoptera: Formicidae) and its possible mimicry by two species of *Pella* (Coleoptera: Staphylinidae). Ann Entomol Soc Am.. 64:589–593.

Kneitel JM, Chase JM. 2004. Trade-offs in community ecology: linking spatial scales and species coexistence. Ecol Lett. 7:69–80.

Koop JA, Huber SK, Clayton DH. 2012. Does sunlight enhance the effectiveness of avian preening for ectoparasite control? J Parasitol. 98:46–48.

Kronauer DJ, Pierce NE. 2011. Myrmecophiles. Curr Biol. 21:R208–R209.

Le Conte Y, Huang ZY, Roux M, Zeng ZJ, Christidès JP, Bagnères AG. 2015. *Varroa destructor* changes its cuticular hydrocarbons to mimic new hosts. Biol Lett. 11:20150233.

Lenoir A, D'Ettorre P, Errard C, Hefetz A. 2001. Chemical ecology and social parasitism in ants. Annu Rev Entomol. 46:573–599.

Lenoir A, Háva J, Hefetz A, Dahbi A, Cerdá X, Boulay R. 2013. Chemical integration of *Thorictus* myrmecophilous beetles into *Cataglyphis* ant nests. Biochem Syst Ecol.. 51:335–342.

Lima SL, Dill LM. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool.. 68:619–640.

Ma Z, Li W, Zhao Y, Wang W, Zhang H, Li Z. 2009. Effects of prey refuges on a predator-prey model with a class of functional responses: the role of refuges. Math Biosci. 218:73–79.

Martin J. 1999. When to come out from a refuge: risk-sensitive and state-dependent decisions in an alpine lizard. Behav Ecol.. 10:487–492.

Martin SJ, Takahashi J, Ono M, Drijfhout FP. 2008. Is the social parasite *Vespa dybowskii* using chemical transparency to get her eggs accepted? J Insect Physiol. 54:700–707.

Messadi D, Helaimia F, Ali-Mokhnache, S, Boumahraz M. 1990. Accurate determination of retention indices in programmed temperature gas chromatography. Chromatographia. 29:429–434.

Mokkonen M, Lindstedt C. 2016. The evolutionary ecology of deception. Biol Rev Camb Philos Soc. 91:1020–1035.

Nash DR, Boomsma JJ. 2008. Communication between hosts and social parasites. In: D'Ettorre P, Hughes DP, editors. Sociobiology of communication: an interdisciplinary perspective. New York (USA): Oxford University Press. p. 55–65.

Nazzi F, Le Conte Y. 2016. Ecology of *Varroa destructor*, the major ectoparasite of the western honey bee, *Apis mellifera*. Annu Rev Entomol. 61:417–432.

Päivinen J, Ahlroth P, Kaitala V, Suhonen J. 2004. Species richness, abundance and distribution of myrmecophilous beetles in nests of *Formica aquilonia* ants. Ann Zool Fenn. 41:447–454.

Parmentier T, Dekoninck W, Wenseleers T. 2014. A highly diverse microcosm in a hostile world: a review on the associates of red wood ants (*Formica rufa* group). Insectes Soc.. 61:229–237.

Parmentier T, Dekoninck W, Wenseleers T. 2015a. Metapopulation processes affecting diversity and distribution of myrmecophiles associated with red wood ants. Basic Appl Ecol.. 16:553–562.

Parmentier T, Dekoninck W, Wenseleers T. 2015b. Context-dependent specialization in colony defence in the red wood ant *Formica rufa*. Anim Behav.. 103:161–167.

Parmentier T, Dekoninck W, Wenseleers T. 2016a. Do well-integrated species of an inquiline community have a lower brood predation tendency? A test using red wood ant myrmecophiles. BMC Evol Biol. 16:12.

Parmentier T, Bouillon S, Dekoninck W, Wenseleers T. 2016b. Trophic interactions in an ant nest microcosm: a combined experimental and stable isotope (δ13C/δ15N) approach. OIKOS. 125:1182–1192.

Parmentier T, Dekoninck W, Wenseleers T. 2016c. Survival of persecuted myrmecophiles in laboratory nests of different ant species can explain patterns of host use in the field (Hymenoptera : Formicidae). Myrmecological News. 23:71–79.

Parmentier T, Dekoninck W, Wenseleers T. 2017. Arthropods associate with their red wood ant host without matching nestmate recognition cues. J Chem Ecol. 43:644–661.

Parmentier T, De Laender F, Wenseleers T, Bonte D. 2018. Data from: prudent behavior rather than chemical deception enables a parasite to exploit its ant host. Dryad Digital Repository. https://doi:10.5061/dryad.f2n09d3

Sasal P, Trouvé S, Müller-Graf C, Morand S. 1999. Specificity and host predictability: a comparative analysis among monogenean parasites of fish. J Anim Ecol.. 68:437–444.

Saul-Gershenz LS, Millar JG. 2006. Phoretic nest parasites use sexual deception to obtain transport to their host's nest. Proc Natl Acad Sci USA. 103:14039–14044.

Steidle JLM, Dettner K. 1993. Chemistry and morphology of the tergal gland of freeliving adult Aleocharinae (Coleoptera: Staphylinidae) and its phylogenetic significance. Syst Entomol.. 18:149–168.

Stevens M, Merilaita S. 2009. Animal camouflage: current issues and new perspectives. Philos Trans R Soc Lond B Biol Sci. 364:423–427.

Stoddard MC, Stevens M. 2010. Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. Proc Biol Sci. 277:1387–1393.

Stoeffler M, Maier TS, Tolasch T, Steidle JL. 2007. Foreign-language skills in rove-beetles? Evidence for chemical mimicry of ant alarm pheromones in myrmecophilous *Pella* beetles (Coleoptera: Staphylinidae). J Chem Ecol. 33:1382–1392.

Stoeffler M, Tolasch T, Steidle JLM. 2011. Three beetles - three concepts. Different defensive strategies of congeneric myrmecophilous beetles. Behav Ecol Sociobiol. 65:1605–1613.

Thomas JA, Elmes GW. 1998. Higher productivity at the cost of increased host-specificity when *Maculinea* butterfly larvae exploit ant colonies through trophallaxis rather than by predation. Ecol Entom. 23:457–464.

Vander Meer RK, Wojcik DP. 1982. Chemical mimicry in the myrmecophilous beetle *Myrmecaphodius excavaticollis*. Science. 218:806–808.

Wisenden BD, Chivers DP, Smith RJF. 1997. Learned recognition of predation risk by *Enallagma* damselfly larvae (Odonata, Zygoptera) on the basis of chemical cues. J Chem Ecol.. 23:137–151.

Zagaja M, Staniec B, Pietrykowska-Tudruj E, Trytek M. 2017. Biology and defensive secretion of myrmecophilous *Thiasophila* spp. (Coleoptera: Staphylinidae: Aleocharinae) associated with the *Formica rufa* species group. J Nat Hist. 00:1–19.

van Zweden JS, d'Ettorre P. 2010. Nestmate recognition in social insects and the role of hydrocarbons. In: Blomquist G, Bagnères A-G, editors. Insect hydrocarbons biology, biochemistry and chemical ecology. New York: Cambridge University Press. p. 222–243.