Survival of persecuted myrmecophiles in laboratory nests of different ant species can explain patterns of host use in the field (Hymenoptera: Formicidae)

Thomas PARMENTIER, Wouter DEKONINCK & Tom WENSELEERS

Abstract
Myrmecophiles or ant associates are able to penetrate and survive inside the heavily defended nests of various ant species. With the exception of some highly specialized species, many of these myrmecophiles elicit a highly aggressive response and are frequently wounded or even killed by their hosts. Many myrmecophiles also appear to strongly prefer particular host species. The factors that allow the myrmecophiles to survive in these hostile environments and cause myrmecophiles to prefer particular host species are largely unknown. The aim of the present study was to examine the impact of the presence or absence of either the preferred host (wood ants of the Formica rufa LINNAEUS, 1761 group) or one of several nonpreferred ant species on the long-term survival of three obligate, unspecialized beetle myrmecophiles, Thiasophila angulata (ERICHSON, 1837), Lypocorcorhe anceps (ERICHSON, 1837), and Amidobia talpa (HEER, 1841), and one facultative myrmecophile, the woodlouse Porcellio scaber LATREILLE, 1804. In addition, we tested whether host specificity was driven by the size of the ant host workers, because host specificity has previously been demonstrated to be inversely related to aggression towards macroparasites. Our results show that despite regular aggressive host interactions, survival of the obligate myrmecophilous beetles over a period of 20 days was not different from a control set-up without ants. By contrast, the facultative ant associate P. scaber hardly provoked any aggressive host response, but its survival was lower in presence of F. rufa workers compared with a control set-up without ants. Furthermore, the data on survival in presence of nine different ant host species show that the three obligate myrmecophile beetles survived better in presence of larger-bodied ant species, and that their survival was highest in presence of their preferred host F. rufa, which also has relatively large workers. The only exception to this trend was the low survival observed in presence of the large-bodied ant Canponotus vagus (SCOPOLI, 1763). Finally, species that were less successful in killing the beetles in our tests are also shown to support more myrmecophilous rove beetles in nature. Overall, our results shed new light on the interaction between ants and various associated macroparasites and on the factors that drive observed host preferences.

Key words: Ant guests, associates, aggression, parasitism, facultative myrmecophile, obligate myrmecophile, diversity, inquiline.

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Introduction
Parasites have an intricate relationship with their host on which they can impose substantial costs (POULIN 2011). However, hosts have evolved an array of defence strategies at the behavioural, immunological and chemical level to counter parasites (HART 1990, CLAYTON & MOORE 1997, SCHMID-HEMPEL 2011). A particularly useful system to test host-parasite interactions can be found in the nests of social insects. Social insect nests harbour a rich diversity of strictly associated symbionts including mutualists, commensals, and parasites (KISTNER 1979, HOLLDOBLER & WILSON 1990, RETTENMEYER & al. 2011). The parasites can have a dramatic effect on host fitness by consuming brood and host resources and inducing queen and worker mortality (HOLLDOBLER & WILSON 1990, SCHMID-HEMPEL 1998, GEISELHARDT & al. 2007, BUSCHINGER 2009, HOVESTADT & al. 2012). The main defence response of social insects to macroparasites is aggression, in which they exhibit biting, stinging, spraying defensive chemicals and chasing of the intruders (HOLLDOBLER & WILSON 1990, AYASSE & PAXTON 2002). Some ant associates or myrmecophiles evolved a specialized biology (symplymes or true guests sensu WASMANN 1894) and employ a plethora of strategies, including advanced behaviours, morphological adaptations, special defensive or appeasement...
glands and chemical mimicry. Such adaptations might lower ant aggression and enable the myrmecophiles to successfully integrate in ant colonies. What is more, they are treated as true colony members as they are fed, groomed and transported by the ants (HOLLDÖBLER & WILSON 1990, AKINO 2008, BLOMQUIST & BAGNÈRES 2010, KRONAUER & PIERCE 2011). However, some myrmecophiles are seemingly unspecialized (synecithrans, i.e., indifferently tolerated guests, and synoeketes, i.e., hostile persecuted guests, sensu WASMANN 1894): They are very similar to their non-myrmecophilous counterparts and lack the aforementioned variety of adaptations (DONISTHORPE 1927, HOLLDÖBLER & WILSON 1990). These myrmecophiles might be exposed to frequent ant aggression (DONISTHORPE 1927, PARMENTIER & al. 2015a), which can lead to an elevated stress response in the myrmecophiles, injuries and ultimately death (HOLLDÖBLER & al. 1981, NELSON & JACKSON 2009; T. Parmentier, W. Dekoninck & T. Wenseleers, unpubl.).

It is surprising how these myrmecophiles succeed to live in association with their host in such a hostile and stressful environment. The long-term effects of the host’s defence response for those unspecialized myrmecophiles are unknown. Therefore, the effect of the association with host ants on the survival of three unspecialized, parasitic and myrmecophilous rove beetles associated with European red wood ants (Formica rufa group) was examined. First, 20-day survival of those myrmecophiles with the preferred host against survival in a control set-up without host workers was tested. The same tests were also done for a facultative myrmecophile (a species that is regularly found in ant nests, but is mainly found not to be associated with ants) to look whether the effect of host ants is similar on them compared with unspecialized myrmecophiles.

Surprisingly, many unspecialized myrmecophiles are associated with only a small group of ants (DONISTHORPE 1927, PAIVINEN & al. 2002, PARMENTIER & al. 2014). The myrmecophiles of this study are restricted to mound building Formica species. It is unclear why these relatively unspecialized myrmecophiles are only associated with mound building Formica species. Hitherto, it is unknown which mechanisms constrain the distribution of these species. A recent study showed that smaller workers in polymorphic red wood ant colonies are more aggressive and more successful in deterring intranidal myrmecophiles (PARMENTIER & al. 2015a). Consequently, the hypothesis under investigation is that ant species with on average smaller workers are more efficient in deterring unspecialized myrmecophiles. Interestingly, the mound building Formica ants have on average relatively large workers compared with other ant species in Europe (SEIFERT 2007) and support many unspecialized myrmecophiles (PARMENTIER & al. 2014). The relatively large mean worker size of these ants compared with other ant species in Europe could play a role in the strict association of many of those Formica associates. For that reason, we also assessed the survival of the three myrmecophilous beetle species in nests of eight other ant species spanning a gradient from one of the smallest to the largest ant species in the study area. We hypothesized that the survival rate of the unspecialized myrmecophiles would be highest in species with relatively large workers and would decrease in colonies of smaller ant species.

Materials and methods

Study species: We collected adults of three myrmecophilous rove beetles (Staphylinidae, Aleocharinae): Thiasophila angulata (ERICHSON, 1837), Lypocrorhie anceps (ERICHSON, 1837), and Amidobia talpa (HEER, 1841) in European red wood ant (Formica rufa group) nests in populations in Northern Belgium and in Northern France during the summer and autumn of 2014 and spring and summer of 2015 (Fig. 1). Two populations (West-Vleteren, Boeschepe) consisted of F. rufa LINNAEUS, 1761 mounds, three of Formica polyctena FORSSTER, 1850 (Beernem, Roksem, Aartrijke) mounds and both species occur sympatrically in the two remaining populations (De Haan and Beisbroek) (map see PARMENTIER & al. 2015b). Beetles were identified following FREUDE & al. (1974). We isolated the myrmecophiles by spreading nest material of F. rufa or F. polyctena nests on a large tray in the field. Ants and their brood were gently put back in the nests afterwards. DONISTHORPE (1927) categorized the three beetle species following the classification of Erich Wasmann as synoeketes, which means that the beetles are rather unspecialized in morphology and behaviour compared with advanced myr-

Fig. 1: Overview of the three myrmecophile beetles with their red wood ant host: (a) Thiasophila angulata with Formica polyctena, (b) Lypocrorhie anceps with Formica rufa, and (c) Amidobia talpa with Formica polyctena. The myrmecophilous spider Thyreostenius biovatus can also be observed in the centre of b.
mechophiles (symphiles) (WASMANN 1894). Synoeketes are not treated as colony members, but mostly ignored by the ants due to their small size and behaviour (WASMANN 1894). However, we found that the three species are detected by the ants and elicited aggression (PARMENTIER & al. 2015a, supplementary videos, Appendix S1: A. talpa, Appendix S2: L. aniceps, and Appendix S3: T. angulata, as digital supplementary material to this article, at the journal’s web pages). Therefore they should rather be categorized in the group of synechtrans (unspecified associates which provoke aggression). The complete life cycle of the beetles probably takes place inside the wood ant mounds (DONISTHORPE 1927). This was supported by the occurrence of the adults in all seasons and the recording of larvae of different stages of the beetles inside the mound from spring to autumn (and raised in the lab to adults for identification). The larvae are free-living scavengers and are not nursed or carried by the workers (T. Parmentier, W. Dekoninck & T. Wenseleers, unpubl.) in contrast with specialized beetle larvae such as Lomechusa and Lomechusoides (see HOLLDOBLER & WILSON 1990). The larvae of T. angulata are very similar to non-myrmechophilous larvae of the Aleocharinae and can be reared in absence of ants (ZAGAJA & al. 2014; M. Zagaja, unpubl.). The adults are both brood predators and kleptoparasites as they prey on ant brood and food brought to the nest (PARMENTIER & al. 2015a, in press). The three beetle species can be found throughout the nest mound (edge and centre of the nest). Lyprocorrhæ aniceps and A. talpa have no nest location preference, whereas T. angulata is attracted to the densely crowded brood chambers (PARMENTIER & al. 2016). Morphological adaptations found in specialized myrmechophiles such as appeasement glands with trichomes are lacking in the three beetles (FREUDE & al. 1974). FREUDE & al. (1974) only report that the segments of the antennae of T. angulata are slightly compressed which could make it more difficult for ants to grab them. Their behaviour is also very similar to non-ant associated rove beetles. They escape from ant aggression by fleeing, hiding or bending their abdomen (DONISTHORPE 1927, PARMENTIER & al. 2015a; Appendices S1 - S3). They probably excrete chemicals from their bent abdomen, which is a general defence strategy of rove beetles (HUTH & DETTNER 1990). The three beetles have a similar aleocharine morphology, but differ in size (T. angulata mean length 10 individuals ± SD = 2.85 mm ± 0.32, L. aniceps mean length 10 individuals ± SD = 2.16 mm ± 0.20, A. talpa mean length 10 individuals ± SD = 1.53 mm ± 0.10, Fig. 1). In spite of their unspecialized myrmechophilous biology, they are very specialized in their host use. Their distribution is mainly restricted to European red wood ants (F. rufa group) (DONISTHORPE 1927, FREUDE & al. 1974, PARMENTIER & al. 2014). There are also some records for all three species in related mound building Formica species. The three species were occasionally observed in nests of Lasius fuliginosus (LATREILLE, 1798) and there is a single record of T. angulata in Lasius brunneus (LATREILLE, 1798) (see references in PARMENTIER & al. 2014), but these are probably infrequently used hosts (T. Parmentier, W. Dekoninck & T. Wenseleers, unpubl.). The three beetles are obligate myrmechophiles, as they cannot be found away from ants. However, a large number of species can occasionally be associated with ants (DONISTHORPE 1927, ROBINSON & ROBINSON 2013, PARMENTIER & al. 2014). The widespread isopod Porcellio scaber LATREILLE, 1804 (adult size: 9 - 13.5 mm, BERG & WUNHOVEN 1997, identified following BERG & WUNHOVEN 1997) lives in a wide variety of habitats without ants (BERG & WUNHOVEN 1997), but can also be very abundant in red wood ant mounds throughout the whole year (ROBINSON & ROBINSON 2013, PARMENTIER & al. 2014). Gravid females and juveniles were regularly observed in the mounds, which indicates that P. scaber is able to reproduce in the mounds. Isopods were collected in the same way as myrmechophilous beetles in red wood ant nests during spring 2015.

Ant aggression towards tested species: First, the interaction of Formica rufa with the three myrmechophilous beetle species and Porcellio scaber was examined. Therefore, a small rectangular plastic arena (length: 8 cm, width: 5.5 cm, height: 5 cm) was filled with ca. 1 cm plaster of Paris and coated with fluon. Forty F. rufa workers (West-Vleteren population) were acclimatized for one hour to the arena and then a myrmechophile found in the same colony was added. Ten seconds after the myrmechophile was introduced, the first twenty interactions with the ants were scored. In spite of these relatively short settling times, ants and myrmechophiles interacted similarly as in conditions where myrmechophiles were already integrated for days in lab nests (T. Parmentier, unpubl.). We also provide data of the effect of longer settling time (one hour) on ant aggression towards seven Thiasophila angulata beetles and compare these with the 10 s settling times (Appendix S4). These data confirm that longer settling times had no significant effect on the interaction between ants and myrmechophiles. Following interactions were observed from the perspective of the ant: ignoring (a worker’s behaviour did not change when her antenna crossed the myrmechophile), showing interest (a worker started to antennate, turned her head or stopped walking or grooming when her antenna crossed the myrmechophile), opening mandibles (a worker aggressively opened her mandibles when her antenna crosses the myrmechophile), biting (a worker snapped with its mandibles and tried to grasp a myrmechophile) and acid spraying (a worker bent her gaster and sprayed acid after her antenna crossed the myrmechophile). Biting and acid spraying often followed directly after opening mandibles. In these cases only the last interaction was recorded. Ant aggression was scored by the proportion of aggressive interactions (acid spraying, biting, opening mandibles) out of the first 20 interactions. From the perspective of the myrmechophiles, the number out of 20 interactions that were directly preceded or followed by abdomen bending were counted. Trials were performed in darkness under red light and were recorded with a video camera (SONY HDR-XR550VE). Videos were subsequently analysed in VirtualDub 1.10.4 (http://www.virtualdub.org) which allowed to watch videos frame by frame.

Survival experiment: In this experiment, 20-day survival of the three beetle species in Formica rufa nests were compared with their survival in a control set-up without ants. Formica rufa workers were collected in a highly polydomous population in Boeschepe, Northern France. In addition, survival of the three beetle species in nests of other ant species, ranging from one of the smallest to the largest ant species in the study region, were tested. Therefore colony fragments of Solenopsis fugax (LATREILLE,
1798) (Eastern bank river Meuse, Dinant), *Tetramorium caespitum* (Linnaeus, 1758) (Duinbosken, Lombardsijde), *Lasius niger* (Linnaeus, 1758) (urban region, Oostende), *Myrmica ruginodis* Nylander, 1846 (St-Sixtusbosken, West-Vleteren), *Formica curculio* Latreille, 1798 (Duinbosken, Lombardsijde), and *Lasius fuliginosus* (Prov. Vinciedomein, Raversijde; Aartrijkskesteeg, Aartrijke) were collected in different sites across Belgium during the summer and autumn of 2014 and spring and summer of 2015. Survival was also tested with *Monomorium pharaonis* (Linnaeus, 1758) and *Camponotus vagus* (Scopolli, 1763) of which we already had established lab colonies. *Monomorium pharaonis* is an indoor pest in Belgium and does not occur outside buildings (Dekoninck et al. 2003). Several colonies of *C. vagus* have only recently established in Belgium and are able to persist outdoors (Dekoninck & Pauly 2002; W. Dekoninck, unpubl.). Ants were identified using the key provided in Seifert (2007).

Within one day after collecting the myrmecophiles, between 9 and 13 individuals of each rove beetle species were placed together in 1 L plastic, cylindrical containers (diameter: 8.5 cm, height: 13.5 cm) with a 1.5 - 2.0 cm bottom of plaster of Paris. The top 5 cm inner wall of the containers were coated with flour to prevent ants and myrmecophiles from escaping through ventilation pin holes made in the container’s lid. Myrmecophiles were collected in different red wood ant populations (*Formica polyctena* and *Formica rufa*) across West Flanders, Belgium and in Boeschepe, France to obtain sufficient numbers of individuals (Appendix S5; Tab.). The tested myrmecophiles do not closely resemble the cuticular hydrocarbon profile of their red wood ant host colony (T. Parmentier, W. Dekoninck & T. Wenseleers, unpubl.). Moreover, conspecific beetles associated with *F. polyctena* or *F. rufa* do not substantially differ in their cuticular chemical profile (T. Parmentier, W. Dekoninck & T. Wenseleers, unpubl.). This lack of chemical adaptation to their host is further confirmed by aggression tests (Parmentier & al. 2016; Additional file; Tab. S3). In these tests, we compared aggression of *F. rufa* workers of one colony (West-Vleteren, description see Parmentier & al. 2015b) towards myrmecophiles collected in the same colony with their aggression towards myrmecophiles found in *F. polyctena* colonies. Interestingly, the aggression response of the *F. rufa* workers was not significantly different towards beetles collected in *F. rufa* or *F. polyctena* colonies. Based on these chemical and behavioural data, we argue that the myrmecophile's colony of origin did not significantly affect the results of the survival experiments. Another confounding factor that might influence myrmecophile survival in our experiments is intra- and interspecific competition. However, no aggression between the beetles was observed. By providing food ad libitum, negative competition effects on survival were minimalized. Depending on the treatment, 100 workers of either *F. rufa*, *F. curculio*, *Lasius fuliginosus*, *L. niger*, *Myrmica ruginodis*, *Tetramorium caespitum*, *Monomorium pharaonis*, or *Solenopsis fugax* were added. Because of their large size, only 50 workers of *Camponotus vagus* were used (cf. Fig. 3). For polymorphic species workers of all worker subcastes were used (Fig. 3). Workers were randomly picked from nests, hence we assume that all worker subcastes (or size cohorts) are represented in numbers similar to their natural distribution. In addition, between 9 and 13 individuals per beetle species were added to containers described as above, but without adding ants. These containers served as controls. Survival of myrmecophiles was monitored every two days for a total period of 20 days. Two cut maggots (larvae of *Phae- nicia sericata*), an Eppendorf tube (1.5 mL) filled with water and one with honey water were provided. The same food sources were offered in the same quantities in the control containers. Eppendorf tubes were sealed with a cotton plug soaked in either water or honey water. Maggots were replaced every two days, honey water every four days. Dead ant workers were replaced by new workers of the corresponding stock colonies every two days. Corpses of myrmecophiles were also removed to prevent contamination. The containers were kept in constant dark and at room temperature (20°C ± 2°C). Every treatment was replicated between eight and ten times (Appendix S5: Tab.) with workers of another colony, except for *S. fugax*, *M. pharaonis* and *C. vagus* where we only had one colony at our disposal. For these species, different workers per replicate were used, but from the same (super)colony. Nest material of ant nests was not added to the containers. However, myrmecophiles were able to hide under dead ants, prey, Eppendorf tubes and cotton made loose by the ants.

Similarly, survival of the facultative nest-inhabitant *Porcellio scaber* was evaluated in *Formica rufa* nests and in a control set-up. Thirty specimens were monitored for 20 days in the plastic containers described above. Individuals were counted every four days. The treatment group with 100 *F. rufa* workers and the control were compared and replicated eight times (in total 8 × 30 = 240 individuals were tested per treatment). Water and honey water as well as two slices of carrot were provided. The latter were replaced every four days. Dead isopods were removed and dead workers were replaced every four days. For this facultative myrmecophile we also compared survival in containers with addition of 25 mL nest material in an additional experiment. We were interested whether we would observe the same effect of the ants on the isopods with much more hiding places in the nests. Nest material was taken of a deserted *F. rufa* nest and was replaced after 10 days. Here, we only counted survivors after 20 days in a treatment with 100 *F. rufa* workers and a in control treatment without workers (in total 9 × 30 = 270 individuals were tested with *F. rufa* and 8 × 30 = 240 were tested in the control treatment).

**Worker size:** Maximum head width of the ant species used in the survival experiment was measured. This allows us to link the mean worker size of ant species with their efficiency in killing the myrmecophilous beetles. For each ant species, maximum head width from a random set of workers was measured. More workers were measured for a given species when it showed a high degree of polymorphism (*N* = 30 for *Solenopsis fugax*, *Tetramorium caespitum*, *Monomorium pharaonis*, and *Myrmica ruginodis*, *N* = 50 for *Formica curculio* and *Lasius niger*, *N* = 100 for *Formica rufa* and *Camponotus vagus*).

**Data analysis:** All tests were two-tailed and a significance level of *α* = 0.05 was used. The proportion of aggressive interactions towards the four associates were modelled with a quasibinomial GLM (family = quasibinomial in function glm) to account for overdispersion and tested with a likelihood ratio test. Subsequently, a set of quasi-
binomial GLMs were conducted to compare post hoc the proportion of aggressive interactions between the four associates. P-values of these six pairwise tests were Bonferroni corrected.

In the survival analyses, survival of the three obligate myrmecophilous beetles subjected to 10 different treatments was evaluated. In particular, the survival per beetle species in nests of *Formica rufa*, in nests of eight other ant species and in a control set-up were tested against each other. Survival data per myrmecophile species were fitted with a mixed-effects Cox proportional-hazards model by using the coxme function implemented in R version 3.2.1 (R CORE TEAM 2012). This package allows the incorporation of random factors (THERNEAU 2012). In the Cox proportional-hazards model, we test whether the hazard ratio of a treatment is significantly different from 1 (COX 1972). The hazard ratio can be interpreted in our experiment as the mortality rate in a particular treatment relative to the mortality rate of a reference treatment. Treatment (i.e., ant species and control) was used as a fixed factor, replicate was modelled as a random factor. In a series of pairwise tests, survival of myrmecophiles in treatments with different ant species and the control set-up against survival in nests of *F. rufa* (reference level) was compared. P-values were estimated with a likelihood ratio test (Anova function in car package) and Bonferroni corrected. In particular, the survival per beetle species in treatments with different ant species and in a control set-up were tested against each other. Survival data per myrmecophile species were fitted with a mixed-effects Cox proportional-hazards model by using the coxme function implemented in R version 3.2.1 (R CORE TEAM 2012). This package allows the incorporation of random factors (THERNEAU 2012). In the Cox proportional-hazards model, we test whether the hazard ratio of a treatment is significantly different from 1 (COX 1972). The hazard ratio can be interpreted in our experiment as the mortality rate in a particular treatment relative to the mortality rate of a reference treatment. Treatment (i.e., ant species and control) was used as a fixed factor, replicate was modelled as a random factor. In a series of pairwise tests, survival of myrmecophiles in treatments with different ant species and the control set-up against survival in nests of *F. rufa* (reference level) was compared. P-values were estimated with a likelihood ratio test (Anova function in car package) and Bonferroni corrected.

For the facultative associate *Porcellio scaber*, survival data of the experiment without nest material (control vs. treatment with 100 *Formica rufa* workers), were similarly fitted with a mixed-effects Cox proportional-hazards model and significance tested with a likelihood ratio test. As we did not count *P. scaber* individuals at regular time intervals in the extra experiment with nest material, we could not do a survival analysis here. In this experiment, we only compared the proportion of surviving isopods (out of 30) after 20 days in a control set-up versus a treatment with 100 *F. rufa* workers with a quasibinomial GLM. Significance was tested with a likelihood ratio test.

### Results

**Aggression of Formica rufa towards beetles and Porcellio scaber:** Ants exhibited frequent aggressive behaviour, such as biting, opening mandibles and acid spraying (proportions can be found in Tab. 1; Appendices S1 - S3). The proportion of aggressive interactions of *Formica rufa* towards the four myrmecophiles was significantly different (quasibinomial GLM, LR Chisq = 262.37, df = 4, P < 0.001). Bonferroni corrected pairwise tests can be found in Appendix S6: Tab. *Thiasphila angulata* elicited most aggression (quasibinomial GLM, LR Chisq = 280.12, df = 4, P < 0.001). *Formica rufa* workers induced a significant mortality (quasibinomial GLM, LR Chisq = 138.01, df = 4, P < 0.001) in the extra experiment with nest material, the proportion of surviving isopods per replicate was also significantly reduced (quasibinomial GLM, LR Chisq = 29.37, df = 4, P < 0.001) compared with a control set-up without workers (mean = 0.51, CI: 0.43 - 0.59).

<table>
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<th>Species</th>
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<th>Showing interest</th>
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<th>Biting</th>
<th>Acid spraying</th>
<th>Proportion aggressive interactions</th>
<th>Abdomen bending</th>
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<td>[0.14 - 0.24]</td>
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<td>Porcellio scaber</td>
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<td>[0.03 - 0.12]</td>
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Tab. 1: Interactions between ant and associates are categorized in different categories. Mean proportions of a particular category out of a total of 20 interactions are given. 95% confidence intervals were calculated by running quasibinomial models for every interaction and with the function confint in R. They are listed in brackets under the means. Aggressive interactions are opening mandibles, biting and acid spraying. Species with a different letter code elicit a significant different proportion of aggressive interactions (Bonferroni corrected pairwise tests). The category "Abdomen bending" refers to the proportions of the 20 interactions that were directly preceded or followed by abdomen bending.
Survival of beetles in nests with other ant species:

There was a large variation in survival of the three beetles when associated with other ant species (Fig. 2a, b, c, Appendix S5: Tab.). In general, the survival ratio of the three beetle species was very similar in nests of the different ant species. Survival of the beetles was highest when associated with *Formica rufa* workers compared with other ant species (Bonferroni corrected pairwise tests listed in Appendix S5: Tab.). *Monomorium pharaonis*, *Solenopsis fugax*, and *Camponotus vagus* killed all rove beetles (*Thiasophila angulata*, *Lyprocorrhe anceps*, *Amidobia talpa*) within the first six days, most of which did not survive the first hours. *Tetramorium caespitum* and *Lasius niger* also significantly reduced survival of all rove beetles compared with survival in *F. rufa* nests (Bonferroni corrected pairwise tests listed in Appendix S5: Tab.). *Myrmica rugi- nodis*, *Lasius fuliginosus*, and *Formica cunicularia* caused reduced survival in one or two beetle species compared with *F. rufa*. While there are records for the three beetle species with *L. fuliginosus* (PARMENTIER et al. 2014), survival of *L. anceps* and *T. angulata* was significantly lower when associated with this ant species compared with their preferred *F. rufa* host.

Relationship of worker size and myrmecophile survival:

In Figure 3, the ln(relative mortality rate) with a particular ant species for the three beetle species vs. the maximum head width size of the ant species was plotted. The ln (relative mortality rate) of the three beetle species initially decreased linearly with larger ant species and reaches its minimum with the large *Formica rufa* species (Fig. 3). However, the extreme efficiency of the largest species *Camponotus vagus* to kill the beetles, deviates from the larger worker-higher survival pattern observed in the other eight ant species.

Discussion

Red wood ants acted aggressively towards three associated rove beetles. These obligate myrmecophiles reacted agitated, often bent their abdomen and fled away. Nevertheless, these short-term antagonistic interactions did not harm the myrmecophiles over a period of 20 days. Interestingly, survival of the common soil-dwelling isopod *Porcellio scab-
ber, which can be highly abundant in red wood ant mounds, did decrease due to red wood ant association. Ants mostly ignored these isopods and were not observed to bite or chase them. However, isopods are reported to have a reduced life time or a lowered reproductive investment when ignored these isopods and were not observed to bite or did decrease due to red wood ant association. Ants mostly exposed to both abiotic and biotic stress (Hörner 2005). The numerous interactions with ants in the experiment with the ants. However, isopods are reported to have a re-

Fig. 3: Relationship between ant species size (maximum head width) and the ln (relative mortality rate) of (a) *Thiasophila angulata*, (b) *Lyprocorrhe anceps*, (c) *Amidobia talpa*. Dots show the mean of the maximum head width and grey bars the range of max head widths. Here, the reference level of the relative mortality rate is the treatment with *F. rufa*. Therefore the ln (relative mortality rate) in nests of *F. rufa* is 0 (ln (mortality rate *F. rufa*/mortality rate *F. rufa* = ln (1) = 0)).

In this study, we show that the general traits (fleeing, hiding, abdomen bending) of these beetles are insufficient for association with most non-host ant species. The impact of different ant species on the myrmecophiles differed dramatically and some ant species even immediately killed the beetles. It is rather surprising that red wood ants, which are commonly assumed as extremely dominant and aggressive towards other ants and arthropods (Mabelis 1979, Skinner & Whittaker 1981, Mabelis 1984, Batchelor & BriiFA 2010), are unsuccessful in killing or harming these beetles. Moreover, it is remarkable that the unspecialized beetles of this study only have a narrow preferred host range, i.e., mound building *Formica* ants. The relatively large size of red wood ants (Seifert 2007) might hamper them to successively detect, attack and / or handle small myrmecophiles and might be more suited to attack larger species, including conspecific competitors. Small animals are harder to detect and are more agile (Blankenhorn 2000) and size constraints can be important in explaining interactions between species. For example, large aerial insectivorous bats either cannot detect small insects, or they detect them too late to allow manoeuvring for capture (Barclay & Brigham 1991). Therefore their diet is constrained to large and less agile insects, whereas small bats effectively detect and hunt small insects (Barclay & Brigham 1991). Small workers in a polymorphic ant colony could have more antennal glomeruli to process olfactory cues as shown in some carpenter ants (Mysore & al. 2009, 2010). Small workers could also be ergonomically more efficient in catching, stinging and biting myrmecophiles that match their size. Moreover, we reported recently that within a red wood ant colony, smaller workers were more aggressive than large workers towards myrmecophiles (Parmentier & al. 2015a). Therefore, we hypothesized that the same size-based aggression response could operate at the species level, whereby species with small workers detect and / or attack these myrmecophiles more easily and efficiently. Interestingly, survival of all three beetles indeed gradually increased with larger ant species and reached its maximum in the relatively large red wood ants (Fig. 3). However, a linear association was violated with the extreme low survival in nests of *Camponotus vagus*, the largest ant.
species known for the study region. Other factors than worker size could affect the efficiency of ants to kill myrmecophiles. For example, polymorphic ant species could have size classes which are more efficient in deterring (PARENTIER et al. 2015a) and killing myrmecophiles. In addition, the defence mechanism (acid spraying vs. stinging), the composition of defence chemicals and behaviour of ant taxa (HOLDDOBLER & WILSON 1990) could affect the mortality rate of myrmecophiles. The observed effect on myrmecomorphic survival of different ant species in our tests are in line with the known diversity of rotate beetle myrmecophiles associated with those ant species / taxa in Northern Europe (PAIVINEN & al. 2002). Red wood ants have most associated myrmecophilous rotate beetles (N = 26) followed by Lasius fuliginosus (N = 21). The subgenus Serviformica (includes Formica curculiaria) (N = 10), Lasius (except L. fuliginosus) (N = 16), and Myrmica species have a moderate number (N = 6). Finally, Tetramorium (N = 2), Camponotus (N = 1), Solenopsis (N = 0) and Monomorium (N = 0) have a very small or no records of associated rotate beetles in Northern Europe (numbers based on PAIVINEN & al. 2002: tab. 1). There are also no records of myrmecophilous rotate beetles associated with Solenopsis and Monomorium in the European myrmecomorph list of WASSMANN 1894 and in the British list of DONISTHORPE (1927). It is postulated that colony size of ants (and corresponding number of niches in nests) is an important factor in predicting myrmecomorphic diversity (KRONAUER & PIERCE 2011). In addition to this rule, we suggest that some ant species are more successful in expelling or killing myrmecophiles, which could constrain myrmecomorph distribution and host range patterns.

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References


KIGHT, S.L. & NEVO, M. 2004: Female terrestrial isopods, Porcellio laevis LATREILLE (Isopoda: Oniscidea) reduce brooding


PARMENTIER, T., DEKONINCK, W. & WENSELEERS, T. 2016: Do well-integrated species of an inquiline community have a lower brood predation tendency? A test using red wood ant myrmecophiles. – BioMed Central Evolutionary Biology 16: art. 12.


ROSENGREN, R., FORTELIS, W., LINDBRÖM, K. & LUTHER, A. 1987: Phenology and caustion of nest heating and thermoregulation in red wood ants of the Formica rufa group studied in coniferous forest habitats in southern Finland. – Annales Zoologici Fennici 24: 147-155.


ZAGAFA, M., STANIEC, B. & PIETRYKOWSKA-TUDRUI, E. 2014: The first morphological description of the immature stages of Thiasophila Kraatz, 1856 (Coleoptera; Staphylinidae) inhabiting ant colonies of the Formica rufa group. – Zootaxa 3774: 301-323.