

1 Dissecting the costs of a facultative symbiosis in an isopod living with ants

2
3 Jens Zarka¹, Frederik C. De Wint², Luc De Bruyn^{2,3}, Dries Bonte¹, Thomas Parmentier^{1,4*}

4
5 ¹Terrestrial Ecology Unit (TEREC), Department of Biology, Ghent University, K.L.
6 Ledeganckstraat 35, B-9000 Gent, Belgium

7 ²Evolutionary Ecology Group, Department of Biology, Universiteit Antwerp,
8 Universiteitsplein 1, 2610 Wilrijk, Belgium

9 ³Research Institute for Nature and Forest (INBO), Havenlaan 88 bus 73, 1000 Brussel,
10 Belgium

11 ⁴Research Unit of Environmental and Evolutionary Biology, Namur Institute of Complex
12 Systems, and Institute of Life, Earth, and the Environment, University of Namur, Rue
13 de Bruxelles 61, 5000 Namur, Belgium

14
15 ***Corresponding author:** Thomas Parmentier: Thomas.Parmentier@unamur.be

16 **Orcid:**

17 Jens Zarka: <https://orcid.org/0000-0001-5492-4493>

18 Frederik C. De Wint : <https://orcid.org/0000-0003-0819-3903>

19 Luc De Bruyn: <https://orcid.org/0000-0002-8968-8862>

20 Dries Bonte: <https://orcid.org/0000-0002-3320-7505>

21 Thomas Parmentier: <https://orcid.org/0000-0002-4082-0922>

22
23 **Author contributions:** JZ, FDW, LDB and TP conceived and designed the experiments. JZ,
24 FDW and TP performed the experiments. LDB and DB participated in the coordination
25 of the study. All authors analyzed the data. JZ and TP wrote the manuscript, FDW, LDB
26 and DB critically revised the manuscript.

27

28 ABSTRACT

29 The balance of costs and benefits is expected to drive facultative associations. Here, we
30 assessed the costs of living in a facultative association, by studying the effect of red wood ants
31 on different fitness correlates of the facultatively associated isopod *Porcellio scaber*.

32 We demonstrated that *P. scaber* frequently occurs in and near hostile red wood ant nests and
33 may even outnumber obligate nest associates. However, the facultative association involved
34 different costs for the isopod. We found that the density of the isopod decreases near the nest
35 with higher ant traffic. Individuals in and near the nest were smaller than individuals further
36 away from the nest. Smaller individuals were also found at sites with higher ant traffic. In
37 addition, a higher proportion of wounded individuals was found closer to the nest and with
38 higher ant traffic. We recorded pregnant females and juveniles in the nest suggesting that the
39 life cycle can be completed inside the nests. Lab experiments showed that females died sooner
40 and invested less in reproduction in presence of red wood ants. Although *P. scaber* rarely
41 provoked a strong aggression response, large numbers of the isopod were carried by the ants
42 as prey to the nest. These preyed isopods were mainly dried out corpses.

43 Our results showed that the ant association involves several costs for a facultative associate.
44 Consequently, red wood ant nests and their surrounding territory may act as an alternative
45 habitat where demographic costs are balanced with stable resource provisioning and
46 protection against enemies.

47 **Key words:** ant associate; commensalism; *Formica*; Isopoda; myrmecophile

48

49 INTRODUCTION

50 Many organisms are associated with other species to get access to resources, shelter,
51 protection and cleaning services (Boucher 1985; Paracer and Ahmadjian 2000). The most
52 intimate associations, known as symbioses, have long fascinated researchers and were key

53 models to explore concepts in evolutionary biology. Experimental and theoretical work
54 significantly enhanced our knowledge of these strong and obligate associations, leading to
55 insight into the dynamics between symbiotic partners and the costs and benefits involved with
56 engaging in a tight symbiotic association (Doebeli and Knowlton 1998; Mueller et al. 2005; van
57 der Heijden et al. 2015). Loose and facultative associations between organisms, which in
58 contrast to symbiotic associations are not vital to the organisms involved, are considerably less
59 studied, but are widespread as well (e.g. in Lycaenid butterflies and ants, Pierce et al. 2002).
60 At present, it is poorly known how these loose associations affect the traits and fitness of the
61 interacting species. Organisms that obligately associate with another organism are expected
62 to gain net benefits from this association. Loose and facultative associations are less stable,
63 and costs are likely to be much more context-dependent (Stadler et al. 2001). In some
64 scenarios, costs might even outweigh potential benefits and associating with another organism
65 might become a non-beneficial strategy (White et al. 2007).

66

67 A diverse fauna of facultative associates can be found particularly in and around the structures
68 created by animals such as burrowing mammals, birds, corals and social insects (Patton 1994;
69 Uppstrom 2010; Kurek et al. 2020; Baardsen et al. 2021; Myczko et al. 2021). The habitats
70 formed by these species may concentrate critical resources, offer protection against enemies,
71 and dampen the adverse effects of physical stressors. Ant nests and ant territories are
72 excellent model systems to study facultative associations. The largest assemblages of
73 facultative ant associates tend to be present in nest sites with high levels of organic matter and
74 refuse, such as those from army ants, *Messor* ants, mound building *Formica* ants and tree-
75 inhabiting *Lasius* species (Donisthorpe 1927; O'Keefe 2000; Witte et al. 2008; Rettenmeyer et
76 al. 2011; Parmentier et al. 2014; Parmentier 2020). Ants are omnipresent ecosystem engineers
77 that provide suitable and long lasting niches in and around their nests (Hölldobler and Wilson
78 1990; Hughes et al. 2008; Parmentier 2020). But ants potentially also exert antagonistic effects
79 on associates by direct attacks or by elevating the level of perceived stress. Stable associated
80 populations will thus only have a positive growth rate when the costs are compensated by the

81 benefits. Otherwise, their facultative association can only be explained by a net flux of
82 immigrants originating from more suitable and less hostile habitat.

83

84 An iconic group of ants in Eurasia are the mound building red wood ants (*Formica rufa* group).
85 They play a vital role as ecosystem engineers that affect both the abiotic and biotic components
86 of forest and heath ecosystems (Frouz et al. 2016; Robinson et al. 2016). Red wood ants may
87 structure communities in and around their nests by predation, competition and the attraction
88 of associated organisms (Gösswald 1989; Stockan et al. 2016; Maák et al. 2021). They are
89 known as opportunistic and non-specific predators that feed on different arthropod species
90 depending on their availability (Skinner 1980; Sørensen and Schmidt 1987; Domisch et al.
91 2009; Parmentier 2010). Different studies pointed out that strong red wood ant predation may
92 control invertebrate groups (Punttila et al. 2004), including some notorious pest species
93 (Adlung 1966; Zingg et al. 2018; Trigos-Peral et al. 2021). Apart from predation, red wood ants
94 may also reduce the numbers of other species by deterrence and competition (Haemig 1992;
95 Reznikova and Dorosheva 2004; Jäntti et al. 2007). Lastly, they attract and interact with a vast
96 array of arthropod species in and around their nests, such as mutualistic tree aphids that
97 provide sugary honeydew (Novgorodova 2005; Depa et al. 2020; Parmentier et al. 2020).

98 A peculiar group of ant-attracted species lives inside red wood ant nests. This community is
99 dominated by beetles and mites and typically exerts neutral to negative effects on the ant host
100 (Parmentier et al. 2014). There are obligate ant nest associates, so-called myrmecophiles, of
101 which we gradually gained a better understanding of their behavioural, chemical and
102 morphological adaptations to the hostile ant nest (Parmentier et al. 2017, 2018). Recent work
103 also demonstrated that the red wood ant host strongly affects myrmecophile distribution in and
104 outside the nest (Parmentier et al. 2021) but exerts no negative effects on their survival
105 (Parmentier et al. 2016a). However, a rich assemblage of common soil organisms such as
106 millipedes, mites, isopods and springtails may facultatively infiltrate red wood ant nests and
107 may also take advantage of the homeostatic, resource-rich and safe nest environment

108 (Robinson and Robinson 2013; Parmentier et al. 2014; Boer 2021). As they seemingly lack
109 any adaptations to the host, it is unknown how they succeed to survive in and around the
110 hostile nest environments. It can be expected that the aggressive host impacts their
111 distribution, behaviour and life history traits, but this is unexplored yet. One of the largest
112 facultative associates is the common rough isopod *Porcellio scaber* Latreille (1804) (De Smedt
113 et al. 2020). Previous inventories showed that *P. scaber* is a frequent associate in red wood
114 ant mounds (Robinson and Robinson 2013; Parmentier et al. 2016b; Boer 2021). Findings of
115 pregnant females as well as juveniles in the nests even suggest that *P. scaber* can breed in
116 red wood ant nests (Robinson and Robinson 2013). The association of *P. scaber* with red
117 wood ant nests is however dual as they are also preyed by red wood ants and might be brought
118 in large quantities to the nest (Driessen et al. 1984; Loones et al. 2008; Parmentier 2010).

119

120 Here, we studied the interaction and effect of living with red wood ants on the facultatively
121 associated isopod *P. scaber* to gain new insights into the costs associated with a facultatively
122 symbiotic biology. As different traits of *P. scaber* and related species are known to be very
123 responsive to various forms of abiotic (Dallinger and Prosi 1988; Bayley and Baatrup 1996;
124 Fischer et al. 1997; Lardies et al. 2004; Calh a et al. 2012) and biotic stress (Castillo and Kight
125 2005; Cazzolla Gatti et al. 2020), we expected that a stressful ant environment will have a
126 considerable impact. More specifically, we assessed whether red wood ants negatively
127 influence the abundance and life history traits (isopod mass, sex ratio, pregnancy, and injuries)
128 of *P. scaber* in the nest and along a spatial gradient away from the nest. We hypothesized that
129 the niches in and near the nest and with high ant densities will be stressful for *P. scaber*. Next,
130 we tested whether red wood ants hamper reproduction of our study species using a controlled
131 lab experiment. Lastly, we studied whether the costs on *P. scaber* can be explained by its
132 behavioural interaction with red wood ants.

133

134 **MATERIAL AND METHODS**

135

136 **Spatial demography and fitness correlates of *P. scaber* in and around red wood**
137 **ant nests**

138 We wanted to compare the distribution of *P. scaber* along a distance gradient characterized
139 by different levels of ant stress. During the summer of 2020, we selected 18 red wood ant nests
140 (*Formica polyctena* and *Formica rufa*). All selected nests were located in the province of West-
141 Flanders in the North-West of Belgium, more specifically in Bruges and Poperinge (Fig. 1a).
142 *Formica polyctena* and *F. rufa* are closely related and may even hybridize, but typically differ
143 in colony organization with *F. polyctena* nests having many queens and *F. rufa* nests a single
144 queen (Seifert 2007). However, the *F. rufa* colony organization in our study region is similar to
145 *F. polyctena* as they were also highly polygynous. Nevertheless, the two species can
146 unambiguously be separated based on their pilosity and no hybrids occur in the studied sites
147 (Dekoninck et al 2012). Because of the similar organization and similarly associated fauna
148 (Parmentier et al. 2015a), we expect no effect of host species on *P. scaber* tolerance. The
149 nests were grouped into seven different clusters, based on similar abiotic conditions and
150 proximity to each other (Appendix 1). The three *F. rufa* nests were located in the same site and
151 were grouped in one cluster. We placed a pitfall in each nest and at three distances outside
152 the nest (1, 5 and 10 meter). Three pitfalls were installed for each distance outside the nest
153 (Fig. 1b). The pitfalls were positioned as much as possible parallel to the forest edge to
154 minimize edge effects of the forest on the spatial distribution of the isopods (De Smedt et al.
155 2018).

156 The pitfalls were plastic, rectangular boxes (25 cm x 7.5 cm x 8 cm) containing an approximate
157 1 cm bottom layer of plaster. To prevent the desiccation of the isopods the plaster was
158 moistened. Debris of the surrounding also got into the pitfalls due to the wind and passing ants,
159 which created extra humidity and offered hiding places for the isopods. By only collecting living
160 isopods, we avoided the collection of dead isopods that were brought into the pitfalls by
161 foraging ant workers. All pitfalls outside of the nests were dug into the ground with the long

162 side of the pitfall parallel to the nest edge and the top part of the pitfall levelled with the ground
163 surface. An elevated roof was placed on the pitfalls. The elevated roof prevented the pitfall
164 from flooding, but it also left a 1.5 cm slit, large enough for isopods to get caught by the pitfall.
165 The pitfalls inside the nest were dug into the nest and covered again with nest material. A
166 plastic roof, kept in place by rubber bands, was also used to prevent nest material from falling
167 into the pitfalls.

168 The pitfalls outside the nests were emptied after one week. The ones inside the nests were
169 emptied every one to two days, because they rapidly filled with nest material brought inside by
170 the ants. The collected isopods were stored per pitfall on 70% ethanol. Ants were not collected,
171 but workers in the pitfalls outside the nests were counted. These numbers were used to
172 determine the degree of ant traffic at the location of the pitfall. In 2019 we also collected
173 *Porcellio scaber* individuals using the same pitfalls in 24 *F. rufa* nests at the Poperinge site, as
174 part of a study on the dispersal of red wood ant myrmecophiles (Parmentier et al. 2021). These
175 unpublished abundance data, allowed us to compare the intranidal densities of *P. scaber* with
176 those of strictly associated myrmecophiles, mostly rove beetles (Parmentier et al. 2021).

177

178 We wanted to correlate different fitness traits with the level of ant stress. Every individual was
179 weighed as a proxy for its size. Isopods were placed in an oven set to 60 degrees for 24 hours.
180 The dry body mass was measured using a microbalance (Brand: OHAUS; accuracy: 0.1
181 milligram).

182 Sex, state of pregnancy and number of intact antennae for the 7814 collected specimens of *P.*
183 *scaber* in the 2020 sampling were determined using a stereomicroscope (Kyowa optical model
184 SD-2P). Sex of *P. scaber* and woodlice in general can be determined by looking at the
185 presence of external male genitalia used for sperm transfer (Sutton 1980). However, these
186 external male genitalia only develop when they become an adult. Therefore, we used the dry
187 body mass at which 50% of the animals had external male genitalia as a cut-off value for
188 determining maturity (juveniles < 1.3 mg, adults \geq 1.3 mg). To assess if a female was pregnant,

189 we checked for a brood pouch or a so-called marsupium holding the eggs (Sutton 1980). To
190 determine if the isopods suffered from an ant attack, their antennae were counted (Ospina et
191 al. 2022). Because *P. scaber* is unable to hide its two antennae under its hard exoskeleton
192 they may have been ripped off during an ant attack. We used this measure as a proxy for the
193 frequency and/or severity of non-lethal attacks as frequently used in ecological studies
194 (Schoener 1979; Ernsting and Fokkema 1983; Ospina et al. 2022)

195 For the 2019 dataset, we did not have info on fitness correlates such as body size, sex, state
196 of pregnancy and number of remaining antennae of *P. scaber*.

197 Statistical analysis

198 *Abundance of adults per pitfall*

199 To analyse the abundance data, we ran a linear mixed model (= LMM) with the fourth-root of
200 the abundances (# isopods / trap) as dependent variable to fulfil normality assumptions. The
201 full model included distance from the nest, ant density (# ants / trap) and the interactions
202 between those two variables as fixed effects. Site and nest were selected as random effects.
203 A likelihood ratio test was used to assess the significance of the predictors.

204

205 *Abundance of *P. scaber* vs obligate rove beetles inside a nest*

206 The abundance of *P. scaber* and obligate rove beetle myrmecophiles in 24 nests of the 2019
207 dataset were pairwise compared with a non-parametric Wilcoxon paired signed rank test.

208

209 *Individual dry mass*

210 The dry mass of adult *P. scaber* individuals around red wood ant nests (excluding individuals
211 caught in the nest) was modelled using a LMM in R. The full model included distance from the
212 nest (three levels: 1 m, 5 m and 10 m), sex, ant density (fourth root transformed) and all two-
213 way interactions as fixed effects. As random effects, site, nest and pitfall were modelled. We
214 determined the optimal fixed effects structure using backwards model selection with

215 the drop1 function. We removed the least significant predictor at each step until none met the
216 criterion $P > 0.10$. The natural logarithm of the body mass of the isopods was taken to fulfil
217 normality assumptions and a post-hoc test was conducted.

218 In addition, we wanted to compare the body mass of individuals found inside the nest with
219 those along the distance gradient outside the nest. As we did not count the ants in the nest
220 pitfalls, we could not control for the factor 'ant density' in this analysis. Eventually, we
221 conducted the same model as described above excluding ant density, but with four distance
222 levels (0 m = intranidal, 1 m, 5 m and 10 m).

223

224 *Proportion of juveniles, females, pregnant females, and individuals with missing antennae*

225 The effects of the predictors distance, ant density and the interaction were assessed on i)
226 proportion of juveniles ii) the proportion of females iii) the proportion of pregnant females, iv)
227 the proportion of individuals with missing antennae. Here we ran four binomial linear mixed
228 models. The full model included distance from the nest, ant density (fourth-root transformed)
229 and the interaction between the variables as fixed effects. As random effects, site and nest
230 were chosen. Overdispersion in these models was checked, but no violations were detected.
231 A post-hoc test was also conducted. .

232

233 *Statistical platform*

234 All models were run in R 4.0.3. For the linear mixed models and binomial mixed models we
235 made use of the lme4 and lmerTest package (Bates et al. 2015, Kuznetsova et al. 2017). For
236 the post-hoc tests we used the package emmeans (Lenth 2021). Overdispersion in the models
237 was checked by using the DHARMA package (Hartig 2020).

238

239 ***Porcellio scaber* as a prey for red wood ants**

240 To assess whether red wood ants preyed on *P. scaber*, we monitored the most crowded ant
241 trail of four nests in Bruges in August, 2021. We identified all prey brought to the nests on each
242 of the four trails (during one hour per trail) and checked if the prey items were alive, died
243 recently or died a while ago (dried out).

244 To test whether red wood ants preferred dead or living *P. scaber* as a prey, we conducted a
245 preference experiment. We installed two pitfalls (dimensions and setup as described above)
246 with ten dead (marked with white enamel dot) and ten living *P. scaber* individuals (marked with
247 green enamel dot) just outside a red wood ant nest in Bruges. Isopods for this experiment were
248 collected in the Bruges site. Dead isopods were obtained by freezing living individuals, and
249 defrosting them one hour before the start of the experiment. After one hour, the individuals
250 were counted to assess how many individuals were taken out of the pitfalls as prey by the ants.
251 The enamel marking allowed us to see whether living individuals were killed and to differentiate
252 the offered isopods from new individuals that fell or were brought into the traps.

253

254 **Lab experiment: costs of ants on reproductive investment**

255

256 We assessed the effect of the presence of red wood ants on reproductive investment (total dry
257 mass of offspring/dry mass of mother) of *P. scaber* in a controlled lab experiment. First, we
258 collected individuals in early winter from a single population (Location: Ostend), males and
259 females were separated into different containers and put in artificial hibernation in a fridge
260 (4°C) for two months. Individuals were then put under room temperature, provided with carrot
261 slices and organic material, and after three weeks we checked the pregnancy status of the
262 females. We discarded the females that showed signs of pregnancy as these had been
263 fertilized in the field. Next, we prepared 17 containers (1L, diameter 8.5 cm, height 13 cm) filled
264 with a plaster bottom and 50 mL of organic material and assigned 15 virgin females and 8
265 males to each of them. In nine of the 17 containers, we added 50 *F. polyctena* workers (ant+

266 treatment). The other eight containers were used as a control without ants (ant- treatment).
267 Containers were placed in a cabinet with an ambient temperature of 24°C and a day night
268 cycle of 16D:8N. In each container, we provided two carrot slices and eppendorf tubes filled
269 with water and sugar water (20%), plugged with cotton as nourishment for the isopods and
270 ants. Organic material and sugar water were replaced every week, carrot slices every 4-5 days.
271 Dead ants were replaced every two days, and dead isopods were removed. We moistened the
272 surface of the containers every 4-5 days. Preliminary experiments indicated that the minimal
273 gestation time was 23 days under the used conditions. Therefore, we isolated the surviving
274 females individually after 22 days, otherwise we would not have been able to assess the
275 gestation time and reproductive investment of each individual female. Females were
276 individually housed in snap lid vials (height 2 cm, diameter = 4.5 cm) with a 0.5 cm bottom
277 layer of plaster. A small slice of carrot was provided, and we added 1 red wood ant worker to
278 the vials with females that were subjected to the ant+ treatment. This prolonged the effect of
279 ant stress on these females. The females were monitored every day to record the gestation
280 time. We ended the trial when the mother gave birth. We assessed the dry mass of the mother
281 and of her offspring.

282 Statistical analysis

283 Female investment (dry mass offspring/dry mass mother) was modelled using a mixed-effects
284 beta regression (package glmmTMB (Brooks et al. 2017)). Treatment (ant+ vs ant-), gestation
285 time and their interaction were included as fixed factors in this model, container as a random
286 factor. Residuals were diagnosed with the DHARMA package, but no violations were detected.
287 We conducted backward variable selection using 'drop1' function and removed the least
288 significant predictor at each step until none met the criterion $P > 0.10$.

289 **Behavioural interaction between red wood ants and *P. scaber***

290

291 We conducted behavioural assays in the lab to assess how red wood ants and *P. scaber*
292 individuals behave when interacting. For this experiment, red wood ants (*Formica polyctena*)
293 and 20 isopod individuals were collected in Bruges.

294

295 The behavioural experiments were conducted in cylindrical plastic containers (diameter = 6.5
296 cm, height = 7 cm). The container had a moistened plaster bottom of ca. 1 cm and the inner
297 side was coated with fluon preventing ants and isopods from escaping. Seven medium sized
298 ants were put into the arenas to acclimatize for 1 hour. The choice for medium sized ants was
299 based on previous research that demonstrated that smaller red wood ant workers react more
300 aggressively (Parmentier et al. 2015b). After the acclimatization period of the ants, an isopod
301 individual was introduced into the arena. Ten seconds following the introduction of the isopod,
302 the interactions were filmed for ten minutes. The videos were later analyzed, frame by frame,
303 with the program "VirtualDub". Interactions were divided in three groups, i.e. initial behaviour
304 of the isopod, behaviour of the ant when contacting the isopod and the response of the isopod
305 on the ant's behaviour. We differentiated the following initial behavioural acts in the isopod: not
306 moving, walking or running. We scored whether the behaviour of the ant was aggressive
307 (opening mandibles, chasing, biting, acid spraying) or not (ignoring, antennation, inspecting).
308 The isopod reacted on the behaviour of the ant by not moving, doing a short stop, walking or
309 running. The first 20 interactions were assessed for each individual. We repeated the
310 behavioural trial with 20 unique individuals and ants.

311

312 Statistical analysis

313 *Effect initial behaviour isopod on ant aggression*

314 A binomial GLMM was modelled including in the full model the initial behaviour of the isopod
315 as a fixed effect and the ID of the isopod as a random effect. No overdispersion was detected,
316 and a post-hoc test was conducted.

317

318 *Effect behaviour of the ants on the response of the isopod*

319 A multinomial model was run to test whether *P. scaber* responded differently (proportion of 4
320 behavioural categories: moving, running, walking, making short stops) to aggressive versus
321 non-aggressive ants.

322 The binomial GLMM was modelled using the packages lme4 and lmerTest, as well as the
323 emmeans package for the post-hoc test. The multinomial model was coded in the nnet
324 package (Venables & Ripley, 2002), and significance was tested with the lsmeans (Russel &
325 Lenth, 2016) package.

326

327 RESULTS

328

329 **Spatial demography and fitness correlates of *P. scaber***

330 *Porcellio scaber* frequently occurred around red wood ant nests, we collected 7814 specimens
331 around red wood ant nests (at 1, 5 and 10 metres distance) in 2020: 493 around three *F. rufa*
332 nests, 7241 around 15 *F. polyctena* nests. A total of 80 *P. scaber* individuals were found in the
333 nests: 40 individuals in the three *F. rufa* nests and 40 individuals distributed over 8 of the 15
334 sampled *F. polyctena* nests

335

336 *Abundance of adults per pitfall*

337 The interaction effect between ant density and sampling distance significantly affected the
338 adult abundance of *P. scaber* per pitfall (LRT = 4.5, df = 2, $P = 0.012$) (Fig. 2). At 1 meter there
339 was a clear decrease of abundance with increasing ant density, while at 5 and 10 meters no
340 such changes were noticed with changing ant densities (Fig. 2). There was a highly positive

341 correlation between the abundance of adults per pitfall and the total biomass per pitfall
342 (Pearson's $r = 0.98$, $P < 0.001$).

343

344 *Abundance of P. scaber vs obligate rove beetles inside a nest*

345 We recorded *P. scaber* in 16 of 24 *F. rufa* nests in the Poperinge site (mean number per nest:
346 12.8, median = 2.5, 0.5 quantile: 0.0-12.8) in 2019. The mean number of obligate rove beetle
347 myrmecophiles in these nests was 10.9 (median = 6.5, 0.5 quantile: 3.0-11.3). No significant
348 differences were found between the number of *P. scaber* and myrmecophilous rove beetles in
349 these nests (Wilcoxon signed rank test with continuity correction, $V = 198.5$, $P = 0.17$). *Porcellio*
350 was more abundant than myrmecophilous rove beetle individuals in 7 of the 24 nests
351 (Appendix 2, Fig. S1).

352

353 *Individual dry mass*

354 The dry mass of a *P. scaber* individual was on average lower with increasing ant densities
355 (LRT= 7.5, $df = 1$, $P = 0.008$) (Fig. 3). Moreover, the dry mass of a *P. scaber* individual was
356 lower close to the nest than further away from the nest (LRT = 9.5, $df = 2$, $P = 0.001$). Both
357 females and males at 1 meter (estimated marginal mean of log mass female at mean ant
358 density \pm SE: $EMM_{1m} = -5.46 \pm 0.07$, males: $EMM_{1m} = -5.46 \pm 0.07$) weighed less than those
359 at 5 and 10 meters (females: $EMM_{5m} = -5.25 \pm 0.06$, $EMM_{10m} = -5.23 \pm 0.06$, $P < 0.001$; males:
360 $EMM_{5m} = -5.34 \pm 0.07$, $EMM_{10m} = -5.28 \pm 0.06$; $P < 0.053$). The mass of individuals at 5 and 10
361 metres were not significantly different in both females ($P = 0.909$) and males ($P = 0.353$).

362 Male mass in the nests (mean log mass = -5.61 ± 0.12) was on average lower than male mass
363 at 5 and 10 meter (mean_{5m} = -5.35 ± 0.08 , mean_{10m} = -5.27 ± 0.08 , $P = 0.029$ & $P = 0.002$,
364 respectively), but was similar to male mass at 1 meter (mean_{1m} = -5.50 ± 0.08 , $P = 0.660$)
365 (Appendix 3: Fig. S2). We found a comparable trend in female body mass, but there was no
366 significant difference in mass between the individuals caught inside the nests and outside the

367 nest (Appendix 3: Fig. S2). Lack of significance here is probably caused by the relatively low
368 number of weighed individuals from inside the nest.

369

370 *Proportion of juvenile individuals*

371 There was also a significant interaction effect between sampling distance and ant density on
372 the proportion of juveniles (LRT = 6.0, df = 2, $P = 0.048$). At 1 meter, we found an increase in
373 the proportion of juveniles with increasing ant density. While at 5 and 10 meters the proportion
374 of juveniles stayed stable with increasing ant density.

375

376 *Proportion of females*

377 The proportion of females significantly increased further away from the nests (LRT = 7.9, df =
378 2, $P = 0.019$) in the adult individuals. But only the difference between 1 meter and 10 meter
379 was significant: 60.4%(95% CI: 57.4-63.4%) of all caught adults were female at 1 meter and
380 64.3% (95% CI: 61.2-66.8%) of them were females at 10 meter ($P = 0.023$).

381

382 *Proportion of pregnant females*

383 The proportion of pregnant females significantly increased with distance away from the nest
384 (LRT = 9.7, df = 2, $P = 0.007$) (Appendix 4, Figure S3). At 10 meters there was a significantly
385 higher proportion of pregnant females than at 1 meter (estimated marginal mean proportion
386 [95% CI] at average ant density: $EMM_{1m} = 0.12$ [0.07-0.18], $EMM_{10m} = 0.17$ [0.11-0.25], $P =$
387 0.005), whereas the proportion of pregnant females at 5 meters ($EMM_{5m} = 0.14$ [0.09-0.22])
388 did not differ from the other sampling distances. The proportion of pregnant females also
389 increased significantly with increasing ant density irrespective of the sampling distance (LRT
390 = 6.2, df = 1, $P = 0.012$) (Appendix 4, Figure S3).

391 We found 12 pregnant females in the nests, but they were not included in the model.

392

393 *Proportion of individuals with missing antennae*

394 There was a significant increase in the number of isopods missing one or two antennae with
395 increasing ant densities (LRT = 3.9, df = 1, $P = 0.047$) (Fig. 4). The proportion of individuals
396 with missing antennae was significantly higher closer to the nest (estimated marginal mean
397 proportions [95% CI] at average ant density: $EMM_{1m} = 0.11$ [0.07-0.16], $EMM_{5m} = 0.07$ [0.05-
398 0.10], $EMM_{10m} = 0.04$ [0.03-0.06], LRT = 43.7, df = 2, $P < 0.001$, post hoc tests: $P_{1/5} = 0.003$;
399 $P_{1/10} < 0.001$; $P_{5/10} = 0.001$) (Fig. 4).

400

401 ***P. scaber* as a prey for red wood ants**

402 *Porcellio scaber* was the most carried prey in four red wood ant nests in Bruges: 65 out of 173
403 collected prey items were *P. scaber* (37.6%). Hymenoptera (solitary bees, other ants...)
404 composed another important part of the diet (33.5%), a minor fraction of the diet composed of
405 Coleoptera (6.4%), Diptera (6.4%), Lepidoptera (5.8%), and diverse groups of other arthropods
406 and invertebrates (10.4%). All of the *Porcellio* individuals were dead: 47 were dry carcasses,
407 18 individuals were not dried out.

408 We found that red wood ants did not take any of the 20 living individuals out of the two pitfalls.
409 By contrast, 18 of the 20 dead isopods were taken by the ants out of the pitfalls.

410

411 **Lab experiment: costs of ants on reproductive investment**

412 Treatment (ant+ or ant-) was the only fixed predictor retained in the final model, gestation time
413 and its interaction with treatment were not included in this final model. Female isopods
414 invested less in offspring (calculated as the dry mass of the released offspring divided by the
415 dry mass of the mother) in presence of ants (17.8%, 95% CI: 15.3-19.0%) than in absence of
416 ants (20.8%, 95% CI: 19.0-22.7%) (LRT = 6.8, df = 1, $P = 0.009$, Fig. 5).

417

418 **Behavioural experiments**

419 *Effect initial behaviour isopod on ant aggression*

420 When *P. scaber* was running, there was a probability of 0.61 that ants would respond
421 aggressively. However, when *P. scaber* was walking or not moving, the probability of ant
422 aggression decreased to 0.17 and 0.11, respectively (LRT = 63.7, df = 2, $P < 0.001$).

423

424 *Effect behaviour of the ants on the response of the isopod*

425 When ants non-aggressively approached *P. scaber*, the isopods mostly did not move
426 (proportion = 0.43), followed by walking away (0.31), running (0.14) or making a short stop
427 (0.12). The isopods response was considerably different when approached aggressively (LRT
428 = 58.1, df = 3, $P < 0.001$). The likelihood of running away then increased towards 0.49, whereas
429 the probability of walking (0.16), not moving (0.33) and short stopping (0.03) decreased in the
430 behaviour of *P. scaber*.

431

432 **DISCUSSION**

433 The facultative ant associate *Porcellio scaber* was frequently recorded in and around hostile
434 red wood ant nests. They constituted a significant proportion of the arthropod fauna in the
435 sampled red wood ant nests and may even outnumber specialized myrmecophilous beetles
436 within these nest fortresses. We found that the loose and facultative association involved
437 multiple costs to the isopod as the ants strongly affect the spatial distribution and some key life
438 history traits of the isopod.

439

440 *Porcellio scaber* adults were considerably smaller closer to the nests of red wood ants.
441 Individuals caught inside the nests also tended to be smaller than those away from the nest.
442 The body mass of adults at a given distance outside the nest was negatively linked to the

443 number of ants passing by. A general pattern found in obligate ant associates is that they are
444 typically small compared to their host ant (Hughes et al. 2008; Witte et al. 2008; Parmentier et
445 al. 2020). Moreover, there is growing evidence that a smaller body size in ant associates
446 decreases their detection by the host (Parmentier et al. 2016a; von Beeren et al. 2021). Many
447 small ant-associated arthropods such as mites, bark lice, springtails and millipedes usually
448 stay undetected in ant nests without resorting to advanced deception strategies (Donisthorpe
449 1927; Kistner 1982; Eickwort 1990; Witte et al. 2008; Uppstrom 2010; Parmentier 2020; Rocha
450 et al. 2020). As a facultative generalist, *P. scaber* does not possess specialist traits such as
451 chemical mimicry or morphological adaptations. The observed effect on body mass is likely
452 the result from larger individuals avoiding high ant densities. In our behavioral trials, we also
453 observed that the isopods avoided the ants. This is a simple and wide-spread strategy to avoid
454 attacks of enemies (Parmentier et al. 2018; Ospina et al. 2022). It was also documented that
455 *Porcellio* species make use of avoidance behaviour when confronted with other adverse
456 conditions, such as the presence of toxins (Zidar et al. 2019) or predator cues (Hegarty and
457 Kight 2014). In addition, they likely benefit from a hard, protective exoskeleton (cf von Beeren
458 et al. 2021) and low concentrations of chemical recognition cues on the cuticle (Parmentier et
459 al. 2017). Juveniles and small adults will likely be less noticed and will better tolerate high ant
460 densities. The relationship between small size and reduced enemy avoidance is not limited to
461 ant interactions (Blanckenhorn 2000) but has been demonstrated in diverse agonistic
462 interactions such as in bats and their prey (Barclay and Brigham 1991). Because of the positive
463 association between body size and detection, it is likely that larger *P. scaber* individuals
464 experience more stress and are more inclined to avoid the nest and sites with high ant densities
465 around the nest. An alternative explanation for the lower body mass in or close by the nest
466 might be that the stress imposed by the hostile ants can lead to lower growth rates in the
467 isopods (Lavy et al. 2001; Dixie et al. 2015). An additional hypothesis for the negative
468 correlation between body mass and ant stress is that larger individuals were detected and
469 predated more by the ants than smaller isopods . The selective predation of larger individuals

470 cannot be ruled out, but needs further testing as we did not observe red wood ants hunting for
471 living isopods in our experiments.

472

473 Previous research demonstrated that ants may strongly affect the spatial distribution of
474 arthropods (Halaj et al. 1997; Hawes et al. 2002; Reznikova and Dorosheva 2004; Cembrowski
475 et al. 2014). Here, we found evidence that ants also drive the distribution of *P. scaber*. The
476 abundance of *P. scaber* adults and the total biomass of all *P. scaber* individuals in a pitfall
477 stayed fairly constant at 5 and 10 meters from the nest with changing red wood ant activity,
478 while at 1 meter, *P. scaber* abundance strongly declined with increasing ant activity. There are
479 fewer hiding places near the nest, as the concentration of ant trails is higher near the nest.
480 This may result in a stronger avoidance response of the isopod when exposed to high levels
481 of ant stress near the nest. The observed size distributions might also be explained by ant
482 predation rather than avoidance by the isopod. The concentration of ants near the nest could
483 lead to a higher predation rate of the isopod, but we did not find support for active hunting of
484 the isopod in this study. Interestingly, there was a higher proportion of juveniles around the
485 nest when ant densities were high, which can be linked to the size-dependence spatial
486 distribution. The corresponding patterns in adult abundance and total biomass in a pitfall were
487 expected as the total pitfall mass is mainly driven by the adult individuals and not by the small
488 juveniles. Crucially, the isopod distribution pattern was opposite to the ones detected in
489 obligate red wood ant associates. The densities of these species increased towards the nest
490 (Parmentier et al. 2021). Moreover, higher densities of obligate red wood ant myrmecophiles
491 were recorded at areas, such as ant trails, with high ant densities (Parmentier et al. 2021).

492

493 The proportion of pregnant females increased away from the nests. Contrary to our
494 expectations that pregnant females would avoid ants because of their reduced mobility and
495 agility, we found that the proportion of pregnant females increased with ant density. A possible
496 explanation is that further away from the nests, pregnant isopods reside along ant trails to
497 avoid predators. In line with Robinson and Robinson 2013, we found large numbers of

498 pregnant females and juveniles inside the red wood ant nests. These observations strongly
499 suggest that the isopod could complete its entire life cycle in the hostile nest environment.
500 However, our controlled lab experiment demonstrated that females in association with red
501 wood ants invest less in reproduction than free-living individuals. Interestingly, an experiment
502 with non-myrmecophilous carabid beetles showed a more drastic decrease in fecundity in
503 presence of red wood ants (Hawes et al. 2013). A previous study also found that the presence
504 of ants may shorten the breeding period of isopods, but this effect was not detected in this
505 study (Castillo and Kight 2005).

506

507 Another indication that the presence of red wood ants can be stressful and costly, is the fact
508 that isopods were more likely to miss one or two antennae closer to the nest and with
509 increasing ant density. *Porcellio scaber* is not able to hide its antennae under its hard
510 exoskeleton so they are exposed to ant attacks. The higher the ant density, the more likely an
511 isopod encounters an ant attack and loses one or two of its antennae.

512

513 *Porcellio scaber* did receive low levels aggression by red wood ants. It was shown that the
514 isopod provoked less aggression than most obligate red wood ant myrmecophiles (Parmentier
515 et al. 2016b). Interestingly, *Porcellio scaber* is also able to infiltrate nests of other social insect
516 species, such as honeybees (Kärcher and Ratnieks 2010), social wasps (pers. observations
517 TP) and *Lasius* ants (pers. observations TP), where it likely provokes low aggression as well.

518

519 It is puzzling that *Porcellio scaber* frequently occurs in and near red wood ants, even though
520 they may form an important component of the allocated prey, as demonstrated in previous
521 studies (Driessen et al. 1984; Loones et al. 2008; Parmentier 2010) and here. However, we
522 observed that all the *P. scaber* individuals brought to the nest were dead, and mostly dried out.
523 So, this observation suggests that red wood ants generally do not hunt for living individuals,
524 but rather scavenge on already dead individuals. Our preference experiment with dead and
525 living *P. scaber* individuals also indicated that only dead individuals were collected. The

526 relatively low aggression towards *P. scaber*, its morphological protection and avoidance
527 behaviour support the idea that ants unlikely directly kill *P. scaber* individuals.

528

529 We found that *P. scaber* isopods associated with red wood ants were smaller, invested less in
530 reproduction, were less numerous in localities with high ant densities near the nest and
531 suffered from more injuries. Previously, it has also been shown that living for a long time in red
532 wood ant lab nests resulted in increased mortality in *P. scaber* (Parmentier et al. 2016a).
533 Overall, all these results indicate that living with the ants involves costs for the studied
534 facultative associate. So why do they live in the nests? They could be forced into the territory
535 and nests of red wood ants because of saturation of suitable habitat nearby. Alternatively, red
536 wood ant nests could act as ecological traps for the isopods if they choose red wood ant nests
537 over more suitable habitat (Robertson and Hutto 2006). In both scenarios, isopods might only
538 persist in association with red wood ants if there is a constant flux of individuals from more
539 suitable habitat. On the other hand, we can expect that the isopod may profit from
540 thermoregulated, moist and protected nests, rich in resources (Hughes et al. 2008; Kronauer
541 and Pierce 2011). Nevertheless, it is much harder to assess the positive effects of an
542 association with red wood ants. At this point, it is unknown whether, and in what context, the
543 negative effects outlined in this study are offset by potential positive effects. Future studies are
544 needed to further improve our understanding of the dynamics of facultative associations.

545

546 ELECTRONIC SUPPLEMENTARY MATERIAL

547 Appendix 1: Table S1. Overview of the sampled nests and grouping into different clusters.

548 Appendix 2: Fig. S1. Comparison of Intranidal abundances of obligate myrmecophilous rove
549 beetles and the facultative myrmecophile *Porcellio scaber*.

550 Appendix 3: Fig. S2. The individual dry mass (g, ln transformed) of female and male *P.*
551 *scaber* individuals inside the nest and at three distances outside the nest.

552 Appendix 4: Fig. S3. The effect of ant density (ants per trap) and sampling distance from the
553 nest on the proportion of pregnant female *P. scaber* individuals.

554

555 **Acknowledgements:** We thank the Flemish Agency for Nature and Forest (ANB) and the
556 provincie West-Vlaanderen for granting permission to sample at their sites

557

558 DECLARATIONS

559 **Funding:** This work was supported by FWO and FNRS (1203020N/30257865 to TP)

560 **Conflicts of interest/Competing interests:** Not applicable

561 **Ethics approval:** Not applicable

562 **Consent to participate:** Not applicable

563 **Consent for publication:** Not applicable

564 **Availability of data and material:** Datasets will be deposited at
565 <https://github.com/tjparmen/Dissecting-the-costs-of-a-facultative-symbiosis> upon
566 acceptance.

567 **Code availability:** The code to run the analyses is available from the corresponding author on
568 reasonable request.

569

570 REFERENCES

571 Adlung K (1966) A critical evaluation of the European research on use of red wood ants
572 (*Formica rufa* group) for protection of forests against harmful insects. Zeitschrift für
573 Angew Entomol 57:167–189

574 Baardsen LF, De Bruyn L, Adriaansen the F, et al (2021) No overall effect of urbanization on

575 nest-dwelling arthropods of great tits (*Parus major*). Urban Ecosyst 24:959–972
576 <https://doi.org/10.1007/s11252-020-01082-3>

577 Barclay RMR, Brigham RM (1991) Prey detection, dietary niche breadth, and body size in
578 bats: Why are aerial insectivorous bats so small? Am Nat 137:693
579 <https://doi.org/10.1086/285188>

580 Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using
581 lme4. J Stat Softw 67:1–48

582 Bayley M, Baatrup E (1996) Pesticide uptake and locomotor behaviour in the woodlouse: An
583 experimental study employing video tracking and 14C-labelling. Ecotoxicology 5:35–45
584 <https://doi.org/10.1007/BF00116322>

585 Blanckenhorn WU (2000) The evolution of body size: what keeps organisms small? Q Rev
586 Biol 75:385–407

587 Boer P (2021) De Nederlandse mieren. <https://www.nlmieren.nl/websitepages/WOODANT>
588 MOUNDS.html. Accessed 26 Oct 2021

589 Brooks M, Kristensen K, van Benthem K, et al (2017) glmmTMB balances speed and
590 flexibility among packages for zero-inflated generalized linear mixed modeling. The R
591 Journal 9:378–4000

592 Boucher D (1985) The biology of mutualism : ecology and evolution. Oxford University Press,
593 New York

594 Calhã CF, Soares AMVM, Loureiro S (2012) Effects on survival and reproduction of
595 *Porcellio dilatatus* exposed to different Cd species. Ecotoxicology 21:48–55
596 <https://doi.org/10.1007/s10646-011-0762-6>

597 Castillo M, Kight S (2005) Response of terrestrial isopods, *Armadillidium vulgare* and
598 *Porcellio laevis* (Isopoda: Oniscidea) to the ant *Tetramorium caespitum*: morphology,
599 behavior and reproductive success. Invertebr Reprod Dev 3:183–190

600 Cazzolla Gatti R, Messina G, Tiralongo F, et al (2020) Learning from the environment: how
601 predation changes the behavior of terrestrial Isopoda (Crustacea Oniscidea). Ethol Ecol
602 Evol 32:29–45 <https://doi.org/10.1080/03949370.2019.1640799>

603 Cembrowski AR, Tan MG, Thomson JD, Frederickson ME (2014) Ants and ant scent reduce
604 bumblebee pollination of artificial flowers. *Am Nat* 183:133–139
605 <https://doi.org/10.1086/674101>

606 Dallinger R, Prosi F (1988) Heavy metals in the terrestrial isopod *Porcellio scaber* Latreille. II.
607 Subcellular fractionation of metal-accumulating lysosomes from hepatopancreas. *Cell*
608 *Biol Toxicol* 4:97–109 <https://doi.org/10.1007/BF00141289>

609 Dekoninck W, Ignace D, Vankerkhoven F, Wegnez P (2012) Verspreidingsatlas van de mieren
610 van België. *Bull la Société R belge d'Entomologie/Bulletin van K Belgische Ver voor*
611 *Entomol* 148:95–186

612 De Smedt P, Baeten L, Berg MP, et al (2018) Desiccation resistance determines distribution
613 of woodlice along forest edge-to-interior gradients. *Eur J Soil Biol* 85:1–3
614 <https://doi.org/10.1016/j.ejsobi.2017.12.002>

615 De Smedt P, Boeraeve P, Arijs G, Segers S (2020) De landpissebedden van België.
616 *Spinicornis*, Bonheiden

617 Depa Ł, Kaszyca-Taszakowska N, Taszakowski A, Kanturski M (2020) Ant-induced
618 evolutionary patterns in aphids. *Biol Rev.* 95: 1574–1589
619 <https://doi.org/10.1111/brv.12629>

620 Dixie B, White H, Hassall M (2015) Effects of microclimate on behavioural and life history
621 traits of terrestrial isopods: Implications for responses to climate change. *Zookeys*
622 2015:145–157 <https://doi.org/10.3897/zookeys.515.9399>

623 Doebeli M, Knowlton N (1998) The evolution of interspecific mutualisms. *Proc Natl Acad Sci*
624 *USA* 95:8676–8680 <https://doi.org/10.1073/pnas.95.15.8676>

625 Domisch T, Finér L, Neuvonen S, et al (2009) Foraging activity and dietary spectrum of wood
626 ants (*Formica rufa* group) and their role in nutrient fluxes in boreal forests. *Ecol Entomol*
627 34:369–377 <https://doi.org/10.1111/j.1365-2311.2009.01086.x>

628 Donisthorpe HSJK (1927) The guests of British ants, their habits and life-histories. George
629 Routledge and Sons, London

630 Driessen GJJ, Van Raalte AT, De Bruyn GJ (1984) Cannibalism in the red wood ant,
631 *Formica polyctena* (Hymenoptera: Formicidae). *Oecologia* 63:13–22

632 Eickwort G (1990) Associations of mites with social insects. *Annu Rev Entomol* 35:469–488
633 <https://doi.org/10.1146/annurev.ento.35.1.469>

634 Ernsting G, Fokkema D (1983) Antennal damage and regeneration in springtails
635 (Collembola) in relation to predation. *Netherlands J Zool* 33:476–484

636 Fischer E, Farkas S, Hornung E, Past T (1997) Sublethal effects of an organophosphorous
637 insecticide, dimethoate, on the isopod *Porcellio scaber* Latr. *Comp Biochem Physiol - C*
638 *Pharmacol Toxicol Endocrinol* 116:161–166 [https://doi.org/10.1016/S0742-](https://doi.org/10.1016/S0742-8413(96)00164-8)
639 [8413\(96\)00164-8](https://doi.org/10.1016/S0742-8413(96)00164-8)

640 Frouz J, Jílková V, Sorvari J (2016) Contribution of wood ants to nutrient cycling and
641 ecosystem function. In: Stockan J, Robinson E (eds) *Wood ant ecology and*
642 *conservation*. Cambridge University Press, pp 207–220

643 Gösswald K (1989) *Die Waldameise Band 2 Die Waldameise im Ökosystem Wald, ihr*
644 *Nutzen und ihre Hege*. Aula-Verlag, Wiesbaden

645 Haemig PD (1992) Competition between ants and birds in a Swedish Forest. *Oikos* 65:479–
646 483 <https://doi.org/10.2307/3545565>

647 Halaj J, Ross DW, Moldenke AR (1997) Negative effects of ant foraging on spiders in
648 Douglas-fir canopies. *Oecologia* 109:313–322 <https://doi.org/10.1007/s004420050089>

649 Hartig F (2020) DHARMA: Residual diagnostics for hierarchical regression models. R
650 package version 0.4.4.

651 Hawes C, Evans HF, Stewart AJA (2013) Interference competition, not predation, explains
652 the negative association between wood ants (*Formica rufa*) and abundance of ground
653 beetles (Coleoptera: Carabidae). *Ecol Entomol* 38:315–322
654 <https://doi.org/10.1111/een.12021>

655 Hawes C, Stewart A, Evans H (2002) The impact of wood ants (*Formica rufa*) on the
656 distribution and abundance of ground beetles (Coleoptera: Carabidae) in a Scots pine
657 plantation. *Oecologia* 131:612–619 <https://doi.org/10.1007/s00442-002-0916-6>

658 Hegarty KG, Kight SL (2014) Do predator cues influence turn alternation behavior in
659 terrestrial isopods *Porcellio laevis* Latreille and *Armadillidium vulgare* Latreille? Behav
660 Processes 106:168–171 <https://doi.org/10.1016/j.beproc.2014.06.005>

661 Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge,
662 Massachusetts

663 Hughes DP, Pierce NE, Boomsma JJ (2008) Social insect symbionts: evolution in
664 homeostatic fortresses. Trends Ecol Evol 23:672–677
665 <https://doi.org/10.1016/j.tree.2008.07.011>

666 Jäntti A, Suorsa P, Hakkarainen H, et al (2007) Within territory abundance of red wood ants
667 *Formica rufa* is associated with the body condition of nestlings in the Eurasian
668 treecreeper *Certhia familiaris*. J Avian Biol 38:619–624
669 <https://doi.org/10.1111/j.2007.0908-8857.03926.x>

670 Kärcher MH, Ratnieks FLW (2010) Honey bee guards recognise allospecific intruders via
671 “different odours” not “harmful-intruder odours.” J Apic Res 49:270–277
672 <https://doi.org/10.3896/IBRA.1.49.3.07>

673 Kistner DH (1982) The social insects’ bestiary. In: H.R. Hermann, ed., Social insects, vol. 3,
674 Academic Press, London. pp 1–244

675 Kronauer DJC, Pierce NE (2011) Myrmecophiles. Curr Biol 21:208–209.
676 <https://doi.org/10.1016/j.cub.2011.01.050>

677 Kurek P, Nowakowski K, Rutkowski T, et al (2020) Underground diversity: Uropodina mites
678 (Acari: Mesostigmata) from European badger (*Meles meles*) nests. Exp Appl Acarol
679 82:503–513 <https://doi.org/10.1007/s10493-020-00563-6>

680 Kuznetsova A, Brockhoff P, Christensen R (2017) lmerTest Package: Tests in linear mixed
681 effects models. J Stat Softw 82:1–26

682 Lardies MA, Carter MJ, Bozinovic F (2004) Dietary effects on life history traits in a terrestrial
683 isopod: The importance of evaluating maternal effects and trade-offs. Oecologia
684 138:387–395 <https://doi.org/10.1007/s00442-003-1447-5>

685 Lavy D, Van Rijn MJ, Zoomer HR, Verhoef HA (2001) Dietary effects on growth,

686 reproduction, body composition and stress resistance in the terrestrial isopods *Oniscus*
687 *asellus* and *Porcellio scaber*. *Physiol Entomol* 26:18–25 [https://doi.org/10.1046/j.1365-](https://doi.org/10.1046/j.1365-3032.2001.00211.x)
688 3032.2001.00211.x

689 Lenth R V (2021) emmeans: Estimated marginal means, aka least-squares means. R
690 package version 1.7.0. <https://CRAN.R-project.org/package=emmeans>

691 Loones J, Maelfait J, Rhijn J Van, et al (2008) De rode bosmier in Vlaanderen: voorkomen,
692 bedreigingen en herstelmaatregelen aan de hand van een detailstudie in de Sixtusbossen
693 (Poperinge-Vleteren). Rapporten van het Instituut voor Natuur- en Bosonderzoek 2008
694 (INBO.R.2008.01). Instituut voor Natuur en Bosonderzoek, Brussel

695 Maák IE, Sondej I, Juhász O, et al (2021) Unexpected distribution of subordinates around
696 nests of the wood ants. *Acta Oecologica* 110:103709. <https://doi.org/10.1016/j.actao.2021.>

697 Mueller UG, Gerardo NM, Aanen DK, et al (2005) The evolution of agriculture in insects.
698 *Annu Rev Ecol Evol Syst* 36:563–595
699 <https://doi.org/10.1146/annurev.ecolsys.36.102003.152626>

700 Myczko Ł, Kurek P, Tryjanowski P, et al (2021) Where to overwinter: burrows of medium-
701 sized carnivores as winter places for invertebrates in temperate environment. *Ecol*
702 *Entomol* 46:1177–1184 <https://doi.org/10.1111/een.13062>

703 Novgorodova TA (2005) Red wood ants (Formicidae) impact on multi-species complexes of
704 aphids (Aphididae) in the forest-park zone of Novosibirsk. *Euroasion Entomol J* 4:117–
705 120

706 O’Keefe ST (2000) Ant-like stone beetles, ants, and their associations (Coleoptera:
707 Scydmaenidae; Hymenoptera: Formicidae; Isoptera). *J New York Entomol Soc* 108:273–
708 303

709 Ospina B, Jonathan J, Lerma JM (2022) Intruders in the nest : Interaction of *Attaphila*
710 *paucisetosa* (Blattodea : Blaberoidea) with *Atta cephalotes* Workers (Hymenoptera:
711 Formicidae). *J Insect Behav.* <https://doi.org/10.1007/s10905-022-09794-4>

712

713 Paracer S, Ahmadjian V (2000) Symbiosis: an introduction to biological associations, 2nd

714 edn. Oxford University Press, New York

715 Parmentier T (2010) Taakverdeling en voedsel­ecologie bij de werksters van *Formica rufa* en
716 *Formica polyctena*. Master thesis, Department of Biology, Ghent University, Ghent,
717 Belgium.

718 Parmentier T (2020) Guests of Social Insects. In: Starr C (ed) Encyclopaedia of Social
719 Insects. Springer, Cham. https://doi.org/10.1007/978-3-319-90306-4_164-1

720 Parmentier T, Claus R, De Laender F, Bonte D (2021) Moving apart together: co-movement
721 of a symbiont community and their ant host, and its importance for community assembly.
722 *Mov Ecol* 9:25. <https://doi.org/10.1186/s40462-021-00259-5>

723 Parmentier T, Dekoninck W, Wenseleers T (2014) A highly diverse microcosm in a hostile
724 world: a review on the associates of red wood ants (*Formica rufa* group). *Insectes Soc*
725 61:229–237 <https://doi.org/10.1007/s00040-014-0357-3>

726 Parmentier T, Dekoninck W, Wenseleers T (2015a) Metapopulation processes affecting
727 diversity and distribution of myrmecophiles associated with red wood ants. *Basic Appl*
728 *Ecol* 16:553–562. <https://doi.org/10.1016/j.baae.2015.04.008>

729 Parmentier T, Dekoninck W, Wenseleers T (2015b) Context-dependent specialization in
730 colony defence in the red wood ant *Formica rufa*. *Anim Behav* 103:161–167
731 <https://doi.org/10.1016/j.anbehav.2015.02.023>

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

735 Parmentier T, Dekoninck W, Wenseleers T (2016b) Do well-integrated species of an inquiline
736 community have a lower brood predation tendency? A test using red wood ant
737 myrmecophiles. *BMC Evol Biol* 16:12 <https://doi.org/10.1186/s12862-016-0583-6>

738 Parmentier T, Dekoninck W, Wenseleers T (2017) Arthropods associate with their red wood
739 ant host without matching nestmate recognition cues. *J Chem Ecol* 43:644–661
740 <https://doi.org/10.1007/s10886-017-0868-2>

741 Parmentier T, De Laender F, Bonte D (2020) The topology and drivers of ant – symbiont

742 networks across Europe. Biol Rev. 95: 1664–1688 <https://doi.org/10.1111/brv.12634>

743 Parmentier T, De Laender F, Wenseleers T, Bonte D (2018) Prudent behavior rather than
744 chemical deception enables a parasite to exploit its ant host. Behav Ecol 29:1225–1233
745 <https://doi.org/10.1093/beheco/ary134>

746 Patton WK (1994) Distribution and ecology of animals associated with branching corals
747 (*Acropora* spp.) from the Great Barrier Reef, Australia. Bull Mar Sci 55:193–211

748 Pierce N, Braby M, Heath A, et al (2002) The ecology and evolution of ant association in the
749 Lycaenidae (Lepidoptera). Annu Rev Entomol 47:733–771

750 Punntila P, Niemelä P, Karhu K (2004) The impact of wood ants (Hymenoptera : Formicidae)
751 on the structure of invertebrate community on mountain birch (*Betula pubescens* ssp .
752 *czerepanovii*). Ann Zool Fennici 41:429–446

753 Rettenmeyer CW, Rettenmeyer ME, Joseph J, Berghoff SM (2011) The largest animal
754 association centered on one species: the army ant *Eciton burchellii* and its more than
755 300 associates. Insectes Soc 58:281–292 <https://doi.org/10.1007/s00040-010-0128-8>

756 Reznikova Z, Dorosheva H (2004) Impacts of red wood ants *Formica polyctena* on the
757 spatial distribution and behavioural patterns of ground beetles (Carabidae). Pedobiologia
758 48:15–21 <https://doi.org/10.1016/j.pedobi.2003.06.002>

759 Robertson BA, Hutto RL (2006) A framework for understanding ecological traps and an
760 evaluation of existing evidence. Ecology 87:1075–1085

761 Robinson EJJ, Stockan JA, Iason GR (2016) Wood ants and their interactions with other
762 organisms. . In: Stockan J, Robinson E (eds) Wood ant ecology and conservation.
763 Cambridge University Press, pp 177–207

764 Robinson NA, Robinson EJJ (2013) Myrmecophiles and other invertebrate nest associates
765 of the red wood ant *Formica rufa* (Hymenoptera Formicidae) in Northwest England. Br J
766 Entomol Nat Hist 26:67–88

767 Rocha F, Lachaud J, Pérez-lachaud G (2020) Myrmecophilous organisms associated with
768 colonies of the ponerine ant *Neoponera villosa* (Hymenoptera: Formicidae) nesting in
769 *Aechmea bracteata* bromeliads: a biodiversity hotspot. Myrmecological News 30:73–92.

770 <https://doi.org/10.25849/myrmecol.news>

771 Russell V. Lenth (2016). Least-Squares Means: The R Package lsmeans. Journal of
772 Statistical Software 69:1-33. doi:10.18637/jss.v069.i01

773 Schoener TW (1979) Inferring the properties of predation and other injury-producing agents
774 from injury frequencies. Ecology 60:1110–1115

775 Seifert B (2007) Die Ameisen Mittel- und Nordeuropas. Iutra Verlags- und
776 Vertriebsgesellschaft, Görlitz

777

778 Skinner GJ (1980) The feeding habits of the wood-ant, *Formica rufa* (Hymenoptera:
779 Formicidae), in limestone woodland in North-West England. J Anim Ecol 49:417–433
780 <https://doi.org/10.2307/4255>

781 Sörensen U, Schmidt GH (1987) Vergleichende Untersuchungen zum Beuteeintrag der
782 Waldameisen (Genus: *Formica*, Hymenoptera) in der Bredstedter Geest (Schleswig-
783 Holstein). J Appl Entomol 103:153–177. [https://doi.org/10.1111/j.1439-](https://doi.org/10.1111/j.1439-0418.1987.tb00974.x)
784 [0418.1987.tb00974.x](https://doi.org/10.1111/j.1439-0418.1987.tb00974.x)

785 Stadler B, Fiedler K, Kawecki TJ, Weisser WW (2001) Costs and benefits for phytophagous
786 myrmecophiles: When ants are not always available. Oikos 92:467–478.
787 <https://doi.org/10.1034/j.1600-0706.2001.920308.x>

788 Stockan JA, Robinson EJH (2016) Wood ant ecology and conservation. Cambridge
789 University Press

790 Sutton SL (1980) Woodlice. Pergamon Press Ltd, England

791 Trigos-Peral G, Juhász O, Kiss PJ, et al (2021) Wood ants as biological control of the forest
792 pest beetles *Ips* spp. Sci Rep 11:1–10. <https://doi.org/10.1038/s41598-021-96990-5>

793 Upstrom KA (2010) Mites (Acari) associated with the ants (Formicidae) of Ohio and the
794 harvester ant, *Messor pergandei*, of Arizona. Master thesis, Ohio State University, USA.

795 van der Heijden MGA, Martin FM, Selosse MA, Sanders IR (2015) Mycorrhizal ecology and
796 evolution: The past, the present, and the future. New Phytol 205:1406–1423

797 <https://doi.org/10.1111/nph.13288>

798 Venables WN, Ripley BD (2002). *Modern Applied Statistics with S*, Fourth edition. Springer,
799 New York.

800 Von Beeren C, Brückner A, Hoenle PO, et al (2021) Multiple phenotypic traits as triggers of
801 host attacks towards ant symbionts: body size, morphological gestalt, and chemical
802 mimicry accuracy. *Front Zool* 1–18 <https://doi.org/10.1186/s12983-021-00427-8>

803 White JW, Grigsby CJ, Warner RR (2007) Cleaning behavior is riskier and less profitable
804 than an alternative strategy for a facultative cleaner fish. *Coral Reefs* 26:87–94
805 <https://doi.org/10.1007/s00338-006-0161-2>

806 Witte V, Leingärtner A, Sabaß L, et al (2008) Symbiont microcosm in an ant society and the
807 diversity of interspecific interactions. *Anim Behav* 76:1477–1486
808 <https://doi.org/10.1016/j.anbehav.2008.05.010>

809 Zidar P, Kos M, Ilič E, et al (2019) Avoidance behaviour of isopods (*Porcellio scaber*)
810 exposed to food or soil contaminated with Ag- and CeO₂- nanoparticles. *Appl Soil Ecol*
811 141:69–78 <https://doi.org/10.1016/j.apsoil.2019.05.011>

812 Zingg S, Dolle P, Voordouw MJ, Kern M (2018) The negative effect of wood ant presence on
813 tick abundance. *Parasites and Vectors* 11:1–9 [https://doi.org/10.1186/s13071-018-2712-](https://doi.org/10.1186/s13071-018-2712-0)
814 0

815

816

817 FIGURE LEGENDS

818 **Fig. 1** a) The two study sites of the sampled nests in the province of West-Flanders in the
819 North-West of Belgium, more specifically Bruges (1) and Poperinge (2). b) Schematic top view
820 of the setup of the pitfalls around a red wood ant nest to assess the spatial and size distribution.
821 Red wood ant nest (circle), pitfalls (filled rectangles) at 1, 5 and 10 meters from the nest and
822 one pitfall in the nest

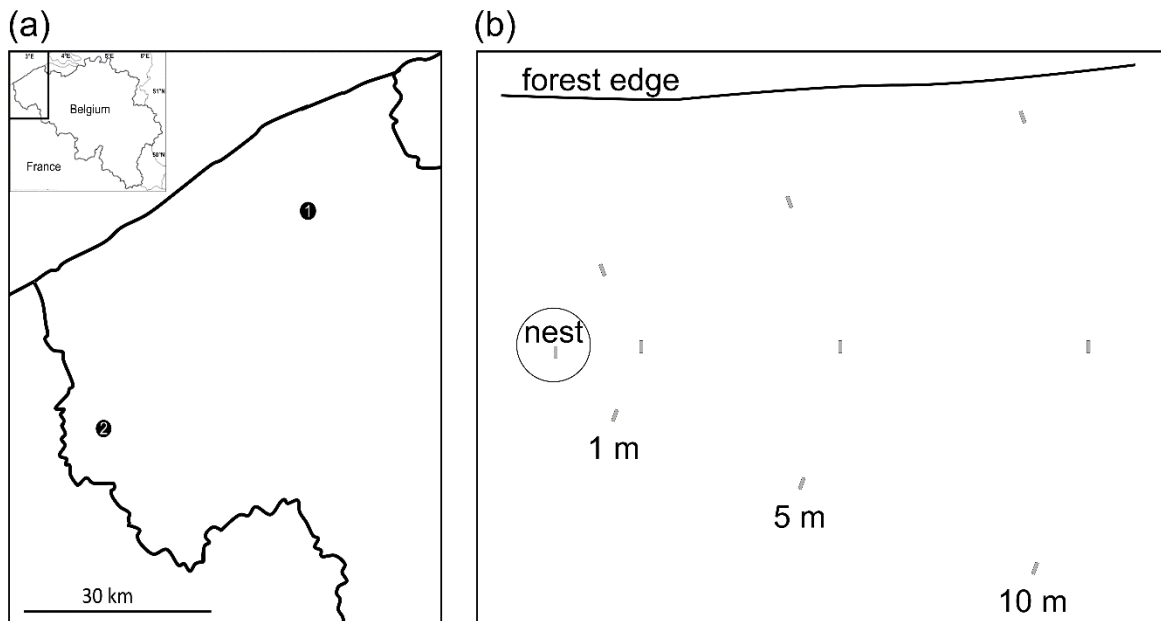
823 **Fig. 2** Effect of ant density (ants/trap, fourth-root transformed) and sampling distance from the
824 nest on the abundance of adult *P. scaber* per pitfall (fourth-root transformed, LMM, mean and
825 95% CI)

826 **Fig. 3** Model estimates displaying the individual dry mass (g, ln transformed) of female and
827 male *P. scaber* individuals at three sampling distances outside the ant nest and with varying
828 ant densities (ants / trap, fourth-root transformed) (LMM, mean and 95% CI)

829

830 **Fig. 4** The effect of ant density (ants/trap, fourth-root transformed) and sampling distance (1
831 m, 5 m and 10 m) on the proportion of *P. scaber* individuals missing one or two antennae
832 (GLMM, mean and 95% CI)

833 **Fig. 5** The female reproductive investment, calculated as the dry mass of the released offspring
834 divided by the dry mass of the mother, in presence and absence of red wood ants (mixed
835 effects beta regression, mean and 95% CI)



837

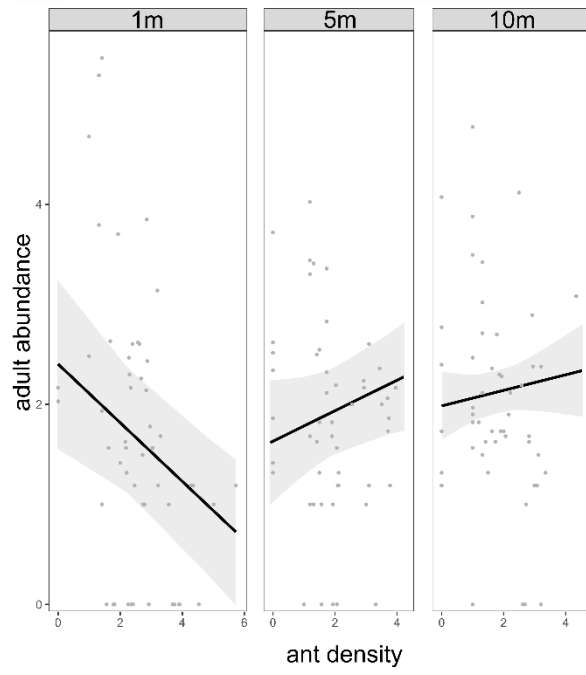
838 **Fig. 1**

839

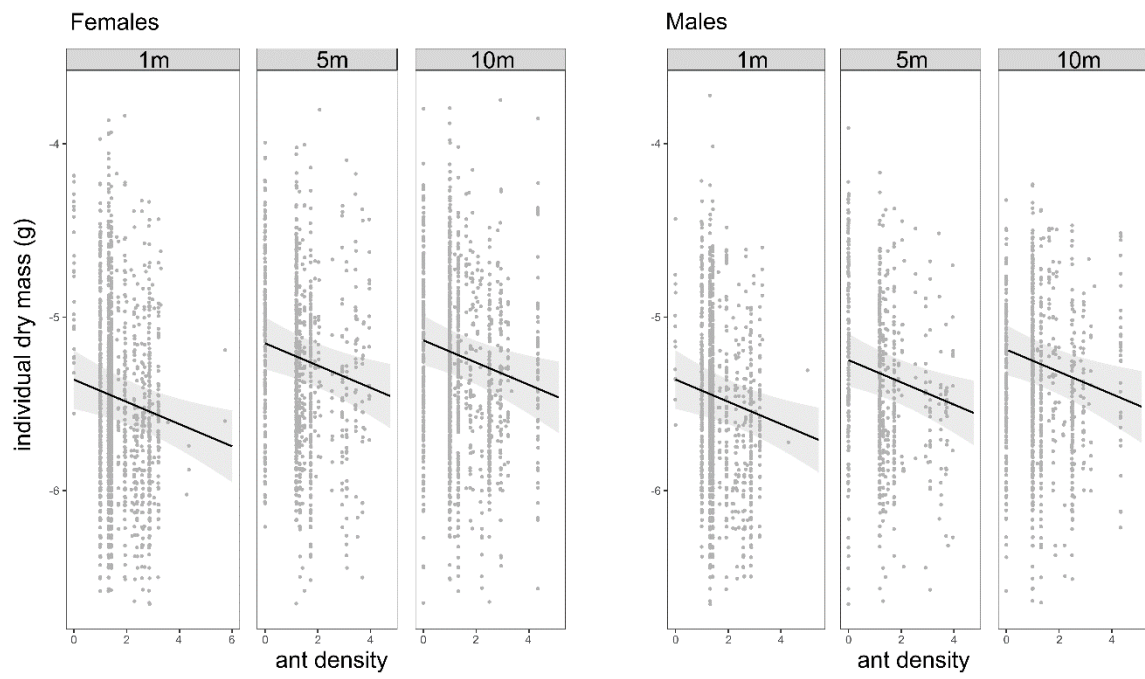
840

841 **Fig. 2**

842

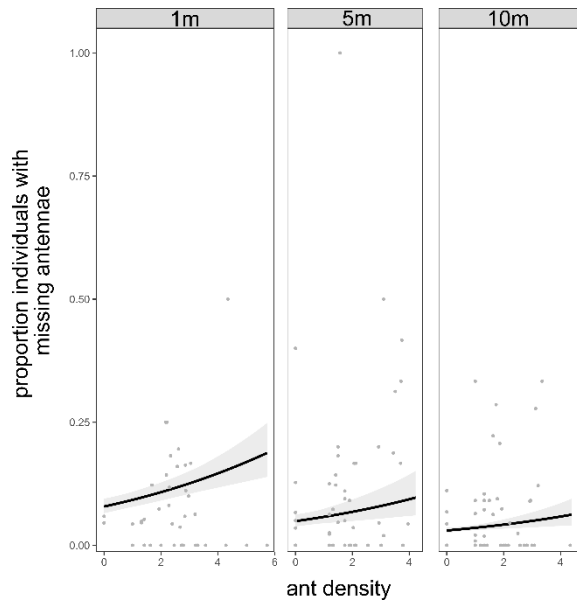


843



844

845 **Fig. 3**

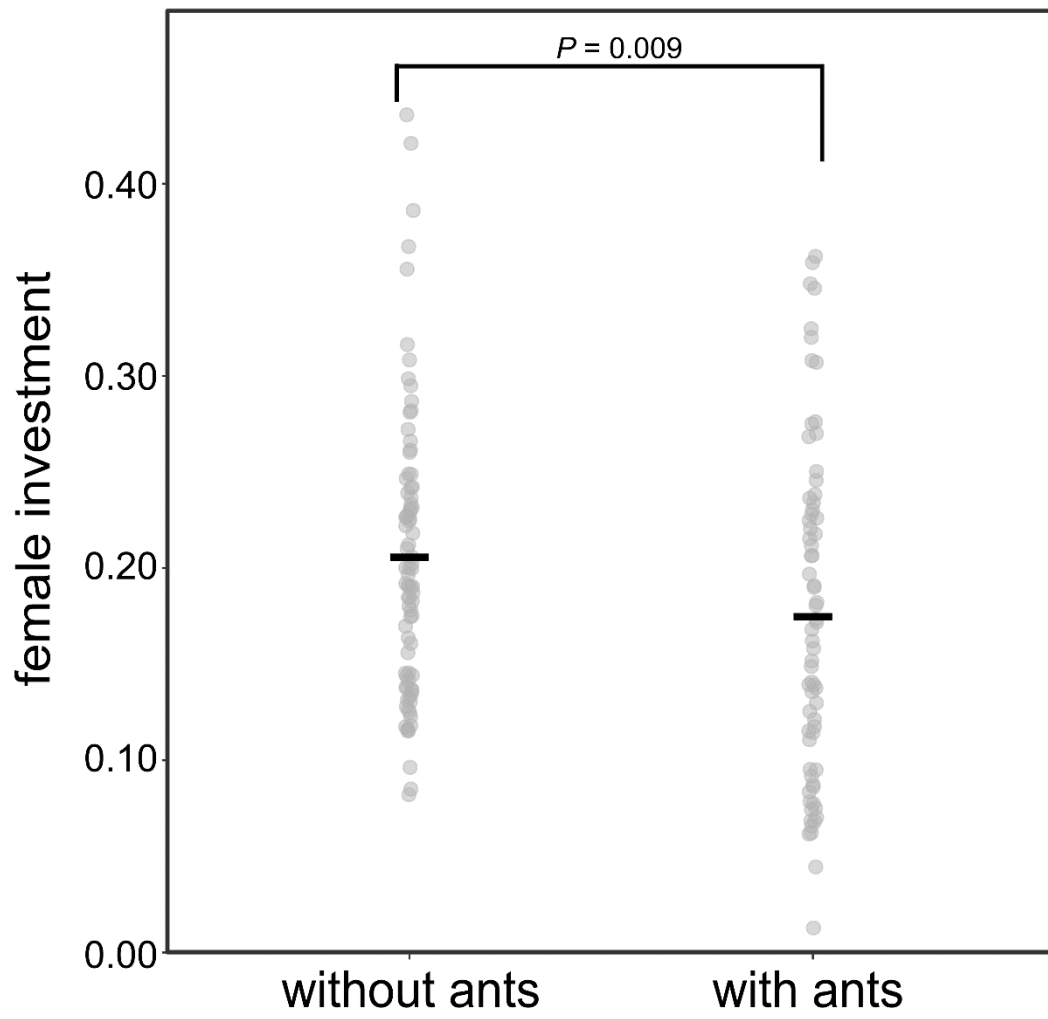


846

847 **Fig. 4**

848

849



850

851 **Fig. 5**

852

853