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### Host following of an ant associate during nest relocation

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22 **Abstract**

23 Ant nests are relatively stable and long-lasting microhabitats that attract a diverse group of  
24 arthropods. Particular stressors, however, can trigger ants to relocate their nest to a new site. It  
25 is unclear how associated arthropods respond to occasional nest moving of their host. Here, I  
26 report field observations which showed that the potentially parasitic larvae of the beetle *Clytra*  
27 *quadripunctata* follow their red wood ant host during nest relocation, either by crawling on  
28 their own or by being carried by the host workers. These observations shed new light on the  
29 spatial dynamics between ants and their associates.

30

## 31 **Introduction**

32 The large group of arthropods that live as guests in the nests of social insects disperse in a  
33 metapopulation context from one suitable host nest to another. Dispersal involves the successful  
34 detection and tracking of their host nest in the landscape, presumably following host-specific  
35 chemical cues (Akre and Rettenmeyer 1968; Hölldobler 1970). The host might periodically  
36 move to new nest sites (McGlynn 2012). Many associated guests freely walk or crawl in the  
37 nest and feed on brood or pilfer retrieved prey. Nest relocation by the host will force these  
38 associates to decide whether to follow the host to a new site or to stay in the abandoned nest.  
39 Following the host will ensure a continuity of food and protection for the guest, but requires  
40 specific adaptations to recognize the onset of nest relocation and to track the new nest.  
41 Successful host following has been widely reported in arthropods associated with nomadic army  
42 ants. Army ant guests can detect the pheromone trails of their host and are well known to run  
43 among the moving horde (Akre and Rettenmeyer 1968; Hölldobler and Wilson 1990).  
44 However, behaviour of their host ants is highly atypical for social insects, as they do not  
45 construct permanent nests and almost continuously migrate to new temporary nest sites.  
46 Consequently, associates of these itinerant hosts evolved advanced tracking behaviour to be  
47 able to keep up. In contrast, social insects with permanent nests only move occasionally and  
48 how guests respond to the infrequent and unpredictable nest relocation events is poorly studied.  
49 So far, it has been anecdotally reported that the rove beetle *Dinarda* and the sowbug  
50 *Platyarthrus hoffmannseggii* followed their *Formica* host to a new nest site (Wheeler 1910;  
51 Donisthorpe 1927).

52

53 European red wood ants construct large conspicuous nests with an aboveground mound of  
54 organic thatch. They can occupy a nest site for many years and even decades (Gösswald 1989).

55 Nevertheless, when nest conditions start to deteriorate, red wood ants are capable of moving  
56 the entire colony to new nest sites (Möglich and Hölldobler 1974). Their nests harbour a rich  
57 community of obligately associated arthropods (Parmentier et al. 2014). The larvae of the leaf  
58 beetle (Chrysomelidae) *Clytra quadripunctata* (Linnaeus, 1758) are common members of this  
59 nest-inhabiting community (Parmentier et al 2015). They are protected by a hard, pear-shaped  
60 case made of excrements and nest material (Fig. 1). Lab tests demonstrated that their larvae  
61 preferentially live in the dense brood chambers of red wood ants (Parmentier et al. 2016a). This  
62 is confirmed by observations in the field, where I mostly detect the larvae in the deep and  
63 thermoregulated brood chambers. The exact effect of these larvae on their host is not fully  
64 understood. Lab tests showed that they readily feed on ant brood and prey collected by the ants  
65 (Parmentier et al. 2016b). It was also argued that they consume organic nest material and debris  
66 (Donisthorpe 1902). Their isotopic  $^{15}\text{N}$  enrichment, which was considerably higher than  
67 expected for a strict decomposer (Parmentier et al. 2016b), and their preferred position in the  
68 brood chambers, however, suggest that they are scavengers with a potential negative effect on  
69 their host. Adult beetles hatch from the case and readily escape out of the nest (Fig. 1A). The  
70 adult beetles settle and feed on plants near wood ant nests. After mating, the female deposits  
71 her eggs covered with a protective case of excrements near or on the nest (Donisthorpe 1902).  
72 It has been suggested that the beetle eggs are picked up by the ant workers and carried into the  
73 nest (Donisthorpe 1902). But possibly, the hatched larvae can locate neighbouring nests and  
74 colonize them on their own. Here I report how the larvae adaptively respond to an occasional  
75 nest relocation event of its red wood ant host.

76

## 77 **Methods**

78 On 02.05.2018 I observed a colony of the red wood ant *Formica polyctena* Förster, 1850  
79 relocating its nest in the nature reserve Hoge Dijken in Oudenburg, Belgium. Colony relocation  
80 in red wood ants can be recognized by massive amounts of workers carrying brood and other  
81 adults (social carrying) in a stereotyped way to another nest site (Möglich and Hölldobler 1974)  
82 (Fig. 1B, video S1). The new nest site was 5 meters away from the old nest and was constructed  
83 on top of a large pile of woodchips originating from chopped exotic trees growing in the reserve  
84 (Fig. 2). A closer look at the emigration column, revealed the presence of crawling larvae of  
85 *Clytra quadripunctata*. The old nest was large (surface area 0.87 m<sup>2</sup>) and very active at least  
86 until the beginning of April 2018 (last visit to this site prior to the nest relocation). It has been  
87 lying there for minimum 12 years (pers. communication Dr. W. Dekoninck). The organic  
88 mound was constructed on a fallen tree branch. When I observed the emigration column on  
89 02.05.2018, the organic mound was disintegrated and the tree branch was exposed. The nest  
90 was no longer repaired and material of the original mound was brought to the new nest.  
91 Probably the peak of nest moving was already going on for some days, and the first relocations  
92 may already started weeks before (cf. Mabelis 1978). I returned to the study site to observe the  
93 progress of the nest relocation and its effect on the behaviour of *C. quadripunctata* on  
94 04.05.2018 and 11.05.2018.

95 On 08.08.2018, I tested the behaviour of red wood ants towards the larvae of *C. quadripunctata*  
96 by placing twenty beetle larvae at the foot of the wood chip pile (50 cm from nest entrance on  
97 the pile) within a very active ant trail.

98 **Results and Discussion**

99 I here report a rare observation of an intranidal ant associate or myrmecophile joining the host  
100 colony relocation to a new permanent nest site. I observed that the larvae of the beetle *Clytra*  
101 *quadripunctata* accompanied their red wood ant host *Formica polyctena* to a new nest site.  
102 Most of the beetle larvae crawled to the new nest on their own during nest relocation. Some,  
103 however, were also carried over a short distance by a host worker.

104 On 02.05.2018, I detected 45 *C. quadripunctata* larvae which were slowly crawling in company  
105 with a moving *F. polyctena* colony towards a new nest site. (Fig. 1B-D, Fig. 2, video S1, Table  
106 1). None of the larvae headed back to the old nest. I also observed 135 beetle larvae crawling  
107 to the top of a pile of wood chips, where the new nest was founded (Fig. 2). I found 21 *C.*  
108 *quadripunctata* larvae on the top of the old nest and in a cavity of a tree branch that supported  
109 this nest. Two days later, the emigration was still ongoing, but considerably less intense. Now  
110 I only detected two immobile larvae in the emigration column, 51 larvae on the pile and 17  
111 larvae on the old nest. I also observed winged sexuals on the new nest. On 11.05.2018, the old  
112 nest was completely deserted, but I still found 15 larvae on top and in the branch. No larvae  
113 were seen between the nests. In addition, I did not find any larvae on the new nest (Table 1).  
114 As the larvae mostly reside in the deep parts of the nest (Parmentier 2016 et al. a, pers.  
115 observations), they probably moved to the core of the new nest. This was confirmed by turning  
116 a wood piece on top of the new nest, where I found three larvae.

117 Remarkably, I also saw three different beetle larvae picked up and dragged by the ants in the  
118 direction of the new site (Fig. 1D and 1E, video S2) on 02.05.2018. Ants, however, did only  
119 transport the larvae during a part of the trajectory between the nests. The transport of the heavy  
120 larvae was hampered by obstacles along the trajectory. When the larvae got stuck, they were  
121 dropped. In one occasion, I saw another worker picking up a dropped beetle larvae and

122 transporting it for some metres. The attractiveness of the larvae to the ants was further  
123 underlined with a small experiment on 08.08.2018. Twenty beetle larvae were placed at the  
124 foot of the wood chip pile (50 cm from nest entrance on the pile) within a very active ant trail  
125 (position indicated with B on Fig. 2). Thirty minutes later, six larvae were carried by the workers  
126 to the top of the pile where the new nest was constructed, four larvae were transported over a  
127 distance of circa 15 cm to the nest, two larvae were dragged for some centimetres and eight  
128 larvae were ignored. Carrying of the covered eggs and larvae was already described in the  
129 beginning of the 20<sup>th</sup> century (Donisthorpe 1902). The case or the larvae could release  
130 appealing substances, a strategy used by other associates that are carried by their ant host  
131 (Hölldobler and Wilson 1990; Witte et al. 2002). Alternatively, the ants might mistake the case  
132 for building material, which is constantly brought to the nest.

133 Accompanying the host to the new nest is an adaptive response, as the beetle will secure its  
134 food provisioning and protection. The beetle larvae are always found in red wood ant nests and  
135 are probably not able to survive in isolation of its host (Donisthorpe 1902). Nest relocations in  
136 social insects mainly occur in response to shifts in microclimatic conditions or after a sudden  
137 disturbance (McGlynn 2012). In other animal groups such as mammals (Lewis 1995) and birds  
138 (Goguen and Mathews 1996), parasite avoidance has been argued as alternative major driver  
139 for nest relocation. So far, parasitic load as a trigger for nest relocation was only demonstrated  
140 in one eusocial insect, i.e. the wasp species *Mischocyttarus labiatus* (Litte 1981), but red wood  
141 ants may also benefit from avoiding parasites in accordance with the parasitic load hypothesis  
142 posed by McGlynn *et al.* (2004). I found that a large fraction of the potential harmful beetle  
143 larvae stayed behind in the old nest (Table 1). Red wood ant nests also harbour many other  
144 brood predators and cleptoparasites (Parmentier et al. 2014, 2016b) and likely a significant part  
145 will not be able to find the new nest site. Consequently by moving to a new site, red wood ants

146 may considerably reduce parasite load. The new nest is only 5 metres away, but relocations to  
147 more remote sites may reduce parasite load even further.

148

149 **Tables**

150 **Table 1.**

date	Larvae in old nest ( <i>N</i> )	Larva crawling between nests ( <i>N</i> )	Larvae carried ( <i>N</i> )	Larvae on pile and new nest ( <i>N</i> )
02.05.2018	21	45	3	135
04.05.2018	17	0 (2 immobile)	0	51
11.05.2018	15	0	0	0

151

152

153 **Supplementary material**

154 Video S1: A crawling *Clytra quadripunctata* larva following the host ant migration column to  
155 the new nest. Note the transport of adult workers (social carrying) in the ant column, typical  
156 behaviour displayed during ant nest relocation.

157 Video S2: *Clytra quadripunctata* carried by the host ant *Formica polycтена*.

158

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161 thank Agentschap voor Natuur en Bos for permission to conduct field work and sampling in de  
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163 **Figures**

164 Fig. 1. *Clytra quadripunctata* and its host *Formica polyctena*. A) A red wood ant worker is  
165 inspecting an adult of *C. quadripunctata* which resides on plants near the nest of its host ant.  
166 B) A beetle larva crawling on its own to the new nest. A worker is transporting another worker  
167 in the background (indicated with arrow). The transported worker folds its legs to the body and  
168 bends its abdomen. This adult transport (social carrying) is typical observed during nest  
169 relocation (see also video S1). C) A larva of *Clytra quadripunctata* is accompanying the  
170 migration column of its host ant *Formica polyctena* during nest relocation. D) The beetle larva  
171 can protect its soft white body by sealing the case with its armoured head (brown). Workers on  
172 the bottom of the figure are inspecting another beetle larva during the nest relocation. E) A  
173 worker is carrying a beetle larva during nest relocation (see also video S2).

174 Fig. 2. Positioning of the old and new nest. The new nest covered with fine thatch lies on top  
175 (indicated with an ellipse) of a pile of woodchips. Crawling larvae between the nest and  
176 transported larvae were observed at the location indicated with A. Larvae were offered at the  
177 the foot of the pile, position indicated with B.

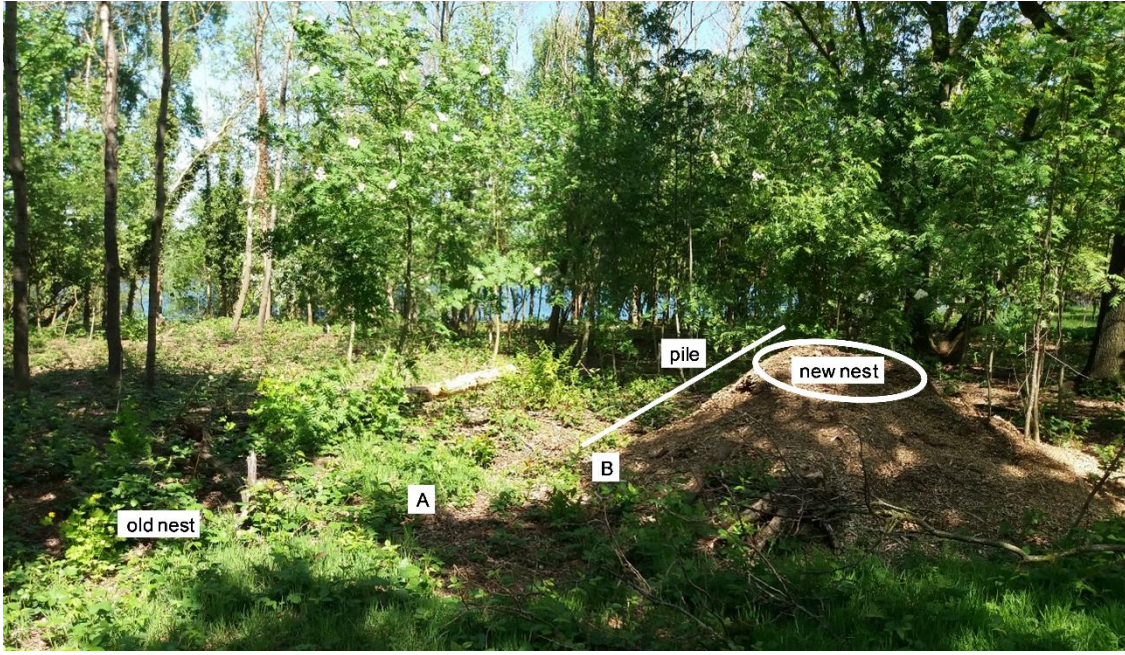
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180

181 Fig. 1



182

183 Fig. 2

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185

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