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Nest mounds of red wood ants (*Formica aquilonia*): hot spots for litter-dwelling earthworms

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Abstract A previously undocumented association between earthworms and red wood ants (*Formica aquilonia* Yarr.) was found during an investigation of the influence of wood ants on the distribution and abundance of soil animals in boreal forest soil. Ant nest mounds and the surrounding soil of the ant territories were sampled. The ant nest mound surface (the uppermost 5-cm layer) harboured a much more abundant earthworm community than the surrounding soil; the biomass of the earthworms was about 7 times higher in the nests than in the soil. *Dendrodrilus rubidus* dominated the earthworm community in the nests, while in soils *Dendrobaena octaedra* was more abundant. Favorable temperature, moisture and pH (Ca content), together with abundant food supply (microbes and decomposing litter) are likely to make a nest mound a preferred habitat for earthworms, provided that they are not preyed upon by the ants. We also conducted laboratory experiments to study antipredation mechanisms of earthworms against ants. The experiments showed that earthworms do not escape predation by avoiding contact with ants in their nests. The earthworm mucus repelled the ants, suggesting a chemical defence against predation. Earthworms probably prevent the nest mounds from becoming overgrown by moulds and fungi, indicating possible mutualistic relationships between the earthworms and the ants.

Key words *Dendrobaena octaedra* · *Dendrodrilus rubidus* · *Formica rufa* nest mound · Predator avoidance · Mutualism

Introduction

The nest building activity of *Formica rufa* group (Hymenoptera, Formicidae) ants generates clearly distinguishable patches in forests. It is not only ants that populate the nest mounds; they live in more or less close association with an array of species of various phyla (Hölldobler and Wilson 1990). The presence of earthworms (Oligochaeta) in inactive ant nests during winter has been reported in one study (Gößwald 1990), but there is no quantitative or qualitative data on the presence of earthworms in active nest mounds. A search of a database (FORMIS 1995; World Wide Web: http://www.public.iastate.edu/~entomology/Formis) with more than 20 000 references on ants resulted in no information on ant-earthworm associations. Kistner (1982) and Hölldobler and Wilson (1990) give extensive lists of ant-associated organisms throughout the world, but there is no reference to earthworms. This is surprising because our recent findings show that earthworms are common in ant nests, where their biomass is much higher than that of any other faunal group, except the ants themselves.

Studies on ant-associated arthropod fauna have revealed several specific adaptations to avoid predation by ants. Similarly, earthworms – as potential prey for the ants – are likely to have evolved an effective predator avoidance mechanism. The protective adaptations of arthropods include protective structure, avoidance by agility, and imitation of ants by producing odours that induce co-operative or alarm behaviour, or by excreting toxic chemicals to deter the ants (Hölldobler and Wilson 1990). In the apparent absence of a protective carapace or the ability to imitate the shape and behaviour of the ants, the most plausible mechanisms for the earthworms to survive in the nest mounds are physical avoidance due to their cryptic lifestyle, and excretion of co-operative or ant-repelling chemicals.

We report data on the existence of earthworms, particularly of two litter dwelling species *Dendrobaena*

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octaedra (Sav.) and *Dendrodrilus rubidus* (Sav.), in nest mounds of *Formica aquilonia* ants in relation to their abundance in the soil close (2–20 m) to the nests. To study the mechanisms underlying co-existence between ants and earthworms in the nests, we conducted laboratory experiments to test the following hypotheses: predator avoidance by the earthworms results from their capability to (1) physically escape predation, or (2) excrete ant repelling chemicals. Possible mechanisms for a mutually beneficial association between earthworms and ants are also discussed.

Material and methods

Field studies

The study site, *c.* 7 ha in size, is situated in Konnevesi, Central Finland (62°37′N, 26°20′E), in a mature *Myrtillus*-type forest dominated by Norway spruce (*Picea abies*), silver birch (*Betula pendula*) and Scots pine (*Pinus sylvestris*). In late July 1993, a total of 60 nest mounds of *F. aquilonia* were counted in the area; ten typical representatives of them were selected for the investigation of the earthworm abundance, substrate pH (H_2O), water content (24 h at +80°C) and organic matter content (5 h at +550°C). Each of the ten nest territories (radius *c.* 20 m) were sampled along two line transects from the nest centre towards territory edges (sampling distances 0, 2, 11 and 20 m from the nest). All samples, including the ones from which the abiotic variables were measured, were taken using a large soil corer (4 cm depth, 17 cm diameter); the nest sample included the top layer of the nest and soil samples included litter, fermentation and humus layers. The earthworms were extracted using a large wet extractor with electric bulbs to heat the sample for 3 hours. After extraction, the earthworms were identified and oven dried (+70°C for 24 h) for dry mass.

Total microbial biomass in the samples was determined by the substrate-induced respiration method (SIR, see Anderson and Domsch 1978) and concentration of Ca was analysed using plasma emission spectrometry. Samples were taken in late July 1995 at 0 and 11 m distance from five of the ten nests. From each of the samples, 500 g fresh mass was homogenised and SIR was measured for four subsamples (4 × 3 g organic material, $n = 5$).

Laboratory studies

We conducted three feeding preference tests of the hypothesis on the chemical defence mechanism, and observed the behaviour of the earthworms and the ants in laboratory conditions to test the contact-avoidance mechanism. The feeding preference tests were designed to explore (1) whether earthworms are used as food, (2) whether the reaction of the ants to a prey differ between earthworm species that are common in the nests (*Dendrobaena octaedra* Eisen) and the ones that are rarely encountered in there (*Lumbricus rubellus* Hoffmeister and *Aporrectodea caliginosa* Sav.), and (3) whether earthworm mucus acts as a repelling agent against the attacks of the ants.

To establish laboratory colonies, we sampled ant nests for workers, larvae, pupae and queens in Konnevesi area in June 1995. Twelve ant colony fragments, originating from six field colonies, were set up in glass terraria (35 × 26 cm, 25 cm in height), each accomodating one to four queens, and several hundred workers from inside and outside the nest. The bottom of the terrarium was composed of a layer (1 cm) of plaster of paris on the bottom to retain moisture, and a layer (1 cm) of turf. In a corner of each terrarium, three separately collected layers of ant nest materials (dry nest centre cavity, moist and dry surface layer materials – total volume 1.1 l) were successively piled to imitate a nest mound structure. The outer glass walls close to the nest mound were covered with a piece of black plastic sheet to avoid light penetration to the nest interior. The ant colonies were kept at room temperature (*c.* 25°C). During the experiment, the colonies were fed with a mixture of honey and water as carbohydrate source, and a mixture of tuna fish, boiled eggs and insects to provide protein for the ants.

In August, 2 months after the establishment of the colonies, feeding preference tests were conducted. Freshly cut pieces (*c.* 2–3 mm) of the three earthworm species were each placed on separate plastic trays, and simultaneously offered as food to six ant colonies. The trays were situated *c.* 20 cm from the edge of the nest mound in an area previously accessible to the ants. The latency time until the ants touched the prey was used a measure of preference. The test was repeated with three nests with the following exeption: to reduce possible extraction of stress-induced chemicals that might affect the prey quality, the worms were anesthezized in warm water prior to cutting.

The role of earthworm mucus as a repelling agent against attack was studied by offering three types of prey items (freshly killed aphids, heteropterans (Miridae) and ant *Lasius niger* (L.) workers) simultaneously on a plastic tray for three ant colonies. Half of the prey items served as untreated controls whereas the other part of the prey was covered with *D. octaedra* mucus by dipping the prey into fresh mucus. The untreated controls and the prey items with earthworm mucus (two prey items per prey type) were randomly placed on one plastic tray (5 × 5 cm), and the mesure for preference was determined as described above.

Direct observations on the behaviour of earthworms in ant nests were conducted by carefully transferring five individuals of *D. octaedra* into six of the nests. The plastic sheet covering the nest from outside was removed one to three times per day for an observation period of *c.* 5 min at a time, and the behaviour of the ants and the earthworms was observed. The observation period lasted 30 days.

Results

Field studies

Abiotic factors

All nests (height 43 ± 4 cm; mean ± SE, bottom diameter 103 ± 8 cm; only the organic, plant-free part of the nest mound considered) had a similar structure: beneath the dry uppermost surface (0.5–1.5 cm thick) was a moist stratum (2–10 cm thick) of more humified material, containing significant amounts of earthworm casts and particles of conifer resin. Most of the sample material was composed of this moist nest material. The nest material below the moist layer gets abruptly drier towards the center of the nest (see also Pokarzhevskij 1981).

The pH and Ca contents of the nest surface (5.42 ± 0.08 and 5.80 ± 0.34 g/kg; mean ± SE, respectively) were significantly higher than that of the soil (pH = 4.69 ± 0.06 and Ca = 3.65 ± 0.72 g/kg). Water content (46 ± 4% of fresh mass) was lower and organic matter content (86 ± 2% of dry mass) higher in the nest samples than in the soil (69 ± 1% and 50 ± 3% for water content and organic matter content, respectively). All above mentioned differences were significant at the $P < 0.05$ level (Student's *t*-test).

Abundance of the earthworms and microbial biomass

Two species of earthworms were recorded in the samples, *Dendrobaena octaedra* and *Dendrodrilus rubidus*. *Dendrobaena octaedra* clearly dominated in the soil, where *Dendrodrilus rubidus* occurred only occasionally. Ant nests harboured a great earthworm abundance and biomass: *D. rubidus* (mean ± SE; in the soil 9 ± 3 individuals/m^2 and the nests 485 ± 211 individuals/m^2) composed 68% of the total earthworm biomass in the nests, the total earthworm biomass being ca. seven times higher in the nests than in the soil (Kruskal-Wallis ANOVA $\chi^2 = 11.23$, $P = 0.011$, Fig. 1). *Dendrobaena. octaedra* tended also to be more numerous in the nests (mean ± SE; 220 ± 87 individuals/m^2) than in the soil (119 ± 12 individuals/m^2).

Total microbial biomass (including fungi and bacteria) was about three times higher in the nest material (4.92 ± 0.33 mg microbial carbon/g dry weight; mean ± SE) than in the soil (1.72 ± 0.30 mg C_{mic}/g dry weight) (Student's *t*-test; $t = 7.2$, $P < 0.001$).

Laboratory studies

Compared to their regular food ants responded differently to earthworms. While the regular prey was almost immediately (<10 s) handled, it took on average 3.5–21 min, depending on earthworm species, to touch the freshly cut pieces, and 7–35 min for the prey that was anesthezized before cutting. In both cases *D. octaedra* was the most repulsive prey (ANOVA, nests as a block effect: $F = 11.01$, $P = 0.003$ (living), and $F = 14.33$, $P = 0.02$ (dead) for prey type factor, block effect not signicant, see Fig. 2). No difference in the handling time between *L. rubellus* and *A. caliginosa* was found. All untreated prey insects were attacked in a few seconds and transported inside the nest. However, when treated with *D. octaedra* mucus, the insect prey was attacked significantly later (ANOVA, nests as a block effect: $F = 284.37$, $P < 0.001$ for the effect of mucus, effect of prey species, prey-mucus interaction or block was not significant, see Fig. 3).

Visual observation of living earthworms during the 30-day period of observations inside the nest mounds gave evidence that the worms do not or cannot avoid physical contact with the ants. The worms were active both in the nest material and inside the nest tunnels, but no signs of aggression by the ants on earthworms could be detected, and the earthworms were never observed at the outer surface of the nest mound.

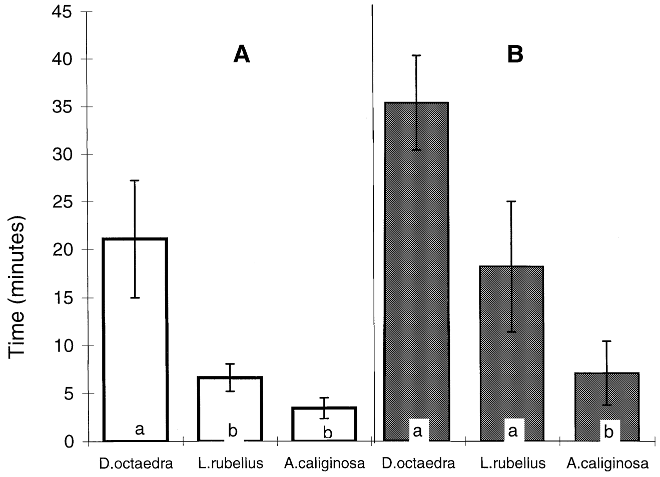

Fig. 2 Mean (± 1 SE; *narrow vertical bars*) time elapsed before *Formica aquilonia* ants touched the prey. **A** Pieces of earthworms that were cut from living specimens, **B** pieces of earthworms cut from anesthesized specimens. *Letters* inside the bars denote significant pairwise difference ($P < 0.05$, Student's *t*-test with Bonferroni correction) between the earthworm species ($n = 6$ for **A** and 3 for **B**)

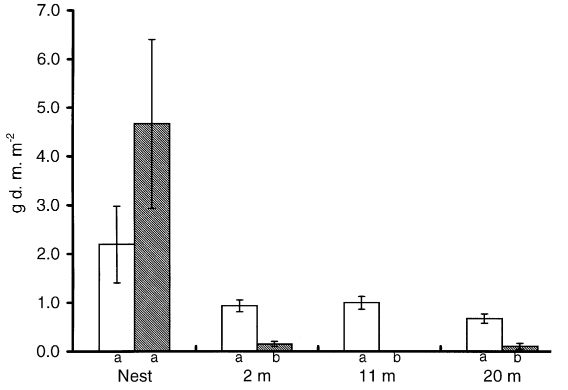

Fig. 1 Biomass (g dry weight m^{-2} of the two earthworm species *Dendrobaena octaedra* (*open bars*) and *Dendrodrilus rubidus* (*shaded bars*) in the surface stratum of ant nests, and in the soil (2, 11 and 20 m from the centre of the nest). Narrow vertical bars are ± 1 SE. *Letters* below the bars denote significant differences ($P < 0.05$, Mann-Whitney *U*-test with Bonferroni correction, $n = 10$ in the nests and 20 outside the nests) in the biomass of each earthworm species between sampling distances

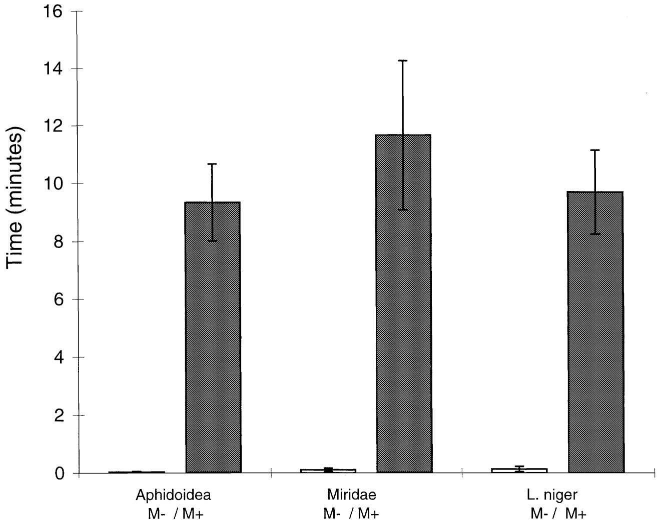

Fig. 3 Effect of earthworm mucus on the mean (± 1 SE) time elapsed before the ants touched the prey (*M*− prey without earthworm mucus, *M*+ prey treated with mucus; $n = 3$)

Discussion

The moist surface stratum of nest mounds of *F. aquilonia* ants is a habitat of high earthworm abundance in the field, and the earthworm biomass in this layer can reach levels close to habitats with much higher productivity, such as alder thickets and deciduous forests (Terhivuo 1989). Sampling of nest mounds of the *F. rufa* species complex gave evidence that earthworms do occur in ant nests from southern Finland (60°N) up to Kuusamo area (66°N, the northernmost place we have collected material) throughout the active period of ants. So far we have identified five species of lumbricids in *F. rufa* nests in Central Finland: *Lumbricus rubellus*, *Aporrectodea caliginosa*, *Dendrobaena octaedra*, *Dendrodrilus rubidus* and *D. subrubicundus*.

The diet of *F. rufa* ants comprises of honey dew (season average 80%) and various animal prey (Skinner 1980). Considering the predatory behaviour of ants it seems surprising that earthworms thrive in nest mounds: this impression most likely being the reason why the ant-earthworm association has gone unnoticed.

From the earthworms' perspective ants provide a "living shelter" against various vertebrate and invertebrate predators (see Cherix and Bourne 1980), provided that the ants do not attack the earthworms. Moreover, the high temperature in the nest surface (close to +20°C throughout the summer; Rosengren et al. 1987, and our own observations) should allow the earthworms to mature and reproduce rapidly. Similarly, the surface layer of the nest mound retains its moisture even during summer droughts, whereas the litter layer may temporarily dry up, thus hindering the growth of the epigeic earthworms (Nurminen 1967; Nordström 1975). In addition, decomposer microbes were more active and abundant in the warm and moist nest layer than in the adjacent soil (see also Pokarzhevskij 1981), providing an abundant food source for the earthworms. It should be noted that the continuous transport of litter and prey items into the nest by the ants fuels the microbes and the microbi-detritivorous earthworms in the nest. A higher pH and Ca content in the nest mounds than in the soil may also affect positively the growth of the earthworms; in his literature review, Persson (1988) reports that liming increases the numbers of *Dendrobaena octaedra* and *Dendrodrilus rubidus* in coniferous forest soils.

The mechanism allowing the earthworms to exist in the nest mounds is unlikely to be associated with the ability of earthworms to locate places in a nest where they can avoid contacts with the ants. Basing on the feeding preference data, we suggest that the earthworms can chemically defend themselves against ant predation. The particular properties of the mucus that are responsible for the repulsiveness to the ants remain, however, unknown.

From the ants' perspective the association seems more complicated: why not prey upon the earthworms and use the extensive energy and nutrient storage in the earthworm biomass? The chemical avoidance mechanism of the earthworms seems to be a reasonable impediment, but not a fully comprehensive one: the mucus only slows down but does not completely prevent feeding on the earthworms by the ants. It is possible that during their evolution the ants have evolved not to feed upon earthworms in cases when the benefits of a living earthworm provides the ant colony exceed the nutritional value of the earthworm tissues. For example, by feeding on fungal mycelia (Persson et al. 1996) earthworms may prevent the nest mounds from becoming overgrown by moulds, decomposer fungi, and fungal pathogens, thus increasing the longevity of the nest and the colony. Since ants are known to show mutualistic relationships with various groups of organisms, a possibility for an indirect mutualistic relationship (*sensu* Boucher et al. 1982) between ants and earthworms seems realistic.

In conclusion, we suggest that the hitherto unexplored association between ants and earthworms in the nest mounds is enabled by the ability of the earthworms to diminish ant predation pressure by secreting ant-repelling mucus. The high density of earthworms in the nest mounds seems to be a consequence of unparalleled richness of resources (litter and microbial biomass), favourable abiotic conditions and low density of earthworm predators. We hypothesize that the association is not commensalistic but is indirectly mutualistic via the benefical effects of earthworms to the nest structure and microbial community composition in the nest.

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