LETTER

Evidence for collective medication in ants

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Abstract

Social organisms are exposed to many pathogens, and have evolved various defence mechanisms to limit the cost of parasitism. Here we report the first evidence that ants use plant compounds as a collective mean of defence against microorganisms. The wood ants *Formica paralugubris* often incorporate large quantities of solidified conifer resin into their nests. By creating resin-free and resin-rich experimental nests, we demonstrate that this resin inhibits the growth of microorganisms in a context mimicking natural conditions. Such a collective medication probably confers major ecological advantages, and may be an unrecognized yet common feature of large, complex and successful societies.

Keywords

Collective medication, *Formica paralugubris*, host defence, parasitism, resin, wood ants.

INTRODUCTION

A major cost of social life is the increased exposure to pathogens (Møller *et al.* 1993; Schmid-Hempel 1998; Nunn *et al.* 2000; Tella 2002). This cost of sociality is expected to be particularly high for social insects, which live in crowded, persistent, warm and resource-rich nests providing ideal conditions for the development of microorganisms (Hölldobler & Wilson 1990). Hence, social insects have evolved a variety of behavioural and physiological defence mechanisms, including antibiotic-producing symbionts (Currie *et al.* 1999), allogrooming (Rosengaus *et al.* 1998), antibiotic secretions (Rosengaus *et al.* 2000; Poulsen *et al.* 2002), removal of wastes and corpses (Hart & Ratnieks 2001), and immune defences (Moret & Schmid-Hempel 2000; Traniello *et al.* 2002). Another potential mechanism of defence may be to add plant compounds with antimicrobial properties to the nest.

Wood ants actively bring solidified resin to their nests, and this particular behaviour has intrigued naturalists since the eighteenth century (de Réaumur 1928). So far, the function of this resin remained speculative, and several non-mutually exclusive hypotheses have been suggested, including nutrition, protection against ultra-violet light, nest consolidation, or water repulsion (Gösswald 1989; Lenoir *et al.* 1999). Because conifer resin probably protects trees against invading bacteria and fungi (Cowan 1999), wood ants may also use resin as a means to suppress the activity of microorganisms in their mounds (Gösswald 1989; Lenoir *et al.* 1999). We tested this hypothesis experimentally by examining whether the presence of resin inhibits the growth of bacteria and fungi in wood ant nests. For this aim, we created resin-rich and resin-free experimental nest units with workers, and monitored the presence of bacteria and fungi.

MATERIALS AND METHODS

Sampling

The study population, located near the Chalet à Roch in the Swiss Jura mountains, consists of hundreds of large mounds forming a supercolony (Chapuisat *et al.* 1997). The density of nests is very high, each mound contains many philopatric queens, and neighbouring mounds are interconnected by trails (Chapuisat & Keller 1999). In November 2001, 2 L of nest material and workers were collected from each of 20 randomly chosen nests along a 1-km transect. The nest material was sampled from within the upper part of the mounds, and consisted of untransformed twigs and needles. All pieces of resin were removed, and the total amount of resin was weighed for each of those samples of nest material.

Experimental nest units

Nest material and workers from the 20 samples were completely mixed, taking advantage of the lack of aggression between workers of this population. The entire mix was subsequently split into 20 homogeneous experimental units. Each experimental unit consisted of 1.6 L of nest material,

and approximately 600 workers were placed in large plastic containers side-coated with fluon to prevent ants from escaping. Eight grams of resin collected from ant nests were added to 10 of the experimental units, whereas the other 10 remained resin-free. Eight grams of resin correspond to approximately 160 pieces of resin. Nest material and resin are continuously mixed by the ants, thus ensuring a homogeneous distribution of resin. Experimental units were kept at 27 °C with regular vaporization of sterile water to maintain high humidity, thus mimicking natural conditions. Ants were fed with 8% sugar solutions and distilled water. After 4 weeks, microbiological tests were conducted.

Microbiological tests

The concentration of microorganisms was assessed by diluting 0.1 g of randomly selected nest material (without resin) from each experimental unit into 5 mL of 0.1% peptone solution and plating dilutions of this solution onto three different nutritive media (Hurst *et al.* 1997): Tryptic Soy Agar (TSA), Eosin Methylene Blue agar (EMB) and Dichloran Glycerol 18 agar (DG18). TSA was supplemented with cycloheximide (500 mg L^{-1}) to inhibit fungal growth. This medium is commonly used for cultivating many kinds of environmental bacteria. EMB is selective for gram-negative bacteria. DG18 is selective for xerophilic fungi. All plates were incubated at 27 °C. Bacterial colonies (TSA, EMB) were counted after 48 h of growth, and fungal colonies (DG18) after 5 days. The three most frequent bacterial species were isolated on the TSA medium and identified with the API system (API 20 NE, BioMérieux, France). Additionally, one of these bacterial species was isolated and plated on a standard medium for susceptibility testing of pathogens (Müller–Hinton Agar). Two pieces of resin were placed on the surface of the plate. One of these pieces had been previously sterilized under UV light, and the other was untreated. After 24 h at 35 °C, the inhibition halos surrounding the resin were measured.

RESULTS

We found conifer (*Picea abies*) resin in every nest sampled. However, the quantity of resin varied greatly among nests (mean = 2.3 g/L of nest material, SE = 0.58, range 0.03–9.3 g L^{-1}, $N = 20$).

The presence of resin in the experimental units had a negative effect on the growth of microorganisms (Fig. 1). The number of colony-forming units was significantly lower in nests containing resin than in resin-free nests for bacteria and fungi (TSA: mean CFU 10^{-4} g^{-1} ± SE = 27740 ± 2370 without resin and 22140 ± 1850 with resin, one-tailed Mann–Whitney U-test $U = 75$, $P = 0.027$; DG18: 319 ± 126 without resin and 90 ± 59 with resin, one-tailed
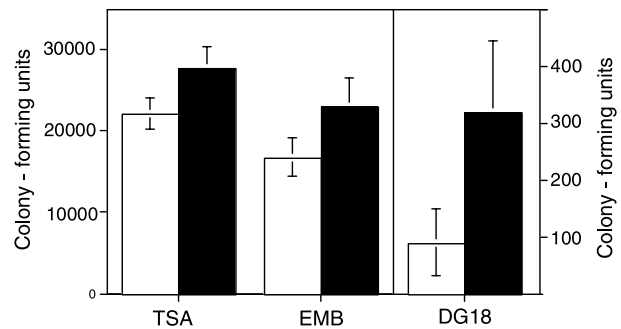

Figure 1 Mean (± SE) number of colony-forming units of bacteria and fungi per 10^{-4} gram of nest material, for nests containing resin (white bars) and resin-free nests (black bars). Tryptic Soy Agar (TSA) is a standard nutritive medium for bacteria, Eosin Methylene Blue agar (EMB) is specific for gram-negative bacteria, and Dichloran Glycerol (18) agar (DG18) is specific for xerophilic fungi.

Mann–Whitney U-test $U = 77$, $P = 0.019$). Moreover, a marginally significant negative effect was detected for gram-negative bacteria (EMB: 23060 ± 3470 without resin and 16760 ± 2590 with resin, one-tailed Mann–Whitney U-test $U = 71$, $P = 0.056$). Altogether, resin significantly inhibited microorganism growth (Fisher's combined probability test, $\chi^2 = 20.91$, d.f. = 6, $P < 0.005$). Three species of bacteria commonly found in samples of nest material from the field were identified: *Pseudomonas luteola*, *Pseudomonas fluorescens* and *Aeromonas hydrophila*. In order to test for a potential effect of resin on bacterial growth, *P. luteola* was isolated and cultivated on the TSA medium. *P. luteola* was clearly inhibited by the pieces of resin *in vitro*, with inhibition halos of 3.2–5.6 mm around both sterile and untreated pieces of resin.

DISCUSSION

We found that the wood ants *Formica paralugubris* incorporate variable but often large amounts of resin into their nests. For example, a large mound can contain as much as 20 kg of resin (3 m^3 of nest material with 7 g of resin per litre). This finding is in accordance with early observations reporting the presence of large quantities of resin in wood ant mounds of various geographical areas (Donisthorpe 1915; de Réaumur 1928; Gösswald 1989). For example, in a Swedish sample, resin constituted from 1 to 44% of the dry weight of mounds (Lenoir *et al.* 1999).

Amounts of resin above the mean but within the range occurring in natural nests inhibited bacterial and fungal growth in experimental units. This effect was particularly pronounced for xerophilic fungi, with a three-fold decrease in the number of colony-forming units per gram of nest material. Altogether, the presence of resin had a significant

negative impact on microorganism growth, as demonstrated when the probabilities from the three assays are combined using Fisher's method.

The three most frequent bacteria found in the present study are opportunistic pathogens in a variety of organisms (Freney *et al.* 1994; Jackson & Phillips 1996; Picot *et al.* 2001). Of special relevance here, *P. fluorescens* is associated with septicaemia in another hymenopteran, the honeybee *Apis mellifera* (Schmid-Hempel 1998). The pronounced antifungal properties of resin may also be important against virulent entomopathogenic fungi such as *Metarhizium* and *Beauveria* (Schmid-Hempel 1998; Wilson *et al.* 2001). Together, the finding of common pathogenic bacteria in wood ant nests, the general inhibition of microorganisms by resin, and the specific inhibition of at least one potentially detrimental bacterium strongly suggest that bringing resin to the nest is an adaptive mechanism of defence against pathogens. However, further experimental studies will be needed to measure the costs and benefits associated with the use of resin, and to fully demonstrate the adaptiveness of this behaviour. In particular, it will be necessary to identify the specific pathogens that are inhibited by resin and to evaluate experimentally the effect of resin on infection dynamics, individual survival and colony productivity.

Our experiment demonstrates that the resin has antimicrobial effects in wood ant nests, in a context close to natural conditions. The antimicrobial activity detected here contrasts with previous work showing no negative impact of resin on fungal biomass when forest litter was incubated without ants (Lenoir *et al.* 1999). This difference highlights the fact that hypotheses of self-medication have to be tested in a natural context, and that experimental tests *in vivo* are needed (Clayton & Wolfe 1993; Lozano 1998). A pharmacological effect of resin in natural conditions is understandable, because conifer resin contains a complex mixture of terpenes (Phillips & Croteau 1999) with some antibacterial, antifungal and antiviral properties (Cowan 1999; Hodgson *et al.* 2002). For example, alpha-pinene and limonene, two major compounds of the oleoresin, are also abundant in terpenoid secretions of *Nasutitermes* termite soldiers, and reduce spore germination of the entomopathogenic fungus *Metarhizium* (Rosengaus *et al.* 2000). Microorganisms are probably inhibited by direct contact with resin as well as by volatile compounds passively diffusing into the nest atmosphere. Several species of birds and mammals also add plants with anti-parasitic effects to their nests (Clark & Mason 1985; Lozano 1998; Hemmes *et al.* 2002; Petit *et al.* 2002). Hence, our experiment strongly suggests that wood ants take advantage of the plant defence. They collect and incorporate into their nests large amounts of a particular environmental resource that confers protection against microorganisms. Such a collective medication may play a crucial role in the extraordinary ecological success of insect societies, and may be an unrecognized yet common feature of large, complex and successful societies. Thus, honeybees collect a resinous compound that has many medicinal properties *in vitro* (Banskota *et al.* 2001) and leaf-cutting ants use antibiotic-producing bacteria to fight a parasitic fungus in their garden (Currie *et al.* 1999).

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