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Mini-review

New insights into symbiotic associations between ants and bacteria

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Abstract – Many ants live in complex mutualistic or parasitic relationships with other insects or plants, some of which are classical examples of the mutual benefit of symbiosis. However, only in the past few years have new insights into the symbiosis of ants and microorganisms been reported. Examples are the symbiosis of ants of the genus *Camponotus* with intracellular bacteria present in their midgut, and the tripartite relationship of ants of the tribe Attini with an extracellular bacterium essential for the cultivation of their fungus gardens. The analysis of the parasitic and mutualistic interactions of these organisms will allow interesting insights into the evolution of symbiosis and possibly lead to novel strategies of pest control. © 2000 Éditions scientifiques et médicales Elsevier SAS

symbiosis / bacteria / endosymbiont / ants

1. Introduction

Mutualism is defined as a reciprocally beneficial interaction between different organisms. Such symbiotic relationships frequently involve the exchange of nutrients or certain services such as protection from enemies or transportation. A real mutualism should therefore result in significant novel properties of the interacting partners, which would be impossible to acquire separately [10]. Social insects such as ants are of particular interest for the understanding of parasitic and mutualistic relationships, because these animals developed numerous interactions with different species at various levels, i.e. individually, but also at the population level. These interspecies interactions can involve bacteria and fungi, but also other insects and even plants. Several examples were described in the book by Hölldobler and Wilson [26]. For example, a classical case of a mutualism with plants is the interaction of ants and trees of the genus *Acacia* in Africa and tropical America. These trees produce thick pairs of thorns which can be used as shelters by the ants. Moreover, the plants secrete sugary liquid from special nectaries which are an important nutrient for ant inhabitants. The ants, in turn, protect the acacias from destructive insects. Many ants adopt aphids, mealybugs or caterpillars to gather honeydew and, in return, they may protect them from predators, sometimes even including them as an integral part of their colonies. Some ant species recruit new members of other ant colonies by force to use them as slaves, in the defense of the nest or for the acquisition of diverse trails. In many cases, it is not clear whether such relationships have a mutualistic basis, according to its strict definition, or whether they benefit only one of the partners. Whereas many such interactions between ants and other animals or plants have been described, only limited knowledge about the interaction of ants with microorganisms has been obtained.

There are many reports in the literature describing symbiotic relationships between microorganisms and insects [10, 17, 19, 22]. Indeed, it was estimated that at least 15–20% of all insects live in symbiotic relationships with

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microorganisms [10]. Symbiosis with the physiologically very versatile bacteria may have allowed access to novel ecological niches and to new unbalanced food resources such as plant sap or blood. Mutualism between insects and microorganisms is clearly one of the motors of evolution of insects and represents one of the keys to the enormous success of this huge group of animals.

Among the insects, several systematic groups are frequently involved in symbiotic interactions with bacteria. These phyla include the Blattaria, Hymenoptera, Homoptera and Coleoptera. Also, among the bacteria, several lineages appear to be especially capable of symbiotic relationships. Many arthropods harbor members of the genus *Wolbachia* [41] which are closely related to pathogenic *Rickettsia* [3, 35] and are classified in the α-subgroup of the *Proteobacteria.* Also, the γ-subgroup harbors symbiotic organisms, some of which are related to important human pathogens such as *Francisella tulariensis, Coxiella burnetii* and some Enterobacteriaceae [17]. Symbionts of mealybugs and of the protist family Trypanosomatidae belong to the β-subgroup of Proteobacteria [34]. The mycetocyte symbionts of cockroaches (*Blattaria*) are members of the *Flavobacterium–Bacteroides* group [4].

2. Bacteria as intracellular endosymbionts in ants

The first observation of 'bacteria-like' structures associated with the ant species *Camponotus ligniperdus* and *Formica fusca* within the subfamily Formicinae (Hymenoptera: Formicidae) was already made by Blochmann in 1887 [9]. At present, we know that these so-called 'Blochmann bodies' [31] are in fact bacteria that are located in specific cells (mycetocytes) of the midgut and in the ovaries of many ants [10, 19, 28, 38]. These bacteria are exclusively found intracellularly [38] and are different from microorganisms present in the digestive tract of ants which consists of a complex population of bacteria and filamentous fungi [11]. In the midgut of *Camponotus* spp. the mycetocytes are intercalated between the normal enterocytes [38]. The presence of the bacteria in the oocytes of queens and workers strongly suggests a maternal transmission route for the *Camponotus* endosymbionts [10, 28, 38]. The membranes of these bacteria are characteristic of Gram-negative bacteria as observed by electron microscopy and staining procedures [19, 28, 38].

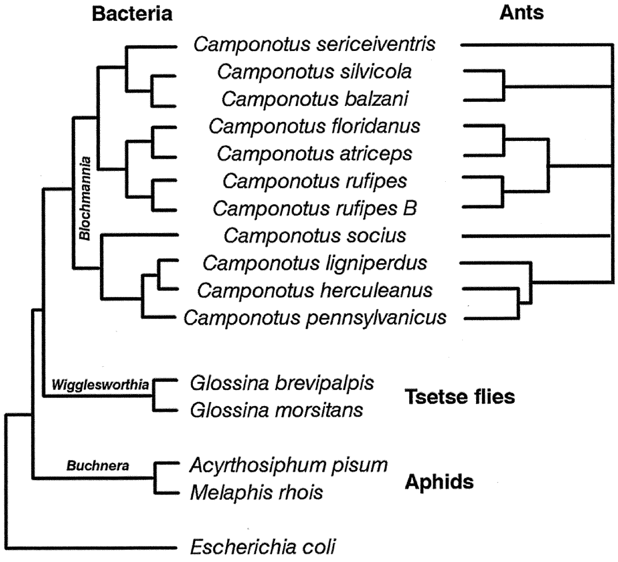

Figure 1. Schematic representation of the cospeciation of endosymbiotic bacteria of ants and their host organisms and their systematic relationships with endosymbionts of tsetse flies and aphids [37]. Please note that the trees are not drawn to scale.

The analysis of the 16S rDNA sequences of the endosymbionts isolated from several American and European *Camponotus* species revealed that the bacteria are most closely related to each other and systematically belong to the γ-subdivision of the Proteobacteria [37, 38]. The *Camponotus* symbionts are now classified in the genus *Blochmannia* (Candidatus *Blochmannia* gen. nov.) [37] and form a sister taxon of the taxa defined by the primary symbionts of aphids classified in the genus *Buchnera* and by the primary symbionts of the tsetse flies classified in the genus *Wigglesworthia* (*figure 1*) [1, 5, 13]. The mycetocyte-associated symbionts of aphids, tsetse flies and ants share a common ancestor and are systematically placed adjacent to the family Enterobacteriaceae (*figure 1*) [1, 5, 38].

The phylogenetic tree derived from the 16S rDNA sequences of the ant symbionts was recently compared with the phylogenetic tree of their host animals, which was based on DNA sequences of the gene coding for the mitochondrial cytochrome oxidase subunit I. The two trees show a very similar branching pattern indicating a cospeciation of the symbionts and their host organisms (*figure 1*) [37, 38]. Once more, this argues for a maternal transmission route of the bacteria. A similar cospeciation of the symbiotic partners and their host animals was noted in several other mycetocyte-associated symbioses including those of the aphids, tsetse flies, mealybugs and cockroaches [4–6, 13, 32, 34].

Buchnera and *Wigglesworthia* species are located intracellularly in so-called symbiosomes within the mycetocytes. In both animals, the mycetocytes form organ-like structures, so-called mycetomes. In contrast to these symbioses, the *Camponotus* symbionts appear to reside free in the cytoplasm of the mycetocytes and no mycetomes are formed [38]. The biological function of the mycetocyte symbiosis of *Camponotus* species is not yet known. However, as in the case of the other mycetocyte symbioses the interaction with the host appears to be obligate for the microorganisms which cannot be cultivated in vitro. In general, it is believed that mycetocyte symbioses have a nutritional basis providing essential nutrients to the host organisms such as aphids or tsetse flies which feed on a poor diet such as phloem sap or blood, respectively [21]. In fact, antibiotic treatment of aphids caused a significant malaise of the animals and the offspring were not viable [5]. It has been shown that the bacteria provide the animals with essential amino acids such as leucine and tryptophan [21]. Interestingly, as an adaptive response, the genes involved in the biosynthesis of these amino acids were found to be amplified and located on plasmids in some of the aphid symbionts [29, 39, 42]. In the case of the ant symbiosis such a nutritional basis is not evident at first sight. The genus *Camponotus* represents an extremely successful group of animals. Its members are found worldwide and in many different ecological niches and the *Camponotus* spp. analyzed so far as harboring the endosymbionts feed on a complex diet. Recently, after antibiotic treatment, aposymbiotic workers of *C. floridanus* could be maintained alive for months without obvious detrimental effects on the adult animals which, however, were fed with a complex diet (Sauer, Hölldobler and Gross, unpublished results). Such aposymbiotic animals will be useful in the future to analyze whether the symbiosis of ants with mycetocyte bacteria also has a nutritional basis.

Due to the availability of fossil aphid records, a molecular clock could be established and related to nucleotide changes in the 16S rRNA of the symbionts of different aphids. Accordingly, the aphid *Buchnera* symbiosis was estimated to have an age of at least 150 million years [30, 32]. Assuming a similar mutation rate for the ant symbionts, the mycetocyte symbiosis of *Camponotus* species may have a similar age and may have been an original attribute of ants which has been maintained only in the subfamily Formicinae.

The intracellular habitat is characterized by an apparently nutrient rich and physically stable environment. A long-lasting adaptation to such an intracellular niche should result in specific genetic and physiological differences, as compared to free-living organisms. For example, the lack of selective pressure on obsolete metabolic pathways or environmental sensing systems may lead to their deletion, resulting in a significant reduction in the genetic diversity of the symbiotic bacteria. Such a reduction in their genetic equipment may well be a reason for the lack of successful cultivation of the mycetocyte bacteria. In fact, similar to several obligate human pathogens such as *Mycoplasma* or *Chlamydia* spp., in the case of *Buchnera* a dramatic shrinkage of its genome has been described [2, 25, 40]. The genome size of *Buchnera* was estimated to be about 650 kb [12], which is in the range of the smallest bacterial genomes known so far [3, 25].

In contrast to free living bacteria, *Buchnera* has retained just one copy of the rRNA encoding genes, which probably reflects an adaptation to the replication cycle of the host cells [5]. The comparison of the 16S rDNA sequences of *Buchnera*, *Wigglesworthia* and *Blochmannia* with the 16S rDNA genes of closely related free-living bacteria revealed a significantly increased mutation rate in the symbionts. These organisms independently accumulated destabilizing mutations in parts of the 16S rRNA molecule which form strongly conserved domains in their free-living relatives [28]. Reasons for this accumulation of mutations may be the lack of significant genetic exchange with other bacteria by these symbionts which are protected but also imprisoned by their eukaryotic host cells. Moreover, the vertical transmission route of the symbiotic bacteria leads to recurrent reductions in their population size and these frequent bottlenecks may favor the accumulation of slightly deleterious mutations [30].

In the case of the aphids and tsetse flies, so-called secondary symbionts were identified which do not appear to have an essential role in the symbiosis and may therefore also be considered as parasites. These bacteria are also located intracellularly either in cells associated with the mycetomes or in enterocytes, respectively. In contrast to the primary symbionts present in the mycetocytes these bacteria can be cultivated in vitro and are closely related to free-living Enterobacteriaceae [1, 5]. A highly related bacterium was recently identified in *C. planatus*, which may indicate that such secondary symbionts are also present in other *Camponotus* species (Sauer, Hölldobler, Gross, unpublished results). Moreover, a recent report demonstrates that many ants may harbor another bacterial companion. In a broad survey in ant species present in Indonesia, bacteria belonging to the *Wolbachia* group have been identified with a high incidence [45]. *Wolbachia* are extremely widespread among insect species and it was estimated that possibly 15% of all insects are infected with these bacteria. Systematically, they belong to the α-subclass of Proteobacteria and are most closely related to *Rickettsia*, well known human and veterinary pathogens [35]. Similar to the mycetocyte bacteria, *Wolbachia* is located intracellularly and is transmitted maternally. In fact, *Wolbachia* is mainly found in the ovaries of infected animals. Interestingly, in contrast to the mycetocyte bacteria, the phylogenetic trees of *Wolbachia* and their host animals are not congruent, indicating repeated events of horizontal exchange [36]. To their own benefit, these bacteria are able to interfere with the reproductive system of their host animals causing phenomena such as cytoplasmic incompatibility, parthenogenesis or feminization of genetic males [41]. The characterization of the effects of *Wolbachia* on the ant biology and, if present also in the genus *Camponotus*, their interference with the mycetocyte symbionts, may provide additional fascinating insights into the biology of these organisms.

3. Bacteria as ‘exo-symbionts’ in the tribe Attini

The tribe Attini (Formicidae: Myrmicinae) includes a taxonomically compact group of 12 genera and about 200 species. It was suggested that the 12 genera can be subdivided into primitive, transitional and advanced groups according to colony size, worker polymorphism and types of substrates [44, 46]. These ants are well known for their obligate symbiosis with a basidiomycete fungus belonging to the family Lepiotaceae, which they use as their food source. It is estimated that the cultivation of fungus gardens by Attini originated more than 50 millions years ago [16, 33].

The mutualism between fungus-growing ants and their fungi is considered to be a classical example of a mutualistic relationship [43]. Until recently, this symbiosis was believed to involve only two mutualists: the ant and the fungus. However, the symbiotic relationship is more complex and the maintenance of a fungal garden in a monoculture requires a recently identified third mutualist, an actinomycete of the genus *Streptomyces*. This microorganism is in fact associated with the cuticle of the ants and helps to avoid the attack of the parasitic fungus *Escovopsis* (Ascomycotina) against the ‘beneficial’ fungus and contributes to ensuring the

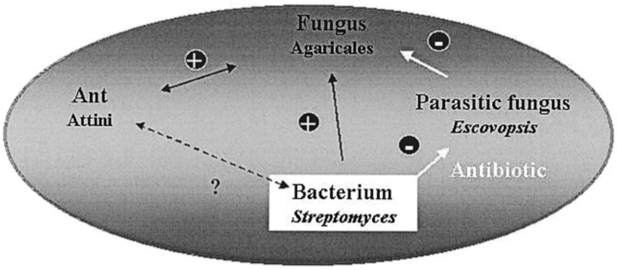

Figure 2. Mutualism in the tribe Attini [18].

stability of the garden (*figure 2*) [18]. The parasite *Escovopsis* is an extremely virulent pathogen apparently specialized in the fungal gardens of Attini. It can cause a rapid devastation of the fungal garden and the death of the ant colony. The localization of *Streptomyces* on the ant integument is specific for different ant genera, indicating that this symbiosis is of very ancient origin. In fact, in a broad survey the bacterium was found on the cuticle of all foundress queens, which indicates a vertical route of transmission of the symbiont. This bacterium synthesizes a powerful antibiotic specifically targeted against *Escovopsis*, but the chemical characteristics of this compound have yet to be analyzed. Moreover, the bacterium appears to promote the growth of the fungus by the production of additional compounds possibly including vitamins or amino acids. Attempts to specifically eliminate the actinomycete from ant cultures may provide some insights into additional roles of this bacterium in symbiosis. The fact that the ants carry the symbiotic *Streptomyces* on their cuticle may also be an advantage for the bacteria because it may provide them with an environment rich in nutrients and with little competition from other microorganisms. In fact, the Attini of the advanced group cultivate their fungus garden on plant leaves which, due to the metabolic activity of the fungus, may lead to the transfer of plant polysaccharides as carbon sources utilizable by the ants and the bacterium [20]. Obviously, the ant–fungus symbiosis is much more complex than previously thought and may even involve additional players which are not yet identified.

4. Ants as vectors for bacterial pathogens

Some ant species including Pharaoh's ants (*Monomorium pharaonis*) may be involved in dangerous infestations of hospitals. Practical experience demonstrates that these insects are extremely difficult to eradicate by conventional treatments [8, 27]. Pharaoh's ants may be carriers of a large spectrum of bacterial species including several pathogenic organisms such as *Serratia marcescens*, *Citrobacter freundii*, *Klebsiella ozaennae*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Staphylococcus epidermidis* and *Yersinia pestis* [27]. To our knowledge, the first report of ant-mediated transmission of a pathogen was reported for *Bordetella bronchiseptica* within a veterinary unit [8]. This bacterium can cause respiratory disease in many animal species [24]. In general, the transmission of bacterial pathogens by arthropod vectors poses an enormous health problem throughout the world and may also be involved in the appearance of new emerging pathogens [17].

5. A role for bacterial symbionts in pest control

In many ecosystems, ants play an extremely important role. In fact, it should be mentioned that ants have many direct benefits for man. As these animals collect vast amounts of insects including beetles and caterpillars while foraging for vegetation, for centuries wood ants were used in Europe to control forest pests [26]. For example, it was shown that a single colony of *Formica rufa* may gather 21 700 sawfly larvae and moth caterpillars in one day [26]. On the other hand, arthropods have long been known to be important competitors for man in agriculture. Devastating locust invasions have been known since the beginning of our historical records. Among the ants, the leaf-cutting species are of particular importance and are a devastating agricultural pest in tropical areas in Latin America [15]. The characterization of the mutualism of these ants, their fungus and the actinomycete and their struggle against attack by the parasitic fungus *Escovopsis* may result in the development of novel strategies to fight this pest [18].

Apart from being competitors, many arthropods represent a threat for human health, as they are important vectors for pathogenic bacterial or viral agents. Many of these arthropod vectors live in symbiotic relationships with bacteria. A well known example is the tsetse fly, which is able to transmit *Trypanosoma gambiense*, the agent of African sleeping sickness. Many attempts were made to eradicate the tsetse flies in the endemic regions with insecticide compounds, which, as in many other cases, in the end led to increased resistance against these compounds but not to the successful defeat of the disease. Endosymbionts of these vector animals may offer new possibilities for interfering with the spread of the pathogenic organisms. Recently, it was shown that the secondary symbionts harbored by the tsetse flies, in addition to their mycetocyte symbionts, can be cultivated in vitro and manipulated genetically, possibly opening up novel strategies for interfering with their host insects [7, 14].

An approach demonstrating the potential of strategies using genetically modified symbionts was recently described for Chagas disease caused by *T. cruzi* affecting 16–18 million people in South and Central America [7]. In this case, the vector insect *Rhodnius prolixus* lives in obligate symbiosis with the extracellular actinomycete *Rhodococcus rhodnii*, which could be transformed in vitro with a gene coding for the cationic peptide antibiotic cecropin A. Paratransgenic insects carrying the genetically modified symbiont were found to be highly resistant to *T. cruzi* infection [23].

Wolbachia is an interesting alternative for controlling undesired arthropod populations and several attempts were made to use its interference with the host reproduction for fighting harmful insects [7]. Other *Rickettsia*-like microorganisms are widespread among arthropods and it is hoped that they will become susceptible to genetic manipulation in the future, possibly allowing their use in pest control [7].

6. Perspectives

Apart from strategies attacking harmful arthropod populations or their pathogenic inhabitants directly by modified symbionts, the example of the fungus-growing Attini demonstrates that characterization of the complex relationship between microorganisms and arthropods could lead to identification of new antimicrobial strategies or compounds which may be extremely useful at a time when antibiotic resistance appears to be throwing us back to the pre-antibiotics era.

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