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Endosymbiotic bacteria associated with nematodes, ticks and amoebae

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Abstract

Endosymbiosis is a mutualistic, parasitic or commensal symbiosis in which one symbiont is living within the body of another organism. Such symbiotic relationship with free-living amoebae and arthropods has been reported with a large biodiversity of micro-organisms, encompassing various bacterial clades and to a lesser extent some fungi and viruses. By contrast, current knowledge on symbionts of nematodes is still mainly restricted to *Wolbachia* and its interaction with filarial worms that lead to increased pathogenicity of the infected nematode. In this review article, we aim to highlight the main characteristics of symbionts in term of their ecology, host-cell interactions, parasitism, and co-evolution, in order to stimulate future research in a field that remains largely unexplored despite the availability of modern tools.
Introduction

Endosymbiosis is a symbiosis in which one symbiont dwells within the body of the other. Usually, when talking about endosymbionts, we refer to bacteria, or, less frequently to fungi living inside the eukaryotic cell or simply inside the body. Interestingly, the endosymbiotic theory first articulated by the Russian botanist Konstantin Mereschkowski in 1905 (1) describes chloroplasts, mitochondria and other organelles as originating from bacterial endosymbionts. Nearly 90 years ago, Paul Buchner, the father of symbiosis research, documented a remarkable array of both endosymbiotic fungal and bacterial associates of arthropods (2). More recently, evidence has also emerged that bacterial symbionts are present in a large variety of additional eucaryotes, including nematodes, amoebae and plants (see Table 1 for a summary of selected discoveries illuminating research on symbionts). In the present review, we will focus on bacterial symbionts associated with nematodes, arthropods and free-living amoebae.

Symbionts of nematodes

Nematodes or ‘roundworms’ form a highly successful and abundant group of organisms found in every ecosystem on Earth. Considering their ubiquity and enormous diversity, it is surprising that only relatively few examples of bacterial endosymbioses have been described in nematodes compared with amoebae and arthropods. Of these few examples only three have been investigated in sufficient detail to unravel some of the biological features of the symbiotic relationship.

The most extensively studied systems are the closely related gamma-proteobacteria, *Photorhabdus* and *Xenorhabdus*, which colonise the guts of *Heterorhabditis* and *Steinemema* nematodes respectively (see 3-5, for in depth reviews). The bacteria have intricate and distinct
roles in the nematode’s life-cycle. On entering its insect prey, the infective juvenile stage of
the nematode regurgitates its bacteria into the haemolymph, which rapidly grows and kills the
insect, releasing nutrients to support the growth and development of the nematode. After the
adults reproduce, a process that is dependent upon symbionts, environmental cues stimulate
the progeny to enter the infective juvenile stage, which becomes re-colonised with one or two
bacteria through maternal inoculation. Therefore, throughout the nematode’s life-cycle, the
bacteria exhibit both pathogenicity to the insect prey and mutualism for the growth and
development of the nematode. Although these symbiotic relationships share many common
features at the whole organism level, the molecular regulation of each phase of the
pathogenic/mutualistic interaction is dependent on both distinct and common pathways and
effector molecules (5). The amenability of these systems to experimental and genetic
manipulation coupled with post-genomic approaches will undoubtedly reveal further insight
into the regulation of pathogenesis and mutualism in these symbiotic associations (3-5).

The other example of a bacterial-nematode mutualism occurs between the
endosymbiont, Wolbachia and members of the Onchocercidae family of filarial nematodes
(Table 2), including medically important parasites of humans and animals (6). Members of
the genus Wolbachia, an alpha-proteobacterial group most closely related to Ehrlichia,
Anaplasma and Rickettsia species, are diverse and abundant endosymbionts of insects and
other arthropods, where they mainly display a parasitic association. Yet in nematodes the
bacterium appears to have become a mutualist, restricted to a sub-group of the family
Onchocercidae (7). Surveys of non-filarial nematodes have failed to detect Wolbachia outside
of this group (8), although some evidence for divergent Wolbachia-like sequences and
structurally distinct bacteria have been reported in the plant parasitic Tylenchid nematode,
Radopholus similis (9). Reports of PCR amplification of Wolbachia sequence from the

metastrongylid nematode, *Angiostrongylus cantonensis* (10) have not been reproduced and appear to be due to laboratory contamination (11). A more in depth survey of sub-families of the *Onchocercidae*, supports the view that *Wolbachia* arose late in the divergence of filarial nematodes. It is absent from all ancestral groups and there are examples of the presence or absence of *Wolbachia* both within nematode genera and species (12). Further evidence of a different tissue tropism and distribution in the more recently acquired Clade F group in *Mansonella* spp., also suggests a more complex evolutionary history and potentially more diverse symbiotic relationships than previously thought (12).

In filarial nematodes that host *Wolbachia*, most studies have naturally focused on the endosymbiont’s relationship with pathogenic nematode species; *Brugia malayi*, a lymphatic filarial parasite of humans, *Onchocerca volvulus*, the cause of human onchocerciasis or ‘river blindness’ and *Dirofilaria immitis*, the cause of dog heartworm disease (6, 13). These, together with the majority of related species and genera host either clade C or D *Wolbachia*, which are ubiquitous in all specimens and stages of the nematode and share a close congruent co-evolution. In these species, *Wolbachia* is an essential requirement for larval and embryonic growth and development, fertility and viability of the nematode host (7).

In species that display an obligate mutualistic association, the bacteria are mostly distributed throughout the syncytial hypodermal chord cells in large numbers (Figure 1) and contained within host-derived vacuoles (7). This tissue tropism develops early in embryonic development, where *Wolbachia* localizes to the posterior of the egg and upon fertilization segregates asymmetrically in a cell-lineage specific pattern (14). Although it was previously assumed that *Wolbachia* enters oocytes through the female germline, a recent observation suggests that the genital primordia remain free of bacteria, which instead appear to translocate
from the hypodermis through the pseudocoelomic cavity and across the ovarial epithelium to infect oocytes at the onset of oocyte development (15). Embryonic development is entirely dependent on *Wolbachia*, with about 70 bacteria being transmitted in each embryo (16). These numbers remain static throughout embryonic development and in the microfilariae and the L2 and L3 larval stages, which develop in the insect vector (17). Only after the L3 larvae have infected the mammalian host does the population of *Wolbachia* rapidly expand to populate the hypodermal tissues with further expansion in reproductively active adult females (17).

The variation in population density between developmental stages and the sensitivity of larval and embryonic development to antibiotic treatment, suggest that *Wolbachia* bacteria are most important during periods of high metabolic activity, presumably through the provision of key nutrients or metabolites to support the rapid growth, organogenesis and development of L4 larvae and embryos. Further evidence in support of this hypothesis comes from observations made on the nematode cellular and nuclear structure following antibiotic depletion of *Wolbachia*. Loss of *Wolbachia* results in extensive and profound apoptosis throughout reproductive cells, embryos and microfilaria, which correlate closely with the tissues and processes initially perturbed following antibiotic therapy. The induction of apoptosis occurs in a non-cell autonomous pattern extending to numerous cells not previously infected with the endosymbiont, implying that a factor derived from *Wolbachia* hypodermal populations is essential for the avoidance of nematode cell apoptosis (16).

Although L4 and embryonic growth and development are the biological processes most sensitive to *Wolbachia* depletion, other phases of the nematode life-cycle including early larval development and transmission through the vector (18) and the viability and longevity of
adult worms (19, 20) are also either partially dependent upon the bacterial endosymbionts or
alternatively, may occur through indirect mechanisms associated with Wolbachia infection.
These include protection from oxidative stress, contribution to the nematodes’ evasion, and
subversion of host immunity.

The molecular basis of the mutualistic role of Wolbachia remains unresolved.
Comparative genomic analysis of B. malayi Wolbachia (wBm), with other Wolbachia
‘strains’ and related rickettsial species together with that of the host nematode, has revealed
that although much of the wBm genome appears degenerate, certain key metabolic pathways
remain intact. These pathways include the biosynthesis of haem, nucleotides, riboflavin and
FAD, which are absent from the host nematode genome and related bacteria (21, 22). How
and when these factors contribute to the mutualistic association is the subject of ongoing
research.

One puzzle, which has confounded the broad acceptance of Wolbachia as an obligate
mutualist, is the apparent secondary loss of the endosymbiont from some of the more
evolutionarily ‘advanced’ species, including the human filaria, Loa loa, the rodent parasite,
Acanthocheilonema viteae and the deer parasite, Onchocerca flexuosa (7). Support for the
secondary loss of the symbiont comes from genomic sequencing, which showed evidence of
Wolbachia gene fragments having been integrated into the host nematode genome through
lateral gene transfer (LGT), facilitated by the close association of the bacteria and germline
cells (23). The process of LGT appears to be common among Wolbachia insect and nematode
hosts, with almost an entire Wolbachia genome inserted into the nuclear genome of
Drosophila ananassae (24). Although evidence for gene transcription has been reported for
some of these LGT events, further work is needed to determine whether they represent a
mechanism by which the nematodes have been able to dispense with the endosymbionts by acquiring the key genes required for obligate mutualism, or if they simply represent a genetic ‘ghosts’ from previous encounters in their evolutionary history.

Another area in which *Wolbachia* has been shown to play an important role is in driving inflammatory disease pathogenesis and inflammatory adverse reactions to anti-nematode drugs in lymphatic filariasis, onchocerciasis and heartworm disease (7, 25). The release of *Wolbachia* bacteria and their products from the nematode has been shown to stimulate innate and adaptive inflammatory immunity through recognition of lipoproteins via Toll-like receptors TLR-2 & TLR-6 (26). This drives the recruitment of inflammatory cells, leading to damage of parasitized tissues, including the cornea and lymphatics (7, 25, 26). Recent work has shown that this TLR-2- and *Wolbachia*-dependent stimulation of inflammation can impart a selective advantage to the parasite, through activating mast cells in the skin, which enhances the establishment of the parasite by increasing vascular permeability (26). Some have even suggested that *Wolbachia*-mediated inflammatory responses may act to block anti-nematode immunity (27) and so contribute indirectly to the unusual longevity of filarial nematodes.

Probably the most important outcome from the discovery of *Wolbachia* mutualism in filarial nematodes has been to create the opportunity to use antibiotics as a novel treatment of filarial diseases (6, 22). Treatment with tetracycline or rifamycin antibiotics results in the clearance of the endosymbiont from the nematode, leading to blockage of embryogenesis, sterilization of adult worms and the eventual death of the adult parasites, an outcome that has remained elusive with existing anti-nematode drugs. Existing treatment regimes require a four-week course of doxycycline to deplete the bacteria. Although this produces a superior therapeutic efficacy compared to existing anti-nematode drugs with benefits to individual
point-of-care treatment, the prolonged course of therapy together with contraindications in
children and pregnant women, restrict its use in widespread community mass drug
administration (MDA) programs. This stimulated the formation of the anti-Wolbachia (A-
WOL) consortium 2007, which was funded by the Bill and Melinda Gates Foundation to
discover and develop new anti-wolbachial drugs suitable for MDA control programs.
Currently, the A-WOL consortium has developed a portfolio of drug discovery projects with
the potential to generate at least one new anti-wolbachial chemotype for eventual deployment
as a macrofilaricide and is evaluating more than 200 ‘hits’ from registered or re-purposed
drugs to improve on existing regimes (http://www.a-wol.com/). The goal of this research is to
deliver anti-wolbachial therapy that can be used in endemic communities, to sustain the
achievements of existing control programmes and provide the means to deliver the
elimination of filarial diseases.

Symbionts of ticks

Ticks are small arachnids in the order Ixodida, subclass Acarina. They are ectoparasites,
living by hematophagy on the blood of mammals, birds, reptiles and amphibians. The lifestyle
of many Ixodid (hard) ticks, that are the important vectors and reservoirs of many human and
veterinary pathogens, encompasses three primary stages of development: larval, nymphal, and
adult. Most ticks take a blood meal only three times in their life (lasting up to 10 years for
some species). This type of feeding (almost always a sterile meal), slows down metabolism
and a very hard chitin covering make ticks the walking “cans” with very limited exchange
with the environment. So, any bacterium found inside the tick should (i) either (directly or
indirectly) kill it in order to exit and pursue its life cycle or, (ii) become endosymbiotic in
order to facilitate coexistence. Specifically integrated in the « can » system, bacteria may be
beneficial or neutral to the host. Symbionts of ticks represent sophisticated systems with an
intimate host/endosymbiont relationship and a specific type of transmission from one
generation to another. Transovarial transmission enables bacterial colonization very early in
the tick life cycle; copulation and egg fertilization could also favour bacterium-tick
associations through possibly infected sperm or the microbiota associated with the female
genital tract (29).

However, surprisingly, no “classical” primary or secondary endosymbionts have been
described for ticks up to date. Moreover, the microbiome of ticks remains largely unexplored.
Only few studies are available that describe the diversity of the microbiota associated with
hard ticks. Most attempts aimed at identifying the bacterial species associated with ticks used
standard culture methods on various solid media (30,31). In almost all studies, only
environmental free-living bacteria were isolated. Most probably, these represent occasional
members of the bacterial microbiota, either ingested or covering the chitin coat of the tick.

Almost all endosymbiotic bacteria are quite difficult to isolate; typical primary
endosymbionts of arthropods were never isolated in pure culture (32-34). In order to identify
bacteria ecologically and evolutionarily associated with ticks, other methods should be used,
such as special cell culture system (tick cell lines), enriched broth, and/or 16S rRNA-based
analysis. The most comprehensive method to characterize bacterial diversity is the bar-coded
16S rRNA pyrosequencing technique. A recent study using this method (35) reports the
presence of bacteria of 121 genera in different tissues and stages of *Rhipicephalus microplus*, an important vector of veterinary pathogens. Most of these were free-living environmental γ-
proteobacteria, Gram-positive cocci and anaerobes without strict association with ticks. These
data confirmed previous culture-based studies (30, 31).

However, several groups of bacteria isolated or identified in ticks are of high interest as
possible endosymbionts or, at least, as closely-associated bacteria (Table 3). Some examples
are highlighted below.
**Coxiella-like bacteria.** The *Coxiella*-like microorganisms comprise a group of genetically similar bacteria that have not yet been isolated in pure culture. These γ-proteobacteria are phylogenetically close to the obligate intracellular *Coxiella burnetii*, the agent of Q fever and the only recognized species of the genus. These bacteria were detected by PCR in many tick species, mostly from the genera *Rhipicephalus*, *Haemaphysalis*, *Amblyomma* and *Dermacentor* (35-38). In most studies, these bacteria are present in almost 100% of ticks of both sexes. In a recent study, Andreotti *et al.* showed the presence of *Coxiella*-like bacteria in ovaries, eggs and adult males of *Rh. microplus* ticks. In ovaries, this constitutes more than 98% of all identified bacterial species. This may indicate that some bacteria of the *Coxiella* genus are tick-associated primary endosymbionts that can be transmitted vertically (35). Interestingly, the reproductive fitness of *Amblyomma americanum* infected with a *Coxiella* spp. endosymbiont was reduced by an antibiotic treatment (39). Moreover, as expected for a tick symbiont, the genome of the *Coxiella*-like bacteria was reduced in size as compared to *C. burnetii* genome, with a lack of several hypothetical proteins of *C. burnetii* including the *recN* gene product involved in DNA repair (38).

**Arsenophonus nasoniae.** Bacteria of the genus *Arsenophonus* are considered as endosymbionts of many insects (hymenoptera, whiteflies, triatomine bugs, hyppoboscids and lice) (40). *A. nasoniae* induces the male killing phenomenon in the wasp *Nasonia vitripennis*, a parasite of several fly species (41). Interestingly, the strain of *A. nasoniae* was identified in hard ticks of the genera *Amblyomma* and *Dermacentor* in the USA (42-43). Recently, a strain almost identical to *A. nasoniae* from wasps was isolated from the nymph of a *Ixodes ricinus* tick collected in Slovakia. Molecular screening of the ticks from the same location showed that 37% of the nymphs contain this bacterium, while only 3.6% of adults do. This suggests that the bacterium is pathogenic toward early developmental stages of the tick, or that its presence in ticks’ bodies depends on the developmental stage. *A. nasoniae* may play a role in
tick fitness and/or development, but data on the precise nature of the bacteria/tick relationship are still lacking. The pathogenicity of *Arsenophonus* spp. for vertebrates is also yet unknown.

*Diplorickettsia massiliensis*. The recently described bacterium *Diplorickettsia massiliensis* was isolated from the hard tick *Ixodes ricinus* (44). It is an obligate intracellular Gram-negative bacterium phylogenetically close to the genus *Rickettsiella*, a clade of intracellular bacteria that infect a wide range of arthropods including insects, crustaceans and arachnids (46). Further, it can be grouped into the Family *Coxiellaceae* and the Order *Legionellales* (γ-proteobacteria). The *Coxiellaceae* Family currently includes three genera: *Diplorickettsia*, *Coxiella* and *Rickettsiella* (45). *Coxiella*-like bacteria, as described above, should be placed in the same family, when isolated and fully characterized.

*D. massiliensis* bacteria are localized within an intracellular compartment (vacuole) in pairs; the internal structure includes crystal-like and multi-layer sheath-like structures of unknown composition and function. The role of *D. massiliensis* in tick natural history and its influence on tick’s fitness is unknown. However, a recent study suggests that this bacterium is pathogenic towards humans (47).

*Spiroplasma ixodetis*. Spiroplasmas (class *Mollicutes*) are helical, motile, wall-less prokaryotes associated with a variety of insects, other arthropods, and some plant hosts (48). They are usually considered as commensal organisms in their arthropod hosts, but several are pathogenic for insects and plants. Several species were associated with a male-killing phenomenon (49). Spiroplasmas have been identified in ticks (*Haemaphysalis leporispalustris, Ixodes pacificus*), blood-sucking members of the *Diptera*, including horseflies (*Tabanus* spp.), deerflies (*Chrysops* spp.), and mosquitoes (*Aedes* spp., *Culex* spp.).

*Spiroplasma ixodetis* was first isolated from *Ixodes pacificus*, a principal vector of Lyme disease on the west coast of the United States of America (USA) (48). Later, a nearly identical bacterium was isolated from a pool of *Ixodes* ticks in North Rhine-Westphalia (Germany).
(50), and another strain of *Spiroplasma* spp. (genetically very close to *S. ixodetis*) was recently isolated on the XTC cell line from a *Ixodes ricinus* tick sampled in Slovakia, where prevalence of tick infection by *Spiroplasma* was 2.5% (Raoult *et al*., unpublished).

Virtually nothing is known about the relationship between *Spiroplasma* and ticks. However, several publications support the pathogenic role of this bacterium towards humans. Thus, Lorenz *et al.* (51) found a *Spiroplasma* sp. infection causing a unilateral cataract in a premature human baby. Spiroplasmas have been reported to be involved in neurodegenerative diseases such as scrapie or Creutzfeldt-Jakob disease (52, 53).

In addition to the 4 potential tick endosymbionts discussed above and to established human pathogens known to be transmitted by ticks (Table 3), several other fastidious intracellular bacteria have been shown to be closely associated with ticks, including *Candidatus* Midichloria mitochondrii (54), *Francisella*-like bacteria (55), *Wolbachia* spp., and different *Rickettsiales*. More studies are needed in this emerging field, whose results may have many applications, including the control of vector-borne diseases of humans and animals. Indeed, the concept of targeting endosymbionts as a mean to control ticks and tick-borne diseases has been tested using a chemotherapeutic approach (56). Novel methods for isolation and characterization of tick-associated bacteria will likely promote new approaches to control ticks by targeting their endosymbionts.

**Symbionts of amoebae**

Amoebae are widespread in the environment, mainly at water-air and water-soil interfaces and these protists are especially prevalent where procaryotes are growing, since they graze on bacteria (57). However, some bacteria are resistant to the microbicidal effectors of amoebae (i) by being either true symbionts, i.e. living in close association during a specific
period of their time-life with amoebae, or (ii) by being true amoebal pathogens able to lyse the amoebae before or after completing an intra-amoebal replication cycle (58, 59). Amoebae may thus be considered as a replicative niche for both amoebal symbionts and amoebal pathogens. However, amoebae are not a neutral replicative site, but a potent evolutionary crib that promotes selection of virulence traits leading to survival against phagocytic cells (60-63). This supports the use of amoebae as a model to assess the bacterial virulence of amoebae-resisting micro-organisms (67). Amoebae also represent protective armour for the internalized bacteria when encysted, and at least for some symbionts, a source of energy and nutrients.

The evidence of the importance of amoebae as a reservoir of *Legionella* spp. led T. Rowbotham to use amoebae as cells in a cell culture system to culture *Legionella* species (71). Since that time, this amoebal co-culture method (see reference 70 for an up-to-date protocol) has proven successful for the recovery by culture of a large biodiversity of amoebae-resisting bacteria (57, 61, 68, 70).

Amoebae are also increasingly considered as an Agora where gene exchanges take place (63-65). This intra-amoebal cross-talk has been corroborated by a recent analysis of gene exchanges occurring between amoebae-resisting microorganisms, whereby as many as 9 horizontal gene transfer events between *Legionella* species, *Chlamydia*-related bacteria and members of the Order *Rickettsiales* (66) were identified. Moreover, the genome of amoebae-resisting bacteria are commonly encoding proteins sharing a domain conserved in eukaryotic proteins (66, 75), suggesting that horizontal transfer may also be at play between the bacterial symbiont and the amoebal host.

Three major groups of amoebae-resisting bacteria have been extensively investigated, the *Legionella*, mycobacteria and *Chlamydia*-related organisms (Figure 2), and several relatively recent reviews are already available (63, 67-69). Here, we thus focus on rickettsial symbionts, and on two other *Candidatus* species for which recently available genomic data
illuminate the biology and their interactions with amoebae: *Odysella thessalonicensis* and *Amoebaphilus asiaticus*.

**Rickettsial symbionts.** Symbionts related to *Rickettsia* have been so far only poorly studied, although *Caedibacter* was already observed in an *Acanthamoeba* amoeba in 1985 (71). The taxonomic position of these rickettsial symbionts was confirmed by coupled 16S rRNA sequencing & FISH approaches (72), *Caedibacter acanthamoebae*, *Paracedibacter acanthamoebae* and *Paraceadibacter symbiosus* sharing (i) only 93.3%, 87.5% and 86.5% 16S rRNA sequence similarity, respectively, with *Caedibacter caryophilus*, their closest neighbour (a symbiont of paramecium) and (ii) 84 to 86% with *Holospora obtusa*. Due to the limited available research reports on rickettsial symbionts, it is likely that a much larger biodiversity of *Rickettsia*-like bacteria remains to be discovered, as suggested by the observation in *Acanthamoeba* of a small rod exhibiting 85.4% 16S rRNA sequence similarity with *Rickettsia sibirica* (74). Future work should thus aim at better defining the distribution, prevalence, host range and pathogenicity towards animals and humans of these amoebal endosymbionts.

**Odysella thessalonicensis.** Like *Rickettsia* spp., *Odysella thessalonicensis* is an alpha-proteobacterium, exhibiting a strict dependency to cells. It has been isolated by amoebal co-culture from an air conditioning system of a Greek hospital in the city of Thessalonika (58). This bacterium could only be grown in *Acanthamoeba* spp. and induced amoebal lysis after 7 and 4 days at 30 and 37°C, respectively. This contrasted with the stability of its symbiotic relationship with the same amoebal strain at 22°C for at least 3 weeks (58). Its biology and potential pathogenicity remain largely unknown.

**Amoebophilus asiaticus.** *Amoebophilus asiaticus* is a strict intracellular symbiont related to *Cardinium hertigii* and both belong to the *Bacteroidetes* group (76). *A. asiaticus* was discovered within an amoeba isolated from sediments of an Austrian lake (75).
analysis of its genome revealed a circular chromosome of 1884 kb, encoding 1557 hypothetical proteins (76). Thus, contrarily to symbionts of arthropods that exhibit small genomes (< 0.8 kb), this amoebal symbiont does not present a highly compact genome, despite the absence of extrachromosomal elements. This suggests that, as observed for *Legionella*, *Chlamydia*-related bacteria and giant viruses (63-65), the sympatric intra-amoebal life of *Amoebophilus asiaticus* has prevented a significant reduction of the genome size. Indeed, mobile elements represent 24% of the whole genome coding capacity of this endosymbiont (76). Moreover, *A. asiaticus* exhibits a reduced number of genes encoding metabolic functions (17% of the coding capacity) and encodes as many as 82 proteins involved in the transmembrane transport of metabolites, a feature expected for an amoebal symbiont (76). Like *Legionella* spp. and *Chlamydia*-related bacteria (see above), *A. asiaticus* encodes different proteins exhibiting eukaryotic domains suggesting that amoebae-resisting bacteria widely use such eukaryotic motifs to manipulate the host cell. These eukaryotic domains include U-box and F-box, leucine-rich repeats (LRRs) and ankyrin repeats, among others. U–box and F-box motifs are likely interfering with the ubiquitin system involved in the degradation of proteins by the proteasome whereas ankyrin proteins are likely controlling the interactions of the intracellular bacteria in its host cell. Finally, the LRRs domain, also largely present in the genome of *Protochlamydia amoebophila* (77), may be involved in decreasing recognition of the bacteria by the innate immune system.

**General conclusions**

We hope that this review on symbionts of nematodes, ticks and amoebae will help the reader to understand the importance of the symbiont in determining the virulence of its host, as exemplified with *Wolbachia* in nematodes; similarly, an amoebal endosymbiont may also be implicated in the pathogenesis of *Acanthamoeba* keratitis, by potentially exacerbating local
inflammation. This review also recaps the importance of the host in the ecology of its endosymbiont, by directly impacting its survival in the environment, its dissemination, and its mode of transmission to humans and animals. This is of paramount importance, since ecology strongly controls the gene content of the symbionts. Sympatric amoebal symbionts exhibit much larger genomes and much more frequent genes exchange events than those living in an allopatric environment in nematodes and ticks. Symbionts have also clearly played an important role by “feeding” eucaryotes with significant amounts of genetic information during evolution, (i) as previously exemplified by the identification of the role of an ancestral member of the *Rickettsiales* in the biogenesis of current mitochondria (78), and (ii) as recently exemplified by the acquisition by a fruit fly of a nearly complete wolbachial genome content (24). The fact that at least one member of the Order *Rickettsiales* has been identified in all three eucaryote lineages discussed in this review further supports the hypothesis that an ancestral rickettsia was already intracellular more than one billion years ago, when it exchanged genes encoding an ADP/ATP transporter with an ancestral *Chlamydiales* (79). Moreover, this explains why rickettsiologists are in the fore-front of research on endosymbiont-host interactions. Other important lessons provided by studying symbionts are that (i) their diverse nature (large biodiversity encompassing several clades) as well as (ii) their intimate relationship with their specific host, provides no guaranty of their innocuousness towards other eukaryotes encountered by chance, for instance in a modified ecosystem such as man-made water networks.
References


**Table 1.** Selected discoveries or hypotheses illuminating research on symbionts*

<table>
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<tr>
<th>Year</th>
<th>Discovery – hypothesis</th>
<th>References</th>
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<td>Concept of symbiosis</td>
<td>(61)</td>
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<tr>
<td>1905, Merezhkovsky</td>
<td>Endosymbiotic bacteria may be at the origin of mitochondria and other eucaryotic organelles</td>
<td>(1)</td>
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<td>1912, Büchner</td>
<td>Description of bacterial and fungal endosymbions of arthropods</td>
<td>(2)</td>
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<td>1967, Jeon</td>
<td>Accidental infection of <em>Amoeba proteus</em>, by a symbiont initially referred as the “x-bacterium” before being renamed <em>Legionella jeonii</em></td>
<td>(80)</td>
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<td>1998, Sullivan</td>
<td>Concept of “symbiosis island” to refer to a genomic island, which is important in mutualistic relationship of a symbiont with its host</td>
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<td>1998, Andersson</td>
<td>An ancestral rickettsial endosymbiont identified to be at the origin of mitochondria</td>
<td>(78)</td>
</tr>
<tr>
<td>2001, Moran</td>
<td>First evidence of strong genome reduction (demonstrated for an aphid symbiont)</td>
<td>(81)</td>
</tr>
<tr>
<td>2003, Greub</td>
<td>Long history of co-evolution of <em>Rickettsiales</em> and <em>Chlamydiales</em> with their hosts (already energy parasites of their hosts &gt; 1 billion year ago)</td>
<td>(79)</td>
</tr>
<tr>
<td>2003, La Scola</td>
<td>Discovery of a giant virus of amoebae</td>
<td>(82)</td>
</tr>
<tr>
<td>2004, Greub</td>
<td>Conjugative DNA transfer may occur between strict intracellular bacteria (between amoebal endosymbionts)</td>
<td>(83)</td>
</tr>
<tr>
<td>2007, Kikuchi</td>
<td>First report of horizontal gene transfer in an insect symbiont</td>
<td>(84)</td>
</tr>
<tr>
<td>2010, Moliner</td>
<td>Reductive evolution among allopatric symbionts (of ticks &amp; nematodes) and large genomes for sympatric symbionts present in amoebae</td>
<td>(65)</td>
</tr>
</tbody>
</table>

* adapted from reference 61.
**Table 2.** Bacteria-nematode mutualism between the *Wolbachia* endosymbiont and filarial nematodes.

<table>
<thead>
<tr>
<th>Effect of <em>Wolbachia</em> endosymbiont</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Provide key nutrients: haem, nucleotides, riboflavine and FAD</td>
<td>(17, 21, 22)</td>
</tr>
<tr>
<td>(especially important during embryogenesis up to L4 larval stages)</td>
<td></td>
</tr>
<tr>
<td>2. Prevent apoptosis of the reproductive cells</td>
<td>(16)</td>
</tr>
<tr>
<td>3. Prevent oxidative stress</td>
<td>(25)</td>
</tr>
<tr>
<td>4. Pro-inflammatory effect:</td>
<td></td>
</tr>
<tr>
<td>- TLR2/TLR6 recognition of wolbachial lipoproteins</td>
<td>(26)</td>
</tr>
<tr>
<td>- Facilitates nematode entry, i.e. represents a mechanism of immune subversion</td>
<td>(27-28)</td>
</tr>
</tbody>
</table>
### Table 3. Bacterial species known to be associated with ticks

<table>
<thead>
<tr>
<th>Species</th>
<th>Ticks</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arsenophonus nasoniae</em></td>
<td><em>Amblyomma</em>, <em>Dermacentor</em>, <em>Ixodes ricinus</em></td>
<td>Endosymbionts of many insects</td>
</tr>
<tr>
<td><em>Borellia</em> spp.</td>
<td><em>Ixodes</em> spp.</td>
<td>Agent of Lyme disease</td>
</tr>
<tr>
<td><em>Bartonella</em> spp.</td>
<td>Uncommonly transmitted by ticks&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Agent of cat-scratch disease, trench fever, bacillary angiomatosis and other bartonella infections</td>
</tr>
<tr>
<td><em>Coxiella burnetii</em></td>
<td>Uncommonly transmitted by ticks</td>
<td>Agent of Q fever</td>
</tr>
<tr>
<td><em>Coxiella</em>-like bacteria</td>
<td><em>Rhipicephalus</em>, <em>Haemaphysalis</em>, <em>Amblyomma</em>, <em>Dermacentor</em></td>
<td>Antibiotic treatment reduces the reproductive fitness of infected ticks</td>
</tr>
<tr>
<td><em>Diplorickettsia massiliensis</em></td>
<td><em>Ixodes ricinus</em></td>
<td><em>Diplorickettsia</em> genus is part of the <em>Coxiellaceae</em> family as <em>Coxiella</em> and <em>Rickettsia</em> genera Possible new pathogen of humans</td>
</tr>
<tr>
<td><em>Ehrlichia/Anaplasma</em> spp.</td>
<td>Various species</td>
<td>Agents of ehrlichiois</td>
</tr>
<tr>
<td><em>Francisella tularensis</em></td>
<td></td>
<td>Agent of tularemia</td>
</tr>
<tr>
<td><em>Francisella</em>-like bacteria</td>
<td>Various species</td>
<td>Unknown human pathogenicity; yet uncultured</td>
</tr>
<tr>
<td><em>Rickettsia</em> spp.</td>
<td>Various species</td>
<td>Agents of various spotted fevers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reductive evolution of the genome associated with increased pathogenicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ticks determine the geographical repartition and the clinical presentation&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Spiroplasma ixodetis</em></td>
<td><em>Ixodes</em> spp.</td>
<td>Belong to the <em>Molliculites</em> class (like <em>Mycoplasma</em>)</td>
</tr>
</tbody>
</table>

<sup>1</sup><br> *Bartonella quintana* is generally transmitted by body lice whereas *Bartonella henseale* is mainly transmitted by cat or dog scratch and to a lesser extend by cat fleas.

<sup>2</sup>Due to the attack strategy of *Amblyomma* ticks, several inoculation eschars are common in the same patient; due to the hair tropism of *Dermacentor* ticks, the inoculation eschar is often missed and first hint for diagnosis of *R. slovacæ* infection is the presence of a loco-regional adenopathy, according to which the disease has been named: tick-borne lymphadenitis (TIBOLA).
Figure legends.

Figure 1. Electron micrograph of Wolbachia (arrows) in the hypodermal chord cell of Brugia malayi (a filarial nematode). Bar represents about 0.5 µm.

Figure 2. A Chlamydia-related bacteria (Criblamydia sequanensis) recently isolated by amoebal co-culture from the Seine river water. The bacteria (here within Acanthamoeba castellani amoebae) exhibits typical star-shaped elementary bodies. Electron microscopy, magnification 20,000x; bar represents 0.2 µm.
Figure 1. Electron micrograph of Wolbachia (arrows) in the hypodermal chord cell of Brugia malayi (a filarial nematode). Bar represents about 500 nm.

130x149mm (300 x 300 DPI)
Figure 2. A Chlamydia-related bacteria (Criblamydia sequanensis) recently isolated by amoebal co-culture from the Seine river water. The bacteria (here within Acanthamoeba castellanii amoebae) exhibits typical star-shaped elementary bodies. Electron microscopy, magnification 20,000x; bar represents 200 nm. 130x97mm (300 x 300 DPI)