PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B (2006) **273**, 2097–2106
doi:10.1098/rspb.2006.3541
Published online 21 April 2006

Review

The emerging diversity of *Rickettsia*

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The best-known members of the bacterial genus *Rickettsia* are associates of blood-feeding arthropods that are pathogenic when transmitted to vertebrates. These species include the agents of acute human disease such as typhus and Rocky Mountain spotted fever. However, many other *Rickettsia* have been uncovered in recent surveys of bacteria associated with arthropods and other invertebrates; the hosts of these bacteria have no relationship with vertebrates. It is therefore perhaps more appropriate to consider *Rickettsia* as symbionts that are transmitted vertically in invertebrates, and secondarily as pathogens of vertebrates. In this review, we highlight the emerging diversity of *Rickettsia* species that are not associated with vertebrate pathogenicity. Phylogenetic analysis suggests multiple transitions between symbionts that are transmitted strictly vertically and those that exhibit mixed (horizontal and vertical) transmission. *Rickettsia* may thus be an excellent model system in which to study the evolution of transmission pathways. We also focus on the emergence of *Rickettsia* as a diverse reproductive manipulator of arthropods, similar to the closely related *Wolbachia*, including strains associated with male-killing, parthenogenesis, and effects on fertility. We emphasize some outstanding questions and potential research directions, and suggest ways in which the study of non-pathogenic *Rickettsia* can advance our understanding of their disease-causing relatives.

Keywords: heritable symbionts; male-killing; reproductive manipulation; *Wolbachia*

1. INTRODUCTION AND GOALS

Bacteria in the genus *Rickettsia* are best known as arthropod-vectored pathogens of vertebrate hosts (Raoult & Roux 1997). *Rickettsia* are intracellular, and are symbionts in the broad sense, having an intimate (but not necessarily beneficial) relationship with their hosts. *Rickettsia* species are the causative agents of numerous diseases of humans, including epidemic typhus, which is thought to have caused up to three million deaths in Russia alone, from 1917 to 1923 (Zinsser 1963). Other *Rickettsia* have received recent attention as potential agents of bioterrorism and as causes of emerging diseases (Azad & Beard 1998). For example, Rocky Mountain spotted fever reached its highest incidence ever in the United States in 2004 (Dumler & Walker 2005). Recent advances in the study of *Rickettsia* include the sequencing and preliminary analyses of seven (and counting) complete rickettsial genomes (Andersson *et al.* 1998; Ogata *et al.* 2001, 2005; McLeod *et al.* 2004; Renesto *et al.* 2005) and the first successful genetic transformation of *Rickettsia* (Qin *et al.* 2004). As one of the closest extant relatives of mitochondria, *Rickettsia* has also been studied for insight into the evolution of organelles (Andersson *et al.* 1998; Emelyanov 2001; Fitzpatrick *et al.* 2006).

Members of the genus *Rickettsia* were traditionally classified into: (i) the spotted fever group, including symbionts transmitted by hard ticks, such as *Rickettsia conorii* and *Rickettsia rickettsii*; and (ii) the typhus group, including *Rickettsia typhi*, the cause of murine typhus, transmitted by fleas and *Rickettsia prowazekii*, the agent of epidemic typhus, transmitted by lice. The typhus group also formerly included the agent responsible for scrub typhus, a bacterium vectored by mites known as *Rickettsia tsutsugamushi*. The advent of molecular phylogenetics has revolutionized *Rickettsia* systematics. First, the causative agent of scrub typhus has been shown to share only approximately 91% sequence similarity at 16S rDNA with *Rickettsia* and has been renamed *Orientia tsutsugamushi* (Tamura *et al.* 1995). Second, surveys of microbial diversity of many disparate hosts, particularly arthropods, are continually uncovering new *Rickettsia*, including many that do not fall into the previously recognized subgroups. These have received much less attention than their relatives in blood-feeding hosts because they are not clearly pathogenic to humans, and in most cases, their effects on their hosts have not been discovered.

The major goal of this review is to highlight the under-appreciated diversity of the *Rickettsia* that have not been identified as pathogens of vertebrates. We focus on the emergence of *Rickettsia* as reproductive manipulators of arthropods, similar to the closely related *Wolbachia*. Phylogenetic analysis suggests multiple transitions between vertically transmitted arthropod symbionts and symbionts that have both horizontal and vertical transmission pathways. This diversity suggests that *Rickettsia* may be an excellent model system for studying the evolution of transmission pathways. We also emphasize some outstanding questions and potential research

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Table 1. *Rickettsia* with no known vertebrate pathogenic effects reported from various invertebrates (excluding ticks).

host organism	phenotype	reference
arthropods		
mites (Acari)		
Tetranychus urticae (Tetranychidae)	not known	Hoy & Jeyaprakash (2005)
beetles (Coleoptera)		
Adalia bipunctata (Coccinellidae)	male-killing	Werren *et al.* (1994)
Adalia decempunctata (Coccinellidae)	male-killing	von der Schulenburg *et al.* (2001)
Brachys tessellatus (Buprestidae)	male-killing	Lawson *et al.* (2001)
Coccotrypes dactyliperda (Scolytidae)	not known	Zchori-Fein *et al.* (in press)
Kytorhinus sharpianus (Bruchidae)	not known	Fukatsu & Shimada (1999)
springtails (Collembola)		
Onychiurus sinensis (Onychiuridae)	not known	GenBank AY712949
flies (Diptera)		
Culicoides sonorensis (Ceratopogonidae)	not known	Campbell *et al.* (2004)
Limonia chorea (Limoniidae)	not known	GenBank AF322443
true bugs (Hemiptera)		
Acyrthosiphon pisum (Aphididae)	reduced fecundity, weight	Chen *et al.* (1996); Sakurai *et al.* (2005)
Bemisia tabaci (Aleyrodidae)	not known	Gottlieb *et al.* (in press)
Empoasca papayae (Cicadellidae)	papaya bunchy top disease	Davis *et al.* (1998)
wasps (Hymenoptera)		
Neochrysocharis formosa (Eulophidae)	parthenogenesis	Hagimori *et al.* (2006)
booklice (Psocoptera)		
Liposcelis bostrychophila (Liposcelididae)	oogenesis	Yusuf & Turner (2004)
other		
leeches (Hirudinida)		
Hemiclepsis marginata (Glossiphoniidae)	not known	Kikuchi *et al.* (2002)
Torix tagoi (Glossiphoniidae)	larger body size	Kikuchi *et al.* (2002), Kikuchi & Fukatsu (2005)
Torix tukubana	larger body size	Kikuchi & Fukatsu (2005)
amoebae		
Nuclearia pattersoni (Nucleariidae)	not known	Dykova *et al.* (2003)

directions, and suggest ways in which study of non-pathogenic *Rickettsia* can advance our understanding of their disease-causing relatives.

2. DIVERSITY OF *RICKETTSIA*

The order Rickettsiales lies in the α-Proteobacteria and is comprised entirely of obligate intracellular symbionts of eukaryotes. The order is currently classified into three families: Anaplasmataceae and Rickettsiaceae, most of whose members live in close association with arthropod hosts, and Holosporaceae, which are mostly protist symbionts (Boone *et al.* 2001). We surveyed the literature and GenBank, focusing on *Rickettsia* species that are either non-pathogenic in vertebrates or hosted by organisms other than blood-feeding arthropods. The survey revealed a great diversity of *Rickettsia* with respect to both host range (including vertebrates, arthropods, annelids, amoebae and plants) and effects on these hosts (table 1). For most taxa that have not been identified as pathogens of vertebrates, only the relatively invariant 16S rDNA sequences are available, so that a phylogenetic analysis that includes other genes is not possible at this time. However, even the 16S rDNA data suggest that there have been transitions between blood-feeding and non-blood-feeding hosts (figure 1). Our analysis recovers clades consisting exclusively of symbionts of blood-feeding arthropods (e.g. spotted fever group, typhus group, *Rickettsia akari*+*Rickettsia australis*), which are also consistently well-supported in other analyses (e.g. Roux & Raoult 1995, 2000; Stothard & Fuerst 1995; Roux *et al.* 1997). To test the hypothesis that there have been transitions between blood-feeding and non-blood-feeding hosts, a Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa 1999) was used to compare the likelihood score of our maximum-likelihood tree with the one in which all *Rickettsia* from the typhus and spotted fever groups (including *R. akari*, *R. australis* and *Rickettsia felis*) are constrained to be monophyletic. The constrained tree had a significantly lower likelihood score (SH test: $p < 0.005$).

3. TICK *RICKETTSIA* WITH NO KNOWN VERTEBRATE PATHOGENICITY

To date, ticks have been sampled for the presence of *Rickettsia* far more than any other hosts (Raoult & Roux 1997). Among the diverse assemblage of *Rickettsia* found across the ticks, several isolates are not associated with pathogenicity in vertebrates. La Scola & Raoult (1997) argued that all *Rickettsia* have the potential to cause disease in vertebrates, and claimed that opportunity for transmission to vertebrate hosts is the limiting factor in determining the probability of disease, but this hypothesis is controversial. In support of this hypothesis, some *Rickettsia* that were previously thought to be non-pathogenic are now known to cause disease in vertebrates (La Scola & Raoult 1997; Shpynov *et al.* 2003). These include *Rickettsia helvetica* and *Rickettsia slovaca* (Raoult *et al.* 1997; Fournier *et al.* 2000), as well as *Rickettsia parkeri*, which was shown to cause rickettsiosis in humans,

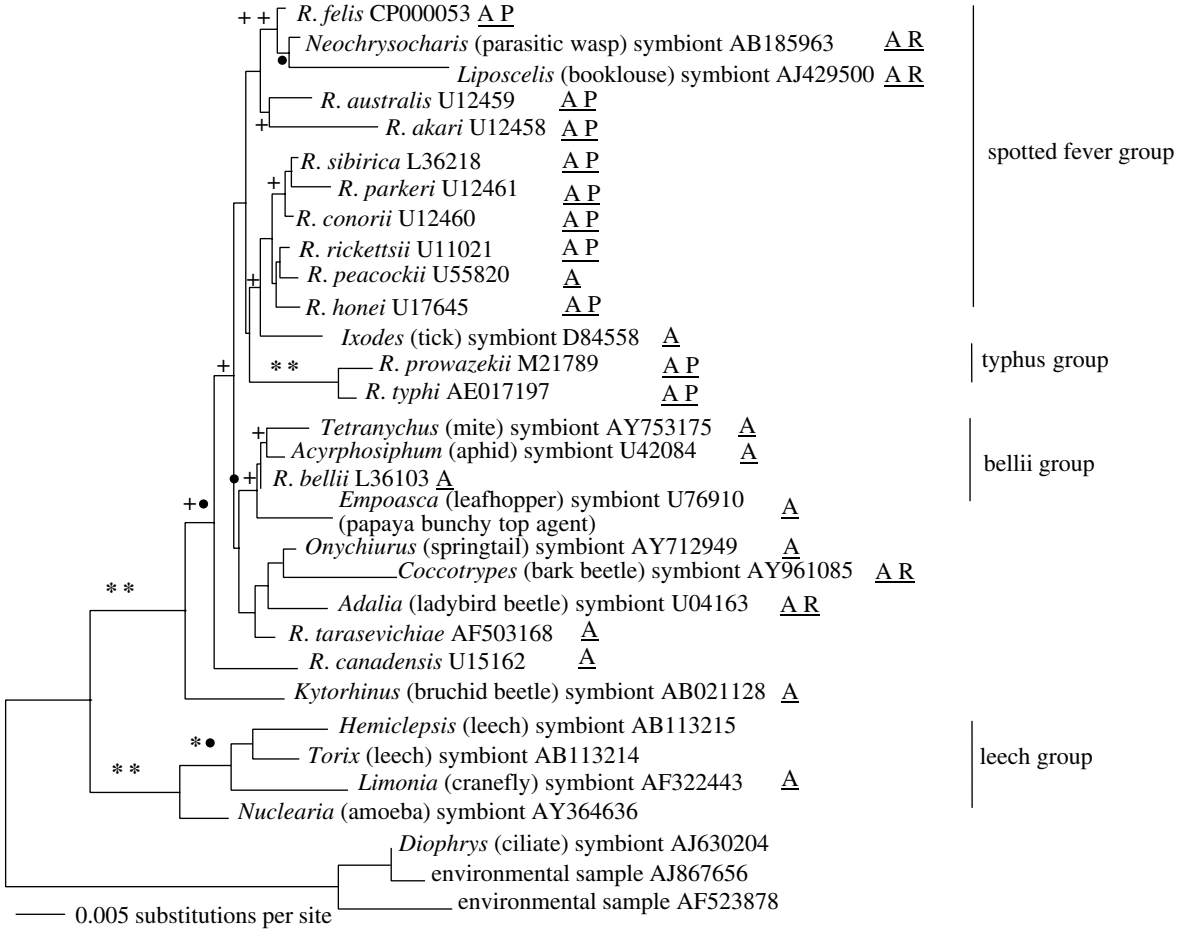

Figure 1. Phylogenetic tree of *Rickettsia* 16S rDNA sequences, using a Kimura 3 parameter distance model of evolution; topology does not differ significantly from maximum parsimony and maximum-likelihood trees. This tree is rooted with 16S sequences from a *Diophrys* ciliate symbiont and two environmental samples. Statistical support for nodes was determined by conducting 500 parsimony and distance bootstraps. Bootstrap support symbols: plus, a node that has greater than 50% bootstrap support; filled circle, a node that has greater than 70% bootstrap support and asterisk, a node that has greater than 90% bootstrap support, with the first symbol referring to parsimony bootstrap and the second to distance bootstrap. *Rickettsia* strain biology: 'A' refers to arthropod symbionts, 'P' refers to symbionts associated with vertebrate pathogenicity, 'R' refers to symbionts associated with reproductive manipulation.

60 years after its discovery (Paddock *et al.* 2004). At this time, however, no tick-associated *Rickettsia* that fall outside of the well-established pathogenic groups (e.g. the typhus and spotted fever groups) have definitively been shown to cause disease in vertebrates (whether *Rickettsia canadensis* causes disease is a matter of dispute; Raoult & Roux 1997; see Bozeman *et al.* 1970; Wenzel *et al.* 1986). These putative non-pathogens currently include symbionts such as *Rickettsia bellii* and *Rickettsia tarasevichiae* that are especially interesting because they are symbionts of ticks whose closest relatives are symbionts of non-blood-feeders. *R. bellii* has long been recognized to be a distinct lineage from the better known typhus and spotted fever group *Rickettsia* (Philip *et al.* 1983; Stothard *et al.* 1994). It is currently unknown whether *R. bellii* and other putative non-vertebrate pathogens colonize the tick salivary glands, but such information will provide a clue to their potential access to the vertebrate host (Munderloh & Kurtti 1995).

4. *RICKETTSIA* ASSOCIATED WITH REPRODUCTIVE MANIPULATION IN ARTHROPODS

Our conception of the genus *Rickettsia* has perhaps been most radically revised by finding several lineages that are reproductive manipulators of arthropod hosts. Reproductive manipulation, also known as reproductive parasitism, refers to a set of distinct life histories that enable some maternally inherited symbionts to spread within host populations. In general, symbionts that are strictly vertically transmitted must either increase the fitness of their host or manipulate host reproduction in ways that benefit their own transmission (Bull 1983; O'Neill *et al.* 1997). Examples of the latter include increasing the frequency of infected females by inducing parthenogenesis, feminizing hosts or killing males. Alternatively, symbionts can decrease the fitness of uninfected females, for example, via cytoplasmic incompatibility (CI), in which uninfected females produce few or no viable offspring when mated with infected males. By far the best known reproductive manipulator is *Wolbachia*, which is in the same order as *Rickettsia* (approx. 86% similar at 16S rDNA) and the only bacterium shown to cause all four major types of reproductive manipulation: CI, feminization, parthenogenesis and male-killing. However, unrelated bacterial lineages have also been found to manipulate host reproduction. *Cardinium*, an intracellular symbiont in the *Bacteroidetes*, has been shown to cause feminization, CI and parthenogenesis (Weeks *et al.* 2001;

Hunter *et al.* 2003; Zchori-Fein *et al.* 2004). Other reproductive manipulators include male-killing *Spiroplasma*, *Arsenophonus*, microsporidia and unnamed members of the Flavobacteria class of the *Bacteroidetes*, as well as feminizing microsporidia (Hurst & Jiggins 2000; Dunn & Smith 2001). Thus, the capability of symbionts to meddle with host reproduction seems to be widespread and generally associated with vertical transmission.

The first *Rickettsia* shown to be involved in reproductive manipulation was also the first to be found in a host that is not a blood feeder. The *Rickettsia* in the ladybird beetle *Adalia bipunctata* is associated with male embryo mortality and was provisionally named the 'AB bacterium' (Werren *et al.* 1994). This discovery prompted the authors to suggest, even at that time, that *Rickettsia* may be primarily arthropod-associated bacteria that do not cause disease in vertebrates, and that the large number of strains associated with mammalian pathogenicity was due to sampling bias. A closely related bacterial strain also kills males of the congener, *Adalia decempunctata* (von der Schulenburg *et al.* 2001). In addition to *Rickettsia*, *A. bipunctata* harbours a *Spiroplasma* and two *Wolbachia* strains, which are all thought to be involved in the male-killing phenotype (Hurst & Jiggins 2000). *Rickettsia* infection is also associated with male embryonic lethality in the leaf-mining beetle *Brachys tessellatus*, as antibiotic treatment increases the number of males that successfully hatch (Lawson *et al.* 2001). These authors reported a low level (approx. 10%) of parthenogenetic reproduction in this beetle as well, and speculated that it may either be a result of selection due to the lack of males, or to symbiont infection itself.

Recently, the first strong evidence for an association between *Rickettsia* and parthenogenetic reproduction was reported. In parthenogenetic populations of the parasitoid *Neochrysocharis formosa* (Hymenoptera: Eulophidae), over 99.5% of individuals are females and all appear infected with *Rickettsia*, but not *Wolbachia*, *Cardinium* or other symbionts (Hagimori *et al.* 2006). Antibiotic treatment results in production of (uninfected) male offspring, suggesting that the symbiont is responsible for parthenogenetic production of females. *Rickettsia* thus joins *Wolbachia* and *Cardinium* as the third bacterial lineage involved in a phenotype that had been thought to be exclusively caused by *Wolbachia* (Stouthamer *et al.* 1999).

The parthenogenetic book louse *Liposcelis bostrychophila* (Psocoptera: Liposcelididae) also harbours *Rickettsia*. Removal of symbionts via rifampicin treatment results in a major reduction in egg hatch rate, as well as offspring production and survival (Yusuf & Turner 2004). It cannot be determined whether this symbiont is parthenogenesis-inducing because viable males are not produced after antibiotic treatment. Alternatively, hosts may depend on the symbiont for egg production or maturation. The symbiont may also be maintained by production of a metabolically stable toxin and a less stable antitoxin, such that symbiont removal would be harmful to the host and/or offspring (Dedeine *et al.* 2003). Similarly, oogenesis was arrested and sterile females produced after antibiotic curing of the bark beetle *Coccotrypes dactyliperda* (Coleoptera: Scolytinae), which harbours both *Rickettsia* and *Wolbachia* (Zchori-Fein *et al.* in press), although the specific role of each symbiont is yet to be determined.

5. *RICKETTSIA* AS SYMBIONTS OF HERBIVOROUS ARTHROPODS

A number of *Rickettsia* that appear closely related to *R. bellii* are symbionts of herbivorous arthropods (figure 1), with the best-studied being the *Rickettsia* infecting the pea aphid *Acyrthosiphon pisum*, referred to as PAR (pea aphid *Rickettsia*). Beside their primary obligate nutritional symbiont *Buchnera aphidicola*, pea aphids harbour several facultative bacterial symbionts, including strains of *Spiroplasma* (Fukatsu *et al.* 2001), *Rickettsia* (Chen *et al.* 1996) and the recently named *Serratia symbiotica*, *Hamiltonella defensa* and *Regiella insecticola* (Moran *et al.* 2005). Some of the pea aphid symbionts other than the *Rickettsia* exert various beneficial effects on their hosts, including resistance to parasitoids and fungi (Oliver *et al.* 2003, 2005; Scarborough *et al.* 2005), heat tolerance (Montllor *et al.* 2002) and host plant utilization (Tsuchida *et al.* 2004). The effect of *Rickettsia* on pea aphids is not clear. On one hand, negative effects such as reduced body weight, lower fecundity and suppressed *Buchnera* densities have been reported (Chen *et al.* 2000; Sakurai *et al.* 2005). On the other hand, frequencies of PAR infection in pea aphid populations can be quite high, reaching up to 48% in California (Chen *et al.* 1996). How then is PAR maintained in nature? Theory predicts there can be no negative fitness effects of a strictly vertically transmitted symbiont in the absence of strong transmission advantage due to reproductive manipulation (Bull 1983). This suggests that the negative fitness effects of PAR may be countermanded by reproductive manipulation or positive effects on other aspects of host fitness (Sakurai *et al.* 2005). Alternatively, PAR may be transmitted horizontally, perhaps via plants, which would radically expand the potential set of relationships with the host under which the symbiont can spread, including pathogenicity.

Horizontal transmission to plants has been demonstrated in a *Rickettsia* infecting *Empoasca* planthoppers that feed on papaya (Davis *et al.* 1998). So far, this is the only *Rickettsia* identified as a plant pathogen, causing papaya bunchy top disease (PBT), and it is not known whether the mechanisms of pathogenicity are related to rickettsial disease in vertebrates. Approximately 50% of adult *Empoasca papayae* collected from infected papaya orchards in Puerto Rico tested positive for the infection; nothing else is known about the *Rickettsia–Empoasca* association. At least one other *Empoasca* species transmits PBT (Haque & Parasram 1973) and it will be important to determine whether it harbours a related or possibly identical *Rickettsia* and if that disease agent can be transmitted horizontally via the plant. Without transmission to leafhoppers from the plant, we would predict that the *Empoasca* symbiont must also benefit its host or manipulate host reproduction. Such a beneficial association has been demonstrated in a recent study of the corn pathogens *Spiroplasma kunkelii* and maize bushy stunt phytoplasma, and their vector, the corn leafhopper *Dalbulus maidis*, with increased vector survival in the presence of either of the plant pathogens (Ebbert & Nault 2001).

The sweet potato whitefly *Bemisia tabaci* harbours a strain of *Rickettsia* that is found in all populations tested, with infection rates ranging from 22 to 100%

(Gottlieb *et al.* in press). This symbiont appears to be widely distributed in various host tissues, such as around the gut and follicle cells, and throughout the haemolymph, but was not detected in plant tissue after whitefly feeding.

Finally, *Wolbachia* has been implicated in hybrid breakdown and CI that result from matings between populations of the herbivorous spider mite *Tetranychus urticae* (Vala *et al.* 2000; Perrot-Minnot *et al.* 2002), suggesting its potential importance in the process of speciation. A more recent study found that four out of six sampled North American populations were infected with *Rickettsia*, as well as *Wolbachia* and *Caulobacter* (Hoy & Jeyaprakash 2005). It would be interesting to determine whether *Rickettsia* infection can account for incompatibilities between different *T. urticae* populations and, thus, whether this microbe may also be implicated in promoting reproductive isolation.

6. OTHER *RICKETTSIA* SYMBIONTS

Virtually nothing is known about the biology of the remaining non-pathogenic *Rickettsia*, including all members of a clade that appears to be the most basal in the genus (figure 1), and is comprised of symbionts associated with craneflies, the fish-parasitic amoeba *Nuclearia*, leeches and *Culicoides* biting midges (Kikuchi *et al.* 2002; Dykova *et al.* 2003; Campbell *et al.* 2004). In two surveys of glossiphoniid leeches, three out of 10 species tested positive for *Rickettsia*, with infection rates varying widely, from 30 to 96% (Kikuchi *et al.* 2002; Kikuchi & Fukatsu 2005). Eggs of the species with high infection rates are all infected, suggesting a high rate of vertical transmission. While Kikuchi *et al.* (2002) speculate that horizontal transmission, perhaps via the blood of the amphibian hosts of these leeches, has been important in the evolution of this symbiont, frogs and fish in the leech habitat were not infected (Kikuchi & Fukatsu 2005). Interestingly, infected *Torix* were larger than uninfected hosts, but whether this reflects the symbiont enhancing host fitness or more frequent opportunities for larger individuals to acquire the symbiont via horizontal transmission, has not yet been determined (Kikuchi & Fukatsu 2005). Another member of this basal clade of *Rickettsia* infects *Nuclearia* amoebae that are parasites of fish, suggesting another possible link to a vertebrate host (Dykova *et al.* 2003). A survey of midgut diversity of *Culicoides* biting midges (Diptera: Ceratopogonidae) uncovered diverse microbes including *Rickettsia*. These flies transmit a variety of viral, protozoan and filarial pathogens to birds and mammals, including humans, but have not been implicated in transmission of pathogenic *Rickettsia*. Other *Rickettsia* with unknown biology include symbionts of *Onychiurus* springtails and *Kytorhinus* bruchid beetles (Fukatsu & Shimada 1999; Czarnetzki & Tebbe 2004).

Finally, a recent study reported the presence of *Rickettsia* in 11 spider species, using primers designed to amplify a portion of the citrate synthase gene (Goodacre *et al.* 2006). However, it is possible that the spider symbionts fall outside the genus, as the citrate synthase sequences are quite divergent from those of tick *Rickettsia* and there are no available sequences from other basal *Rickettsia*.

7. DIVERSITY OF *RICKETTSIA* TRANSMISSION STRATEGIES

It is now clear that a substantial portion of *Rickettsia* diversity falls outside of blood-feeding arthropods, with no demonstrated effects on vertebrates. The data suggest that almost all species within the genus *Rickettsia* are vertically transmitted symbionts of invertebrates. It is likely, moreover, that *Rickettsia* were initially symbionts of invertebrates that secondarily became vertebrate pathogens. First, strictly invertebrate symbionts outnumber vertebrate pathogens in the basal parts of the tree. Second, many related genera, including *Wolbachia*, are arthropod-associated. Indeed, the genus *Rickettsia* itself may pre-date vertebrates.

Most *Rickettsia* also appear to be facultative or 'secondary' symbionts of invertebrates, that is, symbionts that are not obligate for host survival and reproduction. In some primarily blood-feeding hosts, *Rickettsia* species have evolved a horizontal transmission pathway through an alternative host. Horizontal transmission decouples the fate of the bacterium from that of the invertebrate host and allows pathogenic relationships to evolve with both host types.

The diversity of transmission modes of *Rickettsia* is striking, and includes horizontal, vertical (i.e. transovarial) and mixed transmission. It should be noted that we refer here to transmission over ecological, and therefore, epidemiologically relevant, timescales. Over evolutionary time, all facultative symbionts, including all reproductive manipulators, exhibit a small but important component of horizontal transmission; host and symbiont phylogenies are thus incongruent. At the ecological time scale then, the varied transmission pathways of *Rickettsia* are in striking contrast with most related bacteria that are pathogens in vertebrates (e.g. *Anaplasma*, *Ehrlichia* (=*Cowdria*), *Bartonella*), which are not transovarially transmitted in arthropods (Munderloh & Kurtti 1995; Long *et al.* 2003). On the other hand, among reproductive manipulators, *Rickettsia* stands out in the degree of contagious transmission exhibited by some lineages. For example, *Wolbachia* appears to be transmitted strictly transovarially, with one exception (Huigens *et al.* 2000). Other related bacteria with different hosts also exhibit mixed vertical and horizontal transmission, including Holosporaceae, which appear to be exclusively protist symbionts (Kaltz & Koella 2003). Recently, *Neorickettsia* (formerly *Ehrlichia*) *risticii*, the causative agent of Potomac horse fever, was also shown to have a complex natural history, including vertical transmission in trematodes and horizontal transmission in caddisflies and vertebrates (Gibson *et al.* 2005).

Even within the *Rickettsia* found in blood-feeding hosts, a range of biological characteristics and transmission strategies is represented. In general, *Rickettsia* species that are pathogenic to vertebrates appear to have limited fitness costs to their arthropod hosts (although this has not been thoroughly studied), and are transovarially transmitted to the next generation (Azad & Beard 1998). Like other pathogens of animals and plants that are vectored by arthropods, most *Rickettsia* travel in the arthropod host from the gut to the haemocoel and then to the salivary glands where they may be horizontally transmitted to the (in this case vertebrate) host. Vertical transmission appears to maintain the bacterial population when

vertebrate hosts are scarce (Munderloh & Kurtti 1995). Some *Rickettsia* in blood-feeders, such as *Rickettsia peacockii* in the tick *Dermacentor andersoni*, may not be transmitted to vertebrates at all, but apparently remain strictly symbionts of the arthropod hosts, transmitted only vertically (Azad & Beard 1998; Baldridge *et al*. 2004). The most unusual form of transmission appears to be the epidemic typhus agent, *R. prowazekii*, in that it appears to be better adapted to its vertebrate host than its louse host (Azad & Beard 1998). *R. prowazekii* is pathogenic to the louse, generally killing it within two weeks, and is not transovarially transmitted. Unlike the spotted fever group *Rickettsia*, typhus *Rickettsia* do not infect vertebrates directly through the saliva, but through faecal contamination of mucosal surfaces or broken skin. *R. prowazekii* can persist in humans as recrudescent typhus or Brill–Zinsser disease, and can then serve as a source of infection for lice.

Lastly, competitive interactions may be very important in the evolution of virulence and transmission pathways in *Rickettsia*. For example, theory predicts that virulence can evolve under vertical transmission, if the symbiont offers protection from infection by an even more virulent parasite (Lipsitch *et al*. 1996). Coinfections of multiple *Rickettsia* strains, and of *Rickettsia* and other symbionts, are common and we would expect strong competition among symbionts for access to host cells, resulting in trade-offs between transmission modes and tissue specificities. Recent studies of interactions of other vertically transmitted symbionts may give some insight into the kinds of outcomes possible for *Rickettsia* and other symbionts in the same host. For example, Mouton *et al*. (2003) found that densities of three co-infecting *Wolbachia* strains in a parasitoid wasp were regulated independently, such that a triply infected individual had three times the number of symbionts and the largest fitness costs relative to individuals infected with fewer strains. In contrast, both suppression and over-replication of one symbiont in the presence of the other have been reported (Kondo *et al*. 2005; Oliver *et al*. 2006). Other studies showed differential colonization of adult bean beetle tissues by different strains of *Wolbachia* (Ijichi *et al*. 2002). It has long been recognized that *Rickettsia* with no pathogenic effects in vertebrates (as well as other co-infecting micro-organisms) can affect the distribution and dynamics of pathogenic *Rickettsia*, with important public health consequences. For example, the presence of the non-pathogenic *R. peacockii* has been implicated in a decrease in Rocky Mountain spotted fever outbreaks, because this symbiont outcompetes *R. rickettsii* via highly efficient vertical transmission in the tick host *D. andersoni* (Burgdorfer *et al*. 1981). Macaluso *et al*. (2002) recently documented competitive interactions between *Rickettsia rhipicephali* and *Rickettsia montana* in *Dermacentor variabilis*, where the presence of one symbiont inhibited vertical transmission of the other.

8. WHAT ARE *RICKETTSIA* DOING IN THEIR HOSTS?

One of the major questions for future research is what effects do these newly discovered *Rickettsia* have on their hosts? For hosts that are not amenable to antibiotic curing and laboratory investigations, other kinds of studies would help solve these mysteries, including determining the frequency of infection in the host population. Male-killing infections are often at low to medium frequencies (Hurst & Jiggins 2000), but parthenogenesis and CI infections are more likely to sweep to fixation (O'Neill *et al*. 1997). In this light, it is interesting that the tick *Amblyomma rotundatum*, one of the few strictly parthenogenetic tick species, is 100% infected with *R. bellii* (Labruna *et al*. 2004). Interestingly, the first male of this species was recently reported, but it was not determined if it harboured *R. bellii* (Labruna *et al*. 2005). Identifying the location of the bacteria in the hosts would also be helpful in determining the interactions of the two. While obligate symbionts (e.g. nutritional symbionts) and some facultative symbionts are housed in specialized cells such as bacteriocytes, finding the symbionts in salivary glands of ectoparasitic hosts might suggest horizontal transmission. Like other uncultivable bacteria, however, *Rickettsia* pose special challenges for study; they are not easily grown outside of cell lines and therefore, isolation of the bacterium and genetic transformation studies are difficult. Without the ability to demonstrate Koch's postulates, it may be difficult to determine that a particular symbiont causes a certain phenotype. First, hosts often harbour multiple symbionts and without the removal of all but one, it is impossible to determine which symbiont is causing the phenotype. Second, it is often challenging to manipulate the bacterium in the host, for example to experimentally infect an uninfected individual. However, recent studies in which hosts were successfully cured of all but one symbiont (Koga *et al*. 2003; Mouton *et al*. 2004), and in which new infections of uninfected hosts have been attained by microinjection (Chen & Purcell 1997; Oliver *et al*. 2003; Tsuchida *et al*. 2004), suggest these challenges are not insurmountable.

Surprisingly little is known about the effects of vertebrate-pathogenic *Rickettsia* on their arthropod hosts, although the ability to maintain infections for long periods in culture suggests a fairly benign influence of most *Rickettsia* on arthropod host fitness, with two major exceptions. As described earlier, *R. prowazekii* is highly pathogenic to its louse host. *R. rickettsii* also appears to be pathogenic to the tick *D. andersoni*, but this symbiont exhibits a low rate of vertical transmission in addition to horizontal transmission (Niebylski *et al*. 1999). Transmission rates have been quantified in few *Rickettsia* species. In species in which horizontal transmission to other arthropods via the vertebrate host plays a minor or non-existent role in maintenance of the bacterial population, *Rickettsia* that are vertebrate pathogens may adopt some of the strategies of non-pathogenic symbionts. For example, they may be beneficial, and/or may behave like reproductive manipulators, perhaps affecting female fecundity or male-specific lethality. It would be interesting, too, to explore whether certain *Rickettsia* differentially affect male versus female ticks in a similar fashion to the recently discovered transovarially transmitted α-proteobacterial symbiont (approx. 89% similarity to *Rickettsia* at 16S rDNA), which was found to occur only in adult female *Ixodes ricinus* ticks (Beninati *et al*. 2004). Nymphal ticks of both sexes are infected, but intriguingly, adult males appear uninfected. Interestingly, the close relative *O. tsutsugamushi*, the causal agent of scrub typhus, is associated with female-biased sex ratio distortion in its chigger mite hosts,

and may thus represent the first example of a symbiont that is both pathogenic in vertebrates and distorts sex ratios in invertebrates (Roberts *et al.* 1977; Takahashi *et al.* 1997).

Are all Rickettsia potentially pathogenic to vertebrates? Recent advances, spurred by the accumulating set of completely sequenced genomes, have begun to unravel the genetic basis of *Rickettsia* pathogenicity. Newly identified pathogenicity genes will ultimately serve as clues as to whether other *Rickettsia* can potentially cause disease in vertebrates. Pathogenicity will depend on many factors, including the ability to colonize salivary glands (or skin and mucosal cells), for example, by the use of cell secretion systems, and the ability to form actin tails for cell-to-cell motility. For example, in a beautiful study of the non-pathogenic *R. peacockii*, Simser *et al.* (2005) showed that the gene responsible for actin tail polymerization, *rickA*, is non-functional due to the insertion of a mobile element, explaining why *R. peacockii* is not horizontally transmitted. Outer membrane proteins also offer useful clues for helping to infer pathogenicity, with rapid evolution suggesting positive selection to evade host immune response (Jiggins *et al.* 2002), and a reduced number of functional membrane components suggesting selection to present fewer targets for immune response (Blanc *et al.* 2005).

While some have suggested that all *Rickettsia* are potentially pathogenic to vertebrates, perhaps it is also useful to distinguish between specific adaptations that *Rickettsia* use to exploit vertebrate hosts and non-specific responses that may cause disease. Here, we can contrast *Rickettsia* with *Wolbachia*. While they are primarily vertically transmitted, *Wolbachia* are present in many arthropod somatic tissues, including salivary glands (Mitsuhashi *et al.* 2002). However, the only case of vertebrate disease caused by *Wolbachia* appears to result from non-specific vertebrate responses to the *Wolbachia* that are symbionts of filarial nematodes, where the severe host inflammatory response may be due to bacteria being released into the blood when the nematodes die (Taylor *et al.* 2000; Taylor 2003).

9. CONCLUSIONS

At the time that the many ways in which *Wolbachia* manipulates the reproduction of its invertebrate hosts were being revealed, it was speculated that no other bacterium would be found that could induce phenotypes such as CI and parthenogenesis, since these involved complex manipulations of host chromosomes (Stouthamer *et al.* 1999). However, the discovery that the *Bacteroidetes* bacterium *Cardinium* is also capable of inducing CI, parthenogenesis and feminization, refutes that assumption, and provides evidence that even very distantly related bacteria can acquire the mechanisms that will allow them to control their host's reproduction. It appears that *Rickettsia* is emerging as another bacterial genus that is capable of inducing multiple reproductive disorders in their hosts, including male killing and parthenogenesis, and may also on occasion be required for normal reproductive function, as is *Wolbachia*. It also seems likely that some *Rickettsia* will be found to be beneficial to their arthropod hosts. In general, classifications of bacterial lineages, for example as 'nutritional mutualists', 'reproductive parasites' or 'pathogens', may channel our thinking such that we may overlook the full range of potential effects of symbionts on hosts. While the reductions in genome size that accompany the acquisition of a symbiotic lifestyle may eventually constrain the evolutionary potential of some lineages (Moran & Wernegreen 2000), many facultative symbionts and reproductive manipulators like *Rickettsia* appear to be quite labile in function. Considering that only a handful of general surveys for bacterial symbionts have been conducted using universal 16S rDNA primers, it is safe to assume that many symbionts and interesting host phenotypes are waiting to be discovered.

However, even as we expand our understanding of the repertoire of *Rickettsia*, we are unlikely to lose focus on the pathogenic relationships with vertebrates that many of the species have. In fact, these two very different roles, along with the wide range of transmission modes, make *Rickettsia* especially fascinating. *Rickettsia* thus offer an unusual opportunity to answer questions about the origins, mechanisms and transitions involved with manipulation of both arthropods and vertebrates. Is the potential to infect vertebrates common to all *Rickettsia*, as has been suggested (La Scola & Raoult 1997), or do vertebrate pathogens require a special set of genes for transmission and life at the higher temperatures of homeothermic cells that the non-pathogenic members of the genus lack? Conversely, are there genes involved with transovarial transmission that *R. prowazekii* has lost? How do the mechanisms involved in causing plant disease and animal disease compare? Continuing studies that characterize host range and infectivity of different *Rickettsia* would contribute to answering these questions, as would the characterization of the genomes of some *Rickettsia* non-pathogens.

The authors thank Cara Gibson, Tim Kurtti, Kerry Oliver, Jacob Russell and two anonymous reviewers for comments on the manuscript. This research was partially supported by Research grant no. IS-3633-04 R from BARD, the United States—Israel Binational Agricultural Research and Development Fund (E.Z.-F.), by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2001-353-2-10986 to M.S.H. and by a NSERC postdoctoral fellowship to S.J.P.

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