

Research article

Wolbachia: Evolutionary novelty in a rickettsial bacteria

Cort L Anderson*^{1,2} and Timothy L Karr

Address: ¹Department of Organismal Biology and Anatomy, University of Chicago, 1027 E. 57th Street, Chicago, IL 60637 USA and ²Laboratory for Ecological and Conservation Genetics, College of Natural Resources, University of Idaho, Moscow, ID 83843-1136 USA

E-mail: Cort L Anderson* - canders@midway.uchicago.edu; Timothy L Karr - tkarr@midway.uchicago.edu

*Corresponding author

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Abstract

Background: Although closely related, the alpha-proteobacteria *Wolbachia* and the Rickettsiaceae (*Rickettsia* and *Ehrlichia*), employ different evolutionary life history strategies. *Wolbachia* are obligate endocellular symbionts that infect an extraordinary host range and, in contrast to the infectious and pathogenic *Rickettsia* and *Ehrlichia*, profoundly influence host reproductive biology.

Results: Phylogenies of the *Rickettsia*, *Ehrlichia*, and *Wolbachia* were independently inferred from 16S rDNA sequences and GroEL amino acid sequences. Topologies inferred from both sets of sequence data were consistent with one another, and both indicate the genus *Wolbachia* shared a common ancestor most recently with *Ehrlichia*. These two genera are a sister group to the genus *Rickettsia*. Mapping biological properties onto this phylogeny reveals that manipulation of host reproduction, characteristic of *Wolbachia* strains, is a derived characteristic. This evolutionary novelty is accompanied by the loss of the ability to infect vertebrate hosts.

Conclusions: Because of the contrasting transmission strategies employed by each, *Wolbachia* is expected to maximize efficiency of vertical transmission, while *Ehrlichia* and *Rickettsia* will optimize horizontal transfer of infection. *Wolbachia* manipulation of host reproduction could thus be viewed as strategy employed by this bacterium to foster its own propagation via vertical transmission.

Background

In recent years a great deal of excitement has been generated by the discovery that *Wolbachia* infections in Ecdyzoa (arthropods and nematodes) can manipulate reproduction of their hosts in a variety of ways; e.g., induced parthenogenesis, male killing, feminization, and cytoplasmic incompatibility (CI) [1,2]. Superimposing the biological properties of this lineage onto a phylogeny of *Wolbachia* and closely related genera indicates that this reproductive manipulation is an evolutionary novelty.

Initially identified as a rickettsial organism, *Wolbachia pipientis* and related bacterial strains have been extensively documented in arthropods and nematodes, and are now treated as a separate genus within the Rickettsiaceae [1]. *Wolbachia* is clearly part of a monophyletic lineage containing the genera *Rickettsia* and *Ehrlichia*, and all members of this clade share the defining characteristic of being obligate endosymbionts. *Rickettsia* and *Ehrlichia* species are commonly found in arthropods, which serve as reservoirs of infection for a number of diseases of vertebrates, e.g., typhus, caused by *R. prowazekii*, and various ehrlichioses [3–5]. By contrast, *Wolbachia* strains are restricted in nature to ecdyzoan hosts, and

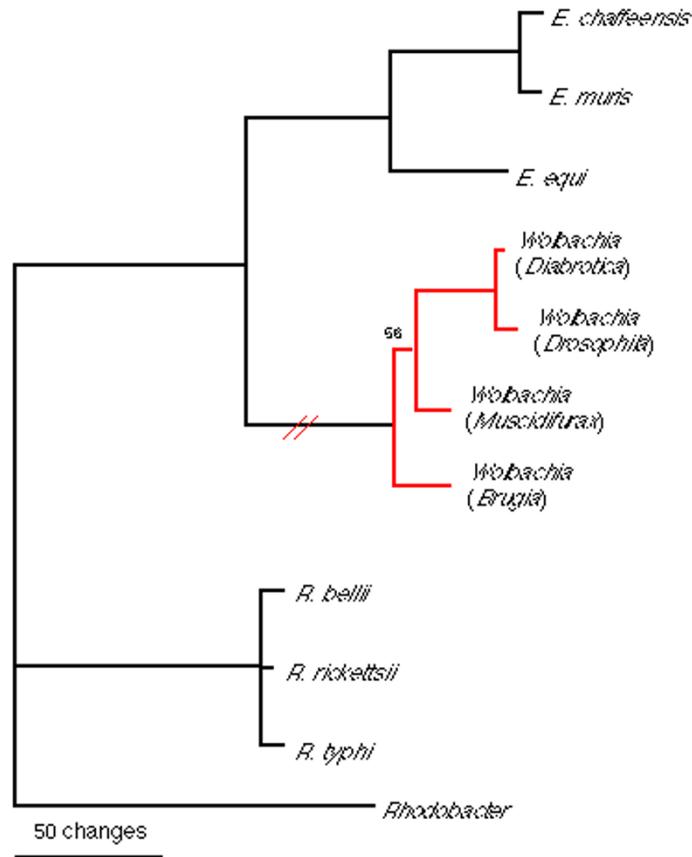


Figure 1

Phylogeny derived from 1.3 kb of 16S rDNA sequence. Parsimony tree (PAUP vers. 4.0b4a). All branches had 100% bootstrap support, except where indicated by numbers on branches. Bold line indicates the lineage which has specialized in manipulating host reproduction, and which has lost the ability to infect vertebrate hosts. *Wolbachia* host genera are shown in parentheses. *Wolbachia* sequences used in this analysis were from infections in coleopteran (*Diabrotica*), dipteran (*Drosophila*), hymenopteran (*Muscidifurax*) and filarial worm (*Brugia*) hosts. Crossed line indicates ancestral loss of the ability to infect vertebrates, and acquisition of reproductive specialization.

have never been linked to vertebrate disease. Mapping the traits which are peculiar to *Wolbachia* onto a phylogeny of representative taxa from the three bacterial genera indicates that these are derived characteristics. Therefore, the manipulation of host reproduction is an evolutionary novelty acquired by the *Wolbachia* lineage, which meanwhile has lost the ability, manifest in *Ehrlichia* and *Rickettsia* species, to infect vertebrate, and in particular, mammalian, hosts.

Results and Discussion

Previous phylogenetic analyses of *Wolbachia* sequence data focused upon relationships among *Wolbachia* strains, but relationships with other members of the

Rickettsiaceae have not been examined in any great detail [6,7]. Other analyses have focused upon relationships within genus *Rickettsia*[8] or genus *Ehrlichia*[9]. In our analysis, we used 16S rDNA sequences from *Rickettsia*, *Wolbachia*, and *Ehrlichia* to specifically and extensively test whether *Wolbachia* is a sister taxon to genus *Ehrlichia* and/or *Rickettsia*. In addition, amino acid sequences from a subset of *Rickettsia*, *Wolbachia*, and *Ehrlichia* species were also analyzed. Our results confirm and augment previously published trees, which indicate that *Wolbachia* and the genus *Ehrlichia* together comprise a sister taxon to the genus *Rickettsia*. This clustering is extremely robust; analyses using different outgroups, and sequences from a variety of *Ehrlichia*, *Rickettsia*, and

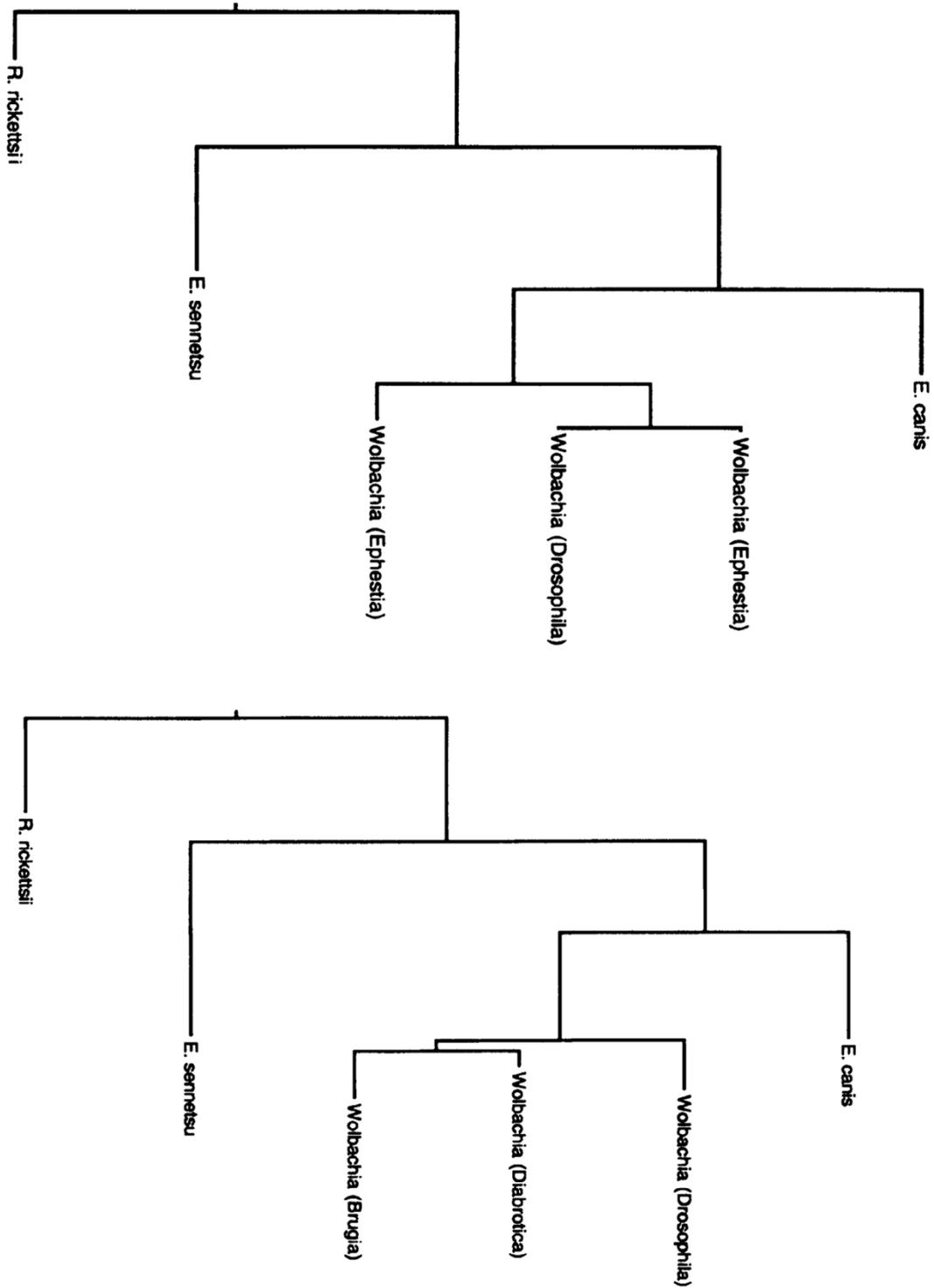


Figure 2

Shortest maximum parsimony trees derived from GroEL amino acid sequence (left) and 16S rDNA sequences (right), showing congruence of 16S rDNA and GroEL trees. Rickettsia and Ehrlichia sequences used to construct both trees are from the same species, Wolbachia sequences used in the GroEL analysis are from different taxa than those used in the 16S analysis. Host genera of Wolbachia strains are shown in brackets.

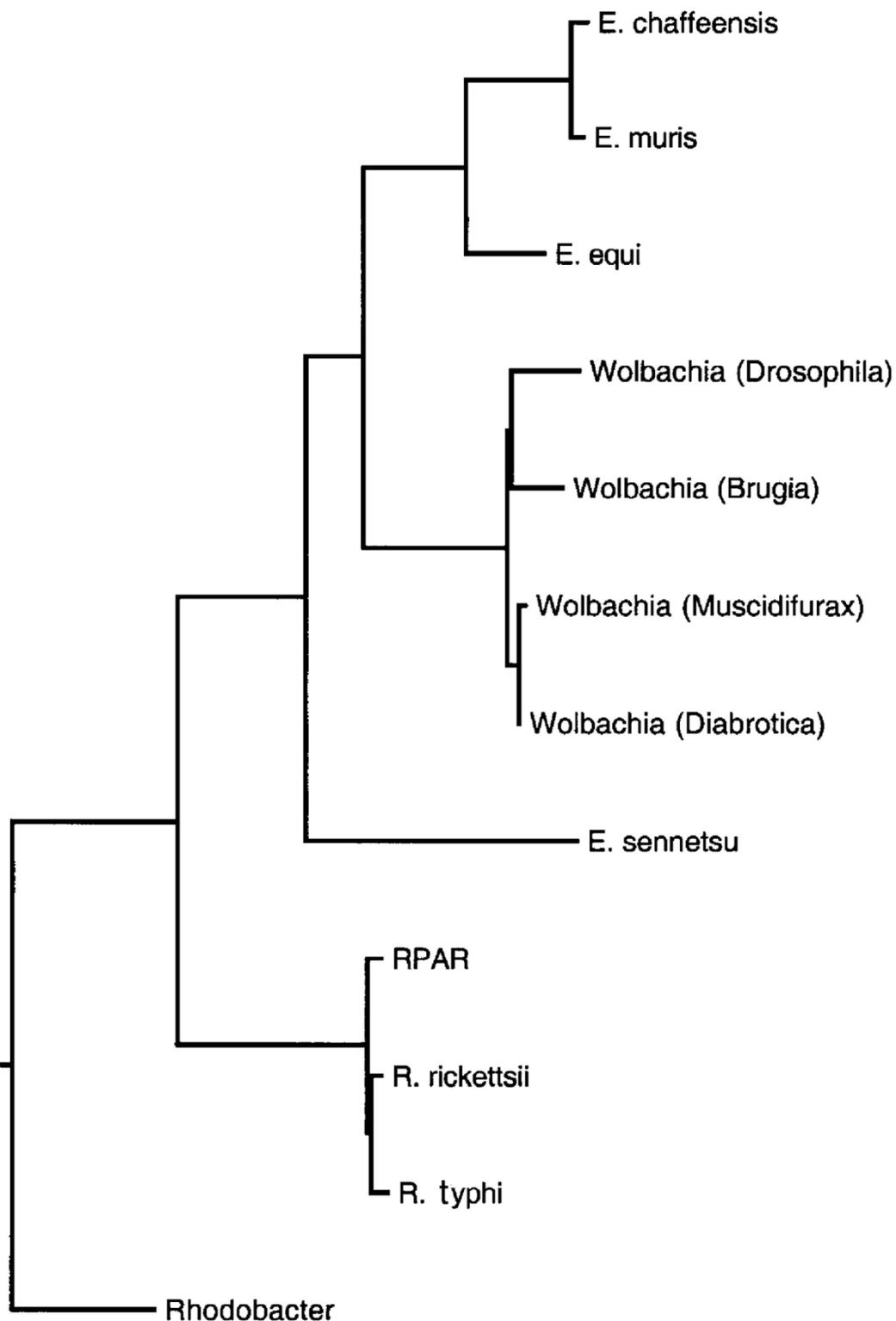


Figure 3
Maximum likelihood tree derived from 16S rDNA sequences. Taxa included are the same as those in Figure 1, with the addition of *E. sennetsu*.

Wolbachia taxa converge upon the same topology, with good resolution of major branches (Figure 1). Different tree building algorithms (maximum likelihood, parsimony) likewise yield similar topologies. A phylogeny derived from amino acid sequences of GroEL proteins is consistent with the phylogeny inferred from rDNA sequences, although the high level of divergence manifest among these genes precludes unambiguous alignment of rickettsial GroEL sequences to homologous sequences from taxa outside the Rickettsiae. Instead, we aligned representative GroEL amino acid sequences from the three rickettsial genera, and constructed an unrooted tree (Figure 2). This tree is congruent with the tree derived from 16S rDNA sequences. Our analyses confirm monophyly for *Wolbachia* taxa, including representatives of *Wolbachia* strains infecting filarial nematodes. When *Ehrlichia sennetsu* is included in the analysis, this genus is shown to be paraphyletic, with *Ehrlichia sennetsu* basal to a node linking *Wolbachia* taxa and all other *Ehrlichia* taxa included in these analyses (Figure 3). These results indicate that *Wolbachia* and *Ehrlichia* lineages shared a common ancestor, and that this ancestral lineage diverged from the genus *Rickettsia* before the *Wolbachia*-*Ehrlichia* split. This conclusion has been implicit in previous studies [10–12]; here we make these relationships explicit, and examine the consequences for our understanding of *Wolbachia* evolution.

These results have important ramifications for future work on members of this clade. As has been previously noted, phylogenetic analyses of diverse *Wolbachia* strains imply horizontal transfer of *Wolbachia* between hosts [1,7,10] and has been demonstrated under laboratory conditions [13,14]. Because the infection frequently results in similar manifestations in taxonomically divergent hosts, this implies bacterial interaction with conserved aspects of host cell and reproductive biology [15]. The obligate endocellular existence is common to all three genera, and presumably represents the ancestral condition of all of these genera. However, the most parsimonious explanation for the evolution and maintenance of traits that alter reproduction, a defining characteristic of the *Wolbachia* lineage, is to assume that this ability appeared as an evolutionary novelty in the direct ancestor of extant *Wolbachia* strains. The alternative hypothesis requires numerous independent acquisitions by an endosymbiont of the capacity to induce CI, alter sex ratios, suppress production of males, and/or feminize host embryos. Hence, the most likely explanation is that a *Wolbachia* ancestor acquired the ability to manipulate arthropod reproduction in a manner that facilitated bacterial transmission into the next generation, and this property persists in surviving lineages.

There are two reported instances of *Rickettsia* species which affect host reproduction, one which causes female-biased sex ratio in mites, and a second which causes male killing in ladybird beetles [16]. These two species are fairly distantly related within the genus *Rickettsia* [8], and intervening members of this genus have not to date been shown to influence or affect reproduction. Therefore, given current information, and in contrast with the situation in the *Wolbachia* clade, we conclude that these instances are most likely independent acquisitions of the capacity to influence reproduction in their respective hosts. Should further investigation of *Rickettsia* host reproduction provide evidence of extensive manipulation of host reproduction, we would have to revisit our conclusions. However, since *Wolbachia* would seem to be selected for efficient vertical transmission, while *Rickettsia* and *Ehrlichia* need to maximize the efficiency of horizontal transfer, this does not seem especially likely. This raises questions of a possible trade-offs in life history strategies – did *Wolbachia* specialization on reproductive manipulation in arthropod hosts cause a loss in the ability to infect vertebrate hosts? Or did loss of the ability to infect vertebrate hosts foster specialization in vertical transmission, and hence, reproductive manipulation? Answers to these questions may come from genetic and molecular studies of the underlying mechanisms involved in *Wolbachia*-mediated alterations in host reproductive biology.

Conclusions

We have used phylogenetic analysis of related rickettsial genera to track the evolution of life-history traits associated with these bacteria. These analyses expand upon and confirm previous phylogenies of this bacterial clade, in that the *Wolbachia* lineage is shown to be a sister taxon to genus *Ehrlichia*, and these genera together form the sister-group to genus *Rickettsia*. Furthermore, analysis of the phyletic distribution of life-history traits indicates that the propensity of *Wolbachia* to influence the reproductive biology of infected hosts is a derived state, an investigation of evolutionary space which apparently optimizes vertical transmission and fosters the spread of *Wolbachia* infection, absent the need for a second host, in contrast with other rickettsial genera.

Materials and Methods

Sequences used in these analyses were obtained from the NCBI database. *Rickettsia* 16S sequences from: pea aphid rickettsia (PAR), accession no. U42084; *R. typhi*, U12463; *R. rickettsii*, U11021; *R. bellii*, U11014. *Ehrlichia* 16S sequences from: *E. canis*, AF156785; *E. chaffeensis*, AF147752; *E. muris*, AB013008; *E. risticii*, AF179351; *E. sennetsu*, M73225. *Wolbachia* 16S sequences from *Wolbachia* endosymbiont in: *Brugia pahangi*, AJ012646; *Diabrotica vergifera*, U83098; *Drosophila mauritiana*, U

17060; *Gryllus integer*, U83096; *Muscidifurax uniraptor*, L02882; *Sphaeroma hookeri*, AJ001610. GroEL amino acid sequences were obtained for: *R. rickettsii*, U96733; *E. canis*, U96731; *E. sennetsu*, U88092; and *Wolbachia* endosymbionts in *Ephestia kuehniella*, ABO02291; *Ephestia cautella*, ABO02290; *Drosophila simulans*, ABO02287.

Sequences were aligned with ClustalW [17], GroEL data incorporates 148 aligned amino acid residues, 16S data incorporates 1320 bp of aligned DNA sequence. Maximum parsimony phylogenetic analysis shown in Figures 1 and 2 was carried out using the Exhaustive Search option of PAUP vers. 4.0b4a [18], ignoring gaps. The maximum likelihood analysis was accomplished using the Branch and Bound search option of PAUP [18], ignoring gaps. Analysis assumes all sites evolve at an equal rate, a transition :transversion ratio of 2:1, molecular clock not enforced.

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