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A review of prevalence and phylogeny of the bacterial symbiont Cardinium in mites (subclass: Acari)

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Abstract

Many species of mites are of agricultural and veterinary importance to human. The bacterial symbiont Cardinium is assumed to be widespread and involved in manipulating its mite host’s reproduction. We investigated Cardinium infections in phylogenetically diverse mite species based on published data. Cardinium was reported in 45 mite species, representing 3 orders and 14 families. Some Cardinium strains can influence their mite hosts’ reproduction via two strategies: cytoplasmic incompatibility and feminization. The phylogeny of the examined mites was well resolved based on the 18S rRNA gene, whereas Cardinium phylogeny was not completely concordant with its associated host phylogeny, raising the possibility that horizontal transmission of Cardinium has occurred among species. In addition, Cardinium phylogeny was associated with the ecology of the hosts. Cardinium cooperating with other endosymbionts influences the ecology and evolution of its host.

Keywords: Mites, bacterial symbionts, reproduction manipulation, horizontal transmission

Introduction

Endosymbiotic bacteria are widespread in arthropods. Traditionally, they can be grouped into primary and secondary symbionts. In 2001, the secondary symbiont Cardinium hertigi was first reported from the parasitoid wasps Encarsia (Weeks et al. 2001; Zchori-Fein et al. 2001). Several screening studies indicated that about 5–10% of arthropods species are infected with Cardinium symbionts (Weeks et al. 2003; Zchori-Fein & Perlman 2004; Duron et al. 2008). Cardinium can manipulate the reproduction of their hosts through various strategies, including parthenogenesis (Zchori-Fein et al. 2001), feminization (Weeks et al. 2001; Zchori-Fein et al. 2004; Giorgini et al. 2009), cytoplasmic incompatibility (CI) (Hunter et al. 2003; Gotoh et al. 2007; Ros & Breeuwer 2009) and altered fecundity (Weeks & Stouthamer 2004). These manipulations play significant roles in the host ecology and evolution (Breeuwer et al. 2012; Kageyama et al. 2012). Thus, Cardinium and other symbionts have received intensive attention.

Mites are the largest group within the arthropod class Arachnida, with over 48,200 described species before 2000 (Halliday et al. 2000) and 55,214 described species by 2013 (Zhang 2013). They live in diverse habitats and act as predators, parasites, herbivores or detritivores (Krantz & Walter 2009). Mites of the family Tetranychidae and Tenuipalpidae are known as serious pests of agricultural crops (Helle & Sabelis 1985), those of the family Sarcoptidae and Dermanyssidae are parasites to animals and humans, acting as direct agents of diseases (Moro et al. 2009a; Mounsey et al. 2013) and those of the family Acaridae are pests of stored products (Palyvos et al. 2008). On the other hand, some mites (belonging to families Phytoseiidae, Laelapidae, Macrochelidae) are known for their beneficial effects, acting as biological control agents against agricultural pests (Gerson et al. 2003).
Using the electron microscope, Hess & Hoy (1982) had observed two morphologically distinct forms of microorganisms in the tissues of the phytoseiid mite Metaseiulus occidentalis, one of which was later identified as Cardinium (Weeks & Breeuwer 2003). Subsequently, many mite species were reported to harbor this symbiont (Duron et al. 2008; Ros et al. 2012; Kopecky et al. 2013; Famah Sourassou et al. 2014; Weinert et al. 2015; Chaisiri et al. 2015). There are several studies reporting Cardinium-induced reproductive alterations in mites (Weeks & Stouthamer 2004; Groot & Breeuwer 2006; Gotoh et al. 2007; Ros & Breeuwer 2009; Liu et al. 2010; Xie et al. 2010; Wu & Hoy 2012; Zhu et al. 2012b; Zhao et al. 2013a; Zhao et al. 2013b; Famah Sourassou et al. 2014), and Cardinium seem to be essential for oviposition in the Ghana population of Neoseiulus paepalivorus (Famah Sourassou et al. 2014). The number of publications on the association mites-Cardinium has substantially increased, a comprehensive study is helpful for understanding the interactions of symbionts and hosts.

In the present paper, the prevalence of Cardinium symbionts and their reproductive effects in mites was reviewed based on published data. In addition, the phylogenies of mites and their associated Cardinium symbionts were presented and compared.

Material and Methods

Available data on Cardinium in mites

In this study, the reports of Cardinium found in mites were summarized by searching from 2 major scientific literature databases, PubMed (www.ncbi.nlm.nih.gov/pubmed) and Web of Science (www.webofknowledge.com). The term ‘mite’ in combination with ‘Cardinium’ or ‘bacteria’ were used as the keywords and applied to the title field or abstracts in those databases. In addition, we considered the unpublished studies on Cardinium in mites that have been described in the GenBank (http://www.ncbi.nlm.nih.gov/genbank).

Phylogenetic analyses of Cardinium and their hosts

Theories suggested that Cardinium and other strict mutualistic maternally inherited symbionts might result in co-evolutionary (phylogenetic) patterns with their hosts (Moran & Baumann 1994; Lo et al. 2003). However, this phylogenetic pattern would be lost if horizontal transmission or recent invasion of endosymbionts occurs. Consequently, comparison of endosymbiont’s and their hosts’ phylogenies could infer their transmission patterns and evolutionary histories.

Previously, the 18S gene of the nuclear ribosomal DNA (18S rRNA) has been widely used for resolving the phylogeny of mites (Cruickshank 2002; Matsuda et al. 2014). Here, we constructed the phylogeny of Cardinium-infected mites based on the available 18S rRNA sequences that were downloaded from GenBank. Similarly, the phylogeny of associated Cardinium was constructed based on the 16S gene of the nuclear ribosomal DNA (16S rRNA) that were obtained from our experiments and GenBank. Accession numbers of all available sequences were shown in Table 1. The analyzed sequences were aligned using the multiple alignment program ClustalW with default setting and edited manually in MEGA 6.0 (Tamura et al. 2013). In total of 33 sequences of mites 18S rRNA were obtained and the final alignment was 1892 nucleotides. In Cardinium 16S rRNA phylogenetic analyses, there were 31 sequences that consisted of 351 nucleotides. Prior to phylogenetic analyses, jModelTest v2.1.6 (Darriba et al. 2012) was used to select the optimal evolution model by Akaike Information Criterion (AIC). Phylogenetic analyses were performed using Bayesian Inference (BI) and Maximum-Likelihood (ML) estimation for mites 18S rRNA and Cardinium 16S rRNA, respectively. For Bayesian inference of mites and Cardinium, analyses were performed in MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) using the GTR+I+G model. Both
Markov chains and samplefreq were set to 1000000 generations and 100, respectively. At the same time, ML trees were constructed using MEGA 6.0, with gamma distributed with invariant sites (G+I), and the method of GTR as genetic distance model; bootstrap replications were set to 1000.

Results and Discussion

Cardinium prevalence in mites

This paper includes a review of 34 papers reporting Cardinium infections in mites. Cardinium infections were reported in 45 mite species belonging to 3 orders and 14 families. Detailed information was present in Table 1. The associations of Cardinium and family Tetranychidae were relatively well documented. Additionally, Cardinium was found in more than one species of family Phytoseiidae, Acaridae, Glycyphagidae, Pyroglyphidae and Tenuipalpidae.

Cardinium is an intracellular symbiotic bacterium that cannot be cultured outside of host cells. Thus, the detection of its presence in mites is done mainly using PCR-based techniques, sometimes aided by bioassays (crossing breeding studies). Available molecular markers for its detection in mites include, but not limited to, the 16S ribosomal RNA (16S rRNA) and the Gyrase B (gyrB) (Hoy & Jeyaprakash 2005; Chigira & Miura 2005; Liu et al. 2006; Novelli et al. 2007; Enigl & Schausberger 2007; Novelli et al. 2008; Chen et al. 2009; Moro et al. 2009b; Jeong et al. 2009; Ros et al. 2012; Zhu et al. 2012b; Zhao et al. 2013a; Zhao et al. 2013b; Hubert et al. 2012; Famah Sourasson et al. 2014). The other techniques used to determine the presence and distribution of Cardinium in mites are transmission electron microscopy (Kitajima et al. 2007) and fluorescence in situ hybridization (Zhu et al. 2012a). Kitajima et al. (2007) presented a visual evidence for the presence of Cardinium in three Brevipalpus species, while Zhu et al. (2012a) demonstrated that Cardinium mainly enters the oocytes with trophic flow from nurse cells, midgut and oviduct in Tetranychus piercei.

Cardinium seems to be restricted to four arthropod lineages: Hymenoptera, Hemiptera, Diptera and Acari (Weeks et al. 2003; Zchori-Fein & Perlman 2004; Duron et al. 2008; Nakamura et al. 2009). Among these, mites probably represent Cardinium hotspots. For example, Weeks et al. (2003) had estimated that about 31.6% mite species should be infected by Cardinium, which seems higher compared with infection rate in others (6.1% in Hemiptera, 10.4% in Hymenoptera). Nine out of 22 spider mite species and 13 astigmatid mite species were reported to harbor Cardinium (Nakamura et al. 2009; Kopecky et al. 2013). Zindel et al. (2012) screening for bacterial symbionts in 21 mite species, including phytophagous, predatory or parasitic species, had discussed their possible importance in biological control. To explain high prevalence of Cardinium in mites, Weinert et al. (2015) speculated that this might reflect evolutionary changes in arthropod immunity, as many of the groups with high Cardinium incidences lack components of immune deficiency (IMD) signal pathway, and IMD is activated by diaminopimelic acid-type peptidoglycan, which is produced by Cardinium (Penz et al. 2012).

Given high prevalence of Cardinium and mite species diversity, the number of infected mite species was obviously underestimated. In addition, the infection in mite species of economic importance appears to receive much attention, as indicated by well-documented data for these species. The screened mite species are only a small proportion, and the bacterial community of mites is influenced by environmental factors (Novelli et al. 2008; Wu & Hoy 2012; Kopecky et al. 2014). It is predicated that more infected species exist on the basis of extensive sampling and sensitive detection approaches. Meanwhile, extensive sampling of mites that have genetic divergence and live in various habitats would allow us to infer how host phylogeny and ecology affect their endosymbiotic bacterial infections.
### TABLE 1. Documented *Cardinium* infections in mite species.

<table>
<thead>
<tr>
<th>Order Family Species</th>
<th>Importance of mites</th>
<th>Cardinium-induced phenotypes</th>
<th>Collection sites</th>
<th>References</th>
<th>GenBank Accession numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mesostigmata</strong></td>
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<td><strong>Dermanyssidae</strong></td>
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<td><strong>Sarcoptiformes</strong></td>
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<tr>
<td><strong>Acariidae</strong></td>
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<td>AF022023</td>
<td>JQ406832^2</td>
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<td>JQ00109</td>
<td>JQ406781^2</td>
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<td>Q64331</td>
<td>JQ406760^3</td>
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<td>EF203768</td>
<td>JQ406755^5</td>
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<td>The Czech Republic</td>
<td>8</td>
<td>DQ025510</td>
<td>JQ406820^3</td>
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<td>JQ406701^2</td>
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<td>Q64327</td>
<td>JQ406713^3</td>
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<td>JQ406809^3</td>
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<td><strong>Echimyopodidae</strong></td>
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<td><em>Blomia tropicalis</em></td>
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<td>AY499555</td>
<td>JQ406725^4</td>
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<td>JQ406843^3</td>
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<td>8</td>
<td>DQ025511</td>
<td>JQ406770^3</td>
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<td>-</td>
<td>-</td>
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<td><strong>Erythraeidae</strong></td>
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<tr>
<td><em>Brevipalpus californicus</em></td>
<td>Plant pest</td>
<td>Feminization</td>
<td>Japan</td>
<td>10</td>
<td>-</td>
</tr>
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</table>

^2 continued on the next page
Superscripts on the mite species indicate Wolbachia-infected ones. Asterisks at the GenBank accession numbers indicate the 16S rRNA sequences used for Cardinium phylogenetic analysis.

1—De Luna et al. (2009); 2—Weeks et al. (2003); 3—Zchori-Fein & Perlman (2004); 4—Hoy & Jeyaprakash (2005); 5—Wu & Hoy (2012); 6—Enigl & Schausberger (2007); 7—Famah Sourassou et al. (2014); 8—Kopecky et al. (2013); 9—Jeong et al. (2009); 10—Gotoh et al. (2007); 11—Groot & Breeuwer (2006); 12—Weeks et al. (2001); 13—Ros et al. (2012); 14—Ros & Breeuwer (2009); 15—Ros et al. (2009); 16—Nakamura et al. (2009); 17—Liu et al. (2010); 18—Xie et al. (2010); 19—Zhao et al. (2013a); 20—unpublished; 21—Zhao et al. (2013b); 22—Zhu et al. (2012).

Cardinium’s reproductive phenotypes in mites

Cardinium is known to affect their hosts in a variety of ways in order to favor their own transmission. These include CI and feminization. For example, Cardinium was found associated with nonreciprocal reproductive incompatibility in M. occidentalis, a phytoseiid mite that is an important predator of spider mite pests of agricultural crops (Wu & Hoy 2012). Cardinium-induced CI was also revealed in six phytophagous mite species, Eotetranychus suginamensis (Gotoh et al. 2007), Bryobia sarothamnii (Ros & Breeuwer 2009), Tetranychus cinnabarinus (Xie et al. 2010), T. piercei (Zhu et al. 2012b), Tetranychus phaseolus (Zhao et al. 2013a) and Tetranychus truncatus (Zhao et al. 2013b). All these CI types were expressed as female mortality, which is the only CI type found for Cardinium so far. Moreover, Groot & Breeuwer (2006) found Cardinium induce thelytoky by feminizing unfertilized haploid eggs in the related species Brevipalpus californicus and B.
*phoenicis*. Through reproductive manipulations, *Cardinium* could strongly impact mite population dynamics and demography as well as the mite genetic diversity (Groot 2007; Ros 2010).

As mentioned previously, factors such as bacterial strains and densities, host genetic background or interactions between bacteria and host species can influence *Cardinium*-induced phenotypes. Gotoh et al. (2007) revealed that phylogenetic similarity of *Cardinium* was not related to their phenotypes, as CI-inducing *Cardinium* strain in *E. suginamensis* is closely related to the non-CI strains found in *T. pueraricola* and *Amphitetranychus quercivorus*, based on the 16S rRNA tree. Furthermore, some studies revealed that *Cardinium* genotype, host genotype and *Cardinium* density may account for variations in CI expression (Xie et al. 2010; Zhu et al. 2012b; Zhao et al. 2013b). To date, studies on *Cardinium*-mediated reproductive alterations are still limited to a few phytophagous, predatory or parasitic species and more related studies especially in synanthropic mites infesting agricultural crops, stored products or acting as allergen are needed.

**Potential roles of Cardinium in mite biological control**

Predatory mites are widely used for biological control of phytophagous mites. *Cardinium*-induced CI in predatory mites *M. occidentalis* has been reported (Johanowicz & Hoy 1998; Wu & Hoy 2012). Weeks & Stouthamer (2004) also reported that three inbred lines of *M. occidentalis* showed a clear and significant increase in fecundity associated with infection by *Cardinium*. In these cases, *Cardinium*-mediated reproductive incompatibility may have significant implications for biological control programs using *M. occidentalis* because the release of *M. occidentalis* strains that are reproductively incompatible with the wild populations (caused by a different status of *Cardinium* infection) may reduce the effectiveness of this predatory mite as a biological control agent.

**Phylogenetic relationship of Cardinium and their mite hosts**

Phylogenetic analyses indicated that the evolutionary relationship among *Cardinium* infecting mites was well resolved and the observed tree showed congruence with the classical taxonomy based on morphology, except for the family Chortoglyphidae and Carpoglyphidae (Fig. 1), suggesting 18S rRNA’s better discriminating ability. Although the resolution of the phylogenetic tree of *Cardinium* using 16S rRNA was low, it can provide information to identify two clusters of closely related *Cardinium* strains. As shown in Fig. 2, cluster I consisted of *Cardinium* from *T. pueraricola*, *T. phaselus*, *T. kanzawai*, *E. suginamensis*, *A. quercivorus*, *A. viennensis*, *Oligonychus illicis*, *B. obovatus*, *Petrobia hartii* and *Balaustium sp*. And the remaining strains formed the cluster II.

Comparisons between the evolutionary relationships among mites and associated *Cardinium* symbionts showed that there is no complete association between the two groups, as several *Cardinium* strains infecting different species were monophyletic. We cannot completely exclude the possibility that there has been repeated loss of infection post-speciation from an infected ancestor. This difference between the two phylogenies indicated that *Cardinium* was not solely acquired vertically, and points to the likelihood of horizontal transmission. Meanwhile, a degree of *Cardinium* specialization was observed, for example, closely related *Cardinium* strains clustered among three closely related *Brevipalpus* species and *Cardinium* infecting family Tetranychidae appeared to be closely related. These results were not surprising and similar trends have been observed in other *Cardinium*-arthropod associations (Weeks et al. 2003; Zchori-Fein & Perlman 2004; Duron et al. 2008; Kopecky et al. 2013) and in other endosymbionts (Weinert et al. 2009; Lachowska et al. 2010; Gerth et al. 2015). In addition, the single molecular marker will fail to assess the accurate phylogeny of *Cardinium* (Weeks et al. 2003; Duron et al. 2008). Thus more rapidly evolving and phylogenetically informative *Cardinium* genes are required in further study.
Correlation between Cardinium infections and mite hosts ecology

Mites exhibit a breadth of ecological interactions unmatched in any other arthropod group (Krantz & Walter 2009). Diversity of habitats allowed us to test whether host ecology affects Cardinium strain infection patterns. Infected mite species can be split into seven types according to their habitats or economic importance. Species of the same type may share common environment or have ecological interactions. In general, mite ecology appeared to shape Cardinium strain similarity (Fig. 2). For example, the phylogenetic analysis revealed that Cardinium from phytophagous mites were more similar to one another. In addition, similar Cardinium strains were observed in mites that inhabiting house environment. These observations suggest that mite species sharing close ecological conditions easily transmit Cardinium among them. Ecologically mediated transmission of bacterial symbionts had also been reported in fruit feeding drosophilids (Haine et al. 2005), mycophagous flies (Stahlhut et al. 2010) and Megalomyrmex social parasites and their fungus-growing ant hosts (Liberti et al. 2015).

FIGURE 1. Bayesian phylogenetic tree of mite species infected with Cardinium symbionts based on 18S rRNA genes. The topology resulting from Maximum Likelihood (ML) method was similar. ML bootstrap values (left numbers) and Bayesian probabilities (right numbers) were presented above the branches. Words on the tree indicated the family in which mite species is placed.
Multiple infections of Cardinium and other endosymbionts in mites

Apart from Cardinium, mites are hosts to other endosymbionts. For instance, Chaisiri et al. (2015) have revealed that mite species were associated with 85 bacterial genera (approximately 150 identified species) belonging to 7 phyla and 25 orders. Among these bacteria, Cardinium, Wolbachia and Orientia are the most prevalent bacteria. Wolbachia is another known reproductive parasite (Werren et al. 2008). Many mite species showed co-infected with Cardinium and Wolbachia (Table 1) and sometimes, the two endosymbionts were found co-infecting within the same individuals of particular species. Population differentiation itself may facilitate the persistence of individual endosymbionts within different populations, whereas the acquisition of superinfections through horizontal transmission may contribute to intra-individual co-infection.
The presence of competing endosymbiont strains is expected to influence the dynamics of the population as a whole, for example, if neighbouring populations are differently infected with one or more bacterial strains with different phenotypes or if a new strain invades one population. An example of this is observed in *Bryobia* spider mites where *Cardinium*, which causes CI, sometimes co-occurs with *Wolbachia* (Ros & Breeuwer 2009). Similarly, either or both *Wolbachia* and *Cardinium* induce CI, as *Wolbachia* induces weak CI, whereas *Cardinium* induces strong CI and doubly-infected ones showed the strongest CI in *T. piercei* (Zhu et al. 2012), *T. phaselus* (Zhao et al. 2013a) and *T. truncates* (Zhao et al. 2013b). In addition, Weeks et al. (2003) and Zchori-Fein & Perlman (2004) reported mixed infections with *Wolbachia* and *Cardinium* in the predatory mite *M. occidentalis*. The poultry red mite *Dermanyssus gallinae* was found to be infected with both *Cardinium* and *Spiroplasma* (De Luna et al. 2009). These observations indicated that *Cardinium* might shape its hosts' ecology and evolution in cooperation with other endosymbionts.

**Conclusions**

Our literature review confirmed that *Cardinium* is widely distributed in mites. As a reproductive parasite, some *Cardinium* strains indeed manipulate their host reproduction. Comparisons of the evolutionary relationships between mites and associated *Cardinium* indicated that there was no complete association between the two, suggesting horizontal transmission of this symbiont, which possibly occurred more readily among species that have close ecological requirements. *Cardinium* is not alone in mites, as there are other symbionts; *Cardinium* may influence the host biology in combination with them. Overall, this work provided basic knowledge for understanding the interaction between endosymbionts and mites.

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