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Evolutionary consequences of reproductive parasites in spider mites

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KEY WORDS
Bryobia*, *Cardinium*, cytoplasmic incompatibility, parthenogenesis, *Wolbachia

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Many arthropods are infected by 'reproductive parasites': parasites that manipulate the reproductive mechanism of their host in order to increase their own transmission. It was recently estimated that around 66% of all arthropod species are infected by *Wolbachia* (Hilgenboecker et al. 2008). *Cardinium*, another reproductive parasite, is currently estimated to infect 7% of arthropod species. In this article, the effects of *Wolbachia* and *Cardinium* on the evolution of spider mites, in particular the genus *Bryobia*, is investigated. Spider mite diversity, the diversity and transmission of *Wolbachia* and *Cardinium*, the origin of asexuality, and the dynamics of multiple infections in a sexually reproducing mite species are discussed.

Introduction

Ever noticed ivy (*Hedera helix*, klimop) in the garden that does not look so shiny, but rather pale/yellowish? Good chance that it is invested by *Bryobia* mites. And an even higher change that these mites are all females. And that they are all infected by *Wolbachia*.

Wolbachia pipientis is an intracellular bacterium that is widespread among arthropods. Recent estimates are that around 66% of all arthropod species are infected by *Wolbachia* (Hilgenboecker *et al.* 2008). That is an extremely high rate. And considering the effects *Wolbachia* may exert on its host, *Wolbachia* potentially plays a large role in the evolution of arthropods.

Wolbachia bacteria are generally considered to be vertically transmitted (i.e., they are passed on from mother to offspring). They are transmitted via the cytoplasm in the eggs, and therefore males, producing sperm that lacks cytoplasm, are dead ends from the bacterial point of view. These bacteria have developed ways to increase their own transmission, by actively manipulating the reproductive mechanism of their host, and increasing the frequency of infected females in the population. Four main types of reproductive manipulation by bacteria are known: parthenogenesis, male-killing, feminization, and cytoplasmic incompatibility (CI) (for reviews see Stouthamer *et al.* 1999, Werren *et al.* 2008).

Bacterium-induced parthenogenesis results in infected virgin females producing daughters only (asexual reproduction). Males are not needed for reproduction. Bacterium-induced parthenogenesis has been observed in some species of wasps, thrips and mites. Male-killing bacteria kill male offspring early in development. Female offspring of infected females have a fitness advantage because they can eat their dead brothers and suffer less from inbreeding and competition. Male-killing has been found in many arthropod species (including ladybird and butterfly species), and is induced by different bacteria. Feminization refers to cases in which genetic males are transformed into functional females during development. This phenomenon has been observed in isopods, butterflies and false spider mites. CI is the most common effect induced by reproductive parasites. Crosses between infected males and uninfected females are incompatible, whereas all other combinations of crosses are compatible. In this way, the fitness of uninfected females in the population is reduced, and the proportion of infected females increases, which enhances the spread of the bacteria. CI is widespread in arthropods.

Wolbachia is not the only reproductive parasite. In 2001, *Cardinium hertigii* was discovered – having similar host effects as

1. The bacteria *Wolbachia* (left) and *Cardinium* (right) co-infecting a cell of the spider mite *Bryobia sarothamni*. Photo: Elliot Kitajima
1. De bacteriën *Wolbachia* (links) en *Cardinium* (rechts) co-infecteren een cel van de spintmijt *Bryobia sarothamni*.

NEV Dissertatieprijs 2009

Tijdens de 21e Nederlandse Entomologendag (Ede, 18 december 2009) is de tweede NEV Dissertatieprijs uitgereikt aan Dr. Vera Ros, voor haar proefschrift 'Evolutionary consequences of reproductive parasites in spider mites', op 25 september 2008 verdedigd aan de Universiteit van Amsterdam. De prijs bestaat uit een geldbedrag plus een oorkonde en wordt jaarlijks toegekend voor het beste proefschrift op het gebied van de entomologie, verdedigd aan een Nederlandse universiteit in het voorgaande academische jaar (1 september – 31 augustus).

In de ogen van de jury scoorde dit proefschrift vooral hoog op het gebied van 'belang voor de entomologie in den brede'. Hoewel al het werk aan een enkele soortengroep is verricht, heeft het proefschrift grote algemene geldigheid doordat reproductieve parasieten in allerlei insectengroepen voorkomen. Het proefschrift is opvallend helder en toegankelijk geschreven.

During the 21st Annual Dutch Entomologists Meeting in Ede, December 18, 2009, the second Netherlands Entomological Society (NEV) Dissertation Award was presented to Dr. Vera Ros, for her thesis 'Evolutionary consequences of reproductive parasites in spider mites', defended on September 25, 2008, at the University of Amsterdam. This price comprises a sum of money and a certificate of appreciation, and is awarded for the best doctoral thesis in the field of entomology, defended at a Dutch university in the preceding academic year (1 September – 31 August).

In the eyes of the jury this thesis scored particularly high in 'importance for entomology in general'. Although all the work described in the thesis has been done on a single species group, the novel findings have a wide validity, because reproductive parasites are known to occur in many different insect groups. In addition, the presentation of the thesis is remarkably clear.

Wolbachia, but being completely unrelated to *Wolbachia* (Weeks *et al.* 2001, Zchori-Fein *et al.* 2001). So far, screening studies indicate that 6-7% of arthropod species are infected with *Cardinium*, and recent studies indicate a higher prevalence in Chelicerates than in insects (Duron *et al.* 2008). *Wolbachia* and *Cardinium* have been found co-infecting the same host (figure 1).

Infections with reproductive parasites have important implications for the evolution of arthropods, as such infections may affect host population genetic structure, population dynamics, or sex determination mechanisms. Moreover, reproductive parasites could play a role in arthropod speciation, by creating reproductive isolation between populations with different infection status. I focused on the evolutionary consequences of the reproductive parasites *Wolbachia* and *Cardinium* in spider mites (family Tetranychidae), with an emphasis on the genus *Bryobia*.

Spider mite diversity

Spider mites (Acari, Tetranychidae) are obligate plant feeders (phytophagous) and have a world-wide distribution. Some 1200 tetranychid species are described, divided into 71 genera. Five genera each include more than 100 species (including *Bryobia* and *Tetranychus*) (Bolland *et al.* 1998). A number of *Tetranychus* species are well-known pests all over the world, causing damage to many agricultural crops. *Bryobia* species are known to infest deciduous fruit trees (e.g., plumb, apple, or cherry trees), causing severe damage (see Box 1). *Bryobia praetiosa* Koch (the clover mite; figure 2), which feeds on grasses and herbaceous plants, has been classified as a 'household pest'. Morphological species identification is problematic in these tiny mites, not only because the number of diagnostic characters is limited, but also because few diagnostic traits are variable. Disagreement exists on the taxonomy and synonyms are common. The taxonomy of the genus *Bryobia* has troubled the mind of acarologists since a long time (see Box 2).

Currently, DNA sequencing is a promising objective tool to aid in description and delineation of species that have few useful taxonomic morphological characters, such as mites. It is also applied in spider mite taxonomy and phylogenetics (Navajas & Fenton 2000, Cruickshank 2002). So far, the central part of the mitochondrial COI (cytochrome oxidase *c* subunit I) region has mainly been used for inferring phylogenetic patterns, and for investigating intra- and interspecific variation. I performed an overall analysis of tetranychid COI sequence information currently available in the GenBank database, revealing new phylogenetic patterns within the Tetranychidae (Ros & Breeuwer 2007). Intraspecific variation appears very high for COI. Additionally, the COI region reveals a high AT content and a biased nucleotide composition at the third codon position.

Box 1

Bryobia biology

The life cycle of *Bryobia* is similar to that of other tetranychid mites, although completion of the cycle takes longer than for most *Tetranychus* species. The mite passes through four active and three resting stages: larva – protochrysalis (first quiescent) – protonymph – deutochrysalis (second quiescent) – deutonymph – teliochrysalis (third quiescent) – adult (Anderson & Morgan 1958). On average the life-cycle takes about a month to complete, depending on temperature and humidity. Life-history traits may however differ substantially between species. Species may have one (e.g., *B. ribis*) or several (e.g., *B. kissophila*) generations per year (Mathys 1957). The occurrence of diapause differs greatly (Helle & Sabelis 1985). Some species hibernate during winter in the egg stage, other species hibernate independent of stage. In some species mites are active during all seasons, whereas in others activity is lowered in winter (e.g., *B. rubriocululus*) or summer (e.g., *B. kissophila*, the 'cool season mite') (Mathys 1957). In contrast to other spider mites, *Bryobia* mites do not produce web (Helle & Sabelis 1985). It does however cover its eggs with bits of dust particles or other material (Anderson & Morgan 1958, personal observations). Eggs are laid separately or in small groups. *Bryobia* mites are all phytophagous, puncturing plant cells with their mouth parts and sucking out the contents. The mites reside on the lower and upper leaf surface as well as on branches and twigs.

2. *Bryobia praetiosa*, adult female. Photo: Jan van Arkel (IBED/UvA)
2. *Bryobia praetiosa*, volwassen vrouwtje.

Box 2

Bryobia taxonomy

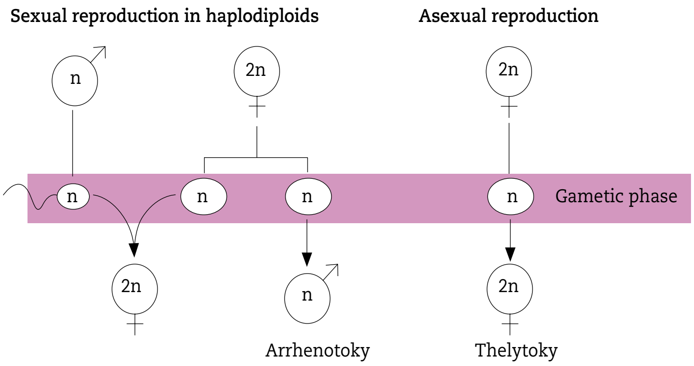
The taxonomy of the genus *Bryobia* has troubled the minds of acarologists for a long time. The first description of a *Bryobia* mite was performed in 1763 by Scopoli. He described it as *Acarus telarius*. At that time all mites were placed in one genus (*Acarus*, first described by Linnaeus) (Baker 1979). In 1836, Koch described *Bryobia praetiosa*. He originally recognized four species, based on behavioral, size and color differences, but these were reunited again in *B. praetiosa*. Various authors have investigated the genus since its erection and many species names have been proposed. Species distinction was mainly based on differences in life history, host plant association and behaviour. As no morphological characters were found to distinguish all species, many of the proposed species names were not accepted. The various forms were considered races belonging to the '*B. praetiosa* species complex' (Georgala 1958). Taxonomists have expressed their difficulties in describing this complex (Roosje & Van Dinther 1953, Van Eyndhoven 1954, 1955, Snertsinger 1963, Smith Meyer 1974). Van Eyndhoven (1955), Prichard & Baker (1955) and Mathys (1957) were the first to distinguish species morphologically. Confusion still exists concerning which characters are useful to separate the *Bryobia* species. Van Eyndhoven (1957), Mathys (1957) and Morgan & Anderson (1957) discovered that the immature stages (especially the larvae) are of great significance in species separation. From then on, species descriptions accumulated, resulting in over 130 species described at present. Main revisions of the genus were performed by Pritchard & Baker (1955) (starting with 13 species), Smith Meyer (1974, 1987), Van Eyndhoven & Vacante (1985), Hatzinikolis & Emmanouel (1991) and Bolland *et al.* (1998) (describing 132 species). Still, disagreement exists on the taxonomy and synonyms are common. Species identification is problematic in these tiny mites, not only because the number of diagnostic characters is limited, but also because the few diagnostic traits are variable. Currently, DNA sequencing is a promising objective tool to aid in description and delineation of species that have few useful taxonomic morphological characters, such as mites (Navajas & Fenton 2000; Cruickshank 2002).

This makes the COI region unsuitable for inferring phylogenetic species relationships, as branching patterns between species remain unresolved. The high intraspecific variation that was detected has serious consequences for the field of DNA barcoding. DNA barcoding is a technique aimed at the identification of pre-defined species using a short standardized DNA sequence (part of the COI gene) (Hebert *et al.* 2003, Moritz & Cicero 2004). It assumes that intraspecific variation is low and smaller than interspecific variation. My study shows that these assumptions are not always valid. The high COI variation found is possibly a result of selective sweeps caused by infection with reproductive parasites: when positive selection acts on the parasites, mutations in mitochondrial DNA (that is female-transmitted along with the parasites) are indirectly selected for as well (Hurst & Jiggins 2005). The use of (additional) nuclear DNA markers might overcome some of the problems encountered with mitochondrial COI sequence data. The challenge is to find suitable nuclear markers (Navajas & Fenton 2000, Cruickshank 2002).

Origin of asexuality

Both sexually and asexually reproducing spider mites are known. Sexually reproducing spider mite species are haplodiploid: females are diploid and develop from fertilized eggs, whereas males are haploid and develop from unfertilized eggs (figures 3 and 4). This phenomenon (males developing from unfertilized eggs) is called arrhenotokous parthenogenesis. Other spider mite species are known that reproduce

3. *Bryobia sarothamni*, adult female (left) and male (right). The male crawls under the female and bends its abdomen upward to mate with the female. Photo: Jan van Arkel (IBED/UvA)
3. *Bryobia sarothamni*, volwassen vrouwtje (links) en mannetje (rechts). Het mannetje kruipt onder het vrouwtje en buigt zijn achterlichaam omhoog om met het vrouwtje te paren.



4. Sexual and asexual reproduction in haplodiploids. In sexual reproduction, females are diploid and develop from fertilized eggs, whereas males are haploid and develop from unfertilized eggs. This asexual production of haploid males is termed 'arrhenotokous parthenogenesis' or 'arrhenotoky'. The term 'arrhenotoky' is however also used as a synonym for sexual reproduction in haplodiploids in general. When females are produced asexually (from unfertilized eggs), this is called 'thelytokous parthenogenesis' or 'thelytoky'. The haploid gametic phase might be absent during thelytoky.
4. Seksuele en aseksuele voortplanting in haplodiploïden. Bij seksuele voortplanting zijn vrouwtjes haploïd en komen voort uit bevruchte eitjes, terwijl mannetjes haploïd zijn en voortkomen uit onbevruchte eitjes. Deze aseksuele productie van haploïde mannetjes wordt 'arrhenotoke parthenogenese' of 'arrhenotokie' genoemd. De term 'arrhenotokie' wordt echter ook gebruikt als synoniem voor seksuele voortplanting in haplodiploïden in het algemeen. Wanneer vrouwtjes op een aseksuele manier ontstaan (vanuit onbevruchte eitjes), wordt dit 'thelytoke parthenogenese' of 'thelytokie' genoemd. De haploïde gametische fase kan afwezig zijn bij thelytokie.

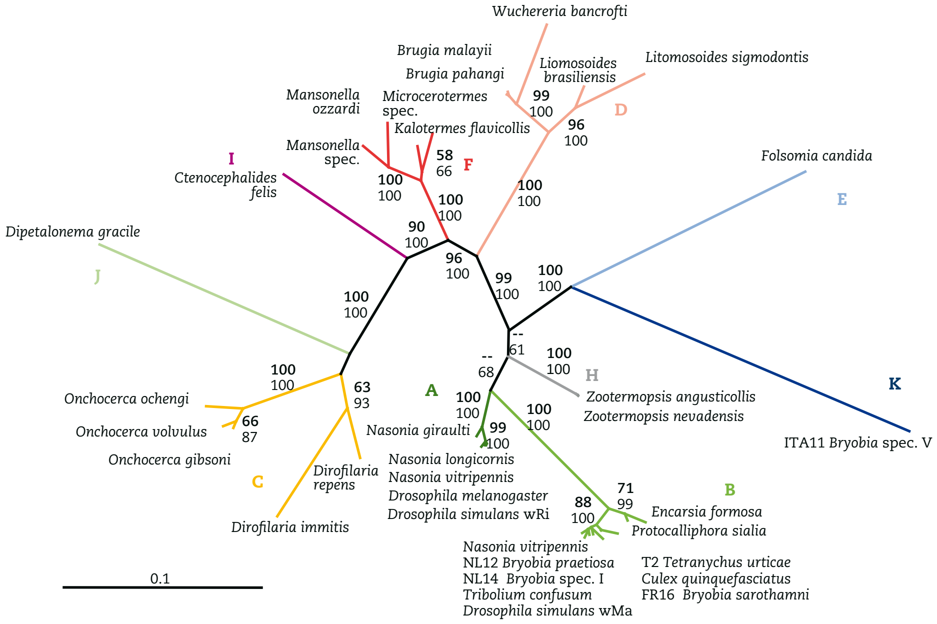
asexually by means of thelytokous parthenogenesis: diploid females develop from unfertilized eggs (males are absent). Here, I use parthenogenesis and asexuality to refer to thelytokous parthenogenesis. Within the Tetranychidae, parthenogenesis is widespread in the sub-family Bryobiinae, which includes the genus *Bryobia*, whereas it is rare in the sub-family Tetranychinae, which includes the genus *Tetranychus* (Helle & Sabelis 1985). Weeks & Breeuwer (2001) showed that in at least two *Bryobia* species, parthenogenesis is induced by *Wolbachia* bacteria. It is not so common in the animal kingdom to find many parthenogenetic species in a single genus, making *Bryobia* an interesting genus to study the evolution of asexuality.

Table 1. Distribution of *Wolbachia* supergroups across nematode and arthropod orders.
Tabel 1. Verdeling van *Wolbachia* 'supergroepen' over de ordes van nematoden en arthropoden.

Taxon	Order[a]	Supergroup
Phylum Arthropoda		
Subphylum Chelicerata		
Class Arachnida		
	Acarina[b]	B,K
	Araneae	A,B, (G)
	Pseudoscorpionida	?[c]
	Scorpiones	F
Subphylum Crustaceae		
Class Ostracoda		
	Podocopida	A,B
Class Malacostraca		
	Amphipoda	B
	Isopoda	B
Subphylum Hexapoda		
Class Entognatha		
	Collembola	B,E
Class Insecta		
	Blattodea	F
	Coleoptera	A,B,F
	Dermaptera	?[c]
	Diptera	A,B,F
	Hemiptera	A,B,F
	Hymenoptera	A,B
	Isoptera	A,B,F,H
	Lepidoptera	A,B
	Mecoptera	?[c]
	Neuroptera	F, ?[c]
	Odonata	B
	Orthoptera	B,F
	Phthiraptera	F
	Psocoptera	A,B
	Siphonaptera	I
	Thysanoptera	B,F
	Thysanura	B
Phylum Nematoda		
Class Secernentea		
	Spirurida	C,D,F,J
	Strongylida	?[c]

[a] Only orders in which *Wolbachia* was found are listed
[b] Oribatida, Prostigmata, Mesostigmata
[c] *Wolbachia* detected, but supergroup undetermined

Obligate asexual reproduction is rare in the animal kingdom. Most asexuals are found as single branches on the tips of the phylogenetic tree (Burt 2000, Butlin 2002). Apparently, asexual species can survive in the short-term, but are doomed to extinction in the long-term. In the short term, asexual reproduction is advantageous because an asexual population has twice the growth rate of a sexual population (no males are produced). With asexual reproduction, there are also no costs related to, e.g., finding a mate, and there is no risk of transmitting a disease. Despite these advantages, sexual reproduction is widespread in the animal- and plant-kingdom (the 'paradox of sex') (Crow 1999). General explanations for this paradox refer to the long-term disadvantages of asexual reproduction: asexuals are less able to adapt to novel environments and are exposed to accumulation of deleterious mutations. This is why asexuals are considered short-lived evolutionary 'dead ends' with limited adaptive potential (Judson & Normark 1996). It also explains the sporadic and low-level phylogenetic distribution of obligate asexual lineages.



5. Phylogenetic tree showing the diversity of *Wolbachia* and its division into 'supergroups'. Concatenated phylogenetic tree (maximum likelihood, unrooted) based on *Wolbachia* sequences of three protein coding genes (*gltA*, *ftsZ*, and *groEL*; 1616 basepairs) is shown. Bacterial strains are characterized by the name of their host species. Each supergroup is indicated with a different color. ML bootstrap values (top number, in bold) based on 100 replicates and Bayesian posterior probabilities (bottom number) are depicted (only values larger than 50 are indicated).

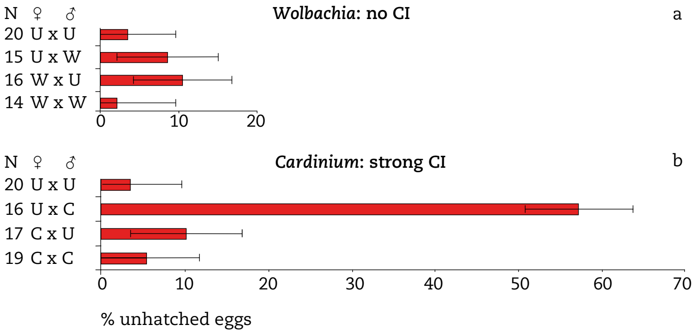
5. Fylogenetische stamboom die de diversiteit van *Wolbachia* en de verdeling ervan in verschillende 'supergroepen' laat zien. Samengestelde fylogenetische boom (maximum likelihood, ongeworteld) gebaseerd op *Wolbachia* sequenties van drie eiwitcoderende genen (*gltA*, *ftsZ* en *groEL*; 1616 baseparen). Bacterie-lijnen zijn aangeduid met hun gastheernaam. Elke supergroep is aangeduid met een verschillende kleur. De waarden op de takken geven 'maximum likelihood bootstrap' waarden weer (100 herhalingen), de waarden onder de takken geven 'Bayesian probabilities' weer (alleen waarden groter dan 50 zijn weergegeven).

The occurrence of many asexuals in one genus (*Bryobia*) is rare and raises questions about the origin and evolution of the asexual lineages. I investigated the phylogenetic history of asexual reproduction in the genus *Bryobia* (Ros *et al.* 2008) and show that asexuality is widespread in *Bryobia*, signifying that some animal taxa do contain a high number of asexuals. Using a combination of mitochondrial (COI gene) and nuclear (28S rDNA gene) sequence data, I argue that asexuality originated multiple times within *Bryobia*. In at least two *Bryobia* species, *Wolbachia* bacteria cause the asexuality. Possibly, *Wolbachia* has infected different *Bryobia* species independently, which is also likely considering the finding of different *Wolbachia* supergroups within *Bryobia* (see below). Additionally, I investigated intraspecific clonal variation by analyzing samples collected on a large geographic scale. Generally, clonal species are thought to harbour little genetic diversity. Within *B. kissophila* Van Eyndhoven, however, high intraspecific clonal diversity and paraphyletic patterns were found at the mitochondrial DNA. These findings are possibly explained by a high mutation fixation rate and past hybridization events. Reproductive parasites like *Wolbachia* and *Cardinium* might influence these processes. Such bacteria could play a role in the evolutionary success of asexual species.

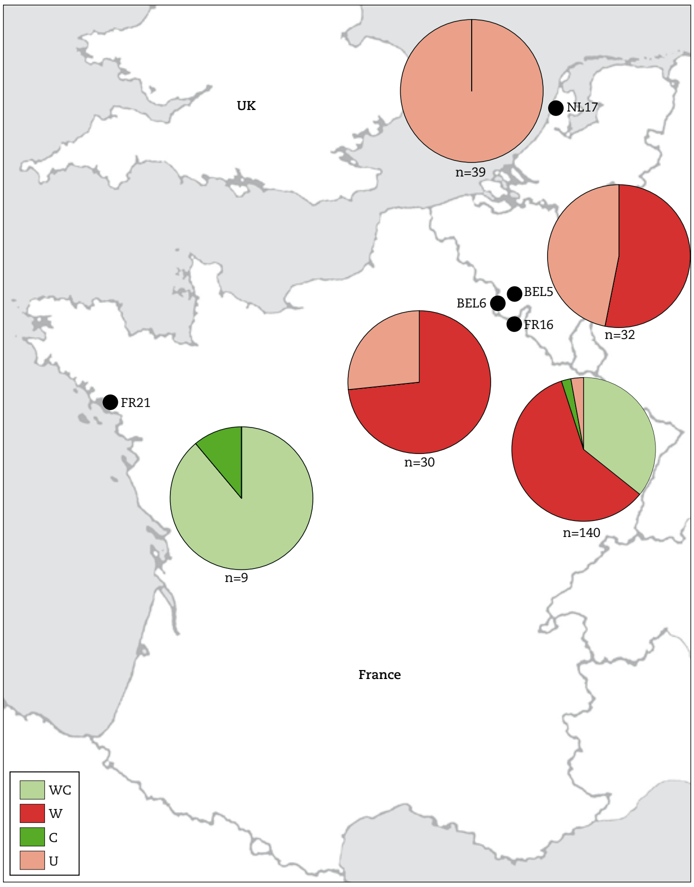
Diversity and transmission of *Wolbachia* and *Cardinium*

The genus *Wolbachia* is genetically highly diverse and has been divided into eight so-called 'supergroups' (A-H) on the basis of sequence data (Lo *et al.* 2007). Supergroup A and B were first described, and are most commonly found among arthropods (table 1). Supergroups C and D are restricted to filarial nematodes. Improved detection methods and a broader screening of host taxa continues to lead to the description of new *Wolbachia* supergroups. I discovered a new *Wolbachia* supergroup, named K, recovered from an unidentified *Bryobia* species (*Bryobia* spec. V), based on the sequences of three protein coding genes (*ftsZ*, *gltA* and *groEL*) and 16S rDNA (Ros *et al.* 2009; figure 5). Other tetranychid mites possess supergroup B *Wolbachia* strains. The discovery of another *Wolbachia* supergroup expands the known diversity of *Wolbachia*, and emphasizes the high variability of the genus. There is still ongoing debate on the status of supergroups (the assignment into supergroups remains somewhat arbitrary), and whether these groups should be considered distinct species or not.

Wolbachia and *Cardinium* are generally believed to be vertically transmitted (from mother to offspring). However, from phylogenetic inferences and genome comparisons, evidence is accumulating that horizontal transmission plays a role as well (Klasson *et al.* 2009). Most diversity studies are performed on diverse samples representing a wide range of host species. I investigated into more detail the diversity of reproductive parasites within a single family (Tetranychidae). Both *Wolbachia* and *Cardinium* were found infecting *Bryobia*, and sometimes they co-infect the same species or even the same individual. Additionally, the recombination rate for *Wolbachia* was inferred. *Wolbachia* is known to recombine, but it is unclear to what extent recombination contributes to *Wolbachia* diversity compared to point mutations. Using sequence data from four genes I show that new alleles are 7.5 to 11 times more likely to be generated by recombination than by point mutation. Such high recombination rates are comparable to rates found for other horizontally transmitted bacteria (Feil *et al.* 2000), suggesting that horizontal transfer of either genes or complete bacteria is substantial within *Wolbachia*. This idea is now supported by the discovery of an extraordinarily high level of mobile elements in the genome of *Wolbachia*, potentially facilitating horizontal gene transfer and recombination (Kent & Bordenstein 2010). No indication for cospeciation of host and *Wolbachia* was found, and there was also a lack of congruence between strain diversity and mtDNA diversity or geographical distribution. *Cardinium* was less frequently found in mites than *Wolbachia*, but also showed a high level of diversity, with eight unique strains detected in 15 individuals on the basis of only two genes. No evidence for recombination was found, although further exploration including more genes is required to sustain this observation. A lack of congruence observed among host and *Cardinium* phylogenies suggests that horizontal transfer is also prevalent for *Cardinium*.



6. *Wolbachia* induces no cytoplasmic incompatibility (CI) in *Bryobia sarothamni* (a), whereas *Cardinium* induces strong CI (b). The percentage of unhatched eggs is shown per cross (mean ± s.e.). N: number of replicates; U: uninfected; W: *Wolbachia* infected; C: *Cardinium* infected.
6. *Wolbachia* induceert geen cytoplasmatische incompatibiliteit (CI) in *Bryobia sarothamni* (a), terwijl *Cardinium* sterke CI induceert (b). Het percentage niet-uitgekomen eieren is weergegeven per kruising (gemiddelde ± standaardfout). N: aantal herhalingen; U: ongeïnfecteerd; W: geïnfecteerd met *Wolbachia*; C: geïnfecteerd met *Cardinium*.



7. Infection frequencies of uninfected, singly infected (*Wolbachia* or *Cardinium*) and doubly infected (*Wolbachia* and *Cardinium*) individuals of *Bryobia sarothamni* in five European populations. n: sample size.
7. Infectiefrequenties van ongeïnfecteerde, enkel geïnfecteerde (met *Wolbachia* of *Cardinium*) en dubbel geïnfecteerde (*Wolbachia* en *Cardinium*) individuen van *Bryobia sarothamni* in vijf Europese populaties. n: monstergrootte.

Cardinium induces cytoplasmic incompatibility in *Bryobia sarothamni*

I discovered that within the genus *Bryobia*, *Wolbachia* is not only found in asexual species, but also in the sexual species *B. sarothamni* (figure 3). Moreover, I found that this species is co-infected with *Cardinium*. Crossing experiments were performed to examine the effect of *Wolbachia* and *Cardinium* in *B. sarothamni* and possible interactions between *Wolbachia* and *Cardinium* were also studied. *Cardinium* causes severe CI in *B. sarothamni* (Ros & Breeuwer 2009; figure 6). This means that crosses between *Cardinium*-infected males and uninfected females yield no offspring (eggs do not hatch). The bacteria 'modify' the sperm in infected males. When these infected males mate with uninfected females, this modification leads to fragmentation of the paternal chromosomes in fertilized eggs. When males are crossed with females infected with the same bacteria, the modification is 'rescued', and eggs develop normally. *Bryobia sarothamni* is the third host species in which *Cardinium*-induced CI is observed and this study reveals the strongest CI effect found for *Cardinium* so far.

In contrast to *Cardinium*, I found no CI effect for *Wolbachia* within *Bryobia*. Also, *Wolbachia* appeared unable to rescue CI induced by *Cardinium*. Even so, CI is not induced when both *Cardinium* and *Wolbachia* infect the same male. I additionally examined field populations for their infection status, and found that infections ranged from completely uninfected to a polymorphic state with uninfected, singly infected and doubly infected individuals co-occurring (figure 7). No population was found to be fixed for infection with one or both bacteria. *Bryobia sarothamni* belongs to the 'Berlesei' group (Van Eyndhoven & Vacante 1985), in which more sexually reproducing *Bryobia* species have been described. The infection status of these species remains to be investigated.

General conclusion and future directions

It is clear that the association between the reproductive parasites *Wolbachia* and *Cardinium* and spider mites is highly dynamic. In many ways, the reproductive parasites play a role in the evolution of the spider mites, e.g., by affecting genetic diversity or reproductive mode. Possibly, they have been involved in processes of reproductive isolation and speciation, and they may even play a role in the evolution of asexuals. Even so, the evolution of the symbionts is influenced by their intracellular lifestyle and interactions with the host and with each other. At least within *Wolbachia*, recombination seems to play an important role in generating diversity, but exact patterns of recombination and modes of transfer require further exploration. Furthermore, additional research has recently shown that *Wolbachia* may exert beneficial effects on their host: *Wolbachia* was found to protect its *Drosophila* host against virus infection (Hedges *et al.* 2008, Teixeira *et al.* 2008). This broadens the range of effects *Wolbachia* can have on its hosts and also implies new potentials for *Wolbachia* as a tool in biocontrol and in the struggle against arthropod-borne diseases. Finally, the discovery of other reproductive parasites (like *Cardinium*), and the increasing potential of comparative genomics and proteomics studies provide new tools to further investigate and clarify mechanisms involved in reproductive manipulations.

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Samenvatting

Evolutionaire consequenties van reproductieve parasieten in spintmijten
Een vlinderpopulatie waarin alle mannelijke nakomelingen doodgaan, een mijtensoort die zich geheel aseksueel voortplant, of een pissebed die eigenlijk man is maar zich voortplant als vrouw – dit zijn enkele voorbeelden van merkwaardige verschijnselen die volop te vinden zijn in het veld, en die vaak door zelfzuchtige bacteriën veroorzaakt worden. Hoe kan dat? Dit betreft bacteriën die de gastheer infecteren en verticaal, van moeder op nakomelingen, worden doorgegeven. Dat gaat dan via de eicellen; in sperma is geen ruimte voor bacteriën. Vanuit bacterieel oogpunt zijn mannetjes overbodig. De bacteriën zien liever veel vrouwtjes, en sommige bacteriesoorten hebben manieren ontwikkeld om hun eigen overleving te vergroten: ze manipuleren het voortplantingsmechanisme van hun gastheer op zo'n manier dat er meer geïnfecteerde vrouwtjes worden geproduceerd. Een viertal manipulaties zijn bekend: 'male-killing' (mannelijke nakomelingen worden gedood), transseksualiteit (genetische mannetjes worden functionele vrouwtjes), aseksualiteit (vrouwtjes planten zich aseksueel voort) en cytoplasmatische incompatibiliteit (paringen van ongeïnfecteerde vrouwtjes met geïnfecteerde mannetjes mislukken).

Naar schatting is ongeveer 60% van alle geleedpotige soorten geïnfecteerd met deze bacteriën. Waarschijnlijk spelen deze bacteriën een grote rol in de evolutie van geleedpotigen en kunnen ze in sommige gevallen zelfs soortsvorming induceren. De meest bekende bacteriën behoren tot de genera *Wolbachia* en *Cardinium*. Dit artikel beschrijft de rol van deze bacteriën in spintmijten (Tetranychidae), met name van het genus *Bryobia*. Er zijn veel aseksuele *Bryobia*-soorten beschreven. Ik heb aangetoond dat *Wolbachia* en *Cardinium* wijdverbreid zijn binnen dit genus en dat de aseksualiteit waarschijnlijk meerdere keren is ontstaan in de loop van de evolutie. Dat is uniek, omdat aseksualiteit niet veel voorkomt in het dierenrijk en zelfs als nadelig wordt beschouwd op de lange termijn. Ook vond ik dat enkele seksuele soorten geïnfecteerd zijn met deze bacteriën. Verder blijkt uit moleculair genetisch onderzoek dat de bacteriën niet altijd strikt verticaal worden doorgegeven, zoals over het algemeen aangenomen wordt, maar ook horizontaal (dat wil zeggen infectueus, tussen onverwante dieren van eenzelfde generatie). Tussen en binnen genen blijkt veel recombinatie op te treden. Ook blijkt de diversiteit binnen de bacteriegenera groter dan verwacht, en heb ik in een *Bryobia*-soort een geheel nieuw *Wolbachia*-type ontdekt ('supergroep' K). Kruisingsexperimenten laten zien dat *Cardinium* cytoplasmatische incompatibiliteit veroorzaakt in de seksuele soort *Bryobia sarothamni*: kruisingen tussen *Cardinium*-geïnfecteerde mannetjes en ongeinfecteerde vrouwtjes leveren geen nakomelingen op. Deze vindingen kunnen verklaren dat de bacteriën zo wijdverbreid zijn en zoveel verschillende gastheren kunnen infecteren.

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