Genetic conflicts between Cytosplasmic bacteria and their Mite Host

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Citation for published version (APA):

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Download date: 11 Sep 2020
1 INTRODUCTION TO THE THESIS

This thesis focuses on the genetic conflicts between cytoplasmically transmitted bacteria and their hosts. The aim is to experimentally test theories on how such conflicts evolve. To this end, I analyzed aspects of the interaction between the two-spotted spider mite *Tetranychus urticae* Koch and the cytoplasmic bacterium Wolbachia.

This introduction is divided in four parts. First, I briefly introduce the host species and review information on manipulation of host reproduction by the endosymbiont. Second, possible outcomes of the co-evolution of Wolbachia and its hosts are discussed in light of current theories on the evolution of symbiosis and genetic conflicts. Third, I summarize the results and possible implications of the results presented in the chapters of this thesis. Fourth, I draw four general conclusions on the evolution of genetic conflicts between Wolbachia and their hosts.

HOST AND SYMBIONT

The two-spotted spider mite

The two-spotted spider mite *T. urticae* is a phytophagous mite that eats many different host plants. Populations of this mite are patchily distributed over host plants, and exhibit local mating structure (Mitchel 1973; McEnroe 1969). Propagation of mite colonies is ensured by adult females that disperse, usually mated, and settle for oviposition on uninfested leaves of the same or another host plant. In this thesis two strains of two-spotted spider mites are used. One strain arises from mites collected from rose plants, another from mites collected from cucumber plants.

Sexual species of spider mites are arrhenotokous haplodiploids (Helle *et al.* 1970), i.e. females are diploid, and develop from fertilized eggs whereas males are haploid and develop from unfertilized eggs. Consequently, un-inseminated females produce males. Both the rose and cucumber populations of the two-spotted spider mite are naturally infected with Wolbachia (Breeuwer & Jacobs 1996).

Wolbachia manipulation of host reproduction

Wolbachia are obligate intracellular α-proteobacteria that manipulate host reproduction in ways that promote replacement of an uninfected host population by an infected one (review by Stouthamer *et al.* 1999). Wolbachia
cannot be cultured outside host cells and essentially depend on their hosts for reproduction. These bacteria are transmitted to offspring of the female host through the egg (the cytoplasm donor gamete). Since infected females produce infected eggs, natural selection on Wolbachia favors mechanisms that increase the relative frequency of infected females (reviewed by Werren 1997). Manipulation of host reproduction by Wolbachia includes parthenogenesis, feminization, male killing and cytoplasmic incompatibility.

In Wolbachia-induced parthenogenesis, unmated infected females produce infected daughters (cf. Stouthamer 1997). Through feminization infected males are converted into reproductively functional phenotypic females, which have to mate to produce offspring (cf. Rigaud 1997). In Wolbachia induced male-killing infected males die and serve as first meals to their sisters, most of which are infected (cf. Hurst et al. 1997). Wolbachia-induced cytoplasmic incompatibility is discussed in detail in the next section. Wolbachia infections that induce parthenogenesis, feminization, and male-killing directly augment the relative fitness of infected females. These infections are expected to increase in frequency in a host population when rare (although this does not mean that they can spread to fixation).

Two further features of Wolbachia biology are important: the role of host genotypes in the infection phenotype, and the occurrence of horizontal transmission. First, closely related hosts harbor infections that are not distinguished based on sequences of Wolbachia genes but that result in different infection phenotypes (cf. Fialho & Stevens 2000). Although homology of gene sequences may arise due to recombination between Wolbachia ‘types’ (Jiggins et al. 2001; Werren & Bartos 2001), the possibility cannot be excluded that host genotype plays a role in the phenotype expressed in a given infection – especially given the results obtained with introgression experiments. Introgression of Wolbachia by microinjection shows that host genotypes may affect infection phenotypes both quantitatively (cf. Boyle et al. 1993; Poinsot et al. 1998) and qualitatively (Fujii et al. 2001). Second, the general absence of correlation between Wolbachia and host phylogenies suggests that horizontal transmission of Wolbachia between host taxa must occur, even though transmission is predominantly vertical (Stouthamer et al. 1999).

**Wolbachia induced cytoplasmic incompatibility**

**Cytoplasmic incompatibility:** Cytoplasmic incompatibility (CI) is expressed in crosses between infected (W) males and uninfected (U) females. If there is CI, ♀U × ♂W crosses are reproductively incompatible (Table 1). This type of CI is called uni-directional because the reverse cross (♀W × ♂U) is compatible. Induction of CI is suppressed if the same Wolbachia strain that is present in the male is also present in the eggs that his sperm fertilizes (review by Hoffmann & Turelli 1997). Thus, ♀W₁ × ♂W₁ is a compatible cross (and ♀U × ♂U is also compatible) (Table 1).
Table 1  Wolbachia-induced uni-directional and bi-directional cytoplasmic incompatibility. W: infected with Wolbachia, subscripts (1,2) indicate infection ‘types’; U: uninfected; X: incompatible cross; V: compatible cross.

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Because infection in the male has to ‘match’ the infection in the female, bi-directional cytoplasmic incompatibility can also occur. In this case both $\varphi W_2 \times \delta W_1$ and the reverse, $\varphi W_1 \times \delta W_2$, are incompatible crosses (whereas $\varphi W_1 \times \delta U$ and $\varphi W_2 \times \delta U$ are compatible) (Table 1).

Cytological studies of CI in *Nasonia* wasps (Reed & Werren 1995) and *Drosophila simulans* (Callaini et al. 1997) showed that incompatible crosses produce haploid or aneuploid embryos. This is because the paternal set of chromosomes fails to segregate properly in mitotic divisions early in embryonic development (Callaini et al. 1997; Reed & Werren 1995). In diploid species, haploid and aneuploid embryos abort, thus CI is expressed as increased F1 mortality. In haplo-diploid species, where females are diploid and males are haploid, haploid eggs develop as males. However, aneuploid eggs may die if haploidization is incomplete. This may explain why in haplodiploids CI results in a bias of F1 sex ratio towards males that is usually accompanied by an increase in F1 (female) mortality (cf. Breeuwer 1997; Vavre et al. 2000; Chapter 2).

Although the molecular details of CI remain unknown, CI is interpreted as involving Wolbachia-mediated modification and rescuing steps (Hoffmann & Turelli 1997; Werren 1997) as follows. Chromosomes from infected males are modified by Wolbachia and become unable to respond properly to cell cycle cues in uninfected eggs (Werren 1997). However, if infection in the fertilized egg ‘matches’ the infection that was present in the father, paternal chromosomes are ‘rescued’, i.e. they segregate properly during mitosis.

**Population dynamics of cytoplasmic incompatibility:** Theory predicts that CI-Wolbachia spread in a panmictic population of hosts because CI reduces the fitness of uninfected females, relative to infected females (Caspari & Watson 1959). This theoretical result is supported by field data on *Drosophila simulans* (Turelli & Hoffmann 1991). Furthermore, for realistic assumptions of that model, imperfect maternal transmission and/or a fecundity cost to infected females, two predictions emerge (Turelli 1994; Hoffmann & Turelli 1997).

First, if there is a reproduction cost to infected females CI cannot increase in frequency when rare (Caspari & Watson 1959). The same result is obtained if transmission of Wolbachia from infected mothers to their offspring is imperfect (Hoffmann et al. 1990). Thus, for imperfect maternal transmission, and/or a fecundity cost to infected females, an infection
frequency exists above which the proportion of infected hosts increases to some prevalence value (stable equilibrium '1') and below which the infection disappears. In other words, there is an unstable equilibrium representing an invasion threshold. This is intuitively easy to understand. If infected mothers produce less infected daughters than uninfected mothers produce uninfected daughters despite CI then the infection cannot spread. CI can only work to reduce the fecundity of uninfected females when sufficient infected males are present in the population. Absence of infection is thus another stable equilibrium.

Second, under imperfect transmission, CI cannot spread to fixation within a host population (Hoffmann et al. 1990). Thus, if transmission is not perfect, the infection will converge to the 'infection prevalence' stable equilibrium where infected and uninfected individuals co-occur.

**CO-EVOLUTION OF WOLBACHIA AND ITS HOSTS**

**Genetic conflicts between Wolbachia and its hosts**

Sexual reproduction with uniparental inheritance of cytoplasmic genes sets the stage for nucleo-cytoplasmic conflicts (Cosmides & Tooby 1981). This conflict arises because cytoplasmic elements are transmitted only via the mother whereas nuclear genes are transmitted through both sexes (Hurst et al. 1996). Most commonly nucleo-cytoplasmic conflicts translate into a conflict over sex ratio: while selection on cytoplasmically transmitted genes favors investment in females (the egg producing sex) selection on nuclear autosomal genes favors investment in both sexes. Conflicts of interest between nuclear genes and genes in organelles (mitochondria and chloroplasts) are 'intragenomic conflicts' because they occur between genes within the genome of an individual. The same type of conflict exists between hosts and cytoplasmically transmitted symbionts like Wolbachia, although here an intragenomic conflict *sensu stricto* is not present since Wolbachia are not part of the genome of their host (Maynard Smith & Szathmary 1995).

In a genetic conflict, the spread of one gene creates the context for the spread of another gene of opposite effect (Hurst et al. 1996). Thus, in nucleo-cytoplasmic conflicts, selection on nuclear genes will favor mechanisms that suppress manipulation by a cytoplasmic element (and *vice versa*) (Hurst et al. 1996). Assume the sex ratio of a population of individuals to be such that nuclear genes in either sex have equal fitness. If sex ratio distortion towards one sex is induced, for example a bias towards females, the fitness of nuclear genes in males increases because males will have more mating opportunities (*cf.* Fisher 1958). Therefore genes favoring the production of the rare sex increase in frequency until the 'original' sex ratio is restored. In populations with female-biasing sex ratio distorters, for example microbe-induced feminization or male-killing, nuclear genes that restore male production in infected females will be positively selected. This selection arises as long as males, which are rare due to the microbe-induced sex ratio bias, are required to produce offspring.

Although CI does not directly result in a sex ratio bias, host suppression of CI is also to be expected. For the endosymbiont, CI provides a mechanism
that increases the relative fitness of infected females. But for nuclear host genes CI means that not all crosses between infected and uninfected individuals will produce viable offspring. Thus, in populations polymorphic for the infection, a nuclear allele that increases compatibility of infected males with uninfected females is expected to increase in frequency when rare (Turelli 1994; Chapter 3). Similarly, an allele that influences the preference of females for males of the same infection type, and thus also results in avoidance of CI, may also be able to invade (Chapter 4).

**The evolution of endosymbiosis: private interests and common good**

A central theme in evolutionary theory is the evolution of obligate endosymbiotic associations. Endosymbionts are organisms that live inside the cells of other organisms. Obligate endosymbionts cannot survive outside their hosts. That obligate endosymbiosis can evolve is a fact demonstrated by associations of mitochondria and chloroplasts, once free-living prokaryotes, with eukaryotic cells (Margulis 1970, 1981). But why do obligate endosymbionts evolve? In other words, why does a partner in a symbiotic association lose its 'evolutionary sovereignty' (Van Baalen & Jansen, in press)?

One possibility is that the association between the two organisms is beneficial from the beginning. If both parties are better off together than alone, it may pay to be together as early in life as possible. Consequently, vertical transmission will be favored by selection once it arises. It is unlikely, however, that two entities that interact for the first time will immediately increase each other's well being (one has to have some knowledge of cats to know which places to scratch). Most probably first contact will not be pleasant [children always pull cat's whiskers first — and cats don't like that (F. Vala, personal observation)].

Another possibility is that the association starts off as antagonistic and evolves to apparent mutualism. If all the new hosts a parasite can infect are offspring of the current host, then less harmful variants of the parasite will replace harmful ones (Yamamura 1993, 1996; Lipsitch *et al.* 1995). In other words, increased vertical transmission favors less virulent parasites. Unquestionably, for the host, infection by a mild parasite is better than infection by a virulent one. However, a parasite remains a parasite — it decreases host fitness. Consequently, selection acting on the host will not favor increased vertical transmission unless the parasite becomes beneficial (Van Baalen & Jansen, in press). How can a parasite become beneficial? One possibility is that a less virulent form of the parasite confers immunity against more virulent forms (for a mini-review see Lipsitch *et al*. 1995). Competition between two parasites for hosts may favor increased vertical transmission in one parasite and, consequently, decreased virulence. Because possessing the less virulent form confers immunity (to the more virulent parasite), selection in the host will also favor increased vertical transmission.

Another mechanism by which selection on hosts and parasites will 'align' in favor of increased vertical transmission is discussed by Law & Dieckmann (1998). These authors consider the evolution of an exploiter-victim system where exploitation occurs when victim and exploiter join forming a
'holobiont'. When in holobionts exploiters receive more help from victims than they give in return, and thereby increase their (exploiters) fitness relative to the free-living form. Holobionts can replicate and each offspring is composed of one exploiter and one victim. Both victims and exploiters can still reproduce in the free-living form. Suppose victims evolve a compensation mechanism (against exploitation when in holobionts) with a cost that is paid also in the free-living form. Then if due to that cost deaths exceed births in the free-living form, victims will also 'prefer' to live in holobionts. At this point, the interaction will be considered 'mutualistic'.

Common good eventually arises from interactions between organisms – including antagonistic ones (Van Baalen & Jansen, in press). In the previous example, holobionts become the common interest of both victim and exploiter. Investment in common good, i.e. cooperation, is favored by selection because that serves the private interests of both partners (Van Baalen & Jansen, in press). In the latter example, free-living forms have decreased fitness, thus the frequency of free-living form may decrease. In individuals that live in partnership traits that allow free-living are not under selection and may be lost. Moreover, selection for better partnerships may result in loss of the ability to live independently. For example, gene transfer from mitochondria to the nucleus may have made for more efficient eukaryotic cells (Maynard Smith & Szathmary 1995). As a consequence one, or both, partners may lose their 'evolutionary sovereignty' and the association becomes 'obligate' (Van Baalen & Jansen, in press).

In the previous example both victim and exploiter replicate when holobionts reproduce. When ancestral mitochondria first joined eukaryotic cells replication probably yielded one cell with several mitochondria. However, in currently living sexually reproducing anisogamous organisms, the situation is different. When individuals reproduce they produce males and females – but only females transmit cytoplasmic elements like mitochondria and Wolbachia. Thus, an essential question is: can co-evolution of Wolbachia and their hosts lead to loss of 'evolutionary sovereignty' of hosts despite the genetic conflict?

Currently, two examples suggest that hosts may indeed lose their evolutionary sovereignty and form a permanent bond with Wolbachia. First, nematode hosts may be unable to survive without Wolbachia (Langworthy et al. 2000). Second, presence of Wolbachia in a parasitic wasp (Asobara tabida) is required for oogenesis (Dedeine et al. 2001). Did the obligate character of the association arise because infection by a 'mild' Wolbachia strains conferred immunity to a 'parasitic' Wolbachia, or because a costly defense arose in the host? To date frequent horizontal transmission of Wolbachia within a species has been described only in parasitoid wasps (Huigens et al. 2000). The fact that at present Wolbachia bacteria spread predominantly vertically (i.e. from mother to offspring) does not confer support to the first possibility (although, the situation may have been different in the past). In the absence of frequent horizontal transmission, a costly compensation mechanism seems a more plausible alternative. In Chapter 5 we provide an example very similar in essence to that discussed by Law & Dieckmann (1998).
INTRODUCTION TO THE THESIS

THIS THESIS

As mentioned above, the focus of this thesis is the genetic conflict between cytoplasmically transmitted bacteria and their hosts. Consequently, I start by assessing how this conflict is expressed in the association of Wolbachia with the two-spotted spider mite.

In Chapter 2 the effects of Wolbachia infection in mites from two different spider mite populations is discussed. This chapter reports on three main results. First, in mites collected from rose plants, the effect of Wolbachia on reproductive incompatibility extends to the F1. Daughters of ♀\text{U} × ♂\text{W} crosses that 'survive' CI have reduced reproductive viability themselves. This effect may well be unique to host species with holokinetic chromosomes. Holokinetic chromosomes have a diffuse centromere, such that microtubules attach anywhere to the chromosome. This property may enhance chromosome fragment survival during induction of CI to the extent that a female will develop, albeit with an incomplete diploid genome (aneuploid females). Aneuploid females will inevitably produce aneuploid, unviable gametes - at least as haploid individuals (males).

Second, it is demonstrated that presence of Wolbachia in rose (R) males aggravates reproductive incompatibility between these males and females from a population of mites originating from cucumber plants. This result is important because of its implications for host race formation, and consequent sympatric speciation, in the host species.

Third, a sex ratio effect was noticed in association with infection in mite females from the cucumber (C) strain. Infected C-females produce more female biased sex ratios than uninfected (cured) females. Increased female production is in the interest of Wolbachia. This effect is investigated further in Chapter 5.

In Chapter 3 (and 4) I investigate whether there exist mechanisms that result in avoidance of CI. As explained above, in a genetic conflict manipulation by one gene creates the context for the spread of another gene of opposite effect. Clearly, if a gene for such a mechanism segregates in our lab cultures it has not spread to fixation because induction of CI is observed (Chapter 2). To look for variation in the effect of Wolbachia on reproductive incompatibility several inbred lines were created and tested. These lines (hereafter 'inbred isofemale lines') were derived from one female and 'inbred' through four generations of mother to son mating. There is one further advantage to use highly inbred isofemale lines. Test for CI involves crossing of infected and uninfected individuals. Uninfected cultures are obtained by curing infected mites with antibiotics. A risk of using cured individuals is that the genetic variability in the uninfected population may not be representative of the genetic variability in the original, infected population. Testing isofemale lines of mites that were highly inbred prior to curing is one way around this problem.

In Chapter 3 I ask whether there may be within and/or between population variation for Wolbachia-induced reproductive incompatibility. Evidence for both was found. First, two isofemale lines from the R strain (R1 and R2) express Wolbachia induced reproductive incompatibility whereas one (R3) does not. The latter infection rescues sperm modified by R1 (thus,
\( W_3 \times \sigma W_1 \) is a compatible cross, but does not modify R3 sperm (thus, \( W_3 \times \sigma W_1 \) and \( \sigma W_3 \times \sigma W_3 \) are also compatible crosses). This result shows that there is variation within the strain for induction of reproductive incompatibility. Second, none of the C-isofemale lines express reproductive incompatibility.

Further to this, two Wolbachia genes from infected C and R mites were sequenced and it was found that sequences were identical. This result supports (or at least is not in contradiction with) the hypothesis that the differences found between the two mite strains are due to genetic differences at the host level. This line of thought was taken further by simulating what would happen in a population of infected and uninfected hosts if a host mutant gene would arise that made sperm 'resistant' to modification by Wolbachia. A Wolbachia infected female with such a genotype can rescue CI. However, as for line R3, an infected male with the mutant allele cannot induce CI. As expected, it is found that this mutant increases in frequency when rare. In doing so it creates conditions for re-invasion by uninfecteds that spread to fixation. Importantly, however, the host population that results is 'immune' to invasion by a Wolbachia using the same type of modification.

In Chapter 4 I focus on the possibility of assortative mating with respect to infection. In Chapter 2 and 3 it was established that presence of Wolbachia in R males could result in CI. In those experiments, females were confined to leaf discs and were offered only one type of male to mate with. Such experiments cannot detect whether hosts avoid CI by choosing compatible mates. According to the experiments presented in this chapter, assortative mating does occur and is manifested in essentially three different ways. First, uninfected females prefer to mate with uninfected males. Second, infected females aggregate their eggs. Third, on average 50% of the females tested prefer to start their own colony. This promotes sib (and thus assortative) mating. Together, these results suggest that panmixis may not apply to populations of spider mites where infected and uninfected individuals co-occur. Panmixia, however, is an important assumption for the claim that CI serves as a mechanism promoting the spread of the infection. This creates a paradox: if hosts can avoid CI, CI cannot be a spreading mechanism. Why, then, is CI commonly observed?

Two possibilities are discussed in Chapter 4. These possibilities have in common that an advantage to individual mites possessing CI (and thus Wolbachia) is evoked. The first possibility concerns competition between mites. Infected mites may have a competitive advantage because CI prevents establishment of uninfecteds in their food-patches. Assuming high transmission efficiency, most uninfected mites will be genetically unrelated to the resident mites in a patch. The second possibility concerns co-adapted genomes. Imagine that efficient exploitation of a food source depends on more than one gene. Co-adapted genomes associated with CI retain their cohesion more efficiently as they will be incompatible with other gene combinations.

In Chapter 5 I report on a Wolbachia infection that causes sex ratio distortion but is not parthenogenesis, male killing or feminization. The most common expression of a nucleo-cytoplasmic incompatibility is a bias of sex
ratio towards females. Upon sex ratio manipulation by a cytoplasmic element, selection on nuclear genes favors mechanisms that counteract it. First it is demonstrated that infected females produce significantly more female biased sex ratios than uninfected (cured) females. Next, it is shown that sex ratio produced by female mites from a culture cured of the infection was not stable and converged in time to the sex ratio produced by females from the infected culture. Finally, evidence is presented that sex ratio is a heritable trait both in presence and absence of the bacteria, and can thus be subjected to selection.

Based on these results, I suggest that upon sex ratio manipulation by Wolbachia compensatory host mechanisms evolved that allow infected females to compensate for the sex ratio manipulation. Curing caused this compensatory effect to become manifest. Subsequently, selection in the uninfected culture favored females that could produce more daughters – thus producing the sex ratio shift observed. This result is interesting because a genotype for a compensatory mechanism of this kind will be selected against unless in association with the symbiont. Consequently, such ‘resistant’ genotypes favor the establishment of permanent bonds with Wolbachia – cf. Section ‘The evolution of endosymbiosis’.

Chapter 6 centres on the problem of invasion by CI-Wolbachia. As explained above, for realistic assumptions CI cannot increase in frequency in a panmictic host population when rare. Typically, drift is evoked to explain how a CI infection reaches frequencies above the unstable equilibrium –from which it can spread. The first important result of Chapter 6 is to show that the probability that a CI infection drifts to the threshold frequency is extremely small – even for small population sizes. Thus, unless horizontal transmission across taxa is very common, (for which there is presently no evidence), drift alone probably cannot account for all CI infections observed. Therefore there must be other mechanisms by which infections increase in frequency when rare.

In Chapter 6 three possibilities are discussed. First, induction of a sex ratio bias towards females by Wolbachia, an effect suggested by the results obtained in Chapter 5, is considered by means of a model. Analytical results show that even small sex ratio biases are sufficient to bring the infection above the ‘CI-threshold’. The second possibility considered is that if fitness measure of the host is the reproduction rate then fecundity costs of infected hosts may be compensated by faster development. Lastly, it is suggested that subdivided population structure of hosts may also aid spread of CI, because most CI-patches cannot be invaded by uninfecteds (whereas the reverse is sometimes true).

**CONCLUSIONS**

I suggest that the evidence presented in this thesis provides empirical support to the following conclusions:

1. the ways in which the conflict of interests between Wolbachia and its hosts are expressed may depend on characteristics of the host (Chapters 2, 3 and 5);
2. in the genetic conflict that results from manipulation of host reproduction by Wolbachia, hosts do not necessarily behave as 'innocent by-standers' (Chapters 3, 4 and 5);
3. evolution of the genetic conflict between Wolbachia and its hosts may work to actually re-enforce the strength of the association between the conflicting parts (Chapter 5).

Furthermore, theoretical analysis suggests that:
4. the probability that genetic drift results in invasion of CI inducing Wolbachia is low, thus other mechanisms must be operating to lift infections above the invasion threshold (Chapter 6).

Acknowledgements I thank H. Breeuwer, D. Claessen, M. Egas, S. Magalhaes and M. Sabelis for helpful remarks on the manuscript and many insightful discussions.

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