Theoretical Aspects of the Evolution of Reproductive Parasites

by

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Declaration

This dissertation represents,
except where specifically mentioned in the text, the results of my own research.

The dissertation is not substantially the same as any that
I have submitted for a degree or other qualification at this or any other university.

No part of my dissertation has already been or is currently submitted
for any such degree or other qualification.

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Abstract

Reproductive parasites are maternally inherited endosymbionts that manipulate the reproduction of their hosts in a way that enhances the transmission of the parasites, but is deleterious to the hosts. In the present thesis, I try to resolve some questions concerning the evolution of reproductive parasites and their hosts by means of theoretical modelling, using a variety of approaches including recurrence equations, optimisation, and stochastic modelling. I first study the question: 'Can male-killing bacteria and meiotic drive elements influence each others' spread and equilibrium frequency in a population?' and demonstrate they can. Following this, I examine two questions with respect to host evolution. First, can male-killing or CI-inducing bacteria facilitate the evolution of haplodiploidy? I conclude that past work on this has been overoptimistic, but that the process is possible. Second, how does the presence of male-killing bacteria affect basic population genetic processes, in particular the interplay between natural selection and random genetic drift? I demonstrate that the host population behaves approximately as if only uninfected individuals existed. I then examine two questions in relation to endosymbiont biology. First, what is the impact of mating systems and segregation on the evolution of new CI-types? I propose outbreeding systems as a context where new CI-types can evolve. Second, how can we expect selection to act on two bacterial strains of reproductive parasites in doubly infected hosts with respect to their density? Finally, I examine how we expect the incidence of reproductive parasites to vary in time within a host clade, and how the phylogenetic history of the host clade affects the pattern of spread and expected incidence of the parasites. I conclude my thesis with a general overview of what is known about the evolution of reproductive parasites both empirically and theoretically, and outline some promising future avenues of research.

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Contents

List of Figures

List of Tables

Chapter 1

Introduction

1.1 Background

Reproductive parasitism is a widespread phenomenon in which microbial endosymbionts manipulate the reproduction of their hosts to their own advantage.[1] Two complementary viewpoints can be taken in attempting to understand reproductive parasitism. The first one is that of classic parasitology, in which the specific pattern of vertical transmission of reproductive parasites explains their manipulations. The second viewpoint puts emphasis on the genetic nature of reproductive parasites, considering them as cytoplasmic genetic elements that are part of the genome, but in conflict to nuclear genes. In this section, I will first set out these two conceptual frameworks, and then merge them into a brief discussion of the nature of reproductive parasitism.

1.1.1 Parasites and vertical transmission

Many symbionts are transmitted horizontally, that is, from one host to another one that is usually unrelated and may even belong to a different species. However, transmission can also be vertical, that is, from parents to offspring. In sexual species, vertical transmission can be maternal and/or paternal. A variety of routes of vertical transmission can be distinguished, including transovarial (within eggs), transovum (on the surface of eggs), and — in mammals — transplacental and transmammary [110].

Some symbionts exhibit a mixture of horizontal and vertical transmission. One of the most important examples is HIV, which is transmitted both sexually and transplacentally. Other examples include some other viruses infecting humans (e.g., herpes and several hepatitis viruses), microsporidian parasites in mosquitoes and silkworms [6, 298], and temperate phages in many bacteria.

In contrast to these symbionts with mixed transmission, many symbionts are transmitted exclusively vertically, usually maternally. Some of these are mutualistic endosymbionts that are often intimately associated with their hosts and provide some nutritional resources. Such mutualistic bacteria are well-known in several in-

[1] Throughout this thesis, I will use the term 'endosymbiont' in a neutral sense, merely denoting microorganisms that live within and in close association with their hosts. Depending on the fitness effects they exert on their hosts, endosymbionts can be mutualists, commensals or parasites.

sect groups, for example *Blattabacterium* in termites and cockroaches, *Buchnera* in aphids, and *Wigglesworthia* in tsetse flies [16, 17, 82, 2]. Organelles like mitochondria and chloroplasts can also be considered mutualistic endosymbionts with strict vertical (usually maternal) transmission. Reproductive parasites are another class of exclusively vertically transmitted endosymbionts.

It has been suggested that horizontally transmitted symbionts tend to be virulent, whereas vertically transmitted symbionts are likely to lie more towards the benign side of a virulence-avirulence continuum [104, 439]. The reasoning for this is that high replication of symbionts within their hosts may often be necessary for effective horizontal transmission, which is harmful to the host. On the other hand, the reproductive success of vertically transmitted symbionts is tightly connected to the reproductive success of their hosts and thus, the symbionts should be selected for low virulence. Although this notion may be true to some extent, it applies strictly to asexual species only, whilst the situation is more complex in sexually reproducing species.

Consider first the case of biparental transmission. We assume a sexual host population that is infected with symbionts that replicate moderately within their hosts and are avirulent. Now consider a mutant symbiont in one of the hosts that replicates at a higher rate than the wildtype symbionts. This overreplication will lead to a certain level of virulence and hence fewer offspring of the host. However, at the same time a higher proportion of offspring of the host will be infected with the mutant strain than with the wildtype strain. Because of the biparental transmission, the mutant strain may spread contagiously in the population and replace the wildtype strain, leading to a virulent population of symbionts which may be susceptible to invasion of mutants with even higher replicating rate and virulence. This reasoning represents a variation of the well known problem of the 'tragedy of the commons', in which the moderate exploitation of a resource (the host) by a group of agents is usually not stable because of an incentive to individuals to exploit the resource more than others.

Consider now the case of uniparental transmission. Most vertically transmitted symbionts are passed on in this way, and usually it is the females that are the sole transmitters. When symbionts are transmitted maternally only, the problem of overreplicating mutants outlined above does not occur because offspring of females with

the wildtype strain cannot become 'infected' with the mutant strain. Indeed, it has been suggested that anisogamy has evolved to prevent biparental transmission of symbionts and thus circumvent the problem of overreplicating symbionts [178]. However, new problems arise with maternal transmission [71]. First, there is no selection on the endosymbionts for low virulence in males, which are reproductive dead ends for them. Why mutualistic maternally inherited bacteria like *Buchnera* apparently function as well in males as they do in females has puzzled biologists for a long time [141]. Second, maternally transmitted endosymbionts are effectively passed on to at most half of the offspring of an infected female (assuming an even primary sex ratio). Even if transmission fidelity is only slightly imperfect, the endosymbionts would go extinct unless they posses some form of 'drive' in their host. This can be a fitness advantage to infected females, as in many bacteria that contribute nutritional functions to their hosts, or reproductive parasitism.

1.1.2 Genetic conflict and selfish genetic elements

Up until the 1970s, genomes were largely viewed as amalgamations of genes that cooperate harmoniously to ensure the survival and reproduction of their hosts. This view had to be gradually revised within the last decades, as more and more examples of 'intragenomic conflict' emerged. Such conflicts occur if different parts of a genome are under different selective pressures. Equivalently, there is intragenomic conflict if "*... the spread of one gene creates the context for the spread of another gene, expressed in the same individual and having the opposite effect*" [180].

For example, competition between alleles at a given locus for transmission to the next generation has led to a phenomenon termed 'meiotic drive' in many species [246, 189]. Here, a 'driving' allele kills gametes carrying the homologous non-driving allele, so that more than 50% of the carrier's offspring will carry the driving allele. Since meiotic drive is often deleterious for the organism as a whole, there is genetic conflict between the driving allele and the rest of the genome. Other examples of such 'selfish genetic elements' include homing endonucleases, transposable elements and B chromosomes (reviewed in [421, 177, 47]).

A particular type of intragenomic conflict arises between nuclear and cytoplasmic genetic entities (e.g., mitochondria, chloroplasts, inherited endosymbionts) [71]. The

basis of this conflict is the different pattern of inheritance: whilst nuclear genes are passed on normally in a Mendelian way (with genes in females and males having a 50% chance of being transmitted), cytoplasmic genes are transmitted maternally only in the vast majority of species. As a consequence, males are reproductive dead ends for cytoplasmic genetic elements, and manipulations that increase transmission through females at the expense of males will be selected for.

One especially important 'bone of contention' between nuclear and cytoplasmic genes is the sex-ratio in which females produce offspring. Usually, nuclear genes are selected to produce offspring of the rarer sex, leading to an even population sex-ratio. This is because the entirety of males contribute the same genetically to the next generation as the entirety of females [83, 113]. By contrast, cytoplasmic genes are selected to produce females only [71, 46], even though males are necessary in the population for fertilisation. Genetic conflict over the sex-ratio is one of the foundations of reproductive parasitism. Another, related manifestation of this conflict is the phenomenon of 'cytoplasmic male sterility', found in many angiosperm plants [341]. Here, selfish mitochondrial variants prevent the formation of male reproductive organs, thereby increasing the amount of resources allocated to female reproductive organs and enhancing their own spread.

1.1.3 Reproductive parasites

The preceding two sections have set the stage for a class of endosymbionts termed 'reproductive parasites'. These endosymbionts are maternally transmitted parasites (or cytoplasmically inherited genetic elements) that manipulate the reproduction of their hosts [415]. Through these manipulations, transmission of the parasites to the next generation is enhanced at a disadvantage to their hosts. Consistent with the theoretical expectations outlined above, it is always the sons of infected females that are exploited in one way or the other to increase the number of infected daughters.

Reproductive parasites are very common in arthropods, in particular insects, spiders, mites and woodlice. A variety of microorganisms induce reproductive manipulations (see Figure 1.1). The best-known and most widespread of these is the alphaproteobacterium *Wolbachia*, which will be the topic of Section 1.2; other groups of reproductive parasites will be introduced in Section 1.3.

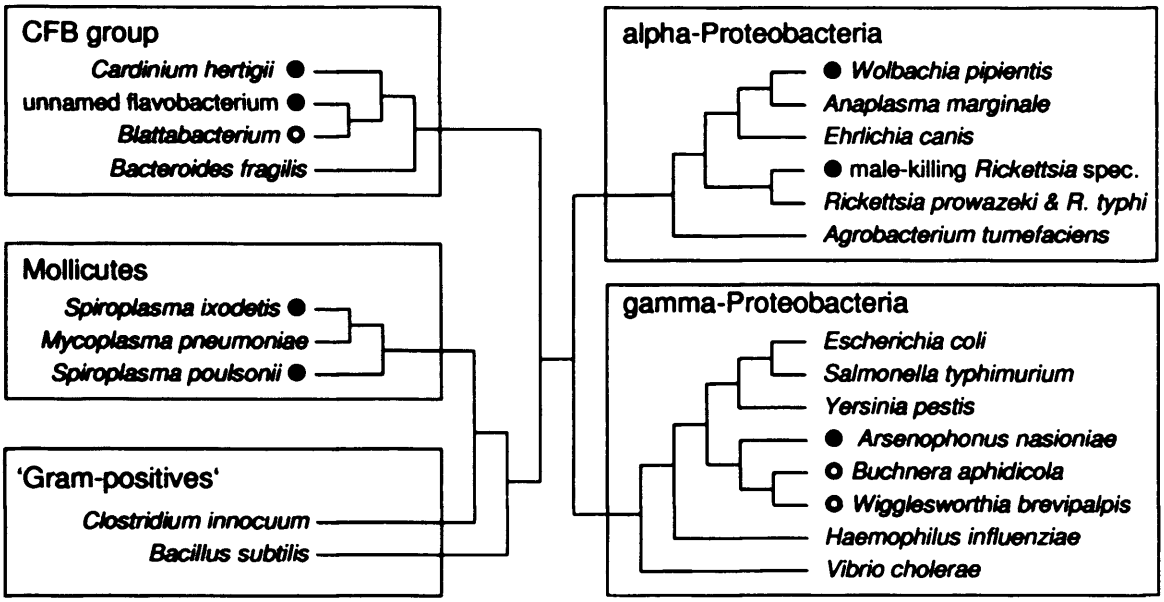

Figure 1.1: Schematic, unscaled phylogenetic relations between bacterial reproductive parasites. Also shown are some other well-known bacteria and some endosymbionts of insects. Reproductive parasites are indicated by black circles, whilst mutualistic endosymbionts are indicated by empty circles. Adapted from Refs. [291, 168, 134, 242, 446].

Four main types of reproductive manipulation can be distinguished. Three of these — feminisation, induction of parthenogenesis, and male-killing — involve a sex-ratio distortion towards females. Here, infected females produce effectively more daughters than uninfected females. The fourth manipulation is cytoplasmic incompatibility (CI). In CI, the sperm of males is modified by the parasites, and unless parasites are present in the egg, the resulting zygotes are killed. Thus, uninfected females will produce fewer offspring than infected females. A detailed description of these four types of reproductive manipulation will be given in Section 1.4.

1.2 *Wolbachia*

Wolbachia is without doubt the paradigm of reproductive parasites. Whilst the existence of other reproductive parasites should not be forgotten (see Section 1.3), this prominent role of *Wolbachia* within the field is justified for two reasons: *Wolbachia* are the most common and widespread reproductive parasites (and indeed, probably the most common endosymbionts in general), and they are the only bacteria that have been reported to induce all four known forms of reproductive manipulation. In this section, I will only give a brief overview on *Wolbachia*; more detailed reviews can be found in Refs. [415, 292, 367, 77, 388].

1.2.1 Brief history of *Wolbachia* research

Wolbachia were discovered in 1924 by Marshall Hertig and S. Burt Wolbach as rodlike structures in the reproductive tissues of the mosquito *Culex pipiens* [150]. Twelve years later, they were formally named *Wolbachia pipientis* by Hertig in honour of his co-worker [149]. However, only in 1971 a connection was drawn between *Wolbachia* and the long-known phenomenon of cytoplasmic incompatibility in *Culex pipiens* [440]. It took again nearly twenty years before it was discovered that *Wolbachia* is also the causative agent of cytoplasmic incompatibility in *Drosophila simulans* [22].

In the 1990s, it became apparent both that *Wolbachia* are responsible for cytoplasmic incompatibility in many more arthropod species, and that they also induce feminisation, parthenogenesis and male-killing [324, 366, 172]. The advent of PCR assays made broad scale surveys for endosymbiotic bacteria possible, which demonstrated that *Wolbachia* are extremely common and widespread in arthropods [425, 428, 424]. Involvement of *Wolbachia* in various host evolutionary processes (see Chapter 8) and possible applications in insect control [80, 357, 443, 358] have further fostered the interest in *Wolbachia*, which has not declined since. In 2004, a new era of *Wolbachia* research was initiated with the determination of the first genome sequence of a *Wolbachia* strain [438].

1.2.2 Biology of *Wolbachia*

Wolbachia are gram-negative eubacteria that live within eukaryotic host cells. The bacteria are either small (0.25–1.3μm), with rodlike or coccoid shapes, or large (1–1.8μm) forms that contain several of the smaller forms [149]. Within host cells, *Wolbachia* are enclosed by three membranes: an outer membrane of host origin, a bacterial cell wall, and an inner bacterial plasma membrane. *Wolbachia* are localised primarily in the ovaries and testes of their hosts, where they can reach high densities. However, in many species, the bacteria can also be detected in somatic tissue, for example the hemocytes, muscles and nervous tissue [149, 22, 79].

The process of maternal transmission of *Wolbachia* is not fully understood. Within the ovaries, *Wolbachia* are most abundant in the nurse cells, where they replicate and from where they enter the oocytes [448, 388]. The localisation of *Wolbachia* within oocytes varies widely between host species and bacterial strains [36, 293, 448, 403, 107]. For example, in *Drosophila melanogaster* the bacteria initially exhibit concentration at the anterior pole of the oocyte, but subsequently disperse throughout the entire egg [107]. This movement appears to involve active transport along microtubules and depends on host motor proteins [107].

Interestingly, *Wolbachia* are also able to migrate through host somatic tissue. When injected into the abdominal cavity of *Drosophila melanogaster* females, the bacteria were observed to cross several somatic tissues and finally entered the germline via the somatic stem cell niche [121]. These exciting findings may help explain the ability of *Wolbachia* to be transmitted occasionally across host species (see below and Section 7.1).

1.2.3 Distribution of *Wolbachia*

Wolbachia are astonishingly widespread in arthropods. According to conservative estimates, about 16-20% of all insect species are infected [425, 428, 424, 410, 445], and a survey using a different PCR-technique even yielded an infection frequency of 76% [195]. *Wolbachia* are equally common in terrestrial isopods, but seem to be rare in aquatic isopods and absent in other crustacean taxa [30]. Finally, within the Chelicerata *Wolbachia* have been detected in about 40% of mite and spider species

screened [35, 334, 129]. A more detailed review of *Wolbachia* incidence in arthropods is given in Section 7.1.1.

Wolbachia are also abundant in a group of filarial nematodes, the Onchocercidae [15, 18]. Here, the bacteria appear not to induce reproductive manipulations, but are obligate symbionts that are necessary for the oogenesis of their hosts. Many of the Onchocercidae are serious human pathogens. For example, *Wuchereria bancrofti* and *Onchocerca volvulus* are the causative agents of elephantiasis and river blindness, respectively. Thus, the discovery of obligate *Wolbachia* endosymbionts has lead to the promising prospect of curing patients infected with these worms by treating them with antibiotics [236, 377]. Unfortunately, some of the symptoms in these diseases are caused by *Wolbachia* following their release from dying nematode hosts [335], so that this therapy is not always tolerable without other measures to suppress the immune response against *Wolbachia*. Since *Wolbachia* in nematodes are not involved in reproductive parasitism, they will not be considered further in this thesis.

It is not known to date whether *Wolbachia* occur in phyla other than arthropods and nematodes. In molluscs, they appear to be absent or rare [342], but to the best of my knowledge, no other negative reports exist.

1.2.4 Phylogeny and systematics of *Wolbachia*

Wolbachia is a genus of alpha-proteobacteria (see Figure 1.1). The proteo- or purple bacteria consist of alpha-, beta-, gamma-, delta- and epsilon-proteobacteria and constitute the largest bacterial phylum. They are a very diverse group of gram-negative eubacteria which include photosynthetic, chemoautotrophic and heterotrophic species. Many (and all alpha-) proteobacteria have specialised in living intracellulary in other organisms. Interestingly in this respect, the ancestor of mitochondria is also thought to belong to the alpha-proteobacteria [5].

Within the alpha-proteobacteria, *Wolbachia* have been placed within the Rickettsiae. Their closest relatives are parasites of mammals like *Anaplasma marginale* and *Ehrlichia canis*. Three species of *Wolbachia* have been described. *Wolbachia pipientis* is the type species of the genus and was described in 1936 by Hertig [149]. Most authors simply refer to this species as *Wolbachia*, a convention that will be followed in this thesis. A second species, *Wolbachia persica*, was demonstrated to

belong to the gamma-proteobacteria genus *Fransicella* and should thus be removed from the genus *Wolbachia*. The relationship of a third species, *Wolbachia melophagi*, to *W. pipientis* has yet to be determined [18]. Neither *W. persica* nor *W. melophagi* will be further considered in this thesis.

Eight phylogenetically distinct supergroups have been identified within the species *Wolbachia pipientis* (see Table 1.1). Members of supergroups A, B, E, G and H have been found in arthropods only, supergroups C and D seem to be restricted to nematodes, and supergroup F consists of *Wolbachia* strains infecting nematodes and arthropods. The phylogenetic relationships between the eight groups are not fully resolved, and new groups are likely to be discovered [53]. However, phylogenetic analyses have revealed that neither nematode nor arthropod infecting *Wolbachia* form a monophyletic group, making multiple horizontal transmission events across phyla likely.

It has also been demonstrated that the phylogeny of *Wolbachia* in arthropods does not correspond to the phylogeny of their hosts [426, 424]. This has lead to the conclusion that horizontal transfer of *Wolbachia* from one species to another one is a frequent event on an evolutionary time scale. In contrast, the phylogeny of *Wolbachia* within the C and D supergroup matches the phylogeny of the nematode hosts, so that here, *Wolbachia* appear to have undergone cospeciation with their hosts [15].

Table 1.1: *Wolbachia* supergroups A-H.

Supergroup	Host groups	References
A	various insects, mites, spiders, woodlice	[426, 424, 334, 129]
B	various insects, mites, spiders	[426, 424, 334, 129]
C	filarial nematodes	[15]
D	filarial nematodes	[15]
E	springtails	[395, 245]
F	termites, weevils, true bugs, filarial nematodes	[245, 315]
G	Australian spiders	[334]
H	termites	[26]

1.2.5 *Wolbachia* genomes

Two *Wolbachia* genome sequences have been completed and published to date, those of the *w*Mel strain from *Drosophila melanogaster* [438], and the *w*Bru strain found in the filarial nematode *Brugia malayi* [115]. Sequencing projects for several other strains are underway, among them *w*Pip (from the mosquito *Culex quinquefasciatus*) and *w*Ri (from *Drosophila simulans*) [106].

*w*Mel has a genome size of about 1.3Mb [438], which is much less than the genome size of free-living bacteria (e.g., 4.2Mb in *Bacillus subtilis*). Thus, there has been substantial genome reduction in *Wolbachia* during their intracellular association with arthropods. This is also evident by the loss of many metabolic genes, which results in reliance of *Wolbachia* on import of amino acids and other molecules from their hosts [438]. On the other hand, *Wolbachia* genomes are considerably larger than those of mutualistic endosymbionts. For example, *Buchnera* bacteria — the primary endosymbionts of aphids — have genomes of less than 0.7Mb size [374, 139]. The relatively large genome of *Wolbachia* compared to the genomes of other obligate endosymbionts might result from the parasitic life-style of *Wolbachia* in arthropods, which can be expected to require retainment of a higher number of genes. However, this explanation may not account for much of the difference observed because the genome of the mutualistic *w*Bru strain of *Wolbachia* (1.1Mb size [115]) is not much smaller than that of *w*Mel.

Perhaps the most exciting result that emerged from the *w*Mel genome sequencing project was the discovery of a high number of genes containing ankyrin-repeat domains [438]. Ankyrin-repeats consist of motifs of 33 amino acids and are generally rare in bacteria. However, they are commonly found in eukaryotic genes, where they are known to mediate protein-protein interactions. Thus, ankyrin-containing genes are good candidates for involvement in interactions with hosts, for example transport within cells along microtubuli or modification of chromosomes as part of reproductive manipulations.

Preceding the completion of the *w*Mel genome project, other *Wolbachia* genomes were known to contain prophage regions [253]. It was also demonstrated that these phages, designated WO, can be active and produce phage particles [254, 359]. *w*Mel also contains three prophages, one of which is thought to be inactive [438]. Interest-

ingly, the above mentioned ankyrin genes are often found within prophage regions. Thus, horizontal transmission of phages between different *Wolbachia* strains (which is known to occur) might drive the evolution of *Wolbachia*-host interactions through transduction of ankyrin containing genes.

1.3 Other reproductive parasites

1.3.1 *Rickettsia*

Rickettsia are a genus of alpha-proteobacteria related to *Wolbachia* (see Figure 1.1). The bacteria are intracellular endosymbionts with a wide range of host taxa, including arthropods, flatworms (leeches), and vertebrates (reviewed in [299]). Most described species of *Rickettsia* are pathogens of vertebrates that are transmitted by blood-sucking arthropods (ticks, lice and mites). Diseases of humans that are caused by *Rickettsia* include epidemic typhus and Rocky Mountain spotted fever.

Recently, it has become apparent that many *Rickettsia* are endosymbionts of non-blood-sucking arthropods [299], begging the question of whether the high proportion of vertebrate pathogens among described *Rickettsia* may only reflect the biased view of a vertebrate. In most Rickettsia-arthropod associations, it is not known what phenotype (if any) the bacteria induce in their arthropod hosts. However, in a few cases, *Rickettsia* have been demonstrated to be reproductive parasites. *Rickettsia* are capable of at least two reproductive manipulations: male-killing (in the ladybird *Adalia bipunctata* and some other beetles [419, 347, 240]), and parthenogenesis induction (in the wasp *Neochrysocharis formosa* [136]).

1.3.2 *Arsenophonus*

Arsenophonus nasoniae is a species of gamma-proeteobacteria that was described in the parasitoid wasp *Nasonia vitripennis* [125], in which the bacteria induce male-killing [360, 422, 11]. Many populations of *N. vitripennis* are infected, and within infected populations the prevalence of *Arsenophonus* is around 10% [11]. Notably, *Arsenophonus* is not transmitted transovarially. Instead, the bacteria are transferred into the host pupae during oviposition, and then taken up through ingestion by the larval offspring [159, 422]. Thus, *Arsenophonus* can also be transmitted horizontally if a single host pupa is parasitised by more than one female of *N. vitripennis*.

Not much is known about the distribution of *Arsenophonus*. Aside from *N. vitripennis*, the bacteria have been detected in the closely related *N. longicornis* and in some other insects [11, 165], but it is not clear whether male-killing or any other reproductive manipulation is induced in these species.

1.3.3 *Spiroplasma*

Spiroplasma is a genus of Mollicutes, also known as mycoplasmas. Mollicutes are among the smallest of bacteria, with diameters ranging from 0.25μm. They do not possess a cell wall and therefore stain as gram-negative; however, phylogenetically they are more closely related to the gram-positive bacteria. All currently recognized Mollicutes are symbionts of animals or plants. Some species are human pathogens. *Mycoplasma pneumoniae*, for instance, causes a mild type of pneumonia.

Spiroplasma are characteristically helical-shaped bacteria. Most species are pathogens of plants, which are often transmitted by arthropods feeding on phloem sap. Two species of *Spiroplasma* have been implicated in reproductive parasitism. Both are maternally transmitted and induce male-killing in their insect hosts. First, strains of *Spiroplasma ixodetis*, the basal group among *Spiroplasma*, have been identified as causative agents of male-killing in the ladybirds *Adalia bipunctata* and *Harmonia axyridis* as well as in the butterfly *Danaus chrysippus* [176, 249, 201]. The second species of male-killers is *Spiroplasma poulsonii*, members of which have been reported in several *Drosophila* species of the willistoni group and also in *Drosophila melanogaster* [431, 432, 276] (see also Takble 2.1). Unlike other reproductive parasites, *Spiroplasma poulsonii* occurs largely extracellularly.

1.3.4 *Cardinium*

Cardinium hertigii is the most recently described species of reproductive parasites [445]. The bacteria belong to the Cytophaga-Flavobacterium-Bacteroides (CFB) group, a diverse, but poorly characterised group of gram-negative, anaerobic eubacteria. *Cardinium* bacteria have been discovered in ovaries of *Encarsia* wasps and are characterised by brush-like microfilament structures [446].

The diversity of reproductive manipulations employed by *Cardinium* is outperformed only by *Wolbachia*: the bacteria induce two different forms of parthenogenesis in the mite *Brevipalpus phoenicis* [409] and several species of the parasitoid wasp genus *Encarsia* [444] as well as cytoplasmic incompatibility in *Encarsia pergandiella* [164]. In broad surveys of arthropods, about 7% of species were infected with *Cardinium*, most of them mites and Hymenoptera [410, 445]. As in *Wolbachia*,

phylogenetic analysis indicates that horizontal transmission frequently occurred on an evolutionary timescale in this group [410, 445]. These surveys also demonstrated that *Cardinium* can co-occur with *Wolbachia*, even within single hosts.

1.3.5 Flavobacteria

Flavobacteria are also members of the above mentioned CFB group. Two closely related strains of unnamed flavobacteria have been reported to induce male-killing in the ladybird species *Adonia variegata* and *Colemegilla maculata* [166, 167]. Interestingly, these male-killers form a monophyletic group together with *Blattabacterium* spec., the beneficial endosymbiont of cockroaches and termites [16, 17, 166].

1.3.6 Microsporidia

In contrast to the reproductive parasites portrayed to date, microsporidia are eukaryotic endosymbionts. They form a group of highly specialised, obligate intracellular parasites that occur in all five vertebrate classes as well as in most invertebrate phyla. Infections of silkworms, honeybees and fish have led to considerable economical damage in the past, and some species are also serious human pathogens, especially in immune deficient people like AIDS patients (reviewed in [214]).

Microsporidia lack mitochondria and peroxisomes, have very small and compact genomes and ribosomes that resemble more those of bacteria than those of other eukaryotes. Because of these peculiar characteristics, they have been proposed as being an ancient 'missing link' between prokaryotes and the 'higher' eukaryotes. However, it is accepted now that microsporidia lost their mitochondria during their evolution and that they are highly reduced rather than primitive eukaryotes. They seem to be related to fungi, but their exact phylogenetic position is still a matter of debate [282].

Most microsporidia are transmitted horizontally. They have developed an elaborate mechanism of invading host cells, in which a polar tube penetrates the cell membrane of the host and the content of the microsporidian cell (the spore) then enters the host cell (reviewed in [119]). However, many species can also be transmitted vertically (transovarially), and some species appear to be transmitted exclusively vertically [385]. Among the latter ones, three species have been demonstrated to in-

duce feminisation in their crustacean host *Gammarus duebeni* (see following section), and there is evidence for microsporidian induced sex-ratio distortion in many more crustaceans [385]. Because microsporidia are eukaryotes, they cannot be killed by antibiotics. Therefore, curing infected hosts is very complicated and experiments to study the effects of microsporidia on their hosts are hard to undertake.

1.4 Phenotypes of reproductive parasites

Four main host manipulations induced by reproductive parasites have been recognized to date. Three of them — feminisation, induction of parthenogenesis and male-killing — involve sex-ratio distortion. Here, the reproductive parasites spread in a population by causing their female hosts to produce more surviving daughters than uninfected females. In the fourth form of manipulation, cytoplasmic incompatibility, infected males are exploited by the parasites to kill offspring of uninfected females.

1.4.1 Feminisation

Conceptually, feminisation is the most straightforward reproductive manipulation that maternally inherited microorganisms induce. Genetically male hosts that have inherited the endosymbionts develop into functional females. Thus, infected females effectively produce a larger number of infected daughters than uninfected females produce daughters, so that inefficient transmission of the microorganisms can be offset. Infection with feminising endosymbionts results in a female biased population sex ratio. Even if infection with the endosymbionts does not impose a physiological cost on their hosts, feminising symbionts are deleterious to their hosts subsequent to their invasion because offspring are produced in an unfavourable sex ratio [83, 113].

Except for the many cases in haplodiploid species where feminisation also involves parthenogenetic reproduction (see the following section), feminisation has only been reported in isopod and amphipod crustaceans and in one butterfly species. The woodlouse *Armadillidium vulgare* (Isopoda) is the first species where feminisation was discovered and is the best studied system (reviewed in [322]). The sex determination of this woodlouse is female heterogamety. Thus, individuals with a WZ sex chromosome constitution develop into females, whereas ZZ individuals normally develop into males. The ZZ state triggers the development of an androgenic gland, which in turn produces a hormone that induces male development. *Wolbachia* appears to be able to disrupt the development of this androgenic gland in ZZ individuals so that they develop into functional females [241].

Wolbachia are passed on from infected *A. vulgare* females to around 90% of their offspring, leading to a strongly female biased sex-ratio in these broods. Most popu-

lations are not infected with *Wolbachia*, but in infected populations, *Wolbachia* can reach a prevalence of up to 64% [210, 323]. *Wolbachia* has profound and fascinating impacts on the sex determination system of *A. vulgare*, which will be discussed in Section 8.6.1.

A second well-studied species infected with feminising reproductive parasites is the shrimp *Gammarus duebini* (Amphipoda). Here, feminisation is induced by several species of microsporidia [186, 385]. The best-studied of these, *Nosema granulosis*, is characterized by a maternal transmission rate of about 75%, prevalence of up to 46% in natural populations, and incomplete feminisation efficiency [384, 216]. Although many microsporidia exhibit elaborate mechanisms of horizontal transmission, *Nosema granulosis* appears to be transmitted maternally only [185]. In contrast to *A. vulgare*, *Gammarus duebeni* normally exhibit environmental sex determination, indicating that feminisation is not confined to sex determination systems with female heterogamety. Nevertheless, as in *A. vulgare*, feminisation is achieved in *G. duebini* by interference of the microsporidia with androgenic gland differentiation [329].

Currently, the only insect species in which feminisation has been reported is the butterfly *Eurema hecabe*, where *Wolbachia* appear to be responsible for the sex ratio distortion [152]. However, the peculiar case of the supposedly feminising *Wolbachia* in the butterfly *Ostrinia scapularis* (which turned out to be a male-killer on closer inspection [211]) makes one sceptical towards the feminiser in *E. hecabe*.

1.4.2 Induction of parthenogenesis

The induction of parthenogenesis (PI) is a widespread host manipulation that occurs in haplodiploid species. In haplodiploidy, males are haploid and females are diploid. In most cases, this is because unfertilised eggs develop into males and fertilised eggs develop into females, a genetic system known as arrhenotoky. In contrast, parthenogenesis in the narrow sense, or thelytoky, denotes the situation where females produce only daughters without fertilisation of their eggs.

Parthenogenesis inducing endosymbionts operate by feminising unfertilised eggs that would normally develop into males, and the same adaptive reasoning as in normal feminisation can be applied. Strains of both *Wolbachia* and *Cardinium* are capable of PI. Table 1.2 lists some of the host species in which parthenogenesis is induced. In

Table 1.2: Examples of species that are infected with parthenogenesis inducing bacteria.

Taxon	Species	Causative agent	References
Insecta			
Hymenoptera	*Trichogramma* spp.	*Wolbachia*	[369, 366, 343]
	Diplolepis spp.	*Wolbachia*	[363, 303, 304]
	Encarsia spp.	*Cardinium*	[444]
Thysanoptera	*Franklinothrips vespiformis*	*Wolbachia*	[7]
Chelicerata			
Acari	*Bryobia* spp.	*Wolbachia*	[408]
	Brevipalpus phoenicisis	*Cardinium*	[409]

some instances, like *Trichogramma deinon* wasps, the endosymbionts only reach low infection frequencies so that males still occur and mate with females [369]. In other species (for example, in all mite systems investigated to date), PI-bacteria are fixed in host populations that consist of asexually reproducing females only. When these females are cured of the bacteria, they start producing males again. However, these males often fail to reproduce, either due to unsuccessful copulations or because their sperm does not fertilise the eggs [447, 7].

The induction of parthenogenesis is achieved by reproductive parasites in at least three different ways. The best (but still poorly) understood mechanism is gamete duplication, which has been found for example in *Trichogramma* spp. and *Diplolepis rosae* [368, 363]. In gamete duplication, the haploid set of chromosomes in the unfertilised egg is doubled during the first mitotic division. The offspring that emerges from such eggs is female and homozygous at all loci (automictic parthenogenesis). Cytologically, gamete duplication seems to result from one of at least two different processes: either, two sets of chromosomes are prevented from being pulled apart during anaphase of the first mitosis, or the two mitotic nuclei fuse after the first mitosis is completed.

The second mechanism of PI has been reported in mite species of the genus *Bryobia* [408]. Here, meiosis of the oocytes appears to be suppressed by the endosymbionts, so that infected eggs never enter a haploid state. Thus, offspring are identical to their mothers and can be heterozygous (amphimictic parthenogenesis). Finally,

in some species of the mite genus *Brevipalpus* feminisation of unfertilised eggs can also be achieved by reproductive parasites without restoring diploidy [409]. Infected unfertilised eggs develop into haploid females, whereas occasionally produced uninfected eggs develop into haploid males. To date, this is the only known case of an animal species that consist entirely of haploid individuals.

1.4.3 Male-killing

In male-killing, infected females produce a strongly female biased sex-ratio because the bacteria kill infected sons. Two distinct types of male-killing can be distinguished. In 'late male-killing', male offspring is killed in a late larval stage. This phenomenon has been reported in several mosquito species and is induced by microsporidian parasites (reviewed in [179]). However, since late male-killing is believed to enhance horizontal transmission, these microsporidia deviate from 'classic' reproductive parasitism and will not be considered further.

In 'early male-killing', which shall be referred to simply as 'male-killing' in what follows, infected males are killed as embryos. Obviously, reproductive parasites have no disadvantage in killing males since these represent a reproductive dead end anyway. However, there is even an advantage to male-killing if infected sisters of killed males benefit from the death of their brothers [414, 179, 174]. Such a benefit would result in infected females having more surviving daughters than uninfected females, so that the male-killers can spread in the population. This crucial reasoning, termed 'fitness compensation', rests upon kin selection: bacteria in males kill their male hosts and themselves to convey a selective advantage to their close relatives in the sisters of these hosts.

Fitness compensation may origin from several sources. First, the death of males may reduce the intensity of competition between siblings and release resources that can be used by the female offspring [174, 191]. Second, daughters may directly benefit from their brothers' death via cannibalism, as commonly occurs in ladybirds [174]. Finally, a reduced rate of inbreeding may be the basis of fitness compensation [414, 174].

Male-killing bacteria are widespread in insects, and an astonishing diversity of microorganisms has been found to induce this reproductive manipulation (see Ta-

ble 1.3). Recently, male-killing *Wolbachia* have also been reported in a pseudoscorpion, *Cordylochernes scorpioides* [449]. The prevalence of male-killer infections ranges from just above 0% in many *Drosophila* species to almost 100% in some butterflies. Consequently, male-killers can sometimes cause extremely female-biased sex-ratios in natural populations [200, 90]. Surprisingly, several different species or strains of male-killers sometimes co-occur within one host species and even host population. For example, a Russian population of the ladybird *Adalia bipunctata* was reported to be infected with male-killing *Rickettsia*, *Spiroplasma* and two strains of *Wolbachia* [248].

Almost nothing is known about how male-killing is achieved by the various bacteria. Only one system, the *Drosophila melanogaster – Spiroplasma poulsonii* association, has been studied molecularly. Here, it was demonstrated that the dosage compensation cascade in males needs to be switched on for male-killing to occur [401]. More precisely, loss of function mutations in any of the five proteins involved in the dosage compensation complex led to survival of males to the third instar larval stage (after which dosage compensation is generally required for survival). By contrast, conversion of genetic females to somatic males does not result in the expression of male-killing [336].

Table 1.3: Examples of species that are infected with male-killing bacteria. An exhaustive list of *Drosophila* species known to be infected with male-killing bacteria is given in Table 2.1.

Taxon	Species	Causative agent	References
Diptera	*Drosophila bifasciata*	*Wolbachia*	[173, 171]
	Drosophila innubila	*Wolbachia*	[88, 89, 191, 372]
	Drosophila melanogaster	*Spiroplasma*	[276, 273, 275, 274]
Coleoptera	*Brachys tesselatus*	*Rickettsia*	[240]
	Adalia bipunctata	several	[419, 172, 248, 348]
	Tribolium madens	*Wolbachia*	[108]
Lepidoptera	*Acraea encedon*	*Wolbachia*	[172, 204, 205]
	Hypolimnas bolina	*Wolbachia*	[91, 90, 61, 156, 60]
	Ostrinia scapulalis	*Wolbachia*	[211]
Hymenoptera	*Nasonia vitripennis*	*Arsenophonus*	[360, 422, 125, 11]

1.4.4 Cytoplasmic Incompatibility

The first note on cytoplasmic incompatibility (CI) dates back to 1938, when Marshall observed that males from one strain of the mosquito *Culex pipiens* were incompatible with females from another strain, but not vice versa [252]. This phenomenon was further investigated in the fifties by Ghelelovitch and in particular Laven [124, 237, 238, 239]. These early investigations demonstrated that the incompatibility trait was inherited maternally, and that beside unidirectional incompatibility also bidirectional incompatibility occurs between strains. In the early seventies a connection was drawn by Yen & Barr [440, 441] between CI and the intracellular bacterium *Wolbachia* that had long been known to infect *Culex pipiens*: unidirectional CI occurs when infected males mate with uninfected females, in which case the offspring suffer a high mortality at early stages of their development. In bidirectional CI, both directions of a cross are incompatible due to infection with different strains of CI-inducing microorganisms.

Today, CI is generally believed to be the most common form of reproductive parasitism. CI-inducing bacteria are not only known to occur in all of the major insect orders, but also in mites and woodlice (see Table 1.4). It has long been believed that CI is induced by *Wolbachia* only, but recently, the unrelated bacterium *Cardinium hertigii* has also been demonstrated to induce CI [164].

CI is currently explained by a modification-rescue principle [415], according to which sperm is modified by the bacteria. The same or a similar strain of bacteria must be present in the eggs to 'rescue' this modification and enable normal development. Of the three different models that have been proposed to explain this pattern, the 'lock-and-key' model fits the empirical findings most parsimoniously [306]. According to this model, the bacteria produce a substance in sperm (the 'lock') that binds to a component of the pronucleus, preventing its normal development. The CI-bacteria in the egg produce another substance (the 'key') which binds to the lock, thereby removing or inactivating it and enabling normal development [415]. However, this model still awaits empirical support from molecular studies. For a more detailed discussion of the different hypotheses on the mechanism of CI I refer to Section 5.1.1 and Ref. [306].

Table 1.4: Examples of species that are infected with CI-inducing bacteria.

Taxon	Species	Causative agent	References
Insecta			
Diptera	*Culex pipiens*	*Wolbachia*	[237, 440, 314, 85]
	Drosophila simulans	*Wolbachia*	[155, 22, 270, 265]
Coleoptera	*Tribolium confusum*	*Wolbachia*	[405, 426, 109]
Lepidoptera	*Ephestia kuehnellia*	*Wolbachia*	[338, 339]
Hymenoptera	*Nasonia* spp.	*Wolbachia*	[36, 37, 300]
	Leptopilina heterotoma	*Wolbachia*	[397, 396, 281]
	Encarsia pergandiella	*Cardinium*	[164, 446]
Chelicerata			
Acari	*Tetranychus* spp.	*Wolbachia*	[34, 394]
Crustacea			
Isopoda	*Cylisticus convexus*	*Wolbachia*	[279]

Cytologically, it has been demonstrated that in incompatible crosses, the paternal chromosomes do not condense, are damaged and finally lost during the first mitotic divisions ([36, 49, 310], see also [388] for an excellent review). In diplodiploid and most haplodiploid species, this haploidisation leads to disrupted development and death of the embryos [34, 49, 397]. In the haplodiploid wasp *Nasonia vitripennis*, the haploid embryos survive and develop into males instead of females [36]. Although cytological and developmental studies provide important clues [388], the molecular mechanism of CI is still elusive. Some progress has been made recently by identifying proteins whose overexpression in males results in a CI-phenotype in embryos [70]. The normal function of these proteins suggest that *Wolbachia* disrupt the balanced expression of key regulators for cytoskeletal processes.

Cytoplasmic incompatibility clearly entails a drive for the bacteria that induce it. By killing offspring of uninfected females, the relative number of infected female offspring in the population is increased. This can offset fitness disadvantages of the bacteria like imperfect transmission or decreased fecundity of infected females, and lead to their spread and maintenance in the host population. Such spread of CI is predicted theoretically [54, 111, 390] and has been observed in the field [390]. Interest-

ingly, as soon as an CI-infection is established in a population, it becomes beneficial for females to be infected, even when carrying the bacteria involves some costs. In males, however, CI-inducing bacteria are serious pathogens that can substantially reduce the fitness of their host due to incompatible matings and possibly reduced fertility [361]. The reader is referred to Section 8.1.2 for a more detailed review of the infection dynamics of CI.

1.5 Scope of this thesis

In this thesis, I will construct mathematical models to resolve a variety of questions concerning the evolutionary biology of reproductive parasites. This is a field that only recently, at the end of the 1990s, began to fully develop and that has produced a fascinating range of observations and new questions. A thorough review of the current thinking about the ecology and evolution of reproductive parasites will be postponed to Chapter 8. In this section, I will introduce the questions that I pose in this thesis and that the subsequent Chapters 2 to 7 aim to answer.

1.5.1 Male-killing and sex chromosome meiotic drive

Several models have been constructed that investigate the spread of reproductive parasites through host populations (see Section 8.1). Some of these models have also considered infections with several strains of parasites, inducing either the same or different host manipulations [332, 117, 313, 120, 101]. However, in addition to reproductive parasites, host population can be infected with genetic 'parasites', nuclear selfish genetic elements that also spread through populations by harming their hosts (see Section 1.1.2). Thus, it is important to ascertain whether selfish genetic elements and reproductive parasites will influence each others' dynamics of spread and the conditions for maintenance in a host population.

Of particular interest are possible interactions between sex-ratio distorting reproductive parasites and X chromosome meiotic drive elements. These are X chromosomes carrying genes that lead to the degeneration of sperm carrying the Y chromosome. Thus, both cytoplasmic sex-ratio distorters and X chromosome drive elements result in a female-biased sex-ratio among the offspring of carriers (female or male, respectively). Following spread, both elements will also result in a female biased population sex-ratio. Because this sex-ratio bias can affect the dynamics of individual elements, it is reasonable to suppose that different elements might interfere.

In Chapter 2, I construct a model for the co-infection dynamics of male-killing endosymbionts and X chromosome drive elements. Both of these elements are common in the Diptera, although to date, no species has been reported to be infected with both (see Section 2.1.3). My model investigates the degree to which interference on

the population level can be expected to occur between male-killing bacteria and X chromosome meiotic drivers.

1.5.2 Reproductive parasites and the origin of haplodiploidy

Parthenogenesis inducing microbes are an impressive example of the way in which reproductive parasites can produce permanent changes in the genetic system of their hosts. Another genetic system that is common in arthropods is haplodiploidy. Here, males transmit their maternally derived genomes only (and often, do not have paternally derived chromosomes), whereas females pass on both maternally and paternally derived chromosome sets. Whereas much is known about the consequences of haplodiploidy, the evolution of this peculiar genetic system is still elusive.

Recently, it was proposed that male-killing bacteria might drive the transition to haplodiploidy [287]. The main assumption of this hypothesis is that endosymbionts kill males by destroying their paternally derived chromosomes. Empirical evidence concerning the habitat and ecology of the ancestors of extant haplodiploid species as well as a mathematical model was presented to support the hypothesis. However, this model suffers from neglect of two important factors: the conditions for invasion and persistence of the male-killing bacteria are not taken into account, and the difference in fitness of males and females resulting from uneven population sex-ratio is also not incorporated into the model. In Chapter 3, I construct a model that includes these factors. This model enables me to investigate the selective forces acting on male-killing endosymbionts and their hosts, and to delineate evolutionary pathways resulting from co-evolution between these two parties. I also construct a second model for the evolution of haplodiploidy mediated by CI-inducing endosymbionts.

1.5.3 The impact of male-killing bacteria on host evolutionary processes

Male-killing bacteria are known to have a strong impact on the population genetics of mitochondrial DNA [208, 169]. Tight linkage of mitochondrial genes with any other maternally transmitted genetic elements will lead to hitchhiking of mitochondrial variants with male-killing bacteria when the latter spread in a host population, and

can result in substantial reduction in mtDNA variability.

By contrast, little is known about the impact of male-killing bacteria on the population genetics of nuclear host genes. In particular, we can ask how basic population genetic variables such as the probability of fixation of alleles or the expected genetic variation will be influenced by the presence of male-killing bacteria. A first approach to this problem might be to use Wright's effective population size for sex-ratio biased populations instead of the actual population size. However, this may be misleading, because it does not take into account the peculiar within population gene flow patterns between infected and uninfected individuals in male-killer infected populations. I argue that mutations that arise in infected individuals are doomed because of these patterns. In Chapter 4 I will investigate this proposition, and derive formulae for fixation probabilities, fixation times and equilibrium genetic variation of nuclear alleles in male-killer infected populations.

1.5.4 The evolution of cytoplasmic incompatibility types

Many different CI-inducing *Wolbachia* strains are known. Some of these render their hosts bidirectionally incompatible, i.e., matings between hosts carrying different strains of infection produce few or no offspring. One unresolved question relates to how new incompatibility types evolve. The problem is that the new type must derive from an ancestral type with which it is not compatible. This question was previously studied theoretically within the lock-and-key framework outlined in Section 1.4.4 [59, 58, 78]. According to these models, the first evolutionary step towards a new CI-type is a mutation in either the modification gene (the lock) or the rescue gene (the key), and the second step is a mutation in the respective other gene. Notably, the second of these possibilities (transition via initial mutation in the rescue gene) is only feasible if the mutant strain occurs as a multiple infection with the wildtype strain [78].

In Chapter 5, I construct a model that incorporates two factors that were not considered in previous models. First, I assume that during maternal transmission, endosymbiont strains can become lost so that the egg is only infected with a subset of the strains that were present in the mother. This 'segregation' of strains is commonly observed in multiple infections [356, 300, 307, 228, 57]. I examine the importance

of this segregation for the evolution of new CI-types. I also examine the role of inbreeding (sibmating) and outbreeding (sibmating avoidance) in the evolution of CI-types. Inbreeding and outbreeding alter the probability of a particular strain interacting with itself, which may therefore alter the probability that 'spiteful' types can spread.

1.5.5 The evolution of endosymbiont density in doubly infected hosts

The development of quantitative PCR methods has allowed accurate measurement of the number of bacteria within hosts. This has permitted workers on *Wolbachia* to make comparisons between different host tissues, host developmental stages, bacterial strains, and infection states with different numbers of strains [281, 262, 183, 184, 280, 234]. However, there is a general lack of theory concerning the evolution of density of reproductive parasites, such that there are few hypotheses that can be tested by empirical studies.

One particular question that I tackle in Chapter 6 is how the density of maternally transmitted endosymbionts (including mutualists and reproductive parasites) can be expected to evolve in doubly infected hosts. Such double infections, especially with different strains of *Wolbachia*, are commonly observed in arthropods (see Section 6.1.1). Multiple infections with horizontally transmitted parasites have been predicted to lead to increased replication and virulence of these parasites because of intra-host competition for transmission to new hosts [290, 257, 10]. This important result is sometimes also applied to maternally transmitted endosymbionts like *Wolbachia* [281, 280, 234]. By contrast, I argue in Chapter 6 that there is no competition between maternally inherited endosymbionts if the double infection is stably maintained in the host population. I construct both a general model and a model of two CI-inducing strains to predict optimal endosymbiont densities in their hosts.

1.5.6 The dynamics of endosymbiont incidence across a clade of host species

Wolbachia and other reproductive parasites are known to spread within arthropods by occasional cross-species transfers (or 'host-switching'), rather than by co-speciation with their hosts. It is also known that *Wolbachia* are heterogeneously distributed across host taxa on different scales (see Section 7.1.1). However, the dynamics of spread and the observed overall incidence of parasites like *Wolbachia* within arthropods have not been modelled, nor have causes of heterogeneity of incidence between host taxa been examined.

In Chapter 7, I construct models to study the process of endosymbiont spread within a clade of host species. These 'first models' have as a main assumption that the probability of successful horizontal transmission between host species declines with increasing genetic distance between donor and recipient host species. This assumption is empirically justified by both transinfection experiments of *Wolbachia* and phylogenetic analyses. By means of computer simulations, I study the influence of different host phylogenies on the 'incidence dynamics' of endosymbionts.

Chapter 2

Male-Killing and Sex Chromosome Meiotic Drive

Abstract. This chapter explores the population genetics of a population infected with male-killing endosymbionts and meiotic drive X chromosomes, another selfish genetic element that is common in insects. Given the abundance of many selfish genetic elements and their diverse modes of action, it is important to study whether different elements can coexist within a population and whether they interfere with each other. Potentially, interference can range from mutual exclusion of the two selfish genetic elements via depression to no interference at all, or even to mutual amplification. I demonstrate that meiotic drive elements and male-killing can interfere antagonistically, so that both elements can force down the equilibrium frequency of the respective other element and even drive it extinct. This has implications also for the likelihood of co-incidence of these two selfish genetic elements.

The main results of this chapter have been published in *Evolution* [100].

2.1 Introduction

2.1.1 What is meiotic drive?

Usually, a certain copy of a gene is passed on, on average, to 50% of the offspring of its carrier. This Mendelian segregation – brought about by the elaborate mechanism of meiosis – represents a 'fair' solution for the inheritance of homologous alleles. However, a gene or a group of closely linked genes that manages to be passed on to more than half of the offspring clearly would have an advantage over its homologous counterpart and could thus spread in a population. This phenomenon has indeed been discovered in natural populations and is termed 'meiotic drive' (or sometimes 'segregation distortion'). Meiotic drive usually occurs in males: the driving element acts by interfering with the spermatogenesis of sperm that does not bear the driver such that mostly sperm carrying the driving element develop to maturity. Along with transposons, B chromosomes and many others, meiotic drive elements have been classified as 'selfish genetic elements' (SGEs) [421, 47].

Meiotic drive elements can be located on autosomes and on sex chromosomes. Examples for autosomal drive are the *SD* system in *Drosophila melanogaster*, the *t*-complex in mice and *spore killer* in the fungus *Neurospora* [246]. Many more cases are known of sex chromosome drive (see below). Here, drive is associated with distorted primary sex-ratios in offspring from males bearing the driving chromosome. Although there are reasons to believe that sex chromosome drive is indeed more common than autosomal drive [182, 246, 189], the greater number of known cases of sex chromosome drive may simply reflect the fact that sex chromosome drive leads to biased offspring sex-ratios and is thus by far easier to detect.

Sex chromosome drive has been reported in three mammals and two dioecious plants (see [189] for references), but the majority of cases and at the same time the ones that are of interest in the present context have been found in insects. Here, interestingly, almost all instances known so far come from the Diptera, with only one possible exception, the butterfly *Eucheira socialis* [392]. The reason for this apparent phylogenetic concentration is unknown. Within the Diptera, X chromosome drive seems to be more common than Y chromosome drive. It has been reported in fourteen species of *Drosophila* (see Table 2.1), four stalk-eyed flies (*Cyrtodiopsis dal-*

manni, *C.whitei*, *Diasemopsis sylvatica* and *Sphyracephala beccarrii*) and the tsetse fly *Glossina morsitans*. Y drive has been reported in the medfly *Ceratitis capitata*, the housefly *Musca domestica* and the two mosquitoes *Aedes aegypti* and *Culex pipiens* (see [189] for references). The fact that all these species belong to extensively studied groups – either because of their role as model organisms in genetic and evolutionary research (*Drosophila*, stalk-eyed flies) or because of their medical or economic importance – implies that a high proportion of species at least within the Diptera harbour sex chromosome drive elements.

In what follows, I will refer to the X chromosome which harbours the meiotic drive element as the X^D chromosome (D=Drive) and to the wild type chromosome as X^S chromosome (S=Standard). Accordingly, I will use the terms X^D males and X^S males.

2.1.2 How are driving X chromosomes balanced?

Without any counter-selection, a sex chromosome drive element would quickly spread to fixation, which would lead to extinction of the population due to lack of males if drive is complete [123, 21]. It has been suggested that this may indeed be what happens in most cases of newly arisen sex chromosome meiotic drive and that the observed cases of balanced meiotic drive elements represent but the 'tip of the iceberg' [52]. Nevertheless, for the extant meiotic drivers an important question concerns the selective forces that maintain their stable polymorphism in populations. Three such forces have been identified so far:

(1) Several studies have shown that driving X chromosomes can have fitness effects on individuals that carry them other than those directly related to drive. These effects are probably due to alleles that are tightly linked to the driving locus within inversions of the chromosome. In *Drosophila pseudoobscura* it has been reported that females homozygous for the driving element have a lower fitness than other females and that X^D males also have a lower fitness than X^S males [407, 75]. Curtsinger & Feldman have demonstrated theoretically that fitness effects in females can lead to a stable polymorphism of an X drive locus. By contrast, frequency independent fitness effects on males are by themselves not sufficient to maintain a polymorphism [75]. The main problem with this explanation for polymorphism is that inversions and linked

deleterious alleles are thought to emerge only gradually and therefore are unlikely to prevent fixation of the driver in the first place. Moreover, in some species where a stable polymorphism was reported, the driving chromosomes do not carry inversions [193, 264].

(2) For males, there may be a direct fitness disadvantage because a substantial proportion of their sperm is being killed. In experiments, X^D males of *Drosophila pseudoobscura* did not exhibit a reduced fertility when mated once with virgin females [437]. However, when they where mated many times with different females, their fertility was decreased compared to that of X^S males [20, 437]. Apparently, males with the meiotic drive element are more readily sperm depleted than males without it. Since it is plausible that males mate more often in a population with a female biased sex-ratio, this could lead to strong selection against the meiotic drive element when it is very common. However, for this frequency dependent fitness effect to entail a stable polymorphism on its own, the relative fertility of males must fall below the proportion of sperm that is not killed by the driver (i.e., below 1/2 in the case of full drive [187]). Another important result from experiments is that X^D males only fertilize a small fraction of eggs when females are remated with X^S males [437, 187, 429]. Reduced abilities in sperm competition is thus another candidate for maintaining a stable polymorphism. Although one might intuitively think that in contrast to sperm depletion this effect should become less and less important with an increasing availability of females in the population, Taylor & Jaenike showed that sperm competition can result in a locally stable polymorphism of X^D [376].

(3) In many species suppressors of drive have been found. Obviously, suppressors are selected for on the Y chromosome. Such Y-linked suppressors would go to fixation if the suppressor Y does not cause a viability or fertility reduction in the males that carry it. If the suppressor Y decreases the viability or fertility of males, a polymorphism of the two Y chromosomes can be maintained [188, 138]. In both cases, the equilibrium frequency of the driving X chromosome will be lowered by the invasion of the suppressor Y. Suppression of drive is also selected for on the autosomes because of the female biased population sex-ratio and the possible deleterious effects of the driving element. It has been demonstrated theoretically that such autosomal suppressors can only evolve if the fitness effects of the driving X on the males that

harbor it are not too severe, and that they can only spread to fixation if there are no such negative fitness effects [399]. The problem with evoking suppression of drive as the cause for polymorphisms of X-drive elements is that in many cases there may not be enough time for suppressors to evolve, because unbalanced driving chromosomes can spread very quickly to fixation.

2.1.3 Co-incidence of male-killers and X chromosome meiotic drive

To date, no species is known to be infected with male-killers and to also harbour driving sex chromosomes. However, this is likely to only reflect the fact that no broad survey has ever been conducted to study the distribution of either of these elements. In fact, many of the cases reported have only been found serendipitously [187, 276]. The best studied group is the genus *Drosophila*, where fourteen species harbour driving X chromosomes and fifteen species (including one ambiguous case) are infected with male-killers (see Table 2.1).

Given that there are several thousand species of *Drosophila* and that most of them have not been studied intensively, these incidences doubtlessly constitute only a tiny proportion of *Drosophila* species that carry male-killers and/or driving chromosomes. For example, Jaenike reported that out of nine mycophagous species of *Drosophila* that he studied (for other reasons), at least five were carrying driving X chromosomes [187]. Moreover, many male-killers in *Drosophila* occur at very low frequencies, so that they are likely to be detected only when large samples are used [198].

These arguments lead to the conclusion that both X chromosome meiotic drivers and male-killers may be very common, at least within certain groups of the Diptera. Both elements seem to have arisen several times independently (via mutation and horizontal transmission, respectively). Within the genus *Drosophila*, several species harbouring X chromosome drivers have closely related species that are infected with male-killers, e.g. *D. simulans* - *D. melanogaster*, *D. bifasciata* - *D. subobscura* and the three species of the quinaria-group listed in Table 2.1. Therefore, it is reasonable to assume that there are species where male-killers and X chromosome drivers encountered within one host population, and it is important to ascertain the outcome of such an encounter.

Table 2.1: Incidence of male-killing (MK) and X chromosome drive in the genus *Drosophila*. Where known, the male-killing agent is given in brackets (Wolb=*Wolbachia*, Spiro=*Spiroplasma*).

Subgenus	Group	Subgroup	Species	MK or drive	Ref.
Sophophora	melanogaster	melanogaster	*melanogaster*	MK (Spiro)	[276]
			simulans	drive	[105]
	obscura	affinis	*affinis*	drive	[404]
			athabasca	drive	[269]
			azteca	drive	[371]
		obscura	*bifasciata*	MK (Wolb)	[173]
			obscura	drive	[123]
			subobscura	drive	[147]
		pseudoobscura	*pseudoobscura*	drive	[407]
			persimilis	drive	[437]
	willistoni	bocainensis	*capricorni*	drive	[271]
			nebulosa	MK (Spiro)	[431]
		willistoni	*equinoxalis*	MK (Spiro)	[431]
			paulistorum	MK (Spiro)	[431]
			willistoni	MK (Spiro)	[431]
Drosophila	cardini		*neocardini*	MK (Spiro)	[272]
	guarani		*ornatifrons*	MK (Spiro)	[272]
	melanica		*paramelanica*	drive	[362]
	quinaria		*innubila*	MK (Wolb)	[191]
			quinaria	drive	[187]
			recens	drive	[187]
	repleta	mercatorum	*mercatorum*	MK (?)	[19]
	robusta	robusta	*robusta*	MK	[309]
	saltans	saltans	*prosaltans*	MK	[56]
	testacea		*neotestacea*	drive	[193]
	tripunctata	ii	*mediopunctata*	drive	[51]
			roehrae	MK	[400]
			paraguayensis	MK (Spiro)	[272]
	virilis	montana	*borealis*	MK	[50]

2.2 The Model

To investigate the interactions between male-killers and X chromosome drive elements I assume an infinitely large population where individuals reproduce sexually in discrete, non-overlapping generations. I assume an XY sex determination system with the heterogametic sex being the male. Without the action of the drive element 50% of the sperm produced by males are assumed to carry the Y chromosome, leading to a primary sex-ratio of 1:1. All individuals are fully characterized by their sex chromosomes and their infection state (uninfected or infected with male-killers).

I consider two types of X chromosomes, the standard, non-driving X^S and the driving X^D . Thus, with respect to the genotype there are three different types of females (X^SX^S, $X^SX^D$and X^DX^D) and two types of males (X^SY and X^DY). Drive is assumed to occur in X^D males only. A fraction d of their sperm is assumed to carry the driving chromosome and $1 - d$ is carrying the Y chromosome ($0.5 \leq d \leq 1$). I assume a maximum of two detrimental effects of the driving element. First, females homozygous for X^D are assumed to have their fitness reduced by s ($0 \leq s \leq 1$). Second, the fertility of X^D males can be reduced due to the fact that a fraction of their sperm is being killed or prevented from maturation. This effect is assumed to take place only when males mate with more than one female. Since females mate only once in my model, the relative fertility of X^D males therefore decreases with increasing ratio of females to males in the population. Figure 2.1 illustrates the relative numbers of offspring of X^S and X^D males.

Females can either be infected with male-killers or uninfected. A fraction t ('transmission rate', $0 \leq t \leq 1$) of their offspring are also infected with the male-killer. All sons which inherit the male-killer are assumed to be killed by the bacteria. The surviving siblings in a brood from an infected mother receive a fitness compensation which increases linearly with the mortality to a maximum value b when all sons are killed ('benefit', $0 \leq b \leq 1$).

The frequencies of uninfected and infected individuals will be denoted by p and q, respectively. Subscripts denote the sex chromosome state, where X stands for the wildtype X^S and D stands for the X^D chromosome. The recursion equations of the

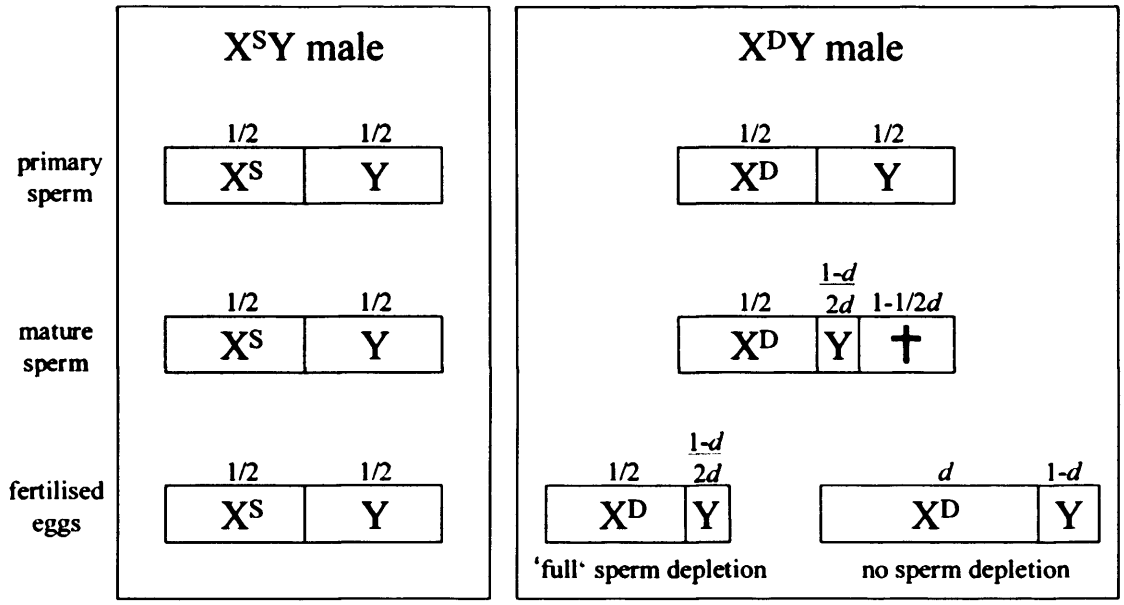

Figure 2.1: Illustration of the offspring production of X^S and X^D males. d is defined as the proportion of females in the offspring of X^D males, which is caused by the death of a fraction $1-1/(2d)$ of their (Y-bearing) sperm. In the model with sperm depletion (fertility function F_2), the relative number of offspring of X^D males will be between $1/(2d)$ and 1, depending on the population sex-ratio.

model are given by

$$
\begin{aligned}
p'_{XX} &= \frac{1}{\overline{W}}\left[2p_{XX}+p_{DX}+(1-t)(2q_{XX}+q_{DX})(1+tb)\right]p_{XY} \\
p'_{DX} &= \frac{1}{\overline{W}}\left[\left(p_{DX}+2p_{DD}+(1-t)(q_{DX}+2q_{DD})(1+tb)\right)p_{XY}+\right. \\
&\quad \left.\left(2p_{XX}+p_{DX}+(1-t)(2q_{XX}+q_{DX})(1+(2-2d)tb)\right)2dF_i(p_{XY},p_{DY})p_{DY}\right] \\
p'_{DD} &= \frac{1-s}{\overline{W}}\left[p_{DX}+2p_{DD}+(1-t)(q_{DX}+2q_{DD})(1+(2-2d)tb)\right]2dF(p_{XY},p_{DY})p_{DY} \\
p'_{XY} &= \frac{1}{\overline{W}}\left[\left(2p_{XX}+p_{DX}+(1-t)(2q_{XX}+q_{DX})(1+tb)\right)p_{XY}+\right. \\
&\quad \left.\left(2p_{XX}+p_{DX}+(1-t)(2q_{XX}+q_{DX})(1+(2-2d)tb)\right)(2-2d)F_i(p_{XY},p_{DY})p_{DY}\right] \\
p'_{DY} &= \frac{1}{\overline{W}}\left[\left(p_{DX}+2p_{DD}+(1-t)(q_{DX}+2q_{DD})(1+tb)\right)p_{XY}+\right. \\
&\quad \left.\left(p_{DX}+2p_{DD}+(1-t)(q_{DX}+2q_{DD})(1+(2-2d)tb)\right)(2-2d)F_i(p_{XY},p_{DY})p_{DY}\right] \\
q'_{XX} &= \frac{1}{\overline{W}}t(2q_{XX}+q_{DX})(1+tb)p_{XY} \\
q'_{DX} &= \frac{1}{\overline{W}}\left[t(q_{DX}+2q_{DD})(1+tb)p_{XY}+\right. \\
&\quad \left.t(2q_{XX}+q_{DX})(1+(2-2d)tb)2dF_i(p_{XY},p_{DY})p_{DY}\right] \\
q'_{DD} &= \frac{1-s}{\overline{W}}t(q_{DX}+2q_{DD})(1+(2-2d)tb)2dF_i(p_{XY},p_{DY})p_{DY}
\end{aligned}
\tag{2.1}
$$

The normalizing factor $\overline{W}$ is given by the sum of all expressions in the Equations 2.1.

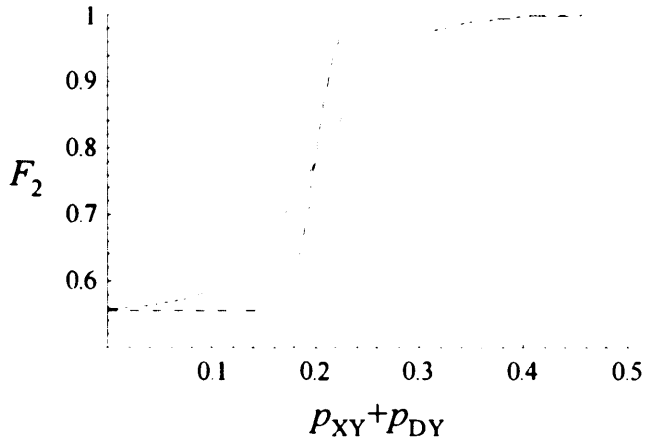

Figure 2.2: Possible courses of the fertility function F_2 (Equation (2.2)). Parameters take the values $d = 0.9$ for all three functions, and $\alpha = 25$, $\beta = 0.2$ (solid line), $\alpha = 100$, $\beta = 0.2$ (dashed line) and $\alpha = 25$, $\beta = 0.3$ (dotted line).

F_i denotes one of the following two functions for the relative fertility of X^D males:

$$\begin{aligned} F_1(p_{XY}, p_{DY}) &:= 1 \\ F_2(p_{XY}, p_{DY}) &:= \frac{1}{2d} + \frac{2d-1}{2d(1+\exp[\alpha(\beta - p_{XY} - p_{DY})])} \end{aligned} \tag{2.2}$$

With F_1 applied, X^D males always have the same fertility as X^S males. F_2 describes a sigmoid fertility function for X^D males which decreases from approximately 1 when the sex-ratio is even to approximately $1/(2d)$ when the sex-ratio is extremely female biased. (The latter term corresponds to the relative amount of functional sperm that X^D males produce compared to X^S males.) The parameters α and β describe how fast fertility declines with decreasing sex-ratio and at what sex-ratio this decline is strongest, respectively. Examples for the function F_2 are given in Figure 2.2.

2.3 Results

2.3.1 Model of drive without male-killers

When no male-killers occur in the population ($q_{XX} = q_{DX} = q_{DD} = 0$), the recursion Equations 2.1 can be simplified to

$$\begin{aligned}
p'_{XX} &= \frac{1}{\overline{W}}(2p_{XX} + p_{DX})p_{XY} \\
p'_{DX} &= \frac{1}{\overline{W}}\left[(p_{DX} + 2p_{DD})p_{XY} + (2p_{XX} + p_{DX})2dF_i(p_{XY}, p_{DY})p_{DY}\right] \\
p'_{DD} &= \frac{1-s}{\overline{W}}(p_{DX} + 2p_{DD})2dF(p_{XY}, p_{DY})p_{DY} \\
p'_{XY} &= \frac{1}{\overline{W}}\left[(2p_{XX} + p_{DX})p_{XY} + (2p_{XX} + p_{DX})(2-2d)F_i(p_{XY}, p_{DY})p_{DY}\right] \\
p'_{DY} &= \frac{1}{\overline{W}}\left[(p_{DX} + 2p_{DD})p_{XY} + (p_{DX} + 2p_{DD})(2-2d)F_i(p_{XY}, p_{DY})p_{DY}\right]
\end{aligned} \tag{2.3}$$

with

$$\overline{W} = 4(p_{XX} + p_{DX} + p_{DD})(p_{XY} + p_{DY}F_i(p_{XY}, p_{DY})) - (p_{DX} + 2p_{DD})2dsp_{DY}F_i(p_{XY}, p_{DY})$$

Let us first consider this model for the fertility function F_1, i.e. when X^D males always have the same fertility as X^S males. Equations (2.3) then represent a special case of a model developed by Curtsinger & Feldman [75], with only a different definition of the parameters. The nontrivial equilibrium is given by

$$\begin{aligned}
\hat{p}_{XX} &= \frac{[1 + 2d(2s-1)]^2}{2ds[4d(1+4s-d)-1]} \\
\hat{p}_{DX} &= \frac{(4d^2-1)[1 + 2d(2s-1)]}{2ds[4d(1+4s-d)-1]} \\
\hat{p}_{DD} &= \frac{(2d-1)^2(1-s)}{s[4d(1+4s-d)-1]} \\
\hat{p}_{XY} &= \frac{[4d(1-d+s)-1][1 + 2d(2s-1)]}{2ds[4d(1+4s-d)-1]} \\
\hat{p}_{DY} &= \frac{4ds(2d-1) - (2d-1)^3}{2ds[4d(1+4s-d)-1]}
\end{aligned} \tag{2.4}$$

It can be demonstrated that this polymorphic equilibrium exists and is stable if $s > (2d-1)/(4d)$ (see [75] for a full analysis). For $s \leq (2d-1)/(4d)$, X^D goes to fixation for any $d > 1/2$. For example, if drive is complete ($d = 1$), the fitness reduction in females homozygous for the driving chromosome must be greater than $1/4$ to maintain a polymorphic equilibrium and prevent the population from extinction.

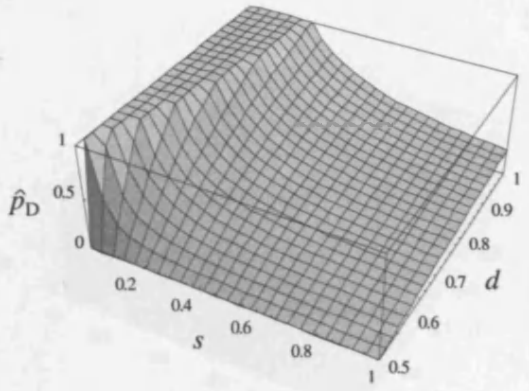

Figure 2.3: Equilibrium frequencies $\hat{p}_D$ in the model without male-killers and with the fertility function $F_1 \equiv 1$, depending on the the fitness reduction s in homozygous females and the strength of drive d.

To visualize the equilibrium of the driving element it is convenient to use the frequency of the X^D chromosome defined as

$$p_D := \frac{p_{DX} + 2p_{DD} + p_{DY}}{2p_{XX} + 2p_{DX} + 2p_{DD} + p_{XY} + p_{DY}} \tag{2.5}$$

The equilibrium frequency $\hat{p}_D$ can be reduced to the simple formula

$$\hat{p}_D = \frac{2d - 1}{4ds}. \tag{2.6}$$

It can be demonstrated that at the same time, $\hat{p}_D$ also equals the equilibrium frequency of X^D among females and among males, i.e.

$$\hat{p}_D = \frac{2\hat{p}_{DD} + \hat{p}_{DX}}{2(\hat{p}_{XX} + \hat{p}_{DX} + \hat{p}_{DD})} = \frac{\hat{p}_{DY}}{\hat{p}_{XY} + \hat{p}_{DY}}. \tag{2.7}$$

Values of $\hat{p}_D$ are shown in Figure 2.5 for all relevant values of the parameters s and d. It can be seen that the equilibrium frequency of the X^D chromosome is an increasing function of the driving strength d and an decreasing function of the cost s in homozygous females.

When the function F_2 is applied, the system could not be solved analytically. Equilibrium frequencies were therefore obtained by computer simulations. A state was considered being in equilibrium if at least 10,000 generations had been simulated and if all frequencies in two succeeding generations differed by less than 10^{-10}.

Values of $\hat{p}_D$ resulting from these simulations are shown in Figure 2.4. Interestingly, the driver can still go to fixation for small values of d, but not for greater values. The reason for this is that the fertility reduction F_2 of males is high only when the population sex-ratio is strongly biased towards the females. For weak meiotic drivers, even a high frequency does lead to only minor sex-ratio distortions, so that there is not sufficient counter-selection to prevent the X^D chromosome from becoming fixed.

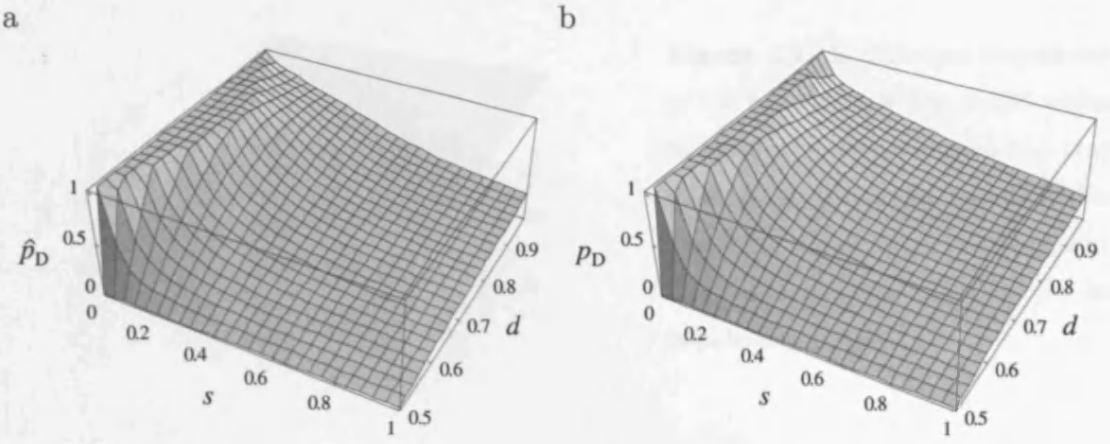

Figure 2.4: Equilibrium frequencies $\hat{p}_D$ in the model without male-killers and with the fertility function F_2, depending on the the fitness reduction s in homozygous females and the strength of drive d. Other parameters take the values $\alpha = 25$ and (a) $\beta = 0.2$, (b) $\beta = 0.3$.

2.3.2 Model of male-killers without drive

As a second preliminary investigation I will consider the case where male-killers, but no driving X chromosomes are present in the population. The model can strongly be simplified to the following recursion equations:

$$\begin{aligned} p'_{XX} = p'_{XY} &= \frac{p_{XX} + (1-t)q_{XX}(1+tb)}{2p_{XX} + (2-t)q_{XX}(1+tb)} \\ q'_{XX} &= \frac{tq_{XX}(1+tb)}{2p_{XX} + (2-t)q_{XX}(1+tb)} \end{aligned} \tag{2.8}$$

Since there is only one type of males in this model and their frequency equals that of uninfected females, the system can be further reduced without loss of information if I only consider the females in the population. If the frequency of infected females is denoted by q, the simple recursion equation

$$q' =: f(q) = \frac{t(1+tb)q}{1+tbq} \tag{2.9}$$

can be obtained. The only nontrivial fixed point of this system can easily be shown to be

$$\hat{q} = \frac{t(1+tb)-1}{tb}. \tag{2.10}$$

Since $\left.\frac{\mathrm{d}f}{\mathrm{d}q}\right|_{q=\hat{q}} = \frac{1}{t(1+tb)}$, this fixed point is stable for

$$t(1+tb) > 1, \tag{2.11}$$

which at the same time is the condition for the fixed point to be positive. Thus, the fitness reduction that the male-killers face because of their inefficient transmission

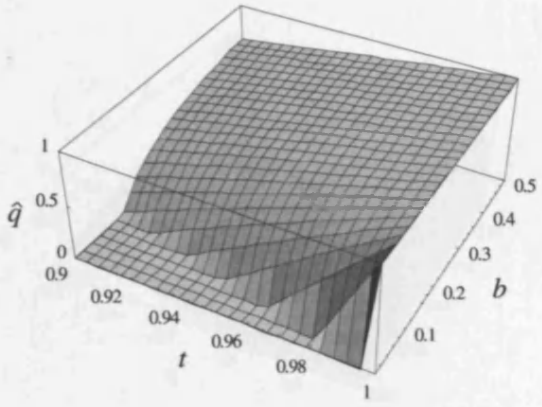

Figure 2.5: Equilibrium frequencies $\hat{q}$ of the male-killer in the model without meiotic drive depending on the transmission rate t and the benefit b. Note that for a subset of parameters b and t, the male-killer cannot invade the host population.

must be offset by the fitness compensation for them to invade and establish in a population. Figure 2.5 shows the fixed point $\hat{q}$ for different values of t and b.

2.3.3 Model of male-killers and drive without sperm depletion

To study the dynamics of a population infected with male-killing bacteria and at the same time harbouring X chromosome meiotic drive elements, I will first assume that X^D males are always as fertile as X^S males, i.e. I will apply the fertility function $F_1 \equiv 1$. The system could not be solved analytically. In all simulations conducted, equilibria could be obtained and were never found to be dependent on the initial frequencies.

Simulations showed that the equilibrium frequency of the male-killers declines with increasing strength d of the meiotic driver (Figures 2.6a and 2.6c). In the case of strong drive and low male-killer transmission rates, the male-killers can even go extinct following invasion of the driver. The reason for this can be found in the female-biased primary sex-ratio in offspring from X^D males: in broods sired by X^D males, there are fewer sons that can be killed by the bacteria, and the resulting fitness compensation for the daughters is lower.

Conversely, the frequency of the meiotic drive element is hardly influenced by the co-occurrence of male-killers (Figures 2.6b and 2.6d). There is however a very slight decrease in the equilibrium frequency of the X driver, which is not discernable in Figures 2.6b and 2.6d. This is due to the following interesting effect. If an infected female mates with an X^S male, nearly half of the offspring are killed and the surviving daughters accordingly receive fitness compensation. This also includes a

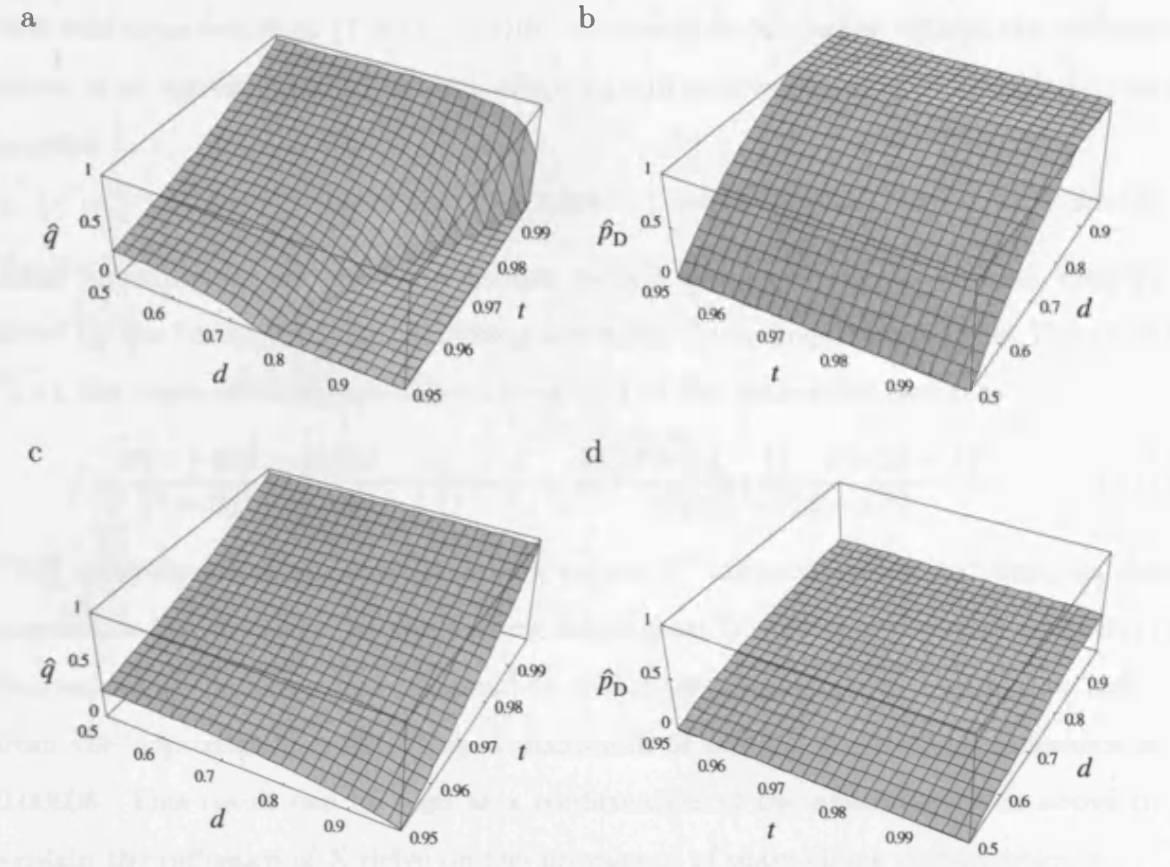

Figure 2.6: Equilibrium frequencies of male-killers (left diagrams a and c) and meiotic drive elements (right diagrams b and d) in the model without sperm depletion. Parameters take the values $b = 0.1$ and (a, b) $s = 0.2$, (c, d) $s = 0.6$. Note that for better visibility, axes are opposite in plots (a) and (c) compared to (b) and (d).

fitness increase for the X^S chromosome the daughters have inherited from their father. However, if an infected female mates with an X^D male, only few or no sons are produced and can be killed. Thus, there is less fitness compensation for the females and also for the X^D chromosome that was passed on from the father to the daughters. In total, the male-killer slightly decreases the relative fitness of the X^D chromosome by increasing the fitness of the X^S more than that of the X^D chromosome via fitness compensation.

Using the results from the previous two sections, I now derive an approximation for the equilibrium frequency of male-killers. Justified by the simulation results and disregarding the small effect just described, I assume that the equilibrium frequency of the X^D chromosome is not altered by the male-killers and is thus given by Equation (2.6). First, let me recall that the amount of fitness compensation that surviving siblings receive depends on the genotype of the father, being either $(1 + tb)$ in case

of a wild-type father or $(1 + (2 - 2d)tb)$ in case of an X^D father. When the meiotic driver is at equilibrium, on average offspring will receive fitness compensation of an amount

$$1 + (1 - \hat{p}_D)tb + \hat{p}_D(2 - 2d)tb = 1 + tb[1 - \hat{p}_D(2d - 1)]. \quad (2.12)$$

After replacing the fitness compensation term $1 + tb$ in the model without meiotic drive by the term (2.12) and inserting the equilibrium frequency $\hat{p}_D$ from Equation (2.6), the approximated equilibrium frequency of the male-killer becomes

$$\hat{q} \approx \frac{t\{1 + tb[1 - \hat{p}_D(2d - 1)]\} - 1}{\{1 + tb[1 - \hat{p}_D(2d - 1)]\} - 1} = \frac{4ds(t^2b + t - 1) - t^2b(2d - 1)^2}{tb[4ds - (2d - 1)^2]}. \quad (2.13)$$

This approximation works well as long as the X^D chromosome is not fixed in the population (in which case $\hat{p}_D$ can become larger than 1). For example, the equilibrium frequencies of the male-killer obtained by computer simulations for Figure 2.6a differ from the approximation (2.13) by a maximum of 0.0547; the average difference is 0.00606. This result can be seen as a confirmation of the reasoning given above to explain the influence of X drive on the prevalence of male-killing endosymbionts.

2.3.4 Model of male-killers and drive with sperm depletion

I now consider the model with the fertility function F_2 applied. This means, I will now assume that sperm depletion occurs in X^D males, reducing their fertility with increasingly female biased sex-ratio (see Equation 2.2 and Figure 2.2). Again, computer simulations were conducted to obtain equilibrium frequencies. In the case of very high values of α, resulting in a very steep decline of F_2 with increasingly biased sex-ratio, no equilibria were reached. Rather, the system was found to converge to cycles of different periods. However, in this study I neglected this type of dynamical behaviour and I will present only data where equilibrium frequencies where obtained. These were always reached irrespective of whether the male-killer or the driver was introduced first in the population.

In Figure 2.7 the equilibrium frequencies of male-killers and meiotic drive element are shown for the same parameters as in Figure 2.6. As can be seen in Figure 2.7a, the frequency of the male-killer can again be decreased considerably by the occurrence of the X^D chromosome and the male-killer can become extinct. However, the decrease

in the equilibrium frequency does only occur for small values of t, whereas it is absent for high transmission rates.

The reason for this becomes clear when looking at Figure 2.7b: the equilibrium frequency of the meiotic driver is strongly reduced for high transmission rates of the male-killer. Apparently, the male-killer decreases the fitness of the driving chromosome by distorting the population sex-ratio towards females. In addition to the sex-ratio distortion that is caused by the driver itself, this leads to further reduction of the relative fertility of X^D males. In some cases, the combined effects of reduced fertility and costs in females homozygous for the driver can thus even lead to extinction of the X^D chromosome (see right edge in Figure 2.7b).

Merging the two results in this section, the interactions on population level between male-killers and X chromosome drivers seem to be antagonistic when sperm depletion occurs, with the presence of one of these elements decreasing the equilibrium frequency of the other and possibly leading to its extinction.

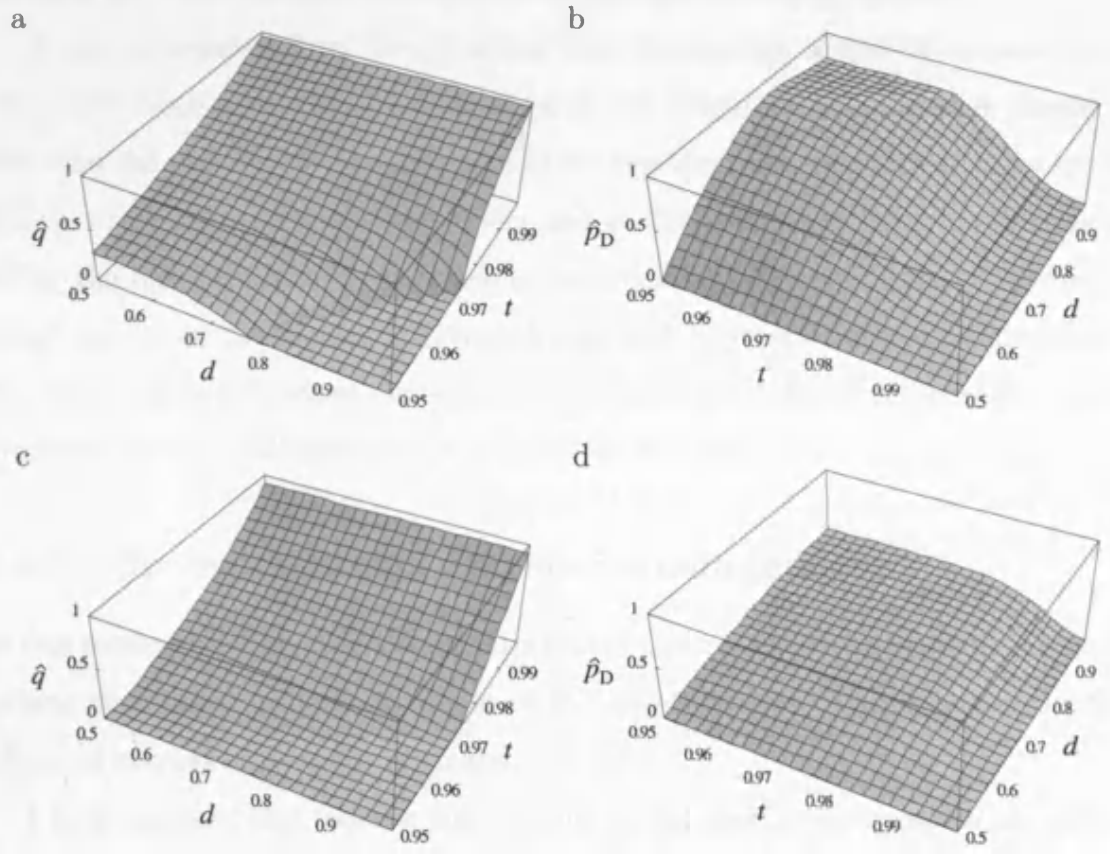

Figure 2.7: Equilibrium frequencies of male-killers (left diagrams a and c) and meiotic drive elements (right diagrams b and d) in the model with sperm depletion. Parameters take the values $\alpha = 25$, $\beta = 0.2$, and (a, b) $b = 0.1$ and $s = 0.2$ and (c, d) $b = 0.05$, $s = 0.6$.

2.4 Discussion

2.4.1 Inferences from the model

I have demonstrated that sex chromosome drive chromosomes can substantially lower the equilibrium frequency of male-killing endosymbionts. This is because the attendant female biased primary sex-ratio in offspring from X^D bearing males leads to fewer sons being killed by the male-killer and therefore, reduced fitness compensation for daughters of infected females. As a result, a population in which X chromosome drive occurs may be immunised by the driver against male-killer infections, and the invasion of a driving X chromosome may lead to the extinction of an existing male-killer. Conversely, male-killers may also decrease the equilibrium frequency of X chromosome driving elements. However, this effect depends crucially on the assumption that the fertility of X^D bearing males declines with increasingly female biased population sex-ratio due to sperm depletion. If this is the case, male-killers can prevent the invasion of X chromosome driving elements or lead to their extinction.

It can be inferred from these findings that the fraction of species or populations that carry both male-killing endosymbionts and driving X chromosomes should be less than the product of the incidences of the two elements. Since there is no species known to be infected with male-killers and at the same time bear X chromosome drive, the data available to date are in accord with this hypothesis. However, no broad survey on the incidence of male-killers and X drivers has been conducted so far, and it seems premature therefore to view the scarce data as corroboration for the predicted antagonistic interference of these two elements.

2.4.2 Sperm depletion and sperm competition

In this section I will discuss the assumptions of the models with regard to the mechanisms that maintain polymorphism of the meiotic driver, especially the complex effects of sperm reduction in X^D males.

I have assumed that females homozygous for the driving chromosome are subject to a fitness reduction. Depending on the strength of drive and the strength of the effects of sperm depletion, a certain threshold value of this fitness reduction has been found to be necessary to prevent the driver from going to fixation. This assumption

has been motivated by empirical findings in populations of *Drosophila pseudoobscura* [407, 75], where X^DX^D females have been found to be less viable and fertile than both X^DX^S and X^SX^S females. Other frequency independent effects of the driver for which empirical evidence exists and that have been used in models include reduced viability in X^D males and reduced or increased fitness in heterozygous females [407, 75]. What impact would the incorporation of such fitness effects have on the results of the model with male-killers and X drivers? Since all these effects do not depend on properties of the population that are affected by the male-killer, for example the population sex-ratio or the secondary sex-ratio in broods, it can be expected that they do not lead to results that are qualitatively different from the ones obtained in the model. Conversely, frequency independent fitness effects can alter the equilibrium frequency of the male-killer only via affecting the equilibrium frequency of the driver, so that again no new types of influences can be expected.

More interesting is the impact of sperm depletion on the joint dynamics of male-killer and X chromosome meiotic driver. It has been found that when mated repeatedly, the fertility of X^D males declines more rapidly than that of wild-type males [435, 436, 187]. Since a female-biased population sex-ratio is likely to lead to an increased male mating rate, I have assumed in the second part of my model that the relative fertility of X^D males decreases with increasing ratio of females to males. In particular, I have assumed that the minimum relative fertility of X^D males is proportional to the amount of sperm that survives. For example, if drive is complete, 50% of sperm would killed by the driver, and the relative fertility of the X^D male would converge towards 0.5 with increasingly female-biased population sex-ratio. The proportionality function is the simplest assumption regarding the relationship between sperm number and fertility, but it is not the only one that is plausible. In other functions that have been proposed, fertility rises asymptotically or sigmoidally towards the maximum with increasing sperm number [144]. It has been shown that the sigmoid function can lead to a stable polymorphism of the driver, whereas the asymptotic function and the linear function used in my model cannot prevent the driver from going to fixation without any other effects [144, 187]. With a monotonous asymptotic function, the fertility of X^D males would always be higher than with the linear function. Therefore, the equilibrium frequency of the driver can be expected

to be higher with this type of function, and the subset of the parameter space where the meiotic driver goes extinct because of an invasion by male-killers becomes more restricted. Conversely, with a sigmoid function applied, the equilibrium frequency of the driving chromosome may be decreased and the driver may become extinct more readily.

In contrast to the assumptions of the model, females may also mate multiply. This could lead to direct sperm competition between wild-type sperm and sperm from X^D males. In *Drosophila pseudoobscura*, the fertility of X^D males is lowest when females are allowed to mate multiply with different males. Similarly, in the stalk-eyed fly *Cyrtodiopsis whitei* the proportion of offspring sired by X^D males was found to be much smaller than that of X^S males if the female was jointly inseminated by both types of males [429]. One might intuitively expect that the adverse effects of sperm competition on X drivers are strongest with an even population sex-ratio and decline with females becoming more frequent. Thus, sperm competition should not result in a stable equilibrium of the driver. However, a recently developed model yielded a polymorphic fixed point for X^D that was locally stable, with stochastic fluctuations in the X^D frequency or the population sex-ratio leading to fixation of the meiotic driver [376]. The reason for this seems to be the (realistic) assumption that the performance of X^D sperm in competition with X^S sperm declines with male mating rate.

What can we expect to happen if we introduce a male-killer into a population in which the polymorphism of X^D is maintained by sperm competition only? Provided that the male-killer can invade and reach a considerable frequency, the population sex-ratio would be further distorted towards the females. This leads to less sperm competition and thus may increase the fitness and equilibrium frequency of the driver, which is in sharp contrast to my findings with sperm depletion effects only. Eventually, the invasion of even weak male-killers might lead to the X driver going to fixation, exposing the population to extinction.

2.4.3 Suppressors of drive

The dynamics of X chromosome drivers co-occurring with both autosomal and Y-linked suppressors have been shown to be complicated [399, 138]. Given the surprising effects that can occur, especially in the case of autosomal suppressors [399], no

attempt shall be made here to predict the outcome if the population is in addition infected with male-killing endosymbionts. However, under some circumstances suppressors of drive can be expected to become fixed in the population [188, 399], as has been found in *Drosophila simulans* [264]. If suppression of drive is not complete, this leads to an effectively weaker driver, the co-dynamics of which with male-killers are readily covered by the model presented here.

2.4.4 Autosomal and Y chromosome drive

Although not as common as X drivers, cases of autosomal and Y chromosome drive are also known in insects (see [189] for references) and it therefore may be important to ascertain whether these types of drivers also interfere with male-killers.

Since autosomal meiotic drivers do not alter the primary sex-ratio, it seems obvious that they do not affect the infection dynamics of male-killers in any way. Conversely, autosomal drivers are thought to be influenced by sperm depletion and sperm competition in the same way as X chromosome drivers. Therefore, male-killers can be expected to decrease the equilibrium frequency of the driver if sperm depletion is important or increase it if sperm competition is important (see Section 2.4.2). Since autosomal drivers do not affect the population sex-ratio, both of these effects may be less pronounced than in the case of X drivers, which by themselves distort the population sex-ratio in the same direction as male-killers do. Of special interest is the case of *Drosophila melanogaster*, which harbours both male-killers of the genus *Wolbachia* and the autosomal meiotic drive element *SD* [276, 151]. However, in this species both male-killers and autosomal drivers seem to occur only at very low prevalence, so that I predict that no mutual inference will be detectable.

Driving Y chromosomes should have the opposite effect on the equilibrium frequency of male-killers as X^D chromosomes. They lead to male-biased primary sex-ratios in broods sired by Y^D males and thereby allow surviving daughters to gain more fitness compensation when sons are killed by the male-killers[1]. Again, the effects of male-killers on Y drivers should be in principle the same as for X and autosomal drivers. Interestingly, populations with male-killers and Y^D chromosomes may ex-

[1]However, if drive is complete, there will be no daughters to receive the fitness compensation! In this case, the frequency of the male-killer will not be influenced by the Y driver.

hibit an even population sex-ratio, although the population size might be severely reduced due to the combined action of these two selfish genetic elements.

2.4.5 Drive and other reproductive parasites

Cytoplasmic incompatibility (CI) is one of the most common phenotypes exhibited by reproductive parasites, especially *Wolbachia* (see Chapter 1.4.4). Can we expect any interference between CI-inducing bacteria and sex chromosome meiotic drive elements? As has been shown, male-killers may affect the equilibrium frequency of X chromosome drivers by distorting the population sex-ratio. Since CI (in diplodiploid species) does not alter the population sex-ratio, CI-inducing endosymbionts cannot be expected to have an impact on driving elements. Conversely, neither primary nor population sex-ratio influence the dynamics of CI-infections, so that meiotic drivers should have no effect on the equilibrium frequency of CI-inducing bacteria. Thus, no interference between meiotic drivers and CI-inducing endosymbionts can be expected, a hypothesis that has been confirmed for some parameters in computer simulations (results not shown).[2]

However, feminising endosymbionts are likely to influence the dynamics of drivers in nearly the same way as male-killers. This is because as we have seen, the main effect that male-killers have on drivers is due to the distorted population sex-ratio. Since feminisation is a more efficient type of reproductive parasitism and should therefore result in higher equilibrium frequencies and more female-biased population sex-ratios, the effect of feminisers on meiotic drive elements is likely to be even stronger than that of male-killers. On the other hand, there is again no reason to believe that meiotic drivers have an impact on the equilibrium frequency of feminising endosymbionts.

[2]Interestingly, CI-inducing endosymbionts and X chromosome drivers may interfere indirectly in a synergistic way: since CI-inducing endosymbionts can exclude male-killing endosymbionts from a population [101], they prevent X chromosome frequency to be reduced by male-killers. *Vice versa*, by keeping male-killer frequency low, X chromosome drivers may facilitate the invasion of CI-inducing bacteria.

Chapter 3

Reproductive Parasites and the Origin of Haplodiploidy

Abstract. In this chapter, I investigate theoretically whether haplodiploidy might evolve as an outcome of the co-evolution between maternally inherited endosymbionts and their hosts. First, I extend a previously developed model [287] that involves maternally inherited endosymbionts that kill male offspring by eliminating the paternal genome. Second, I develop a model that involves bacteria that induce CI. Based on these models, I explore the co-evolutionary events that might occur between hosts and symbionts. My results suggest that both with male-killers and CI-inducing endosymbionts, the hosts are likely to develop increased viability of haploid males, which can be considered a pre-adaptation to haplodiploidy. In addition, populations with haploidising male-killers can in some cases evolve directly towards a genetic system of paternal genome elimination, a special form of haplodiploidy. Combining these results with empirical findings, it appears that the ecological setting in which the extant haplodiploid species evolved favoured male-killing bacteria, whilst from the mechanistic point of view, CI-inducing bacteria seem better candidates in mediating the evolution of haplodiploidy.

An abridged version of this chapter has been published in the *Journal of Evolutionary Biology* [97].

3.1 Introduction

One of the most fascinating possible impacts of reproductive parasites on their hosts concerns the transition between different genetic systems, as exemplified most convincingly by the induction of parthenogenesis (see Section 1.4.2). Another interesting transition that might have been caused or facilitated by reproductive parasites is the transition to haplodiploidy.

Haplodiploidy is a genetic system in which males transmit only their maternal genome, whilst females transmit both maternally and paternally inherited chromosomes as in diplodiploid species. The most common form of haplodiploidy is arrhenotokous haplodiploidy, sometimes referred to as arrhenotoky or just haplodiploidy. Here, males develop from unfertilised eggs and are haploid throughout their lives. The second form of haplodiploidy is paternal genome elimination (PGE). Here, males develop from fertilised, diploid eggs, but the paternal genome is not passed on to their offspring. The paternal chromosomes are eliminated either in the germ line only, or they are eliminated in the soma and the germ line. Thus, with PGE, males can be diploid or haploid. Note that with respect to inheritance, there is no difference between arrhenotokous haplodiploidy and the different forms of PGE.

After providing some background on the incidence of the different forms of haplodiploidy in animals and the existing theories on the origin and evolution of haplodiploidy, I will develop and analyse two models that scrutinise two new hypothesis on the origin of haplodiploidy. The first hypothesis, proposed by Normark [287], claims that maternally inherited bacteria that selectively kill males by destroying their paternally derived chromosomes can directly lead to PGE. The second hypothesis that I develop argues that haplodiploidy arose due to maternally inherited bacteria inducing CI. The results of both models will be discussed in the light of our current ecological and cellular biological understanding of male-killing and CI.

3.1.1 Incidence of haplodiploidy

Table 3.1 gives a list of the distribution of haplodiploidy among animals. Compared with diplodiploidy, parthenogenis and hermaphroditism, haplodiploidy is an unusual genetic system in animals, whilst it seems to be altogether absent in other eukaryotes.

Table 3.1: Incidences of haplodiploidy in arthropods (Acari and Hexapoda), nematodes and rotifers (Monogononta). Remarks: [1] arrhenotoky alternates with parthenogenesis, [2] it has not been demonstrated yet that the eliminated genome in males is the paternal one, [3] PGE is a consequence rather than the cause of male development, [4] diploid individuals are hermaphrodites in some species, [5] According to the time when the paternal genome is eliminated during male development, three different types of PGE have been distinguished in this group.

Group	Incidence	Type	References
Hexapoda			
Coleoptera	Xyleborini & Dryocoetini (bark beetles)	arrhenotoky	[288]
	Hypothenemus hampei (bark beetle, 'coffee borer')	PGE	[44]
	Micromalthus debilis	arrhenotoky[1]	[349, 308]
Diptera	Cecidomyiidae (gall midges)	probably PGE[2]	[43]
	Sciaridae (fungal gnats)	PGE[3]	[268, 148]
Hemiptera	Aleyrodidae (whiteflies)	arrhenotoky	[364]
	Iceryini (scale insects)	arrhenotoky[4]	[345]
	most Neococcoidea (scale insects)	PGE[5]	[43]
Hymenoptera	probably all sexual species	arrhenotoky	
Thysanoptera	probably all sexual species	arrhenotoky	[353]
Acari			
Mesostigmata	Antennophorina	arrhenotoky	[289]
	most Dermanyssina	both	[289]
Prostigmata	Eleutherengona	arrhenotoky	[289]
	Eupodina	arrhenotoky	[289]
Sarcopteriformes	some Brachypylina	arrhenotoky	[289]
	some Astigmata	arrhenotoky	[289]
Rotifera			
Monogononta	all	arrhenotoky[1]	[427]
Nematoda			
Oxyuridae (pin worms)	all	arrhenotoky	[1]

In insects, haplodiploidy has evolved at least ten times independently. By far the largest and most diverse group of haplodiploids are the Hymenoptera, of which — with only a few exceptions — all species are arrhenotokous. A second insect order that is completely arrhenotokous is the order Thysanoptera (thrips). In some groups, haplodiploidy occurs as a mixture with other genetic systems. For example, the beetle *Micromalthus debilis* (a phylogenetically very isolated and basal species within the Coleoptera) usually reproduces parthenogenetically as larvae and only under poor conditions produces haploid male and diploid female imagoes that reproduce sexually [308]. Some species of the arrhenotokous scale insect tribe Iceryini (Hemiptera: Sternorrhyncha: Coccoidea) consist of hermaphroditic individuals and males, which represents the only known case of hermaphroditism in insects [345]. As indicated in the table, there is also a great diversity among PGE systems in insects. In some groups, the paternal chromosomes are eliminated in the germ line of males, whilst in others the paternal chromosomes are eliminated in the soma.

Haplodiploidy is also very common in mites, where apparently it has evolved several times independently [289]. However, only comparatively few species have been investigated with regards to their karyotype or genetic system. Since the phylogenetic relations among mites are also largely unresolved, it is premature to speculate on how often haplodiploidy has evolved within the Acari.

Outside the arthropods, only two groups of animals are known to be haplodiploid. The Monogononta — one of the four groups of rotifers — have reproductive cycles in which parthenogenesis alternates with arrhenotokous haplodiploidy [427]. The Oxyuridae (pinworms), an order within the nematodes, reproduce entirely as arrhenotokous haplodiploids [1].

3.1.2 The origin of haplodiploidy

To date there is no accepted route to the evolution of haplodiploidy. Several hypotheses have been put forward, and will be discussed shortly in this section. In different taxa different factors may have led to haplodiploidy, and the following reasonings are not necessarily mutually exclusive (see the discussion in Section 3.4.4). The following hypotheses differ markedly in their elaborations from mere propositions to well developed models.

(1) Maternal transmission advantage. One of the oldest hypotheses on the origin of haplodiploidy is based on the observation that sons that transmit only their maternal genes to their offspring confer a twofold fitness benefit to females that manage to produce them[1] [42, 143, 45]. Thus, so long as haploid males are at least 50% as viable and fertile as diploid males, an allele that allows females to produce haploid males may spread in a population. Both arrhenotokous haplodiploidy and PGE might evolve because of this maternal transmission advantage. Further selection increasing the viability and fertility of haploid males can then be expected to occur on a larger time scale.

(2) Effective clearance of deleterious recessive alleles. An important difference between haploid and diploid organisms is the way selection operates. Whereas in haploids, all alleles are subject to selection, alleles in diploids can be recessive and may thus be masked from selection when occurring in the heterozygous state. As a result, deleterious recessive alleles in haploids are eliminated by natural selection at a much higher rate than in diploids. It has been suggested that this process might facilitate the evolution of haplodiploidy. A theoretical analysis has demonstrated that for low levels of recombination between a locus where deleterious recessive alleles arise by mutation and a locus determining the ploidy of males, arrhenotokous haplodiploidy can evolve [127]. However, for high recombination between these loci, the 'masking' advantage in diploid males overcomes the 'purging' advantage in haploid males, and haplodiploidy is impeded. Notably, this hypothesis on the origin of haplodiploidy can only explain arrhenotokous haplodiploidy and systems of PGE where the paternal genome is (functionally) eliminated in the somatic line.

(3) Parthenogenetic male production. Arrhenotoky may also be favoured because offspring can be produced without fertilisation of eggs [143]. This enables females to reproduce that did not find males to mate with, and females that did not receive sufficient sperm to fertilise all of their eggs will increase their offspring production when unfertilised eggs develop into males. If females live long enough to mate with their sons, arrhenotoky may also enable unfertilised females to colonise new areas. This phenomenon of 'spanandric males' is known in some mites [297].

[1]The reasoning here is essentially the same as for the 'twofold cost of sex' [430, 258].

(4) Maternal sex-ratio control. Another hypothesis emphasises that in arrhenotokous haplodiploidy, females may control the sex-ratio of their offspring much more readily than in sex determination systems based on diplodiploidy [140, 28]. This may especially be of importance in species with high inbreeding and local mate competition, where female biased sex-ratios are selected for [140]. For example, the parasitoid wasp *Nasonia vitripennis* is able to adjust the sex-ratio of their offspring according to how many other females have laid their eggs in the same host [412, 413]. However, such mating systems might also be a consequence rather than a cause of haplodiploidy. Another situation where maternal sex-ratio control is likely to be an important factor is when X chromosome meiotic drive elements occur in the population [140]. This is because males will be the rarer sex in such a population, and fertilising eggs yields the risk of producing only daughters. The evolutionary dynamics of X driving elements and 'arrhenotoky-alleles' remain yet to be explored.

(5) Feminizing endosymbionts. Hamilton suggested that maternally inherited endosymbionts that eliminate the chromosomes in the sperm of their male hosts might lead to haplodiploidy [141]. At the beginning of an ongoing process, the endosymbionts would only attack the Y chromosome, thus leading to all female offspring of infected males. Such a behaviour would enhance the fitness of the endosymbionts when inbreeding is common, because it leads to close relatives of the endosymbionts in males being transmitted to more daughters. The Y chromosome would then eventually get lost from the population, and one of the autosomes might adopt the function of a sex chromosome. The process of elimination of the Y chromosome might then be repeated again and again, until the last chromosome is eliminated and the males have become haploid.

Having discussed these possible advantages of haplodiploidy, it is warranted to also point out the many obstacles that may prevent haplodiploidy from evolving and elucidate the importance of these impediments under different circumstances. Most importantly, there are likely to be many initial developmental and physiological barriers. In the case of arrhenotokous haplodiploidy, eggs must commence development into an embryos without the signal of fertilisation. Embryogenesis must then proceed

smoothly, which is likely to require a substantial up-regulation of gene expression. Finally, in the adult males, meiosis during spermatogenesis must be suppressed and replaced by mitotic production of sperm. This last requirement might be easier to achieve when the number of chromosomes is low, as an 'attempted meiosis' can be expected to lead less often to chromosome loss than with many chromosomes [42]. A genetic barrier might be the revelation of deleterious recessive alleles in haploid males. It has been suggested therefore that inbreeding and the adaptation to it might be a necessary condition for the evolution of haplodiploidy [42]. Moreover, the ancestral sex determination system is likely to play a crucial role [46], an issue that will further be discussed in Section 3.4.3.

To conclude this section about the evolution of haplodiploidy, I will set out in short a notion about transitions between different forms of haplodiploidy, known as the Schrader-Hughes-Schrader hypothesis [346]. According to this hypothesis, the several forms of haplodiploidy evolve in a sequence of four steps (see also Figure 3.9). In a first step, a switch in the genetic system leads from zygogenetic diplodiploidy to PGE where the paternal genome is eliminated during spermatogenesis. The second step is a switch in the karyotype to a species where the paternal genome is eliminated in the somatic and the germ line, so that the males are haploid. In the next stage, males are produced from unfertilised eggs and are haploid (arrhenotokous haplodiploidy). Finally, without a change in the genetic system, the karyotype in males may again switch to a diploid state due to duplication of the maternal chromosome set. This state, termed 'diploid arrhenotoky', is only known from some scale insects. Although Schrader and Hugh-Schrader put forward their hypothesis more than 70 years ago, it has largely been ignored. Only one empirical study has been undertaken so far, in which the phylogenetic distribution of arrhenotoky and PGE among the mite group Dermanyssina was demonstrated to support the Schrader-Hughes-Schrader hypothesis [74].

3.2 Male-killing and haplodiploidy

The first model that I present is an extension of a previous model by Normark [287]. This model assumed a strain of endosymbionts that kill males by destroying the paternally derived genome, and the conditions were derived for such a male-killer to become beneficial for female carriers. However, Normark's model did not take into account that in a sex-ratio biased population, males and females have different fitness. Moreover, the conditions for invasion and stable maintenance of the male-killer were not considered, so that some predictions made by this model are not valid.

My model is elaborated such that (1) it includes conditions for the male-killing bacteria to spread and persist in the host population, and (2) the differential fitness of males and females due to the distorted population sex-ratio is taken into account. As will be demonstrated, these extensions allow a more precise understanding of the co-evolution between male-killing bacteria and their hosts.

3.2.1 Infection dynamics

I assume an infinitely large panmictic host population with discrete, non-overlapping generations. The primary sex-ratio is assumed to be 1:1. Females can be either infected with male-killing bacteria or uninfected. The frequency of infected females among all females will be denoted by p. The male-killing endosymbionts are passed on to a fraction α of the offspring of infected females, whilst $1-\alpha$ of the offspring will be uninfected. All infected males are assumed to be haploidised by the endosymbionts. A fraction ν of these haploid males is assumed to be viable. However, they are not necessarily as fertile as diploid males, but rather have a fertility f relative to a fertility 1 of diploid males.

In order to be consistent with Normark, I will adopt a slightly different approach to fitness compensation than I have used in Chapter 2 (cf. equations (2.1)), which has also been used in previous models on male-killing [179]. An infected female will have a fraction $\alpha(1-\nu)$ of their male offspring killed by the male-killers. A fraction β of the resources that these males would normally use is now reallocated to the surviving siblings and increase their fitness. Since all female and $1-\alpha(1-\nu)$ of the male offspring survive, the relative fitness R of surviving offspring of infected females

becomes

$$R = 1 + \frac{\beta\alpha(1-\nu)}{2-\alpha(1-\nu)} = \frac{2-\alpha(1-\nu)(1-\beta)}{2-\alpha(1-\nu)}. \tag{3.1}$$

The recursion equation for the infection frequency in females is then given by

$$p' = \frac{\alpha R p}{[\alpha R p] + [1 - p + p(1-\alpha)R]} = \frac{\alpha R p}{1 + p(R-1)}. \tag{3.2}$$

It is easy to see that this dynamical system has two fixed points,

$$\hat{p}_1 = 0, \qquad \text{and} \qquad \hat{p}_2 = \frac{\alpha R - 1}{R - 1}. \tag{3.3}$$

Figure 3.1a shows numerical values for the nontrivial fixed point $\hat{p}_2$, depending on transmission rate α and viability of haploidised males ν. To determine the stability of the two fixed points, the derivation of p' is calculated as

$$\frac{dp'}{dp} = \frac{\alpha R}{[1 + p(R-1)]^2}. \tag{3.4}$$

Evaluating this term at the position of the two fixed points demonstrates that $\hat{p}_1$ is unstable and $\hat{p}_2$ is stable if $\alpha R > 1$, which at the same time is the condition for $\hat{p}_2$ to be positive. Thus, the fitness reduction of the male-killers caused by inefficient transmission must be outweighed by the fitness increase that infected females receive due to the death of their brothers. Inserting the formula for R (Equation 3.1) into this inequality and solving for the viability of haploid males yields

$$\nu < \frac{3\alpha - 2 - \alpha^2(1-\beta)}{\alpha[1-\alpha(1-\beta)]} \tag{3.5}$$

as a necessary and sufficient condition for the male-killing endosymbionts to invade a population and reach the stable equilibrium $\hat{p}_2$.

3.2.2 Calculating the population sex-ratio

As a next step, I will derive the population sex-ratio when the infection frequency is at equilibrium, a quantity that will be needed in the following section to determine the fitness of infected and uninfected females. In calculating the sex-ratio, I will weigh each male with his fertility. That this is necessary becomes clear when interpreting the relative fertility f of haploid males as the proportion of haploid males that are fully fertile, and $1 - f$ as the proportion of haploid males that are totally infertile.

In terms of the population sex-ratio as needed in the following section, these latter sterile males can also be considered dead and should therefore not be counted.

Let thus Θ be the proportion of fertile males in a population where the male-killing bacteria are at equilibrium. Using the recursion Equation 3.2 and the analogous, straightforward recursion equation for males, Θ can be calculated as

$$\begin{aligned}\Theta &= \frac{\hat{p}_2 \times [(1-\alpha)R + \alpha\nu f R] + (1-\hat{p}_2) \times 1}{\hat{p}_2 \times [(1-\alpha)R + \alpha\nu f R + R] + (1-\hat{p}_2) \times 2} \\ &= 1 - \frac{1 + \hat{p}_2(R-1)}{2 - \hat{p}_2[2 - 2R + \alpha R(1-\nu f)]}. \end{aligned} \tag{3.6}$$

Figure 3.1b shows values of Θ for a wide range of parameters α and ν.

3.2.3 When can male-killers be beneficial?

Equipped with the equilibrium infection frequency and the equilibrium population sex-ratio, we can now proceed to determine the conditions under which haploidising male-killers are beneficial for their female hosts. Since the population sex-ratio is biased and the different classes of offspring (male/female, infected/uninfected) themselves produce different numbers of offspring, it seems appropriate to employ the number of grandchildren rather than the number of children for measuring the fitness of females [83, 113, 103]. In addition to that, it must be considered that a given maternal allele is always passed on to grandchildren by haploidised sons, whereas

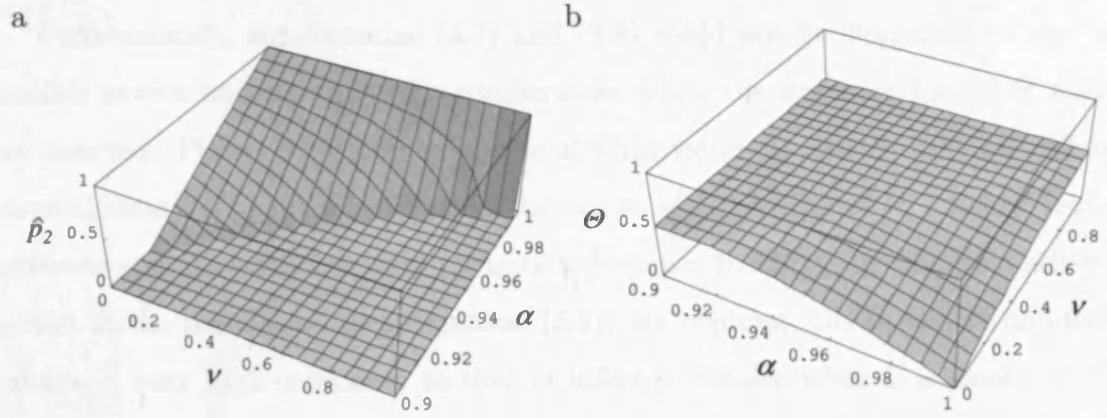

Figure 3.1: (a) Equilibrium frequency $\hat{p}_2$ of the haploidising male-killers in females among all females, depending on the transmission rate α and the viability of haploid males ν. (b) Equilibrium population sex-ratio Θ — given as the fraction of males — that corresponds to the equilibrium infection frequency. In both plots, $\beta = 0.1$ and $f = 1$. Note that for better visibility the axes are exchanged in (a) and (b).

diploid sons only pass on half of the maternal genes. Thus, the grandchildren sired by haploid sons must be counted twice when calculating an estimate for the fitness of females[2]. The fitness estimates for infected and uninfected females will be denoted by T_i and T_u, respectively, and are given by

$$\begin{aligned} T_i &= \alpha R \times \frac{1}{1-\Theta} \times [\alpha R + (1-\alpha)R + \alpha\nu R + (1-\alpha)R] \\ &\quad +(1-\alpha)R \times \frac{1}{1-\Theta} \times 2 \\ &\quad +\alpha\nu R \times \frac{1}{\Theta} \times 2f[\hat{p}_2 R(2-\alpha(1-\nu)) + 2(1-\hat{p}_2)] \\ &\quad +(1-\alpha)R \times \frac{1}{\Theta} \times [\hat{p}_2 R(2-\alpha(1-\nu)) + 2(1-\hat{p}_2)] \qquad (3.7) \\ T_u &= 1 \times \frac{1}{1-\Theta} \times 2 \\ &\quad +1 \times \frac{1}{\Theta} \times [\hat{p}_2 R(2-\alpha(1-\nu)) + 2(1-\hat{p}_2)]. \qquad (3.8) \end{aligned}$$

In the formula for T_i, the four addends represent fitness components of an infected female due to infected daughters, uninfected daughters, infected sons and uninfected sons (in this order). Each addend consists of three factors (separated by the $\times$ symbol), giving (1) the expected number of offspring in the respective class, (2) their relative contribution to the following generation according to their sex, and (3) the expected number of children from these children of the infected mother. The formula for T_u is written in an analogous way, giving the expected number of grandchildren from daughters plus the expected number of grandchildren from sons.

Unfortunately, the formulae (3.7) and (3.8) could not be simplified to any reasonable extent and become very cumbersome when the formulae for R, Θ and $\hat{p}_2$ are inserted. Figure 3.2 shows examples of what values T_i and T_u take for different survival rates ν of haploidised males. As can be seen, the fitness of infected females increases steadily with increasing ν until ν becomes too high for the male-killers to persist in the population (see Equation (3.5). By contrast, the fitness of uninfected females is very high compared to that of infected females when ν is small. This is due to the distorted population sex-ratio that makes male offspring (of which uninfected females have by far more) very precious. With increasing ν, the population

[2]Note, that even when counting the grandchildren, this is only an approximation for the fitness of females because the fitness of the grandchildren also varies between infected females and uninfected females.

sex-ratio becomes more even and T_u decreases until it reaches a minimum. From then on, T_u again increases slightly and becomes constant at the value $T_u = 8$ when the male-killers cannot persist in the population. The slight increase is explained by the fact that the reproductive success of male offspring from uninfected females depends on how many infected females there are in the population (see last factor in the last addend of Equation (3.8)).

To determine the conditions where male-killers are beneficial for their female hosts, the inequality

$$T_i > T_u \tag{3.9}$$

can be solved for the parameter of interest. Numerically derived results, combined with the conditions for the male-killers to persist in a population (Equation (3.5)), are shown in Figure 3.3. The plots show that for high viabilities ν of haploid males, the haploidising endosymbionts can indeed be beneficial for their female hosts. However, in contrast to Normark's predictions [287], male-killing is never beneficial when ν is small. In addition to ν, the fertility f of haploid males must also be high in order for the male-killers to be beneficial.

It can also be seen that the conditions under which male-killers can exist in a population strongly delimit the subset of the parameter space where male-killing is beneficial. For example, Inequality 3.5 reveals that even if $\nu = 0$ and $\beta = 1$ — that is, under 'optimal conditions' for the male-killer — the transmission rate α must be

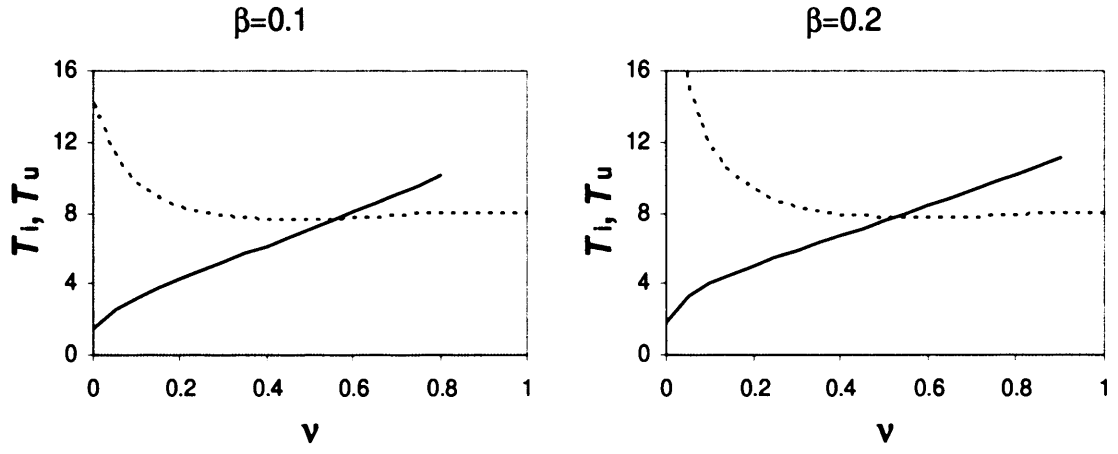

Figure 3.2: Examples for the fitness approximations T_i for infected females (solid lines) and T_u for uninfected females (dotted lines), depending on the survival rate ν of haploid males. Other parameters take the values $\alpha = 0.99$ and $f = 1$. Note that for high values of ν the male-killers cannot persist in the population and T_i therefore is not defined.

larger than 2/3 in order for the male-killer to persist in a population. Thus, two of the three graphs given in Normark's treatment (where $\alpha = 0.01$ and $\alpha = 0.5$) are void because under these conditions there would be no male-killer in the population to benefit females.

3.2.4 Coevolution between hosts and male-killers

Based on the results of the previous sections, I will now try to explore the possible outcomes of coevolution between the haploidising endosymbionts and their hosts. After briefly discussing how natural selection might influence the fertility of haploid males f and the resource allocation parameter β, I will concentrate on the evolution of the viability of haploid males ν and the transmission rate α, since these are the two parameters where we can expect different selective pressure on hosts and symbionts at different points in the parameter space.

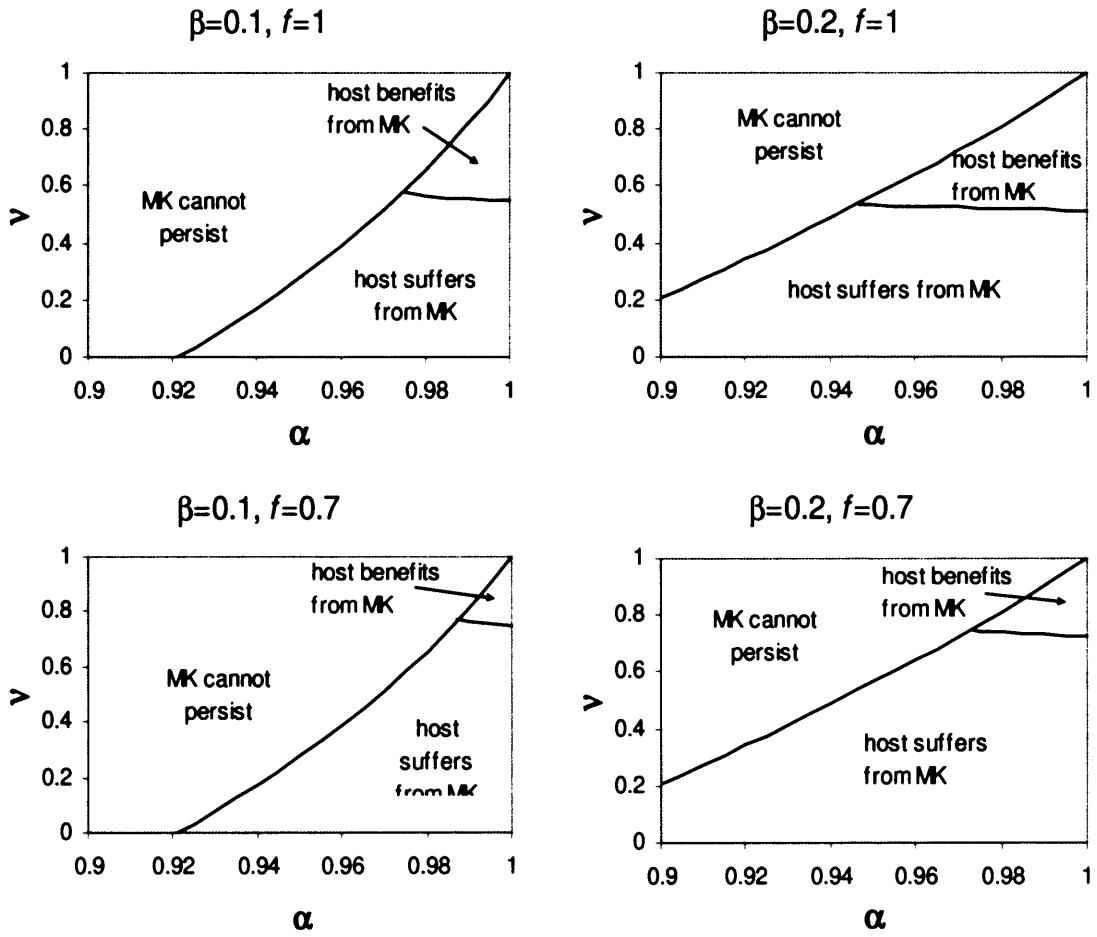

Figure 3.3: Subsets of the parameter space where male-killers (MK) are detrimental or beneficial for their female hosts and where they cannot persist in the population.

Obviously, increased fertility f of haploid males is favoured by natural selection on the hosts if some of these males survive, i.e. $\nu > 0$. On the other hand, there is no selection pressure on the endosymbionts with regards to f, if fitness compensation stems from resource allocation. Therefore, the fertility of haploid males can be expected to increase with time[3]. The fraction β of resources that become available to surviving siblings because of their brothers' death can be expected to increase under selection on both the hosts' and the endosymbionts' side. For example, increasing values of β may be the result of selection on the endosymbionts for optimisation of the time in the development of males when the latter are killed. On the other hand, the female hosts might maximise β by increasing their clutch size [175].

The selective pressures on hosts and endosymbionts with regards to transmission rate α and viability of haploid males ν are illustrated in Figure 3.4a. Whereas the hosts are always selected for high viabilities of haploid males ν and the endosymbionts are always selected for low values of ν and high transmission rates α, the selection pressure on the hosts with regards to α varies: depending on whether the male-killers are detrimental or beneficial for their female hosts, natural selection favours an increase or decrease in the transmission rate.

Building upon these findings on selection, we can now identify some trajectories for the parameters α and ν that may be the result of coevolution between hosts and symbionts (Figure 3.4b). In the beginning of the association, the viability of haploid males ν is likely to be very close to zero (state A in Figure 3.4b). Increasing viability of haploid males due to selection on the hosts may then lead to extinction of the endosymbionts (state B), in particular, if the hosts succeed in decreasing the transmission rate. Alternatively, when the transmission rate is still high enough, the endosymbionts may become beneficial for their female hosts. At this point, both hosts and symbionts are selected for increased transmission rate α. However, further increasing of ν may still lead to extinction of the male-killers (state C), and selection on the bacteria for increased mortality among male offspring may also make the endosymbionts detrimental again (state D). Finally, when the transmission rate α

[3]If inbreeding avoidance plays an important role in fitness compensation, natural selection on the endosymbionts favours low fertilities of haploid males. Thus, there are divergent selective pressures on hosts and symbionts in this case, and the fertility of haploid males may or may not increase during coevolution.

increases fast enough towards one and the viability of haploid males also increases — but more slowly — towards one, the population might end up in a state where all individuals are infected with the haploidising endosymbionts and all males survive and are haploid (state E): a genetic system of endosymbiotically induced paternal genome elimination has arisen.

Although — given the high risk of extinction of the endosymbionts — this latter outcome does not seem very likely, the above reasoning suggests that the process of haploidising male-killers leading to PGE as proposed by Normark [287] can work. Moreover, even if this state is not reached, the selection caused by the endosymbionts towards more viable and fertile haploid males may well facilitate the emergence of haplodiploidy out of other reasons (see Section 3.1.2) and can thus lead to pre-adaptation.

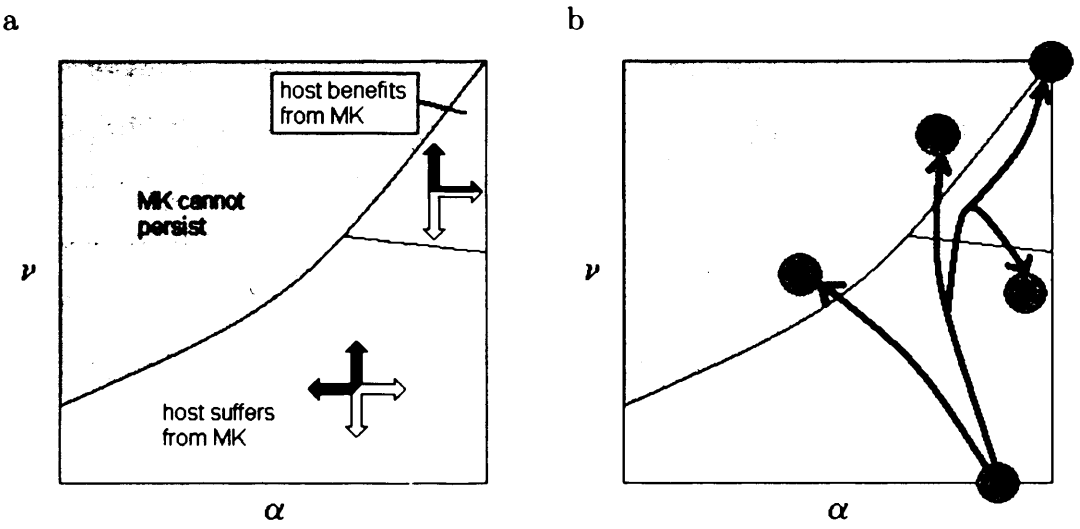

Figure 3.4: Figure (a) illustrates the direction of selection on hosts (black arrows) and endosymbionts (white arrows) with regard to the transmission rate α and the viability of haploid males ν in different areas of the $\alpha - \nu$ plane. In (b), some possible co-evolutionary trajectories are shown (see text for detailed explanations). Note that the shape of the different areas does not correspond to specific parameter values of β and f, but is to be understood merely as a stylised version of one of the plots in Figure 3.3.

3.3 Cytoplasmic incompatibility and haplodiploidy

In this section, I will construct a second model to determine whether the evolution of haplodiploidy can also be facilitated by maternally inherited endosymbionts that induce cytoplasmic incompatibility (CI). Since it is well known that in CI, the paternal chromosomes are modified by the bacteria and lost when not rescued, this seems a promising avenue of investigation.

3.3.1 Infection dynamics

As in the previous model, I assume an infinitely large population of hosts which reproduce in discrete, non-overlapping generations and a primary sex-ratio of 1:1. Individuals can either be infected with CI-inducing endosymbionts or uninfected. A fraction α of the offspring of an infected female inherits the infection, whilst $1-\alpha$ are uninfected.

Incompatible matings occur when an infected male mates with an uninfected female. A fraction l ('CI-level') of the offspring from such matings are assumed to have their paternal genome destroyed. A fraction ν of these haploid offspring survive to adulthood, and $1-\nu$ of the haploid offspring do not survive. It is assumed that the surviving haploid individuals always develop into males. For example, this assumption would be reasonable with an XY or X0 sex determination system where the absolute number of X chromosomes determines the sex.

In contrast to the previous model I will not use a parameter for the fertility of males in this model. This proceeding is vindicated by the fact that here, viability and fertility operate exactly in a multiplicative way, so that these two parameters can be merged into one. Thus, although for reasons of simplicity I will refer to ν as the viability of haploid males, this parameter can be interpreted either as viability of fully fertile haploid males, as fertility of fully viable haploid males, or as a product of viability and fertility.

I denote by q the fraction of infected females among all females, and by r the fraction of infected males among all males. Note that although there are three types of males in this model (infected, uninfected diploid and uninfected haploid males), the fractions of the two uninfected types need not to be distinguished when analysing

the infection dynamics. The recursion equations of q and r are given by

$$\begin{aligned} q' &= \frac{\alpha q}{\alpha q + [(1-\alpha)q + (1-q)](1-lr)} = \frac{\alpha q}{1 - lr(1-\alpha q)} \\ r' &= \frac{\alpha q}{\alpha q + [(1-\alpha)q + (1-q)][1 + rl(2\nu - 1)]} = \frac{\alpha q}{1 - lr(1-\alpha q)(1-2\nu)}. \end{aligned} \tag{3.10}$$

In case of no survival of haploid males (i.e., $\nu = 0$), these equations represent simplified versions of previously developed models on CI in diplodiploid host species [111, 389]. In accordance with these earlier models, the system of recursion equations (3.10) has three fixed points $(\hat{q}, \hat{r})$, which are given by

$$\begin{aligned} (\hat{q}_1, \hat{r}_1) &= (0,0) \\ (\hat{q}_2, \hat{r}_2) &= \left(\frac{l - \sqrt{l^2 - 4l(1-\alpha)(\alpha + 2\nu - 2\alpha\nu)}}{2\alpha l}, \frac{l - \sqrt{l^2 - 4l(1-\alpha)(\alpha+2\nu-2\alpha\nu)}}{2l(\alpha+2\nu-2\alpha\nu)} \right) \\ (\hat{q}_3, \hat{r}_3) &= \left(\frac{l + \sqrt{l^2 - 4l(1-\alpha)(\alpha + 2\nu - 2\alpha\nu)}}{2\alpha l}, \frac{l + \sqrt{l^2 - 4l(1-\alpha)(\alpha+2\nu-2\alpha\nu)}}{2l(\alpha+2\nu-2\alpha\nu)} \right) \end{aligned} \tag{3.11}$$

To determine the stability of these fixed points, I derived the Jacobian matrix of the function defining the recursion equations (3.10) as

$$\mathbf{J} = \begin{pmatrix} \frac{\partial q'}{\partial q} & \frac{\partial q'}{\partial r} \\ \frac{\partial r'}{\partial q} & \frac{\partial r'}{\partial r} \end{pmatrix} = \begin{pmatrix} \frac{\alpha(1-lr)}{[1-lr(1-\alpha q)]^2} & \frac{\alpha l q(1-\alpha q)}{[1-lr(1-\alpha q)]^2} \\ \frac{\alpha[1-lr(1-2\nu)]}{[1-lr(1-\alpha q)(1-2\nu)]^2} & \frac{\alpha l q(1-\alpha q)(1-2\nu)}{[1-lr(1-\alpha q)(1-2\nu)]^2} \end{pmatrix}. \tag{3.12}$$

It can easily be seen that

$$\mathbf{J}|_{(q,r)=(\hat{q}_1,\hat{r}_1)=(0,0)} = \begin{pmatrix} \alpha & 0 \\ \alpha & 0 \end{pmatrix}. \tag{3.13}$$

Thus,

$$\det(\mathbf{J}|_{(0,0)} - \lambda I) = \det \begin{pmatrix} \alpha - \lambda & 0 \\ \alpha & -\lambda \end{pmatrix} = \lambda(\lambda - \alpha), \tag{3.14}$$

and it is apparent that the Jacobian matrix at the position of the fixed point $(\hat{q}_1, \hat{r}_1) = (0,0)$ has the two eigenvalues $\lambda_1 = 0$ and $\lambda_2 = \alpha$. If we assume $\alpha < 1$, both eigenvalues are less than one, so that this fixed point is stable. Since $\hat{q}_1 = 0 < \hat{q}_2 < \hat{q}_3$ and $\hat{r}_1 = 0 < \hat{r}_2 < \hat{r}_3$, it seems rather natural that $(\hat{q}_2, \hat{r}_2)$ is an unstable and $(\hat{q}_3, \hat{r}_3)$ again is a stable fixed point (as has been shown in the very similar one-dimensional models on CI [111]). However, I was not able to prove this contention, but could only confirm it using some numerical investigations (not shown).

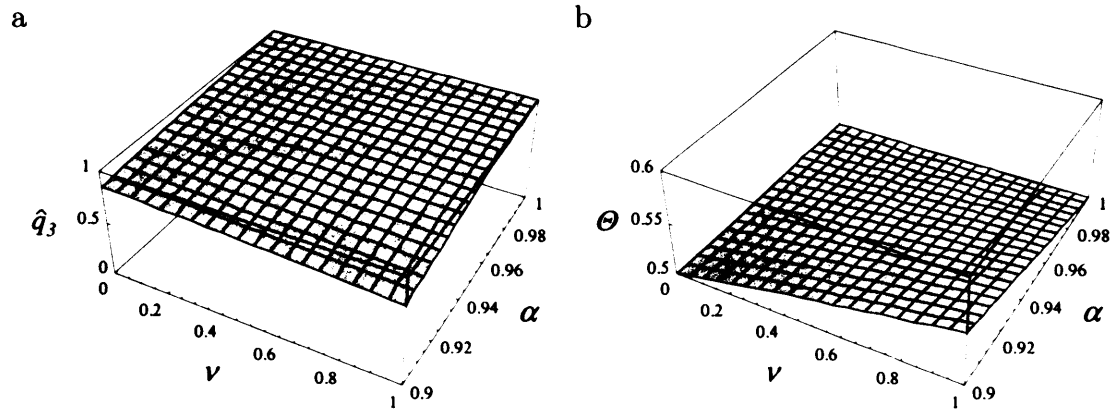

Figure 3.5: (a) Equilibrium frequency $\hat{q}_3$ of the CI-inducing endosymbionts in females among all females, depending on the transmission rate α and the viability of haploid males ν. (b) Equilibrium fraction Θ of males in the population, corresponding to equilibrium infection frequencies. In both plots, the CI-level takes the value $l = 0.5$.

Under the assumption that the fixed point $(\hat{q}_3, \hat{r}_3)$ is always stable where it exists, I now determine when the CI-inducing bacteria can persist in a population. This will be the case when $(\hat{q}_3, \hat{r}_3)$ exists (as a pair of real numbers), so that the subset of the parameter space where the term under the roots of $\hat{q}_3$ and $\hat{r}_3$ (see equations 3.11) is non-negative needs to be determined. After applying simple arithmetics to this problem,

$$\nu < \frac{l - 4\alpha(1-\alpha)}{8(1-\alpha)^2} \tag{3.15}$$

is found to be the condition for the endosymbionts to persist in a population. Figure 3.5a shows equilibrium frequencies $\hat{q}_3$ for a range of parameters α and ν. It can be seen that these equilibrium frequencies are always relatively high. Because of the invasion threshold, they do not approach zero, but stop to exist abruptly at a high level when the transmission rate becomes too small compared to viability of haploid males and CI-level (not shown).

3.3.2 Calculating the population sex-ratio

As in the previous model, the population sex-ratio is needed to determine when the CI-inducing endosymbionts are beneficial for their hosts. We can make advantage of the fact that the absolute number of infected females and infected males in a population will always be the same (because both are produced by infected females only and experience the same level of mortality). Denoting this number of infected

females and infected males in the population by M, and the total number of females and males by N_f and N_m, respectively, we get for the fraction Θ of males in the population at the stable polymorphic equilibrium

$$\Theta = \frac{N_m}{N_m + N_f} = \frac{\frac{M}{\hat{r}_3}}{\frac{M}{\hat{r}_3} + \frac{M}{\hat{q}_3}} = \frac{\hat{q}_3}{\hat{q}_3 + \hat{r}_3}. \tag{3.16}$$

Inserting the equilibria $\hat{q}_3$ and $\hat{r}_3$ from Equation (3.11) yields after some simplification

$$\Theta = \frac{\alpha + 2\nu(1-\alpha)}{2\alpha + 2\nu(1-\alpha)} = \frac{1}{2} + \frac{\nu(1-\alpha)}{2\alpha + 2\nu(1-\alpha)}. \tag{3.17}$$

From the second formula, it can be seen that there will always be at least as many males as females in the population. When $\nu = 0$ (all haploid males die), the population sex-ratio will be even. When $\nu > 0$, the population sex-ratio will be male-biased and become $1 - \alpha/2$ in the case of fully viable haploid males. With respect to the transmission rate, the sex-ratio will also be even when $\alpha = 1$ (perfect transmission), simply because in this case all individuals are infected and no incompatible matings occur. Figure 3.5b shows values of the equilibrium sex-ratio Θ for a slice of the parameter space. Note that in contrast to a population infected with male-killing bacteria, the population sex-ratio can be expected to be only mildly biased in this model.

3.3.3 When are CI-inducing endosymbionts beneficial?

This question will be treated in the same way as in the previous model: the number of grandchildren that infected and uninfected females can be expected to have will be determined and compared, with the surviving haploid sons being counted twice. Again, I denote by T_i and T_u the transmission success of maternal genes of infected and uninfected females, respectively. These quantities are given by

$$\begin{aligned} T_i &= \alpha \times \frac{1}{1-\Theta} \times [1 - l\hat{r}_3(1-\nu)(1-\alpha)] \\ &+ (1-\alpha)(1-l\hat{r}_3) \times \frac{1}{1-\Theta} \times [1 - l\hat{r}_3(1-\nu)] \\ &+ \alpha \times \frac{1}{\Theta} \times [1 - l(1-\alpha\hat{q}_3)] \\ &+ (1-\alpha)(1-l\hat{r}_3) \times \frac{1}{\Theta} \times 1 \\ &+ (1-\alpha)2\nu l\hat{r}_3 \frac{1}{\Theta} \times 2, \end{aligned} \tag{3.18}$$

$$\begin{aligned} T_u &= (1 - l\hat{r}_3) \times \frac{1}{1-\Theta} \times [1 - l\hat{r}_3(1-\nu)] \\ &+ (1 - l\hat{r}_3) \times \frac{1}{\Theta} \times 1 \\ &+ 2\nu l\hat{r}_2 \times \frac{1}{\Theta} \times 2 \end{aligned} \tag{3.19}$$

Similar to the male-killing model, the five addends in the formula for T_i represent offspring through infected daughters, uninfected daughters, infected sons, uninfected diploid sons and uninfected haploid sons, in this order. In the formula for T_u, the three addends stand for offspring through uninfected daughters, uninfected diploid sons and uninfected haploid sons. The three factors in each addend of the Formulae 3.18 and 3.19 constitute the relative number of the respective type of offspring, their weighting according to the population sex-ratio, and the expected representation of maternal genes in the offspring of these offspring.

Figure 3.6 shows how the fitness approximations T_i and T_u for infected and uninfected females depend on the survival rate ν of haploidised males. As can be seen, the pattern is reversed to that found in the male-killing scenario (compare Figure 3.2: for low viabilities of haploid males, the fitness of infected females is much higher than that of uninfected females). This is because uninfected females have a high chance of mating with an infected male and in this case, will produce only few offspring. Only when the viability of haploid males is high does the fitness of uninfected females exceed that of infected females. That this can happen is explained again by the double transmission advantage of maternal alleles through haploid sons.

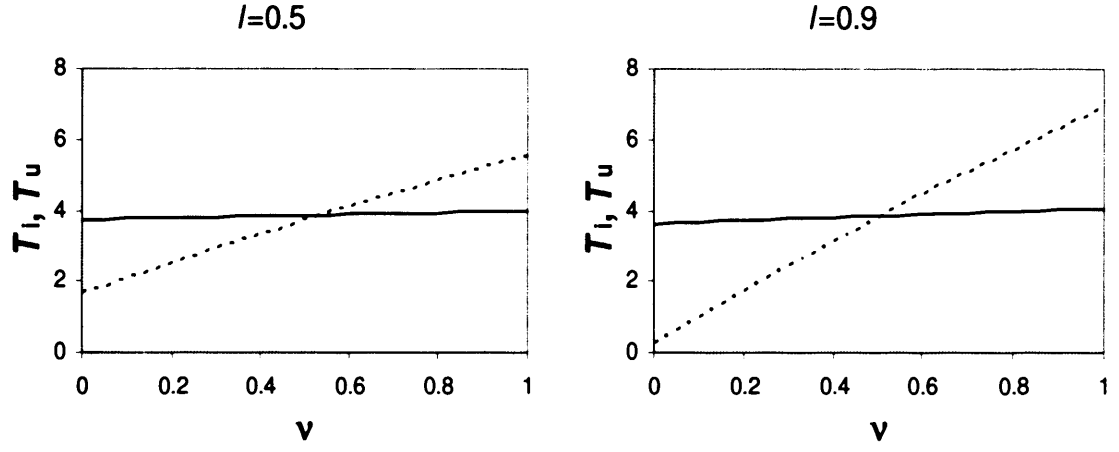

Figure 3.6: Examples for the fitness approximations T_i for infected females (solid lines) and T_u for uninfected females (dotted lines), depending on the survival rate ν of haploid males. The transmission rate in both plots is fixed at $\alpha = 0.95$.

The intersection of the two fitness curves is found to be approximately at $\nu = 1/2$, which is not surprising given that if half of the haploidised offspring survive, their double transmission advantage roughly offsets the death of their siblings. This consideration is, however, confounded by the slightly distorted sex-ratio as well as by the fact that not all matings of uninfected females are with infected males.

To establish more systematically the region of the parameter space where CI-inducing endosymbionts are beneficial or detrimental for their female hosts, the inequality $T_i > T_u$ needs to be solved for the parameters of interest. Unfortunately, as in the male-killing model, this inequality could not be solved analytically. Figure 3.7 gives numerically derived results on regions of the $\nu - \alpha$ plane where CI-inducing bacteria are beneficial, detrimental or where they cannot persist in the host population. In accordance with the above reasonings and in contrast to the male-killer model, high viabilities of haploid males make the CI-inducing bacteria detrimental, whilst low viabilities result in a beneficial relationship.

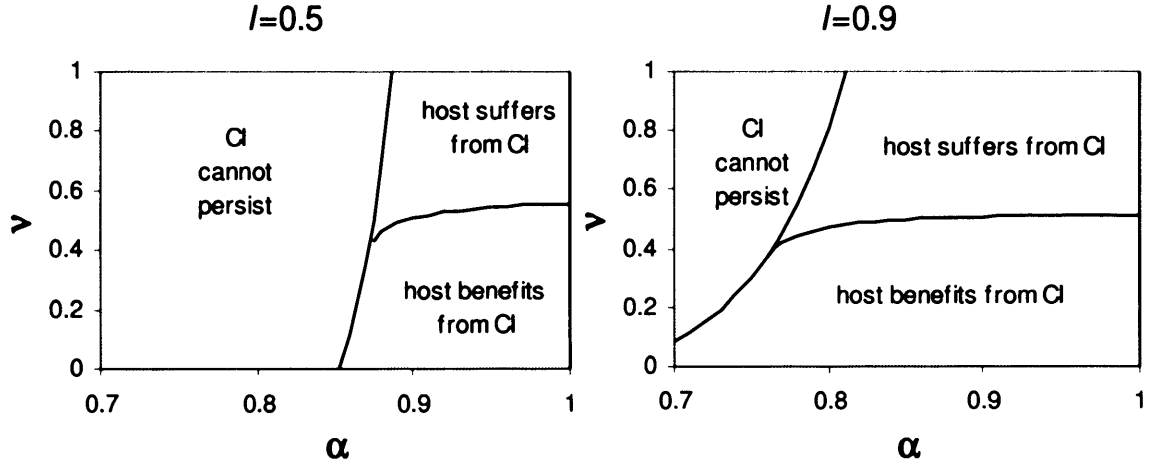

Figure 3.7: Subsets of the parameter space where CI-inducing endosymbionts are detrimental or beneficial for their female hosts and where they cannot persist in the population.

3.3.4 Coevolution between CI-inducing endosymbionts and their hosts

The results of the previous sections enable me to make predictions on possible evolutionary trajectories of CI-inducing endosymbionts and their hosts. After a brief discussion of the CI-level l, I will focus on the transmission rate α and the viability of haploidised hosts.

Male hosts can be expected to be selected for decreasing CI-levels as long as the CI-inducing bacteria are not fixed in the population. This is simply because with a certain positive probability, males will mate with uninfected females, in which case their chances of their genes being lost are given by the CI-level. The selection acting on the bacteria with respect to the CI-level is more difficult to predict. In an infinitely large panmictic population as assumed in my model, there is no selection acting on the CI-level because the fitness of the bacteria in males is zero irrespective of whether and how strongly they induce CI [311, 389, 181]. However, population structure and the resulting kin selection can be expected to lead to selection for high CI-levels [116].

The selection pressures with respect to transmission rate and viability of haploidised offspring are schematically shown in Figure 3.8a. The transmission rate α is

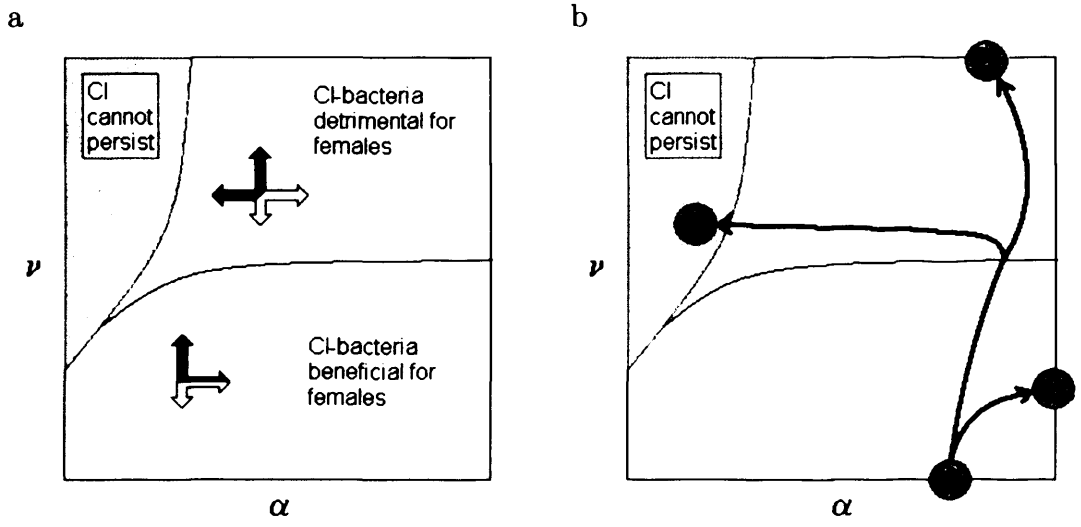

Figure 3.8: Figure (a) illustrates the direction of selection on hosts (black arrows) and endosymbionts (white arrows) with regard to the transmission rate α and the viability of haploid males ν in different areas of the $\alpha - \nu$ plane. In (b), some possible co-evolutionary trajectories are shown (see text for detailed explanations). Note that the shape of the different areas is to be understood merely as a stylised version of one of the plots in Figure 3.7.

immediately associated with the fitness of the endosymbionts, and like male-killers (and in fact all vertically transmitted symbionts), CI-inducing endosymbionts will be selected for high transmission rates. Selection on the hosts for bacterial transmission will depend on whether the bacteria are beneficial or detrimental. Thus, as derived in the previous section, for low viabilities of haploidised offspring, a high transmission rate will be selected for in hosts, whilst high viabilities will lead to selection for low transmission rates.

How will selection influence the viability ν of haploidised offspring? For the hosts, it is clear that higher viabilities will always be selected for when incompatible matings occur. The strength of selection, however, will decrease with increasing transmission rate because fewer and fewer offspring will be haploidised. On the other hand, the selection pressure acting on the bacteria is expected to be similar to the selection with respect to CI-level: in a panmictic population, no selection for lower or higher ν can be expected, whilst in a structured population, kin selection may result in selection for low viability of haploidised offspring.

At the onset of an association between CI-inducing endosymbionts and their hosts we can suppose a very low or zero viability of haploidised offspring, and a fairly high transmission rate (point A in Figure 3.8b). Selection on both hosts and symbionts may then lead to an increasing transmission rate α, and possibly also an increasing viability ν of haploidised individuals. The population may then end up with perfect endosymbiont transmission and some viability of haploid individuals (point B in Figure 3.8b). Such a state in host-endosymbiont co-evolution can be expected to be stable, because no incompatible matings occur anymore and hence, selection for higher viability of haploid offspring has ceased.

Alternatively, the viability of haploidised males may become sufficiently high before perfect transmission is reached, such that the CI-inducing bacteria become detrimental for the females that carry them. In this case, the hosts commence to be selected for low a transmission rate, which eventually may lead to extinction of the CI-inducing bacteria (point C in Figure 3.8b). At the same time, the hosts are still selected for high viability of haploids, so that complete viability (and fertility) of haploid males can also result from co-evolution (point D). However, with high viability of haploid males, no outcome apart from extinction of the endosymbionts

can be considered stable, because there will always be conflict between hosts and endosymbionts over transmission rate.

Most importantly, within the frame-work of the model, co-evolution between CI-inducing endosymbionts and their hosts cannot be expected to directly lead to a genetic system of PGE. The basic reason for this is that the number of infected males will always equal the number of infected females. From this it follows that the number of incompatible matings will always be limited, so that haploid males will always co-occur with diploid males. This finding is in contrast to the model with haploidising male-killers. Nevertheless, as in the male-killer model, the increasing viability of haploid males that can result from the co-evolution may facilitate the evolution of haplodiploidy.

3.4 Discussion

I have constructed and analysed two models for the origin of haplodiploidy that involve haploidising male-killers and CI-inducing bacteria. In both models, presence of the endosymbionts leads to selection for more viable and fertile haploid males, which can be regarded as a pre-adaptation to haplodiploidy. In addition, co-evolution between male-killing endosymbionts and their hosts can lead directly to a new genetic system of 'paternal genome elimination' (PGE), a special type of haplodiploidy.

In what follows, I will discuss the ecological and mechanistical evidence for the two hypotheses presented and speculate on the ancestral sex determination system of haplodiploid species. I conclude with a general discussion of the various hypotheses on the origin of haplodiploidy and their conceptual interrelations.

3.4.1 Ecological evidence

Male-killing bacteria are thought to spread through benefits that females receive through the death of their brothers. Thus, ecological properties of host species such as laying eggs in clutches, low dispersal of offspring, strong competition or cannibalism among siblings will favour male-killing bacteria. Normark noted that these features are believed to be part of the ancestral ecology of most of the extant haplodiploid insect taxa [287], lending support to the hypothesis that male-killing is involved in the evolution of haplodiploidy. In contrast, there are no ecological features known to substantially facilitate or impede endosymbionts inducing CI.

It is also known that many of the taxa exhibiting PGE rely on inherited microorganisms for their nutrition [141, 287]. This observation has been evoked as another argument for the involvement of maternally inherited endosymbionts in the evolution of PGE in general [141], and for the male-killing hypothesis in particular [287].

Indeed, bacteria that are nutritionally beneficial to their hosts could also evolve to induce male-killing. However, it is well known that maternally inherited bacteria are widespread and common in arthropods in general, in particular, reproductive parasites like *Wolbachia* (see Sections 1.2 and 7.1.1). This general high incidence of endosymbiotic bacteria with a well-known potential to evolve male-killing leads me to conclude that the common occurrence of beneficial endosymbionts in the ancestors of

haplodiploid species contributed little to the probability of a haploidising male-killing strain to evolve.

3.4.2 Mechanistic evidence

To date, the molecular mechanism of both male-killing and CI is unknown. In the case of male-killing, the only system that has been studied in this respect is the *Spiroplasma* - *Drosophila melanogaster* system. Here, it has been demonstrated that the bacteria require a functional dosage compensation complex in order to recognise and kill males [401]. There is no evidence that in this or in other species male-killing bacteria operate by haploidising males. However, given that at least one male-killing species, *Wolbachia*, is known to interfere with host chromosomes when inducing CI or parthenogenesis, it is feasible that *Wolbachia* male-killers kill males by destroying the paternally inherited chromosomes.

By contrast, CI-inducing *Wolbachia* are known to modify the chromosomes of infected males, which leads to their subsequent loss in the zygote if this modification is not rescued (see Section 1.4.4). Thus, the mechanism proposed here is in agreement with empirical findings. Moreover, in the wasp *Nasonia vitripennis* it has been established that not only do zygotes from incompatible matings survive and develop normally into haploid males, but also that a recessive 'conversion gene' is responsible for the survival of these males. By contrast, such a conversion gene is absent in the sister species *N. giraulti*. Although *Nasonia* wasps are haplodiploid and therefore haploidy does not pose a problem for development *per se*, these observations demonstrate that *Wolbachia* induced partial elimination of the paternal chromosomes can be 'rescued' by host factors that completely eliminate the paternal chromosomes.

3.4.3 Sex determination

In both models, it was assumed that haploidised individuals — if they survive — develop into males. This is a rather strong assumption which does not hold for many sex determination systems found in arthropods. Three different sex determination systems involving sex chromosomes have been identified where haploid individuals always develop into males [46]:

(1)	XY (X0), X → m XX → f	(2)	ZZ, Z, W → m ZW, WW, ZZ → f	(3)	X_iX_j $(i \neq j)$, X_i → m X_iX_i → f

(In these schematic representations, a single letter denotes that the organism is haploid; in (3), an arbitrary number of sex chromosomes is possible.)

Male heterogametic systems like (1) are very common among animals, and XY and X0 systems are also thought to be the ancestral system of all haplodiploid insect clades [287]. However, in most species that have been studied in detail with this respect, haploid individuals carrying only one X chromosome would develop into females instead of males. For example, in the well-studied system of *Drosophila melanogaster*, sex is determined by the ratio of the number of X chromosomes relative to the number of autosomes. Thus, if the paternal chromosomes are eliminated, this ratio is 1:1 as in normal diploid individuals with two X chromosomes, and the haploid individuals would, if viable, develop into females. Likewise, systems where male sex is determined by dominant factors on the Y chromosome (as in most mammals), individuals deprived of their paternally inherited chromosomes would develop into females. Unfortunately, the precise sex determination systems of species closely related to extent haplodiploid species have not been studied so far.

Likewise, female heterogametic systems are commonly found in animals. However, there is no indication that female heterogamy (let alone precisely mechanism (2) of the above scheme) was ancestral for any haplodiploid clade. System (3) is interesting in that it is the complementary sex determination system found in some Hymenoptera, although again, it does not seem that the ancestors of haplodiploid taxa had such a system.

In summary, the evolution of PGE from diplodiploid ancestors as analysed in the two models of this chapter is severely constrained by the sex determination system of these ancestors. However, this is a problem that all hypotheses on the evolution of haplodiploidy (PGE or arrhenotoky) face. Sex determination as a major obstacle in the evolution of haplodiploidy may thus be an answer to the question why haplodiploidy evolved only a few times [46].

3.4.4 Relationship with other hypotheses

In this section I will attempt to clarify the relationship of the two hypotheses put forward here with other hypotheses on the evolution of haplodiploidy, as detailed in the introduction of this chapter (Section 3.1.2).

Figure 3.9 shows the different types of haplodiploidy, their sequence according to the Schrader-Hughes-Schrader hypothesis, and which transitions the various hypotheses on the origin of haplodiploidy might help explain. The hypotheses can be classified into three groups, according to the particular aspect of haplodiploidy they are related to. First, two hypotheses (H3 and H4) attempt to explain the evolution of arrhenotoky with an advantage to the asexual production of males. Clearly, these hypotheses are not in any conflict with the two hypotheses I dealt with in this chapter (H6 and H7), as the former ones relate to arrhenotoky and the latter ones to PGE only.

The second group (H1, H5, H6, H7) consists of hypotheses that are based on the elimination of paternally derived chromosomes in males. H1, the 'maternal transmission advantage' hypothesis [42], is not exactly an alternative to the two hypotheses involving male-killing and CI-inducing bacteria. Rather, it forms the basis upon which it is decided when the endosymbionts are selected for or against in the host nuclear genome. On the other hand, H1 is sufficient to explain the transition from zygogenesis to PGE or arrhenotoky without relying on other hypotheses [42, 143, 45, 46]. The three hypotheses involving maternally transmitted endosymbionts (H5,H6,H7) are alternative explanations for the transition from zygogenesis to PGE with haploid males. Notably, these three hypotheses are in conflict with the Schrader-Hughes-Schrader hypothesis in that they propose a direct transition from diplodiploid zygogenesis to haplodiploid PGE, rather than an intermediate form of PGE with diploid males.

Finally, one explanation on the evolution of haplodiploidy (H2) is based on the haploid state of males in (normal) arrhenotoky. Closely related to the general theory of ploidy levels, this hypothesis states the conditions when haploidy in males is favoured due to more effective purging of deleterious mutations. Although only the transition from zygogenesis to arrhenotoky was investigated theoretically [127], other transitions from systems with diploid to systems with haploid males may also be

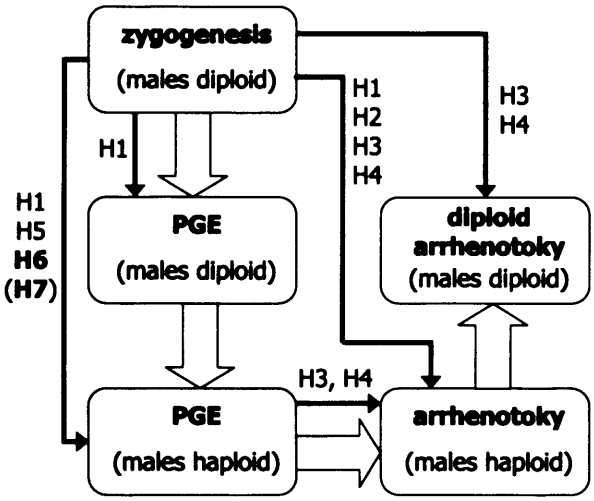

Figure 3.9: The different varieties of haplodiploidy and their sequence according to the Schrader-Hughes-Schrader hypothesis. Also shown are the transitions that different hypotheses on the evolution of haplodiploidy might explain. H1: Maternal transmission advantage; H2: clearance of deleterious recessive alleles; H3: parthenogenetic male production; H4: sex-ratio adjustment; H5: feminising endosymbionts; H6 and H7 (in bold) are the two hypotheses proposed in this chapter involving male-killing or CI-inducing endosymbionts.

partly explained by hypotheses of this type[4]. With respect to the hypotheses put forward in this chapter, recessive deleterious alleles and their expression in haploidised males can be expected to have an impact on the evolutionary trajectories proposed, although the exact influence is hard to predict.

[4]Interestingly, even the transition from PGE with haploid to PGE with diploid males where both genomes are expressed may be a consequence of selection against deleterious mutants, namely when the advantage of masking deleterious alleles outweighs that of purging them more efficiently. By contrast, the transition from arrhenotoky to diploid arrhenotoky cannot be explained by this type of hypotheses, as the diploid chromosome set in males arises by duplication of the maternally derived chromosomes.

Chapter 4

The Impact of Male-Killing Bacteria on Host Evolutionary Processes

Abstract. Male-killing bacteria are thought to play an important role in shaping the ecology and evolution of their hosts. One rather basic and important aspect of host evolution is the impact on the hosts' population genetics, i.e., the influence of male-killing bacteria on host genes whose function is not related to the male-killer infection. In this chapter, I investigate how fundamental population genetic processes, in particular the interplay between selection and random genetic drift, are affected by male-killer infections in the population. I demonstrate that as a good approximation, a male-killer infection behaves as it would if it consisted of the uninfected individuals only. I derive and corroborate formulae for the fixation probability of mutant alleles, rate of allele substitution, mean times to fixation and/or extinction, and heterozygosity for varying male-killer prevalence. My results demonstrate that infections with male-killing bacteria impede the spread of beneficial alleles, facilitate the spread of deleterious alleles, and reduce genetic variation.

An abridged version of this chapter has been accepted for publication in *Genetics* [99].

4.1 Introduction

Male-killing bacteria are commonly found in arthropods and can assert a variety of influences on their hosts biology (see Section 1.4.3 and Chapter 8). It has been recognised that male-killers will have a profound impact on mitochondrial DNA [208], which can confound population and phylogeographic studies of infected populations [169]. However, to date the effect of male-killers on nuclear host genes has not been investigated. In this introductory section, I will first summarize previous work on population genetics in male-killer infected populations. I will then give a short introduction to random genetic drift and how the diffusion equation methodology has been applied to study stochastic processes in evolutionary biology.

4.1.1 Previous work on host population genetics in male-killer infected populations

Most studies on the impact of male-killing bacteria on their hosts' population genetic processes are concerned with mitochondrial DNA (mtDNA). Because mitochondria and male-killers are both maternally inherited, there is tight linkage between these two genetic elements[1] and thus, male-killing bacteria may strongly influence mtDNA population genetics [208]. Consequently, studies that use mtDNA markers to reconstruct the biogeographical history are likely to produce misleading results if the species under investigation is infected with male-killing bacteria [208, 169]. On the other hand, analysing mtDNA can be very useful in drawing inferences on the history and the infection dynamical nature of a male-killer infection [348, 197, 88, 207].

Two major effects of male-killers on mtDNA can be distinguished [208]. First, during the spread of a male-killer infection, the mitochondrial haplotype associated with the first infected female will spread in a 'hitchhiking' manner along with the male-killer infection in the host population. This will lead to a reduction in mtDNA variation, the extend of which depends on the prevalence that the male-killer infection reaches. If the hitchhiking mtDNA happens to be selectively deleterious, population

[1]Interestingly, the same linkage is found between male-killing bacteria and the W chromosome in female heterogametic species like butterflies. Although this effect has been recognised [197], to date no studies on W chromosome variation in male-killer infected species have been undertaken.

mean fitness will be reduced during the spread (beyond the reduction caused by the male-killer *per se*).

The second effect comes into play when the male-killing bacteria have reached an equilibrium in the population. If this equilibrium is maintained (by the offsetting effects of imperfect transmission and positive selection through fitness compensation), mitochondrial haplotypes in the uninfected part of the population will be continuously replaced by haplotypes from infected females that have not transmitted their infection. Thus, subsequent to the rapid decline in mtDNA variation during the spread of the male-killer infection, mtDNA will not recover to its original value, but may remain low. The reduction in standing mtDNA diversity caused by male-killers will be the stronger the lower the prevalence, the lower the transmission rate, and the higher the fitness benefit for the male-killer is [208].

To date, only one study has been reported that is concerned with the impact of male-killing bacteria on the population genetics of nuclear genes [378]. In this theoretical investigation, a scenario with an infected and an uninfected population with bidirectional migration was analysed. It was demonstrated that the distorted gene flow caused by the different population sex ratios results in impeded adaptation on the infected island. To some extent, this also includes impeded spread of genes that confer resistance to the male-killer infection.

4.1.2 Random genetic drift and diffusion equations

Random genetic drift is the process of random fluctuations in gene frequencies due to random sampling of gametes in a population[2]. The importance of random genetic drift (then known as the 'Hagedoorn Effect') was recognized right at the onset of population genetics in Fisher's seminal article [112], and it continued to be regarded as a major force in evolution during the last century, for example within the frameworks of Wright's 'Shifting Balance Theory' [433], Kimura's 'Neutral Theory of Molecular Evolution' [221], and coalescence theory [225].

Several approaches have been used to analyse populations subject to random

[2]Note that random fluctuations in directional evolutionary forces such as selection, mutation and migration may also lead to random fluctuations in gene frequencies, and such processes are sometimes also included under the term 'random genetic drift' in a wider sense.

genetic drift, including Markov chains, integral equations, branching processes, and diffusion equations. In populations that reproduce in non-overlapping generations, the most exact approach is probably that of Markov chains. However, in many respects, diffusion equations have proved to be the more fruitful methodology when it comes to the interplay between random genetic drift and other evolutionary forces.

Fisher, in his 1922 paper, was the first to apply diffusion equations to investigate the effects of random genetic drift [112]. These equations were similar to the ones developed by Einstein and Smoluchowski to describe Brownian motion in physics. In 1931, Kolmogorov laid the firm mathematical foundation of diffusion processes in general [230]. Kolmogorov's fundamental results have been applied by (amongst others) Kolmogorov himself [231], Wright [434] and especially Kimura (e.g., [218, 219, 224]) to various problems in population genetics. Most notably, Kimura succeeded in obtaining the complete solution for the process of gene frequency change under random genetic drift alone [218], and a general formula for the probability of allele fixation [219].

Perhaps the most important diffusion equation is the Kolmogorov forward equation, also known as the Fokker-Planck equation in physics. In the context of random genetic drift, this partial differential equation takes the form

$$\frac{\partial \phi}{\partial t} = \frac{1}{2}\frac{\partial^2}{\partial x^2}(V_{\delta x}\phi) - \frac{\partial}{\partial x}(M_{\delta x}\phi). \tag{4.1}$$

In this equation, $\phi = \phi(p, x, t)$ is the probability density that the gene frequency is x at time t, provided the gene frequency was p at time $t = 0$. $M_{\delta x}$ and $V_{\delta x}$ are the mean and the variance in gene frequency change per generation, respectively. Both $M_{\delta x}$ and $V_{\delta x}$ will in most cases be functions of x, and both can also be functions of t. The Kolmogorov forward equation 4.1 can be derived from two fundamental assumptions concerning gene frequency change, as is demonstrated in [220]. First, it is assumed that the process of gene frequency change is a continuous stochastic process. This means, roughly speaking, that the probability of gene frequency change becomes infinitesimally small as the time interval under consideration becomes small. Second, it is assumed that the probability distribution of gene frequencies at given time t depends on the distribution at a previous time $t_0 < t$, but not on the history of gene frequency change before t_0. This second assumption is the time continuous analogue to the main assumption in Markov chains.

4.2 Model of selection and drift in populations infected with male-killing bacteria

I assume a single, unstructured host population of diploid individuals that reproduce sexually in discrete, nonoverlapping generations. Each individual is characterised by its sex, its genotype and its infection state (infected or not infected with male-killing bacteria). For the genotype, I consider one autosomal locus with two alleles, the wildtype and a mutant allele. Starting with N adult individuals, I assume a life cycle that consists of the following steps:

1. Random mating. Genotype-cytotype frequencies of the zygotes in the next generation are obtained. I assume fair meiosis, i.e. the genotype frequencies in the gametes are the same as in the parent population. The primary sex-ratio is assumed to be 1:1. Transmission of the male-killing bacteria is strictly maternal. Uninfected females produce uninfected eggs only, whilst infected females produce a proportion α of infected eggs and $1 - \alpha$ uninfected eggs.

2. Male-killing. All infected male zygotes are killed by the male-killing bacteria.

3. Fitness compensation. Following Hurst [179], the fitness of siblings of killed males is increased by a factor $[1 + b\alpha/(2 - \alpha)]$. Here, I assume that the resources freed by the death of males are equally distributed among the surviving siblings and that fitness increases linearly with the amount of resources. The parameter b gives

Table 4.1: Parameters used in the model.

Parameter	Description
N	population size at the stage of reproducing adults ($N > 0$)
s	selection coefficient ($s \geq -1$)
h	dominance level; heterozygotes have fitness of $1 + hs$ ($0 \leq h \leq 1$)
α	transmission rate of the male-killing bacteria ($0.67 \leq \alpha \leq 1$)
b	fitness compensation parameter; gives the amount of resources that are reallocated from dead males to surviving siblings ($0 \leq b \leq 1$)
μ	mutation rate per allele per generation ($0 \leq \mu \leq 1$)

Table 4.2: Variables used in the model. Note that in the model with perfect transmission, y is used as a parameter rather than as a variable.

Variable	Description
y, y^*	actual and equilibrium prevalence of the male-killing bacteria (i.e., fraction of infected females among all females in the population)
u_0, u_{MK}	fixation probability of newly arisen mutations in uninfected and male-killer infected populations, respectively
$\hat{t}_0$, $\hat{t}_{MK}$	expected times until fixation of new mutations provided fixation occurs
$\tilde{t}_0$, $\tilde{t}_{MK}$	expected times until either fixation or extinction of new mutations
H_0,H_{MK}	expected equilibrium heterozygosity

the fraction of resources freed by the death of males that are reallocated to increase the surviving siblings' fitness, and the transmission rate α gives the proportion of male zygotes that are killed.

4. Selection according to genotype. I assume that individuals that are homozygous for the mutant allele have their fitness altered by the factor $1 + s$, and heterozygous individuals have their fitness altered by $1 + hs$, relative to a fitness 1 of individuals homozygous for the wildtype allele. s can take both negative and positive values, reflecting deleterious and beneficial mutations, respectively. h is the level of dominance and can take values between zero and one[3]. For example, with $h = 0$, the mutant allele is completely recessive, whilst with $h = 0.9$, it is almost completely dominant.

5. Random survival. Based upon the genotype-cytotype frequencies obtained in the above steps of the life cycle, N new mature adult individuals are randomly drawn. Thus, I assume that the prevalence of the male-killing bacteria does not affect total population size.

The parameters of the model are summarized in Table 4.1, and the variables that I will use to describe properties of the host population are given in Table 4.2.

[3]Over- and underdominant mutant alleles, i.e., $h > 1$ and $h < 0$, respectively, are not considered in my model.

4.3 Results I: Perfect transmission

Throughout this section, I will assume that all offspring of infected females are again infected (i.e., $\alpha = 1$), and that daughters from infected mothers do not receive any fitness benefit through the death of their brothers (i.e., $b = 0$). The assumption of perfect transmission is not only convenient for the derivation of analytic approximations, but is also a situation found in some natural populations infected with male-killing bacteria [205, 90]. Moreover, I will assume that the prevalence of the male-killing bacteria and the population sex-ratio remain constant over the time frame under consideration.

4.3.1 The probability of fixation of a mutant allele

Following Kimura [219], the fixation probability of an allele that is at frequency p in an uninfected population with even sex-ratio can be approximated by

$$u(p) = \frac{\int_0^p G(x)\mathrm{d}x}{\int_0^1 G(x)\mathrm{d}x}, \qquad \text{with} \qquad G(x) = e^{-4Nshx-2Ns(1-2h)x^2}. \tag{4.2}$$

Accordingly, the fixation probability of a newly arisen allele is

$$u_0 = \frac{\int_0^{1/2N} G(x)\mathrm{d}x}{\int_0^1 G(x)\mathrm{d}x}. \tag{4.3}$$

I now consider the case where the population is infected with a male-killing parasite with perfect maternal transmission. I assume that the prevalence of the male-killer is fixed at a proportion y of infected females among all females and the sex-ratio is distorted accordingly. The relative proportions of infected females, uninfected females and uninfected males in the adult population are then given by $y/(2-y)$, $(1-y)/(2-y)$, and $(1-y)/(2-y)$, respectively.

A newly arisen nuclear mutant allele will be found either in an infected female (with probability $y/(2-y)$) or in an uninfected individual (with probability $(2-2y)/(2-y)$). However, if the mutant allele emerges in an infected female, the chances of quick extinction are extremely high unless the allele confers a very large fitness advantage to its carrier in the heterozygous state. This is simply because nuclear genes in infected females have *a priori* a fitness that is only about half that of the same allele in uninfected females (see Ref. [372] for a similar argument).

Thus, it can be assumed that neutral alleles, detrimental alleles and slightly or moderately beneficial alleles will invariably become extinct when they arise in infected females. Therefore, only alleles that arise in the uninfected proportion of the population have a chance of becoming fixed in the population. Since alleles can only spread from uninfected males to infected females, but not from infected females to uninfected individuals, the dynamics of new alleles are completely determined by the uninfected individuals in the population. Thus, building upon Equation 4.3, we can approximate the fixation probability of a newly arisen allele by

$$u_{\mathrm{MK}} = \frac{2-2y}{2-y} \times \frac{\int_0^{(2-y)/4N(1-y)} G(x)\mathrm{d}x}{\int_0^1 G(x)\mathrm{d}x} \tag{4.4}$$

with

$$G(x) = \exp\left[-8N\frac{1-y}{2-y}shx - 4N\frac{1-y}{2-y}s(1-2h)x^2\right].$$

In this approximation, I take into account the probability that a mutation occurs in an uninfected individual by replacing the population size N in Equation 4.3 with the number of uninfected individuals in the population, $2N(1-y)/(2-y)$. Note that the sex-ratio within the uninfected part of the population is even, so that no sex-ratio distortion needs to be taken into account.

Unfortunately, in general formula 4.4 cannot be simplified. However, we can shed light on the fixation probability of nuclear alleles in populations infected with male-killers by making two specific assumptions about the selection and dominance coefficients s and h.

Neutral alleles. With $s = 0$, the function $G(x)$ greatly simplifies to $G(x) \equiv 1$, yielding

$$u_{\mathrm{MK}} = \frac{2-2y}{2-y} \times \frac{\int_0^{(2-y)/4N(1-y)} \mathrm{d}x}{\int_0^1 \mathrm{d}x} = \frac{1}{2N} \tag{4.5}$$

As expected from neutral theory, this is the same fixation probability as for a neutral allele in an uninfected population. (The reason for this result is that two effects exactly cancel out: the probability that the mutant arises in an individual from which it can spread through the population is decreased when the population is infected with male-killers, but at the same time, the allele can spread more easily because of the reduced relevant population size.)

Semidominant alleles. For a semidominant allele, the fixation probability in an infected population simplifies to

$$u_{MK} = \frac{2-2y}{2-y} \times \frac{1-\exp(-s)}{1-\exp[-4Ns(1-y)/(2-y)]} \tag{4.6}$$

In what follows, I will prove that for beneficial alleles ($s > 0$), u_{MK} is always less than u_0 (see Equation 4.3). Let $A := 2Ns$ ($A > 0$) and $B := (2-2y)/(2-y)$ ($0 < B < 1$). Then, $u_{MK} < u_0$ is equivalent to

$$1 - B - e^{-AB} + Be^{-A} > 0. \tag{4.7}$$

The left side of this inequality is zero for $A = 0$. Moreover, the derivative of the left side with respect to A, $B\exp(-AB) - B\exp(-A)$, is always greater than zero. From this it follows that Inequality 4.7 is true for all $A > 0$ and $0 < B < 1$, which proves the assertion. Analogously, it can be demonstrated that $u_{MK} > u_0$ holds for $s < 0$.

Thus, I have proven that within the model framework employed, male-killing bacteria will decrease the fixation probability of beneficial semidominant alleles and increase the fixation probability of deleterious semidominant alleles. For large values of Ns, $u_{MK} \approx u_0(2-2y)/(2-y)$ holds, i.e., the probability that a beneficial allele becomes fixed in a sufficiently large population is approximately the fraction of uninfected individuals multiplied by the probability of fixation of the same allele in an uninfected population of the same size.

Figure 4.1 shows estimates of the fixation probability in populations carrying a male-killer relative to fixation probabilities in uninfected populations (i.e., u_{MK}/u_0) for different slices of the parameter space. Also shown are the average fixation rates observed in computer simulations, in which I ran 10^6 simulations per data point. These simulation results are in very good agreement with the analytic predictions.

Finally, Figure 4.1 gives the predictions for the fixation probability derived from the application of Wright's equation relating effective population size to sex ratio, $N_e = 4N_mN_f/(N_m + N_f)$ [433]. Here, the resulting formula in terms of the actual population size N and the male-killer prevalence y, $N_e = 4N(1-y)/(2-y)^2$, was inserted into the function $G(x)$ instead of N in Equation 4.3. As can be seen, the prediction based on the skewed sex-ratio is not appropriate for male-killer infected populations.

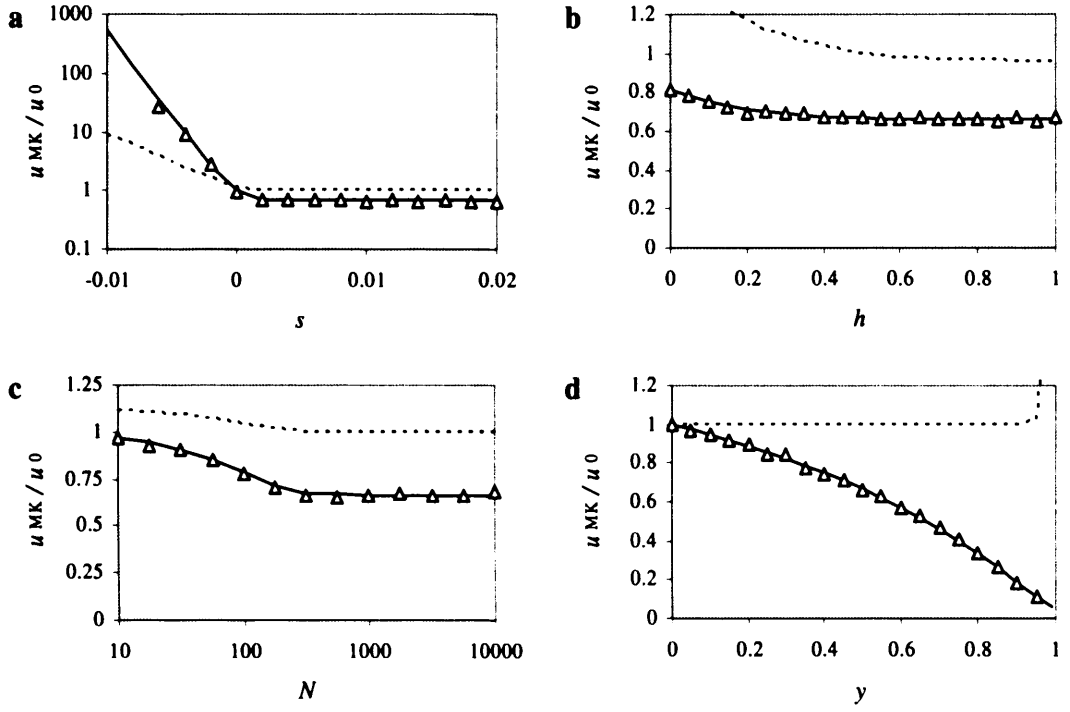

Figure 4.1: Fixation probabilities of newly arisen nuclear alleles in male-killer infected populations, relative to fixation probabilities in uninfected populations. The solid curve gives the analytic approximation, empty triangles represent fixation rates averaged over (a) 10^7 or (b-d) 10^6 simulations per datum. The dotted lines give fixation probability estimates based on Wright's approximation for effective population size in populations with biased sex-ratio. Unless varied, parameters take the values $N = 1000$, $s = 0.01$, $h = 0.5$, $y = 0.5$.

For semidominant alleles I have demonstrated analytically that a male-killer infection increases the fixation probability of deleterious alleles, but decreases the fixation probability of beneficial ones (see above). To test whether this result also holds for arbitrary dominance levels, I conducted further computer simulations with randomly chosen parameters s, h, N, and y. The results shown in Figure 4.2 confirm that the male-killer infection makes the fixation of deleterious alleles more likely, and the fixation of beneficial alleles less so, across all parameter values.

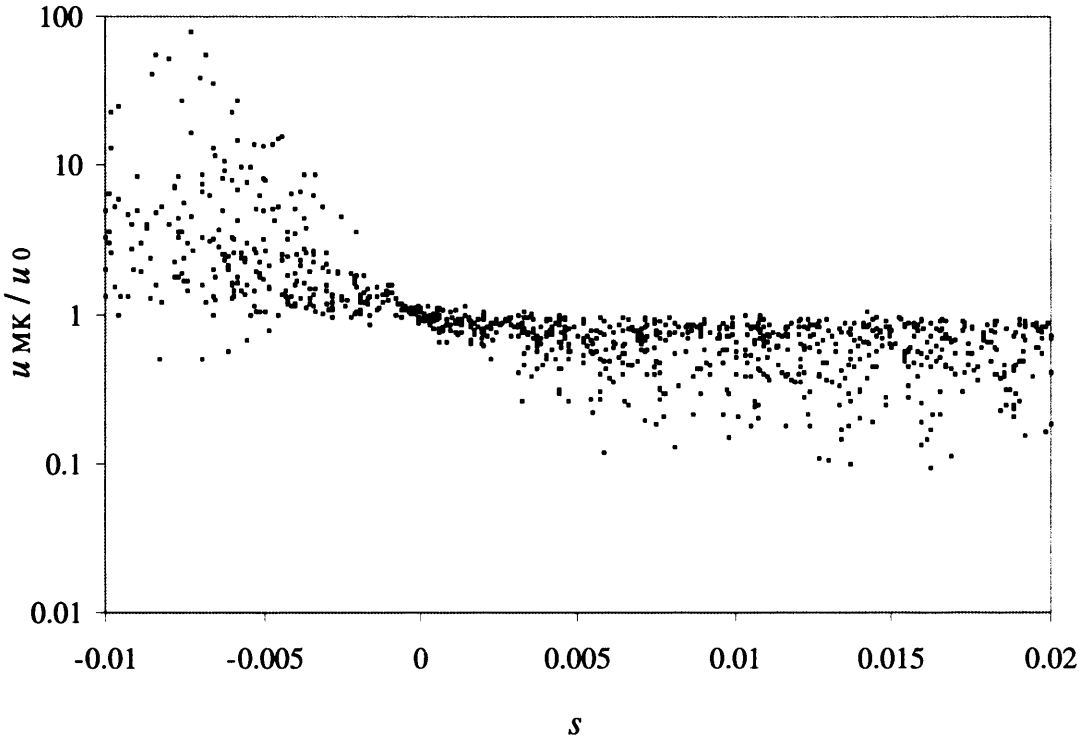

Figure 4.2: Relative fixation probabilities of newly arisen nuclear alleles in male-killer infected populations (i.e., u_{MK}/u_0). The plot is based on fixation rates from 10^6 computer simulations with an infected population and 10^6 simulations with an uninfected population. Parameters for the 1000 sets of simulations conducted were chosen randomly, with selection coefficients s as shown, dominance levels h in-between 0 and 1, population sizes N in between 100 and 500, and male-killer infection frequencies y in between 0.2 and 0.99. Note that for 30 out of 1000 parameter combinations, simulations results could not be included in the plot because the alleles never became fixed in the uninfected population during the 10^6 simulations conducted.

4.3.2 The rate of allele substitution

The rate of allele substitution in an uninfected population of size N is given by $k_0 = 2\mu N u_0$, where μ is the mutation rate per locus per generation. In a population infected with male-killing bacteria, the substitution rate is accordingly $k_{MK} = 2\mu N u_{MK}$. After inserting the above formula for u_{MK} (Equation 4.4), it becomes apparent that the rate of allele substitution in a population carrying a male-killer is identical to that in an uninfected population of the same size as the uninfected portion of the male-killer infected population. In other words, the rate of allele substitution is completely determined by the size of the uninfected part of a population, and additional infected individuals have no impact.

The relative rate of allele substitution in an infected compared to an uninfected population is the same as the relative fixation probability ($k_{MK}/k_0 = u_{MK}/u_0$), so that the results obtained in the previous section also hold for the rate of allele substitution (see also Figures 4.1 and 4.2). In particular, the substitution rate of deleterious mutations is higher in male-killer infected populations than in uninfected populations, and *vice versa* for beneficial mutations.

4.3.3 Expected time until fixation

The expected time until fixation for a mutant allele in a population of size N was estimated by Kimura & Ohta [224] as

$$\hat{t}_0(N) = \int_{1/2N}^{1} \psi(x)u(x)[1-u(x)]\mathrm{d}x + \frac{1-u_0}{u_0}\int_0^{1/2N} \psi(x)u^2(x)\mathrm{d}x, \tag{4.8}$$

where

$$\psi(x) = \frac{4N\int_0^1 G(z)\mathrm{d}z}{x(1-x)G(x)} \tag{4.9}$$

and $u(x)$, u_0 and $G(x)$ are defined as above (Equations 4.2 and 4.3). As has become apparent in the previous section, for a mutant allele to spread and become fixed in a male-killer infected population, it is necessary that the mutant arises and spreads in the uninfected individuals of the population. Fixation within the uninfected individuals will be followed quickly by fixation of the allele within infected females, because of the strong influx of the allele each generation through males. Hence, we can estimate the expected time until fixation in an infected population simply as that found in an uninfected population with size equal to the uninfected fraction of the infected population,

$$\hat{t}_{MK}(N,y) = \hat{t}_0\left(\frac{2-2y}{2-y}N\right). \tag{4.10}$$

For a selectively neutral allele, the expected time until fixation in an uninfected population is approximately $\hat{t}_0 \approx 4N$ [224]. Accordingly, in an infected population we have

$$\hat{t}_{MK}(N,y) \approx \frac{1-y}{2-y}8N. \tag{4.11}$$

for neutral alleles. Thus, the expected time until fixation of neutral alleles is decreased in infected compared to uninfected populations by a factor given by the proportion of uninfected individuals in the population.

4.3.4 Expected time until fixation or loss

Aside from this conditional time until fixation, we can also estimate the expected time for a mutant allele to either become fixed or be lost. From the formulae given by Kimura & Ohta [224], the following approximation can be derived for this quantity in an uninfected population:

$$\tilde{t}_0(N) = u_0 \int_{1/2N}^{1} \psi(x)[1-u(x)]\mathrm{d}x + (1-u_0)\int_0^{1/2N} \psi(x)u(x)\mathrm{d}x \qquad (4.12)$$

To derive an approximation for the unconditional time a mutant allele stays polymorphic in a male-killer infected population, it is again useful to consider the different individuals the mutant allele might arise in. With probability $(2-2y)/(2-y)$, the mutant arises in an uninfected individual, in which case the expected time until fixation or loss can be estimated by $\tilde{t}_0((2-2y)/(1-y)N)$. To get an estimate for the expected time until fixation or loss when the mutant arises in an infected female, I will not attempt an approximation based on the diffusion equation, but adopt a more direct approach. I will assume that the fitness effects of the allele itself are negligible compared to the deleterious effect that the male-killing bacteria have on the allele's fitness, namely a fitness reduction by 1/2. The probability distribution of the frequency of the mutant allele in infected females is described by a Markov chain with transition matrix

$$(\mathbf{P})_{ij} = \left(\binom{M/2}{i} \left(\frac{j}{M}\right)^i \left(\frac{M-j}{M}\right)^{M/2-i} \right)_{ij}, \qquad i,j \in \left\{0,1,...,\frac{M}{2}\right\} \qquad (4.13)$$

where $M := 2Ny/(2-y)$ is the number of alleles present in the infected females. $(\mathbf{P})_{ij}$ is the probability that i mutant alleles are present in the infected females provided that j mutant alleles were present in the previous generation. Note that the maximum number of mutant alleles present in infected females is $M/2$ only, because every infected female inherits one wildtype allele from her father.

The probability that the allele is extinct at generation k after the mutation event is given by $(\mathbf{P}^k)_{01}$. In my approximation, I will only consider the first two generations, as the probability that the mutant allele survives longer than that is very low.

Putting all the above considerations together, we get as an estimate for the expected time until fixation or loss of a mutant allele in a population infected with

male-killers

$$\begin{aligned}
\tilde{t}_{\text{MK}} &= \frac{2-2y}{2-y} \times \tilde{t}_0 \left(\frac{2-2y}{2-y} N \right) + \frac{y}{2-y} \times \sum_{k=0}^{\infty} k(\mathbf{P}^k)_{01} \\
&\approx \frac{2-2y}{2-y} \times \tilde{t}_0 \left(\frac{2-2y}{2-y} N \right) + \frac{y}{2-y} \times \sum_{k=0}^{2} k(\mathbf{P}^k)_{01} \\
&= \frac{2-2y}{2-y} \times \tilde{t}_0 \left(\frac{2-2y}{2-y} N \right) + \frac{y}{2-y} \times \left(\frac{M-1}{M} \right)^{M/2} \\
&\quad + \frac{y}{2-y} \times 2 \sum_{l=0}^{M/2} \binom{M/2}{l} \left(\frac{1}{M} \right)^{l} \left(\frac{M-1}{M} \right)^{M/2-l} \left(\frac{M-l}{M} \right)^{M/2} \qquad (4.14)
\end{aligned}$$

Estimates for both the expected conditional time until fixation and the expected time until fixation or extinction of mutant alleles in male-killer infected populations are shown in Figure 4.3, again relative to the respective expected times in uninfected populations (i.e., $\hat{t}_{\text{MK}}/\hat{t}_0$ and $\tilde{t}_{\text{MK}}/\tilde{t}_0$). Both expected times are lower in infected than in uninfected populations. Moreover, both $\hat{t}_{\text{MK}}$ and $\tilde{t}_{\text{MK}}$ decrease with increasing frequency of the male-killer. Unfortunately, my attempts at numerical integration failed for large values of s. Also shown in Figure 4.3 are simulation results for the relative expected times until fixation and fixation or extinction, obtained by the same simulations as for Figure 4.1. For both the expected conditional time until fixation and the expected time until fixation or extinction, prediction and simulation results are in good agreement. For the expected time until fixation, strongly diverging values for deleterious alleles are readily explained by large confidence intervals (out of 10^6 simulations, there were only 12 and 1 fixation events for $s = -0.004$ and $s = -0.006$, respectively).

4.3.5 Genetic variation

One measure of genetic variation in a population is the level of heterozygosity H, the proportion of heterozygous individuals in a population. This quantity is equal to $1 - F$, where F is the proportion of homozygous individuals in the population. Consider a locus where mutations occur at rate μ and each mutation leads to a new allele not previously present in the population ('infinite alleles model' [223]). Assuming that the actual population size equals the effective population size, and that all alleles are selectively neutral, the expected equilibrium heterozygosity has

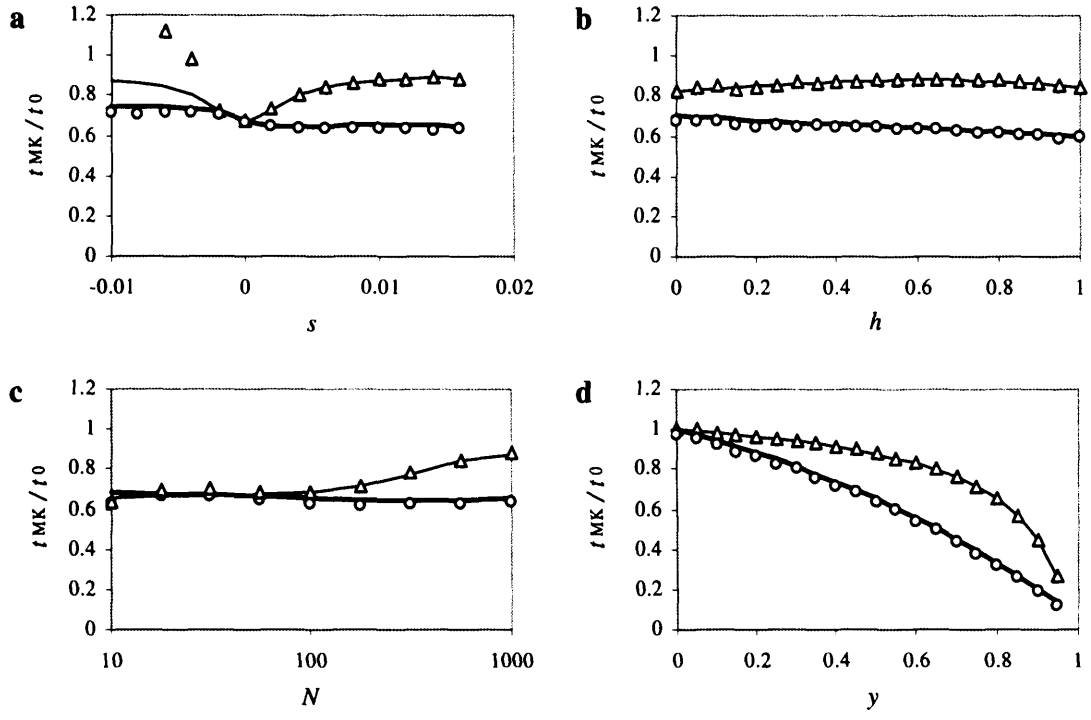

Figure 4.3: Times until fixation (for alleles that fix) and times until fixation or extinction in a population infected with a male-killer infected relative to an uninfected population. The solid curves gives the approximation for the relative time until fixation, the bold curve gives the approximation for the relative time until fixation or extinction. Triangles and circles give the respective simulation results for these quantities. Simulation results are based on the same data and parameters take the same values as in Figure 4.1.

then been estimated [223] to be

$$H_0 = 1 - F_0 = 1 - \frac{1}{4N\mu + 1}. \tag{4.15}$$

Applying this fundamental result to a population infected with male-killing bacteria, we first note that the equilibrium heterozygosity in the uninfected part of the population will be equal to the equilibrium heterozygosity of an uninfected population that has the same size as the uninfected part of the infected population. This is simply because there is no gene flow from infected females to uninfected individuals. Thus,

$$H^{\text{uninf}} = 1 - \frac{1}{4[(2-2y)N/(2-y)]\mu + 1} = 1 - \frac{2-y}{8(1-y)N\mu + 2 - y}. \tag{4.16}$$

Because newly arisen mutations in infected females will quickly disappear from the population, the genetic composition of infected females will be approximately the same as that of uninfected females. Therefore, it may be predicted that the total heterozygosity in an infected population will be approximately the same as the heterozygosity in the uninfected fraction of that population, as given in formula 4.16.

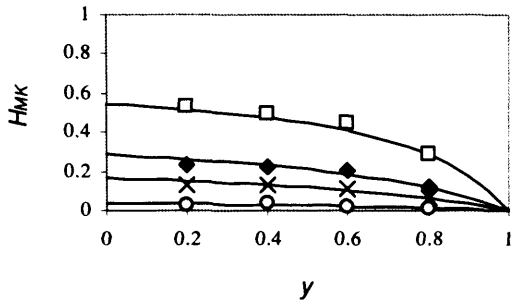

Figure 4.4: Predicted equilibrium heterozygosity (lines) derived from Equation 4.17, and corresponding simulation results (symbols), depending on the prevalence of the male-killer infection. In the simulations, I initiated the population with one allele fixed, and took values of heterozygosity every 1000 generations. Each datum is an average of 300 values of heterozygosity. In all simulations, $N = 100$. The mutation rate μ takes values of 0.0001 (grey circles), 0.0005 (crosses), 0.001 (black diamonds) and 0.003 (white squares).

Given this reasoning, the expected equilibrium heterozygosity in a male-killer infected population is approximately

$$H_{\text{MK}} = H^{\text{uninf}} = \frac{8(1-y)N\mu}{8(1-y)N\mu + 2 - y} \tag{4.17}$$

Clearly, the expected genetic variability in a male-killer infected population as measured by heterozygosity is smaller than in an uninfected population of the same size. Figure 4.4 shows how the expected equilibrium heterozygosity in male-killer infected populations declines with increasing prevalence of the male-killers. The decline is most notable for prevalence values in excess of 0.6; here marked reductions in heterozygosity are observed. Also shown are the results of computer simulations that confirm my analytical approximations.

4.4 Results II: Imperfect transmission

In the previous section, I assumed perfect transmission of the bacteria. However, in many cases, infected females also produce some uninfected offspring. Transmission rates ranging from 80% to 100% have been reported, although they are usually in the range of 95% to 100% [174, 205, 88]. Thus, let us now assume that only a proportion α ($0.67 < \alpha < 1$) of the offspring of infected females inherit the infection, and $(1 - \alpha)$ of the offspring are uninfected ($\alpha > 0.67$ is required for male-killer invasion). I will also assume that surviving siblings of infected females receive a fitness compensation benefit b due to the death of their brothers, and relax my previous assumption of a constant prevalence of the male-killing bacteria. I do not attempt to derive approximations for the case of imperfect male-killer transmission, but rather determine by computer simulations the extent to which fixation probabilities and times until fixation and/or extinction deviate from the approximations derived for perfect transmission.

In a deterministic model, the polymorphic equilibrium frequency of the male-killing bacteria (proportion of infected females among all females) is given by

$$y^* = \frac{3\alpha - 2 - \alpha^2(1-b)}{\alpha b}, \tag{4.18}$$

and this equilibrium is always stable when it exists ([179], see also Equation 3.3). This equilibrium corresponds to the expected frequency of the male-killing bacteria in my stochastic model, as long as the male-killer does not become extinct and the population does not go extinct due to lack of males.

How can we expect inefficient transmission to affect the population genetics of host alleles? First, given a certain frequency of the male-killing bacteria within females, decreasing transmission rates will both increase the fitness of infected females and lead to gene flux from infected females to uninfected individuals. This will result in fixation probabilities and expected times to fixation and/or extinction that lie in-between the predictions for an uninfected population and an infected population with perfect male-killer transmission. Second, one also needs to take into account that the male-killing bacteria themselves will be subject to random genetic drift. For a given equilibrium male-killer frequency, drift will become stronger with increasing transmission rate and correspondingly decreasing fitness compensation benefit

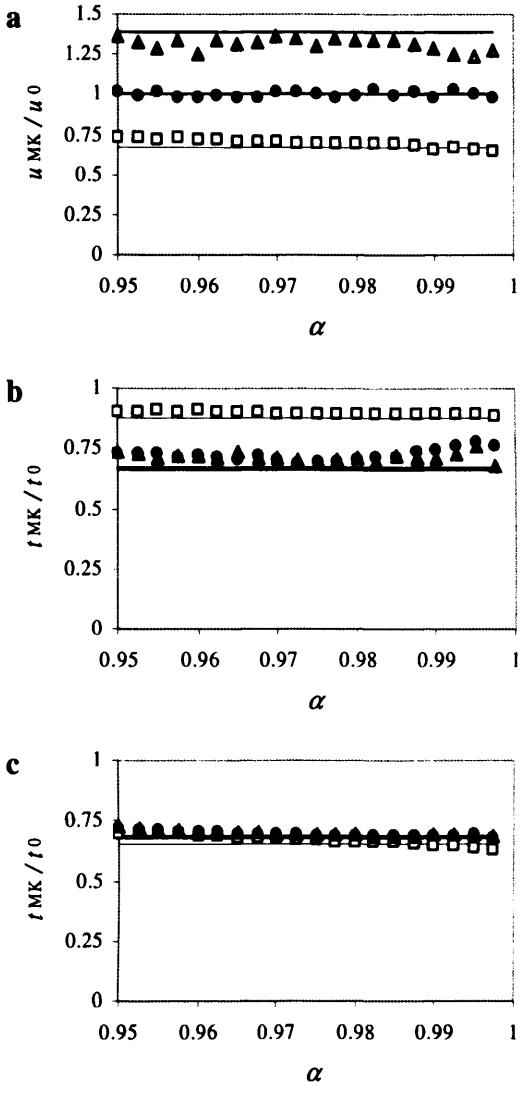

Figure 4.5: The effect of relaxing the assumption of perfect vertical transmission. Simulation results are given with varying rates of imperfect transmission. The plots show simulation estimates for (a) relative probability of fixation of alleles, (b) relative times to fixation, and (c) relative times to fixation or extinction, all depending on the transmission rate of the male-killing bacteria α. Three different allele classes have been assumed in these simulations: a beneficial semidominant allele with $s = 0.01$ and $h = 0.5$ (white squares), a neutral allele (black circles) and a deleterious recessive allele with $s = -0.001$ and $h = 0$ (grey triangles). Each datum represents an average of 3×10^6 simulations. Solid lines show analytical predictions based on perfect transmission. Other parameters take the values $N = 1000$ and $y^* = 0.5$; the fitness compensation parameter b was varied according to transmission rate to keep equilibrium male-killer frequency constant (see Equation 4.18).

b. The impact of endosymbiont drift on the drift of the nuclear alleles is thus not straightforward to predict.

I obtained simulation estimates for fixation probabilities of alleles, expected times until fixation and expected persistence times with different selection coefficients s and dominance levels h for varying transmission efficiencies, α (Figure 4.5). In these simulations, the expected frequency of the male-killing bacteria y^* was kept at a constant value with varying α by adjusting the fitness compensation parameter b according to formula 4.18. Each simulation was initiated with the male-killing bacteria at their expected frequency.

As can be seen in Figure 4.5a, the predictions based on perfect transmission give fairly good approximations for a wide range of transmission rates below 1. For low transmission rates, estimates for the fixation probabilities u_{MK} are slightly closer to u_0 than predicted by the estimates for perfect transmission and fixed male-killer

frequency. This is in accord with the above reasoning. For the expected conditional time until fixation and the expected time until fixation or extinction (Figures 4.5b and c), the deviations of the simulation results from the predictions based on perfect transmission again are not severe, and the direction of the deviations is towards the expected times in an uninfected population.

To determine the regions of parameter space where deviations in fixation probabilities from the analytical predictions are highest, I performed simulations with randomly chosen parameters N, s, α, and b. Plotting relative fixation probabilities against the selection coefficient s yields a graph very similar to that in Figure 4.2 (not shown), confirming that imperfect transmission does not qualitatively alter my fundamental result of increased and decreased fixation probabilities of deleterious and beneficial alleles, respectively.

Figure 4.6 shows relative deviations of fixation rates yielded from these simulations from the analytical predictions with perfect transmission. For beneficial alleles (Figure 4.6a), most deviations are relatively small, and tend to be positive, at least

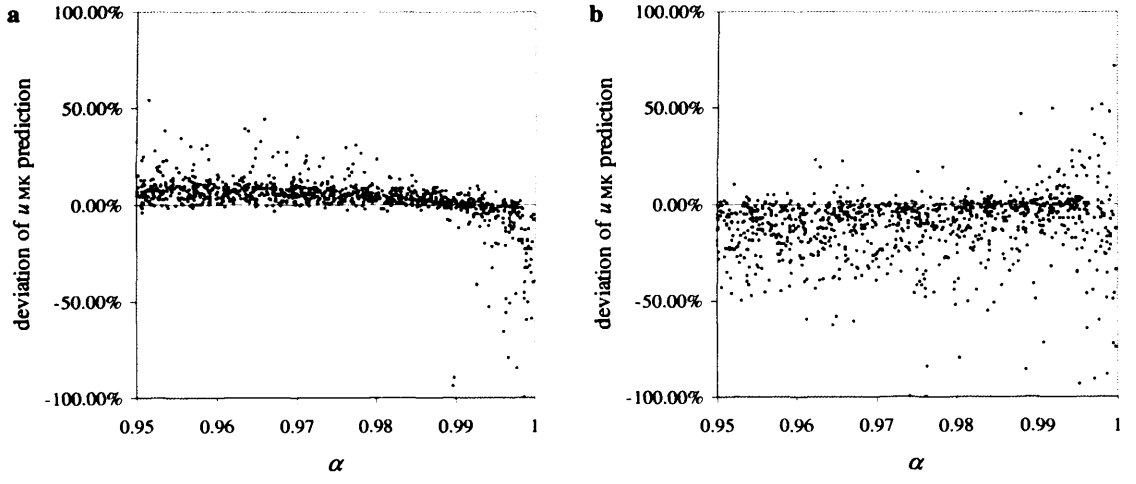

Figure 4.6: Relative deviations of fixation rates derived from simulations with imperfect transmission from predictions based on the assumption of perfect transmission. Each datum in the two plots represents an average of 10^6 simulations. For each of the 1000 points per plot, parameters were chosen randomly, with transmission rate α in-between 0.95 and 1, equilibrium frequency y^* in-between 0 and 1, population size N in between 100 and 500, and selection coefficients s in-between (a) 0 and 0.02 (beneficial alleles) and (b) -0.001 and 0 (deleterious alleles). The fitness compensation parameter b was chosen according to Equation 4.18, but only simulations with biologically reasonable values of $0 \leq b \leq 1$ are included in the plots. Semidominance (h=0.5) was assumed in all simulations.

for low transmission rates. This means that the actual fixation rates are higher than the predictions based on the assumption of perfect transmission, and thus closer to fixation probabilities in uninfected populations. However, for very high transmission rates, deviations become negative, meaning that fixation rates are even lower than predicted. The strongest negative deviations are observed with high transmission rate and high equilibrium frequency (i.e., when both α and b are high). This may be explained by the surmise that with male-killing bacteria drifting at high frequencies, the frequencies above the equilibrium will have a much stronger impact than frequencies below the equilibrium. The reasoning behind this conjecture is an analogy with populations of fluctuating size, in which it has been demonstrated that the effective population size is given by the harmonic mean of population sizes, and thus, smaller than the arithmetic mean [222].

For deleterious alleles (Figure 4.6b), deviations of fixation rates from the predictions tend to be higher than for beneficial alleles, as can be expected from the fact that such fixation events are very rare and thus the variance of fixation rates in a finite set of simulations is much higher than for beneficial alleles. Most deviations are negative, i.e. fixations occurred less often than predicted. Again, this is explained by the fact that with imperfect transmission, there is allele flux from infected females to uninfected individuals, and thus more efficient purging of deleterious mutations.

4.5 Discussion

I have studied basic aspects of the population genetics of populations infected with male-killing bacteria. My results suggest that when maternal transmission of the bacteria is perfect and their prevalence in the population stable, the population behaves approximately as if it consisted of the uninfected part of the population only. This includes increased fixation probabilities for deleterious alleles and decreased fixation probabilities for beneficial alleles, decreased times to fixation and to either fixation or extinction, and reduced genetic variation. When transmission of the male-killing bacteria is imperfect, the effect is somewhat mitigated, but still strong.

In contrast to what might be expected at first glance, the distorted sex ratio in populations infected with male-killing bacteria is not primarily connected to these results, and approximations based on Wright's effective population size in populations with uneven sex ratios [433] yield misleading predictions. Rather, the reason for the effects observed is that alleles that arose in an infected female cannot spread, because (1) infected females have only about half the fitness of uninfected females, and (2) the nucleotype of infected females is tightly linked to the male-killing cytotype. Thus, although reproducing, the infected females in the population can be regarded as 'living deads' (to use a metaphor framed for individuals of inferior genotype in asexual populations [318]), and their number has almost no impact on the population genetics of nuclear genes.

It is well known that selection for or against alleles is not completely determined by the phenotype that the allele induces, but also by linkage to other alleles. For example, recurrently arising deleterious mutations can reduce genetic variability at linked neutral loci, a process known as 'background selection' [66]. The effect of male-killing endosymbionts is similar to background selection, as both stem from linkage to deleterious genetic elements. However, provided that their prevalence is sufficiently high, male-killing bacteria can be expected to have a stronger impact on genetic variability than deleterious nuclear alleles. This is because first, linkage of all newly arisen alleles in infected females to the male-killing element is complete or at least very high. Linkage here corresponds to the vertical transmission rate of the male-killing bacteria, for which quantity values in the range of 0.8 to 1 have been reported in natural and laboratory populations of various insect species [174, 205, 88]. Second,

despite their being strongly deleterious to nuclear alleles, male-killing bacteria carry a drive and can be stably maintained in populations at high frequencies [90, 88]. This is in contrast to deleterious nuclear alleles, which are constantly purged and usually kept at low frequencies. Combined, these two aspects are expected to result in very strong selection against all alleles (even beneficial ones) when they arise in infected females, and correspondingly in a strong reduction in neutral genetic variation and adaptability.

Although I have focused on 'classical' population genetics (i.e., I considered alleles as the basic unit of evolution), some inferences may also be drawn regarding molecular evolution. For example, let us consider a certain gene and let us assume that synonymous nucleotide substitutions are neutral, and that most nonsynonymous nucleotide substitutions are deleterious. Because deleterious alleles have an increased probability of fixation in male-killer infected populations, whilst neutral alleles have the same fixation probability in infected and uninfected populations, we would expect an increased ratio $\omega = dN/dS$ in male-killer infected populations compared to uninfected populations of similar size.

4.5.1 Male-killing bacteria and population dynamics

One assumption in my model is that changes in the prevalence do not affect the total population size. In contrast, there may either be a positive or a negative impact of male-killer prevalence on population size, depending on the type of density dependent population regulation [146, 226].

Male-killer infections can influence population size in two ways. First, the population size when the male-killer is at equilibrium frequency in the host population may be different from that if the population is uninfected. This is important when one is interested in how the infection of a particular population with male-killing bacteria changed the population genetics of that population. On the other hand, if one is interested in how the population genetics of a male-killer infected population deviate from the expectations if it were not known that the population is infected, it is irrelevant how the equilibrium male-killer frequency affects the population size. (The former case is a comparison prior and after infection, the latter a comparison of an infected and an uninfected population of the same size.) Second, and independent

of the type of question asked, fluctuations in population size as a result of fluctuations in the male-killer prevalence need to be taken into account. Fluctuations of this kind are likely to make the effects found in my model more severe, as the effective population size will be decreased below the population size where the male-killer is at equilibrium frequency.

4.5.2 Other sex-ratio distorters and non-autosomal genes

In my model, I have only studied the impact of male-killing bacteria on population genetic properties of autosomal alleles. It may also be of interest to make some conjectures on the impact of male-killers on alleles located on sex chromosomes, and the impact of sex ratio distorters other than male-killers on their hosts' population genetics.

Feminisation is another common strategy of reproductive parasitism that is found in arthropods. The best studied example is the woodlouse *Armadillidium vulgare*, which is infected by feminising *Wolbachia* (reviewed in [322]). In some populations, the ancestral sex determination of female heterogamety has been replaced by a system where all infected individuals are females, and all uninfected individuals are males; the W chromosome has become extinct, so that all individuals are 'genetic males' with a ZZ constitution. Thus, females produce offspring in a female-biased sex ratio that corresponds to the transmission rate of *Wolbachia*. Correspondingly, as with male-killer infections, the population sex-ratio is female biased. However, in contrast to a male-killer infection, there is unrestricted gene flow between infected and uninfected individuals (i.e., females and males). Therefore, the effect of selection and drift on nuclear alleles will be the same as in any uninfected population with the same population sex ratio, and applying Wright's [433] formula for the effective population size should be adequate. Similarly, Wright's equations should be sufficient for describing cases of X chromosome meiotic drive.

How can we expect male-killing bacteria to affect the population genetics of sex chromosomes? My results for autosomal genes can readily be extended to X and Y chromosomes in male heterogametic species, and Z chromosomes in females heterogametic species. Again, we can expect the number of infected females in the population to be unimportant, and the number of uninfected individuals in the population to be

the determining factor. Analogously to standard population genetics in uninfected populations, the number of sex chromosomes present in the uninfected individuals needs to be taken into account, so that the effective population size will be 3/4, 1/4, and 3/4 times the number of uninfected individuals for X, Y, and Z chromosomes, respectively.

W chromosomes in female heterogametic species, like mitochondrial genes, are closely linked to maternally inherited endosymbionts, and the impact of male-killers on these genetic elements has already been been discussed in the introduction of this chapter (see Section 4.1.1). One noteworthy aspect when comparing the impact of male-killers on autosomal with that on mitochondrial or W linked genes is that the relevant fraction of the population are the uninfected individuals for autosomal, but the infected individuals for maternally inherited alleles. For example, with increasing male-killer prevalence, standing genetic variation will decrease for autosomal, but increase for maternally inherited DNA.

4.5.3 Implications for empirical and theoretical studies

My results imply that caution needs to be taken when estimating population genetic properties such as fixation rates or genetic variability in arthropod populations with biased sex ratio. Clearly, it is not sufficient to apply Wright's formula for the effective population size without determining the reason for the distorted sex ratio. In studies comparing mitochondrial with nuclear DNA variation in male-killer infected populations [197, 88, 207], this may lead to overestimates of mitochondrial genetic variability relative to nuclear variability and correspondingly, the age of a male-killer infection may be overestimated. For example, Dyer & Jaenike studied DNA variation of mitochondrial and nuclear genes in the male-killer infected *Drosophila innubila* [88]. These authors concluded that *Wolbachia* infection is evolutionarily old, because the level of polymorphism of mtDNA was not significantly different from that of autosomal nuclear DNA. However, this analysis was based on the assumption that the effective population size of the host population is given by Wright's formula. The results presented in this chapter demonstrate that the actual effective population size is smaller than predicted by Wright's formula. Thus, there might be less mtDNA variation than expected at equilibrium, and the male-killer infection might have occurred

more recently than predicted.

Moreover, I would like to caution against disregarding the peculiar population genetics of male-killer infected populations in ESS models. For example, based on the results of an ESS model, it has been hypothesised that male-killing bacteria can select for increased clutch-size [175]. In this model, the mean fitness effect caused by a trait (the clutch size), averaged over all individuals (infected and uninfected), was considered and maximised. By contrast, whether a beneficial gene will spread or not will largely depend on its fitness impact in uninfected individuals. Taking the average of the fitness effect in infected and uninfected individuals will often produce misleading conclusions. Explicit population genetic modelling, or averages weighted according to different baseline fitness of infected and uninfected individuals in an ESS model are needed to produce accurate predictions.

Future work should ascertain whether the predictions made by my model hold true for real populations. Although some hypotheses may be hard to test (e.g., those related to fixation probabilities and times), my prediction that male-killer infections should lead to decreased nuclear genetic variability should be straightforward to test. One good system to study would be the butterfly *Hypolimnas bolina*, because of the high variation of male-killer prevalence both in time and space, and the extremely high prevalence (up to 99% of females infected) that the male-killers can reach. In populations where it is known that male-killers have persisted for a long time (as in *Drosophila innubila* [88]), it would also be very interesting to determine if, as suggested above, the male-killing bacteria have had an impact on the dN/dS ratio.

Chapter 5

The Evolution of Cytoplasmic Incompatibility Types

Abstract. In this chapter, I develop and analyse a model for the evolution of new types of cytoplasmic incompatibility (CI). This model extends previous theoretical approaches by including segregation of bacterial strains during maternal transmission, and a continuum of breeding systems ranging from strong inbreeding (complete sibmating) to outbreeding (complete avoidance of matings between siblings). My results indicate that (1) evolution is unlikely to lead to new CI-types that co-occur as a double infection with the pre-existing one, (2) inbreeding impedes the evolution of new CI-types, and (3) outbreeding facilitates the evolution of new CI-types. The model confirms that new CI-types are likely to arise via a mutant that induces a novel sperm modification in males, but can still rescue the wildtype modification prevalent in the population. In contrast, the proposed pathway via a mutant that differs from the wildtype in its rescue ability could not be confirmed in the presence of segregation. Interesting future avenues of investigation include the incorporation of population structure into the models, and a detailed study of the interplay between random genetic drift and segregation.

Most parts of this chapter have been published in *Genetics* [95].

5.1 Introduction

Cytoplasmic incompatibility (CI) is arguably the most common manipulation induced by reproductive parasites, and at the same time the conceptually most complicated one (see Section 1.4.4). CI-inducing strains of bacteria are remarkably diverse with regards to the modification and rescue type. Many species harbour different strains that are bidirectionally incompatible [266, 356, 300], and even closely related strains can be completely incompatible [63]. One particularly well studied system is *Drosophila simulans*, in which five different strains of *Wolbachia* have been found to date. Three of these strains induce CI (bidirectionally incompatible with the respective other strains), one does not induce CI, but is able to rescue the modification induced by another strain, and one strain neither induces CI nor has any rescue ability (reviewed in Ref. [265]).

CI does not appear to be a trait that can evolve easily, so that it seems most parsimonious to assume that CI itself has evolved only a few times, and that most of the CI-types found today have evolved from ancestors that also induced CI. Therefore, it is important to ascertain how transitions from one CI-type to another proceed, what intermediate types might arise or be necessary, and what conditions favour or inhibit such transitions.

In this chapter, I will propose and analyse a model on the evolution of CI-types that includes two components that have not been studied previously, namely segregation of bacterial strains during maternal transmission, and breeding systems other than panmixis. In the remainder of this introduction, I will first set out an important assumption with regards to the genetic basis of CI, upon which the investigations of this chapter rest. I will then discuss previous attempts to understand the evolution of new CI-types, and give a short introduction to segregation, as well as inbreeding and outbreeding.

5.1.1 Hypotheses for the mechanism of CI

Three hypotheses have been put forward to account for the mechanism of CI. The first hypothesis, the 'lock-and-key' hypothesis, was first proposed by Breeuwer & Werren [36]. It states that during spermatogenesis, the bacteria in males produce a certain detrimental substance that 'locks in' the paternal chromosomes. This leads to improper chromosome condensation in the zygote and loss of the chromosomes. Bacteria present in the eggs produce another substance, the 'key', that specifically binds to and removes the 'lock', so that the paternal chromosomes can segregate normally during the first mitotic divisions of the zygote. The crucial feature of this hypothesis is that it assumes two different molecular substances and correspondingly, two different genes that encode them.

According to the second, the 'titration-restitution' hypothesis [415], the bacteria remove binding factors normally present on the chromosomes during gametogenesis. In uninfected zygotes, this again leads to loss of the paternal chromosomes. In infected zygotes, the removed factor is released and binds to the chromosomes, resulting in normal development. Empirical support for this hypothesis comes from experiments with antibodies selected against *Wolbachia* extracts. These antibodies have been found to bind not only to the bacteria, but also to some extent to chromosomes in uninfected eggs and to histone-like proteins [235].

Finally, the 'slow-motion' hypothesis assumes that the bacteria produce a factor that binds to the chromosomes and delays entry into the first mitosis of the zygote [49]. In an uninfected zygote, this results in the maternal chromosomes entering mitosis before the paternal chromosomes, which leads to loss of the paternal chromosomes. In infected zygotes, the entry point into mitosis of both chromosomes is equally delayed (even if the factor is not present in the sperm), so that development can proceed normally.

Naturally, these three hypotheses on the mechanism of CI can explain the basic features of unidirectional CI. There are, however, many more observations concerning CI that can serve as tests for these hypotheses and uncover difficulties inherent to them. The following discussion of these problems is largely based on a review by Poinsot *et al.* [306], to which I refer for a more detailed treatment.

'Lock-and-key' hypothesis. Although there is no molecular evidence that supports the 'lock-and-key' hypothesis, it readily accounts for all observations that have been made with respect to CI.

'Titration-restitution' hypothesis. One difficulty with this hypothesis is how to account for the diversity of distinct and independent mod-resc systems found in natural populations. If one assumes that each bacterial strain removes a different binding factor, the number of different mod-resc systems should be very limited. To circumvent this problem, one has to assume that different strains titrate and subsequently release different subsets of binding factors. A more severe problem is posed by the existence of strains that can rescue a modification induced by another strain, but do not induce a modification. How can the binding factor be released by the bacteria, if they have not titrated it before? A sex-specific removal of the binding factor for these resc-only strains must be assumed, i.e., the binding factors are removed in females only.

Slow-motion hypothesis. This hypothesis faces problems similar to that of the titration-restitution hypothesis. In particular, the additional assumption of sex-specific expression of the slowing-down factor must be made to account for the resc-only phenotype. Moreover, observations involving several CI-inducing strains (bidirectional CI, additive mortality with multiple infection of males) make it a necessary assumption that different slowing-down factors bind at different sites of the chromosomes.

It can be concluded from this discussion that all three hypotheses may be valid, but that the 'lock-and-key' hypothesis is the most parsimonious. Therefore, this hypothesis — or a more general version in which mod and resc functions are determined by different genes — will be the foundation of the remainder of the chapter.

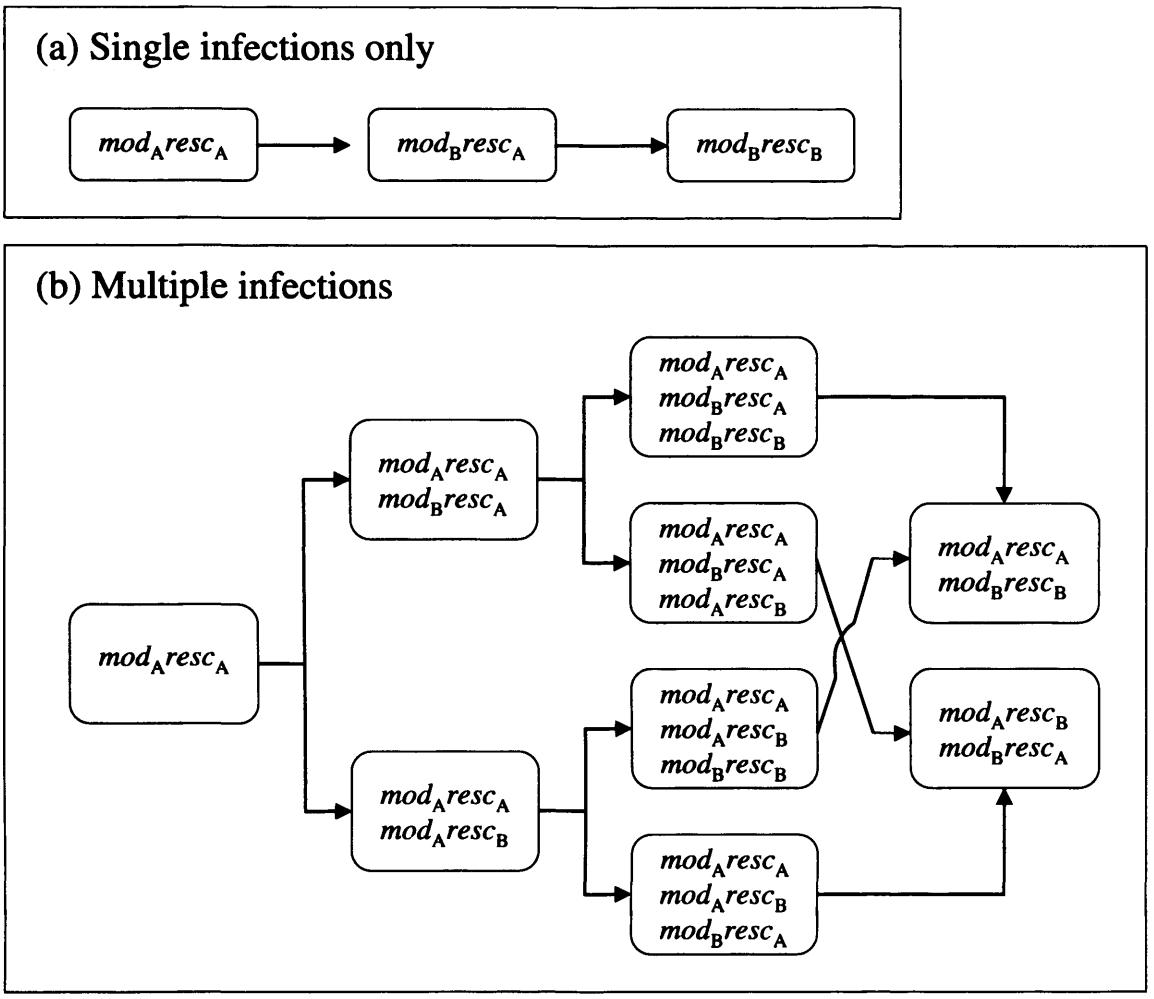

Figure 5.1: Schematic representation of different hypotheses on the evolution of new CI-types. In (a), mutations of bacterial strains lead to individuals only infected with the mutant strains, i.e., there are no multiply infected individuals. In these models [59, 58], a new $mod_B resc_B$ CI-type can only evolve via a $mod_B resc_A$ intermediate strain. In (b), mutation events in bacterial strains lead to individual hosts that are multiply infected. The third and last step in the different pathways represents loss of one of the bacterial strains. A new $mod_B resc_B$ CI-type can evolve either through a $mod_B resc_A$, or through a $mod_A resc_B$ intermediate.

5.1.2 Current understanding of the evolution of CI-types

Previous models have established that under the 'lock-and-key' hypothesis, new CI-types can evolve via a strain that induces a new sperm modification that can be rescued by neither the wildtype nor the mutant strain [59, 58]. If we denote the wildtype strain in the population by $mod_A resc_A$, such a mutant can be denoted a $mod_B resc_A$ strain. In a panmictic population, a $mod_B resc_A$ mutant is selectively neutral because in a male, the mutant causes incompatibility in all crosses and hence is equally detrimental for all females. The $mod_B resc_A$ mutant therefore can spread by

genetic drift in the population [59, 58]. If it has reached a sufficiently high frequency in the population, the $mod_{B}resc_{A}$ mutant permits a second, $mod_{B}resc_{B}$, mutant to be selectively favoured and spread in the population, replacing the $mod_{A}resc_{A}$ wildtype and the $mod_{B}resc_{A}$ mutant. By contrast, a $mod_{A}resc_{B}$ mutant, occurring as a single infection in a wildtype $mod_{A}resc_{A}$ infected population, will be strongly selected against and will go extinct rapidly.

In the framework discussed so far, all individuals in the population bear no more than a single strain of CI-inducing bacteria. However, new mutations can be expected to lead to the co-occurrence of wildtype and mutant strains within single individuals. In a recent theoretical treatment [78], multiple infections were allowed to coexist and it was demonstrated that the pathway via a $mod_{B}resc_{A}$ mutant is still possible. In addition, new CI-types may also evolve via a $mod_{A}resc_{B}$ mutant, because such a mutant is neutral when co-occurring with the $mod_{A}resc_{A}$ wildtype in this model. Apart from a novel $mod_{B}resc_{B}$ CI-type that co-occurs with the wildtype $mod_{A}resc_{A}$ strain, an infection type with $mod_{A}resc_{B}$ and $mod_{B}resc_{A}$ co-occuring in the same individuals was also produced by this model. Note that this latter infection type is phenotypically indistinguishable from the ($mod_{A}resc_{A}$ & $mod_{B}resc_{B}$) co-infection.

Figure 5.1 summarizes the different pathways of the evolution of new CI-types that have been put forward so far. A more detailed scheme for the transitions involving multiple infections can be found in Ref. [78].

5.1.3 Segregation of strains

In the context of maternally inherited endosymbionts, segregation is defined as the loss of a subset of endosymbiont strains during maternal transmission. This phenomenon is known to occur for example in the wasp *Nasonia vitripennis* [300], the mosquito *Aedes albopictus* [228], *Drosophila simulans* [356, 307] and *D. sechellia* [57]. In fact, to the best of my knowledge, the 'opposite' of segregation — perfect co-transmission of all strains without overall perfect transmission — has never been reproted.

5.1.4 Inbreeding and outbreeding

Whilst all previous models on the evolution of CI — and, for that matter, all previous models on CI — have assumed a population of randomly mating individuals, it is well known that mating may be non-random in natural populations. Important deviations from panmixis are inbreeding and outbreeding. Inbreeding is defined as matings between individuals that are more closely related than randomly chosen individuals from the (appropriately defined) population, whilst outbreeding is defined conversely as breeding between individuals more distantly related than a randomly chosen pair.

Inbreeding is commonly found in haplodiploid species, as first pointed out by Hamilton, who gives an extensive list of inbreeding species in his seminal article on extraordinary sex-ratios [140]. For example, in many haplodiploid mite species, males usually mate with their sister only, and matings between females and their sons are also commonly found. In diplodiploid species, inbreeding usually takes less extreme forms, and is documented in butterflies [255, 135, 41] and spiders [319].

Outbreeding, resulting from pre- or post-copulatory inbreeding avoidance, has been reported for example in the cricket *Gryllus bimaculatus* [354, 355, 40], cactophilic *Drosophila* [250, 251] and the mite *Phytoseiulus persimilis* [102].

5.2 Model of the evolution of new CI-types

In what follows, I will develop a model for the evolution of new CI-types. This model will include an arbitrary number of strains of bacteria that can induce complex patterns of CI. In contrast to previous models, my model will include a continuum of breeding systems and segregation of bacterial strains during transmission of multiple infections.

I assume a finite host population in which individuals reproduce in discrete, nonoverlapping generations. Each individual can be infected by a maximum of n different strains of bacteria. Each host is characterized by its infection state $\mathbf{i}$, where $\mathbf{i} = (i_1, i_2, ..., i_n) \in \{0,1\}^n$. In this notation, $i_k = 1$ denotes that strain k is present in the individual, whilst $i_k = 0$ means that strain k is absent.

5.2.1 Transmission of strains

The bacterial strains are transmitted maternally only. Consider first the case when only one strain k is present in a female. I assume that a certain number m of bacteria is present in a female zygote and that for successful transmission of the strain, at least one of these bacteria (or their descendents) must be transmitted through the germ line to a given egg of that female. I further assume that transmission of the bacteria occurs independently. Under these assumptions, and denoting by b_k the probability that a single bacterium is transmitted from zygote to egg, the probability that at least one of the bacteria is transmitted is given by

$$t_k = 1 - (1 - b_k)^m. \tag{5.1}$$

t_k will be referred to as the transmission rate[1] of strain k. Now consider the case when a female is multiply infected with σ different strains. Since the main scope of the model is the fate of recently arisen mutations that lead to multiple strains within hosts, it is straightforward to assume that the replication of these strains is still regulated as a single quorum. Hence, I will assume that the total number of

[1]Note that the total number m of bacteria, the minimum number of bacteria needed for successful transmission (i.e., 1), and the transmission probability b_k of an individual bacterium are merely used to derive the following function τ and do not play a role in model. The parameters used will be the t_k only.

Table 5.1: Transmission rates resulting from the function τ in the case of $n = 2$ (i.e., two strains of bacteria). Numbers above the columns denote the infection state of the mother, and numbers on the left of the rows denote the infection state of the offspring.

	$(0,0)$	$(1,0)$	$(0,1)$	$(1,1)$
$(0,0)$	1	$1-t_1$	$1-t_2$	$\sqrt{1-t_1}\sqrt{1-t_2}$
$(1,0)$	0	t_1	0	$(1-\sqrt{1-t_1})\sqrt{1-t_2}$
$(0,1)$	0	0	t_2	$\sqrt{1-t_1}(1-\sqrt{1-t_2})$
$(1,1)$	0	0	0	$(1-\sqrt{1-t_1})(1-\sqrt{1-t_2})$

bacteria within hosts is the same irrespective of the number of strains present. For simplicity, I assume that the number of bacteria from each of the strains is the same, so that there will be m/σ bacteria from each strain present in the zygotes of infected females. The probability that a strain k among these is successfully transmitted is then given by

$$1-(1-b_k)^{m/\sigma} = 1-(1-t_k)^{1/\sigma}. \tag{5.2}$$

I assume that all strains are transmitted independently, i.e., the probability of transmission of one of the strains in a multiply infected female is not affected by whether other strains are transmitted or not. Hence, the expected fraction of offspring with infection state $\mathbf{i}$ that a mother with infection state $\mathbf{g}$ produces can be obtained by multiplying all the respective probabilities in Equation 5.2,

$$\tau(\mathbf{g},\mathbf{i}) = \prod_{k=1}^{n}[(1-g_k)(1-i_k)+g_k i_k - g_k(2i_k-1)(1-t_k)^{1/\max\{1,\sigma(\mathbf{g})\}}]. \tag{5.3}$$

In this formula, $\sigma(\mathbf{g})$ is the number of strains present in the mother. For the important case of a double infection ($n = 2$), the fractions of offspring produced by mothers are given in Table 5.1.

5.2.2 CI-induced mortality

To incorporate the effects of CI, I first define a matrix **L**, in which the coefficients L_{kl} denote the viability of offspring only infected with strain l that were sired by a father only infected with strain k. In this matrix, the indices k and l run from 0 to n, where '0' denotes the uninfected state.

A few examples may be helpful for illustrating the variety of CI-systems that can be described by the matrix **L**:

$$\mathbf{L1} := \begin{pmatrix} 1 & 1 & 1 \\ 0.1 & 1 & 0.1 \\ 0.5 & 0.5 & 1 \end{pmatrix} \quad \mathbf{L2} := \begin{pmatrix} 1 & 1 & 1 \\ 0.1 & 1 & 1 \\ 0.5 & 0.5 & 1 \end{pmatrix} \quad \mathbf{L3} := \begin{pmatrix} 1 & 1 & 1 \\ 0.1 & 0.1 & 0.9 \\ 0.5 & 0.9 & 0.5 \end{pmatrix}$$

In all examples, strain 1 induces rather strong CI (90% mortality). In matrix **L1**, strain 2 induces intermediately strong CI (50% mortality) and is bidirectionally incompatible to strain 1. In **L2**, strain 2 again induces intermediate CI, but can fully rescue the modification induced by strain 1. Finally, in **L3**, both strains are unable to rescue their own modification, but can almost fully rescue the respective other strain's modification.

In the next step, $\lambda(\mathbf{h}, \mathbf{i})$, the viability of offspring with infection state **i** sired by a father with infection state **h** will be defined. As this notation makes clear, I will assume that only the infection state in the zygote, but not the maternal infection state, determines the mortality of the zygote. I will further assume that for a given modification induced by bacteria in the father, the strain in the zygote with the best ability to rescue the modification will determine the survival of the zygote. In other words, if the father is infected with strain k, the viability reduction caused by the sperm modification of this strain in a zygote with infection state **i** will be

$$\max\{L_{k0}, i_1 L_{k1}, i_2 L_{k2}, ..., i_n L_{kn}\}. \tag{5.4}$$

To determine the overall viability of zygotes, I assume that all modification-rescue systems act independently, so that these viability reductions can be multiplied. Also considering the cases where strains are not present in the father, this gives

$$\lambda(\mathbf{h}, \mathbf{i}) = \prod_{k=1}^{n} [(1 - h_k) + h_k \max\{L_{k0}, i_1 L_{k1}, i_2 L_{k2}, ..., i_n L_{kn}\}]. \tag{5.5}$$

5.2.3 Mating system

At the time of reproduction, the host population is assumed to consist of N_f mated females. The proportion of females with infection state $\mathbf{g}$ mated with a male with infection state $\mathbf{h}$ is denoted by $p_{\mathbf{gh}}$. Accordingly, the offspring from these females can be divided into several breeding classes $(\mathbf{g}, \mathbf{h})$. Each female in such a breeding class $(\mathbf{g}, \mathbf{h})$ gives birth to a relative number of $\tau(\mathbf{g}, \mathbf{i})\lambda(\mathbf{h}, \mathbf{i})$ daughters with infection state $\mathbf{i}$. To include inbreeding and outbreeding in the model, I define the following three quantities. Consider a focal brood within the breeding class $(\mathbf{g}, \mathbf{h})$. First, the proportion of male offspring with infection state $\mathbf{i}$ in this focal brood is given by

$$q_{\mathbf{ghi}} = \frac{\tau(\mathbf{g}, \mathbf{i})\lambda(\mathbf{h}, \mathbf{i})}{\sum_{\mathbf{j}\in\{0,1\}^n} \tau(\mathbf{g}, \mathbf{j})\lambda(\mathbf{h}, \mathbf{j})}. \tag{5.6}$$

Second, the proportion of male offspring with infection state i in the remainder of the population (i.e., excluding the focal brood) is given by

$$r_{\mathbf{ghi}} = \frac{\max\left\{0, \sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k}, \mathbf{i})\lambda(\mathbf{l}, \mathbf{i}) - \frac{1}{N_f}\tau(\mathbf{g}, \mathbf{i})\lambda(\mathbf{h}, \mathbf{i})\right\}}{\sum_{\mathbf{j}\in\{0,1\}^n} \max\left\{0, \sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k}, \mathbf{j})\lambda(\mathbf{l}, \mathbf{j}) - \frac{1}{N_f}\tau(\mathbf{g}, \mathbf{j})\lambda(\mathbf{h}, \mathbf{j})\right\}}. \tag{5.7}$$

Finally, the proportion of female offspring with infection state $\mathbf{i}$ in the breeding class of the focal brood among all offspring in the population can be calculated as

$$s_{\mathbf{ghi}} = \frac{p_{\mathbf{gh}}\tau(\mathbf{g}, \mathbf{i})\lambda(\mathbf{h}, \mathbf{i})}{\sum_{\mathbf{k},\mathbf{l},\mathbf{j}\in\{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k}, \mathbf{j})\lambda(\mathbf{l}, \mathbf{j})}. \tag{5.8}$$

To model the mating system, I assume that a proportion χ of the females mate with one of their brothers (randomly chosen within their broods), whilst $(1 - \chi)$ of the females choose a single male from one of the other broods. Thus, for $\chi = 0$, sibmating is avoided entirely, for $\chi = 1$ females mate with their brothers only, and $\chi = 1/N_f$ corresponds to a panmictic population.

The recursion equation for the proportions of breeding classes from one generation to the next can now be written as

$$p'_{\mathbf{gh}} = \sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} s_{\mathbf{klg}}[\chi q_{\mathbf{klh}} + (1 - \chi)r_{\mathbf{klh}}]. \tag{5.9}$$

5.2.4 Methods

The model described above was analysed by iterating Equation 5.9. Although the variable iterated is the proportion of breeding classes, $p_{\mathbf{gh}}$, I will present only the frequencies of the host individuals with infection state $\mathbf{i}$, defined as

$$y_{\mathbf{i}} = \sum_{\mathbf{h}\in\{0,1\}^n} p_{\mathbf{ih}}. \tag{5.10}$$

Moreover, all simulations were initialised with starting frequencies $y_{\mathbf{i}}$, and the first frequencies of breeding classes were obtained under the assumption of panmictic reproduction.

5.3 Results

5.3.1 Consistency with other models

In what follows, I will demonstrate that for $\chi = 1/N_f$ and $N_f \to \infty$, the model is equivalent to a panmictic model with an infinitely large host population. When in addition $n = 1$ holds (only one strain present), the model is identical to previously developed models on CI in panmictic host populations [111, 389].

Starting from the recursion Equation 5.9, we get for $\chi = 1/N_f$ and $N_f \to \infty$

$$\begin{aligned} p'_{\mathbf{gh}} &= \sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} s_{\mathbf{klg}}[\chi q_{\mathbf{klh}} + (1-\chi) r_{\mathbf{klh}}] = \sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} s_{\mathbf{klg}} r_{\mathbf{klh}} \\ &= \frac{\left(\sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k},\mathbf{g})\lambda(\mathbf{l},\mathbf{g})\right)\left(\sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k},\mathbf{h})\lambda(\mathbf{l},\mathbf{h})\right)}{\left(\sum_{\mathbf{k},\mathbf{l},\mathbf{j}\in\{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k},\mathbf{j})\lambda(\mathbf{l},\mathbf{j})\right)^2}. \end{aligned} \tag{5.11}$$

After defining

$$x_{\mathbf{i}} := \frac{\sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k},\mathbf{i})\lambda(\mathbf{l},\mathbf{i})}{\sum_{\mathbf{k},\mathbf{l},\mathbf{j}\in\{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k},\mathbf{j})\lambda(\mathbf{l},\mathbf{j})}, \tag{5.12}$$

we get

$$p'_{\mathbf{gh}} = x_{\mathbf{g}} x_{\mathbf{h}}, \tag{5.13}$$

and therefore

$$x'_{\mathbf{i}} = \frac{\sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} p'_{\mathbf{kl}}\tau(\mathbf{k},\mathbf{i})\lambda(\mathbf{l},\mathbf{i})}{\sum_{\mathbf{k},\mathbf{l},\mathbf{j}\in\{0,1\}^n} p'_{\mathbf{kl}}\tau(\mathbf{k},\mathbf{j})\lambda(\mathbf{l},\mathbf{j})} = \frac{\sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} x_{\mathbf{k}} x_{\mathbf{l}}\tau(\mathbf{k},\mathbf{i})\lambda(\mathbf{l},\mathbf{i})}{\sum_{\mathbf{k},\mathbf{l},\mathbf{j}\in\{0,1\}^n} x_{\mathbf{k}} x_{\mathbf{l}}\tau(\mathbf{k},\mathbf{j})\lambda(\mathbf{l},\mathbf{j})}. \tag{5.14}$$

Thus, the 2^{2n}-dimensional system of recursion equations of breeding classes $p_{\mathbf{gh}}$ has been simplified to a 2^n-dimensional system of infection state frequencies $x_{\mathbf{i}}$. This reduced model is similar to a previous model on multiple CI-infections [117]; differences arise for example because of my 'one quorum' assumption and the resulting function τ for maternal transmission (see Section 5.2.1).

Let now n equal 1, i.e. I consider only one strain of CI-inducing endosymbionts. I further assume unidirectional incompatibility with incompatibility level $H := L_{10}$ and full rescue when the egg is infected ($L_{11} = 1$). Denoting by $x := x_1$ the fraction of infected females (and males) in the population and by $t := t_1$ their transmission rate, Equation 5.14 simplifies to

$$\begin{aligned} x' &= \frac{x(1-x)t + x^2 t}{(1-x)^2 + (1-x)xH + x(1-x)(1-t) + x^2(1-t)H + x(1-x)t + x^2 t} \\ &= \frac{xt}{1 - x(1-H)(1-xt)}. \end{aligned} \tag{5.15}$$

This last recursion equation is identical to recursion equations in previous models on CI, with only a different notation [111, 389].

5.3.2 Infection dynamics with in- and outbreeding

In this section, I will briefly analyse the infection dynamics of unidirectional CI inducing endosymbionts in inbreeding and outbreeding host populations. Although not the main focus of this chapter, this is an issue interesting on its own that has not been studied previously.

I found that neither moderate inbreeding nor outbreeding alters the infection dynamics in a qualitative way compared to models with infinite panmictic host populations. However, it could be observed that the invasion threshold — the minimum frequency of infected females in the population for the infection to spread — increases with increasing χ (increasing level of inbreeding), whilst the stable equilibrium frequency decreases with increasing χ (Figure 5.2). The reason for the decreasing invasion ability with increasingly inbreeding hosts is that uninfected females mate increasingly with uninfected males (their brothers). Therefore, fewer and fewer incompatible matings occur and the benefit for infected females decreases until at a certain level of inbreeding, the benefit is not sufficient to compensate for imperfect transmission.

It can also be seen on Figure 5.2 that there is a maximum level of inbreeding above

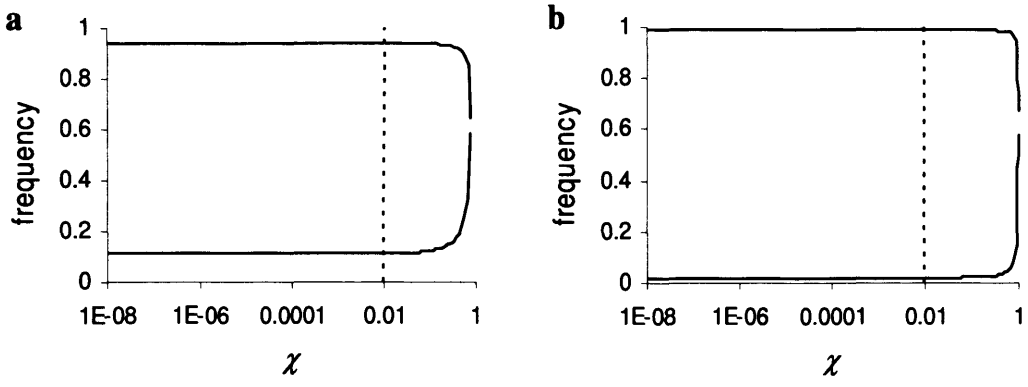

Figure 5.2: Invasion threshold (lower curve) and stable equilibrium frequency (upper curve) of a single CI inducing strain under inbreeding and outbreeding. Parameters: $N_f = 100$, $L_{10} = 0.5$, (a) $t_1 = 0.95$, (b) $t_1 = 0.99$. The dotted lines indicate the value of χ where reproduction is panmictic (i.e., $\chi = 1/N_f$).

which a CI inducing strain of bacteria cannot persist in the population. The results of a more systematic investigation on this issue are given in Figure 5.3, which shows maximal inbreeding levels for different transmission rates and CI-levels. To obtain these values, simulations were initialised with all individuals being infected, and it was tested whether a stable positive equilibrium was obtained for different values of χ. Perhaps not unexpectedly, the maximum value of χ at which the CI-inducing bacteria can persist in a population decreases with decreasing transmission rate and decreasing mortality in offspring from incompatible matings.

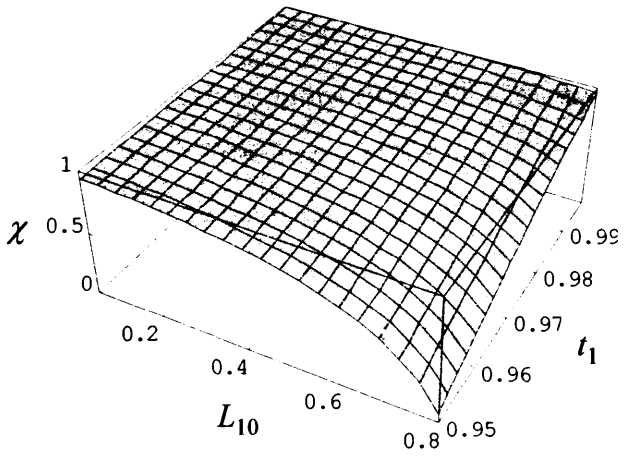

Figure 5.3: Maximum values of χ at which a CI-inducing strain can persist in the population, depending on its transmission rate t_1 and the viability L_{10} of offspring from incompatible matings.

5.3.3 The evolution of CI-types I: via $mod_B resc_A$

The first hypothesis to explain the evolution of CI-types that I scrutinize was proposed by Charlat and co-workers [59], who noted that in a population with stable infection of a CI strain ($mod_A resc_A$), a mutant strain inducing a different modification ($mod_B resc_A$) would be selectively neutral. This mutant strain could then spread by random genetic drift to a high frequency, and a second mutant rescuing the new modification ($mod_B resc_B$) could then arise and spread due to positive selection (see Figure 5.1). Subsequent analyses have confirmed that this process can work under the assumptions of the respective models [78, 58].

Assuming no differences between the strains in the intensity of modification or in the ability to rescue a modification, this scenario of three strains ($mod_A resc_A$,

$mod_{\mathrm{B}}resc_{\mathrm{A}}$, and $mod_{\mathrm{B}}resc_{\mathrm{B}}$) is represented in my model by the matrix

$$\mathbf{L} := \begin{pmatrix} 1 & 1 & 1 & 1 \\ \eta & 1 & 1 & \eta \\ \eta & \eta & \eta & 1 \\ \eta & \eta & \eta & 1 \end{pmatrix},$$

where η denotes viability in incompatible crosses. (Recall that the columns in this matrix denote the single infection of the offspring, whilst the rows correspond to the single infection state of the father. The first column and the first row stands for the uninfected state.)

How can we expect inbreeding and outbreeding to affect the spread of a $mod_{\mathrm{B}}resc_{\mathrm{A}}$ strain? In an inbreeding population, a $mod_{\mathrm{B}}resc_{\mathrm{A}}$ strain can be expected to be selected against. This is because females infected with $mod_{\mathrm{B}}resc_{\mathrm{A}}$ are more likely to mate with males that are also infected with $mod_{\mathrm{B}}resc_{\mathrm{A}}$ than females infected with $mod_{\mathrm{A}}resc_{\mathrm{A}}$ only, and hence the offspring from females with the mutant strain suffer from a higher mortality than offspring from females carrying the wildtype strain. Conversely, in an outbreeding population, we expect a $mod_{\mathrm{B}}resc_{\mathrm{A}}$ strain to be selected for. This is because a female infected with the mutant $mod_{\mathrm{B}}resc_{\mathrm{A}}$ strain is less likely to mate with a male infected with the mutant strain than females infected with the wildtype $mod_{\mathrm{A}}resc_{\mathrm{A}}$ strain. Therefore, average offspring production of females with the wildtype strain is more strongly reduced by mod_{B} than offspring production of females carrying the $mod_{\mathrm{B}}resc_{\mathrm{A}}$ strain.

Figure 5.4 gives an example of the spread of a $mod_{\mathrm{B}}resc_{\mathrm{A}}$ mutant into a relatively small, outbreeding population ($\chi = 0, N_f = 100$) infected with $mod_{\mathrm{A}}resc_{\mathrm{A}}$, followed by the spread of a $mod_{\mathrm{B}}resc_{\mathrm{B}}$ mutant. The simulation was initiated with the wildtype infection state ($mod_{\mathrm{A}}resc_{\mathrm{A}}$ only) being at its equilibrium frequency. Then, a double infection ($mod_{\mathrm{A}}resc_{\mathrm{A}}$ & $mod_{\mathrm{B}}resc_{\mathrm{A}}$) at frequency $1/N_f = 10^{-2}$ in females was introduced. It can be seen in Figure 5.4a that the $mod_{\mathrm{B}}resc_{\mathrm{A}}$ mutant can spread deterministically, although this spread is very slow. It should be noted that it is the single infection state ($mod_{\mathrm{B}}resc_{\mathrm{A}}$ only) that spreads; the double infection state goes rapidly extinct. This is because, due to segregation of the two strains, the double infection state has a lower effective transmission rate than the single infection state.

At generation 500 the second mutant, $mod_{\mathrm{B}}resc_{\mathrm{B}}$, was introduced. Again, this

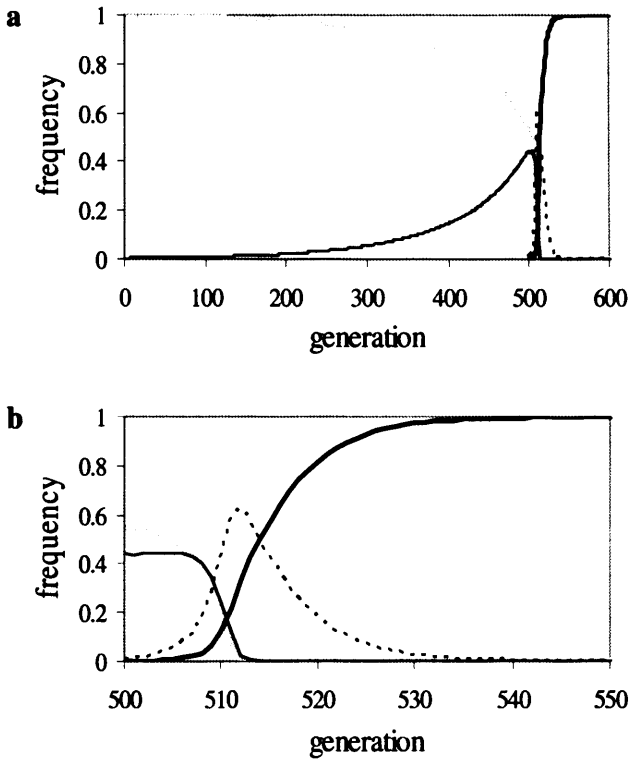

Figure 5.4: Deterministic invasion of a $mod_{\mathrm{B}}resc_{\mathrm{A}}$ mutant (strain 2) into an outbreeding population infected with a $mod_{\mathrm{A}}resc_{\mathrm{A}}$ wildtype (strain 1), followed by invasion of a $mod_{\mathrm{B}}resc_{\mathrm{B}}$ mutant (strain 3). Both plots show the same simulation results, with magnification of the time axis in plot (b). The frequencies of females with the following infection states are shown: y_{100} ($mod_{\mathrm{A}}resc_{\mathrm{A}}$ strain only) with bold grey line, y_{010} ($mod_{\mathrm{B}}resc_{\mathrm{A}}$ strain only) with solid line, y_{011} ($mod_{\mathrm{B}}resc_{\mathrm{A}}$ and $mod_{\mathrm{B}}resc_{\mathrm{B}}$) with dotted line, y_{001} ($mod_{\mathrm{B}}resc_{\mathrm{B}}$ only) with bold line. y_{000} is not shown in the plots, whilst y_{110} is present, but not discernable on plot (a). Parameters take the values $\chi = 0$, $N_f = 100$, $t_1 = t_2 = t_3 = 0.99$, $\eta = 0.1$.

mutant was introduced as a double infection, but this time together with $mod_{\mathrm{B}}resc_{\mathrm{A}}$. In Figure 2b, it can be seen that the double infection state ($mod_{\mathrm{B}}resc_{\mathrm{A}}$ & $mod_{\mathrm{B}}resc_{\mathrm{B}}$) spreads quickly through the population, driving extinct both other infection states (($mod_{\mathrm{A}}resc_{\mathrm{A}}$ only) and ($mod_{\mathrm{B}}resc_{\mathrm{A}}$ only)). Because of the decreasing proportion of mod_{A} males in the population, the double infection state soon loses its selective superiority over the single infection state ($mod_{\mathrm{B}}resc_{\mathrm{B}}$ only). As a consequence, the double infection also becomes extinct, and the ($mod_{\mathrm{B}}resc_{\mathrm{B}}$ only) infection state prevails, spreading to a high equilibrium frequency. This simulation shows that transition from one CI-type to another is possible even without random genetic drift or the assumption that the mutant CI-strain increases the fitness of their female hosts.

It is obvious that the first step in this scenario - the invasion of the $mod_{\mathrm{B}}resc_{\mathrm{A}}$ mutant - is the crucial one, whereas the subsequent invasion of the $mod_{\mathrm{B}}resc_{\mathrm{B}}$ mutant is straightforward. To determine the conditions when a $mod_{\mathrm{B}}resc_{\mathrm{A}}$ mutant can spread in a population, I performed scans of the parameter space spanned by N_f and χ (Figure 5.5). For each combination of these parameters tested, I started with a

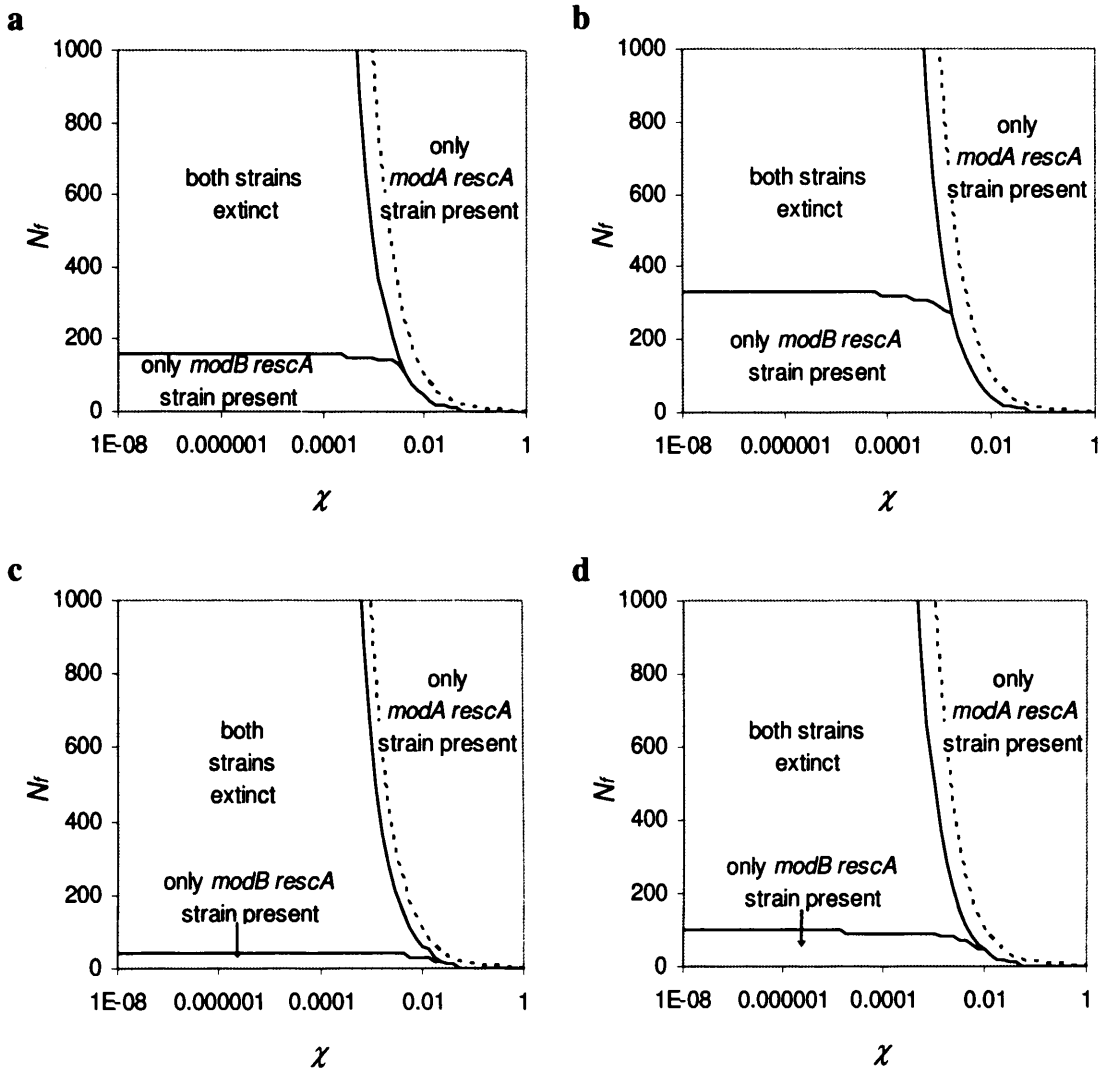

Figure 5.5: Different outcomes of an invasion of a $mod_{\mathrm{B}}resc_{\mathrm{A}}$ mutant into a population infected with $mod_{\mathrm{A}}resc_{\mathrm{A}}$ at equilibrium, depending on population size and breeding system in the population. Other parameters take the values (a) $\eta = 0.1, t_1 = t_2 = 0.99$, (b) $\eta = 0.1, t_1 = t_2 = 0.995$, (c) $\eta = 0.5, t_1 = t_2 = 0.99$, (d) $\eta = 0.5, t_1 = t_2 = 0.995$ The dotted line indicates parameter combinations of χ and N_f where reproduction is panmictic.

population with the infection state ($mod_{\mathrm{A}}resc_{\mathrm{A}}$ only) at equilibrium. I then introduced a $mod_{\mathrm{B}}resc_{\mathrm{A}}$ mutant into females (again at frequency $1/N_f$) and simulated for 10^5 generations.

Three different outcomes could be observed (Figure 5.5). In inbreeding populations, the $mod_{\mathrm{B}}resc_{\mathrm{A}}$ mutant was unable to invade the population. More precisely, when χ was roughly larger than $1/N_f$, the $mod_{\mathrm{B}}resc_{\mathrm{A}}$ mutant went extinct. (The slightly higher minimum values of χ compared to the expected value $1/N_f$ arise because of an invasion threshold for the $mod_{\mathrm{B}}resc_{\mathrm{A}}$ mutant in an outbreeding, but close to panmictic population.) In outbreeding populations, the $mod_{\mathrm{B}}resc_{\mathrm{A}}$ mutant could

invade the population, driving the $mod_{A}resc_{A}$ wildtype extinct. As in the previously discussed simulation, invasion of $mod_{B}resc_{A}$ always occurred as a single infection, whilst the double infection state ($mod_{B}resc_{A}$ & $mod_{A}resc_{A}$) always went extinct. The final outcome after invasion of $mod_{B}resc_{A}$ was found to depend on population size relative to the breeding coefficient χ: whilst the $mod_{B}resc_{A}$ mutant went extinct in large populations, it could be maintained in smaller populations.

This latter result can be explained as follows. In an outbreeding population, the fitness advantage of the $mod_{B}resc_{A}$ variant over uninfected cytotypes stems from the fact that an uninfected female is more likely to mate with a male infected with the $mod_{B}resc_{A}$ strain than an infected female. However, the number of potential mates that are infected differ only by the number of brothers an infected female has, so that with increasing population size, the probabilities of infected and uninfected females mating with an infected male converge. Thus, to overcome the fitness reduction due to inefficient transmission, the host population must be sufficiently small, and in larger populations, a $mod_{B}resc_{A}$ variant cannot be maintained. As can be expected by this reasoning, the maximum population size for the $mod_{B}resc_{A}$ strain to persist in the population for a given breeding coefficient χ increases with increasing transmission rate of the bacteria (compare Figures 5.5a and b and 5.5c and d). The same is true for the intensity of sperm modification, i.e. the maximum population size where $mod_{B}resc_{A}$ can persist increases with decreasing η (compare 5.5a and c and 5.5b and d).

Aside from a $mod_{B}resc_{B}$ mutant originating from the initial $mod_{B}resc_{A}$ mutant, a mutation might also occur in the resc-function of the wildtype strain, leading to a $mod_{A}resc_{B}$ mutant that co-occurs with the wildtype $mod_{A}resc_{A}$ strain (see Figure 5.1). The respective matrix $\mathbf{L}$ for this scenario is

$$\mathbf{L} := \begin{pmatrix} 1 & 1 & 1 & 1 \\ \eta & 1 & 1 & \eta \\ \eta & \eta & \eta & 1 \\ \eta & 1 & 1 & \eta \end{pmatrix}.$$

Figure 5.6 shows the simulation results for the invasion of such a mutant, after the initial invasion of the $mod_{B}resc_{A}$ mutant in an outbreeding population. As can be seen, the double infection of wildtype and second mutant strain ($mod_{A}resc_{A}$ &

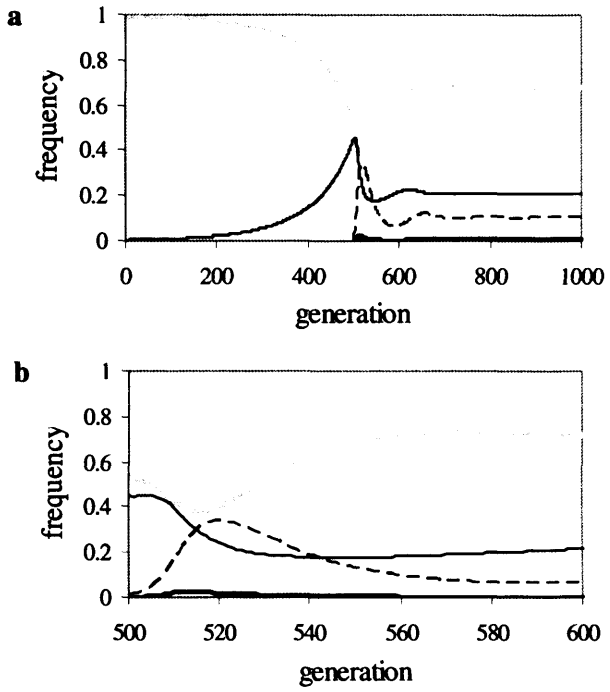

Figure 5.6: Invasion of a $mod_{\mathrm{B}}resc_{\mathrm{A}}$ mutant (strain 2) into an outbreeding population infected with a $mod_{\mathrm{A}}resc_{\mathrm{A}}$ wildtype (strain 1), followed by invasion of a $mod_{\mathrm{A}}resc_{\mathrm{B}}$ mutant (strain 3). Both plots show the same simulation results, with magnification of the time axis in plot (b). The frequencies of females with the following infection states are shown: y_{100} ($mod_{\mathrm{A}}resc_{\mathrm{A}}$ strain only) with bold grey line, y_{010} ($mod_{\mathrm{B}}resc_{\mathrm{A}}$ strain only) with solid line, y_{011} ($mod_{\mathrm{A}}resc_{\mathrm{A}}$ and $mod_{\mathrm{A}}resc_{\mathrm{B}}$) with dashed line, y_{001} ($mod_{\mathrm{A}}resc_{\mathrm{B}}$ only) with bold line. Parameters take the values $\chi = 0$, $N_f = 100$, $t_1 = t_2 = t_3 = 0.99$, $\eta = 0.1$.

$mod_{\mathrm{A}}resc_{\mathrm{B}}$) is stable in this case, and the end result is a stable polymorphism of this double infection and the single infections of the three strains involved. Further simulations (not shown) suggest that at this polymorphic equilibrium, a $mod_{\mathrm{B}}resc_{\mathrm{B}}$ mutant cannot invade the population, neither when originating from the $mod_{\mathrm{B}}resc_{\mathrm{A}}$ nor from the $mod_{\mathrm{A}}resc_{\mathrm{B}}$ strain. Thus, this peculiar pathway appears to be an impasse in the evolution of new CI-types.

In summary, my results suggest that the evolution of a new CI-type via a $mod_{\mathrm{B}}resc_{\mathrm{A}}$ mutant is weakly favored by natural selection in outbreeding populations (especially small ones), but selected against in inbreeding populations. Infection states with more than one strain are expected to occur transiently only, and not as a final, stable outcome (but see the above pathway that does not lead to a new CI-type). It is also clear that infection states with more than one strain do not aid transitions, except for the case where transmission is perfect and segregation of the strains does not occur.

5.3.4 The evolution of CI-types II: via $mod_{B}resc_{A}$ with partial compatibility

Partial compatibility between the new mod_{B} and the $resc_{A}$ function has been determined to play an important role in the likelihood of the evolution of a new CI-type [58]. To assess the impact of partial compatibility in the present treatment, I will use the matrix

$$\mathbf{L} := \begin{pmatrix} 1 & 1 & 1 \\ \eta & 1 & 1 \\ \eta & (1-\eta)\pi + \eta & (1-\eta)\pi + \eta \end{pmatrix}.$$

In this matrix the degree of compatibility between mod_{B} and $resc_{A}$ is a linear function of π, with $\pi = 0$ yielding complete incompatibility and $\pi = 1$ resulting in full compatibility. Simulations demonstrated that the parameter space with regard to χ and N_f in which the $mod_{B}resc_{A}$ mutant can invade the population deterministically is not affected by π (results not shown).

However, the selective advantage of the $mod_{B}resc_{A}$ mutant in an outbreeding population decreases with increasing π, becoming zero for full compatibility ($\pi = 1$). This is because the selective advantage for the $mod_{B}resc_{A}$ mutant stems from its adverse effects on the $mod_{A}resc_{A}$ wildtype, and this decreases with increasing compatibility between mod_{B} and $resc_{A}$; at $\pi = 1$ (complete compatibility) the $mod_{B}resc_{A}$ 'mutant' is essentially identical to the wildtype and thus neutral. This decreasing selective advantage of the $mod_{B}resc_{A}$ mutant with increasing π results in decreasing tempo of

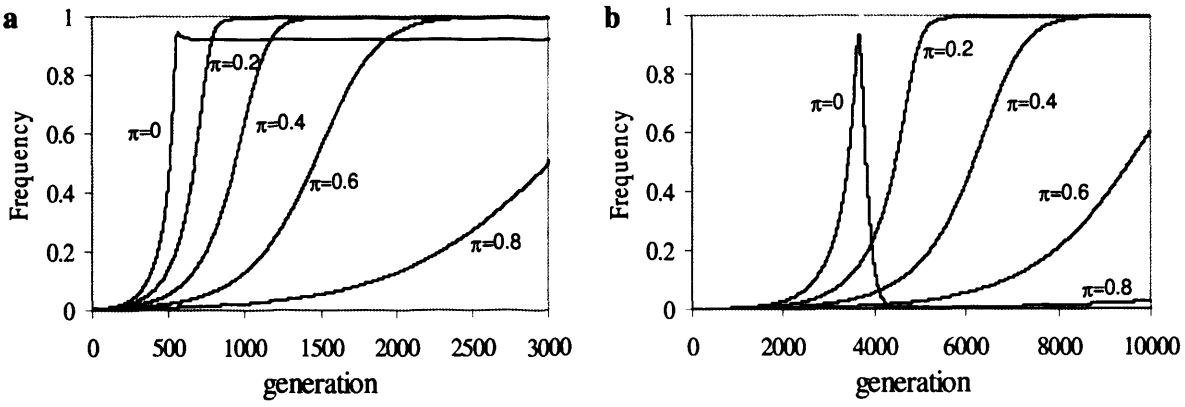

Figure 5.7: Invasion of a $mod_{B}resc_{A}$ mutant into a population with $mod_{A}resc_{A}$ at equilibrium, for varying degrees of partial compatibility π between *modB* and *rescA*. Other parameters take the values $\chi = 0$, $\eta = 0.1$, (a) $N_f = 100$ and (b) $N_f = 500$

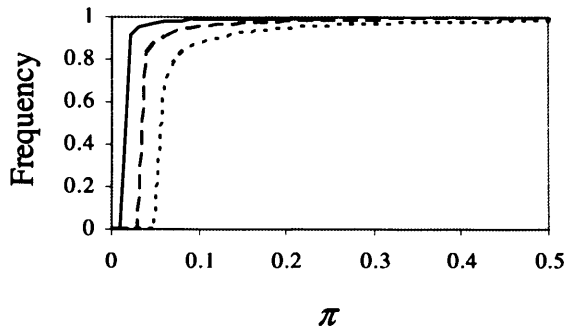

Figure 5.8: Equilibrium frequency of a $mod_B resc_A$ mutant after invasion into a population infected with $mod_A resc_A$ at equilibrium, depending on the degree π of partial compatibility of mod_B with $resc_A$. Parameters take the values $\chi = 0$ (complete sibmating avoidance), $N_f = 500$, $t_1 = t_2 = 0.99$, $\eta = 0.1$ (solid line), $\eta = 0.3$ (dashed line), and $\eta = 0.5$ (dotted line).

invasion, as shown in Figure 5.7.

In addition to making $mod_B resc_A$ males and $mod_A resc_A$ females more compatible, slowing down the invasion of the mutant, increasing π also makes $mod_B resc_A$ males and $mod_B resc_A$ females more compatible. As a consequence, both the conditions where the $mod_B resc_A$ mutant can be maintained in the population and its equilibrium frequency after its invasion and exclusion of the wildtype are affected by partial compatibility (Figures 5.7 and 5.8). Surprisingly, even a very small degree of partial compatibility can result in stable maintenance of $mod_B resc_A$ at high equilibrium frequency.

Taken together, these results imply that partial compatibility has an ambiguous effect on the transition from one CI-type to another one. When mod_B is partially compatible with $resc_A$, selection for a $mod_B resc_A$ mutant is weaker in an outbreeding population, but at the same time, a $mod_B resc_A$ infection is much more stable once this strain has spread and driven the $mod_A resc_A$ extinct. In addition, increased stability of $mod_B resc_A$ due to partial compatibility may also result from higher stability of the host population itself [58]. The trade-off between the decreased invasion ability and the higher stability is beyond the capacity of my model. However, I conjecture that in total, partial compatibility tends to facilitate the transition to new CI-types because selective pressures for $mod_B resc_A$ mutants are only weak in outbreeding and absent in panmictic populations and hence, the invasion of such mutants will be determined largely by drift.

5.3.5 The evolution of CI-types III: via $mod_{A}resc_{B}$

Recently, Dobson [78] proposed a new hypothesis for the evolution of new CI-types (see Figure 5.1b). According to his model, a $mod_{A}resc_{B}$ mutant could arise in a population infected with a $mod_{A}resc_{A}$ strain. Because this $mod_{A}resc_{B}$ strain would then occur together with the wildtype $mod_{A}resc_{A}$ as a double infection within the same individual, it would be 'protected' by the $mod_{A}resc_{A}$ from mod_{A}-modified sperm. Thus, the $mod_{A}resc_{B}$ mutant could be maintained as a neutral element in the population, its frequency determined by random genetic drift only. After a new mutation in a doubly infected female, leading to a $mod_{B}resc_{B}$ strain, the triple infection state would be selected for and spread in the population. The 'intermediate' $mod_{A}resc_{B}$ strain may then go extinct, leading to a population with a stable ($mod_{A}resc_{A}$ & $mod_{B}resc_{B}$) double infection. (Alternatively, the second mutation could also lead to a $mod_{B}resc_{A}$ strain, with a stable ($mod_{B}resc_{A}$ & $mod_{A}resc_{B}$) double infection as a final outcome.)

The neutrality of the ($mod_{A}resc_{A}$ & $mod_{A}resc_{B}$) infection state depends crucially on perfect co-transmission of the two strains, an implicit assumption in Dobson's model. In contrast, if we assume that doubly infected females also produce singly infected offspring, we would expect the double infection state to have a transmission disadvantage compared to a single infection state with the same phenotype (i.e., $resc_{A}$). It therefore is expected that due to segregation of the two strains, the ($mod_{A}resc_{A}$ & $mod_{A}resc_{B}$) infection state is not stably maintained in a population, a supposition that has been confirmed in several simulations of my model (not shown).

To understand this principle of segregation in more detail, consider the case where the two strains $mod_{A}resc_{A}$ and $mod_{A}resc_{B}$ have the same transmission rate t_s when occurring as a single infection, whilst the proportion of doubly infected offspring that a doubly infected mother produces is denoted by t_d. Since the ($mod_{A}resc_{A}$ & $mod_{A}resc_{B}$) infection state does not differ from the ($mod_{A}resc_{A}$ only) infection state in its capability to rescue mod_{A}, the double infection state can be maintained in the population only if $t_d \geq t_s$ holds. In the present model, $t_d = \tau((1,1),(1,1)) = (1-\sqrt{1-t_s})^2$, and it can be demonstrated easily that this term is always less than t_s for $t_s < 1$. A somewhat simpler assumption that has been used frequently in previous models on multiple *Wolbachia* infections [120, 117, 101] is $t_d = t_s^2$, which again is always less than t_s when $t_s < 1$. In conclusion, unless transmission is perfect, a

($mod_A resc_A$ & $mod_A resc_B$) infection state is unlikely to be maintained in a population. Because of strong selection against the ($mod_A resc_B$ only) infection state, this suggests that the evolution of a new CI-type is unlikely to occur via a $mod_A resc_B$ mutant when transmission is not perfect.

5.3.6 The emergence of CI

As decribed in Section 5.3.3, a $mod_B resc_A$ strain can be maintained at a stable frequency in an outbreeding population of otherwise uninfected individuals (see Figure 5.4). This is equivalent to a situation where a strain only modifies sperm in males, but has no rescue capability (in other words, a 'mod-only' strain). Interestingly, this provides an explanation of how CI could have evolved in the first place, based on mutation and selection only. Consider an uninfected population where individuals reproduce with a certain level of inbreeding avoidance. A new strain of maternally transmitted endosymbionts that modify the sperm of their male hosts in a detrimental way would then be positively selected, because infected males harm uninfected females more than infected ones. Therefore, the mod-only mutant could spread in the population, provided its transmission rate is sufficiently high. A new mutant with the additional ability to rescue the modification could then arise, be strongly selected for and replace the mod-only strain, similar to the $mod_B resc_B$ strain replacing the $mod_B resc_A$ strain discussed above.

Analogously, the invasion of the mod-only strain is the crucial step, whilst the subsequent invasion of the rescuing mutant can then be expected to occur always and quickly, even with partial rescue ability only. To scrutinize my hypothesis, I therefore performed simulations that determined the threshold and stable equilibrium frequency of a mod-only strain, depending on population size, transmission rate and modification intensity (Figure 5.9). In accord with the results presented in Figure 5.5, spread is only possible up to a maximum population size that depends on the transmission rate and modification intensity. The threshold frequency is low for small populations and remains constant up to a certain population size, from where on it increases rapidly. The observed equilibrium frequencies are high, making a strong impact on the hosts' population dynamics likely (see Section 5.4.4).

These results demonstrate that my proposed scenario of the evolution of CI can

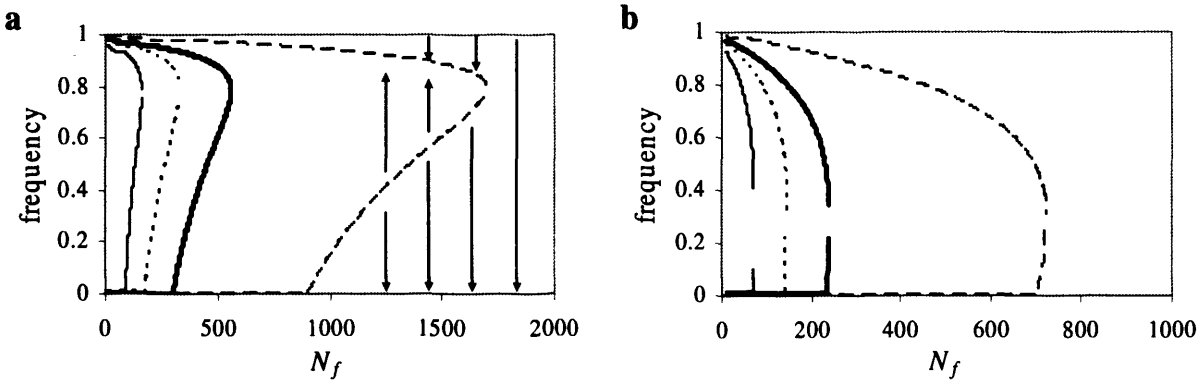

Figure 5.9: Invasion threshold and stable equilibrium frequency of a mod-only strain in an outbreeding population. Transmission rates of $t_1 = 0.99$ (solid lines), $t_1 = 0.995$ (dotted lines), $t_1 = 0.997$ (bold lines) and $t_1 = 0.999$ (dashed lines) were used; other parameters take the values $\chi = 0$, and (a) $L_{10} = L_{11} = 0.1$, (b) $L_{10} = L_{11} = 0.3$. Note the different scales of the N_f-axes in plots (a) and (b).

work in theory, but small population sizes and high transmission rates are important requirements. I would like to stress that the selective pressures leading to invasion of the mod-only mutant are in general rather weak, as is indicated by the high numbers of generations it takes until the equilibrium frequency is reached (not shown).

5.4 Discussion

The main purpose of the model presented in this chapter was to investigate how new CI-types can evolve from preexisting ones under the assumption that mod and resc are two genetically independent functions. Moreover, the model proved useful to make predictions on the infection dynamics of CI-inducing bacteria under inbreeding and outbreeding and provided a novel explanation for the evolution of CI itself.

In this discussion, I will first try to elucidate the impact of the two novel components that were incorporated into my model, segregation and different breeding systems. I will then discuss aspects that have been demonstrated previously to be important for the evolution of CI-types, or may be conjectured to be important, but were not part of my model.

5.4.1 The impact of segregation

The main consequence of segregation of bacterial strains during transmission is that double infections that arise after mutation events disappear quickly from the population. In particular, the double infections arising from the first mutation event, ($mod_{\mathrm{A}}resc_{\mathrm{A}}$ & $mod_{\mathrm{B}}resc_{\mathrm{A}}$) and ($mod_{\mathrm{A}}resc_{\mathrm{A}}$ & $mod_{\mathrm{A}}resc_{\mathrm{B}}$), go extinct rapidly. With regards to the results obtained by Dobson [78], this has the somewhat dramatic consequence that none of the proposed pathways for the evolution of CI-types were viable in my model (see Figure 5.1 and also Figure 1 in Ref. [78]).

The extinction of the two crucial infection states arising from an initial mutation, ($mod_{\mathrm{A}}resc_{\mathrm{A}}$ & $mod_{\mathrm{B}}resc_{\mathrm{A}}$) and ($mod_{\mathrm{A}}resc_{\mathrm{A}}$ & $mod_{\mathrm{A}}resc_{\mathrm{B}}$), is due to two factors. First, the two infection states have the same fitness as single infection states co-occurring in the same population (($mod_{\mathrm{B}}resc_{\mathrm{A}}$ only) and ($mod_{\mathrm{A}}resc_{\mathrm{A}}$ only), respectively). Secondly, the double infection states have a transmission disadvantage compared to these single infection states. Combined, these two factors yield a net disadvantage. Both the fitness and the transmission component are the result of certain assumptions of the model and deserve some closer attention.

With regards to the fitness of the double infection state (the number of surviving daughters that females with the respective infection state produce), it is clear that if the double infection state would confer increased fecundity to its female host com-

pared to singly infected females, this could stabilise the double infection. However, it seems unlikely that a mutation affecting the *mod* or *resc* function of a bacterial strain should at the same time result in increased fecundity of doubly infected individuals. On the contrary, in the case of the ($mod_A resc_A$ & $mod_A resc_B$) double infection, one might assume that (unlike in my model) the rescue ability of this double infection with respect to mod_A is decreased compared to the $mod_A resc_A$ single infection, because not all of the bacteria contribute to the rescue activity. This would make the double infection go extinct even more rapidly. Thus, the assumption of my model that the rescue ability of a strain does not depend on how many other strains are present within the same egg can be considered conservative with respect to the fate of multiple infections.

The transmission disadvantage of multiple infections arises rather naturally from the assumptions that (1) transmission of a particular strain is independent of whether other strains are transmitted and (2) the transmission rate of a particular strain is the same or less in a multiple infection state than in a single infection state. Both of these assumptions are implicitly made in the model developed here and also in all previous models on multiple infections with CI-inducing bacteria [117, 101, 120], as already discussed in Section 5.3.5.

However, the second of these assumptions has not been confirmed in an empirical study on the *w*Ha and *w*No double infection of *Drosophila simulans* [307]. From the data given in this study, it can be calculated that the total transmission rate of the two strains (i.e., the proportion of offspring infected with the strain) is 0.943 (*w*Ha) and 0.909 (*w*No) for single infections, but 0.974 (*w*Ha) and 0.957 (*w*No) for double infections. The proportion of doubly infected offspring from doubly infected females was estimated to be 0.939, which is considerably greater than the transmission rate of *w*No in the singly infected state. One possible explanation for these findings is that the bacterial density of the two strains is higher in the double than in the single infection state. This is a situation, however, that I would not expect to occur in a double infection recently arisen through mutation in the mod-resc-system, but only in double infections that are old enough for differential regulation of bacterial density to evolve. In conclusion, I believe that the (scarce) data on transmission rates in multiple infections do not invalidate the assumptions I have made about segregation, because

they were observed in well-established and not in newly arisen double infections.

5.4.2 The impact of inbreeding and outbreeding

The main consequence of breeding systems that depart from panmixis is that a $mod_{B}resc_{A}$ mutant in a $mod_{A}resc_{A}$ wildtype population is no longer selectively neutral. With inbreeding, such a mutant will be selected against, because females infected with the mutant will mate more often with $mod_{B}resc_{A}$ males than wildtype females and therefore their offspring will suffer greater mortality. On the other hand, outbreeding will selectively favour $mod_{B}resc_{A}$ mutants. As a variation of this theme, mod-only strains — possible ancestors to 'real' CI-inducing strains with a rescue function — will also be selectively favoured in uninfected populations.

Obviously, the relevance of these results depends on how common inbreeding and outbreeding are in natural populations of arthropods. As argued in the introductory Section 5.1.4, inbreeding appears to be common in certain groups of insects and mites. My results predict that such species are 'coldspots' for the evolution of new CI-types. Unfortunately, this prediction will be very hard to test.

Easier to test will be the prediction that CI-inducing bacteria should be less common in inbreeding species in general, because the conditions for invasion and persistence of CI are more stringent than in panmictic populations. Species with extreme inbreeding should harbour no or very few CI-inducing endosymbionts. In apparent contrast to this prediction, *Wolbachia* are extremely common in fig wasps that exhibit extensive inbreeding [352, 137]. Moreover, no correlation between inbreeding and either prevalence or presence/absence of *Wolbachia* has been found in this group [352]. However, it is not known if *Wolbachia* induce CI in fig wasps, and some of the prevalence data reported suggest that they do not [352].

Outbreeding has been reported from few arthropod species only, although this may only be indicative of the fact that it is intrinsically more difficult to detect outbreeding than inbreeding. Nevertheless, given that outbreeding has only a limited capacity of facilitating the evolution of new CI-types, and that it is probably not very common in arthropods, a cautious view would be that outbreeding plays only a minor role in the evolution of new CI-types.

5.4.3 Random genetic drift

The present study has focused on the selective forces that are important for the evolution of new CI-types, and the model presented is entirely deterministic. However, random genetic drift has been conjectured [59] and demonstrated theoretically [58] to be potentially very important for the evolution of new CI-types. Thus, it is desirable to determine how genetic drift will interfere with the results obtained from my model.

Genetic drift is known to become an important factor when the genetic element in question is selectively neutral or almost neutral relative to the population size. Two such elements have been identified in this and previous studies. First, the ($mod_{\mathrm{B}}resc_{\mathrm{A}}$ only) infection state is neutral in a panmictic population, so that its fate is entirely determined by genetic drift [59, 58]. In an inbreeding population, this infection state is selected against, so that only in very small populations this infection state might spread due to drift. Finally, in an outbreeding population, ($mod_{\mathrm{B}}resc_{\mathrm{A}}$ only) is positively selected. As has been demonstrated, this selection becomes the stronger the smaller the population is. However, genetic drift also becomes stronger with decreasing population size. Therefore, genetic drift will always be important in determining whether the ($mod_{\mathrm{B}}resc_{\mathrm{A}}$ only) infection state spreads.

The second type of genetic elements that can be assumed to be strongly influenced by drift are the double infections ($mod_{\mathrm{A}}resc_{\mathrm{A}}$ & $mod_{\mathrm{B}}resc_{\mathrm{A}}$) and ($mod_{\mathrm{A}}resc_{\mathrm{A}}$ & $mod_{\mathrm{A}}resc_{\mathrm{B}}$). I have demonstrated that these double infections have a transmission disadvantage compared to the $mod_{\mathrm{A}}resc_{\mathrm{A}}$ or $mod_{\mathrm{B}}resc_{\mathrm{A}}$ single infection states, whilst selection is equal for double and single infection states. However, this transmission disadvantage may only be weak, especially when the transmission rates are high. Therefore, in a stochastic model the double infections may be maintained in the population and spread due to genetic drift. This would allow the evolution of a new CI-type to occur via the ($mod_{\mathrm{A}}resc_{\mathrm{A}}$ & $mod_{\mathrm{A}}resc_{\mathrm{B}}$) double infection, as hypothesised by Dobson [78].

5.4.4 Host population dynamics

The spread of CI-inducing bacteria, and of the various mutations studied in this chapter, can be expected to have an influence on host population size. As an extreme, consider the case where a $mod_B resc_A$ mutant inducing complete mortality in incompatible crosses becomes fixed in a population: all females will mate with a male inducing mod_B, no viable offspring will be produced and the population inevitably goes extinct.

Indeed, the invasion of a $mod_B resc_A$ mutant (as a double infection with $mod_A resc_A$) has been predicted to have a profound impact on the population size [78]. More specifically, it was demonstrated that host population size could be strongly reduced during the spread of the $mod_B resc_A$ mutant, and extinction could occur. On the other hand, with scramble-type competition and a sufficiently high baseline reproductive rate, the equilibrium population size could also increase as a result of the invasion of the mutant.

In another study, the population dynamic impact of a cytoplasmic element that increases the mortality of offspring of carrier males was investigated [94]. In the context of the present chapter, such an element is equivalent to a mod-only mutant in an uninfected population, as has been discussed in Section 5.3.6. In this stochastic model, the underlying assumption for the population dynamics was the logistic equation [256]. Again it was found that both reduced population size (including extinction) and increased population size could be the result of an invasion of a mod-only mutant. In addition, it was demonstrated that the invasion of the mod-only mutant could have stabilising effects on the population dynamics.

With respect to the present chapter, these results imply that population extinction may prevent the evolution of a new CI-type, or the evolution of CI itself. The likelihood of such an event depends on the parameters of the hosts population dynamics (basic reproductive rate, type of competition), and the mortality induced by the $mod_B resc_A$ or mod-only mutant.

Chapter 6

The Evolution of Endosymbiont Density in Doubly Infected Hosts

Abstract. Multiple infection of individual hosts with several species or strains of maternally inherited endosymbionts is commonly observed in arthropods. In this chapter, I address theoretically the effect of co-infection on the optimal density of endosymbionts in doubly infected hosts. My analysis is based on the observation that a maternally inherited double infection is only stable if doubly infected females produce more doubly infected daughters than singly infected or uninfected females produce daughters. I consider both a general model and a model involving two endosymbionts inducing bidirectional cytoplasmic incompatibility. I demonstrate that the optimal endosymbiont density of endosymbionts in doubly infected hosts can be expected to be similar to or below the optimal endosymbiont density in singly infected hosts.

An abridged version of this chapter has been accepted for publication in the *Journal of Evolutionary Biology* [96].

6.1 Introduction

6.1.1 Multiple infections

Infections of individuals hosts with more than one strain of endosymbionts are commonly observed in insects (see Table 6.1 for some well-studied examples). In the case of reproductive parasites, several surveys have demonstrated that many species are infected by more than one strain of *Wolbachia* [425, 424, 428, 195]. For example, in the first broad *Wolbachia* survey in arthropods, 5.7% of all species were doubly infected with two different *Wolbachia* strains [425]. However, in this and other surveys, multiple infections could only be detected when the strains were very divergent (i.e., belonged to different supergroups of *Wolbachia*). Thus, many cases of multiple infections with more closely related *Wolbachia* strains would not have been detected, and the reported frequency of multiple infections is only an estimate for the lower limit of the total frequency of multiple *Wolbachia* infections. Screenings for the bacterium *Cardinium hertigii* also revealed that double infections of *Cardinium* and *Wolbachia* occur commonly [410, 445].

Theoretical work has demonstrated that multiple infection with different strains of reproductive parasites can be stable if these strains induce bidirectional CI or different reproductive manipulations (e.g., CI and male-killing) [117, 101]. Indeed, in many

Table 6.1: Some examples of multiple infection with several strains or species of endosymbionts. Note that in all these cases, multiple infection has been found within single *individuals*. Host *species* infected with several strains of endosymbionts, but in which no multiple infections on the individual level occur, are also known (e.g., the butterfly *Hypolimnas bolina* [60]), but are not the focus of this study.

Species	Endosymbionts	Ref.
Cinara cedri (aphid)	*Buchnera*, secondary symbiont, *Wolbachia*	[128]
Glossina morsitans (tsetse fly)	*Wigglesworthia*, secondary symbiont, *Wolbachia*	[67, 328]
Drosophila melanogaster (fly)	CI-inducing *Wolbachia* & male-killing *Spiroplasma*	[275]
Formica exsecta (ant)	up to five strains of *Wolbachia*	[316]
Leptopilina heterotoma (wasp)	three strains of CI-inducing *Wolbachia*	[396]
Nasonia vitripennis (wasp)	two strains of CI-inducing *Wolbachia*	[300]
Asobara tabida (wasp)	three *Wolbachia* strains	
	(two CI-inducing, one required for oogenesis)	[76]

cases it has been demonstrated that *Wolbachia* strains co-occurring within single individuals induce bidirectional cytoplasmic incompatibility [266, 356, 300, 396, 76], and in *Drosophila melanogaster* CI-inducing *Wolbachia* and male-killing *Spiroplasma* co-occur within individual hosts [275].

Aside from the reproductive parasites *Wolbachia* and *Cardinium*, multiple infections are also well known in mutualistic host-symbiont relationships. In aphids, the obligate symbiotic bacterium *Buchnera* often co-occurs with one of several secondary symbiont strains [82]. Likewise, the mutualistic *Wigglesworthia* in tsetse flies can be found commonly together with secondary symbionts of the genus *Sodalis* [2]. In both cases, it is clear that the secondary symbionts are also transmitted maternally, but their (fitness) effects on the hosts are largely unknown. Finally, *Wolbachia* also co-occur with *Buchnera* and the secondary endosymbionts in aphids, and with *Wigglesworthia* in tsetse flies [128, 67].

6.1.2 Competition among parasites

The question of optimal within-host replication is intimately connected with the evolution of virulence, an area which has been explored by numerous theoretical studies (e.g., see Refs. [290, 257, 10, 243]). A crucial quantity in this respect is the 'reproductive ratio' R_0 of parasites, defined as the number of new infections caused by an infected host in an otherwise uninfected population [3]. In simple scenarios, R_0 can be regarded as a measure of parasite fitness, and must be greater than one for a parasite to invade and persist in a host population. R_0 is influenced by the mode of transmission (horizontal or vertical), the efficiency with which transmission occurs, the mortality of infected hosts, and the density of the uninfected population when transmission has a horizontal component.

Another factor that has been recognized as important for the evolution of virulence is the number of strains or species of parasites that co-occur within a host population. Several models have been developed for this scenario, especially for purely horizontally transmitted parasites [290, 257, 10]. If individual hosts can only be infected by one strain of parasite, the strain with the highest R_0 will outcompete all other strains and drive them extinct [39, 290]. However, when multiple infections at the individual level occur, co-existence of several strains is possible. Two extreme cases have been

studied for an arbitrary number of parasite strains. In the first model ('superinfection' model) it was assumed that only the most virulent strain that infects an individual host can be transmitted to other hosts [290]. In the second, the 'coinfection' model, all parasites within a host are transmitted independently of the presence or absence of other parasite strains [257]. In both cases, analyses of the model demonstrated that intra-host competition among the parasites selects for increasing levels of virulence.

This fundamental result appears sometimes to be mistaken as a general principle that applies as well to maternally transmitted endosymbionts like *Wolbachia* [281, 280, 234]. For example, Mouton *et al.* propose that '... *strain-specific density regulation* [by the hosts] *protects bacteria from competition, and thus could avoid their evolution towards excess virulence*' [281]. In what follows, I will argue that competition between maternally transmitted strains of endosymbionts does not occur when these strains can persist stably in a population. I will then construct two models that rest on this argument and yield predictions for optimal endosymbiont densities.

6.2 Preliminaries: What should be optimised?

In a population that is infected with only one strain of endosymbionts, the symbionts are selected to optimise the number of daughters from infected females that are also infected. Therefore, the endosymbiont should maximise the proportion of eggs that are infected and, at the same time, maximise the lifetime number of eggs that infected females lay [4, 104, 389]. However, since both of these quantities are likely to depend on the number of endosymbionts that are present in the germ cells of the infected females, the two components may not be independent. Rather, it seems likely that a high density in the adult leads to high transmission to eggs, but at the same time will trade off against deleterious effects on the female hosts' fecundity. Therefore, the optimal growth rate of the endosymbionts within their female hosts may result in a transmission rate and relative fecundity both below one.

Consider now a population that is co-infected with two different strains of maternally transmitted endosymbionts such that some hosts in the population are doubly infected. Provided that a doubly infected female produces more doubly infected daughters than any of the other types of females produce daughters, the double infection can be expected to be stably maintained within a panmictic host population. In such a situation, doubly infected females can produce daughters that again are doubly infected, daughters that are infected with only one of the strains, and daughters that are not infected. Conversely, only doubly infected females can give birth to doubly infected daughters, since transmission is strictly maternal. From this, it follows that in the case where the frequency of doubly infected females in a population is at equilibrium, all endosymbiont genotypes that happen to be transmitted without endosymbionts of the other strain will eventually vanish from the population. Appendix A at the end of this chapter gives a mathematical formulation and Fig. 6.1 an illustration of this argument.

It follows that, in contrast to the above considerations in a singly infected population, each endosymbiont strain in a doubly infected female should be selected not for a high number of daughters that are infected with the same strain, but for a high number of daughters that are infected with both strains. Singly infected strains created by inefficient transmission are less fit and are doomed to vanish from the population. Thus, the evolutionary interests of the two endosymbiont strains are in complete

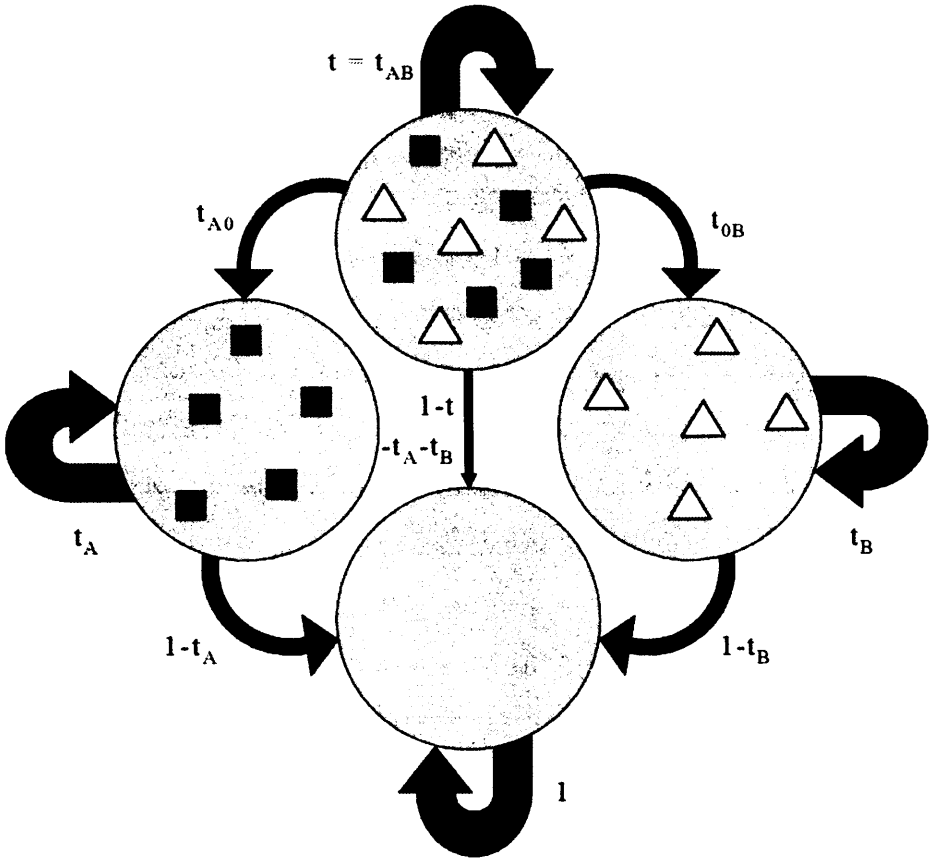

Figure 6.1: Illustration of the transmission dynamics in a population with two strains of endosymbionts and imperfect maternal transmission. Doubly infected hosts produce doubly and both types of singly infected as well as uninfected offspring. Singly infected hosts produce singly and uninfected offspring, and uninfected hosts produce uninfected offspring only. Rates of transmission are given as used in the main model and in the appendices.

agreement as long as the double infection is maintained in the population: coupling of the fitness of the two endosymbiont strains removes any competition between them. Provided the necessary genetic variation is available, selection is expected to lead to endosymbiont numbers that can be predicted by a simple optimisation approach rather than a game theoretic one.

I would like to note at this point that, save for the case of sex ratio distorting symbionts, host and endosymbiont interests are also congruent, and the modifier affecting the density of strain A could occur equally in strain A, strain B or the host genome (see also the discussion in Section 6.5.1).

6.3 General Model

6.3.1 The main function describing endosymbiont fitness

The considerations of the previous section will now be modelled concretely for a very general scenario of a stable double infection with two different strains of endosymbionts, denoted as A and B. Let E be the number of doubly infected daughters that a doubly infected female produces, relative to the number of daughters that an uninfected female produces. It is clear from the above argumentation that E is the function that we can expect to be maximised by natural selection. I assume that the only variable quantity upon which natural selection can act is the number of endosymbionts that are present in the germ line cells that develop to eggs. These numbers will be measured by n_A and n_B for the two strains, respectively. I will also assume that n_A and n_B are strain specific traits that depend only on the replication rate of the endosymbionts, but not on how many endosymbionts have been initially transmitted from mother to egg.

The relative number of doubly infected daughters that a doubly infected female produces can be partitioned into three components. First, due to inefficient transmission only a proportion t of the daughters of doubly infected females will also be doubly infected. t is assumed to increase with the numbers of the two endosymbionts, n_A and n_B. Second, the fecundity of doubly infected females is reduced with increasing number of endosymbionts present in the germ cells, so that doubly infected females have a relative fecundity f. Third, a certain phenotype induced by the endosymbionts is assumed that stably maintains the double infection within the host population. This is introduced into the model as the parameter b, the number of daughters a doubly infected female produces relative to an uninfected female, barring the fecundity reduction f. To keep the calculations simple, b is assumed not to depend on the numbers of endosymbionts n_A and n_B. However, I have also obtained data (not shown) from a model in which I assumed that b increases monotonously with increasing bacterial numbers, approaching asymptotically a maximum value. None of my qualitative results was affected by using a function $b(n_A, n_B)$ instead of a fixed value b, hence presentation of the most simple formulation. I assume that these three components act independently of each other, so that the relative number

of doubly infected daughters a doubly infected female produces is given by

$$E(n_A, n_B) = t(n_A, n_B) f(n_A, n_B) b. \tag{6.1}$$

6.3.2 Transmission and fecundity

I assume that transmission of a single endosymbiont of a strain from mother to offspring is sufficient to ensure transmission of that strain. For successful transmission of both strains, at least one endosymbiont from each strain must be transmitted. I further assume that transmissions of all endosymbionts occur independently. These assumptions lead to the formula

$$t(n_A, n_B) = [1 - (1 - \tau_A)^{n_A}][1 - (1 - \tau_B)^{n_B}], \tag{6.2}$$

where τ_A and τ_B are the probabilities for single endosymbionts from the respective strain to be transmitted.

I assume the following function for the relative fertility of doubly infected females:

$$f(n_A, n_B) = \max\{0, 1 - (\varphi_A n_A + \varphi_B n_B)^{\gamma}\}. \tag{6.3}$$

In this formula, every endosymbiont of a strain contributes a quantity φ_A or φ_B, respectively, to the physiological load on its host, and the effects of the two strains are also assumed to be additive. I then allow the total physiological load, given by $(\varphi_A n_A + \varphi_B n_B)$, to influence the relative fecundity in a variety of ways, depending on the parameter γ (see Figure 6.2). In all of these functions, the relative fecundity of uninfected females is one, and decreases with increasing number of endosymbionts to zero when the physiological load $(\varphi_A n_A + \varphi_B n_B)$ becomes one.

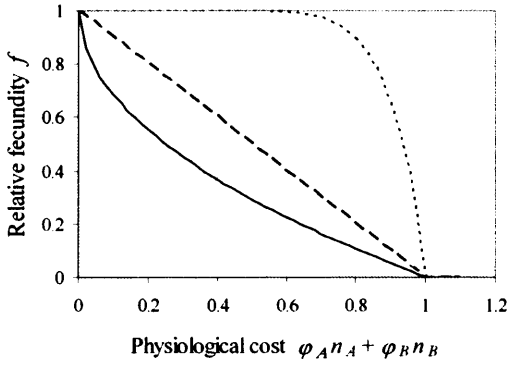

Figure 6.2: Variation in fecundity, f, as a function of the 'physiological cost', which in turn is a linear function of the numbers n_A and n_B of endosymbionts of the two strains. Depending on the parameter γ, f can be a concave ($\gamma > 1$), linear ($\gamma = 1$) or convex ($\gamma < 1$) function. In the examples, γ takes the values 0.5 (solid line), 1 (dashed line) and 5 (dotted line).

6.3.3 Optimal replication rate in singly infected hosts

In order to assess the optimal numbers of each endosymbiont in doubly infected hosts, it is desirable to compare them to the respective numbers expected in singly infected hosts. Based on the assumptions of the previous sections, and denoting bacterial numbers in the single infection by m, the reproductive success of endosymbionts in these single infections is given by

$$E_i(m) = t_i(m)f_i(m)b_i = [1 - (1 - \tau_i)^n] \times \max\{0, 1 - (\varphi_i m)^\gamma\} \times b_i, \qquad (6.4)$$

where $i = A, B$. The maximum of this function could not been determined analytically. Numerically derived optimal endosymbiont numbers m^* that maximise $E_i(m)$ are shown in Figure 6.3. As can be seen, the optimal number of endosymbionts decreases with increasing transmission rate of single endosymbionts τ and also with increasing physiological cost φ that a single endosymbiont imposes on its host. The shape of $m^*(\tau, \varphi)$ is very similar for $\gamma = 0.5$ and $\gamma = 5$ (not shown).

6.3.4 Optimal endosymbiont numbers in doubly infected hosts

The optimal number of endosymbionts in doubly infected hosts is given by the values of n_A and n_B that maximise the function $E(n_A, n_B) = t(n_A, n_B)f(n_A, n_B)b$. These optimal numbers can be derived either for a fixed endosymbiont number of the respective other strain (i.e., as functions $n_A^*(n_B)$ and $n_B^*(n_A)$), or as overall maximum numbers of both strains (i.e., a pair of values (n_A^*, n_B^*)). Again, I determined these values numerically only. Figure 6.4 shows optimal endosymbiont numbers for different parameter combinations and compares them to the optimal numbers in the singly

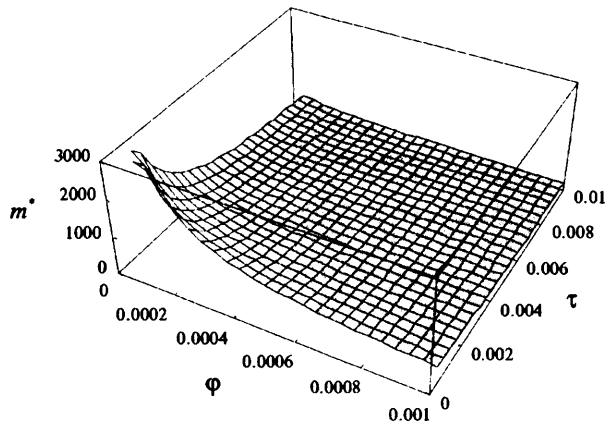

Figure 6.3: Optimal endosymbiont numbers m^* in singly infected hosts with varying transmission rates τ of single bacteria and varying contributions φ to the physiological load. All values were derived with $\gamma = 1$.

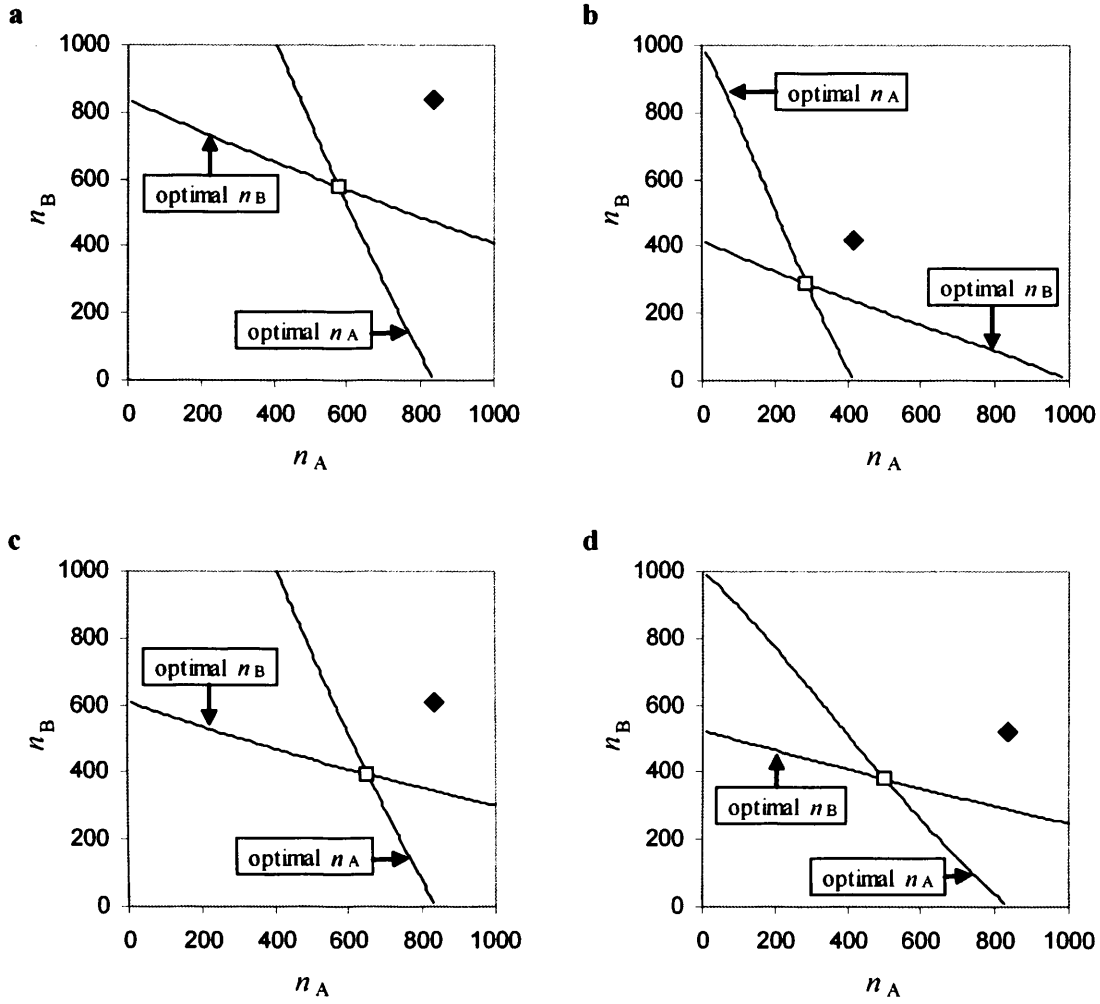

Figure 6.4: For given sets of parameters, the plots show optimal numbers of particular endosymbionts in the doubly infected state for a given endosymbiont number of the respective other strain (lines), with the optimal endosymbiont number of both strains (white squares) at the intersection of the lines. The optimal endosymbiont numbers in the singly infected state (black diamonds) are given for reference. Parameters take the values (a) $\tau_A = \tau_B = 0.005$, $\varphi_A = \varphi_B = 0.0005$, (b) $\tau_A = \tau_B = 0.01$, $\varphi_A = \varphi_B = 0.001$, (c) $\tau_A = 0.005$, $\tau_B = 0.01$, $\varphi_A = \varphi_B = 0.0005$, (d) $\tau_A = \tau_B = 0.005$, $\varphi_A = 0.0005$, $\varphi_B = 0.001$. In all plots, $\gamma = 5$.

infected strain. For the parameter combinations tested, the optimal endosymbiont number of a given strain decreases with the endosymbiont number of the respective other strain. The overall maximum of the function $E(n_A, n_B)$ is attained at the intersection of the two curves $n_A^*(n_B)$ and $n_B^*(n_A)$ that give the optimal number of the two strains for fixed numbers of the other strain.

How do optimal endosymbiont numbers (n_A^*, n_B^*) in the doubly infected state change compared to the singly infected state? In order to address this question, it

is convenient to consider the term n_A^*/m_A^*, where n_A^* is the optimal number of endosymbionts of strain A in the doubly infected state (when strain B has also attained its optimal number) and m_A^* is the optimal number of strain A in the singly infected state derived in the previous section. Values of the quotient n_A^*/m_A^* greater then one imply that strain A is selected to replicate more in the doubly than in the singly infected state, whereas $n_A^*/m_A^* < 1$ means that a lower titre is favoured in the doubly than in the singly infected state. Figure 6.5 shows values of n_A^*/m_A^* for a wide range of parameters. For the majority of parameter combinations tested, $n_A^*/m_A^* < 1$ was found to be true. In particular, for values of $\gamma > 1$ I found that the optimal endosymbiont number was always less in the doubly than in the singly infected state. Thus, if we assume a fertility function f that decreases more strongly with increasing number of endosymbionts, the endosymbionts are selected for reduced replication in the doubly compared to the singly infected host.

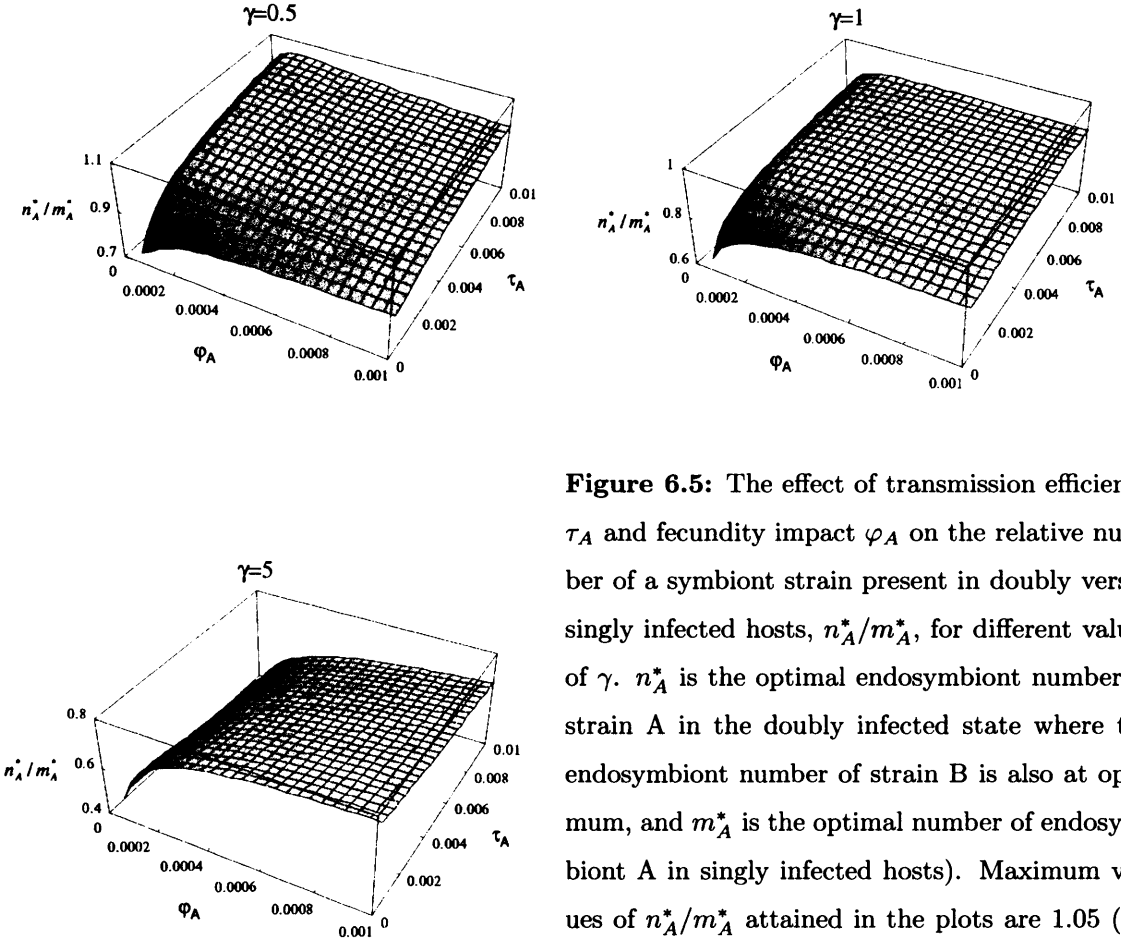

Figure 6.5: The effect of transmission efficiency τ_A and fecundity impact φ_A on the relative number of a symbiont strain present in doubly versus singly infected hosts, n_A^*/m_A^*, for different values of γ. n_A^* is the optimal endosymbiont number of strain A in the doubly infected state where the endosymbiont number of strain B is also at optimum, and m_A^* is the optimal number of endosymbiont A in singly infected hosts). Maximum values of n_A^*/m_A^* attained in the plots are 1.05 (for $\gamma = 0.5$), 0.89 (for $\gamma = 1$) and 0.75 (for $\gamma = 5$). τ_B and φ_B are fixed at $\tau_B = 0.005$ and $\varphi_B = 0.0005$. Note the shifting of the scale in the plots.

6.4 Model with two CI-inducing strains

Building upon the general model, I will now analyse the important case in which both endosymbionts induce cytoplasmic incompatibility (CI). As in the general model, the quantity that we can expect to be optimised by natural selection acting upon the two endosymbionts in doubly infected hosts is the function

$$E(n_A, n_B) = t(n_A, n_B) f(n_A, n_B) b(n_A, n_B). \tag{6.5}$$

The crucial extension to the general model is that with CI, the fitness benefit b that doubly infected females have compared to uninfected females also depends on the number of endosymbionts present in the host individuals. This is because the fitness advantage of doubly infected females stems from a protection against the sperm modification in infected males. Thus, the fitness benefit b depends on the infection frequencies in males and hence, on transmission rate and relative fecundity of infected females, which in turn depend on the endosymbiont numbers n_A and n_B in host individuals. Moreover, experiments have demonstrated that bacterial density in eggs correlates negatively with incompatibility levels [37]. Thus, I will also take into account that the ability of the endosymbionts to rescue a modification may decline with decreasing endosymbiont numbers.

6.4.1 Model assumptions

I make the same basic assumptions and use the same functions and parameters for fecundity and transmission as in the general model. In addition, I define the CI-level l_A as the mortality of eggs not infected with strain A that are fertilised by males infected with strain A. The CI-level l_B is defined analogously. When a male is doubly infected, the level of offspring reduction is assumed to occur multiplicatively. The rescue ability of the two strains is given by the functions

$$r_i(n) := 1 - \exp\left(-\rho_i \frac{n\tau_i}{1-(1-\tau_i)^n}\right), \tag{6.6}$$

where $i = A, B$. Here, the rescue ability increases from 0 when the egg is uninfected towards 1 with increasing number of endosymbionts present in the eggs. The term $n\tau_i/[1-(1-\tau_i)^n]$ gives the expected number of endosymbionts present in an

infected egg, and the parameter ρ_i determines how strongly rescue ability increases with increasing endosymbiont number.

6.4.2 Deriving the function $b(n_A, n_B)$

I will now incorporate all assumptions with regard to CI to derive the fitness benefit b of doubly infected females compared to uninfected females. I assume that the frequencies of singly and doubly infected males - denoted by $\tilde{p}_A$, $\tilde{p}_B$, and $\tilde{p}_{AB}$ - are at equilibrium in the population. (Note that these frequencies equal the respective frequencies in females.) The infection dynamic model that forms the basis of these equilibrium frequencies is set out in Appendix B at the end of this chapter.

Barring fecundity reduction and losses through imperfect transmission, a doubly infected female will on average produce a relative offspring number of

$$\begin{aligned} b(n_A, n_B) &= 1 - \tilde{p}_A l_A(1 - r_A) - \tilde{p}_B l_B(1 - r_B) \\ &\quad -\tilde{p}_{AB}[l_A(1 - r_A) + l_A(1 - r_A) - l_A(1 - r_A) l_B(1 - r_B)] \end{aligned} \quad (6.7)$$

In this formula, offspring number is reduced according to the probability of mating with the three types of infected males. Note that although omitted in the notation, the terms $\tilde{p}_A$, $\tilde{p}_B$, $\tilde{p}_{AB}$, r_A and r_B are functions of n_A and/or n_B. Also note that in contrast to the general model, the respective term for uninfected females is not equal to, but less than, one.

6.4.3 Optimal replication rates

I will now determine optimal numbers of CI-inducing endosymbionts in the doubly infected state by finding the maximum of the function $E(n_A, n_B)$ (Equation 6.5). As in the general model, it is desirable to compare these optimal endosymbiont numbers to the optimal endosymbiont numbers in the singly infected state. The latter can be determined by maximising the following function that follows naturally from the above considerations:

$$\begin{aligned} E_i(n) &:= t_i(n) f_i(n) b_i(n) \\ &= [1 - (1 - \tau_i)^n] \times \max\{0, 1 - (\varphi_i n)^\gamma\} \times [1 - \tilde{p}_i(n) l_i(1 - r_i(n))] \end{aligned} \quad (6.8)$$

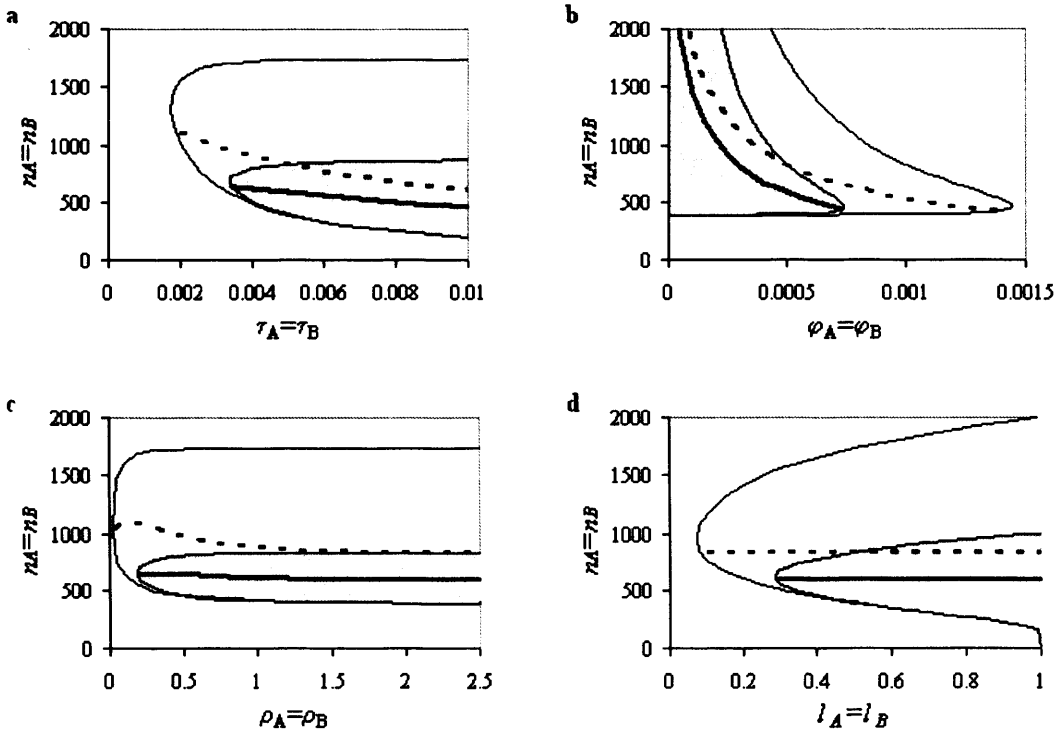

Figure 6.6: Optimal endosymbiont numbers in the singly infected state (dotted lines) and in the doubly infected state (solid lines) in the CI model. Also shown are the ranges of endosymbiont numbers where a single endosymbiont strain can stably exist within a host population (light grey areas) and where a double infection is stable (dark grey areas). Unless varied, parameters take the values $\tau_A = \tau_B = 0.005$, $\varphi_A = \varphi_B = 0.0005$, $l_A = l_B = 0.5$, $\rho_A = \rho_B = 2$.

Figure 6.6 shows numerically determined maxima of the functions $E(n_A, n_B)$ and $E_i(n)$ for the case when both endosymbiont strains have the same properties in terms of transmission, fecundity reduction, CI-level induced and rescue ability (i.e., $\tau_A = \tau_B$, $\varphi_A = \varphi_B$, $l_A = l_B$, and $\rho_A = \rho_B$). As in the general model, for $\gamma = 5$ (entailing a concave fertility function) the optimal endosymbiont numbers are always lower in the doubly than in the singly infected state. The optimal numbers both in the singly and doubly infected state appear to be most sensitive to changes in the fecundity parameter φ, and do not depend at all on the CI-level l.

Also shown in Figure 6.6 is the range of endosymbiont numbers where existence of the endosymbiont strain in the doubly and singly infected state is possible. It can be seen that for a subset of the parameter space and independent of endosymbiont numbers, persistence of a single CI-strain is possible, whereas stable co-existence of two such strains inducing bidirectional CI is not. For another subset of the parameter

space, persistence of both one and two strains is possible, although the range of endosymbiont numbers is more restricted for the co-existence of two strains. Note that the minimum endosymbiont number in the doubly infected state is always the same or very close to the minimum number in the singly infected state, whereas the maximum numbers in the doubly infected state are considerably lower than the maximum numbers in the singly infected state. This leads me to conclude that, at least for the set of parameters chosen, the degree of decreased host fecundity in the doubly compared to the singly infected state is more crucial than decreases in net transmission rate.

Interestingly, the optimal endosymbiont numbers in the singly infected state are too high to allow stable co-existence of the two strains in a subset of the parameter space. This may have the following consequences. Consider two CI-inducing strains that occur separately in different species or populations of the same species and have attained their optimal replication within their hosts. When, for example through horizontal transmission, a doubly infected host comes into being, such a double infection may not be stable because the two endosymbiont strains replicate too strongly. Thus, for new double infections to become established in a population it is necessary that either the two original strains have a sufficiently low replication rate or that they can evolve quickly to a low replication rate when they come together in a single host. (Another, well-known obstacle for new double infections is the invasion threshold, a minimum frequency that must be obtained for the double infection to spread [117]. This threshold, however, might be overcome more easily by random genetic drift.)

6.5 Discussion

I have developed and studied a model that predicts optimal densities of maternally transmitted endosymbionts in doubly infected hosts. My approach is based on the observation that where double infections are stably maintained in a population, natural selection should act on both symbiont strains to increase the number of daughters of their hosts that are again doubly infected. I analysed both a general model and a specific model where both endosymbiont strains induce cytoplasmic incompatibility (CI). I demonstrated that, depending on the parameters of the model, the expected number for any particular symbiont strain in the doubly relative to the singly infected state ranges from slightly increased to substantially reduced. Under the realistic assumption that the lifetime fecundity of infected females decreases more and more strongly with increasing number of endosymbionts, the optimal endosymbiont numbers of a given strain were always found to be lower in the doubly compared to the singly infected state.

In what follows, I will discuss the assumptions made in my models, relate my results to empirically obtained data on *Wolbachia* density in multiply infected hosts, and conclude with some remarks on the density with CI-inducing bacteria.

6.5.1 Assumptions of the models

One of my key assumptions is that the greater the number of endosymbionts in the females, the higher their transmission rate and the stronger the reduction in host fecundity. This assumption is corroborated by a number of studies on the bacterium *Wolbachia*. For example, it has been demonstrated that after transinfection of the *Wolbachia* strain popcorn from its native host *Drosophila melanogaster* to *D. simulans*, decrease of bacterial numbers in the ovaries over 9 generations corresponded to increased fecundity of infected females [262]. The assumption is also supported by the observation that among the two CI-inducing Wolbachia strains that infect the mosquito *Aedes albopictus*, the one with the higher transmission rate is also the one with the higher bacterial density [228, 87].

In my model on CI-inducing endosymbionts, the strength of CI induced by the bacteria in males was assumed not to depend on the bacterial density in females.

Several empirical studies have demonstrated that the CI-level is at least partly influenced by the bacterial density in males [32, 37, 31, 183]. Whilst bacterial density may be regulated independently in males and females as assumed in my model, there may also be a correlation between the two densities, so that the level of CI induced may be influenced by the selection on bacterial densities in females. The empirical reality of this notion is corroborated by an experiment in the wasp *Nasonia vitripennis*, in which selection for high CI-levels lead to increased *Wolbachia* density in eggs [301]. Consequently, selection on the bacteria for reduced density in females, as observed for some parameters in my model, might lead to CI-levels that are too low to maintain the infection (see also Ref. [181]). However, modelling such trajectories requires a more explicit population genetic framework than employed in this chapter, so that I leave this problem to future theoretical studies.

Throughout my analysis, I have assumed that endosymbiont density is regulated by the bacteria only. However, it is known that endosymbiont density can also be influenced by the hosts' nuclear genetic background [32, 305, 261], and it is therefore important to determine whether the optimal replication rates from the bacteria's and the hosts' point of view are the same or not, i.e., whether there is intragenomic conflict about replication rates. The answer to this question depends on the mechanism by which the endosymbionts are maintained in the population. For bacteria that confer some kind of fitness advantage to their hosts, e.g. through providing essential nutritional substances, optimal rates for bacteria and for the hosts will be the same. This is also true for CI-inducing bacteria, as these can also be considered beneficial in the particular environment they have created (i.e., many males with modified sperm). On the other hand, endosymbionts that act through sex ratio distortion (male-killing or feminisation) reduce the fitness of their female hosts by decreasing the number of male offspring they produce. Here, the optimal replication rate for the hosts is zero, and hence resistance alleles that decrease the replication rate of the bacteria below the optimal density for endosymbiont persistence can be expected to spread.

In addition to endosymbionts and hosts, the density of the endosymbionts may also be influenced by parasites that infect them. Results reported in a recent study by Bordenstein *et al.* [24] suggest that the temperate phage WO-B can negatively affect *Wolbachia* density. The selective forces and the population and evolutionary

dynamics of the tripartite system arthropod host - *Wolbachia* - phage are difficult to predict at the present state of knowledge; clearly, more empirical and theoretical work is needed.

6.5.2 Empirical data on *Wolbachia* density in multiply infected hosts

Several studies in different insect host species have investigated the interactions between different strains of *Wolbachia* by curing multiply infected hosts of a subset of their strains or transinfecting strains, and then comparing bacterial densities in hosts with different numbers of strains [281, 184, 280, 234]. In most cases, the *Wolbachia* strains were observed to have the same density within their hosts irrespective of whether they co-occur with one or two other strains. In the two cases where differences in density were observed, there was one case of a *Wolbachia* strain exhibiting slightly increased replication in triply compared to singly infected female hosts [280], and one showing decreased density [234].

These results are in accord with my predictions and indicate that usually, *Wolbachia* replication is regulated more or less independently of the number of other strains present. However, because of the experimental setup (matings with uninfected males only in some experiments) and the low number of generations elapsed since the establishment of these lines, the bacterial numbers obtained may not reflect optimal replication rates of the bacteria in the host strains that were cured of one or two of their endosymbiont strains. It would be desirable to conduct an experimental evolution experiment in which host populations with different numbers of endosymbiont strains could evolve for many generations, after which the bacterial densities could be compared. One complication which must be controlled for is that not only the bacterial numbers might be adjusted by natural selection (as assumed in my model), but also other properties of the endosymbionts that influence transmission, fecundity reduction, and CI-induced mortality.

6.5.3 Multiple infections of CI-inducing *Wolbachia*

Several multiple infections with more than two (up to five) CI-inducing *Wolbachia* strains have been reported in natural populations [396, 194, 232, 316]. These findings raise the question as to the maximum number of CI-strains that can be stably maintained in a host population. Although in my model I considered only two strains, my results also allow some conjectures on what may limit the possible number of *Wolbachia* strains. First, my results demonstrate that in some cases, a double infection may not be stable even when the two component bidirectional CI-inducing strains can themselves be stably maintained as single infections. With increasing number of strains, the parameter space with regard to transmission rates, fecundity reduction and CI-level of each strain that allows stable multiple infection of the strains in a host population will become narrower and narrower, so that for given properties of the strains, there will be a maximum number of strains that can persist in a host population. (The maximal number of strains found to date is five [194, 316]).

Second, the number of strains may also be limited because the optimal bacterial replication rate of a number of strains within their hosts may exclude the possibility that another strain can be added and stably maintained, even if stable co-existence would be possible with lower replication rates. The importance of this second limit on strain number depends on the rate at which the replication rate of the bacterial strains present can be adjusted by mutation and natural selection.

Appendix A: Infection dynamics in the general model

I denote by p_A and p_B the frequencies of singly infected females in the population, and by p_{AB} the frequency of doubly infected females. As in the main text, t_A and t_B are the transmission rates, f_A and f_B are the relative fecundities and b_A and b_B are the increases in the number of daughters of singly infected females. A doubly infected female has a relative fecundity f_{AB} (denoted by f in the main text) and has a relative number of daughters b_{AB} (barring fecundity losses). A proportion t_{AB} (= t in the main text) of the offspring from doubly infected females is again doubly infected, whilst fractions t_{A0} and t_{B0} are infected with strain A and B only, respectively. Assuming an infinitely large panmictic host population with discrete, nonoverlapping generations, the recursion equations for the three types of females are given by

$$\begin{aligned} p'_A &= \frac{1}{\overline{W}}[p_A t_A f_A b_A + p_{AB} t_{A0} f_{AB} b_{AB}] \\ p'_B &= \frac{1}{\overline{W}}[p_B t_B f_B b_B + p_{AB} t_{B0} f_{AB} b_{AB}] \\ p'_{AB} &= \frac{1}{\overline{W}}[p_{AB} t_{AB} f_{AB} b_{AB}] \end{aligned}$$

where

$$\overline{W} = 1 + p_A(f_A b_A - 1) + p_B(f_B b_B - 1) + p_{AB}(f_{AB} b_{AB} - 1). \tag{6.9}$$

The fixed points of this dynamical system can be determined as

$$\begin{aligned} P_1 &= (0,0,0) \\ P_2 &= \left(\frac{t_A f_A b_A - 1}{f_A b_A - 1}, 0, 0\right) \\ P_3 &= \left(0, \frac{t_B f_B b_B - 1}{f_B b_B - 1}, 0\right) \\ P_4 &= \Big(\frac{Q_B t_{A0} f_{AB} b_{AB}(t_{AB} f_{AB} b_{AB} - 1)}{Z}, \\ &\quad \frac{Q_A t_{B0} f_{AB} b_{AB}(t_{AB} f_{AB} b_{AB} - 1)}{Z}, \\ &\quad \frac{Q_A Q_B (t_{AB} f_{AB} b_{AB} - 1)}{Z}\Big) \end{aligned}$$

with

$$\begin{aligned} Q_A &= t_{AB} f_{AB} b_{AB} - t_A f_A b_A \\ Q_B &= t_{AB} f_{AB} b_{AB} - t_B f_B b_B \end{aligned}$$

$$\begin{aligned} Z &= Q_A Q_B (f_{AB} b_{AB} - 1) + Q_A t_{B0} f_{AB} b_{AB} (f_B b_B - 1) \\ &\quad + Q_B t_{A0} f_{AB} b_{AB} (f_B b_B - 1) \end{aligned}$$

To determine the stability of P_4, i.e. the conditions when doubly infected individuals can occur stably within the population, I evaluated the Jacobian matrix of the system at the position of the fixed point P_4 and found its eigenvalues to be

$$\lambda_1 = \frac{1}{t_{AB} f_{AB} b_{AB}}, \quad \lambda_2 = \frac{t_A f_A b_A}{t_{AB} f_{AB} b_{AB}}, \quad \text{and} \quad \lambda_3 = \frac{t_B f_B b_B}{t_{AB} f_{AB} b_{AB}}.$$

Since for the fixed point to be stable, $|\lambda| < 1$ must hold for all eigenvalues, it can be seen that the conditions for stable persistence of doubly infected individuals in the population are

$$\begin{aligned} &(1)\ t_{AB} f_{AB} b_{AB} > 1, \\ &(2)\ t_{AB} f_{AB} b_{AB} > t_A f_A b_A, \\ &(3)\ t_{AB} f_{AB} b_{AB} > t_B f_B b_B \end{aligned} \tag{6.10}$$

Thus, as can be expected intuitively, the fitness of the doubly infected cytotype - given by the product of transmission rate, relative fecundity and fitness increase due to the induced effect - must be larger than the fitness of the uninfected and the two singly infected cytotypes for the double infection to be stable.

Appendix B: Infection dynamics in the CI-model

I assume an infinite panmictic host population. I use all parameters and variables as defined in the main text and Appendix A. The recursion equations for the fractions of singly and doubly infected females in the population are given by

$$\begin{aligned} p'_A &= \frac{1}{\overline{W}} (p_A t_A f_A + p_{AB} t_{A0} f_{AB}) \\ &\quad \times \{1 - p_A l_A (1 - r_A) - p_B l_B - p_{AB} [1 - (1 - l_A (1 - r_A))(1 - l_B)]\} \\ p'_B &= \frac{1}{\overline{W}} (p_A t_A f_A + p_{AB} t_{A0} f_{AB}) \\ &\quad \times \{1 - p_A l_A - p_B l_B (1 - r_B) - p_{AB} [1 - (1 - l_A)(1 - l_B (1 - r_B))]\} \\ p'_{AB} &= \frac{1}{\overline{W}} p_{AB} t_{AB} f_{AB} \\ &\quad \times \Big\{ 1 - p_A l_A (1 - r_A) - p_B l_B (1 - r_B) \\ &\quad - p_{AB} [1 - (1 - l_A (1 - r_A))(1 - l_B (1 - r_B))] \Big\} \end{aligned} \tag{6.11}$$

$\overline{W}$ is the average fitness of a cytotype, given by the sum of the numerators in the above equations and the numerator in the corresponding equation for uninfected females.

I was not able to determine all fixed points of the system analytically. The fixed points where the population is infected with endosymbiont strain A only are given by

$$\begin{aligned} P_1 &= (0,0,0) \\ P_2 &= \left(\frac{u-\sqrt{u^2-2v(1-f_A t_A)}}{v},0,0\right) \\ P_3 &= \left(\frac{u+\sqrt{u^2-2v(1-f_A t_A)}}{v},0,0\right) \end{aligned}$$

with $u = 1 - f_A - l_A - t_A f_A l_A (1 - r_A)$ and $v = 2l_A(1 - f_A + t_A f_A r_A)$. The formulae for the fixed points P_4 and P_5, where only strain B exists in the population, are given analogously to the ones in P_2 and P_3. The fixed points P_2 and P_4 are unstable and referred to as the invasion threshold of CI-inducing bacteria in the literature, whereas P_3 and P_5 are stable [111]. There are two more fixed points P_6 and P_7 without double infections in which both strains occur in the population, both are unstable (formulae not shown). When doubly infected individuals occur within the population, there is one stable fixed point $P_8 = (\hat{p}_A, \hat{p}_B, \hat{p}_{AB})$, values of which could be determined numerically only. P_8 is the fixed point used in the main text to determine optimal endosymbiont numbers. In addition, there are several other unstable fixed points. For more detailed analyses of the infection dynamics with multiple strains of CI-inducing bacteria, see Refs. [332, 117].

Chapter 7

The Dynamics of Endosymbiont Incidence across a Clade of Host Species

It is well known that some endosymbionts, especially reproductive parasites like *Wolbachia*, occasionally switch from one host species to another and thus spread within a host clade. However, the patterns of spread and the observed heterogeneity in endosymbiont incidence between host taxa are not well understood. Here, I develop a simple stochastic model as a first attempt to understand these 'incidence dynamics'. Based on the empirically supported assumption that the probability of successful transmission from an infected to a new host species declines with increasing genetic distance between them, I study the impact of different phylogenetic histories of the host clade on the pattern of spread and the average incidence of the parasites. My results suggest that host phylogeny alone can lead to heterogeneous endosymbiont incidence. I also investigate some extensions of my model that include two endosymbiont strains and refractoriness.

Some of the work reported in this chapter has been published in a slightly broader context in *Evolutionary Ecology* [98].

7.1 Introduction

A great body of theory has been developed to explain how the prevalence of symbionts — their frequency within a host population — changes over time. By contrast, little is known about the dynamics of incidence, i.e., how the frequency of a certain group of symbionts within a clade of host species varies over time, and what influences these dynamics. This is intriguing especially because, whilst it is known that endosymbionts like *Wolbachia* are heterogeneously distributed within their arthropod hosts, there is no general explanation of what might cause this heterogeneity.

The incidence of endosymbionts depends on the balance between loss of infection (associated with host extinction or endosymbiont extinction) and emergence of new infected host species. New infected host species can emerge either when an infected host undergoes speciation and both daughter species inherit the endosymbionts (a process known as cospeciation or, in a phylogenetic context, cocladogenesis) or when endosymbionts switch from an infected species to a previously uninfected species, possibly only distantly related to the original host, and establish on it. The relative contribution of cospeciation and host switching varies greatly between different endosymbionts. High levels of cospeciation have been documented in many (nutritionally or otherwise) beneficial endosymbionts, including *Blattabacterium* in cockroaches and termites [244], the endosymbionts of whiteflies [386] and *Wolbachia* in nematodes [15]. Host switching is predominant in reproductive parasites like *Wolbachia* and *Cardinium* in arthropods [426, 343, 450, 410, 445].

In this chapter, I make an attempt to understand the 'incidence dynamics' of endosymbionts across a clade of host species. I develop and analyse a simple stochastic model in which incidence is affected by host switching and parasite extinction only (not by cospeciation), and this process is carried on in host clades with different phylogenetic history. Several methods have been developed to compare and match the phylogenetic relationships between species that are closely associated with each other, and some of these also include host switching (e.g., [331, 296, 64, 65]). However, there has been no investigation into how the incidence of an endosymbiont species that can spread in a host clade by host switching changes over time, nor is there any explanation for the processes that lead to heterogeneity in incidence of particular endosymbionts across host taxa.

My main assumption is a decreasing probability of successful host switching with increasing genetic distance between host species. This is not only intuitively sensible, but also well supported by empirical studies. Intuitively, parasites need to be adapted in some way to the physiology of their hosts. They are thus most likely to prosper on naïve hosts of similar physiology to their origin, and similarity in host physiology is likely to covary negatively with genetic distance between the donor and recipient hosts. Empirically, this assumption is corroborated by observations of transinfection success in *Wolbachia*, as will be seen in Section 7.1.2. Moreover, phylogenetic studies both in *Wolbachia* and *Cardinium* have demonstrated that although host and endosymbiont trees are not concordant, closely related strains of endosymbionts often cluster in closely related host species, suggesting frequent horizontal transfers across short genetic distances [199, 445].

In the remainder of this introduction, I will elaborate on two important points mentioned above. First, I will set out what is known about the incidence of *Wolbachia* within their arthropod hosts and show that incidence is heterogeneously distributed among host clades (Section 7.1.1). Second, the results from transinfection experiments will be described in some detail, corroborating my assumption that horizontal transmission probability declines with increasing genetic distance between hosts (Section 7.1.2).

7.1.1 The incidence of *Wolbachia*

Wolbachia is by far the best studied reproductive parasite in terms of incidence, and it is also the most widespread. In this section, I will use *Wolbachia* as an example to demonstrate that incidence can be heterogeneously distributed in a given host clade. Another widespread reproductive parasite, *Cardinium*, also appears to be heterogeneously distributed in arthropods [410, 445], but will not be further discussed here because not much data is available yet.

Table 7.1 gives an overview of incidences of *Wolbachia* reported in different arthropod groups. To date, the highest incidence of *Wolbachia* (64%) has been reported in fig wasps [352, 137]. Interestingly, two independent surveys — one in central America and one in Australia — produced very similar results, despite the fact that no species was tested in both studies. Thus, this high incidence appears to be a property of the taxonomic group Agaonidae and not that of a geographically defined group of species. High incidence of *Wolbachia* has also been reported in ants, again in two independent studies [411, 29]. On the other extreme, aphids seem to be almost entirely devoid of *Wolbachia*; to the best of my knowledge, only one aphid species has been reported to be infected [128]. *Wolbachia* incidence in beetles is also relatively low (13%), but given the huge number of species, further surveys are needed to ascertain the distribution of the bacteria in this order.

The incidence data in Table 7.1 suggest a heterogeneous distribution of *Wolbachia* on the scale of orders and families. In addition, not listed in this table, *Wolbachia* incidence is also significantly heterogeneous among 19 families of the Heteroptera (true bugs) [217]. Incidence can also be heterogeneous within families of host species: within gall wasps (Cynipidae), three tribes were found to have a very high incidence of *Wolbachia* (up to 60%), whereas in the tribe Cynipini, only 9.4% of the species tested were infected.

Table 7.1: Incidence of *Wolbachia* in various groups of arthropods. Where several surveys have been conducted, numbers represent averages. Note that surveys that studied a certain taxonomic group were also used to obtain estimates for subgroups if a reasonable number of species were tested. All surveys cited ascertained the presence or absence of *Wolbachia* via PCR amplification of bacterial DNA. One study [195] has been excluded because a different PCR method ('Long PCR') was used that may have produced false positives. Only data from surveys on *Wolbachia* are given, whilst reports of *Wolbachia* in a particular host species are not included.

Group	Incidence	Species	References
Arachnida	28.6%	49	[425, 424, 410, 445]
Acari	38.3%	60	[35, 410, 445]
Phytoseidae (predatory mites)	36.4%	11	[35]
Tetranychidae (spider mites)	22.4%	58	[35, 130]
Aranaea	42.3%	163	[334, 129]
Crustacea	25.9%	85	[30]
Isopoda	34.9%	63	[30]
Hexapoda	19.7%	671	[425, 428, 424, 410, 445]
Coleoptera	13.3%	135	[425, 424, 410, 445]
Diptera	23.7%	59	[425, 424, 410, 445]
Culicidae (mosquitoes)	28.1%	89	[227]
Diopsidae (stalk-eyed flies)	23.5%	17	[142]
Drosophilidae	10%	40	[420, 31]
Psychodidae (sand flies)	26.7%	15	[294]
Hemiptera	18.8%	85	[425, 428, 424, 410, 445]
Aleyrodidae (whiteflies)	30.8%	13	[284, 410]
Aphidoidea (aphids)	0%	28	[284, 428]
Heteroptera (true bugs)	34.3%	137	[217]
Hymenoptera	22.9%	218	[425, 428, 424, 410, 445]
Agaonidae (fig wasps)	63.7%	105	[352, 137]
Cynipidae (gall wasps)	28.3%	92	[304, 330]
Formicidae (ants)	56.1%	57	[411, 29]
Lepidoptera	29.7%	162	[425, 428, 424, 410, 445, 373]
Gracilariidae (leaf-miners)	38.1%	21	[428]
Odonata	12.1%	33	[387]

7.1.2 Genetic distance and transinfection studies

The key assumption of the model analysed in this chapter will be that the probability of successful horizontal transmission of endosymbionts from one host species to another one declines with increasing genetic distance between these species. Empirical support for a declining probability of horizontal transmission with genetic distance comes from transinfection experiments with *Wolbachia*. In these experiments, *Wolbachia* strains from one host species were transferred to another host species, usually by microinjection into the eggs. Most such studies involved transinfections from or into several *Drosophila* species; the reported success or failure of these experiments

Table 7.2: Transinfection experiments of *Wolbachia* in *Drosophila*. Successful transinfection and stable infection is denoted by '+', failure of transinfection by '-'. 'o' denotes that transinfection was successful, but the infection was not stable. Two symbols (e.g., o/-) stand for different *Wolbachia* strains for which transinfections have been attempted. The genus name is omitted for the species *Drosophila*. 'He.' and 'Hy.' stand for Hemiptera and Hymenoptera, respectively.

Donor	Recipient	Success?	References
Within subgroup			
mauritiana	*simulans*	+	[126]
melanogaster	*simulans*	+/+	[305, 261]
simulans	*mauritiana*	+	[126]
simulans	*melanogaster*	+	[32]
simulans	*santomea*	+	[442]
sechellia	*simulans*	+	[62]
simulans	*teisserii*	+	[442]
simulans	*yakuba*	+	[442]
Between subgroups			
bifasciata	*melanogaster*	-	[402]
simulans	*serrata*	o	[68]
Outside genus			
Aedes albopictus (Diptera)	*simulans*	+	[33]
Ceratitis cerasi (Diptera)	*simulans*	o/-	[320]
simulans	*Laodelphax striatellus* (He.)	+	[213]
Muscidifurax uniraptor (Hy.)	*serrata*	o	[263]

is listed in Table 7.2.

All transinfections between members of the melanogaster subgroup of the genus *Drosophila* were successful and resulted in stable infections. By contrast, of the two transinfection attempts between *Drosophila* species of different subgroups (but still within the group melanogaster), one failed completely and one resulted in an unstable infection only due to low transmission rate and reduced host fecundity. Out of five published attempts to transinfect *Wolbachia* from other species into *Drosophila simulans* or *vice versa*, only two were successful[1].

Similar results have been obtained for transinfections of *Wolbachia* between woodlouse species. Transinfections in both directions between *Armadillidium vulgare* and *A. nasatum* were successful and produced stable infections [325, 327]. In contrast, transinfections between more distantly related woodlouse species (*Cylisticus convexus* to *A. vulgare*; *A. vulgare* and *A. nasatum* to *Oniscus asselus* and *Porcellio saber*; *Chaetophiloscia elongata* to *A. vulgare*) were successful, but the bacteria were either not or poorly transmitted to the next generation and failed to induce the reproductive manipulation that they induce in their native host species [325, 279, 327].

Taken together, these results imply that whilst long-distance transinfections can work (even between host species belonging to different orders), the success of transinfections appears to decline with increasing genetic distance between the host species.

[1]Note that successful transinfections are much more likely to be published than failures. However, there is no reason to assume that the proportion of unreported failures of transinfections is higher when transinfection were attempted between closely than between distantly related species.

7.2 Basic model of incidence dynamics

In this section, I will describe and analyse the model that is the core of this chapter. In two subsequent sections, I will study extensions of this basic model, the first one of which involves refractoriness to infection, and the second one two strains of endosymbionts.

7.2.1 Description of the model

I consider a clade of n potential host species. The genetic distance between these species is given by the symmetric matrix $\mathbf{D} = (d_{ij})_{i,j\in\{1,2,...,n\}}$, where $d_{ij} > 0$. The infection state of a species i is denoted by the Boolean variable s_i. Each species can either be infected ($s_i = 0$) or uninfected ($s_i = 1$), and no multiple infections occur. The infection state of the clade at a given time t is thus represented by the vector $\mathbf{s}_t = (s_{t,1}, s_{t,2}, ..., s_{t,n}) \in \{0,1\}^n$.

Within one time step, one of two events can take place for each species, depending on whether the species is uninfected or infected. First, an uninfected species j can become infected due to horizontal transmission from a species i that was infected in the previous time step. The probability for such an event is given by the function

$$g(d_{ij}) := \gamma 2^{-\left(\frac{d_{ij}}{\beta}\right)^{\kappa}} \tag{7.1}$$

In this function, γ ($0 \leq \gamma \leq 1$) is the probability for transmission from one species to a hypothetical identical species, i.e., γ gives the transmission probability for genetic distance $d_{ij} = 0$. The parameter β ($\beta > 0$) describes how fast the transmission probability declines with increasing genetic distance between hosts: where $d_{ij} = \beta$, the probability of successful transmission is $\gamma/2$. For $\beta \to \infty$, the transmission probability g becomes γ for all genetic distances. Finally, the parameter κ ($\kappa \in \{1,2,3,...\}$) determines the shape of the function g. For $\kappa = 1$, g is a exponentially declining function of d_{ij}. For $\kappa \geq 2$, g is a sigmoidally declining function whose steepness $d_{ij} = \beta$ increases with increasing κ. I assume that transmission events occur independently from each other.

The second event that can take place is extinction of the endosymbionts in a host species. The probability η for this event is assumed to be constant in time and across the clade of host species. Again, it is assumed that extinction events occur

independently from each other, and are also independent of horizontal transmission events.

The main scope of the model is to ascertain how different host phylogenies, i.e., different matrices **S**, affect the dynamics of incidence and the expected incidence of the endosymbionts in the clade. The incidence at a given time is given simply by

$$\text{incidence} := \frac{1}{n}\sum_{i=1}^{n} s_i. \tag{7.2}$$

Aside from the incidence, it is also of interest to consider the probability that the endosymbionts went extinct from the entire clade of host species, and also what the distribution of the endosymbionts within the clade is. Two quantities will be defined that are informative with regards to endosymbiont distribution. The first quantity is defined as

$$\text{distribution centre} := \frac{\sum_{i=1}^{n}(i-1)s_i}{(n-1)\sum_{i=1}^{n} s_i}. \tag{7.3}$$

The 'distribution centre' gives the average species position of all infected species and takes values in-between 0 (when only species 1 is infected) to 1 (when only species n is infected).

The second quantity describing the distribution of the endosymbionts is the average relatedness of all infected species,

$$\text{distance coefficient} := \frac{\sum_{1\le i<j\le n} s_i s_j d_{ij}}{\tilde{d}\sum_{1\le i<j\le n} s_i s_j}. \tag{7.4}$$

In this definition, $\tilde{d}$ is the average genetic distance between two randomly chosen species. The distance coefficient takes values close to one when the average genetic distance between all infected species is similar to the overall average genetic distance in the tree, but takes small values when infected species tend to be more related to each other.

7.2.2 Host phylogenies

In my analysis of the model, I will always assume four basic tree types that are meant to cover some extremes of all possible phylogenies. Miniature versions of the four trees are shown in Figure 7.1. Two of these trees are perfectly symmetrical ('EvenBush' and 'ShorteningBush'), one is strongly asymmetrical ('Ladder'), and one consists of

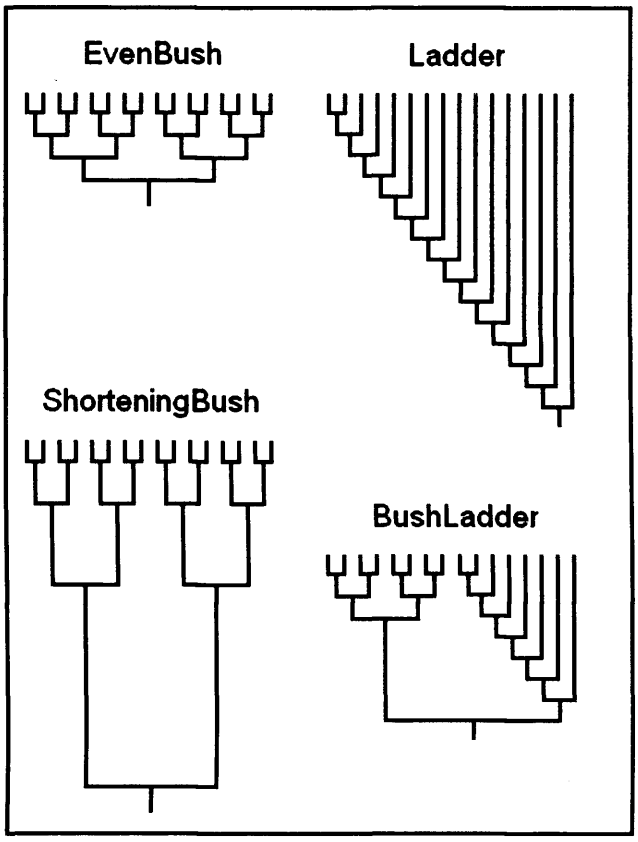

Figure 7.1: Basic tree types used in the model, with $n = 16$ species. The genetic distance between two species is proportional to the vertical distance that is covered when going from one to the other. For example, the genetic distance between the first two species equals 2 in all tree types. In the full trees used when investigating the model (n=128 species), mean (and variance) in genetic distance between randomly chosen species for each of the trees are 12.1102 (6.4445) for 'EvenBush', 170.0 (3612.0) for 'Ladder', 170.0 (8160.0) for 'ShorteningBush' and 88.0315 (2534.25) for 'BushLadder'.

a symmetrical and an asymmetrical branch ('BushLadder'). The 'EvenBush' tree corresponds to an adaptive radiation.

What follows is a list of the distance matrices of these four tree types. Note that the matrix for 'BushLadder' is not given in its general form, but as an example with $n = 8$.

$$\mathbf{D}_{\text{EvenBush}} := \begin{pmatrix} 0 & 2 & 4 & 4 & 6 & 6 & 6 & 6 & \cdots \\ 2 & 0 & 4 & 4 & 6 & 6 & 6 & 6 & \cdots \\ 4 & 4 & 0 & 2 & 6 & 6 & 6 & 6 & \cdots \\ 4 & 4 & 2 & 0 & 6 & 6 & 6 & 6 & \cdots \\ 6 & 6 & 6 & 6 & 0 & 2 & 4 & 4 & \cdots \\ 6 & 6 & 6 & 6 & 2 & 0 & 4 & 4 & \cdots \\ 6 & 6 & 6 & 6 & 4 & 4 & 0 & 2 & \cdots \\ 6 & 6 & 6 & 6 & 4 & 4 & 2 & 0 & \cdots \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \ddots \end{pmatrix}$$

$$\mathbf{D}_{\text{Ladder}} := \begin{pmatrix} 0 & 2 & 4 & 6 & 8 & 10 & 12 & 14 & \cdots \\ 2 & 0 & 4 & 6 & 8 & 10 & 12 & 14 & \cdots \\ 4 & 4 & 0 & 6 & 8 & 10 & 12 & 14 & \cdots \\ 6 & 6 & 6 & 0 & 8 & 10 & 12 & 14 & \cdots \\ 8 & 8 & 8 & 8 & 0 & 10 & 12 & 14 & \cdots \\ 10 & 10 & 10 & 10 & 10 & 0 & 12 & 14 & \cdots \\ 12 & 12 & 12 & 12 & 12 & 12 & 0 & 14 & \cdots \\ 14 & 14 & 14 & 14 & 14 & 14 & 14 & 0 & \cdots \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \ddots \end{pmatrix}$$

$$\mathbf{D}_{\text{ShorteningBush}} := \begin{pmatrix} 0 & 2 & 6 & 6 & 14 & 14 & 14 & 14 & \cdots \\ 2 & 0 & 6 & 6 & 14 & 14 & 14 & 14 & \cdots \\ 6 & 6 & 0 & 2 & 14 & 14 & 14 & 14 & \cdots \\ 6 & 6 & 2 & 0 & 14 & 14 & 14 & 14 & \cdots \\ 14 & 14 & 14 & 14 & 0 & 2 & 6 & 6 & \cdots \\ 14 & 14 & 14 & 14 & 2 & 0 & 6 & 6 & \cdots \\ 14 & 14 & 14 & 14 & 6 & 6 & 0 & 2 & \cdots \\ 14 & 14 & 14 & 14 & 6 & 6 & 2 & 0 & \cdots \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \ddots \end{pmatrix}$$

$$\mathbf{D}_{\text{BushLadder}} := \begin{pmatrix} 0 & 2 & 4 & 4 & 8 & 8 & 8 & 8 \\ 2 & 0 & 4 & 4 & 8 & 8 & 8 & 8 \\ 4 & 4 & 0 & 2 & 8 & 8 & 8 & 8 \\ 4 & 4 & 2 & 0 & 8 & 8 & 8 & 8 \\ 8 & 8 & 8 & 8 & 0 & 2 & 4 & 6 \\ 8 & 8 & 8 & 8 & 2 & 0 & 4 & 6 \\ 8 & 8 & 8 & 8 & 4 & 4 & 0 & 6 \\ 8 & 8 & 8 & 8 & 6 & 6 & 6 & 0 \end{pmatrix}$$

The expected genetic distance $\tilde{d}$ of two different, randomly chosen species is given by the arithmetic mean of all entries of the distance matrices barring the main diagonal. The resulting formulae are

$$\begin{aligned}
\tilde{d}_{\text{EvenBush}} &= \frac{\frac{1^2\times 2\times n}{2} + \frac{2^2\times 4\times n}{4} + \frac{4^2\times 6\times n}{8} + \ldots + \frac{(n-1)^2\times 2\log_2 n\times n}{n}}{1+2+3+\ldots+(n-1)} \\
&= \frac{\sum_{i=1}^{\log_2 n} \frac{(2^{i-1})^2\times 2i\times n}{2^i}}{\sum_{i=1}^{n-1} i} = \frac{1}{n-1}\sum_{i=1}^{\log_2 n} 2^i i \\
&= \frac{2}{n-1}(1-n+n\log_2 n), \qquad (7.5)
\end{aligned}$$

$$\tilde{d}_{\text{Ladder}} = \frac{2}{n(n-1)}\sum_{i=1}^{n-1} 2i^2 = \frac{4n-2}{3}, \qquad (7.6)$$

$$\begin{aligned}
\tilde{d}_{\text{ShorteningBush}} &= \frac{2}{n(n-1)}\sum_{i=1}^{\log_2 n} \frac{(2^{i-1})^2\times 2(2^i-1)\times n}{2^i} \\
&= \frac{1}{n-1}\sum_{i=1}^{\log_2 n} 2^i(2^i-1) = \frac{4n-2}{3}, \qquad (7.7)
\end{aligned}$$

$$\begin{aligned}
\tilde{d}_{\text{BushLadder}} &= \frac{2}{n(n-1)}\left[\sum_{i=1}^{\log_2 n-1} \frac{(2^{i-1})^2\times 2i\times n/2}{2^i} + \frac{n^3}{4} + \sum_{i=1}^{n/2-2} 2i^2\right] \\
&= \frac{n\log_2 n + n^2}{2(n-1)} + \frac{n-8}{6}. \qquad (7.8)
\end{aligned}$$

Note that $\tilde{d}_{\text{Ladder}} = \tilde{d}_{\text{ShorteningBush}}$, i.e., the average genetic distances are the same in 'Ladder' and 'ShorteningBush'. I will only consider clades with $n = 128$ host species. The resulting average genetic distances $\tilde{d}$ for the four tree types, and also the variances in genetic distances are given in the legend of Figure 7.1.

7.2.3 An analytic approximation without distance-dependence

Mathematically, the model described in the previous section is a Markov chain with 2^n possible states (the infection states of the clade) and, correspondingly, a $2^n \times 2^n$ transition matrix. For any reasonable number n of species, let alone $n = 128$, this Markov chain does not appear to be tractable analytically. In this section, I will derive a simpler model from the full model that does not include the dependency of horizontal transmission probability on genetic distance. This simpler model will then

serve to yield some fundamental insights into the incidence dynamics and will also produce approximations to validate the simulation results of the full model.

As in the full model, n will denote the number of host species in the clade, and η is the probability that an infected species becomes uninfected within one time step. The probability that the endosymbionts are horizontally transmitted from an infected to an uninfected species is given by γ, the value of the transmission probability function g without distance dependence (see Equation (7.1)).

The number of infected species in the clade at a given time t will be denoted by m_t. Within one time step, extinction and horizontal transmission take place. The probability that j previously infected species become uninfected through extinction of the endosymbionts, provided that k species were infected before extinction, is given by

$$P(m_{\text{after extinction}} = k - j | m_t = k) = \binom{k}{j} \eta^j (1-\eta)^{k-j} \tag{7.9}$$

Similarly, the probability that j species become newly infected through horizontal transmission, provided that k species were infected previously, is

$$P(m_{\text{after transmission}} = k + j | m_t = k) = \binom{n-k}{j} \left(1 - (1-\gamma)^k\right)^j \left((1-\gamma)^k\right)^{n-k-j} \tag{7.10}$$

Both distributions are binomial, so that the expected number of species that lose their infection and the expected number of species that become infected (expressed as functions of numbers k of previously infected species) are

$$E_{\text{loss}}(k) = k\eta, \tag{7.11}$$

$$E_{\text{gain}}(k) = \left(1 - (1-\gamma)^k\right)(n-k) \tag{7.12}$$

Taking these expected values as averages, the stochastic model can be transformed into a deterministic one where the recursion equation for the number of infected species is given by

$$\begin{aligned} m_{t+1} &= m_t - E_{\text{loss}}(m_t) + E_{\text{gain}}(m_t) \\ &= m_t - m_t\eta + \left(1 - (1-\gamma)^{m_t}\right)(n - m_t) \end{aligned} \tag{7.13}$$

Since for small values of γ, $(1-\gamma)^{m_t} \approx 1 - \gamma m_t$, the recursion Equation 7.13 can

further be simplified to

$$m_{t+1} \approx m_t(1-\eta) + m_t\gamma(n-m_t) \tag{7.14}$$

It may be worth pointing out that this recursion equation is essentially the same as a simple epidemiological model that describes the dynamics of a horizontally transmitted parasite in a finite population of (constant) size n. It can be seen that the rate of change in incidence consists of a negative component (extinction) and a positive component (transmission) that follows the standard mass-action assumption frequently employed in epidemiological models.

The recursion Equation 7.14 has the two fixed points 0 and

$$m^* = n - \frac{\eta}{\gamma} \tag{7.15}$$

This nontrivial fixed point is obviously always less than n, and it is positive if $\eta < n\gamma$. It can be demonstrated with standard methods that if this condition holds, 0 is always an unstable fixed point, whilst m^* is always stable. Thus, starting from any incidence $0 < m_0 \leq n$, the incidence will converge to the equilibrium incidence m^*. Since $g < \gamma$, m^* can be regarded as an upper limit for the expected infection frequency in the full, stochastic model, as will be demonstrated in the following section.

7.2.4 Simulation results with $\kappa = 1$

In this section, I will explore the incidence dynamics of the full model using computer simulations. I will always assume an exponentially declining function g of horizontal transmission probability in this section (i.e., $\kappa = 1$); other functions will be considered in the following section.

Figure 7.2 shows an example for the incidence dynamics for each of the four trees. I started the simulations with the leftmost species in the phylogeny being infected, whilst all other species are uninfected (note, this point of initial infection gives the maximum probability of infection establishment in each tree, so represents the best case scenario). With the chosen set of parameters, the infection spreads very quickly in the 'EvenBush' tree, leading to a state where on average all or almost all species are infected (bold line in Figure 7.2a). The distribution centre increases even more quickly from 0 (when only the first species is infected) to 0.5, showing that even

when only about 50% of the species are infected, the infected species are evenly spread within the clade. The values of the distance coefficient are found to be always close to 1, which means that the infected species are on average as closely related to each other as any randomly chosen species pair. Note that the distribution coefficient can also take values higher than one, although this is not to be expected on average.

Incidence dynamics in the 'EvenBush' tree contrast with dynamics in the 'Ladder' tree (Figure 7.2b). In the 'Ladder' tree, the infection spreads more slowly and does not reach a high frequency. The low values of the distribution centre show that the majority of infected species can be found to the left side of the tree. This can readily be explained by the topology of 'Ladder': since the genetic distance of a species i to any of the species $j < i$ (to the left in the tree) increases with i and is even larger to a species $j > i$, the probability of a species to become infected decreases the more to the right of the tree that species sits. In the 'ShorteningBush' tree, a stepwise pattern

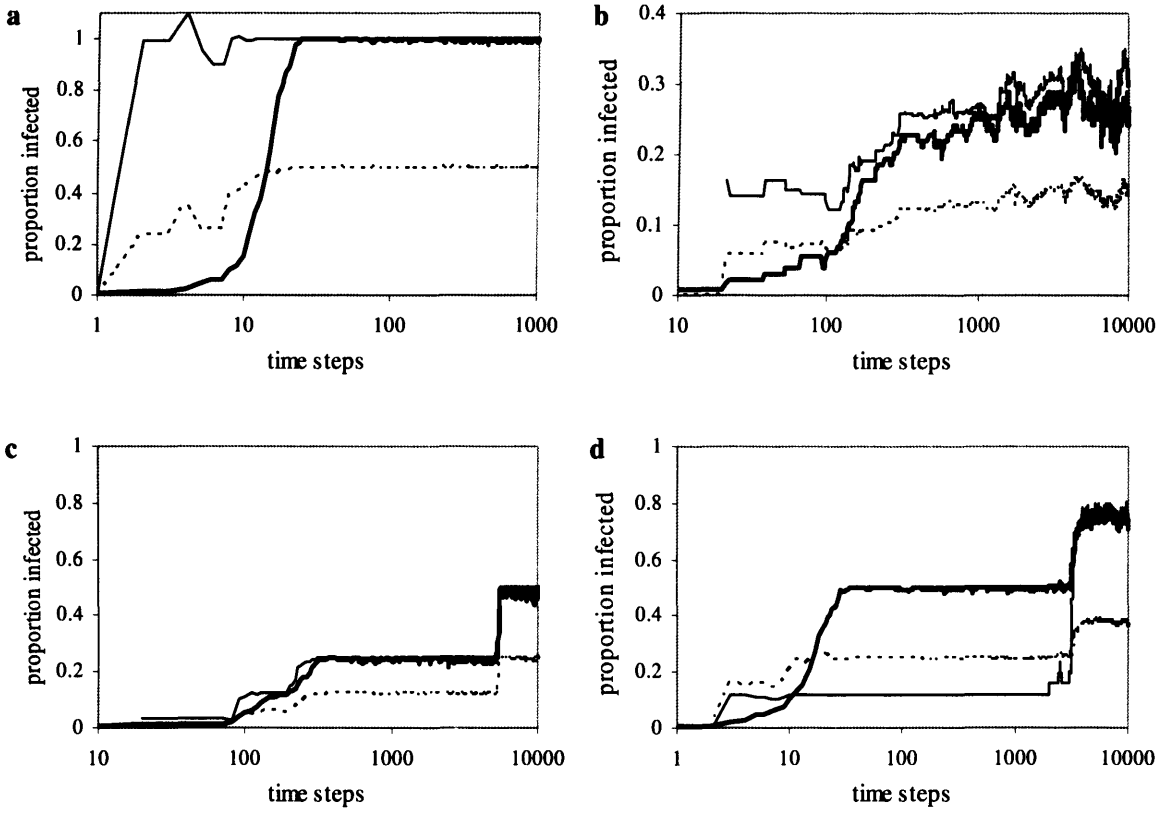

Figure 7.2: Examples for the infection dynamics over time in the basic model. The plots show the fractions of species that are infected at each time step (bold lines), the distribution centres (dotted lines) and the distance coefficients (solid lines) for the tree types (a) 'EvenBush', (b) 'Ladder', (c) 'ShorteningBush' and (d) 'BushLadder'. Parameters take the values $\gamma = 0.01$, $\beta = 8$ and $\eta = 0.001$. Note the different scale on the y-axis of plot (b).

of spread can be observed (Figure 7.2c). The course of the distance coefficient shows that the infection spreads first in the first left quarter of the tree, and after about 5000 time steps swaps over into the second quarter, where it spreads almost to fixation within a few hundred time steps. However, even after 100,000 time steps (only the first 10,000 are shown), the infection did not get into the second half of the tree.

Finally, in the 'BushLadder' tree the infection spreads initially as in the 'EvenBush' tree very quickly, until the first, symmetric half of the tree is almost completely infected (Figure 7.2d). Then, after about 3000 time steps, the infection also spreads in the second, asymmetric half of the tree. This lag in the infection of the ladder-like half of the tree can be explained by the fact that in order to establish an infection in this part of the tree, its first few species must be infected. Since all the species in the asymmetric half of the tree are equally distant from any species in the symmetric half, many attempts to colonize the second half fail because the first species that become infected in the asymmetric half are too distantly related to other species in this subtree and therefore prove bad candidates for further promoting the incidence.

In a more systematic set of simulations I explored how the steepness parameter β of the transmission probability function affects the incidence dynamics (Figure 7.3). For each value of β I performed 1000 simulations with 10,000 time steps each. In each simulation, initially species 1 (leftmost in Figure 7.1) was infected, whereas all other species were uninfected. As can be expected, the number of species that become infected increases and the proportion of simulations where the endosymbionts become extinct decreases with increasing β (Figure 7.3a and 7.3b). Comparing the results for the different tree types, it can be seen that the endosymbionts attain highest incidence in the 'EvenBush' and 'BushLadder' tree, whereas in 'Ladder' and 'ShorteningBush' they can spread to substantial incidence only where genetic distance is relatively unimportant for establishment (high β).

Figure 7.3c shows that for 'EvenBush' the distribution centre takes values close to 0.5, implying that infections are evenly spread across the tree, even for low overall infection frequencies. By contrast, for the other tree types the infections tend to be concentrated on the left side of the tree unless β becomes considerably high. In case of the completely symmetrical 'ShorteningBush' this is because in most simulations an equilibrium has not been reached: since the genetic distance between branches

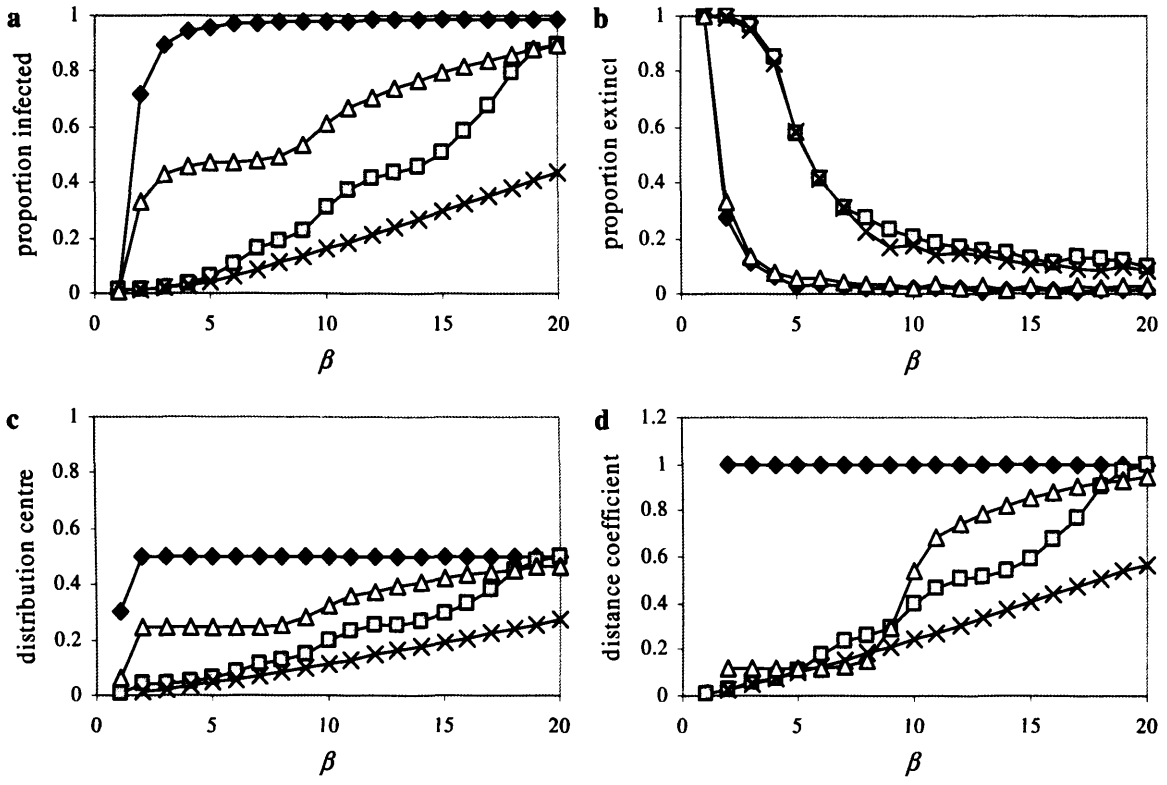

Figure 7.3: Simulation results for the infection dynamics of the basic model. For different values of the parameter β, 1000 simulations were run for each of the four tree types 'EvenBush' (black diamonds), 'Ladder' (crosses), 'ShorteningBush' (grey squares) and 'BushLadder' (empty triangles). Per simulation, 10,000 time steps were run. The plots show (a) the fraction of species that are infected (averaging over all simulations where the endosymbionts did not go extinct), (b) the fraction of simulations where the endosymbionts became extinct in the clade, (c) the 'distribution centre', and (d) the 'distance coefficient'. Other parameters are fixed at $\gamma = 0.001$ and $\eta = 0.001$.

of the tree increases exponentially with increasing branch size in this tree, the endosymbionts often did not manage to establish in the second half of the tree (or the second quarter, second eighth and so on) during the 10,000 time steps simulated. In case of 'Ladder', low values of the distribution centre do not seem to be due to such effects, but to the increasing isolation of species to the right of the tree. Thus, these species are increasingly unlikely to become infected, whereas the probability of losing an infection remains constant. Finally, in 'BushLadder', we observe that the distribution centre remains constant at about 0.25 over a range of small values of β, after which it increases. This shows that for low values of β, the infection is evenly spread in the symmetric part of that tree, but is not established in the asymmetric half, and colonization of the asymmetric half of the tree is likely only where genetic distance is relatively unimportant for establishment (high values of β).

The distance coefficient was found to be always less than 1 or very close to 1, meaning that infected species are never less strongly related than randomly chosen species pairs (Figure 7.3d). In the case of 'EvenBush', the distance coefficient is always close to one, which shows that when uninfected species occur, these do not tend to form closely related clusters, but are rather widely spread in the tree because the infection can spread easily to all members of the clade. The low distance coefficients for 'BushLadder' found for small values of β can again be explained by the fact that the infection spreads only in the symmetric part of this tree. (Note that two species from the symmetric part of the tree are on average much more closely related than two species drawn randomly from the whole tree.) The low distance coefficients found for 'ShorteningBush' and 'Ladder' are readily explained by the same reasoning as for the distribution centre.

For increasing values of β, the function for the probability of horizontal transmission g becomes increasingly flat and converges to the baseline probability γ. Thus, for increasing β we would expect the impact of host phylogeny on endosymbiont incidence to decrease and, at the limit $\beta \to \infty$, vanish. This expectation was confirmed in another set of simulations, as shown in Figure 7.4. For these simulations, I chose a much smaller baseline probability γ, and initiated the simulations with all species infected. Clearly, with increasing β the average proportion of infected species converges to the same value for all trees (Figure 7.4a). Likewise, the distribution centre was found to converge to 0.5 for all trees (Figure 7.4b), indicating that host phylogeny

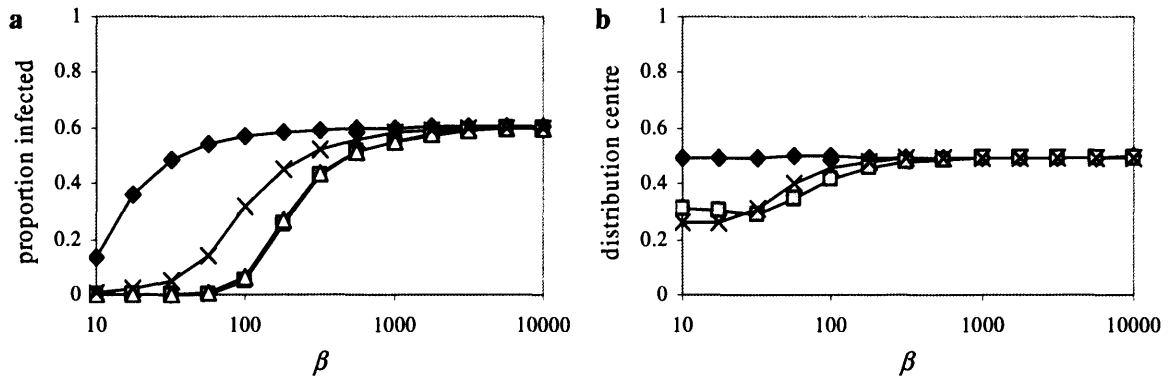

Figure 7.4: Average proportion of infected species for $\gamma = 0.00002$ and $\eta = 0.001$. Each datum represents the mean of 1000 simulations, each of which was initiated with all species being infected and run for 10,000 time steps. For the four tree types, the same symbols as in the previous figures are used.

also becomes unimportant for the average distribution of the endosymbionts within the tree of host species.

For very high values of β, i.e., low impact of host phylogeny, the simulation results should be approximately the same as predicted by the analytic model without distance dependence. For the parameters used in Figure 7.4, Equation 7.15 yields the fixed point $m^* = 78.0$. This corresponds to an incidence of about 61%, a value that is very close to the simulation results for $\beta = 10,000$. Further simulations (not shown) with $\beta = 10,000$, $\eta = 0.001$ and a range of different values for γ have also confirmed that the analytic model produces very good approximations when horizontal transmission does not depend on genetic distance between donor and recipient host species.

7.2.5 Simulation results with $\kappa > 1$

To what extent do the above results depend on the shape of the function g relating the probability of horizontal transmission to genetic distance? To investigate this question, I ran simulations with $\kappa > 1$, which gives a sigmoidally declining function g (see Equation 7.1).

Figure 7.5 shows how this influences the average incidence and overall extinction rates within host clades in the four trees. As can be seen, when progressing from $\kappa = 1$ (Figure 7.3a) to $\kappa = 2$ to $\kappa = 5$ (Figures 7.5a and c), higher values of β are required to lead to similar average incidence for all trees. Thus, although $\kappa > 1$ entails higher transmission probabilities over short genetic distances, overall spread in the trees is increasingly impeded by the strong decrease in the probability of horizontal transmission over large genetic distances. Note that with increasing exponent κ, transmission becomes increasingly restricted to genetic distances lower than β. This effect can be seen in Figure 7.5c, where for small values of β the average incidences in the 'EvenBush' and 'BushLadder' trees roughly correspond to almost complete infection in the leftmost subclade, where the maximum genetic distance between species is less than β, whilst all other species are uninfected. This interpretation is corroborated by the distribution centres obtained in the respective simulations (not shown).

With increasing κ, again higher values of β are required for the overall extinction rates to drop to a given value (Figures 7.5b and d), which appears to be a natural consequence of the low incidences found for low values of β.

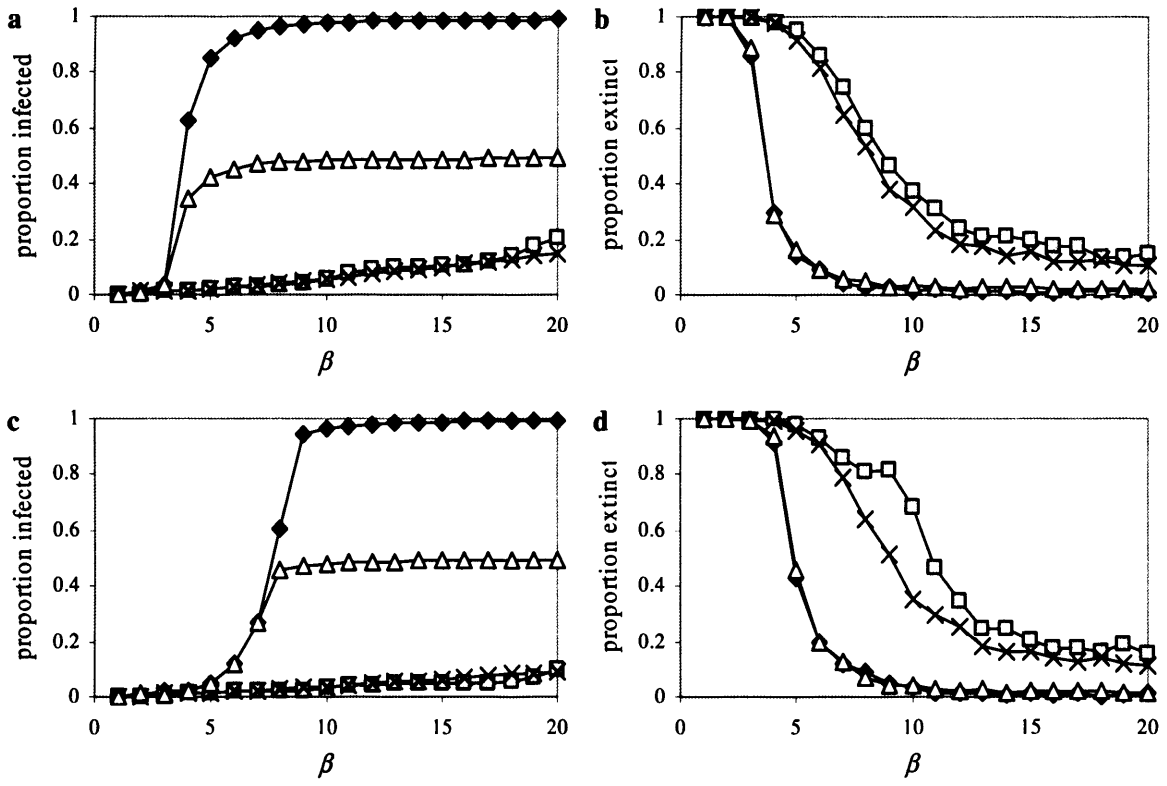

Figure 7.5: Average proportion of species infected and overall extinction rates with sigmoidal functions g relating horizontal transmission probability to genetic distance, (a,b) $\kappa = 2$ and (c,d) $\kappa = 5$. 1000 simulations were run for each of the four tree types 'EvenBush' (black diamonds), 'Ladder' (crosses), 'ShorteningBush' (grey squares) and 'BushLadder' (empty triangles). Per simulation, 10,000 time steps were run. Other parameters and starting conditions are as in Figure 7.3.

7.3 Models with refractoriness

In this section, I will study extensions of the basic model in which host species may be or become refractory to infection with the endosymbionts. In the first extension, refractoriness of species will be fixed, whereas in the second extension, species can become refractory in the process of losing the endosymbiont infection and stay refractory for some time. Throughout, I will use the same four tree types as in the basic model, with 128 species in each tree, and assume $\kappa = 1$, i.e. an exponentially declining function g for the probability of horizontal transmission.

7.3.1 Fixed refractoriness

First, I will assume that not all species in the tree can be infected by the endosymbionts. This can be interpreted either as a situation where some species are refractory or unsuitable for the endosymbionts, or as subtraction of species from the tree (and a resulting altered host phylogeny). I assume that the refractory species are randomly distributed within the clade, and in the simulations I randomly chose one of

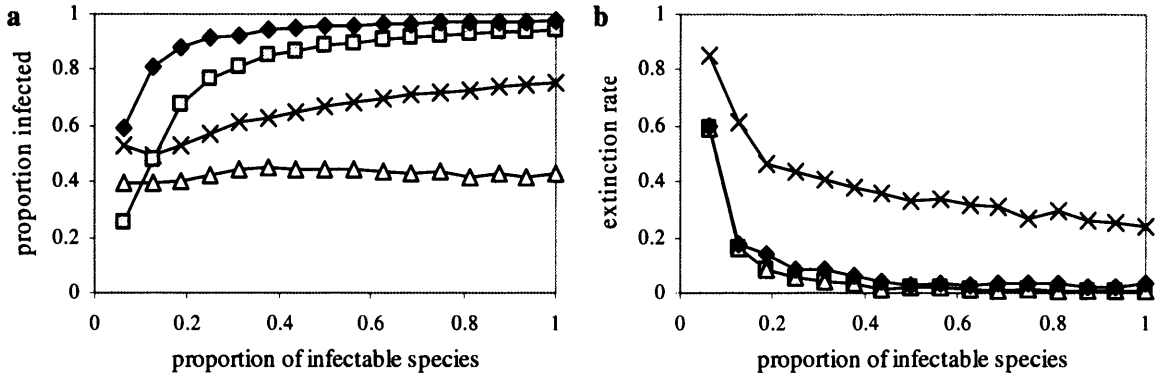

Figure 7.6: Simulation results for the extension of the model with fixed refractoriness. For each simulation, a fixed number of randomly chosen species (out of the 128 species in the clade) were assigned refractory status, whilst the other species were assigned susceptible status. Plot (a) shows the mean proportion of infected species within the susceptible class, averaged over the simulations were the endosymbionts did not go extinct. Plot (b) shows the fraction of simulations were the endosymbionts went extinct. 1000 simulations were run for each of the four tree types 'EvenBush' (black diamonds), 'Ladder' (crosses), 'ShorteningBush' (grey squares) and 'BushLadder' (empty triangles). Per simulation, 10,000 time steps were run. Parameters take the values $\gamma = \eta = 0.001$, $\beta = 8$ for the 'EvenBush' and 'BushLadder' trees, and $\gamma = 0.01$, $\eta = 0.001$, $\beta = 20$ for the 'Ladder' and 'ShorteningBush' trees.

the susceptible species to be infected at the beginning of each simulation.

Figure 7.6 shows how the average proportion of infected species and the proportion of simulations with complete extinction depend on the number of susceptible species in the clade. In the two symmetric trees, the fraction of infected species among all non-refractory species remains more or less constant when many species remain susceptible. However, as the number of susceptible species decreases, the fraction of species infected shows an accelerating rate of decline (Figure 7.6a). By contrast, in the 'Ladder' tree, the average incidence among all susceptible species decreases more linearly as species are removed. Finally, in the 'BushLadder' tree, the average incidence remains constant with decreasing number of susceptible species. This may be explained by the observation that the infection can spread in the bush-like part of the tree only for the parameters chosen (see Figure 7.3). Therefore, on average only half of the non-susceptible species will be of importance for impeding the spread of the endosymbionts.

It can also be seen that in all tree types, the proportion of simulations where the endosymbionts went extinct increases only moderately with decreasing number of susceptible species as long as this number is above a certain value. However, below this value, the extinction rate increases strongly (Figure 7.6b). This suggests that clades which consist of very few susceptible species only are unlikely to maintain a stable infection without frequent horizontal transmission from other clades.

In summary, these results suggest that my observations in the previous sections are robust to small changes in the number of susceptible species, including small deviations in the tree topology. However, when the clade consists of few species only, we expect both decreased average incidence and an increased probability of overall endosymbiont extinction compared to larger clades. Where host clades are of similar age, but differ in the number of species present, I expect the fraction of species infected to be lower in the species-poor than in the species-rich clade.

7.3.2 Acquired refractoriness

Loss of parasitic endosymbionts may be the result of selection in the hosts against them. In a second extension of my model with regards to refractoriness, I will therefore assume that all losses of endosymbionts are due to the host species becoming

refractory. Refractory species cannot become infected, but they are assumed to lose their refractoriness with probability λ in each time step due to neutral evolution or selection against costly resistance alleles.

As in the basic model, I first tried to understand some elementary properties of this extension by looking directly at examples of the dynamics (Figure 7.7). As can be seen in all plots, an initial increase in the incidence of the endosymbionts is followed by a decrease due to the accumulation of refractory species. In 'ShorteningBush', we can again observe a stepwise proceeding of the endosymbionts into a new branch of the tree. However, even after 100,000 time steps only the first half of the tree is infected in this tree type.

Figure 7.8 shows the results of an exploration of the parameter space with respect to λ, the probability that a refractory species becomes susceptible again. In

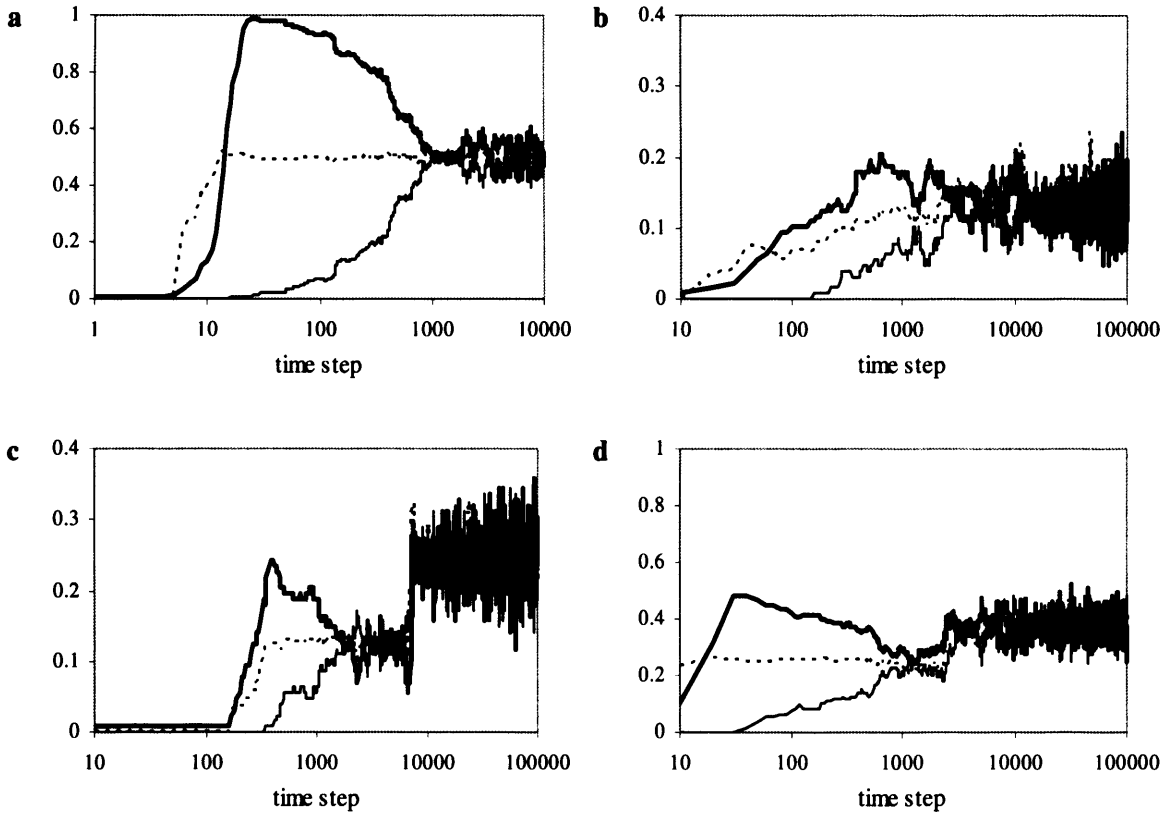

Figure 7.7: Examples for the infection dynamics over time in the model with acquired refractoriness in the tree types 'EvenBush' (a), 'Ladder' (b), 'ShorteningBush' (c) and 'BushLadder'(d). Each simulation was initiated with all species being susceptible, and only species 1 being infected. Shown are the fractions of infected species (bold lines), the fractions of refractory species (solid lines) and the distribution centre of the infected species (dotted lines). Parameters take the values $\gamma = 0.01$, $\eta = \lambda = 0.001$, $\beta = 8$.

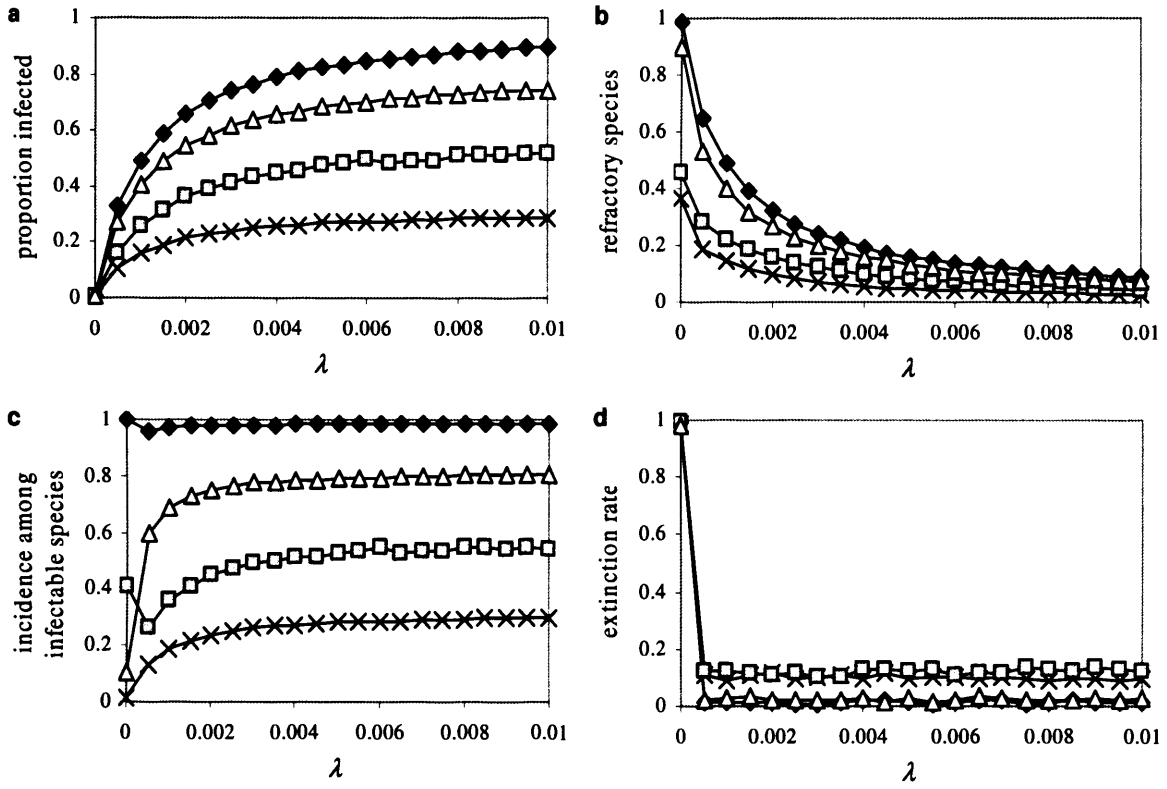

Figure 7.8: Simulation results of the model with acquired refractoriness in the tree types 'Even-Bush' (black diamonds), 'Ladder' (crosses), 'ShorteningBush' (grey squares) and 'BushLadder' (empty triangles). Each data point represents an average of 1000 simulations, each of which was run for 10,000 time steps. Parameters take the values $\gamma = \eta = 0.001$, $\beta = 16$.

all tree types the average infection frequency of the clade increases with increasing λ, approaching the values obtained in the model without refractoriness (see Figure 7.3a at $\beta = 16$). As expected, when $\lambda = 0$, the endosymbionts went extinct within the 10,000 time steps simulated in the great majority of simulations because a high proportion of species become (and remain) refractory. In 'EvenBush' and 'BushLadder', this happened only after most of the species had become refractory, while in 'Ladder' and 'ShorteningBush', extinction on average occurred when only less than half of the species had become refractory.

We can further observe that in all tree types, the proportion of simulations where the endosymbionts went extinct drops strongly when the rate of return to susceptibility to infection λ is slightly increased above 0 and remains constant when λ is further increased such that susceptibility is restored rapidly. It appears that apart from a strong effect when refractoriness is persistent (low λ), the probability of loosing refractoriness has no impact on how likely extinction of the endosymbionts is.

7.4 Model with two endosymbionts

7.4.1 Model description

To date, I have assumed that the host clade is infected by one strain or species of endosymbionts only. In this section, I will analyse a second extension of the basic model that includes two endosymbiont strains. I assume that one strain of endosymbionts induces cytoplasmic incompatibility (CI), and the other strain male-killing (MK). Whilst the CI-inducing endosymbionts can infect all species in the clade, the MK-inducing endosymbionts may only be able to infect a subset of species. This is motivated by the observation that certain ecological properties of host species like gregarious broods and antagonistic sibling interactions are usually required for male-killer infections to be stably maintained [174].

It is well known that species can be multiply infected with different endosymbionts. For example, multiple infections with up to five different strains of *Wolbachia* are commonly observed in arthropods [425, 424, 217, 316]. Multiple infections involving different species of endosymbionts are also common [410, 445, 128, 129]. Different populations of a host species may be infected with CI- and MK-inducing endosymbionts, and theory predicts that these two types of reproductive parasites may also co-occur within a single host population [120, 101].[2]

Thus, apart from uninfected species, species infected with CI-inducing and species infected with MK-inducing endosymbionts, I will also allow species to be infected with both endosymbionts. I assume that extinction events of the two endosymbiont strains in a doubly infected host species occurs independently and with the same probability as in singly infected species. The probability of horizontal transmission from a species infected with one of the two strains to a new host species is assumed not to depend on whether the native species is also infected with the respective other strain. However, I assume that the probability of horizontal transmission of the MK-inducing endosym-

[2]More precisely, coexistence is possible if doubly infected individuals occur within the population. Invasion of MK-inducing endosymbionts in singly infected hosts into a CI-infected population is virtually impossible, but invasion can occur readily when the MK-inducing endosymbionts invade as a co-infection with CI-inducing endosymbionts. Conversely, CI-inducing endosymbionts can invade a MK-infected population both as a single and as a double infection, but the MK-inducing endosymbionts may impede invasion compared to an uninfected population [101].

biont will be reduced by a factor ζ_{CI} when the new host species is infected with the CI-inducing endosymbionts. Conversely, the probability of horizontal transmission of CI-inducing endosymbionts is reduced by a factor ζ_{MK} when the new host species is infected with MK-inducing endosymbionts. For example, if $\zeta_{CI} = \zeta_{MK} = 0$, each endosymbiont strain prevents infection of the species with the respective other strain, and no doubly infected species will arise. On the other extreme, if $\zeta_{CI} = \zeta_{MK} = 1$, the probabilities of horizontal transmission are independent of the infection state of the recipient species. Successful horizontal transmission of a strain to a species that is already infected by the other strain will always lead to a doubly infected species, i.e., the new strain will never drive the other one extinct.

To keep the model simple, I will assume that — barring interactions with the respective other strain — the two endosymbionts have the same extinction rate and horizontal transmission properties. Even under this premise, the model as described above has two additional parameters (ζ_{CI} and ζ_{MK}) and an additional structure on the host clade determining which species are susceptible to male-killer infection, making the parameter space rather cumbersome. I will therefore focus on only a few interesting aspects and will not attempt to analyse the entire parameter space in detail. In particular, I will only consider the 'EvenBush' tree and the exponentially declining probability function g, i.e., $\kappa = 1$.

7.4.2 Monophyletic susceptibility to male-killer infection

In this section I will assume that during the evolution of the host clade, the susceptibility to infection with the MK-inducing endosymbionts either arose or was lost only once. In other words, all host species that are susceptible or all host species that are not susceptible to MK infection form a monophyletic clade. Barring equivalencies, there are 14 different constellations of susceptibilities in the 'EvenBush' tree, namely when the first 1, 2, 4, 8, 16, 32, 64, 96, 112, 120, 124, 126, 127 and 128 species are susceptible to male-killer infections.

I have run simulations for these different susceptibility constellations and different invading abilities ζ_{CI} and ζ_{MK} of the two endosymbionts into host species infected with the respective other strain (Figures 7.9 and 7.10). In all these simulations, I started with species 1 being infected with the male-killing endosymbionts, and with species

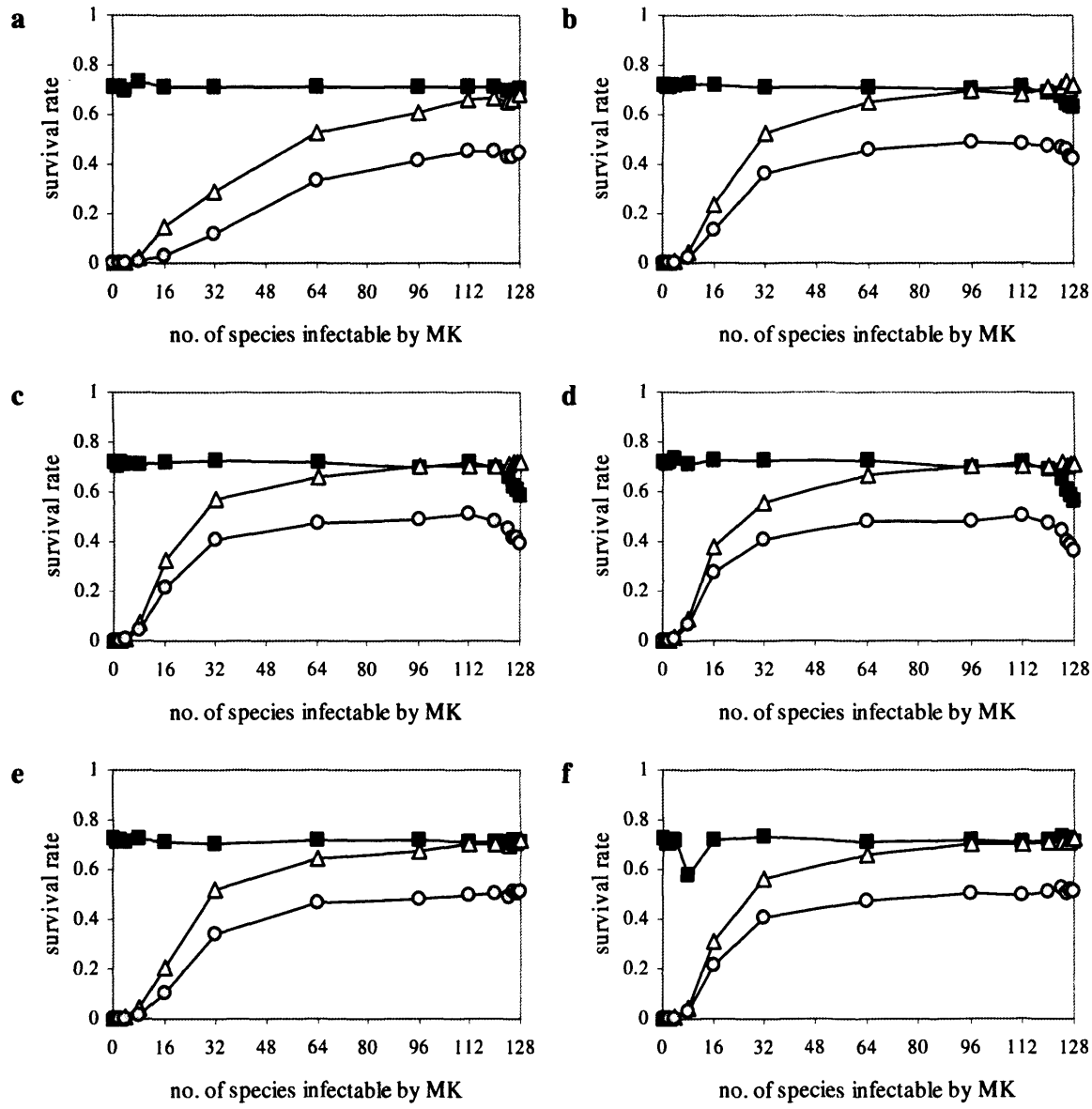

Figure 7.9: Overall survival rates of endosymbionts in host clades where all species susceptible or refractory to male-killer infection form a monophyletic subclade. Black squares show survival rates for the CI-endosymbionts, white triangles survival rates of the male-killers, and grey circles the fraction of simulations where both endosymbionts survive. Each datum is an average of 5000 simulations. Each simulation was initiated with species 1 and 128 being infected with CI- and MK-inducing endosymbionts, respectively, and run for 10,000 time steps. The underlying tree type is 'EvenBush' for all plots, and parameters take the values $\gamma = \eta = 0.001$, $\beta = 2$, and (a) $\zeta_{CI} = \zeta_{MK} = 0$, (b) $\zeta_{CI} = 0.5$ and $\zeta_{MK} = 0$, (c) $\zeta_{CI} = 0.8$ and $\zeta_{MK} = 0$, (c) $\zeta_{CI} = 1.0$ and $\zeta_{MK} = 0$, (e) $\zeta_{CI} = \zeta_{MK} = 0.5$, (f) $\zeta_{CI} = \zeta_{MK} = 1.0$.

128 infected with the CI-inducing endosymbionts. Note that this starting condition is the optimal one for the spread of both endosymbionts, as early 'encounters' are least likely.

Figure 7.9 shows the overall survival rates of the two endosymbionts (i.e., the fraction of simulations where the endosymbionts did not go extinct within the entire clade of host species). Also shown is the fraction of simulations where both endosymbionts survived. Not surprisingly, the survival rate of the MK-inducing endosymbiont increases with increasing number of susceptible host species. Analogously to the results of the previous extension of the model (Section 7.3.1), a minimum number of susceptible host species is required to lead to survival rates substantially above zero.

Comparing different invasion abilities ζ_{CI} and ζ_{MK}, it can be seen that survival rates of the MK-inducing endosymbionts are lowest and increase most slowly with increasing number of susceptible species when $\zeta_{CI} = \zeta_{MK} = 0$, i.e., when neither strain can invade a species infected with the other strain. The highest survival rates were found in the case where the MK-inducing endosymbionts can invade CI-infected species with the same probability as uninfected species ($\zeta_{CI} = 1$), but when the CI-inducing endosymbionts cannot invade male-killer infected species ($\zeta_{MK} = 0$). At least when the MK-inducing symbionts reach a high prevalence within a population, this may well be the most realistic scenario [101]. It appears that the survival rate of the male-killing inducing endosymbionts not only increases with increasing invasion ability into CI-infected host species, but also with decreasing invasion ability of CI-inducing endosymbionts into male-killer infected species.

The survival rate of the CI-inducing endosymbionts was found in general not to depend very much on the number of host species that are susceptible to male-killer infection. This is especially true with symmetric invasion abilities of both endosymbionts (Figure 7.9a, e and f). However, when the CI-inducing endosymbionts cannot invade species that are already infected with male-killing inducing endosymbionts (Figure 7.9b-d) and when all or almost all species are susceptible to male-killer infections, the survival rates of the CI-inducing endosymbionts are slightly reduced. This phenomenon is readily explained by the low average incidences of the CI-inducing endosymbionts in these cases (see below).

The average infection frequencies of the two endosymbionts are shown in Figure 7.10. Not surprisingly, the incidence of the male-killing inducing endosymbionts always increases with increasing number of susceptible species. The higher the invasion ability into species infected with CI-inducing endosymbionts, the stronger is this

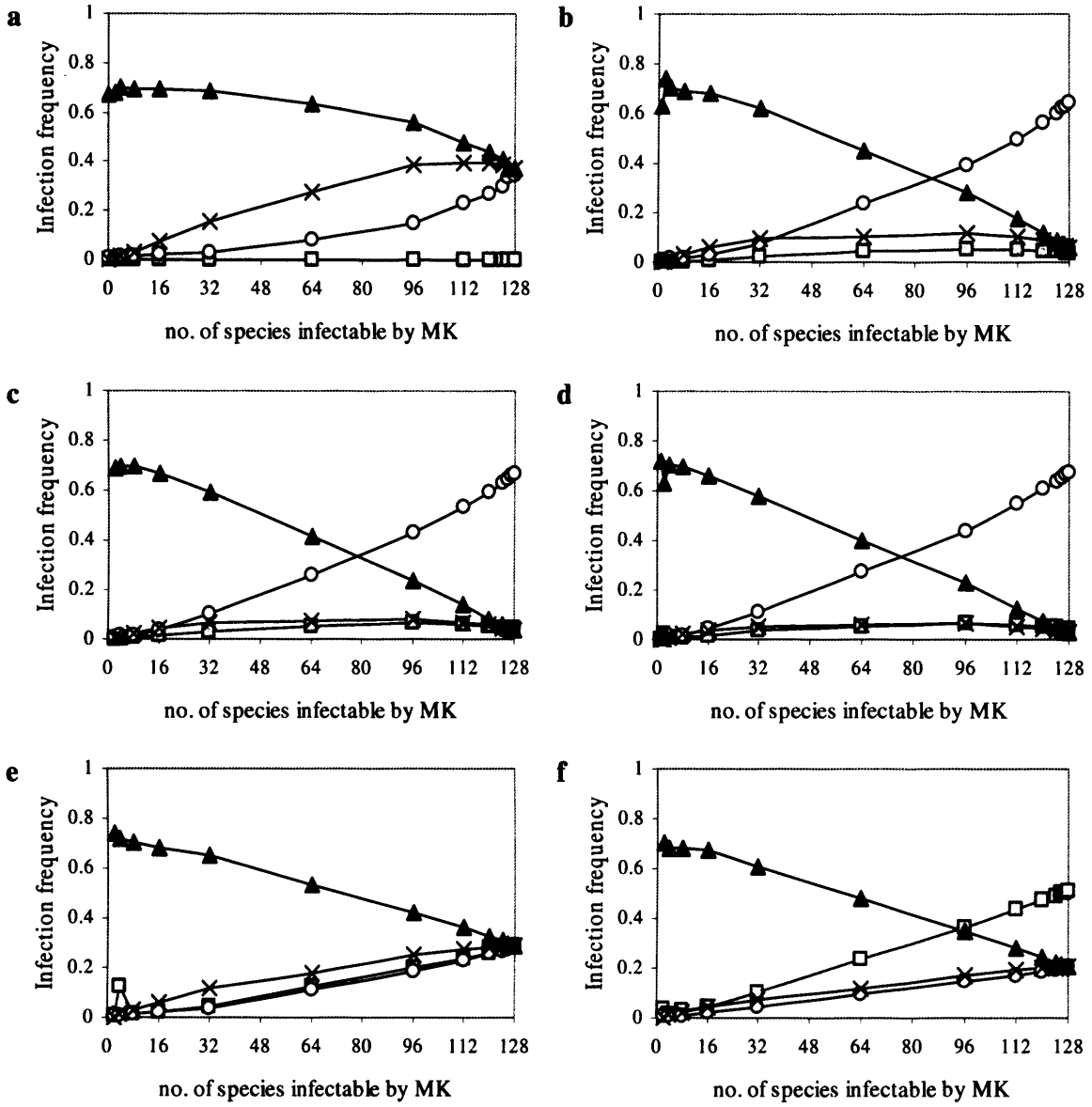

Figure 7.10: Average infection frequencies of endosymbionts in host clades where all species susceptible or refractory to male-killer infection form a monophyletic subclade. Shown are average incidences of the CI-inducing endosymbionts (black triangles), MK-inducing endosymbionts (white circles), doubly infected species (grey squares), and CI-inducing endosymbionts among all species susceptible to MK-infection (crosses). Each datum is an average of 5000 simulations. Each simulation was initiated with species 1 and 128 being infected with CI- and MK-inducing endosymbionts, respectively, and run for 10,000 time steps. The underlying tree type is 'EvenBush' for all plots, and parameters take the values $\gamma = \eta = 0.001$, $\beta = 2$, and (a) $\zeta_{CI} = \zeta_{MK} = 0$, (b) $\zeta_{CI} = 0.5$ and $\zeta_{MK} = 0$, (c) $\zeta_{CI} = 0.8$ and $\zeta_{MK} = 0$, (c) $\zeta_{CI} = 1.0$ and $\zeta_{MK} = 0$, (e) $\zeta_{CI} = \zeta_{MK} = 0.5$, (f) $\zeta_{CI} = \zeta_{MK} = 1.0$.

increase. Interestingly, when the CI-inducing endosymbionts cannot invade species infected with male-killers (plots 7.10b-d), the incidence of the latter depends hardly upon the invasion ability ζ_{CI} into species infected with CI-inducing endosymbionts.

The incidence of the CI-inducing endosymbionts was found to decrease with increasing number of species susceptible to male-killer infection. This decline is strongest when the male-killing endosymbionts can invade species infected with the CI-inducing endosymbionts, but not *vice versa* (plots 7.10b-d). In this case, the MK-inducing endosymbionts have a higher overall rate of horizontal transmission (including invasion into species infected with the CI-inducing endosymbionts), whilst extinction rates are equal. As a consequence, if this advantage is not offset by a substantially lower number of susceptible species, the CI-inducing endosymbionts can be expected to be constrained to low incidences. With symmetric invasion abilities (plots 7.10a, e, and f), the incidences of the two endosymbionts converge. High frequencies of doubly infected species are only seen when both endosymbionts can invade species infected by the respective other endosymbiont. The reason for this observation is that with asymmetric invasion abilities, the CI-inducing endosymbionts will be strongly restricted in their spread and achieve low overall infection frequencies only.

7.4.3 Random susceptibility to male-killer infection

In this section I will assume that the host species that are susceptible to male-killer infection are randomly distributed over the clade of host species. This can be interpreted such that gain and loss of susceptibility to male-killer infection occur frequently during the evolution of the host clade.

Aside from this assumption of random susceptibility, the simulations I performed are the same as the ones in the previous section. Figure 7.11 shows the overall survival rates of the two endosymbionts, and 7.12 shows their average incidence. The plots are very similar to the ones for monophyletic susceptibility, and the same interpretations as given in the previous section apply to most of the patterns that can be observed. Thus, in what follows I will focus on the differences in these plots.

With respect to overall survival rates of the two endosymbionts (Figure 7.11), the crucial difference to the 'monophyletic susceptibility' scenario is that a substantially higher number of susceptible species is needed to ensure survival of the male-killing inducing endosymbionts. The reason for this is that the spread of the male-killing inducing endosymbionts is encumbered when the susceptible species do not form one closely related cluster. By contrast, the survival rates of the CI-inducing endosym-

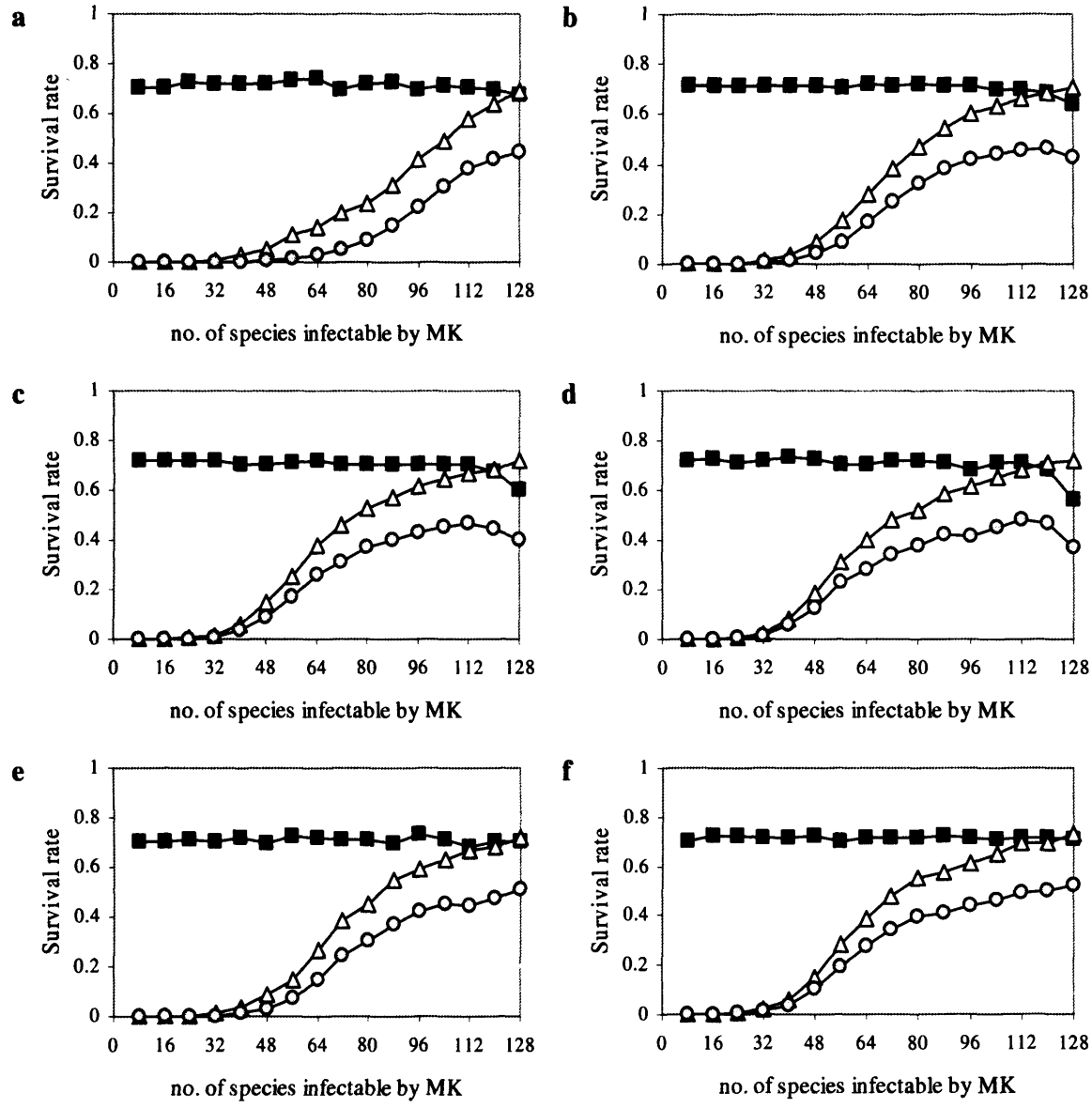

Figure 7.11: Overall survival rates of endosymbionts in host clades where species susceptible to male-killer infection were randomly chosen. Black squares show survival rates for the CI-endosymbionts, white triangles survival rates of the male-killers, and grey circles the fraction of simulations where both endosymbionts survive. Each datum is an average of 5000 simulations. Each simulation was initiated with species 1 and 128 being infected with CI- and MK-inducing endosymbionts, respectively, and run for 10,000 time steps. The underlying tree type is 'EvenBush' for all plots, and parameters take the values $\gamma = \eta = 0.001$, $\beta = 2$, and (a) $\zeta_{CI} = \zeta_{MK} = 0$, (b) $\zeta_{CI} = 0.5$ and $\zeta_{MK} = 0$, (c) $\zeta_{CI} = 0.8$ and $\zeta_{MK} = 0$, (c) $\zeta_{CI} = 1.0$ and $\zeta_{MK} = 0$, (e) $\zeta_{CI} = \zeta_{MK} = 0.5$, (f) $\zeta_{CI} = \zeta_{MK} = 1.0$.

bionts are not affected by whether the species susceptible to male-killer infection form a monophyletic group or are randomly distributed.

The problems that the MK-inducing endosymbionts face when the susceptible host

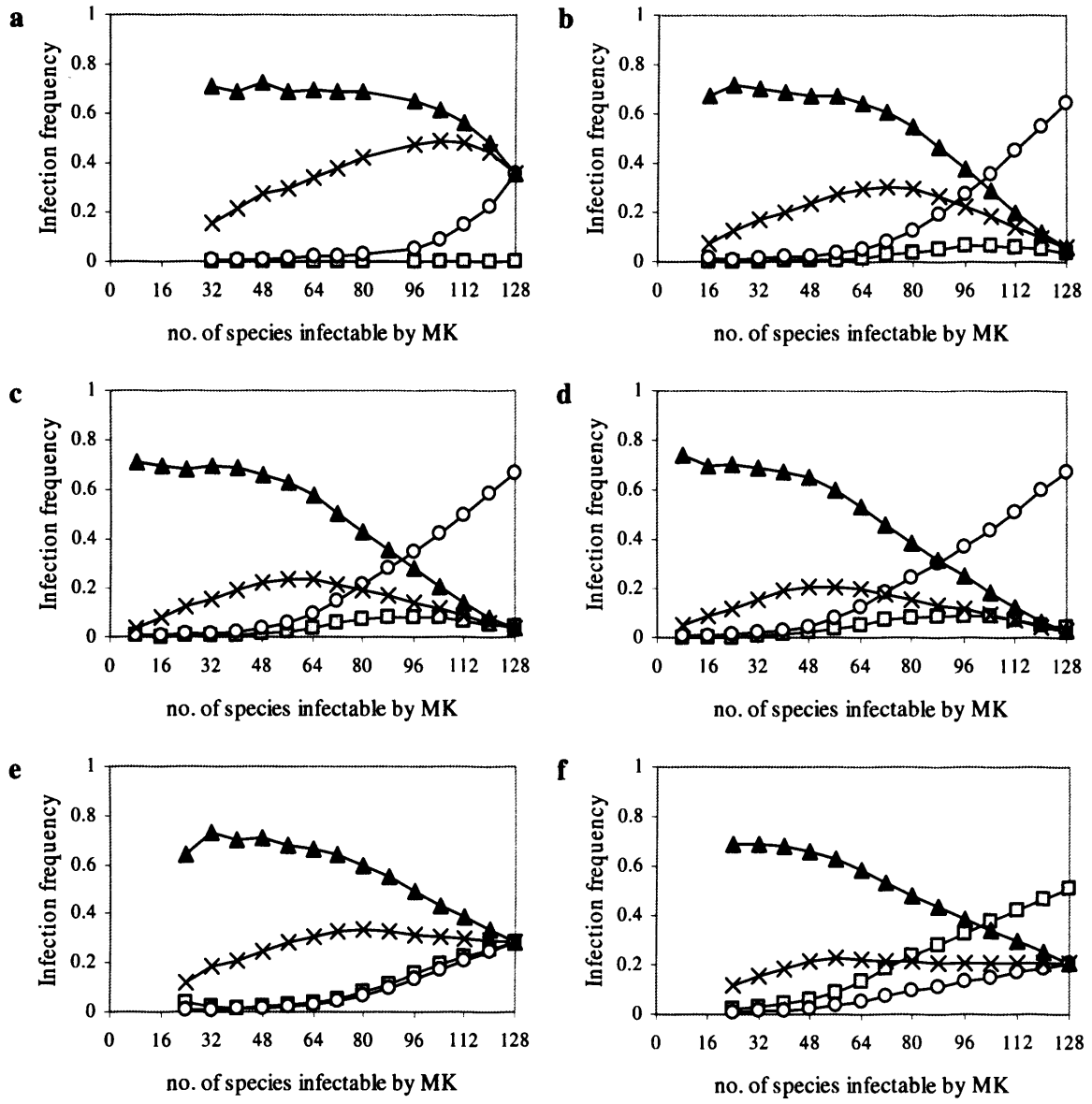

Figure 7.12: Average infection frequencies of endosymbionts in host clades where species susceptible to male-killer infection were randomly chosen. Shown are average incidences of the CI-inducing endosymbionts (black triangles), MK-inducing endosymbionts (white circles), doubly infected species (grey squares), and CI-inducing endosymbionts among all species susceptible to MK-infection (crosses). Each datum is an average of 5000 simulations. Each simulation was initiated with species 1 and 128 being infected with CI- and MK-inducing endosymbionts, respectively, and run for 10,000 time steps. The underlying tree type is 'EvenBush' for all plots, and parameters take the values $\gamma = \eta = 0.001$, $\beta = 2$, and (a) $\zeta_{CI} = \zeta_{MK} = 0$, (b) $\zeta_{CI} = 0.5$ and $\zeta_{MK} = 0$, (c) $\zeta_{CI} = 0.8$ and $\zeta_{MK} = 0$, (c) $\zeta_{CI} = 1.0$ and $\zeta_{MK} = 0$, (e) $\zeta_{CI} = \zeta_{MK} = 0.5$, (f) $\zeta_{CI} = \zeta_{MK} = 1.0$.

species do not form a monophyletic cluster can also be seen in the average incidences (Figure 7.12). The increase in average incidence of the male-killing endosymbionts is very weak when fewer than about one half of the species are susceptible to male-killer

infections. As a consequence of the low male-killer infection frequencies, the incidence of the CI-inducing endosymbionts is generally higher than in the 'monophyletic susceptibility' scenario, especially for low number of species susceptible to male-killer infection. Moreover, the effect that the incidence of the CI-inducing endosymbionts among the species susceptible to male-killer infection is highest for intermediate numbers of susceptible species is much more pronounced. The average number of doubly infected species is not substantially altered with random susceptibility.

In summary, stable coexistence of the two strains is possible if (1) both strains are symmetric in their invasion abilities, or (2) reduced invasion ability of the CI-inducing strain is offset by a reduced number of species susceptible to the male-killing inducing strain. High numbers of doubly infected species are only expected if both endosymbiont strains can invade species already infected with the other strain. Monophyletic susceptibility favours the spread of the male-killing inducing endosymbionts compared to randomly distributed susceptible species, and as a consequence, the average incidence of the CI-inducing endosymbionts is reduced in the former compared to the latter scenario.

7.5 Discussion

7.5.1 Assumptions of the model

In this section I will discuss some of the assumptions implicit in my models and how these assumptions might affect the predictions of the model. First, I assumed that while host species may become infected or uninfected, the number of host species and their genetic distances remain the same over time. This assumption seems justified only when gains, adaptation and losses of the endosymbionts occur on a much smaller time scale than the evolution of the host species. Otherwise, genetic differentiation, extinction of host species and speciation need to be taken into consideration to obtain a more realistic idea of the incidence dynamics. It should also be noted that the infection state of a species may have an impact on the probability of extinction. For example, sex-ratio distorting endosymbionts may lead to the extinction of their hosts due to lack of males [140, 146], and uninfected species may have a higher chance of extinction than species infected with nutritionally beneficial endosymbionts. On the other hand, endosymbionts may also have an impact on speciation rates of their hosts. In particular, cytoplasmic incompatibility inducing *Wolbachia* may facilitate speciation events of their arthropod hosts [416, 25, 23, 382].

Second, the probability of transmission of the endosymbionts was assumed to depend only on the genetic distance of the donor and recipient host species. By contrast, we can expect a variety of factors to be important for horizontal transmission. I will discuss these factors in the light of the different stages in the process of horizontal transmission in the following section.

Finally, in the extension of my model with CI- and male-killing inducing endosymbionts, I assumed that the phenotype induced does not change over time and does not depend on the host species. By contrast, it has been reported that after transinfection of CI-inducing *Wolbachia* into a new host, the endosymbionts induced male-killing [340]. This result may be interpreted in two ways. First, because of differences in the host physiology, the endosymbionts might 'accidentally' induce male-killing. Second, and more convincingly, the endosymbionts might induce both male-killing and CI in their native hosts, but male-killing is suppressed by the hosts. This second interpretation is corroborated by the discovery of resistance to male-killing in Southeast Asian

butterflies *Hypolimnas bolina*. Here, *Wolbachia* also induces CI when male-killing is suppressed, but induces male-killing in a nuclear background devoid of the resistance allele [157]. Moreover, a theoretical study has shown that a mutant strain of *Wolbachia* that induces both CI and male-killing can invade a population consisting of CI-inducing *Wolbachia* only [170]. It is a tempting avenue of inquiry to construct a model that includes such changes in phenotype and the evolution of host resistance to male-killing.

7.5.2 The process of horizontal transmission

One important assumption of the model is that the probability of horizontal transmission depends only on the genetic distance between donor and recipient species. By contrast, a variety of factors can be expected to be important for horizontal transmission. To discuss these factors it is useful to distinguish three different stages in the process of horizontal transmission:

1. Horizontal transmission itself. One or several endosymbionts from an infected host of species A must be transmitted to a host from an uninfected species B. Such events may occur frequently when the mode of transmission of the endosymbionts within their host population is horizontal, but may be very rare when the endosymbionts are transmitted vertically only. With vertical transmission in particular, close contact of host individuals from species A and B may be required for successful transmission. For example, this could be the result of a predator-prey or parasite-host relationship, or if both A and B are themselves parasites of the same host. More generally, an overlap in the geographical distributions of species A and B appears to be necessary. Also, the density of the two species and the prevalence of the endosymbionts in the donor species A can be expected to be important. In general, there seems to be no obvious relationship between the genetic distance of the host species and the probability of physical transfer. Whilst it might be argued that many forms of intimate contact take place between unrelated species (predator and prey, host and parasite), it is also true that closely related species are most likely to share predators, or share parasitoids. Although the former process promotes long distance movement, there are ecological reasons also to produce physical transfer be-

tween closely related species (e.g. on the exterior of a parasitoid's ovipositor with more than one host species, or likewise on the mandibles of a predator).

2. Survival and reproduction in new host. After being transferred to a host of the new species B, the endosymbionts need to cope with the new environment in order to survive and reproduce within their new host. This may for example involve elevated immune responses from the new host, or failure to integrate into host physiology. We expect that similar physiologies of species A and B can substantially facilitate the survival of the parasites in their new host, as indicated by many transinfection studies of *Wolbachia* (see Section 7.1.2). Therefore, the probability that the endosymbionts surmount this stage of horizontal transmission will commonly decline with increasing genetic distance between the species A and B.

3. Spread in host population. Finally, the endosymbionts must spread into their new host species. This involves both sufficiently high transmission rates between individual hosts and sufficiently low virulence. These requirements can be expected to be more likely fulfilled in closely than in distantly related hosts, but there may also be heterogeneity in the suitability of host species that my model does not account for. For instance, the frequency of asexual reproduction in aphids acts as a barrier to the spread of reproductive parasites in individual aphid species, which is likely to be a more powerful force in determining the absence of these endosymbionts in aphids than their tempo and pattern of speciation. In summary, I suspect that genetic distance between the donor and recipient host species is an important indicator for the likelihood that this stage can be overcome, but other factors may also be important.

I conclude that my approach of determining the transmission probability solely by genetic distance can be expected to be appropriate only when the last two of these stages are the limiting ones. The more important the first stage, physical transfer, the more we can expect ecological factors to obscure the effect of host phylogeny. In addition, where genetic and ecological factors are diversifying rapidly, and strongly influence the ability of functioning reproductive parasites to spread, this may further obscure the effect of phylogeny on incidence as related species become uncorrelated in their tendency to allow symbiont spread. However, I have demonstrated that heterogeneity in phylogenetic history alone, with the simple assumption of a reliance

of establishment on genetic distance, can create heterogeneity in incidence between clades, notwithstanding the importance of other factors.

7.5.3 Testing the predictions of the model using *Wolbachia*

This study was motivated mainly by the incidence patterns of *Wolbachia*, in particular, the observations that *Wolbachia* spreads through host species predominantly through horizontal transmission rather than co-speciation, and that the probability of horizontal transmission events appears to decline with increasing genetic distance between donor and recipient host species. Further, the heterogeneous distribution of *Wolbachia* in arthropods (see Section 7.1.1) begs the question of whether there is a universal factor that might help explain this heterogeneity.

Since my model uses hypothetical, rather extreme phylogenetic trees of host species only, it does not aim to make predictions about the distribution of *Wolbachia* in specific arthropod taxa. However, the model does corroborate the heuristic point that under realistic assumptions, host phylogeny alone can have a strong impact on the incidence of *Wolbachia*. In addition, the model makes several general predictions that may be tested empirically:

- Host clades that have undergone adaptive radiation (symmetric trees with short branches) should have a higher incidence than host clades that consist predominantly of isolated species (asymmetric trees with long branches).

- Species-rich host clades (or clades with many species susceptible to infection) should have a higher incidence than clades consisting of few (susceptible) species only.

- Within trees, isolated species should be less likely to be infected than species with many closely related relatives.

To test these predictions, it is desirable to reduce the impact of factors other than host phylogeny on the incidence dynamics. I propose the following two criteria for choosing host clades to be screened. First, host clades should be as distinct as possible. This means, the genetic distance of any member of the clade to the most closely related species outside that clade should be sufficiently large. This will make

the incidence dynamics depend largely on transmission within the host clade, rather than on transmission from other clades. To test whether a host clade is sufficiently distinct, it may be useful to ascertain the phylogenetic position of the *Wolbachia* strains infecting the host clade and to determine the relative contribution of intra- to interclade transmission.

Second, the species within the clade should preferably be very similar, for example with respect to population sizes and densities, breeding systems, and geographic distribution. This will minimize the impact of factors aside from host phylogeny on the probability of horizontal transmission, and it also makes it more likely that extinction rates of the endosymbionts are similar among host species.

7.5.4 Conclusions and future directions

I have developed a simple stochastic model as a first attempt to understand the 'incidence dynamics' of endosymbionts that spread in a clade of host species by occasional horizontal transmission. Based on the empirically supported assumption that the probability of successful transmission from an infected to a new host species declines with increasing genetic distance between them, I studied the impact of different phylogenetic histories of the host clade on the pattern of spread and the average incidence of the endosymbionts.

My results suggest that host phylogeny alone can lead to heterogeneous endosymbiont incidence. Symmetric host clades with short branches (as can be expected in a clade that has undergone recent adaptive radiation) can be expected to have a higher endosymbiont incidence than asymmetric clades or symmetric clades with very long branches. The spacial-temporal pattern of spread is also strongly influenced by the host phylogeny. Refractoriness — either as a constant property of host species or as an acquired resistance — can strongly reduce the probability that the endosymbionts can spread in a host clade, and also their average incidence if they spread. When the host clade is infected by two distinct strains or species of endosymbionts, antagonistic effects can be expected if infection of a host species with one endosymbiont reduces the probability that the other endosymbiont is successfully transmitted to that host. In particular, when invasion abilities of the two strains are asymmetric, the strain with the higher invasion ability can strongly reduce the incidence of the other strain,

or even drive it extinct from the host clade.

The models presented in this chapter are clearly not more than a rather primitive first attempt to understand incidence dynamics. One of the most important aspects that needs to be integrated into future models is the evolution of the host clade, which was assumed to be constant in my model. In particular, co-speciation of host and endosymbionts are known to occur commonly in some endosymbionts and should be incorporated. This line of investigation would strongly benefit from previous models that are designed to reconcile host and parasite phylogenetic trees [295]. Another important aspect that I have not studied is how factors other than genetic distance influence the probability of horizontal transmission of endosymbionts. However, almost nothing is known about the natural routes of horizontal transmission (but see [325, 161, 160]), so that it appears premature to develop sophisticated models in this direction before more empirical data is available.

The last decade has seen a substantial increase in knowledge about the incidence of *Wolbachia* in arthropods (see Table 7.1). It would be interesting to analyse these and future data with respect to the predictions of my model. More generally, it may be hoped that beyond accumulation of incidence data, the next decade will witness the development of further and more detailed theoretical predictions on endosymbiont incidence, and surveys specifically designed to test these predictions.

How about simple model that diffs due to diffs in rate of gain & loss (and # of spp?)

Chapter 8

The Ecology and Evolution of Reproductive Parasites

Abstract. In this chapter I will review our current understanding of the ecology and evolution of reproductive parasites. Theoretical results, including the ones presented in the preceding chapters, will be prominent, but empirically obtained data will also be reviewed. The sections in this chapter are presented roughly in the order of increasing time frames. I will start my discussion with the basic question of how reproductive parasites spread in their host populations, and continue with their impact on host population dynamics and host population genetics. I will then discuss influences of reproductive parasites on their hosts' behaviour and the long-term evolutionary dynamics arising from co-evolution between hosts and parasites. Transitions to novel sex determination systems are among the most interesting impacts of reproductive parasites and will be covered in the following section. I conclude this chapter with macroevolutionary events involving hosts and reproductive parasites, including host speciation and extinction. Throughout, I will point to areas of ignorance and try to identify promising avenues of future research.

8.1 Infection dynamics

The most basic challenge in the evolutionary biology of reproductive parasites is to understand how these endosymbionts spread in natural populations, and the factors that determine infection dynamics. Consequently, this was also the focus of the early theoretical work on reproductive parasites, with the first model on CI dating back to the late fifties [54].

In contrast to the modelling of horizontally transmitted parasites, a population genetic framework has most commonly been employed in studying the infection dynamics of reproductive parasites. Infinitely large, panmictic host populations are assumed, and infection dynamics are described by recursion (=difference) equations. The reproductive parasites are treated like a single genetic entity, and within host population dynamics are ignored.

Empirical studies have delineated three parameters to be important, which are incorporated in many models. These parameters are (1) the penetrance of the induced effect (e.g., CI-induced mortality in incompatible crosses), (2) the transmission rate of the reproductive parasites (i.e., the proportion of offspring from infected females that are also infected), and (3) the fecundity reduction caused by the infection in infected females. Note that whilst penetrance and fecundity reduction correspond to fitness effects in standard population genetic models, the transmission rate is more specific to reproductive parasites and is more closely related to mutation rates.

I will first consider the infection dynamics of cytoplasmic sex-ratio distorters (feminising, male-killing and parthenogenesis inducing endosymbionts) and proceed with the more complicated dynamics of CI-inducing bacteria. I will then discuss the joint dynamics of different types of reproductive parasites, and the joint dynamics of reproductive parasites and nuclear selfish genetic elements.

8.1.1 Cytoplasmic sex-ratio distorters

Models for the basic infection dynamics of cytoplasmic sex-ratio distorters are very simple. If infected females produce more infected daughters than uninfected females produce normal daughters, the reproductive parasites will spread in the population [46, 414]. If this condition is fulfilled, two equilibria exist: one that represents the

uninfected population and is unstable, and a second, stable equilibrium that is always attained irrespective of the initial frequency. This second equilibrium will be polymorphic if maternal transmission is imperfect, whilst with perfect maternal transmission, the endosymbionts will become fixed [179, 365, 322].

For feminising and parthenogenesis inducing parasites, calculating the number of infected daughters that infected females produce is straightforward for given parameters. Male-killing bacteria, however, rely on a more complicated process termed 'fitness compensation'. Here, the fitness of surviving siblings in a brood from an infected female is increased due to the death of their brothers. The most common assumption is that a certain fraction of resources freed by the death of males is evenly distributed among the surviving siblings and increases their fitness linearly ([179, 312, 313, 287], see also Chapters 3 and 4). Other formulations for fitness compensation have also been employed (see Chapter 2 and Refs. [414, 120, 170, 101]), and the choice of different formulations has only minor influences on the outcome of the models.

The simple model framework outlined above predicts that infections with several sex-ratio distorters are unstable [313]. More precisely, the strain that induces infected females to produce the greatest number of infected daughters should outcompete all other strains and drive them extinct. In contrast to this prediction, the ladybird *Adalia bipuntata* is infected by at least three species (and even more strains) of male-killing bacteria [172, 176, 248, 207], and some of the polymorphisms reported appear to be evolutionary stable [207]. The butterfly *Acraea encedon* is also infected by two strains of male-killing *Wolbachia* [204].

Two hypotheses have been put forward to account for such polymorphisms of different sex-ratio distorters. First, it has been proposed that spatial structure will facilitate coexistence of different strains, a claim that has been corroborated by computer simulations of a lattice model [132]. Unfortunately, it appears that populations of *Adalia bipunctata* are rather unstructured, with long-distance dispersal of both sexes [247]. Likewise, butterflies like *Acraea* are unlikely to have structured populations. Second, modelling predicts that co-existence of several male-killers can be the result of male-killer specific resistance alleles, when these alleles are very costly (see also Section 8.5.3). Again, there is no evidence for such resistance alleles in either

Adalia bipunctata or *Acraea encedon* [205], suggesting that as yet unknown selective forces maintain infection polymorphism in these species.

8.1.2 CI-inducing reproductive parasites

The simplest case of CI — unidirectional incompatibility caused by a single strain of bacteria in an unstructured, panmictic host population — has been investigated theoretically in several studies that provide a good null model for the infection dynamics of CI in general [54, 111, 154, 179, 389].

A crucial feature that emerged from these simple models is that whenever transmission of the CI-inducing endosymbionts is imperfect and/or the fecundity of infected females is reduced, there is an 'invasion threshold' for the bacteria. In deterministic models, when the starting frequency of infected females is above this threshold, the CI-inducing endosymbionts will spread in the host population, whilst starting frequencies below this threshold result in symbiont extinction. Following spread, the CI-inducing parasites will attain a stable equilibrium frequency. This equilibrium will be polymorphic if maternal transmission is imperfect and CI-induced mortality is less than 100%. Otherwise, the parasites will become fixed in the population (and the phenotype of CI will no longer be evident). It is not known how the invasion threshold can be overcome in natural populations; suggested mechanisms include random genetic drift, additional effects of the reproductive parasites on host fitness or sex-ratio, and population structure [92, 398].

Several minor extensions of this basic framework have been constructed to account for deviations of the hosts' biology from the null model. In haplodiploid species, for example, the invasion threshold for CI-inducing bacteria is higher than in diplodiploid species [397]. This is because males develop from unfertilised eggs and hence, are not affected by CI. Fitness compensation — an early boost in fitness that surviving offspring receive through the death of their siblings — has an ambiguous effect on invasion threshold and equilibrium frequency, as it benefits surviving siblings from both uninfected and infected mothers [120]. Finally, inbreeding has been demonstrated to increase the invasion threshold and decrease the equilibrium frequency of CI-inducing endosymbionts (see Section 5.3.2). In extreme cases of inbreeding, invasion or persistence of CI may not be possible at all.

An important factor in the dynamics of CI is space. For example, CI-inducing *Wolbachia* have been reported to have spread in Californian *Drosophila simulans* at a rate of more than 100km per year [390]. This process has been modelled using a continuous reaction-diffusion framework and empirically obtained parameter estimates [390]. However, discrepancies between the predicted wave speed and dispersal estimates of *Drosophila* led the authors to conclude that the assumptions underlying the model are not realistic for this system. Subsequent modelling has confirmed that the quantitative outcome of this and other models depends very much upon the specific assumptions of host dispersal patterns [344], indicating that good field data on these patterns are needed to bring theory and reality into closer contact.

Another open issue concerns the question of whether population subdivision facilitates or impedes the spread of CI-inducing bacteria. Wade & Stevens studied a model in which the host population was subdivided and completely mixed back in each generation [406]. In this model, population subdivision always slowed down the spread of the endosymbionts. However, another model that incorporated random genetic drift yielded the opposite result: because drift becomes stronger in subdivided populations, the rate and likelihood of invasion of CI-inducing endosymbionts increased with decreasing migration rate and was highest at intermediate deme sizes [317]. Finally, a deterministic two-island model demonstrated that infection polymorphism (one subpopulation infected, the other uninfected) can be a stable state when migration between islands is sufficiently rare [114], an outcome that did not occur in the two models previously mentioned. A clear need is apparent for a reconciliating theoretical framework that can reproduce all of the above mentioned results under different conditions, thus clarifying the impact of population structure on the infection dynamics of CI. Even more importantly, further empirical studies on the spread of CI-inducing endosymbionts in subdivided populations are needed.

Many species are infected with two or more strains of *Wolbachia* that induce bidirectional CI [237, 293, 27, 267, 192, 76]. Two different situations need to be distinguished. First, different CI-strains may be present in a population, but each host individual is infected by a single strain only. It has been demonstrated theoretically that in a panmictic population, a stable polymorphism of bidirectionally incompatible strains is not possible [332]. However, in a structured host population,

infection polymorphism can be stable even in the face of high migration between subpopulations [381, 215, 383].

Second, different *Wolbachia* strains may be present within single host individuals. This situation is also commonly found in natural populations [27, 76, 266, 333], and up to five *Wolbachia* strains have been reported to co-occur within their hosts [194, 316]. Modelling has also demonstrated that multiple infection with bidirectional CI-inducing endosymbionts can be stable in a single panmictic population, as long as hosts occur that are infected with all CI-strains [117].

8.1.3 Joint infection dynamics of different reproductive parasites

The conditions for stable coexistence of sex-ratio distorting and CI-inducing reproductive parasites are rather similar to those for the coexistence of two CI-inducing strains. Stable persistence of male-killing and CI-inducing endosymbionts in a panmictic population is only possible if some host individuals are infected with both strains [120, 101]. The same qualitative result applies to coexistence of parthenogenesis and CI-inducing endosymbionts [101], and can most certainly be extended to feminisers as well.

To date, there are only very few reports of multiple infections with sex-ratio distorting and CI-inducing endosymbionts [275, 36, 11]. This may reflect the fact that there are indeed only few infections with these different types of reproductive parasites. Alternatively, multiple infection of this kind may have been overlooked because when females are infected with CI- and sex-ratio inducing endosymbionts, few or no infected male offspring will be produced, so that the CI-effect is 'masked' by the action of the sex-ratio distorters.

All reports of multiple infections involve CI- and male-killing inducing bacteria, and they are consistent with theoretical predictions. In two cases — *Drosophila melanogaster* and *Nasonia vitripennis* — individuals can be infected with both reproductive parasites. However, the male-killer in *N. vitripennis* is known to be transmitted horizontally following host sharing, so that this system does not represent a 'classical' scenario as envisaged by the models. In a third case, the butterfly *Hypolimnas bolina*, no doubly infected individual have been found, and the complementary

distribution of the two strains across South Pacific islands appears to confirm the theoretical prediction of mutual exclusion [60].

8.1.4 Interference of reproductive parasites with other selfish genetic elements

There are numerous genetic elements that spread in a host population by harming their hosts, and that consequently have been classified as 'selfish genetic elements' (SGEs) [421, 177, 47]. In many cases, no interference between reproductive parasites and SGEs can be expected, but there are some interesting exceptions.

Meiotic drive elements increase their transmission to future generations above the Mendelian rate of 50% by killing gametes (usually sperm) that do not carry the element [246, 189]. Since males carrying a meiotic drive element have only half as many sperm as normal males, such males can become sperm depleted more quickly than normal males when mating with several females [20, 436]. As I have argued in Chapter 2, if a population is infected with male-killing bacteria, the resulting female biased sex-ratio may lead to higher mating rates of males, so that sperm depletion results in decreased fitness of males carrying the meiotic drive element. As a consequence, male-killing bacteria may decrease the equilibrium frequency of meiotic drive elements and even drive them extinct. Conversely, meiotic drive elements located on the X chromosome can also decrease the equilibrium frequency of male-killing bacteria and drive them extinct. This is because the average primary sex-ratio in the population will be more female biased, so that fewer male offspring can be killed and the fitness advantage for the male-killers declines. Taking these two results together, an antagonistic relationship between X chromosome elements and male-killers emerges, which should lead to less than expected joint incidence.

Another selfish genetic element is the PSR ('paternal sex ratio') chromosome, found in several haplodiploid arthropods [423]. When in males, this supernumerary chromosome destroys the chromosomes in sperm, so that fertilised eggs are haploid and develop into males instead of females. In the parasitoid wasp *Trichogramma kaykai*, both PSR and parthenogenesis inducing *Wolbachia* co-occur [370]. If a *Wolbachia* infected female mates with a PSR carrying male, rather paradoxically all fertilised eggs will develop into males (due to PSR), whilst all unfertilised eggs

develop into females (due to *Wolbachia*). Thus, PSR decreases the number of daughters that *Wolbachia* infected females produce, which is a likely explanation for the low frequency of *Wolbachia* in this species [370].

A common feature of all SGEs is that their spread and maintenance in a population rely on the sexual reproduction of their hosts [177]. For example, the otherwise ubiquitous transposable elements of the LINE and GYPSY group appear to be absent in the Bdelloidea, an ancient asexual group of rotifers [9]. Thus, we can expect parthenogenesis inducing reproductive parasites to impede the spread of SGEs, and fixation of the former may lead to extinction (or degeneration) of the latter.

8.2 Impact on host population dynamics

Reproductive parasites can be expected to have a considerable impact on the dynamics of their host population, either through increased mortality among host offspring (CI), through distortion of the population sex ratio (feminisation and parthenogenesis induction), or through a combination of both of these effects (male-killing). However, surprisingly little is known both theoretically and empirically about the impact of reproductive parasites on host population dynamics. Given that suppression of damaging insect populations is one of the promising areas where reproductive parasites might find a useful application, this lack of knowledge is particularly notable.

8.2.1 Cytoplasmic sex-ratio distorters

The spread of cytoplasmic sex-ratio distorters results in a female-biased population sex-ratio. Since the reproductive capacity of a population is usually more strongly determined by the number of reproducing females than reproducing males, we can expect a higher intrinsic population growth rate with increasing prevalence of the sex-ratio distorters, which may result in an increased equilibrium population size. On the other hand, the opposite effect can be expected if male mating capacities are limited, as the number of unmated females will increase with increasing prevalence of the reproductive parasites. (This second effect does not apply, of course, to parthenogenesis inducing bacteria.)

The impact of feminising reproductive parasites on host population dynamics has been analysed theoretically with a deterministic and a stochastic model [146]. Both of the above described effects have been found. When male mating rate was not limited, equilibrium population size increased with increasing prevalence of the feminiser. However, when an upper limit to male mating rate was assumed, equilibrium population size declined with increasingly prevalent feminisers, which eventually can lead to extinction. Notably, extinction was possible even with intermediate prevalence of the feminising parasites. One interesting outcome of the stochastic model was that during the decline of the host population, extinction of the feminising parasites was sometimes observed, resulting in population recovery.

Male-killing bacteria can be expected to have similar effects on host population

dynamics. The main difference is that male-killers decrease the total offspring number of infected females, so that the number of female offspring is only slightly increased (depending on fitness compensation). However, these dynamics have not yet been investigated in a systematic way. Some preliminary results suggest another interesting effect. With overcompensating density dependence (or time delays), increases in the intrinsic growth rate of the population (through a female-biased population sex-ratio) can make population dynamics less stable and even chaotic [226]. This may constitute another cause of host population extinction that operates even in the absence of male mating rate constraints.

8.2.2 CI-inducing reproductive parasites

CI does not normally lead to distorted population sex-ratios (except for some minor effects in haplodiploid species) but entails a reduction in the total number of offspring produced. The effect of CI on host population dynamics has been studied theoretically with a deterministic model [80]. This model produced a decline in population size during the spread of CI-inducing endosymbionts, followed by partial recovery of the host population when the reproductive parasites reached their equilibrium frequency. This effect is readily explained by the number of incompatible matings that occur and the resulting total mortality among offspring. Total mortality will be highest when 50% of the population are infected and declines when CI-prevalence increases above 50%.

The population size eventually attained after the spread of the CI-inducing bacteria depends on their equilibrium frequency. If the reproductive parasites are fixed in the population, no incompatible matings will occur, and population size will not be influenced by CI-induced mortality. However, if the equilibrium frequency is lower than one, incompatible matings may slightly reduce equilibrium population size (depending on the presence/absence of other density dependent processes). In addition, fitness effects associated with the infection may lead to diminished host population size. In total, the model demonstrated that if the infection is at equilibrium, host population size is expected to be only mildly decreased compared to the size of the uninfected population [80].

Substantial and persistent reduction in host population size has been predicted to

be the consequence of recurrent sweeps of new strains of CI-inducing bacteria that are incompatible with the other strains [80]. This theoretical result implies a promising strategy for the suppression of harmful insect populations [80, 77]. Empirical studies of the effects of CI on host population size are needed to validate this and other model predictions.

8.3 Impact on host population genetics

8.3.1 Mitochondrial genes

Like reproductive parasites, mitochondria are predominantly transmitted maternally. Thus, there is tight or even complete linkage between these types of cytoplasmic elements, and a strong impact of reproductive parasites on the population genetics of mitochondrial DNA (mtDNA) can be expected. Inferences from mtDNA variation in arthropod populations can therefore be confounded by the presence of reproductive parasites [169], and conversely, mtDNA variation can provide useful information about reproductive parasites and the history of their relationship with the hosts [283, 391, 197, 88, 207].

During the spread of a reproductive parasite in a host population, the mitotype that happens to be associated with the infection will also spread by indirect selection ('hitchhiking') with the endosymbionts. Such selective sweeps, caused by infection with CI-inducing *Wolbachia*, have been observed both in the lab and in the wild [212, 391]. As a result, mtDNA diversity can be strongly reduced, especially if the reproductive parasite reaches high prevalence [259]. If the presence of reproductive parasites is disregarded, low mtDNA diversity might easily be mistaken for recent host demographic processes, such as founder or bottleneck events [169].

Once a reproductive parasite has reached an equilibrium frequency in a population, and provided that no further selective sweeps occur, mtDNA diversity can be expected to gradually rebuild. However, if transmission of the endosymbionts is imperfect, there will be mtDNA gene flow from infected to uninfected individuals, but not into the other direction. As a consequence, mitotypes in uninfected individuals will continuously be replaced by those in infected females, so that the original mitotypes in uninfected individuals will eventually be lost [391, 208]. This also means that effective population size with respect to mtDNA will be reduced to a value closer to the number of infected females than to the total number of females. Thus, under a mutation-random drift process, genetic diversity will not recover to the original value of the uninfected population, but to a lower equilibrium [208].

Analysing mitotypes in infected and uninfected individuals of a host population can be very informative with respect to transmission of reproductive parasites. A

good example is in the butterfly *Acraea encedon*, which is infected with two strains of male-killing *Wolbachia* [202]. mtDNA diversity in uninfected females of this species was reported to be high, and the mitotype associated with one of the *Wolbachia* strains was very rare within uninfected females [197]. This suggests that maternal transmission rate is perfect or extremely high in *A. encedon*, a prediction that is in accord with direct measurements [205]. By contrast, transmission efficiency in the closely related *A. encedana* is only about 96% [200], and correspondingly, all uninfected individuals were found to have the same mitotype as the infected females [197]. Finally, rare mitotypes in infected individuals that are otherwise prevalent in uninfected individuals can indicate events of paternal or horizontal transmission [283, 391].

mtDNA can also be used to estimate the date when a reproductive parasite spread through a host population. One approach is based on the slow recovery of mtDNA diversity following the spread of the reproductive parasite (see above). Here, mtDNA variation is compared to the expected mtDNA variation at equilibrium, which in turn is estimated using neutral nuclear DNA variation. For example, high variation of mtDNA has been reported in male-killer infected *Drosophila innubila*, suggesting that the infection is evolutionarily old [88]. One caveat with this approach is that reproductive parasites may also have an impact on the population genetics of nuclear DNA, which must be taken into account (see Chapter 4 and the following section).

8.3.2 Nuclear genes

The impact of reproductive parasites on evolutionary processes in nuclearly encoded genes has been studied to a much lesser extent than that on mtDNA. One reason for this may be that *a priori*, there is no linkage between cytoplasmic and nuclear genetic elements[1], so that the population genetics of nuclear genes is often considered to be unaffected by reproductive parasites.

[1]The only exception to this rule is the W chromosome in female heterogametic species like butterflies and some woodlice. The W chromosome will be linked like mitochondria to the reproductive parasites, so that usually the same population genetic effects as discussed in the previous section can be expected. However, with feminising endosymbionts, the dynamics are entirely different (see Section 8.6.1).

This reasoning is approximately true for CI-inducing bacteria. For example, a neutral allele introduced together with CI-inducing bacteria at low frequency will become slightly less frequent in the population during the spread of the endosymbionts. This arises because infected males have a much lower than average fitness when the infection is rare, whereas infected females have only a slightly increased fitness. Thus, on average the cytoplasmic genetic background of the new allele is deleterious, and frequency of this allele will drop. However, since nuclear and cytoplasmic genes are not linked, the CI-inducing bacteria and the new allele will become dissociated within a few generations, so that background fitness differences will have only a minor effect [69].

By contrast, as I have demonstrated in Chapter 4, male-killing bacteria have an interesting and potentially severe effect on basic population genetic processes of their hosts. Because infected females produce only (or mainly) infected daughters, there will be tight linkage not only between the male-killing bacteria and mitochondrial DNA, but also between male-killers and nuclear genes. As a consequence of this linkage and the low fitness of infected females, it is the case that (almost) only alleles that arise in uninfected individuals can spread in the population. Thus, the population genetics in a male-killer infected population is largely determined by the uninfected individuals in the population, a situation in direct contrast to the population genetics of mtDNA. As a consequence, the spread of beneficial alleles is impeded, the spread of deleterious alleles is facilitated, and neutral genetic variation can be substantially reduced.

The same logic also applies to some extent to feminising endosymbionts. However, the effect can be expected to be less strong because females infected with feminising endosymbionts have a higher fitness than male-killer infected females (when the population sex-ratio is the same). In cases where all infected individuals are females and all uninfected individuals are males (like in some populations of the woodlouse *Armadillidium vulgare* [326]), the population genetics will be the same as for an uninfected population with biased sex-ratio.

The most drastic change in host population genetics can be expected with parthenogenesis inducing endosymbionts. Two situations can be distinguished. First, several host species are fixed for parthenogenesis inducing endosymbionts [447, 304, 7, 408,

409]. In this case, the population will consist entirely of asexually reproducing females, with well-known population genetic consequences (e.g., increased accumulation of deleterious mutations and a slower rate of adaptive evolution). Second, the host population can be polymorphic for the parthenogenesis inducing endosymbionts, so that some males exist and mate successfully [369, 304]. A crucial determinant of the population genetic properties in such a situation is how many females will remain unmated and produce unfertilised eggs only. If, on one extreme, all females fertilise as many eggs as they would without the parthenogenesis-inducing infection, the population resembles that of a population infected with male-killing or feminizing endosymbionts. On the other extreme, if only few females mate, the population will behave more like an asexual one, with only viscous 'gene flow' between individual lineages. As yet, neither theoretical nor empirical investigations have been conducted on these issues.

Finally, when there is an infection polymorphism of reproductive parasites across different host subpopulations connected by migration, gene flow between these subpopulations will be altered by the reproductive parasites. In the case of CI-inducing endosymbionts, this effect has important consequences for CI-mediated host speciation and will be discussed in some detail below (see Section 8.7.1). Another scenario is that of an infection polymorphism for male-killing inducing bacteria, as found for example on different island populations of the butterfly *Hypolimnas bolina* [61, 60]. Here, male migrants from the uninfected subpopulation can contribute substantially genetically to the infected population because of the female biased population sex ratio. Thus, gene flow from uninfected to infected subpopulations can be strongly increased [378]. As a consequence, local adaption in the infected subpopulation is impeded, and even resistance alleles against the male-killer may not be able to spread in the population.

8.4 Impact on host behaviour

8.4.1 Mating preference

When a population is polymorphic for infection with reproductive parasites, individuals with different infection states will have different fitness. Thus, in theory we can expect the evolution of mating preference, such that males choose to mate with females with the infection state that confers the higher fitness, and *vice versa* [312]. In practice, however, it is not clear if males can distinguish between females of different infection state, or *vice versa.*

One compelling case has been reported in the woodlouse *Armadillidium vulgare.* This species is infected with *Wolbachia* that induce feminisation by interfering with the development of the androgenic gland in genetic males (see section 1.4.1). An investigation into mating behaviour in infected populations of this species revealed that a higher than expected proportion of unmated females were infected [278]. When males were given the choice to mate between a 'real' female and a genetic male that has been feminised by *Wolbachia*, the males made more mating attempts with and transferred more sperm to real females than to 'neo-females' [277]. This preference may be due to differences in the pheromones produced by the two types of females. Moreover, neo-females often responded inappropriately to mating, resulting in premature termination of mating.

These result suggests that feminisation is somewhat incomplete in neo-females, making them less attractive and less receptive. As *A. vulgare* is a female heterogametic species, one possible explanation is that some of the genes coding for female specific pheromones or behaviour are located on the W chromosome. Alternatively, choosiness of males could also be the result of selection acting on males to prefer real females. This kind of selection would occur because (1) mating attempts with real females are more successful and (2) such matings would produce males, which have a high fitness in a female biased population. However, to date there is no evidence for adaptive evolution of choosiness in *A. vulgare.*

The system of *Acraea encedon* was initially considered another good case study of this phenomenon. This African butterfly species harbours male-killing *Wolbachia* at a high prevalence of up to 90% [202, 172, 203]. Many females remain virgins, but

uninfected females were significantly more likely to have mated than infected females [203]. This suggested that males choose to mate with uninfected females, although other explanations are possible (see below and [203]). However, further observation failed to show evidence for this preference, and the result that unmated females were more likely to having mated could not be reproduced [205].

Clearly, choosing uninfected females would substantially increase the fitness of males (especially with high male-killer prevalence), because they would produce offspring of the rare male sex. Indeed, it has been demonstrated theoretically that a male mate choice gene can spread in a male-killer infected population under broad conditions, even when costs for choosiness and errors in mate choice are included in the model [312]. The presence of such a mate choice allele will lead to a decreased prevalence of the male-killing bacteria and may prevent fixation of male-killers even when maternal transmission is perfect.

Mate choice can also be expected to be selected for in host populations infected with CI-inducing endosymbionts, as long as the infection has not spread to fixation. In this case, incompatible matings will occur, so that males will be selected to mate with infected females, and females will be selected to mate with uninfected males. In a model of the latter scenario, a preference allele for uninfected males was found to always invade the population and slow down or even prevent the invasion of the CI-inducing endosymbionts [73]. However, choosiness was assumed to be cost-free in this model, and the important case of the fate of a newly arisen preference mutation when the CI-endosymbionts were at equilibrium frequency was not studied. Both of these factors are likely to considerably reduce the subset of the parameter space in which a preference allele can invade. Empirically, mate choice according to infection with CI-inducing endosymbionts is supported in a study of the spider mite *Tetranychus urticae* [393]. Here, infected females laid their eggs preferentially in the vicinity of infected eggs, and uninfected females laid their eggs in the vicinity of uninfected eggs, thus reducing the probability of incompatible matings among their offspring. Moreover, mate choice experiments demonstrated that uninfected females mated preferentially with uninfected males.

A neat variation of the theme of mate choice selected for by reproductive parasites is selection for inbreeding. In the case of cytoplasmic sex-ratio distorters, males are

selected to mate with their sisters, because the very existence of a male makes is very likely that his sisters are uninfected. In the case of CI-inducing endosymbionts, matings between siblings have a much higher chance to being compatible than matings between unrelated individuals, so that again, sibmating may be favoured. In the above-mentioned study on *T. urticae*, females were reported to lay their eggs in a way that promotes sibmating among their offspring (i.e., removed from other clutches) [393]. However, since no comparison with an uninfected population was made, it is not clear to what extent this behaviour represents an adaptation to the presence of CI-inducing *Wolbachia*. In terms of theory, the potentially complicated co-dynamics of a 'sibmating allele', prevalence of the reproductive parasite, and inbreeding depression, still await exploration.

8.4.2 Other influences on host reproductive behaviour

In most species, males compete for matings. This is because usually, males have a higher reproductive potential than females, resulting in a male-biased operational sex-ratio [93]. In the male-killer infected butterfly *Acraea encedon*, a sex role reversal has been reported: females aggregate on the top of hills to obtain matings by one of the few males present in the population [203]. This can be either explained as an result of adaptation to male-killer infection, or simply as a behaviour in which females gather on the top of hills to obtain matings as in uninfected species, but remain there for a long time because they do not find a male to mate with.

In parasitoid *Encarsia* wasps, fertilized eggs (which will develop into females) are usually laid in 'primary' hosts, whilst unfertilized eggs (developing into males) are laid in 'secondary' hosts. Unmated females do not normally lay eggs in primary hosts, because these would fail to develop. However, in populations of different *Encarsia* species infected with parthenogenesis inducing bacteria (*Wolbachia* or *Cardinium*), unfertilised eggs were reported to be readily laid in primary hosts [163, 444]. This is adaptive for both the wasps and the reproductive parasites, as it ensures development of the offspring 'feminised' by the bacteria through chromosome duplication. Surprisingly, in *Encarsia pergandiella*, the change in the wasps' oviposition behaviour appears to be directly determined by infection with *Cardinium*, as antibiotic treatment produced females that refused to lay unfertilised eggs in primary hosts [444].

The selective advantage of male-killing is thought to be a fitness benefit that infected females receive through the death of their brothers [414, 179, 174, 191]. A crucial requirement for this benefit is that eggs are laid in clutches so that siblings will interact with each other in an antagonistic way (through competition, cannibalism, or sibmating that is deleterious for females). Consequently, the fitness benefit for male-killing bacteria can be expected to decrease with decreasing clutch-size. In the limit of singly laid eggs, no fitness benefit is gained (unless there are other forms of antagonistic kin interactions), and male-killing bacteria cannot invade a host population. Thus, a male-killer infected population in which eggs are laid in clutches may be cured of its infection if females would lay eggs singly. However, selection on individuals can be expected to work just in the opposite direction [175]. Infected females will have all or most of their sons killed independently of clutch-size, but can obtain a greater number of surviving daughters by laying eggs in clutches. As a result, infected females are selected to increase their clutch size, leading to even higher male-killer prevalence.

Unfortunately, a model constructed to predict evolutionary stable clutch-sizes disregarded the peculiar population genetics that are at work in male-killer infected populations ([175], see Chapter 4, in particular Section 4.5.3). Integrating this important factor into the model would probably produce at best a slightly increased clutch-size. This is expected because the fate of a new 'clutch-size increasing' allele depends largely on its fitness effects in uninfected females, whose optimal clutch-size is not altered by the presence of male-killers in the population.

8.5 Evolutionary dynamics

8.5.1 Evolution of CI

Investigations into the evolution of CI have been severely limited by ignorance about the genetic basis and the mechanism of CI. The most important question in this respect is the number of loci that are involved. Theoretically, it is possible that both modification of sperm ('mod-function') and rescue of eggs ('resc') are controlled by a single locus [235, 49, 415]. However, several findings concerning more complex patterns of CI (e.g., bidirectional CI, resc-only phenotypes) make it more parsimonious to assume that two loci are involved [306]. One of these loci is assumed to cause the modification of sperm (sometimes referred to as the 'lock'), whilst the other codes for gene products that rescue the modification in fertilised eggs (the 'key', fitting to a particular 'lock') [36, 415, 306]. In the following discussion of the evolution of CI, I will assume such a two component system of a mod- and a resc-locus to be the genetic basis for CI.

The first question that arises when thinking about the evolution of CI is: how can such a two-component system evolve in the first place? Clearly, without the existence of males in a population that have their sperm modified, a resc-function has no effect and does not confer any selective advantage to endosymbionts expressing it. Conversely, the mod-function alone leads to a 'suicidal' phenotype that is not normally selected for either.

Two different scenarios for the evolution of CI seem possible under these premises. In the first scenario, the ancestral endosymbionts might have been maintained in the host population by some other form of drive (e.g., by providing nutrients to their hosts), or by horizontal transmission. Some or all of these endosymbionts incidentally could have had a resc-function that never came into play, or that served some other purpose. A mutation could then lead to a mod-function, which would cause positive selection on endosymbionts carrying the resc-function. The problem with this explanation is that the mod-function is not normally selected upon (but see below), so that among those endosymbionts that have the resc-function, those that in addition have the mod-function could spread by random genetic drift only.

In a second scenario, the ancestral endosymbionts could have had a mod-function

only. Although clearly deleterious for males that carry them, such a strain of endosymbionts would not have a selective disadvantage because of its maternal transmission. Thus, the mod-only strain could spread in the population by random genetic drift. Recently, it has been demonstrated that a mod-only strain can even be weakly selected for in outbreeding populations (e.g., if the hosts avoid sibmating) [94]. This is because under outbreeding, infected males are more likely to decrease the offspring number of uninfected than of infected females, thus boosting the infection. Once the mod-only strain has spread in the host population through drift and/or selection, a resc-mutation would be strongly selected for and could spread in the population, replacing the mod-only endosymbionts. Both of these scenarios for the evolution of CI involve random genetic drift as an important factor, and it is hard to assess which one is more likely.

What is the evolutionary fate of CI-inducing endosymbionts once they have established within a host population? I will first consider evolutionary pathways that arise from selection on the endosymbionts only, disregarding the hosts. As outlined above, in a panmictic population there is no direct selection on the mod-function. The situation resembles the well-known 'tragedy of the commons': whilst it is advantageous for the CI-inducing endosymbionts if all of them induce the mod-function, there is no benefit for any single endosymbiont to express mod. Indeed, expression of mod may even be costly, for example if gene expression in males and females is coupled. It has been suggested therefore, that the mod-function might deteriorate over time, or that a mutation that does not induce mod may spread by selection [389, 311, 181]. As a consequence, the resulting endosymbionts with no or decreased mod-activity may not be selected for strongly enough to be maintained in the population and become extinct [181].

Alternatively, if the host population is structured, the mod-function may be under positive (kin) selection [116]. This is because the mortality in incompatible crosses caused by infected males will benefit related infected females of these males more than unrelated females. Therefore, in structured populations the mod-function may be actively maintained or even improved towards higher mortality in incompatible crosses.

How can we expect host populations infected with CI-inducing endosymbionts to

respond to the infection in evolutionary terms? One peculiar feature of CI is that once the infection is established, the endosymbionts become 'beneficial' because of the particular environment they create (i.e., many infected males that have their sperm modified). Consequently, there will be selection in females to provide the bacteria with sufficient resources and to transmit them to as many of their offspring as possible. This selective pressure for high maternal transmission rates may explain why CI-inducing *Wolbachia* are fixed in many host species [420, 233, 397].

However, when CI-inducing endosymbionts are not fixed in a population (or in a hybrid zone where two bidirectionally incompatible CI-strains co-occur), there will be selection on infected males to resist modification of sperm, and selection on uninfected females to rescue modifications [389]. Both of these types of resistance will lead to decreased mortality in incompatible crosses. The dynamics of the spread of alleles coding for decreased modification in infected males and increased rescue in uninfected females will be the same, because gene frequencies will be the same in males and females [389]. During the spread of such an allele, the frequency of the CI-inducing endosymbionts will decline. This leads to positive frequency dependence for the resistance allele: as even more incompatible matings occur, selection for the resistance allele will become stronger. During the spread of the resistance allele, the average mortality in incompatible crosses will decline. Therefore, the selective advantage to the endosymbionts arising from CI will also decline. For some parameter combinations (low transmission rates, high costs of infection), the drive can become too low, such that the endosymbionts will go extinct. Alternatively, with higher transmission efficiency and/or low cost, the resistance allele may spread to fixation and result in a decreased equilibrium frequency of the CI-inducing bacteria only [229].

8.5.2 Evolution of new CI-types and pluripotence

Many species are infected with different strains of endosymbionts that render their hosts bidirectionally incompatible. This is explained by distinct mod-resc-systems or 'CI-types' in different CI-strains. An important question that only recently has received attention is how such new CI-types can evolve (see Chapter 5 and Refs. [59, 78, 58]).

The most plausible evolutionary pathway towards a new CI-type is via a mutant

that induces a new mod-function that cannot be rescued by its own resc [59]. Such a mutant is neutral in panmictic populations, selected against in inbreeding ones and selected for in cases of outbreeding (see Ref. [59] and Section 5.3.3). Once the mutant strain has spread by selection and/or random genetic drift, a new mutant that can rescue the new mod is selected for and can spread in the population. Two different outcomes are possible. First, the resc-mutant can occur in one of the endosymbionts with the new mod-function, in which case a new CI-type has arisen. Second, the resc-mutation may occur in one of the endosymbionts with the wildtype-mod, leading to a 'suicidal polymorphism' of two strains that can mutually rescue their respective modifications (see Refs. [59, 58] and Section 5.3.3).

A second pathway that has been proposed commences with a mutation concerning the resc-function [78]. In a single infection state (infection of females with only the mutant strain), such a mutation would be strongly selected against [59]. However, if the mutant strain co-occurs with the wildtype strain as a double infection, it may be protected against the mod-function it cannot rescue. This strain may then spread by random genetic drift, and a subsequent new mod-mutation may then lead to a new CI-type, co-occurring as a double infection with the wildtype strain [78]. The problem with this scenario is that if maternal transmission of the strains involved is imperfect, segregation is likely to lead to loss of the resc-mutant before it can reach considerable frequencies in the population (see Section 5.3.5).

Finally, another evolutionary trajectory for CI-inducing bacteria might be a switch in reproductive manipulation towards sex-ratio distortion. Modelling has demonstrated that a CI-inducing endosymbiont can be invaded by a mutation that induces male-killing in addition to CI [170]. Interestingly, invasion does not always lead to a stable equilibrium frequency of the new 'pluripotent' strain, but sometimes also to its extinction. This is because CI is 'masked' by male-killing, so that fewer males in the population have their sperm modified, and the pluripotent strain needs to be maintained in the population mainly via fitness compensation resulting from male-killing.

Recently, such a pluripotent *Wolbachia* strain has been reported in the butterfly *Hypolimnas bolina* [157]. Here, infected females normally induce male-killing. However, in Southeast Asian populations of this butterfly, a resistance allele has recently spread that suppresses male-killing [156]. In the presence of the suppressor,

infected males are formed, and these are observed to induce CI. Thus, it can be concluded that male-killing has masked CI. Further support for the notion of pluripotent strains comes from transinfection studies of the almond moth *Cadra cautella* [340]. This species is naturally infected with *Wolbachia* that induce CI in their native hosts, but induce male-killing after transinfection into another host, the flour moth *Ephestia kuehniella*. Again, this suggests that *Wolbachia* is capable of both forms of manipulations, one of which is suppressed by host factors.

8.5.3 Resistance against sex-ratio distortion

Cytoplasmic sex-ratio distorters (feminising, male-killing, and parthenogenesis inducing endosymbionts) lead to a female biased population sex-ratio. As a consequence, infected females will have a lower fitness than uninfected females, because they produce few or no offspring of the rare, male sex [83, 113]. In addition, male-killing endosymbionts strongly decrease the fitness of their female hosts by killing about half of their offspring. Finally, the fitness of infected females may be reduced by a physiological burden put upon them by the endosymbionts (which could, e.g., lead to fecundity reduction).

Given all these deleterious effects of sex-ratio distorting endosymbionts, we can expect resistance against them to evolve [414, 375, 313, 55]. Two types of resistance mechanisms can be distinguished. First, hosts may evolve means to entirely suppress growth or transmission of the endosymbionts. Second, only the manipulation induced by the endosymbionts or, equivalently, transmission to males may be suppressed.

Both of these types of resistance have been modelled for the case of a male-killer infected population [313]. In this model, a resistance allele could invade the population even under a broad range of costs associated with it, and always reached an equilibrium frequency. Following the spread of this allele, the frequency of the male-killer could be strongly reduced. Note that as long as there is a cost to resistance, the resistance allele will never spread to fixation in the population. This is because the more frequent the resistant allele, the less frequent will the male-killer become, so that the selective advantage of the resistance allele declines. Thus, in contrast to CI, selection for resistance is negatively frequency dependent.

Recently, the first record of male-killer suppression was reported in the butterfly

Hypolimnas bolina [156]. This species is infected with the *Wolbachia* strain *w*Bol1, which kills males in many South Pacific island populations, but not in Southeast Asian population. It was demonstrated that survival of males in Southeast Asia is caused by dominant zygotic suppression of male-killing. Moreover, historical data of sex-ratio distortion in these populations suggest that the suppressor gene(s) have spread rapidly within the past 30 years.

Similar dynamics of resistance alleles can be expected for populations infected with feminising or parthenogenesis inducing endosymbionts. Resistance against feminising endosymbionts has been studied theoretically for the system of the *Wolbachia*-infected woodlouse *Armadillidium vulgare* [375, 55]. Since resistance alleles here are effectively 'sex ratio' or 'sex determination' genes, these studies will be discussed below in the context of sex determination (see Section 8.6.1).

8.5.4 Parthenogenesis induction and 'virginity' mutants

A rather interesting form of 'resistance' has been proposed if a haplodiploid population is infected with parthenogenesis inducing reproductive parasites [162]. Because males are rare when the endosymbionts are prevalent, it is advantageous for females to produce more sons. Suppose there is a mutation that causes females to fertilise fewer of their eggs, or that causes females not to mate at all. Such a 'virginity' mutant can be expected to spread in a population, because, unless infected, unfertilised eggs will develop into males.

Paradoxically, the spread of a 'virginity' allele will lead to an increasing prevalence of the parthenogenesis inducing endosymbionts [162]. This is because both infected and uninfected females that carry the 'virginity' allele will produce fewer uninfected daughters, whilst infected females will still produce the same number of infected daughters.

The reality of 'virginity' mutations is corroborated by studies of species where sexual populations co-occur allopatrically with asexual populations in which *Wolbachia* was fixed [302, 8]. Males were produced by curing infected females of the asexual populations with antibiotics. These males then readily produced offspring with females from the sexual populations, but failed to mate successfully with females from the asexual populations.

An alternative explanation for these results could be that female mating functions deteriorated during their asexual association with *Wolbachia*. However, this hypothesis cannot explain why female, but not male mating functions deteriorated. The 'virginity' mutation hypothesis thus appears to be a more likely explanation for female mating failure. Deterioration of alleles influencing female mating, fertilisation, male specific traits and the like, are nevertheless expected to gradually deteriorate once parthenogenesis inducing endosymbionts have become fixed in a population, eventually rendering the parasites 'mutualists'.

8.6 Host sex determination and genetic systems

Arthropods exhibit a wealth of different sex determinations and genetic systems [46, 286], suggesting frequent transitions between different systems. It has been proposed that genetic conflict is responsible for many of these transitions [418]. Reproductive parasites exemplify one type of intragenomic conflict and clearly have the potential to influence their hosts genetic system or sex determination. Feminising endosymbionts can override the sex determination of their hosts, parthenogenesis inducing endosymbionts induce a switch in the genetic system of their hosts from haplodiploidy to asexual reproduction (thelytoky), and male-killing bacteria target one sex and thus have some interaction with the host sex determination system. Are these influences merely transient abnormalities that quickly vanish with loss of the endosymbionts, or can we expect more permanent modifications or transition in sex determination and genetic system?

As already discussed (see Section 8.5.4), parthenogenesis inducing bacteria may become fixed and obligate in their host population. This represents a clear-cut example of a permanent transition between genetic systems. In what follows, I will consider two other types of transitions that can be caused by reproductive parasites.

8.6.1 Feminisation in *Armadillidium vulgare*

The best studied system with regards to reproductive parasite mediated evolution of sex determination is the common pill woodlouse *Armadillidium vulgare*. This species normally has female heterogametic sex chromosomes, i.e. WZ individuals develop into females and ZZ individuals into males. However, ZZ individuals infected with *Wolbachia* develop into females, because *Wolbachia* interfere in some way with the production of an androgenic gland that induces male development (see Section 1.4.1).

Modelling predicts rapid extinction of the W chromosome in such a system, because ZZ neo-females produce more daughters than 'real' WZ females [46, 414, 375, 55]. Indeed, in many populations of *A. vulgare*, all individuals are genetic males (ZZ) [322], so that *Wolbachia* has become indispensable for the hosts as the sole factor that determines female development. Because of the scarcity of males, autosomal resistance alleles that reduce transmission of *Wolbachia* can spread, but only as long

as the population sex-ratio is female-biased [55].

Aside from transmission reducing resistance, two evolutionary pathways have been proposed that might ensue the loss of the W chromosome. First, a masculinising mutation (termed M) might arise that overrides the action of *Wolbachia*. The fate of such a mutant that is dominant has been studied theoretically [55]. If M is not costly, it will spread until the population sex-ratio is even. If there is a cost associated with M, the allele will still spread and increase the proportion of males in the population, but an even sex-ratio is not restored. In both cases, the equilibrium frequency of *Wolbachia* is not influenced by the spread of M.

Notably, the genetic constitutions after the spread of M somewhat resemble that of a new male heterogametic (XY) system: Mm individuals are always males, mm individuals are males or females (depending on their infection state), and MM individuals do not occur. Thus, if *Wolbachia* become fixed in the population, a 'pseudo-XY' system has arisen, with half of the population being Mm males and the other half mm females. The crucial difference to a real neo-XY system is, of course, that the sex of females is still determined by the cytoplasmic factor *Wolbachia*.

A second pathway involves another feminizing element termed f. In contrast to M, this element actually occurs in several populations of *A. vulgare*, sometimes with and sometimes without *Wolbachia*[2] [326]. Transmission of this element is also mainly maternal, but transmission is much more irregular than with *Wolbachia*. The nature of the f factor is not clear, but it appears to be a part of the *Wolbachia* genome, e.g., a phage or a transposable element. The f factor can arise spontaneously in *Wolbachia* infected females and sometimes integrates stably into the Z chromosome, leading to a neo-W chromosome [209]. On the basis of these findings a cyclic process has been proposed that consists of the spread of *Wolbachia*, loss of the W chromosome, spread of the f-factor, emergence of a neo-W chromosome through integration of the f factor and loss of *Wolbachia* [209, 326]. However, the population genetics of this process have not been studied. In particular, it is not clear why the neo-W should spread and why *Wolbachia* should go extinct.

[2]Rather confusingly, another masculinising factor, also termed M, exists in *A. vulgare*. However, unlike the hypothetical M factor assumed in Ref. [55] and discussed above, this factor overrides feminisation of the f factor only, but not feminisation induced by *Wolbachia* [326].

In conclusion, *Armadillidium vulgare* offers a fascinating opportunity to study transitions in sex determination systems that are mediated by a reproductive parasite. Sex chromosomes have been lost in some populations of this species, new sex chromosomes have come into existence, and transitions from female to male heterogamety might have occurred. Open issues include the physical nature of the f factor, the interactions between this factor and *Wolbachia*, and a population genetic exploration of the formation of novel ZW or XY sex determination systems.

8.6.2 Reproductive parasites and the origin of haplodiploidy

Haplodiploidy is a genetic systems where males are transmit their maternally derived genome only and are usually haploid, whilst females are diploid and transmit both of their genomes in a Mendelian fashion. Three different hypotheses have been proposed with respect to the role of reproductive parasites in driving the transition from normal diplodiploidy to haplodiploidy.

First, Hamilton postulated the existence of endosymbionts that when in males, destroy chromosomes in sperm [141]. Initially, these endosymbionts would destroy only the Y chromosome in the sperm of infected males, such that all offspring of these males would be female. If the hosts exhibit inbreeding, such male-mediated feminisation would be beneficial for the endosymbionts, because close relatives of the 'sperm feminisers' would find themselves more often in females. As a result of the endosymbiont manipulation, the Y chromosome is thought to become lost from the population and one of the autosomes adopts the function of male determination (neo-Y). This process of elimination of neo-Ys is then assumed to continue until all males are haploid.

The most serious problem with this hypothesis is that the postulated reproductive manipulation (sex chromosome elimination in sperm) has never been reported. However, reproductive parasites like *Wolbachia* are well-known to manipulate their hosts chromosomes (in CI and parthenogenesis induction), so that the proposed mechanism is at least feasible. A second potential problem is that the population genetic details of Y chromosome loss during the process described above have not been worked out, so that it is not clear if the transition to haplodiploidy is a likely outcome.

A second hypothesis on the evolution of haplodiploidy involves male-killing bac-

teria that kill males by destroying their paternally derived chromosomes (see Ref. [287] and Chapter 3). If a host population is infected with such a type of male-killer, selection can be expected to lead to increasing viability and fertility of the haploidised males. Crucially for the argument, such surviving and fertile haploidised males are genetically very precious to their mothers, because they transmit their maternal genome to all of their offspring [42, 287]. Consequently, once the haploidised males are sufficiently viable and fertile, the haploidising endosymbionts become beneficial for their female hosts (see Ref. [287] and Section 3.2). Selection for higher transmission rate on both the hosts' and the endosymbionts' side may then lead to fixation of the endosymbionts. The result of this process may be a population in which all males are haploidised. This genetic system is a special type of haplodiploidy known as 'paternal genome elimination' that has been reported in several groups of insects and mites (see Table 3.1) and might be a precursor to haplodiploidy in the narrow sense ('arrhenotokous haplodiploidy').

Again, it is as yet not known whether there are endosymbionts with the proposed mechanism of male-killing through haploidisation. However, as I have demonstrated theoretically in Section 3.2, co-evolution of haploidising male-killers and their hosts can indeed under some conditions lead to haplodiploidy. In addition, it has been argued that the ancestors of the extent haplodiploid species lived under conditions that made prospering of male-killing endosymbionts likely [287]. These conditions include a diet of phloem sap or wood (making nutritionally beneficial endosymbionts necessary) and gregarious broods (enabling fitness compensation for surviving females if their brothers are killed).

A third hypothesis predicts that CI-inducing endosymbionts facilitated the evolution of haplodiploidy (see Chapter 3). The main appeal of this hypothesis is that CI-inducing symbionts are known to modify chromosomes in sperm in a way that leads to their loss in embryos if the modification is not 'rescued'. The model developed in Section 3.3 demonstrates that indeed, CI-inducing bacteria will select for increasing viability and fertility of haploidised males, which can be seen as a pre-adaptation to the evolution of haplodiploidy. However, in contrast to the two previously discussed hypotheses, direct transition to one or another form of haplodiploidy appears not to be possible through CI-inducing endosymbionts.

8.7 Macroevolution

8.7.1 Host speciation

Species are commonly defined as reproductively isolated sets of populations [260]. Since CI is a reproductive incompatibility between hosts with different infection states of reproductive parasites, it is perhaps not surprising that simultaneously with its discovery in the mosquito *Culex pipiens*, CI has been implicated in promoting host speciation [237, 239]. Another reproductive manipulation that has been implicated in speciation more recently is parthenogenesis induction [416]. Excellent reviews on speciation mediated by reproductive parasites are available [416, 23], so that here, I will cover only CI-induced speciation and restrict the account to the main points and more recent developments.

A first condition that must be fulfilled for CI-mediated speciation to commence is an infection polymorphism across different host populations. This may, for example, be an infected and an uninfected population, or two populations infected with endosymbiont strains inducing bidirectional CI. Both types of infection polymorphism have been reported in several species. For example, species in which some populations are infected with CI-inducing *Wolbachia* and other populations uninfected include *Drosophila melanogaster* [153], the bug *Laodelphax striatellus* [158], the butterfly *Hypolimnas bolina* [60], and the mite *Tetranychus urticae* [35]. Examples of host species polymorphic for infections with bidirectional CI-inducing *Wolbachia* are the mosquito *Culex pipiens* [237, 239, 133, 85], *Drosophila simulans* [293, 267, 192] and the cherry fruit fly *Rhagoletis cerasi* [321, 320].

A second requirement if CI-inducing endosymbionts are to facilitate or trigger speciation is that infection polymorphisms are stable upon contact of the two populations. Modelling predicts that an infection polymorphism with two bidirectional CI-inducing endosymbionts can be stable even for high migration rates [383]. For example, with CI-induced mortalities higher than 70% (as is commonly observed), critical migration rates above which the infection polymorphism collapses were predicted to be at least 10%. Systems of unidirectional CI (i.e., one infected and one uninfected population) are much less stable [114]. In this case, stability is highest for intermediate levels of CI-induced mortality. This is because with high mortality,

the CI-inducing endosymbionts are more likely to invade the uninfected population, whilst with low mortality, they may go extinct.

Empirically, it is not known for most of the examples given above how stable polymorphisms are (or would be if there was any contact between the respective populations). One exception is the well-studied species complex of the mosquito *Culex pipiens*, where a surprising number of CI-inducing variants of *Wolbachia* have been described [237, 239, 133, 85]. CI-induced mortality is usually very high in bidirectionally incompatible crosses of *C. pipiens*, potentially leading to high stability of the infection polymorphism. Unpublished data suggest that some of the infection polymorphisms in *Culex pipiens* can stably persist for at least a few decades [86].

Third, nuclear divergence between host populations must be maintained in spite of migration between these populations. This issue has been studied using population genetic models of two populations connected by migration [380, 381, 379]. One nuclear locus with two alleles was considered, with each allele being locally beneficial in one of the populations. This yields (if migration rates are not too high) stable allele frequencies in the two populations that are maintained by a migration/selection balance. Gene flow in the presence of CI-inducing bacteria was then measured by the 'effective migration rate', defined as the migration rate that in the absence of CI-inducing bacteria leads to the same allele frequencies as when CI is present.

For unidirectional CI, a scenario has been investigated in which migration occurred from an infected to an uninfected population, but not into the opposite direction [379]. In this case, gene flow can be substantially reduced, even though only the offspring of infected male migrants suffer from CI. This can be understood when it is recognised that infected female migrants produce predominantly infected offspring, the males among which again suffer from CI. In the extreme case of perfect maternal transmission and 100% CI-induced mortality, there would be no gene flow from the infected to the uninfected population because all migrant genes would be infinitely diluted in the uninfected population. With bidirectional CI, models with migration in one and in both directions have been studied [380, 381]. Here, gene flow can be strongly reduced in both directions, especially if CI-induced mortality is high.

Currently, the best empirical support for CI-mediated reduction of gene flow comes from investigations of three wasp species of the genus *Nasonia*. All three species are

bidirectionally incompatible due to infection with different strains of *Wolbachia* [36, 25]. Unfortunately, in addition to CI, the sympatrically co-occurring *N. vitripennis* and *N. giraulti* exhibited several severe forms of nuclear incompatibility [38, 27]. It has not yet been possible to determine if CI preceded these nuclear incompatibilities (and could thus have been responsible for speciation) or not. CI has been reported to be the main reproductive isolation for a second, younger species pair *N. giraulti* and *N. longicornis* [25]. However, since these species do not co-occur sympatrically, *Wolbachia* may not have actively promoted speciation by restricting gene flow.

Finally, it has been suggested that speciation initiated by CI-inducing endosymbionts may be completed through selection for premating isolation. In the context of nuclear incompatibilities, this idea goes back to Dobzhansky and has been coined 'reinforcement' [81, 72, 285, 350]. Telschow *et al.* have studied theoretically the fate of a female preference allele for males carrying a locally adaptive allele and compared situations where incompatibilities are caused by cytoplasmic elements and nuclear alleles [382, 379]. The models demonstrated that with high levels of incompatibility, bidirectional CI can select for premating isolation and is much more effective in doing so than epistatic two-locus nuclear incompatibilities [382]. Unidirectional CI, with migration from an infected to an uninfected population, can also select for premating isolation [379]. However, the effect is weaker here because the infection polymorphism is not maintained with high levels of CI-induced mortality.

Some asymmetric and incomplete mate discrimination in the above-mentioned species pair *Nasonia giraulti* and *N. longicornis* has been reported [25], but it is not clear if this pre-mating isolation evolved through selection to avoid incompatible matings. The best empirical evidence for CI-induced reinforcement comes from a recent study of the species pair *Drosophila recens–D. subquinaria* [190]. *D. recens* is infected with CI-inducing *Wolbachia*, whilst *D. subquinaria* is uninfected. Thus, matings between *D. recens* males and *D. subquinaria* females result in high offspring mortality, whilst all other crosses are compatible [351]. There is some overlap in the geographical distributions of these species, such that allopatric and sympatric populations of both species occur. Mass population choice and no-choice trials demonstrated that *D. quinaria* females from sympatric populations exhibited significantly stronger mating discrimination against *D. recens* males than did allopatric *D. subquinaria* females

[190]. By contrast, mate discrimination against *D. subquinaria* males did not differ significantly between allopatric and sympatric *D. recens* females. These results, combined with data on general genetic differentiation among populations, strongly suggest that mate discrimination in *D. subquinaria* females results from selection against mating with CI-infected *D. recens* males.[3]

In summary, theoretical models have demonstrated that both uni- and bidirectional CI can promote speciation under some conditions. However, empirical support is scarce, largely because of an absence of intensive studies rather than failure to detect any influence of CI on speciation. Two exceptions are the well-studied systems of the wasp genus *Nasonia*, and the *Drosophila subquinaria–D. recens* species pair. Investigations of both of these systems generally support the view of *Wolbachia* mediated speciation. More empirical studies investigating the geographical distribution of CI-inducing bacteria as well as the impact of CI on host gene flow and reinforcement are needed. On the theoretical side, detailed models of the interaction of CI-induced reproductive isolation with nuclear incompatibilities appear a promising avenue of investigation.

8.7.2 Host extinction

For obvious reasons, extinction is a notoriously difficult subject to study in natural populations. Thus, it is perhaps not surprising that virtually nothing is known about the impact of reproductive parasites on host extinction patterns and rates. Here, I outline some theoretical routes towards host extinction caused or facilitated by reproductive parasites, and assess their likelihood.

The most direct threat to extinction can be expected to be posed by feminising or male-killing inducing endosymbionts. With increasing prevalence of either of these reproductive parasites, the population sex ratio will become increasingly

[3]Interestingly, the data on *D. quinaria* mating preference also provide a novel mode of *Wolbachia* induced speciation that has not been anticipated by theory. *D. subquinaria* females are not only reluctant to mate with *D. recens* males, but also with allopatric *D. subquinaria* males. This may be interpreted such that mate choice does not function as discrimination against *D. recens* males *per se*, but as discrimination against all males other than sympatric *D. subquinaria* males. Thus, reinforcement has lead to behavioural isolation between different populations of *D. subquinaria*, implicating that CI may promote speciation even within species that are uninfected.

female-biased, and due to limited mating capacity of males, some females may remain unmated throughout their lives. At a certain threshold sex ratio, not enough females may be fertilised by the few males present to keep the population size stable, and extinction of the host population ensues. This process has been modelled for the case of feminising endosymbionts [146]. The necessary number of male matings to keep the population viable was demonstrated to rise rapidly to unrealistic values for parasite transmission rates above 0.9. Thus, feminizing endosymbionts with high transmission rates make extinction of the host population likely. However, this model assumed that the efficiency of feminisation is 100%; incomplete penetrance of feminisation (as is often found in microsporidian induced feminisation) will decrease the subset of the parameter space where the host population goes extinct.

Male-killing endosymbionts were not considered in this model, but the qualitative effects will be very similar. Quantitative comparison is not straightforward, however. On the one hand, male-killers are less efficient reproductive manipulators than feminizing endosymbionts and need a higher transmission rate and fitness compensation to reach similar prevalence. On the other hand, however, male-killers directly reduce the population size at least at the larval stage, so that the risk of host extinction may be even higher than with feminizing endosymbionts. In addition, male-killing is usually very efficient, and in many cases all sons of infected mothers die. This contrasts with feminisation, whose penetrance and transmission efficiency may be lower, such that even at high prevalence, significant numbers of males are produced.

An interesting empirical case is the butterfly *Hypolimnas bolina*, where an extreme sex-ratio biases of 1:100 due to male-killer infection has been observed on Independent Samoa [90]. Surprisingly, the host population appears to have been viable under this sex-ratio for at least 100 years (400 generations). Apparently, the population could persist because of an extremely high lifetime mating rate of males (estimated to be around 50 females), combined with a high intrinsic growth rate of this species and potentially, with the absence of any competition or other natural enemies on isolated oceanic islands. It remains to be seen how much of an exception *Hypolimnas bolina* is with respect to its ability to resist male-killer mediated extinction.

CI-inducing endosymbionts are unlikely to cause host extinction. Although the host population size may be somewhat reduced during the spread of CI, this reduc-

tion does not normally appear sufficiently severe to cause population extinction [80]. However, host extinction may occur more readily when a mod-only mutant spreads in the population, i.e. a strain that modifies sperm, but cannot rescue its own modification. As discussed above (Section 8.5.1) and in Chapter 5, such a strain can spread in a population either neutrally (in panmictic populations) or driven by selection (in outbreeding populations). In a simulation study on the evolution of CI, extinction of the host population caused by such a mod-only strains was observed frequently, at least when population sizes were small [58]. Likewise, in a theoretical study entirely devoted to the mod-only phenotype (without any other CI-strains), host population extinction following invasion of the mod-only strain was observed for a large range of parameter values [94].

The above considerations apply to extinction of populations infected with reproductive parasites, but not necessary to entire host species. Clearly, local extinction (before the reproductive parasite can spread to other populations) followed by recolonisation are plausible. Some light has been shed on this issue by a lattice model of a male-killer infected population [132]. Here, even male-killers with perfect vertical transmission did not cause global, but only local extinction.

In contrast to feminising, male-killing, and the hypothetical mod-only variant of CI-inducing endosymbionts, parthenogenesis inducing bacteria are not expected to directly lead to host extinction. This is simply because no mortality is induced and lack of males is readily tolerated. However, parthenogenesis inducing endosymbionts may increase extinction rates of their hosts indirectly by depriving them of the benefits of sexual reproduction. This view is corroborated by the scattered distribution of asexual species at the tips of phylogenetic trees, suggesting that asexual taxa appear occasionally, but are evolutionarily short-lived [48].

8.7.3 Dynamics of reproductive parasite incidence

Reproductive parasites, in particular *Wolbachia* are extremely widespread and common in arthropods. Several surveys have demonstrated that *Wolbachia* infect about 20% of all host species, but there are significant differences in *Wolbachia* incidence across different arthropod groups (see Section 7.1.1). Phylogenetic analyses have demonstrated that *Wolbachia* spread predominantly by horizontal transfer from one

to another species [426, 343, 450], but the patterns of these transfers are not understood. What factors influence the probability of horizontal transmission of reproductive parasites and other endosymbionts? What determines the frequency of 20% *Wolbachia* incidence within arthropods? And how can we explain the heterogeneous incidence of *Wolbachia* and other reproductive parasites across different host taxa?

A first theoretical attempt to answer such questions has been made in Chapter 7. Here, I have assumed a fixed clade of host species and a horizontal transmission probability of endosymbionts that declines with increasing genetic distance between donor and recipient host species. My results suggest that host phylogeny alone can lead to heterogenous incidence of reproductive parasites or other endosymbionts. Host clades with symmetric phylogeny or clades that have undergone recent adaptive radiation can be expected to have a higher incidence than clades with asymmetric phylogenies. I have also studied the effect of refractoriness to infection, which can considerably lower the probability of endosymbiont invasion into the host clade. Finally, I investigated the effect of an additional strain of endosymbionts that infects the host clade and found that the strains can antagonistically affect the respective other strain's incidence.

As horizontal transmission events are rare, future models should also include evolution of the host clade. In particular, possible influences of reproductive parasites on extinction and speciation rates of their host species (see previous two sections) should be incorporated. In some host groups where *Wolbachia* incidence is very high (e.g., fig wasps and ants [352, 137, 411, 29]) or very low (e.g., aphids), specific explanation based on the particular biology of these host species may be found [411].

As yet, empirical research on the patterns of spread of *Wolbachia* in arthropods has been limited by the fact that the vast majority of phylogenetic trees constructed for *Wolbachia* were based on the sequence of a single gene (usually *wsp* or *ftsZ*). Because *Wolbachia* are known to recombine frequently when two strains co-occur within hosts [206, 417, 13], these gene trees are not reflective of the 'true phylogeny' of the bacteria. Recently, a multilocus sequence typing (MLST) system has been proposed for *Wolbachia*, involving five *Wolbachia* genes that have been selected according to their variability and proximity in known *Wolbachia* genomes [14, 12]. If accepted by the research community (despite its higher demands in terms of workload and costs),

this MLST system promises to provide fascinating insights into transmission routes of *Wolbachia* and its patterns of global spread within arthropods.

Bibliography

[1] Adamson ML. Evolutionary biology of the Oxyurida (Nematoda): biofacies of a haplodiploid taxon. Advances in Parasitology 28, 175–228 (1989)

[2] Aksoy S. Symbiosis in Tsetse. In: Insect Symbiosis, Bourtzis K, Miller TA (eds), CRC Press, Boca Raton, pp. 53–65 (2003)

[3] Anderson RM, May RM. Population Biology of Infectious Diseases I. Nature 280, 361–367 (1979)

[4] Anderson RM, May RM. The population dynamics of microparasites and their invertebrate hosts. Philosophical transactions of the Royal Society of London 291, 451–524 (1981)

[5] Andersson SGE, Zomorodipour A, Andersson JO, Sicheritz-Pontén T, Alsmark UCM, Podowski RM, Näslund AK, Eriksson AS, Winkler HH, Kurland CG. The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. Nature 396, 133–140 (1998)

[6] Andreadis TG. Life-cycle, epizootiology, and horizontal transmission of *Amblyospora* (Microspora: Amblyosporidae) in a univoltine mosquito *Aedes stimulans*. Journal of Invertebrate Pathology 46, 31–46 (1985)

[7] Arakaki N, Miyoshi T, Noda H. *Wolbachia*-mediated parthenogenesis in the predatory thrips *Franklinothrips vespiformis* (Thysanoptera: Insecta). Proceedings of the Royal Society of London Series B-Biological Sciences 268, 1011–1016 (2001)

[8] Arakaki N, Noda H, Yamagishi K. *Wolbachia*-induced parthenogenesis in the egg parasitoid *Telenomus nawai*. Entomologia Experimentalis et Applicata 96, 177–184 (2000)

[9] Arkhipova I, Meselson M. Transposable elements in sexual and ancient asexual taxa. Proceedings of the National Academy of Science of the USA 97, 14473-14477 (2000)

[10] Baalen M van, Sabelis MW. The Dynamics of Multiple Infection and the Evolution of Virulence. American Naturalist 146, 881- 910 (1995)

[11] Balas MT, Lee MH, Werren JH. Distribution and fitness effects of the son-killer bacterium in *Nasonia*. Evolutionary Ecology 10, 593–607 (1996)

[12] Baldo L (curator). Website of the *Wolbachia* multilocus sequence typing system: http://www.pubmlst.org/wolbachia/

[13] Baldo L, Bordenstein SR, Wernegreen JJ, Werren JH. Widespread recombination throughout *Wolbachia* genomes. Molecular Biology and Evolution 23, 437–449 (2006)

[14] Baldo L, Dunning Hotopp JC, Jolley KA, Bordenstein SR, Biber SA, Choudhury RR, Hayashi C, Maiden MCJ, Tettelin H, Werren JH. A Multilocus Sequence Typing System for the Endosymbiont *Wolbachia*. Applied and Environmental Microbiology, in press.

[15] Bandi C, Anderson TJC, Genchi C, Blaxter ML. Phylogeny of *Wolbachia* in filarial nematodes. Proceedings of the Royal Society of London Series B-Biological Sciences 265, 2407–2413 (1998)

[16] Bandi C, Damiani G, Magrassi L, Grigolo A, Fani R, Sacchi L. Flavobacteria as intracellular symbionts in cockroaches. Proceedings of the Royal Society of London Series B-Biological Sciences 257, 43–48 (1994)

[17] Bandi C, Sironi M, Damiani G., Magrassi L., Nalepa CA, Laudani U, Sacchi L. The establishment of intracellular symbiosis in an ancestor of cockroaches and termites. Proceedings of the Royal Society of London Series B-Biological Sciences 259, 293–299 (1995)

[18] Bandi C, Trees AJ, Brattig NW. *Wolbachia* in filarial nematodes: evolutionary aspects and implications for the pathogenesis and treatment of filarial diseases. Veterinary Parasitology 98, 215–238 (2001)

[19] Barros R. Um caso de alterao na proporo entre os sexos em *Drosophila mercatorum pararepleta*. Cien. Cult. 1, 107–110 (1949)

[20] Beckenbach AT. The 'sex-ratio' trait in *Drosophila pseudoobscura*: fertility relations of males and meiotic drive. American Naturalist 112, 97–117 (1978)

[21] Beckenbach AT. Selection and the 'sex-ratio' polymorphism in natural populations of *Drosophila pseudoobscura*. Evolution 50, 787–794 (1996)

[22] Binnington KC, Hoffmann AA. *Wolbachia*-like Organisms and Cytoplasmic Incompatibility in *Drosophila simulans*. Journal of Invertebrate Pathology 54: 344-352 (1989)

[23] Bordenstein SR. Symbiosis and the Origin of Species. In: Bourtzis K, Miller TA (eds) Insect Symbiosis, CRC Press: Boca Raton, 283–304 (2003)

[24] Bordenstein SR, Marshall ML, Fry AJ, Kim U, Wernegreen JJ. The tripartite associations between bacteriophage, *Wolbachia*, and arthropods. PLoS Pathogens 2, e43 (2006)

[25] Bordenstein SR, O'Hara FP, Werren JH. *Wolbachia*-induced incompatibility precedes other hybrid incompatibilities in *Nasonia*. Nature 409, 707–710 (2001)

[26] Bordenstein SR, Rosengaus RB. Discovery of a novel *Wolbachia* supergroup in isoptera. Current Microbiology 51, 393–398 (2005)

[27] Bordenstein SR, Werren JH. Effects of A and B *Wolbachia* and Host Genotype on Interspecies Cytoplasmic Incompatibility in *Nasonia*. Genetics 148, 1833–1844 (1998)

[28] Borgia G. Evolution of haplodiploidy: models for inbred and outbred systems. Theoretical Population Biology 17, 103–128 (1980)

[29] Borm S van, Wenseleers T, Billen J, Boomsma JJ. *Wolbachia* in leafcutter ants: a widespread symbiont that may induce male killing or incompatible matings. Journal of Evolutionary Biology 14, 805–814 (2001)

[30] Bouchon D, Rigaud T, Juchault P. Evidence for widespread *Wolbachia* infection in isopod crustaceans: molecular identification and host feminization. Proceedings of the Royal Society of London Series B-Biological Sciences 265, 1081–1090 (1998)

[31] Bourtzis K, Nirgianaki A, Markakis G, Schaefer PW. *Wolbachia* Infection and Cytoplasmic Incompatibility in *Drosophila* Species. Genetics 144, 1063–1073 (1996)

[32] Boyle L, O'Neill SL, Robertson HM, Karr TL. Interspecific and intraspecific horizontal transfer of *Wolbachia* in *Drosophila*. Science 260, 1796–1799 (1993)

[33] Braig HR, Guzman H, Tesh RB, O'Neill SL. Replacement of the natural *Wolbachia* symbiont of *Drosophila simulans* with a mosquito counterpart. Nature 367, 453–455 (1994)

[34] Breeuwer JAJ. *Wolbachia* and Cytoplasmic Incompatibility in the Spider Mites *Tetranychus urticae* and *T. turkestani*. Heredity 79, 41–47 (1997)

[35] Breeuwer JAJ, Jacobs G. *Wolbachia*: Intracellular manipulators of mite reproduction. Experimental and Applied Acarology 20, 421–434 (1996)

[36] Breeuwer JAJ, Werren JH. Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. Nature 346, 558–560 (1990)

[37] Breeuwer JAJ, Werren JH. Cytoplasmic Incompatibility and Bacterial Density in *Nasonia vitripennis*. Genetics 135, 565–574 (1993)

[38] Breeuwer JAJ, Werren JH. Hybrid Breakdown between Two Haplodiploid Species: The Role of Nuclear and Cytoplasmic Genes. Evolution 49, 705–717 (1995)

[39] Bremermann HJ, Thieme HR. A competitive-exclusion principle principle for pathogen virulence. Journal of Mathematical Biology 27, 179–190 (1989)

[40] Bretman A, Wedell N, Tregenza T. Molecular evidence of post-copulatory inbreeding avoidance in the field cricket *Gryllus bimaculatus*. Proceedings of the Royal Society of London Series B-Biological Sciences 271, 159–164 (2004)

[41] Britten HB, Glasford JW. Genetic population structure of the Dakota skipper (Lepidoptera: *Hesperia dacotae*): A North American native prairie obligate. Conservation Genetics 3, 363–374 (2004)

[42] Brown SW. Automatic frequency response in the evolution of male haploidy and other coccid chromosome systems. Genetics 49, 797–817 (1964)

[43] Brown SW, Chandra HS. Chromosome Imprinting and the Differential Regulation of Homologous Chromosomes. In: Cell Biology - A Comprehensive Treatise, Vol. 1, Goldstein L, Prescott DM (eds), Academic Press, New York, pp. 110–189 (1977)

[44] Brun LO, Borsa P, Gaudichon V, Stuart JJ, Aronstein K, Coustau C, ffrench-Constat RH. 'Functional' haplodiploidy. Nature 374, 506 (1995)

[45] Bull JJ. An advantage for the evolution of male haploidy and systems with similar transmission. Heredity 43, 361–381 (1979)

[46] Bull JJ. The evolution of sex determination mechanisms. The Benjamin / Cummings Publishing Company, Inc., Menlo Park (1983)

[47] Burt A, Trivers R. Genes in conflict: The biology of selfish genetic elements. Belknap Press (2006)

[48] Butlin R. The costs and benefits of sex: new insights from old asexual lineages. Nature Reviews Genetics 3, 311–317 (2002)

[49] Callaini G, Dallai R, Riparbelli MG. *Wolbachia*-induced delay of paternal chromatin condensation does not prevent maternal chromosomes from entering anaphase in incompatible crosses of *Drosophila simulans*. Journal of Cell Science 110, 271–280 (1997)

[50] Carson HL. A female-producing strain of *Drosophila borealis* Patterson. Drosophila Information Service 30, 109–110 (1956)

[51] Carvalho AB, Peixoto AA, Klaczko LB. Sex-ratio in *Drosophila mediopunctata.* Heredity 71, 546–551 (1989)

[52] Carvalho AB, Vaz SC. Are *Drosophila* SR drive chromosomes always balanced? Heredity 83, 221–228 (1999)

[53] Casiraghi M, Bordenstein SR, Baldo L, Lo N, Beninati T, Wernegreen JJ, Werren JH, Bandi C. Phylogeny of *Wolbachia pipientis* based on gltA, groEL and ftsZ gene sequences: clustering of arthropod and nematode symbionts in the F supergroup, and evidence for further diversity in the *Wolbachia* tree. Microbiology 151, 4015–4022 (2005)

[54] Caspari E, Watson GS. On the Evolutionary Importance of Cytoplasmic Sterility in Mosquitoes. Evolution 13, 568–570 (1959)

[55] Caubet Y, Hatcher MJ, Mocquard JP, Rigaud T. Genetic conflict and changes in heterogametic mechanisms of sex determination. Journal of Evolutionary Biology 13, 766–777 (2000)

[56] Cavalcanti AGL, Falcão DN, Castro LE. "Sex-ratio" in *Drosophila prosaltans* - A character due to interaction between nuclear genes and cytoplasmic factors. American Naturalist 91, 327–329 (1957)

[57] Charlat S, Bonnavion P, Merçot H. *Wolbachia* segregation dynamics and levels of cytoplasmic incompatibility in *Drosophila sechellia.* Heredity 90, 157–161 (2003)

[58] Charlat S, Calmet C, Andrieu O, Merçot H. Exploring the evolution of *Wolbachia* compatibility types: a simulation approach. Genetics 170, 495–507 (2005)

[59] Charlat S, Calmet C, Merçot H. On the mod resc model and the evolution of *Wolbachia* compatibility types. Genetics 159, 1415–1422 (2001)

[60] Charlat S, Engelstädter J, Dyson EA, Hornett EA, Duplouy AMR, Tortosa P, Davis N, Roderick GK, Wedell N, Hurst GDD. Competing selfish genetic elements in the butterfly *Hypolimnas bolina*. Current Biology, in press.

[61] Charlat S, Hornett EA, Dyson EA, Ho PPY, Loc NT, Schilthuizen M, Davis N, Roderick GK, Hurst GDD. Prevalence and penetrance variation of male-killing *Wolbachia* across Indo-Pacific populations of the butterfly *Hypolimnas bolina*. Molecular Ecology 14, 3525–3530 (2005)

[62] Charlat S, Nirgianaki A, Bourtzis K, Merçot H. Evolution of *Wolbachia*-induced cytoplasmic incompatibility in *Drosophila simulans* and *D. sechellia*. Evolution 56, 1735–1742 (2002)

[63] Charlat S, Riegler M, Baures I, Poinsot D, Stauffer C, Merçot H. Incipient Evolution of *Wolbachia* Compatibility Types. Evolution 58, 1901–1908 (2004)

[64] Charleston MA. Jungles: a new solution to the host/parasite phylogeny reconciliation problem. Mathematical Biosciences 149, 191–223 (1998)

[65] Charleston MA, Robertson DL. Preferential Host Switching by Primate Lentiviruses Can Account for Phylogenetic Similarity with the Primate Phylogeny. Systematic Biology 51, 528–535 (2002)

[66] Charlesworth B, Morgan MT, Charlesworth D. The Effect of Deleterious Mutations on Neutral Molecular Variation. Genetics 134, 1289–1303 (1993)

[67] Cheng Q, Ruel TD, Zhou W, Moloo SK, Majiwa P, O'Neill SL, Aksoy S. Tissue distribution and prevalence of *Wolbachia* infections in tsetse flies, *Glossina* spp. Medical and Veterinary Entomology 14, 44–50 (2000)

[68] Clancy DJ, Hoffmann AA. Behavior of *Wolbachia* endosymbionts from *Drosophila simulans* in *D. serrata*, a novel host. American Naturalist 149, 975–988 (1997)

[69] Clark AG. Natural selection with nuclear and cytoplasmic transmission. I. A deterministic model. Genetics 107: 679-701 (1984)

[70] Clark ME, Heath BD, Anderson CL, Karr TL. Induced paternal effects mimic cytoplasmic incompatibility in *Drosophila*. Genetics 173, 727–734 (2006)

[71] Cosmides LM, Tooby J. Cytoplasmic Inheritance and Intragenomic Conflict. Journal of Theoretical Biology 89, 83–129 (1981)

[72] Coyne JA, Orr HA. The evolutionary genetics of speciation. Philosophical transactions of the Royal Society of London B 353, 287–305 (1998)

[73] Crespigny FEC de, Butlin R, Wedell N. Can cytoplasmic incompatibility inducing *Wolbachia* promote the evolution of mate preferences? Journal of Evolutionary Biology 18, 967–977 (2005)

[74] Cruickshank RH, Thomas RH. Evolution of haplodiploidy in dermanyssine mites (Acari: Mesostigmata). Evolution 53, 1796–1803 (1999)

[75] Curtsinger JW, Feldman MW. Experimental and Theoretical Analysis of the "Sex-Ratio" Polymorphism in *Drosophila pseudoobscura*. Genetics 94, 445-466 (1980)

[76] Dedeine F, Vavre F, Shoemaker DD, Boulétreau M. Intra-individual coexistence of a *Wolbachia* strain required for host oogenesis with two strains inducing cytoplasmic incompatibility in the wasp *Asobara tabida*. Evolution 58, 2167–2174 (2004)

[77] Dobson SL. *Wolbachia pipientis*: Impotent by Association. In: Bourtzis K, Miller TA (eds), Insect Symbiosis. CRS, pp. 199–215 (2003)

[78] Dobson SL. Evolution of *Wolbachia* cytoplasmic incompatibility types. Evolution 58, 2156–2166 (2004)

[79] Dobson SL, Bourtzis K, Braig HR, Jones BF, Zhou WG, Rousset F, O'Neill SL. *Wolbachia* infections are distributed throughout insect somatic and germ line tissues. Insect Biochemistry and Molecular Biology 29, 153–160 (1999)

[80] Dobson SL, Fox CK, Jiggins CD. The effect of *Wolbachia*-induced cytoplasmic incompatibility on host population size in natural and manipulated systems.

Proceedings of the Royal Society of London Series B-Biological Sciences 269, 437–445 (2002)

[81] Dobzhansky T. Genetics and the origin of species. Columbia University Press, New York (1937)

[82] Douglas AE. *Buchnera* Bacteria and Other Symbionts of Aphids. In: Bourtzis K, Miller TA (eds) Insect Symbiosis, CRC Press: Boca Raton. pp 165–176 (2003)

[83] Düsing K. Die Factoren, welche die Sexualität entscheiden. Jenaische Zeitschrift für Naturwissenschaft 16, 428–464 (1883)

[84] Dunn AM, Adams J, Smith JE. Transovarial transmission and sex-ratio distortion by a microsporidian parasite in a shrimp. Journal of Invertebrate Pathology 61, 248–252 (1993)

[85] Duron O, Bernard C, Unal S, Berthomieu A, Berticat C, Weill M. Tracking factors modulating cytoplasmic incompatibilities in the mosquito *Culex pipiens*. Molecular Ecology 15, 3061–3071 (2006)

[86] Duron O *et al.*. Manuscript in preparation.

[87] Dutton TJ, Sinkins SP. Strain-specific quantification of *Wolbachia* density in *Aedes albopictus* and effects of larval rearing conditions. Insect Molecular Biology 13, 317–322 (2004)

[88] Dyer KA, Jaenike J. Evolutionarily Stable Infection by a Male-Killing Endosymbiont in *Drosophila innubila*. Genetics 168, 1443–1455 (2004)

[89] Dyer KA, Minhas MS, Jaenike J. Expression and modulation of embryonic male-killing in *Drosophila innubila*: Opportunities for multilevel selection. Evolution 59, 838–848 (2005)

[90] Dyson EA, Hurst GDD. Persistence of an extreme sex-ratio bias in a natural population. Proceedings of the National Academy of Sciences of the United States of America 101, 6520–6523 (2004)

[91] Dyson EA, Kamath MK, Hurst GDD. *Wolbachia* infection associated with all-female broods in *Hypolimnas bolina* (Lepidoptera: Nymphalidae): evidence for horizontal transmission of a butterfly male killer. Heredity 88, 166–171 (2002)

[92] Egas M, Vala F, Breeuwer JAJ. On the evolution of cytoplasmic incompatibility in haplodiploid species. Evolution 56, 1101–1109 (2002)

[93] Emlen ST, Oring LW. Ecology, sexual selection and evolution of mating systems. Science 197, 215–223 (1977)

[94] Engelstädter J, Charlat S. Outbreeding selects for spiteful cytoplasmic elements. Proceedings of the Royal Society of London Series B-Biological Sciences 273, 923–929 (2006)

[95] Engelstädter J, Charlat S, Pomiankowski A, Hurst GDD. The evolution of cytoplasmic incompatibility: integrating segregation, inbreeding and outbreeding. Genetics 172, 2601–2611 (2006)

[96] Engelstädter J, Hammerstein P, Hurst GDD. The evolution of endosymbiont density in doubly infected host species. Journal of Evolutionary Biology, in press.

[97] Engelstädter J, Hurst GDD. Can maternally inherited endosymbionts facilitate the evolution of haplodiploidy? Journal of Evolutionary Biology 19, 194-202 (2006)

[98] Engelstädter J, Hurst GDD. The dynamics of parasite incidence across host species. Evolutionary Ecology, in press.

[99] Engelstädter J, Hurst GDD. The impact of male-killing bacteria on host evolutionary processes. Genetics, in press.

[100] Engelstädter J, Montenegro H, Hurst GDD. To what extent do different types of sex ratio distorters interfere? Evolution 58, 2382–2386 (2004).

[101] Engelstädter J, Telschow A, Hammerstein P. Infection dynamics of different *Wolbachia*-types within one host population. Journal of Theoretical Biology 231, 345–355 (2004)

[102] Enigl M, Schausberger P. Mate choice in the predaceous mite *Phytoseiulus persimilis*: evidence of self-referent phenotype matching? Entomologia Experimentalis et Applicata 112, 21–28 (2004)

[103] Eshel I. Selection on sex ratio and the evolution of sex determination. Heredity 34, 351–361 (1975)

[104] Ewald PW. Transmission modes and evolution of the parasite-mutualist continuum. Annals of the New York Academy of Sciences 503, 295–305 (1987)

[105] Faulhaber SH. An abnormal sex ratio in *Drosophila simulans*. Genetics 56, 189–213 (1967)

[106] Fenn K, Blaxter M. *Wolbachia* genomes: revealing the biology of parasitism and mutualism. Trends in Parasitology 22, 60–65 (2006)

[107] Ferree PM, Frydman HM, Li JM, Cao J, Wieschaus E, Sullivan W. *Wolbachia* utilizes host microtubules and Dynein for anterior localization in the *Drosophila* oocyte. PLoS Pathogens 1, e14 (2005)

[108] Fialho RF, Stevens L. Male-killing *Wolbachia* in a flour beetle. Proceedings of the Royal Society of London Series B-Biological Sciences 267, 1469–1473 (2000)

[109] Fialho RF, Stevens L. Molecular evidence for single *Wolbachia* infections among geographic strains of the flour beetle *Tribolium confusum*. Proceedings of the Royal Society of London Series B-Biological Sciences 264, 1065–1068 (1997)

[110] Fine PEM. Vectors and Vertical Transmission: An Epidemiologic Perspective. Annals of the New York Academy of Science 266, 173–194 (1975)

[111] Fine PEM. On the Dynamics of Symbiote-Dependent Cytoplasmic Incompatibility in Culicine Mosquitoes. Journal of Invertebrate Pathology 30, 10–18 (1978)

[112] Fisher RA. On the dominance ratio. Proceeding of the Royal Society of Edinburgh 50, 321–341 (1922)

[113] Fisher RA. The Genetical Theory of Natural Selection. Oxford University Press, Oxford (1930)

[114] Flor M, Hammerstein P, Telschow A. Dynamics and stability of *Wolbachia*-induced unidirectional cytoplasmic incompatibility in parapatric host populations. submitted.

[115] Foster J, Ganatra M, Kamal I, Ware J, Makarova K, Ivanova N, Bhattacharyya A, Kapatral V, Kumar S, Posfai J, Vincze T, Ingram J, Moran L, Lapidus A, Omelchenko M, Kyrpides N, Ghedin E, Wang S, Goltsman E, Joukov V, Ostrovskaya O, Tsukerman K, Mazur M, Comb D, Koonin E, Slatko B. The *Wolbachia* genome of *Brugia malayi*: Endosymbiont evolution within a human pathogenic nematode. PLoS Biology 3, 599–614 (2005)

[116] Frank SA. Cytoplasmic Incompatibility and Population Structure. Journal of Theoretical Biology 184, 327–330 (1997)

[117] Frank SA. Dynamics of Cytoplasmic Incompatability with Multiple *Wolbachia* Infections. Journal of Theoretical Biology 192, 213–218 (1998)

[118] Frank SA, Hurst LD. Mitochondria and male disease. Nature 383, 224 (1996)

[119] Franzen C. Microsporidia: How can they invade other cells? Trends in Parasitology 20, 274–279 (2004)

[120] Freeland SJ, McCabe BK. Fitness compensation and the evolution of selfish cytoplasmic elements. Heredity 78, 391–402 (1997)

[121] Frydman HM, Li JM, Robson DN, Wieschaus E. Somatic stem cell niche tropism in *Wolbachia*. Nature 441, 509–512 (2006)

[122] Fujii Y, Kageyama D, Hoshizaki S, Ishikawa H, Sasaki T. Transfection of *Wolbachia* in Lepidoptera: the feminizer of the adzuki bean borer *Ostrinia scapulalis* causes male killing in the Mediterranean flour moth *Ephestia kuehniella*. Proceedings of the Royal Society of London Series B-Biological Sciences 268, 855–859 (2001)

[123] Gershenson S. A New Sex-Ratio Abnormality in *Drosophila obscura*. Genetics 13, 488–507 (1928)

[124] Ghelelovitch S. Sur le determinisme génétique de la sterilitée dans le croisement entre differentes souches de *Culex autogenicus* Roubaud. C. R. Acad. Sci. Paris 24, 238688 (1952)

[125] Ghema RL, Werren JH, Weisburg W, Cote R, Woese CR, Mandelco, L, Brenner DJ. *Arsenophonus nasoniae* gen. nov., sp. nov., the causative agent of the son-killer trait in the parasitic wasp *Nasonia vitripennis.* International Journal of Systematic Bacteriology 41, 563–565 (1991)

[126] Giordano R, O'Neill SL, Robertson HM. *Wolbachia* Infections and the Expression of Cytoplasmic Incompatibility in *Drosophila sechellia* and *D. mauritiana.* Genetics 140, 1307–1317 (1995)

[127] Goldstein DB. Deleterious Mutations and the Evolution of Male Haploidy. American Naturalist 144, 176–183 (1994)

[128] Gomez-Valero L, Soriano-Navarro M, Perez-Brocal V, Heddi A, Moya A, Garcia-Verdugo M, Latorre A. Coexistence of *Wolbachia* with *Buchnera aphidicola* and a Secondary Symbiont in the Aphid *Cinara cedri.* Journal of Bacteriology 186, 6626–6633 (2004)

[129] Goodacre SL, Martin OY, Thomas CFG, Hewitt GM. *Wolbachia* and other endosymbiont infections in spiders. Molecular Ecology 15, 517–527 (2006)

[130] Gotoh T, Noda H, Hong X-Y. *Wolbachia* distribution and cytoplasmic incompatibility based on a survey of 42 spider mite species (Acari: Tetranychidae) in Japan. Heredity 91, 208–216 (2003)

[131] Grenier S, Pintureau B, Heddi A, Lassablière F, Jager C, Louis C, Khatchadourian C. Successful horizontal transfer of *Wolbachia* symbionts between *Trichogramma* wasps. Proceedings of the Royal Society of London Series B-Biological Sciences 265, 1441–1445 (1998)

[132] Groenenboom MAC, Hogeweg P. Space and the persistence of male-killing endosymbionts in insect populations. Proceedings of the Royal Society of London Series B-Biological Sciences 269, 2509–2518 (2002)

[133] Guillemaud T, Pasteur N, Rousset F. Contrasting levels of variability between cytoplasmic genomes and incompatibility types in the mosquito *Culex pipiens*. Proceedings of the Royal Society of London Series B-Biological Sciences 264, 245–251 (1997)

[134] Gupta RS. The phylogeny of proteobacteria: relationships to other eubacterial phyla and eukaryotes. Fems Microbiology Reviews 24, 367–402 (2000)

[135] Haag DL, Dearaujo AM. Inbreeding, genetic load and morphometric variation in natural populations of *Dryas iulia* (Lepidoptera, Nymphalidae). Revista Brasileira de Genetica 17, 35–39 (1994)

[136] Hagimori T, Abe Y, Date S, Miura K. The first finding of a *Rickettsia* bacterium associated with parthenogenesis induction among insects. Current Microbiology 52, 97–101 (2006)

[137] Haine ER, Cook JM. Convergent incidences of *Wolbachia* infection in fig wasp communities from two continents. Proceedings of the Royal Society of London Series B-Biological Sciences 272, 421–429 (2005)

[138] Hall DW. Meiotic drive and chromosome cycling. Evolution 58, 925–931 (2004).

[139] Ham RCHJ von, Kamerbeek J, Palacios C, Rausell C, Abascal F, Bastolla U, Fernández JM, Jiménez L, Postigo M, Silva FJ, Tamames J, Viguera E, Latorre A, Valencia A, Moran F, Moya A. Reductive genome evolution in *Buchnera aphidicola*. Proceedings of the National Academy of Sciences of the United States of America 100, 581–586 (2003)

[140] Hamilton WD. Extraordinary Sex Ratios. Science 156, 477–488 (1967)

[141] Hamilton WD. Inbreeding in Egypt and in this book: a childish perspective. In: The natural history of inbreeding and outbreeding: theoretical and empirical perspectives. Thornhill NW (eds), University of Chicago Press, Chicago , pp. 429–450 (1993)

[142] Hariri AR, Werren JH, Wilkinson GS. Distribution and reproductive effects of *Wolbachia* in stalk-eyed flies (Diptera: Diopsidae). Heredity 81, 254–260 (1998)

[143] Hartl DL, Brown SW. The origin of male haploid systems and their expected sex ratio. Theoretical Population Biology 1, 165–190 (1970)

[144] Hartl DL. Population dynamics of sperm and pollen killers. Theoretical and Applied Genetics 42, 81–88 (1972)

[145] Hatcher MJ. Persistence of selfish genetic elements: population structure and conflict. Trends in Ecology & Evolution 15, 271–277 (2000)

[146] Hatcher MJ, Taneyhill DE, Dunn AM, Tofts C. Population dynamics under parasitic sex ratio distortion. Theoretical Population Biology 56, 11–28 (1999)

[147] Hauschtschek-Jungen E, Maurer B. Sperm dysfunction in sex-ratio males of *Drosophila subobscura*. Genetica 46, 459–477 (1976)

[148] Herrick G, Seger J. Imprinting and paternal genome elimination in insects. In: Genomic imprinting: An interdisciplinary approach. Ohlsson R (Ed.), Springer, Berlin, pp. 41–71 (1999)

[149] Hertig M. The richettsia, *Wolbachia pipientis* (gen. et sp. n.) and associated inclusions of the mosquito *Culex pipiens*. Parasitology 28, 453-86 (1936)

[150] Hertig M, Wolbach SB. Studies on Rickettsia-like Organisms in Insects. Journal of Medical Research 44, 329–374 (1924)

[151] Hiraizumi Y, Sandler L, Crow JF. Meiotic drive in natural populations of *Drosophila melanogaster* III. Populational implications of the segregation-distorter locus. Evolution 14, 433–444 (1960)

[152] Hiroki M, Kato Y, Kamito T, Miura K. Feminization of genetic males by a symbiotic bacterium in a butterfly, *Eurema hecabe* (Lepidoptera: Piriedae) Naturwissenschaften 89, 167–170 (2002)

[153] Hoffmann AA, Clancy DJ, Merton E. Cytoplasmic incompatibility in Australian populations of *Drosophila melanogaster*. Genetics 136, 993-999 (1994)

[154] Hoffmann AA, Turelli M, Harshman LG. Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. Genetics 126, 933–948 (1990)

[155] Hoffmann AA, Turelli M, Simmons GM. Unidirectional Incompatibility Between Populations of Drosophila simulans. Evolution 40, 692–701 (1986)

[156] Hornett EA, Charlat S, Duplouy AMR, Davies N, Roderick GK, Wedell N, Hurst GDD. Evolution of male-killer suppression in a natural population. PLoS Biology 4, e282 (2006)

[157] Hornett EA, *et al.*, manuscript in preparation.

[158] Hoshizaki S, Shimada T. PCR-based detection of *Wolbachia*, cytoplasmic incompatibility microorganisms, infected in natural populations of *Laodelphax striatellus* (Homoptera: Delphacidae) in central Japan: has the distribution of *Wolbachia* spread recently? Insect Molecular Biology 4, 237-243 (1995)

[159] Huger AM, Skinner SW, Werren JH. Bacterial infections associated with the son-killer trait in the parasitoid wasp *Nasonia* (= *Mormoniella*) *vitripennis.* Journal of Invertebrate Pathology 46, 272–80 (1985)

[160] Huigens ME, Almeida RPd, Boons PAH, Luck RF, Stouthamer R. Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing *Wolbachia* in *Trichogramma* wasps. Proceedings of the Royal Society of London Series B-Biological Sciences 271, 509–515 (2004)

[161] Huigens ME, Luck RF, Klaassen RHG, Maas MFPM, Timmermans MJTN, Stouthamer R. Infectious parthenogenesis. Nature 405, 178–179 (2000)

[162] Huigens ME, Stouthamer R. Parthenogenesis associated with *Wolbachia.* In: Insect Symbiosis, Bourtzis K, Miller TA (eds), CRC Press, Boca Raton, pp. 247–266 (2003)

[163] Hunter MS. The influence of parthenogenesis-inducing *Wolbachia* on the oviposition behaviour and sex-specific developmental requirements of autoparasitoid wasps. Journal of Evolutionary Biology 12, 735–741 (1999)

[164] Hunter MS, Perlman SJ, Kelly SE. A bacterial symbiont in the Bacteroidetes induces cytoplasmic incompatibility in the parasitoid wasp *Encarsia pergandiella.* Proceedings of the Royal Society of London Series B-Biological Sciences 270, 2185–2190 (2003)

[165] Hurst GDD. Unpublished results.

[166] Hurst GDD, Bandi C, Sacchi L, Cochrane AG, Bertrand D, Karaca I, Majerus MEN *Adonia variegata* (Coleoptera : Coccinellidae) bears maternally inherited Flavobacteria that kill males only. Parasitology 118, 125–134 (1999).

[167] Hurst GDD, Hammarton TC, Bandi C, Majerus TMO, Bertrand D, Majerus MEN. The diversity of inherited parasites of insects: the male- killing agent of the ladybird beetle *Coleomegilla maculata* is a member of the Flavobacteria. Genetical Research 70, 1–6 (1997)

[168] Hurst GDD, Jiggins FM. Male-killing bacteria in insects: Mechanisms, incidence, and implications. Emerging Infectious Diseases 6, 329–336 (2000)

[169] Hurst GDD, Jiggins FM. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proceedings of the Royal Society of London Series B-Biological Sciences 272, 1525–1534 (2005)

[170] Hurst GDD, Jiggins FM, Pomiankowski A. Which Way to Manipulate Host Reproduction? *Wolbachia* That Cause Cytoplasmic Incompatibility Are Easily Invaded by Sex Ratio-Distorting Mutants. American Naturalist 160, 360–373 (2002)

[171] Hurst GDD, Jiggins FM, Robinson SJW. What causes inefficient transmission of male-killing *Wolbachia* in *Drosophila*? Heredity 87, 220–226 (2001)

[172] Hurst GDD, Jiggins FM, Schulenburg JH Graf von der, Bertrand D, West SA, Goriacheva II, Zakharov IA, Werren JH, Stouthamer R, Majerus MEN. Male-killing *Wolbachia* in two species of insect. Proceedings of the Royal Society of London Series B-Biological Sciences 266, 735–740 (1999)

[173] Hurst GDD, Johnson AP, Schulenburg JH Graf von der, Fuyama Y. Male-killing *Wolbachia* in *Drosophila*: A temperature-sensitive trait with a threshold bacterial density. Genetics 156, 699–709 (2000)

[174] Hurst GDD, Majerus MEN. Why do maternally inherited microorganisms kill males? Heredity 71, 81–95 (1993)

[175] Hurst GDD, McVean GT. Parasitic male-killing bacteria and the evolution of clutch-size. Ecological Entomology 23, 350–353 (1998)

[176] Hurst GDD, Schulenburg JH Graf von der, Majerus TMO, Bertrand D, Zakharov IA, Baungaard J, Volkl W, Stouthamer R, Majerus MEN. Invasion of one insect species, *Adalia bipunctata*, by two different male-killing bacteria. Insect Molecular Biology 8, 133–139 (1999)

[177] Hurst GDD, Werren JH. The role of selfish genetic elements in eukaryotic evolution. Nature Reviews Genetics 2, 597–606 (2001)

[178] Hurst LD. Parasite diversity and the evolution of diploidy, multicellularity and anisogamy. Journal of Theoretical Biology 144, 429–443 (1990)

[179] Hurst LD. The incidences and evolution of cytoplasmic male killers. Proceedings of the Royal Society of London Series B-Biological Sciences 244, 91–99 (1991)

[180] Hurst LD, Atlan A, Bengtsson BO. Intragenomic conflict. Quaterly Review of Biology 71, 317–364 (1996)

[181] Hurst LD, McVean GT. Clade selection, reversible evolution and the persistence of selfish elements: the evolutionary dynamics of cytoplasmic incompatibility. Proceedings of the Royal Society of London Series B-Biological Sciences 263, 97–104 (1996)

[182] Hurst LD, Pomiankowski A. Causes of sex ratio bias may account for unisexual sterility in hybrids: a new explanation of Haldane's rule and related phenomena. Genetics 128, 841–858 (1991)

[183] Ikeda H, Ishikawa H, Sasaki A. Infection density of *Wolbachia* and level of cytoplasmic incompatibility in the Mediterranean flour moth, *Ephestia kuehniella*. Journal of Invertebrate Pathology 84, 1–5 (2003)

[184] Ikeda H, Ishikawa H, Sasaki A. Regulation of *Wolbachia* Density in the Mediterranean Flour Moth, *Ephestia kuehniella*, and the Almond Moth, *Cadra cautella*. Zoological Science 20, 153–157 (2003b)

[185] Ironside JE, Dunn AM, Rollinson D, Smith JE. Association with host mitochondrial haplotypes suggests that feminizing microsporidia lack horizontal transmission. Journal of Evolutionary Biology 16, 1077–1083 (2003)

[186] Ironside JE, Smith JE, Hatcher MJ, Sharpe RG, Rollinson D, Dunn AM. Two species of feminizing microsporidian parasite coexist in populations of *Gammarus duebeni*. Journal of Evolutionary Biology 16, 467–473 (2003)

[187] Jaenike J. Sex-Ratio Meiotic Drive in the *Drosophila quinaria* Group. American Naturalist 148, 237–254 (1996)

[188] Jaenike J. Suppression of sex-ratio meiotic drive and the maintenance of Y-chromosome polymorphism in *Drosophila*. Evolution 53, 164–174 (1999)

[189] Jaenike J. Sex Chromosome Meiotic Drive. Annual Review of Ecology and Systematics 32, 25–49 (2001)

[190] Jaenike J, Dyer KA, Cornish C, Minhas MS. Asymmetrical reinforcement and *Wolbachia* infection in *Drosophila*. PLoS Biology 4, e325 (2006)

[191] Jaenike J, Dyer KA, Reed LK. Within-population structure of competition and the dynamics of male-killing *Wolbachia*. Evolutionary Ecology Research 5, 1023–1036 (2003)

[192] James AC, Ballard JWO. Expression of cytoplasmic incompatibility in *Drosophila simulans* and its impact on infection frequencies and distribution of *Wolbachia pipientis*. Evolution 54, 1661–1672 (2000)

[193] James AC, Jaenike J. "Sex Ratio" Meiotic Drive in *Drosophila testacea*. Genetics 126, 651–656 (1990)

[194] Jamnongluk W, Kittayapong P, Baimai V, O'Neill SL. *Wolbachia* infections of tephritid fruit flies: Molecular evidence for five distinct strains in a single host species. Current Microbiology 45, 255–260 (2002)

[195] Jeyaprakash A, Hoy MA. Long PCR improves *Wolbachia* DNA amplification: *wsp* sequences found in 76% of sixty-three arthropod species. Insect Molecular Biology 9, 393–405 (2000)

[196] Jiggins FM. The rate of recombination in *Wolbachia* bacteria. Molecular Biology and Evolution 19, 1640–1643 (2002)

[197] Jiggins FM. Male-killing *Wolbachia* and mitochondrial DNA: Selective sweeps, hybird introgression and parasite population dynamics. Genetics 164, 5–12 (2003)

[198] Jiggins FM, Bentley JK, Majerus MEN, Hurst GDD. How many species are infected with Wolbachia? Cryptic sex ratio distorters revealed to be common by intensive sampling. Proceedings of the Royal Society of London, Series B: Biological Sciences 268, 1123–1126 (2001)

[199] Jiggins FM, Bentley JK, Majerus MEN, Hurst GDD. Recent changes in phenotype and patterns of host specialization in *Wolbachia* bacteria. Molecular Ecology 11, 1275–1283 (2002)

[200] Jiggins FM, Hurst GDD, Dolman CE, Majerus MEN. High-prevalence male-killing *Wolbachia* in the butterfly *Acraea encedana*. Journal of Evolutionary Biology 13, 495–501 (2000)

[201] Jiggins FM, Hurst GDD, Jiggins CD, Schulenburg JH Graf von der, Majerus MEN. The butterfly *Danaus chrysippus* is infected by a male-killing *Spiroplasma* bacterium. Parasitology 120, 439–446 (2000)

[202] Jiggins FM, Hurst GDD, Majerus MEN. Sex ratio distortion in *Acraea encedon* (Lepidoptera : Nymphalidae) is caused by a male-killing bacterium. Heredity 81, 87–91 (1998)

[203] Jiggins FM, Hurst GDD, Majerus MEN. Sex-ratio-distorting *Wolbachia* causes sex-role reversal in its butterfly host. Proceedings of the Royal Society of London Series B-Biological Sciences 267, 69–73 (2000)

[204] Jiggins FM, Hurst GDD, Schulenburg JH Graf von der, Majerus MEN. Two male-killing *Wolbachia* strains coexist within a population of the butterfly *Acraea encedon*. Heredity 86, 161–166 (2001)

[205] Jiggins FM, Randerson JP, Hurst GDD, Majerus MEN. How can sex ratio distorters reach extreme prevalences? Male-killing *Wolbachia* are not suppressed

and have near-perfect vertical transmission efficiency in *Acraea encedon*. Evolution 56, 2290–2295 (2002)

[206] Jiggins FM, Schulenburg JH Graf von der, Hurst GDD, Majerus MEN. Recombination confounds interpretations of *Wolbachia* evolution. Proceedings of the Royal Society of London Series B-Biological Sciences 268, 1423–1427 (2001)

[207] Jiggins FM, Tinsley MC. An Ancient Mitochondrial Polymorphism in *Adalis bipunctata* Linked to a Sex-Ratio-Distorting Bacterium. Genetics 71, 1115–1124 (2005)

[208] Johnstone RA, Hurst GDD. Maternally inherited male-killing microorganisms may confound interpretation of mitochondrial DNA variability. Biological Journal of the Linnean Society 58, 453–470 (1996)

[209] Juchault P, Mocquard JP. Transfer of a parasitic sex factor to the nuclear genome of the host - A hypothesis on the evolution of sex-determining mechanisms in the terrestrial isopod *Armadillidium vulgare* Latr. Journal of Evolutionary Biology 6, 511–528 (1993)

[210] Juchault P, Rigaud T, Mocquard JP. Evolution of sex determination and sex ratio variability in wild populations of *Armadillidium vulgare* (Latr) (Crustacea, Isopoda): a case study in conflict resolution. Acta Oecologia 14, 547-562 (1993)

[211] Kageyama D, Traut W. Opposite sex-specific effects of *Wolbachia* and interference with the sex determination of its host *Ostrinia scapulalis*. Proceedings of the Royal Society of London Series B-Biological Sciences 271, 251–258 (2004)

[212] Kambhampati S, Rai KS, Verleye DM. Frequencies of Mitochondrial DNA Haplotypes in Laboratory Cage Populations of the Mosquito, *Aedes albopictus*. Genetics 132, 205–209 (1992)

[213] Kang L, Cai L, Sun L, Zhu H, Chen X, Shen D, Zhao S, Li C. Superinfection of *Laodelphax striatellus* with *Wolbachia* from *Drosophila simulans*. Heredity 90, 71–76 (2003)

[214] Keeling PJ, Fast NM. Microsporidia: Biology and Evolution of Highly Reduced Intracellular Parasites. Annual Review of Microbiology 56, 93–116 (2002)

[215] Keeling MJ, Jiggins FM, Read JM. The invasion and coexistence of competing *Wolbachia* strains. Heredity 91, 382–388 (2003)

[216] Kelly A, Dunn AM, Hatcher MJ. Incomplete feminisation by the microsporidian sex ratio distorter, *Nosema granulosis*, and reduced transmission and feminisation efficiency at low temperatures. International Journal for Parasitology 32, 825–831 (2002)

[217] Kikuchi Y, Fukatsu T. Diversity of *Wolbachia* endosymbionts in heteropteran bugs. Applied and Environmental Microbiology 69, 6082–6090 (2003)

[218] Kimura M. Solution of a process of random genetic drift with a continuous model. Proceedings of the National Academy of Sciences of the U.S.A. 41, 144–150 (1955)

[219] Kimura M. On the probability of fixation of mutant genes in a population. Genetics 47, 713–719 (1962)

[220] Kimura M. Diffusion models in population genetics. Journal of Applied Probability 1, 177-232 (1964)

[221] Kimura M. The neutral theory of molecular evolution. Cambridge University Press, Cambridge (1983)

[222] Kimura M, Crow JF. The Measurement of Effective Population Number. Evolution 17, 279–288 (1963)

[223] Kimura M, Crow JF. The number of alleles that can be maintained in a finite population. Genetics 49, 725–738 (1964)

[224] Kimura M, Ohta T. Average number of generations until fixation of a mutant allele in a finite population. Genetics 61, 763–771 (1969)

[225] Kingman JFC. The coalescent. Stochastic Processes and Applications 13, 235–248 (1982)

[226] Kirkby P, Hurst GDD. Unpublished results.

[227] Kittayapong P, Baislay KJ, Baimai V, O'Neill SL. Distribution and diversity of *Wolbachia* infections in Southeast Asian mosquitoes (Diptera: Culicidae). Journal of Medical Entomology 37, 340–345 (2000)

[228] Kittayapong P, Baisley KJ, Sharpe RG, Baimai V, O'Neill SL. Maternal transmission efficiency of *Wolbachia* superinfections in *Aedes albopictus* populations in Thailand. American Journal of Tropical Medicine and Hygiene 66, 103–107 (2002)

[229] Koehnke A, Telschow A, Werren JH, Hammerstein P., unpublished manuscript.

[230] Kolmogorov A. Über die analytischen Methoden in der Wahrscheinlichkeitsrechnung. Mathematische Annalen 104, 415–458 (1931)

[231] Kolmogorov A. Deviations from Hardy's formula in partial solution. C.R. de l'Acad. des Sciences de l'U.R.S.S. 3, 129–132 (1935)

[232] Kondo N, Ijichi N, Shimada M, Fukatsu T. Prevailing triple infection with *Wolbachia* in *Callosobruchus chinensis* (Coleoptera : Bruchidae). Molecular Ecology 11, 167–180 (2002)

[233] Kondo N, Shimada M, Fukatsu T. High prevalence of *Wolbachia* in the azuki bean beetle *Calosobruchus chinensis* (Coleoptera, Bruchidae). Zoological Science 16, 955-962 (1999)

[234] Kondo N, Shimada M, Fukatsu T. Infection density of *Wolbachia* endosymbiont affected by co-infection and host genotype. Biology Letters 1, 488–491 (2005)

[235] Kose H, Karr TL. Organization of *Wolbachia pipentis* in the *Drosophila* fertilized egg and embryo revealed by an anti-*Wolbachia* monoclonal antibody. Mechanisms of Development 51, 275–288 (1995)

[236] Langworthy NG, Renz A, Mackenstedt U, Henkle-Dührsen K, Bronsvoort MBdC, Tanya VN, Dennelly MJ, Trees AJ. Macrofilaricidal activity of tetracycline against the filarial nematode *Onchocerca ochengi*: elimination of *Wolbachia* precedes worm death and suggests a dependent relationship. Proceedings of the Royal Society of London Series B-Biological Sciences 267, 1063–1069 (2000)

[237] Laven H. Crossing Experiments with *Culex* Strains. Evolution 5, 370–375 (1951)

[238] Laven H. Vererbung durch Kerngene und das Problem der außerkaryotischen Vererbung bei *Culex pipiens*. II. Außerkaryotische Vererbung. Zeitschrift für Vererbungslehre 88, 166–175 (1957)

[239] Laven H. Speciation by Cytoplasmic Isolation in the *Culex pipiens*-Complex. Cold Spring Harbour Symposium of Quantitative Biology 24, 166–175 (1959)

[240] Lawson ET, Mousseau TA, Klaper R, Hunter MD, Werren JH. *Rickettsia* associated with male-killing in a buprestid beetle. Heredity 86, 497–505 (2001)

[241] LeGrand J-J, LeGrand-Hamelin E, Juchault P. Sex determination in Crustacea. Biological Reviews 62, 439–470 (1987)

[242] Lerat E, Daubin V, Moran NA. From gene trees to organismal phylogeny in prokaryotes: The case of the gamma-proteobacteria. PLoS Biology 1, 101–109 (2003)

[243] Lipsitch M, Siller S, Nowak MA. The Evolution of Virulence in Pathogens with Vertical and Horizontal Transmission. Evolution 50, 1729–1741 (1996)

[244] Lo N, Bandi C, Watanabe H, Nalepa C, Beninati T. Evidence for Cocladogenesis Between Diverse Dictyopteran Lineages and Their Intracellular Endosymbionts. Molecular Biology and Evolution 20, 907–913 (2003)

[245] Lo N, Casiraghi M, Salati E, Bazzocchi C, Bandi C. How Many *Wolbachia* Supergroups Exist? Molecular Biology and Evolution 19, 341–346 (2002)

[246] Lyttle TW. Segregation distorters. Annual Review of Genetics 25, 511–557 (1991)

[247] Majerus MEN. Ladybirds. Harper Collins, London (1994)

[248] Majerus MEN, Schulenburg JH Graf von der, Zakharov IA. Multiple causes of male-killing in a single sample of the two-spot ladybird, *Adalia bipunctata* (Coleoptera : Coccinellidae) from Moscow. Heredity 84, 605–609 (2000)

[249] Majerus TMO, Schulenburg JH Graf von der, Majerus MEN, Hurst GDD. Molecular identification of a male-killing agent in the ladybird *Harmonia axyridis* (Pallas) (Coleoptera : Coccinellidae). Insect Molecular Biology 8, 551–555 (1999)

[250] Markow TA. Ecological Genetics and Evolution: The Cactus-Yeast-*Drosophila* Model System, Barker JSF, Starmer WT (eds), Academic, New York, pp. 273–287 (1982)

[251] Markow TA. Assortative mating in *Drosophila*. Proceedings of the National Academy of Sciences of the United States of America 97, 7756–7760 (1997)

[252] Marshall JF. In: The British Mosquitoes. London, pp. 253–254 (1938)

[253] Masui S, Kamoda S, Sasaki T, Ishikawa H. Distribution and evolution of bacteriophage WO in *Wolbachia*, the endosymbiont causing sexual alterations in arthropods. Journal of Molecular Evolution 51, 491–497 (2000)

[254] Masui S, Kuroiwa H, Sasaki T, Inui M, Kuroiwa T, Ishikawa H. Bacteriophage WO and virus-like particles in *Wolbachia*, an endosymbiont of arthropods. Biochemical and Biophysical Research Communications 283, 1099–1104 (2001)

[255] Mauricodasilva L, Dearaujo AM. The genetic structure of *Heliconius eratus* populations (Lepidoptera, Nymphalidae). Revista Brasileira de Genetica 17, 19–24 (1994)

[256] May RM. Biological Populations with Nonoverlapping Generations: Stable Points, Stable Cycles, and Chaos. Science 186, 645–647 (1974)

[257] May RM, Nowak MA. Coinfection and the evolution of parasite virulence. Proceedings of the Royal Society of London Series B-Biological Sciences 261, 209–215 (1995)

[258] Maynard Smith J. The Evolution of Sex. Cambridge University Press, Cambridge (1978)

[259] Maynard Smith J, Haigh J. The hitch-hiking effect of a favourable gene. Genetical Research 23, 23–35 (1974)

[260] Mayr E. Animal species and evolution. Belknap Press, Cambridge, MA (1963)

[261] McGraw EA, Merritt DJ, Droller JN, O'Neill SL. *Wolbachia*-mediated sperm modification is dependent on the host genotype in *Drosophila*. Proceedings of the Royal Society of London Series B-Biological Sciences 268, 2565–2570 (2001)

[262] McGraw EA, Merritt DJ, Droller JN, O'Neill SL. *Wolbachia* density and virulence attenuation after transfer into a novel host. Proceedings of the National Academy of Sciences of the United States of America 99, 2918–2923 (2002)

[263] Meer MMM van, Stouthamer R. Cross-order transfer of *Wolbachia* from *Muscidifurax uniraptor* (Hymenoptera: Pteromalidae) to *Drosophila simulans* (Diptera: Drosophilidae). Heredity 82, 163–169 (1999)

[264] Merçot H, Atlan A, Jacques M, Montchamp-Moreau C. Sex-Ratio distortion in *Drosophila simulans*: co-occurence of a meiotic drive and a suppressor of drive. Journal of Evolutionary Biology 8, 283–300 (1995)

[265] Merçot H, Charlat S. *Wolbachia* Infections in *Drosophila melanogaster* and *D. simulans*: Polymorphism and Levels of Cytoplasmic Incompatibility. Genetica 120, 51–59 (2004)

[266] Merçot H, Llorente B, Micheline J, Atlan A, Montchamp-Moreau C. Variability Within the Seychelles Cytoplasmic Incompatibility System in *Drosophila simulans*. Genetics 141, 1015–1023 (1995)

[267] Merçot H, Poinsot D. *Wolbachia* transmission in a naturally bi-infected *Drosophila simulans* strain from New-Caledonia. Entomologia Experimentalis et Applicata 86, 97–103 (1998)

[268] Metz CW. Chromosome Behavior, Inheritance and Sex Determination in *Sciara*. American Naturalist 72, 485–520 (1938)

[269] Miller DD, Voelker RA. Salivary gland chromosome variation in the *Drosophila affinis* subgroup. IV. The short arm of the X chromosome in "western" and "eastern" *Drosophila athabasca*. Journal of Heredity 60, 307–311 (1969)

[270] Montchamp-Moreau C, Ferveur JF, Jacques M. Geographic distributition and inheritance of 3 cytoplasmic incompatibility types in *Drosophila simulans*. Genetics 129, 399–407 (1991)

[271] Montenegro H., personal communication.

[272] Montenegro H, Hatadani LM, Medeiros HF, Klaczko LB. Male killing in three species of the tripunctata radiation of *Drosophila* (Diptera: Drosophilidae). Journal of Zoological Systematics and Evolutionary Research 44, 130–135 (2006)

[273] Montenegro H, Klaczko LB. Low temperature cure of a male-killing agent in *Drosophila melanogaster*. Journal of Invertebrate Pathology 86, 50–51 (2004)

[274] Montenegro H, Petherwick AS, Hurst GDD, Klaczko LB. Fitness effects of *Wolbachia* and *Spiroplasma* in *Drosophila melanogaster*. Genetica 127, 207–215 (2006)

[275] Montenegro H, Solferini VN, Klaczko LB, Hurst GDD. Male-killing *Spiroplasma* naturally infecting *Drosophila melanogaster*. Insect Molecular Biology 14, 281–287 (2005)

[276] Montenegro H, Souza WN, Da Silva Leite D, Klaczko LB. Male-killing selfish cytoplasmic element causes sex-ratio distortion in *Drosophila melanogaster*. Heredity 85, 465–470 (2000)

[277] Moreau J, Bertin A, Caubet Y, Rigaud T. Sexual selection in an isopod with *Wolbachia*-induced sex reversal: males prefer real females. Journal of Evolutionary Biology 14, 388–394 (2001)

[278] Moreau J, Rigaud T. Operational sex ratio in terrestrial isopods: interaction between potential rate of reproduction and *Wolbachia*-induced sex ratio distortion. Oikos 91, 477–484 (2000)

[279] Moret Y, Juchault P, Rigaud T. *Wolbachia* endosymbiont responsible for cytoplasmic incompatibility in a terrestrial crustacean: effects in natural and foreign hosts. Heredity 86, 325–332 (2001)

[280] Mouton L, Dedeine F, Henri H, Bouletreau M, Profizi N, Vavre F. Virulence, Multiple Infections and Regulation of Symbiotic Population in the *Wolbachia-Asobara tabida* Symbiosis. Genetics 168, 181–189 (2004)

[281] Mouton L, Henri H, Bouletreau M, Vavre F. Strain-specific regulation of intracellular *Wolbachia* density in multiply infected insects. Molecular Ecology 12, 3459–3465 (2003)

[282] Müller M. What are the microsporidia? Parasitology Today 13, 455–456 (1998)

[283] Nigro L, Prout T. Is there selection on RFLP differences in mitochondrial DNA? Genetics 125: 551-555 (1990)

[284] Nirgianaki A, Banks GK, Frohlich DR, Veneti Z, Braig HR, Miller TA, Bedford ID, Markham PG, Savakis C, Bourtzis K. *Wolbachia* infections of the whitefly *Bemisia tabaci*. Current Microbiology 47, 93–101 (2003)

[285] Noor MAF. Reinforcement and other consequences of sympatry. Heredity 83, 503–508 (1999)

[286] Normark BB. The evolution of alternative genetic systems in insects. Annual Reviews in Entomology 48, 397–423 (2003)

[287] Normark BB. Haplodiploidy as an outcome of co-evolution between male-killing cytoplasmic elements and their hosts. Evolution 58, 790–798 (2004)

[288] Normark BB, Jordal BH, Farrell BD. Origin of a haplodiploid beetle lineage. Proceedings of the Royal Society of London, Series B: Biological Sciences 266, 2253–2259 (1999)

[289] Norton RA, Kethley JB, Johnston DE, O'Connor BM. Phylogenetic Perspectives on Genetic Systems and Reproductive Modes of Mites. In: Evolution and Diversity of Sex Ratio in Insects and Mites. Wrensch DL, Ebber MA (eds), Chapman & Hall, New York and London, pp. 8–99 (1993)

[290] Nowak MA, May RM. Superinfection and the Evolution of Parasite Virulence. Proceedings of the Royal Society of London Series B-Biological Sciences 255, 81–89 (1994)

[291] O'Neill SL, Giordano R, Colbert AME, Karr TL, Robertson HM. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proceedings of the National Academy of Sciences of the United States of America 89, 2699–2702 (1992)

[292] O'Neill SL, Hoffmann AA, Werren JH. (eds) Influential Passengers: Inherited microorganisms and arthropod reproduction. Oxford University Press, New York (1997)

[293] O'Neill SL, Karr TL. Bidirectional incompatibility between conspecific populations of *Drosophila simulans*. Nature 348, 178–180 (1990)

[294] Ono M, Braig HR, Munstermann LE, Ferro C, O'Neill SL. *Wolbachia* infections of phlebotomine sand flies (Diptera : Psychodidae). Journal of Medical Entomology 38, 237–241 (2001)

[295] Page RDM. Tangled Trees : Phylogeny, Cospeciation, and Coevolution. University of Chicago Press (2002)

[296] Page RDM. Parallel Phylogenies: Reconstructing the History of Host-Parasite Assemblages. Cladistics 10, 155–173 (1994)

[297] Palmer SC, Norton RA. Taxonomic, geographic and seasonal distribution of thelytokous parthenogenesis in the Desmonomata (Acari). Experimental and Applied Acarology 12, 67–81 (1991)

[298] Pasteur L. Études sur la Maladie des Vers à Soie. Paris (1870)

[299] Perlman SJ, Hunter MS, Zchori-Fein E. The emerging diversity of *Rickettsia*. Proceedings of the Royal Society of London Series B-Biological Sciences 273, 2097–2097 (2006)

[300] Perrot-Minnot MJ, Guo LR, Werren JH. Single and Double Infections with *Wolbachia* in the Parasitic Wasp *Nasonia vitripennis*: Effects on Compatibility. Genetics 143, 961–972 (1996)

[301] Perrot-Minnot MJ, Werren JH. *Wolbachia* infection and incompatibility dynamics in experimental selection lines. Journal of Evolutionary Biology 12, 272–282 (1999)

[302] Pijls JWAM, Steenbergen HJv, Alphen JJMv. Asexuality cured: the relations and differences between sexual and asexual Apoanagyrus diversicornis. Heredity 76, 506–513 (1996)

[303] Plantard O, Rasplus J-Y, Mondor G, Le Clainche I, Solignac M. *Wolbachia*-Induced Thelytoky in the Rose Gallwasp *Diplolepis spinosissimae* (Giraud) (Hymenoptera: Cynipidae), and Its Consequences on the Genetic Structure of Its Host. Proceedings of the Royal Society of London Series B-Biological Sciences 265, 1075–1080 (1998)

[304] Plantard O, Rasplus J-Y, Mondor G, Le Clainche I, Solignac M. Distribution and phylogeny of *Wolbachia* inducing thelytoky in Rhoditini and 'Aylacini' (Hymenoptera: Cynipidae). Insect Molecular Biology 8, 185–191 (1999)

[305] Poinsot D, Bourtzis K, Markakis G, Savakis C, Merçot H. *Wolbachia* Transfer from *Drosophila melanogaster* into *D. simulans*: Host Effect and Cytoplasmic Incompatibility Relationships. Genetics 150, 227–237 (1998)

[306] Poinsot D, Charlat S, Merçot H. On the mechanism of *Wolbachia*-induced cytoplasmic incompatibility: Confronting the models with the facts. BioEssays 25, 259–265 (2003)

[307] Poinsot D, Montchamp-Moreau C, Merçot H. *Wolbachia* segregation rate in *Drosophila simulans* naturally bi-infected cytoplasmic lineages. Heredity 82, 191–198 (2000)

[308] Pollock DA, Normark BB. The life cycle of *Micromalthus debilis* LeConte (Coleoptera: Archostomata: Micromalthidae): Historical review and evolutionary perspective. Journal of Systematic and Evolutionary Research 40, 105–112 (2002)

[309] Poulson DF. Further cases of maternal SR in *Drosophila* species. Drosophila Inform. Serv 41, 77 (1966)

[310] Presgraves DC. A Genetic Test of the Mechanism of *Wolbachia*-Induced Cytoplasmic Incompatibility in *Drosophila*. Genetics 154, 771–776 (2000)

[311] Prout T. Some Evolutionary Possibilities for a Microbe that Causes Incompatibility in its Host. Evolution 48, 909–911 (1994)

[312] Randerson JP, Jiggins FM, Hurst LD. Male killing can select for male mate choice: a novel solution to the paradox of the lek. Proceedings of the Royal Society of London Series B-Biological Sciences 267, 867–874 (2000)

[313] Randerson JP, Smith NGC, Hurst LD. The evolutionary dynamics of male-killers and their hosts. Heredity 84, 152–160 (2000)

[314] Rasgon JL, Scott TW. *Wolbachia* and Cytoplasmic Incompatibility in the California *Culex pipiens* Mosquito Species Complex: Parameter Estimates and Infection Dynamics in Natural Populations. Genetics 165, 2029–2038 (2003)

[315] Rasgon JL, Scott TW. Characterization of *Wolbachia* symbionts infecting *Cimex lectularis* L. and *Oeciacus vicarius* Horvath (Hemiptera: Cimicidae). Journal of Medical Entomology 41, 1174-1178 (2004)

[316] Reuter M, Keller L. High Levels of Multiple *Wolbachia* Infection and Recombination in the Ant *Formica exsecta*. Molecular Biology and Evolution 20, 748–753 (2003)

[317] Reuter M, Lehmann L, Guillaume F. The spread of incompatibility-inducing parasites in subdivided populations. submitted.

[318] Rice WR. Experimental test for the adaptive significance of sexual recombination. Nature Reviews Genetics 3, 241–251 (2002)

[319] Riechert SE, Roeloffs RM. Evidence for and consequences of inbreeding in the cooperative spiders. In: The Natural History of Inbreeding and Outbreeding, Thornhill NW (eds), The University of Chicago Press, Chicago, (1993)

[320] Riegler M, Charlat S, Stauffer C, Merçot H. *Wolbachia* Transfer from *Rhagoletis cerasi* to *Drosophila simulans*: Investigating the Outcomes of Host-Symbiont Coevolution. Applied and Environmental Microbiology 70, 273–279 (2004)

[321] Riegler M, Stauffer C. *Wolbachia* infections and superinfections in cytoplasmically incompatible populations of the European cherry fruit fly *Rhagoletis cerasi* (Diptera, Tephritidae). Molecular Ecology 11, 2425–2434 (2002)

[322] Rigaud T. Inherited microorganisms and sex determination of arthropod hosts. In: O'Neill SL, Hoffmann, AA, Werren JH (eds) Influential Passengers. Oxford University Press (1997)

[323] Rigaud T, Bouchon D, Souty-Grosset C, Raimond R. Mitochondrial DNA Polymorphism, Sex Ratio Distorters and Population Genetics in the Isopod *Armadillidium vulgare*. Genetics 152, 1669–1677 (1999)

[324] Rigaud T, Juchault P. Genetic control of the vertical transmission of a cytoplasmic sex factor in *Armadillidium vulgare* Latr. (Crustacea, Oniscidea). Heredity 68, 47–52 (1992)

[325] Rigaud T, Juchault P. Success and failure of horizontal transfers of feminizing *Wolbachia* endosymbionts in woodlice. Journal of Evolutionary Biology 8, 249–255 (1995)

[326] Rigaud T, Juchault P, Mocquard JP. The evolution of sex determination in isopod crustaceans. BioEssays 19, 409–416 (1997)

[327] Rigaud T, Pennings PS, Juchault P. *Wolbachia* Bacteria Effects after Experimental Interspecific Transfers in Terrestrial Isopods. Journal of Invertebrate Pathology 77, 251–257 (2001)

[328] Rio RVM, Wu Y-N, Filardo G, Aksoy S. Dynamics of multiple symbiont density regulation during host development: tsetse fly and its microbial flora. Proceedings of the Royal Society of London Series B-Biological Sciences in press, (2006)

[329] Rodgers-Gray TP, Smith JE, Ashcroft AE, Isaac RE, Dunn AM. Mechanisms of parasite-induced sex reversal in *Gammarus duebeni*. International Journal for Parasitology 34, 747–753 (2004)

[330] Rokas A, Atkinson RJ, Nieves-Aldrey J-L, West SA, Stone GN. The incidence and diversity of *Wolbachia* in gallwasps (Hymenoptera; Cynipidae) on oak. Molecular Ecology 11, 1815–1829 (2002)

[331] Ronquist F, Nylin S. Process and Pattern in the Evolution of Species Associations. Systematic Zoology 39, 323–344 (1990)

[332] Rousset F, Raymond CS, Kjellberg F. Cytoplasmic incompatibilities in the mosquito *Culex pipiens*: How to explain a cytotype polymorphism? Journal of Evolutionary Biology 4, 69–81 (1991)

[333] Rousset F, Solignac M. Evolution of single and double *Wolbachia* symbiosis during speciation in the *Drosophila simulans* complex. Proceedings of the National Academy of Sciences of the United States of America 92, 6389–6393 (1995)

[334] Rowley SM, Raven RJ, McGraw EA. *Wolbachia pipientis* in Australian Spiders. Current Microbiology 49, 208–214 (2004)

[335] Saint André Av, Blackwell MN, Hall LR, Hoerauf A, Brattig NW, Volkmann L, Taylor MJ, Ford L, Hise AG, Lass JH, Diaconu E, Pearlman E. The Role of Endosymbiotic *Wolbachia* Bacteria in the Pathogenesis of River Blindness. Science 5561, 1892–1985 (2002)

[336] Sakaguchi B, Poulson DF. Interspecific transfer of the "sex-ratio" condition from *Drosophila willistoni* to *D. melanogaster*. Genetics 48, 841 (1963)

[337] Saumitou-Laprade P, Cuguen J, Vernet P. Cytoplasmic male sterility in plants: molecular evidence and the nucleocytoplasmic conflict. Trends in Ecology & Evolution 9, 431–435 (1994)

[338] Sasaki T, Ishikawa H. *Wolbachia* infections and cytoplasmic incompatibility in the almond moth and the Mediterranean flour moth. Zoological Science 16, 739-744 (1999)

[339] Sasaki T, Ishikawa H. Transinfection of *Wolbachia* in the Mediterranean Flour Moth, *Ephestia kuehniella*, by Embryonic Microinjection. Heredity 85, 130–135 (2000)

[340] Sasaki T, Kubo T, Ishikawa H. Interspecific transfer of *Wolbachia* between two lepidopteran insects expressing cytoplasmic incompatibility: A *Wolbachia* variant naturally infecting *Cadra cautella* causes male killing in *Ephestia kuehniella*. Genetics 162, 1313–1319 (2002)

[341] Saumitou-Laprade P, Cuguen J, Vernet P. Cytoplasmic male sterility in plants: molecular evidence and the nucleocytoplasmic conflict. Trends in Ecology & Evolution 9, 431–435 (1994)

[342] Schilthuizen M, Gittenberger E. Screening mollusks for *Wolbachia* infection. Journal of Invertebrate Pathology 71, 268–270 (1998)

[343] Schilthuizen M, Stouthamer R. Horizontal transmission of parthenogenesis-inducing microbes in *Trichogramma* wasps. Proceedings of the Royal Society of London Series B-Biological Sciences 264, 361–366 (1997)

[344] Schofield P. Spatially explicit models of Turelli-Hoffmann *Wolbachia* invasive wave fronts. Journal of Theoretical Biology 215, 121–131 (2002)

[345] Schrader F, Hughes-Schrader S. Haploidy in *Icerya purchasi.* Zeitschrift für wissenschaftliche Zoologie 128, 182–200 (1926)

[346] Schrader F, Hughes-Schrader S. Haploidy in metazoa. The Quaterly Review in Biology 6, 411–438 (1931)

[347] Schulenburg JH Graf von der, Habig M, Sloggett JJ, Webberley KM, Bertrand D, Hurst GDD, Majerus MEN. Incidence of male-killing *Rickettsia* spp. (alpha-proteobacteria) in the ten spot ladybird *Adalia decempunctata* L. (Coleoptera: Coccinellidae). Applied and Environmental Microbiology 67, 270–277 (2001)

[348] Schulenburg JH Graf von der, Hurst GDD, Tetzlaff D, Booth GE, Zakharov IA, Majerus MEN. History of Infection With Different Male-Killing Bacteria in the Two-Spot Ladybird Beetle *Adalia bipunctata* Revealed Through Mitochondrial DNA Sequence Analysis. Genetics 160, 1075–1086 (2002)

[349] Scott A. Haploidy and aberant spermatogenesis in a coleopteran, *Micromalthus debilis* LeConte. Journal of Morphology 59, 485–515 (1936)

[350] Servedio MR. Reinforcement and the genetics of nonrandom mating. Evolution 54, 21–29 (2000)

[351] Shoemaker DD, Katju V, Jaenike J. *Wolbachia* and the evolution of reproductive isolation between *Drosophilla recens* and *Drosophila subquinaria.* Evolution 53, 1157–1164 (1999)

[352] Shoemaker DD, Machado CA, Molbo D, Werren JH, Windsor DM, Herre EA. The distribution of *Wolbachia* in fig wasps: correlations with host phylogeny, ecology and population structure. Proceedings of the Royal Society of London Series B-Biological Sciences 269, 2257–2267 (2002)

[353] Shull AF. Sex determination in *Anthothrips verbasci.* Genetics 2, 480–488 (1917)

[354] Simmons LW. Kin Recognition and its influence on mating preference in the field cricket, *Gryllus bimaculatus* (Degeer). Animal Behaviour 38, 68–77 (1989)

[355] Simmons LW. Female choice and the relatedness of mates in the field cricket, *Gryllus bimaculatus.* Animal Behaviour 41, 493–501 (1991)

[356] Sinkins SP, Braig HR, O'Neill SL. *Wolbachia* Superinfections and the Expression of Cytoplasmic Incompatibility. Proceedings of the Royal Society of London Series B-Biological Sciences 261, 325–330 (1995)

[357] Sinkins SP, Godfray HCJ. Use of *Wolbachia* to drive nuclear transgenes through insect populations. Proceedings of the Royal Society of London Series B-Biological Sciences 271, 1421–1426 (2004)

[358] Sinkins SP, Gould F. Gene drive systems for insect disease vectors. Nature Reviews Genetics 7, 427–435 (2006)

[359] Sinkins SP, Walker Th, Lynd AR, Stephen AR, Makepeace BL, Godfray HCJ, Parkhill J. *Wolbachia* variability and host effects on crossing type in *Culex* mosquitoes. Nature 436, 257–260 (2005)

[360] Skinner SW. Son-killer: A third extrachromosomal factor affecting the sex-ratio in the parasitoid wasp, *Nasonia* (=*Mormoniella*) *vitripennis.* Genetics 109, 745–759 (1985)

[361] Snook RR, Cleland SY, Wolfner MF, Karr TL. Offsetting Effects of *Wolbachia* Infection and Heat Shock on Sperm Production in *Drosophila simulans*: Analy-

ses of Fecundity, Fertility and Accessory Gland Proteins. Genetics 155, 167–178 (2000)

[362] Stalker HD. The Genetic System Modifying Meiotic Drive in *Drosophila paramelanica*. Genetics 46, 177–202 (1961)

[363] Stille B, Dävring L. Meiosis and reproductive strategy in the parthenogenetic gall wasp *Diplolepis rosae*. Hereditas 92, 353–362 (1980)

[364] Stoll NR, Shull AF. Sex determination in the white fly. Genetics 4, 251–260 (1919)

[365] Stouthamer R. *Wolbachia*-induced parthenogenesis. In: O'Neill SL, Hoffmann AA, Werren JH (eds) Influential passengers: Inherited Microorganisms and Invertebrate Reproduction. Oxford University Press, New York, pp. 102–124 (1997)

[366] Stouthamer R, Breeuwer JAJ, Luck RF, Werren JH. Molecular identification of microorganisms associated with parthenogenesis. Nature 361, 66–68 (1993)

[367] Stouthamer R, Breeuwer JAJ, Hurst GDD. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. Annual Review of Microbiology 53, 71–102 (1999)

[368] Stouthamer R, Kazmer DJ. Cytogenetics of microbe-associated parthenogenesis and its consequences for gene flow in *Trichogramma* wasps. Heredity 73, 317–327 (1994)

[369] Stouthamer R, Luck RF, Hamilton WD. Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera, Trichogrammatidae) to revert to sex. Proceedings of the National Academy of Sciences of the United States of America 87, 2424–2427 (1990)

[370] Stouthamer R, Tilborg M van, de Jong JH, Nunney L, Luck RF. Selfish element maintains sex in natural populations of a parasitoid wasp. Proceedings of the Royal Society of London Series B-Biological Sciences 268, 617–622 (2001)

[371] Sturtevant AH, Dobzhansky T. Geographical Distribution and Cytology of "Sex Ratio" in *Drosophila pseudoobscura* and Related Species. Genetics 21, 473–490 (1936)

[372] Sullivan J, Jaenike J. Male-killing *Wolbachia* and male mate choice: a test with *Drosophila innubila*. Evolutionary Ecology Research 8, 91–102 (2006)

[373] Tagami Y, Miura K. Distribution and prevalence of *Wolbachia* in Japanese populations of Lepidoptera. Insect Molecular Biology 13, 359–364 (2004)

[374] Tamas I, Klasson L, Canback B, Näslund AK, Eriksson AS, Wernegreen JJ, Sandström JP, Moran NA, Andersson SGE. 50 million years of genomic stasis in endosymbiotic bacteria. Science 296, 2376–2379 (2002)

[375] Taylor DR. Evolutionary consequences of cytoplasmic sex ratio distorters. Evolutionary Ecology 4: 235–248 (1990)

[376] Taylor JE, Jaenike J. Sperm Competition and the Dynamics of X Chromosome Drive: Stability and Extinction. Genetics 160, 1721-1731 (2002)

[377] Taylor MJ, Makunde WH, McGarry HF, Turner JD, Mand S, Hoerauf A. Macrofilaricidal activity after doxycycline treatment of *Wuchereria bancrofti*: a double-blind, randomised placebo-control led trial. Lancet 365, 2116–2121 (2005)

[378] Telschow A, Engelstädter J, Yamamura N, Hammerstein P, Hurst GDD. Asymmetric gene flow and constraints on adaptation caused by sex ratio distorters. Journal of Evolutionary Biology 19, 869–878 (2006)

[379] Telschow A, Flor M, Hammerstein P, Werren JH. *Wolbachia* induced Unidirectional CI as a Promotor of Speciation: Mainland-Island Model. Submitted.

[380] Telschow A, Hammerstein P, Werren JH. Effects of *Wolbachia* on Genetic Divergence Between Populations: Mainland-Island Model. Integrative and Comparative Biology 42, 340–351 (2002)

[381] Telschow A, Hammerstein P, Werren JH. The Effect of Wolbachia on Genetic Divergence between Populations: Models with Two-Way Migration. American Naturalist 160, S54–S66 (2002)

[382] Telschow A, Hammerstein P, Werren JH. The effect of *Wolbachia* versus genetic incompatibilities on reinforcement and speciation. Evolution 59, 1607–1619 (2005)

[383] Telschow A, Yamamura N, Werren JH. Bidirectional cytoplasmic incompatibility and the stable coexistence of two *Wolbachia* strains in parapatric host populations. Journal of Theoretical Biology 235, 265–274 (2005)

[384] Terry RS, Smith JE, Dunn AM. Impact of a novel feminizing microsporidian parasite on its crustacean host. Journal of Eukaryotic Microbiolgogy 45, 497-501 (1998)

[385] Terry RS, Smith JE, Sharpe RG, Rigaud T, Littlewood DTJ, Ironside JE, Rollinson D, Bouchon D, MacNeil C, Dick JTA, Dunn AM. Widespread vertical transmission and associated host sex-ratio distortion within the eukaryotic phylum Microspora. Proceedings of the Royal Society of London Series B-Biological Sciences 271, 1783–1789 (2004)

[386] Thao ML, Baumann P. Evolutionary Relationships of Primary Prokaryotic Endosymbionts of Whiteflies and Their Hosts. Applied and Environmental Microbiology 70, 3401–3406 (2004)

[387] Thipaksorn A, Jamnongluk W, Kittayapong P. Molecular evidence of *Wolbachia* infection in natural populations of tropical odonates. Current Microbiology 47, 314–318 (2003)

[388] Tram U, Ferree PA, Sullivan W. Identification of *Wolbachia*-host interacting factors through cytological analysis. Microbes and Infections 5, 999–1011 (2003)

[389] Turelli M. Evolution of incompatibility-inducing microbes and their hosts. Evolution 48, 1500–1513 (1994)

[390] Turelli M, Hoffmann AA. Rapid spread of an inherited incompatibility factor in California *Drosophila*. Nature 353, 440–442 (1991)

[391] Turelli M, Hoffmann AA, McKechnie SW. Dynamics of cytoplasmic incompatibility and mtDNA variation in natural *Drosophila simulans* populations. Genetics 132, 711-723 (1992)

[392] Underwood DA, Shapiro AM. A male-biased primary sex-ratio and larval mortality in *Eucheira socialis* (Lepidoptera: Pieridae). Evolutionary Ecology Research 1, 703–717 (1999)

[393] Vala F, Egas M, Breeuwer JAJ, Sabelis MW. *Wolbachia* affects oviposition and mating behaviour of its spider mite host. Journal of Evolutionary Biology 17, 692–700 (2004)

[394] Vala F, Weeks AR, Claessen D, Breeuwer JAJ, Sabelis MW. Within- and between-population variation for *Wolbachia*-induced reproductive incompatibility in a haplodiploid mite. Evolution 56, 1331–1339 (2002)

[395] Vandekerckhove TTM, Watteyne S, Willems A, Swing JG, Mertens J, Gillis M. Phylogenetic analysis of the 16S rDNA of the cytoplasmic bacterium *Wolbachia* from the novel host *Folsomia candida* (Hexapoda, Collembola) and its implications for wolbachial taxonomy. Fems Microbiology Letters 180, 279–286 (1999)

[396] Vavre F, Dedeine F, Quillon M, Fouillet P, Fleury F, Boulétreau M. Within-species diversity of *Wolbachia*-induced cytoplasmic incompatibility in haplodiploid insects. Evolution 55, 1710–1714 (2001)

[397] Vavre F, Fleury F, Varaldi J, Fouillet P, Boulétreau M. Evidence for Female Mortality in *Wolbachia*-Mediated Cytoplasmic Incompatibility in Haplodiploid Insects: Epidemiologic and Evolutionary Consequences. Evolution 54, 191–200 (2000)

[398] Vavre F, Fouillet P, Fleury F. Between- and Within-Host species Selection on Cytoplasmic Incompatibility-Inducing *Wolbachia* in Haplodiploids. Evolution 57, 421–427 (2003)

[399] Vaz SC, Carvalho AB. Evolution of Autosomal Suppression of the *Sex-Ratio* Trait in *Drosophila*. Genetics 166, 265–277 (2004)

[400] Vaz SC, Vibranovski, MD, Carvalho, AB. Sex-ratio citoplasmico em *Drosophila roehrae*. Genetics and Molecular Biology 21 (Suppl.):260 (1998)

[401] Veneti Z, Bentley JK, Koana T, Braig HR, Hurst GDD. A Functional Dosage Compensation Complex Required for Male Killing in *Drosophila*. Science 307, 1461–1463 (2005)

[402] Veneti Z, Bourtzis K. Personal communication (2005)

[403] Veneti Z, Clark ME, Karr TL, Savakis C, Bourtzis K. Heads or Tails: Host-Parasite Interactions in the *Drosophila-Wolbachia* System. Applied and Environmental Microbiology 70, 5366–5372 (2004)

[404] Voelker RA. Preliminary Characterization of "Sex Ratio" and Rediscovery and Reinterpretation of "Male Sex Ratio" in *Drosophila affinis*. Genetics 71, 597–606 (1972)

[405] Wade MJ, Stevens L. Microorganism Mediated Reproductive Isolation in Flour Beetles (Genus Tribolium). Science 227, 527–528 (1985)

[406] Wade MJ, Stevens L. The Effect of Population Subdivision on the Rate of Spread of Parasite-Mediated Cytoplasmic Incompatibility. Journal of Theoretical Biology 167, 81–87 (1994)

[407] Wallace B. Studies on "sex ratio" in *Drosophila pseudoobscura*. I. Selection and "sex ratio". Evolution 2, 189–217 (1948)

[408] Weeks AR, Breeuwer JAJ. *Wolbachia*-induced parthenogenesis in a genus of phytophagous mites. Proceedings of the Royal Society of London Series B-Biological Sciences 268, 2245–2251 (2001)

[409] Weeks AR, Marec F, Breeuwer JAJ. A mite species that consists entirely of haploid females. Science 292, 2479–2482 (2001)

[410] Weeks AR, Velten R, Stouthamer R. Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthropods. Proceedings of the Royal Society of London Series B-Biological Sciences 270, 1857–1865 (2003)

[411] Wenseleers T, Ito F, van Borm S, Huybrechts R, Volkaert F, Billen J. Widespread occurrence of the microorganism *Wolbachia* in ants. Proceedings of the Royal Society of London Series B-Biological Sciences 265, 1447–1452 (1998)

[412] Werren JH. Sex ratio adaptations to local mate competition in a parasitic wasp. Science 208, 1157–1160 (1980)

[413] Werren JH. Sex ratio evolution under local mate competition in a parasitic wasp. Evolution 37, 116–124 (1983)

[414] Werren JH. The coevolution of autosomal and cytoplasmic sex-ratio factors. Journal of Theoretical Biology 124, 317–334 (1987)

[415] Werren JH. Biology of *Wolbachia*. Annual Review of Entomology 42, 587–609 (1997)

[416] Werren JH. *Wolbachia* and speciation. In: Howard DJ, Berlocher SH (eds) Endless Forms: Species and Speciation, Oxford University Press: New York, 245–260 (1998)

[417] Werren JH, Bartos JD. Recombination in *Wolbachia*. Current Biology 11, 431–435 (2001)

[418] Werren JH, Beukeboom LW. Sex determination, sex ratios, and genetic conflict. Annual Review of Ecology and Systematics 29, 233–261 (1998)

[419] Werren KH, Hurst GDD, Zhang W, Breeuwer JAJ, Stouthamer R, Majerus MEN. Rickettsial relative associated with male killing in the ladybird beetle (Adalia bipunctata) Journal of Bacteriology 176, 388–394 (1994)

[420] Werren JH, Jaenike J. *Wolbachia* and cytoplasmic incompatibility in mycophagous *Drosophila* and their relatives. Heredity 75, 320–326 (1995)

[421] Werren JH, Nur U, Wu CI. Selfish genetic elements. Trends in Ecology & Evolution 3, 297–302 (1988)

[422] Werren JH, Skinner SW, Huger AM. Male-killing bacteria in a parasitic wasp. Science 231, 990–992 (1986)

[423] Werren JH, Stouthamer R. PSR (paternal sex ratio) chromosomes: the ultimate selfish genetic elements. Genetica 117, 85–101 (2003)

[424] Werren JH, Windsor DM. *Wolbachia* infection frequencies in insects: evidence of a global equilibrium? Proceedings of the Royal Society of London Series B-Biological Sciences 267, 1277–1285 (2000)

[425] Werren JH, Windsor D, Guo LR. Distribution of *Wolbachia* among neotropical arthropods. Proceedings of the Royal Society of London Series B-Biological Sciences 262, 197–204 (1995)

[426] Werren JH, Zhang W, Guo LR. Evolution and phylogeny of *Wolbachia*: Reproductive parasites of arthropods. Proceedings of the Royal Society of London Series B-Biological Sciences 261, 55–63 (1995)

[427] Wesenberg-Lund C. Contributions to the biology of Rotifera. Mem. Academ. Roy. Sci. Danemark, Copenhague, Sect. Sci. (8) 4, 189 (1923)

[428] West SA, Cook JM, Werren JH, Godfray HCJ. *Wolbachia* in two insect host-parasitoid communities. Molecular Ecology 7, 1457–1465 (1998)

[429] Wilkinson GS, Fry CL. Meiotic drive alters sperm competitive ability in stalk-eyed flies. Proceedings of the Royal Society of London, Series B: Biological Sciences 268, 2559–2564 (2001)

[430] Williams GC. Sex and Evolution. Princeton University Press, New Jersey (1975)

[431] Williamson DL, Poulson DF. Sex ratio organisms (Spiroplasmas) of *Drosophila*. In: Whitcomb RF, Tully JG (eds) The Mycoplasmas, vol 3 edn. Academic Press, New York, pp 175–208 (1979)

[432] Williamson DL, Sakaguchi B, Hackett KJ, Whitcomb RF, Tully JG, Carle P, Bové JM, Adams JR, Konai M, Henegar RB. *Spiroplasma poulsonii* sp. nov., a new species associated with male-lethality in *Drosophila willistoni* a neotropical species of fruit fly. International Journal of Systematic Bacteriology 49, 611–618 (1999)

[433] Wright S. Evolution in Mendelian Populations. Genetics 16, 97–159 (1931)

[434] Wright S. The differential equation of the distribution of gene frequencies. Proceedings of the National Academy of Sciences 31, 382–389 (1945)

[435] Wu C-I. Virility Deficiency and the Sex-Ratio Trait in *Drosophila pseudoobscura*. I. Sperm Displacement and Sexual Selection. Genetics 105, 651–662 (1983)

[436] Wu C-I. Virility Deficiency and the Sex-Ratio Trait in *Drosophila pseudoobscura*. II. Multiple Mating and Overall Virility Selection. Genetics 105, 663–679 (1983)

[437] Wu C-I, Beckenbach AT. Evidence for Extensive Genetic Differentiation between the Sex-Ratio and the Standard Arrangement of *Drosophila pseudoobscura* and *D. persimilis* and Identification of Hybrid Sterility Factors. Genetics 105, 71–86 (1983)

[438] Wu M, Sun LV, Vamathevan J, Riegler M, Deboy R, Brownlie JC, McGraw EA, Martin W, Esser C, Ahmadinejad N, Wiegand C, Madupu R, Beanan MJ, Brinkac LM, Daugherty SC, Durkin AS, Kolonay JF, Nelson WC, Mohamoud Y, Lee P, Berry K, Young MB, Utterback T, Weidman J, Nierman WC, Paulsen IT, Nelson KE, Tettelin H, O'Neill SL, Eisen JA. Phylogenomics of the reproductive parasite *Wolbachia pipientis* wMel: A streamlined genome overrun by mobile genetic elements. PLoS Biology 2, 327–341 (2004)

[439] Yamamura N. Vertical Transmission and Evolution of Mutualism from Parasitism. Theoretical Population Biology 44, 95–109 (1993)

[440] Yen JH, Barr AR. New hypothesis of the cause of cytoplasmic incompatibility in *Culex pipiens* L. Nature 232, 657–658 (1971)

[441] Yen JH, Barr AR (1973). The etiological agent of cytoplasmic incompatibility in *Culex pipiens*. Journals of Invertebrate Pathology 22, 242–250 (1973)

[442] Zabalou S, Charlat S, Nirgianaki A, Lachaise D, Merçot H, Bourtzis K. Natural *Wolbachia* Infections in the *Drosophila yakuba* Species Complex Do Not Induce Cytoplasmic Incompatibility but Fully Rescue the wRi Modification. Genetics 167, 827–834 (2004)

[443] Zabalou S, Riegler M, Theodorakopoulou M, Stauffer C, Savakis C, Bourtzis K. *Wolbachia*-induced cytoplasmic incompatibility as a means for insect pest population control. Proceedings of the National Academy of Sciences of the United States of America 101, 15042–15045 (2004)

[444] Zchori-Fein E, Gottlieb Y, Kelly SE, Brown JK, Wilson JM, Karr TL, Hunter MS. A newly discovered bacterium associated with parthenogenesis and a change in host selection behavior in parasitoid wasps. Proceedings of the National Academy of Sciences of the United States of America 98, 12555–12560 (2001)

[445] Zchori-Fein E, Perlman SJ. Distribution of the bacterial symbiont *Cardinium* in arthropods. Molecular Ecology 13, 2009–2016 (2004)

[446] Zchori-Fein E, Perlman SJ, Kelly SE, Katzir N, Hunter MS. Characterization of a 'Bacteroidetes' symbiont in *Encarsia* wasps (Hymenoptera: Aphelinidae): proposal of 'Candidatus *Cardinium hertigii*'. International Journal of Systematic and Evolutionary Microbiology 54, 961–968 (2004)

[447] Zchori-Fein E, Roush RT, Hunter MS. Male production induced by antibiotic treatment in *Encarsia formosa* (Hymenoptera: Aphelinidae), an asexual species. Experientia 48, 102–105 (1992)

[448] Zchori-Fein E, Roush RT, Rosen D. Distribution of parthenogenesis-inducing symbionts in ovaries and eggs of *Aphytis* (Hymenoptera : Aphelinidae). Current Microbiology 36, 1–8 (1998)

[449] Zeh JA, Zeh DW. Male-killing *Wolbachia* in a live-bearing arthropod: Brood abortion as a constraint on the spread of a selfish microbe. Journal of Invertebrate Pathology 92, 33–38 (2006)

[450] Zhou W, Rousset F, O'Neill SL. Phylogeny and PCR-based classification of *Wolbachia* strains using wsp gene sequences. Proceedings of the Royal Society of London Series B-Biological Sciences 265, 509–515 (1998)