Chapter 1

General Introduction

Sexual versus Asexual Reproduction

Sexual reproduction is the predominant mode of reproduction among eukaryotes. Therefore, it is generally thought that sexual reproduction is the optimal strategy for reproduction. There are many theories that explain the success of sexual reproduction. These theories fall into two broad categories (Maynard Smith 1978, Bell 1982). Mutational theories argue that sexual recombination facilitates the shedding of deleterious mutations from the genome, while under asexual reproduction these mutations accumulate and cause a genetic load (Muller 1964, Kimura & Maruyama 1966, Maynard Smith 1978, Kondrashov 1988, Crow 1994). Ecological theories suggest that sexual reproduction allows faster adaptation during antagonistic coevolution with other organisms, such as parasites, predators and competitors (Van Valen 1973, Glesener & Tilman 1978, Bell 1982, 1985, Hamilton *et al.* 1990, Lively *et al.* 1990, Scheu & Drossel 2007). All of these theories support the general thought that sexual reproduction allows faster adaptation to a complex environment than asexual reproduction. In addition, over a long period of time sexual reproduction will be more advantageous than asexual reproduction.

However, sexual reproduction also has a lot of costs. Given the large fitness disadvantage of sexual reproduction, known as the twofold cost of sex (Maynard Smith 1978), it is remarkable that it is the predominant mode of reproduction in eukaryotes. The twofold cost of sex describes the fitness advantages of asexual reproduction (and fitness disadvantages of sexual reproduction), both from a population dynamical view and from a genetical view. Because an asexual female only produces daughters, all of her offspring will contribute to the next generation (in theory, each individual will produce at least one individual in the next generation). In contrast, only half of the offspring (with a 50/50 sex ratio) of a sexually reproducing female will contribute to the next generation (two individuals, a male and a female, are needed for one individual in the next generation). Therefore, an asexual population can grow faster than a sexual population and when they are in competition, the asexual population should outcompete the sexual one (Maynard Smith 1978). Also,

asexual females transmit all of their genome to each offspring, while a sexual female transmits only half of her genome to each offspring. Therefore, an asexual female has a higher fitness than a sexual female and a higher mother-offspring relatedness should favour asexual reproduction (Maynard Smith 1978).

Given the advantages and disadvantages of sexual and asexual reproduction, it can be predicted that asexual reproduction should be more advantageous in simplified environments with few biotic interactions and/or over a short period of time, while sexual reproduction should be more advantageous in complex environments and/or over a long period of time. Although sexual reproduction is the predominant mode of reproduction, asexual reproduction may be relatively common in simplified environments (Glesener & Tilman 1978, Bürger 1999, Haag & Ebert 2004, Scheu & Drossel 2007, Becks & Agrawal 2010). In support of this idea, recent studies on invertebrates have shown a prevalence of asexual species and populations in agricultural and human-disturbed (simplified) environments, but not in natural (complex) environments (Haack *et al.* 2000, Hoffmann *et al.* 2008, Foucaud *et al.* 2009, Gilabert *et al.* 2009).

Asexual reproduction in invertebrates is often induced by infection with cytoplasmatically inherited microorganisms, for example *Wolbachia* (Huigens & Stouthamer 2003), *Cardinium* (Zchori-Fein & Perlman 2004) and *Rickettsia* (Perlman *et al.* 2006). In this thesis, I will focus on the microorganism *Wolbachia*.

Wolbachia

Wolbachia are intracellular, symbiotic bacteria belonging to the order Rickettsiales within the α-Proteobacteria. *Wolbachia* are known to infect a wide range of arthropods, including insects, spiders, mites, scorpions and isopods, and have also been found in nematodes (Rousset *et al.* 1992, Werren 1997, Stouthamer *et al.* 1999, Werren *et al.* 2008). The type species for the *Wolbachia* genus is *Wolbachia pipientis*, which was first described in the mosquito *Culex pipiens* (Hertig 1936). The genus *Wolbachia* can be divided into six, and possibly eight, major clades or supergroups (A-H) (Lo *et al.* 2002, Casiraghi *et al.* 2005, Baldo & Werren 2007, Werren *et al.* 2008). Supergroup C and D are only found in filarial nematodes. The other supergroups are primarily found in arthropods, in which supergroup A and B are the most common. It has been estimated that 66% of all insect species is (partly) infected with *Wolbachia* (Hilgenboecker *et al.* 2008). Various insect species harbour multiple *Wolbachia* strains, which may even belong to different supergroups (Werren *et al.* 1995, Vavre *et al.* 1999, Werren & Windsor 2000).

Wolbachia are maternally inherited, because a sperm cell contains too little cytoplasm to harbour the bacteria. Therefore, vertical transmission (from mother to daughter) is the main transmission mode of *Wolbachia* within established hosts. However, incongruence between the phylogenies of *Wolbachia* and their hosts suggests widespread horizontal transmission of *Wolbachia* between hosts (O'Neill *et*

al. 1992, Rousset *et al.* 1992, Werren *et al.* 1995, Schilthuizen & Stouthamer 1997, Vavre *et al.* 1999). Horizontal transmission has been achieved experimentally both within and between species (Grenier *et al.* 1998, Heath *et al.* 1999, Huigens *et al.* 2000, 2004) and a recent field study (Kraaijeveld *et al.* 2011a, Kremer & Huigens 2011) found evidence for horizontal transmission during the early stages of *Wolbachia* infection in a parasitoid wasp. This suggests that horizontal transmission not only plays a role in the spread of *Wolbachia* from one species to another, but also between individuals within newly infected species.

Because *Wolbachia* are maternally inherited, they benefit from female-biased sex ratios in its hosts. To enhance its own transmission, *Wolbachia* can induce various alterations of the reproduction mechanism of its host, such as cytoplasmic incompatibility, feminization, male-killing and parthenogenesis (Rousset *et al.* 1992, Werren 1997, Stouthamer *et al.* 1999, Werren *et al.* 2008).

Cytoplasmic incompatibility (CI) is the most widespread effect of *Wolbachia* infection and has been described in mites, isopods and many insect orders, such as Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera and Orthoptera (Werren 1997, Stouthamer *et al.* 1999, Werren *et al.* 2008). CI is an alteration of the reproduction mechanism that causes incompatibility between sperm and egg cells. CI-*Wolbachia* modify the sperm of *Wolbachia*-infected males during spermatogenesis. Eggs of CI-*Wolbachia*-infected females harbour a rescue mechanism for this sperm modification. However, when the right rescue mechanism is not present in the egg, because the female is not infected or infected with a different *Wolbachia* strain than the male, sperm and egg will be incompatible. When the sperm modification is not rescued, an asynchrony between the male and female pronuclei occurs during the first embryonic mitotic division. In diploid species this will result in embryonic death. In haplodiploid species only male offspring will develop (Werren 1997, Stouthamer *et al.* 1999, Werren *et al.* 2008). Two forms of CI exist. Unidirectional incompatibility occurs when the sperm of a *Wolbachia*-infected male fertilizes an egg of an uninfected female. The egg of a *Wolbachia*-infected female and sperm of an uninfected male are compatible. Bidirectional incompatibility occurs when a male and a female are infected with different strains of *Wolbachia* that are mutually incompatible. In this case, the sperm and egg cell are only compatible when a male and a female are infected with the same *Wolbachia* strain (Werren 1997, Stouthamer *et al.* 1999, Werren *et al.* 2008).

Feminization-inducing *Wolbachia* have been found in isopods and the insect orders Hemiptera and Lepidoptera (Werren 1997, Bouchon *et al.* 1998, Stouthamer *et al.* 1999, Hiroki *et al.* 2002, Negri *et al.* 2006, Werren *et al.* 2008). *Wolbachia*-induced feminization causes genetic males to be converted into functional females, for example by suppression of the androgenic glands (Werren 1997, Stouthamer *et al.* 1999, Werren *et al.* 2008).

Wolbachia-induced male-killing has been described in pseudoscorpions and the

insect orders Coleoptera, Diptera and Lepidoptera (Hurst *et al.* 1999, Stouthamer *et al.* 1999, Dyer & Jaenike 2004, Zeh *et al.* 2005, Werren *et al.* 2008). *Wolbachia*-infected males are killed during embryogenesis, possibly to provide more nutrients to the female offspring (Stouthamer *et al.* 1999, Werren *et al.* 2008).

Parthenogenesis-inducing (PI) *Wolbachia* have so far only been found in haplodiploid organisms, such as hymenopterans, thrips and mites (Stouthamer *et al.* 1990a, Werren 1997, Stouthamer *et al.* 1999, Arakaki *et al.* 2001, Weeks & Breeuwer 2001, Huigens & Stouthamer 2003, Werren *et al.* 2008). In uninfected haplodiploid organisms, fertilized eggs develop into diploid daughters and unfertilized eggs develop into haploid sons (arrhenotoky). PI-*Wolbachia* cause diploidization of the haploid eggs by disrupting the segregation of the homologous chromosomes during the first mitotic division after meiosis (Stouthamer & Kazmer 1994, Huigens & Stouthamer 2003, Pannebakker *et al.* 2004a) resulting in the production of daughters from unfertilized eggs (thelytoky).

In this thesis, I will focus on PI-*Wolbachia* in hymenopterans. More specifically, I studied the dynamics, causes and consequences of *Wolbachia*-induced parthenogenesis in two parasitoid wasp species, *Tetrastichus coeruleus* and *Asobara japonica*.

Consequences of *Wolbachia*-induced parthenogenesis

In most cases of *Wolbachia*-induced parthenogenesis, the infection is fixed and the entire host population consists of females (Huigens & Stouthamer 2003). In the absence of males and sexual reproduction, genes involved in sexual reproduction are not actively maintained by selection. Accumulation of neutral mutations or selection against the maintenance of costly sexual traits may lead to their loss or deterioration (Carson *et al.* 1982, Pijls *et al.* 1996, Pannebakker *et al.* 2005, Kraaijeveld *et al.* 2009). Because male traits are not expressed in parthenogenetic populations, they are likely to degenerate due to neutral mutations. Female traits, which are expressed but not used, may also be selected against when they are costly to maintain (Pijls *et al.* 1996). In addition, females may loose the ability to reproduce sexually due to 'functional virginity mutations' that may spread concomitantly with the *Wolbachia* infection through a population (Stouthamer *et al.* 2010, King & Hurst 2010). Mutations that prevent females from fertilizing their eggs will have a selective advantage in the presence of PI-*Wolbachia*-infected females because they induce the bearer of these mutations to produce more sons which will have many mating opportunities. Virginity mutations arise during the early stages of PI-*Wolbachia* infection and affect traits in females involved in sexual reproduction, e.g. mating or egg fertilization. Accumulation of neutral mutations or selection against the maintenance of costly traits arise after a longer period of parthenogenetic reproduction and can potentially affect all traits involved in sexual reproduction, both in males and females, e.g. courtship behaviour or pheromone production.

Sexual traits may evolve to be costly when the reproductive interests of males and

females are different. Sexual conflict may result in sexually antagonistic coevolution, in which males evolve adaptations that manipulate female behaviour and females evolve resistance to male manipulation (Rice 1996, Chapman *et al.* 2003, Arnqvist & Rowe 2005). In the absence of males, selection on manipulative male traits and costly female resistance traits will be absent. Adaptations evolved under sexually antagonistic coevolution are released from selection and alleles that were favoured can be replaced by others due to accumulation of random mutations (Carson *et al.* 1982, Pijls *et al.* 1996) or antagonistic pleiotropy (Pijls *et al.* 1996, Pannebakker *et al.* 2005). Therefore, parthenogenetic females may be either more receptive to mating when they have been selected in the absence of males or not receptive at all when they have been selected in the presence of a small number of males.

When deterioration or loss of genes involved in sexual reproduction can evolve over a long period of time, asexual species or populations may no longer be capable of sexual reproduction. Many insect species with female-only populations exhibit deterioration or loss of female sexual traits, as expected by theory (e.g. Carson *et al.* 1982, Pijls *et al.* 1996, Gottlieb & Zchori-Fein 2001, Kraaijeveld *et al.* 2009).

Tetrastichus coeruleus

Tetrastichus coeruleus (Hymenoptera: Eulophidae) is a gregarious egg-larval parasitoid of the common asparagus beetle (*Crioceris asparagi*). *T. coeruleus* both feeds on and parasitizes the eggs of *C. asparagi* (Capinera & Lilly 1975a, van Alphen 1980). This (and own observations) indicates that *T. coeruleus* is synovigenic, meaning that it produces eggs during all or most of its adult life (Heimpel & Collier 1996).

C. asparagi lives on the asparagus plant (*Asparagus officinalis*), which grows on sandy soils, such as coastal dune areas, and as a crop in monoculture on agricultural fields. *A. officinalis* is native to western Asia, Europe and northern Africa and has been cultured for thousands of years (Audas & Heywood 1981, Weeda *et al.* 1991). *C. asparagi* is known as a pest species in asparagus agriculture and *T. coeruleus* can be used as a biological control agent against these beetles (Capinera & Lilly 1975b). *A. officinalis* has been introduced to the United States for culturing (Weeda *et al.* 1991) and in 1859 *C. asparagi* was first noticed in northeastern United States (Capinera & Lilly 1975a). Later, probably already in 1863, but certainly in 1909, *T. coeruleus* also was recorded and was noticed to control the asparagus beetle population by feeding on and ovipositing in the eggs of *C. asparagi* (Fernald 1909, Russell & Johnston 1912, Johnston 1915, Capinera & Lilly 1975a, 1975b). Russell & Johnston (1912) and Johnston (1915) only recorded females of *T. coeruleus* in the population, both in the field and when they were reared in the lab for multiple generations, showing that *T. coeruleus* occurs in parthenogenetic populations on agricultural fields in northeastern United States. Moreover, previous work in our lab suggested that several *T. coeruleus* populations in The Netherlands are infected with *Wolbachia* that cause parthenogenesis (B. Wielaard, pers. comm.).

Asobara japonica

Asobara japonica (Hymenoptera: Braconidae) is a solitary larval-pupal parasitoid of drosophilid flies (Ideo *et al.* 2008). *A. japonica* naturally occurs in Japan. Populations of *A. japonica* on the temperate main islands of Japan exhibit highly female-biased sex ratios (92.7% - 99.2% females), whereas population sex ratios on the smaller subtropical southern islands are not biased (Mitsui *et al.* 2007). The populations on the main islands are infected with parthenogenesis-inducing *Wolbachia*, while the populations on the smaller southern islands are not (Kremer *et al.* 2009).

Wolbachia in *T. coeruleus* and *A. japonica*

Interestingly, both *T. coeruleus* and *A. japonica* have populations that are infected with *Wolbachia*, while they also have populations that are not infected (*T. coeruleus*: chapter 2, Reumer *et al.* 2010; *A. japonica*: Mitsui *et al.* 2007, Kremer *et al.* 2009). In both species, *Wolbachia*-infected populations reproduce through parthenogenesis, whereas uninfected populations reproduce sexually. Also, in both species a small number of male offspring is regularly produced in the otherwise parthenogenetically reproducing populations (*T. coeruleus*: chapter 2, Reumer *et al.* 2010; *A. japonica*: Mitsui *et al.* 2007, chapter 6, Reumer *et al.* 2012).

Only a few other parasitoid wasp species are known in which both sexual and parthenogenetic populations occur. In the parasitoid wasps *Apoanagyrus diversicornis* (Pijls *et al.* 1996), *Telenomus nawai* (Arakaki *et al.* 2000) and *Leptopilina clavipes* (Pannebakker *et al.* 2004b) PI-*Wolbachia*-infected and uninfected populations occur allopatrically. Mixed populations of PI-*Wolbachia*-infected and uninfected individuals are limited to a number of species of the genus *Trichogramma* (Stouthamer *et al.* 1990a, 1990b, 2001). In most cases of *Wolbachia*-induced parthenogenesis, the infection is fixed and the entire host population consists of females that reproduce parthenogenetically (Huigens & Stouthamer 2003).

In this thesis, I will investigate the dynamics of *Wolbachia*-infected and uninfected populations of *T. coeruleus* and *A. japonica*. In addition, I will study the occasional male production in otherwise parthenogenetic populations of *A. japonica*.

Thesis Overview

In this thesis, I investigated *Wolbachia*-induced parthenogenesis in two parasitoid wasp species, *Tetrastichus coeruleus* (chapter 2, 3, 4 & 5) and *Asobara japonica* (chapter 6 & 7). I studied the population genetics of infected and uninfected populations of both species in order to determine population dynamics and shed more light on the origin of the *Wolbachia* infection (chapter 3 & 6). I studied the differences between infected and uninfected populations, in terms of *Wolbachia* infection frequency, mode of reproduction, ecology and life history traits (chapter

2 & 4). In addition, I studied the consequences of a PI-*Wolbachia* infection by investigating the sexual functionality in *T. coeruleus* females (chapter 5) and *A. japonica* males (chapter 7).

In **chapter 2**, I tested whether there is a correlation between ecology and mode of reproduction in populations of *T. coeruleus*. Classical ecological theories for the maintenance of sexual reproduction state that sexual reproduction facilitates adaptation to complex environments with many biotic interactions, whereas simplified environments are expected to favour asexual reproduction. Because *T. coeruleus* occurs both in natural (complex) and agricultural (simplified) environments, I investigated whether different reproductive modes can be found in different habitats and whether the possible correlation followed the classical theories. In addition, I investigated whether *Wolbachia* infection caused parthenogenesis in female-biased populations.

I sampled 13 populations of *T. coeruleus* in The Netherlands, France and Massachusetts, USA. In contrast to the general pattern, in Dutch and French natural areas I found *Wolbachia*-infected, highly female-biased populations that reproduce parthenogenetically (thelytoky), while populations on Dutch agricultural fields were not infected with *Wolbachia*, showed higher frequencies of males and reproduced sexually (arrhenotoky). However, I also found a female-only, *Wolbachia*-infected population on agricultural fields in Massachusetts. All *Wolbachia*-infected populations were infected with the same *Wolbachia* strain.

At this moment, I do not have a convincing explanation for the deviation of *T. coeruleus* populations in The Netherlands and France from the general association between ecology and mode of reproduction. The fixation of *Wolbachia* in the population from Massachusetts (where the species was introduced) may be due to founder effect and lack of uninfected, sexual source populations.

In **chapter 3**, I studied the population genetics of *Wolbachia*-infected and uninfected populations of *T. coeruleus*. Because *Wolbachia* are maternally inherited, vertical transmission is the main transmission mode within established hosts. However, horizontal transmission plays a major role in the spread of *Wolbachia* to novel hosts and within some newly infected host populations. I investigated the transmission mode with which *Wolbachia* has spread through the populations of *T. coeruleus*. In addition, I looked for evidence of recent gene flow between populations.

I studied the population genetics of 13 populations of *T. coeruleus*, using nuclear microsatellite markers and mitochondrial DNA. Two major genetic clusters were evident: a thelytokous cluster containing all the *Wolbachia*-infected populations and an arrhenotokous cluster containing all the uninfected populations.

Within the thelytokous cluster, there was no variation in mitochondrial DNA, suggesting that initially only one female became infected with *Wolbachia* and ver-

tical transmission was the main transmission mode for *Wolbachia* spread within *T. coeruleus*. However, nuclear markers displayed considerable genetic variation, suggesting that infected females mated with males, which would introduce nuclear DNA variation into the mitochondrial lineage. Thelytokous populations showed significant genetic substructure. Within the arrhenotokous cluster, both nuclear and mitochondrial DNA variation was present, but no population structure was recognized.

Several females from otherwise thelytokous populations were uninfected and/or heterozygous for one or more microsatellite loci. No infected females were found in any of the arrhenotokous populations. This suggests occasional migration from arrhenotokous to thelytokous populations, but not vice versa. I conclude that *Wolbachia* has spread via vertical transmission through the populations of *T. coeruleus*. The nuclear genetic variation in the *Wolbachia*-infected populations is due to occasional sex and not to horizontal transmission of *Wolbachia*.

In **chapter 4**, I studied the differences between two populations of *T. coeruleus* that differ in ecology, *Wolbachia* infection and mode of reproduction. Differences between populations may result in differences in life history traits and strategies in order to optimize reproduction and survival.

I quantified several life history traits in two populations of *T. coeruleus*, one from a natural (Meijendel) and one from an agricultural (Brabant) environment: clutch size, life span, female weight and nutrient concentrations (proteins, lipids, sugars and glycogen).

Females from Brabant laid larger clutches, had longer life spans and were heavier than females from Meijendel. There was no difference in the relative amounts of proteins, lipids or sugars, but females from Meijendel had relatively more glycogen than females from Brabant.

The two populations therefore exhibit markedly different life history strategies. Females from Brabant invest relatively more in survival, body size and clutch size, whereas females from Meijendel seem to be more active or fly longer distances. Further studies are needed to determine if and how these life history differences are related to differences in ecology, *Wolbachia* infection or mode of reproduction.

In **chapter 5**, I studied the consequences of a PI-*Wolbachia* infection in *T. coeruleus* females. In most cases of *Wolbachia*-induced parthenogenesis, the infection is fixed and the entire host population consists of females. In the absence of males and sexual reproduction, genes involved in sexual reproduction are not actively maintained by selection. Accumulation of neutral mutations or selection against the maintenance of such traits may lead to their loss or deterioration. In addition, females may loose the ability to reproduce sexually due to 'functional virginity mutations'.

First, I examined whether mating is costly to females. Then, I compared the sex-

ual functionality of arrhenotokous and thelytokous *T. coeruleus* females. I measured their attractiveness and receptiveness to males and I compared the morphology of their spermathecae.

Mated females had a shorter life span than virgin females, showing that either mating or sperm storage was costly. Several sexual traits of thelytokous females have degraded compared to arrhenotokous females. Arrhenotokous and thelytokous females were equally attractive to males, but thelytokous females were unreceptive to males and there was a clear difference in spermathecal morphology between arrhenotokous and thelytokous females.

Selection against the maintenance of costly sexual traits appears to have resulted in the degradation of receptivity and spermathecal morphology of thelytokous females. However, I cannot exclude that these traits have degraded due to functional virginity mutations or accumulation of neutral mutations.

In **chapter 6**, I investigated the origin of male offspring production by PI-*Wolbachia*-infected females in *A. japonica*. Because all females in the field are infected and infected *A. japonica* females are not capable of sexual reproduction, male production seems to be maladaptive in thelytokous populations. In addition, I studied the population genetics of *Wolbachia*-infected and uninfected populations of *A. japonica*.

I tested three hypotheses for the origin of male production: high rearing temperatures could result in higher offspring sex ratios (more males), low *Wolbachia* titer of the mother could lead to higher offspring sex ratios and/or the *Wolbachia* infection is of relatively recent origin and not enough time has passed to allow complete co-adaptation between *Wolbachia* and host.

One third of the *Wolbachia*-infected females produced males and more than half of these males were also infected with *Wolbachia*. Neither offspring sex ratio nor male infection frequency were affected by rearing temperature or *Wolbachia* concentration of the mother. The mitochondrial DNA sequence of one of the uninfected populations was identical to that of two of the infected populations. Therefore, the initial *Wolbachia* infection of *A. japonica* must have occurred recently. Mitochondrial sequence variation among the infected populations suggests that the spread of *Wolbachia* through the host populations involved horizontal transmission.

I conclude that the occasional male production by *Wolbachia*-infected *A. japonica* females is most likely a maladaptive side-effect of incomplete co-evolution between symbiont and host in this relatively young infection.

In **chapter 7**, I studied the consequences of a PI-*Wolbachia* infection in *A. japonica* males. Recent studies have shown that male-killing-*Wolbachia* strains can induce cytoplasmic incompatibility (CI) when introgressed into a resistant host. Phylogenetic studies suggest that transitions between CI and other *Wolbachia* phenotypes

have occurred frequently, raising the possibility that latent CI may be widespread among *Wolbachia*. I investigated whether a PI-*Wolbachia* strain also can induce CI.

PI-*Wolbachia*-infected *A. japonica* females regularly produce male offspring, which may either be infected or not. First, I tested whether infected males and females harboured the same *Wolbachia* strain. Then, mating experiments were performed between uninfected arrhenotokous females and males that were either infected or uninfected. Uninfected males were obtained naturally from infected thelytokous populations and through removal of *Wolbachia* using antibiotics. In addition, males from a naturally uninfected arrhenotokous *A. japonica* population were used.

Infected males and females harboured the exact same *Wolbachia* strain, indicating that the *Wolbachia* strain that induces parthenogenesis in females also was present in infected males. Uninfected arrhenotokous females that had mated with *Wolbachia*-infected males produced a slightly more male-biased sex ratio than females that had mated with uninfected males. This effect was strongest in females that had mated with males with a relatively high *Wolbachia* concentration. Because *Wolbachia*-infected males did not show higher ratios of nuclear versus mitochondrial DNA content than uninfected males, I concluded that *Wolbachia* does not cause diploidization of cells in infected males.

I conclude that the *Wolbachia* strain that induced parthenogenesis in females induced CI in infected males. However, the effect was very small. This suggests that if CI is induced in *A. japonica* males by the PI-*Wolbachia* strain, the ability of CI-induction may have degenerated through the accumulation of mutations. Although the results are consistent with CI, other alternatives such as production of abnormal sperm by *Wolbachia*-infected males cannot completely be ruled out.

In **chapter 8**, I summarized the most important results presented in this thesis and I compared the findings about *Wolbachia*-induced parthenogenesis for the two studied parasitoid wasp species: *T. coeruleus* and *A. japonica*. The main conclusions of this thesis are that different scenarios may occur for the spread of a parthenogenesis-inducing *Wolbachia* infection in different host species, different barriers may prevent migration and gene flow between *Wolbachia*-infected and uninfected populations in different host species, different ages of the PI-*Wolbachia* infection may have different consequences for the host species, and a PI-*Wolbachia* infection can have severe consequences for the sexual functionality of infected males and females.