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Review

Insect Sex Determination Manipulated by Their Endosymbionts: Incidences, Mechanisms and Implications

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Abstract: The sex-determining systems of arthropods are surprisingly diverse. Some species have male or female heterogametic sex chromosomes while other species do not have sex chromosomes. Most species are diploids but some species, including wasps, ants, thrips and mites, are haplodiploids (n in males; 2n in females). Many of the sexual aberrations, such as sexual mosaics, sex-specific lethality and conversion of sexuality, can be explained by developmental defects including double fertilization of a binucleate egg, loss of a sex chromosome or perturbation of sex-determining gene expression, which occur accidentally or are induced by certain environmental conditions. However, recent studies have revealed that such sexual aberrations can be caused by various groups of vertically-transmitted endosymbiotic microbes such as bacteria of the genera *Wolbachia*, *Rickettsia*, *Arsenophonus*, *Spiroplasma* and *Cardinium*, as well as microsporidian protists. In this review, we first summarize the accumulated data on endosymbiont-induced sexual aberrations, and then discuss how such endosymbionts affect the developmental system of their hosts and what kinds of ecological and evolutionary effects these endosymbionts have on their host populations.

Keywords: endosymbiont; feminization; insect; male killing; *Wolbachia*; sex determination

1. Introduction

In arthropods, the fundamental system of sex determination is generally considered to be genetic, and this is supported by the strict concordance in the dimorphism of sexual phenotypes and karyotypes (*i.e.*, sex chromosomes) and the rare occurrence of gynandromorphs (chimeric individuals having tissues with male and female genotypes). However, the sex determination and differentiation of arthropods can be perturbed by the endosymbionts or parasites they harbor. The most striking effects on sexual phenotype are induced by microbes that are transmitted from mothers to offspring (often called maternally transmitted or cytoplasmic parasites). Since males cannot transmit cytoplasmic parasites to their offspring, parasites in the male cytoplasm are essentially dead from the evolutionary viewpoint. To make up for these shortcomings, some parasites adopt a variety of tactical strategies such as killing of males, converting males into females or inducing parthenogenesis.
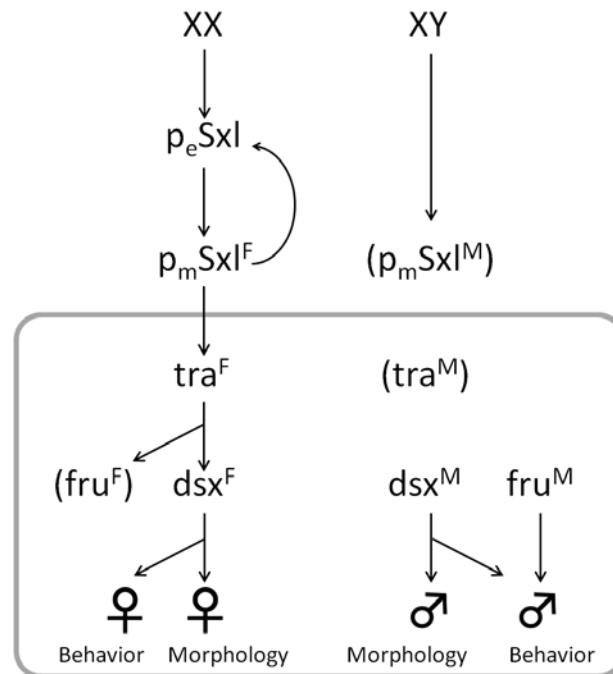
In this article, we briefly overview the diversity of the sex-determining mechanisms in insects, summarize the incidences of maternally transmitted microbes that affect or may affect sex determination, and discuss the possible mechanism of such phenomena. We also discuss the ecological and evolutionary effects that such microbes potentially have on their host populations.

2. Diversity and Common Features of Insect Sex Determination

In the majority of insects, sex is strictly determined according to the genotype. For example, many of the dipteran insects (flies and mosquitoes) have a male-heterogametic sex chromosome constitution (*i.e.*, XX: female; XY: male) while many of the lepidopteran insects (butterflies and moths) have a female-heterogametic chromosomal constitution (*i.e.*, ZZ: male; ZW: female). On the other hand, many of the hymenopteran insects (ants, bees and wasps) do not have sex chromosomes. Instead, they have a haplodiploid sex-determination system, in which fertilized diploid (2n) eggs become females and unfertilized haploid (n) eggs develop into males [1–3]. The molecular mechanisms underlying sex determination and differentiation in the model insect *Drosophila melanogaster* (Diptera; Drosophilidae) are well understood. At a very early embryonic stage, each cell determines its sex independently, and once determined, the sex of each cell is maintained during later development through a gene expression cascade consisting of *Sex-lethal* (*Sxl*), *transformer* (*tra*), *doublesex* (*dsx*) and other genes, in which sex-specific mRNA splicing plays an important role [4–6]. In the honeybee *Apis mellifera*, the *complementary sex determiner* (*csd*) gene affects sex through allelic combination, whereas the *feminizer* (*fem*) gene induces sex-specific splicing, producing a functional protein only in females. Comparisons between the sex-determination pathways of *A. mellifera*, *Ceratitis capitata* and *D. melanogaster* suggest that the *tra*/*dsx* pathway is conserved among insects and is likely to be ancestral [7] (Figure 1). Sex determination in a cell-autonomous manner is also believed to be widespread among insects on the basis that sexually mosaic individuals often occur in a diverse array of insects [8].

Similar to insects, some of the non-insect arthropods are also considered to have genetically based sex determination. However, they differ from insects because their sexual differentiation is deeply affected by sex hormones that are secreted by particular organs (e.g., the androgenic gland in crustaceans) and circulate in the body together with hemolymph.

Figure 1. The sex determination cascade in *Drosophila melanogaster*. PeSxl indicates *Sxl* transcript from the early promoter. PmSxl indicates *Sxl* from the late promoter. The gray box shows the conserved part of the cascade. (Adapted from [7].)

3. Sexual Aberrations of Insects

In arthropods, sexually mosaic or gynandromorphic individuals are repeatedly observed in natural and laboratory populations of numerous species [9]. Most of them are considered to arise through accidental, very rare developmental defects such as double fertilization of a binucleate egg, loss of a sex chromosome or upregulation/downregulation of sex-determining genes.

On the other hand, some conditions can stably generate sexual aberrations in certain insects. In some mosquitoes of the genus *Aedes*, a high temperature condition can cause males to develop into morphological females. When the number of days of exposure to high temperature was lessened, various degrees of intersexes, *i.e.*, individuals having both male and female characters, appeared [10–12]. Crosses between geographical races of the gypsy moth *Lymantria dispar* also resulted in the appearance of intersexes [13–15]. In some crossing combinations, genetically male offspring (ZZ) showed mosaic color patterns in their wings, which is clearly distinguishable between sectors of male color and female color. In other combinations, genetically female offspring (ZW) showed homogeneous in their wing color, which is intermediate between male and female [14]. Sexual mosaics were also found in crosses between tetraploid females and diploid males in the psychid moth *Solenobia triquetrella* [16]. The resulting triploid offspring with two Z chromosomes and three sets of autosomes developed into sexual intergrades owing to the intermediate ratio (2:3) between the Z chromosomes and autosome sets, which is neither typically female (1:2) nor typically male (1:1). Although it was used to be considered that all the aberrations of sexuality were attributed to environmental disturbances or stochastic effects, recent investigations revealed that some of them can be caused by endosymbionts, another organism living inside the body.

4. Involvement of Endosymbiotic Microorganisms

It has been increasingly recognized that various forms of sex aberrations in arthropods can be caused by symbiotic microorganisms. It was first recognized that *Wolbachia pipientis* (belonging to Alphaproteobacteria) exerts various types of manipulation on the reproductive system of arthropods [17–19]. *Wolbachia*-induced reproductive manipulation can be classified into four major categories: feminization, male killing, parthenogenesis induction and cytoplasmic incompatibility. By using these manipulations, *Wolbachia* can spread among host populations at the expense of host fitness. Following the succession of discoveries regarding *Wolbachia*-induced host manipulations, several other bacteria such as *Cardinium*, *Spiroplasma* and *Arsenophonus*, which belong to distantly related taxonomic groups, were also found to manipulate host reproduction in similar ways to *Wolbachia* (Tables 1 and 2; Table S1).

Similar to other symbiotic bacteria such as *Buchnera* [20] and *Wigglesworthia* [21], *Wolbachia* resides in the cytoplasm of the host cells and mainly relies on maternal inheritance within arthropod lineages. However, in contrast to mutualistic symbionts, phylogenetic analyses suggest that *Wolbachia* have moved horizontally between distantly related insect lineages multiple times possibly through host–parasite or predator–prey interactions [22–24].

Table 1. Endosymbionts that are capable of manipulating host sexuality and reproduction.

Endosymbiont				Hosts	Phenotype
Kingdom	**Phylum**	**Class**	**Species**		
Bacteria	Proteobacteria	α-Proteobacteria	*Wolbachia pipientis*	insects, crustaceans, arachnids, nematodes	CI, MK, PI, FM
			Rickettsia sp.	insects	MK, PI
		γ-Proteobacteria	*Arsenophonus nasoniae*	insects	MK
	Firmicutes	Mollicutes	*Spiroplasma poulsonii*	insects	MK
			Spiroplasma ixodetis relative	insects	MK
	Cytophaga-Flavobacterium-Bacteroides	Bacteroidetes	*Cardinium hertigii*	insects, arachnids, nematodes	CI, PI, FM
			Flavobacteria relative	insects	MK
Eukaryotes	Microsporidia	Dihaplophasea	*Octosporea effeminans*	crustaceans	FM
			Thelohania herediteria	crustaceans	FM
			Nosema granulosis	crustaceans	FM
			Dictyocoela duebenum	crustaceans	FM
			Amblyospora spp.	insects	MK
			Parathelohania legeri	insects	MK
			Parathelohania obesa	insects	MK
	Nematoda	Adenophorea	*Gasteromermis* sp.	insects	FM
Viruses	Unknown	Unknown	Unknown	crustaceans	MS
			Unknown (RNA virus)	insects	MK

Abbreviations: CI, cytoplasmic incompatibility; MK, male killing; PI, parthenogenesis induction; FM, feminization; MS, masculinization.

Table 2. A List of endosymbionts and their hosts, where feminization, male killing and parthenogenesis induction were described.

Endosymbiont	Class	Order	Species	Reference
(a) Feminizing bacteria				
Wolbachia pipientis	Insecta	Hemiptera	*Zyginidia pullula*	[28]
		Lepidoptera	*Eurema hecabe*	[27]
			E. madarina	[25,26,129]
	Malacostraca	Isopoda	*Armadillidium vulgare*	[130–132]
			A. nasatum	[132–134]
			Chaetophiloscia elongata	[135]
			Porcellionides pruinosus	[135,136]
			Sphaeroma rugicauda	[137]
Cardinium hertigii	Insecta	Hymenoptera	*Encarsia hispida*	[44]
	Arachnida	Trombidiformes	*Brevipalpus phoenicis*	[40]
			B. californicus	[42]
(b) Feminizing microsporidia				
Octosporea effeminans	Malacostraca	Amphipoda	*Gammarus duebeni*	[46]
Thelohania hered021teria			*G. duebeni*	[138]
Nosema granulosis			*G. duebeni*	[48]
Dictyocoela duebenum			*G. duebeni*	[52]
(c) Other feminizers				
Gasteromermis sp.	Insecta	Ephemeroptera	*Baetis bicaudatus*	[53]
f factor (unknown)	Malacostraca	Isopoda	*Armadillidium vulgare*	[33]
(d) Male-killing bacteria				
Wolbachia pipientis	Insecta	Coleoptera	*Adalia bipunctata*	[139]
			Tribolium madens	[140]
		Diptera	*Drosophila bifasciata*	[141]
			D. borealis	[142]
			D. innubila	[143]
		Lepidoptera	*Acraea encedon*	[128]
			A. encedana	[144]
			Hypolimnas bolina	[69]
			Ostrinia furnacalis	[64]
			O. orientalis	[145]
			O. scapulalis	[63,146]
			O. zaguliaevi	[145]
	Arachnida	Pseudoscorpionida	*Cordylochernes scorpioides*	[147]
Spiroplasma ixodetis relatives	Insecta	Coleoptera	*Adalia bipunctata*	[148,149]
			Anisosticta novemdecimpunctata	[150]
			Harmonia axyridis	[151,152]
			Menochilius sexmaculatus	[153]
		Hemiptera	*Acyrthosiphon pisum*	[77,79]
		Lepidoptera	*Danaus chrysippus*	[154]
			Ostrinia zaguliaevi	[155]

Table 2. *Cont.*

Endosymbiont	Class	Order	Species	Reference
(d) Male-killing bacteria				
Spiroplasma poulsonii		Diptera	*Drosophila nebulosa*	[156]
			D. neocardini	[157]
			D. melanogaster	[158]
			D. ornatifrons	[157]
			D. paraguayensis	[157]
			D. willistoni	[159]
Rickettsia spp.	Insecta	Coleoptera	*Adalia bipunctata*	[149,160,161]
			A. decempunctata	[162]
			Brachys tesselatus	[163]
			Propylea japonica	[164]
Flavobacteria	Insecta	Coleoptera	*Adonia variegate*	[165]
			Coccinula sinensis	[166,167]
			Coleomegilla maculata	[168]
Arsenophonus nasoniae	Insecta	Hymenoptera	*Nasonia vitripennis*	[169,170]
(e) Male-killing microsporidia				
Parathelohania legeri		Diptera	*Anopheles quadimaculatus*	[171]
Parathelohania obesa			*A. quadimaculatus*	[172]
Amblyospora spp.			*Aedes* spp.	[173,174]
Amblyospora spp.			*Culex* spp.	[174–177]
Amblyospora spp.			*Culiseta* spp.	[174]
(f) Other male killers				
Unknown virus	Insecta	Lepidoptera	*Homona magnanima*	[59,178]
			Armadillidium vulgare	[56]
			Porcellio dilatatus	[56]
			P. laevis	[56]
(g) Parthenogenesis-inducing bacteria				
Wolbachia pipientis	Insecta	Hymenoptera	*Aphytis diaspidis*	[179,180]
			A. lignaensis	[179,181]
			Aponanagyrus diversicornis	[91]
			Asobara japonica	[92]
			Diplolepis rosae	[182]
			Encarsia formosa	[90]
			Eretmocerus mundus	[183]
			Gronotoma micromorpha	[184]
			Muscidifurax uniraptor	[185,186]
			Telenomus nawai	[187]
			Trichogramma brevicapilliun	[188]
			T. chilonis	[189]
			T. cordubensis	[188,189]
			T. deion	[93,188,189]
			T. embryophagum	[188,189]
			T. evanescens	[188,189]
			T. kaykai	[190]
			T. oleae	[132,189]

Table 2. *Cont.*

Endosymbiont	Class	Order	Species	Reference
(g) Parthenogenesis-inducing bacteria				
			T. platneri	[188,189]
			T. pretiosum	[93,188,189]
	Insecta	Thysanoptera	*Franklinothrips vespiformis*	[88]
	Arachnida	Trombidiformes	*Bryobia praetiosa*	[40]
			Bryobia sp.	[40]
Cardinium hertigii	Insecta	Hymenoptera	*Encarsia hispida*	[44]
			E. pergandiella	[191]
			E. protransvena	[191,192]
	Arachnida	Trombidiformes	*Brevipalpus phoenicis*	[43]
Rickettsia spp.	Insecta	Hymenoptera	*Neochrysocharis formosa*	[96,193]
			Pnigalio soemius	[194]

5. Endosymbiont-Induced Feminization: Examples Are Scarce but May Potentially Be More Common
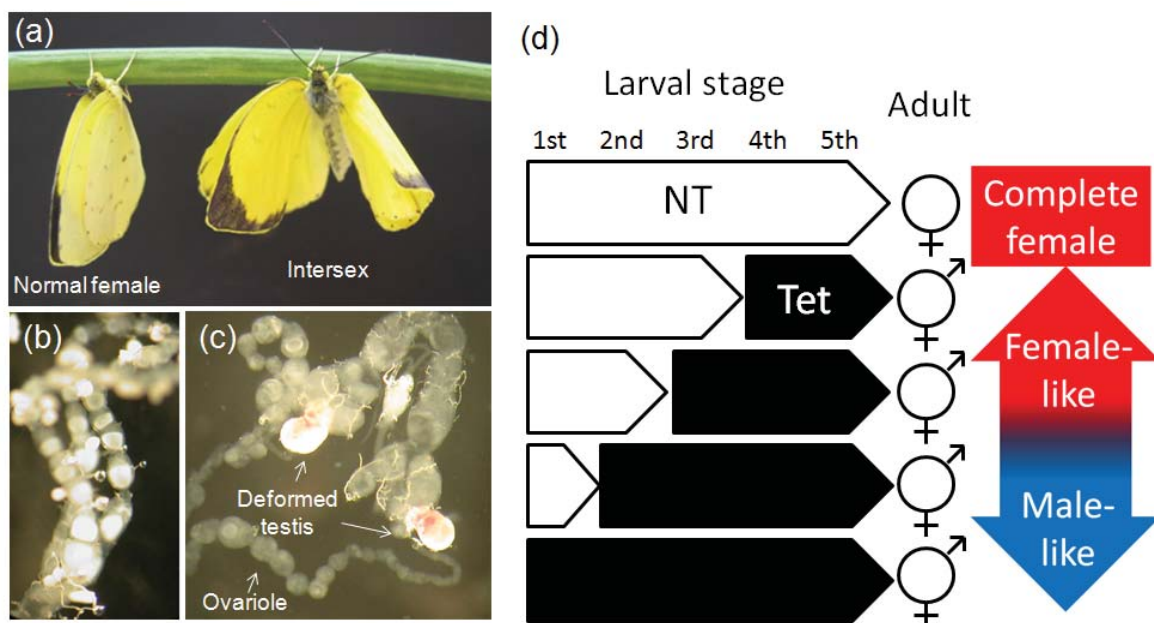
For maternally transmitted microbes, males are an evolutionary dead end. To circumvent this problem, some microbes convert males into functional females so that they can be transmitted to subsequent generations. In insects, microbe-induced feminization has only been found in butterflies and leafhoppers, wherein *Wolbachia* is the causal agent of feminization. Among non-insects, woodlice, mites and shrimps are known to be feminized by *Wolbachia*, *Cardinium* and microsporidians (e.g., *Octosporea*), respectively (Table 2).

5.1. Feminization of Butterflies by Wolbachia

In two islands located in the southern part of Japan (Tanegashima Island and Okinawa Island), some females of the butterfly *Eurema mandarina* (Lepidoptera; Pieridae) are known to have a chromosomal constitution of males (ZZ) instead of females (ZW). This incongruence between chromosomal and phenotypic sex can be explained by feminization of genetic males induced by *Wolbachia* [25,26]. Two distinct strains of *Wolbachia*, *w*CI and *w*Fem, have been found in *E. mandarina* inhabiting these islands. Females having male chromosomes (ZZ) are consistently infected with both *w*CI and *w*Fem, while females singly infected with *w*CI are true females (ZW). Despite having complete male chromosomes, ZZ females are morphologically and behaviorally completely female and fully fertile. Moreover, when such individuals are treated with tetracycline hydrochloride, a bacteriostatic antibiotic, during larval development, they develop as butterflies with intersexual morphology. Before carrying out this experiment, the appearance of intersexes in the treated generation was not expected because perturbation of sex determination was believed to occur during early embryogenesis when the sex-determining genes start to be expressed. However, the appearance of intersexes clearly shows that *Wolbachia* needs to be present during larval development for complete feminization. The fact that butterflies with more male-like characters were generated after longer antibiotic treatments shows that *Wolbachia* acts continuously during larval development [26] (Figure 2). The presence of *Wolbachia* during the embryonic stage seems to be necessary for complete feminization since antibiotic treatment

during the whole larval stages also generated intersexes instead of complete males. We recently found that the splicing pattern of the sex-determining gene *doublesex* (*dsx*) changes according to the *Wolbachia* infection status (Narita *et al.*, in preparation). *Wolbachia*-induced feminization has also been found in *Eurema hecabe*, a sibling species of *E. mandarina*. The two *Wolbachia* strains found in feminized *E. hecabe* are indistinguishable from *w*CI and *w*Fem derived from *E. mandarina* based on multi-locus sequence typing [27].

Figure 2. Effects of the antibiotic on the sexual phenotype of the butterfly *Eurema mandarina* infected with two strains of *Wolbachia* [26]. (**a**) A normal female and an intersex generated by antibiotic treatment. The intersex has both male and female features in external morphology. (**b**) Ovarioles of normal females. (**c**) The reproductive organs of the intersex. Both male-specific organs (testes) and female-specific organs (ovarioles) occur in an individual treated with tetracycline hydrochloride during larval stages. (**d**) Schematic illustration showing the effect of antibiotic treatment of larvae on adult sexual phenotype. The longer the treatment, the more male-like phenotype appears. NT: non-treated. Tet: tetracycline-treated.

5.2. Feminization of Leafhoppers by Wolbachia

A second case of *Wolbachia*-induced feminization in diploid insects has been found in the leafhopper *Zyginidia pullula* (Homoptera; Cicadellidae) [28]. In *Z. pullula*, females have two X chromosomes (2n = 8AA + XX) while males have only one X chromosome (2n = 8AA + X0). This is contrary to the situation of the *Eurema* butterflies wherein females have one Z chromosome and males have two Z chromosomes. When *Wolbachia*-infected *Z. pullula* females collected in northern Italy were mated with males, they produced exclusively female broods. Close inspection of these female broods revealed that about half of them had an intersexual morphology (*i.e.*, showing upper pygofer appendages, a typical male secondary sexual character), while the rest of the broods had the normal female phenotype. While the karyotypes of the normal females were XX, those with the upper pygofer

appendages were X0 and were thus feminized males. Administration of tetracycline hydrochloride to *Wolbachia*-infected females (phenotypically normal female) resulted in a nearly 1:1 sex ratio in the subsequent generation [28]. The incongruence between the phenotypic sex and genomic sex implies the presence of epigenetic modification. Negri *et al.* [29] compared the DNA methylation patterns between intersexual and normal *Z. pullula* by performing a methylation-sensitive random PCR and found that females with the male upper pygofer appendages showed a female methylation pattern. On the other hand, some rare feminized males bore testes instead of ovaries. And these individuals showed a male methylation pattern. These findings suggest that *Wolbachia* induces feminization by disrupting male imprinting.

5.3. Feminization of Woodlice by Wolbachia

Wolbachia can also cause feminization in non-insect arthropods. *Wolbachia*-induced feminization in the woodlouse *Armadillidium vulgare* (Isopoda; Armadillidiidae) has a long research history [30]. As known in several insect species, the *A. vulgare Wolbachia* appear to be quite sensitive to high temperatures. Very young *Wolbachia*-infected females of *A. vulgare* reared at 30 °C gradually acquire a male phenotype [31,32]. In addition to *Wolbachia*, a non-bacterial feminizing factor (f) can also force chromosomal males of *A. vulgare* to become phenotypic functional females. The f factor is suspected to be a genetic element derived from the *Wolbachia* genome that becomes inserted into the host nuclear genome [33]. Genes resisting the feminizing effects or the transmission of feminizing elements have been found in natural populations of *A. vulgare* [34,35], thus illustrating the conflict between the feminizing elements and the rest of the host genome.

Unlike insects, androgenic hormone, a sex hormone secreted from a specific male organ called the androgenic gland, profoundly affects male sexual differentiation in *A. vulgare* [36,37]. In this respect, sex determination in *A. vulgare* seems to be more labile compared with that in insects. It is considered that *Wolbachia* feminize genetic males by disrupting the secretion of androgenic hormone. Before the discovery of feminization in *Eurema* butterflies, *Wolbachia* was assumed to be incapable of inducing complete feminization in insects [31]. This assumption appeared reasonable considering the substantial differences in the sex-determining systems between insects and non-insects. The actual occurrence of *Wolbachia*-induced feminization in both insects and non-insects may imply the presence of some common mechanism of sex determination that is targeted by *Wolbachia*. Alternatively, the feminization found in insects and non-insects may have distinct underlying mechanisms and only be superficially similar.

5.4. Feminization of Mites and Wasps by Cardinium Bacteria

The false spider mite, *Brevipalpus phoenicis* (Acarina; Tenuipalpidae), along with two closely related species, *Brevipalpus obovatus* and *Brevipalpus californicus*, is known to reproduce by thelytokous parthenogenesis [38]. Surprisingly, *B. phoenicis* females have a haploid genome, which was confirmed by fluorescence microscopy and variations at nine microsatellite loci [39]. Treatment of adult females with tetracycline hydrochloride led to a drastic increase in the proportion of male offspring (from 5.6% to 50.6%), suggesting that haploid individuals were feminized by a bacterium [40]. This bacterium is now known to be a member of the genus *Cardinium* [41]. Haploid individuals of the

other species, *B. obovatus* and *B. californicus*, were also found to be feminized by *Cardinium* [42,43]. A feminizing effect of *Cardinium* was also found in the wasp *Encarsia hispida* (Hymenoptera; Aphelinidae), in which thelytokous parthenogenesis is known [44] (see Section 7.1).

5.5. Feminization of Shrimps by Microsporidian Parasites

Sex determination of the amphipod crustacean *Gammarus duebeni* (Amphipoda; Gammaridae) is more complex compared with that of insects. Basically, males and females are determined by a balance of a polyfactorial system of allelic sex genes located on several pairs of chromosomes, but the photoperiod can profoundly affect the sex determination [30]. Moreover, based on detailed breeding experiments, cytoplasmic factors were considered to act as female determiners in *G. duebeni* [45]. Subsequently, it was found that several microsporidian parasites such as *Octosporea effeminans*, *Nosema granulosis* and *Dictyocoela duebenum* living in the host cytoplasm alter male *G. duebeni* to functional females [46–52]. Rodgers-Gray *et al.* [50] demonstrated that *Nosema* manipulate the sex differentiation of *G. duebeni* by preventing androgenic gland differentiation, androgenic gland hormone production and consequently male differentiation. This is in agreement with observations of *Wolbachia*-induced feminization in *A. vulgare*. Although taxonomically unrelated (eukaryotes and prokaryotes), these feminizers may manipulate their crustacean hosts through a common mechanism.

5.6. Feminization Induced by Non-Microbes

Feminization can also be caused by non-microbes. According to Vance [53], more than 10% of wild-caught adults of the mayfly *Baetis bicaudatus* (Ephemeroptera; Baetidae) are parasitized by nematode *Gasteromermis* sp. (Nematoda; Mermithidae). None of the parasitized individuals (n = 126) contained visible eggs, ovaries or testes. Among them, the external morphology of 82 individuals (65%) was indistinguishable from that of normal females. The remaining 44 parasitized individuals showed an array of intersexual morphologies. Moreover, measurement of the DNA contents by flow cytometry suggested that the parasitized individuals having intersexual morphologies ("parasitized intersexes") were genetically male individuals while parasitized individuals visibly indistinguishable from normal females ("parasitized females") were composed of both genetically female and genetically male individuals [53]. The behaviors of the mayfly are also changed by the nematode. Unparasitized males form swarms near the river and do not return to the water after they have emerged. Vance [53] found that all 418 swarming individuals were unparasitized males and that the parasitized individuals showed ovipositing behavior, which is never seen in unparasitized males. Laboratory studies demonstrated that parasitized individuals (both parasitized females and parasitized intersexes) became very agitated shortly after emergence as adults. Within 3–6 hours, all the parasitized mayflies had crawled into the water down the side of the rock. The nematodes could then be seen escaping through a puncture wound in the mayfly's abdomen [53]. Therefore, the *Gasteromermis* nematode can horizontally transmit to a new host. The mayflies were killed by the emergence of the nematode. The feminization in this case seems to be an adaptive strategy for the *Gasteromermis* nematodes, which do not have a vertical route of transmission.

Although less conspicuous than the case of the mayfly, perturbation of the development of secondary sexual characters by endosymbionts or parasites has also been found in various insects [54,55], in which the adaptive role of feminization remains unclear.

5.7. Masculinization Induced by Viruses

In woodlice such as *Porcellilo dilatatus*, *Porcellio laevis* and *A. vulgare*, an intersexual trait in genetic females is known to be transmitted from both parents. Viruses are considered to be the causal agent of the masculinization in genetic females because male characters disappeared after heat treatment, male characters appeared after injection of a 0.22-μm-filtered tissue extract and intersexuality was correlated with the presence of cytoplasmic viral particles [56]. The adaptive significance of the masculinizing effect remains unknown.

6. Endosymbiont-Induced Male Killing: An Easily Evolved Trait?

Compared with feminization, male killing is more common in insects (Table 2). Moreover, male killers have been found in taxonomically diverse microorganisms (e.g., bacteria in the genus *Spiroplasma*, *Wolbachia*, *Rickettsia*, *Arsenophonus*; unicellular eukaryotes in the phylum Microsporidia; and RNA viruses). Therefore, male killing may be easy to evolve [57]. Among them, *Wolbachia* and *Spiroplasma* are prevalent male killers among insects. In bacteria-induced male killing, only males are typically killed during embryonic or early larval stages which is called "early" male killing. On the other hand, in male killing induced by microsporidia and RNA viruses, the males are killed during later development (typically during the last stage of larva) which is called "late" male killing [58,59].

6.1. Function of Dosage Compensation is Necessary for Male Killing

Drosophila have two X chromosomes in females and only one X chromosome in males. By hypertranscribing the X-linked genes in males, *Drosophila* dissolve the imbalance in the gene dosage of the X-linked genes, which is often called dosage compensation. This process requires the formation of the dosage compensation complex (DCC), which consists of MSL-1, MSL-2, MSL-3, MLE and MOF [60].
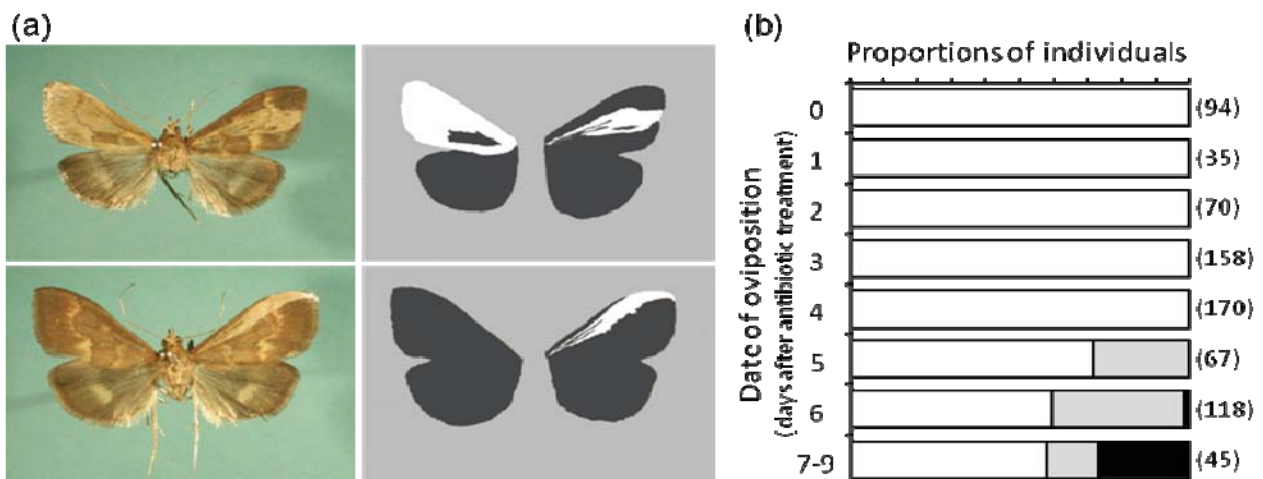
How do male killers discriminate between males and females? Using classic genetic experiments, Veneti *et al.* [61] tested the ability of *Spiroplasma* to kill *D. melanogaster* males carrying mutations in genes encoding the DCC. Interestingly, *Spiroplasma* failed to kill males lacking any of the five protein components (MSL-1, MSL-2, MSL-3, MLE and MOF) of the DCC. Therefore, although the direct mechanism of male killing is elusive, their study clearly showed that the presence of the functional DCC is necessary for *Spiroplasma* to cause male killing in *D. melanogaster*.

6.2. Male Killing by Lethal Effect of Feminization?

A mechanistic implication of male killing was obtained in our study on *Wolbachia*-induced male killing in the moth *Ostrinia scapulalis* (Lepidoptera; Crambidae), namely that males are killed by the feminizing effect of *Wolbachia*. Similar to other lepidopteran species, the sex chromosome constitution of *O. scapulalis* is ZZ in males and ZW in females. *Wolbachia*-infected females produce both ZZ and

ZW offspring, but only ZZ offspring die during late embryonic and larval development. Interestingly, when adult females were fed with sucrose containing an antibiotic (tetracycline hydrochloride) prior to oviposition, they produced eggs that developed as intersexes as well as normal males and females [62–64] (Figure 3). The forewings of these intersexes showed a clear mosaic pattern of male-color and female-color sectors. Some of the intersexes had the bursa copulatrix, the female organ which accepts spermatophores from males during mating. Surprisingly, none of the tissues of the intersexes, including the bursa copulatrix, had a W chromosome, thus representing the pure male genotype (ZZ). Therefore, it is clear that the bursa copulatrix in these intersexes shows a female phenotype under a male genotype. Based on these findings, we concluded that *Wolbachia* has a feminizing effect on *Ostrinia* males but its full expression, which occurs in the natural condition, is lethal for genetic males. A reduction in the *Wolbachia* density during embryogenesis may result in partially feminized individuals (intersexes), probably due to the attenuated expression of the feminizing effect. The sex-determining gene *dsx* of these intersexes was shown to exhibit both male and female splicing patterns [65]. Reported very recently was conclusive evidence for the feminization as a mechanism of male killing in *Ostrinia*. In *Wolbachia*-infected *O. scapulalis*, female-specific splicing in *dsx* was observed in all the embryos including those with male genotype (ZZ) which were destined to die [66].

> **Figure 3.** Effects of antibiotics on sexual phenotype of the moth *Ostrinia scapulalis* infected with *Wolbachia* [62]. (**a**) Intersexual individuals generated by tetracycline treatment. Black and white depict male-like color and female-like color, respectively. (**b**) The proportion of phenotypic females (white), intersexes (gray) and phenotypic males (black) among individuals whose mothers were treated with tetracycline hydrochloride prior to oviposition.

In addition, this case of male killing in *Ostrinia* showed another aspect when *Wolbachia* was completely eliminated. While other cases of male killing exhibit a normal 1:1 sex ratio after elimination of the male killers in their mothers, the elimination of *Wolbachia* from *Ostrinia* females by treatment with tetracycline hydrochloride during the whole larval stages resulted in the appearance of only males in the subsequent generation. This finding was erroneously interpreted as evidence for the *Wolbachia*-induced feminization [67,68]. However, later studies clearly showed that half of these offspring having female karyotype (ZW) die during late embryonic and larval development [63,64].

These individuals were recently shown to exhibit male-specific splicing in *dsx* [66], which implies that a female-determining factor, possibly located on W chromosome is degraded in *Ostrinia* and the *Wolbachia* substitutes the sex-determining role.

6.3. Hidden Male Killing

In some cases, male killing is masked or suppressed. *Wolbachia*-induced male killing was reported in the butterfly *Hypolimnas bolina* (Lepidoptera; Nymphalidae) [69,70]. In *H. bolina*, a strong female-biased sex ratio has been maintained for more than 100 years [71]. Surprisingly, in some populations, host resistance to male killing has recently spread and reached fixation [72–74]. In these populations, *Wolbachia* ceased to kill males but was not excluded from the host population. Hornett *et al.* [75] revealed that the same *Wolbachia* was inducing cytoplasmic incompatibility in the butterfly. This finding can explain the persistence of *Wolbachia* in the *H. bolina* population without inducing male killing.

In *Drosophila recens*, the sex ratios are not affected by naturally-occurring *Wolbachia*. This *Wolbachia* causes strong cytoplasmic incompatibility (see below) in *D. recens* and the infection frequency of *Wolbachia* in this species is 98%. On the other hand, none of the individuals are infected with *Wolbachia* in *Drosophila subquinaria*. Interestingly, introgression of the *D. recens Wolbachia* into *D. subquinaria* by hybridization and backcrossing resulted in the expression of male killing [76]. Furthermore, crossing experiments have demonstrated that the resistance to male killing is dominant, autosomal, multigenic and dependent on the zygotic, not maternal, genotype [76]. Similar to the case of *H. bolina*, male killing was masked by the fixation of resistant genes in *D. recens*. If there were no sister species, we would not have been able to reveal the potential for male killing in this insect.

The pea aphid *Acyrthosiphon pisum* is known to exhibit cyclical parthenogenesis. Previously, infection of *Spiroplasma* was only investigated under the asexual reproduction mode [77,78]. The effects of various endosymbionts in *A. pisum* under the sexual reproduction mode were recently analyzed and *Spiroplasma* was revealed to cause male killing [79]. The fact that *Spiroplasma* found in *A. pisum* was monophyletic with other male-killing *Spiroplasma* in ladybird beetles, butterflies and moths may indicate a common origin of the male-killing ability.

6.4. Is Timing of Male Killing Important?

Why do these microbes kill males? Killing only males during early development (early male killing) is considered advantageous for maternally transmitted microbes for three possible reasons that are not mutually exclusive [80,81]: (i) females can gain extra resources that were to be allocated to their brothers (resource reallocation); (ii) mating with sibling can be avoided (inbreeding avoidance); and (iii) microbes can be transmitted from dead (or dying) males to females by oral intake (horizontal transmission by cannibalism). On the other hand, resource reallocation cannot explain late male killing because males would consume nearly all the resources necessary for their own development before they are killed. Microsporidian parasites that cause late male killing in mosquitoes have intermediate hosts (copepods). Killing the mosquito larvae of large body size (late male killing) is considered to be an optimal strategy for the microsporidians because they can maximize their number and thereby increase their probability of transmission to copepod hosts [58,82].

Thus, the classification of male-killing phenotypes is based not only on the timing of the action, but also on the evolutionary strategies adopted by the symbionts and microbial agents responsible for the phenotypes (*i.e.*, bacteria versus eukaryotes and viruses). Therefore, it has been assumed that early and late male killing are fundamentally different phenomena whose underlying mechanisms are also entirely different [83,84]. In *Drosophila*, however, a *Spiroplasma* strain that normally cause early male killing was also found to induce late male killing depending on the maternal host age, in that male-specific mortality of larvae and pupae was more frequently observed in the offspring of young females [85]. Since the lowest *Spiroplasma* density and occasional male production were also associated with newly emerged females, we proposed a density-dependent hypothesis for the expression of the early and late male-killing phenotypes. Early male killing and late male killing could be regarded as alternative strategies adoptable by microbial reproductive manipulators [85].

The timing of male killing can be artificially changed in the *Wolbachia*-infected butterfly, *H. bolina*. Treatment of *Wolbachia*-infected adult females with tetracycline, a bacteriostatic antibiotic, produced a delay in the timing of male death. On the other hand, treatment of the surviving larvae with rifampicin, a bactericidal antibiotic, rescued the males. Based on these findings, it was hypothesized that *Wolbachia* possesses the ability to kill males through bacterial activity during larval development [86]. This phenomenon argues against the view that male killing is achieved by specifically targeting an early developmental process within the sex determination pathway. This is in line with the case of *Wolbachia*-induced feminization in the butterfly *E. mandarina*, wherein *Wolbachia* needs to be present during larval development for complete feminization [26].

7. Parthenogenesis Induction: Conversion of Genetic Males to Genetic Females or Feminization Following Diploidization?

Microbe-induced thelytokous parthenogenesis has been reported in haplodiploid arthropods, such as wasps, thrips and mites [19,87,88] (Table 2). On the other hand, there is no conclusive evidence for the microbe-induced parthenogenesis in diplodiploid organisms, although involvement of *Wolbachia* in thelytokous parthenogenesis in springtails and booklice is suspected based on the presence of *Wolbachia* [89]. In most of the known cases, microbe-induced parthenogenesis is achieved by altering the male genotype (haploid) to the female genotype (diploid), *i.e.*, restoration of diploidy. In this sense, the phenomenon can also be referred to as conversion of genetic males to genetic females [49]. At least in the wasp *E. hispida*, however, the restoration of diploidy is not sufficient for female development, and thus diploidization and feminization can be separate processes [44]. Frequently, certain species or populations are fixed for parthenogenesis-inducing microbes. In such cases, selection will not act on host genes involved in male and female sexuality (e.g., mating behaviors, sperm production, or sex pheromone production). Thus, mutations in such genes are expected to accumulate, resulting in eventual degeneration of sexuality and irreversible parthenogenesis. This process appears to be occurring in species of wasps [90–92] and thrips [88]. In contrast, sexual functions are not degenerated in *Trichogramma deion*, wherein parthenogenesis-inducing *Wolbachia* is not fixed and genetic exchange occurs between sexual and asexual individuals [93].

7.1. Mechanisms of Microbe-Induced Parthenogenesis

There are various mechanisms underlying microbe-induced parthenogenesis. Gamete duplication appears to be common in *Wolbachia*-infected hymenopteran wasps [93,94]. In wasps such as *Trichogramma* species and *Leptopilina clavipes*, meiosis is normal, but during the first mitotic division, the chromosomes fail to segregate in metaphase, resulting in diploidization of the nucleus [93,94]. In *Muscidifurax uniraptor*, on the other hand, meiosis and the first mitotic division are normal, but during the second mitotic division, diploid females are produced by the fusion of two cell nuclei [95]. Each offspring produced by gamete duplication is a homozygote at all loci and is not a genomic copy of its mother. On the other hand, the genotype of all offspring was indistinguishable from their mothers in *Rickettsia*-induced parthenogenesis occurring in *Neochrysocharis formosa* (Hymenoptera; Eulophidae) based on the polymorphisms in a microsatellite locus [96]. Moreover, by excluding the possibility of automictic parthenogenesis with central fusion, which has been observed in some ants [97,98], and using cytogenetic observations, Adachi-Hagimori *et al.* [96] concluded that apomictic parthenogenesis is the underlying mechanism wherein eggs do not undergo meiosis. Similarly, in the *Wolbachia*-induced parthenogenesis in the mite *Bryobia praetiosa*, the genotypes of the mothers and offspring are indistinguishable based on the polymorphisms in three microsatellite loci [40]. Therefore, apomictic parthenogenesis is the likely mechanism for this phenomenon as Weeks *et al.* [40] assumed, but another possibility of automictic parthenogenesis with central fusion cannot be completely excluded owing to the lack of cytogenetic observations.

It has been naturally assumed that diploidization induced by microbes automatically leads to female development. However, a recent finding indicates that, in the wasp *E. hispida*, *Cardinium*-induced parthenogenesis induction does not occur by diploidization alone. Feeding antibiotics to infected adult *E. hispida* females resulted in uninfected male offspring. By karyotype observations and flow cytometry analyses, Giorgini *et al.* [44] demonstrated that these males were diploid. This finding indicates that at least in *E. hispida*, diploidy restoration is necessary but not sufficient for female development. Thus, *Cardinium* is required to feminize diploid male embryos [44]. In this sense, this example should also be added to the list of microbe-induced feminization. As Giorgini *et al.* [44] argues, it might be necessary to consider the possibility that the mechanism of *Wolbachia*-induced parthenogenesis is also comprised of two separate steps, *i.e.*, diploidization and feminization.

8. Other Phenotypes

Here we briefly overview the other conspicuous effects caused by the above endosymbionts (Table S1).

8.1. Cytoplasmic Incompatibility

Cytoplasmic incompatibility is the most common phenotype of *Wolbachia* and *Cardinium* infection (Table S1). In diploid organisms, cytoplasmic incompatibility is an embryonic lethality that results in sperm and eggs having different cytoplasmic contents [17,99,100]. The effect arises from changes in the gamete cells caused by cytoplasmic (intracellular) parasites like *Wolbachia* and *Cardinium*, which infect a wide range of insect species. Cytoplasmic incompatibility occurs when a *Wolbachia*-infected

male mates with a female that is either uninfected (unidirectional cytoplasmic incompatibility) or infected by another *Wolbachia* strain (bidirectional cytoplasmic incompatibility). Any other combinations of crosses are compatible. An infected female is compatible with an uninfected male or any infected male of the same *Wolbachia* strain. On the other hand, an uninfected female is only compatible with an uninfected male.

Cytoplasmic incompatibility in haplodiploid hosts may lead to haploid (male) offspring. Cytoplasmic incompatibility produces distinct phenotypes in three closely related haplodiploid hymenopteran species of the genus *Nasonia*, namely mortality in *Nasonia longicornis* and *Nasonia giraulti*, and conversion to male development in *Nasonia vitripennis* [101].

8.2. Beneficial Effects of Wolbachia *and* Spiroplasma *on Hosts*

In addition to arthropods, some nematodes are also hosts of *Wolbachia* [102]. In filarial nematodes, *Wolbachia* does not seem to exhibit selfish behaviors like reproductive manipulations. Instead, *Wolbachia* is necessary for the host nematodes. Elimination of *Wolbachia* from filarial nematodes generally results in either death or sterility of the nematodes [103]. Consequently, current strategies for the control of filarial nematode diseases include elimination of *Wolbachia* via treatment with the antibiotic doxycycline rather than far more toxic anti-nematode medications [102].

In the bedbug *Cimex lectularius* (Hemiptera; Cimicidae), *Wolbachia* appears to be an obligate nutritional mutualist. *Wolbachia* is specifically localized in the bacteriomes, a specialized organ for endosymbiotic bacteria, and vertically transmitted via the somatic stem cell niche of germalia to oocytes. The transmitted *Wolbachia* infects the incipient symbiotic organ at an early stage of embryogenesis. Elimination of *Wolbachia* results in retarded growth and sterility of the host insect. However, these deficiencies are rescued by oral supplementation of B vitamins. These findings suggest that *Wolbachia* provides essential nutrition for this host [104].

In the parasitic wasp *Asobara tabida* (Hymenoptera; Braconidae), a strain of *Wolbachia* is necessary for oogenesis [105]. It is also known that *Wolbachia* rescues the oogenesis defect in a *Sex-lethal* mutant of *D. melanogaster* [106].

Increasing attention has been paid to the recent findings that *Wolbachia* can protect *Drosophila* against pathogenic RNA viruses such as *Drosophila* C virus, Cricket Paralysis virus, Flock House virus, Nora virus [107–109] and West Nile virus [110], as well as the fungus *Beauveria bassiana* [111]. Interestingly, in mosquitoes, the presence of transinfected *Wolbachia* interferes with a wider range of pathogens including nematodes and bacteria [112], Dengue virus and Chikungunya virus [113,114], as well as the avian and rodent malaria parasites *Plasmodium gallinaceum* [114] and *Plasmodium berghei* [115]. Biological control of mosquito-borne diseases is at the testing stage [116–118].

Spiroplasma also has similar effects on its hosts. *Drosophila neotestacea*, a fly that feeds on mushrooms, suffers complete loss of fecundity when parasitized by the nematode *Howardula aoronymphium* (Allantonematidae; Tylenchida). However, flies infected with *Spiroplasma* have near normal fecundity when parasitized by the nematode [119].

9. Transition of the Biological Systems of Arthropods by Sex-Associated Microbes
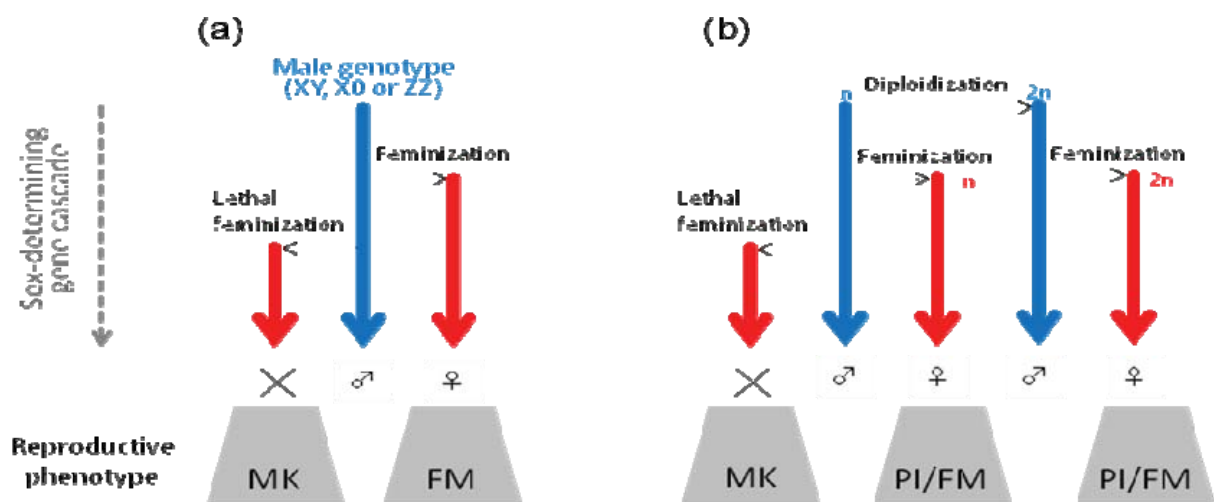
Despite the diversity of the sex-determining systems of arthropods and the diversity of microbial agents, the types of reproductive manipulation seem to be limited. For example, male killing occurs in male heterogametic insects, female heterogametic insects and haplodiploid insects. Moreover, the causal agents of male killing include a wide variety of bacteria as well as some eukaryotic organisms. Similarly, feminization occurs in arthropods having a ZW-ZZ system (butterflies, woodlice and shrimps), XX-X0 system (leafhoppers) and haplodiploid system (mayflies), and the causal agents of feminization also include bacteria and eukaryotes. Considering the stability of vertical transmission, these endosymbiotic microbes can be assumed to be sex-determining elements in the cytoplasm that work together with other sex-determining genes in the nucleus. It can also be assumed that the effects of these cytoplasmic sex-determining elements are relatively well conserved among arthropods.

Moreover, the presence of resistance genes against male killers and feminizers has been documented in some arthropods [73,76,120]. These resistance genes against microbial effects might be recognized as sex-determining genes. Although speculative, some of the sex-determining genes, such as *Sex-lethal*, *intersex*, *daughterless* and *sisterless* in *D. melanogaster*, could originally have arisen as resistance genes against the effects of the microbes and become fixed within the species. The diversity and complexity of the sex-determining systems of insects might have been generated by an arms race involving repeated invasion and/or mutation of various microbes and other genetic elements, which may not necessarily exist at the present time [2].

Currently, almost nothing is known about the molecular mechanisms of these acquired sex-determining systems. Regardless of the actual mechanisms, however, we can hypothesize a possible link between the microbial effects and the diversity of arthropod sex determination. As shown in Figure 4, one can place the possible effects of endosymbionts in the informational flow of sex-determining signals. To cause feminization, for example, endosymbionts need to simultaneously affect the sex determination in the germline, somatic line and central nervous system, each of which is considered to depend on a distinct expression cascade consisting of different genes. To achieve this, the endosymbionts may interfere with certain elements in the upstream of the sex-determining gene cascade that play pivotal roles, like *Sxl* in *Drosophila*, in sex determination in different lineages [4,121]. On the other hand, at least in *Ostrinia* moths, male killing may involve interactions with downstream genes, which may result in simultaneous expression of sexually antagonistic, male-specific and female-specific, genes and lead to lethality [63,65]. Although it was previously assumed that diploidization is the only effect of the parthenogenesis inducers in haplodiploid insects, recent findings in the *E. hispida* wasps imply that we need to take into account the possibility that endosymbionts may cause two distinct effects in order, *i.e.*, diploidization and feminization, the latter of which can be integrated into the feminization phenomena found in diploid insects. It is tempting to consider that, although endosymbionts work very simply (e.g., by only producing some proteins), the diversity and complexity of the host biology generate the diverse effects on the phenotype. A straightforward and frequently-used approach to elucidate the mechanisms of microbial effects on their hosts would be to compare the gene expression profiles between infected and uninfected insects. However, despite various attempts to compare the gene expression profiles [122–125], the mechanisms of reproductive manipulation still remain unclear. We consider that the mechanisms of microbe-induced reproductive

manipulation will be clarified by a deeper understanding of insect development and physiology. Moreover, genomic data of endosymbionts and their hosts together with sophisticated mutagenesis techniques may allow reverse genetics approaches to elucidate the precise mechanism of reproductive manipulations.

Figure 4. A parsimonious explanation of male killing (MK), feminization (FM) and parthenogenesis induction (PI). Here, MK, FM and PI are all assumed to be associated with the sex-determining process. The hypothetical flows of the male-determining and female-determining information are shown with blue and red arrows, respectively. Horizontal arrows indicate manipulation by endosymbionts. Note that this explanation is a hypothesis and may not necessarily reflect the actual mechanisms.

10. Population-Level Effects of Endosymbiotic Microbes on Their Hosts

The spread and fixation of male killers and feminizers should lead to the absence of males and the extinction of the sexually reproducing hosts [126]. Moreover, a strongly-biased population sex ratio leads to a drastic reduction in the effective population size, which may inevitably cause inbreeding. Furthermore, inbreeding may cause a reduction in fecundity, which may increase the probability of extinction. In fact, a decrease in heterozygosity, an indication of inbreeding, was shown to increase the risk of extinction of island populations of the butterfly *Melitaea cinxia* [127]. However, endosymbiont-driven extinction has not been empirically demonstrated thus far.

Instead, in some butterflies, a *Wolbachia*-induced extreme bias in the population sex ratio was shown to be maintained for substantially long periods of time [71,128]. Moreover, resistance genes against male killers and feminizers have been documented [34,35]. Is this a usual reaction of hosts against the invasion of male killers and feminizers? We should be aware, however, that it is practically difficult to show the occurrence of population extinction caused by the spread and fixation of male killers and feminizers. Under the evolutionary timescale, the time required for sex-ratio distorters to spread among the host population can be too short for resistance genes to evolve. Owing to the short timeframe, the probability that we can observe a gradual decline in a population size is extremely low, even if endosymbiont-driven extinctions occur repeatedly in natural populations.

11. Conclusions

The accumulating data demonstrate that a variety of sexual aberrations in arthropods is imposed by endosymbionts (e.g., bacteria, protists, nematodes, insects and viruses). Although reducing the diverse phenomena to simple fundamental mechanisms may be dangerous and is not completely possible at present, some of the sexual aberrations can be explained by the interference with the sex-determining systems: for instance, male killing can be induced by the lethal effect of *Wolbachia*-induced feminization in a moth; parthenogenesis can be induced by *Cardinium* that feminizes haploid males in a mite; and diploid males are transformed by *Cardinium* into parthenogenetic females in a wasp. Importantly, these endosymbionts may profoundly affect host behavior, ecology and population structure. At present, the molecular mechanisms of any of the sexual aberrations are largely unknown. The deeper understanding of the arthropod physiology and development as well as arthropod and microbial genomes may allow us to elucidate the mechanism of the sexual aberrations, which will benefit both applied sciences (e.g., management of agricultural and medical pests and mass rearing of beneficial organisms) and other areas of biological sciences.

References

1. Bull, J.J. *Evolution of Sex Determining Mechanisms*; The Benjamin/Cummings Publishing Company, Inc.: Menlo Park, CA, USA, 1983; p. 316.
2. Werren, J.H.; Beukeboom, L.W. Sex determination, sex ratios, and genetic conflict. *Ann. Rev. Ecol. Evol. Syst.* **1998**, *29*, 233–261.
3. Heimpel, G.E.; de Boer, J.G. Sex determination in the hymenoptera. *Ann. Rev. Entomol.* **2008**, *53*, 209–230.
4. Schütt, C.; Nöthiger, R. Structure, function and evolution of sex-determining systems in Dipteran insects. *Development* **2000**, *127*, 667–677.
5. Nöthiger, R.; Steinmann-Zwicky, M. A single principle for sex determination in insects. *Cold Spring Harb. Symp. Quant. Biol.* **1985**, *50*, 615–621.
6. Hoy, M.A. *Insect Molecular Genetics*, 2nd ed.; Academic Press/Elsevier: San Diego, CA, USA, 2003; p. 560.
7. Verhulst, E.C.; van de Zande, L.; Beukeboom, L.W. Insect sex determination: It all evolves around transformer. *Curr. Opin. Genet. Dev.* **2010**, *20*, 376–383.
8. Laugé, G. Sex determination: Genetic and epigenetic factors. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 1. Embryogenesis and Reproduction*; Kerkut, G.A., Gilbert, L.I., Eds.; Pergamon Press: Oxford, UK, 1985; pp. 295–318.

9. Narita, S.; Pereira, R.A.S.; Kjellberg, F.; Kageyama, D. Gynandromorphs and intersexes: Potential to understand the mechanism of sex determination in arthropods. *Terrestrial Arthropod Rev.* **2010**, *3*, 63–96.

10. Brust, R. Temperature-induced intersexes in Aedes mosquitoes: Comparative study of species from Manitoba. *Can. Entomol.* **1968**, *100*, 879–891.

11. Horsfall, W.R.; Anderson, J.F. Suppression of male characteristics of mosquitoes by thermal means. *Science* **1961**, *133*, 1830.

12. Horsfall, W.R.; Anderson, J.F. Thermally induced genital appendages on mosquitoes. *Science* **1963**, *141*, 1183.

13. Goldschmidt, R. *Lymantria. Bibliogr. Genet.* **1934**, *11*, 1–186.

14. Mosbacher, G.C. Die Intersexualität bei Lymantria dispar L. (Lepidoptera). *Z. Morphol. Tiere* **1973**, 76.

15. Mosbacher, G.C. Sex specific cell differentiation in different types of intersexes of *Lymantria dispar* L. In *Intersexuality in the Animal Kingdom*; Reinboth, R., Ed.; Springer: Berlin, Germany, 1975; pp. 146–157.

16. Seiler, J. Sexuality as developmental process. In *Genetics Today. Proc. XI Int. Congress of Genetics, The Hague 1963*; Geerts, S.J., Ed.; Pergamon: Oxford, UK, 1965; pp. 199–207.

17. Werren, J.H. Biology of *Wolbachia. Ann. Rev. Entomol.* **1997**, *42*, 587–609.

18. Werren, J.H.; Baldo, L.; Clark, M.E. *Wolbachia*: Master manipulators of invertebrate biology. *Nat. Rev. Microbiol.* **2008**, *6*, 741–751.

19. Stouthamer, R.; Breeuwer, J.A.J.; Hurst, G.D.D. Microbial Manipulator of Arthropod Reproduction. *Ann. Rev. Microbiol.* **1999**, *53*, 71–102.

20. Douglas, A.E. Nutritional interactions in insect-microbial symbioses: Aphids and their symbiotic bacteria *Buchnera. Ann. Rev. Entomol.* **1998**, *43*, 17–37.

21. Weiss, B.L.; Wang, J.; Aksoy, S. Tsetse immune system maturation requires the presence of obligate symbionts in larvae. *PLoS Biol.* **2011**, *9*, e1000619.

22. Baldo, L.; Ayoub, N.A.; Hayashi, C.Y.; Russell, J.A.; Stahlhut, J.K.; Werren, J.H. Insight into the routes of *Wolbachia* invasion: High levels of horizontal transfer in the spider genus *Agelenopsis* revealed by *Wolbachia* strain and mitochondrial DNA diversity. *Mol. Ecol.* **2008**, *17*, 557–569.

23. Russell, J.A.; Goldman-Huertas, B.; Moreau, C.S.; Baldo, L.; Stahlhut, J.K.; Werren, J.H.; Pierce, N.E. Specialization and geographic isolation among *Wolbachia* symbionts from ants and lycaenid butterflies. *Evolution* **2009**, *63*, 624–640.

24. Stahlhut, J.K.; Desjardins, C.A.; Clark, M.E.; Baldo, L.; Russell, J.A.; Werren, J.H.; Jaenike, J. The mushroom habitat as an ecological arena for global exchange of *Wolbachia. Mol. Ecol.* **2010**, *19*, 1940–1952.

25. Hiroki, M.; Tagami, Y.; Miura, K.; Kato, Y. Multiple infection with *Wolbachia* inducing different reproductive manipulations in the butterfly *Eurema hecabe. Proc. R. Soc. Lond. B* **2004**, *271*, 1751–1755.

26. Narita, S.; Kageyama, D.; Nomura, M., Fukatsu, T. Unexpected mechanism of symbiont-induced reversal of insect sex: Feminizing *Wolbachia* continuously acts on the butterfly *Eurema hecabe* during larval development. *Appl. Environ. Microbiol.* **2007**, *73*, 4332–4341.

27. Narita, S.; Kageyama, D.; Hiroki, M.; Sanpei, T.; Hashimoto, S.; Kamitoh, T.; Kato, Y. *Wolbachia*-induced feminisation newly found in *Eurema hecabe*, a sibling species of *Eurema mandarina* (Lepidoptera: Pieridae). *Ecol. Entomol.* **2011**, *36*, 309–317.

28. Negri, I.; Pellecchia, M.; Mazzoglio, P.J.; Patetta, A.; Alma, A. Feminizing *Wolbachia* in *Zyginidia pullula* (Insecta, Hemiptera), a leafhopper with an XX/X0 sex-determination system. *Proc. R. Soc. Lond. B* **2006**, *273*, 2409–2416.

29. Negri, I.; Franchini, A.; Gonella, E.; Daffonchio, D.; Mazzoglio, P.J.; Mandrioli, M.; Alma, A. Unravelling the *Wolbachia* evolutionary role: The reprogramming of the host genomic imprinting. *Proc. R. Soc. Lond. B* **2009**, *276*, 2485–2491.

30. Legrand, J.J.; Legrand-Hamelin, E.; Juchault, P. Sex determination in Crustacea. *Biol. Rev.* **1987**, *62*, 439–470.

31. Rigaud, T. Inherited microorganisms and sex determination of arthropod hosts. In *Influential Passengers*; O'Neill, S.L., Hoffmann, A.A., Werren, J.H., Eds.; Oxford University Press: Oxford, UK, 1997; pp. 81–101.

32. Thierry, R.; Juchault, P.; Mocquard, J.P. The evolution of sex determination in isopod crustaceans. *BioEssays* **1997**, *19*, 409–416.

33. Juchault, P.; Mocquard, J.P. 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. *J. Evol. Biol.* **1993**, *6*, 511–528.

34. Rigaud, T.; Juchault, P. Genetic control of the vertical transmission of a cytoplasmic sex factor in *Armadillidium vulgare* Latr. (Crustacea, Oniscidea). *Heredity* **1992**, *68*, 47–52.

35. Rigaud, T.; Juchault, P. Conflict between feminizing sex ratio distorters and an autosomal masculinizing gene in the terrestrial isopod *Armadillidium vulgare* Latr. *Genetics* **1993**, *133*, 247–252.

36. Martin, G.; Sorokine, O.; Moniatte, M.; Bulet, P.; Hetru, C.; Van Dorsselaer, A. The structure of a glycosylated protein hormone responsible for sex determination in the isopod, *Armadillidium vulgare*. *Eur. J. Biochem.* **1999**, *262*, 727–736.

37. Okuno, A.; Hasegawa, Y.; Ohira, T.; Katakura, Y.; Nagasawa, H. Characterization and cDNA cloning of androgenic gland hormone of the terrestrial isopod *Armadillidium vulgare*. *Biochem. Biophys. Res. Comm.* **1999**, *264*, 419–423.

38. Helle, W.; Bolland, H.R.; Heitmans, W.R.B. Chromosomes and types of parthenogenesis in the false spider mites (Acari: Tenuipalpidae). *Genetica* **1980**, *54*, 45–50.

39. Weeks, A.R.; Marec, F.; Breeuwer, J.A.J. A mite species that consists entirely of haploid females. *Science* **2001**, *292*, 2479–2482.

40. Weeks, A.R.; Breeuwer, J.A.J. *Wolbachia*-induced parthenogenesis in a genus of phytophagous mites. *Proc. R. Soc. Lond. B* **2001**, *268*, 2245–2251.

41. Zchori-Fein, E.; Perlman, S.J.; Kelly, S.E.; Katzir, N.; Hunter, M.S. Characterization of a "Bacteroidetes" symbiont in *Encarsia* wasps (Hymenoptera: Aphelinidae): Proposal of "Candidatus Cardinium hertigii." *Int. J. Syst. Evol. Microbiol.* **2004**, *54*, 961.

42. Chigira, A.; Miura, K. Detection of "Candidatus Cardinium" bacteria from the haploid host *Brevipalpus californicus* (Acari: Tenuipalpidae) and effect on the host. *Exp. Appl. Acarol.* **2005**, *37*, 107–116.

43. Groot, T.V.M.; Breeuwer, J.A.J. *Cardinium* symbionts induce haploid thelytoky in most clones of three closely related *Brevipalpus* species. *Exp. Appl. Acarol.* **2006**, *39*, 257–271.

44. Giorgini, M.; Monti, M.M.; Caprio, E.; Stouthamer, R.; Hunter, M.S. Feminization and the collapse of haplodiploidy in an asexual parasitoid wasp harboring the bacterial symbiont *Cardinium*. *Heredity* **2009**, *102*, 365–371.

45. Traut, W. Zur Geschlechtsbestimmung bei *Gammarus duebeni* und verwandten Arten. *Z. wiss. Zool.* **1962**, *167*, 1–72.

46. Bulnheim, H.P.; Vávra, J. Infection by the Microsporidian *Octosporea effeminans* sp. n., and its sex determining influence in the amphipod *Gammarus duebeni*. *J. Parasitol.* **1968**, *54*, 241–248.

47. Dunn, A.M.; Rigaud, T. Horizontal transfer of parasitic sex ratio distorters between crustacean hosts. *Parasitology* **1998**, *117*, 15–19.

48. Terry, R.S.; Smith, J.E.; Dunn, A.M. Impact of a Novel, Feminising Microsporidium on its Crustacean Host. *J. Euk. Microbiol.* **1998**, *45*, 497–501.

49. Dunn, A.M.; Hatcher, M.J.; Terry, R.S.; Tofts, C. Evolutionary ecology of vertically transmitted parasites: Transovarial transmission of a microsporidian sex ratio distorter in *Gammarus duebeni*. *Parasitology* **1995**, *111*, S91–S109.

50. Rodgers-Gray, T.P.; Smith, J.E.; Ashcroft, A.E.; Isaac, R.E.; Dunn, A.M. Mechanisms of parasite-induced sex reversal in *Gammarus duebeni*. *Int. J. Parasitol.* **2004**, *34*, 747–753.

51. Dunn, A.; Adams, J.; Smith, J. Transovarial transmission and sex-ratio distortion by a microsporidian parasite in a shrimp. *J. Invertebr. Pathol.* **1993**, *61*, 248–252.

52. Terry, R.S.; Smith, J.E.; Sharpe, R.G.; Rigaud, T.; Littlewood, D.T.J.; Ironside, J.E.; Rollinson, D.; Bouchon, D.; MacNeil, C.; Dick, J.T.; Dunn, A.M. Widespread vertical transmission and associated host sex-ratio distortion within the eukaryotic phylum Microspora. *Proc. R. Soc. Lond. B* **2004**, *271*, 1783–1789.

53. Vance, S.A. Morphological and Behavioural sex reversal in mermithid-infected mayflies. *Proc. R. Soc. Lond. B* **1996**, *263*, 907–912.

54. Baudoin, M. Host castration as a parasitic strategy. *Evolution* **1975**, *29*, 335–352.

55. Kathirithamby, J. The effects of stylopisation on the sexual development of *Javesella dubia* (Kirschbaum) (Homoptera: Delphacidae). *Biol. J. Linn. Soc.* **1978**, *10*, 163–179.

56. Juchault, P.; Louis, C.; Martin, G.; Noulin, G. Masculinization of female isopods (Crustacea) correlated with non-Mendelian inheritance of cytoplasmic viruses. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 10460–10464.

57. Hurst, G.D.; Jiggins, F.M. Male-killing bacteria in insects: Mechanisms, incidence, and implications. *Emerg. Inf. Dis.* **2000**, *6*, 329–336.

58. Hurst, L.D. The incidences and evolution of cytoplasmic male killers. *Proc. R. Soc. Lond. B* **1991**, *244*, 91.

59. Nakanishi, K.; Hoshino, M.; Nakai, M.; Kunimi, Y. Novel RNA sequences associated with late male killing in *Homona magnanima*. *Proc. R. Soc. Lond. B* **2008**, *275*, 1249–1254.

60. Gilfillan, G.D.; Dahlsveen, I.K.; Becker, P.B. Lifting a chromosome: Dosage compensation in *Drosophila melanogaster*. *FEBS Lett.* **2004**, *567*, 8–14.

61. Veneti, Z.; Bentley, J.K.; Koana, T.; Braig, H.R.; Hurst, G.D.D. A functional dosage compensation complex required for male killing in *Drosophila*. *Science* **2005**, *307*, 1461–1463.

62. Kageyama, D.; Ohno, S.; Hoshizaki, S.; Ishikawa, Y. Sexual mosaics induced by tetracycline treatment in the *Wolbachia*-infected adzuki bean borer, *Ostrinia scapulalis*. *Genome* **2003**, *46*, 983–989.

63. Kageyama, D.; Traut, W. (2004) Opposite sex-specific effects of *Wolbachia* and interference with the sex determination of its host *Ostrinia scapulalis*. *Proc. R. Soc. Lond. B* **2004**, *271*, 251–258.

64. Sakamoto, H.; Kageyama, D.; Hoshizaki, S.; Ishikawa, Y. Sex-specific death in the Asian corn borer moth (*Ostrinia furnacalis*) infected with *Wolbachia* occurs across larval development. *Genome* **2007**, *50*, 645–652.

65. Sugimoto, T.N.; Fujii, T.; Kayukawa, T.; Sakamoto, H.; Ishikawa, Y. Expression of a *doublesex* homologue is altered in sexual mosaics of *Ostrinia scapulalis* moths infected with *Wolbachia*. *Insect Biochem. Mol. Biol.* **2010**, *40*, 847–854.

66. Sugimoto, T.N.; Ishikawa, Y. A male-killing Wolbachia carries a feminizing factor and is associated with degradation of the sex-determining system of its host. *Biol. Lett.* **2012**, doi: 10.1098/rsbl.2011.1114.

67. Kageyama, D., Hoshizaki, S., Ishikawa, Y. Female-biased sex ratio in the Asian corn borer, *Ostrinia furnacalis*: Evidence for the occurrence of feminizing bacteria in an insect. Heredity **1998**, *81*, 311–316.

68. Kageyama, D.; Nishimura, G.; Hoshizaki, S.; Ishikawa, Y. Feminizing *Wolbachia* in an insect, *Ostrinia furnacalis* (Lepidoptera: Crambidae). *Heredity* **2002**, *88*, 444–449.

69. Dyson, E.A.; Kamath, M.K.; Hurst, G.D.D. *Wolbachia* infection associated with all-female broods in *Hypolimnas bolina* (Lepidoptera: Nymphalidae): Evidence for horizontal transmission of a butterfly male killer. *Heredity* **2002**, *88*, 166–171.

70. Mitsuhashi, W.; Saiki, T.; Wei, W.; Kawakita, H.; Sato, M. Two novel strains of *Wolbachia* coexisting in both species of mulberry leafhoppers. *Insect Mol. Biol. Biol.* **2002**, *11*, 577–584.

71. Dyson, E.A.; Hurst, G.D.D. Persistence of an extreme sex-ratio bias in a natural population. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 6520–6523.

72. Hornett, E.A.; Charlat, S.; Duplouy, A.M.R.; Davies, N.; Roderick, G.K.; Wedell, N.; Hurst, G.D. Evolution of male-killer suppression in a natural population. *PLoS Biol.* **2006**, *4*, e283.

73. Charlat, S.; Hornett, E.A.; Fullard, J.H.; Davies, N.; Roderick, G.K.; Wedell, N.; Hurst, G.D. Extraordinary flux in sex ratio. *Science* **2007**, *317*, 214.

74. Mitsuhashi, W.; Ikeda, H.; Muraji, M. Fifty-year trend towards suppression of *Wolbachia*-induced male-killing by its butterfly host, *Hypolimnas bolina*. *J. Insect Sci.* **2011**, *11*, 1–15.

75. Hornett, E.A.; Duplouy, A.M.R.; Davies, N.; Roderick, G.K.; Wedell, N.; Hurst, G.D.; Charlat, S. You can't keep a good parasite down: Evolution of a male-killer suppressor uncovers cytoplasmic incompatibility. *Evolution* **2008**, *62*, 1258–1263.

76. Jaenike, J. Spontaneous emergence of a new *Wolbachia* phenotype. *Evolution* **2007**, *61*, 2244–2252.

77. Fukatsu, T.; Tsuchida, T.; Nikoh, N.; Koga, R. *Spiroplasma* symbiont of the pea aphid, *Acyrthosiphon pisum* (Insecta: Homoptera). *Appl. Environ. Microbiol.* **2001**, *67*, 1284–1291.

78. Tsuchida, T.; Koga, R.; Shibao, H.; Matsumoto, T.; Fukatsu, T. Diversity and geographic distribution of secondary endosymbiotic bacteria in natural populations of the pea aphid, *Acyrthosiphon pisum*. *Mol. Ecol.* **2002**, *11*, 2123–2135.

79. Simon, J.C.; Boutin, S.; Tsuchida, T.; Koga, R.; Le Gallic, J.F.; Frantz, A. Outreman Y, Fukatsu T. Facultative symbiont infections affect aphid reproduction. *PLoS One* **2011**, *6*, e21831.

80. Elnagdy, S.; Majerus, M.E.N.; Handley, L.-J.L. The value of an egg: Resource reallocation in ladybirds (Coleoptera: Coccinellidae) infected with male-killing bacteria. *J. Evol. Biol.* **2011**, *24*, 2164–2172.

81. Hurst, G.D.D.; Majerus, M.E.N. Why do maternally inherited microorganisms kill males? *Heredity* **1993**, *71*, 81–95.

82. Dunn, A.M.; Smith, J.E. Microsporidian life cycles and diversity: The relationship between virulence and transmission. *Microb. Inf.* **2001**, *3*, 381–388.

83. O'Neill, S.L.; Hoffmann, A.A.; Werren, J.H. *Influential Passengers*; Oxford University Press: Oxford, UK, 1997; p. 214.

84. Bourtzis, K.; Miller, T. *Insect Symbiosis*; CRC Press: Boca Raton, FL, USA, 2003; p. 368.

85. Kageyama, D.; Anbutsu, H.; Shimada, M.; Fukatsu, T. *Spiroplasma* infection causes either early or late male killing in *Drosophila*, depending on maternal host age. *Naturwissenschaften* **2007**, *94*, 333–337.

86. Charlat, S.; Davies, N.; Roderick, G.K.; Hurst, G.D.D. Disrupting the timing of *Wolbachia*-induced male-killing. *Biol. Lett.* **2007**, *3*, 154–156.

87. Stouthamer, R. *Wolbachia*-induced parthenogenesis. In *Influential Passengers*; O'Neill, S.L., Hoffmann, A.A., Werren, J.H., Eds.; Oxford University Press: Oxford, UK, 1997; pp. 102–124.

88. Arakaki, N.; Miyoshi, T.; Noda, H. *Wolbachia*-mediated parthenogenesis in the predatory thrips *Franklinothrips vespiformis* (Thysanoptera: Insecta). *Proc. R. Soc. Lond. B* **2001**, *268*, 1011–1016.

89. Koivisto, R.K.K.; Braig, H.R. Microorganisms and parthenogenesis. *Biol. J. Linn. Soc.* **2003**, *79*, 43–58.

90. Zchori-Fein, E.; Roush, R.T.; Hunter, M.S. Male production induced by antibiotic treatment in *Encarsia formosa* (Hymenoptera: Aphelinidae), an asexual species. *Experientia* **1992**, *48*, 102–105.

91. Pijls, J.W.A.M.; Steenbergen, H.J.V.A.N.; Van, J.J.M. Asexuality cured: The relations and differences between sexual and asexual *Apoanagyrus diversicornis*. *Heredity* **1996**, *76*, 506–513.

92. Kremer, N.; Charif, D.; Henri, H.; Bataille, M.; Prévost, G.; Kraaijeveld, K.; Vavre, F. A new case of *Wolbachia* dependence in the genus *Asobara*: Evidence for parthenogenesis induction in *Asobara japonica*. *Heredity* **2009**, *103*, 248–256.

93. Stouthamer, R.; Kazmer, D.J. Cytogenetics of microbe-associated parthenogenesis and its consequences for gene flow in *Trichogramma* wasps. *Heredity* **1994**, *73*, 317–327.

94. Pannebakker, B.A.; Pijnacker, L.P.; Zwaan, B.J.; Beukeboom, L.W. Cytology of *Wolbachia*-induced parthenogenesis in *Leptopilina clavipes* (Hymenoptera: Figitidae). *Genome* **2004**, *47*, 299–303.

95. Gottlieb, Y.; Zchori-Fein, E.; Werren, J.H.; Karr, T.L. Diploidy restoration in *Wolbachia*-infected *Muscidifurax uniraptor* (Hymenoptera: Pteromalidae). *J. Invertebr. Pathol.* **2002**, *81*, 166–174.

96.   Adachi-Hagimori, T.; Miura, K.; Stouthamer, R. A new cytogenetic mechanism for bacterial endosymbiont-induced parthenogenesis in Hymenoptera. *Proc. R. Soc. Lond. B* **2008**, *275*, 2667–2673.

97.   Verma, S.; Ruttner, F. Cytological analysis of thelytokous parthenogenesis in the Cape honeybee (*Apis mellifera capensis* Escholtz). *Apidologie* **1983**, *17*, 47–58.

98.   Pearcy, M.; Hardy, O.; Aron, S. Thelytokous parthenogenesis and its consequences on inbreeding in an ant. *Heredity* **2006**, *96*, 377–382.

99.   Bourtzis, K., Braig, H., Karr, T. Cytoplasmic incompatibility. In *Insect Symbiosis*; Bourtzis, K., Miller, T., Eds.; CRC Press: Boca Raton, FL, USA, 2003; pp. 217–246.

100.   Poinsot, D.; Charlat, S.; Merçot, H. On the mechanism of *Wolbachia*-induced cytoplasmic incompatibility: Confronting the models with the facts. *BioEssays* **2003**, *25*, 259–265.

101.   Tram, U.; Fredrick, K.; Werren, J.; Sullivan, W. Paternal chromosome segregation during the first mitotic division determines *Wolbachia*-induced cytoplasmic incompatibility phenotype. *J. Cell Sci.* **2006**, *119*, 3655–3663.

102.   Taylor, M.J.; Bandi, C.; Hoerauf, A. *Wolbachia* bacterial endosymbionts of filarial nematodes. *Adv. Parasitol.* **2005**, *60*, 245–284.

103.   Hoerauf, A.; Mand, S.; Fischer, K.; Kruppa, T.; Marfo-Debrekyei, Y.; Debrah, A.Y.; Pfarr, K.M.; Adjei, O.; Büttner, D.W. Doxycycline as a novel strategy against bancroftian filariasis-depletion of *Wolbachia* endosymbionts from *Wuchereria bancrofti* and stop of microfilaria production. *Med. Microbiol. Immunol.* **2003**, *192*, 211–216.

104.   Hosokawa, T.; Koga, R.; Kikuchi, Y.; Meng, X.-Y.; Fukatsu, T. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 769–774.

105.   Dedeine, F.; Vavre, F.; Fleury, F.; Loppin, B.; Hochberg, M.E.; Bouletreau, M. Removing symbiotic *Wolbachia* bacteria specifically inhibits oogenesis in a parasitic wasp. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 6247–6252.

106.   Starr, D.J.; Cline, T.W. A host–parasite interaction rescues *Drosophila* oogenesis defects. *Nature* **2002**, *418*, 76–79.

107.   Hedges, L.M.; Brownlie, J.C.; O'Neill, S.L.; Johnson, K.N. *Wolbachia* and virus protection in insects. *Science* **2008**, *322*, 702.

108.   Osborne, S.E.; Leong, Y.S.; O'Neill, S.L.; Johnson, K.N. Variation in antiviral protection mediated by different *Wolbachia* strains in *Drosophila simulans*. *PLoS Pathog.* **2009**, *5*, e1000656.

109.   Teixeira, L.; Ferreira, A.; Ashburner, M. The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biol.* **2008**, *6*, e2.

110.   Glaser, R.L.; Meola, M.A. The native *Wolbachia* endosymbionts of *Drosophila melanogaster* and *Culex quinquefasciatus* increase host resistance to West Nile virus infection. *PLoS One* **2010**, *5*, e11977.

111.   Panteleev, D.I.; Goriacheva, I.I.; Andrianov, B.V.; Reznik, N.L.; Lazebnyĭ, O.E.; Kulikov, A.M. The endosymbiotic bacterium *Wolbachia* enhances the nonspecific resistance to insect pathogens and alters behavior of *Drosophila melanogaster*. *Genetika* **2007**, *43*, 1277–1280.

112.   Kambris, Z.; Cook, P.; Phuc, H.; Sinkins, S. Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes. *Science* **2009**, *326*, 134–136.

113. Bian, G., Xu, Y., Lu, P., Xie, Y., Xi, Z. The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. *PLoS Pathog.* **2010**, *6*, e1000833.

114. Moreira, L.A.; Iturbe-Ormaetxe, I.; Jeffery, J.A.; Lu, G.; Pyke, A.T.; Hedges, L.M.; Rocha, B.C.; Hall-Mendelin, S.; Day, A.; Riegler, M.; Hugo, L.E.; Johnson, K.N.; Kay, B.H.; McGraw, E.A.; van den Hurk, A.F.; Ryan, P.A.; O'Neill, S.L. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and Plasmodium. *Cell* **2009**, *139*, 1268–1278.

115. Kambris, Z.; Blagborough, A.M.; Pinto, S.B.; Blagrove, M.S.C.; Godfray, H.C.J.; Sinden, R.E., Sinkins, S.P. *Wolbachia* stimulates immune gene expression and inhibits plasmodium development in *Anopheles gambiae*. *PLoS Pathog.* **2010**, *6*, e1001143.

116. Hoffmann, A.A.; Montgomery, B.L.; Popovici, J.; Iturbe-Ormaetxe, I.; Johnson, P.H.; Muzzi, F.; Greenfield, M.; Durkan, M.; Leong, Y.S.; Dong, Y.; Cook, H.; Axford, J.; Callahan, A.G.; Kenny, N.; Omodei, C.; McGraw, E.A.; Ryan, P.A.; Ritchie, S.A.; Turelli, M.; O'Neill, S.L. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* **2011**, *476*, 454–457.

117. Walker, T.; Johnson, P.H.; Moreira, L.A.; Iturbe-Ormaetxe, I.; Frentiu, F.D.; McMeniman, C.J.; Leong, Y.S.; Dong, Y.; Axford, J.; Kriesner, P.; Lloyd, A.L.; Ritchie, S.A.; O'Neill, S.L.; Hoffmann, A.A. The *w*Mel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature* **2011**, *476*, 450–453.

118. Iturbe-Ormaetxe, I.; Walker, T.; O' Neill, S.L. *Wolbachia* and the biological control of mosquito-borne disease. *EMBO Rep.* **2011**, *12*, 508–518.

119. Jaenike, J.; Unckless, R.; Cockburn, S.N.; Boelio, L.M.; Perlman, S.J. Adaptation via symbiosis: Recent spread of a *Drosophila* defensive symbiont. *Science* **2010**, *329*, 212–215.

120. Rigaud, T.; Juchault, P.; Mocquard, J.-P. The evolution of sex determination in isopod crustaceans. *BioEssays* **1997**, *19*, 409–416.

121. Hashiyama, K.; Hayashi, Y.; Kobayashi, S. *Drosophila Sex lethal* gene initiates female development in germline progenitors. *Science* **2011**, *333*, 885–888.

122. Clark, M.E.; Heath, B.D.; Anderson, C.L., Karr, T.L. Induced paternal effects mimic cytoplasmic incompatibility in *Drosophila*. *Genetics* **2006**, *173*. 727.

123. Xi, Z.; Gavotte, L.; Xie, Y.; Dobson, S. Genome-wide analysis of the interaction between the endosymbiotic bacterium *Wolbachia* and its *Drosophila* host. *BMC Genomics* **2008**, *9*, 1.

124. Nakamura, Y.; Gotoh, T.; Imanishi, S.; Mita, K.; Kurtti, T.J.; Noda, H. Differentially expressed genes in silkworm cell cultures in response to infection by *Wolbachia* and *Cardinium* endosymbionts. *Insect Mol. Biol.* **2011**, *20*, 279–289.

125. Hughes, G.L.; Ren, X.; Ramirez, J.L.; Sakamoto, J.M.; Bailey, J.A.; Jedlicka, A.E.; Rasgon, J.L. *Wolbachia* infections in *Anopheles gambiae* cells: Transcriptomic characterization of a novel host-symbiont interaction. *PLoS Pathog.* **2011**, *7*, e1001296.

126. Hatcher, M.J.; Taneyhill, D.E.; Dunn, A.M.; Tofts, C. Population dynamics under parasitic sex ratio distortion. *Theor. Pop. Biol.* **1999**, *56*, 11–28.

127. Saccheri, I.; Kuussaari, M.; Kankare, M.; Vikman, P.; Hanski, I. Inbreeding and extinction in a butterfly metapopulation. *Nature* **1998**, *45*, 1996–1999.

128. Jiggins, F.M.; Hurst, G.D.; Majerus, M.E. Sex-ratio-distorting *Wolbachia* causes sex-role reversal in its butterfly host. *Proc. R. Soc. Lond. B* **2000**, *267*, 69–73.

129. Narita, S.; Nomura, M.; Kageyama, D. Naturally occurring single and double infection with *Wolbachia* strains in the butterfly *Eurema hecabe*: Transmission efficiencies and population density dynamics of each *Wolbachia* strain. *FEMS Microbiol. Ecol.* **2007**, *61*, 235–245.

130. Martin, G.; Juchault, P.; Legrand, J. Mise en évidence d'un microorganisme intracytoplasmique symbiote de l'oniscoïde Armadillidium vulgare Latr., dont la présence accompagne l'intersexualité ou la féminisation totale des mâles génétiques de la lignée thélygène. *C. R. Acad. Sci.* **1973**, *276*, 2313–2316.

131. Rigaud, T.; Souty-Grosset, C.; Raimond, R.; Mocquard, J.P.; Juchault, P. Feminizing endocytobiosis in the terrestrial crustacean *Armadillidium vulgare* Latr. (Isopoda): Recent acquisitions. *Endocytobiosis Cell Res.* **1991**, *7*, 259–273.

132. Rousset, F.; Bouchon, D.; Pintureau, B.; Juchault, P.; Solignac, M. *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. *Proc. R. Soc. Lond. B* **1992**, *250*, 91–98.

133. Juchault, P.; Legrand, J.J. Analyse génétique et physiologique de la détermination du sexe dans une population du crustacé Oniscoïde—*Armadillidium nasatum* Budde-Lund. *Arch. Zool. Exp. Gén.* **1979**, *120*, 25–43.

134. Juchault, P.; Legrand, J.J. Sex determination and monogeny in terrestrial isopods *Armadillidium vulgare* (Latreille, 1804) and *Armadillidium nasatum* (Budde-Lund, 1885). *Monogr. Monit. Zool. Italian.* **1989**, *4*, 359–375.

135. Juchault, P.; Frelon, M.; Bouchon, D.; Rigaud, T. New evidence for feminizing bacteria in terrestrial isopods: Evolutionary implications. *C. R. Acad. Sci.* **1994**, *317*, 225–230.

136. Rigaud, T.; Antoine, D.; Marcadé, I.; Juchault, P. The effect of temperature on sex ratio in the isopod *Porcellionides pruinosus*: Environmental sex determination or a by-product of cytoplasmic sex determination? *Evol. Ecol.* **1997**, *11*, 205–215.

137. Martin, G.; Gruppe, S.G.; Laulier, M.; Bouchon, D.; Rigurd, D.; Juchault, P. (1994) Evidence for *Wolbachia* spp. in the estuarine isopod *Sphaeroma rugicauda* (Crustacea): A likely cytoplasmic sex ratio distorter. *Endocytobiosis Cell Res.* **1994**, *10*, 215–225.

138. Bulnheim, H.-P. Interaction between genetic, external and parasitic factors in sex determination of the crustacean amphipod *Gammarus duebeni*. *Helgoländer Wissenschaftliche Meeresuntersuchungen* **1978**, *31*, 1–33.

139. Hurst, G.D.D.; Jiggins, F.M.; Schulenburg, J.H.; Bertrand, D.; West, S.A.; et al. Male-killing *Wolbachia* in two species of insect. *Proc. R. Soc. Lond. B* **1999**, *266*, 735–740.

140. Fialho, R.F.; Stevens, L. Male-killing *Wolbachia* in a flour beetle. *Proc. R. Soc. Lond. B* **2000**, *267*, 1469–1473.

141. Hurst, G.D.; Johnson, A.P.; Schulenburg, J.H.; Fuyama, Y. Male-killing *Wolbachia* in *Drosophila*: A temperature-sensitive trait with a threshold bacterial density. *Genetics* **2000**, *156*, 699–709.

142. Sheeley, S.L.; McAllister, B.F. Mobile male-killer: Similar *Wolbachia* strains kill males of divergent *Drosophila* hosts. *Heredity* **2009**, *102*, 286–292.

143. Jaenike, J.; Dyer, K.A.; Reed, L.K. Within-population structure of competition and the dynamics of male-killing *Wolbachia*. *Evol. Ecol. Res.* **2003**, *5*, 1023–1036.

144. Jiggins, F.M.; Hurst, G.D.D.; Dolman, C.E.; Majerus, M.E.N. High-prevalence male-killing *Wolbachia* in the butterfly *Acraea encedana*. *J. Evol. Biol.* **2000**, *13*, 495–501.

145. Kageyama, D.; Nishimura, G.; Ohno, S.; Takanashi, T.; Hoshizaki, S.; Ishikawa, Y. *Wolbachia* infection and an all-female trait in *Ostrinia orientalis* and *Ostrinia zaguliaevi*. *Entomol. Exp. Appl.* **2004**, *111*, 79–83.

146. Kageyama, D.; Nishimura, G.; Hoshizaki, S.; Ishikawa, Y. Two kinds of sex ratio distorters in a moth, *Ostrinia scapulalis*. *Genome* **2003**, *46*, 974–982.

147. Zeh, D.W.; Zeh, J.A.; Bonilla, M.M. *Wolbachia*, sex ratio bias and apparent male killing in the harlequin beetle riding pseudoscorpion. *Heredity* **2005**, *95*, 41–49.

148. Hurst, G.D.; Schulenburg, J.H.; Majerus, T.M.; Bertrand, D.; Zakharov, I.A.; Baungaard, J.; Völkl, W.; Stouthamer, R.; Majerus, M.E. Invasion of one insect species, *Adalia bipunctata*, by two different male-killing bacteria. *Insect Mol. Biol.* **1999**, *8*, 133–139.

149. Majerus, M.E.; Schulenburg, J.H.; Zakharov, I.A. Multiple causes of male-killing in a single sample of the two-spot ladybird, *Adalia bipunctata* (Coleoptera: Coccinellidae) from Moscow. *Heredity* **2000**, *84*, 605–609.

150. Tinsley, M.C.; Majerus, M.E.N. A new male-killing parasitism: *Spiroplasma* bacteria infect the ladybird beetle *Anisosticta novemdecimpunctata* (Coleoptera: Coccinellidae). *Parasitology* **2006**, *132*, 757–765.

151. Matsuka, M.; Hashi, H.; Okada, I. Abnormal sex ratio found in the lady beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). *Appl. Entomol. Zool.* **1975**, *10*, 84–89.

152. Majerus, T.M.; Schulenburg, J.H.; Majerus, M.E.; Hurst, G.D. Molecular identification of a male-killing agent in the ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). *Insect Mol. Biol.* **1999**, *8*, 551–555.

153. Niijima, K.; Nakajima, K. Abnormal sex-ratio in Menochilus sexmaculatus (Fabricius). *Bull. of the Faculty of Agriculture-Tamagawa Univ (Japan)* **1981**, *21*, 59–67.

154. Jiggins, F.M.; Hurst, G.D.; Jiggins, C.D.; Schulenburg, J.H.; Majerus, M.E. (2000) The butterfly *Danaus chrysippus* is infected by a male-killing *Spiroplasma* bacterium. *Parasitology* **2000**, *120*, 439–446.

155. Tabata, J.; Hattori, Y.; Sakamoto, H.; Yukuhiro, F.; Fujii, T.; Kugimiya, S.; Mochizuki, A.; Ishikawa, Y.; Kageyama, D. Male killing and incomplete inheritance of a novel spiroplasma in the moth *Ostrinia zaguliaevi*. *Microb. Ecol.* **2011**, *61*, 254–263.

156. Bentley, J.K.; Veneti, Z.; Heraty, J.; Hurst, G.D.D. The pathology of embryo death caused by the male-killing *Spiroplasma* bacterium in *Drosophila nebulosa*. *BMC Biol.* **2007**, *5*, 9.

157. Montenegro, H.; Hatadani, L.M.; Medeiros, H.F.; Klaczko, L.B. Male killing in three species of the *tripunctata* radiation of *Drosophila* (Diptera: Drosophilidae). *J. Zool. Syst. Evol. Res.* **2006**, *44*, 130–135.

158. Pool, J.E.; Wong, A.; Aquadro, C.F. Finding of male-killing *Spiroplasma* infecting *Drosophila melanogaster* in Africa implies transatlantic migration of this endosymbiont. *Heredity* **2006**, *97*, 27–32.

159. Williamson, D.L.; Sakaguchi, B.; Hackett, K.J.; Whitcomb, R.F.; Tully, J.G.; Carle, P.; Bové, J.M.; Adams, J.R.; Konai, M.; Henegar, R.B. *Spiroplasma poulsonii* sp. nov., a new species associated with male-lethality in *Drosophila willistoni*, a neotropical species of fruit fly. *Int. J. Syst. Bacteriol.* **1999**, *49*, 611–618.

160. Hurst, G.D.D.; Majerus, M.E.N.; Walker, L.E. The importance of cytoplasmic male killing elements in natural populations of the two spot ladybird, *Adalia bipunctata* (Linnaeus) (Goleoptera: Goccinellidae). *Biol. J. Linn. Soc.* **1993**, *49*, 195–202.

161. Werren, J.H.; Hurst, G.D.; Zhang, W.; Breeuwer, J.A.; Stouthamer, R.; Majerus, M.E. Rickettsial relative associated with male killing in the ladybird beetle (*Adalia bipunctata*). *J. Bacteriol.* **1994**, *176*, 388–394.

162. Schulenburg, J.H.; Habig, M.; Sloggett, J.J.; Webberley, K.M.; Bertrand, D.; Hurst, G.D.; Majerus, M.E. Incidence of male-killing *Rickettsia* spp. (alpha-proteobacteria) in the ten-spot ladybird beetle *Adalia decempunctata* L. (Coleoptera: Coccinellidae). *Appl. Environ. Microbiol.* **2001**, *67*, 270–277.

163. Lawson, E.T.; Mousseau, T.A.; Klaper, R.; Hunter, M.D.; Werren, J.H. *Rickettsia* associated with male-killing in a buprestid beetle. *Heredity* **2001**, *86*, 497–505.

164. Majerus, T.M.; Majerus, M.E. Discovery and identification of a male-killing agent in the Japanese ladybird *Propylea japonica* (Coleoptera: Coccinellidae). *BMC Evol. Biol.* **2010**, *10*, 37.

165. Hurst, G.D.; Bandi, C.; Sacchi, L.; Cochrane, A.G.; Bertrand, D.; Karaca, I.; Majerus, M.E. *Adonia variegata* (Coleoptera: Coccinellidae) bears maternally inherited flavobacteria that kill males only. *Parasitology* **1999**, *118*, 125–134.

166. Majerus, T.M.O. *The Evolutionary Genetics of Male killing in the Coccinellidae*; PhD thesis, Department of Genetics, University of Cambridge: Cambridge, UK, 2001.

167. Majerus, M.E.N.; Majerus, T.M.O. Female-biased sex ratio due to male-killing in the Japanese ladybird *Coccinula sinensis*. *Ecol. Entomol.* **2000**, *25*, 234–238.

168. Hurst, G.D.D.; Hammarton, T.C.; Bandi, C.; Majerus, T.M.O.; Bertrand, D.; Majerus, M.E.N. The diversity of inherited parasites of insects: The male-killing agent of the ladybird beetle *Coleomegilla maculata* is a member of the Flavobacteria. *Genet. Res.* **1997**, *70*, 1–6.

169. Werren, J.H.; Skinner, S.W.; Huger, A.M. Male-killing bacteria in a parasitic wasp. *Science* **1986**, *231*, 990–992.

170. Ferree, P.M.; Avery, A.; Azpurua, J.; Wilkes, T.; Werren, J.H. A bacterium targets maternally inherited centrosomes to kill males in *Nasonia*. *Curr. Biol.* **2008**, *18*, 1409–1414.

171. Hazard, E.I.; Weiser, J. Spores of *Thelohania* in adult female *Anopheles*: Development and transovarial transmission, and redescriptions of *T. legeri* Hesse and *T. obesa* Kudo. *J. Euk. Microbiol.* **1968**, *15*, 817–823.

172. Avery, S. Horizontal transmission of (Protozoa: Microsporida) to (Diptera: Culicidae). *J. Invertebr. Pathol.* **1989**, *53*, 424–426.

173. Andreadis, T.G. Life cycle, epizootiology, and horizontal transmission of (Microspora: Amblyosporidae) in a univoltine mosquito. *J. Invertebr. Pathol.* **1985**, *46*, 31–46.

174. Kellen, W.; Chapman, H.; Clark, T.; Lindegren, J. Host-parasite relationships of some from mosquitoes (Nosematidae: Microsporidia). *J. Invertebr. Pathol.* **1965**, *7*, 161–166.

175. Kellen, W.R.; Lipa, J.J. *Thelohania californica* n. sp. a microsporidian parasite of *Culex tarsalis* Coquillet. *J. Insect. Pathol.* **1960**, *2*, 1–12.

176. Kellen, W.R.; Wills, W. The transovarian transmission of *Thelohania californica* Kellen and Lipa in *Culex tarsalis* Coquillet. *J. Insect Pathol.* **1962**, *4*, 321–326.

177. Andreadis, T.G.; Hall, D.W. Development, ultrastructure, and mode of transmission of *Amblyospora* sp. (Microspora) in the mosquito. *J. Protozool.* **1979**, *26*, 444–452.

178. Morimoto, S.; Nakai, M.; Ono, A.; Kunimi, Y. Late male-killing phenomenon found in a Japanese population of the oriental tea tortrix, *Homona magnanima* (Lepidoptera: Tortricidae). *Heredity* **2001**, *87*, 435–440.

179. Zchori-Fein, E.; Faktor, O.; Zeidan, M.; Gottlieb, Y.; Czosnek, H.; Rosen, D. Parthenogenesis-inducing microorganisms in *Aphytis* (Hymenoptera: Aphelinidae). *Insect Mol. Biol.* **1995**, *4*, 173–178.

180. Gottlieb, Y.; Zchori-Fein, E.; Faktor, O.; Rosen, D. Phylogenetic analysis of parthenogenesis-inducing *Wolbachia* in the genus *Aphytis* (Hymenoptera: Aphelinidae). *Insect Mol. Biol.* **1998**, *7*, 393–396.

181. Zchori-Fein, E., Rosen, D., Roush, R. Microorganisms associated with thelytoky in *Aphytis lingnansensis compere* (Hymenoptera: Aphelinidae). *Int. J. Insect. Morphol. Embryol.* **1994**, *23*, 169–172.

182. Schilthuizen, M.; Stouthamer, R. Distribution of *Wolbachia* among the guild associated with the parthenogenetic gall wasp *Diplolepis rosae*. *Heredity* **1998**, *81*, 270–274.

183. Barro, P.J.; Hart, P.J. Antibiotic curing of parthenogenesis in *Eretmocerus mundus* (Australian parthenogenic form). *Entomol. Exp. Appl.* **2001**, *99*, 225–230.

184. Arakaki, N.; Oishi, T.; Noda, H. Parthenogenesis Induced by *Wolbachia* in *Gronotoma micromorpha* (Hymenoptera: Eucoilidae). *Entomol. Sci.* **2001**, *4*, 9–15.

185. Stouthamer, R.; Breeuwert, J.A.; Luck, R.F.; Werren, J.H. Molecular identification of microorganisms associated with parthenogenesis. *Nature* **1993**, *361*, 66–68.

186. Stouthamer, R.; Lukoe, S.; Mak, F. Influence of parthenogenesis *Wolbachia* on host fitness. *Nor. J. Agric. Sci. (Suppl.)* **1994**, *6*, 117–122

187. Arakaki, N.; Noda, H.; Yamagishi, K. *Wolbachia*-induced parthenogenesis in the egg parasitoid *Telenomus nawai*. *Entomol. Exp. Appl.* **2000**, *96*, 177–184.

188. Stouthamer, R.; Werren, J.H. Microbes associated with parthenogenesis in wasps of the genus *Trichogramma*. *J. Invertebr. Pathol.* **1993**, *61*, 6–9.

189. Stouthamer, R.; Luck, R.F.; Hamilton, W.D. Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera/Trichogrammatidae) to revert to sex. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 2424–2427.

190. Schilthuizen, M.; Honda, J.; Stouthamer, R. Parthenogenesis-inducing *Wolbachia* in *Trichogramma kaykai* (Hymenoptera: Trichogrammatidae) originates from a single infection. *Ann. Entomol. Soc. Am.* **1998**, *91*, 410–414.

191. Zchori-Fein, E.; Gottlieb, Y.; Kelly, S.E.; Brown, J.K.; Wilson, J.M.; Karr, T.L.; Hunter, M.S. A newly discovered bacterium associated with parthenogenesis and a change in host selection behavior in parasitoid wasps. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 12555–12560.

192. Giorgini, M. Induction of males in thelytokous populations of *Encarsia meritoria* and *Encarsia protransvena*: A systematic tool. *BioControl* **2001**, *46*, 427–438.

193. Hagimori, T.; Abe, Y.; Date, S.; Miura, K. The first finding of a *Rickettsia* bacterium associated with parthenogenesis induction among insects. *Curr. Microbiol.* **2006**, *52*, 97–101.

194. Giorgini, M.; Bernardo, U.; Monti, M.M.; Nappo, A.G.; Gebiola, M. *Rickettsia* symbionts cause parthenogenetic reproduction in the parasitoid wasp *Pnigalio soemius* (Hymenoptera: Eulophidae). *Appl. Environ. Microbiol.* **2010**, *76*, 2589–2599.

195 Kondo, N.; Ijichi, N.; Shimada, M.; Fukatsu, T. Prevailing triple infection with *Wolbachia* in *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Mol. Ecol.* **2002**, *11*, 167–180.

196 Keller, G.P.; Windsor, D.M.; Saucedo, J.M.; Werren, J.H. Reproductive effects and geographical distributions of two *Wolbachia* strains infecting the Neotropical beetle, *Chelymorpha alternans* Boh. (Chrysomelidae, Cassidinae). *Mol. Ecol.* **2004**, *13*, 2405–2420.

197 Giordano, R.; Jackson, J.J.; Robertson, H.M. The role of *Wolbachia* bacteria in reproductive incompatibilities and hybrid zones of *Diabrotica* beetles and *Gryllus* crickets. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 11439–11444.

198 Blickenstaff, C.C. Partial Inter sterility of Eastern and Western U. S. Strains of the Alfalfa Weevil. *Ann. Entomol. Soc. Am.* **1965**, *58*, 523–526.

199 Hsiao, C; Hsiao, T. *Rickettsia* as the cause of cytoplasmic incompatibility in the alfalfa weevil, *Hypera postica. J. Insect Pathol.* **1985**, *45*, 244–246.

200 O'Neill, S.L.; Giordano, R.; Colbert, A.M.; Karr, T.L.; Robertson, H.M. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 2699–2702.

201 Fialho, R.; Stevens, L. *Wolbachia* Infections in the Flour Beetle *Tribolium confusum*: Evidence for a Common Incompatibility Type across Strains. *J. Insect Pathol.* **1996**, *67*, 195–197.

202 Fialho, R.F.; Stevens, L. Molecular evidence for single *Wolbachia* infections among geographic strains of the flour beetle *Tribolium confusum. Proc. R. Soc. Lond. B* **1997**, *264*, 1065–1068.

203 Macdonald, W. Mosquito genetics in relation to filarial infections. *Symposia of the British Society of Parasitology* **1976**, *14*, 1–24.

204 Wright, J.D.; Barr, A.R. The ultrastructure and symbiotic relationships of *Wolbachia* of mosquitoes of the *Aedes scutellaris* group. *J. Ultra. Res.* **1980**, *72*, 52–64.

205 Wright, J.; Wang, B. Observations on *Wolbachia*e in mosquitoes. *J. Insect Pathol.* **1980**, *35*, 200–208.

206 Trpis, M.; Perrone, J.; Reissig, M.; Parker, K. Control of cytoplasmic incompatibility in the *Aedes scutellaris* complex. *J. Heredity* **1981**, *72*, 313–317.

207 Tesfa-Yohannes, T.M.; Rozeboom, L.E. Experimental crossing of *Aedes (S.) polynesiensis* Marks and *A. scutellaris malayensis* Colless (Diptera: Culicidae). *J. Med. Entomol.* **1974**, *11*, 323–331.

208 Beckett, E.B.; Boothroyd, B.; Macdonald, W.W. A light and electron microscope study of rickettsia-like organisms in the ovaries of mosquitoes of the *Aedes scutellaris* group. *Ann. Trop. Med. Parasitol.* **1978**, *72*, 277–283.

209 Meek, S.R. Occurrence of rickettsia-like symbionts among species of the *Aedes scutellaris* group (Diptera: Culicidae). *Ann. Trop. Med. Parasitol.* **1984**, *78*, 377–381.

210 Meek, S.; Macdonald, W. Crossing relationships among seven members of the group of *Aedes scutellaris* (Walker)(Diptera: Culicidae). *Bull. Entomol. Res.* **1984**, *74*, 65–78.

211 Smith-White, S.; Woodhill, A. The nature and significance of non-reciprocal fertility in *Aedes scutellaris* and other mosquitoes. *Proc. Linn. Soc. N. S. W.* **1954**, *79*, 163–176.

212 Jamnongluk, W.; Kittayapong, P.; Baisley, K.J.; O'Neill, S.L. *Wolbachia* infection and expression of cytoplasmic incompatibility in *Armigeres subalbatus* (Diptera: Culicidae). *J. Med. Entomol.* **2000**, *37*, 53–57.

213 Laven, H. Speciation and evolution in Culex pipiens. In *Genetics of insect vectors of disease*; Wright, J., Pal, P., Eds.; Elsevier: Amsterdam, The Netherlands,1967; pp. 251–275.

214 Barr, A.R. Cytoplasmic incompatibility in natural populations of a mosquito, *Culex pipiens* L. *Nature* **1980**, *283*, 71–72.

215 Magnin, M.; Pasteur, N.; Raymond, M. Multiple incompatibilities within populations of *Culex pipiens* L. in southern France. *Genetica* **1987**, *74*, 125–130.

216 Irving-Bell, R.J. Cytoplasmic incompatibility within and between *Culex molestus* and *Cx. quinquefasciatus* (Diptera: Culicidae). *J. Med. Entomol.* **1983**, *20*, 44–48.

217 O'Neill, S.L.; Paterson, H.E. Crossing type variability associated with cytoplasmic incompatibility in Australian populations of the mosquito *Culex quinquefasciatus* Say. *Med. Vet. Entomol.* **1992**, *6*, 209–216.

218 Bourtzis, K.; Nirgianaki, A.; Markakis, G.; Savakis, C. *Wolbachia* infection and cytoplasmic incompatibility in *Drosophila* species. *Genetics* **1996**, *144*, 1063–1073.

219 Hoffmann, A.A.; Turelli, M. Unidirectional incompatibility in *Drosophila simulans*: Inheritance, geographic variation and fitness effects. *Genetics* **1988**, *119*, 435–444.

220 Holden, P.R.; Brookfield, J.F.; Jones, P. Cloning and characterization of an *ftsZ* homologue from a bacterial symbiont of *Drosophila melanogaster*. Mol. Genet. *Genomics* **1993**, *240*, 213–220.

221 Werren, J.H.; Jaenike, J. *Wolbachia* and cytoplasmic incompatibility in mycophagous *Drosophila* and their relatives. *Heredity* **1995**, *75*, 320–326.

222 Giordano, R.; O'Neill, S.L.; Robertson, H.M. *Wolbachia* infections and the expression of cytoplasmic incompatibility in *Drosophila sechellia* and *D. mauritiana*. *Genetics* **1995**, *140*, 1307–1317.

223 Hoffmann, A.A.; Turelli, M.; Harshman, L.G. Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. *Genetics* **1990**, *126*, 933–948.

224 Tagami, Y; Doi, M.; Sugiyama, K.; Tatara, A.; Saito, T. *Wolbachia*-induced cytoplasmic incompatibility in *Liriomyza trifolii* and its possible use as a tool in insect pest control. *Biol. Cont.* **2006**, *38*, 205–209.

225 Kassem, H.A.; Hassan, A.N.; Abdel-Hamid, I.; Osman, G.; El Khalab, E.M.; Madkour, M.A. *Wolbachia* infection and the expression of cytoplasmic incompatibility in sandflies (Diptera: Psychodidae) from Egypt. *Ann. Trop. Med. Parasitol.* **2003**, *97*, 639–644.

226 Boller, E.F.; Russ, K.; Vallo, V.; Bush, G.L. Incompatible races of European cherry fruit fly, *Rhagoletis cerasi* (Diptera: Tephritidae), their origin and potential use in biological control. *Entomol. Exp. Appl.* **1976**, *20*, 237–247.

227 Riegler, M.; Stauffer, C. *Wolbachia* infections and superinfections in cytoplasmically incompatible populations of the European cherry fruit fly *Rhagoletis cerasi* (Diptera, Tephritidae). *Mol. Ecol.* **2002**, *11*, 2425–2434.

228 Vasquez, C.J.; Stouthamer, R.; Jeong, G.; Morse, J.G. Discovery of a CI-inducing *Wolbachia* and its associated fitness costs in the biological control agent *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae). *Biol. Cont.* **2011**, *58*, 192–198.

229 Dedeine, F.; Vavre, F.; Shoemaker, D.D.; 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* **2004**, *58*, 2167–2174.

230 Mochiah, M.B.; Ngi-Song, A.J.; Overholt, W.A.; Stouthamer, R. *Wolbachia* infection in *Cotesia sesamiae* (Hymenoptera: Braconidae) causes cytoplasmic incompatibility: Implications for biological control. *Biol. Cont.* **2002**, *25*, 74–80.

231 White, J.A.; Kelly, S.E.; Perlman, S.J.; Hunter, M.S. Cytoplasmic incompatibility in the parasitic wasp *Encarsia inaron*: Disentangling the roles of *Cardinium* and *Wolbachia* symbionts. *Heredity* **2009**, *102*, 483–489.

232 Noda, H. Cytoplasmic incompatibility in a rice planthopper. *J. Heredity* **1984**, *75*, 345–348.

233 Rousset, F.; Bouchon, D.; Pintureau, B.; Juchault, P.; Solignac, M. *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. *Proc. R. Soc. Lond. B* **1992**, *250*, 91–98.

234 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 Mol. Biol.* **1995**, *4,* 237–243.

235 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* **2000**, *54*, 191–200.

236 Vavre, F.; Dedeine, F.; Quillon, M.; Fouillet, P.; Fleury, F.; Boulétreau, M. Within-species diversity of *Wolbachia*-induced cytoplasmic incompatibility in haplodiploid insects. *Evolution* **2001**, *55*, 1710–1714.

237 Mouton, L.; Henri, H.; Boulétreau, M.; Vavre, F. Multiple infections and diversity of cytoplasmic incompatibility in a haplodiploid species. *Heredity* **2005**, *94*, 187–192.

238 Machtelinckx, T.; van Leeuwen, T.; Vanholme, B.; Gehesquière, B.; Dermauw, W.; Vandekerkhove, B.; Gheysen, G.; De Clercq, P. *Wolbachia* induces strong cytoplasmic incompatibility in the predatory bug *Macrolophus pygmaeus*. *Insect Mol. Biol.* **2009**, *18*, 373–381.

239 Breeuwer, J.A.; Werren, J.H. Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. *Nature* **1990**, *346*, 558–560.

240 Breeuwer, J.A.; Stouthamer, R.; Barns, S.M.; Pelletier, D.A.; Weisburg, W.G.; Werren J.H. Phylogeny of cytoplasmic incompatibility micro-organisms in the parasitoid wasp genus *Nasonia* (Hymenoptera: Pteromalidae) based on 16S ribosomal DNA sequences. *Insect Mol. Biol.* **1992**, *1*, 25–36.

241 Bordenstein, S.R.; O'Hara, F.P.; Werren, J.H. *Wolbachia*-induced incompatibility precedes other hybrid incompatibilities in *Nasonia*. *Nature* **2001**, *409*, 707–710.

242 Saul, G.B. An analysis of non-reciprocal cross incompatibility in *Mormoniella vitripennis* (Walker). *Zeitschrift für Vererbungslehre* **1961**, *92*, 28–33.

243 Watanabe, M.; Miura, K.; Hunter, M.S.; Wajnberg, E. Superinfection of cytoplasmic incompatibility-inducing *Wolbachia* is not additive in *Orius strigicollis* (Hemiptera: Anthocoridae). *Heredity* **2011**, *106*, 642–648.

244 Narita, S.; Shimajiri, Y.; Nomura, M. Strong cytoplasmic incompatibility and high vertical transmission rate can explain the high frequencies of *Wolbachia* infection in Japanese populations of *Colias erate poliographus* (Lepidoptera: Pieridae). *Bull. Entomol. Res.* **2009**, *99*, 385–391.

245 Sasaki, T.; Ishikawa, H. *Wolbachia* Infections and Cytoplasmic Incompatibility in the Almond Moth and the Mediterranean Flour Moth. *Zool. Sci.* **1999**, *16*, 739–744.

246 Narita, S.; Nomura, M.; Kato, Y.; Yata, O.; Kageyama, D. Molecular phylogeography of two sibling species of *Eurema* butterflies. *Genetica* **2007**, *131*, 241–253.

247 Hiroki, M.; Ishii, Y.; Kato, Y. Variation in the prevalence of cytoplasmic incompatibility-inducing *Wolbachia* in the butterfly *Eurema hecabe* across the Japanese archipelago. *Evol. Ecol. Res.* **2005**, *7*, 931–942.

248 Narita, S.; Nomura, M.; Kato, Y.; Fukatsu, T. Genetic structure of sibling butterfly species affected by *Wolbachia* infection sweep: Evolutionary and biogeographical implications. *Mol. Ecol.* **2006**, *15*, 1095–1108.

249 Kamoda, S.; Masui, S.; Ishikawa, H.; Sasaki, T. *Wolbachia* infection and cytoplasmic incompatibility in the cricket *Teleogryllus taiwanemma*. *J. Exp. Biol.* **2000**, *203*, 2503–2509.

250 Moret, Y.; Juchault, P.; Rigaud, T. *Wolbachia* endosymbiont responsible for cytoplasmic incompatibility in a terrestrial crustacean: Effects in natural and foreign hosts. *Heredity* **2001**, *86*, 325–332.

251 Legrand, J.; Martin, G.; Artault, J. Corrélation entre la présence d'un symbiote bactérien dans les ovocytes de *Porcellio dilatatus petiti*, et la stérilité du croisement *P. d. petiti* mâle × *P. d. dilatatus* femelle. *Inst. Pasteur. Tunis.* **1978**, *55*, 507–514.

252 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* **2003**, *91*, 208–216.

253 Gotoh, T.; Noda, H.; Fujita, T.; Iwadate, K.; Higo, Y.; Saito, S.; Ohtsuka, S. *Wolbachia* and nuclear-nuclear interactions contribute to reproductive incompatibility in the spider mite *Panonychus mori* (Acari: Tetranychidae). *Heredity* **2005**, *94*, 237–246.

254 Tsagkarakou, A.; Guillemaud, T.; Rousset, F.; Navajas, M. Molecular identification of a *Wolbachia* endosymbiont in a *Tetranychus urticae* strain (Acari: Tetranychidae). *Insect Mol. Biol.* **1996**, *5*, 217–221.

255 van Opijnen, T.; Breeuwer, J.A. High temperatures eliminate *Wolbachia*, a cytoplasmic incompatibility inducing endosymbiont, from the two-spotted spider mite. *Exp. Appl. Acarol.* **1999**, *23*, 871–881.

256 Gotoh. T.; Sugasawa, J.; Noda, H.; Kitashima, Y. *Wolbachia*-induced cytoplasmic incompatibility in Japanese populations of *Tetranychus urticae* (Acari: Tetranychidae). *Exp. Appl. Acarol.* **2007**, *42*, 1–16.

257 Hunter, M.S.; Perlman, S.J.; Kelly, S.E. A bacterial symbiont in the Bacteroidetes induces cytoplasmic incompatibility in the parasitoid wasp *Encarsia pergandiella*. *Proc. R. Soc. Lond. B* **2003**, *270*, 2185–2190.

258 de Luna, C.J.; Moro, C.V.; Guy, J.H.; Zenner, L.; Sparagano, O.A.E. Endosymbiotic bacteria living inside the poultry red mite (*Dermanyssus gallinae*). *Exp. Appl. Acarol.* **2009**, 48, 105–113.

259 Ros, V.I.D.; Breeuwer, J.A.J. The effects of, and interactions between, *Cardinium* and *Wolbachia* in the doubly infected spider mite *Bryobia sarothamni*. *Heredity* **2009**, 102, 413–422.

260 Xie, R.; Zhou, L.; Zhao, Z.; Hong, X.; Xiao-Yue, H. Male age influences the strength of *Cardinium*-induced cytoplasmic incompatibility expression in the carmine spider mite *Tetranychus cinnabarinus*. *Appl. Entomol. Zool.* **2010**, *45*, 417–423.

261 Kambris, Z.; Blagborough, A.M.; Pinto, S.B.; Blagrove, M.S.C.; Godfray, H.C.J.; Sinden, R.E.; Sinkins, S.P. *Wolbachia* stimulates immune gene expression and inhibits *plasmodium* development in *Anopheles gambiae*. *PLoS Pathog.* **2010**, *6*, e1001143.

262 Moreira, L.A.; Iturbe-Ormaetxe, I.; Jeffery, J.A.; Lu, G.; Pyke, A.T.; Hedges, L.M.; Rocha, B.C.; Hall-Mendelin, S.; Day, A.; Riegler, M.; *et al*. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell* **2009**, *139*, 1268–1278.

263 Kambris, Z.; Cook, P.E.; Phuc, H.K.; Sinkins, S.P. Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes. *Science* **2009**, *326*, 134–136.

264 Bian, G.; Xu, Y.; Lu, P.; Xie, Y.; Xi, Z. The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. *PLoS Pathog.* **2010**, *6*, e1000833.

265 Glaser, R.L.; Meola, M.A. The native *Wolbachia* endosymbionts of *Drosophila melanogaster* and *Culex quinquefasciatus* increase host resistance to West Nile virus infection. *PloS One* **2010**, *5*, e11977.

266 Fytrou, A.; Schofield, P.G.; Kraaijeveld, A.R.; Hubbard, S.F. *Wolbachia* infection suppresses both host defence and parasitoid counter-defence. *Proc. R. Soc. Lond. B* **2006**, *273*, 791–796.

267 Osborne, S.E.; Leong, Y.S.; O'Neill, S.L.; Johnson, K.N. Variation in antiviral protection mediated by different *Wolbachia* strains in *Drosophila simulans*. *PLoS Pathog.* **2009**, *5*, e1000656.

268 Panteleev, D.I.; Goriacheva, I.I.; Andrianov, B.V.; Reznik, N.L.; Lazebny, O.E.; Kulikov, A.M. The endosymbiotic bacterium *Wolbachia* enhances the nonspecific resistance to insect pathogens and alters behavior of *Drosophila melanogaster*. *Genetika* **2007**, *43*, 1277–1280.

269 Hedges, L.M.; Brownlie, J.C.; O'Neill, S.L.; Johnson, K.N. *Wolbachia* and virus protection in insects. *Science* **2008**, *322*, 702.

270 Teixeira, L.; Ferreira, A.; Ashburner, M. The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biol.* **2008**, *6*, e2.

271 Walker, T.; Johnson, P.H.; Moreira, L.A.; Iturbe-Ormaetxe, I.; Frentiu, F.D.; McMeniman, C.J.; Leong, Y.S.; Dong, Y.; Axford, J.; Kriesner, P.; *et al*. The *w*Mel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature* **2011**, *476*, 450–453.

272 Jaenike, J.; Unckless, R.; Cockburn, S.N.; Boelio, L.M.; Perlman, S.J. Adaptation via symbiosis: Recent spread of a *Drosophila* defensive symbiont. *Science* **2010**, *329*, 212–215.

273 Hosokawa, T.; Koga, R.; Kikuchi, Y.; Meng, X-Y.; Fukatsu, T. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 769–774.

274 Supali, T.; Djuardi, Y.; Pfarr, K.M.; Wibowo, H.; Taylor, M.J.; Hoerauf, A.; Houwing-Duistermaat, J.J.; Yazdanbakhsh, M.; Sartono, E. Doxycycline treatment of *Brugia malayi*-infected persons reduces microfilaremia and adverse reactions after diethylcarbamazine and albendazole treatment. *Clin. Infect. Dis.* **2008**, *46*, 1385–1393.

275   Rao, R.; Well, G.J. In vitro effects of antibiotics on *Brugia malayi* worm survival and reproduction. *J. Parasitol.* **2002**, *88*, 605–611.

276   Bandi, C.; Trees, A.J.; Brattig, N.W. *Wolbachia* in filarial nematodes: Evolutionary aspects and implications for the pathogenesis and treatment of filarial diseases. *Vet. Parasitol.* **2001**, *98*, 215–238.

277   Bandi, C.; McCall, J.W.; Genchi, C.; Corona, S.; Venco, L.; Sacchi, L. Effects of tetracycline on the filarial worms *Brugia pahangi* and *Dirofilaria immitis* and their bacterial endosymbionts *Wolbachia*. *Int. J. Parasitol.* **1999**, *29*, 357–364.

278   Genchi, C.; Sacchi, L.; Bandi, C.; Venco, L. Preliminary results on the effect of tetracycline on the embryogenesis and symbiotic bacteria (*Wolbachia*) of *Dirofilaria immitis*. An update and discussion. *Parassitologia* **1998**, *40*, 247–249.

279   Hoerauf, A.; Nissen-Pähle, K.; Schmetz, C.; Henkle-Dührsen, K.; Blaxter, M.L.; Büttner, D.W.; Gallin, M.Y.; Al-Qaoud, K.M.; Lucius, R.; Fleischer, B. Tetracycline therapy targets intracellular bacteria in the filarial nematode *Litomosoides sigmodontis* and results in filarial infertility. *J. Clin. Invest.* **1999**, *103*, 11–18.

280   Hoerauf, A.; Volkmann, L.; Nissen-Paehle, K.; Schmetz, C.; Autenrieth, I.; Büttner, D.W.; Fleischer, B. Targeting of *Wolbachia* endobacteria in *Litomosoides sigmodontis*: Comparison of tetracyclines with chloramphenicol, macrolides and ciprofloxacin. *Trop. Med. Int. Health* **2000**, *5*, 275–279.

281   Townson, S.; Hutton, D.; Siemienska, J.; Hollick, L.; Scanlon, T.; Tagboto, S.K.; Taylor, M.J. Antibiotics and *Wolbachia* in filarial nematodes: Antifilarial activity of rifampicin, oxytetracycline and chloramphenicol against *Onchocerca gutturosa*, *Onchocerca lienalis* and *Brugia pahangi*. *Ann. Trop. Med. Parasitol.* **2000**, *94*, 801–816.

282   Langworthy, N.G.; Renz, A.; Mackenstedt, U.; Henkle-Dührsen, K.; de Bronsvoort, M.B.; Tanya, V.N.; Donnelly, M.J.; Trees, A.J. Macrofilaricidal activity of tetracycline against the filarial nematode *Onchocerca ochengi*: Elimination of *Wolbachia* precedes worm death and suggests a dependent relationship. *Proc. R. Soc. Lond. B* **2000**, *267*, 1063–1069.

283   Hoerauf, A.; Mand, S.; Adjei, O.; Fleischer, B.; Büttner, D.W. Depletion of *Wolbachia* endobacteria in *Onchocerca volvulus* by doxycycline and microfilaridermia after ivermectin treatment. *Lancet* **2001**, *357*, 1415–1416.

284   Hoerauf, A.; Mand, S.; Fischer, K.; Kruppa, T.; Marfo-Debrekyei, Y.; Debrah, A.Y.; Pfarr, K.M.; Adjei, O.; Büttner, D.W. Doxycycline as a novel strategy against bancroftian filariasis-depletion of *Wolbachia* endosymbionts from *Wuchereria bancrofti* and stop of microfilaria production. *Med. Microbiol. Immunol.* **2003**, *192*, 211–216.

285   Zchori-Fein, E.; Borad, C.; Harari, A.R. Oogenesis in the date stone beetle, *Coccotrypes dactyliperda*, depends on symbiotic bacteria. *Physiol. Entomol.* **2006**, *31*, 164–169.

286   Son, Y.; Luckhart, S.; Zhang, X.; Lieber, M.J.; Lewis, E.E. Effects and implications of antibiotic treatment on *Wolbachia*-infected vine weevil (Coleoptera: Curculionidae). *Agr. Forest. Entomol.* **2008**, *10*, 147–155.

287 Timmermans, M.J.T.N.; Ellers, J. *Wolbachia* endosymbiont is essential for egg hatching in a parthenogenetic arthropod. *Evol. Ecol.* **2008**, *23*, 931–942.

288 Starr, D.J.; Cline, T.W. A host parasite interaction rescues *Drosophila* oogenesis defects. *Nature* **2002**, *418*, 76–79.

Supplementary Materilas

Table S1. Other phenotypes induced by *Wolbachia*, *Spiroplasma* and *Cardinium*.

Phenotype	Endosymbiont	Class	Order	Species	Reference
CI	*Wolbachia*	Insecta	Coleoptera	*Callosobruchus chinensis*	[195]
CI	*Wolbachia*	Insecta	Coleoptera	*Chelymorpha alternans*	[196]
CI	*Wolbachia*	Insecta	Coleoptera	*D. virgifera*	[197]
CI	*Wolbachia*	Insecta	Coleoptera	*D. virgifera virgifera*	[197]
CI	*Wolbachia*	Insecta	Coleoptera	*D. virgifera zeae*	[197]
CI	*Wolbachia*	Insecta	Coleoptera	*Hypera postica*	[198-200]
CI	*Wolbachia*	Insecta	Coleoptera	*Tribolium confusum*	[201,202]
CI	*Wolbachia*	Insecta	Diptera	*Aedes albopictus*	[200]
CI	*Wolbachia*	Insecta	Diptera	*A. cooki*	[209,204]
CI	*Wolbachia*	Insecta	Diptera	*A. kesseli*	[205,206]
CI	*Wolbachia*	Insecta	Diptera	*A. malayensis*	[207,208]
CI	*Wolbachia*	Insecta	Diptera	*A. polynesiensis*	[207,208]
CI	*Wolbachia*	Insecta	Diptera	*A. pseudoscutellaris*	[209,210]
CI	*Wolbachia*	Insecta	Diptera	*A. s. scutellaris*	[209,211]
CI	*Wolbachia*	Insecta	Diptera	*Armigeres subalbatus*	[212]
CI	*Wolbachia*	Insecta	Diptera	*Culex pipiens*	[213–215]
CI	*Wolbachia*	Insecta	Diptera	*C. pipiens quinquefasciatus*	[216,217]
CI	*Wolbachia*	Insecta	Diptera	*Drosophila auraria*	[218]
CI	*Wolbachia*	Insecta	Diptera	*D. melanogaster*	[219,220]
CI	*Wolbachia*	Insecta	Diptera	*D. recens*	[221]
CI	*Wolbachia*	Insecta	Diptera	*D. sechellia*	[222]
CI	*Wolbachia*	Insecta	Diptera	*D. simulans*	[219,224]
CI	*Wolbachia*	Insecta	Diptera	*Liriomyza trifolii*	[224]
CI	*Wolbachia*	Insecta	Diptera	*Phlebotomus papatasi*	[225]
CI	*Wolbachia*	Insecta	Diptera	*Rhagoletis cerasi*	[226,227]
CI	*Wolbachia*	Insecta	Hymenoptera	*Aphytis melinus DeBach*	[228]
CI	*Wolbachia*	Insecta	Hymenoptera	*Asobara tabida*	[229]
CI	*Wolbachia*	Insecta	Hymenoptera	*Cotesia sesamiae*	[230]
CI	*Wolbachia*	Insecta	Hymenoptera	*Encarsia inaron*	[231]
CI	*Wolbachia*	Insecta	Hemiptera	*Laodelphax striatellus*	[232–234]
CI	*Wolbachia*	Insecta	Hemiptera	*Leptopilina heterotoma*	[235–237]
CI	*Wolbachia*	Insecta	Hemiptera	*Macrolophus pygmaeus*	[238]

Table S1. *Cont.*

Phenotype	Endosymbiont	Class	Order	Species	Reference
CI	*Wolbachia*	Insecta	Hemiptera	*Nasonia giraulti*	[239,240]
CI	*Wolbachia*	Insecta	Hemiptera	*N. longicornis*	[240,241]
CI	*Wolbachia*	Insecta	Hemiptera	*N. vitripennis*	[240,242]
CI	*Wolbachia*	Insecta	Hemiptera	*Orius strigicollis*	[243]
CI	*Wolbachia*	Insecta	Lepidoptera	*Colias erate poliographus*	[244]
CI	*Wolbachia*	Insecta	Lepidoptera	*Ephestia cautella*	[245]
CI	*Wolbachia*	Insecta	Lepidoptera	*E. kuehniella*	[246]
CI	*Wolbachia*	Insecta	Lepidoptera	*Eurema hecabe*	[247]
CI	*Wolbachia*	Insecta	Lepidoptera	*E. madarina*	[248,249]
CI	*Wolbachia*	Insecta	Orthoptera	*Gryllus assimilis*	[197]
CI	*Wolbachia*	Insecta	Orthoptera	*G. integer*	[197]
CI	*Wolbachia*	Insecta	Orthoptera	*G. ovisopis*	[197]
CI	*Wolbachia*	Insecta	Orthoptera	*G. pennsylvanicus*	[197]
CI	*Wolbachia*	Insecta	Orthoptera	*G. rubens*	[197]
CI	*Wolbachia*	Insecta	Orthoptera	*Teleogryllus taiwanemma*	[249]
CI	*Wolbachia*	Malacostraca	Isopoda	*Cylisticus convexus*	[250]
CI	*Wolbachia*	Malacostraca	Isopoda	*Porcellio dilatatus*	[233,251]
CI	*Wolbachia*	Arachnida	Trombidiformes	*Oligonychus gotohi*	[252]
CI	*Wolbachia*	Arachnida	Trombidiformes	*Panonychus mori*	[252,253]
CI	*Wolbachia*	Arachnida	Trombidiformes	*Tetranychus urticae*	[253,254]
CI	*Cardinium*	Insecta	Hymenoptera	*Encarsia pergandiella*	[257]
CI	*Cardinium*	Arachnida	Mesostigmata	*Dermanyssus gallinae*	[258]
CI	*Cardinium*	Arachnida	Trombidiformes	*Bryobia sarothamni*	[289]
CI	*Cardinium*	Arachnida	Trombidiformes	*Eotetranychus suginamensis*	[256]
CI	*Cardinium*	Arachnida	Trombidiformes	*Tetranychus cinnabarinus*	[260]
RP [a]	*Wolbachia*	Insecta	Diptera	*Anopheles gambiae*	[261]
RP [b]	*Wolbachia*	Insecta	Diptera	*Aedes aegypti*	[262–264]
RP [c]	*Wolbachia*	Insecta	Diptera	*Culex quinquefasciatus*	[265]
RP [d]	*Wolbachia*	Insecta	Diptera	*Drosophila simulans*	[266,267]
RP [e]	*Wolbachia*	Insecta	Diptera	*D. melanogaster*	[265,268–270]
RP [f]	*Spiroplasma*	Insecta	Diptera	*D. hydei*	[271]
RP [g]	*Spiroplasma*	Insecta	Diptera	*D. neotestacea*	[272]
M	*Wolbachia*	Insecta	Hemiptera	*Cimex lectularius*	[273]
M	*Wolbachia*	Secernentea	Spirurida	*Brugia malayi*	[274–271]
M	*Wolbachia*	Secernentea	Spirurida	*B. pahangi*	[277]
M	*Wolbachia*	Secernentea	Spirurida	*Dirofilaria immitis*	[277,278]
M	*Wolbachia*	Secernentea	Spirurida	*Litomosoides sigmodontis*	[279,280]
M	*Wolbachia*	Secernentea	Spirurida	*Onchocerca gutturosa*	[281]
M	*Wolbachia*	Secernentea	Spirurida	*O. lienalis*	[281]
M	*Wolbachia*	Secernentea	Spirurida	*O. ochengi*	[282]
M	*Wolbachia*	Secernentea	Spirurida	*O. volvulus*	[283]
M	*Wolbachia*	Secernentea	Spirurida	*Wuchereria bancroft*	[284]
O	*Wolbachia*	Insecta	Hymenoptera	*Asobara tabida*	[236]

Table S1. *Cont.*

Phenotype	*Endosymbiont*	Class	Order	*Species*	Reference
O	*Wolbachia*	Insecta	Coleoptera	*Coccotrypes dactyliperda*	[285]
O	*Wolbachia*	Insecta	Coleoptera	*Otiorhynchus sulcatus*	[286]
O	*Wolbachia*	Insecta	Collembola	*Folsomia candida*	[287]
O	*Wolbachia*	Insecta	Diptera	*Drosophila melanogaster* (*Sxl*)	[288]

CI, cytoplasmic incompatibility; RP, resistance to pathogen; M mutualism; O; oogenesis.

[a] Confer resistance to *Plasmodium berghei* and *P. falciparum*

[b] Confer resistance to *Plasmodium gallinaceum,* Dengue, Chikungunya and *Brugia pahangi*

[c] Confer resistance to West Nile virus

[d] Confer resistance to *Drosophila* C virus, Flock House virus, Dengue

[e] Confer resistance to *Drosophila* C virus, Nora virus, Flock House virus, West Nile virus and *Blauveria bassiana*

[f] Confer resistance to the parasitic wasp *Leptopilina heterotoma*

[g] Confer resistance to the nematode *Howardula aoronymphium*