Review

The impact of endosymbionts on the evolution of host sex-determination mechanisms

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The past years have revealed that inherited bacterial endosymbionts are important sources of evolutionary novelty for their eukaryotic hosts. In this review we discuss a fundamental biological process of eukaryotes influenced by bacterial endosymbionts: the mechanisms of sex determination. Because they are maternally inherited, several endosymbionts of arthropods, known as reproductive parasites, have developed strategies to convert non-transmitting male hosts into transmitting females through feminization of genetic males and parthenogenesis induction. Recent investigations have also highlighted that endosymbionts can impact upon host sex determination more subtly through genetic conflicts, resulting in selection of host nuclear genes resisting endosymbiont effects. Paradoxically, it is because of their selfish nature that reproductive parasites are such powerful agents of evolutionary change in their host sex-determination mechanisms. They might therefore represent excellent models for studying transitions between sex-determining systems and, more generally, the evolution of sex-determination mechanisms in eukaryotes.

Endosymbionts as a source of evolutionary novelty

The most intimate interaction between organisms is endosymbiosis, a type of symbiosis (*sensu lato*, including both parasites and mutualists) in which a microbial partner lives within its host cells [1,2]. Obligate intracellular bacterial endosymbionts exclusively replicate inside the cytoplasm of mostly eukaryotic host cells and they typically have no extracellular state. Endosymbionts have played a key role in the emergence of major lifeforms on Earth and in the generation of biological diversity. However, appreciation of endosymbiosis as an important source of evolutionary novelty has developed relatively recently [1,2]. The evolutionary significance of endosymbiosis is perhaps best exemplified by the evolution of mitochondria and chloroplasts, both of which result from endosymbiotic events involving α-proteobacterial and cyanobacterial ancestors, respectively [3]. Over the past years evidence has been accumulating that bacterial endosymbionts further affect animal biology in many ways, such as nutrition [1,4], defense against natural enemies [5] and immunity [6,7]. In this review we focus on another crucial evolutionary process influenced by bacterial endosymbionts: the mechanisms of sex determination of their eukaryotic hosts. We discuss recent investigations that have highlighted the deep impact endosymbionts can have upon host sex determination via direct manipulation and indirectly through genetic conflicts. Finally, we explore the broader evolutionary consequences of these interactions.

Direct manipulation of host sex determination

Diverse types of sex-determination mechanisms have been identified in animals [8,9]. Generally, sexual differences between males and females are genetically determined by chromosomal sex factors, commonly carried by the sex chromosomes. In some animals, however, sex is determined after conception by environmental factors such as temperature (in reptiles, fish and crustaceans), photoperiod (in crustaceans), crowding (in nematodes) or behavior (in fish). Sex determination can also be affected by inherited bacterial endosymbionts. Disrupting the mode of sex determination of their hosts could be advantageous for endosymbionts because they are predominantly transmitted vertically through the female egg cytoplasm, and not via male sperm. Thus, males represent dead-ends for such microorganisms. Consequently, any effect of the endosymbiont that distorts the host sex ratio towards females will be selectively advantageous for the endosymbiont.

Several endosymbionts of arthropods, known as reproductive parasites, have evolved such a selfish evolutionary strategy consisting of manipulating their host reproduction [10] (Table 1). Recent surveys showed that $> 30\%$ of sampled arthropods are infected by the most common reproductive parasites [11]. Among them, *Wolbachia* is not only the most common bacterial endosymbiont involved in reproductive parasitism [12], but is also the only one known to date to induce all four commonly recognized types of reproductive manipulations: (i) cytoplasmic incompatibility (sperm–egg incompatibility leading to post-zygotic sterility between infected males and females that are uninfected or infected with a different endosymbiont strain) (Box 1), (ii) male killing (sex-ratio distortion towards females through targeted death of male progeny) (Box 2), (iii) feminization of genetic males (sex-ratio distortion towards females through conversion of genetic males into functional females) (Figure 1a), and (iv) parthenogen-

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Table 1. Bacterial endosymbionts associated with reproductive parasitism

Endosymbiont	Bacterial group	Infected arthropod host groups	Manipulation phenotypes[a]
Wolbachia	α-Proteobacteria	Insects, crustaceans, mites, spiders	F, PI, CI, MK
Cardinium	Bacteroidetes	Insects, mites, spiders	F, PI, CI
Rickettsia	α-Proteobacteria	Insects, spiders	PI, MK
Spiroplasma	Mollicutes	Insects	MK
Flavobacteria	Mollicutes	Insects	MK
Arsenophonus	γ-Proteobacteria	Insects	MK

[a]F, feminization of genetic males; PI, parthenogenesis induction; CI, cytoplasmic incompatibility; MK, male killing.

esis induction (sex-ratio distortion towards females through induction of asexual daughter development) (Figure 1b). Here we focus on the two reproductive manipulations that directly affect host sex determination (i.e. they convert non-transmitting males into transmitting females): feminization of genetic males and parthenogenesis induction.

Feminization in crustaceans

In the isopod crustacean *Armadillidium vulgare*, genetic sex determination follows female heterogamety (ZZ males and ZW females). Nevertheless, some *A. vulgare* females produce highly female-biased progenies without differential mortality between sexes. The causative agents of this maternally-inherited sex-ratio distortion are *Wolbachia* endosymbionts [13–17]. In *A. vulgare*, all zygotes inheriting *Wolbachia* develop a female phenotype. In particular, ZZ genetic males harboring *Wolbachia* are converted into functional phenotypic females which, in turn, produce female-biased broods [13–17] (Figure 1a). One important outcome of this evolution is the elimination of the W female sex chromosome in populations harboring *Wolbachia* endosymbionts [15–17]. This is because feminized ZZ individuals produce females without transmitting any W chromosome (Figure 1a). Thus, W chromosome frequency decreases at each generation until eventual loss from the population. Consequently, in populations in which *Wolbachia* are present, infected females actually are ZZ genetic males sexually converted by *Wolbachia*. Sex determination is therefore under the control of *Wolbachia* in infected populations: individuals inheriting *Wolbachia* develop as

Box 1. Cytoplasmic incompatibility: favoring endosymbiont transmission without host sex-ratio distortion

Among the different reproductive manipulations induced by bacterial endosymbionts, cytoplasmic incompatibility (CI) is the most widespread phenotype: it has been described in mites, isopods and insects and is caused by *Wolbachia* and *Cardinium* (Table 1). Unlike feminization (Figure 1a), parthenogenesis induction (Figure 1b) and male killing (Box 2), CI does not induce host sex-ratio distortion. Instead, CI favors infected female reproduction and, thereby, transmission of maternally inherited endosymbionts. CI has been particularly studied in *Wolbachia* [12]. CI *Wolbachia* are thought to cause sperm modification in infected males. Although modified sperm can be rescued by infected oocytes and lead to viable progeny, uninfected females fail to rescue modified sperm and abort. CI-induced sterilization of uninfected females thus leads to the spread of females carrying *Wolbachia* in uninfected populations [76]. Bidirectional CI is also observed when both sexes are infected by different *Wolbachia* strains which are not able to rescue the sperm modification induced by the other strain [77]. Several studies have shown that bidirectional CI between diverging populations could promote speciation that can be reinforced by pre-mating isolation [78–81].

Although CI molecular mechanisms remain largely unknown, cytological studies in different insect and isopod species have described asynchrony in the development of male and female pronuclei, leading to defects of the first mitotic division of the embryo [82,83]. Delay of histone H3.3 phosphorylation in the male pronucleus, which is required for the initiation of mitosis, induces late male chromosome condensation during the first metaphase and exclusion during the following anaphase, leading to embryonic lethality [84].

Because CI efficiently leads infected cytoplasm to invade host populations, it is not surprising that *Wolbachia* has attracted considerable interest for potential applications in agricultural pest and disease vector control [85,86]. The first study that made credible the use of *Wolbachia* as an environmentally-friendly biocontrol agent described the dramatic decrease of an uninfected population of *Ceratitis capitata* (a worldwide fruit pest) when only infected males carrying a CI *Wolbachia* strain were introduced into population cages [87]. The recent demonstration in *Drosophila melanogaster* and *Aedes aegypti* of *Wolbachia*-induced resistance to RNA virus infection, and to the establishment of human pathogens such as dengue and Chikungunya viruses, provides further evidence for the ability of these bacteria to interfere with pathogen propagation [86].

Box 2. Male killing: sex-ratio distortion without manipulation of host sex determination

Male killing (MK) is an adaptation to maternal transmission used by several bacterial endosymbionts such as *Wolbachia*, *Rickettsia*, *Spiroplasma*, *Arsenophonus* and *Flavobacteria*, that are found in five insect orders and acari [88] (Table 1). Embryonic male killers (i.e. early male killers) are found in host species with environmental, XO, XY, ZW and haplodiploid sex-determination systems. Contrary to feminization and parthenogenesis induction, which both consist of converting non-transmitting males into transmitting females, MK involves death of the sex that does not vertically transmit endosymbionts (i.e. males). In ladybird beetles, which are 'hotspots' for male killers [69], MK-induced death of males benefits their infected sisters by sibling egg consumption [89], decreased intensity of antagonistic interactions between siblings, and reduced levels of inbreeding [88]. As a result of this fitness compensation, infected females produce daughters with a higher probability of survival than uninfected ones, allowing endosymbionts to spread in the population [88]. However fitness compensation is generally imperfect (death of male progeny commonly increases sister host survival probability by 10% or less). Selection for a MK endosymbiont is therefore much smaller than that for a feminizing endosymbiont, such that male killer prevalence in the majority of hosts is lower than 40%, although high-prevalence infections do occur [90]. The low drive also increases sensitivity of male killer prevalence to environmental conditions [91].

The mechanism by which the sex specificity of virulence is achieved has been demonstrated in the association between *Spiroplasma poulsonii* and *Drosophila melanogaster* [92]. A functional dosage-compensation complex, a major component of the sex-determination pathway in *Drosophila,* is required for MK by *S. poulsonii*. Endosymbionts failed to kill males lacking any of the five protein components of the dosage-compensation complex. This result can be exploited to yield further insights into the MK mechanism, which still remains unknown.

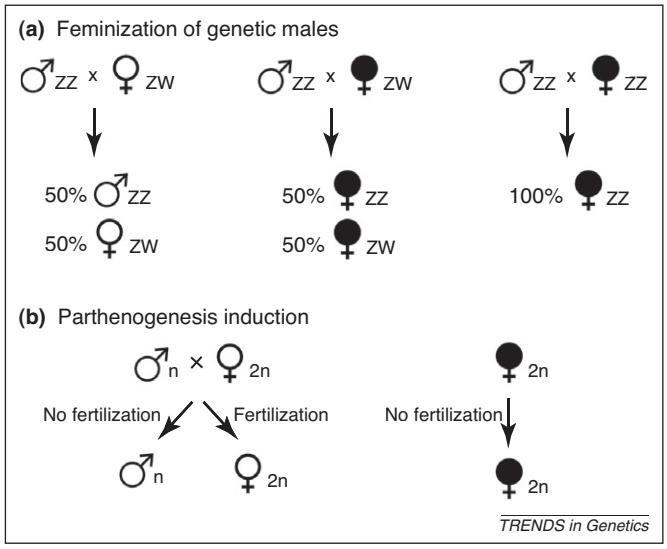

Figure 1. Endosymbiont-mediated reproductive manipulations directly impacting host sex determination. **(a)** Feminization of genetic males, characterized by sex-ratio distortion towards females through conversion of genetic ZZ males into phenotypic ZZ females. **(b)** Parthenogenesis induction, characterized by sex-ratio distortion towards females through conversion of genetic (haploid) males into genetic (diploid) females. Black (white) coloration: individual carries (does not carry) endosymbionts. ZZ/ZW, homo/heterogametic status of individual. *n*/2*n*, haploid/diploid status of individual. To simplify, the endosymbiont transmission rate from mother to offspring is assumed to be 100%.

females whereas males are uninfected individuals. Thus, the *A. vulgare*/*Wolbachia* model is a perfect example of cytoplasmic sex determination (Figure 2). Because *Wolbachia* transmission is ~90% efficient, the sex ratio of infected broods is highly biased towards females.

The precise molecular mechanism of *Wolbachia* feminization in *A. vulgare* is unknown. However, evidence indicates that it is achieved by preventing androgenic gland differentiation during post-embryonic sexual differentiation [15–17]. In *A. vulgare*, both sexes apparently possess the genetic programs necessary for expression of the opposite sex. The W chromosome is thought to be a Z chromosome carrying an additional 'female' gene that inhibits the activity of the 'male' gene located on the Z chromosome. In fact, the cue for sex determination and differentiation in crustaceans appears to be the 'male' gene, which controls the development of the androgenic gland. This organ is responsible for producing the androgenic hormone which causes male sex differentiation after the third moult of young isopods and maintains secondary male characters in adults [18]. In genetic females, the 'female' gene inhibits the activity of the 'male' gene, allowing female sex differentiation. *Wolbachia* could also target the 'male' gene or act at a later stage of sexual development, but the end result is that androgenic gland never differentiates in ZZ genetic males infected by *Wolbachia*. A *Wolbachia* dosage effect could be involved in the feminization process because incomplete feminization sometimes occurs, presumably due to insufficient *Wolbachia* density to inhibit androgenic gland differentiation, but sufficient to target androgenic hormone receptors in adults and lead to partial feminization [19].

Many isopods carry *Wolbachia* and feminization is strongly suspected in many species [13,17,20]. However, it has been formally demonstrated only in a few species [13–17,21]. Feminization has also been described or is suspected in several amphipod crustaceans, although the causative agents are not bacteria but unicellular eukaryotes (i.e. microsporidia) [22,23]. More generally, female-biased sex ratios and intersexuality have been reported in many crustacean species [15] and *Wolbachia* have recently been found in non-isopod crustaceans [20,24,25], suggesting that feminization is probably more widespread in crustaceans than is currently recognized. Interestingly, microsporidia feminize their amphipod hosts by preventing androgenic gland differentiation, as feminizing *Wolbachia* do in *A. vulgare* [22]. This suggests that phylogenetically distantly-related microorganisms could manipulate their crustacean host sex determination using a common strategy.

Feminization in insects and acari

Feminization has long been thought to be restricted to crustaceans owing to the labile sex determination of these organisms, which exclusively depends on the production of a circulating sexual hormone: the androgenic hormone produced by the androgenic gland [15–18]. By contrast, sex determination in insects is a cellular process. Consequently, endosymbionts have to infect all host cells and interact with the genetic control of sex determination in all somatic cells for feminization to be effective. This view changed, however, with the discovery of *Brevipalpus phoenicis* mites feminized by the bacterial endosymbiont *Cardinium* [26] (Table 1). Cases of feminization in insects have also been discovered recently, mediated by *Wolbachia* in the butterfly *Eurema hecabe* [27,28] and the leafhopper *Zyginidia pullula* [29,30], and by *Cardinium* in the wasp *Encarsia hispida* [31].

In *E. hecabe* and *Z. pullula*, *Wolbachia*-infected females produce all-female broods in laboratory conditions and antibiotic treatment leads to male-biased progenies, which suggested that these phenotypic females possess a male genotype [27,29]. In *E. hecabe*, genetic sex determination follows female heterogamety (ZZ males and ZW females) and cytological observations have confirmed that *Wolbachia*-infected females are in fact ZZ genetic males inverted into phenotypic females [27,28]. In *Z. pullula*, antibiotics restore a balanced sex ratio in the next generation, but most females harbor male secondary sexual characters [29]. This species has an XX/X0 sex-determination system and cytological observations on female intersexes showed that nuclei have male genotypes and, thus, are X0 genetic males inverted into phenotypic females [29]. However, no study has yet shown whether these females are functional (i.e. fertile) or not.

Given the widely different sex-determination processes involved in insects and crustaceans, host genes targeted by *Wolbachia* might be different in both groups. In insects, *Wolbachia* could interact with key genes that control somatic sex determination, such as *Drosophila melanogaster doublesex* or *transformer* homologs. *Doublesex* (a switch gene at the bottom of the sex-determining cascade) and *transformer* (responsible for female-specific splicing of *doublesex*) have been identified in several insect orders [32]. In the moth *Ostrinia scapulalis*, *Wolbachia*

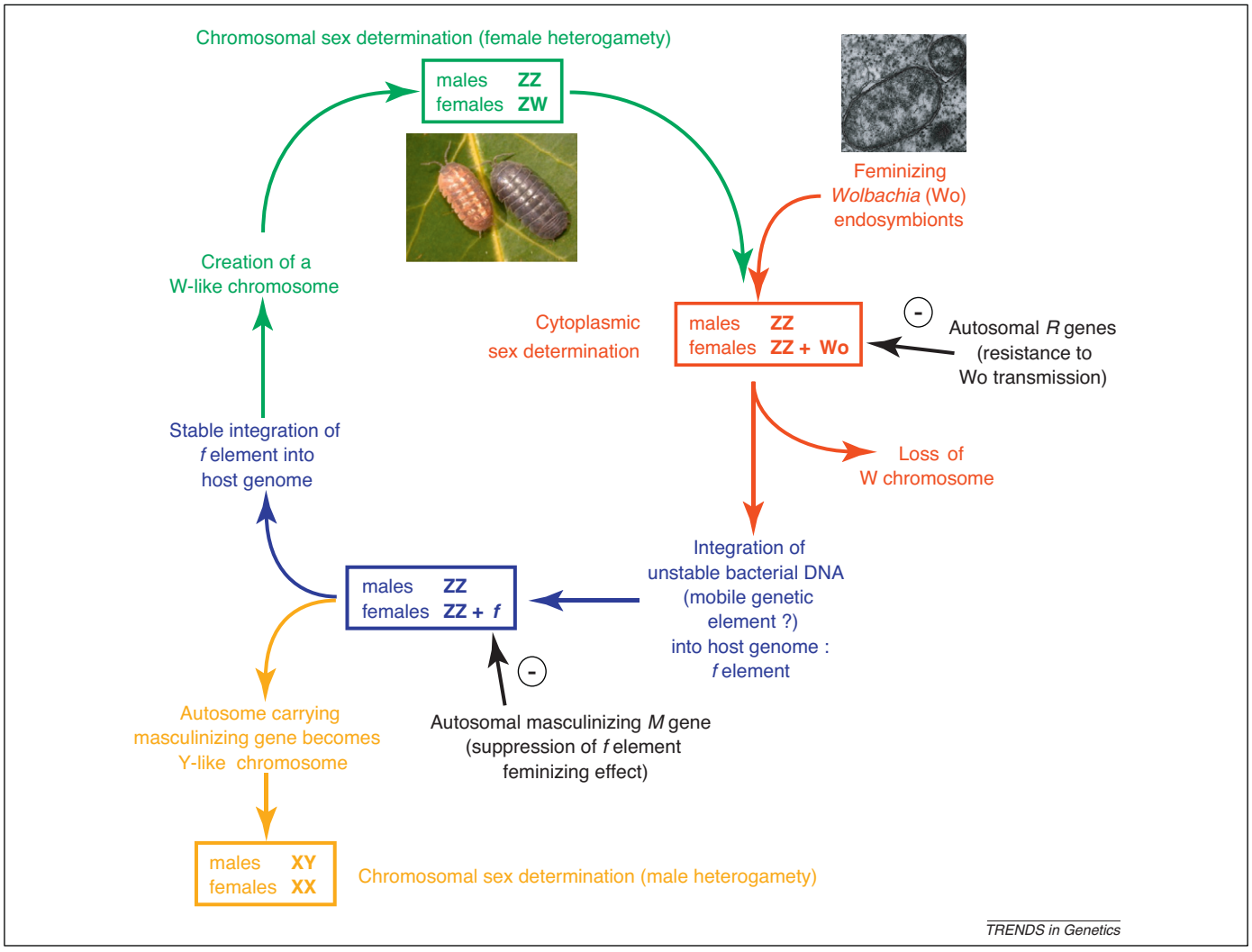

Figure 2. The 'extended sex-determination system' of the isopod *Armadillidium vulgare*. Chromosomal sex determination follows female heterogamety (ZZ males and ZW females) in *A. vulgare* (green). Introduction of feminizing *Wolbachia* endosymbionts in an *A. vulgare* population leads to a shift from chromosomal to cytoplasmic sex determination (red). *Wolbachia* subsequently gives rise to the *f* element, a non-Mendelian feminizing sex factor (blue). The *f* element ultimately becomes stably integrated into a Z chromosome, hence generating a W-like chromosome and restoring chromosomal sex determination with female heterogamety (green). Genetic conflicts the host genome and between feminizing *Wolbachia* or the *f* element result in selection of nuclear resistance genes (black). Under particular conditions, selection of a masculinizing suppressor gene can trigger a shift in heterogamety type (orange). Male heterogamety is not observed in *A. vulgare*, but is found in the closely related isopod *A. nasatum*.

endosymbionts typically induce male killing (Box 2) but, at lower density, they also have a feminizing effect on genetic males [33]. It was recently shown that *Wolbachia* can manipulate *O. scapulalis* sex determination by interfering with sex-specific splicing of the *doublesex* homolog or with an upstream gene in the sex-determination cascade [34]. In crustaceans, *transformer* and *doublesex* homologs have only been identified in the freshwater branchiopod *Daphnia magna* [35,36]. In *D. magna*, *transformer* does not present sex differences in expression or splicing patterns and it does not seem to be involved in sex determination [35]. In addition, *doublesex* is not regulated at the level of pre-mRNA splicing but it rather exhibits different expression levels between males and females [36]. Alternatively, it has been hypothesized that *Wolbachia* could modulate host sexual phenotypes by interacting with hormonal pathways involving ecdysteroids [37]. Overall, although the mechanisms of *Wolbachia*-mediated feminization largely remain elusive, it is suspected that bacterial endosymbionts are able to interact with several different molecular pathways to achieve feminization of their arthropod hosts.

Induction of parthenogenesis in insects and acari

Feminizing endosymbionts drive female development to enhance their vertical transmission. However, successful endosymbiont transmission not only requires production of males and females, but also mating. From an endosymbiont perspective, the ultimate manipulation would consist in driving female development while making males superfluous. This strategy is used by at least three endosymbionts: *Wolbachia*, *Cardinium* and *Rickettsia* [38–40] (Table 1). In all cases, these endosymbionts induce parthenogenesis in haplodiploid insects (hymenopterans and thrips) and acari [38–40]. In these taxa, sex is normally regulated by the ploidy of the embryo: males develop from unfertilized haploid eggs and females develop from fertilized diploid eggs (Figure 1b). Parthenogenesis-inducing

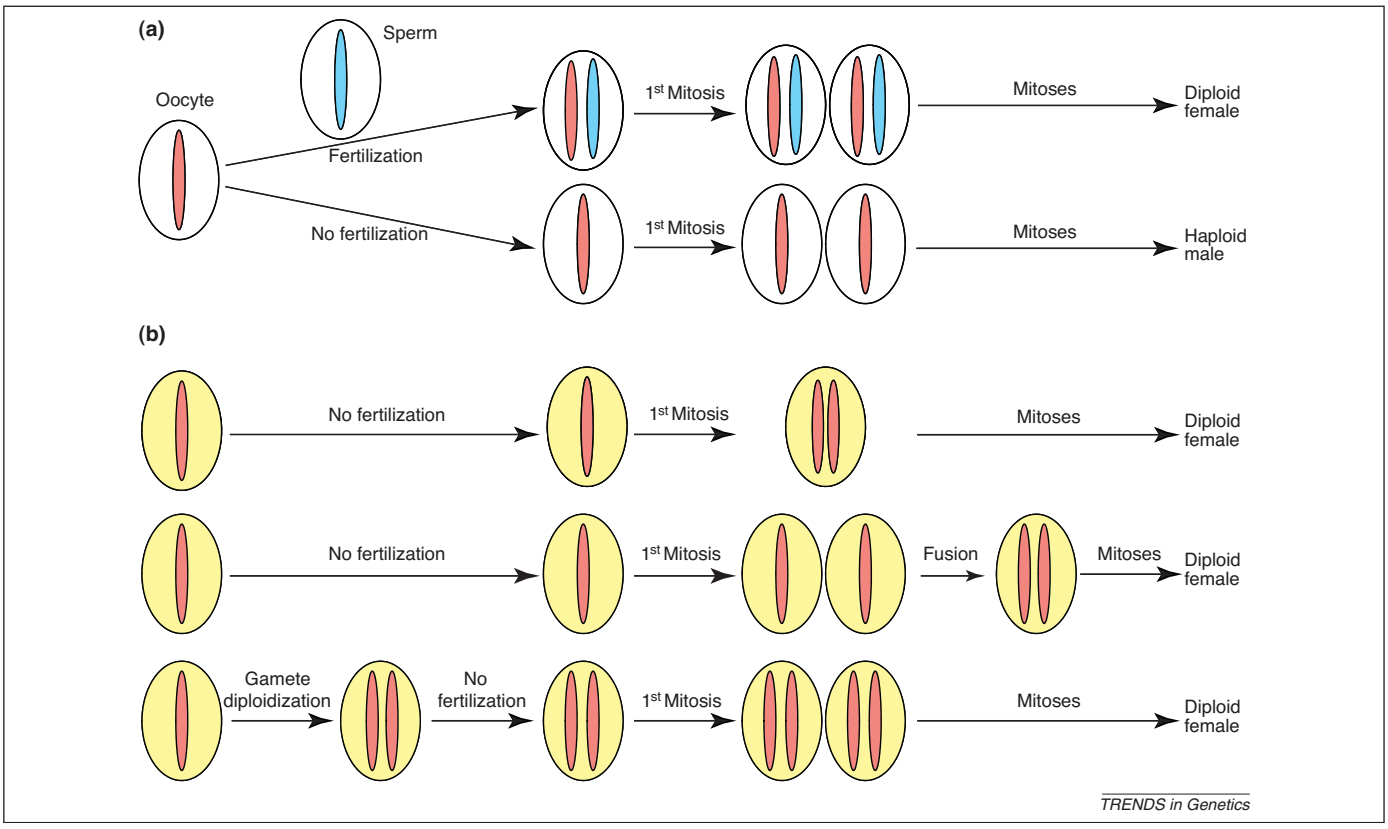

Figure 3. Schematic representation of the mechanisms of male and female development in haplodiploid species lacking or carrying parthenogenesis-inducing endosymbionts. **(a)** In the absence of endosymbionts (white cells), normal diploid females develop following oocyte fertilization (top), which result in diploid eggs containing maternal (pink) and paternal (blue) sets of chromosomes. Normal haploid males develop from unfertilized eggs (bottom), which only contain the maternal set of chromosomes. **(b)** In the presence of parthenogenesis-inducing endosymbionts (yellow cells), diploid females are produced without fertilization through at least three different mechanisms: (i) abnormal separation of haploid chromosome sets during the first mitotic division, as in *Trichogramma* wasps (top), (ii) fusion of cell nuclei after completion of the first mitotic division, as in *Muscidifurax uniraptor* (middle), and (iii) gamete diploidization before egg development, as in *Bryobia* mites (bottom).

(PI) endosymbionts are able to convert non-transmitting males into transmitting females by enabling unfertilized eggs to develop as females. This is achieved through the doubling of chromosome number in unfertilized eggs, rendering them diploid [38]. As a result, infected parthenogenetic females are in turn able to produce endosymbiont-transmitting female progeny without the need for sexual reproduction, egg fertilization and, thus, males.

As for feminization, the precise molecular mechanisms underlying parthenogenesis induction are unknown. However, cytogenetic observations have shown that parthenogenesis is induced by endosymbionts in at least three different ways (Figure 3). In hymenopterans, endosymbiont-mediated diploidization is caused by disruption of the cell cycle during early embryonic development in two different ways: (i) in *Trichogramma* wasp species, the two haploid sets of chromosomes do not separate during the anaphase of the first mitotic division, resulting in one diploid nucleus with two identical sets of haploid chromosomes instead of two haploid nuclei [38], and (ii) in the wasp *Muscidifurax uniraptor*, the first mitotic division is normal, leading to two cells with haploid nuclei, and diploidy is restored by fusion of the two cell nuclei after completion of the first mitotic division [41]. A third mechanism occurs in *Bryobia* mites, in which *Wolbachia* induces parthenogenesis by meiotic modification in infected eggs, resulting in diploid gametes [42].

Similar to feminizing endosymbionts, PI endosymbionts sometimes produce intersex phenotypes when some tissues become diploid whereas others remain haploid during embryogenesis [40]. This is influenced by the rearing temperature of the mothers, which is thought to affect the density of endosymbionts (which are thermosensitive). For example, in *Trichogramma* species infected by *Wolbachia*, all-female progenies are produced at temperatures below 26 °C because endosymbiont density is high enough to diploidize unfertilized (haploid) eggs. By contrast, higher temperatures (>30 °C) eliminate *Wolbachia* endosymbionts. As a result, unfertilized (haploid) eggs are not diploidized, which leads to the production of all-male progenies [40]. At intermediate temperatures, the *Wolbachia* titer is moderate and many infected females produce males, females and gynandromorphs (i.e. individuals in which some tissues are male whereas others are female) from unfertilized (haploid) eggs [40]. In gynandromorphs, the gender of the tissue is determined by the level of ploidy of the cell from which the tissue is derived. In these individuals, *Wolbachia*-mediated diploidization could be somewhat repressed, and this does not take place during the first mitotic division but at a later stage in a subset of cells.

Overall, the outcomes of parthenogenesis induction and feminization of genetic males are fairly similar in many respects: they both convert males into females and occasionally produce intersexes. The main difference is that

feminizing endosymbionts invert genetic males into phenotypic females, whereas PI endosymbionts invert genetic males into genetic females.

Genetic conflict and evolution of host resistance genes

Genetic conflict occurs when different components of a genetic system are subject to selection in opposite directions, in other words, when favoring one genetic component causes a loss of fitness in other components. This concept is linked to the development of two other important concepts in evolutionary biology: (i) selection can operate at different levels [43] from genes to groups of organisms, and (ii) some genetic elements can be selfish or parasitic [44,45], such as transposable elements and reproductive parasites. Typically, the presence of sex-ratio-distorting endosymbionts in a host induces a situation in which two components of the cell are in conflict over sex-ratio and their inheritance pattern: cytoplasmic microbial genes are maternally inherited and selected to favor a female-biased host sex-ratio whereas nuclear genes are mostly bi-parentally inherited and selected to favor a balanced sex-ratio [9]. In female-biased populations, males have a higher reproductive success than females on average because they are rarer than females. Because they produce few or no offspring of the male sex, females carrying sex-ratio-distorting endosymbionts have a lower fitness than uninfected females. Hence, any gene that prevents the transmission and/or suppresses the action of the endosymbionts can be selected in the host because it promotes the production of males. These genes may not necessarily be viewed as sex-determining genes *per se* (for example, genes preventing endosymbiont transmission). However, resistance genes, together with other components such as formal sex-determining genes and endosymbionts, can broadly be considered as parts of the 'extended sex-determination system' of the species because they all ultimately participate and interact to determine whether the progeny develop into males or females (Figure 2). This view is analogous to the widely accepted concept of the 'extended phenotype' [46], in which a phenotype is not merely the result of a gene expressed as part of a biological process, but is extended to include all the effects of a gene on its environment, inside or outside the body of an individual organism.

Empirical evidence for the occurrence of genetic conflicts in response to reproductive parasites has been accumulating in the past years. Below we discuss in greater detail several cases of resistance genes selected to counter the action of sex-ratio-distorting endosymbionts in arthropods. These examples illustrate how bacterial endosymbionts can subtly affect host sex determination through the genetic conflicts that their presence and selfishness indirectly induce.

Resistance to feminization

A polygenic system of resistance genes (*R* genes) preventing feminization has been identified in the isopod *A. vulgare*, by selecting for lines infected by feminizing *Wolbachia* in which females produced male-biased progenies [47]. Using crossing and *Wolbachia*-inoculation experiments, it was shown that *R* genes prevent feminization by resisting *Wolbachia* transmission to offspring [47]. Because *A. vulgare* individuals inheriting *Wolbachia* develop as females whereas males are uninfected individuals, *R* genes contribute to restore males in the population by reducing the rate of *Wolbachia* transmission. Thus, *R* genes indirectly impact upon sex determination in *A. vulgare* (Figure 2).

In addition to *Wolbachia*, *A. vulgare* hosts another feminizing agent known as the *f* element [48] (Figure 2). The *f* element is thought to be a part of the *Wolbachia* genome that carries feminization information and that has been transferred into the host nuclear genome [48]. The precise nature of the *f* element is unknown but it could be a mobile genetic element [48]; this contention is plausible because mobile genetic elements are particularly frequent and mobile in *Wolbachia* [25,49,50] and lateral transfer of genetic material occurs frequently between *Wolbachia* and their host nuclear genomes [51–53]. Because of its selfish inheritance, the feminizing *f* element is in conflict with other genetic components of the cell. This has resulted in the selection of the dominant autosomal masculinizing *M* gene, which can restore the male sex in the presence of the *f* element, but is inefficient against the feminizing effect of *Wolbachia* [54] (Figure 2).

The *f* element illustrates particularly well how parasitic sex factors have the potential to drive the evolution of host sex-determining mechanisms. In addition to triggering genetic conflicts and repressor selection in some *A. vulgare* lines, the *f* element has acquired a stable Mendelian inheritance pattern in other *A. vulgare* lines, and this has restored a stable, balanced sex ratio [55]. Crossing experiments demonstrated that *f* element stabilization in these lines occurred on a Z male chromosome, thus effectively leading to the creation of a W-like female chromosome [55]. Overall, these observations suggest a circular model of evolution of the 'extended sex-determination system' in the isopod *A. vulgare*, outlining the prime influence of *Wolbachia* endosymbionts and illustrating how genetic conflicts could catalyze evolutionary changes in sex-determination mechanisms (Figure 2) [15–17,55].

Resistance to parthenogenesis

In the haplodiploid wasp *Trichogramma kaykai* from the Mojave Desert (USA), PI *Wolbachia* are found at a low frequency in females (<30%) [56]. This frequency remains stable despite the fact that PI *Wolbachia* are expected to spread in the population. It has been shown that *Wolbachia* infection is maintained at a low frequency owing to the presence of a paternal sex-ratio (PSR) chromosome [56,57]. The PSR chromosome is an exclusively paternally-inherited B chromosome that converts diploid fertilized eggs into haploid eggs by destroying the paternal chromosome set, with the exception of itself. This is achieved by condensation of the paternal chromosome set into a dense chromatin mass during the first mitotic division after egg fertilization [58]. As a result, fertilized eggs do not develop as normal diploid females but instead develop as haploid males carrying the maternal chromosome set and the paternal PSR chromosome. Thus, any chromosome in contact with the PSR chromosome is doomed to extinction. In summary, the extremely selfish PSR chromosome restores males despite the presence of PI *Wolbachia* in the *T. kaykai*

population, keeps PI *Wolbachia* infection at low frequency, and maintains normal sexual reproduction [56,57].

Resistance to male killing

Similarly to feminization of genetic males and parthenogenesis induction, male killing (MK) is an adaptation to maternal transmission used by different bacterial endosymbionts that leads to sex-ratio distortion towards females (Box 2). MK endosymbionts are more costly than their feminizing and PI counterparts. Indeed females harboring MK endosymbionts produce fewer offspring than uninfected females because about half of their offspring are killed. Therefore, it is not surprising that MK suppressors have been identified in several systems [59,60].

The best documented example of MK suppression is that of the butterfly *Hypolimnas bolina* [59]. This species is infected with a *Wolbachia* strain (*w*Bol1) which kills males in Polynesian populations. By contrast, in Southeast Asian populations, both males and females naturally harbor *w*Bol1 and infected females produce progenies with a balanced sex ratio. It has been shown that the MK effect in Southeast Asia is completely suppressed by a single, dominant autosomal host gene, and this allows male survival in presence of MK endosymbionts [59]. This indicates that host suppression can completely silence reproductive manipulations induced by endosymbionts. Thus, some species that do not currently express reproductive manipulations might well have done so in the past. If so, the number of species which biology has been affected by reproductive parasites could be far greater than is currently recognized.

The spread of the suppressor has been monitored in Samoan islands, where the *H. bolina* sex-ratio shifted from 99% of females to parity within barely 10 generations [61]. This makes the spread of MK suppression one of the most rapid evolutionary and ecological changes observed in the wild. The spread of suppressor genes is expected to reduce endosymbiont prevalence [62]. Even so, *w*Bol1 occurs at high frequency where suppressed [59,61]. This is because, in males in which MK is suppressed, *w*Bol1 induces cytoplasmic incompatibility (CI) [63], a widespread reproductive manipulation that favors the spread of endosymbionts (Box 1). CI has not evolved *de novo* after MK suppression in *H. bolina*. Instead, the ability to induce CI was already present in *w*Bol1 and immediately became expressed upon survival of infected males [63]. This example illustrates that some endosymbionts have the ability to induce multiple reproductive manipulations. The molecular genetic basis of these manipulations in *w*Bol1 is currently unknown, but the evolutionary maintenance of CI in a system in which it is conditionally expressed suggests a link with MK or other traits under selection [63]. The ability to induce multiple manipulative phenotypes could represent an adaptation of endosymbionts in a permanent arms race with their hosts. Indeed, genetic conflicts favor the evolution of mechanisms in endosymbionts that enhance their transmission, just as they favor the evolution of host resistance genes to endosymbionts. Thus, being able to induce a second reproductive manipulation when the first has been suppressed by a host gene is advantageous for endosymbionts, which can thus continue to spread in host populations.

Broader evolutionary consequences for sex-determination mechanisms

We now consider the broader evolutionary impact hypothesized for endosymbionts upon various aspects of the evolution of sex-determination mechanisms, as illustrated by the evolution of sex chromosomes and heterogametic systems, evolution of haplodiploidy, and evolution of obligate asexuality.

Evolution of sex chromosomes and heterogametic systems

In isopod crustaceans, genetic sex determination generally follows female (ZZ/ZW) or male (XX/XY) heterogamety [15,16,64]. Interestingly, sex chromosomes usually show no or very slight heteromorphy (morphological differentiation), and both heterogametic systems are sometimes found in closely related species within the same genus or even within the same species [15,16,64]. Furthermore, sex inversion can easily be obtained by simple experimental manipulations, and unusual combinations such as WW females or YY males are viable and fertile, indicating that both sexes have identical or nearly identical genetic programs [15,16]. Altogether, these observations suggest that in many isopods the evolution of sex chromosomes is at an incipient stage of the specialization of a pair of ancestral autosomes carrying sex determinants [8,64,65]. In the case of a masculinizing nuclear gene resulting from genetic conflicts with sex-ratio distorters (such as the *M* gene, which has become the male sex-determining gene in the presence of the *f* element in *A. vulgare* [54]), a system similar to male heterogamety could be selected, and the autosome pair carrying the masculinizing gene would then become the new sex chromosome pair [66]. If this happens in a species with ancestral female heterogamety it would effectively cause a switch between male and female heterogametic systems [66] (Figure 2). In the long term, repeated changes in sex-determining genes caused by feminizing endosymbionts and ensuing genetic conflicts could constantly relocate the position of sex-determining genes on different autosomal pairs and, thus, constantly generate incipient sex chromosomes [15,16,64]. This could explain why sex chromosomes generally show very limited heteromorphy in isopods [15,16,64].

Evolution of haplodiploidy

Haplodiploidy has evolved at least ten times independently in insects, which raises the questions of the origin and adaptive significance of this sex-determination system [67]. It has been suggested that haplodiploidy could have originated from coevolution between MK endosymbionts and their hosts [67]. According to this hypothesis, MK endosymbionts would operate through destruction of the paternal chromosome-set in diploid males, thus killing male embryos by haploidizing them. This would favor the selection of host genes rescuing haploid embryos as viable males. Modeling studies have shown that haploidizing endosymbionts can become beneficial for their female hosts under different conditions [67–70]. In such circumstances, endosymbionts can become fixed in the population, and this effectively results in a haplodiploid system of paternal chromosome elimination.

Evolution of obligate asexuality

Parthenogenesis has evolved multiple times from sexual lineages in both invertebrates and vertebrates, and this raises the question of how transitions to asexuality might occur [71]. Interestingly, PI endosymbionts can invade haplodiploid species without causing population extinction because females can produce progeny without males. This is indeed the case in some wasp populations in which PI *Wolbachia* infection is fixed [38]. Many of these populations have lost the ability to reproduce sexually and now rely on obligate parthenogenesis [72–74]. Concomitantly, *Wolbachia* status has shifted from facultative to obligate partner in these populations. The inability for sexual reproduction is due to loss of sexual function in females [72–74]. This can be explained by an elegant hypothesis known as the 'virginity mutation' hypothesis [38,72,75]. In female-biased populations, producing males is advantageous. Because males are produced from unfertilized eggs in haplodiploids, virginity (or any other mechanism reducing egg fertilization rate) is beneficial for uninfected females. Consequently, genetic conflicts could result in selection of 'virginity' mutations in nuclear genes that disable any trait required for successful sexual reproduction in females [75]. The model also predicts irreversible loss of sexual reproduction and complete reproductive dependence of hosts on endosymbionts [75]. Recent empirical evidence on the wasp *Trichogramma pretiosum* has shown that a single dominant nuclear effect is sufficient to explain loss of female sexual function [74]. Thus, endosymbionts could facilitate transitions from facultative sexuality to obligate asexuality.

Concluding remarks

Paradoxically, because of their selfish nature, reproductive parasites are powerful agents of evolutionary change in their host partners. Their considerable impact on host sex determination initially stems from the ability of these bacteria to directly manipulate a fundamental biological process of eukaryotes. Endosymbiont manipulation of host sex determination generates genetic conflicts which lead to the selection of host resistance genes and could ultimately drive shifts in sex-determination systems. Thus, reproductive parasites represent excellent models for studying transitions between sex-determining systems and, more generally, the evolution of sex-determination mechanisms. However, recent discoveries have produced at least as many questions as answers. An exciting area for future investigations concerns the elucidation of the mechanisms of endosymbiont–host molecular interactions, which will provide fundamental cues on the mechanistic bases of reproductive phenotypes. An essential aspect to this question relates to the molecular genetic bases of the manipulations and associated sex-determining factors, such as resistance genes. Presently, no such gene has been characterized. The latest molecular genetic technologies, such as next-generation DNA sequencing, are likely to provide an unprecedented opportunity to fill this gap in our knowledge.

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