Evolutionary and ecological signals in *Wolbachia*-beetle relationships: A review

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Abstract. Intracellular bacteria of the genus *Wolbachia* are the most abundant endosymbionts infecting many arthropods, with Coleoptera being the most diverse hosts in terms of taxonomy and ecology. There has been great progress in studies on the relations between *Wolbachia* and beetles, however, only some of the research details the consequences of infection. In this review, I summarise the knowledge on the evolutionary relations or ecological associations between *Wolbachia* and its beetle hosts. These bacteria often cause cytoplasmic incompatibility in the infected hosts and are responsible for a selective sweep of the mitochondrial genomes in some beetles. *Wolbachia* can manipulate the sex ratio or reproduction of some species of beetles, however, it does not induce parthenogenesis, with the possible rare exception of some Naupactini. Proof of the co-evolution of *Wolbachia* with beetles is missing, but some aquatic groups seem to be prone to co-speciation, unlike terrestrial taxa. On the other hand, there is a growing number of studies indicating or proving horizontal transmission of *Wolbachia* among beetle hosts, mostly via common host plants or the foraging substrate (such as dung). *Wolbachia* is not alone in infecting beetles as other endosymbiotic bacteria occur in beetles (*Rickettsia, Spiroplasma, Cardinium, Arsenophorus*), which have often been reported as interchangeable, suggesting the infection by various bacteria is dynamic. Nonetheless, there are still many issues associated with *Wolbachia* that are not yet been described in beetles (like the provision of nutrition or protection against pathogens) and high-throughput sequencing should be used to improve our understanding of *Wolbachia*-Coleoptera relations.

INTRODUCTION

Evolution is a complex process that is shaped by numerous biotic and abiotic factors, acting on the genetic and phenotypic constitution, traits characterising plasticity and adaptations of organisms, as well their behaviour and factors that differentiate them from other taxa or enable mixing with congeners in the case of ongoing hybridisation, and determine their geographic distribution and association with specific habitats and food resources (Sexton et al., 2009). An increasing number of studies indicate that interactions between organisms determine diversity or inter-kingdom interactions. The most important in this respect being microorganisms (Zilber-Rosenberg et al., 2008). Bacteria and other microorganisms, like fungi and algae increase the fitness of their host species and in some cases also contribute to speciation (Richardson, 2017), make up a large part of the host's 'body' (holobiont theory; Simon et al., 2019) and have numerous effects on the host's genomic and phenotypic condition (Rosenberg & Rosenberg, 2016). Among these microorganisms are numerous antagonistic taxa classified as pathogens, parasites or parasitoids of the infected species (Guerrero et al., 2013). However, another large group of microorganisms cooperate with their hosts in a more or less direct way, as either occasional, facultative or obligatory symbionts (Parmentier & Michel, 2013). The most pronounced are those microorganisms that provide their hosts with nutrients, which favours these ecological associations (Shapira, 2016). Other relations are, e.g., providing defence against some pathogens (like viruses) (Hamilton & Perlman, 2013). Consequently, if the evolutionary timescale of such interactions is long, the microorganisms and their hosts are likely to show some level of phylogenetic congruence (co-evolution) (Groussin et al., 2020). This is the case for both antagonistic and mutualistic microorganisms. A particularly interesting group are endosymbionts, microorganisms living within the cells of their hosts (White et al., 2013) as their interactions with their hosts are often very complex.

Among the endosymbiotic bacteria are phylogenetically unrelated taxa that have similar effects on the host's reproductive processes (O'Neill et al., 1992; Duron et al., 2008). These bacteria, which are sometimes called 'male-killers', for a long time were considered as pathogens, mostly infect Arthropods (Hurst & Jiggins, 2000). They are intracellular and long believed to be transmitted only vertically, that is matrilineally (Correa et al., 2016). Matrilineal mode

of transmission enabled them to manipulate the host's reproductive function by changing the sex ratio (by either feminising males, killing them or promoting unisexual modes of reproduction like parthenogenesis) (Stouthamer et al., 1999; Engelstädter & Hurst, 2007; Kageyama et al., 2012). They are known to cause cytoplasmic incompatibility (Poinsot et al., 2003) in hosts that are infected and non-infected, or when the hosts are infected by different strains (Hoffman et al., 1997; Werren et al., 2008). These effects were initially recognised as harmful, however, later studies indicate they can be also beneficial, which led to these bacteria being classified as symbionts, rather than pathogens or parasites (Weeks et al., 2007). An increasing body of exemplary studies indicate that these bacteria play a crucial role in the evolution of many Arthropods (Charlat et al., 2003), however, patterns of co-speciation are rarely reported and mostly in closely-related hosts. The weak cophylogenetic relations are attributed the horizontal transmission of these bacteria (Vavre et al., 1999; Gonella et al., 2015). It is known that direct interactions, like predation and parasitism, or a common environment, e.g., host plants and food sources, are likely to promote the transmission of these bacteria between phylogenetically unrelated hosts (Caspi-Fluger et al., 2012; Chrostek et al., 2017). There are several bacterial taxa classified as 'male-killers': *Rickettsia* (Rickettsiaceae), *Spiroplasma* (Tenericutes), *Cardinium* (Bacteroidetes), *Arsenophorus* (Enterobacteriaceae) and *Wolbachia* (Rickettsiaceae) (Duron et al., 2008). Most of these taxa are poorly studied and only infest hosts occasionally, or are known from only a small percentage of the examined host taxa (e.g., *Rickettsia* and *Spiroplasma)* (Bové, 1997; Perlman et al., 2006). Currently *Wolbachia* are the most studied and widespread intracellular bacteria (Werren & Windsor, 2000) and are present in from 20–70% of the host species (mostly insects) screened (Hilgenboecker et al., 2008; Zug & Hammerstein, 2012). Its prevalence differs greatly in different taxonomic, geographic and ecological groups of insects, but is common everywhere. Preliminary studies aimed at determining the prevalence of *Wolbachia* in insects probably strongly underestimated it since usually only one individual per species was examined. Later more detailed studies indicate that it is present in only a fraction of individuals (Hancock et al., 2011; Huang et al., 2015). In addition, there are several phylogenetically distant supergroups of these bacteria, some of which are regarded as separate species (Ramirez-Puebla et al., 2015). This taxonomic distinction, however, is questioned due to the recombination between strains belonging to various supergroups (Lindsey et al., 2016). Finally, both demographic and theoretical studies indicate that *Wolbachia* changes its distribution and abundance in hosts, which is increased or reduced many times at both evolutionary and demographic scales (Adekunle et al., 2019; Sanaie et al., 2020). The dynamics of *Wolbachia* (and possibly other bacteria with similar effects on hosts), make studies on the relations with hosts challenging, but such studies are interesting and important for a proper understanding of the evolutionary relations and ecological associations.

Beetles (Coleoptera) are one of the most diverse group of Arthropods with approximately 400,000 species living in almost all parts of the world, in various habitats and at all trophic levels (Ślipiński et al., 2011). There are numerous evolutionary and ecological issues related to beetles that need to be examined, many of which are likely to be related to their interactions with microorganisms. This highly diversified group (both in terms of taxa richness and ecology) makes them especially interesting in terms of studies on their relations with 'male-killers'. A recent review reports that *Wolbachia* is present in approximately 40% of beetle taxa (Kajtoch & Kotásková, 2018) and large-scale screening indicates a low infection rate (below 30%) (Kajtoch et al., 2019). Currently, three supergroups of *Wolbachia* are associated with beetles of which the most widespread is supergroup A, slightly less frequent but still abundant is supergroup B and rarely they are infected by supergroup F (Kajtoch & Kotásková, 2018).

All the information on beetles involving *Wolbachia* (up to 2017) is summarized by Kajtoch & Kotásková (2018). The overall progress, methodologies and aims are summarised in this review and the diversity of *Wolbachia* infecting Coleoptera presented. The increasing number of studies on microorganisms (incl. *Wolbachia*) and improvements in screening for bacteria and determining diversity, the analytical tools available for detailed studies of evolutionary and ecological relations (Wang et al., 2020) now make it possible to summarize the current state of knowledge of specific issues, which is crucial for understanding the relations between beetles and *Wolbachia*. Therefore, in this article I aim to summarise and present crucial aspects of the evolutionary relationships and ecological associations of *Wolbachia* and its beetle hosts. This knowledge is still fragmentary. For example, there are no studies on the effects of *Wolbachia* on beetle hosts in terms of increasing their fecundity (Browlie et al., 2009), conferring protection against pathogens (Hedges et al., 2008; Braquart-Varnier et al., 2015), supplementing nutrition (Hosokava et al., 2020) or mediating host plant specialisation (Jiggins et al., 2022). There is a great number of studies on the use of *Wolbachia* as a biological control agent of pathogens or pests, but as these relations are artificial, they are not considered in this review. Therefore, in this review I focus on the following subjects that are well documented with many examples in the literature: (i) effect of *Wolbachia* on the reproduction of beetles (with an emphasis on parthenogenesis); (ii) cospeciation of Coleoptera and *Wolbachia*; (iii) horizontal transmission pathways of *Wolbachia* among Coleoptera (and other hosts) and (iv) co-existence of *Wolbachia* with other bacteria (particularly other 'male-killers') infecting common beetles.

LITERATURE SEARCH

I used all the literature on *Wolbachia* in Coleoptera cited in the previous systematic review (Kajtoch & Kotásková, 2018). In addition, I used the same search strategy for the

years 2018–2021 (to 31.12.2021) and searched the scientific literature in the Web of Knowledge database, using the following combination of keywords linked by AND, OR (the Boolean search term to stipulate that the record should contain this AND/OR the next term): '(beetle OR Coleoptera) AND Wolbachia'. This was repeated for all the years until 31.12.2021 using '(beetle OR Coleoptera) AND Wolbachia AND microbiome', in order to include studies on the whole bacterial community in beetle taxa. The first group of articles included 322 papers, the second an additional 79 papers and third 161. After removing irrelevant papers, the numbers were: 86, 45 and 12. Each result was inspected to determine whether or not it contained information on the subject. The articles that had no relevance (e.g. any reports that were not about *Wolbachia*-Coleoptera evolutionary / phylogenetic or ecological relations) were excluded. The same was done for unoriginal reports (e.g. review papers). After the removal of duplicates, this resulted in 46 + 14 + 8 articles (68 in total), which are considered in this review (Table S1). I am aware that this search strategy possibly omitted some studies that could be relevant for the topic, as it is possible that the Web of Sciences search could omit some articles. However, this is not a systematic review, and as there are so many studies, it was not possible to include all of them in a description of the currently known evolutionary and ecological relations between *Wolbachia* and Coleoptera.

TOPICS

1. Effect of *Wolbachia* on Coleoptera reproduction

1.1. Cytoplasmic incompatibility and selective sweep.

Information on the effect on the reproduction of infected beetles was found in many articles (Table S1). However, the majority describe an uncertain (or presumed) cytoplasmic incompatibility, linkage disequilibrium and/or cases of selective sweep. Cytoplasmic incompatibility (CI) (Poinsot et al., 2003) occurs when infected males mate with uninfected females (unidirectional CI) or when their mates harbour different *Wolbachia* (or other 'male-killing' bacteria) strains (bidirectional CI). CI is caused by a mismatch in gametes, which enables or reduces the formation of viable offspring (usually death of the progeny occurs during early embryogenesis) (Werren et al., 2008). In some cases, viable and fertile progeny are produced by infected females when they mate with infected or uninfected males (Hoffmann et al., 1997). Therefore, a reduction in fecundity is observed only for infected females (Kageyama et al., 2012). Consequently, the prevalence of *Wolbachia* can increase within the host population leading to an equilibrium. Among the other consequences of CI could be selective sweep (SS) (Santiago & Caballero, 2005). *Wolbachia* and other similar bacteria could be the agents of SS. The effects of SS are mostly visible in mitochondrial genomes, which are inherited matrilineally along with *Wolbachia* (Jiggins, 2003). A strong signature of selective sweep is often an excess of linkage disequilibrium (Kim et al., 2004).

In the case of beetles, there are reports of CI in many species of Chrysomelidae (*Altica lythri*, Jäckel et al., 2013; *Brontispa longissimi*, Takano et al., 2017; *Callosobruchus analis*, Numajiri et al., 2017; *Callosobruchus chinensis*, Kondo et al., 2002; *Chelymorpha alternans*, Keller et al., 2004; *Diabrotica barberi*, Roehrdanz & Levine, 2007; *Diabrotica virgifera virgifera*, Giordano et al., 1997) and Curculionidae (*Pantomorus postfasciatus*, Elias-Costa et al., 2019; Rodriguero et al., 2021). For some other species, CI is only presumed (e.g. Curculionidae: *Aramigus conirostris*, Rodriguer et al., 2010a; *Eusomus ovulum*, Mazur et al., 2016; *Euwallacea interjectus* and *E. validus*, Kawasaki et al., 2016; *Xyleborinus* spp., Kawasaki et al., 2016; Micromalthidae: *Micromalthus debilis*, Perotti et al., 2016). There are also detailed reports that reject CI in Chrysomelidae (*Calligrapha* spp., Gómez-Zurita, 2019; *Altica cirsicola, A. fragariae* and *A. viridicyanea*; Xue et al., 2011) and Curculionidae (*Strophosoma* spp., Kotásková et al., 2018). SS is reported in only a few species like *Altica lythri* (Jäckel et al., 2013), *Aphthona nigriscutis* (Roehrdanz et al., 2006), *Eusomus ovulum* (Mazur et al., 2016), *Naupactus cervinus* (Rodriguero et al., 2010b), *Polydrusus inustus* and *P. pilifer* (Kajtoch et al., 2012) and *Hypera postica* (Ruda et al., 2021). But as in the previous case, there is also research which rejects SS in Chrysomelidae (*Calligrapha* spp., Gómez-Zurita, 2019) and Coccinelidae (*Adalia bipunctata*, Shaikevich et al., 2021). Only in some studies is CI or the associated SS linked with an actual distortion in the reproduction of beetles infected with *Wolbachia* (*Altica lythri*, Jäckel et al., 2013).

In summary, there is a growing amount of evidence that *Wolbachia* could be responsible for numerous changes in the genetic composition of its beetle hosts. Infection by *Wolbachia*, particularly various strains of this bacterium, causes incompatibility of gametes, which affects the reproductive process (see below), but the associated selective sweep of mitochondrial genomes by several strains of *Wolbachia* could also lead to speciation (see below). Moreover, *Wolbachia* infection in some populations could lead to erroneous identification or species delimitation using DNA barcoding (Smith et al., 2012). A selective sweep could lead to a reduction in the diversity of mtDNA resulting in the loss or infrequent occurrence of some mitochondrial lineages in populations. The opposite effect is also possible. Therefore, the fixation of different mitochondrial variants in populations infected by different (incompatible) strains could artificially increase the number of species delimited when using only mtDNA (Hurst & Jiggins, 2005). There are also reports that an amplification of the host cox-1 barcode could be accompanied by an unintentional amplification of the *Wolbachia* coxA gene. This could have consequences for evolutionary studies, however, proper management of data can resolve this problem as there are large differences in the cytochrome oxidase sequences (Smith et al., 2012). It is also known that *Wolbachia* infection could mimic speciation (there are examples of this in insects such as butterflies: Ritter et al., 2013, but currently not in beetles).

1.2. Sex ratio distortion and parthenogenesis

A basic question of some of the studies investigating *Wolbachia*-beetle relations is does this bacterium cause parthenogenesis. Parthenogenetic taxa are reported mostly in weevils (particularly the subfamily Entiminae) (Takenouchi et al., 1986), where numerous Old-World genera (like *Otiorhynchus, Polydrusus, Euromus, Strophosoma, Brachyderes, Brachysomus, Parafoucartia*) (Saura et al., 1993) and New-World genera (*Naupactus, Aramigus, Pantomorus*) (Lanteri et al., 1995) include parthenogenetic species. Some Scolytinae are also parthenogenetic (e.g. *Xyleborinus, Euwallacea*) (Jordal, 1998). However, parthenogenetic species are very rare in other beetle families and are only reported in some Chrysomelidae (e.g. *Calligrapha*; Robertson, 1996) and Micromalthidae (e.g. *Micromalthus*; Perotti et al., 2016). Studies on *Wolbachia* in beetles have generally rejected the idea that these bacteria can induce parthenogenesis. This idea is rejected for both Old-World (e.g. *Polydrosus*, Kajtoch et al., 2012; *Strophosoma*, Kotásková et al., 2018) and New-World leaf beetles (*Calligrapha* spp., Gómez-Zurita, 2019). For these species, it is reported that *Wolbachia* is not responsible for inducing parthenogenesis, although it benefits from reproduction within parthenogenetic lineages and possibly reinforces unisexual reproduction. In these beetles, parthenogenesis evolved via hybridisation between congeneric species or distinct evolutionary units, which resulted in polyploidy (in the case of weevils). In some cases, it is speculated that parthenogenesis induction has occurred, as in the weevil *Eusomus ovulum*, in which there are only parthenogenetic populations (Mazur et al., 2016). *Wolbachia* inducing parthenogenesis is also postulated in the case of parthenogenetic bark beetles (*Xyleborinus, Euwallacea*; Kawasaki et al., 2016). Probably, the only examples of parthenogenetic beetles originating via *Wolbachia* induction are the New-World Entiminae. For *Aramigus conirostris* (Rodriguer et al., 2010a) and *Naupactus cervinus* (Rodriguero et al., 2010b), this mechanism is proposed, but a hybrid origin and further infection and reinforcement of unisexual reproduction cannot be ruled out. Detailed examination of the genetic variability (Elias-Costa et al., 2019) of *Pantomorus postfasciatus* and experimental studies (Rodriguero et al., 2021), support the *Wolbachia* induction of parthenogenesis, but these studies are again not conclusive. An interesting case of parthenogenetic paedogenesis is reported for *Micromalthus debilis* (Perotti et al., 2016), which is infected by *Wolbachia* (and *Rickettsia*). These bacteria (or one of them) are most probably responsible for a sex ratio bias (deficiency of males in artificially induced imago), that must predate the loss of adults and the induction of unisexual reproduction of the larvae.

There are other confirmed or presumed effects of *Wolbachia* on the reproduction of beetles. A sex ratio distortion is reported in *Altica lythri* (Jäckel et al., 2013) and males are killed by *Wolbachia* in *Altica* (Wei et al., 2021), *Adalia bipunctata* (Majerus et al., 2000, Shaikevich et al., 2021) and *Tribolium madens* (Fialho & Stevens, 2000) where the females are also more frequently infected (as in *Polygra-*

phus proximus, Bykov et al., 2020; and *Paederus fuscipes*, Maleki-Ravasan et al., 2019). In some species of beetles, sex determination is affected by *Wolbachia* infection (e.g. *Hypothenemus hampei*, Vega et al., 2002). *Wolbachia* can modify the sperm in *Chelymorpha alternans* (Clark et al., 2008) or affect oogenesis in *Coccotrypes dactyliperda* (Zchori-Fein et al., 2006), *Otiorhynchus sulcatus* (Son et al., 2008), and *Lissorhoptrus oryzophilus* (Chen et al., 2012). In addition, Roehrdanz et al. (2006) show that *Wolbachia* reduces the genetic diversity of infected *Aphthona nigriscutis*. Numajiri et al. (2017) report a decline in fitness of infected *Callosobruchus analis*, whereas Heddi et al. (1999) report the production of more viable offspring, indicating a better adaptation to the environment of infected *Sitophilus*. In some cases, these modifications of reproduction are known to have demographic effects, such as, a bottleneck in *Naupactus cervinus* (Rodriguero et al., 2010b).

In summary, among the possible effects that *Wolbachia* could have on the beetle host, the most common are a distortion of reproduction or changes in the sex ratio of infected populations. On the other hand, there is almost no proof for the induction of parthenogenesis in beetles, with the possible exception of some American Naupactini, but even for them the data is not conclusive and needs further study (Elias-Costa et al., 2019; Rodriguero et al., 2021). It is more probable that in all unisexual beetle taxa (or forms), *Wolbachia* benefits just from being within clonally reproducing hosts, which increase its spread to further generations of hosts. In some parthenogenetic beetles, *Wolbachia* not being an inducer could even reinforce the mechanisms of unisexuality (Kotásková et al., 2018; Gómez-Zurita, 2019). Therefore, the effects of *Wolbachia* on the sex ratio or reproduction in its beetle hosts could have consequences not only on the contemporary diversity and demography of infected populations, but could also contribute to the evolution of both the strains of bacteria and the beetles harbouring endosymbionts.

2. Co-speciation of *Wolbachia* and beetles

Surprisingly, there are few articles that refer directly to the co-diversification or co-speciation of beetle hosts with *Wolbachia*. Unfortunately, in most of these studies, co-speciation is only briefly examined and just report the phylogenetic patterns that indicate such a relation and only two statistically tested co-speciation with specific *Wolbachia* strains (Sontowski et al., 2015; Kajtoch et al., 2019). The majority of the reports simply reject co-speciation of beetles with *Wolbachia*, as in the case of *Altica* (Jäckel et al., 2013; Wei et al., 2021), *Euwallacea, Xyleborus* and *Xylosandrus* (Kawasaki et al., 2016), *Nanos* (Miraldo & Duplouy, 2019), *Cyanapion* (Kajtoch et al., 2017, 2019), *Strophosoma* (Kotásková et al., 2018), *Rhinusa* (Toševski et al., 2015), *Crioceris* (Kajtoch et al., 2019), *Aphodius* (Kajtoch et al., 2019) and *Paederus/Paederidus* (Kajtoch et al., 2019). Nonetheless, after detailed studies of some groups of beetles, co-speciation with *Wolbachia* remains controversial (e.g. *Polydrusus*, Kajtoch et al., 2012; *Oreina*, Montagna et al., 2014; *Altica*, Xue et al., 2011; *Monochamus*, Plewa et al., 2018), as the recorded patterns could

not be easily assigned to either co-speciation or horizontal transmission among congeners (see below). Co-speciation is reported only in the cases of Hydraenidae (Sontowski et al., 2015), Gerridae (Castillo et al., 2020) and *Bembidion* (Kajtoch et al., 2019). It is interesting that these three groups are either freshwater beetles (Hydraenidae, Gerridae) or inhabit riverine channels (*Bembidion*). Sontowski et al. (2015) argue that it is probable that *Wolbachia* infecting freshwater hosts could be prone to co-speciate, contrary to the strains found in terrestrial beetles. The reason for this could be the habitat, as the spread of *Wolbachia* in water could be limited to vertical pathways (matrilineally) and horizontal transmission is less possible due missing vectors. There are very few herbivorous species of beetles, so host plants are not likely to mediate transmission and there are also no hymenopteran parasites/parasitoids that are known as important vectors in terrestrial communities.

There is little evidence of co-speciation of beetle hosts and *Wolbachia*. Large-scale analyses (Kajtoch et al., 2019) reject co-speciation in terrestrial beetles, but signs of a parallel evolution in aquatic species (Sontowski et al., 2015; Castillo et al., 2020), however, these could still be single-group cases. Apparently, *Wolbachia* has not been present in beetles for a long enough time (on an evolutionary scale) for the development of close associations that would enable co-diversification along with the evolution of their hosts (Correa & Ballard, 2016). Indeed, recent studies indicate that *Wolbachia* infections are unstable and frequently change within infected hosts in which some strains are lost and others gained, and as a consequence the infection is in epidemiological equilibrium (Bailly-Bechet et al., 2017). The most probable reason for the lack of co-speciation is frequent horizontal transmission, which is rarely considered in beetle-*Wolbachia* studies.

3. Horizontal transmission of *Wolbachia* in beetles

For a long time, the transmission of intracellular endosymbiotic bacteria (incl. *Wolbachia*) was only from ancestral to descendent hosts via gametes (almost exclusively eggs) (Jaenike, 2009). This point of view was questioned as a consequence of finding the same strains of bacteria in various, unrelated hosts (Gonella et al., 2015). Such patterns of distribution among strains of *Wolbachia* had to be caused by other means of transmission; i.e. horizontal transmission (Chrostek et al., 201). It was first speculated and then proven in some cases that *Wolbachia* could survive (most probably only for a limited time) outside arthropod hosts. The currently known vectors for horizontal transmission of *Wolbachia* are: plants, parasitoids and direct contact (Caspi-Fluger et al., 2012; Chrostek et al., 2017). However, in the case of beetles, the information is rather superficial and often speculative. Most horizontal transmission is assumed and rarely tested. Such presumed horizontal transmission is reported for herbivorous leaf beetles of the genera *Altica* (Jäckel et al., 2013; Xue et al., 2011), *Diabrotica* (Clark et al., 2001) and Naupactini weevils (Rodriguer et al., 2010a). In addition, *Asparagus* host plants are the vectors for *Wolbachia* between two steppe European leaf beetles *Crioceris quaterdecimpunc-*

tata and *C. quinquepunctata* (Kolasa et al., 2017). Steppe plants are probably also the vectors of *Wolbachia* in several weevil species (*Polydrosus, Eusomus, Parafoucartia* and *Strophosoma*; Lachowska-Cierlik et al., 2010; Kotásková et al., 2018). This is confirmed for the tree *Alnus glutinosa*, which is the host plant of several leaf beetles (*Calligrapha, Chrysomela, Altica*) in North America (Cardoso & Gómez-Zurita, 2020). Horizontal transmission is also likely in wood-boring Xyleborini (Kawasaki et al., 2016) and *Ips* (Chakraborty et al., 2020). Ants are proposed as *Wolbachia* vectors for myrmecophilous beetles (*Dendrophilus pygmaeus, Leptacinus formicetorum, Monotoma angusticollis, Myrmechixenus subterraneus, Ptenidium formicetorum* and *Thiasophila angulata*) (Kaczmarczyk et al., 2020). It is also interesting that a common food substrate like dung can be a pathway for the transmission of *Wolbachia* in scarab beetles *Onthophagus* (possible, Parker et al., 2020), *Aphodius* (possible, Kajtoch et al., 2019) and *Nanos* (confirmed, Miraldo & Duplouy, 2019). A similar pattern is reported for riverine predatory beetles (Carabidae, Staphylinidae) that feed on common invertebrates (nematodes, invertebrate larvae, etc.) (Kolasa et al., 2018a).

The increasing evidence for horizontal transmission of *Wolbachia* support the concept that these bacteria are common in beetles, however, they are not abundant as only a fraction of beetle populations harbour these bacteria (Kajtoch & Kotásková, 2018; Kajtoch et al., 2019). That is why studies on *Wolbachia* in beetles should not be restricted to screening a few individuals per species, but include many from various sites. It also seems that the most important horizontal transmission route is via host plants, which could also be vectors for *Wolbachia* (Kolasa et al., 2017; Cardoso & Gómez-Zurita, 2020). However, this could be simply the visible picture due to the limitations of the available studies, which mostly do not include beetles in other trophic groups, except for sapro-coprophages (Miraldo & Duplouy, 2019; Parker et al., 2020) for which dung is the most probable transfer route. It is probable that further studies on groups at other trophic levels will reveal that predatory species share *Wolbachia* with their prey, parasitoids gain *Wolbachia* from parasitized species and beetles sharing the same habitat are infected by common strains. Therefore, it is important not only to screen for the presence of Wolbachia in other species and more individuals, but also to analyse the presence of this bacterium and the possibility of horizontal transfer between beetles (e.g. via host plants, dung, prey or the environment in general). Moreover, it is known that *Wolbachia* "do not walk alone" (Duron et al., 2008) and that other bacteria are known to co-infect and spread together, including other endosymbionts that affect the host's reproductive processes.

4. Co-infection of beetles with *Wolbachia* and other male-killing bacteria

The relations among beetles and *Wolbachia* should be considered along with that of other co-infecting bacteria, particularly other 'male-killing' bacteria. A relatively large number of species of beetles infected with *Rickettsia* and *Spiroplasma*, and *Cardinium* and *Arsenophonus* are only

reported in one beetle taxon (Kolasa et al., 2018b, 2019; Castillo et al., 2020). The study of the effect on beetles of co-infection with *Wolbachia*, *Rickettsia* and/or *Spiroplasma*, as well as *Cardinium* could be important for understanding the effects on the host's reproductive processes, demography or speciation, as an infection by all of these bacteria could have similar consequences. So far, co-infection by *Wolbachia* and *Rickettsia* is reported for *Micromalthus debilis* (Perotti et al., 2016), *Sitona obsoletus* (White et al., 2015), *Ips* (Chakraborty et al., 2020), *Oulema melanopus* (Wielkopolan et al., 2021), *Calligrapha, Chrysomela, Altica* (Cardoso & Gómez-Zurita, 2020), *Bembidion punctulatum, Argoptohus quadrisignatus* and *Eusomus ovulum* (Kolasa et al., 2019), *Calvia quattuordecimguttata, Coccidula rufa, Coccinella septempunctata, Halyzia sedecimguttata* and *Rhizobius litura* (Weinert et al., 2007), *Luperus longicornis*, and *Smaragdina affinis* (Brunetti et al., 2022), *Aphthona venustula, Luperus luperus, Mecinus pascuorum, Phyllobius glaucus, Pseudoprotapion ergenense, Rhinusa tetra* and *Sitona suturalis* (Kolasa et al., 2018b). *Wolbachia* and *Spiroplasma* can co-occur in the following beetles: *Harmonia axyridis* (Li et al., 2020), *Paederus riparius* and *Crioceris quatuordecimpunctata* (Kolasa et al., 2019), and *Chilocorus bipustulatus* (Weinert et al., 2007). Whereas, *Wolbachia* and *Spiroplasma* only co-occur in *Cassida viridis* and *Sibinia pellucens* (Kolasa et al., 2018b). There are also several examples of taxa being infected by three bacteria (*Wolbachia*, *Rickettsia* and *Spiroplasma*): *Paederus limnophilus, Polydrosus inustus* (Kolasa et al., 2019), *Adalia bipunctata* (Majerus et al., 2000), *Harmonia axyridis* (Dudek et al., 2017), *Curculio sikkimensis* (Toju & Fukatsu, 2011), *Aleochara bilineata*

and *A. bipustulata* (Bili et al., 2016). Cases of a triple infection with *Wolbachia*, *Spiroplasma* and *Cardinium* are exceptional (Kolasa et al., 2018b) and there are no reports of a triple infection with *Wolbachia*, *Rickettsia* and *Cardinium*.

Interestingly, some studies report 'male-killing' bacteria other than *Wolbachia* in beetle hosts, e.g., only *Rickettsia* infecting the following leaf beetles: *Hispa atra, Clytra quadripunctata, Labidostomis longimana Smaragdina affinis, Chrysolina fastuosa* and *Luperus longicorni* and *Wolbachia* in: *Chaetocnema hortensis, Dicladispa testacea, Donacia obscura, Exosoma thoracicum, Pachybrachis exclusus, Plateumaris consimilis, Prasocuris phellandrii* and *Zeugophora flavicollis* (Brunetti et al., 2022). Only *Rickettsia* infections are reported for *Anthaxia nitidula, Bembidion articulatum, Dasytes plumbeus, Derocrepis rufipes, Dinoptera collaris, Dolichosoma lineare, Nivellia sanguinosa, Obrium brunneum, Otiorhynchus perdix, O. riessi, Pidonia lurida, Pyrrhidium sanguineum* and *Rhyzobius chrysomeloides* (Kolasa et al., 2018b). There are also some species infected with *Spiroplasma* but not *Wolbachia*, e.g., *Crioceris paracenthesis* (Brunetti et al., 2022) *Cantharis rustica, Chrysolina polita, Ch. varians* and *Paederus caligatus* (Kolasa et al., 2018b). *Phyllobius brevis* and *Pseudomechoris aethiops* are the only beetles infected with only *Cardinium* (Kolasa et al., 2018b). In the water strider *Rheumatobates bergrothi, Rickettsia, Spiroplasma* (and *Cardinium*) occur, but not *Wolbachia*, whereas in other water striders (*Rheumatobates ornatus, Potamobates tridentatus* and *P. assimetricus*) it is the reverse (Castillo et al., 2020). On the other hand, among 24 European species of beetles of various families, there is no
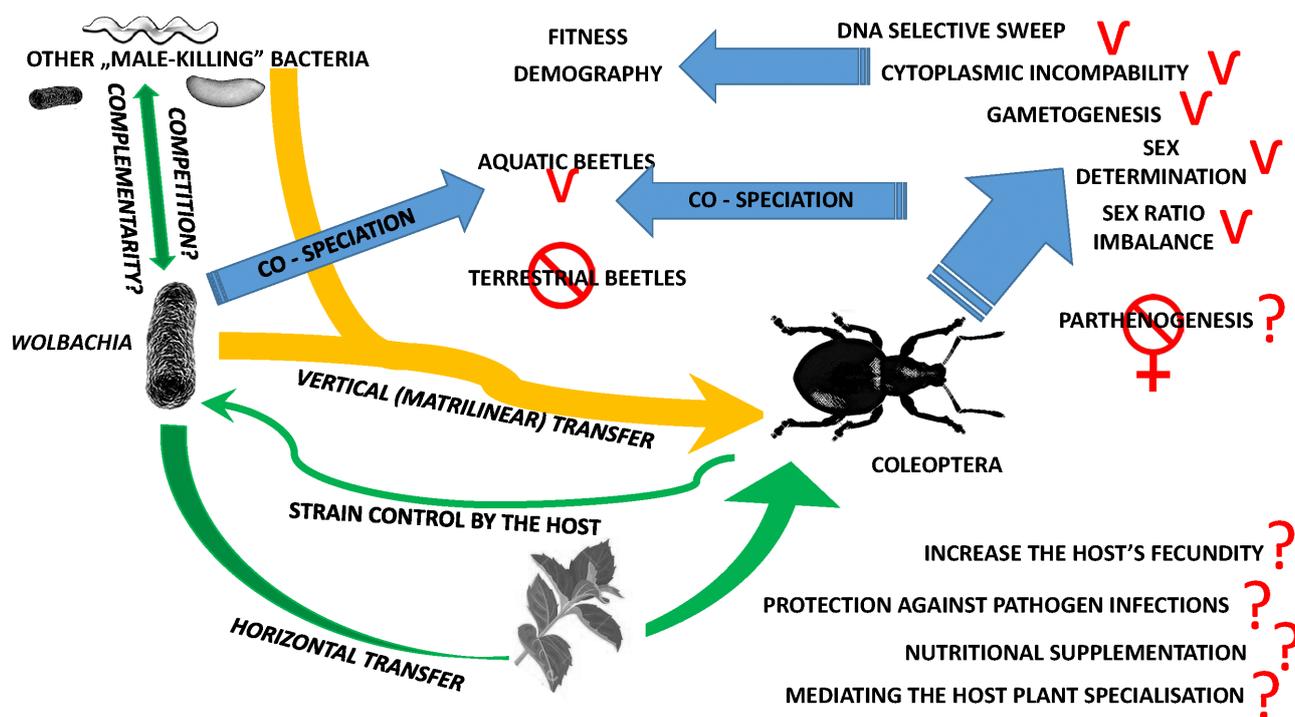

Fig. 1. Diagram of evolutionary relations and ecological associations that are known for *Wolbachia* and its beetle hosts. V indicate confirmed effects of bacteria on beetle hosts, whereas missing (or not-examined) effects are indicated by question marks.

correlation between the abundance of *Wolbachia* and *Rickettsia* (Kolasa et al., 2019). However, most of the species of beetles infected with endosymbiotic bacteria other than *Wolbachia* are based on very few samples, usually one or a few individuals, therefore, it is possible that co-infection was simply overlooked.

The studies on multiple infections by various 'male-killing' bacteria in beetles and insects in general are still preliminary. The data only indicate that *Wolbachia* could be present, or could be more abundant in hosts not infected by other 'male-killers', in particular *Rickettsia* and *Spiroplasma*, as only these bacteria seem to be relatively frequently present in beetles (Kolasa et al., 2018b). This might indicate competition between these bacteria if they are present in the same host. There are many examples of species of beetles infected by several species of bacteria, but the majority are based on just the detection (presence/absence) of bacteria and not on their relative or absolute abundance in these hosts. The differences in the presence of different 'male-killers' may not be due to competition, but selective sweep in hosts infected by one species of bacteria, which prevents the infection with another taxa having a similar effect on the host. Complementarity of these bacteria should also be considered, however, there are no studies examining such relations in beetles. The co-distribution (or avoidance) of endosymbiotic bacteria is just the 'tip of the iceberg' of the possible combinations of bacterial relations and their diversity in beetles, as studies on the complete microbiome are still limited and unsatisfactory.

CONCLUSIONS

Currently, only some of the various evolutionary or ecological interactions between hosts and *Wolbachia* are described for beetles (Fig. 1). Some of the undescribed relationships are likely to have been overlooked due to the lack of appropriate studies or absent, for example, *Wolbachia* infecting Coleoptera could not result in some of the effects recorded in other hosts, due to the constraints of the genotypic or phenotypic traits of beetles. *Wolbachia* is known to induce cytoplasmic incompatibility in many species of beetles, which have consequences for the genomic composition of the infected hosts and in some taxa result in disturbances in their reproduction including an unequal sex ratio. *Wolbachia* is not known to induce parthenogenesis in beetles, with the possible exception of some Naupactini, whereas it could reinforce unisexual reproduction. These effects on the genetic variability and reproduction of beetle hosts have rarely resulted in speciation of both bacteria and their hosts (although this is possibly more pronounced in aquatic than terrestrial species). On the other hand, there is a growing amount of evidence for horizontal transmission, suggesting that this is major force shaping the occurrence and diversity of *Wolbachia* in beetles. *Wolbachia* is not alone in inhabiting beetles, as other 'male-killing' bacteria are also known to infect these insects and they are often interchangeable.

There is a need for further studies on *Wolbachia* in Coleoptera and other insect hosts. In the age of next-generation sequencing, these high-throughput based methods for bacteria screening are also becoming standard in *Wolbachia* studies, but have mainly focused on meta-barcoding of microbiomes using only 16S rDNA. *Wolbachia* is highly diverse and such genomic studies could result in a better understanding of the diversity of strains and its prevalence in beetle populations. Regarding the topics that still need to be addressed, there are many open questions about the mechanisms causing cytoplasmic incompatibility, selective sweeps of bacteria and mitogenomes, sex ratio distortions and the induction or reinforcement of parthenogenesis. It is very likely that *Wolbachia* and/or other similar bacteria have an important role in the evolution of beetles, however, it is likely that co-speciation of these bacteria and beetles mainly occurred over short periods, so such signs should be looked for in groups of young and closely related species. An interesting question is whether aquatic beetles are indeed more prone to co-evolve with *Wolbachia* than terrestrial ones. Another large and open topic is whether horizontal transfer of *Wolbachia* has occurred between beetles and other invertebrates. This could be studied in both natural populations and by tracking strains across the environment, as well as using some experimental approaches. Finally, the studies on 'male-killers' in beetles should be extended to include other bacteria having similar effects on the reproduction of their hosts. Some of the patterns are unlikely to be caused by a *Wolbachia* infection and the effect of *Rickettsia*, *Spiroplasma*, *Cardinium* and *Arsenophonus* should be verified along with that of *Wolbachia*.

Beetles, due to their extremely high diversity, both in terms of their taxonomy/phylogeny and ecology/trophy, are excellent subjects for further studies on the evolutionary and ecological associations with *Wolbachia*, the most widespread intracellular endosymbiont in the world.

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Supplementary Table S1 follows on next page.

Table S1. Articles describing ecological associations or evolutionary relations between *Wolbachia* and its beetle hosts. V - confirmed; ? - uncertain; 1 – *Bembidion*; 2 – Hydraenidae; 3 – via *Alnus*; 4 – via *Asparagus*; 6 – via common habitat / preys?; 7 - via common steppe plants; 8 - via dung. For full references see the main article.

Citation	Examined- infected species	Taxonomy	Parthenogenesis	Cytoplasmic incompatibility	Selective sweep / linkage disequilibrium	Other effects	Co-speciation	Horizontal transmission	Coexistence with *Rickettsia*	Coexistence with *Spiroplasma*	Coexistence with *Cardinium*
Arthofer et al., 2009	*Pityogenes chalcographus*	Curculionidae						V			
Bili et al., 2016	*Aleochara bilineata* and *A. bipustulata*	Staphylinidae							V	V	
Brunetti et al., 2021	30 beetle species (Donacinae, Cassidinae)	Chrysomelidae							V	V	
Bykov et al., 2020	*Polygraphus proximus*	Curculionidae				females more infected than males					
Cardoso & Gómez-Zurita, 2020	6 beetle species	Chrysomelidae					V (3)	V			
Carvalho et al., 2014	*Sitophilus oryzae* and *S. zeamais*	Curculionidae						V			
Castillo et al., 2020	6 beetle species	Gerridae					V		V	V	
Chakraborty et al., 2020	*Ips*	Curculionidae						V			
Chen et al., 2012	*Lissorhoptrus oryzophilus*	Curculionidae				necessary for oocyte production					
Clark et al., 2008	*Chelymorpha alternans*	Chrysomelidae				modification of sperm					
Clark et al., 2001	*Diabrotica virgifera virgifera*	Chrysomelidae		?							
Clark et al., 2001	*Diabrotica* (3 species)	Chrysomelidae					?	?			
Dudek et al., 2017	*Harmonia axyridis*	Coccinellidae							V	V	
Elias-Costa et al., 2019	*Pantomorus postfasciatus*	Curculionidae	V								
Fialho & Stevens, 2000	*Tribolium madens*	Tenebrionidae				male-killing					
Fialho & Stevens, 1996	*Tribolium confusum*	Tenebrionidae		V							
García-Vázquez & Ribera, 2016	*Deronectes angelinii* (6 species)	Dytiscidae						no			
Giordano et al., 1997	*Diabrotica virgifera virgifera*	Chrysomelidae		V							
Gómez-Zurita, 2019	*Calligrapha*	Chrysomelidae	no		no						
Heddi et al., 1999	*Sitophilus* (3 species)	Curculionidae				production of more viable offspring					
Jäckel et al., 2013	*Altica lythri*	Chrysomelidae		V	V	sex ratio distortion		no	?		
Jensen, 2011	*Popillia japonica*	Scarabaeidae		?							
Kaczmarczyk et al., 2020	6 beetle species	Staphylinidae, Histeridae, Monotomidae, Ptiliidae, Tenebrionidae						?			
Kajtoch et al., 2012	*Polydrusus inustus* and *P. pilifer*	Curculionidae	no	V			?				
Kajtoch et al., 2017	*Cyanapion* (6 species)	Curculionidae					no				
Kajtoch et al., 2019	297 beetle species						V (1)				
Kawasaki et al., 2016	*Euwallacea, Xyleborus, Xylosandrus* (6 sp.)	Curculionidae	?				no	?			
Keller et al., 2004	*Chelymorpha alternans*	Chrysomelidae		V							
Kolasa et al., 2017	*Crioceris* (2 species)	Chrysomelidae					V (4)				
Kolasa et al., 2018a	*Bembidion, Paederidus, Paederus*	Carabidae, Staphylinidae					V (6)				
Kolasa et al. 2019	24 beetle species	Carabidae, Staphylinidae, Curculionidae, Chryso-melidae, Scarabaeidae						V			
Kolasa et al.. 2018b	297 beetle species								V	V	V
Kondo et al. 2002	*Callosobruchus chinensis*	Chrysomelidae		V		strains controled by the host					
Kotaskova et al., 2018	*Strophosoma*	Curculionidae	no				no				
Lachowska-Cierlik et al., 2010	40 beetle species	Curculionidae					V (7)				
Li et al., 2016	*Tribolium confusum*	Tenebrionidae		V							
Li et al., 2020	*Harmonia axyridis*	Coccinelidae								V	
Majerus et al., 2000	*Adalia bipunctata*	Coccinellidae				killing of male embryo			V	V	
Maleki-Ravasan et al., 2019	*Paederus fuscipes*	Staphylinidae				females more infected than males					
Mariño et al., 2017	*Hypothenemus hampei*	Curculionidae		V							
Mazur et al., 2016	*Eusomus ovulum*	Curculionidae	?		V						
Ming et al., 2015	*Tribolium confusum*	Tenebrionidae		V							
Miraldo & Duplouy, 2019	*Nanos*	Scarabaeidae					no	V (8)			
Montagna et al., 2014	*Oreina* (6 species)	Chrysomelidae					?				
Numajiri et al., 2017	*Callosobruchus analis*	Chrysomelidae		V		fitness decline					
Parker et al., 2020	*Onthophagus*	Scarabaeidae						?			
Perotti et al., 2016	*Micromalthus debilis*	Micromalthidae	?						V		
Plewa et al., 2018	*Monochamus*	Cerambycidae					?				
Rodriguer et al., 2010a	29 beetle species	Curculionidae	?					?			
Rodriguero et al., 2010b	*Naupactus cervinus*	Curculionidae	no		V	host bottleneck					
Rodriguero et al., 2021	*Pantomorus postfasciatus*	Curculionidae	V								
Roehrdanz & Levine, 2007	*Diabrotica barberi*	Chrysomelidae		V							
Roehrdanz et al., 2006	*Aphthona nigriscutis*	Chrysomelidae			V	reduction of host diversity					
Shaikevich et al., 2021	*Adalia bipunctata*	Coccinelidae			no	male-killing					
Sharaf et al., 2010	*Oryzaephilus surinamensis*	Sylvanidae		?							
Son et al., 2008	*Otiorhynchus sulcatus*	Curculionidae				necessary for egg developnment					
Sontowski et al., 2015	35 beetle species	Dytiscidae, Gyrinidae, Haliplidae, Hydraenidae, Hydrophilidae, Noteridae					V (2)				
Takano et al., 2017	*Brontispa longissima*	Chrysomelidae		V							
Toju & Fukatsu, 2011	*Curculio sikkimensis*	Curculionidae							V	V	
Toševski et al., 2015	*Rhinusa* (3 species)	Curculionidae					no				
Tuda et al., 2021	*Hypera postica*	Curculionidae			V						
Vega et al., 2002	*Hypothenemus hampei*	Curculionidae				possible sex determination					
Wei et al., 2021	*Altica*	Chrysomelidae			no	male-killing		no			
Weinert et al., 2007	*Calvia quattuordecimguttata*	Coccinellidae							V	V	
White et al., 2015	*Sitona obsoletus*	Curculionidae							V		
Wielkopolan et al., 2021	*Oulema melanopus*	Chrysomelidae							V		
Xue et al., 2011	*Altica* (3 species)	Chrysomelidae			no		?	?			
Zchori-Fein et al., 2006	*Coccotrypes dactyliperda*	Curculionidae				role in oogenesis					
Zhang et al., 2010	*Conotrachelus nenuphar*	Curculionidae		?							