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REVIEW ARTICLE

Distribution and Evolutionary Impact of *Wolbachia* on Butterfly Hosts

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Abstract *Wolbachia* are maternally inherited endosymbiotic alpha-proteobacteria found in terrestrial arthropods and filarial nematodes. They are transmitted vertically through host cytoplasm and alter host biology by inducing various reproductive alterations, like feminization, parthenogenesis, male killing (MK) and cytoplasmic incompatibility. In butterflies, some effects especially MK and sperm-egg incompatibility are well established. All these effects skew the sex ratio towards female and subsequently favor the vertical transmission of *Wolbachia*. Some of the insects are also infected with multiple *Wolbachia* strains which may results in some complex phenomenon. In the present review the potential of *Wolbachia* for promoting evolutionary changes in its hosts with emphasis on recent advances in interactions of butterfly–*Wolbachia* is discussed. In addition to this, strain diversity of *Wolbachia* and its effects on various butterfly hosts are also highlighted.

Keywords Symbiosis · *Wolbachia* · Phenotypic effects · Butterfly · Evolutionary impact

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Introduction

Symbiosis is the phenomenon in which different types of organisms are living together and called as symbionts. These are either ectosymbiont or endosymbiont [1, 2]. The evidences over the years prove that endosymbionts have important role in their hosts for immunity, nutrition and in defense [1]. Mode of transmission of these symbionts from one host to another, generally decides the effect cause by them. Vertical transmission from mother to offspring is commonly observed in mutualistic symbionts and it generally leads to the long term association. Horizontal transfer from one host to another is observed in parasitic symbionts [3]. In order to maintain their effects, some of the symbionts are capable of manipulating host reproduction and hence also known as reproductive manipulator. Inherited endosymbiont, *Wolbachia* is one of such master manipulator known to alter biology of their host by various ways [4]. *Wolbachia* have ability to infect very diverse hosts and showed various long term associations with their hosts (ranging from mutualism to parasitism) making them vital candidate to study.

History, Distribution and Diversity of *Wolbachia*

Wolbachia was first detected in mosquito host *Culex pipiens* and later described as *Wolbachia pipientis* [5]. This bacterium was found to infect large variety of insects and nematode and hence receiving the great interest of the many ecologists. Meta-analyses by Hilgenboecker et al. [6] showed that *Wolbachia* infect at least 66 % of all insect species making them one of the most abundant intracellular bacterial genus. A remarkable genetic diversity of *Wolbachia* divided them in 11 supergroups (A–K) [7].

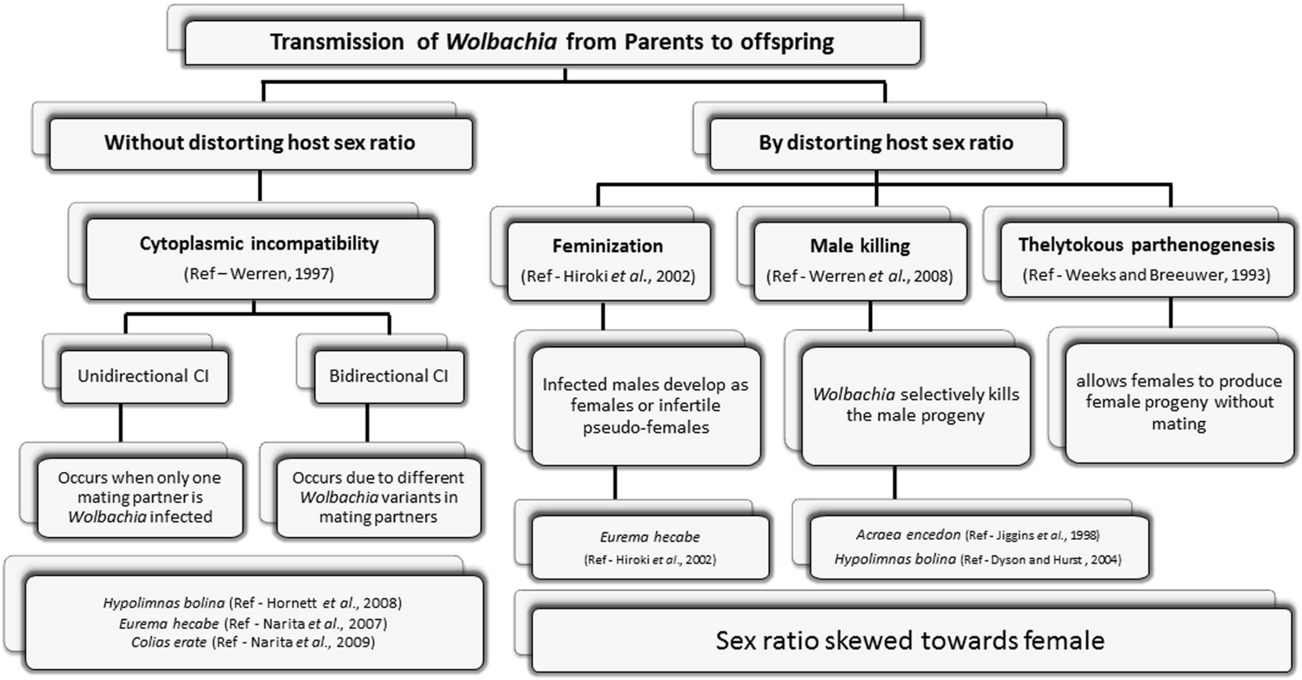

Fig. 1 Illustration showing mode of transmission of *Wolbachia* from parents to progeny with examples of butterflies

Supergroups A and B are found in arthropods whereas supergroups C and D are found in nematodes [8]. Springtails, *Mesaphorura* and *Collembola* are reported to harbor E supergroup [9]. Supergroup G was reported from spiders but has been suggested for temporary removal [10]. H Supergroup is found in two species of *Zootermopsis* [11]. Two fleas *Ctenocephalides* and *Orchopeas* harbors I supergroup, while supergroup J is found in *Dipetalonema gracile* [7]. *Bryobia* sp. is reported for K supergroup [7]. Supergroup F is highly diverse and found to break renowned barrier by infecting both nematode *Mansonella* and arthropods like termites, butterflies, cockroaches, etc. [12–16].

The multi locus sequence typing (MLST) system provides a standardized and rigorous framework to study *Wolbachia* strains diversity [17]. Studies of *Wolbachia* MLST systems have demonstrated power of these approaches in accurately characterizing and identifying various *Wolbachia* strains. Extensive sampling and MLST approach has been successfully applied to *Wolbachia* strains from butterflies, termites, etc. [13, 15].

Phenotypic Effects of *Wolbachia* Induce in Their Hosts

Wolbachia are known for manipulating cellular and reproductive processes in host, still capable to live within the host cell which suggest ancient history of their evolution. Along with *Wolbachia* several endosymbionts of arthropods like *Cardinium*, *Spiroplasma*, *Flavobacteria*, *Arsenophonus* are known as reproductive parasites [18]. These endosymbiont employs some effects like cytoplasmic incompatibility (CI), male killing (MK), feminization and parthenogenesis. *Wolbachia* is unique among them as it is able to show all the four effects in their diverse host. In the present review, we summarized some of the effects cause by *Wolbachia* in their hosts (Fig. 1).

CI is a phenomenon, which modulate host and it became ineffectual to produce viable offspring. CI is the most widespread and, perhaps, the most comprehensively studied *Wolbachia*-induced phenotype [19]. *Wolbachia* gain attention when Yen and Barr [20] confirmed *Wolbachia* as a causative agent behind CI occurs in mosquito *C. pipiens*. Later on CI has been reported in many insect orders like Lepidoptera, Coleoptera, Diptera, Hymenoptera, etc. [21]. CI is either unidirectional or bidirectional. In unidirectional CI, fertilization ceases when infected males mate with uninfected female. However, the crosses between both, male and female which are either infected or uninfected lead to the normal reproduction. This phenomenon gives reproductive advantages to infected females over uninfected and favors the vertical transmission of the *Wolbachia*. Bidirectional CI is incompatibility which occurs because of different *Wolbachia* variants in mating partners [22].

Feminization is a phenomenon in which *Wolbachia* infected males develop as females or infertile pseudo-females. *Wolbachia* induced feminization is a strategy to produce more females since males are dead ends for their vertical transmission. This phenomenon was first observed in terrestrial crustaceans [23] in which they showed that infected female produce twice as many daughters as their uninfected counterpart. Later *Wolbachia* induced feminization was also demonstrated in two different insect species *Eurema hecabe* (Lepidoptera) and *Zyginidia pullula* (Hemiptera) [24, 25].

Thelytokous parthenogenesis is a type of parthenogenesis in which unfertilized eggs produces females. *Wolbachia* is reported to induce this phenomenon in haplodiploid species like Hymenoptera, Thysanoptera and Acari [26]. It allows females to produce female progeny without mating and favors transmission of *Wolbachia*. Since this phenomenon is recorded only for haplodiploid species, it cannot occur in butterflies.

MK is a phenomenon in which *Wolbachia* selectively kills the male progeny of the infected female before hatching [4]. This phenomenon has been reported in Lepidoptera [27], Coleoptera, Diptera and Pseudoscorpiones [4]. This is undisruptive to the bacterium, because its transmission is favor by female and helps in its spreading.

Interaction of *Wolbachia* and Butterfly Hosts

Butterflies are among the most beautiful and fascinating animals in nature. These are mainly day-flying insects with large scaly wings. They belong to order Lepidoptera, comprising the true butterflies, skippers and moth. The phenomenon like genetic polymorphisms, mimicry and aposematism are observed in butterflies. Some butterflies are known to develop parasitic as well as symbiotic relationships with social insects, such as ants [28]. Butterflies serve as important plant pollinators and known to be involve in pollination of more than 50 economically important crop plants [29]. Some species in their larval stages are pests and can damage domestic leaf of crops or trees [30]. Lots of study has been done on butterfly–*Wolbachia* interaction. In the present review, we highlighted about current investigation about the impact of this interaction and various phenotypic effects of *Wolbachia* found in butterfly hosts.

Wolbachia Diversity in the Butterflies

Wolbachia have been detected in five butterfly families (Nymphalidae, Papilionidae, Pieridae, Lycaenidae and Hesperiidae), while the family Riodinidea is not reported for infection so far. *Wolbachia* in butterflies have been concerned in basic biological evolutions such as sex ratio distortion, sperm-egg incompatibility and speciation [31, 32]. Molecular data and phenotypic effects of *Wolbachia* from some butterfly species are reported, which showed presence of supergroup A and B *Wolbachia* [15, 33–38]. Supergroup A was observed in *Hypolimnas bolina*, *Ornipholidotos peucetia*, *Jamides alecto* and *Iraota rochana.* While supergroup B was observed in a wide range of the butterfly hosts.

Currently, the MLST database has a record for 24 *Wolbachia* strains types (STs) for butterfly hosts. Among these, ST41 is extremely diverse and found in different hosts of all three families [15]. ST125 and ST146 were found in Nymphalidae and Lycaenidae while ST40 was shared between Pieridae and Lycaenidae. Apart from this, family Lycaenidae was reported to harbor ten unique STs, while Nymphalidae and Pieridae are reported for six and four unique STs, respectively. Recently Salunke et al. [15] extensively surveyed the samples of butterflies ($n = 118$) representing 56 species belonging to five families. Out of which 28 species under study are found to be infected with *Wolbachia*. In this paper, they also characterized 13 STs which were new to the MLST database (http://pubmlst.org/Wolbachia/).

Blue Moon Butterfly, *H. bolina* (Nymphalididae) have been extensively studied and is excellent model to discuss about *Wolbachia*–butterfly relationships. This species is distributed in the tropical parts of Madagascar, South and South-East Asia, Australia, Saudi Arabia, New Zealand, South Pacific islands like French Polynesia and Samoa. In twentieth century, entomologists extensively collected and reared the *H. bolina* species because of attractive polymorphism of wing pattern found in females. Surprisingly, they found all female progeny from this species in the Fiji Islands during decade of 1920s [39]. Though this phenomenon was transfer from mother to daughter, it was not because of parthenogenesis [39]. Clarke et al. [40] had proven the persistent of this phenomenon in Fiji even after 150 generation by re-survey and breeding experiments. However, the precise mechanism was unknown till 2002, when Dyson et al. [34] proven *Wolbachia* strain wBol1 as a driving force behind this alter sex ratio.

In 2004, Dyson and Hurst [41] had shown that this extreme sex bias is persistent at least 400 generation after its first record in Independent (Western) Samoa. They further concluded that, sex bias is because of high prevalence of MK *Wolbachia* and not because of parthenogenesis. Here an interesting question arises. How this interaction has persisted without the extinction of host population or the host evolving arrangement to compete with the sex-ratio alteration? To answer this question, they compare their results with other island with lower *Wolbachia* infection in *H. bolina*. They interpret that low male frequency leads to 57 % loss of reproductive output [41]. They further found that male spermatophores of *H. bolina* from Independent Samoa were almost half the diameter of spermatophores produce by *H. bolina* from neighboring island. They also hypothesis that, this small size spermatophores might be due to tiredness of the males due to multiple mating and this might be the revised mechanism to overcome the effect of fever males due to this phenomenon. Charlat et al. [42] studied the *H. bolina* population from several locations and they were expecting that female mating frequency should be lower because of a reduced number of available male partners. In contrast, their experiments suggest that females were mating more repeatedly and male were investing less per copulation. Hence they suggest that bias sex ratio increase female proficiency to mate multiple males, at the same time it decreases male productivity.

Beside all these, *H. bolina* from Japan and South East Asia, have evolved a mechanism, which is able to suppress MK effect of wBol1 [37]. Mitsuhashi et al. [43] showed that this MK suppression trend was observed in natural population of *H. bolina*. This evolution might be the outcome of host response to suppressed destructive effects of the parasite and represented the evolutionary elasticity in host–*Wolbachia* interactions. When MK phenomenon is suppressed by the host response, *Wolbachia* strain reaches towards fixation. However it is not the case with wBol1. Hornett et al. [44] showed that when MK is suppressed in *H. bolina*, then wBol1 strain immediately expresses CI phenotype. They also suggest that CI worked as a backup policy when MK is suppressed and it helps the parasite by avoiding their fixation. This was the first report for two different phenotypes induced by the same *Wolbachia* strain in same host. In 2006, Charlat et al. [45] discovered new *Wolbachia* strain wBol2, which exhibit equal occurrence of male and female. Their results further confirmed that the strain wBol2 causes male induce CI in host and is not the sex ratio distorter. Two more *Wolbachia* strains are reported so far from the host *H. bolina*. Out of these Hbol_B_wBol is reported from Western Ghats, India [15] while wBol_B_wBol3 is reported from Japan [43] however phenotypes induce by these *Wolbachia* were not recorded.

These findings lead to another question. Is this MK phenotype useful to butterfly host and is it responsible to cause any behavioral changes in them? In 1972, Chanter and Owen [46] observed that breeding of nymphalid butterfly *Acraea encedon* in laboratory yielded all female siblings. Jiggins et al. [47] demonstrated that this effect is maternally inherited and mediated by *Wolbachia*. They also point out that MK *Wolbachia* favors sister by reallocation of resources from dead brothers either through sexual cannibalism or through a reduction in competition for food. It also decreases the possibility of inbreeding. In many animals, male are remaining as a group and female select the mating partner. But Jiggins et al. [33] showed that *Wolbachia* infection is responsible to rework the phenomenon in *A. encedon* in which lekking behavior was observed in female while male selected the partner among them, which prevent the population from extinction. Phylogenetically distinct strains of *Wolbachia* where observed in *A. encedon* population from Uganda and Tanzania which are also showing the MK phenomenon [48].

The well-established MK phenotypes in butterfly host gained attention of many ecologist and they were looking for other effects of this bacterium in butterflies. Hiroki et al. [24, 36] have extensively studied the *E. hecabe* butterfly from Okinawa-jima Island, Japan for *Wolbachia* infection. They had detected two different strains of *Wolbachia* which were having ability to induce Feminization and CI independently. They also found that CI strain was similar with MK strain reported earlier in *Hypolimnas* and *Acraea* [34, 48] which suggested possible horizontal transfer among host. Specific interactions among host and symbiont might be responsible for this alter phenotypic effect.

Narita et al. [31] further studied this *E. hecabe* population. They showed that these butterflies are in fact infected with two different strains out of which one is feminization causing while other is CI causing strain. Their experiments also suggest that feminizing *Wolbachia* continuously acts during larval development on the host for suppression of male phenotype in genetically males [31]. Further studies showed that CI inducing *Wolbachia* infection frequency is 100 % in pierid butterfly *Colias erate* and these *Wolbachia* increase the survival rate of the host if compared to their antibiotic cured counterpart in laboratory condition [49]. Recently Kodandaramaiah et al. [50] observed *Wolbachia* infection in an entire population of *Polygonia c-album* (comma butterfly) suggesting existence of strong CI. Ankola et al. [51] reported B supergroup *Wolbachia* in *Talicada nyseus* and *Papilio polymnestor*. They further found that *Wolbachia* infected *T. nyseus* showing female biased sex ratio and they suggest the possibility of *Wolbachia* driven MK or feminization as a cause behind this distortion.

Conclusion

Exceptionally diverse associations of *Wolbachia* and their host are ranging from extreme sex manipulation to mutualism. Mechanism behind this association tells the success of its wide spread distribution making it most dominant symbiont. There is rapid development in the *Wolbachia* research, particularly in the areas of cell biology, genomics and molecular biology. Other methods like, transfer of *Wolbachia* strains from one host to other, genome sequencing, MLST and cell culturing are currently adding more information to the existing knowledge of the research community. Now day's occurrence and characterization of

Wolbachia strains from different butterfly species is underway. However, there is massive scope for further exploring and understanding the fascinating interactions between *Wolbachia* and butterfly. Since many of the butterfly species either are reported or supposed to be infected with *Wolbachia*, the butterfly–*Wolbachia* is emerging as a good model to study host–parasite interactions.

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