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Wolbachia-mediated Reproductive Alterations in Arthropod Hosts and its use for Biocontrol Program

Elahe Rostami, Hossein Madadi*, Habib Abbasipour[1] and Shiva Sivaramakrishnan[2]

Department of Plant Protection, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran
[1]Department of Plant Protection, Faculty of Agricultural Sciences, Shahed University, Tehran, Iran
[2]Department of Biotechnology and Genetic Engineering, Bharathidasan, University of Tiruchirappalli - 620 024, Tamil Nadu, India

볼바키아 세균에 의한 절지동물 기주의 생식적 변화와 생물적방제 프로그램에 이용 방안

엘라히 로스타미 · 후세인 마다디* · 하비브 아바시포르[1] · 쉬바 시바라마크리쉬난[2]

부알리 시나 대학교, 농업부, 식물보호학과, [1]샤헤드 대학교, 농업과학부, 식물보호학과, [2]바라티다산 대학교, 유전생명공학과, 티루치라팔리

ABSTRACT: The alpha-proteobacterium *Wolbachia* is one of the most important intracellular symbionts of arthropods. This Gram-negative bacterium is involved in many biological processes and is currently considered as a potential tool for biological control. *Wolbachia* is a cytoplasmic bacterium, maternally transferred through generations, and to facilitate its success, it has evolved several strategies that manipulate its host reproductive system to increase the number of infected individuals in the host population. The variety of *Wolbachia* was first recognized using genes *wsp*, *16S rRNA*, *ftsZ*, *gltA* and *groEL* as molecular markers while strain genotypes of Wolbachia are determined of Multilocus sequence typing (MLST) and sequence of amino acid in region, hyper variable regions (HVRs) in protein WSP. Possible uses of the bacteria and their predominant phenotypes in control programs for agricultural pests and human disease vectors have been considered. Phenotypes are known to induce cytoplasmic incompatibility (CI), parthenogenesis induction (PI), feminization (F) and male killing (MK). Finally, applications of the bacterium in control programs of agricultural and medical insect pests have been discussed.

Key words: *Wolbachia*, Intracellular symbiosis, Molecular marker, Biological control

초 록: 알파 프로테박테리아(α-proteobacterium)인 볼바키아(Wolbachia) 세균은 절지동물 세포내의 중요한 공생균 중의 하나이다. 그람 음성 세균인 이 공생균은 기주동물의 여러 생물적 과정에 관여하고 있으며, 현재 생물적 방제 수단으로 주목 받고 있다. 볼바키아는 기주 세포의 세포질에 서식하는 세균인데 암컷을 통하여 세대간 전염된다. 볼파키아의 감염 개체 밀도를 높이기 위해 기주의 생식방식을 조작하는 다양한 전략을 발달시켰다. 볼바키아 유전자형 계통은 볼바키아 표면 단백질(WSP)의 고변이영역 아미노산 서열과 복합좌위 서열 타이핑(Multilocus sequence typing, MLST)으로 결정된다. 상이한 유전계통 판별은 *wsp*, *16S rRNA*, *ftsZ*, *gltA*, *groEL* 등 유전자 분자표지를 이용하게 된다.. 이 계통 볼바키아 세균과 그들의 우월한 표현형이 농업해충과 인간의 질병매개 곤충에 대한 방제 프로그램에서 이용 가능성이 고려되고 있다. 볼바키아 표현형들은 세포질불일치(cytoplasmic incompatibility, CI), 단성생식 유도(parthenogenesis induction, PI), 여성화(feminization, F), 수컷치사(male killing, MK) 등을 유발하는 것으로 알려져 있다. 기타 볼바키아 세균의 농업과 위생곤충 방제 프로그램에서 응용 방안을 고찰하였다.

검색어: 볼바키아, 세포내 공생, 분자표지, 생물적 방제

Wolbachia (Alpha-Proteobacteria; Rickettsiales; Rickettsiaceae) is an obligatory intracellular bacterium found in most arthropods (insects, mites, isopods, crustaceans, spiders, springtails) and in filarial nematodes, causing unbiased sex ratio (Hoerauf et al., 2003; Tsillassie and Legesse, 2007). Six out of sixteen *Tetranychus* spp., four out of seven predatory mites, 35% of

***Corresponding author:** madadiho@gmail.com
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terrestrial isopods and nine out of ten filarial nematode species are infected with *Wolbachia* (Stouthamer et al., 1999; Islam, 2007). Seventy-six percent of Nearctic arthropods (Jeyaprakash and Hoy, 2000), 50% of Indonesian ants, 100% of Panamanian leafcutter ants (van Borm et al., 2001) and 44.9% of butterflies (Tagami and Miura, 2004) have been reported to carry *Wolbachia*. Globally, 20-76% of insect species are infected with *Wolbachia* (Weeks et al., 2002; Islam, 2007). By altering their host reproductive system, *Wolbachia* provides a suitable ground for its own vertical transmission (Werren, 1997; Stouthamer et al., 1999; Bandi et al., 2001; Islam, 2007). This review focuses on the mechanisms induced by *Wolbachia* in arthropods and its potential role in the control of pests and vector-borne diseases. Despite significant progress in the field of ecology and *Wolbachia* population genetics, the use of the bacterium as a new tool in biological control of arthropods of medical and agricultural importance is still challenged by several strategic problems, such as the unknown effect of speciation, the difficulty of mass production, the absence of the bacterium in some species and the unknown adaptive outcomes of transfection of the bacterium to novel hosts (both at the phenotypic and cellular levels).

The species of *Wolbachia pipientis* Herting & Wolbach (1936) is the only identified species of *Wolbachia* genus in arthropods and nematodes (Lo et al., 2007). The nearest relatives of *Wolbachia* are *Cowdria* and *Anaplasma* genus which are obligatory intracellular bacteria causing disease in mammals. The association of this bacterium with arthropods varies from parasitism to symbiosis. In 2009, McMeniman et al. reported the successful transfer of *w*MelPop, a life-shortening strain of *Wolbachia* from *Drosophila melanogaster*, into the major mosquito vector of dengue, *Aedes aegypti*. This example points out the parasitic role of *Wolbachia* in arthropods.

Wolbachia classification and nomenclature

Nowadays, old microbiological methods are not suitable to identify and study *Wolbachia*. In recent decades, molecular techniques such as polymerase chain reaction (PCR) methods have been used to screen for *Wolbachia*. Applying these methods helped to identify different strains of bacteria with unique gene sequences and to associate them with differently expressed phenotypes in a wide variety of hosts. The use of PCR together with sequencing techniques allows the identification of the *Wolbachia* strains present in the samples of interest. The genes *wsp*, *16SrDNA*, *ftsZ*, *groEL*, *coxA*, *fbpA*, *hcpA*, *gatB*, *dnaA*, *gltA* are commonly used in this purpose and for phylogenetic studies (Chai et al., 2011). Based on identified genes, the bacteria are currently subdivided into 13 super-groups from A to F and H to N (Augustinos et al., 2011). Super-group G is considered as a recombinant between A and B (Bandi et al., 1998). The super-groups of A and B are identified from crustaceans, Hexapoda and Chelicerata, super-groups C and D from filarial nematodes, super-group E from some Collembola (Lo et al., 2002; Augustinos et al., 2011), super-group F from Isopoda, Insecta and some Onchocerid nematodes (Augustinos et al., 2011), super-group G from Australian spider (Rowely et al., 2004), super-group H from Isoptera (*Zootermopsis* spp.) (Augustinos et al., 2011), super-group I in *Ctenocephalides* and *Orchopeas* fleas (Augustinos et al., 2011), the super-group J from *Dipetalonema gracile* Rudolphi, super-group K from Brown mite, *Bryobia* sp., super-group L from a nematode, *Radopholus similis* Cobb, and aphids, and finally the super-groups M and N from aphids (Augustinos et al., 2011). Currently, multi-locus sequence typing (MLST) is used to identify the genotype of strains of *Wolbachia*, in which several genes are identified simultaneously in addition to gene of *wsp* (*Wolbachia*-specific protein) which is a surface protein of cell (Islam, 2007; Chai et al., 2011). With the use of standard molecular techniques, 22 species of Lepidoptera were screened, 19 of which were infected by *Wolbachia*. All samples that were positive for *16S rDNA* also tested positive for *fbpA* (Hamm et al., 2014).

Parvizi et al. (2010) used *16S rDNA* and *wsp* to detect and probe *W. pipientis* bacterium in *Phlebotomus papatasi* Scopoli. To determine the presence of bacterium in *P. papatasi*, a batch of about 550 base pairs was reproduced using the general primer of *wsp*: 81F (forward, 5′TGGTCCAATAAGTGATGAAGAAAC3′) and primer 691R (reverse, 5′AAAAATTAAACGCTACTCCA3′). For *16S rDNA*, 99F (forward, 5′TTGTAGCCTGCTATGGTA TAACT3′) and 99R (reverse, 5′GAATAGGTATGATTTTC ATGT3′) were used (Parvizi et al., 2010).

With the identification of a wide variety of *Wolbachia* strains, a clear nomenclature system to discriminate strains had to be defined. Currently three methods are commonly used: after the common *"w"* referring to *Wolbachia*, (I) the author

may choose to add the three initial letters of host species (Rousset and Stordeur, 1994), which was proposed by Zhou et al. (1998), with combination of the initial letter of genus and two letters from the species (Charlat et al., 2002), (II) the strain may be named in regard to its collection place. This nomenclature was for example used to differentiate the strains present in *Drosophila simulans* Stutevant; *w*Ri refers to a strain of bacteria in flies collected from Riverside in California, while *w*Ha, *w*Ma, *w*Au, *w*Ki refer to strains of bacteria in flies collected from Hawaii, Madagascar, Australia and Kilimanjaro in Tanzania, respectively (Stouthamer et al., 1999; Islam, 2007).

Bacterium transfer

Wolbachia bacterium transfers via vertical and horizontal routes. Vertical gene transfer is a way to transfer gene from a generation to another or mother cell to daughter cell, while horizontal gene transfer is a way to transfer gene from a living thing to another without having parental relationship or mother-daughter cell relationship. The bacterium is transferred naturally or through injection with slender needles to the hosts not located in a species without any kin relationship (Stouthamer, 1993). It is determined from the evidence and genealogic investigations that this bacterium must be transferred horizontally among different species because the strains of bacterium with genetic similarity have been found in hosts which are non-kin in terms of taxonomy (Stouthamer, 1993; Werren et al., 1995). The results from the researches on the injection of bacterium *Wolbachia* with micropipette and specific slender needles to the embryonic stage of bacterium-free hosts indicate the success in horizontal transfer within species of *Drosophila* and inter-species transfer in a type of mosquito and *D. melanogaster* (Braig et al., 1994; Rosset and Stordeur, 1994). In several species of Isopoda, horizontal transfers occur naturally after injection of oocytes of animal with intricate tools (Rigaud and Juchault, 1995). Injections are however not always successful. For example, researchers were not able to transfer *Wolbachia* into parasitoids such as *Muscidifurax uniraptor* Kogan & Legner (Hym.: Pteromalidae), and *Nasonia vitripennis* Walker (Hym.: Pteromalidae) (van Meer et al., 1996). The reason might be attributed to bacterial concentrations or puniness of parasitoid eggs. It seems that especially for egg parasitoids, use of multiparasitism might be more useful.

Furthermore, the presence of similar *ftsZ* gene sequences in unrelated Lepidoptera, Hymenoptera, Diptera and Coleoptera species suggested horizontal transfers between the parasitoid wasp *Nasonia giraulti* and its host (Beard et al., 1993; Werren, 1997). By tracing bacterium in *Trichogramma brassicae* Westwood with PCR using specific primers of gene *wsp* of ITS-2 area and subsatellite TTG49, successful horizontal transfer of the bacterium were also demonstrated (Farrokhi et al., 2010).

Reproductive manipulations induced by *Wolbachia*

Wolbachia has evolved strategies to modify its host reproductive system, thus in the selfish goal of improving its own spread across its host population. This feature causes the bacterium to be known as a reproductive parasite, and the observed phenotypes include cytoplasmic incompatibility (CI), male killing (MK), feminization and thelytokous parthenogenesis (Stouthamer et al., 1999; Augustinos et al., 2011). We will describe these phenotypes and their consequences from both host and *Wolbachia*.

Cytoplasmic incompatibility

One of the most widespread manipulation observed in some insects, mites and crustaceans infected with *Wolbachia*, is cytoplasmic incompatibility (CI). It was first observed and described in *C. pipiens*. Then, other researchers investigated the phenomenon carefully in species of *Drosophila* sp. and parasitoid wasp, *Nasonia* (Hym.: Peteromalidae), and to some extent in other insects. The *Wolbachia* bacterium exists in a free form, but not transferred to other hosts. In fact, this bacterium exits in sperm structure during spermatogenesis stage, while it has affected sperm before or during spermatogenesis. When the process of cytoplasmic incompatibility occurred, the infected male mates with uninfected female (Uni-directional cytoplasmic incompatibility), or infected male mates with female carrying another strain (bidirectional cytoplasmic incompatibility). In this case, the father chromosomes are destroyed in the first mitotic division in produced eggs. Consequently, the fertilized egg will die (Diplodiploids and Haplodiploids) or turn in male individuals (in Haplodiploids). Other mating compositions

such as intersection of males and females infected to a similar strain are completely compatible and produced eggs will survive (Werren, 1997; Stouthamer et al., 1999).

Uni-directional cytoplasmic incompatibility

Uni-directional cytoplasmic incompatibility occurs when intact ovule cell is inoculated by *Wolbachia*-infected sperm. However, an inoculation of disinfected sperm or inoculation with similar strains will be compatible (Hoffman and Turelli, 1988; Tram et al., 2003) (Fig. 1).

Bi-directional cytoplasmic incompatibility

Incompatibility occurs when both males and females are infected to bacterium (of course, different strains) and the eggs produced are induced with each other. If each individual with similar strain is infected, the inoculation is compatible (Tram et al., 2003, 2006) (Fig. 2).

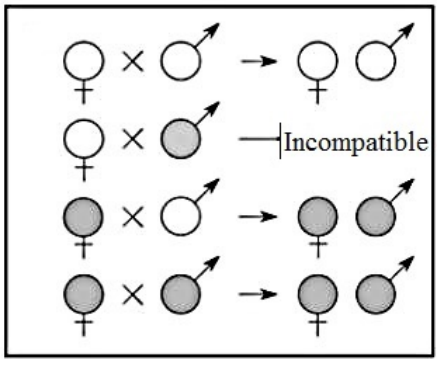

Fig. 1. Uni-directional cytoplasmic incompatibility. Uninfected individuals are shown in white, infected individuals in gray (Brelsfoard and Dobson, 2009).

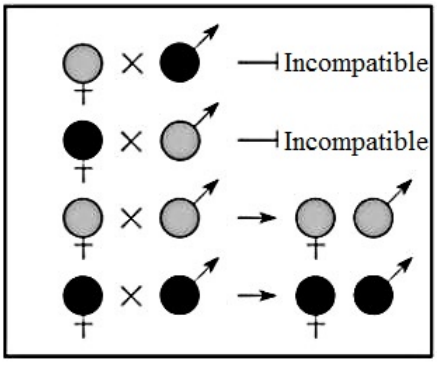

Fig. 2. Bi-directional cytoplasmic incompatibility. Infected strain type 1 is shown in gray, infected strain type 2 in black (Brelsfoard and Dobson, 2009).

Super infections

In this case, if ovule is intact, but the sperm is infected with one or two strains of *Wolbachia*, the inoculation become incompatible. If sperm and ovule both are infected with the same strain, or sperm infects with one strain and ovule infects with two strains (one of them is the same strain of infected sperms), the inoculation would be compatible. Additionally, the inoculation would be compatible, if the male is *Wolbachia*-free but the female is not (Tram et al., 2006) (Fig. 3).

Regarding the principle of cytoplasmic incompatibility, the male plays a main role in incompatibility and the female determines the compatibility or incompatibility of inoculation. Thus, *Wolbachia* is transmitted from female to male and the inoculation will be compatible when male and female are same in terms of infection to *Wolbachia*. In other words, if male is healthy, inoculation is always compatible (whether female is healthy or infected to one or two strains). If male is infected to a strain of *Wolbachia*, then inoculation will be compatible under a condition that female is infected to the same strain or if female is infected to another strain common to male. Some models have been presented for mechanisms of cytoplasmic incompatibility by bacterium (Mehrabadi and Bandani, 2008). Although there is not any specified mechanism for bacterium mode of action, this bacterium, apparently, disturbs the mitosis division based on the models that have been presented so far to

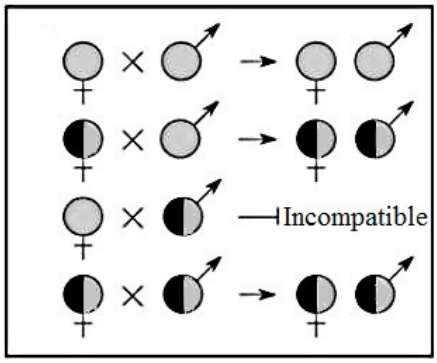

Fig. 3. Multiple infections (super infections) cytoplasmic incompatibility. Infected strain type 1, and infected strain type 2 are shown in gray and black, respectively (Brelsfoard and Dobson, 2009).

show the mechanism.

Lock and key theory

Based on the lock and key theory, during spermatogenesis in infected males, some factors named lock (mod) are produced with this bacterium incorporated into the father chromosomes. If infected sperms inoculate healthy ovules, cytoplasmic incompatibility occurs, as in this case father chromosomes are locked and cannot work properly. In contrast, the ovules infected to *Wolbachia* are productive for these sperms (compatible) as in infected ovules, a factor is produced (key or rescue factor) and helps the lock to open in father chromosome (Rescue function) (Poinsot et al., 2003) (Fig. 4).

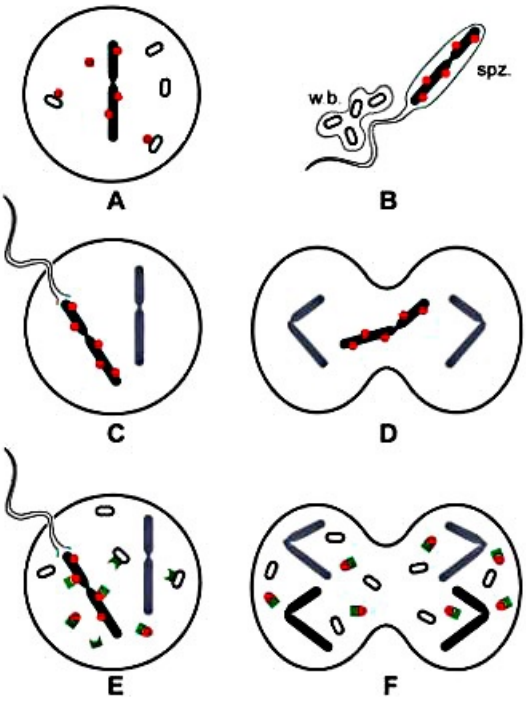

Fig. 4. Lock and key model. A: *Wolbachia* (white symptom) produce a lock, (red circle) binding, for example to paternal chromosomes (large black bar). B: The bacteria are, then, saved in a waste-bag structure (w.b.) with most of the cytoplasm, so, being absent from the mature spermatozoon (spz). C: The sperm cell transporting locked paternal chromosomes enters an uninfected egg meeting unmodified maternal chromosomes (grey tape). D: In the absence of a key to remove the lock, paternal chromosomes are not functional and only maternal chromosomes participates normally in mitosis, resulting in Cytoplasmic incompatibility (CI). E: In an infected oocyte, *Wolbachia* produce a key (green symptom). F: The lock is, so, removed from parental chromosomes and mitosis takes place normally, rescuing the embryo (Poinsot et al., 2003).

Sink or titration–restitution theory

Based on sink or titration-restitution theory, *Wolbachia* produces some proteins (ex, histone-like protein) surrounding host chromosomes at spermatogenesis stages in male individuals infected to this bacterium. *Wolbachia* possibly returns the separated proteins to the whole chromosomes after inoculation. Variation and rescuing factors must be coded with the similar gene or genes, changing the imperfect to ordinary chromosomes in host or expressed with different genes with two titration and restitution codes (Poinsot et al., 2003) (Fig. 5).

Slow–motion theory

Based on slow-motion theory, *Wolbachia* does not prevent

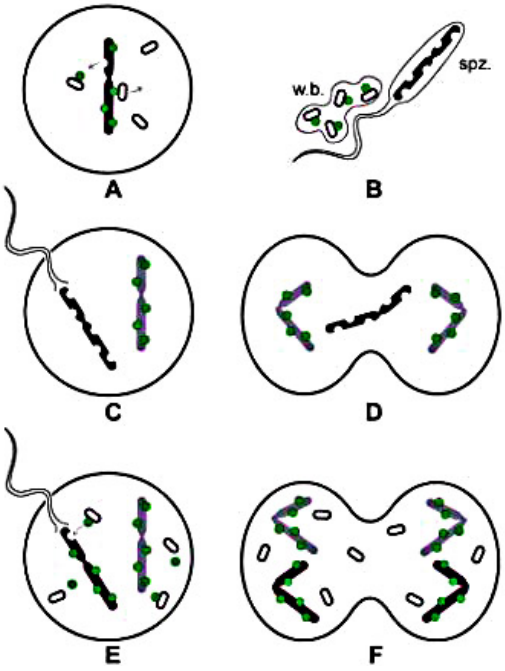

Fig. 5. Titration-restitution model. A, B: Spermatogenesis in an infected male. A: *Wolbachia* (white symptom) titrate-out a host protein (green circles) normally related to chromosomes. B: The titrated protein is, then, expelled from the cell when *Wolbachia* are shed from the maturing spermatocyte, with most of the cytoplasm, being in a waste bag structure (w.b.). C, D: Incompatible cross between an infected male and an uninfected female. C: Paternal chromosomes (black tape) in the mature spermatozoon (spz) are not functional because of missing the protein. D: Paternal chromosomes are not functional normally in mitosis resulting in Cytoplasmic incompatibility (CI). E, F: Compatible cross between two infected individuals. E: In an infected oocyte, the *Wolbachia* give back to maternal and paternal chromosomes the host protein previously titrated-out. F: mitosis can now proceed normally, rescuing the embryo (Poinsot et al., 2003).

the first mitosis division, but it changes and delays its stages. This mechanism is operated at two phases. First, *Wolbachia* produces factors in spermatogenesis incorporated into paternal chromosomes and reduces the first mitotic division rate. This action makes parental chromosomes be in compatible in mitosis stage. The second stage, in infected ovules, occurs on mother chromosomes and reduces the movement speed of maternal chromosomes, equaling to that of father chromosomes. At this stage, inoculation will be incompatible, because both series of parental chromosomes enter the mitotic divisions with delay. The important point is that division takes a longer time than normal state. If the sperm originated from an infected male inoculates ovule of non-infected female, paternal chromosomes have then lower movement than maternal ones. Thus it cannot enter the division in mitosis stages simultaneously with mother chromosomes and incompatibility happens (Poinsot et al., 2003) (Fig. 6).

Precise investigation on first mitotic division in embryo of *D. simulans* and *N. vitripennis*, in which incompatibility has occurred, shows that *Wolbachia* manipulates the proteins related to cellular cycle (Tram et al., 2003). In the preparation process of first mitotic division, mother and father nuclei migrate toward and embed adjacent into each other without being mingled. The mother and father nuclei are not blended with each other until the end of telophase stage. In *D. melanogaster*, it has been demonstrated that the remains of nucleus coating prevent the mixing of mother and father nucleus during metaphase stage. Consequently the spindle apparatus obtained consists of two series of separate microtubules during the first karyokinesis, each of them connected with father and mother chromosomes and is responsible for their motion. For a nucleus to exist with both series of father and mother chromosomes, it is necessary for mother and father chromosomes to enter and exit the division process simultaneously. Thus, any disturbance in harmony of chromosomes leads to incompatibility (Tram et al., 2003, 2006) (Fig. 7).

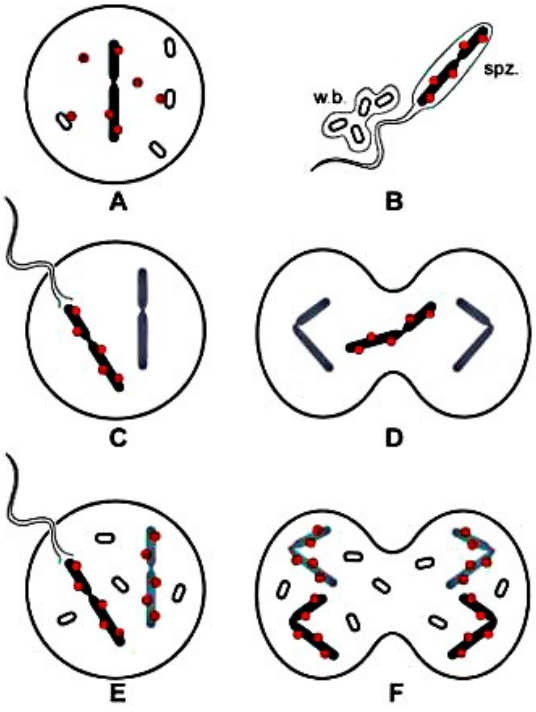

Fig. 6. Slow-motion model. A: *Wolbachia* (white symptom) produce a slowing -down factor (red circles). B: The bacteria are infusing from the maturing spermatocyte, with most of the cytoplasm, being in a waste-bag structure (w.b.). E: The sperm cell bearing slowed-down paternal chromosomes enters an oocyte infected maternal by *Wolbachia*. F: Since maternal chromosome sets are synchronous, and the first mitosis proceeds normally (Poinsot et al., 2003).

Parthenogenesis induction

Some *Wolbachia* strains interfere with the productive haplodiploid systems of insects at chromosome differentiation stage and enable the virgin female to produce female offspring (2n) through thelytokous parthenogenesis (in contrast with arrhenotokous parthenogenesis through which virgin females only produce male offspring with haploid chromosomes). In the ovules of infected females, the set of mother chromosome doubles in a process named gamete duplication during the mitosis division or interphase and before the second division. Therefore, these embryos are developed to homozygote diploid female offspring after growth (Gottlieb et al., 2002).

It seems that the main prerequisite for inducing parthenogenesis by *Wolbachia* is the haplodiploid reproduction system of the host. Various groups of arthropods including Hymenoptera, Acari, Thysanoptera and some Coleoptera (Snout beetles) have been shown that these kinds of reproduction and the inoculated or non- inoculated offspring become female or male individuals, respectively.

The induced parthenogenesis is first suggested when bias in sex ratio is observed, as in of *Habrolepis rouxi* Compere (Hym.: Encyrtidae) population along with temperature rising from 26.6℃

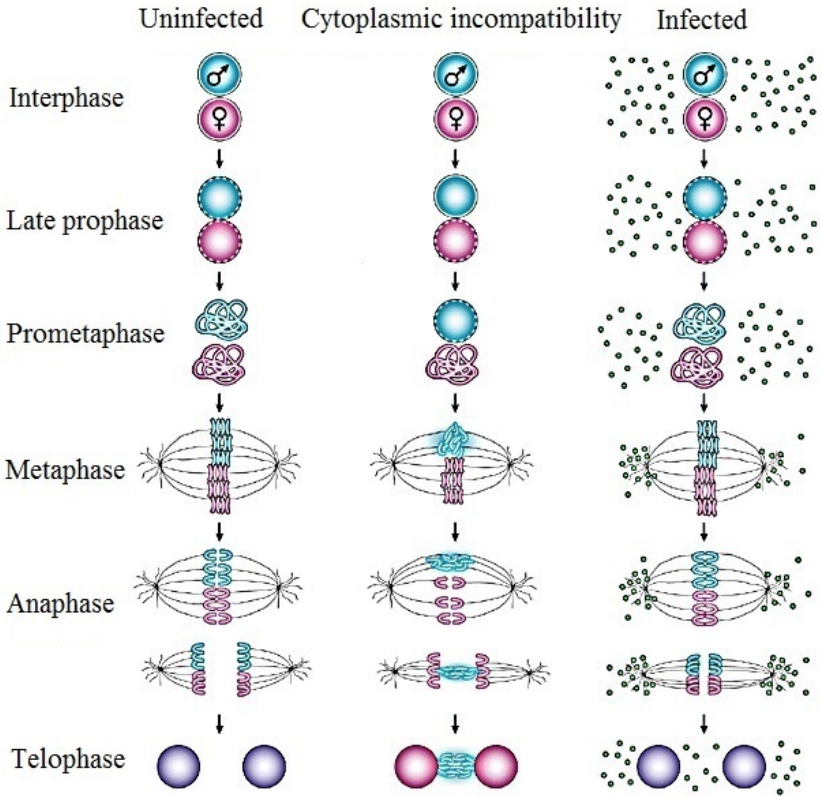

Fig. 7. In a cytoplasmic incompatibility cross (middle column), asynchrony can be observed in the development of paternal (blue) and maternal (pink) pronuclei at the first embryonic mitotic division. Breakdown of the nuclear envelope in the male pronuclei, also chromatin condensation, lag behind the female pronuclei. At metaphase, paternal chromosomes are not fully condensed, and at anaphase, paternal chromosomes do not properly segregate. Pronuclear synchrony and normal development are restored in an infected embryo (right column) (Werren et al., 2008).

to 32.2℃. Furthermore, when parthenogenetic *Trichogramma* sp. individuals are fed with antibiotic provides one of the most strongly documented evidence about the intervention of microbial factors in parthenogenesis (Flanders, 1965; Stouthamer et al., 1990). Feeding female bees of single sex with honey solution containing antibiotics led to production of male offspring and returning reproduction from parthenogenesis to bisexual mating mode. Also, application of thermal treatments showed similar effect.

Parthenogenesis induction (PI) by bacteria is dependent on bacterial density. As seen in cytoplasmic incompatibility (CI) of density (titer) effect, this issue has been explained in *Muscidifrurax uniraptor* Koganand Legner such as increasing dose of antibiotics raised the production of male individuals (Zchori-Fein et al., 2000).

In *Trichogramma*, the parthenogenesis induced by *Wolbachia* is different from what is seen in other hosts. In this hymenopteran species, the infection does not reach a fixed level in the population. In all species of this parasitoid wasp, except two cases, the infection frequency is very low in the population, leading to mixed presence of infected and non-infected individuals. In populations of *T. cordubensis* Vargas and Cabello and *T. oleae* Voegele and Pointel (Hym.: Trichogrammatidae), bacterial infection is fixed or universal form. Furthermore, infection in *T. cacoeciae* Marchal has a genetic form and there is no reversal to normal state in antibiotic containing treatments or high temperature ones (Gottlieb et al., 2002; Vavre et al., 2004).

About 10% of males in population of *T. kaykai* Pinto & Stouthamer, bear chromosome paternal sex ratio (PSR) (Stouthamer et al., 2001), which is a kind of chromosome B, identified in *N. vitripennis* (Werren, 1991). This so-called paternal sex ratio (PSR) chromosome transmits only through sperm and shortly after fertilization triggers degeneration of the paternal genome, while keeping itself intact. This chromosome

does not follow Mendellian segregation. When female wasps mate with the male bearing PSR, the father chromosome in induced egg are destroyed and only the mother chromosome and PSR remain. These induce eggs, which bear a set of mother natural chromosomes and father PSR chromosomes are indeed haploid eggs turning to male offspring. In fact, this chromosome causes the male wasps exit from fertilized eggs of infected and non-infected eggs. The presence of this chromosome and such a mating structure as sustainability factor in mixed populations are of great importance (Stouthamer et al., 2001).

Male-killing

In some species, *Wolbachia* strains destroy the male embryos. Male-killing is seen in some insect orders (e.g. Coleoptera, Lepidoptera and Diptera). The factors inherited from mother cause the male offspring of *Adalia bipunctata* Linnaeus (Col.: Coccinellidae) to die at embryonic stage (Lusis, 1947). The infection rate in populations and different insects varied, for instance 20-30% of Russian population of *A. bipunctata* females and 80% of *Acraea encedon* Linnaeus, female are infected (Hurst et al., 1997). In infected populations, the bacterium induces a bias sex ratio toward females. Such phenotype allows more resources to be allocated to females and fitness of host reproduction and then bacterium transfer increases (Hurst et al., 1997).

Feminization

Wolbachia is known as a feminizing agent in some Amphipoda, Isopoda and in butterflies (Bouchon et al., 1998; Kageyama et al., 2002). Both groups of butterflies and crustaceans have a set of specific sexual chromosomes in which females have heterogametic state (males ZZ and females ZW). Feminization strains of *Wolbachia* in male individuals help to induce female genetic system and turn into fertile females. Therefore, there are a good opportunity for transfer of symbiotic bacterium from mother and daughters of next generation. In *Armadillidium vulgare* Latreilla (Isopoda: Armadillidae), this bacterium prevents from the formation of glands generating sexual hormone and changes the host's reaction to male sexual hormone. It is estimated that about 25% of Isopoda are infected by feminization-inducing bacteria, which often belong to super group B (Bouchon et al., 1998).

Application of *Wolbachia* to control insect pests

Molecular studies have major contribution in today's knowledge about the effects of *Wolbachia* on insects and other arthropods. It seems that *Wolbachia* is considered to be an effective agent in evolution and speciation influential on sex determining among arthropods with different mechanisms. Also, in an applied view, researchers have become interested in the role of different strains of *Wolbachia* in two fields; first, biological control as a carrier for spread of genetic modifier (Sinikin et al., 1997), or increase parasitoids efficiency (measured as parasitism success)(Stouthamer et al., 1993). And second, *Wolbachia* seems to play an important role in the pathogenesis of filarial diseases. Treatments aimed at reducing *Wolbachia* density in filarial worms could thus become useful; for example, in reducing the side effects of microfilaricidal treatments, for therapy of filarial diseases (Beard et al., 1993; Bandi et al., 2001).

Releasing incompatible males of *C. pipiens* and *Rhagoletis cerasi* to control populations of these insect pests are two new applications of this bacterium against medical vector and agricultural pest (Tram et al., 2003). The existence of bacterium in *Aedes aegypti* Linnaeus which is the vector of dengue fever brings about cytoplasmic incompatibility (Beard et al., 1993). Using the bacterium *Wolbachia* as a transgene of targeted gene among the population of *P. papatasi*, we can control the vector population and prevent the spread of disease *Leishmania major* (Parvizi et al., 2010).

The possibility of controlling *Liriomysa trifolii* Burgess (Dip: Agromyziidae) with cytoplasmic incompatibility mechanism and in a smaller scale, releasing incompatible males to control population of *Cadra cautella* Walker (Lep.: Pyralidae), are among the activities in the field of plant protection. In all above cases, releasing incompatible males lead to the reduction of pest population (Werren et al., 1995; Tram et al., 2006).

Wolbachia can be used in a variety of ways for disease suppression by decreasing the size of a vector population through (i) the release of *Wolbachia*-infected males that are incompatible with females (O'Connor et al., 2012; Hoffmann

et al., 2015) or (ii) the invasion of a *Wolbachia* strain that produces deleterious fitness effects particularly under seasonally variable environments (Rašić et al., 2014), and particularly by (iii) decreasing the ability of the vector population to transmit diseases through the invasion of a *Wolbachia* strain that interferes directly with transmission (Teixeira et al., 2008; Kambris et al., 2009; Moreira et al., 2009; Walker et al., 2011). The third way is considered particularly promising because it may not require ongoing management by health authorities; once a *Wolbachia* strain blocking disease transmission has invaded a target vector population by altering host reproduction, the *Wolbachia* strain should stay at a high frequency in that population without further releases being required (Hoffmann et al., 2011). It is also important to note that the three strategies are not mutually exclusive but rather complementary.

Wolbachia infections are being introduced into mosquito vectors of human diseases following the discovery that they can block transmission of disease agents. This requires mosquitoes infected with the disease-blocking *Wolbachia* to successfully invade populations lacking the infection. While this process is facilitated by features of *Wolbachia*, particularly their ability to cause cytoplasmic incompatibility, blocking *Wolbachia* may produce deleterious effects, such as reduced host viability or fecundity, that inhibit successful local introductions and subsequent spatial spread. Hoffmann and Turelli (2015) showed that outline an approach to facilitate the introduction and spread of *Wolbachia* infections by coupling *Wolbachia* introduction to resistance to specific classes of insecticides. The approach takes advantage of very high maternal transmission fidelity of *Wolbachia* infections in mosquitoes, complete incompatibility between infected males and uninfected females, the widespread occurrence of insecticide resistance, and the widespread use of chemical control in disease-endemic countries. This approach is easily integrated into many existing control strategies, provides population suppression during release and might be used to introduce *Wolbachia* infections even with high and seasonally dependent deleterious effects, such as the wMelPop infection introduced into *A. aegypti* for dengue control.

Kean et al. (2015) considered the use of endosymbiotic bacteria such as *Wolbachia*, which in some cases have proven to be remarkably efficient in disrupting arbovirus transmission by mosquitoes. Finally, they discussed the use of paratransgenesis as well as entomopathogenic fungi, which are also proposed strategies to control vector competence.

In nematode *Onchocerca volvulus* Bickel, bacterium removal by antibiotics leads to disturbance in the growth and development stages and eventually kills the nematode. Therefore, by manipulating genetically, we can prevent the humans to be inflicted by this worm or at least decrease the infection level (Hoerauf and Rao, 2007). *Wolbachia* is involved in producing vitamin B in bedbug, *Cimex lectularius* Latreille (Hem.: Cimicidae), consequently helps to increase fertility rate. In the case of bacterium removal, vitamin synthesis is disturbed and the growth and development do not become complete making the host death occur (Hosokawa et al., 2010).

The presence of bacterium *Wolbachia* in all populations of *Cinara cedri* Mimeur has been proved and increased the asexual reproduction. Moreover, it was observed that the members of Lachninae (Hemiptera) tend to be infected with *Wolbachia* (Augustinos et al., 2011). This bacterium induces parthenogenesis in the population of *B. praetiosa* Koch (Acari, Trombidiformes: Tetranychidae). In all individuals of *Bryobia* spp. having asexual reproduction, the presence of the bacterium has been proved. *Wolbachia* (super group B and K) has been detected in at least six asexual and one sexual *Bryobia* species and strains (Ros et al., 2009). Super group K is a new super group that has only been detected in *Bryobia* spp. so far (Ros et al., 2012). The researchers reported the presence of the bacterium in *Dacus ciliatus* Loew, *R. cerasi* Linnaeus, *Ceratitis capitata* Wiedemann, *Myiopardalis pardalina* Bigot and *Carypomyia vesuviana* Costa (Dip.: Tephritidae). They showed different levels of infection by two new strains of *Wolbachia* named *w*Cv1 and *w*Rc1. The rate of symbiosis had different values at two species, in such a way that in the population of *R. cerasi*, it was fixed, while it was variable in *C. vesuviana* population (Karimi and Darsouei, 2012).

Quantitative RT-PCR in *D. simulans* showed that the transcript of an important odorant receptor gene *or83b* in flies with fast olfactory response was significantly more than those with slow olfactory response. These results suggest that *Wolbachia* might increase olfactory response of flies by regulating the expression of olfaction-related genes in hosts (Peng and Wang, 2009). The effect of *Wolbachia* on *T.*

cordubensis and *Trichogramma deion* Pinto and Oatman under laboratory and greenhouse conditions exhibited that non-infected female wasps, had more dispersal ability in laboratory. On the other hand, bacteria-infected wasps showed higher parasitism rates under greenhouse conditions versus bisexual wasps of their conspecifics (Silva et al., 2000).

Recently, a novel approach to control mosquitoes by transinfection of life shortening maternally transmitted endosymbiont *Wolbachia* wMelPop strain from fruit fly *Drosophila* into mosquito population has been developed by researchers (Guruprasad et al., 2014).

Wolbachia has been identified from many Arthropod species including vectors of human diseases, crop and veterinary pests. Additionally, there are increasing interests toward the use of *Wolbachia* in biocontrol programs. Furthermore, *Wolbachia* can be used as a gene transfer tool or the transgenic insects. In this way, the target genes design and synthesize by genetic engineering then move into *Wolbachia* gene and appeared within insect population.

The specialists are very interested to produce offspring by unisexual reproduction in parasitoids. Unlike sexual wasps, Unisexual ones offsprings all is female. Accordingly, release of the parthenogenesis population cause to decrease release rate per area unit and releasing will be more affordable and predictable. Parthenogenic populations infected with the bacteria do not mate, then in biological control they preserve better in nature. One of the major applications of *Wolbachia* is *Trichogramma* unisexual populations that do not produce male eggs therefore increasing population rapidly and reduce mass production costs.

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