MARINA MAGALHÃES MOREIRA

NEGATIVE FITNESS EFFECTS OF *Wolbachia* INFECTION IN *Drosophila sturtevanti*

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Magister Scientiae*.

Orientadora: Karla Suemy Clemente Yotoko

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A meus pais, avós e irmã, que de perto ou de longe sempre me apoiaram
e cuidaram de mim.

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Resumo

MOREIRA, Marina Magalhães, M.Sc., Universidade Federal de Viçosa, fevereiro de 2020. **Negative fitness effects of W*olbachia* infection in *Drosophila sturtevanti***. Orientadora: Karla Suemy Clemente Yotoko.

A Seleção Natural favorece os indivíduos mais aptos em uma determinada população, enquanto a deriva genética pode aumentar ou diminuir a frequência de um determinado caracter por acaso. Nesse sentido, tanto a seleção natural quanto a deriva genética podem alterar a prevalência de endossimbiontes. *Wolbachia* é uma alfa-proteobactéria que infecta 52 % dos artrópodes. É principalmente transferida das mães para os filhos (transferência vertical). *Wolbachia* permanece em uma dada população de hospedeiros, aumentando seu *fitness* ou através de fenótipos reprodutivos que manipulam os hospedeiros, como a incompatibilidade citoplasmática (IC) ou a morte de embriões machos (MK). Como alternativa, *Wolbachia* pode permanecer por acaso através de uma transmissão vertical perfeita (100 %). Neste trabalho, investigamos a relação entre *Wolbachia* e *Drosophila sturtevanti*, uma espécie neotropical que presumivelmente adquiriu a infecção por transferência horizontal. Em nosso primeiro esforço de amostragem, entre 2015 e 2016, encontramos 100 % dos indivíduos infectados por *w*Stv MI, a mesma cepa encontrada em algumas linhagens de *D. sturtevanti* co- letadas no Panamá. O sucesso do tratamento com antibióticos mostrou que *D. sturtevanti* não depende da bactéria para sobreviver e se reproduzir. Assim, cruzamos diferentes combinações de indivíduos infectados e não infectados com o mesmo background genético para medir os componentes de aptidão da infecção e testar se wStvMI induz fenótipos reprodutivos em *D. sturtevanti*. Não encontramos evidências de IC ou MK. Além disso, *Wolbachia* parece reduzir o *fitness* dos hospedeiros infectados. Diante destes resultados, realizamos novas amostragens em 2019 na população da UFV para investigar a taxa de transmissão vertical. Para nossa surpresa, apenas 50 % dos indivíduos coletados estavam infectados e apresentaram transmissão vertical quase perfeita. Esse resultado sugere que a prevalência de *Wolbachia* está diminuindo na população de *D. sturtevanti* da UFV. Por

outro lado, dada a conectividade das populações da espécie, a prevalên- cia da bactéria pode flutuar devido à chegada de indivíduos infectados de outras populações. Novas amostragens de campo, por longos períodos de tempo, responderão se a infecção está diminuindo na população de UFV *D. sturtevanti* ou se a prevalência de infecção varia devido à chegada de indivíduos infectados de outras populações.

Palavras-chave: Drosófila Neotropical. Cruzamentos controlados. Amostragem de campo. Caracterização molecular.

Abstract

MOREIRA, Marina Magalhães, M.Sc., Universidade Federal de Viçosa, February, 2020. **Negative fitness effects of W*olbachia* infection in *Drosophila sturtevanti***. Advisor: Karla Suemy Clemente Yotoko.

Natural Selection favors the fittest individuals in a given population, while the genetic drift can increase or decrease by chance the frequency of a given character. In this sense, both natural selection and genetic drift affect the prevalence of endosymbionts. *Wolbachia* is an alpha-proteobacteria that infects 52 % of arthropods. It is primarily transferred from the mothers to their offspring (vertical transfer). *Wolbachia* remains in a given population of hosts by increasing their fitness or through reproductive phenotypes that manipulate the hosts, such as the cytoplasmic incompatibility (CI) or male-killing (MK). Alternatively, it can stay by chance through a perfect (100 %) vertical transmission. In this work, we investigated the relation- ship between *Wolbachia* and *Drosophila sturtevanti*, a Neotropical species that presumably acquired the infection through horizontal transfer. In our first sampling effort, performed in several sampling points in Minas Gerais, between 2015 and 2016, we found 100 % of individuals infected by *w*Stv MI, the same strain found in some *D. sturtevanti* lineages sampled in Panama. The success of antibiotic treatment showed that *D. sturtevanti* does not depend on the bacteria to survive and reproduce. We thus crossed different combinations of infected and non-infected individuals with the same genetic background to measure the fitness components of the infection and test whether *w*Stv MI induces reproductive phenotypes in *D. sturtevanti*. We found no evidence of CI or MK. Also, *Wolbachia* seems to reduce the fitness of infected hosts. Given these results, we performed a new sampling in the UFV population in 2019 to investigate the rate of vertical transmis- sion. Surprisingly, only 50 % of the samples were infected and presented an almost perfect vertical transmission. Such a result suggests that the prevalence of *Wolbachia* is declining in the UFV *D. sturtevanti* population. On the other hand, given the connectivity of *D. sturtevanti* populations, *Wolbachia* prevalence may fluctuate due to the arrival of infected individu-

als from other populations. New field samplings, over long time intervals, and the investigation of the effects of *Wolbachia* in a populational approach will help us to understand the epidemiological dynamics of *Wolbachia* in the UFV *D. sturtevanti* population.

Keywords: Neotropical Drosophila. Controled Crosses. Field Sampling. Molecular Characterization.

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1.1 Introduction

Natural Selection and Genetic Drift change the frequency of biological traits in a population: the first through differential survival of individuals with higher fitness, and the second due to chance (KIMURA, 1968). Such mechanisms usually describe the changes in the frequency of alleles that arise in a population through mutation or immigration. However, it is possible to apply them in studies involving the prevalence of endosymbionts in a given population. This interaction may generate new characters that also undergo selection and drift in the host populations (TURELLI, 1994).

Wolbachia, a bacterium of the order Ricketziales, is a common intracellular symbiont infecting about 52 % of arthropods (WEINERT et al., 2015). Like a mitochondrial genome, mothers vertically transmit *Wolbachia* to their offspring. To ensure its presence in the next generation, *Wolbachia* induces reproductive phenotypes that increase the fitness of infected females (WERREN; BALDO; CLARK, 2008). Such phenotypes include parthenogenesis (STOUTHAMER; LUCK; HAMILTON, 1990; WEEKS; BREEUWER, 2001; ARAKAKI; MIYOSHI; NODA, 2001); feminization of male embryos (WERREN; BEUKEBOOM, 1998; NARITA et al., 2007; NEGRI et al., 2008); death of male embryos (DYER; JAENIKE, 2004; KAGEYAMA; TRAUT, 2004), and cytoplasmic incompatibility (CI), that reduces the offspring of infected males and uninfected females (WERREN, 1997; BECKMANN; RONAU; HOCHSTRASSER, 2017).

However, the inconsistency between phylogenies of hosts and *Wolbachia* suggests that this bacteria can also invade new hosts through horizontal transmission (WERREN; WINDSOR; GUO, 1995; BALDO et al., 2006a). It means that infections took place after the diversification of hosts, *i.e.*, most hosts did not inherit the bacteria from their common ancestors (BAILLY-BECHET et al., 2017). Therefore, new infections should target the germinative cells of the new host (FRYDMAN et al., 2006) and cause CI to increase their frequency in the host populations (TURELLI, 1994). Since CI is harmful to infected males (DUFFY et al., 2018), natural selection can suppress it, which could result in a loss of the bacteria in the host population (KOEHNCKE et al., 2009). Thus, to remain in the host population, *Wolbachia* should increase the fitness of its specific host (MEANY et al., 2019) or behave like a neutral trait, maintained by perfect maternal transmission (CHARLAT; BALLARD; MERCOT, 2004).

In this work, we aimed to study the *Wolbachia* infection found in *Drosophila sturtevanti* Duda, 1927 (Diptera: Drosophilidae), collected in Minas Gerais, Brazil. *D. sturtevanti* belongs to the *Drosophila saltans* group and lives in different environments of the Neotropical region. This species probably occurs in large populations once it is one of the most abundant species among the native taxa at field samples over time (DOBZHANSKY; PAVAN, 1950; GOTTSCHALK et al., 2007; DAMATA; MCGEOCH; TIDON, 2008; CHAVES; TIDON, 2008). In 2016, Miller and Riegler showed that this species hosts *Wolbachia* strains related to *w*Stv. Such strains are phy-

logenetically distant from strains infecting other *saltans* species and have a high identity with the *w*Whi strain found in *Lutzomyia shannoni* (Dyar 1929) (Diptera: Psychodidae) and *Lutzomyia whitmani* (Antunes and Coutinho, 1939). Ono et al. (2001) suggested a horizontal transfer of *w*Whi between *L. shannoni* and *L. whitmani* because such strain is identical at these two distantly related species.

Once Miller and Riegler (2006) studied *D. sturtevanti-Wolbachia* interaction at Central America, we wonder 1. whether Brazilian populations, especially in the Viçosa region, are also infected, 2. which strain or strains are present. 3. whether *D. sturtevanti* depends on *Wolbachia* to survive and reproduce; 4. if the strain or strains increase hosts fitness, through CI or increasing the fertility or longevity; and 5. what is the rate of vertical transmission of the bacteria in a local population.

1.2 Materials and Methods

1.2.1 Sampling and maintenance of fly lines

In 2015, we started our studies on the association between *Wolbachia* and *D. sturtevanti*. Our objective was to sample such species in the town of Viçosa (Universidade Federal de Viçosa, UFV population) and neighboring locations. Therefore, we placed traps (MEDEIROS; KLACZKO, 1999), using banana or jackfruit baits, since both are efficient baits to attract *D. sturtevanti* (DOBZHANSKY; PAVAN, 1950). Initially, we sampled *D. sturtevanti* in five locations, as specified in Table 1.1. The traps were lived at each point for 48 h. After that, we sent them to the lab to search for females of the *saltans* group. Such females were individually put in tubes containing banana-barley culture medium in the laboratory at 23 , with a controlled 12h dark/12h light photoperiod for oviposition and obtaining F_1 males, which which were properly identified (MARKOW; O'GRADY, 2005). Those lines identified as *D. sturtevanti* were maintained in the lab for further experiments.

1.2.2 Detection of *Wolbachia*

From each isofemale line identified as *D. sturtevanti*, we extracted DNA from a set of five females following the standard protocol of the Wizard Genomic DNA Purification kit (Promega A1120). We measure the quality of the extracted DNA with a standard PCR (annealing at 55) with the primers COI 106F 5'-ATTCAGAATATGTTCAG-3' and COI 154R 5'-TTTAATTTTACCTGGATTTGG-3' (Wolfgang Miller, personal communication), which generated a 650 bp fragment of the COI mitochondrial gene. We discard all samples that showed no bands at agarose gel (1.0 %). The remaining samples were tested for *Wolbachia* infection with a standard PCR (annealing at 67) using primers WSP-81F 5'-TGGTCCAATAAGTGATGA AGAAACTAGCTA -3' and WSP-691R 5'-AAAAATTAAACGCTACTCCAG CTTCTGCAC-3' (modified de Zhou *et al.*, 1998) which amplifies a 365 bp

base pair fragment of the *wsp* gene (*Wolbachia* Surface Protein). The visualization of the band in 1.0 % agarose gel was interpreted as evidence of infection in high titer while non-visualization was interpreted as an absence or undetectable levels of infection.

All PCR reactions included, in addition to the studied samples, a positive control, and three negative controls. The positive control is a sample of *Drosophila willistoni* (Sturtevant, 1916) DNA infected by *Wolbachia* (JS 6.3 - Miller and Riegler, 2006). As negative controls, we used DNA samples of non-infected lineages: 1118 of *Drosophila melanogaster* Meigen, 1830; STC of *Drosophila simulans* Sturtevant, (1919); or will3 of *D. willistoni* (MILLER; EHRMAN; SCHNEIDER, 2010). We also used a blank for the DNA extraction process (without biological material), and a blank for the PCR reaction (no DNA sample).

1.2.3 Strain(s) identification

To identify the strain or strains of *Wolbachia* infecting *D. sturtevanti*, we used the primers wsp-F1 5-'GTCCAATARSTGATGARGAAAC-3' and wsp-R1 5-'CYGCACCAAYAGYRCTRTAAA-3' (BALDO et al., 2006b) in standard PCRs (annealing to 69) to amplify and sequence a 546 bp fragment of the *wsp* gene (*Wolbachia* Surface Protein). We also used five pairs of MLST primers (Multilocus Sequence Typing System - https: // pubmlst.org/ *Wolbachia*).

1.2.4 Antibiotic treatment

Females of the st8 line, collected in 2016 at UFV population, were placed to lay eggs in culture medium prepared with 0.01 % antibiotic Rifampicin. Such treatment was maintained for four generations (LI et al., 2014). The fifth-generation was transferred and maintained in an antibiotic-free medium for at least four generations. Such recovery time minimizes problems caused by antibiotic treatment, such as the reduction of gut biota (DE-CRESPIGNY; PITT; WEDELL, 2008; LI et al., 2014). At each generation of treatment and recovery, we used the *Wolbachia* detection protocol previously described to confirm the efficiency of the treatment. We called the line treated with Rifampicin as st8R.

1.2.5 Larval density control

The evaluation of fitness components requires the control of larval density to obtaining the adults for the posterior crosses. Such control is essential to avoid comparisons between the offspring of adults with different levels of nutrition in the larvae phase, which may skew the results (OHBA, 1961). Therefore, we transferred sets of 50 first stage larvae to tubes containing 10 ml of banana-barley culture medium (SNOOK et al., 2000), and used the resulting adults in the further experiments.

1.2.6 Evaluation of fitness components

Fertility assessment

At the beginning of the fifth generation of recovery (after the antibiotic treatment), we set up 15 replica of each the four possible crosses with the lines st8 and st8R: (1) ♀st8 X ♂st8, (2) ♀st8 X ♂st8R, (3) ♀st8R X ♂st8 and (4) ♀st8R X ♂st8R. Each cross contained a one-day-old virgin couple. Such couples were individually placed at Petri dishes containing culture medium composed of grape juice (30 %), agar (0.8 %), and a thin layer of yeast. During 14 days, we searched for eggs and placed each couple on a new plate. We inferred fertility using the number of eggs deposited daily on the plates. We discard plates without eggs after 24h, and those with eggs were kept for more 48h to count the number of larvae present in each plate. We compared the four different crosses using the GLMM with the lme4 package (BATES et al., 2015) in the R program (RCORETEAM, 2019) .

Cytoplasmic incompatibility test

Cytoplasmic incompatibility (CI) stems from the lower fertility of crosses involving uninfected females and infected males (BOURTZIS; BRAIG; KARR, 2003). We estimated CI by comparing the number of eggs and larvae of the incompatible cross (♀st8R x ♂st8) with the remaining crosses. A significant lower ratio at cross 3 would indicate that *Wolbachia* causes CI in *D. sturtevanti*. We performed statistical analysis using GLM (quasi-binomial family). An ANOVA was performed on the model using the Turkey test for pairwise comparisons in the R program (RCORETEAM, 2019) .

F_1 adult count and sex ratio

To assess the total offspring and the sex ratio of infected (st8) and treated (st8R) lines, we set up 25 replicas of the crossing 5 (♀st8 X ♂st8) and 21 of the crossing 6 (♀st8R X ♂st8R) in tubes containing culture medium. Each tube contained one female and two six-day-old virgin males. We kept the adults together for 48 h for copulation, and after that period, we removed the males and maintained the females in the tubes for another 24 hours for oviposition. We counted and removed the adults from the tubes daily. We froze the adults at -20 °C in 100 % alcohol for possible further analyzes. As male-killing is one of the possible phenotypes to occur in drosophilids (HURST et al., 2000; WERREN; BALDO; CLARK, 2008), we obtained the sex ratio from the total number of males and females that emerged in each tube. We analyzed the data with GLMM using the lme4 package (BATES et al., 2015) in the R program (RCORETEAM, 2019).

Life cycle

We compared the development time of the st8 and st8R lines by counting the number of days until we saw the first egg, first larva (crosses 1 and 4),

first pupa, and first adult (crosses 5 and 6). Analyzes were performed with GLM using the R program (RCORETEAM, 2019).

Longevity

An essential component of fitness is the longevity of adults since longer they live, more opportunities they have to copulate, and generate offspring. We thus determined the longevity of males and females newly emerged from the lines st8 and st8R. We set up seven tubes containing banana-barley culture medium with five males and five females of st8 and ten of such tubes of st8R. We searched for dead individuals and removed them for the tubes every day. To avoid generation overlaps, we transferred adults to new tubes every five days. To assess longevity, we chose to show survival curves, obtained with the Kaplan-Meier method (KAPLAN; MEIER, 1958) and the Log-rank test (BLAND; ALTMAN, 2004), from the Survival package (THERNEAU, 2015) implemented in R (RCORETEAM, 2019).

Vertical transmission rate

Estimation of the vertical transmission rate of *Wolbachia* in a given host requires new field samplings (CHARLAT; BALLARD; MERCOT, 2004) that were performed on 2019 February and March at UFV population. After obtained the F_1 to confirm the identification of *D. sturtevanti* females (See 0.2.1 - Sampling and maintenance of fly lines), we tested them for the presence of *Wolbachia* (item 1.2.2 - Detection of *Wolbachia*)- using a single fly DNA extraction). For those isofemale lineages infected by *Wolbachia*, we also tested five males and five females from the F_1 and F_5 for the presence of *Wolbachia*.

1.3 Results

1.3.1 Fly sampling and infection prevalence

Table 1.1 shows the samplings made in 2015 and 2016 in Viçosa (UFV population); Araponga (Serra do Brigadeiro border); and Juiz de Fora, with the number of *D. sturtevanti* lineages maintained at the lab. All the lineages evaluated were infected with *Wolbachia* in high titer, resulting in a 100 % prevalence of infection in the sampled points.

1.3.2 *Wolbachia ID*

We sequenced the *wsp* of at least one individual of each sampling site. All sequences obtained were identical to the *w*Stv MI strain (accession numbers AY620215.1 and DQ412110.1), found in other specimens of *D. sturtevanti*. Miller and Riegler (2006) documented AY620215.1 on samples collected in Maria Eugenia, Panama, while Mateos et al. (2006) documented DQ412110 (unfortunatelly without origin). The wStvMI sequence is identical to allele 89 at the MLST database (Public databases for molecular typing and

Table 1.1: Collections of *Drosophila sturtevanti* in the cities of Viçosa (V), Araponga (A), and Juiz de Fora (JF) in the years 2015 and 2016 with the number (N) of isofemale lines initiated, all infected in high titer

Date	Site	Lat S	Long	Bait	N
Jan/15	UFV population* (V)	20°45′48″	42°51′53′	Banana	5
Feb/15	UFV population* (V)	20°48′10″	42°51′48″	Banana	2
Apr/15	Brigadeiro (A)	20°40′23″	42°29′28″	Banana	2
	UFV population (V)*	20°45′34″	42°52′14″	Banana	1
	Juiz de Fora (JF)	21°48′06″	43°23′16″	Jackfruit	2
May/15	UFV population* (V)	20°45′34″	42°52′14″	Jackfruit	1
	UFV population* (V)	20°45′35″	42°52′09″	Banana	1
Aug/15	UFV population* (V)	20°45′48″	42°51′53″	Banana	10
Aug/16	UFV population* (V)	20°45′48″	42°51′53″	Banana	11

*Universidade Federal de Viçosa - UFV Population.

microbial genome diversity), obtained from an unknown host collected in Panama. This sequence is usually divided into four regions (HVR1 - HVR4), with alleles 54, 28, 62 and 60, respectively. Table 1.2 shows the identification of the MLST alleles obtained by sequencing a sample collected at UFV population (**Genbank XXX)** as well as the three *Wolbachia* strains that best match with the alleles found here.

Table 1.2: Alleles identification (Id) for the MLST loci found in *w*Stv MI (collected at UFV) and the three *Wolbachia* strains that best match with it, their host, and sampling sites (when available).

Strain	Id	wsp	gatB	coxA	hcpA	ftsz	fbpA	Host
*w*Stv MI†		89	23	2	86	21	26	*D. sturtevanti*
Aspa-A‡	3	5	23	2	26*	21	26	*Acromis sparsa*
A-PanAA‡	91	89	23	60*	86	21	26	-

*Alleles that differ from *w*Stv MI.
† Sampled in Viçosa - MG.
‡ Sampled in Panama

1.3.3 Antibiotic treatment

From the second generation of treatment, it was no longer possible to detect the infection in the line called st8R using the *wsp* PCR (Item 1.2.2 - Detection of *Wolbachia*). The treatment took place between February and June 2018, and the lineage remains in the lab (February/2020). Periodic checks confirm the absence or low titer of the infection.

1.3.4 Fecundity

We found no significant difference in fecundity or larvae production between crosses 1 (♀st8 X ♂st8) and 2 (♀st8 X ♂st8R), involving infected females, or between crosses 3 (♀st8R X ♂st8) and 4 (♀st8R X ♂st8R), involving uninfected females. However, females treated with antibiotics showed higher fecundity and produced more larvae over the days (Figure 1.1).

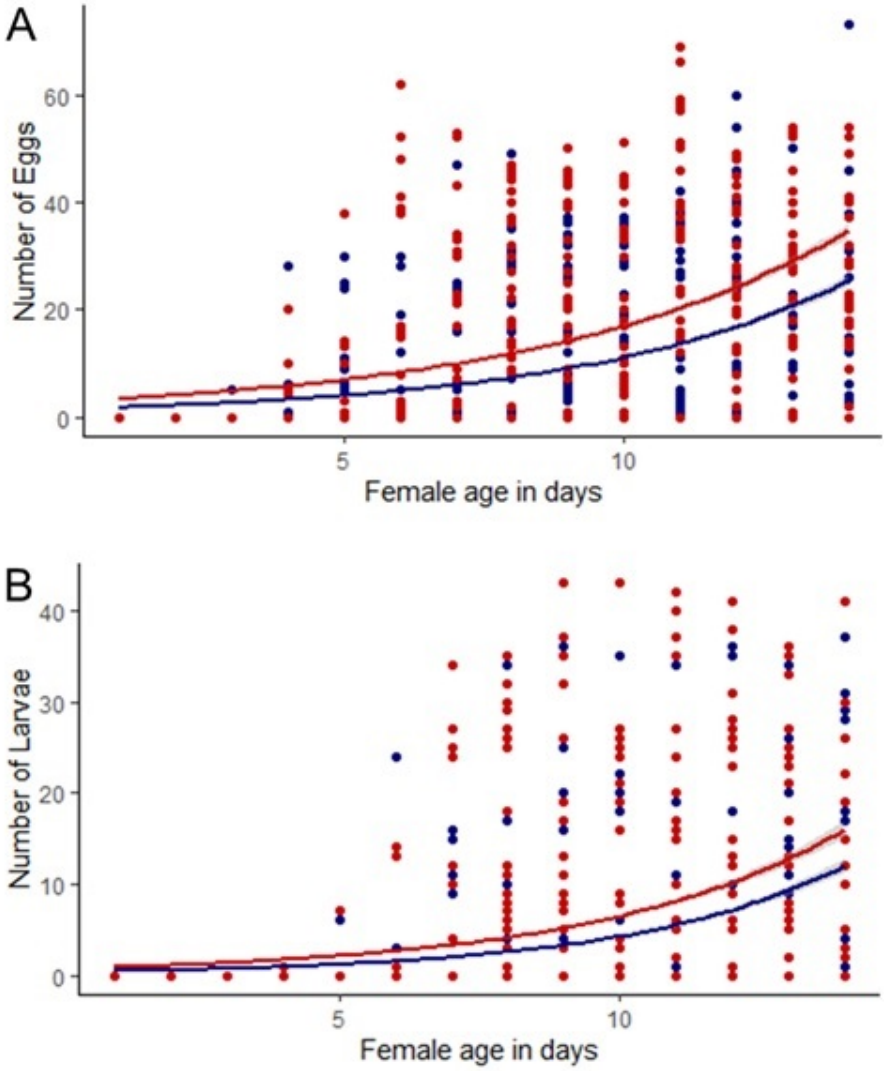

Figure 1.1: (A) Fecundity and (B) larvae production of crosses with *Drosophila sturtevanti* females infected by *Wolbachia* (st8, blue) and treated with Rifampicin (st8R, red). The difference between lines was significant in A ($N = 60, LRT : \chi^2_1 = 14.564$, $p = 0.0001355$) and B ($N = 60, LRT : \chi^2_1 = 20.585$, $p = 5.703e^{-06}$).

1.3.5 Cytoplasmic incompatibility (CI)

CI occurs when the larvae/egg ratio is lower at crossing 3 (♀st8R X ♂st8) than at the other crosses. We found no significant difference between the four crosses assembled ($F_{1,406} = 1.073$, $p = 0.3009$), indicating that *w*Stv MI did not cause CI in *D. sturtevanti*.

1.3.6 F_1 counting and sex ratio

The offspring produced by crosses 5 (♀st8 X ♂st8) and 6 (♀st8R X ♂st8R) showed no differences in terms of the number of adults ($N = 46, LRT : \chi^2_1 = 2.38145$, $p = 0.1228$) or sex ratio ($N = 46, LRT : \chi^2_1 = 0.34352$, $p = 0.5578$). Females and males emerged concomitantly in both lines ($N = 46, LRT : \chi^2_1 = 1.63480$, $p = 0.2010$).

1.3.7 Life cycle

The lines st8 and st8R took an average of 6.44 days until the first egg [$\chi^2_{(1,N=32)} = 140.94$, $p = 0.1432$], 8.15 days until the first larva [$\chi^2_{(1,N=20)} = 5.64$, $p = 0.6768$], and 22.45 days until the first adult [$\chi^2_{(1,N=46)} = 7.52$, $p = 0.5491$]. However, pupae arose significantly earlier at st8R ($\bar{x} = 12.8$ days) than at st8 ($\bar{x} = 13.88$ days) $F_{1,46} = 7.20$, $p = 0.01021$ (Figure 1.2).

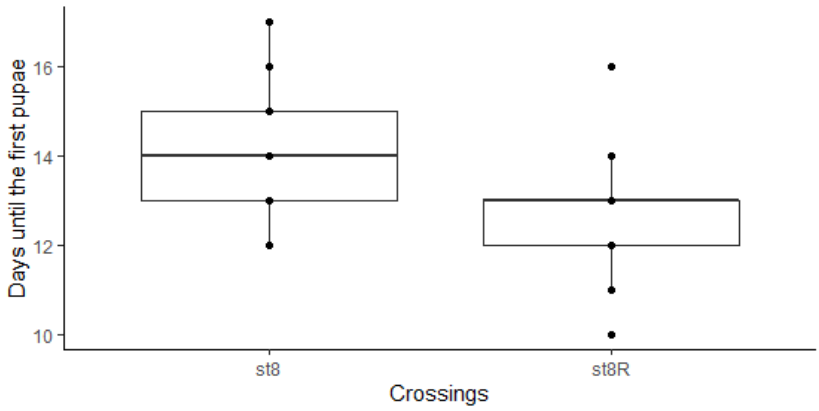

Figure 1.2: Number of days until the first pupae of lines st8, infected by *Wolbachia* and st8R, treated with Rifampicin at *D. sturtevanti* ($p = 0.01021$).

1.3.8 Longevity

We found no significant difference in the longevity of the lines st8 and st8R ($p = 0.6969$). However, in both lines, males lived longer than females ($p = 0.00010$) (Figure 1.3).

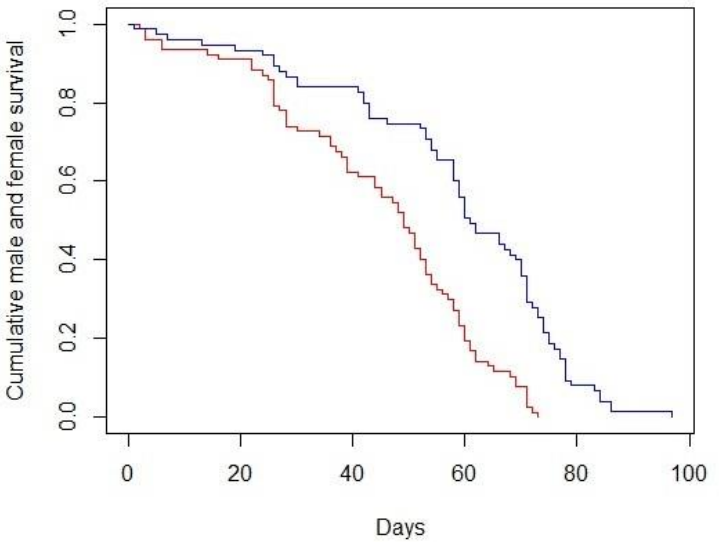

Figure 1.3: Survival curves of females (red) and males (blue) of *Drosophila sturtevanti* infected with *Wolbachia* and treated with Rifampicin.

1.3.9 Vertical trasmission rate

From the 12 *D. sturtevanti* females sampled in 2019 in the UFV population (Table 1.3), only six were infected with *Wolbachia*, resulting in a prevalence of 50 %. The st70 line generated an uninfected male at F_1 and an uninfected female at F_5, indicating that vertical transmission is not perfect in this population. The *wsp* sequence was identical to that found in the 2015 and 2016 samples.

Table 1.3: Amplification of the *wsp* sequence in lines of *D. sturtevanti* sampled in 2019. Sample 1 obtained in February and sample 2 obtained in March at UFV population. G_0 corresponds to the infection status of the founder female and F_1 and F_5 corresponds to the number of infected from 10 tested individuals at the first and the fifth generation.

Sample	Lineage	G_0	F_1	F_5
Sample 1	st04	w^+	10	10
	st05	w^-	0	0
	st12	w^-	0	0
	st14	w^-	0	0
	st21	w^-	0	0
	st22	w^+	*	10
Sample 2	st54	w^+	10	10
	st58	w^-	0	0
	st66	w^+	10	10
	st68	w^-	0	0
	st69	w^+	10	10
	st70	w^+	9	9

* In the first generation st22 did not produce enough individuals to test.

1.5 Discussion

All samples of *D. sturtevanti* collected in 2015 and 2016 at Minas Gerais state hosted *Wolbachia* (Table 1.1) of the same strain, *w*Stv MI, which differs from the pattern found by Miller and Riegler (2006) in Central America, where some populations of *D. sturtevanti* were uninfected, while others harbored different but similar strains of *Wolbachia*, such as *w*Stv SG and *w*Stv Pan6. The *wsp* sequence of *w*Stv MI also has high similarity with strains found in other species of insects sampled in the American continent such as *L. whitmani*, sampled in Bahia, Brazil; *L. shannoni*, sampled in Puerto Bocaya, Colombia (ONO et al., 1992); *Belonocnema treatae* Mayr, 1881 (*Hymenoptera*: Cynipidae: Cynipini), sampled in South USA (SCHULER et al., 2018); and *Lasioglossum hitchensi* Gibbs 2012 (*Hymenoptera*: Halictidae: Halictini), sampled in Central Kentucky, USA.

Table 1.2 shows that the MLST alleles profile of *w*Stv MI is very similar to those found in hosts sampled in America. According to the MLST database (https://pubmlst.org/*Wolbachia*/), the alleles found in four of the five loci studied here (23 for *gatB*, 86 for *hcpA*, 21 for *ftsZ*, and 26 for *fbpA*), were found exclusively in samples collected in Panama, Colombia, and the United States. More samples of strains harbored by Neotropical insects will help to elucidate whether the strain described in this work originated in Panama or is part of a set of ordinary strains in the Neotropical region.

Since we found *Wolbachia* in every *D. sturtevanti* sampled in 2015 and 2016, we we asked whether this species depends on this endosymbiont to survive, as found in *Asobara tabida* (Ness van Esembeck, 1834) (Hy-

menoptera: Braconidae), where the antibiotic treatment resulted in ovaries development faults (DEDEINE; BOULÉTREAU; VAVRE, 2005); or *Cimex lectularius* Linnaeus, 1758 (Hemiptera: Cimidae), that depends on *Wolbachia* to produce vitamin B (NIKOH et al., 2014). However, the success of the treatment with Rifampicin told us the contrary: *D. sturtevanti* does not depend on high-titer *Wolbachia* to survive or reproduce in the laboratory. Indeed, beyond the lines used in this work, st8 and st8R, which had controlled larvae density, other six infected, and one antibiotic-treated lines of our lab, have been successfully maintained. One of our lines, st11, sampled in 2016 at the UFV population, lost the infection or reduced it after 15 generations in the laboratory with no antibiotic treatment.

Since *D. sturtevanti* does not depend on *w*Stv MI, we questioned whether this strain causes cytoplasmic incompatibility (CI), an ingenious strategy used by *Wolbachia* to enhance the infected female fertility and spread in its host populations (TURELLI; HOFFMANN, 1991; TURELLI; HOFFMANN, 1995; OTE; YAMAMOTO, 2020). However, our results show no evidence of CI in st8 (the four combinations of crosses presented the same fertility). Considering that CI is usually stronger in the laboratory than in the field (HOFFMANN; TURELLI; HARSHMAN, 1990; HOFFMANN; HERCUS; DAGHER, 1998; TURELLI; HOFFMANN, 1995), CI in this population is unlikely. Following Turelli (1994), some strains do cause CI, and others do not because the incidence of CI depends on the time of the relationship of the strain with its host, *i.e.*, after some time, because of natural selection acting in favor of infected males, the strains lose the ability to induce CI in their hosts (DUFFY et al., 2018). Indeed, Martinez *et al.* (2015) injected *w*Stv into uninfected embryos of *D. simulans* and detected CI, showing that such strain does cause CI in another species host. Unfortunately, they did not specify the *w*Stv used in their experiment.

Although rare in *Drosophila* (MONTENEGRO et al., 2006) male-killing, or death of male embryos, are other phenotypes induced by *Wolbachia* that can increase its prevalence in a host population (HURST et al., 2000; DYER; JAENIKE, 2004) since infected females produce more female offspring than uninfected ones (WERREN, 1997). We tested for this possibility comparing the sex ratio of the treated and infected lineages and found no differences. Indeed, the sex ratio was not significantly different from 1.0, indicating that the *w*Stv MI strain does not bias the sex ratio in *D. sturtevanti*.

Another explanation for the high prevalence of *w*Stv MI in *D. sturtevanti* is an improvement in the fitness of infected hosts. Most of our results show no differences between infected and treated lines regarding the production of adults, the development time (until the first egg, larvae, or adult), or the adults' longevity.

Instead, some of our results indicate that *w*Stv MI has harmful effects on *D. sturtevanti* because it decreases fertility and slows down the pupation processes of infected females. Figure 1.1 reveals that treated (st8R) produced more eggs and larvae than infected females (st8). Notwithstanding, we evaluated eggs and larvae numbers over 14 days, and found that the differences between st8 and st8R increased over time. In contrast, we counted the adults after 72 hours of oviposition (See F_1 adult count and

sex ratio) from females that copulated at six days old. Therefore, younger females of both lineages produce the same number of eggs, larvae, and adults, allowing us to infer that the fitness decrease found in st8 is more relevant under laboratory conditions than in the field. Figure 1.2 indicates that the pupation process of st8R larvae started some days before than of st8 ones, reducing their development time (RODRIGUES et al., 2015), their risk of predation, and avoiding problems with depletion resources (KRIJGER; PETERS; SEVENSTER, 2001).

Maintaining high *Wolbachia* prevalence requires CI (TURELLI, 1994) and a high rate of vertical transmission (CHARLAT; BALLARD; MERCOT, 2004) or an improvement in the host's fitness (MEANY et al., 2019). As already discussed, we found no reproductive phenotypes and detected a slight fitness reduction of infected individuals. Thus, we questioned whether the high prevalence found in *D. sturtevanti* populations from Minas Gerais sampled in 2015/2016 resulted from the combination of perfect transmission of the bacteria to the offspring and genetic drift. In this specific case, we speculated whether the infection causes a nearly neutral phenotype (OHTA, 1992; CHARLAT; BALLARD; MERCOT, 2004). Notwithstanding, this hypothesis requires a small and isolated host population, which hardly applies to *D. sturtevanti*, a species known to be abundant (DOBZHANSKY; PAVAN, 1950; GOTTSCHALK et al., 2007; DAMATA; MCGEOCH; TIDON, 2008; CHAVES; TIDON, 2008). Also, since the Minas Gerais populations share *w*Stv MI with a population from Panama, the isolation of any *D. sturtevanti* is very unlikely.

Accordingly, our vertical transmission measures based on newly collected females in 2019 showed that vertical transmission was not perfect, and we realized that the prevalence dropped to 50 %. Such imperfect transmission explains the infection lost in the st11 strain (collected in 2016) and the reduced prevalence of *Wolbachia* in the UFV *D. sturtevanti* population.

Together, our results indicate that the 100 % prevalence found in 2015-2016 was temporary, and *w*StvMI may leaving this population of hosts. According to the models proposed by Turelli (1994) and Koehncke *et al.* (2009), the association *D. sturtevanti* - *w*StvMI would be in an intermediate stage. *w*StvMI no longer causes CI in *D. sturtevanti*, indicating that the species may have become resistant, as predicted by the models. Also, the transmission rate is high in *D. sturtevanti*, but insufficient to maintain the high prevalence in the study population, mainly because *Wolbachia* harms its host. Accordling, Bailly-Bechet *et al.* (2017) proposed that *Wolbachia* infections in arthropods tend to be temporary, suggesting that horizontal transmission between species is essential to maintain the global *Wolbachia* pandemic. Therefore, it is possible to infer that *Wolbachia* prevalence fluctuates due to the arrival of infected individuals. Further collections at the UFV population will show us whether *D. sturtevanti* is losing *Wolbachia* or whether this interaction fluctuates over time.

Bibliography

ARAKAKI, N.; MIYOSHI, T.; NODA, H. *Wolbachia*–mediated parthenogenesis in the predatory thrips *Franklinothrips vespiformis* (thysanoptera: Insecta). **Proceedings of the Royal Society of London Series B: Biological Sciences**, v. 268, p. 1011–1016., 2001.

BAILLY-BECHET, M.; MARTINS-SIMÕES, P.; SZÖLLOSI, G. J.; MIALDEA, G.; SAGOT, M. F.; CHARLAT, S. How long does *Wolbachia* remain on board? **Molecular Biology and Evolution**, v. 34, p. 1183–1193., 2017.

BALDO, L.; BORDENSTEIN, S.; WERNEGREEN, J. J.; WERREN, J. H. Widespread recombination throughout *Wolbachia* genomes. **Molecular Biology and Evolution**, v. 23, p. 437–449., 2006.

BALDO, L.; HOTOPP, J. C. D.; JOLLEY, K. A.; WERREN, J. H. Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. **Applied and Environmental Microbiology**, v. 72, p. 7098–7110., 2006.

BATES, D.; MAECHLER, M.; BOLKER, B.; WALKER, S. Fitting linear mixed-effects models using lme4. **Journal of Statistical Software**, v. 67, p. 1–48., 2015.

BECKMANN, J.; RONAU, J.; HOCHSTRASSER, M. A *Wolbachia* deubiquitylating enzyme induces cytoplasmic incompatibility. **Nature Microbiology**, v. 2, p. 17007., 2017.

BLAND, J. M.; ALTMAN, D. G. The logrank test. **BMJ**, v. 328, p. 1073., 2004.

BOURTZIS, K.; BRAIG, H. R.; KARR, T. L. Cytoplasmic incompatibility. in insect symbiosis. **CRC Press**, p. 217–246., 2003.

CHARLAT, S.; BALLARD, J. W. O.; MERCOT, H. What maintains noncytoplasmic incompatibility inducing *Wolbachia* in their hosts: a case study from a natural *Drosophila yakuba population*. **Journal of Evolutionary Biology**, v. 17, p. 322–330., 2004.

CHAVES, N. B.; TIDON, R. Biogeographical aspects of drosophilids (diptera, drosophilidae) of the brazilian savanna. **Revista Brasileira de Entomologia**, v. 52, p. 340–348., 2008.

DAMATA, R. A.; MCGEOCH, M.; TIDON, R. Drosophilid assemblages as a bioindicator system of human disturbance in the brazilian savanna. **Biodiversity and conservation**, v. 17, p. 2899., 2008.

DECRESPIGNY, F. E. C.; PITT, T. D.; WEDELL, N. Increased male mating rate in *Drosophila* is associated with *Wolbachia* infection. **Journal of Evolutionary Biology**, v. 17, p. 2899., 2008.

DEDEINE, F.; BOULÉTREAU, M.; VAVRE, F. *Wolbachia* requirement for oogenesis: occurrence within the genus *Asobara* (hymenoptera, braconidae) and evidence for intraspecific variation in *A. tabida*. **Heredity**, v. 95, p. 394–400., 2005.

DOBZHANSKY, T.; PAVAN, C. Local and seasonal variations in relative frequencies of species of *Drosophila* in brazil. **Journal of Animal Ecology**, v. 19, p. 1–14, 1950.

DUFFY, E.; ARCHER, C. R.; SHARMA, M. D.; PRUS, M.; JOAG, R. A.; RADWAN, J.; HOSKEN, D. J. *Wolbachia* infection can bias estimates of intralocus sexual conflict. **Ecology and evolution**, v. 9, p. 328–338., 2018.

DYER, K. A.; JAENIKE, J. Evolutionarily stable infection by a male-killing endosymbiont in *Drosophila innubila*: molecular evidence from the host and parasite genomes. **Genetics**, v. 168, p. 1443–1455, 2004.

FRYDMAN, H. M.; LI, J. M.; ROBSON, D. N.; WIESCHAUS, E. Somatic stem cell niche tropism in *Wolbachia*. **Nature**, v. 441, p. 509–512., 2006.

GOTTSCHALK, M. S.; TONI, D. C. D.; VALENTE, V. L.; HOFMANN, P. R. Changes in brazilian *Drosophilidae* (diptera) assemblages across an urbanisation gradient. **Neotropical entomology**, v. 36, p. 848–862., 2007.

HOFFMANN, A. A.; HERCUS, M.; DAGHER, H. Population dynamics of the *Wolbachia* infection causing cytoplasmic incompatibility in *Drosophila melanogaster*. **Genetics**, v. 148, p. 221–231., 1998.

HOFFMANN, A. A.; TURELLI, M.; HARSHMAN, L. G. Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. **Genetics**, v. 126, p. 933–948., 1990.

HURST, G. D.; JOHNSON, A. P.; SCHULENBURG, J. H.; FUYAMA, Y. Male-killing *Wolbachia* in *Drosophila*: a temperature-sensitive trait with a threshold bacterial density. **Genetics**, v. 156, p. 699–709., 2000.

KAGEYAMA, D.; TRAUT, W. Opposite sex-specific effects of *Wolbachia* and interference with the sex determination of its host *Ostrinia scapulalis*. **Proceedings of the Royal Society of London**, v. 271, p. 251–258., 2004.

KAPLAN, E.; MEIER, P. Nonparametric estimation from incomplete observations. **Journal of the American Statistical Association**, v. 53, p. 457–481., 1958.

KIMURA, M. Evolutionary rate at the molecular level. **Nature**, v. 217, p. 624–626, 1968.

KOEHNCKE, A.; TELSCHOW, A.; WERREN, J. H.; HAMMERSTEIN, P. Life and death of an influential passenger: *Wolbachia* and the evolution of ci-modifiers by their hosts. **PLOS ONE**, v. 4, p. e4425, 2009.

KRIJGER, C. L.; PETERS, Y. C.; SEVENSTER, J. G. Competitive ability of neotropical *Drosophila* predicted from larval development times. **Oikos**, v. 92, p. 325–332., 2001.

LI, Y.; FLOATE, K. D.; FIELDS, P. G.; PANG, B.-P. Review of treatment methods to remove *Wolbachia* bacteria from arthropods. **Symbiosis**, v. 62, p. 1–15., 2014.

MARKOW, T. A.; O'GRADY, P. *Drosophila*: a guide to species identification and use. **Elsevier.**, 2005.

MEANY, M. K.; CONNER, W. R.; RICHTER, S. V.; BAILEY, J. A.; TURELLI, M.; COOPER, B. S. Loss of cytoplasmic incompatibility and minimal fecundity effects explain relatively low *Wolbachia* frequencies in *Drosophila mauritiana*. **Evolution**, v. 73, p. 1278–1295., 2019.

MEDEIROS, H.; KLACZKO, L. A weakly biased *Drosophila* trap. ***Drosophila* Information Service**, v. 82, p. 100–102, 1999.

MILLER, W. J.; EHRMAN, L.; SCHNEIDER, D. Infectious speciation revisited: Impact of symbiont-depletion on female fitness and mating behavior of *Drosophila paulistorum*. **PLOS Pathogens**, v. 6, p. e1001214., 2010.

MONTENEGRO, H.; HATADANI, L. M.; MEDEIROS, H. F.; KLACZKO, L. B. Male killing in three species of the tripunctata radiation of *Drosophila* (diptera: Drosophilidae). **Journal of Zoological Systematics and Evolutionary Research**, v. 44, p. 130–135., 2006.

NARITA, S.; KAGEYAMA, D.; NOMURA, M.; FUKATSU, T. Unexpected mechanism of symbiont-induced reversal of insect sex: feminizing *Wolbachia* continuously acts on the butterfly *Eurema hecabe* during larval development. **Applied and Environmental Microbiology**, v. 73, p. 4332–4341., 2007.

NEGRI, I.; FRANCHINI, A.; MANDRIOLI, M.; MAZZOGLIO, P. J.; ALMA, A. The gonads of *Zyginidia pullula* males feminized by *Wolbachia pipientis*. **Bulletin of Insectology**, v. 61, p. 213–214., 2008.

NIKOH, N.; HOSOKAWA, T.; MORIYAMA, M.; OSHIMA, K.; HATTORI, M.; FUKATSU, T. Evolutionary origin of insect–*Wolbachia* nutritional mutualismo. **PNAS**, v. 111, p. 10257–10262., 2014.

OHBA, S. Analytical studies on the experimental population of *Drosophila*. 1.the effect of larval population density upon the pre-adult growth in *D. melanogaster* and *D. virilis* with special reference to their nutritional conditions. **Biology Journal of Okayama University**, v. 7, p. 87–125., 1961.

OHTA, T. The nearly neutral theory of molecular evolution. **Annual Review of Ecology and Systematics**, v. 23, p. 263–286., 1992.

ONO, M.; BRAIG, H. R.; MUNSTERMANN, L. E.; FERRO, C.; O'NEILL, S. L. *Wolbachia* infections of phlebotomine sand flies (diptera: Psychodidae). **Journal of Medical Entomology**, v. 38, p. 237–241., 1992.

OTE, M.; YAMAMOTO, D. Impact of *Wolbachia* infection on *Drosophila* female germline stem cells. **Current opinion in insect science**, v. 37, p. 8–15., 2020.

RCORETEAM. R: A language and environment for statistical computing. **R Foundation for Statistical Computing, Vienna, Austria.**, https://www.R-project.org/., 2019.

RODRIGUES, M. A.; NELSON, E.; MARTINS, N. E.; BALANCÉ, L. F.; BROOM, L. N.; DIAS, A. J. S.; FERNANDES, A. S. D.; RODRIGUES, F.; SUCENA, E.; MIRTH, C. K. *Drosophila melanogaster* larvae make nutritional choices that minimize developmental time. **Journal of Insect Physiology**, v. 81, p. 69–80., 2015.

SCHULER, H.; EGAN, S. P.; HOOD, G. R.; BUSBEE, R. W.; DRISCOE, A. L.; OTT, J. R. Diversity and distribution of *Wolbachia* in relation to geography, host plant affiliation and life cycle of a heterogonic gall wasp. **BMC Evolutionary Biology**, v. 18, p. 37., 2018.

SNOOK, R. R.; CLELAND, S. Y.; WOLFNER, M. F.; KARR, T. L. Offsetting effects of *Wolbachia* infection and heat shock on sperm production in *Drosophila simulans*: Analyses of fecundity, fertility and accessory gland proteins. **Genetics**, v. 155, p. 167–178., 2000.

STOUTHAMER, R.; LUCK, R. F.; HAMILTON, W. D. Antibiotics cause parthenogenetic *Trichogramma* (hymenoptera/trichogrammatidae) to revert to sex. **Proceedings of the National Academy of Sciences**, v. 87, p. 2424–2427., 1990.

THERNEAU, T. A package for survival analysis in r. **version 2.38**, https://CRAN.R-project.org/package=survival., 2015.

TURELLI, M. Evolution of incompatibility-inducing microbes and their hosts. **Evolution**, v. 48, p. 1500–1513, 1994.

TURELLI, M.; HOFFMANN, A. A. Rapid spread of an inherited incompatibility factor in california *Drosophila*. **Nature**, v. 353, p. 440–442., 1991.

_____. Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and parameter estimates from natural populations. **Genetics**, v. 140, p. 1319–1338., 1995.

WEEKS, A. R.; BREEUWER, J. A. J. *Wolbachia*-induced parthenogenesis in a genus of phytophagous mites. **Proceedings of the Royal Society B: Biological Sciences**, v. 268, p. 2245–2251., 2001.

WEINERT, L. A.; ARAUJO-JNR, E. V.; AHMED, M.; WELCH, J. J. The incidence of bacterial endosymbionts in terrestrial arthropods. **Proceedings of the Royal Society B: Biological Sciences**, v. 282, p. 20150249., 2015.

WERREN, J. H. Biology of *Wolbachia*. **Annual Review of Entomology**, v. 42, p. 587–609., 1997.

WERREN, J. H.; BALDO, L.; CLARK, M. E. *Wolbachia*: master manipulators of invertebrate biology. **Nature Reviews Microbiology**, v. 6, p. 741–751, 2008.

WERREN, J. H.; BEUKEBOOM, L. W. Sex determination, sex ratios, and genetic conflict. **Annual Review of Ecology and Systematics**, v. 29, p. 233–261, 1998.

WERREN, J. H.; WINDSOR, D.; GUO, L. Distribution of *Wolbachia* among neotropical arthropods. **Proceedings of the Royal Society of London B**, v. 262, p. 197–204, 1995.