Scientific Research Publishing

Open Journal of Animal Sciences, 2019, 9, 324-340
http://www.scirp.org/journal/ojas
ISSN Online: 2161-7627
ISSN Print: 2161-7597

Intracellular Interactions in the Reproduction Control of Introgressed Strains Involving Species from *Drosophila saltans* Group (*Sophophora* Subgenus) with Emphasis to the Effect of *Wolbachia* Infection

Thais de França Patarro, Hermione E. M. de Campos Bicudo*

Departamento de Biologia, Instituto de Biociências Letras e Ciências Exatas (Ibilce), Câmpus São José do Rio Preto, Universidade Estadual Paulista (Unesp), São José do Rio Preto, Brasil
Email: *hermione.bicudo@unesp.br

How to cite this paper: de França Patarro, T. and de Campos Bicudo, H.E.M. (2019) Intracellular Interactions in the Reproduction Control of Introgressed Strains Involving Species from *Drosophila saltans* Group (*Sophophora* Subgenus) with Emphasis to the Effect of *Wolbachia* Infection. *Open Journal of Animal Sciences*, **9**, 324-340.
https://doi.org/10.4236/ojas.2019.93027

Received: June 6, 2019
Accepted: July 12, 2019
Published: July 15, 2019

Abstract

Interspecific hybrids and constructed research models have provided information on intracellular interactions. We used two introgressed, chromosomally differentiated strains (H4 and H2) derived from F1 hybrids of crosses between *D. prosaltans* females and *D. saltans* males. In H4, the *D. prosaltans* chromosomes were eliminated. In H2, a mixture was maintained, preserving the entire genome of *D. prosaltans* (except the Y chromosome) and parts of the *D. saltans* genome. The IIR arm and a segment of chromosome III were eliminated. A third strain, used for comparison, was a normal *D. prosaltans* strain (P). This study aimed primarily to analyze the effect on the reproductive characteristics productivity (number of progeny) and sex-ratio caused by *Wolbachia* infection in interaction with different chromosome constitutions. For this, infected and uninfected flies were used in intrastrain cross combinations. Firstly, we analyzed the productivity of intracrosses of uninfected parents, in each strain, in order to detect the effects of intracellular interactions, in flies carrying different chromosome constitutions and sharing a *Wolbachia*-free, *D. prosaltans* cytoplasm. Data indicated that the chromosome parts that were eliminated, in H2, carry the isolating genes that impair productivity in hybrids of the two species, and suggested the occurrence of a nuclear/nuclear interaction. The analysis of *Wolbachia*-infected flies showed that the three strains presented different responses, depending on the chromosome constitution. As to productivity, the infection was harmful in P strain, in H2 behaved as mutualistic, and, in H4, produced the effect cytoplasmic incompati-

bility. As to sex-ratio, intracrosses showed significant differences in P and H4 strains. These results, associated with the cytological characteristics of the strains, pointed to the fundamental importance of host chromosome constitution to define the interactive process host/*Wolbachia*, and showed the flexibility of the endosymbiont manifested in different forms of self-preservation.

Keywords

Isolating Genes, Harmful Interaction, Mutualistic Interaction, Cytoplasmic Incompatibility of *Wolbachia*

1. Introduction

It has been known, for a long time, that interactions between nucleus and cytoplasm are essential for normal cell physiology. Many basic cellular processes such as protein synthesis and maturation of DNA, RNA and proteins are dependent on these interrelations. The interplay for carrying out these processes and many others requires compatibility between nuclear and cytoplasmic material.

Hybrids have often been successfully used to understand the intracellular interactions and their mechanisms. The joining of cell components of different species breaks the harmony, impairing cell functions, and is used to indicate what is going on. The characteristics productivity and viability are among the ones that are affected in interspecific hybrids. The harmful effects may be due to abnormal interplay nuclear-nuclear DNA, resulting from accumulation of genes that cause incompatible epistatic interactions between the species intercrossed. These genes were called speciation genes [1] [2] [3].

The literature has also focused on detrimental effects caused in hybrids by failure in the interaction between mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) of the species intercrossed. Products of both components are required for normal cell physiology. Most of the proteins present in mitochondria are produced from nuclear genes, but some are made from mtDNA. Both kinds of proteins constitute subunits of the electron transport chain that perform the oxidative phosphorylation (OXPHOS). It is necessary that mtDNA and nDNA work in harmony to provide energy in eukaryotes [4] [5] [6] [7].

Intracellular interactions that cause effects on reproduction have also been described in organisms infected by endosymbionts. From the last decade of the twentieth century, an extensive literature has shown the great variability of effects on host reproduction, resulting from *Wolbachia* infection, and how these bacteria interact with host cell structures to produce those effects.

Wolbachia are maternally inherited intracellular bacteria that infect the cytoplasm of a wide range of arthropods and nematodes. This bacterium is supposed to be carried by more than 16% of Neotropical insect species with a potential for 25% to 70% hosts, considering all insects [8] [9].

Wolbachia manipulate their hosts causing, among other effects, cytoplasmic

incompatibility (CI), feminization and male killing [10]. CI is considered the main detrimental effect of *Wolbachia* infection, causing embryonic lethality in crosses involving non-infected females and infected males. This and the other effects that impair males are considered processes selected to ensure the wide spreading of *Wolbachia* in the populations through the favoring of infected females [11] [12].

The study of *Wolbachia* infected organisms has multiple importance. It allows better knowledge of biological aspects of host-symbiont interaction [12]. It is also important from the evolutionary point of view because CI is considered to be involved in the speciation process, reinforcing incipient isolation among populations, in nature [13] [14]. Besides, *Wolbachia* infection has shown to be a viable strategy to modify mosquito populations aiming pest control [15] [16] [17] [18] or as a method for treatment of diseases caused by filarial nematodes [19] [20].

It is considered that the effects of *Wolbachia*-hosts interaction on reproduction are due mainly to the interplay of the bacteria with nuclear DNA and the host mitochondria. However, the variation of results produced by different infected organisms point to different strategies used by these bacteria, most of which still require understanding.

With the aim of studying the interaction between *Wolbachia* and their hosts, in the manipulation of productivity and sex ratio, we used a *Drosophila* model consisting of two introgressed strains, involving species from the *saltans* subgroup and a normal strain of *D. prosaltans* taken from the stocks.

Considering separately the crosses performed between uninfected *Wolbachia* males and females, in each strain, it was possible to obtain information relative to the effect of intracellular interactions on productivity of flies sharing *D. prosaltans* cytoplasm, but carrying nuclei with different chromosome constitutions, in the absence of the microorganism interference. These results and the results obtained in the analysis of *Wolbachia* infected flies furnished data on aspects of the reproductive effect of intracelular interactions in species from the *saltans* group.

2. Materials and Methods

2.1. Species and Strains Used in the Experiments

We used two introgressed, chromosomally differentiated strains (H4 and H2) that were derived from F1 hybrids of crosses between *D. prosaltans* females and *D. saltans* males. These hybrids were maintained by serial transfer technique [21] [22]. *Drosophila prosaltans* strain (P76) used for obtaining the hybrids was a mixture of two Brazilian isofemale lines, being one originated from Eldorado, RS and other from Belem, PA. *Drosophila saltans* strain was from Huychiauyan, México. Besides H2 and H4, a normal *D. prosaltans* strain (P), from Cachoeira dos Monteiros, BA, Brazil, taken from the stocks, was used for comparison.

The two introgressed strains are part of a group of four laboratory populations prepared simultaneously and in the same way with F1 flies from intercrosses of

D. prosaltans females with *D. saltans* males. In this direction, the intercrosses yield F1 fertile females and males, while in the reciprocal direction they yield fertile females and sterile males [23]. Chromosome analyses of the populations were performed in the salivary gland cells of third instar larvae, at intervals, till the populations had fixed a chromosome constitution. This occurred about 151 weeks after preparing the populations [21].

Drosophila prosaltans and *D. saltans* have three chromosome pairs, being two metacentric (X and II) and one acrocentric (III). Over time, the population H4 fixed a chromosome constitution formed exclusively by *D. saltans* chromosomes, having thus eliminated the *D. prosaltans* chromosomes. Differently, H2 maintained the complete set of *D. prosaltans* chromosomes (except the Y that was absent in the original F1 hybrids) and parts of *D. saltans* genome, including, besides the Y, the IIL chromosome arm recombined with the IIR of *D. prosaltans* and several types of recombinant chromosomes III, bearing a mixture of parts from both species. The segment of chromosome III derived from *D. saltans*, encompassing from the first band of section 79 to the first band of section 85 had been eliminated. These strains had been maintained in laboratory for about 37 years, before the present study was carried out (**Table 1**).

The diagnosis of the specific origin of the chromosomes, in the flies of the H2 and H4 introgressed strains, made by [21] was based on the degree of pairing of the polytene chromosomes, in the salivary glands. Regions with constant asynapsis denote interspecific heterozygosis. Besides, flies from these strains were intercrossed with *D. saltans* and *D prosaltans* and their progeny analyzed to confirm heterozygosis. The presence of interspecific inversions, known from previous studies of both species [24], were also used for diagnosis of chromosome composition. The chromosome constitution of both introgressed strains persisted over time, having been analyzed again by [25] and by the present authors before starting the study.

2.2. Maintenance of Strains in the Laboratory

The strains and experiments were kept at 20˚C ± 1˚C, in a constant temperature room, located at the Department of Biology, IBILCE-UNESP. The usual culture medium of banana-agar was used.

Table 1. Specific origin of the chromosomes in the strains used. P = *Drosophila prosaltans*; S = *Drosophila saltans*; H4 and H2 = Introgressed strains.

Strain	Chromosomes				
	X	IIL	IIR	III	Y
P	P	P	P	P	P
H4	S	S	S	S	S
H2	P,S	P,S	P	P,S*	S

*The segment of chromosome III from *D. saltans*, encompassing the first band of section 79 till the first band of section 85 was eliminated.

2.3. Analyses of the *Drosophila* Strains for Detecting *Wolbachia* Infection

First of all, the stocks had to be analyzed for the presence of *Wolbachia* infection. The screening of *Wolbachia* involved the following steps:

2.3.1. DNA Extraction

DNA extractions were conducted according to [26] with modifications. Samples containing 10 flies were homogenized in 160 µl of solution I (10 mM Tris, 60 mM NaCl, 5% sucrose, 10 mM EDTA, pH 7.8). 200 µl of solution II (300 mM Tris, 1.25% SDS, 5% sucrose, 10 mM EDTA, pH 8.0) were added to each sample, mixed and incubated in water bath at 65˚C for 30 minutes. Then, 60 µl of potassium acetate 3 M (pH 5) were added to the samples, which were cooled at −20˚C for 20 minutes. After centrifuging at 13,000 rpm for 20 minutes, the supernatants were transferred to new tubes containing 400 µl of isopropanol and left to rest at room temperature for 5 minutes. Pellets were obtained after applying 500 µl 70% ethanol, centrifuging samples at 13,000 rpm for 10 minutes and air drying. The DNA was resuspended in 100 µl ultra-pure water and maintained at −20 ˚C.

2.3.2. Amplification of DNA Sequences

Specific primers were used for amplifications of the locus *Wsp* (*Wolbachia* Surface Protein) [27] as insertion sequences of the transposon IS5 in two loci (IS5-WD0516/7 and IS5-WD1310), and *in tandem* sequences VNTR-105 and VNTR-141 ([28]). All primer sequences are listed in **Table 2**. The reactions were prepared according to specific protocols for each primer. *Wsp*-PCR mix *r*eactions were prepared as described in [29] and amplifications according to [27]. IS5-PCR was conducted as described in [30]. *VNTR*–PCR was performed as described in [29].

2.3.3. Identification of the Amplification Products

The amplified samples were subjected to electrophoresis using 8% polyacrylamide

Table 2. *Primer* sequences used for *Wolbachia* screenings in *Drosophila* strains.

Primer	Sequences
Wsp-F	5-TGGTCCAATAAGTGATGAAGAAACTAGCTA-3
Wsp-R	5-AAAAATTAAACGCTACTCCAGCTTCTGCAC-3
IS5-WD0516/7-foward	5-CCATCAAGGTCTCTTTCA -3
IS5-WD0516/7-reverse	5-TGCAAGGAAAACTAAACCAG-3
IS5-WD1310-foward	5-AGGAGAACTGGTCTACGC-3
IS5-WD1310-reverse	5-TGTTGCTGAGCTTTGCT-3
VNTR-105-foward	5-GCAATTGAAAATGTGGTGCC-3
VNTR-105-reverse	5-ATGACACCTTACTTAACCGTC-3
VNTR-141-foward	5-GGAGTATTATTGATATGCG-3
VNTR-141-reverse	5 –GACTAAAGGTTAGTTGCAT-3

gel (30% bisacrylamide, TBE 10X Glycerin 10% ammonium persulfate and TEMED). 10 µl of each sample were subjected to electrophoresis at 90 volts for 3 hours in the presence of 1X TBE buffer solution (Tris, boric acid and 0.5 M EDTA pH 8.0). The fragments were visualized after fixation (10% ethanol, 0.75% Glacial Acetic Acid), and staining of the gel with silver nitrate followed by development (sodium hydroxide and formaldehyde). The gels were air dried and preserved wrapped in cellophane paper [31].

2.4. Preparation of Aposymbiotic Strains (*Wolbachia*-Infection Free)

After observing that the strains in the stocks were infected, it was necessary to prepare uninfected (or aposymbiotic) strains. With this aim, the strains were maintained during three generations in culture medium treated with tetracycline (Sigma-Aldrich) 0.03%. To prepare the medium, a stock solution of tetracycline diluted in 98% ethanol was used at 30 mg/ml concentration and kept at −20˚C. After the three generations of treatment, the flies were maintained for other three generations in culture medium without tetracycline, in order to exclude possible remains of the antibiotic that could interfere in the results. The use of this process has been considered secure in relation to the influence of the antibiotics on the results. Previous studies tested the effect of tetracycline treatment, confirming its efficiency for *Wolbachia* elimination and showing no significant effect on host fitness [32] [33] [34].

The Experiments

After performing the analysis for detecting the presence of *Wolbachia* in the stocks and preparing the strains in the infected and uninfected (treated) conditions, the experiments could be carried out.

Experiments were performed using intracrosses prepared with three replicas, each with five virgin couples aged 7 to 9 days. The intracrosses were maintained in flasks and transferred once to new flasks containing recently prepared culture medium. In each strain, all the combinations of *Wolbachia* infected and uninfected flies were prepared.

The characteristics studied for detecting interaction effects on reproduction were productivity (number of progeny) and sex-ratio. First of all, the analyses were made considering only the crosses of uninfected parents, in the three strains, aiming to analyze the intra cell interaction in the absence of *Wolbachia infection*. These flies carried *D. prosaltans* cytoplasm and different chromosome constitutions. The effect of *Wolbachia* infection was analyzed considering the four combinations of infected and uninfected flies in each strain.

In the analysis of F1 progeny, males and females were computed separately till the last fly emerged in the crosses.

2.5. Statistics

Data were analyzed by Chi-square tests. Sex-ratio was calculated using the ex-

pression: number of males times 100 divided by the number of females, as proposed by [35].

3. Results

3.1. Results of Experiments for Detecting the Presence of *Wolbachia* Infection in the Strains

The five loci used in the amplification tests to detect the presence of *Wolbachia* in the *Drosophila* strains produced a single fragment (amplicon), indicating that each of them houses a single type of *Wolbachia* (**Figure 1**). The literature reports several cases of multiple *Wolbachia* infections in species of *Drosophila* and other organisms [36] [37] [38] [39] [40]. In these cases, the trials show more than one amplicon in the same host strain. This was not observed in the strains used in the present work.

The screenings for the characterization of the *Wolbachia* strains present in the *Drosophila* strains used in this study were inconclusive. However, considering that, unlike the nuclear genes, which are inherited from both mother and father, cytoplasmic structures and *Wolbachia* are passed on along the female line, it is expected that H2 and H4, which were originated from the same F1 progeny, share the same cytoplasmic constitution, including the same mitochondrial genome and the same *Wolbachia* strain originated from *D. prosaltans*.

Most likely, the *Wolbachia* strain present in H2 and H4 is the same present in

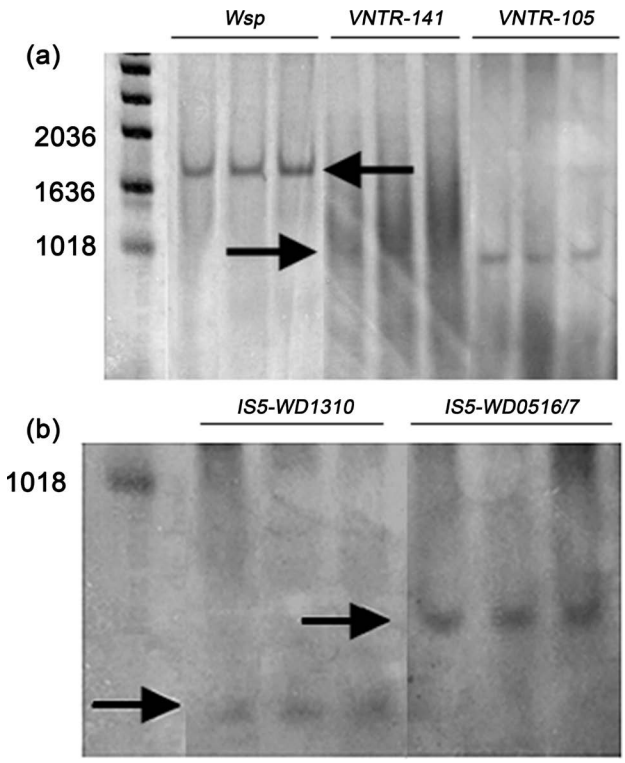

Figure 1. DNA fragments produced by the screenings for *Wolbachia* presence in H2, H4 and P strains (in the sequence of columns), using the primers *Wsp*, *VNTR*-141, *VNTR*-105 (a); *IS*5-*WD*1310 and *IS*5-*WD*0516/7 (b).

P strain, since we suspect that the transmission of the endosymbiont among strains from the *saltans* group have occurred horizontally through mites that parasite the flies, in the long time they have been maintained in the stocks. There is also the idea that this might be happened in nature on the basis of data obtained by [41]. Those authors suggested that P elements have entered *D. melanogaster* genome by horizontal transmission from other species. In order to identify the potential donor of the horizontal transfer process, those authors performed an extensive study on the genus *Drosophila*, using Southern blot analysis, showing that P-homologous sequences are essentially confined to the subgenus *Sophophora*, to which also belong the species in this study. This finding may reinforce the possibility of horizontal transference also for endosymbionts.

3.2. Data Obtained in the Analysis of *Wolbachia*-Free Intrastrain Crosses

Productivity of intracrosses of both uninfected parents was analyzed, in each strain in order to detect the effect of inner cell interactions in flies carrying different chromosome constitutions and sharing a *Wolbachia*-free, *D. prosaltans* cytoplasm.

Comparison of data among the three strains showed high and similar progeny numbers in P2 (with complete *D. prosaltans* genome) and H2 strain (with the *D. prosaltans* genome plus one partial *D. saltans* genome), and much lower productivity in H4 (with *D. saltans* genome) (Table 3).

We also compared H2 productivity with data previously obtained for F1 hybrids

Table 3. F1 productivity and sex ratio in the three replicas of intracrosses involving *Wolbachia* infected (I) and treated (uninfected) (T) flies. P= *D. prosaltans*; H2 and H4= introgressed strains; F= Females, M= Males; To = total of progeny.

Strain	Crosses			Replicas									Sum			Sex ratio
	F		M	1			2			3						
				F	M	To	F	M	To	F	M	To	F	M	To	♂×100/♀
P	T	X	T	115	140	255	55	70	125	1	0	1	171	210	381	122,81
	I	X	I	26	23	49	0	0	0	0	0	0	26	23	49	88,46
	I	X	T	42	40	82	0	0	0	46	69	115	88	109	197	123,86
	T	X	I	31	41	72	51	67	118	23	45	68	105	153	258	145,71
H2	T	X	T	0	0	0	113	91	204	45	65	110	158	156	314	98,73
	I	X	I	0	0	0	18	15	33	213	165	378	231	180	411	77,92
	I	X	T	41	37	78	181	184	365	190	151	341	412	372	784	90,29
	T	X	I	132	122	254	201	195	396	124	102	226	457	419	876	91,68
H4	T	X	T	10	6	16	0	0	0	0	0	0	10	6	16	60
	I	X	I	0	0	0	66	47	113	8	14	22	74	61	135	82,43
	I	X	T	45	25	70	32	36	68	0	0	0	77	61	138	79,22
	T	X	I	6	2	8	1	1	2	0	0	0	7	3	10	42,86

of crosses between *D. prosaltans* females and *D. saltans* males [23]. In F1 flies, 25 among 50 pair mating crosses were fertile and produced 326 descendants (a mean of 13.04 per female) and 50% sterility among all crosses. In H2, 314 descendants were produced by 10 fly pairs (one of the replicas was sterile) giving a mean of 31.40 descendant per female), and cross sterility was about 30% (one of the three replicas was sterile).

3.3. Data on Productivity and Sex-Ratio of the Strains Infected by *Wolbachia*

The effects on productivity and sex-ratio were analyzed in the strains infected by *Wolbachia*, carrying *D. prosaltans* cytoplasm and different nuclear chromosome constitutions (**Table 3**).

3.3.1. Productivity

Productivity differences among cross combinations were significant in the three strains (for P, $X^2 = 258.2090$; for H2, $X^2 = 381.5392$ and for H4, $X^2 = 204.3445$; in every strain $p = 0.000000$). The results were as follows:

P strain: The intercrosses of both infected parents showed low productivity when compared to the other intracrosses, being the intracross of both treated (uninfected) parents (*Wolbachia*-free) the one that yielded the greatest number of progeny. The crosses between treated females *versus* infected males showed higher productivity than the reciprocal ones. Crosses of both infected parents, in addition to being the least productive, showed a single fertile replica.

H2 strain: All combinations were highly productive, but those involving one infected and another uninfected parent showed productivity about the double or more, relatively to the crosses of both uninfected or both infected parents.

H4 strain: The less productive intracrosses involved both treated parents and treated females *versus* infected males. One or two replicas were sterile in each of the combinations, and the number of offspring was much lower than that in the combinations of both infected sexes or infected females *versus* treated males. The productivity, much higher in IF X IM than in the reciprocal crosses, characterizes the process named cytoplasmic incompatibility (CI) in operation.

3.3.2. Sex-Ratio

Data on sex-ratio of F1 progeny in the three strains showed the following results:

P strain: In this strain, the difference among combinations was significant ($X^2 = 33.12171$; $p < 0.000000$). The intercrosses of infected parents produced number of males lower than 1:1 proportion, while the other three combinations produced male proportions higher than 1:1.

H2 strain: In this strain the differences among combinations were not significant ($X^2 = 6.526458$; $p < 0.088627$). However, in the light of the numbers, the combination that involved both infected parents showed the lower proportion of male progeny.

H4 strain: In this strain, sex-ratio differed significantly among combinations

(X^2 = 56.05493; p = 0.000000). Crosses involving both treated parents and involving treated females with infected males showed the lowest male progeny percentages, respectively 60% and 43%.

4. Discussion

Aiming to analyze intracellular interactions, we used two introgressed strains (H4 and H2) derived from F1 hybrids of crosses between *D. prosaltans* females and *D. saltans* males, chromosomally differentiated over time [21], and a *D. prosaltans* strain (P strain) taken from the stocks, used for comparison.

We started the study analyzing productivity (number of descendants) in the intracrosses of uninfected flies, in the three strains. This allowed studying the effects of intra cell interactions, in flies containing different chromosome constitutions and sharing a *Wolbachia*-free cytoplasm from *D. prosaltans*. The results showed that the strain with interspecific hybrid genome (H2) and the normal *D. prosaltans* (P) were similar in productivity, while the strain with only *D. saltans* chromosomes had a low performance.

The low productivity of H4 may be explained by a high degree of nucleus/cytoplasm incompatibility due to the presence of chromosomes exclusively of one species in the cytoplasm of another. However, the similar degrees of productivity shown by P and H2 strains were unexpected, given their difference in chromosome structure. We expected that productivity in a situation of hybrid chromosome structure (H2) would be impaired.

Because of this, we considered interesting to compare H2 productivity with data previously obtained for F1 hybrids of crosses between *D. prosaltans* females and *D. saltans* males [23]. The production of descendants per fly, in H2, was more than double that in F1 flies.

Since the chromosome difference between F1 hybrids and H2 strain was the absence of part of the *D. saltans* genomic material in H2, we considered that the chromosome parts eliminated might contain incompatible genes that act on the reproductive isolation between *D. prosaltans* and *D. saltans,* and that their elimination has caused the improvement of H2 productivity. Loss of the same *D. saltans* chromosome parts occurred in another population (H3), also started with F1 hybrids and prepared simultaneously to H2 by [21] reinforced this idea. Those authors mentioned that the parallelism of the two populations, H2 and H3, could be indicative that the elimination of chromosome parts of *D. saltans* was not casual, and that they could carry genomic material modified in the species divergence. Because the two strains shared the *D. prosaltans* cytoplasm, it seems that the intracellular interaction enabling the elimination of the *D. saltans* chromosome material was nDNA/nDNA.

Nuclear/nuclear interactions affecting reproduction, in hybrids, have been considered as involving autosomal chromosomes of one species and the X chromosome of the other. For example, the sterility of *D. melanogaster*/*D. simulans* hybrids was explained by interaction of autosomic loci of *D. simulans*

with loci of *D. melanogaster* X chromosome [2]. Progeny decrease in *D. prosaltans/D. saltans* F1 hybrids may also be promoted by inadequate interaction involving the *D. saltans* autosome regions with the *D. prosaltans* X chromosome, but this is a possibility that remain to be verified.

Inadequate nuclear-mitochondrial integration that causes energy crisis in cells, changes drastically cell physiology [42] [43]. The literature shows that deleterious reproductive phenotypes may also result from such incompatibility, in animal and plant hybrids [44] [45] [46] [47]. Harmful mtDNA/nDNA interaction might explain the low productivity of flies we observed in H4 strain, that have *D. prosaltans* cytoplasm and chromosomes exclusively from *D. saltans*.

Male F1 hybrids produced in interspecific crosses, in laboratory, are frequently sterile in one cross direction, and fertile in the other. Several studies of this kind of asymmetrical results have indicated that it is due to male sterility factors present in the cytoplasm of one species that conflicts with the genome of the other species. The cytoplasmic incompatibility factors have been mapped to the mitochondrial genome and interact with nuclear genes related to the mitochondrial functions. In *Saccharomyces,* the nuclear gene *AEP*2, located in the chromosome 13, codes a regulatory protein involved in the translation of the mitochondrial gene *OLI*1 that codes a subunit of the complex of ATP synthesis [48].

Intercrosses between *D. saltans* females and *D. prosaltans* males produce sterile male progeny in contrast to the reciprocal ones [23]. Since mtDNA/nDNA incompatibility is widely indicated to explain asymmetrical sterility (for example [49] in yeast; [5] in *Drosophila*; [47] in mice), we consider the possibility that in intercrosses *D. prosaltans versus D. saltans,* the same interaction process is operating.

The presence of the endosymbiont *Wolbachia* in the flies of the three strains studied, in the present work, affects the reproductive success of the host organisms. It is already known that *Wolbachia* causes multiple effects on their hosts. They exhibit a spectrum of relationships with their hosts, ranging from parasitism to mutualism. In the maximum degree, the relationship between host and symbiont becomes obligate. In this case, if they are separated from each other they die because the endosymbiont takes part of essential host processes, frequently causing a metabolic complementation [33] [50] [51].

Through the process named cytoplasm incompatibility (CI), *Wolbachia* promotes its own spreading in the population, favoring infected females. CI operates in intercrosses between treated (uninfected) females and infected males causing complete sterility or low number of progeny while, in the reciprocal crosses, the number of progeny is normal. In these intercrosses, *Wolbachia* causes cell cycle damages in the male pronucleus that lead embryos to death, in early stages. In the reciprocal crosses, eggs of infected females have the power to rescue modified sperm and embryos develop normally [52]. The involvement of *Wolbachia* in the origin of these problems was demonstrated in crosses of *Drosophila subquinaria* with *D. recens*. When the crosses involved *D. subquinaria*

females (uninfected) and *D. recens* males (infected), mating occurred readily, but only some few viable hybrids were produced. Treatment of *D. recens* with antibiotic restored viability of hybrids in this cross direction, indicating that the strong progeny reduction was due to *Wolbachia* infection [53] [54].

In the present study, the effect of *Wolbachia* infection on productivity and sex-ratio, of the strains used showed strong dependence on the host nuclear chromosomes. The three strains with their particular chromosome constitutions and cytoplasm of *D. prosaltans* showed different interaction effects.

Let's consider first the strains H4 and H2, since because of their common origin they must share the same cytoplasm, including mitochondria and *Wolbachia*. H4 strain, with exclusively *D. saltans* chromosomes, manifested the CI effect: intercrosses TF x IM produced very low number of progeny in comparison with the reciprocal crosses (IF × TM), in which productivity was about 14 times greater. In crosses involving both infected parents, the number of descendants was also as high as in IF × TM, indicating that interaction of *Wolbachia* with the host chromosome content is favoring the feature productivity. Thus, in this strain, we have two fountains of infected flies for spreading *Wolbachia*: the crosses of both infected parents and the crosses of infected females with treated males. It is also interesting to note the degree of sterility of the replicas; in the four combinations of this strain, at least one replica didn't yield progeny. Apparently, in H4, the presence of *Wolbachia* has contributed to maintain the strain alive, in the stocks of our laboratory, in the course of time. As to the sex-ratio, in H4, crosses of treated parents and crosses TF x IM males showed, parallel to the lowest number of progeny, the lowest male percentages, (60% and 43%, respectively).

H2, with a mixture of chromosomes from both species faced well the *Wolbachia* infection. The crosses of both infected parents showed progeny number higher than the crosses of both treated parents. But, the highest performance was shown in crosses in which one of the parents was infected, in both directions, with productivity about twice or more than that in crosses of treated parents. The interspecific mixture in the host nuclear content seems to favor positive interaction with *Wolbachia*, characterizing a mutualistic relationship. For *Wolbachia,* an increase of population size is a guarantee for their survival. Regarding sex ratio, in H2, the difference of 1:1 proportion was not significant. However, in the light of numbers, the crosses of both infected parents showed the lower male proportion (about 78%).

Mutualism in *Wolbachia* has been described, intraspecifically, for strains of several *Drosophila* species such as *D. suzukii* [55], *D. simulans* [56] and *D. melanogaster* [34] [57]. A stock of *Drosophila mauritiana*, infected with a native *Wolbachia* strain, produced about four times more eggs than the non-infected strain. In cellular studies, the authors found increase of the mitotic activity of the germline stem produced cells (GSC) and decrease in programmed cell death of the germarium, both induced by *Wolbachia* [58].

Our results for *D. prosaltans* (P strain) showed the opposite, since *Wolbachia* infection was harmful for productivity. In crosses of both infected parents, the number of progeny was significantly lower than that in the other three combinations, being the higher productivity found in crosses of both infection-free parents. The results on sex-ratio showed some impair of males in intercrosses of infected parents but males were favored in crosses involving one or both uninfected parents with some higher advantage in crosses TF x IM. The results showed that lower total productivity and lower female productivity may be considered part of the harmful effect of *Wolbachia* infection in this strain.

5. Conclusion

The present study using species from the *Drosophila saltans* group points to some forms of intracellular interactions revealed or suggested by the hybrid constitution involving *D. prosaltans and D. saltans*, in introgressed strains. Productivity of *Wolbachia* uninfected flies reinforced the hypothesis that the segments of *D. saltans* chromosomes eliminated in H2 are carrier of interspecific isolating genes. Interactions nDNA/nDNA and mtDNA/nDNA were also suggested, considering both the cytological characteristics of the strains and the results on productivity. In the crosses involving *Wolbachia* infected flies, the effect on productivity varied among the three strains, being harmful (P strain), mutualistic (H2), or CI inductor (H4), emphasizing the importance of the host chromosome constitution in defining the effect of the endosymbiont activity and showing flexibility of these bacteria to use interaction processes for achieving self-preservation.

Acknowledgements

We are indebted to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for fellowship given to Thais de França Patarro, to Dr. Wanderli Pedro Tadei for allowing to use the data included in Tadei and Bicudo (1981) and to Dr. Luis Fernando Segala for statistical counselling.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Orr, H.A. (1995) The Population Genetics of Speciation: The Evolution of Hybrid Incompatibilities. *Genetics*, **39**, 1805-1813.

[2] Presgraves, D.C. (2003) A Fine-Scale Genetic Analysis of Hybrid Incompatibilities in *Drosophila*. *Genetics*, **163**, 955-972.

[3] Orr, H.A., Masly, J.P. and Presgraves, D.C. (2004) Speciation Genes. *Current Opinion in Genetics & Development*, **1**, 675-679. https://doi.org/10.1016/j.gde.2004.08.009

[4] Ballard, J.W.O., Matthew, D. and Dean, M. (2001) The Mitochondrial Genome: Mutation, Selection and Recombination. *Current Opinion in Genetics & Develop-*

ment, **11**, 667-672. https://doi.org/10.1016/S0959-437X(00)00251-3

[5] Meiklejohn, C.D., Holmbeck, M.A., Siddiq M.A., *et al*. (2013) An Incompatibility between a Mitochondrial tRNA and Its Nuclear-Encoded tRNA Synthetase Compromises Development and Fitness in *Drosophila*. *PLoS Genetics*, **9**, e1003238. https://doi.org/10.1371/journal.pgen.1003238

[6] Holmbeck, M.A., Donner, J.R., Villa-Cuesta, E. and Rand, D.M. (2015) A *Drosophila* Model for Mito-Nuclear Diseases Generated by an Incompatible Interaction between tRNA and tRNA Synthetase. *Disease Models & Mechanisms*, **8**, 843-854. https://doi.org/10.1242/dmm.019323

[7] Chang, C.C., Rodriguez, J. and Ross, J. (2016) Mitochondrial–Nuclear Epistasis Impacts Fitness and Mitochondrial Physiology of Interpopulation *Caenorhabditis briggsae* Hybrids. *G3*: *Genes*, *Genomes*, *Genetics*, **6**, 209-219. https://doi.org/10.1534/g3.115.022970

[8] Hoerauf, A. and Rao, R.U. (2007) The Discovery of *Wolbachia* in Arthropods and Nematodes—A Historical Perspective. In: Kozek, W.J. and Rao, R.U., Ed., *Wolbachia*: A Bug's Life in Another Bug, Karger Publishers, Basel, 1-14. https://doi.org/10.1159/000104228

[9] Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A. and Werren, J.H. (2008) How Many Species Are Infected with *Wolbachia*?—A Statistical Analysis of Current Data. *FEMS Microbiology Letters*, **281**, 215-220. https://doi.org/10.1111/j.1574-6968.2008.01110.x

[10] O'Neill, S.L., Pettigrew, M.M., Sinkins, S.P., *et al*. (1997) *In Vitro* Cultivation of *Wolbachia pipientis* in an *Aedes albopictus* Cell Line. *Insect Molecular Biology*, **6**, 33-39. https://doi.org/10.1046/j.1365-2583.1997.00157.x

[11] Serbus, L.R., Casper-Lindley, C.F., Landmann, F. and Sullivan, W. (2008) The Genetics and Cell Biology of *Wolbachia*-Host Interactions. *Annual Review of Genetics*, **42**, 683-707. https://doi.org/10.1146/annurev.genet.41.110306.130354

[12] Pietri, J.E., DeBruhl, H. and Sullivan, W. (2016) The Rich Somatic Life of *Wolbachia*. *Microbiology Open*, **5**, 923-936. https://doi.org/10.1002/mbo3.390

[13] Telschow, A., Flor, M., Kobayashi, Y., Hammerstein, P. and Werren, J.H. (2007) *Wolbachia*-Induced Unidirectional Cytoplasmic Incompatibility and Speciation: Mainland-Island Model. *PLoS ONE*, **2**, e701. https://doi.org/10.1371/journal.pone.0000701

[14] Lamelza, P. and Ailion, M. (2017) Cytoplasmic–Nuclear Incompatibility between Wild Isolates of *Caenorhabditis nouraguensis*. *G3*: *Genes*, *Genomes*, *Genetics*, **7**, 823-834. https://doi.org/10.1534/g3.116.037101

[15] Zabalou, S., Riegler, M., Theodorakopoulou, M., *et al*. (2004) *Wolbachia*-Induced Cytoplasmic Incompatibility as a Means for Insect Pest Population Control. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 15042-15045. https://doi.org/10.1073/pnas.0403853101

[16] Blagrove, M.S.C., Arias-Goeta, C., Failloux, A.B., Steven, P. and Sinkins, S.P. (2012) *Wolbachia* Strain *wMel* Induces Cytoplasmic Incompatibility and Blocks Dengue Transmission in *Aedes albopictus*. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 255-260. https://doi.org/10.1073/pnas.1112021108

[17] Jiggins, F.M. (2017) The Spread of *Wolbachia* through Mosquito Populations. *PLOS Biology*, **15**, e2002780. https://doi.org/10.1371/journal.pbio.2002780

[18] Joubert, D.A. and O'Neill, S.L. (2017) Comparison of Stable and Transient *Wolbachia* Infection Models Dirk Albert in *Aedes aegypti* to Block Dengue and West Nile

Viruses. *PLOS Neglected Tropical Diseases*, **11**, e0005275. https://doi.org/10.1371/journal.pntd.0005275

[19] Slatko, B.E., Luck, A.N., Dobson, S.L. and Foster, J.M. (2014) *Wolbachia* Endosymbionts and Human Disease Control. *Molecular and Biochemical Parasitology*, **195**, 88-95. https://doi.org/10.1016/j.molbiopara.2014.07.004

[20] Hughes, G.L., Rivero, A. and Rasgon, J.L. (2014) *Wolbachia* Can Enhance *Plasmodium* Infection in Mosquitoes: Implications for Malaria Control? *PLOS Pathogens*, **10**, e1004182. https://doi.org/10.1371/journal.ppat.1004182

[21] Tadei, W.P. and Bicudo, H.E.M.C. (1981) Experimental Populations of *Drosophila prosaltans*, *D. saltans*, and Their Hybrids. I. Chromossomal Evolution. *Revista Brasileira de Genética*, **4**, 549-570.

[22] Ayala, F.J. (1965) Relative Fitness of Populations of *Drosophila serrata* and *Drosophila birchii*. *Genetics*, **51**, 527-544.

[23] Bicudo, H.E.M.C. (1973) Reproductive Isolation in the *Saltans* Group of *Drosophila*. I. The *Saltans* Subgroup. *Genetica*, **44**, 313-329. https://doi.org/10.1007/BF00161311

[24] Bicudo, H.E.M.C. (1973) Chromossomal Polymorphism in the *Saltans* Group of *Drosophila*. I. The Saltans Subgroup. *Genetica*, **44**, 520-552. https://doi.org/10.1007/BF00116808

[25] Freschi, A.P.P. (2002) Uso de marcadores de AFLP no estudo de polimorfismo de DNA em *Drosophila saltans*, *Drosophila prosaltans* e seus híbridos em populações experimentais. Master's Dissertation, Instituto de Biociências, Letras e Ciências Exatas, São José do Rio Preto.

[26] Dellaporta, S.L., Wood, J. and Hicks, J.B. (1983) A Plant DNA Minipreparation: Version II. *Plant Molecular Biology Reporter*, **1**, 19-21. https://doi.org/10.1007/BF02712670

[27] Jeyaprakash, A. and Hoy, M.A. (2000) Long PCR Improves *Wolbachia* DNA Amplification: *Wsp* Sequences Found in 76% of Sixty-Three Arthropod Species. *Insect Molecular Biology*, **9**, 393-405. https://doi.org/10.1046/j.1365-2583.2000.00203.x

[28] Riegler, M., Sidhu, M., Miller, W.J. and O'Neill, S.L. (2005) Evidence for a Global *Wolbachia* Replacement in *Drosophila melanogaster*. *Current Biology*, **15**, 1428-1433. https://doi.org/10.1016/j.cub.2005.06.069

[29] Miller, W.J., Ehrmn, L. and Schneider, D. (2010) Infectious Speciation Revisited: Impact of Symbiont-Depletion on Female Fitness and Mating Behavior of *Drosophila paulistorum*. *PLoS Pathogens*, **6**, e1001214. https://doi.org/10.1371/journal.ppat.1001214

[30] Iturbe-Ormaetxe, I. and O'Neill, S.L. (2007) *Wolbachia*-Host Interactions: Connecting Phenotype to Genotype. *Current Opinion in Microbiology*, **10**, 221-224. https://doi.org/10.1016/j.mib.2007.05.002

[31] Ceron, C.R., Santos, J.R. and Campos-Bicudo, H.E.M. (1992) The Use of Gelatin to Dry Cellophane Wound Slab Gels In an Embroidering Hoop. *Revista Brasileira de Genética*, **15**, 201-203.

[32] Poinsot, D. and Merçot, H. (1997) *Wolbachia* Infection in *Drosophila simulans*: Does the Female Host Bear a Physiological Cost? *Evolution*, **51**, 180-186. https://doi.org/10.1111/j.1558-5646.1997.tb02399.x

[33] Dedeine, F., Vavre, F., Fleury, F., Loppin, B., Hochberg, M.E. and Boulétreau, M. (2001) Removing Symbiotic *Wolbachia* Bacteria Specifically Inhibits Oogenesis in a Parasitic Wasp. *Proceedings of the National Academy of Sciences of the United*

States of America, **98**, 6247-6252. https://doi.org/10.1073/pnas.101304298

[34] Fry, A.J., Palmer, M.R. and Rand, D.M. (2004) Variable Fitness Effects of *Wolbachia* Infection in *Drosophila melanogaster*. *Heredity*, **93**, 379-389. https://doi.org/10.1038/sj.hdy.6800514

[35] Darwin, C (1871) The Descent of Man, and Selection in Relation to Sex. D. Appleton & Company, New York. https://doi.org/10.5962/bhl.title.24784

[36] Rousset, F.R. and Solignac, M. (1995) Evolution of Single and Double *Wolbachia* Symbioses during Speciation in the *Drosophila simulans* Complex. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 6389-6393. https://doi.org/10.1073/pnas.92.14.6389

[37] Rousset, F., Braig, H.R. and O'neill, S.L. (1999) A Stable Triple *Wolbachia* Infection in *Drosophila* with Nearly Additive Incompatibility Effects. *Heredity*, **82**, 620-627.

[38] James, A.C., Dean, M.D., McMahon, M.E. and Ballard, J.W. (2002) Dynamics of Double and Single *Wolbachia* Infections in *Drosophila simulans* from New Caledonia. *Heredity*, **88**,182-189. https://doi.org/10.1038/sj.hdy.6800025

[39] Reuter, M. and Keller, L. (2003) High Levels of Multiple *Wolbachia* Infection and Recombination in the Ant *Formica exsecta*. *Molecular Biology and Evolution*, **20**: 748-753. https://doi.org/10.1093/molbev/msg082

[40] Mouton, L., Dedeine, F., Henri, H., *et al.* (2004) Virulence, Multiple Infections and Regulation of Symbiotic Population in the *Wolbachia-Asobara tabida* Symbiosis. *Genetics*, **168**, 181-189. https://doi.org/10.1534/genetics.104.026716

[41] Daniels, S.B., Peterson, K.R., Strausbaugh, L.D., Kidwell, M.G. and Chovnick, A. (1990) Evidence for Horizontal Transmission of the P Transposable Element between *Drosophila* Species. *Genetics*, **124**, 339-355.

[42] Guantes, R., Rastrojo, A., Neves, R., *et al.* (2015) Global Variability in Gene Expression and Alternative Splicing Is Modulated by Mitochondrial Content. *Genome Research*, **25**, 633-644. https://doi.org/10.1101/gr.178426.114

[43] Muir, R., Diot, A. and Poulton, J. (2016) Mitochondrial Content Is Central to Nuclear Gene Expression: Profound Implications for Human Health. *Bioessays*, **38**, 150-156. https://doi.org/10.1002/bies.201500105

[44] Ellison, C.K. and Burton, R.S. (2008) Genotype-Dependent Variation of Mitochondrial Transcriptional Profiles in Interpopulation Hybrids. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 15831-15836. https://doi.org/10.1073/pnas.0804253105

[45] Sambatti, J.B., Ortiz-Barrientos, D., Baack, E.J. and Rieseberg, L.H. (2008) Ecological Selection Maintains Cytonuclear Incompatibilities in Hybridizing Sunflowers. *Ecology Letters*, **11**, 1082-1091. https://doi.org/10.1111/j.1461-0248.2008.01224.x

[46] Aalto, E.A., Koelewijn, H.P. and Savolainen, O. (2013) Cytoplasmic Male Sterility Contributes to Hybrid Incompatibility between Subspecies of *Arabidopsis lyrata*. *G3*: *Genes*, *Genomes*, *Genetics*, **3**, 1727-1740. https://doi.org/10.1534/g3.113.007815

[47] Ma, H., Gutierrez, N.M., Morey, R., *et al.* (2016) Incompatibility between Nuclear and Mitochondrial Genomes Contributes to Interspecies Reproductive Barrier. *Cell Metabolism*, **24**, 283-294. https://doi.org/10.1016/j.cmet.2016.06.012

[48] Finnegan, P.M., Payne, M.J., Keramidaris, E. and Lukins, H.B. (1991) Characterization of a Yeast Nuclear Gene, *AEP2*, Required for Accumulation of Mitochondrial mRNA Encoding Subunit 9 of the ATP Synthase. *Current Genetics*, **20**, 53-61. https://doi.org/10.1007/BF00312765

[49] Lee, H.Y., Chou, J.Y., Cheong, L., *et al.* (2008) Incompatibility of Nuclear and Mi-

tochondrial Genomes Causes Hybrid Sterility between Two Yeast Species. *Cell*, **135**, 1065-1073. https://doi.org/10.1016/j.cell.2008.10.047

[50] Nikoh, N., Hosokawa, T., Moriyama, M., *et al*. (2014) Evolutionary Origin of Insect-*Wolbachia* Nutritional Mutualism. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 10257-10262. https://doi.org/10.1073/pnas.1409284111

[51] Sullivan, W. (2017) *Wolbachia*, Bottled Water, and the Dark Side of Symbiosis. *Molecular Biology of the Cell*, **28**, 2343-2346. https://doi.org/10.1091/mbc.e17-02-0132

[52] Landmann, F., Orsi, G.A., Loppin, B. and Sullivan, W. (2009) *Wolbachia*-Mediated Cytoplasmic Incompatibility Is Associated with Impaired Histone Deposition in the Male Pronucleus. *PLoS Pathogens*, **5**, e1000343. https://doi.org/10.1371/journal.ppat.1000343

[53] Shoemaker, D.D., Katju, V. and Jaenike, J. (1999) *Wolbachia* and the Evolution of Reproductive Isolation between *Drosophila recens* and *Drosophila subquinaria*. *Evolution*, **53**, 1157-1164. https://doi.org/10.2307/2640819

[54] Fry, A.J. and Rand, D.M. (2002) *Wolbachia* Interactions that Determine *Drosophila melanogaster* Survival. *Evolution*, **56**, 1976-1981. https://doi.org/10.1111/j.0014-3820.2002.tb00123.x

[55] Mazzetto, F., Gonella, E. and Alma, A. (2015) *Wolbachia* Infection Affects Female Fecundity in *Drosophila suzukii*. *Bulletin of Insectology*, **68**,153-157.

[56] Weeks, A.R., Turelli, M., Harcombe, W.R., Reynolds, K.T. and Hoffmann, A.A. (2007) From Parasite to Mutualist: Rapid Evolution of *Wolbachia* in Natural Populations of *Drosophila*. *PLoS Biology*, **5**, e114. https://doi.org/10.1371/journal.pbio.0050114

[57] Olsen, K., Reynolds, K. and Hoffmann, A. (2001) A Field Cage Test of the Effects of the Endosymbiont *Wolbachia* on *Drosophila melanogaster*. *Heredity*, **86**, 731-737. https://doi.org/10.1046/j.1365-2540.2001.00892.x

[58] Fast, E.M., Toomey, M.E., Panaram, K., *et al*. (2011) *Wolbachia* Enhance *Drosophila* Stem Cell Proliferation and Target the Germline Stem Cell Niche. *Science*, **334**, 990-992. https://doi.org/10.1126/science.1209609