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# FIELD PREVALENCE OF CONSERVED *WOLBACHIA PIPIENTIS* IN BRAZILIAN POPULATIONS OF *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

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**Abstract** *Wolbachia pipientis* has been suggested to control insect vectors. In this context, studies on the prevalence and diversity of this bacterium in wild populations are relevant for future interventions. Here, we evaluated the diversity of two *W. pipientis* genes (*ftsZ* and *wsp*) and the prevalence of this endosymbiont in wild *A. albopictus*. Our results showed that 99.3% of *A. albopictus* individuals were superinfected with *Wolbachia* when screened by seminested PCR. In regard to genetic diversity, the two genes showed no variation within or among *A. albopictus* populations. Among the hypotheses may explain the conservation of *wsp* and *fts*Z in *Wolbachia* from *A. albopictus,* there is the crucial role of these genes in CI phenotype, causing a high selective pressure that may inhibit the emergence of new strains. Analysis of other *Wolbachia* markers may help to clarify the symbiotic relationship between these bacteria and their hosts, and thus aid on the development of novel mosquito control strategies.

**Key Words** Mosquitoes, intracellular bacterium, genetic diversity, *fts*Z, *wsp*

## INTRODUCTION

*Wolbachia pipientis*, an intracellular bacterium, infects about 16 to 66% from screened insect species (Hilgenboecker et al., 2008). Part of the success of *Wolbachia* prevalence may be explained by unusual dispersion mode through the manipulation of reproductive processes in arthropods, being cytoplasmic incompatibility (CI) the most common phenotype. CI occurs privileging *Wolbachia*-infected females enhance their maternal transmission into the next generation (Hoffmann, 2005).

In this context, the use of *Wolbachia* as a tool for gene drive has become a subject of interest among the scientific community, especially considering its potential application to control agriculture pests and insect vectors (Bourtzis, 2008). Field trials to release *A. aegypti* infected with *w*MelPop *Wolbachia* strain that shortens insect longevity (*w*MelPop) are scheduled for 2011 in Australia, where the community has been consulted about this issue (Popovici et al., 2010).

Among the mosquitoes of medical importance that are naturally infected with *Wolbachia* is *Aedes albopictus* (Zhou et al., 1998), competent vector to a number of arboviruses, including dengue (de Albuquerque et al., 2000). Although *A. albopictus* has not been related to dengue epidemics in Brazil, larvae naturally infected with dengue virus have been found in Campos Altos city (Minas Gerais State) (Serufo et al., 1993).

In the present study, the prevalence and diversity of *W. pipientis* in wild *A. albopictus* from Brazil were studied through the analysis of two *W. pipientis* genes (*ftsZ* and *wsp*). Considering the interest on the use of *Wolbachia* as a genetic drive tool or as a controlling agent of insect vectors, information about its prevalence and/or diversity in natural insect populations is extremely relevant, nevertheless scarce.

## MATERIALS AND METHODS

### Mosquito Samples

To analyze the prevalence of *W. pipientis* in *A. albopictus* 150 mosquitoes originated from three neighborhoods (Dois Irmãos-DI, Engenho do Meio-EM and Morro da Conceição-MC) of Recife-PE, Brazil, were assayed. For

the analysis of *W. pipientis* diversity, 64 mosquitoes from six neighborhoods of Recife and eight individuals from other cities of PE were assayed. *A. albopictus* from other states were also included in the analysis: two individuals from Rio de Janeiro (Rio de Janeiro city – Manguinhos and Jacarepaguá neighborhoods) and one from Minas Gerais (Passos city). Adult mosquitoes were separated by sex and total DNA was individually extracted according to the protocol described by Ayres et al. (2002).

***Wolbachia pipientis* Prevalence in Wild *A. albopictus***

**Standard PCR.** *W. pipientis* infection diagnosis was performed in 150 individual DNA samples of *A. albopictus* collected in Recife-PE using primers supergroup-specific to the *ftsZ* gene described by Baldo et al. (2006). Negative samples for *W. pipientis* were assayed with primers that amplify a region of 122 bp of the *rpL*8 gene (Lan and Fallon, 1992). Samples that were not amplified by *rpl8* primers were excluded from analysis.

**Seminested PCR.** All the negative samples by standard PCR and infected samples with only one strain of *Wolbachia* were further analyzed through seminested PCR assays. The general *Wolbachia*-specific primers, *wsp*81F and *wsp*691R (618-632 bp), were used in the first PCR and the PCR product was used in the second PCR utilizing primers previously described by Zhou et al. (1998), *wsp*136F and *wsp*691R (577 bp) to supergroup A and *wsp*81F and *wsp*522R (449 bp) to supergroup B.

***Wolbachia pipientis* Diversity in Wild *A. albopictus***

Twenty *A. albopictus* from the three neighborhoods of Recife (DI, EM, and MC) and all samples from the other localities were used. These mosquitoes were previously diagnosed as being infected with both strains (A and B) of *W. pipientis*. Diversity was assessed by analyzing fragments of *ftsZ* and *wsp* genes. Bioinformatics analyses were performed through CodonCode Aligner version 3.6.1 and BioEdit/Clustal W (Hall, 1999) for multiple alignment and manual editing. Sequence identity was confirmed by BLAST search.

**Statistical Analysis**

For comparative analysis of *Wolbachia* infection among populations, chi-square and Fisher's exact test were performed. In all cases, a significant level of 5% was considered. The software utilized for these analyses were Excel 2000 and R v2.10.0. (Zar, 1996).

## RESULTS AND DISCUSSION

***Wolbachia pipientis* Prevalence in Wild *A. albopictus***

Out of the 150 *A. albopictus*, seven were excluded from analysis since in these samples the *rpl8* gene (endogenous control) was not amplified. Of the remaining 143 samples screened through standard PCR, 91.61% were PCR positive for *Wolbachia* (Table 1). There was a significant difference between the percentages of infected males versus females. While 82.76% of males were positive, 97.65% of females were infected ($P$= 0.0043).

On the other hand, when these samples were examined by seminested PCR, 99.3% of the 143 individuals were superinfected, differing significantly from the 67.13% obtained by standard PCR ($P$= 1.078e-12). Using seminested PCR, no mosquito was found negative for *Wolbachia* and only one female from EM (1,18%) was diagnosed positive for only one strain (supergroup B). Regarding the relationship between infected males and females, the seminested PCR did not show any significant difference ($P$ = 1).

Our results showed that 98.82% of *A. albopictus* females were superinfected with *W. pipientis* A and B strains, similarly to what was found in *A. albopictus* females from Thailand by Kittayapong et al. (2002) (97.93%; $P$= 0.8728, when compared to 98.82% found here). Unlike seminested PCR, through standard PCR a lower *W. pipientis* superinfection rate was found in males. According to Berticat et al. (2002), *Wolbachia* density may vary between females and males, being lower in the latter. It may in part explain the lower superinfection rate in males, since there is a DNA threshold in the template to allow amplification by regular PCR when compared to nested (Dutton and Sinkins, 2004).

Besides the general results data obtained with seminested PCR is corroborated by studies showing that *Wolbachia* superinfection in *A. albopictus* females is a common and stable event (Kittayapong et al., 2002). Moreover, the seminested PCR-based method seemed to be efficient for surveying *Wolbachia* prevalence and is recommended for further screening of negative individuals diagnosed as such by traditional PCR.

**Table 1.** Comparison between standard PCR and seminested PCR methods used to assess *Wolbachia* prevalence in *Aedes albopictus* from Recife.

| Status of infection | Number of positive males and females for *Wolbachia* strains with % of positive individuals in parenthesis. | | | |
|---|---|---|---|---|
| | Standard PCR | | Seminested PCR | |
| | Males | Females | Males | Females |
| AB | 22 (37.9) | 74 (87.1) | 58 (100.0) | 84 (98.8) |
| A | 3 (5.1) | 7 (2.4) | 0 (0) | 0 (0) |
| B | 23 (39.7) | 2 (8.2) | 0 (0) | 1 (1.2) |

***Wolbachia pipientis* Diversity in Wild *A. albopictus***

Seventy sequences of *ftsZ* A, 65 of *ftsZ* B, 40 of *wsp* A, and 71 of *wsp* B genes were analyzed of the 14 populations. Results showed no variation in the nucleotide sequences among individuals of the same population (inter-population) or from different populations (intra-population). The nucleotide sequences of *wsp* and *ftsZ* were 100% identical to those published by Zhou et al. (1998), Baldo et al. (2006) and Werren *et al*. (1995).

Armbruster et al. (2003) analyzed *wsp* sequences in 18 *A. albopictus* individuals collected in 14 regions throughout the new and old world, and did not find variation in supergroups A and B. Although, the lack of diversity could be the result of the sampling methodology (only one individual per locality) in the present study we did not find any variation of *wsp* among individuals from the same population or among populations. On the other hand, Reuter and Keller (2003) observed a high recombination rate of *wsp* among *Formica exsecta* individuals from the same population. As for *fts*Z gene, another study six different sequences were found among 11 populations of the spider *Hylyphantes graminicola* collected from distinct geographic regions (Yun et al., 2010).

## CONCLUSIONS

Ayres et al. (2002) found high genetic variability and divergence among *A. albopictus* populations from different regions of Brazil. Notwithstanding, results presented here and in other studies (Armbruster et al., 2003) indicate that *Wolbachia* strains infecting *A. albopictus* are stable and highly conserved, independent of the divergence degree among mosquito populations. Between the hypotheses that may explain the conservation of *fts*Z and *wsp* in *Wolbachia* infecting *A. albopictus* is the crucial role of these genes in CI phenotype, causing a high selective pressure that may inhibit the emergence of new strains. It is clear that this hypotheses must be well studied and, additionally, analysis of other *Wolbachia* markers, such as intergenic sites (Petridis and Chatzidimitriou, 2011), may help to clarify the symbiotic relationship between these bacteria and their arthropod hosts, thus aiding on the development of novel mosquito control strategies.

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