FIELD PREVALENCE OF WOLBACHIA IN THE MOSQUITO VECTOR

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Abstract. The endosymbiotic bacteria in the genus Wolbachia have been proposed as a potential candidate to deliver pathogen-blocking genes into natural populations of medically important insects. The successful application of Wolbachia in insect vector control depends on the ability of the agent to successfully invade and maintain itself at high frequency under field conditions. Here, we evaluated the prevalence of Wolbachia infections in a field population of the Wolbachia-infected mosquito Aedes albopictus. A field prevalence of 100% (n = 1,016) was found in a single population in eastern Thailand via polymerase chain reaction (PCR) testing of Wolbachia both from individual parent females and their corresponding F1 offspring. This is the first report of accurate Wolbachia prevalence in a field population of an insect disease vector. The prevalence of superinfection was estimated to be 99.41%. All single-infected individual mosquitoes (n = 6) were found to harbor group A Wolbachia. For this particular population, no was found to be single-infected with group B Wolbachia. Our results also show that PCR testing of field materials alone without checking F1 offspring overestimated the natural prevalence of single infection. Thus, the confirmation of infection status by means of F1 offspring was critical to the accurate estimates of Wolbachia prevalence under field conditions.

INTRODUCTION

The mosquito Aedes albopictus (Skuse) is native to Asia and the South Pacific and has recently been introduced into the continental United States and South America. This species has been implicated as one of the important vectors of endemic dengue in Southeast Asia. Under experimental conditions, it has been shown to be an efficient vector, and dengue virus has been recovered from field mosquitoes. For example, Ae. albopictus was reported to be naturally infected with dengue virus during the 1995 outbreak in Mexico. Similarly, Ae. albopictus was observed to participate in viral transmission during dengue hemorrhagic fever outbreaks in Singapore and on the island of Samui, Thailand.

Wolbachia infection was discovered in Ae. albopictus by Wright and Barr. They reported the presence of Wolbachia in the ovaries of mosquitoes collected from Thailand. Later, the expression of Wolbachia-mediated cytoplasmic incompatibility in laboratory-bred Ae. albopictus was studied by Kambhampati and others. In 1995, the presence of Wolbachia superinfections was detected in 12 laboratory populations of Ae. albopictus by restriction fragment length polymorphism of both the 16S rRNA polymerase chain reaction (PCR) products when digested with XbaI and the ftsZ PCR products when digested with EcoRV. Results showed that all colonies except for the Mauritius and Koh Samui strains appeared to be superinfected with strains of A and B group Wolbachia. Individuals from these single-infected colonies exhibited Wolbachia-mediated cytoplasmic incompatibility when crossed with other mainland colonies of the same species.

Wolbachia-induced cytoplasmic incompatibility has been proposed as a potential mechanism to introduce and spread transmission-blocking genes into natural populations of insect vectors in an attempt to modify the vector competence of these populations. The success of this long-term goal for disease control is critically dependent on the ability of Wolbachia to invade a host population and to establish a stable equilibrium prevalence within the target population that is high enough to have a significant impact on disease transmission. Previous studies have indicated that in Drosophila simulans field populations infected with wRi Wolbachia, this stable equilibrium frequency is commonly 96–97%. Although this infection frequency is quite high, it may not be high enough to eliminate disease transmission in an insect vector population, even if all Wolbachia-infected insects were genetically altered so as to be completely unable to transmit pathogens. However, very few reliable data exist in species outside of the genus Drosophila to indicate whether infection frequencies reported in Drosophila are typical for other insect species. A number of studies have reported infection frequencies of different insect species that are based on PCR surveys, but these results are notoriously unreliable because of the common occurrence of false-negative results in the PCR assays used to detect Wolbachia in insects.

In this study, we examined the stable field infection frequency of a natural Wolbachia infection in the vector mosquito, Ae. albopictus, in a region of endemic dengue transmission. In order to circumvent the problems of previous studies in accurately measuring this frequency, we used a combination of PCR detection of Wolbachia in field collected adults as well as in the laboratory-reared F1 progeny of these same individuals.

MATERIALS AND METHODS

Mosquito specimens. Aedes albopictus mosquitoes were collected weekly for 6 months from August 1999 to January 2000 in their natural habitats in Hua Samrong Subdistrict, Phaeng Yao District, Chachoengsao Province, eastern Thailand. The method of collection was the standard mosquito landing catch. Live mosquitoes were brought back to the laboratory at Mahidol University in Bangkok, where individuals were identified to species level by use of the morphological keys of Buei and of Rattanarithikul and Panthusiri.

Individual mosquitoes were blood-fed from hamsters and
Prevalence of Wolbachia infection in natural Aedes albopictus population sampling from Chachoengsao, eastern Thailand

<table>
<thead>
<tr>
<th>Date of collection</th>
<th>Total no.</th>
<th>Infected, n (%)</th>
<th>AB</th>
<th>A only</th>
<th>B only</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 1999</td>
<td>180</td>
<td>145 (100.00)</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>September 1999</td>
<td>255</td>
<td>232 (99.15)</td>
<td>2</td>
<td>0.85</td>
<td>0.00</td>
</tr>
<tr>
<td>October 1999</td>
<td>267</td>
<td>259 (99.23)</td>
<td>2</td>
<td>0.77</td>
<td>0.00</td>
</tr>
<tr>
<td>November 1999</td>
<td>142</td>
<td>137 (98.56)</td>
<td>2</td>
<td>1.44</td>
<td>0.00</td>
</tr>
<tr>
<td>December 1999</td>
<td>161</td>
<td>161 (100.00)</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>January 2000</td>
<td>76</td>
<td>76 (100.00)</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>1,081</td>
<td>1,010 (99.41)</td>
<td>6</td>
<td>0.59</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* Total number of field females that laid eggs and screened via PCR for the presence of Wolbachia before excluding samples negative for the Wolbachia outer surface protein (wsp) and the synaptic vesicle nuclear protein (SVNP) genes.
† Percentage infection of Wolbachia based on combined polymerase chain reaction (PCR) results of field females and their corresponding F1 progeny.

RESULTS

A total of 1,568 individuals of Ae. albopictus were collected from 3 adjacent locations in Hua Samrong Subdistrict, Plaeng Yao District, Chachoengsao Province, eastern Thailand. Out of 1,107 blood-fed females, 1,081 laid eggs and were screened via PCR to determine their Wolbachia infection status by use of general wsp primers. Sixty-five individuals were negative for both wsp and SVNP primers and were excluded from the data set. Table 1 shows the frequency of double and single infections of Wolbachia in the Ae. albopictus population sampled from Chachoengsao, eastern Thailand. Our PCR results showed that 100% of the mosquitoes sampled were infected with the A group Wolbachia strain. Prevalence of double infection was determined to be 99.41%. Individuals carrying a single infection of group A Wolbachia were observed at a very low rate, ranging 0.77–1.44% during the months September–November. Over the entire sampling period, the mean percentage of individuals carrying only the group A infection was 0.59%. No individuals were found carrying a single infection of group B Wolbachia in this study. Similarly, no uninfected individuals were sampled.

According to the PCR results of female parents with no F1 confirmation, a total of 995 (97.93%) of 1,016 samples were determined to be superinfected with both A and B strains of Wolbachia, whereas single A-infected and single B-infected individuals were estimated to be 1.48 and 0.59%, respectively (Table 2). However, when the infection status of each female parent was confirmed by PCR testing of her F1 offspring, we found that the PCR result underestimated the superinfected individuals in the field by 1.48%. A female that PCR tested as single infected was considered to be actually superinfected if any of her progeny were superinfected. All male and female F1 offspring of individual parent females that were previously screened as single B-infected

Table 1

<table>
<thead>
<tr>
<th>Date of collection</th>
<th>Infection status</th>
<th>Total no.</th>
<th>AB</th>
<th>A only</th>
<th>B only</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 1999</td>
<td>AB infected</td>
<td>0.979 (995/1,016)</td>
<td>0.994 (1,010/1,016)</td>
<td>1.48</td>
<td></td>
</tr>
<tr>
<td>September 1999</td>
<td>A infected</td>
<td>0.015 (15/1,016)</td>
<td>0.006 (6/1,016)</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>October 1999</td>
<td>B infected</td>
<td>0.006 (6/1,016)</td>
<td>0.000 (0/1,016)</td>
<td>0.59</td>
<td></td>
</tr>
</tbody>
</table>

Table 2

Comparison of Wolbachia infection rate between field-collected female mosquitoes without F1 versus those with F1 confirmation

<table>
<thead>
<tr>
<th>Infection status</th>
<th>Without F1</th>
<th>With F1</th>
<th>Inaccuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB infected</td>
<td>0.979 (995/1,016)</td>
<td>0.994 (1,010/1,016)</td>
<td>1.48</td>
</tr>
<tr>
<td>A infected</td>
<td>0.015 (15/1,016)</td>
<td>0.006 (6/1,016)</td>
<td>0.89</td>
</tr>
<tr>
<td>B infected</td>
<td>0.006 (6/1,016)</td>
<td>0.000 (0/1,016)</td>
<td>0.59</td>
</tr>
</tbody>
</table>
were found to be superinfected with both A and B groups of *Wolbachia*. After combining the PCR data of both parents and F1 offspring, the more accurate superinfection prevalence in this natural population was estimated to be 99.41% (1,010 of 1,016). In addition, only 6 single A-infected individuals were present in this population. No single B-infected individual or uninfected individual was detected, even though a large population of *Ae. albopictus* was sampled in this study (*n* = 1,016).

**DISCUSSION**

Our results show a *Wolbachia* infection frequency of 100% in natural populations of the mosquito vector *Ae. albopictus*. Of these mosquitoes, 99.41% were superinfected, and the remainder were infected with group A *Wolbachia* infection. This prevalence is higher than previous studies that have measured infection prevalence in *Drosophila* populations. It is possible that infection with 2 different strains of *Wolbachia* may contribute to the high fidelity of maternal transmission of *Wolbachia* in natural populations of this mosquito species.

Many different species of mosquito vectors have been reported to be infected with different strains of *Wolbachia*. Therefore, in order to use *Wolbachia* to drive transmission blocking genes into these species, *Wolbachia* superinfections would be needed. The results of this study show that mosquito populations are capable of being superinfected with *Wolbachia* at extremely high frequency and suggest that segregation of single infected strains from superinfected mothers is not a common event.

In this study, no confirmed uninfected individuals were encountered despite extensive sampling. However, we did find 8 females that were negative with specific primers of both *Wolbachia* A and B groups. Unfortunately, all of these females died before laying eggs, so we could not check F1 progeny to confirm their infection status. However, these samples were not positive after PCR testing with the SVNP control primers and were finally excluded from our analysis. If they were truly negative, the rate of uninfected individuals would have been maximally estimated at 0.78% (8 of 1,024), which is still extremely low.

As indicated by Turelli and Hoffmann, data on natural infection frequency is critical in order to evaluate the potential to use *Wolbachia* as a vehicle to modify insect vector populations. Our data provide evidence that *Wolbachia* is a better candidate to use for genetic control experiments of mosquitoes than previous studies focused on *Drosophila* suggest.

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**REFERENCES**


