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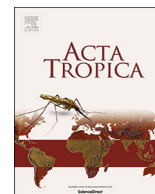
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Screening of natural *Wolbachia* infection in *Aedes aegypti*, *Aedes taeniorhynchus* and *Culex quinquefasciatus* from Guadeloupe (French West Indies)



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ABSTRACT

Guadeloupe islands are threatened by several mosquito-borne viruses such as Dengue, Chikungunya, Zika and West Nile virus. It appears essential to look for alternative mosquito control methods such as the incompatible insect technique (ITT) aiming at sterilizing wild females by inundative releases of incompatible males. Before considering the implementation of such a strategy, the characterization of genetic diversity of the endocellular bacterium *Wolbachia* regarding the local mosquito populations is a critical issue. Here, for the first time, we describe the prevalence and diversity of *Wolbachia* in natural populations of three mosquito species from Guadeloupe: *Aedes aegypti*, *Aedes taeniorhynchus* and *Culex quinquefasciatus*. The detection of *Wolbachia* in natural *Ae. aegypti*, *Ae. taeniorhynchus* and *Cx. quinquefasciatus* populations was conducted by studying *Wolbachia* 16S ribosomal RNA gene using a TaqMan quantitative real-time PCR and results were confirmed by conventional PCR and sequencing. In addition, molecular typing of wPip strains in *Cx. quinquefasciatus* was done by PCR-RFLP. We did not find *Wolbachia* infection in any of *Ae. aegypti* and *Ae. taeniorhynchus* studied populations. Natural *Wolbachia* infection was detected in *Cx. quinquefasciatus* with prevalence varying from 79.2% to 95.8%. In addition, no polymorphism was found between the *Wolbachia* strains infecting *Cx. quinquefasciatus* specimens, all carrying an infection from the same *Wolbachia* genetic wPip-I group. These results pave the way for the evaluation of the feasibility of IIT programs to fight against these medically-important mosquito species in Guadeloupe.

1. Introduction

Since the last three decades, the Guadeloupe islands (French West Indies) have been facing mosquito-borne diseases: (i) dengue is hyper-endemic with epidemics occurring every 2–3 years (Larrieu et al., 2014), (ii) unprecedented chikungunya and Zika outbreaks started in 2013 and 2016 respectively (CIRE Antilles Guyane, 2014; CIRE Antille Guyane, 2016), and (iii) West Nile virus (WNV) circulation have been repeatedly detected since 2002 in horses and chickens (Lefrançois et al., 2005). So far, traditional vector control strategies (i.e. use of chemical insecticides) have failed to prevent the diffusion of vector borne epidemics in naïve human populations and have led to the selection of resistance (Goindin et al., 2017). This fact clearly evidence the urgent need for the implementation of innovative and more efficient vector

control strategies.

The Incompatible Insect Technique (IIT) based on the use of the endosymbiotic *Wolbachia* bacterium has drawn increasing attention in the last years and is currently under development in several countries (Slatko et al., 2014). *Wolbachia* are maternally inherited and in mosquitoes, they induce cytoplasmic incompatibility (CI), a form of embryonic death resulting from sperm-egg incompatibility when *Wolbachia*-infected males mate with uninfected females or with females harboring an incompatible *Wolbachia* strain (Serbus et al., 2008). CI can be either bidirectional when the embryos die in both reciprocal crosses (i.e. males and females infected with different *Wolbachia* strains), or unidirectional. Unidirectional CI appears between infected males and uninfected females (or females infected with a different *Wolbachia* strain), while the reciprocal cross (i.e. infected females and uninfected

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males) is viable. CI can thus be exploited as a source of sterility, which was first deployed in 1967 in a promising pilot trial carried out in Burma against *Cx. quinquefasciatus* (Laven, 1967). This latter mosquito species is naturally infected with different *Wolbachia* strains (*wPip* strains) and the prevalence is high in natural populations (Dumas et al., 2013). The *wPip* strains can be grouped in five distinct subclades or groups (*wPip*-I to *wPip*-V) (Atyame et al., 2011). Interestingly, most *Wolbachia* strains from the same group are often compatible while those from different groups generally show CI (Atyame et al., 2014), highlighting the importance of knowing the *wPip* diversity in order to evaluate the feasibility of IIT against *Cx. quinquefasciatus* in a specific environmental context. *Wolbachia*-based approaches can also be very interesting for the control of mosquito species such as *Ae. aegypti* which are not naturally infected with *Wolbachia* (McGraw and O'Neill, 2013).

In Guadeloupe, *Ae. aegypti* is the main vector of dengue, chikungunya, and Zika viruses (Chouin-Carneiro et al., 2016). *Culex quinquefasciatus* are the most abundant mosquitoes in urban environments after *Ae. aegypti* (Rosine, 1999). This species is highly anthropophilic and have been shown to be competent to transmit WNV in the Americas (Richards et al., 2014). Finally, the mosquito *Aedes taeniorhynchus* is considered as an important nuisance in Guadeloupe and in the Americas (Rey et al., 2012). In addition, this species have been involved in Venezuelan encephalitis epidemics in Americas (Aguilar et al., 2011), and have the ability to transmit West Nile virus (Eastwood et al., 2013). In this study we have undertaken for the first time the screening for the presence and the diversity of *Wolbachia* in three local and medically important mosquito species in Guadeloupe: *Ae. aegypti*, *Ae. taeniorhynchus* and *Cx. quinquefasciatus*. This screening being a prerequisite for the evaluation of the feasibility of IIT against these mosquito vectors.

2. Materials and methods

2.1. Ethics statement

This study has been approved by the board and direction of the Institute Pasteur of Guadeloupe. Anubis Vega Rúa (author of the study) provided a written consent for blood donation in order to artificially feed mosquitoes.

2.2. Mosquito collection and rearing

Mosquito specimens belonging to *Ae. aegypti* (five populations), *Ae. taeniorhynchus* (three populations) and *Cx. quinquefasciatus* (six populations) species were collected as adults or larvae and pupae in different localities in Guadeloupe from January 2016 to April 2017 (Table 1, Fig. 1). Adult mosquitoes were collected using BG-sentinel® traps (with BG-Lure® attractant, CDC-light® traps and an Improved Prokopack Aspirator® (John W. Hocks, Gainesville, USA) closed to human habitations except in the locations îlet à Cochons (IC) and Port-Louis (PL) where trapping were done in natural sites. Traps were set for 48 h. In order to

produce the first generation (F1) of IC1 *Ae. aegypti*, mosquitoes were reared in insectaries under controlled conditions (Goindin et al., 2017) and the emerging females were allowed to blood feed using a Hemotek feeding system (Hemotek Ltd. Great Britain, United Kingdom).

2.3. *Wolbachia* detection

Total DNA of 21–24 mosquito females per population was extracted individually using Cetyltrimethylammonium bromide technique (Lardeux et al., 2008). The detection of *Wolbachia* in natural *Ae. aegypti*, *Ae. taeniorhynchus* and *Cx. quinquefasciatus* populations was conducted via the screening of *Wolbachia* 16S ribosomal RNA gene using a TaqMan quantitative real-time PCR, following the manufacturer's instructions (Applied Biosystems, California, USA) and according to Rao et al. and Mee et al. (Rao et al., 2006; Mee et al., 2015). The quality of the DNA template was checked by the amplification of the sodium channel gene (Martinez-torres et al., 1999) for the individuals in which *Wolbachia* DNA could not be amplified.

2.4. *Wolbachia* sequencing

Sequencing of *Wolbachia* 16S ribosomal RNA gene was done to confirm the results obtained with TaqMan quantitative real-time PCR. The amplification was done using DreamTaq Green PCR Master Mix following the manufacturer's instructions (Thermo Fisher Scientific) and according to Mee et al., 2015. The amplified products were separated by agarose gel electrophoresis and were purified with QIAquick Gel Extraction Kit (Qiagen, Redwood city, CA, USA). Purified and non-purified direct PCR products were sequenced on both strands by Eurofins sequencing platform (Paris, France). The sequencing was based on Sanger method using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) on an ABI 3730xl DNA analyzer. The results were analyzed by SnapGene Viewer software and NCBI Blast online.

2.5. *wPip* strains identification

The genotyping of *wPip* strains infecting *Cx. quinquefasciatus* mosquitoes was performed through PCR-RFLP tests on two ankyrin-domain genes, *ank2* and *pk1*, as previously described (Atyame et al., 2011). These ankyrin genes were found to be robust to distinguish the five *wPip* groups (*wPip*-I to *wPip*-V) (Atyame et al., 2011). The PCR reactions were conducted using a DreamTaq Green PCR Master Mix (Thermo Fisher Scientific). Then, the *Hinf*I (Thermo Fisher Scientific) restriction enzyme was used for the *ank2* gene, whereas a combination of *Taq*I and *Pst*I (Thermo Fisher Scientific) enzymes was used for the *pk1* gene (Dumas et al., 2013). The Digested DNA fragments were separated on 2% agarose gel electrophoresis.

Table 1
Mosquito populations used.

Mosquito population	Collection site	Coordinates	Life stage collected	Mosquito species used	Mosquito generation	Environment
SF	Saint-François	16°17'23.2"N 61°17'01.0"W	Adult females	AA/CQ	F0	urban
M	Le Moule	16°18'53.9"N 61°22'54.8"W	Adult females	CQ	F0	urban
SA	Sainte-Anne	16°14'06.4"N 61°23'19.9"W	Adult females	CQ	F0	urban
G	Gosier	16°13'00.8"N 61°28'59.9"W	Adult females	AA/CQ	F0	urban
PL	Port-Louis	16°25'01.5"N 61°30'59.8"W	Adult females	AT	F0	rural (coastal swamp)
PB	Petit-Bourg	16°10'19.6"N 61°36'49.0"W	Adult females	AA/CQ	F0	urban
DH	Deshaies	16°18'38.6"N 61°47'24.0"W	Adult females	AA/CQ	F0	urban
IC1	Îlet à cochons site 1	16°12'55.0"N 61°32'14.5"W	Larvae, pupae	AA	F1	rural (coastal forest)
IC2	Îlet à cochons site 2	16°12'49.6"N 61°32'11.3"W	Adult females	AT	F0	rural (coastal forest)
IC3	Îlet à cochons site 3	16°13'03.7"N 61°32'32.2"W	Adult females	AT	F0	rural (coastal forest)

AA: *Aedes aegypti*; CQ: *Culex quinquefasciatus*; AT: *Aedes taeniorhynchus*.

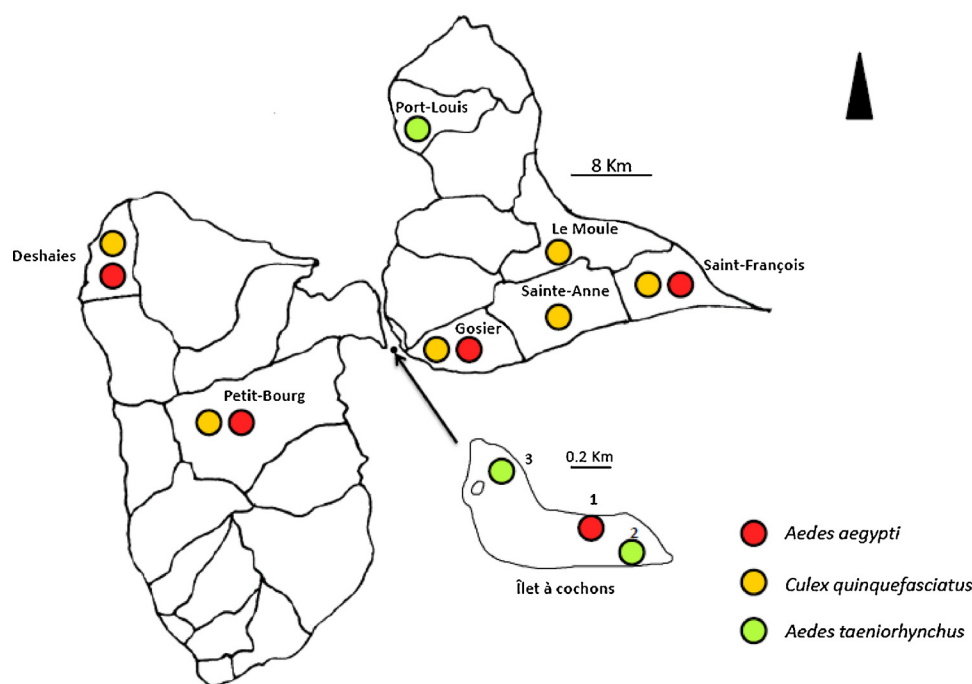


Fig. 1. Collection sites of *Culex quinquefasciatus*, *Aedes aegypti* and *Aedes taeniorhynchus* mosquitoes in Guadeloupe, Map source: drawn by the authors.

3. Results and discussion

Aedes aegypti, the major vector of dengue, chikungunya and Zika viruses is not considered as naturally infected by *Wolbachia* (Ricci et al., 2002), although one study has recently detected *Wolbachia* in a natural population of *Ae. aegypti* from Florida (Coon et al., 2016). In the present investigation, no evidence of *Wolbachia* infection was found in any *Ae. aegypti* population by quantitative PCR and/or sequencing (Table 2). As previously mentioned, these negative results obtained for *Ae. aegypti* in our study are in agreement with the majority of studies conducted about this subject (Nugapola et al., 2017). Finally, the *Wolbachia* natural infection of *Ae. taeniorhynchus* has never been characterized to the best of our knowledge. This first investigation shows that *Wolbachia* does not naturally infect *Ae. taeniorhynchus* populations from Guadeloupe.

By contrast, it is accepted that mosquitoes belonging to the *Culex pipiens* complex are naturally infected by *Wolbachia* endobacteria

Table 2

Prevalence of *Wolbachia* detected in the three mosquito species *Culex quinquefasciatus*, *Aedes aegypti* and *Aedes taeniorhynchus*.

Collection sites	Mosquito species	Number of tested mosquitoes	<i>Wolbachia</i> prevalence (%)
Saint-François	<i>Cx. quinquefasciatus</i>	24	91.7
	<i>Ae. aegypti</i>	22	0.0
Le Moule	<i>Cx. quinquefasciatus</i>	24	87.5
	<i>Cx. quinquefasciatus</i>	24	91.7
Sainte-Anne	<i>Cx. quinquefasciatus</i>	24	91.7
	<i>Ae. aegypti</i>	24	0.0
Gosier	<i>Cx. quinquefasciatus</i>	24	91.7
	<i>Ae. aegypti</i>	24	0.0
Port-Louis	<i>Ae. taeniorhynchus</i>	22	0.0
	<i>Cx. quinquefasciatus</i>	24	95.8
Petit-Bourg	<i>Ae. aegypti</i>	23	0.0
	<i>Cx. quinquefasciatus</i>	24	79.2
Deshaies	<i>Ae. aegypti</i>	21	0.0
	<i>Ae. taeniorhynchus</i>	24	0.0
Îlet à cochons (site 2)	<i>Ae. taeniorhynchus</i>	24	0.0
Îlet à cochons (site 3)	<i>Ae. taeniorhynchus</i>	24	0.0
Îlet à cochons (site 1)	<i>Ae. aegypti</i>	24	0.0

(Rasgon and Scott, 2003). TaqMan quantitative PCR conducted on *Wolbachia* 16S ribosomal RNA gene revealed high prevalence of infection for all tested *Cx. quinquefasciatus* populations, with values ranging from 79.2% (Deshaies) to 95.8% (Petit-Bourg) (Table 2). The *Wolbachia* infection rates (WIR) obtained in our study are slightly inferior when compared to other reports (WIR = 100%; Tmimi et al., 2017) but remain plausible, as even lower WIR (around 30%–60%) have already been found in natural *Cx. quinquefasciatus* populations of Sri Lanka and Iran (Karami et al., 2016, Nugapola et al., 2017). These differences on the WIR reported for *Cx. quinquefasciatus* populations could be explained by a contrasted sensibility of the techniques employed for *Wolbachia* detection, or by real *Wolbachia* prevalence variations according to mosquito population characteristics (i.e. mating behavior, *Wolbachia* vertical transmission rates).

The molecular typing of *Wolbachia pipiensis* (*wPip*) was conducted by RFLP-PCR using *Cx. quinquefasciatus* mosquitoes (24 individuals/population) from Saint-François, Le Moule, Sainte-Anne, Gosier, Petit-Bourg and Deshaies locations. Visualization of the agarose gel revealed a combination of the three enzymatic digestions highlighting *Wolbachia* endosymbionts in *Cx. quinquefasciatus* from Guadeloupe belong to the *wPip*-I genetic group (Atyame et al., 2011). Our results are in agreement with the global typing conducted in 2013 where the *wPip*-I and *wPip*-III were detected in Americas (Dumas et al., 2013). This low *wPip* diversity encourages the development of IIT against *Cx. quinquefasciatus* in Guadeloupe. For this purpose, the next step of this study will be the identification of a *Cx. quinquefasciatus* line whose males are able to sterilize *Cx. quinquefasciatus* females from Guadeloupe. So, cytoplasmic incompatibility phenotypes will be characterized in the laboratory through reciprocal crosses between *Cx. quinquefasciatus wPip*-I populations from Guadeloupe and *Cx. quinquefasciatus* mosquitoes from different geographic origins and infected with other *wPip* group (*wPip*-II, *wPip*-III, *wPip*-IV and *wPip*-V). Indeed, It has been previously demonstrated that CI generally occurs in crosses between mosquitoes infected with genetically divergent *wPip* group while most of the crosses between mosquitoes infected with the same *wPip* group are fertile (Atyame et al., 2014). Such extensive crosses could allow the identification of a *wPip* group that results in bidirectional CI with *Cx. quinquefasciatus* populations from Guadeloupe; and, in contrast to unidirectional CI, the bidirectional CI will prevent the establishment of

exogenous wPip strain in Guadeloupe.

4. Conclusion

In this study we characterized for the first time the natural *Wolbachia* infection and prevalence in three medically important mosquito species from Guadeloupe. *Ae. aegypti* and *Ae. taeniorhynchus* mosquitoes are not naturally infected by this endosymbiotic bacterium, while *Cx. quinquefasciatus* populations displayed a high prevalence of *Wolbachia* infection from the same genetic group (wPip-I). Now that the type of *Wolbachia* infecting the local *Cx. quinquefasciatus* populations in Guadeloupe has been identified, it is possible to go further in the evaluation of IIT program feasibility for this mosquito species by identifying a mosquito line that sterilizes *Cx. quinquefasciatus* females from Guadeloupe.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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