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Aedes aegypti lines for combined sterile insect technique and incompatible insect technique applications: the importance of host genomic background

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Abstract

Aedes aegypti L. (Diptera: Culicidae), being the primary vector of pathogenic arboviruses, is a target for the development of novel genetic approaches to complement current conventional vector control strategies such as the combined sterile insect and incompatible insect technique (SIT/IIT). A transinfected line of *Ae. aegypti* carrying the *w*AlbB *Wolbachia* strain (WB2) was introgressed into two genomic backgrounds, Brazil and Mexico, producing two new *Ae. aegypti* strains (WB2-BRA and WB2-MEX). These strains were evaluated with respect to several life-history traits such as fecundity, fertility, longevity, pupa size, pupation curve, and male mating competitiveness, as well as their response to irradiation. Our results show that the impact of *Wolbachia* infection depends on the genomic background and that the Brazilian one had no significant effect, whereas the Mexican one negatively affected fertility, longevity, and pupal size. Interestingly, *Wolbachia*-infected *Ae. aegypti* lines required a lower irradiation dose to achieve complete female sterility than the uninfected ones. The present findings are discussed given the potential use of *Wolbachia*-infected *Ae. aegypti* lines in combined SIT/IIT population suppression programs.

Introduction

Aedes aegypti L. (Diptera: Culicidae) is the primary vector of major human arboviral pathogens including dengue, chikungunya, yellow fever, and Zika (Bhatt et al., 2013; Kraemer et al., 2015). According to the World Health Organization, these pathogens cause diseases resulting in hundreds of thousands of deaths, and the affected countries face medical service overload and severe economic impacts (Carrasco et al., 2011; Halasa et al., 2011; Martelli et al., 2015). Due to the lack of effective drugs and vaccines, disease management efforts mainly focus on vector control, which largely relies on sanitation and insecticides. However, the anthropophilic behavior of *Ae. aegypti*, its excellent adaptation in urban areas, its increasing resistance to several groups of insecticides, and the difficulty to eliminate its breeding sites (particularly the cryptic ones) suggest that conventional control methods may not be sustainable (Forattini, 2002; Lees et al., 2015; Bourtzis et al., 2016).

Several genetic control methods have been suggested as potential tools for the population control of *Ae. aegypti*

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and its associated diseases, and some of them are currently being tested in the field. These methods aim either at replacing a target population with a strain having reduced vector competence or at suppressing an *Ae. aegypti* natural population below the threshold required for disease transmission (Hoffmann et al., 2011; Bourtzis et al., 2014, 2016; Carvalho et al., 2014; Gato et al., 2014; Lees et al., 2015). Both the replacement and the suppression methods have pros and cons (for a brief review see Bourtzis et al., 2016). The population suppression genetic methods, such as the sterile insect technique (SIT), the *Wolbachia*-based incompatible insect technique (IIT), and the transgenic release of insects carrying a dominant lethal (RIDL) gene, are based on male releases which aim to introduce sterility or lethality in the target population (Dyck et al., 2005; Vreysen et al., 2007; Black et al., 2011; Entwistle, 2011; Bourtzis et al., 2016). All three methods face the major challenge of sex separation given that there is currently no efficient and robust method of sex separation or genetic sexing strains for the major mosquito vector species such as *Ae. aegypti* and *Aedes albopictus* (Skuse) during their mass rearing (Papathanos et al., 2009, 2018; Gilles et al., 2014). Therefore, SIT and RIDL face the risk of releasing potentially disease-transmitting females (even if they are sterile or carrying a dominant lethal gene), whereas IIT faces the risk of the inadvertent release of fertile females which may result in population replacement instead of suppression of the target population (Bourtzis et al., 2016).

In the absence of an efficient and robust method of sex separation for *Aedes* species, it was recently suggested that irradiation (SIT) and *Wolbachia* (IIT) can be combined, which may eliminate the risks associated with the presence of a few females in sterile male batches being released in the field to suppress a target population (Bourtzis et al., 2014, 2016; Lees et al., 2015). The concept of this idea was developed in *Ae. albopictus*, and it was recently validated in a small-scale open field trial in China (Bourtzis et al., 2014, 2016; Zhang et al., 2015a,b, 2016; Lees et al., 2015; Zheng et al., 2019). Briefly, the natural populations of *Ae. albopictus* in Guangzhou, China (GUA strain) are naturally double infected (*w*AlbA and *w*AlbB). The *Ae. albopictus* GUA population was used to establish a triple-infected strain (HC strain) as a donor. Males of the triple-infected *Ae. albopictus* HC strain (*w*AlbA, *w*AlbB, and *w*Pip) have the ability to induce high levels of cytoplasmic incompatibility in the appropriate crosses (Zhang et al., 2015b). Females of the *Ae. albopictus* HC strain have the ability to significantly reduce the transmission of arboviruses such as dengue and Zika, as previously reported for transinfected lines of *Ae. aegypti* (Zhang et al., 2015a; Zheng et al., 2019). In addition, we recently showed that irradiation levels as low as 28 gray (Gy) could completely sterilize *Ae. albopictus* HC females (Zhang et al., 2015a). Thus, during the implementation of a combined SIT and IIT approach, even if a few females are released, these will be fully sterile (due to the irradiation) and will have significantly reduced capacity of pathogen transmission, if any at all (due to the presence of *Wolbachia*). Males are fully sterile due to the presence of the *w*Pip *Wolbachia* strain and the low irradiation doses (Zhang et al., 2015a,b, 2016).

The effectiveness of any male release-based approach, including the combined SIT/IIT for the population suppression of a mosquito vector, depends on the availability of large numbers of high-quality males. Any strain which is a candidate for sterile male releases should undergo quality control analysis and be tested with respect to its productivity and the quality of the males, particularly regarding their mating competitiveness. In the case of the *Ae. albopictus* HC strain, currently used for combined SIT/IIT applications in the field, it was shown that *w*Pip transinfection and irradiation did not affect its life-history traits, including the survival and mating competitiveness of sterile males (Zhang et al., 2015b, 2016). However, mating behavior, as well as vector competence, may be influenced by many factors including genetic background (Beard et al., 1993; Bennett et al., 2002; Menge et al., 2005; Cox et al., 2011; Guo et al., 2013; Campbell et al., 2017). Therefore, it is recommended that mosquito strains developed for release programs should be integrated into the local genomic background to minimize the potential effects associated with their vector competence, mating behavior, and/or insecticide resistance properties.

Natural populations of *Ae. aegypti* are free of *Wolbachia*, although sporadic infections have been reported recently (Coon et al., 2016; Balaji et al., 2019). The present study aimed to assess the potential of the *Wolbachia* transinfected strain *Ae. aegypti* WB2 (*w*AlbB) to be used in population suppression programs using the combined SIT/IIT approach. We introgressed *Wolbachia w*AlbB strain into two genetic backgrounds and used a thorough quality control analysis to assess the impact of *Wolbachia*, irradiation, and genomic background on life-history traits including productivity and male mating competitiveness.

Materials and methods

Mosquito strains and rearing conditions

The following *Ae. aegypti* strains were used in the present study: WB2, BRA, and MEX. The WB2 strain was developed by transferring the *Wolbachia w*AlbB strain from *Ae. albopictus* HOU strain into *Ae. aegypti* Waco strain (Xi, 2005). The transfer was done with microinjections of cytoplasm from donor to recipient early embryos at the Michigan State University according to a protocol described

previously (Xi et al., 2005). The wild-type strain BRA was kindly provided by Prof. Margaret Capurro (University of São Paulo, Brazil). The wild-type strain MEX was kindly provided by the Regional Investigation Centre for Public Health of the National Public Health Institute (Tapachula, Chiapas, Mexico). This latter strain was originally established from field mosquitoes collected in 12 areas of Chiapas using ovitraps in 2016. The *Ae. aegypti* WB2, BRA, and MEX mosquitoes were maintained in the IPCL (Insect Pest Control Laboratory, Vienna, Austria) for at least seven generations before they were used in the experiments.

All three *Ae. aegypti* strains as well as the introgressed lines were maintained in a climate-controlled room at 27 ± 1 °C, 80 ± 10% r.h., and L12:D12 photoperiod. The feeding regime was as described previously (Puggioli et al., 2013) with the following modifications: 26.26 g (35%) bovine liver powder (MP Biomedicals, Santa Ana, CA, USA), 37.5 g (50%) tuna meal (TC Union Agrotech, Bangkok, Thailand), and 11.24 g (15%) brewer yeast powder (Sigma Aldrich, St. Louis, MO, USA) mixed in 1 l of deionized water. Pupae were collected and sexed, although sex was also confirmed upon adult emergence. Blood feeding was as described previously using an artificial membrane system and animal (mainly porcine and bovine) blood from an authorized slaughterhouse following European Union (EU) laws and regulations (Zhang et al., 2015b). Eggs were collected by placing a cup into the adult cage with the inner wall covered with filter paper and half-filled with distilled water. In all subsequent experiments, 3- to 5-day-old mosquitoes were used.

Introgression of *Wolbachia* infection into a local genomic background

For the initial introgression cross, 300 WB2 females and 100 BRA (or MEX) males were placed inside a 30 × 30 × 30 cm adult cage (BugDorm-1; MegaView, Taichung, Taiwan) and were provided with 10% sugar solution ad libitum. Females were first mated and then received a blood meal around 7 days post-emergence. First generation (F1) eggs were collected as described above. The introgressed lines were named WB2-BRA and WB2-MEX. The next generation (F2) was produced by crossing F1 WB2-BRA (or WB2-MEX) females with BRA (or MEX) wild-type males. The introgression continued until it reached almost 100% genomic replacement in the 10th generation, as depicted in Figure S1.

After the first generation, reciprocal crosses (50 BRA or MEX females mated with 25 WB2-BRA or WB2-MEX males, respectively) were set up in adult cages to monitor for the expression of cytoplasmic incompatibility. After mating, females were blood fed; eggs were collected, dried, and hatched using appropriate hatching solution as described in Zhang et al. (2015).

Wolbachia infection status

The *Wolbachia* infection status of the mosquitoes used in the introgression experiment was regularly confirmed by a *Wolbachia*-specific PCR assay (Augustinos et al., 2011). At least 10 individuals were randomly selected in each generation. DNA extraction was done using the ExtractMe kit following the manufacturer's manual. The PCR assay was based on the amplification of part of the 16S *rRNA* gene (438 bp) using the Wspec pair of primers (F: 5'-YAT ACC TAT TCG AAG GGA TAG-3' and R: 5'-AGC TTC GAG TGA AAC CAA TTC-3') under the following conditions: 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s, and a final step at 72 °C for 10 min. Amplification of part of the ribosomal gene 12S *rRNA* (420 bp) was used as control for DNA quality (F: 5'-GAG AGT GAC GGG CGA TAT-3' and R: 5'-AAA CCA GGA TTA GAT ACC CTA TTA T-3') using the following PCR conditions: 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and a final step at 72 °C for 10 min. The PCR products were analyzed in a 1.5% agarose electrophoresis gel (with ethidium bromide) run at 100 V for approximately 60 min in 1X TAE buffer.

Life-history traits analysis

Fecundity was assessed by counting the eggs produced individually by 30 fully engorged and mated females for two gonotrophic cycles. After drying for 1 or 2 weeks at room temperature, the eggs were placed in a plastic Petri dish with some drops of water and counted under a stereomicroscope. Each counted egg paper was placed in a plastic cup (200 ml max. volume) with 40-50 ml water and 3 ml of hatching solution as described previously. Fertility was assessed by the number of recorded larvae (L3 or L4) divided by the total number of eggs.

To determine the pupation curve and morphometrics, 100 larvae were reared in trays as described above. Due to the synchronization of hatching, pupation started on the 7th day post-hatching, and the collection stopped when there were no more pupae in the trays. Pupae were sorted using the glass-plate separator, which separates males and females based on their size (Fay & Morlan, 1959). The number of pupae and their gender was recorded every 24 h. The gender was also confirmed upon emergence. The measurement of the cephalothorax longer transversal line (the longest transversal distance near the respiratory trumpet) was performed using a stereomicroscope. Pictures of the cephalothorax were taken using a CC-12 camera, and its measurements were performed using the analysis B software (Olympus Soft Imaging Solutions, Munster, Germany). Fecundity, fertility, pupation curve, and the size of the cephalothorax were assessed in the 5th generation of introgression for all strains.

To determine longevity, recently emerged adults (less than 12 h) were placed in cages in groups of 50 insects per cage (BugDorm-1) with access to 10% sucrose solution. Males and females were kept separately, and the number of dead mosquitoes was recorded daily for up to 30 days. This experiment was performed in the 7th and 8th generations of WB2-MEX and WB2-BRA, respectively.

Male mating competitiveness

Male mating competitiveness was performed as described previously (Zhang et al., 2016). Briefly, *Wolbachia*-infected and uninfected males were placed in BugDorm-1 cages for at least 60 min before uninfected virgin females were placed inside the cage. Two densities were tested (1:1:1 and 1:1:10, indicating the ratio of uninfected female-to-uninfected male-to-infected and irradiated male), and the irradiation dose was determined as the lowest dose to achieve 100% sterility in females obtained during the female dose-response curve experiment. The male mating competitiveness was conducted between WB2-BRA vs. BRA males and WB2-MEX vs. MEX males. The male mating competitiveness (Fried index) was calculated according to the formula: c = (Hn-Ho)/(Ho-Hs)*(N/S) (Fried, 1971), where Hn is the hatch rate of wild-type males, Ho is the observed hatch rate from competitiveness, Hs is the hatch rate of the sterile/*Wolbachia*-infected males, N is the number of wild-type males, and S is the number of sterile/*Wolbachia*-infected males. The induced sterility index (ISI) was estimated by the formula: ISI = 100 – (Hn/Ho) (Oliva et al., 2012). The expected hatch rate (E) was calculated by the formula provided by Fried (1971) as follows: E = N(Hn)+S(Hs)/N + S. This experiment was performed in three replicates during the 15th generation for all strains.

Irradiation dose-response curve for female sterility

Thirty female pupae, more than 40 h old, were irradiated using gamma rays at 0, 30, 35, 40, 45, and 50 gray (Gy). After irradiation, they were placed in a cage and adult mosquitoes were provided with 10% sucrose solution ad libitum. Non-irradiated males were added to a cage for at least 24 h to mate with irradiated females at a 1:1 ratio (mass cross). Mated females received a blood meal. Females that had not fed were excluded from the analysis. A suitable egg position container was placed inside the cage 3 days after a blood meal. Fecundity and fertility were determined as described above. This experiment was performed in the 9th and 10th generations of WB2-MEX and WB2-BRA, respectively, in three replicates each.

Data analysis

All experimental datasets were analyzed using the R environment and RStudio software (RStudio Team, 2016; R Core Team, 2018). Statistical analysis for fecundity, fertility, and cephalothorax morphometry was carried out using generalized linear models (GLM), with strains and/or irradiation doses as independent variables ($\alpha = 0.05$). Residual fertility for the female dose-response was carried out with a linear regression model with a transformed hatch rate to obtain the highest R^2, using the complementary log-log (CLL) function for transformation. Kruskal–Wallis test was carried out to analyze the female dose-response fecundity, and pupation rates were analyzed with the Wilcoxon rank-sum test for the different strains and gender. The Malcolm-Cox log-rank test and the chi-squared test were used to analyze the longevity and survival data. Male mating competitiveness (Fried index, c) and induced sterility index (ISI) are reported as geometric means (Fried, 1971; Hooper & Horton, 1981). The R code and packages used for the analysis and graph plots presented in this study are available as supplementary material (Statistical summary report).

Results

Introgression

Using the genetic crosses scheme (Figure S1), the *w*AlbB infection was transferred from the nuclear background of the *Ae. aegypti* WB2 strain (originating from Waco, TX, USA) into the nuclear background of the *Ae. aegypti* BRA and MEX strains, originating from Juazeiro (Brazil) and Chiapas (Mexico), respectively. PCR analysis confirmed that the *w*AlbB *Wolbachia* infection was successfully established in the two new strains, *Ae. aegypti* WB2-BRA and *Ae. aegypti* WB2-MEX (Figure S2). The reciprocal cross between virgin wild-type non-infected females (BRA or MEX) and virgin *w*AlbB-infected males (WB2-BRA or WB2-MEX) produced no offspring, confirming the complete expression of cytoplasmic incompatibility (CI).

Life-history traits

The impact of the *w*AlbB *Wolbachia* infection and the genomic background on the life-history traits (fecundity, fertility, pupation curve, cephalothorax size, longevity, and male mating competitiveness) of the *Ae. aegypti* WB2-BRA and WB2-MEX strains were studied. With respect to fecundity, there was no difference between the *Ae. aegypti* BRA and WB2-BRA strains, as each one produced, on average, 74-75 eggs per female (GLM: $F_{1,157} = 0.058$, $P>0.05$; Figure 1A). However, the *w*AlbB *Wolbachia* infection appeared to have a significant cost in the Mexican genomic background, as the number of eggs produced per female was, on average, 76 for the *Ae. aegypti* MEX strain whereas it was only 57 for the *Ae. aegypti* WB2-MEX strain ($F_{1,150} = 48.184$, $P<0.001$; Figure 1A).

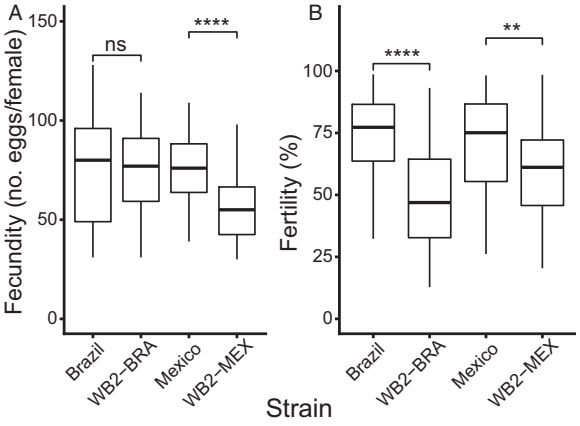

Figure 1 (A) Fecundity (no. eggs per female) and (B) fertility (100% × no. L3 or L4 larvae/ total no. eggs) of uninfected (Brazil, Mexico) and *Wolbachia w*AlbB-infected (WB2-BRA, WB2-MEX) *Aedes aegypti* strains. The whiskers indicate the variability outside the upper and lower quartiles (represented as the upper and lower boxes, respectively). The thick horizontal line represents the median and the dot indicates a possible outlier. The asterisks indicate significant pairwise differences: **0.001<P≤0.01, ***0.0001<P≤0.001, ****P≤0.0001; ns: P>0.05.

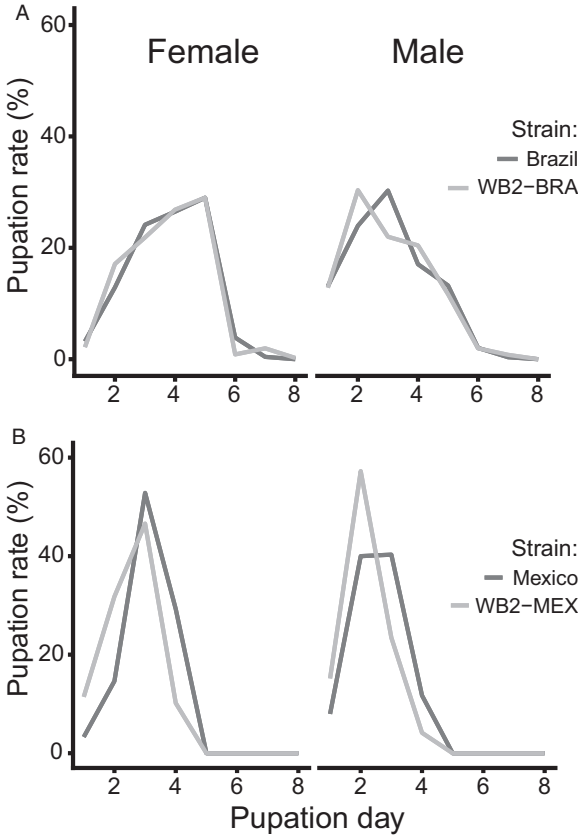

Figure 2 Pupation curves of females and males of uninfected (Brazil, Mexico) and *Wolbachia w*AlbB-infected (WB2-BRA, WB2-MEX) *Aedes aegypti* strains. Pupation rate is represented as the daily percentage of collected pupae over the entire collection period (maximum up to 8 days).

The *w*AlbB *Wolbachia* infection had a strong negative impact on the fertility in both the Brazilian and the Mexican genomic backgrounds as the egg hatch rate was, on average, 73.6% for the *Ae. aegypti* BRA strain and it was only 49.8% for the *Ae. aegypti* WB2-BRA strain (GLM: $F_{1,159} = 66.27$, P<0.001; Figure 1B). Similarly, the egg hatch rate was 69.8% for the *Ae. aegypti* MEX strain and only 59.8% for the *Ae. aegypti* WB2-MEX strain ($F_{1,150} = 10.42$, P<0.01; Figure 1B).

The pupation was monitored at 24-h intervals for both genders and all strains, from the time of appearance of the first pupa until the last pupa was collected. There was no difference in the pupation curve of the uninfected *Ae. aegypti* BRA and the *Wolbachia w*AlbB-infected *Ae. aegypti* WB2-BRA males (Wilcoxon rank-sum test: W = 33.5, P>0.05; Figure 2A). The peak was observed on day 2 after the onset of pupation for the WB2-BRA strain and on day 3 for BRA strain, whereas 100% of the pupae were collected by day 7 for both strains (Figure 2). In contrast, there was no difference in the pupation curve of the BRA and WB2-BRA females (W = 32, P>0.05; Figure 2). The peak was observed on day 5 after the onset of female pupation, for both strains, and 100% of the pupae were collected by days 7 (BRA) and 8 (WB2-BRA) (Figure 2A). Regarding the Mexican strains, there was no difference in the pupation curve of the MEX and WB2-MEX males (W = 32, P>0.05; Figure 2). Like the Brazilian strains, the peak was observed on days 2 and 3 after the onset of pupation, for the WB2-MEX and MEX strains, respectively, whereas 100% of the pupae were collected by day 4 for both strains (Figure 2B). There was also no difference in the pupation curve of the MEX and WB2-MEX females (W = 32, P>0.05; Figure 2B). The peak was observed on day 3 after the onset of pupation for both strains, whereas 100% of the pupae were collected by day 4 (Figure 2B).

Our analysis of the impact of the *w*AlbB *Wolbachia* infection and the genomic background on the size of the pupal cephalothorax showed that there was no difference between the *Ae. aegypti* BRA and WB2-BRA males, which were 1.049 ± 0.0051 and 1.048 ± 0.0052 mm, respectively (GLM: $F_{1,116} = 0.008$, P>0.05; Figure 3). Similarly, there was no difference between the *Ae. aegypti* BRA and WB2-BRA females, which had a mean cephalothorax size of 1.275 ± 0.0081 and 1.269 ± 0.0082 mm, respectively

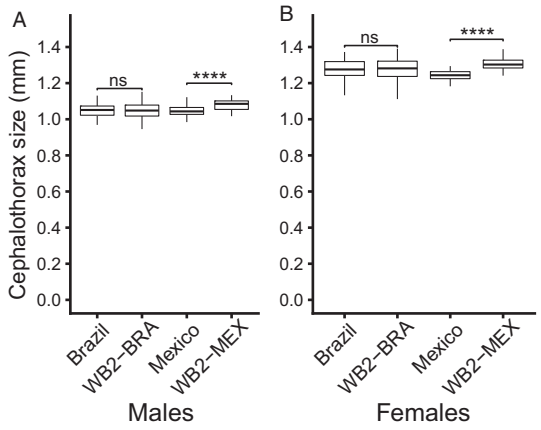

Figure 3 Size of pupal cephalothorax of (A) males and (B) females of uninfected (Brazil, Mexico) and *Wolbachia w*AlbB-infected (WB2-BRA, WB2-MEX) *Aedes aegypti* strains. The whiskers indicate the variability outside the upper and lower quartiles (represented as the upper and lower boxes, respectively). The thick horizontal line represents the median and the dots indicate possible outliers. The asterisks indicate significant pairwise differences: ****P≤0.0001; ns: P>0.05.

($F_{1,116}$ = 0.247, P>0.05; Figure 3). In contrast, significant differences were detected between the uninfected *Ae. aegypti* MEX and the *Wolbachia w*AlbB-infected *Ae. aegypti* WB2-MEX strains, for both males and females. The mean cephalothorax size for MEX males was 1.044 ± 0.0039 mm, whereas that of WB2-MEX males was 1.079 ± 0.0038 mm ($F_{1,116}$ = 46.78, P<0.001; Figure 3). Similarly, the mean cephalothorax size for MEX females was 1.245 ± 0.0043 mm, whereas that of WB2-MEX females was 1.309 ± 0.0050 mm ($F_{1,116}$ = 94.06, P<0.001; Figure 3).

Our data suggest that there was no difference between the *Ae. aegypti* BRA and WB2-BRA strains with respect to male longevity, with a mean survival rate of 32 and 40%, respectively (χ^2 = 0.6, d.f. = 1, P = 0.44; Figure 4A). Female longevity did not differ between the same strains either, with a mean survival rate of 15 and 11%, respectively (χ^2 = 2.4, d.f. = 1, P = 0.66; Figure 4B). Also the longevity of MEX and WB2-MEX males did not differ with a mean survival rate of 94 and 96%, respectively (χ^2 = 0.2, d.f. = 1, P = 0.12; Figure 4C). However, there was a small, marginally significant effect on female longevity in the Mexican strains: the MEX and WB2-MEX strains had a mean survival rate of 8 and 5%, respectively (χ^2 = 3.9, d.f. = 1, P = 0.048; Figure 4D).

Radiation dose-response curve for female sterility

The dose-response for the BRA and WB2-BRA strain showed that there was a difference in the number of eggs produced per female for doses 30-50 Gy in comparison to the control dose (0 Gy) for both strains (Figure 5; see the Statistical summary report in Supplementary Material). Females from BRA treated with 35-50 Gy produced fewer eggs than those treated with 0 or 30 Gy (Kruskal–Wallis test: χ^2 = 38.6, d.f. = 5, P = 2.8e-7) and WB2-BRA females treated with 40-50 Gy produced fewer eggs than those treated with 0, 30, or 35 Gy (χ^2 = 57.9, d.f. = 5, P = 3.3e-11). Also, the number of eggs produced per female differed between the strains for each dose (GLM: $F_{1,140}$ = 7.65, P<0.05).

Similar to BRA and WB2-BRA, the MEX strain showed a difference in the number of eggs produced per female in the same range of doses (40–50 Gy), compared to the other doses (χ^2 = 47.3, d.f. = 5, P = 5e-9), and the same was found for the WB2-MEX strain (χ^2 = 57.6, d.f. = 5, P = 3.9e-11). The number of eggs produced per female for each dose tested differed between strains ($F_{1,140}$ = 10.02, P<0.05; Figure 5; Statistical summary report in Supplementary Material).

A linear regression was calculated to predict the hatch rate (fertility) based on the strain and dose. For Brazil and WB2-BRA, predicted transformed hatch rate is equal to 2.0 + 0.14 (strain) – 0.05 (dose), where strain is coded as 1 = Brazil, 2 = WB2-BRA, and dose is measured in Gy (R^2 = 0.885; $F_{3,116}$ = 297.2, P<0.01). Both dose and strain were significant predictors of transformed fertility. The dose of 50 Gy was found as the minimum dose required to induce 100% female sterility for BRA whereas 45 Gy was required for WB2-BRA strain (Figure 6A).

Similarly, for Mexico and WB2-MEX, predicted transformed hatch rate is equal to 2.0 – 0.13 (strain) – 0.05 (dose), where strain is coded as 1 = Mexico, 2 = WB2-MEX ($F_{3,116}$ = 290.1, P<0.01; R^2 = 0.882). As with the Brazilian strains, a 50 Gy dose was needed to induce 100% female sterility in the MEX strain whereas only 45 Gy was required to fully sterilize females of the WB2-MEX strain (Figure 6B; linear model: $F_{1,116}$ = 45.87, P<0.05). For both infected populations, a dose of 45 Gy was used for WB2-BRA and WB2-MEX male sterility during the male mating competitiveness experiment.

Male mating competitiveness under laboratory conditions

The analysis of male mating competitiveness of the *Ae. aegypti* WB2-BRA and WB2-MEX strains was conducted with cages containing uninfected male-to-infected and irradiated male ratios of 1:1 or 1:10 (BRA:WB2-BRA and MEX:WB2-MEX) in comparison to their respective fertile (cage with only uninfected males and females) and sterile (cage only with infected males and uninfected females) controls. The WB2-BRA test cages had a mean number of eggs per female of 46.8 with a hatch rate of 64.8% at the 1:1

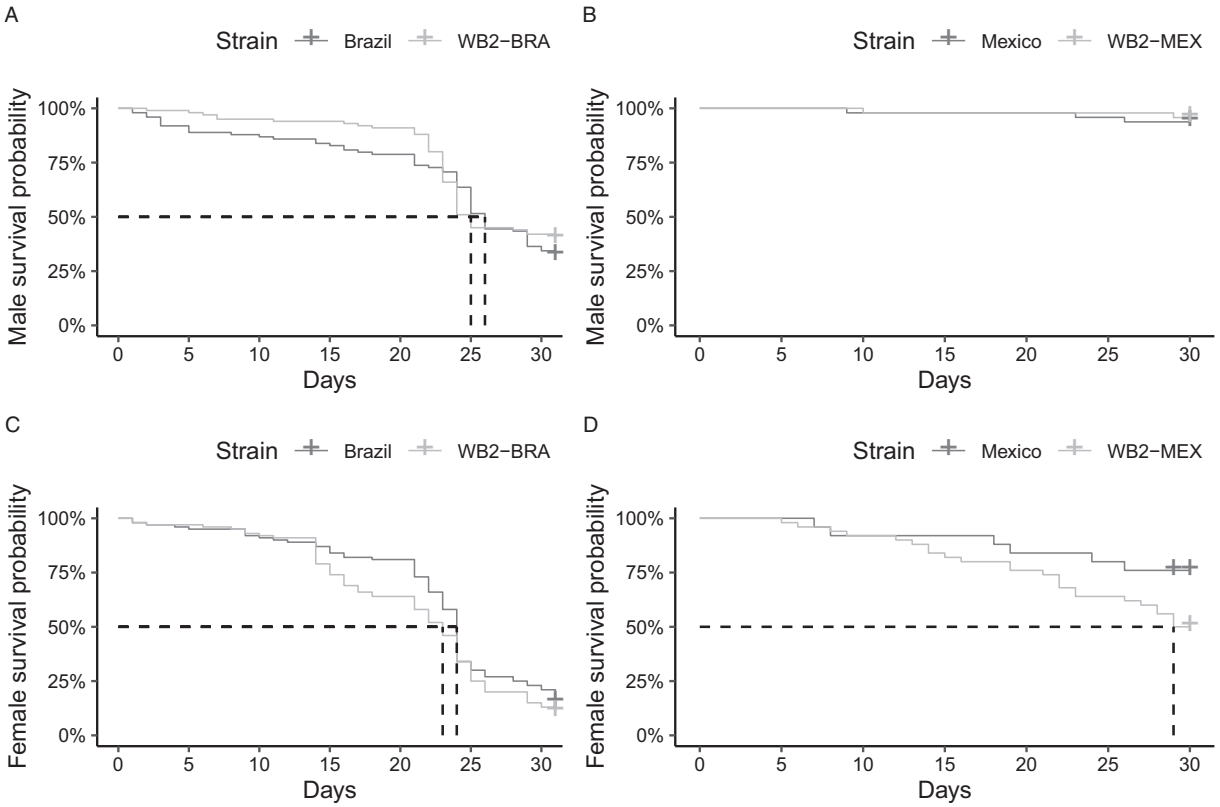

Figure 4 Survival curves of (A,B) males and (C,D) females of uninfected (Brazil, Mexico) and *Wolbachia w*AlbB-infected (WB2-BRA, WB2-MEX) *Aedes aegypti* strains. Survival curve established according to the Malcolm-Cox log-rank test of males and females with sucrose solution access and kept virgins for up to 30 days. Dashed lines indicate the median survival (i.e., 50% survival)

ratio of uninfected male-to-infected and irradiated male, and 46.2 eggs per female with a hatch rate of 9.7% at 1:10 ratio (Table 1). These results reflect induced sterility of 28.0 and 89.3 for 1:1 and 1:10 ratio, respectively, with a corresponding Fried index of 0.40 and 0.88 (Table 2).

The WB2-MEX test cages had a mean number of eggs per female of 44.4, with a hatch rate of 56.0%, and 44.1 eggs per female with 10.2% hatch rate at the 1:1 and 1:10 ratio of uninfected male-to-infected and irradiated male, respectively (Table 1). These results reflect induced sterility of 36.1 and 88.3 for 1:1 and 1:10 ratio, respectively, and a corresponding Fried index of 0.57 and 0.79 (Table 2). For both introgressed strains, the ratio 1:10 presented the best performance regarding induced sterility compared to the other ratios evaluated.

Discussion

The transfer of the *w*AlbB infection from its original *Ae. aegypti* genomic background (Waco, TX, USA) into two new genomic backgrounds affected life-history traits in the Mexican, but not in the Brazilian background. The Brazilian genomic background did not affect any of the life-history traits studied, whereas the Mexican one had a major impact on fertility, pupal size, and longevity. Taken together, our data clearly show that the same *Wolbachia* strain may have a different impact on the fitness and life-history traits of a strain depending on the host's genomic background. Given that several *Wolbachia* strains are currently injected and/or integrated via genetic crosses in various mosquito vector species and strains for population suppression and/or population replacement-based vector control strategies, this finding is of major applied importance as key parameters such as induced sterility, productivity, male mating competitiveness, and vector competence may be affected, positively or negatively, depending on host genomic background. However, it should also be noted that differences which may be observed between laboratories may also be related to different experimental settings and conditions, such as the source of blood, introgression approach, and generations during which the experiment was done.

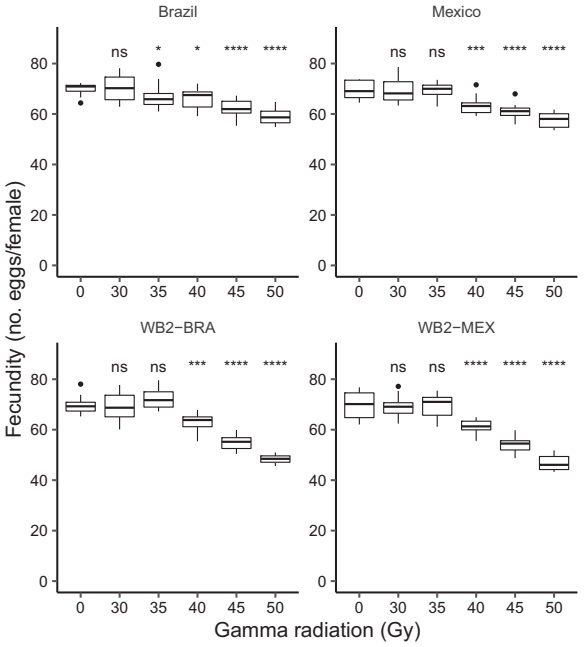

Figure 5 Fecundity (no. eggs per female) at a range of gamma radiation doses for uninfected (Brazil, Mexico) and *Wolbachia* *w*AlbB-infected (WB2-BRA, WB2-MEX) *Aedes aegypti* females. The whiskers indicate the variability outside the upper and lower quartiles (represented as the upper and lower boxes, respectively). The thick horizontal line represents the median and the dots indicate possible outliers. The asterisks indicate significant pairwise differences in comparison to the respective 0 Gy dose: *0.01<P<0.05, **0.001<P<0.01, ***0.0001<P<0.001, ****P<0.0001; ns: P>0.05

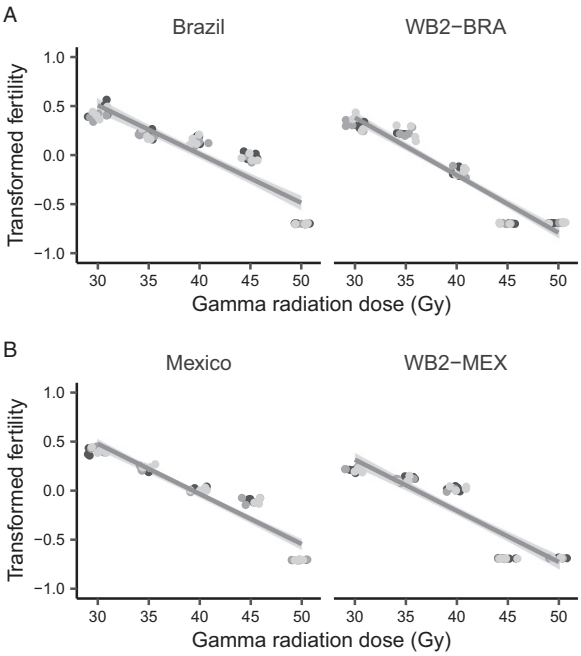

Figure 6 Linear regression of the transformed (complementary log-log) residual fertility on a range of gamma radiation doses for *Aedes aegypti* trains from (A) Brazil and (B) Mexico, both uninfected (Brazil, Mexico) and *Wolbachia* *w*AlbB-infected (WB2-BRA, WB2-MEX). Dots represent the transformed data and intensity of grey its different replicates. The light grey area around the line represents the confidence interval of the linear model

Table 1 Mean (± SE) number of eggs per female and hatch rate (%) of each cross ratio of the *Aedes aegypti* WB2-BRA and WB2-MEX strains, representing wild-type non-infected females: wild-type non-infected males: introgressed *w*AlbB infected and irradiated males

Strain	Cross ratio	No. eggs/female	Hatch rate (%)
WB2-BRA	1:0:1	44.4 ± 0.97	0
	1:1:1	46.8 ± 1.27	64.8 ± 3.6
	1:1:10	46.2 ± 1.37	9.7 ± 1.14
	1:1:0	46.1 ± 1.01	92.4 ± 0.79
WB2-MEX	1:0:1	43.6 ± 0.87	0
	1:1:1	44.4 ± 0.60	56.0 ± 1.12
	1:1:10	44.1 ± 1.46	10.2 ± 1.26
	1:1:0	46.4 ± 0.97	88.0 ± 0.94

The importance of both the *Wolbachia* strain and host genomic background on the expression of cytoplasmic incompatibility (CI) as well as on fitness and life-history traits has been indicated in several studies. Positive and negative effects on the expression of cytoplasmic incompatibility, as well as on fecundity, fertility, and longevity have previously been reported for various combinations of hosts and *Wolbachia* strains (Hoffmann & Turelli, 1997; McGraw et al., 2002; Veneti et al., 2004; Xi et al., 2005; Weeks et al., 2007; Joubert et al., 2016). The embryonic cytoplasmic transfer of *w*AlbB from *Ae. albopictus* to the *Ae. aegypti* Waco background (WB2 strain) did not affect host fitness, although the egg hatch rate was significantly reduced in the first transinfected *Ae. aegypti* line established carrying the same symbiont strain (WB1) into the same genomic background (Axford et al., 2016). An adverse effect on fertility, as well as on longevity and pupal size, was also observed in the present study but only in the Mexican genomic background (WB2-MEX). Single *Wolbachia* infections – *w*Mel, *w*MelPop, and *w*AlbB – on various life-history traits of *Ae. aegypti* showed no effect on fecundity, fertility, mating success, and adult size; however, and similar to our *Ae. aegypti* WB2-MEX strain, a significant negative effect was observed on the longevity

Table 2 Mean (± SE) male mating competitiveness (Fried index) and induced sterility index (ISI; %) of the *Aedes aegypti* WB2-BRA and WB2-MEX strains in two cross ratios, representing wild-type non-infected females: wild-type non-infected males: introgressed *w*AlbB infected and irradiated males

	WB2-BRA		WB2-MEX	
	1:1:1	1:1:10	1:1:1	1:1:10
Male mating competitiveness (Fried index)	0.40 ± 0.09	0.88 ± 0.13	0.57 ± 0.04	0.79 ± 0.12
ISI (%)	28.02 ± 4.43	89.34 ± 1.3	36.08 ± 1.56	88.32 ± 1.44

of females (Moretti & Calvitti, 2013). Another study showed that a double infection of *w*Mel and *w*AlbB *Wolbachia* strains did not affect the fecundity of the *Ae. aegypti* host but significantly reduced fertility and longevity of males and females (Chambers et al., 2011).

Interestingly, the embryonic cytoplasmic transfer of *w*Pip from *Culex pipiens* L. into *Ae. albopictus* and the establishment of the triple-infected HC strain (*w*AlbA, *w*AlbB, and *w*Pip) did not affect the fecundity, fertility, size, longevity, or male mating competitiveness (Zhang et al., 2015b, 2016). It is also worth noting that the male mating competitiveness of the *Ae. albopictus* HC strain was not affected at irradiation doses required for the induction of complete female sterility (Zhang et al., 2015a, 2016). No major effect on male mating competitiveness was observed in single-infected lines of *Ae. albopictus* (*w*Pip), *Ae. aegypti* (*w*Mel), or *Aedes polynesiensis* Marks (Chambers et al., 2011). The range of values of the male mating competitiveness index detected in these studies was similar or higher than the values found for the *Ae. aegypti* WB2-BRA and WB2-MEX strains in the present study. However, lower male mating competitiveness values were reported for a *w*Pip-infected *Ae. albopictus* strain irradiated at 35 Gy, a dose that is usually used to induce complete male sterility (Atyame et al., 2016). Interestingly, low male mating competitiveness, varying from 0.031 to 0.059, has been observed in the transgenic *Ae. aegypti* OX513A strain during open-field trials in Brazil and Cayman Islands (Harris et al., 2012; Carvalho et al., 2015).

The applied significance of *Wolbachia*, particularly with respect to vector and disease control, is due to its ability to induce cytoplasmic incompatibility and block the transmission of major human pathogens (Kondo et al., 2005; Moreira et al., 2009; Hoffmann et al., 2011; Hughes et al., 2012; Bourtzis et al., 2014, 2016; Dodson et al., 2014; Lees et al., 2015; Zhang et al., 2015a,b, 2016; King et al., 2018). Both of these extended phenotypes depend on *Wolbachia* density levels, which in turn can be affected by the host genomic background (Berticat et al., 2002; Reynolds et al., 2003; Veneti et al., 2004; Kondo et al., 2005; Duron et al., 2006; Hughes et al., 2012; Dodson et al., 2014, 2017; Axford et al., 2016; Ahmad et al., 2017; Amuzu et al., 2018; King et al., 2018; Schultz et al., 2018). The *Ae. aegypti* WB2-BRA and WB2-MEX strains used in the present study are infected with *w*AlbB, which has been shown to block the major human pathogens in both *Anopheles* and *Aedes* species (Joubert et al., 2016; Joshi et al., 2017). In addition, a recent study showed that the density levels of *Wolbachia* strains such as *w*AlbB are critical for its interaction with the mosquito innate immune system and the Toll and IMD pathways, which in turn may be associated with the pathogen blocking phenotype (Pan et al., 2017). Therefore, the assessment of the impact of host genomic background becomes very important when *Wolbachia*-infected mosquito strains are to be used in open-field releases for population control. If the host genomic background reduces *Wolbachia* density levels, this would also result in reduced pathogen blocking. The assessment of the vector competence of *Wolbachia*-infected mosquito lines, with or without irradiation, should be investigated prior to any open-field releases given the recent reports that some *Wolbachia* infections may be enhancing the transmission of major human pathogens (Dodson et al., 2014; Hughes et al., 2014; Amuzu et al., 2018; King et al., 2018).

Open-field trials for mosquito population control depend on the development, mass rearing, and release of selected strains with desired properties. However, laboratory development and/or domestication and mass rearing of a strain commonly result in inbreeding. Consequently, this leads to the reduction of genetic diversity of a strain, compromising its biological properties including its productivity, breeding site selection, host preference for blood feeding, mating behavior, and/or vector competence (Dyck et al., 2005; Dodson et al., 2014, 2017). Therefore, any mosquito strain released in nature for vector and disease control should be sexually compatible with the target population and, in addition, it should ideally have similar or reduced vector competence. The best way to avoid undesirable complications and at the same time have an efficient and cost-effective operational program is to release a strain that has the same genetic background as the

target population (Dyck et al., 2005). In addition, maintaining the local genomic background increases biosafety and biosecurity, facilitates regulatory approvals, and increases the chances for public and stakeholders' acceptance.

Conclusions

The combined SIT/IIT approach was recently suggested as a safe and sustainable approach to suppress populations of mosquito vector species below the threshold required for disease transmission (Lees et al., 2015; Zhang et al., 2015a, b, 2016; Bourtzis et al., 2016). Proof of concept has been provided for *Ae. albopictus* under laboratory, semi-field, and field conditions (Zhang et al., 2016, 2017; Zheng et al., 2019). This study presented the characterization of two *w*AlbB-infected *Ae. aegypti* strains, WB2-BRA and WB2-MEX, suitable for the application of the combined SIT/IIT approach against *Ae. aegypti*. An additional double *Wolbachia*-infected (*w*AlbA and *w*AlbB) *Ae. aegypti* has been developed and successfully tested in a small-scale population suppression trial in Thailand (Kittayapong et al., 2018). Given the lack of efficient and robust sex separation methods, there are two critical components for the safe and successful implementation of the combined SIT/IIT approach: (1) the *Wolbachia* infection and its ability to block transmission of pathogens, as discussed earlier and (2) the integration of irradiation that should induce complete female sterility to avoid the release of fertile females, which lead to undesirable population replacement (Lees et al., 2015; Bourtzis et al., 2016). In the case of *Ae. albopictus*, it was shown that low irradiation doses can induce complete female sterility without affecting the productivity or male mating competitiveness (Zhang et al., 2015a,b, 2016). In general, low irradiation doses are expected to have minimal or no effect on the biological quality of the insects. However, the combined effect of *Wolbachia* infection and irradiation on the biological quality of mosquito strains should be studied on a case-by-case basis, including their effect on vector competence, given the effect that the host genomic background may have on these factors, as shown in the present and previous studies.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Scheme of the genetic crosses in order to introgress the local genomic background from Brazil and Mexico into the WB2 strain infected with *Wolbachia*.

Figure S2. Agarose gel (1%) with amplification fragments to confirm the *Wolbachia* infection status over generations (2, 4, 6, and 8) for the strains WB2-BRA and WB2-MEX. The 12S fragment amplification was used for each strain to evaluate the presence and quality of the DNA. Each fragment is around 430 bp as expected. The letter B stands for Brazil (uninfected), M for Mexico (uninfected), and N for negative control of the reaction.

Data S1. Statistical summary report, R code and statistical analysis for *Wolbachia* introgressed lines.

Data S2. Data bank.