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BRIEF REPORT

ENVIRONMENTAL MICROBIOLOGY

Wolbachia strain *w*AlbB shows favourable characteristics for dengue control use in *Aedes aegypti* from Burkina Faso

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Abstract

Dengue represents an increasing public health burden worldwide. In Africa, underreporting and misdiagnosis often mask its true epidemiology, and dengue is likely to be both more widespread than reported data suggest and increasing in incidence and distribution. *Wolbachia*-based dengue control is underway in Asia and the Americas but has not to date been deployed in Africa. Due to the genetic heterogeneity of African *Aedes aegypti* populations and the complexity of the host-symbiont interactions, characterization of key parameters of *Wolbachia*-carrying mosquitoes is paramount for determining the potential of the system as a control tool for dengue in Africa. The *w*AlbB *Wolbachia* strain was stably introduced into an African *Ae. aegypti* population by introgression, and showed high intracellular density in whole bodies and different mosquito tissues; high intracellular density was also maintained following larval rearing at high temperatures. No effect on the adult lifespan induced by *Wolbachia* presence was detected. Moreover, the ability of this strain to strongly inhibit DENV-2 dissemination and transmission in the host was also demonstrated in the African background. Our findings suggest the potential of harnessing *Wolbachia* for dengue control for African populations of *Ae. aegypti*.

INTRODUCTION

Aedes mosquito-transmitted diseases represent a major global health challenge, with incidence increasing considerably over the past 50 years. Reported cases of dengue, the world's most common arboviral disease, have increased over 8-fold, with a dramatic increase in reported deaths over the last decade (Wilson & Chen, 2015; World Health Organization, 2023). Although the epidemiology of dengue in Africa has been less characterized than in other areas of the globe, laboratory-confirmed reports indicate that dengue virus (DENV) is present in 34 African countries (Amarasinghe et al., 2011; Buchwald et al., 2020; Gainor et al., 2022; Wilson & Chen, 2015), with all 4 DENV serotypes circulating (Shah et al., 2020). Over the past decade (2011–2021), outbreaks of dengue have occurred in a number of African countries throughout the continent (reviewed in 2). Since 2013, an increasing number of dengue outbreaks have been registered in West-Central African countries, with Burkina Faso reporting a localized outbreak of 1061 cases, most of which had progressed to dengue haemorrhagic fever (Ridde et al., 2016; Sondo et al., 2021; Tarnagda et al., 2018), indicating that the true incidence of infections with no hospitalization was much higher.

A significant aspect to consider when evaluating dengue epidemiology in Africa is the potential for under

recognition and the underreporting of cases, due to the common diagnostic bias towards classifying most febrile illnesses as malaria, even when patients do not respond to antimalarial drugs. For instance, between 2014 and 2017, a surveillance campaign on children in Kenya demonstrated that >40% of undifferentiated febrile illnesses had evidence of dengue viremia (Shah et al., 2020). Nevertheless, the burden of malaria has concentrated vector control interventions on *Anopheles* mosquito species; therefore, the epidemiological risks related to dengue fever have often been overlooked. For the majority of arboviral diseases, there are currently no approved vaccines or specific antiviral therapies, with efforts relying heavily on vector control strategies. Moreover, an increased rate of urbanization, people and goods exchange, as well as global warming and ecological imbalance, specifically favours the increase and invasion of mosquito species responsible for dengue transmission (Benedict et al., 2007; Lambrechts et al., 2010; Ryan et al., 2019). For these reasons, there is a critical need to develop alternative and effective approaches for dengue control (Ridde et al., 2014).

Aedes aegypti mosquitoes are the primary vectors of the dengue virus and several other arboviruses across the globe; they show high transmission efficiency strongly anthropophilic behaviour, and an ability to breed in close proximity to urban human settings (Gloria-Soria et al., 2016). *Ae. aegypti* is considered responsible for historic epidemics of Yellow Fever virus (Barrett & Higgs, 2007; Ellis & Barrett, 2008) and Chikungunya virus (Thonnon et al., 1998) in West Africa. The species is believed to have originated in Africa from a sylvatic, zoophilic tree-hole breeding ancestor, and evolved into a domestic subspecies (Brown et al., 2011; Gloria-Soria et al., 2016; Gloria-Soria et al., 2018; Kotsakiozi et al., 2018) followed by a global range expansion (Crawford et al., 2017). Increasing urbanization and the absence of reproductive isolation between African subspecies led to independent domestication events in some areas and the evolution of two subspecies; hybridization has subsequently produced genetic admixture of the subspecies (Brown et al., 2011; Crawford et al., 2017; Kotsakiozi et al., 2017). There is higher genetic heterogeneity in African *Ae. aegypti* populations than is found in the globally dispersed form (Failloux et al., 2002; Gloria-Soria et al., 2016; Kotsakiozi et al., 2018; Lorimer et al., 1976). Although entomological reports are incomplete, *Ae. aegypti* is known to occur in all countries of Sub-Saharan Africa, colonizing a broad range of environments, from sylvan to urban (Weetman et al., 2018).

Studies on vector competence of sylvatic African populations of *Ae. aegypti* indicated that their susceptibility to arboviruses is extremely variable and greatly depends on the specific interaction between the host genetic background and the viral isolate (Diallo et al., 2008; Dickson et al., 2014; Gaye et al., 2014; Vazeille et al., 2013). Such genotype-by-genotype specificity and interactions have been documented with several flaviviruses in *Ae. aegypti* and in other host-pathogen systems, with important implications for arbovirus control approaches (Aubry et al., 2020; Dickson et al., 2014).

Vector control and surveillance programmes in West Africa mainly rely on the widespread use of insecticides, the operational limitations of which are increasingly apparent, including increasing levels of insecticide resistance. Among novel dengue control strategies, *Wolbachia*-mediated interventions are currently being deployed in many endemic areas. This maternally inherited obligate symbiont can inhibit virus transmission following transfer into *Ae. aegypti*, a non-native host (Ant et al., 2018; Frentiu et al., 2010; Moreira et al., 2009; Terradas & McGraw, 2017). In addition, to spread through populations, *Wolbachia* can manipulate host reproduction using cytoplasmic incompatibility (CI) (Huang et al., 2015; Martinez et al., 2021; Ruang-areerate & Kittayapong, 2006; Yuan et al., 2012), which generates a frequency-dependent reproductive advantage for *Wolbachia*-carrying females over uninfected counterparts. CI induction occurs when *Wolbachia*-induced sperm modifications induce lethal defects in the fertilized embryo generated from crosses between *Wolbachia*-carrying males and either *Wolbachia*-free females or females harbouring a different symbiont strain. The CI modification and rescue phenotypes are generated by *cifB-cifA* gene pairs (Beckmann et al., 2019; LePage et al., 2017; Shropshire et al., 2018; Shropshire & Bordenstein, 2019).

Several successful field trials for population replacement have been carried out in various endemic areas and two *Wolbachia* strains, *w*AlbB and *w*Mel, are now being deployed for dengue control (Frentiu et al., 2014; Hoffmann et al., 2014; Jiggins, 2017; Nazni et al., 2019; Utarini et al., 2021). The strain *w*AlbB is native to *Ae. albopictus* and was previously artificially transferred into an *Ae. aegypti* colony originating in Malaysia (Ant et al., 2018). The initial characterization of the *w*AlbB-carrying line showed minimal effects on host fitness, unidirectional CI, and strong antiviral activity against major arboviruses (Ant et al., 2018). In addition, exposure of larvae to high tropical temperatures (with peaks of 36°C) did not impact intracellular density and ability to inhibit DENV replication (Mancini et al., 2021; Ross et al., 2020; Ulrich et al., 2016). When considering field *Wolbachia* interventions, it is crucial to understand the specific interactions between the *Wolbachia* strain, and the endemic *Ae. aegypti* population and the local environmental conditions, like temperature, on which the ability of *Wolbachia* to establish may depend. Open field trials started in 2017, demonstrated the

establishment of *w*AlbB-*Ae. aegypti* at high frequency in wild populations in several local dengue transmission hotspots in urban Kuala Lumpur, Malaysia, resulting in significant reductions in dengue incidence (Ahmad et al., 2021). Two years after its deployment in Malaysia, the genomic analysis of the *w*AlbB strain from recaptured mosquitoes showed no evidence of symbiont genomic evolution compared to the originally transinfected strain (Martinez et al., 2022).

Introgression of *Wolbachia* into local mosquito backgrounds represents a key step prior to commencing releases, followed by determining mosquito performance and thus the likely efficacy of the intervention. Effects on fitness traits and variations in vector competence, reproductive incompatibility, and mating preferences are linked to differences in the genomic background of mosquito populations (Leftwich et al., 1818). In the context of *Wolbachia*-based releases, the lack of insecticide-resistant alleles in released mosquitoes carrying *Wolbachia w*Mel had a direct deleterious effect on the establishment of released mosquitoes in Rio de Janeiro, Brazil (Garcia et al., 2019; Garcia et al., 2020). Moreover, *Wolbachia* establishment can be affected by local climate and environmental factors, like high diurnal tropical temperatures or very long dry seasons. The phenotypic stability—including viral blockage capacity—of *Wolbachia* strains, like variants of strain *w*Mel (native to *Drosophila melanogaster*), was shown to be impaired by exposure to high tropical temperatures, in particular during larval stages (Mancini et al., 2021; Ross et al., 2017; Ross et al., 2019). In addition, long egg desiccation conditions can impose substantial fitness costs on *w*Mel and *w*AlbB-carrying females (Ross et al., 2021a; Ross et al., 2023; Ross & Hoffmann, 2022). Therefore, the evaluation of the balance between *Wolbachia-conferred* costs and benefits to the hosts, and the interactions with environmental factors, will be decisive for strain selection and will ensure the long-term viability of the strategy.

Our study aims to phenotypically characterize the major life traits and dengue virus competence of the *w*AlbB *Wolbachia-carrying* line, introgressed for the first time into an African background of *Ae. aegypti* from Burkina Faso, and to investigate the overall potential of this line in laboratory settings for use in dengue control programmes.

EXPERIMENTAL PROCEDURES

Mosquito collection

Aedes aegypti were collected as larvae in urban natural breeding sites in Bobo Dioulasso, Burkina Faso, in October 2018. The larvae were transported to the laboratory of the Institut de Recherche en Sciences de la Santé (IRSS), reared to adults, and blood-fed to generate eggs to start a colony, hereon named BF_WT. Adult mosquitoes were kept in the local insectary and maintained at 28°C with 70%–80% relative humidity and a 12:12-h photoperiod. Eggs were collected on filter paper and stored. Eggs from the third generation (G3) of the field-collected line (G0) were shipped to the Centre for Virus Research in Glasgow, UK.

Wolbachia-carrying lines and introgression

The *Ae. aegypti* line carrying *Wolbachia w*AlbB was previously generated in a colonized wild-type line from Selangor State, Malaysia (Ant et al., 2018). The G4 of the wild-type field-collected *Ae. aegypti* (BF_WT) was used for backcrossing wild-type males with *w*AlbB-carrying females for 6 consecutive generations. Cohorts of more than 500 adults were used for each backcross. *Wolbachia* transmission through generations was confirmed using end-point PCR with strain-specific primers for every generation of backcrossing. gDNA was extracted from whole bodies of 12 randomly selected individuals using STE buffer (10 μM Tris HCl pH 8, 100 mm NaCl, 1 mm EDTA) and quantified using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). PCR reactions were prepared using 1x Taqmaster mix (Vazyme), according to the manufacturer's protocol, and *w*AlbB-specific primers. DNA was amplified with an initial denaturation at 94°C for 3 min, followed by 30 cycles consisting of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and a final step at 72°C for 10 min. 1% agarose gel stained with SYBR-safe DNA Gel Stain (ThermoScientific, UK) was used to visualize the samples. Sequences of primer sets are listed in Table S1.

The same protocol was followed for generating an introgressed *w*Mel *Ae. aegypti* line in the Burkina Faso background. Females of the original *w*Mel-carrying *Ae. aegypti*, established in a lab-adapted Malaysian colony (Ant et al., 2018), were crossed with age-matched males from the wild-type colony from Burkina Faso for six generations. *Wolbachia* transmission fidelity was assessed every generation by end-point PCR using *w*Mel-specific primers, as above.

The newly generated lines (BF_wAlbB and BF_wMel), the originally transinfected wAlbB-*Wolbachia* line (LS_*w*AlbB), and the WT lines (BF_WT and LS_WT) were maintained at 28°C, 70%–80% RH, and a 12:12 h light: dark cycle, with *ab libitum* access to 5% sucrose solution. Females were fed using an artificial blood-feeding system (Hemotek, UK) on human blood (Scottish National Blood Transfusion Service,

UK). Eggs used for the assays were collected on wet filter paper (Grade 1 filter paper, Whatman plc, GE Healthcare, UK), desiccated for only 5–7 days (unless otherwise stated), and hatched in deionized water containing 1 g/L bovine liver powder (MP Biomedicals, Santa Ana, California, USA). Larvae were maintained using tropical fish pellets (Tetramin, Tetra, Melle, Germany).

Wolbachia *w*AlbB density in whole bodies and tissues

Wolbachia intracellular density was quantified in female adults of the stabilized colony (after G6) using relative quantification of the *Wolbachia* 16S rDNA gene against the homothorax gene (*HTH*) as a reference gene (Glazov et al., 2005), on 5-day-old females. 2x SYBR-Green master mix (Biotool, Houston, Texas, USA) with a BioRad CFX-96 real-time PCR detection system (Bio-Rad, Hercules, California, USA) was used for the amplification reaction. The reaction was 95°C for 5 min, 40× cycles of 95°C for 15 s, and 60°C for 30 s, followed by a melt-curve analysis. In addition, *Wolbachia* density was also assessed on mosquito tissues. Ovaries, midguts, and salivary glands (6 pools with 3 sets of tissues) of 5-day-old females were dissected in sterile PBS and transferred in STE buffer for DNA extraction. Quantitative analysis was performed as described above.

Cytoplasmic incompatibility

Patterns of cytoplasmic incompatibility and rescue between BF_*w*AlbB and BF_WT were also assessed by crossing 25 3-day-old virgin females and males of each line. After mating for 3–5 days, females were blood-fed and individualized. The resulting eggs were desiccated for 5 days at standard insectary conditions, counted, and hatched in water containing 1 g/L bovine liver powder. Viable and non-viable eggs were counted, and the hatch rate percentage was measured.

Viral challenge

Four groups of *Ae. aegypti* mosquitoes were challenged with DENV2: (i) BF_WT (ii) LS_WT, (iii) BF_*w*AlbB, and (iv) LS_*w*AlbB. LS_WT and LS_*w*AlbB lines have a lab-adapted Malaysian genetic background where the original transinfected lines have been generated. Together with the corresponding wild-type group, the laboratory *Wolbachia* line was included in the viral challenge assay for a direct comparison of DENV susceptibility between different *Ae. aegypti* genetic backgrounds. Five-day-old females of each strain were infected by feeding through a pork intestine as a membrane on an artificial feeding system. The infectious meal consisted of two-thirds washed rabbit erythrocytes, one-third virus suspension, and ATP (as a phagostimulant). Dengue virus was serotype 2 (-Bangkok Strain 1974, Accession Number MK268692) and at a final concentration of 10^7 FFU/ml in the blood meal. The clinical isolate was passaged two times in *Ae. albopictus* C6/36 cells, two times in *Toxorhynchites amboinensis*, and once in *Ae. aegypti* by intrathoracic inoculation. Viral stocks were obtained by inoculating C6/36 cells, as previously described (Vazeille-Falcoz et al., 1999).

After 14 days, the heads and thoraxes of females were homogenized with glass beads in Trizol (Sigma-Aldrich, MA, USA) on a Precellys 24 homogenizer (VWR) for viral quantification. RNA was extracted with Trizol according to the manufacturer's guidelines and diluted to 100 ng/μL with ddH_2O. cDNA was synthesized using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, MA, USA). DENV-2 was quantified by qPCR using DENV2-specific primers (Table S1). Values were normalized to the host ribosomal RpS17 gene as a reference and viral loads were quantified by relative expression (Pfaffl method).

Wolbachia strain stability at high temperatures

For a direct comparison of *Wolbachia* stability at high temperatures, batches of eggs from BF_*w*AlbB and BF_*w*Mel lines were hatched and exposed to two cycles of fluctuating temperatures in a Panasonic MLR-352-H Plant Growth Chamber incubator (Panasonic, Osaka, Japan). The replicated temperature, named as 33°C_peak diurnal cycle, reflected the average of water temperature readings recorded over 3 months (July, August, and October 2021) during the rainy season in Bobo Dioulasso, Burkina Faso, where BF_WT mosquitoes were originally collected. In addition, a higher temperature cycle, 38°C_peak diurnal cycle, was also tested. Cycles are displayed in panels B and D of Figure 3. Water temperatures were continuously monitored using a data logger (Hobo Water Temperature Pro V2, Bourne, MA, USA). Mosquitoes under control conditions were stably maintained at 27°C in standard insectary conditions, as described above. Pupae from both conditions were sexed according to size, introduced into cages, and maintained during the adult stage at 27°C. Groups exposed to high temperatures and controls had consistent larval density (200 larvae per 500 mL of water) and food provision. Five-day-old adults from each experimental group, control and high temperature-treated, were sampled and *Wolbachia* density was quantified as described before.

Fitness traits of the newly introgressed *w*AlbB-carrying line

Survival, fecundity, and fertility of adult females in the new genetic background were assessed.

Twenty-five females and 25 males of BF_*w*AlbB and BF_WT were maintained in small rearing cages under standard insectary conditions, as described above. Mortality was monitored daily until no live mosquitoes were found in the cages.

Female fecundity was assessed using 5-day-old, blood-fed, fully engorged individualized- females from BF_wAlbB and BF_WT lines. Single oviposition experiments were set with up-turned cups on top of filter paper, and the total number of eggs laid per female was counted.

Additionally, the impact of desiccation on egg survival was measured on collected eggs from blood-fed 5-day-old females. Sections of filter papers containing 200–300 eggs were stored at 27°C and 70% relative humidity. After 5, 10, 15, and 20 days post oviposition, sections were floated in water containing 1 g/L bovine liver powder. Hatch rates were assessed by discriminating between viable and non-viable eggs.

To assess maternal transmission fidelity in newly introgressed lines, 50 individual progeny from *Wolbachia*-carrying females were backcrossed with wild-type males, blood-fed, and their offspring tested at the L4 larval stage with strain-specific PCR, as described above.

Statistics

Graphics were generated using Prism Software (version 9). Statistical analyses were conducted in Prism and R software v. 3.2.3. A Shapiro–Wilk test was used for assessing the normality distribution of data, and parametric and nonparametric tests were selected accordingly for virus titres and *Wolbachia* density after high temperatures exposure. Multiple comparisons were calculated using the Bonferroni method for *p*-value adjustment. *Wolbachia* density was analysed with linear models (*lme4* package) after $\log_{10}$ transformation to meet assumptions of normality. Post hoc pairwise multiple comparisons were performed with the function *emmeans* (R package emmeans) and *p* values adjusted using the Bonferroni method. Multiple comparisons were performed using Multiple t-tests and one-way ANOVA using Holm-Šídák and Bartlett tests. Survival analysis was conducted using a proportional hazard ratio model (Log-rank–Mantel-Cox test).

RESULTS

Wolbachia intracellular density

Wolbachia intracellular density can show variation between different host genotypes. *Wolbachia* density in whole bodies of females was quantified by qPCR at 5 and 10 days post-eclosion (DPE), as shown in Figure 1A. A significant increase in the overall density over time was observed (Age effect::$\chi^2_{df=1} = 0.46$, $p < 0.0001$), which was particularly evident in the African background ($p = 0.01$). *Wolbachia* *w*AlbB reached a similar intracellular density in whole bodies of female mosquitoes in the two genetic backgrounds (Line effect: $\chi^2_{df=1} = 0.02$, $p = 0.43$). The interaction of background and age revealed a significant difference between the groups (Background-by-Age effect: $\chi^2_{df=3} = 0.48504$, $p < 0.001$).

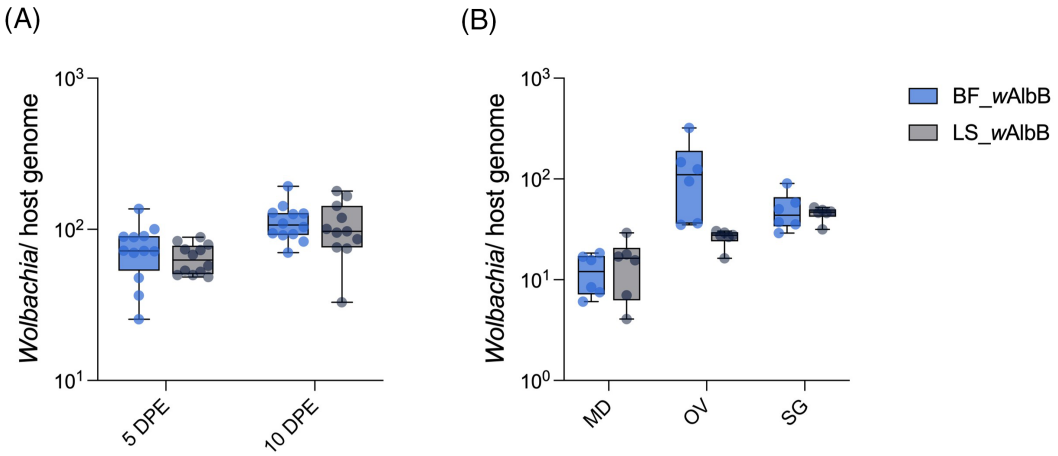

FIGURE 1 *Wolbachia* density in whole bodies and dissected organs of the wAlbB-carrying line in lab strain (LS) and the Burkina Faso (BF) genetic background. (A) *Wolbachia* density was determined in whole bodies of adult females 5 and 10 days post eclosion by qPCR ($N = 12$). (B) Six biological replicates of three sets of midguts (MD), ovaries (OV), and salivary glands (SG) of 5-day-old females were analysed. Boxplots show median and interquartile range.

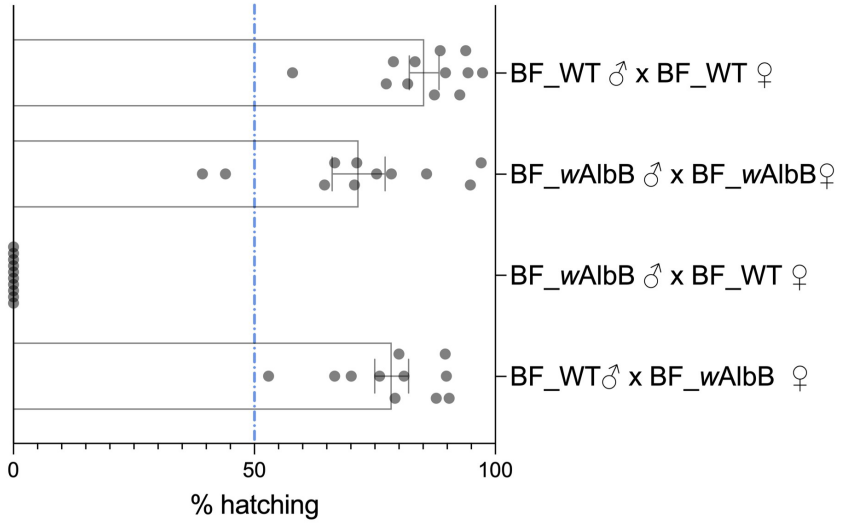

FIGURE 2 Cytoplasmic incompatibility. Percentage hatch rates of eggs were measured from individualized females ($N = 10–12$) from the different crosses between WT and *w*AlbB-carrying males and females in the Burkina Faso background (BF).

To investigate the symbiont tropism, mosquito midguts, ovaries, and salivary glands were dissected and assessed for tissue-specific *Wolbachia* density (Figure 1B). As expected, higher intracellular density of *Wolbachia* was observed in female ovaries and salivary glands, compared to midguts (Organ effect: $\chi^2_{df=2} = 2.9327$, $p < 0.0001$) in both genetic backgrounds. When the effect of the different genetic backgrounds was assessed, no significant differences were observed between the two groups (Background effect: $\chi^2_{df=1} = 0.27$, $p = 0.18$).

Cytoplasmic incompatibility and virus inhibition

Figure 2 shows the pattern of cytoplasmic incompatibility assessed from crosses between *w*AlbB-carrying and wild-type females and males in the African genetic background. Crosses between *Wolbachia*-carrying males and wild-type females confirmed the ability of the *w*AlbB strain to induce fully penetrant CI, generating inviable progeny (0% hatching). Similar hatching rates were observed between WT and *w*AlbB self-crosses, indicating full CI rescue ($p = 0.5$, Kruskal–Wallis, Dunn Post hoc test).

The ability of *Wolbachia w*AlbB to inhibit DENV-2 transmission was previously demonstrated in laboratory challenges using the original lab-adapted transinfected line, also blocking DENV-1 in Malaysian field-collected *w*AlbB-carrying *Ae. aegypti* mosquitoes were shown using viremic blood from patient donors (Ahmad et al., 2021; Ant et al., 2018). Here, we assessed the DENV-2 competence of *w*AlbB-carrying and wild-type mosquitoes in the introgressed African background and the Malaysian lab line in parallel. After the viral challenge, both *Wolbachia*-carrying lines showed a highly significant reduction in viral RNA load in heads and thoraxes compared to wild-type counterparts ($\chi^2 = 75.63$, df = 3, $p < 0.0001$, Kruskal–Wallis test) (Figure 3). Vector competence was not affected by the host genetic background: no statistically significant difference was detected for the comparison of the viral

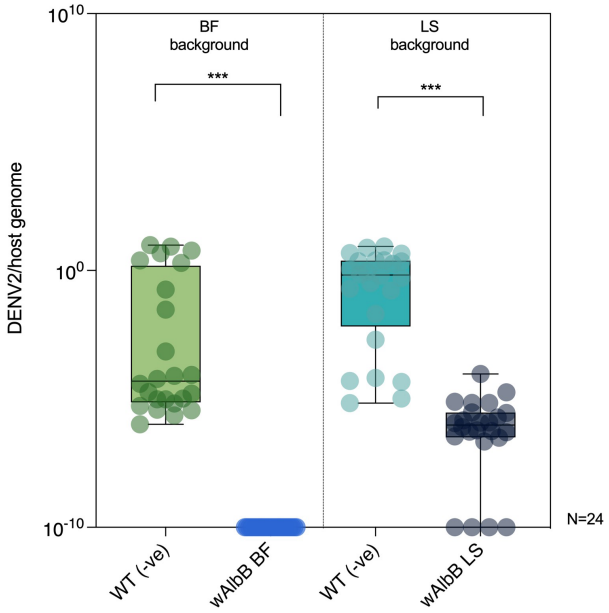

FIGURE 3 DENV2 challenges of *w*AlbB-*Ae. aegypti* in different genetic backgrounds. DENV2 genome copies were quantified by RT-qPCR in the heads and thoraxes of infected females ($N = 24$ for each group), 14 days post blood meal. Groups are wild-type and wAlbB-carrying *Ae. aegypti* in the Burkina Faso (BF) and laboratory (LS) background. Dots represent samples of heads and thoraxes from individual mosquitoes and boxplots indicate median values. ***$p < 0.0001$.

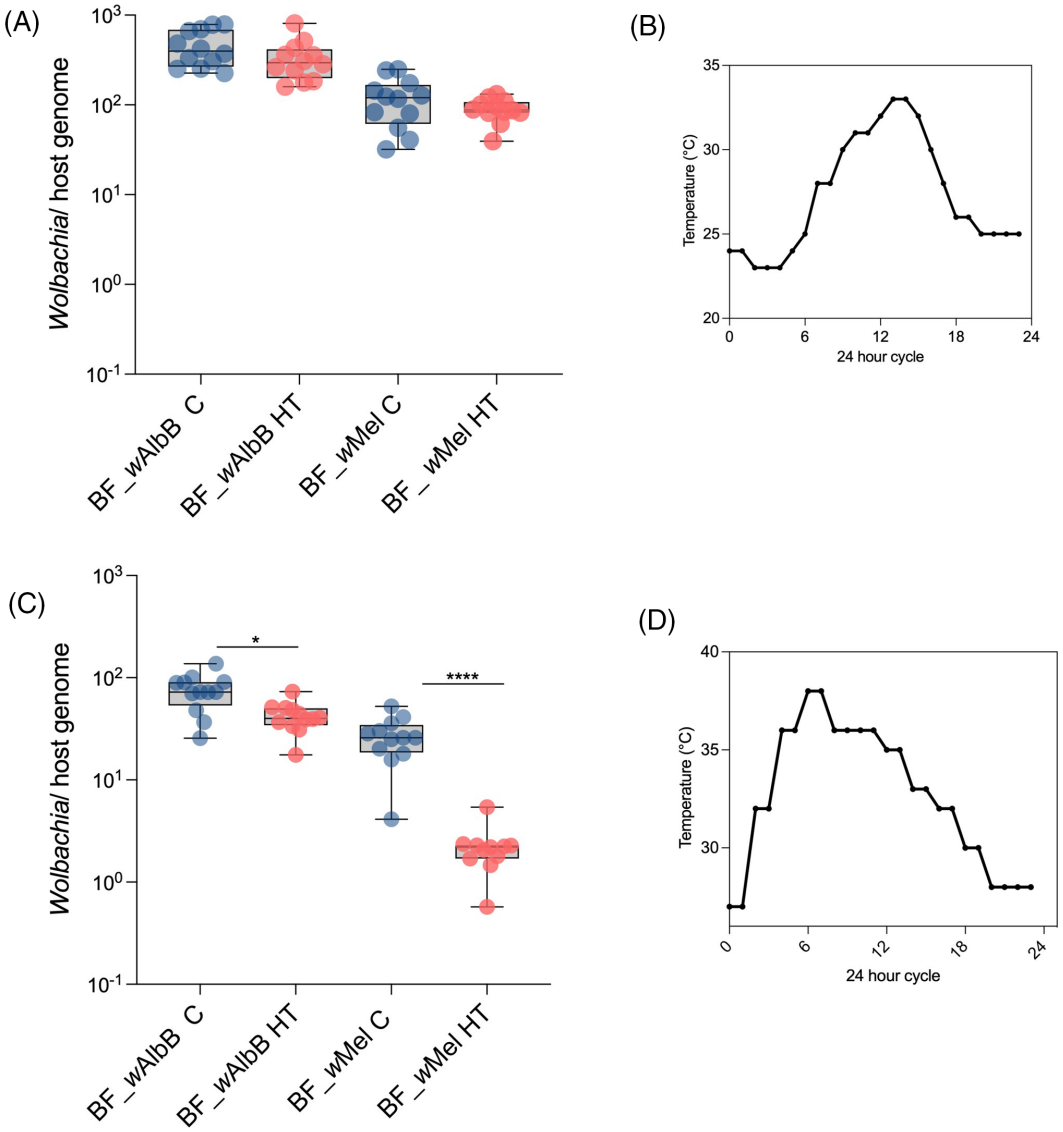

FIGURE 4 Exposure to high temperatures during larval development. (A) Density of *w*AlbB and *w*Mel ($N = 24$) strains introgressed in the Burkina Faso genetic background (BF_wAlbB, BF_wMel) after larval exposure to a diurnal cycle of the high-temperature cycle (HT), shown in (B). (B) Diurnal 24-h cycle of the simulated field temperature, having a Tmax = 33°C. (C) Density of *w*AlbB and *w*Mel ($N = 12$) strains introgressed in the Burkina Faso genetic background (BF_wAlbB, BF_wMel) after larval exposure to a higher diurnal cycle of high temperature (HT), shown in (D). (D) Diurnal 24-h cycle of the simulated field temperature, having a Tmax = 38°C. Controls (C) were maintained at a constant 27°C. Medians are shown. *$p = 0.01$; ***$p < 0.0001$. Non-significant differences are not indicated.

loads between wild-types having the African and the Asian genotypes ($p = 0.8$, Kruskal–Wallis, Dunn Post hoc test). However, the virus inhibition by *w*AlbB in the Burkina Faso background was even stronger than in the Malaysian background ($p = 0.02$, Kruskal–Wallis, Dunn Post hoc test), despite no significant difference in the *Wolbachia* density between the two lines.

Wolbachia phenotypic stability under field-like temperatures

Exposure to cyclical high temperatures during larval developmental stages of mosquitoes is known to result in substantial reductions in intracellular density of some *Wolbachia*: an example is the *w*Mel *Wolbachia* strain (Ant et al., 2018; Mancini et al., 2021; Martinez et al., 2022). To examine *w*AlbB stability at non-standard rearing temperatures in the introgressed African background, BF_*w*AlbB and BF_*w*Mel larvae were exposed to field-like temperatures in two diurnal cycles (Figure 4B,D). The quantitative analysis on *Wolbachia* density of 5-day-old females exposed to the 33°C-peak temperature cycle confirmed that both *w*AlbB and *w*Mel maintained a stable density ($p = 0.1$ and $p = 0.2$, respectively; Mann–Whitney *U* test) (Figure 4A). A reduction in density was however observed after exposure to a cycle reaching higher temperatures with a

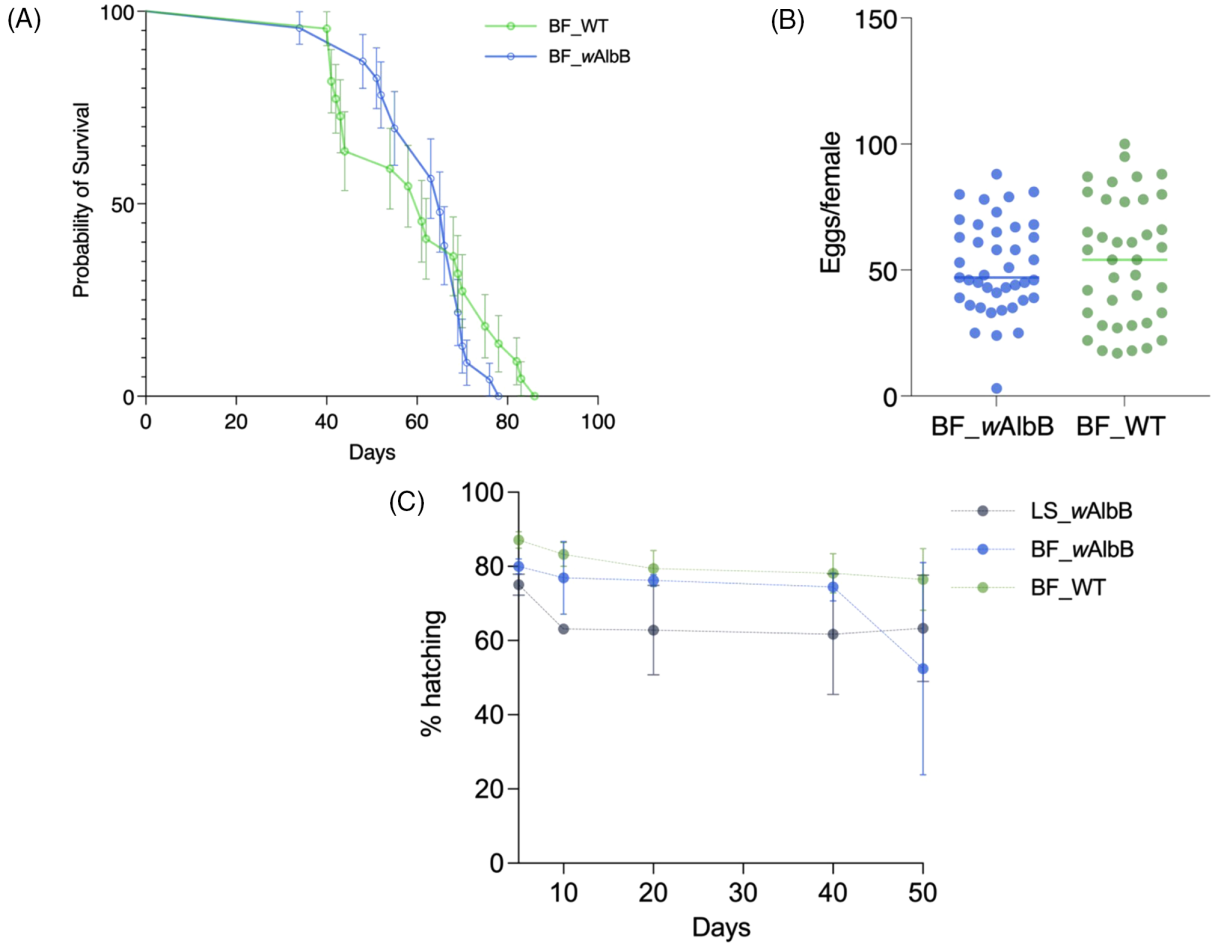

FIGURE 5 Major life history traits of the introgressed *w*AlbB-*Ae. aegypti* line. (A) Survival of adult females of *w*AlbB-infected lines (BF_wAlbB), compared to wild-type (BF_WT). Curves show percentage survival with whiskers indicating 95% confidence intervals from 2 replicate cages for each line each containing a starting number of 25 adult females. (B) Fecundity of females from *w*AlbB-infected line (BF_wAlbB) and wild-type (BF_WT) over the first gonotrophic cycle ($N = 39$, BF_WT; $N = 41$, *w*AlbB_BF. (C) Percentage hatch rates of *w*AlbB-carrying eggs in both genetic backgrounds (Burkina Faso, BF and laboratory, LS), compared to wild. type (BF_WT) after 5, 10, 20, 40, and 50 days of desiccated quiescence at standard rearing conditions. For each time point, the number of eggs assessed varied from 200 to 500. The average ± SD of two independent biological replicates is displayed.

peak at 38°C for a prolonged period of time: *w*AlbB experienced a mild drop ($p = 0.01$, Mann–Whitney *U* test), while the reduction in *w*Mel was significantly more pronounced ($p < 0.0001$, Mann–Whitney *U* test) (Figure 4C). These findings are consistent with previous studies exploring the effects of diurnal high temperatures on the density of the same *w*AlbB and *w*Mel variants in the original Asian genetic background of *Ae. aegypti* (Ant et al., 2018; Mancini et al., 2021).

Major life history parameters of BF_*w*AlbB

The effects of the interaction of the *w*AlbB strain and the African host background were assessed on the major life parameters of introgressed mosquitoes. Maternal transmission fidelity was assessed from blood-fed females individualized for oviposition; the resulting eggs were hatched as single batches and a random selection of the progeny from each family was assessed for *Wolbachia*-infection status using end-point PCR with *Wolbachia*-specific primers. The maternal transmission of *Wolbachia* to the offspring was complete (100%) from the initial crosses and remained stable in standard laboratory conditions.

Potential effects of *Wolbachia* presence were investigated by assessing the survival rate of adult females and males of BF_*w*AlbB and BF_WT *Ae. aegypti*. Females of the BF_*w*AlbB-carrying line showed a similar lifespan to the wild-type group, with a median survival of 61 and 65 days, respectively (Figure 5A) ($p = 0.59$, Log-rank–Mentel-Cox test). In males, a significant reduction in longevity of *Wolbachia carriers* compared to wild-type males was observed (*w*AlbB, $p < 0.0001$) (Figure S1). In addition, the effects of *Wolbachia* strain infection were investigated on fecundity of

BF_*w*AlbB females (Figure 5B). The assessment of the total number of eggs laid by single individualized females showed no impact of *Wolbachia* on fecundity ($p = 0.78$, Mann–Whitney test). To test the effect of quiescence, the hatching rate of eggs stored in standard conditions was assessed after 5, 10, 20, 40, and 50 days. No significant effects of the background (BF_wAlbB vs. Lab_*w*AlbB; $\chi^2_{df=1} = 3.28$, $p < 0.07$) and the *Wolbachia* infection (BF_wAlbB vs BF_WT (−ve); $\chi^2_{df=1} = 1.58$, $p < 0.2$) were observed (Figure 5C).

DISCUSSION

The *w*AlbB *Wolbachia*-carrying line, originally generated in a lab-adapted Asian genetic background, was stably introgressed into a field-collected African *Ae. aegypti* population from Burkina Faso. Mosquito backcrossing and introgression into local genetic backgrounds represent a crucial step for developing effective field-applied mosquito control strategies: they can improve the fitness and competitiveness of the released mosquitoes and reduce any possible reproductive barriers in the field between the released and wild mosquitoes. Given the intrinsic complexity of symbiont-host systems, understanding the interactions between *Wolbachia* strains and hosts is useful. Several transinsfections of Wolbachia have disclosed variations in Wolbachia-conferred phenotypes in the different introduced hosts, suggesting that symbiotic interactions are driven by co-evolution and adaptation.

This becomes particularly valid within the heterogeneous and complex African genetic background of *Ae. aegypti* is crucial for exploring the implementation of *Wolbachia*-deployed strategies in the African continent (Hoffmann et al., 2015). The founders of the BF_WT colony were collected in urban natural breeding sites, showing high human specialization and anthropophilic behaviour.

By direct comparison, the intracellular density of the *w*AlbB strain in the Burkina Faso background and the original transinfected Malaysian line showed substantial similarity in whole bodies over time and similar tissue tropism. In addition, the introgressed lines showed perfect maternal transmission from individualized mothers, generating progeny with stable 100% *Wolbachia* infection.

We confirmed the absence of major detrimental costs in terms of survival, fertility, and fecundity for *w*AlbB, compared to the wild-type counterpart, as previously demonstrated in the original and other genetic backgrounds (Ant et al., 2018; Axford et al., 2016; Ross et al., 2021b). Nevertheless, it is also known that *w*AlbB can induce diverse fitness costs in its new host *Ae. aegypti*, including reduced fertility after a long time of quiescence and reduced quiescent egg viability, most likely determined by the host background (Lau et al., 2021; Lau et al., 2022; Ross & Hoffmann, 2022). *Wolbachia*-induced reproductive fitness costs could be explained by a competition for nutritional resources provided by the host, to which *Wolbachia* is highly dependent (Allman et al., 2020).

Quantitative data on viral RNA in isolated heads and thoraxes only provides a proxy for the transmission potential of mosquitoes. However, given that no viral particles were detected, we can conclude that *w*AlbB provides strong inhibition of DENV-2 transmission in the Burkina Faso genetic background. Susceptibility or refractoriness of *Ae. aegypti* populations to DENV infection show variability between *Ae. aegypti* populations and genetic factors were demonstrated to underly such variability (Fansiri et al., 2013; Lambrechts et al., 2009). In addition, variability in the strength of *w*AlbB blocking capacity was observed, highlighting that the combination of the *Wolbachia* strains and the host backgrounds importantly contribute to the overall transmission capacity. The functional mechanisms behind natural vector competence are still challenging to pinpoint, however studies show that the interaction between *Ae. aegypti* and dengue is a dynamic co-evolutionary process balancing between host antiviral defences and the virus ability to survive within the host through adaptive selection (Koo et al., 2018). Since genotype–genotype interactions between the host and viral lineages are known to greatly impact vectorial capacity (Aubry et al., 2020), further viral challenges with viremic blood from local dengue cases can be employed for assessing lines competence and their interaction with the endemic circulating viral lineages.

Although *Wolbachia*-based control approaches have already been used in several endemic areas, the evaluation of their efficacy must also consider various aspects related to the environment and geography in which the target *Ae. aegypti* populations have adapted. The long-term success of *Wolbachia* population replacement studies depends on its stability in field populations. There is ample evidence that different *Wolbachia* strains in *Ae. aegypti* respond differently to heat stress; however, *w*AlbB-carrying mosquitoes maintain a high *Wolbachia* density, a high maternal transmission rate, and a robust ability to block DENV when exposed to high tropical temperatures (Mancini et al., 2021). Here, we used cycles of fluctuating temperatures, based on field-collected temperature recordings, and we showed that *w*AlbB density in African *Ae. aegypti* remained stable in a field-like scenario for Burkina Faso. In addition, we demonstrated that the density of the *w*Mel strain, known to be susceptible to very high-temperature ranges, is not affected when larvae are exposed to temperature cycles reaching lower peaks (i.e. 33°C in our case). Based on previous evidence, this finding reinforces the hypothesis of a potential *w*Mel threshold to temperature susceptibility set

between 32 and 33°C, considering a similar period of exposure.

Altogether, our data demonstrate the phenotypic stability of *Ae. aegypti* introgressed into an African genetic background carrying a *Wolbachia* strain in a laboratory setting. The BF_*w*AlbB line displayed strong DENV inhibition, stable *Wolbachia* infections, and no major detrimental fitness costs. These findings represent the initial and fundamental steps for developing tailored *Wolbachia*-based control approaches to support DENV control programmes in African populations of *Ae. aegypti*.

AUTHOR CONTRIBUTIONS

Steven P. Sinkins: Writing – original draft; funding acquisition; writing – review and editing; project administration; supervision; conceptualization. **Maria Vittoria Mancini:** Conceptualization; investigation; writing – original draft; formal analysis. **Shivan M. Murdochy:** Investigation; formal analysis; writing – review and editing. **Etienne Bilgo:** Investigation; writing – review and editing. **Thomas H. Ant:** Investigation; writing – review and editing. **Daniel Gingell:** Investigation. **Edounou Jacques Gnambani:** Investigation. **Abdoulaye Diabate:** Supervision. **Anna-Bella Failloux:** Investigation.

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CONFLICT OF INTEREST STATEMENT

The authors declare that no competing interests exist.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Enlighten Research Data repository (University of Glasgow) at https://doi.org/10.5525/gla.researchdata.1579.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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