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Supplemental Information

Wolbachia Genome Stability and mtDNA Variants in *Aedes aegypti* Field Populations Eight Years after Release

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Table S1. Number of ***Aedes aegypti*** samples used for mtDNA analysis. Related to Figure 2.

Location (Suburb name)	Year *Wolbachia* infected mosquitoes released	Infection status	Year of collection		
			2011	2018	2019*
			Amplicon sequencing		Mosquito sequencing
Gordonvale	2011	+		12	30
Yorkeys Knob	2011	+	10†	12	5
Edge Hill	2013	+		20	25
		-		2	
Parramatta Park and Cairns North	2013, 2014 and 2017	+		17	28
		-		12	
Parramatta Park and Portsmith	2014 and 2017	+		20	12
Bungalow	2014	-		3	
Caravonica	No releases	+			2
		-			5

*The samples used for the 2019 analysis are the same as those used for the genome sequencing of *Wolbachia*

†Original 2011 samples from Yeap et al (Yeap et al., 2016) were included to represent the field release stock.

Table S2. Haplotype combinations for *CO*I region. Haplotypes are numbered in the first column on the left. Positions of SNPs in the 750 bp fragment, 1: **282**, 2: **531**, 3: **744;** Position underlined is the single nucleotide polymorphism that is associated with the *Wolbachia* infection. Related to Figure 2.

	1	2	3
	C	G	T
1	-	A	G
2	-	-	-
3	T	-	G

Transparent methods

Sample collection. A *w*Mel-infected colony of *Ae. aegypti* was initially started from mosquitoes collected from Cairns, Queensland, Australia in 2013, in areas where the *w*Mel infection had established. To maintain this colony, *Wolbachia*-infected females have been crossed 1-2 times a year to uninfected males sourced from recently collected field material.

Eggs of *Ae. aegypti* were collected using ovitraps deployed on 2-4 April 2019 in 6 release suburbs in Cairns and one isolated suburb where *Wolbachia* had not been released (Fig 1). Eggs from each ovitrap were hatched and larvae reared at 26 ^{o}C and 12:12 L:D. On days 5-7 post emergence, adults from each site were immobilised on wet ice, sorted into pools of up to 5 females and frozen at -80 ^{o}C. The mtDNA variation was analysed in *Ae. aegypti* individuals from the above source as well as field collections of uninfected and infected mosquitoes (Table S1) originating from eggs from ovitraps collected in 2018, with emerging adults then screened for *Wolbachia* (as in Ross et al. (Ross et al., 2020)). Note that original samples from Yeap et al (Yeap et al., 2016) were also included to represent the field release stock.

Sample processing and analysis: *w*Mel. *Wolbachia* DNA was purified from the pooled *Ae. aegypti* field material (Iturbe-Ormaetxe et al., 2011). There was a total of 23 field samples with mosquito numbers per pool of 2-5, with a mean of 4.4. A subcellular fraction was then prepared from the lysed mosquitoes which was enriched for *Wolbachia* (Iturbe-Ormaetxe et al., 2011), and DNA was extracted using the QIAamp Viral RNA extraction kit (Qiagen). Nextera XT libraries were prepared from the DNA and these were sequenced on an Illumina NextSeq500 (paired end 2 x 150 nt), obtaining approximately 5-15 million total reads per sample. The genomes were assembled from the Illumina data using *w*Mel (NC_002978.6) of total size 1,267,782 nt as a reference (Wu et al., 2004). For field samples, genome coverage varied from 99.7 – 100%. Pools from 22 of the sites in release suburbs, the positive Caravonica pool and a colony control sample produced a minimum genome coverage of 99.97% for at least one sample for each suburb. A single nucleotide polymorphism (SNP) analysis tool from the Geneious package (Kearse et al., 2012) was used to find differences between the sampled genomes and the reference. This tool was set at a minimum depth of 30 reads and a minimum frequency of 0.25 (default). Where a polymorphism was detected, this site was further analyzed in all samples to determine the within-sample nucleotide frequency.

Sample processing and analysis: mtDNA. The COI amplicon sequencing is based on Yeap et al. (Yeap et al., 2016) who identified SNP variation associated with the *Wolbachia* infection in this region (9). Samples were analysed for a 750bp region within the COI region (positions 1994-2743 on GenBank: EU352212.1) using forward primers 5'AGTTTTAGCAGGAGCAATTACTAT3' and reverse primers 5'TCCAATGCACTAATCTGCCATATTA3'. PCR amplicons from individuals were sequenced in both forward and reverse directions using Sanger Sequencing (Macrogen, Inc., Geumcheongu, Seoul, South Korea). The sequence 970bp region was analysed using Geneious package (Kearse et al., 2012) to investigate SNP variation among samples. We also lined up the rest of the mtDNA genome for the 2019 field samples where full sequence data was available. The mtDNA genomes were assembled from the Illumina data using the *A. aegypti* LVP_AGWG mitochondrial genome (NC_035159), of total size 16,790 nt, as a reference. Low-coverage positions (coverage < 3) were masked. Sequence regions within the COX2 (174bp), ATP6 (582

bp), ND1 (683 bp), ND3 (240 bp), ND4 (719 bp), ND5 (902 bp) and CYTB (968 bp) genes were analysed for SNP variation among samples.

Supplemental references

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