Fine-scale landscape genomics helps explain the slow spread of *Wolbachia* through the *Aedes aegypti* population in Cairns, Australia

Thomas. L. Schmidt*, Igor. Filipović, Ary A. Hoffmann, Gordana Rašić

Affiliation: School of BioSciences, Bio21 Institute, University of Melbourne, 30 Flemington Road, Parkville, 3010 VIC, Australia

***Correspondence**: T.L. Schmidt, E-mail: t.ludovic.schmidt@gmail.com

Keywords: *Aedes aegypti*, dispersal, *Wolbachia*, kinship, genome-wide single-nucleotide polymorphisms (SNPs), landscape genomics

Running Title: Dispersal and *Wolbachia* loss in *Aedes aegypti*

Abstract

The endosymbiotic bacterium *Wolbachia* suppresses the capacity for arboviral transmission in the mosquito *Aedes aegypti*, and can spread through wild mosquito populations following local introductions. Recent introductions in Cairns, Australia have demonstrated slower than expected spread, that could be due to: i) barriers to *Ae. aegypti* dispersal; ii) leptokurtically distributed dispersal distances; and iii) intergenerational loss of *Wolbachia*. We investigated these three potential causes using genome-wide single-nucleotide polymorphisms (SNPs) and an assay for the *Wolbachia* infection *w*Mel in 161 *Ae. aegypti* collected from Cairns in 2015. We observed a significant barrier effect of Cairns highways on *Ae. aegypti* dispersal using distance-based redundancy analysis and patch-based simulation analysis. We detected putative full-siblings in ovitraps 1312m apart, suggesting long-distance female movement likely mediated by human transport. Finally, we found a pair of full-siblings of different infection status, suggesting loss of *Wolbachia* in the field. While the long-distance movement and *Wolbachia* loss currently represent single observations, these findings together with the identified dispersal barriers can contribute to the slow spread of *Wolbachia* through the *Ae. aegypti* population in Cairns. Our landscape genomics approach can be extended to other host/symbiont systems that are being considered for biocontrol.

Introduction

The mosquito *Aedes aegypti* (Diptera, Culicinae) is the primary vector of arboviruses such as dengue, Zika and chikungunya, that impose an increasing burden on human health worldwide [1, 2]. Conventional approaches to combatting these viruses have involved the suppression of *Ae. aegypti* populations through source reduction or insecticide-based programs, but these have had limited efficacy [3, 4]. One alternative strategy involves the release of *Ae. aegypti* carrying an infection of the virus-inhibiting, endosymbiotic bacteria *Wolbachia* into wild *Ae. aegypti* populations [5]. Once they are widespread in host populations, *Wolbachia* are expected to diminish the disease transmission rate enough to prevent outbreaks [6]. Although *Ae. aegypti* does not naturally carry *Wolbachia,* several *Wolbachia* strains have been successfully transferred into *Ae. aegypti* from other hosts [7, 8].

The *Wolbachia* strain *w*Mel, originating from *D. melanogaster*, has proven suitable for field deployments in *Ae. aegypti* given its viral blockage [6], moderate fitness costs [7], high intergenerational transmission fidelity [9] and complete cytoplasmic incompatibility [7]. Cytoplasmic incompatibility (CI) describes the phenomenon that offspring of uninfected females mated with *Wolbachia*-infected males are unviable, while offspring of *Wolbachia*-infected females are viable and will carry the infection regardless of male infection status [10]. When *Wolbachia*-infected males are common, CI greatly reduces the relative fitness of uninfected females, which ensures that future generations will be increasingly comprised of *Wolbachia*-infected individuals [11]. However, *Wolbachia* also impose frequency-independent fitness costs on hosts, which for *w*Mel in *Ae. aegypti* include a shorter lifespan [7] and reduced larval competitive ability [9, 12-14]. The interaction between costs and benefits produces a critical frequency of *Wolbachia* infection ($\hat{p}$) that needs to be exceeded for *Wolbachia* to invade the mosquito population [11, 15, 16].

Releases of *w*Mel-infected *Ae. aegypti* into two sites near Cairns, Australia, confirmed that *Wolbachia* can establish stably in quasi-isolated habitat patches [9, 17]. A subsequent study with releases centred within continuous mosquito habitat demonstrated the successful spread of the invasion into surrounding habitat, so that the infection region grew ≈70-85% over the ensuing 18 months [18]. However, the rate of spread of approximately 100-200m per year is close to the lower bound of predicted spread speeds for this host/*Wolbachia* system [19]. This suggests that various potential biological and environmental processes could be operating to restrict the speed or extent of the spread [19, 20].

This study investigates three potential causes for the slow spread observed in Cairns: i) barriers to mosquito dispersal; ii) leptokurtic distribution of dispersal distances; and iii) occasional intergenerational loss of *Wolbachia* (designated as μ). Identifying causes of slow spread in Cairns will help future *Wolbachia* deployments to achieve regional invasion faster and at lower cost. We screened *Ae. aegypti* from Cairns for the *w*Mel transinfection and genotyped individuals at genome-wide single nucleotide polymorphisms (SNPs) using double digest restriction-site-associated DNA sequencing (ddRADseq [21]). SNP datasets produced with ddRADseq have been used to elucidate genetic structure in *Ae. aegypti* within cities [22, 23], and have power superior to microsatellites when inferring relationships between *Ae. aegypti* individuals and populations [24].

Fine-scale dispersal of *Ae. aegypti* is mostly accomplished through short-range flight [25-30], which may make highways and other geographical features effective barriers to movement. In Cairns, a Mark-Release-Recapture (MRR) study with releases centred next to a 20m-wide road

recorded lower recapture rates at traps across the road [28]. Similarly, patches separated by a 120 m-wide highway in Trinidad had different frequencies of mitochondrial haplotypes [31]. Observing *Wolbachia* invasion dynamics around highways also allows for indirect inference of highway barrier effects. Specifically, *Wolbachia* failed to invade a region across a highway in Gordonvale, Queensland after several years [19], and similar dynamics were recently observed in urban Cairns [18]. In this study we explicitly tested the hypothesis that the genetic structure of *Ae. aegypti* in Cairns is affected by highways acting as barriers to dispersal. This was tested alongside the hypotheses that genetic structure reflected a simple isolation-by-distance (IBD [32]) pattern, and that it was affected by the recent patchy and asynchronous releases of *Wolbachia*-infected mosquitoes throughout the region (see Schmidt et al. [18]).

If host dispersal distances follow a high-kurtosis, leptokurtic distribution, *Wolbachia* spread can proceed up to ≈4 times slower than if the distribution has low kurtosis [19]. This is because high-kurtosis distributions have greater numbers of short-distance and long-distance dispersers [33]. Longer-than-average dispersal distances increase the likelihood that a *Wolbachia*-infected individual will move to an uninvaded region, where they will receive no frequency-dependent fitness benefits and thus contribute less to the invasion. Passive dispersal of *Ae. aegypti* by humans, such as when mosquitoes enter vehicles, may lead to higher kurtosis. Passive movement may often be orders of magnitude higher than the typical 50-100m flight range of *Ae. aegypti* [25-30], leading to higher kurtosis of the dispersal kernel. Passive dispersal is commonly observed in immatures [34], however, movement of imagoes may also occur. *Aedes aegypti* females lay batches of eggs over multiple gonotrophic cycles [35], and through multiple acts of "skip" oviposition within a gonotrophic cycle [36], and thus full-sibling immatures can be found at different sites. In this study we use the distance of separation between sampled full-siblings to indicate female flight ranges or passive dispersal distances within one or two gonotrophic cycles.

Slow *Wolbachia* spread can also result from intergenerational loss of *Wolbachia* (i.e. $\mu > 0$), which will lead to an increase in the critical infection frequency $\hat{p}$ [16]. Loss of *w*Mel has not been detected in laboratory-reared *Ae. aegypti* [7, 13]. However, laboratory populations subjected to high, fluctuating temperatures similar to those of Cairns showed significant decreases in *w*Mel density [37, 38], which could lead to loss of the infection [39, 40]. We looked for evidence of infection loss in Cairns *Ae. aegypti* by first identifying all matrilineages in which the infection was present, then looking for uninfected individuals within these matrilineages. These uninfected individuals will have lost or failed to inherit the infection.

Methods

Study site and sample collection

We deployed 110 ovitraps within properties of consenting householders in Cairns, Australia between 13 and 16 April, 2015. Traps covered a 3.3km x 1.9km region of central Cairns, which we partitioned into 6 "plots" for reference: Cairns North West (CNW), Cairns North East (CNE), Parramatta Park North (PPN), Parramatta Park South (PPS), Westcourt (WC) and Bungalow (BN) (Fig 1). Partitions were defined by geographic location, location of highways, and the release history of *w*Mel. Each of these had three possible assignments: the locational groupings of Cairns North [CNW, CNE],

Parramatta Park [PPN, PPS] and Westcourt/Bungalow [WC, BN]; the highway groupings of southeast of Bruce Highway [PPS, BN], west of both highways [CNW, PPN, WC], or northeast of Captain Cook Highway [CNE]; and the *Wolbachia* release groupings of releases in 2013 [PPN, WC], releases in 2014 [CNW, BN], or no release history [CNE, PPS] (see Schmidt et al. [18]).

Our sampling period of mid-April was at the end of the region's monsoonal wet season, when *Ae. aegypti* are abundant [18]. Each ovitrap consisted of a 9.3L black plastic bucket filled halfway with an infusion of water and alfalfa pellets to attract gravid female *Ae. aegypti* [41], which oviposit on strips of red felt clipped to the bucket and extending into the liquid. Traps were left in place for 5-7 days, then the felt strips were removed and dried. Dried strips of mosquito eggs were transferred to the laboratory and hatched by immersion into vessels filled with reverse osmosis water, 2-3 grains of yeast and one quarter of a crushed tablet of tropical fish food. The water, food and yeast were replaced after three days. Emerging virgin imagoes were transferred to freezing ethanol and stored at -20°C until DNA extraction.

We extracted genomic DNA using Roche DNA Isolation Kit for Cells and Tissues (Roche, Pleasanton, CA, USA), with an additional step of RNase treatment. Of the 110 ovitraps deployed, 74 produced *Ae. aegypti* imagoes, from which we selected 161 individuals for sequencing. As we expected ovitraps to contain many full-siblings from the same oviposition [9, 24, 42], which can bias analyses of population structure [43], we limited the number of samples per ovitrap to three individuals. This ensured that, after removing closely related individuals, we retained a large enough sample for a powerful analysis of genetic structure.

ddRADseq library preparation and SNP discovery

We applied the method of Rašić et al. [24] for ddRADseq library preparation, but selected a smaller size range (350–450bp) of genomic fragments to accommodate more individuals per library. Three libraries containing 161 *Ae. aegypti* in total were sequenced in three Illumina HiSeq2500 lanes using 100bp paired-end chemistry.

We processed raw fastq sequences within a customized pipeline [24], retaining reads with phred scores ≥ 13 and trimming them to 90bp. High-quality reads were aligned to the *Ae. aegypti* nuclear genome assembly AaegL1 [44] with the program Bowtie [45]. We allowed for up to three mismatches in the alignment seed, and uniquely aligned reads were analysed using Stacks [46], which we used to call genotypes at RAD stacks of a minimum depth of five reads.

We used the Stacks program *populations* to export VCF files, which were filtered with the program VCFtools [47]. We first removed individuals with >20% missing data, leaving 134 individuals. We then removed all loci not in Hardy-Weinberg equilibrium and with minor allele frequencies <0.05. To avoid using markers in high linkage disequilibrium, we applied thinning to ensure no SNP was within 250kbp of another. As *Aedes* genome is thought to contain approximately 2.1 megabases per cM [48], 250kbp roughly corresponds to eight SNPs per map unit, a sampling density that has been shown to largely eradicate the effects of linkage in SNPs [49]. We retained 3,784 unlinked and informative SNPs for analyses of relatedness and genetic structure.

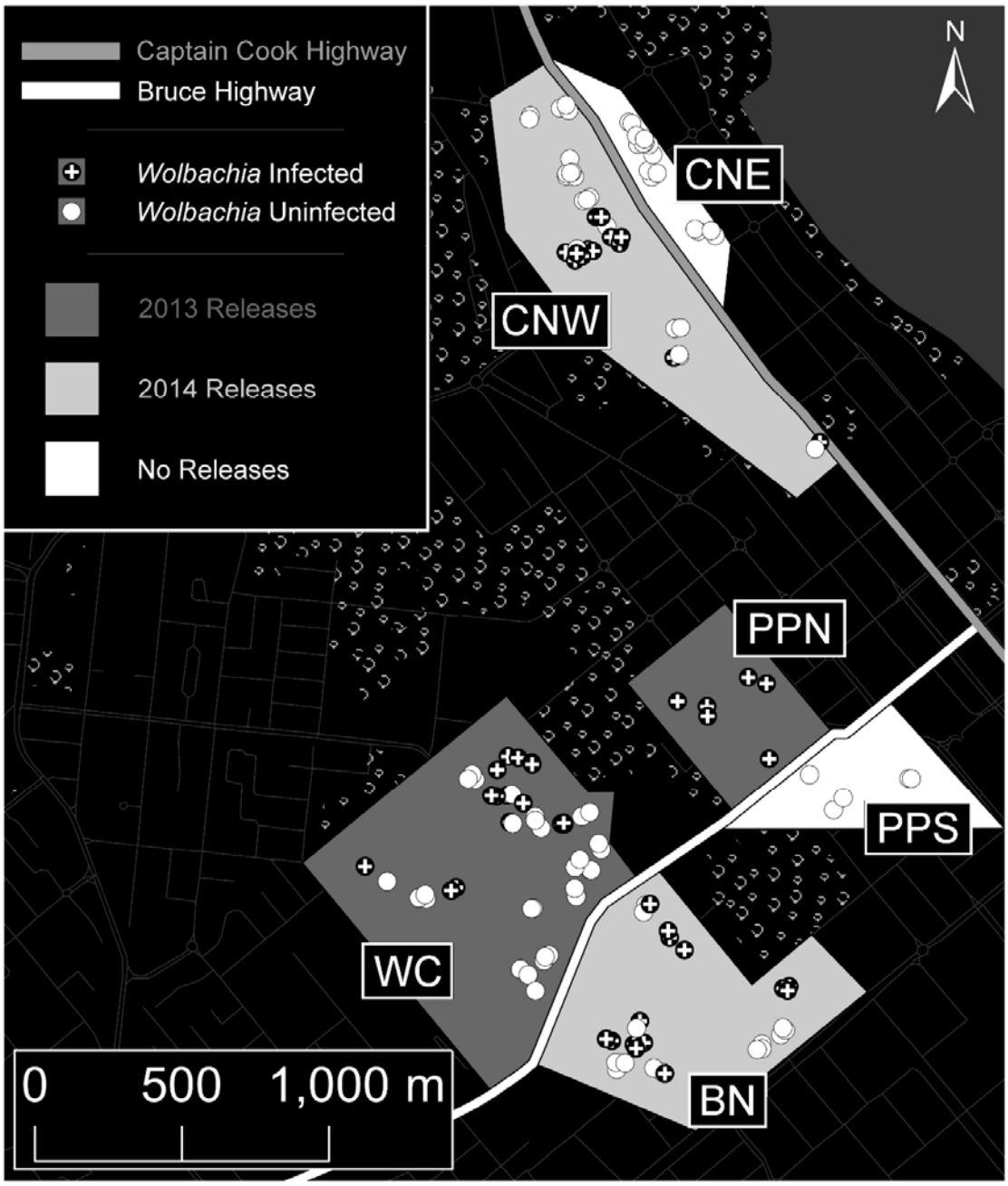

Fig 1: Sampling locations of the mosquitoes analysed with ddRADseq, set within the six sampling plots. Each sample was assigned a *Wolbachia* infection status, a score indicating its position relative to the two highways, and a score indicating when *Wolbachia* releases were carried out in the area. Plot abbreviations are: CNW (Cairns North West), CNE (Cairns North East), PPN (Parramatta Park North), PPS (Parramatta Park South), WC (Westcourt) and BN (Bungalow). (The underlying road network is derived from "Australia Oceania Continent Roads" made available by MapCruzin.com and OpenStreetMap.org under the Open Database License [https://opendatacommons.org/licenses/ odbl/1.0/].)

171 *Assessing barriers to dispersal: Landscape resistance modelling*
172 We tested the hypotheses that highways affect *Ae. aegypti* genetic structure in Cairns (H_1), and that
173 genetic structure was affected by recent releases of *Wolbachia* (H_2). For H_1, each individual mosquito
174 was assigned a score (0, 1, 2) based on its position southeast of Bruce Highway, west of both
175 highways, or northeast of Captain Cook Highway (Fig 1). The difference between a pair of scores
176 represented the number of highways between the two individuals; we called this variable
177 "Highways". For testing H_2 each individual was assigned a score (0, 1, 2) based on the *Wolbachia*
178 release history in the area where it was sampled; we called this variable "Releases". Plots having
179 undergone releases in 2013 (0) were considered more 'distant' from those never having undergone
180 releases (2) than those with releases in 2014 (1). We assumed that potential structuring effects such
181 as selection or reductions in diversity would display a greater effect over time. Treating the
182 geographical or temporal separation of individuals as additive variables analogous to landscape
183 resistance surfaces avoids issues associated with detecting discrete barriers [50].
184 We performed partial distance-based redundancy analyses (dbRDA [51]), to test H_1 and H_2 as
185 explicit hypotheses, while controlling for the potentially confounding effects of IBD and latitudinal
186 and longitudinal patterns. We sampled one individual from each matrilineage (see below) with the
187 lowest percentage of missing data, retaining 100 individuals. Easting and Northing UTM coordinates,
188 and a binary variable indicating *Wolbachia* infection status for each individual, called "Infection
189 Status", were treated as potentially confounding variables and were placed inside a conditional
190 matrix. The dependent variable was a distance matrix of Rousset's *a* scores [52] calculated for each
191 pair of individuals using the program SPAGeDi [53].
192 All remaining model procedures were performed in the package VEGAN [54]. The dbRDA
193 models were built using the function *capscale*. For all models, we applied the effects of the
194 conditional matrix described above, and assessed the significance of "Highways" and "Releases". We
195 built three models: one implementing both predictor variables and the other two implementing
196 each variable in isolation. We assessed the marginal significance of each predictor variable with the
197 function *anova.cca,* using 99999 permutations. We then repeated the procedure with "Infection
198 Status" used as a predictor variable alongside "Highways" and "Releases". While constructing
199 models, we calculated variance-inflation factors (VIF) to check for multicollinearity between
200 predictor variables, using the function *vif.cca*. All VIFs were < 1.1, so none were rejected.
201 We tested the sensitivity of the variables used to investigate H_1 and H_2 by performing
202 additional dbRDAs that followed the above procedure but with modifications made to "Highways"
203 and "Releases". In each case, instead of a given barrier being 100% the strength of the other, it was
204 assigned strengths of 150% and 200%. Thus, instead of scores of (0, 1, 2) for a given variable, these
205 scores were (0, 1.5, 2.5) or (0, 1, 2.5) for 150% strengths, and (0, 2, 3) or (0, 1, 3) for 200% strengths.
206
207
208 *Assessing barriers to dispersal: Type I error testing*
209 Although ordination methods such as dbRDA can detect genetic structure with greater power than
210 traditional Mantel tests [55, 56], they can also generate more Type I errors [57]. Type I errors are
211 common when hypotheses of geographical structure are only proposed against null hypotheses of
212 panmixia, rather than against alternative geographical hypotheses [58, 59]. We considered IBD an
213 alternative hypothesis of genetic structure. A Mantel test performed with the function *mantel* in the
214 R package VEGAN showed a weak correlation between geographical and genetic distances ($r = 0.047$,
215 $P < 0.05$), and stronger correlations between geographical distance and both "Highways" ($r = 0.424$,

216 $P < 0.001$) and "Releases" ($r = 0.305$, $P < 0.001$). When genetic structure exhibits IBD, spatial
217 dependence of barrier variables and inadequate sampling can lead to significance being observed
218 when no barrier effect exists [60]. Therefore, in order to confirm any significance among variables in
219 dbRDA, further analyses were required to eliminate IBD as a hypothesis of genetic structure.
220 We adapted the method of Kierepka and Latch [60] to evaluate whether the spatial
221 distribution of our traps relative to barriers would lead to a high risk of Type I error. We used CDPOP
222 [61] to simulate the field site without any highways, *Wolbachia* infections, or mosquito release
223 histories, thus creating an artificial environment in which only IBD could explain the pattern of
224 structuring. Parameters and methodology of our CDPOP simulations are detailed in Supplementary
225 Information A and our CDPOP input file is supplied in Supplementary Information B. To summarise:
226 we used the method of Kimura and Crow [62] to calculate the number of effective alleles in our
227 empirical data set, as effective allele counts have been found to correspond well with analytical
228 power across different types of genetic data [63, 64]. We assigned genotypes with equivalent allele
229 counts to spatially-referenced individuals corresponding to the 100 individuals used in empirical
230 analyses, and ran simulations for a sufficient number of generations to produce IBD of similar
231 strength to that in the empirical data ($r_{simulated}$, $\bar{x} = 0.082$, $\sigma = 0.022$, all $P < 0.05$; $r_{empirical} = 0.047$, P
232 < 0.05). We constructed new dbRDA models using the same parameters as the empirical data, and
233 calculated the marginal significance of "Highways" and "Releases" with ANOVAs. If more than 5 of
234 the 100 simulated samples showed significance for a variable then observations regarding that
235 variable would be at elevated risk of Type I error.
236
237
238 *Long-distance host movement and infection loss: Estimating relatedness/kinship*
239 We used SPAGeDi to calculate Loiselle's *k* [65] among individuals. First-degree kin relations (full-
240 sibling or parent/offspring) can be ascertained with hundreds of SNPs [66-68], and with the
241 thousands of SNPs available through ddRADseq this confidence can be extended to include second-
242 degree relations (half-sibling or grandparent/grandchild) [24, 69-71]. Considering that the 5-7 day
243 sampling period provided by our cross-sectional study design was shorter than the 14 days required
244 for *Ae. aegypti* development [35], we assumed that related individuals were of the same generation.
245 Therefore, we considered pairs with first-degree levels of relatedness to be full-siblings and those
246 with second-degree relatedness to be half-siblings.
247 We assigned each pair to the most likely kinship category, so that pairs with kinship
248 coefficient $k > 0.1875$ represented putative full-siblings, and those with $0.1875 > k > 0.09375$
249 represented putative half-siblings [72]. We considered half-siblings to be paternal because polyandry
250 is much rarer than polygyny in wild *Ae. aegypti* [73]. Therefore, we assumed full-siblings to come
251 from the same matrilineage, and half-siblings from different matrilineages.
252 As our investigations of long-distance movement and *Wolbachia* loss both require precise
253 assignment to kinship categories, we also used the program ML-Relate [74] to run specific
254 hypothesis tests of putative relationships. These were run for all pairs of putative full-siblings of
255 different infection status, and for all individual pairs with $k > 0.09375$ collected from different
256 ovitraps. For each pair we ran one standard test that estimated the relationship assuming that the
257 kinship category assigned using *k* was more likely than the next most likely kinship category,
258 followed by a conservative test that assumed that the kinship category assigned using *k* was less
259 likely to be correct. All tests were run using 10,000,000 simulations.

We considered the distance of separation between full-siblings to indicate movement of adult females. Full-siblings collected from the same ovitrap would likely be from the same gonotrophic cycle, while those collected from different ovitraps could be from one or two gonotrophic cycles. Although passive dispersal cannot be directly inferred from kinship observations, a study near Cairns estimated the average daily movement speed of female *Ae. aegypti* as 17-25m/day [26] and the maximum time between ovitrap deployment and sampling in this study was 7 days. Nevertheless, the distinction between active and passive dispersal is irrelevant to *Wolbachia* invasion dynamics, which depend on the summation of all movement across all life history stages [19, 20]; in this sense, our definition of dispersal is derived from Howard [75]. We thus consider full-sibling separation distances as composites of active flight and passive movement, which are more informative for understanding *Wolbachia* invasion dynamics than active flight alone.

Long-distance host movement and infection loss: Wolbachia infection screening

Mosquitoes were screened for *Wolbachia* using the protocol of Lee et al. [76]. The infection is diagnosed with PCRs run on the Roche LightCycler® 480 system (384-well format). For each mosquito, PCR was performed using three primer sets, *Aedes* universal primers (*mRpS6*_F/*mRpS6*_R), *Ae. aegypti*-specific primers (*aRpS6*_F/*aRpS6*_R) and *Wolbachia*-specific primers (*w1*_F/*w1*_R). A sample was scored as *Wolbachia* positive when there was robust amplification of all three primer sets *mRpS6*, *aRpS6* and *w1,* while an *Ae. aegypti* sample that was *Wolbachia* negative amplified only *mRpS6*, and *aRpS6.* Each PCR was run using three positive *Wolbachia* controls and three negative *Wolbachia* controls. *Wolbachia* titre, defined as the ratio of *Wolbachia* gene copies to host gene copies, was estimated using $2^{[\mathrm{cp(A)}-\mathrm{cp(W)}]}$, where cp(A) is the crossing point of the *aRpS6* marker and cp(W) is the crossing point of the *w1* marker [76].

We performed three PCR replicates for each individual to confirm its *w*Mel infection status. No inconsistencies in assigning *Wolbachia* infection status were observed across replicates of samples and controls, and similar titres were also observed across replicates (single factor ANOVA *F*-value = 0.341, P = 0.711). Considering the relative consistency of titres across replicates, we used the mean of the three scores to attain final estimates of *Wolbachia* titre for each individual.

From our *Wolbachia*-infection screening and relatedness analysis, we identified *w*Mel-infected matrilineages as being any group of full-siblings containing at least one individual infected with *Wolbachia*. An uninfected individual within an infected matrilineage was considered to have either failed to inherit the infection from its mother or to have lost it during development. We estimated the rate of infection loss per generation (μ) by comparing the number of these individuals with the total number of full-siblings from infected matrilineages.

Results

Barriers to dispersal

ANOVAs performed on partial dbRDA models showed that only the "Highways" variable was predictive of genetic structure (Table 1; 1.55 ≤ F-value ≤ 1.64; P < 0.05), and was significant irrespective of whether other variables were included. Based on partial Eta squared (η_p^2 [77]), "Highways" explained 1.7% of the variance in each model. Of the 100 CDPOP simulations, only two

showed significant structuring by "Highways" at the P < 0.05 level, and inclusion of the "Releases" variable in the models did not change this observation. This suggests that the effect of "Highways" observed in the real data is unlikely a Type I error caused by sampling bias or residual autocorrelation. Sensitivity analyses demonstrated robust results for "Highways", with η_p^2 effect sizes in the range 1.54-1.90%.

Table 1: Results of ANOVAs testing marginal significance of "Highways" and "Releases" variables in dbRDA. The two variables were each analysed in isolation in separate models, then together in a single model. In every case, "Highways" was predictive of genetic structure while "Releases" was not. Partial Eta squared (η_p^2) showed that "Highways" accounted for 1.7% of the variation within each model.

		sum of squares	F-value	P	η_p^2
analysed in isolation	**HIGHWAYS**	0.021	1.635	**0.021**	0.017
	RELEASES	0.016	1.241	0.163	0.013
analysed together	**HIGHWAYS**	0.020	1.555	**0.032**	0.017
	RELEASES	0.015	1.165	0.231	0.012

Although $\eta_p^2 = 0.017$ suggests only a small structuring effect, *Wolbachia* invasions with bistable dynamics are particularly sensitive to barrier effects [20]. Sensitivity increases exponentially as $\hat{p}$ approaches 0.5, and the $\hat{p} \approx 0.35$ of *w*Mel in *Ae. aegypti* [19] indicates such an invasion can be stopped by a barrier half the strength of that required to stop an invasion with $\hat{p} \approx 0.25$ [20]. Likewise, higher $\hat{p}$ means that slight disruptions to dispersal can slow an advancing wave considerably [19, 20]. To demonstrate this, we used a patch-based simulator derived from Nemo2 [78] and empirical data from Schmidt et al. [18] to simulate the spread of *w*Mel in the absence of barriers. Parameters, methodology and results are detailed in Supplementary Information C. Our simulations showed that, when no barrier effect was set, simulations overestimated infection frequencies in patches separated by highways relative to empirical infection frequencies recorded in Schmidt et al. [18], with an average overestimation across treatments of 6.5%. In comparison, no overestimation of infection frequencies was observed in patches not separated by highways. Overall, simulation of the *Wolbachia* invasion progress south of Bruce Highway (Supplementary Information C) indicated that barrier strength corresponded to an added 30-35 m of separation.

Estimates of long-distance movement

We detected 31 putative full-sibling groups that contained 43 full-sibling pairs, 41 of which were found within single ovitraps. Two pairs were spread between different plots (Fig 2): one was split between CNW and CNE (239 m separation, $k = 0.203$); the other between CNW and PPS (1312 m separation, $k = 0.235$). Standard maximum-likelihood simulations gave strong support to both pairs being full-siblings (both P < 0.0001). However, conservative simulations were unable to reject the hypotheses that either pair represented half-siblings (P < 0.0001 and P < 0.02 respectively), but rejected them as unrelated (both P = 1).

Eight of the 27 putative half-sibling pairs were found across multiple traps (47 - 560 m separation) but not across different plots. Of the eight pairs, only the most closely related pair (k = 0.1412, 54m separation, P < 0.07) was confidently determined to be half-siblings and not unrelated using conservative maximum likelihood estimation.

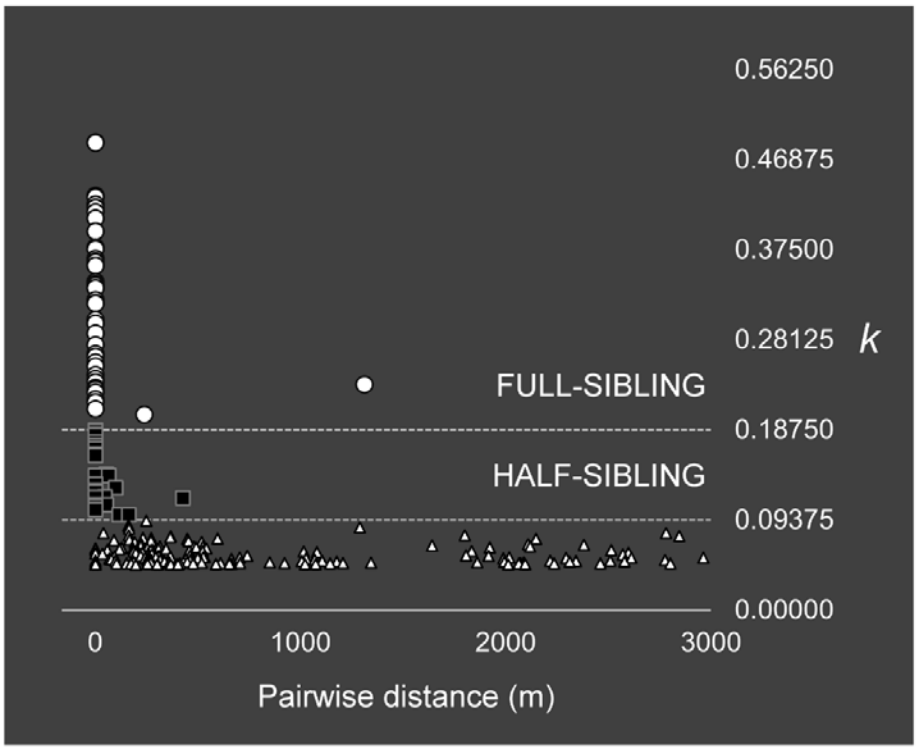

Fig 2: Loiselle's *k* estimates for sample pairs of relatedness *k* > 0.046875. Pairs of 0.09375 < k < 0.1875 are most likely half-sibs, those of k < 0.1875 are most likely full-sibs. Most related pairs were found within the same trap, but separation distances of up to 1312 m were observed.

Loss of Wolbachia

Triplicate PCR runs for *w*Mel detection indicated that 60 of the 161 mosquitoes were infected with *Wolbachia.* The six individuals from PPN were all infected, none of PPS and CNE were infected, and the remaining plots had a mixture of infection rates (Fig 1). We detected 10 *Wolbachia*-infected matrilineages, containing 21 individuals. Importantly, we recorded a single case of infection loss in CNW. The sample from this matrilineage consisted of a pair of putative full-siblings (k = 0.376), one of which carried the infection (titre = 6.15) and one of which did not. Maximum likelihood simulation confirmed that this pair represented full-siblings (P < 0.0001) and rejected the alternative hypotheses (both P = 1). We calculated a tentative probability of infection loss among offspring within infected matrilineages, giving a likelihood of loss of one in 21 (μ = 0.048), although the 95% binomial confidence intervals around this estimate were large (0.001, 0.238).

Supplementary Information D describes a comparison between *Wolbachia* titres in the lab-reared Cairns sample and that of a field-collected sample from the neighbouring town of Gordonvale. The field-collected sample had titres that were almost three times higher than Cairns on

average, but also three times as dispersed ($\bar{x}$ = 18.4, σ = 10.8; compared with $\bar{x}$ = 6.6, σ = 3.8 in Cairns), with one field-reared individual having a titre of only 1 *Wolbachia* gene copy for every 3 *Aedes* gene copies. The lowest titre in the Cairns samples was more than 3 times this amount. The highest titre in Gordonvale was 56 *Wolbachia* gene copies for each *Aedes* gene copy, more than double the highest recorded in Cairns.

Discussion

Our study produced three main findings: i) highways exert a significant influence on *Ae. aegypti* genetic structure; ii) gravid *Ae. aegypti* females can travel > 1 km; and (iii) *Ae. aegypti* in Cairns may occasionally lose the *w*Mel infection ($\mu \approx 0$). Each phenomenon is expected to have a slowing effect on *w*Mel invasion [19, 20]. Because ii) and iii) were based on single observations, we cannot confidently predict their frequency among *Ae. aegypti* in Cairns. However, field observations of slow spread of *w*Mel through Cairns [18, 19] are congruent with some long-distance movement and occasional infection loss.

Highways act as dispersal barriers

While previous studies suggested that roads could be barriers to *Ae. aegypti* dispersal [28, 31], we provide the first evidence for such an effect through explicit hypothesis testing within a landscape genetics framework. We detected a small but statistically significant barrier effect of highways, corresponding to 1.7% of dbRDA variance in genetic distance between individuals. Our simulations of *Wolbachia* invasion progress south of Bruce Highway (Supplementary Information C) showed that barrier strength corresponded to an added 30-35m of separation. The *w*Mel invasion observed at PP was slow (100-200m per year [18]), with the infection frequencies at the wave front being only slightly above the critical frequency $\hat{p} \approx 0.35$ [19]. Therefore, even a small added "cost" to cross the highway could slow the invasion considerably [20]. If the restrictive effect of highways on dispersal increase with highway width and traffic levels, then many urban highways in cities earmarked for future *w*Mel releases would likely be effective barriers to spread.

On the other hand, the restrictive effects of highways on dispersal can strengthen the invasion within the area they enclose. The habitat patches along these highway boundaries will have increased infection frequencies relative to patches in regions that are not subdivided, and could fortify the local invasion from the counteracting effect of uninfected immigrants. Following the 2013 *Wolbachia* releases in Cairns, the largest and most successfully invaded release site at Edge Hill/Whitfield recorded an influx of uninfected *Ae. aegypti* at the start of the 2014/2015 wet season [18]. This site was deemed particularly vulnerable to such reinvasion, due to its greater connectivity to surrounding uninfected regions and very low dry-season mosquito density. Ideally, future release sites in areas with low dry-season densities should be positioned adjacent to dispersal barriers such as highways, as this may help reduce the threat of reinvasion.

Long-distance movement of Aedes aegypti adult females

410 Analyses of local kinship patterns have provided several useful inferences regarding *Ae. aegypti*
411 ecology. The pair of probable full-siblings collected from ovitraps 1312m apart indicates movement
412 of a single female between oviposition events, and represents either the extreme end of the flight
413 range in *Ae. aegypti* or some combination of active and passive dispersal. Previous maximum
414 dispersal estimates of gravid *Ae. aegypti* females have been less than a kilometre in a single
415 gonotrophic (egg producing) cycle [79, 80]. In our study, 7 days passed between deployment and
416 sampling of the two ovitraps 1312m apart, which is long enough for an additional unobserved egg
417 deposition somewhere between them [35]. If we assume this distance to reflect active dispersal, an
418 additional gonotrophic cycle is likely. The length of a single such cycle is rarely more than a few days,
419 particularly when temperatures exceed 30°C [35] as they did during our sampling. If the distance was
420 crossed in a single gonotrophic cycle, the average daily speed would have been an order of
421 magnitude greater than the previous estimates of average female flight speed near Cairns of 17-
422 25m/day [26]. Therefore, passive, human-assisted transport is likely to have occurred. Adults of a
423 related mosquito *Aedes albopictus,* have been found in cars stopped at major roads in Barcelona,
424 Spain (David Roiz, pers. comm.)
425 Using a continuous coefficient of relationship such as *k* to assign pairs of individuals to
426 discrete kinship categories can be problematic when scores are close to the critical cut-off values.
427 Pairing this method with conservative maximum likelihood estimation resolved some of the clearer
428 distinctions (i.e. the full-siblings exhibiting *Wolbachia* loss) but not others (i.e. the putative full-
429 siblings exhibiting long-distance movement). However, the *k* scores of putative full-siblings and
430 putative half-siblings were clearly separated from each other relative to the variability in *k* scores
431 within each category. This reflects the power of genome-wide SNPs for inferring relationships [66-
432 68]. Inferring dispersal from relatedness also avoids potential biases resulting from lab-raised *Ae.*
433 *aegypti* used in MRR studies failing to develop experience in local conditions that will inform their
434 future oviposition choices [81, 82]. Our findings are broadly consistent with the results of several
435 MRR studies showing potential for long distance movement in *Ae. aegypti* imagoes [79, 80], and
436 suggest that this method provides an alternative to MRR for studying dispersal.
437
438
439 *Infection loss in Cairns Aedes aegypti*
440 We found the first evidence in support of intergenerational infection loss (μ = 4.8%) in the *Ae.*
441 *aegypti*/*w*Mel system, albeit from a single data point. It is worth noting that a comparable loss of
442 3.3% was recorded for the *w*AlbA infection in field-collected *Ae. albopictus* [83]. Transmission of
443 *w*Mel in *Ae. aegypti* has previously been estimated as perfect or quasi-perfect in the field, but this
444 was based on assaying eggs that were oviposited in the laboratory [9]. By comparison, samples in
445 this study were eclosed from eggs that had spent days in the field before collection, potentially
446 exposing them to stressors such as high heat fluctuations, which have been observed to affect titre
447 of *w*Mel transmission in laboratory populations [37]. Fluctuating high temperatures could be the
448 reason for the very low titres recorded for some Gordonvale individuals (Supplementary Information
449 D), though this could also be due a reduction in titre with age, which has been recorded for *w*AlbA in
450 *Ae. albopictus* males [84]. The inclusion of blood-fed females in the Gordonvale sample may explain
451 the occurrence of some very high *w*Mel titres, which are known to double in blood-fed *Ae. aegypti*
452 [85].
453 The *w*Mel strain successfully invaded *Ae. aegypti* populations in Gordonvale and Yorkey's
454 Knob, two quasi-isolated release sites near Cairns, in 2011 [17] and these areas have maintained

infection rates close to 95% for years without reaching fixation [9]. Under the assumptions of $\mu = 0$, high migration rates of uninfected gravid females would be required to maintain the observed frequencies of infection of 0.03 into Gordonvale and 0.06 into Yorkey's Knob [9]. Alternatively, the failure to reach fixation in these areas could be due to a combination of migration and infection loss, which would mean that migration rates of uninfected, gravid females may be lower than 0.06.

Conclusions

Our study has provided empirical evidence for three processes predicted to slow down the spread of *w*Mel in *Ae. aegypti*, using a landscape genomics analytical framework and molecular assays of *Wolbachia* infection. This approach could be extended to other host/*Wolbachia* systems that are increasingly considered for the biocontrol of disease vectors and pests. Non-perfect maternal transmission of *w*Mel in *Ae. aegypti* may not occur in other *Wolbachia* strains such as *w*AlbB, whose density shows greater constancy under fluctuating high temperatures [37]. Also, it is as yet unclear whether the observed transmission failure of *w*Mel occurs at a high enough frequency to affect invasion dynamics, and a more extensive field test of transmission fidelity will be necessary to derive an accurate estimate of μ. On the other hand, regardless of the *Wolbachia* strain deployed, presence of barriers like highways and leptokurtic dispersal are potential challenges for any *Wolbachia* invasion strategy requiring spatial spread [19].

Acknowledgements

We would like to thank Eliminate Dengue Cairns, particularly field officer Angela Caird, for assistance with ovitrap deployment. We thank the Cairns householders participating in the study for granting permission to deploy ovitraps on their property, as well as Scott Ritchie and Christopher Paton from the Centre for Biosecurity in Tropical Infectious Diseases, James Cook University, for assisting with the processing of field collections. The National Health and Medical Research Council provided funding for this research through a Program grant and Fellowship grant to A.A. Hoffmann.

482 **Data Archiving**

483 Demultiplexed fastq files have been deposited at NCBI SRA under [name].

484

Competing Interests

We declare no competing interests.

Author Contributions

A.A. Hoffmann, G. Rašić, and T. L. Schmidt conceived of and designed the study.

T. L. Schmidt, G. Rašić, and I. Filipović collected and dried the samples.

T. L. Schmidt performed the laboratory work and conducted the analyses, with assistance from G. Rašić, and computational support from I. Filipović.

T. L. Schmidt wrote the manuscript with assistance from A.A. Hoffmann and G. Rašić.

493 ## References

494

495

496 1. Weaver SC, Lecuit M. Chikungunya virus and the global spread of a mosquito-borne disease.
497 N Engl J Med. 2015;372(13):1231-9.
498 2. Bogoch II, Brady OJ, Kraemer M, German M, Creatore MI, Kulkarni MA, et al. Anticipating the
499 international spread of Zika virus from Brazil. Lancet. 2016;387(10016):335-6.
500 3. Focks DA, Brenner RJ, Hayes J, Daniels E. Transmission thresholds for dengue in terms of
501 *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. Am J Trop
502 Med Hyg. 2000;62(1):11-8.
503 4. Ballenger-Browning KK, Elder JP. Multi-modal *Aedes aegypti* mosquito reduction
504 interventions and dengue fever prevention. Trop Med Int Health. 2009;14(12):1542-51.
505 5. McGraw EA, O'Neill SL. Beyond insecticides: new thinking on an ancient problem. Nat Rev
506 Microbiol. 2013;11(3):181-93.
507 6. Ferguson NM, Kien DTH, Clapham H, Aguas R, Trung VT, Chau TNB, et al. Modeling the
508 impact on virus transmission of *Wolbachia*-mediated blocking of dengue virus infection of *Aedes*
509 *aegypti*. Sci Transl Med. 2015;7(279):279ra37-ra37.
510 7. Walker T, Johnson P, Moreira L, Iturbe-Ormaetxe I, Frentiu F, McMeniman C, et al. The *w*Mel
511 *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. Nature.
512 2011;476(7361):450-3.
513 8. Xi Z, Khoo CC, Dobson SL. *Wolbachia* establishment and invasion in an *Aedes aegypti*
514 laboratory population. Science. 2005;310(5746):326-8.
515 9. Hoffmann AA, Iturbe-Ormaetxe I, Callahan AG, Phillips BL, Billington K, Axford JK, et al.
516 Stability of the *w*Mel *Wolbachia* infection following invasion into *Aedes aegypti* populations. PLoS
517 Negl Trop Dis. 2014;8(9):e3115.
518 10. Hoffmann A, Turelli M. Cytoplasmic incompatibility in insects. In: SL ON, AA H, JH W, editors.
519 Influential passengers: inherited microorganisms and arthropod reproduction. Oxford: Oxford
520 University Press; 1997. p. 42-80.
521 11. Caspari E, Watson G. On the evolutionary importance of cytoplasmic sterility in mosquitoes.
522 Evol. 1959;13(4):568-70.
523 12. Suh E, Dobson SL. Reduced competitiveness of *Wolbachia* infected *Aedes aegypti* larvae in
524 intra- and inter-specific immature interactions. J Invertebr Pathol. 2013;114(2):173-7.
525 13. Ross PA, Endersby NM, Hoffmann AA. Costs of three *Wolbachia* infections on the survival of
526 *Aedes aegypti* larvae under starvation conditions. PLoS Negl Trop Dis. 2016;10(1):e0004320.
527 14. Turley AP, Zalucki MP, O'Neill SL, McGraw EA. Transinfected *Wolbachia* have minimal effects
528 on male reproductive success in *Aedes aegypti*. Parasit Vectors. 2013;6(1):36.
529 15. Hoffmann AA, Turelli M, Harshman LG. Factors affecting the distribution of cytoplasmic
530 incompatibility in *Drosophila simulans*. Genetics. 1990;126(4):933-48.
531 16. Turelli M. Cytoplasmic incompatibility in populations with overlapping generations. Evol.
532 2010;64(1):232-41.
533 17. Hoffmann A, Montgomery B, Popovici J, Iturbe-Ormaetxe I, Johnson P, Muzzi F, et al.
534 Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission.
535 Nature. 2011;476(7361):454-7.
536 18. Schmidt TL, Barton NH, Rašić G, Turley AP, Montgomery BL, Iturbe-Ormaetxe I, et al. Local
537 introduction and heterogeneous spatial spread of dengue-suppressing *Wolbachia* through an urban
538 population of *Aedes aegypti*. PLoS Biol. 2017;15(5):e2001894.
539 19. Turelli M, Barton NH. Deploying dengue-suppressing *Wolbachia*: robust models predict slow
540 but effective spatial spread in *Aedes aegypti*. Theor Popul Biol. 2017;115:45-60.
541 20. Barton N, Turelli M. Spatial waves of advance with bistable dynamics: cytoplasmic and
542 genetic analogues of Allee effects. Am Nat. 2011;178(3):E48-E75.

543 21. Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. Double digest RADseq: an
544 inexpensive method for de novo SNP discovery and genotyping in model and non-model species.
545 PLoS One. 2012;7(5):e37135.
546 22. Rašić G, Schama R, Powell R, Maciel-de Freitas R, Endersby-Harshman NM, Filipović I, et al.
547 Contrasting genetic structure between mitochondrial and nuclear markers in the dengue fever
548 mosquito from Rio de Janeiro: implications for vector control. Evol Appl. 2015;8(9):901-15.
549 23. Rašić G, Endersby-Harshman N, Tantowijoyo W, Goundar A, White V, Yang Q, et al. *Aedes*
550 *aegypti* has spatially structured and seasonally stable populations in Yogyakarta, Indonesia. Parasit
551 Vectors. 2015;8(1):610.
552 24. Rašić G, Filipović I, Weeks AR, Hoffmann AA. Genome-wide SNPs lead to strong signals of
553 geographic structure and relatedness patterns in the major arbovirus vector, *Aedes aegypti*. BMC
554 Genomics. 2014;15(1).
555 25. McDonald P. Population characteristics of domestic *Aedes aegypti* (Diptera: Culicidae) in
556 villages on the Kenya Coast I. Adult survivorship and population size. J Med Entomol. 1977;14(1):42-
557 8.
558 26. Muir LE, Kay BH. *Aedes aegypti* survival and dispersal estimated by mark-release-recapture
559 in northern Australia. Am J Trop Med Hyg. 1998;58(3):277-82.
560 27. Harrington LC, Scott TW, Lerdthusnee K, Coleman RC, Costero A, Clark GG, et al. Dispersal of
561 the dengue vector *Aedes aegypti* within and between rural communities. Am J Trop Med Hyg.
562 2005;72(2):209-20.
563 28. Russell RC, Webb C, Williams C, Ritchie S. Mark–release–recapture study to measure
564 dispersal of the mosquito *Aedes aegypti* in Cairns, Queensland, Australia. Med Vet Entomol.
565 2005;19(4):451-7.
566 29. Maciel-de-Freitas R, Souza-Santos R, Codeço C, Lourenço-de-Oliveira R. Influence of the
567 spatial distribution of human hosts and large size containers on the dispersal of the mosquito *Aedes*
568 *aegypti* within the first gonotrophic cycle. Med Vet Entomol. 2010;24(1):74-82.
569 30. Maciel-De-Freitas R, Codeco CT, Lourenco-De-Oliveira R. Daily survival rates and dispersal of
570 *Aedes aegypti* females in Rio de Janeiro, Brazil. Am J Trop Med Hyg. 2007;76(4):659-65.
571 31. Hemme RR, Thomas CL, Chadee DD, Severson DW. Influence of urban landscapes on
572 population dynamics in a short-distance migrant mosquito: evidence for the dengue vector *Aedes*
573 *aegypti*. PLoS Negl Trop Dis. 2010;4(3):e634.
574 32. Wright S. Isolation by distance. Genetics. 1943;28(2):114-38.
575 33. Clark JS, Fastie C, Hurtt G, Jackson ST, Johnson C, King GA, et al. Reid's paradox of rapid plant
576 migration: dispersal theory and interpretation of paleoecological records. Biosci. 1998;48(1):13-24.
577 34. Reiter P, Sprenger D. The used tire trade: a mechanism for the worldwide dispersal of
578 container breeding mosquitoes. J Am Mosq Control Assoc. 1987;3(3):494-501.
579 35. Christophers S. *Aëdes aegypti* (L.) the yellow fever mosquito: its life history, bionomics and
580 structure. London: Cambridge University Press; 1960.
581 36. Reiter P. Oviposition, dispersal, and survival in *Aedes aegypti*: implications for the efficacy of
582 control strategies. Vector Borne Zoonotic Dis. 2007;7(2):261-73.
583 37. Ross PA, Wiwatanaratanabutr I, Axford JK, White VL, Endersby-Harshman NM, Hoffmann AA.
584 *Wolbachia* infections in *Aedes aegypti* differ markedly in their response to cyclical heat stress. PLoS
585 Pathog. 2017;13(1):e1006006.
586 38. Ulrich JN, Beier JC, Devine GJ, Hugo LE. Heat Sensitivity of *w*Mel *Wolbachia* during *Aedes*
587 *aegypti* Development. PLoS Negl Trop Dis. 2016;10(7):e0004873.
588 39. Clancy DJ, Hoffmann AA. Environmental effects on cytoplasmic incompatibility and bacterial
589 load in *Wolbachia*-infected *Drosophila simulans*. Entomol Exp Appl. 1998;86(1):13-24.
590 40. Ikeda T, Ishikawa H, Sasaki T. Infection density of *Wolbachia* and level of cytoplasmic
591 incompatibility in the Mediterranean flour moth, *Ephestia kuehniella*. J Invertebr Pathol.
592 2003;84(1):1-5.

593 41. Ritchie SA. Effect of some animal feeds and oviposition substrates on *Aedes* oviposition in
594 ovitraps in Cairns, Australia. J Am Mosq Control Assoc Mosq News. 2001;17(3):206-8.
595 42. Apostol B, Black Wt, Miller B, Reiter P, Beaty B. Estimation of the number of full sibling
596 families at an oviposition site using RAPD-PCR markers: applications to the mosquito *Aedes aegypti*.
597 Theor Appl Genet. 1993;86(8):991-1000.
598 43. Goldberg CS, Waits LP. Quantification and reduction of bias from sampling larvae to infer
599 population and landscape genetic structure. Mol Ecol Resour. 2010;10(2):304-13.
600 44. Nene V, Wortman JR, Lawson D, Haas B, Kodira C, Tu ZJ, et al. Genome sequence of *Aedes*
601 *aegypti,* a major arbovirus vector. Science. 2007;316(5832):1718-23.
602 45. Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of
603 short DNA sequences to the human genome. Genome Biol. 2009;10(3):R25.
604 46. Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. Stacks: an analysis tool set for
605 population genomics. Mol Ecol. 2013;22(11):3124-40.
606 47. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, et al. The variant call
607 format and VCFtools. Bioinformatics. 2011;27(15):2156-8.
608 48. Brown S, Severson D, Smith L, Knudson D. Integration of the *Aedes aegypti* mosquito genetic
609 linkage and physical maps. Genetics. 2001;157(3):1299-305.
610 49. Cho K, Dupuis J. Handling linkage disequilibrium in qualitative trait linkage analysis using
611 dense SNPs: a two-step strategy. BMC Genet. 2009;10(1):44.
612 50. Landguth E, Cushman S, Murphy M, Luikart G. Relationships between migration rates and
613 landscape resistance assessed using individual-based simulations. Mol Ecol Resour. 2010;10(5):854-
614 62.
615 51. Legendre P, Anderson MJ. Distance-based redundancy analysis: testing multispecies
616 responses in multifactorial ecological experiments. Ecol Monogr. 1999;69(1):1-24.
617 52. Rousset F. Genetic differentiation between individuals. J Evol Biol. 2000;13(1):58-62.
618 53. Hardy OJ, Vekemans X. SPAGeDi: a versatile computer program to analyse spatial genetic
619 structure at the individual or population levels. Mol Ecol Notes. 2002;2(4):618-20.
620 54. Oksanen J, Kindt R, Legendre P, O'Hara B, Stevens MHH, Oksanen MJ, et al. The vegan
621 package. Community Ecology Package. 2007;10:631-7.
622 55. Legendre P, Fortin MJ. Comparison of the Mantel test and alternative approaches for
623 detecting complex multivariate relationships in the spatial analysis of genetic data. Mol Ecol Resour.
624 2010;10(5):831-44.
625 56. Cushman SA, Shirk AJ, Landguth EL. Landscape genetics and limiting factors. Conserv Genet.
626 2013;14(2):263-74.
627 57. Kierepka E, Latch E. Performance of partial statistics in individual-based landscape genetics.
628 Mol Ecol Resour. 2015;15(3):512-25.
629 58. Cushman SA, Landguth EL. Spurious correlations and inference in landscape genetics. Mol
630 Ecol. 2010;19(17):3592-602.
631 59. Cushman SA, McKelvey KS, Hayden J, Schwartz MK. Gene flow in complex landscapes:
632 testing multiple hypotheses with causal modeling. Am Nat. 2006;168(4):486-99.
633 60. Kierepka E, Latch E. Fine-scale landscape genetics of the American badger (*Taxidea taxus*):
634 disentangling landscape effects and sampling artifacts in a poorly understood species. Heredity.
635 2016;116(1):33-43.
636 61. Landguth EL, Cushman S. CDPOP: a spatially explicit cost distance population genetics
637 program. Mol Ecol Resour. 2010;10(1):156-61.
638 62. Kimura M, Crow JF. The number of alleles that can be maintained in a finite population.
639 Genetics. 1964;49(4):725-38.
640 63. Wang J. Informativeness of genetic markers for pairwise relationship and relatedness
641 inference. Theor Popul Biol. 2006;70(3):300-21.

642 64. Hauser L, Baird M, Hilborn R, Seeb LW, Seeb JE. An empirical comparison of SNPs and
643 microsatellites for parentage and kinship assignment in a wild sockeye salmon (*Oncorhynchus nerka*)
644 population. Mol Ecol Resour. 2011;11(s1):150-61.
645 65. Loiselle BA, Sork VL, Nason J, Graham C. Spatial genetic structure of a tropical understory
646 shrub, *Psychotria officinalis* (Rubiaceae). Am J Bot. 1995:1420-5.
647 66. Tokarska M, Marshall T, Kowalczyk R, Wojcik J, Pertoldi C, Kristensen T, et al. Effectiveness of
648 microsatellite and SNP markers for parentage and identity analysis in species with low genetic
649 diversity: the case of European bison. Heredity. 2009;103(4):326.
650 67. Weinman LR, Solomon JW, Rubenstein DR. A comparison of single nucleotide polymorphism
651 and microsatellite markers for analysis of parentage and kinship in a cooperatively breeding bird.
652 Mol Ecol Resour. 2015;15(3):502-11.
653 68. Blouin MS. DNA-based methods for pedigree reconstruction and kinship analysis in natural
654 populations. Trends Ecol Evol. 2003;18(10):503-11.
655 69. Bateson ZW, Hammerly SC, Johnson JA, Morrow ME, Whittingham LA, Dunn PO. Specific
656 alleles at immune genes, rather than genome-wide heterozygosity, are related to immunity and
657 survival in the critically endangered Attwater's prairie-chicken. Mol Ecol. 2016;25(19):4730-44.
658 70. Munshi-South J, Zolnik CP, Harris SE. Population genomics of the Anthropocene:
659 urbanization is negatively associated with genome-wide variation in white-footed mouse
660 populations. Evol Appl. 2016.
661 71. Shultz AJ, Baker AJ, Hill GE, Nolan PM, Edwards SV. SNPs across time and space: population
662 genomic signatures of founder events and epizootics in the House Finch (*Haemorhous mexicanus*).
663 Ecol Evol. 2016;6(20):7475-89.
664 72. Iacchei M, Ben-Horin T, Selkoe KA, Bird CE, García-Rodríguez FJ, Toonen RJ. Combined
665 analyses of kinship and FST suggest potential drivers of chaotic genetic patchiness in high gene-flow
666 populations. Mol Ecol. 2013;22(13):3476-94.
667 73. Richardson JB, Jameson SB, Gloria-Soria A, Wesson DM, Powell J. Evidence of limited
668 polyandry in a natural population of *Aedes aegypti*. Am J Trop Med Hyg. 2015;93(1):189-93.
669 74. Kalinowski ST, Wagner AP, Taper ML. ML-Relate: a computer program for maximum
670 likelihood estimation of relatedness and relationship. Mol Ecol Notes. 2006;6(2):576-9.
671 75. Howard WE. Innate and environmental dispersal of individual vertebrates. Am Midl Nat.
672 1960:152-61.
673 76. Lee SF, White VL, Weeks AR, Hoffmann AA, Endersby NM. High-throughput PCR assays to
674 monitor *Wolbachia* infection in the dengue mosquito (*Aedes aegypti*) and *Drosophila simulans*. Appl
675 Environ Microbiol. 2012;78(13):4740-3.
676 77. Tabachnick BG, Fidell LS. Using multivariate statistics. 3rd ed. New York: Harper Collins;
677 1996.
678 78. Guillaume F, Rougemont J. Nemo: an evolutionary and population genetics programming
679 framework. Bioinformatics. 2006;22(20):2556-7.
680 79. Reiter P, Amador MA, Anderson RA, Clark GG. Dispersal of *Aedes aegypti* in an urban area
681 after blood feeding as demonstrated by rubidium-marked eggs. Am J Trop Med Hyg. 1995;52(2):177-
682 9.
683 80. Honório NA, Silva WdC, Leite PJ, Gonçalves JM, Lounibos LP, Lourenço-de-Oliveira R.
684 Dispersal of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in an urban endemic dengue
685 area in the State of Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz. 2003;98(2):191-8.
686 81. Kaur J, Lai Y, Giger A. Learning and memory in the mosquito *Aedes aegypti* shown by
687 conditioning against oviposition deterrence. Med Vet Entomol. 2003;17(4):457-60.
688 82. Ruktanonchai N, Lounibos L, Smith D, Allan S. Experience-and age-mediated oviposition
689 behaviour in the yellow fever mosquito *Stegomyia aegypti* (= *Aedes aegypti*). Med Vet Entomol.
690 2015;29(3):255-62.

691 83. Kittayapong P, Baisley KJ, Sharpe RG, Baimai V, O'Neill SL. Maternal transmission efficiency
692 of *Wolbachia* superinfections in *Aedes albopictus* populations in Thailand. Am J Trop Med Hyg.
693 2002;66(1):103-7.
694 84. Tortosa P, Charlat S, Labbé P, Dehecq J-S, Barré H, Weill M. *Wolbachia* age-sex-specific
695 density in *Aedes albopictus*: a host evolutionary response to cytoplasmic incompatibility? PLoS One.
696 2010;5(3):e9700.
697 85. Frentiu FD, Zakir T, Walker T, Popovici J, Pyke AT, van den Hurk A, et al. Limited dengue virus
698 replication in field-collected *Aedes aegypti* mosquitoes infected with *Wolbachia*. PLoS Negl Trop Dis.
699 2014;8(2):e2688.

700