Short-term suppression of *Aedes aegypti* using genetic control does not facilitate *Aedes albopictus*

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Abstract

BACKGROUND: Under permit from the National Biosafety Commission for the use of genetically modified organisms, releases of a genetically engineered self-limiting strain of *Aedes aegypti* (OX513A) were used to suppress urban pest *Ae. aegypti* in West Panama. Experimental goals were to assess the effects on a coexisting population of *Ae. albopictus* and examine operational parameters with relevance to environmental impact.

RESULTS: *Ae. albopictus* populations were shown to be increasing year upon year at each of three study sites, potentially reflecting a broader-scale incursion into the area. *Ae. albopictus* abundance was unaffected by a sustained reduction in *Ae. aegypti* by up to 93% through repeated releases of OX513A. Males accounted for 99.99% of released OX513A, resulting in a sustained mating fraction of 75%. Mean mating competitiveness of OX513A was 0.14. The proportion of OX513A in the local environment decreased by 95% within 25 days of the final release.

CONCLUSIONS: There was no evidence for species replacement of *Ae. aegypti* by *Ae. albopictus* over the course of this study. No unintentional environmental impacts or elevated operational risks were observed. The potential for this emerging technology to mitigate against disease outbreaks before they become established is discussed.

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Keywords: mosquito; OX513A; transgenic; dengue; chikungunya; Panama

1 INTRODUCTION

For some mosquito-borne viral diseases, conventional vector control techniques are failing to prevent or contain their impact on public health. The implications of human migration and virus evolution for the expansion of diseases into naive areas where they have not previously posed a significant threat are of major concern. Dengue and chikungunya, both transmitted by *Aedes* mosquitoes, are two such diseases for which incidence has increased markedly in recent decades.1,2 Both are primarily transmitted by the yellow fever mosquito, *Aedes aegypti* (Linnaeus), and the Asian tiger mosquito, *Aedes albopictus* (Skuse), potentially even leading to the prospect of simultaneous dengue and chikungunya transmission.3

Dengue is an acute febrile illness with a broad clinical spectrum from flu-like symptoms to the potentially fatal complications of severe dengue haemorrhagic fever and dengue shock syndrome. The disease is caused by the dengue virus complex, which consists of four genetically and immunogenically distinct serotypes, compounding the complexities of either predicting or preventing severe outbreaks.4 A recent publication in *Nature* modelled the annual global number of infections as 390 million.5 In 2013, the Republic of Panama (Panama) suffered 4781 reported dengue cases, more than treble the number in 2012 (1320). On 5 February 2014, following 1703 dengue cases and six deaths since the beginning of the year (as of 3 February 2014), the Panamanian Ministry of Health declared the whole country to be a dengue epidemic area. The year 2014 culminated in a total of 4485 dengue cases, 19 cases of severe dengue and eight deaths (Panamanian Ministry of Health, unpublished). The associated economic costs of dengue in Panama are also significant; the average cost per hospitalised dengue case during 2010 was estimated at US$1336.6

Chikungunya typically presents as severe body cramps and acute joint pain, with a proportion of patients developing persistent arthralgia after acute infection. Since 2005, a single chikungunya genotype is thought to be responsible for over 1 million cases...
across Asia, Africa and the Indian Ocean islands, with a number of autochthonous cases in Europe. Although both \textit{Ae. aegypti} and \textit{Ae. albopictus} have been shown to be efficient vectors of all chikungunya genotypes, there is evidence to suggest that \textit{Ae. aegypti} may have historically been the primary vector. More recently, the virus–vector relationships appear to have been dynamic and possibly exemplify evolutionary convergence as different chikungunya strains have independently acquired an adaptive mutation that favours replication in \textit{Ae. albopictus}.\textsuperscript{8,9} It appears that this specificity may be due to a single amino acid substitution, potentially posing a new and significant threat of chikungunya in areas recently colonised by \textit{Ae. albopictus}.\textsuperscript{10} Chikungunya virus became an increasing global concern following epidemics in Africa, Asia and the Indian Ocean from 2004. In late 2013, the first incidence of locally transmitted chikungunya virus in the Americas was identified,\textsuperscript{2} which according to the Centres for Disease Control (CDC) has since amplified to over 1 million cases.\textsuperscript{11} Panama first documented autochthonous chikungunya transmission in 2014,\textsuperscript{12} although the number and spread of cases to date have been relatively restricted (Panamanian Ministry of Health, unpublished).

There is currently no vaccine or therapeutic medication available for the treatment of dengue or chikungunya, which leaves vector control as the primary line of defence available to public health authorities. Open releases of a genetically engineered strain of \textit{Ae. aegypti}, OX513A, have been shown to reduce significantly native populations of \textit{Ae. aegypti} in field trials in the Cayman Islands and Brazil.\textsuperscript{13–15} The method involves releasing male OX513A mosquitoes to mate with wild females, which pass a gene to their offspring that causes them to die before adulthood.\textsuperscript{16} With such novel and unique modes of action, it is essential to evaluate genetic control technologies not only in terms of their efficacy and potential benefits for human health but also for their wider environmental impacts and operational safety. To these ends, and following a laboratory-based evaluation that confirmed the compatibility of OX513A with wild \textit{Ae. aegypti} from Panama, this manuscript reports on specific environmental considerations of an open release evaluation in a suburb of Arraijan District, West Panama (CNB-01-2014, 14 January 2014). In doing so, it represents the first study of \textit{Ae. albopictus} in an area undergoing treatment with OX513A \textit{Ae. aegypti}.

## 2 EXPERIMENTAL METHODS

### 2.1 Study location

Panama has a tropical maritime climate; temperature and relative humidity are generally high with minimal seasonal variation, and diurnal ranges are typically low. Rainfall is largely restricted to the wet season, which is usually from May to November. Panama City, in the province of Panama, is centrally located along the Panamanian Pacific coastline. The mosquito production facility (sited at the Gorgas Memorial Institute for Human Health) lies near the centre of Panama City, and the study area was 23 km away within the neighbouring westerly province (West Panama) and district of Arraijan (Fig. 1a).

The treated site was a residential neighbourhood within a suburb of Arraijan called ‘Nuevo Chorrillo’. It had a moderate human population density and marked seasonality of \textit{Ae. aegypti}.\textsuperscript{17} Data obtained from the treated site were compared with those for two nearby untreated neighbourhoods, located in similar residential estates called ‘Lluvia de Oro’ and ‘Princesa Mia’ (Fig. 1b). All three sites were of equal size (~10 ha).

### 2.2 OX513A insect strain

OX513A contains a self-limiting gene insertion that is controllable using tetracycline or its analogues. In the absence of tetracycline, expression of this transgene produces a cellular protein (tTAV) in quantities that cause cell malfunction and >95% mortality before adulthood. However, when OX513A is reared in the presence of tetracycline, expression of tTAV is repressed, allowing normal cell function and survival.\textsuperscript{16} OX513A was formed via introgression into a wild-type genetic background through five generations of backcrossing, prior to a selection process for homozygous individuals. This was the same strain previously used for field evaluations in the Cayman Islands and Brazil.\textsuperscript{13–15} Individuals of this strain can be visually identified during the immature stages owing to the expression of a fluorescent protein (DsRed2) driven by a promoter that gives a characteristic spotted pattern of fluorescence in OX513A larvae.\textsuperscript{16}

#### 2.3 Insect rearing

##### 2.3.1 Production of eggs

A colony of homozygous OX513A established and maintained in the United Kingdom under controlled environmental conditions (26–29 °C; >70% RH; 12 h light cycle) was used to generate sufficient eggs. Egg batches (5–7 million eggs batch\textsuperscript{−1}) were packed and sent to Panama on a monthly basis to ensure a supply of fresh, high-quality eggs of a standardised age.

##### 2.3.2 Eggs to pupae

At the Gorgas Memorial Institute for Human Health, mosquitoes for release were reared from OX513A eggs to pupae under controlled environmental conditions (26–29 °C; >70% RH; 12 h light cycle). Eggs were hatched in water by maintaining them for 1 h at reduced atmospheric pressure using a vacuum chamber and pump. This promoted rapid, synchronous egg hatch and facilitated batches of larvae where all individuals were of a consistent age. Freshly hatched larvae were placed in rearing trays at a density of 2.25 larvae mL\textsuperscript{−1} tap water. Chlortetracycline hydrochloride (Sigma Aldrich, St Louis, MO) was added to each tray at a final concentration of 30 μg mL\textsuperscript{−1}. The larvae were fed commercially available Tetramin® fish flakes (Tetra GmbH, Melle, Germany) throughout the growth period according to a predetermined regime to ensure a consistent growth rate both within and between batches. Eight days after egg hatch, when the majority of larvae had developed into pupae, groups of up to 120 000 pupae were mechanically sorted by size using proprietary equipment to remove remaining larvae and female pupae. Following the sorting procedure, a minimum of 1000 male pupae from every group were individually checked using a microscope to ensure ≤0.2% female contamination; any group that exceeded this threshold was re-sorted. This threshold was imposed on every group as a quality control measure, ensuring that the percentage of adults released that were males was consistently high at all times. This was important not only for maintaining efficacy but also for mitigating against the release of adult females, which could then potentially acquire and/or transmit diseases.

##### 2.3.3 Pupae to adults

Male pupae were aliquoted into 1.8 L clear plastic cylindrical pots (Produtos Prafesta® , Mairiporã, Brazil) covered with a fine cotton mesh to allow ventilation. Each pot contained approximately
1000 male pupae in 100 mL of water. After 48 h, when adults had eclosed, any remaining water was drained from the pots. To facilitate quality control checks, six pots were randomly identified, and the numbers of dead insects and uneclosed pupae in each were recorded. Adults were provided with cotton wool soaked in 10% sucrose solution from which to feed, and all adults released were between 1 and 5 days old.

2.4 Field procedures

2.4.1 OX513A adult releases
The period of study was throughout the wet season, when annual *Ae. aegypti* levels were typically high. Although the chosen number of OX513A adults for release was relative to the estimated size of the target *Ae. aegypti* population, wild populations of *Ae. aegypti* are most closely associated with human populations, and therefore the release rate (or dose rate) is described as 'number of OX513A males per person'. A target dose rate of 67 OX513A males per person was chosen. As there were 900 inhabitants living at the treated site, a requirement of 60 000 per release was determined. To ensure a constant presence of OX513A males, a treatment frequency of 3 times per week was used.

Ventilated plastic pots (see Section 2.3.3) containing male OX513A mosquitoes were packed into airtight, insulated transport boxes and driven to the release site. On arrival, they were allowed to settle and rest for a short period as final preparations were made. The release vehicle then proceeded along a predetermined route at a consistent speed (30–35 km h$^{-1}$). Adult male mosquitoes were released by opening one plastic container (≈1000 male OX513A) at each of 60 premarked points, shaking gently so that the adult males were efficiently dispersed. After the release, the six pots previously identified for quality control checks (see Section 2.3.3) were assessed, and the numbers of insects remaining in each (dead and alive) were recorded. This release pattern was repeated 3 times per week (Monday, Wednesday and Friday) from Friday 25th April 2014 to Friday 31st October 2014 inclusive.

2.4.2 Vector surveillance – egg traps
Egg traps (known as ovitraps) are a commonly used system for monitoring *Aedes* mosquitoes, mimicking natural breeding sites in which females lay eggs.$^{18,19}$ The ovitraps consisted of a pot containing water and a wooden paddle to act as a substrate on which females could lay eggs, and were used to sample *Ae. albopictus* and *Ae. aegypti* populations at the treated and untreated sites. Ovitraps were placed outside residential houses, in sheltered locations preferred by aedine species (e.g. under tables, in corners, near water storage). The verbal consent of each property owner/occupant was sought before placement. At each of the three sites, 60 ovitraps, equivalent to approximately six per hectare, were distributed between 30 different properties (two per property). Several ovitrap locations were subsequently discounted, primarily because of restricted access, yielding final ovitrap numbers of 56, 54 and 55 at UT1, UT2 and T sites respectively (Fig. 1b). All ovitraps were examined and replaced weekly, returning the substrate, including any eggs, to the laboratory for inspection.

Once at the laboratory, any eggs collected were air dried for a minimum of 72 h and hatched in tap water. At the first instar stage (L$_1$), all hatched larvae were screened for the presence of the OX513A fluorescence marker using a stereomicroscope (Leica MZ10F; Leica Microsystems AG, Wetzlar, Germany) at a wavelength of 561 nm, and numbers of fluorescent larvae were recorded. All non-fluorescent larvae were retained and reared to the third-instar stage (L$_3$), at which point each individual was identified taxonomically to species level. Ovitrack data were used to assess: *Ae. albopictus* and *Ae. aegypti* abundance (numbers of eggs); *Ae. albopictus* and *Ae. aegypti* presence (percentage of positive traps); OX513A mating fraction (the proportion of females mated by OX513A males) using the proxy of proportion of fluorescent *Ae. aegypti* larvae.

2.4.3 Vector surveillance – adult traps
BG-Sentinel® traps (Biogents, Regensburg, Germany) were used to trap adult mosquitoes. These are particularly attractive to *Aedes* spp. and utilise both olfactory and visual cues. In a similar manner to ovitraps, BG-Sentinel® traps were deployed externally, near domestic houses at locations considered to be attractive to *Ae. albopictus* and *Ae. aegypti*. The consent of each property owner/occupant was sought before placement. At each of the treated and one of the untreated sites (UT1), ten BG traps were deployed. These were examined and replaced daily, 4 times per week, returning the catches to the laboratory for inspection.
Adult mosquitoes caught in BG traps were identified to species level and counted, and their sex was determined using a stereomicroscope. Adult trap data contributed to population estimates for OX513A and native Ae. aegypti.

### 2.5 Statistics

Unless otherwise stated, confidence intervals (CIs) are reported at the 95% level, and data are presented to two significant figures. Significant differences were classified by non-overlapping 95% confidence intervals.

For both abundance and presence of Ae. albopictus and Ae. aegypti, four-week moving averages relative to the same period at each untreated site were calculated according to the equation

\[
M = (T_u / U_u)(T_b / U_b) - 1,
\]

where \(M\) is the population change, \(T_u\) is mean eggs trap\(^{-1}\) (or larval index) in the treated area after release (value is moving), \(U_u\) is mean eggs trap (or larval index) in the untreated area after release (value is moving); \(T_b\) = mean eggs trap\(^{-1}\) (or larval index) in the treated area before release (value is constant) and \(U_b\) is mean eggs trap\(^{-1}\) (or larval index) in the untreated area before release (value is constant).

This was done by comparing data combined across four consecutive weeks on a weekly basis against baseline data obtained across the 4 weeks prior to the evaluation period (Ae. albopictus) or the entire 2 year dataset (Ae. aegypti). The corresponding 95% CIs were calculated by a 10 000-loop bootstrap for each period.\(^{20}\)

The proportion of larvae that were observed to be fluorescent (i.e. carried the OX513A fluorescence gene) was stable until 3 weeks after the final release. Therefore, an endpoint of 2 weeks after the final release [200 days after first treatment (DAT1)] was used.

Mating competitiveness was derived from the ratio of OX513A to wild adult males and the corresponding proportion of fluorescent larvae hatched from Ae. aegypti eggs collected in ovitraps. These estimates are used widely in sterile insect technique (SIT) programmes and followed the equation

\[
C = PM_w/(1 - P)M_b,
\]

where \(C\) is the mating competitiveness, \(P\) is the proportion of larvae that were fluorescent, \(M_w\) is the number of wild mosquitoes and \(M_b\) is the number of OX513A mosquitoes.\(^{21}\) Corresponding 95% confidence intervals were calculated by a 10 000-loop bootstrap.\(^{20}\)

### 3 RESULTS

#### 3.1 Climatic conditions

Looking across a 3 year dataset for the Arraijan District, the period of OX513A releases (25 April 2014 to 31 October 2014) was largely typical in terms of maximum and minimum temperatures and rainfall amounts (Fig. 2). Daily temperatures during the release period ranged from 22.1 to 30.8 °C, and the mean daily rainfall was 1.28 mm (SD = 0.81) (data provided by Weather Analytics, Bethesda, MD).

![Figure 2. Minimum and maximum temperatures (°C) and rainfall amounts (mm) in Arraijan, Panama, over a 3 year period (1 January 2012 to 1 December 2014). The period of OX513A releases (25 April 2014 to 31 October 2014) is indicated by the shaded area. Data provided by Weather Analytics.](image-url)

#### 3.2 Background Ae. albopictus populations

Monitoring of abundance and presence of Ae. albopictus (Fig. 3) showed that the native population increased year upon year at all three sites from 2012 to 2014. Peak abundance across the three sites rose from 4.4 larvae trap\(^{-1}\) (2012) to 16.3 larvae trap\(^{-1}\) (2013), to 23.8 larvae trap\(^{-1}\) (2014). Peak presence charted an increasing Ae. albopictus distribution across the three sites from 17% positive ovitraps (2012) to 62% (2013), to 75% (2014). Such a volatile period for Ae. albopictus populations within the area prevented the calculation of a statistically valid long-term baseline for this species.

#### 3.3 Operational parameters

##### 3.3.1 OX513A releases

Releases of OX513A Ae. aegypti took place on Monday, Wednesday and Friday of each calendar week, beginning on 25 April 2014 and finishing 189 DAT1 (days after first treatment) on 31 October 2014 (Fig. 4a). Only on one occasion (115 DAT1) did a release not happen, in that case because the production batch of OX513A died prematurely in the laboratory owing to a missed dose of chlortetracycline. Overall, there were 81 releases spanning 189 days. The total number of OX513A adults released was 4 249 951. The mean number released on each occasion was 52 469 (SD = 13 539), equating to 87% of the target release rate.

##### 3.3.2 Gender of OX513A adults released

The intention was to release only male OX513A adults. This was strategic, as male Ae. aegypti do not bite and so cannot transmit diseases, but also as male-only releases increase efficiency by...
denying OX513A males the opportunity to mate with OX513A females. Female *Ae. aegypti* may bite humans, so it was important to minimise the number of female OX513A mosquitoes released. Although OX513A females are unlikely to have been exposed to dengue or other viruses prior to release, if released in sufficient numbers they could potentially have contributed to local disease transmission.

For every group of male pupae that were mechanically sorted (i.e. separated) from females, a sample of at least 1000 individuals were manually checked by visual inspection to confirm the accuracy of the sorting procedure. Multiple groups contributed to a single batch of pupae produced on any given day. A minimum threshold for pupal sorting accuracy of 99.8% males per group was imposed; any group below this threshold was re-sorted until it was above 99.8%. Data showed that for any single release batch the minimum percentage of pupae that were males was calculated as 99.91% (105 and 142 DAT1), and the mean value for males in a single release batch was 99.99% (SD = 0.019). For 59 of the 81 release batches, no females were detected (Fig. 4b).

### 3.3.3 OX513A mating fraction and persistence

The proportion of wild females mated by OX513A males (as opposed to wild males) was termed the mating fraction. This was estimated by calculating the percentage of *Ae. aegypti* larvae caught in ovitraps as eggs that expressed the OX513A fluorescent phenotype. Higher mating fractions therefore reflected fewer matings between wild males and wild females. It was estimated that, to exert a positive and ongoing control pressure on the wild population, a sustained target fluorescence (mating fraction) of >50% was required.

Releases of OX513A adults began on 25 April 2014, and the first traps containing fluorescent individuals were recovered at 4 DAT1 (Fig. 5). The fluorescence level rose steadily until it reached 100% in the traps collected at 67 DAT1, marking the first complete week during which no wild homozygous *Ae. aegypti* were recorded in any of the 55 ovitraps of the treated site. Traps collected at 179 DAT1 did not yield a value, as this was the first complete week when no *Ae. aegypti* (neither wild nor fluorescent) were detected at the treated site. Prior to this, fluorescence was sustained over a 23 week period at a mean value of 75% (SD = 17).

Monitoring for OX513A larvae (i.e. insects fathered by OX513A males) also enabled an assessment of the level of persistence in the environment. In principle, any progeny carrying OX513A genes would die and could not reproduce or pass on their genes. Therefore, once OX513A adult releases had ceased, the presence of the transgene would be expected to decline sharply and disappear following the subsequent generation (i.e. within several weeks). The data obtained show that at approximately 3 weeks post-release there was a marked fall in the presence of OX513A genes within a
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7 day period, from 88% of all *Ae. aegypti* individuals at 207 DAT1 to 5% at 214 DAT1. A subsequent 8 week monitoring period from 273 DAT1 to 327 DAT1 at site T1 recovered 2329 *Ae. aegypti*, of which no individuals (0%) were fluorescent (i.e. carried OX513A genes).

3.4 Abundance of *Aedes* spp.

Abundance of *Ae. albopictus* and *Ae. aegypti* populations in the treated and untreated areas was estimated using the numbers of larvae that hatched from eggs caught in ovitraps. All eggs recovered were reared through to larvae to enable identifications at species level.

3.4.1 *Aedes aegypti*

Four-week moving averages show that treatment with OX513A resulted in significant changes in *Ae. aegypti* abundance (signified by CIs not overlapping with those for 4 DAT1). Reductions of 78% (CI = −54 to −87) at 116 DAT1 and 86% (CI = −67 to −93) at 109 DAT1 occurred relative to both UT1 and UT2 respectively. These were then largely maintained for the remainder of the study period, culminating in 91% (CI = −79 to −98) and 95% (CI = −89 to −99) reductions at 200 DAT1 in the treated population relative to UT1 and UT2 respectively (Fig. 6). From 116 to 200 DAT1, 635 wild *Ae. aegypti* were recovered from the treated site, 13 237 (21 times more) were recovered from UT1 and 10 515 (17 times more) were recovered from UT2.

For three separate calendar weeks (those ending 81, 179 and 186 DAT1), no wild *Ae. aegypti* were detected at the treated site. Contrastingly, during the same 7 day period, 425 and 311 *Ae. aegypti* larvae were recovered from UT1 and UT2 respectively.

3.4.2 *Aedes albopictus*

Because *Ae. albopictus* appeared to be in the process of colonising the study area (see Section 3.4), it is conceivable that, even given their close geographical locations, populations at the three study sites were potentially changing at different rates. It was
As for abundance data, data for presence of *Ae. aegypti* included both wild and fluorescent larvae. Changes in presence of *Ae. aegypti* were largely aligned with changes in *Ae. aegypti* abundance. For one calendar week (week ending 179 DAT1), *Ae. aegypti* presence in the treated site was 0% (i.e. *Ae. aegypti* were not detected in any trap); this compared to 48% in each of the untreated sites (i.e. 48% of traps detected *Ae. aegypti*). Calculated four-week moving averages culminated in significant reductions

### Table 1. Monthly proportions of total *Aedes aegypti* adults and larvae collected that carried the OX513A transgene and the corresponding monthly OX513A mating competitiveness estimates (C). All values are presented to three significant figures

| Calendar month | Proportion of adults | Proportion of larvae | C value (95% CI) |
|----------------|---------------------|----------------------|-----------------|
| May            | 0.988               | 0.571                | 0.016 (0.011–0.021) |
| June           | 0.974               | 0.871                | 0.178 (0.132–0.230) |
| July           | 0.975               | 0.945                | 0.444 (0.303–0.607) |
| August         | 0.991               | 0.652                | 0.016 (0.008–0.025) |
| September      | 0.976               | 0.639                | 0.043 (0.024–0.067) |
| October        | 0.974               | 0.860                | 0.165 (0.093–0.272) |
| Mean           | 0.980               | 0.756                | 0.144 (0.05–0.28) |

therefore important to assess the effects of releasing OX513A *Ae. aegypti* on *Ae. albopictus* populations by specifically examining the period during which *Ae. aegypti* had been suppressed. To this end, *Ae. albopictus* populations at all three sites were monitored for changes in abundance from 109 DAT1 to the end of the study (200 DAT1).

*Ae. albopictus* abundance was relatively stable, with relative changes at UT1 and UT2 being significantly different from zero on only a limited number of occasions (Fig. 7). Over this 91 day period, changes in mean larvae per ovitrap culminated in increases of 2% (CI = −29 to 50), 17% (CI = −20 to 69) and 12% (CI = 23–70) at UT1, UT2 and T respectively. Site T was not significantly different to either UT1 or UT2 at any time point.

### 3.5 Presence of *Aedes* spp.

One method used routinely by some vector control agencies to quantify mosquito presence is the percentage of ovitraps that caught at least one individual (i.e. were considered to be positive). As both *Ae. aegypti* and *Ae. albopictus* were present and it is not possible to discriminate between these two species at the egg stage, it was necessary to hatch all recovered eggs and rear through to the larval stage to complete species identifications.

#### 3.5.1 *Aedes aegypti*

As for abundance data, data for presence of *Ae. aegypti* included both wild and fluorescent larvae. Changes in presence of *Ae. aegypti* were largely aligned with changes in *Ae. aegypti* abundance. For one calendar week (week ending 179 DAT1), *Ae. aegypti* presence in the treated site was 0% (i.e. *Ae. aegypti* were not detected in any trap); this compared to 48% in each of the untreated sites (i.e. 48% of traps detected *Ae. aegypti*). Calculated four-week moving averages culminated in significant reductions

**Figure 5.** Percentage of *Aedes aegypti* larvae that were fluorescent. Fluorescent larvae could only have been fathered by an OX513A male, and therefore the percentage fluorescence represented the mating fraction. The final release of OX513A was 189 days after first treatment (DAT1) and is indicated by the dotted line; the endpoint of the study (200 DAT1) is indicated by the dashed line.

**Figure 6.** Four-week moving averages showing percentage change in *Aedes aegypti* abundance at the treated site, measured by mean number of *Ae. aegypti* larvae per trap relative to (a) UT1 and (b) UT2. The final release of OX513A (highlighted by the dotted line) was 189 days after first treatment.
Figure 7. Four-week moving averages showing abundance (mean numbers per ovitraps) of Ae. albopictus during the period of Ae. aegypti suppression (from 123 days after first treatment until the end of the study period) at sites (a) UT1, (b) UT2 and (c) T. The final release of OX513A (highlighted by the dotted line) was 189 days after first treatment.

at 200 DAT1 of 79% (CI = −58 to −95) and 88% (CI = −76 to −97) against UT1 and UT2 respectively (Fig. 8).

3.5.2 Ae. albopictus

Ae. albopictus presence was monitored at all three sites from 109 DAT1 to the end of the study (200 DAT1). In contrast to data for Ae. aegypti presence, across this 91 day period Ae. albopictus populations were generally stable. The number of positive ovitraps increased by 18% (CI = −5 to 44) at UT2, and decreased by 6% (CI = −21 to 9) and 12% (CI = −23 to 70) at UT1 and T respectively (Fig. 9). Site T was not significantly different to either UT1 or UT2 at any time point.

4 DISCUSSION

The monitoring for Ae. albopictus highlighted an increasing population at all three sites since 2012. This concurs with other reports of population expansion for this species within the local and wider region. Interestingly, while documenting a significant influx of Ae. albopictus within the study area, there was no evidence of this negatively affecting the residing Ae. aegypti population, the levels of which were typically high in 2014. Although somewhat preliminary, these observations are not suggestive of a high level of interspecific competition. The mean relative changes documented in the target Ae. aegypti population against UT1 and UT2 showed that at 116 DAT1 there had been a significant reduction of 82% (SD = 5.7) in abundance of the treated Ae. aegypti population, and by 200 DAT1 that reduction was 93% (SD = 2.8). This demonstrated that the numbers of OX513A deployed were sufficient to exert control on the population and realistically represented an operational scale of release. In contrast, there was no evidence of a significant change in either Ae. albopictus abundance or presence. It may be considered unlikely that the exploitation of vacated niches resulting from removal of Ae. aegypti would occur over the course of this relatively short study, and that assessing the full potential for interactions with coexisting species will only be possible as longer-term datasets at a range of geographical scales also become available. Nonetheless, with a requirement to advance such pioneering studies in a stepwise manner, it was evident that significant suppression of Ae. aegypti by >82% for an 84 day period did not result in an increase in Ae. albopictus at these sites.

The accuracy of the OX513A male production process resulted in extremely low numbers of female Ae. aegypti being released. The equivalent of less than one female OX513A per person per year was released at the treated site. Calculated against the monitoring data retrieved just prior to the last release (186 DAT1), the proportion of individuals at the treated site that carried the transgene declined by 95% in 28 days (Fig. 9). In conjunction with the absence of detectable transgene from 84 days...
A value of 0.14. This compares favourably with a previously completed field study of OX513A (Caceres L, unpublished). Over the course of this field study in caged laboratory experiments against Panamanian wild and wild males would have been deemed equally competitive; if = 1, OX513A would be deemed more competitive than wild. If > 1, OX513A would be deemed less competitive than wild; if < 1, OX513A would be deemed more competitive than wild. A value of = 0.9 for OX513A had been previously obtained (Wiedermann), have also reported variable C values ranging from 0.0001 and 0.17. In this study there was also noticeable variation between the mating competitiveness values obtained across different months, potentially reflecting a number of variables, including (but not limited to) OX513A male fitness, the wild population level and environmental conditions. The urban landscape at the treated site during this study was typified by large verandas that shaded the front of many properties lining the roadsides. Both adult traps and ovitraps were frequently located under these verandas, potentially offering a strong correlation between the catch data they provided.

A key consideration for public health agencies is maintaining vector populations below the levels required for sustained disease transmission. Such disease transmission thresholds are dynamic and dependent upon multiple factors. These factors vary spatially and temporally, making predictions for specific localities difficult. However, using a published temperature-dependent model, generic predictions for dengue transmission thresholds that are related to initial serotype prevalence (also termed herd immunity) are possible. Mark–release–recapture statistics, relevant ambient temperatures and known numbers of OX513A males released at the treated site were used to estimate local population sizes and dengue transmission thresholds (Fig. 10). It was found that OX513A treatment maintained Ae. aegypti levels throughout the wet season at below the transmission threshold predicted even for a fully susceptible human population without any prior immunity (seroprevalence rate of 0%). Contrastingly, the model predicted an increased risk of a dengue outbreak at an untreated site (UT1), with abundance of Ae. aegypti rising during the wet season to above the transmission threshold predicted for when one-third of the human population is immune.

In summary, no significant change in either Ae. albopictus prevalence or spread was observed over the course of the study. Therefore, there was no evidence to support the theory that Ae. albopictus fills vacant niches when the local population of Ae. aegypti is largely removed from the environment. Such observations have not been possible hitherto, and they should continue as evaluations of such highly targeted technologies broaden in scale and scope. Indeed, in addition to contributing to assessments of biosafety, and regardless of the technology deployed, an understanding of the likely environmental impacts can contribute in the long term to improvements in efficiency and performance. The sex-sorting procedure employed resulted in estimated numbers of released OX513A females of well under one per person for the duration of the study. As OX513A releases suppressed the local pest population by over 90% to below the modelled disease transmission threshold for dengue, it is likely that the risk of being bitten by a disease-carrying mosquito in the treated area was significantly reduced. In addition, the disappearance of OX513A from the environment was observed shortly after releases of OX513A stopped, supporting the assertion that OX513A does not persist in the environment and is self-limiting. Monitoring will continue at the three sites to detect any ongoing effects on either Ae. aegypti or Ae. albopictus populations in the medium to long term.

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