nature biotechnology

# Field performance of engineered male mosquitoes

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**Dengue is the most medically important arthropod-borne viral disease, with 50–100 million cases reported annually worldwide[1]. As no licensed vaccine or dedicated therapy exists for dengue, the most promising strategies to control the disease involve targeting the predominant mosquito vector, *Aedes aegypti*. However, the current methods to do this are inadequate. Various approaches involving genetically engineered mosquitoes have been proposed[2–4], including the release of transgenic sterile males[5–10]. However, the ability of laboratory-reared, engineered male mosquitoes to effectively compete with wild males in terms of finding and mating with wild females, which is critical to the success of these strategies, has remained untested. We report data from the first open-field trial involving a strain of engineered mosquito. We demonstrated that genetically modified male mosquitoes, released across 10 hectares for a 4-week period, mated successfully with wild females and fertilized their eggs. These findings suggest the feasibility of this technology to control dengue by suppressing field populations of *A. aegypti*.**



The Sterile Insect Technique (SIT) is a species-specific and environmentally friendly method of pest control based on the mass release of sterile insects to mate with, and thereby control, the population size of their wild counterparts. SIT has been used successfully for decades to control several agricultural pest insects[11]. However, despite its attractive features and several trials in the 1970s and 1980s, SIT is not in operational use against mosquitoes[5,12]. This is in part due to the damaging effect on the mosquitoes of sterilizing doses of radiation, which makes irradiated males less able to compete for mates[5,12,13]. We have proposed that a genetics-based approach termed RIDL (Release of Insects carrying a Dominant Lethal genetic system)[14] could remove the need for radiation-mediated sterilization and make sterile-male methods an attractive option for mosquito control[5,15]. Prototype RIDL strains have been reported previously[6,7].

A critical issue that needs to be addressed before attempts to implement RIDL on a large scale concerns whether genetic modification itself and/or rearing the insects in a laboratory environment might impose fitness costs, such that the sterile males would be ineffective. Irradiated sterile males with a fluorescent marker were found to be equivalent to an unmodified comparator[16]. However, evidence on the fitness cost of genetic engineering from laboratory studies is mixed[17–20], though the relevance of such studies to the field situation is also questionable.

We therefore set out to examine this in the field, using as the RIDL system OX513A, an engineered strain of *A. aegypti*, the principal vector of dengue. OX513A carries a repressible lethal genetic system to ensure that mosquitoes can be reared to maturity in the presence of tetracycline in the laboratory, although their offspring will not survive to adulthood in the absence of the repressor. The strain also expresses a fluorescent marker gene to allow detection[7,21–23]. We selected a field site on Grand Cayman—the largest of the Cayman Islands, a British Overseas Territory in the Caribbean—on the basis of several criteria outlined in Online Methods. Dengue is rare on Grand Cayman but common in the region and an ongoing threat as long as the mosquito vector is present. High levels of insecticide resistance in *A. aegypti* in Grand Cayman[24] and neighboring regions have highlighted the need for alternative methods of control.

The success of releasing sterile male insect pests relies critically on, first, the ability of RIDL males to compete successfully with wild males for mating with wild females in the field, and second, the ability of the inherited RIDL construct to kill some or all of the progeny of such matings. In principle, field strains might vary in their propensity to mate RIDL males or to be killed by the transgene; strong mating barriers or substantial resistance to the transgene could compromise field effectiveness[7]. Therefore, before undertaking releases, we examined under laboratory conditions the effect of the Cayman strain background on both male competitiveness and penetrance of the lethal phenotype.

We tested mating competitiveness by exposing ten Cayman females simultaneously to ten RIDL males and ten Cayman males in a laboratory cage (Online Methods) and determining which type of male each female mated. From five replicate experiments, we obtained data from 31 females (after excluding those females that failed to mate, blood feed and lay viable eggs). Of these 31, 17 (55%) had mated a RIDL male, indicating that no strong mating barrier exists between the strains, at least in this laboratory assay.

We tested penetrance of the lethal phenotype of the transgene in $F_1$ hybrids between OX513A homozygous males and Cayman females. Such hybrids are expected to arise in the field after release of OX513A males; modeling suggests that >90% of these need to die before reproductive maturity for local elimination to be achievable in the absence of immigration[7]. Eggs from a laboratory cross of OX513A homozygous males with Cayman wild-type females, and from control crosses, were hatched and first instar larvae counted into rearing trays. These were reared in the presence and absence of tetracycline and the number of

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Received 28 June; accepted 27 September; published online 30 October 2011; doi:10.1038/nbt.2019

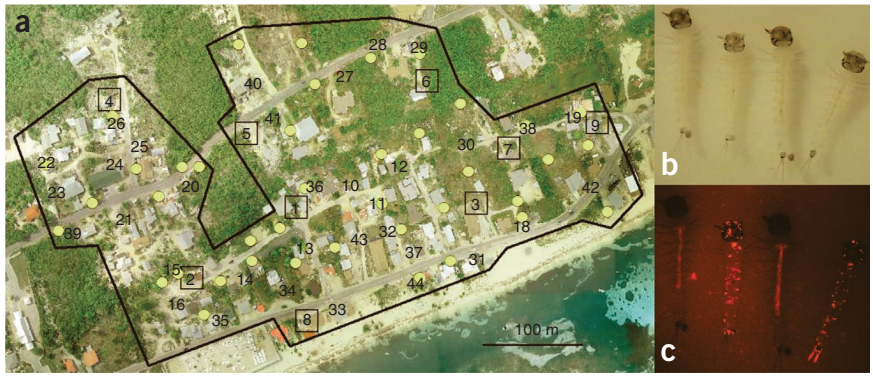


**Figure 1** Field site and larval fluorescence. (**a**) Aerial photomap illustrating the trial site in East End, Grand Cayman. The 10-ha release area is outlined; this area includes ~97 substantial buildings including 89 houses. Yellow circles indicate pupal release locations. The BG Sentinel adult trap locations are indicated by numbers 1–9 in boxes and the ovitraps by numbers 10–44. Trap numbers correspond with those in **Tables 1** and **2**. Additional traps were placed outside of the release area (not shown). Trapped eggs were hatched and scored for fluorescence. (**b**,**c**) Four wild-type and OX513A larvae, from left to right: wild type, OX513A, wild type, OX513A visualized in white light (**b**) or under conditions for fluorescence photomicroscopy (**c**). OX513A shows a distinctive and readily identifiable punctate pattern of red fluorescence but is indistinguishable from wild type under normal light.



emerging adults was counted (dietary tetracycline represses the lethal genetic system[7]). Mortality of 96.5% (exact 95% confidence interval (CI): 95.1–97.6%) was observed ($n$ = 913), consistent with previous analysis of this strain[7]. Mortality of 100% (exact 95% CI: 99.3–100%) was observed in OX513A homozygotes ($n$ = 524), which carry two rather than one copy of the lethal transgene, and 1–2% mortality for all other controls (OX513A and OX513A/Cayman hybrids reared in the presence of 30 μg/ml tetracycline ($n$ = 292 and 932, respectively), Cayman wild-type reared with or without tetracycline ($n$ = 300 and 123, respectively)). These data provide no indication that the penetrance of the OX513A-induced lethal phenotype is reduced or inadequate in hybrids with Cayman-derived *A. aegypti*. These experiments were conducted under ideal and relatively benign laboratory conditions. Our observations that the survivors were weak and short-lived (data not shown) led us to suspect that survival would be even lower in the field, although this is not necessary for the method to be effective.

OX513A males were released in a 10-hectare (ha) area at an average release rate of 465 males/ha/week for 4 weeks, starting on Nov. 16, 2009 (**Figs. 1a**, **2b** and Online Methods). There are several compelling reasons to release only males[5,25], one being that only female mosquitoes bite humans. We monitored the mosquito population, comprising both OX513A and wild insects, using ovitraps, which mimic natural oviposition sites in which females lay eggs. We hatched these eggs under laboratory conditions, and screened the resulting larvae for fluorescence to determine paternity. Fluorescent larvae had OX513A fathers, whereas nonfluorescent larvae had wild-type fathers (**Fig. 1b**,**c**).

PCR analysis of DNA of males from adult traps (Dec. 1–14) showed that we recaptured 20 released OX513A males and 105 wild-type males. This indicates that OX513A males represented ~16% (95% bootstrap confidence interval (CI): 8.6–34%) of the total (RIDL plus wild) adult males in this period, assuming equal trapping efficiency for each type (**Table 1** and **Fig. 2c**). Statistically significant clustering of the OX513A males was observed relative to the wild males (Fisher's exact test $P < 0.001$). This may represent uneven distribution of OX513A males due to the point-release method used and/or uneven distribution of the wild population. Ovitrap data (**Fig. 1a**) and adult capture rates (1.6 female *A. aegypti* per 24 h trap period) indicate fairly large populations of wild *A. aegypti* during the release period relative to the population densities known for areas infested with *A. aegypti*[26–28].

We hatched eggs obtained from ovitraps under laboratory conditions and scored fluorescence of the larvae. At the time of the initial release, older females would have mated (*A. aegypti* females typically mate only once) and so only newly emerged females would be available to mate the OX513A males. An equilibrium will be approached when the OX513A male population has accumulated to a steady state (new introductions matched by deaths), and similarly for females emerging late enough to have been exposed to these OX513A males. This equilibrium is itself transient as death of OX513A heterozygous offspring will eventually start to have an impact on the number of wild mosquitoes, depending on the numbers of RIDL males released and their mating competitiveness compared with wild males. Females additionally have to blood feed and find oviposition sites. Owing to these various factors, we expected a time delay of the order of a week or so between release of OX513A males and the appearance in ovitraps of eggs that they had fathered. Conversely, such eggs were expected

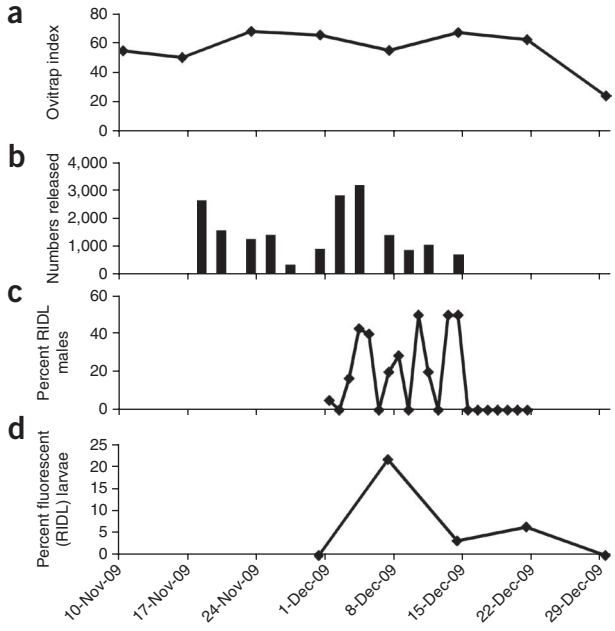


**Figure 2** OX513A release and recapture data. (**a**) The wild mosquito population density in the release area was consistently high throughout the release period. Ovitrap index is the proportion of ovitraps containing at least one *A. aegypti* egg after a week in the field. (**b**) Pupae were placed in the field in release devices and the number of males exiting calculated for each of 12 releases. (**c**) Adult mosquitoes were trapped and the relative proportions of OX513A and wild-type males determined. (**d**) Eggs from wild females were obtained from ovitraps, hatched and scored for fluorescence to determine the relative OX513A and wild-type paternity.

**Table 1 Trapping adult males**

| Trap number | OX513A males | Total tested |
|---|---|---|
| 1 | 1 | 21 |
| 2 | 4 | 22 |
| 3 | 1 | 1 |
| 4 | 0 | 4 |
| 5 | 3 | 5 |
| 6 | 4 | 9 |
| 7 | 4 | 13 |
| 8 | 3 | 50 |
| 9 | 0 | 0 |
| Total | 20 | 125 |

Adults were found in eight of nine BG Sentinel traps in the release area (Dec. 1–14). DNA from each male was analyzed by PCR for the presence of the transgene. Of the 125 males captured, 20 were OX513A, indicating that OX513A males represented about 16% (95% bootstrap confidence interval (CI): 8.6–34%) of the total (RIDL plus wild) adult males in this period. The OX513A mosquitoes were significantly clustered in traps relative to wild type (Fisher's exact test $P < 0.001$). Thus, the trap-based bootstrap confidence interval is reported. Location of adult traps is shown in **Figure 1a**.

**Table 2 Investigating mating outcomes by ovitrapping**

| Trap number | OX513A larvae recovered | Total tested |
|---|---|---|
| 10 | 0 | 10 |
| 11 | 1 | 2 |
| 12 | 0 | 9 |
| 13 | 0 | 11 |
| 14 | 0 | 136 |
| 15 | 0 | 168 |
| 16 | 0 | 50 |
| 17 | 43 | 44 |
| 18 | 0 | 1 |
| 19 | 0 | 9 |
| 20 | 12 | 42 |
| 21 | 64 | 139 |
| 22 | 0 | 69 |
| 23 | 0 | 4 |
| 24 | 0 | 9 |
| 25 | 0 | 4 |
| 26 | 6 | 28 |
| 27 | 0 | 23 |
| 28 | 0 | 49 |
| 29 | 0 | 22 |
| 30 | 0 | 13 |
| 31 | 0 | 29 |
| 32 | 0 | 29 |
| 33 | 0 | 34 |
| 34 | 0 | 253 |
| 35 | 0 | 129 |
| Total | 126 | 1,316 |

Ovitraps were placed in the field and serviced weekly. Eggs from each trap were hatched and scored for fluorescence. Fluorescent larvae were detected from ovitraps recovered on Dec. 7, 14 and 21 (weeks 3, 4 and 5 relative to first release date of Nov. 16). The OX513A eggs and/or larvae were significantly clustered in traps (Fisher's exact test $P < 0.001$). Thus, the trap-based bootstrap confidence interval is reported in the text. A further nine traps (36–44) caught no hatchable eggs. Ovitrap locations are shown in **Figure 1a**.

still to appear for a short period after releases ceased. Both of these effects were observed. Fluorescent larvae were detected from ovitraps recovered on Dec. 7, 14 and 21, corresponding to weeks 3–5 measured from the first release date (**Table 2** and **Fig. 2d**). As these traps were serviced weekly, they represent the eggs laid over the preceding week. In total, we observed 126 fluorescent larvae from five ovitraps. This represents 9.6% (95% bootstrap CI: 0.7–23%) of the 1,316 larvae scored from ovitraps collected in this period. In principle, fluorescent larvae could have had an OX513A mother, rather than father. To avoid this, we manually screened all pupae after sex separation to remove any remaining females (Online Methods). We also analyzed fluorescent larvae for the presence of a wild-type allele at the OX513A insertion site. We would expect that if any OX513A females were accidentally released, they would likely mate a co-released OX513A male. If so, some of the fluorescent larvae would be homozygous rather than heterozygous for the OX513A insertion. All fluorescent larvae analyzed were heterozygous ($n = 24$).

The roughly twofold difference between this progeny fraction (9.6%) and the RIDL OX513A adult male fraction in the field (16%) is not statistically significant and may therefore result from random variation. Nonetheless there are several reasons why one might expect these two ratios to differ. These include unequal trapping efficiency for the two types of male, unequal trapping efficiency for the two types of eggs, reduced mating competitiveness of OX513A males relative to wild males, imperfect distribution of OX513A males relative to the wild mosquito population or movement of mosquitoes across the boundary of the release area. Imperfect mating competitiveness of the OX513A males relative to wild males could in turn be due to one or more of several factors, including environmental and genetic consequences of colonization and mass rearing, physical effects of handling and distribution, and any negative effect of the transgene on adult male performance. One limitation in this analysis is that we can only sample eggs or larvae; it is difficult to estimate the relationship between the number of eggs analyzed and the number of females from which they derived.

We define field competitiveness ($C$) as the relationship between the numerical density of wild-type ($N$) and sterile ($S$) insects and the relative mating success, such that $C = PN/S\ (1 - P)$, where $P$ is the proportion of sterile matings[29]. Using this definition, we estimate $C$ for OX513A in this experiment as 0.56 (95% bootstrap CI: 0.032–1.97), where 1.0 would represent exact equivalence with wild males. For comparison, estimates of $C$ for classical SIT programs have been derived, estimating mating success by measuring induced sterility in field-collected embryos, which is similar to the method used in the present study. For the New World screwworm (*Cochliomyia hominivorax*), field competitiveness was estimated at 0.29–0.43 at smaller scales, decreasing to 0.1 for larger programs[29,30]. An estimated $C = 0.17$ has been reported for a small-scale trial of irradiated Mediterranean fruitfly (Medfly, *Ceratitis capitata*), increasing to 0.42 if the males were exposed to ginger root oil[31]. However, of four other field trials, only one gave a $C$ value >0.01. In one large trial in a successful control program, no significant induced sterility was observed until sterile/wild ratios reached 100:1 or higher[32], with peak values of $P$ around 70% requiring release ratios ~500:1 or higher; $C = 0.0001–0.001$ was estimated for this trial[31].

These data also allow us tentatively to estimate how many OX513A mosquitoes might need to be released in this area to suppress the target population. Briefly, models indicate that a mating fraction of 13–57% is required for suppression[7]. Based on the proportion (9.6%) of fluorescent offspring observed in the experiment described here, this would require sustained release at ~1.4–12 times the release rate of the experiment described here. Combined use of appropriate conventional control methods in an integrated vector management system would reduce this number.

To our knowledge, there have not been any other experimental demonstrations of mating between released transgenic mosquitoes and wild mosquitoes. Release and recapture data gave measures of the released/wild male ratio and mating outcomes resulting from the release of a known number of OX513A male mosquitoes. The estimated field competitiveness, albeit with wide confidence bounds, suggests that OX513A males can compete well for mates in the field. Additional data to refine estimates of $C$ would be desirable, as would other parameters not addressed here such as longevity and dispersal of the released males, and the extent to which each of these may vary between locations. Nonetheless our data

suggest the likely feasibility of suppressing field populations of *A. aegypti* by sustained release of engineered sterile male mosquitoes.

## METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturebiotechnology/.

*Note: Supplementary information is available on the Nature Biotechnology website.*

### ACKNOWLEDGMENTS

We thank Z. Ebanks, I. Black, Z. Curtis, C. Phillips, A. Miles and T. Matthews for technical and logistical assistance, G. Labbé and P. Gray for strain development, and G. Labbé and N. Morrison for manuscript review. We are grateful to the Lands & Survey Department of the Cayman Islands Government for permission to use imagery and data. A.F.H. thanks Adapco, Bayer and Central Life Sciences for supporting her PhD studentship. C.A.D. thanks the UK Medical Research Council for Centre funding.

### AUTHOR CONTRIBUTIONS

L.A., A.R.M., C.B., A.F.H., D.N. and W.D.P. conceived and supervised the project. A.F.H., D.N., N.K. and S.S. conducted the experiments. A.R.M., C.A.D. and L.A. analyzed the data and wrote the paper. All authors discussed the results and commented on the manuscript.

### COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/.

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## ONLINE METHODS

**Competition between OX513A and Cayman males.** Both RIDL and Cayman strain *A. aegypti* were reared separately to pupae and allowed to emerge into individual 30 ml vials. The Cayman Islands strain of *A. aegypti* originated from larvae collected in the George Town and West Bay areas of Grand Cayman in January 2008; larvae were collected from multiple water containers, pooled and reared to adulthood in the insectaries at the Mosquito Research and Control Unit (MRCU). Ten 4-d-old Cayman female *A. aegypti* were released into a 30 cm × 30 cm × 30 cm cage (Mega View Science); 10% sucrose solution was provided. Ten Cayman strain and ten RIDL males were transferred from their tubes into a plastic cup to allow them to mix before being released into the cage along with the females. Five replicates were performed. After 48 h females were removed to individual tubes, allowed to blood feed and oviposit. The resulting eggs were hatched, and the larvae screened for presence of the fluorescent marker indicative of a RIDL father.

**Field site selection and description.** MRCU approached Oxitec in 2008 to discuss the potential use of Oxitec's RIDL technology for control of *A. aegypti* in the Cayman Islands. MRCU, established in 1965, is an agency of the Cayman Islands government responsible for mosquito research as well as control, and scans the horizon for new methods and technologies potentially of use in the Cayman Islands. Potential sites for field evaluation of the technology were then considered against a predefined set of biological, sociological and logistical criteria[33], including the following.

- Significant presence of target *Aedes* species. For *A. aegypti,* which is highly anthropophilic, this means an infested human settlement.
- Preferably absence of the closely related *A. albopictus*, or predominance of *A. aegypti* over *A. albopictus*.
- Logistically viable. Reasonably accessible with infrastructure for establishing a field station with a small team of workers.
- Cooperation and support of local population and health authorities.
- Ecologically stable.
- Geographically isolated (from a mosquito perspective), to limit the impact of immigration of mosquitoes from neighboring communities. *A. aegypti* has a limited dispersal range, seldom flying >400 m in a lifetime.
- Presence of comparable locations, which can act as suitable comparison sites for the release trial.

It was additionally decided to use a site that did not have routine control of *A. aegypti*. An initial long list of potential sites was drawn up based on aerial imagery and field experience of MRCU field staff. This was followed by site visits by Oxitec and MRCU staff (Sept. 10, 2009). After taking all factors into consideration East End and Bodden Town were shortlisted as the preferred, potential, treated and untreated 'control' sites for an evaluation of OX513A. East End is ~15 km east of Bodden Town, and 25 km east of George Town, the capital of the Cayman Islands.

East End comprises ~200 houses in an area of ~0.5 $km^2$. It provides excellent ecological isolation with regard to *Aedes* mosquitoes, located on the sparsely populated east of the island and bounded by unmanaged natural habitat and ocean. Population consists of a traditional community of Caymanians and immigrant workers with a mix of brick and open wooden housing. Housing is serviced with paved roads, piped water and electricity.

Bodden Town, ~15 km west of East End, is the nearest sizable community to East End. It is slightly larger, more dispersed with less defined boundary and isolation than East End. However, it has very similar environment, housing type and community to East End, providing an ideal untreated site for comparison. (For the experiments described here, internal comparisons are more relevant than external controls. However, adult and ovitrap data indicated that the *A. aegypti* populations in Bodden Town were similar to those of East End during the period of this trial, for example, **Fig. 1a** (data not shown)).

There were no existing data on *Aedes* presence in these sites as routine monitoring by MRCU did not extend to East End or Bodden Town. Weekly ovitrapping was initiated on Sept. 21, 2009 to establish the presence of *Aedes* and species composition. An initial survey confirmed the presence of *A. aegypti* and the absence of *A. albopictus* (small pockets of *A. albopictus* were present further west in Grand Cayman). Absence of *A. albopictus* considerably simplifies the monitoring process. Both species are closely related, occupy similar ecological niches and require an additional identification step in standard monitoring systems, which generally trap both species.

**Community engagement and regulatory affairs.** A pest risk analysis (PRA) is designed for the analysis of risks to the environment and biological diversity in the area and the risk management measures that could be employed to manage those risks. A PRA was conducted according to the standard protocol defined by the International Plant Protection Convention (IPPC - ISPM 11, adapted as *A. aegypti* is not a plant pest and also incorporating elements of Cartagena Biosafety Protocol Annex III on Risk Assessment)[34]. It was concluded that the proposed release was short in duration, limited in scope and the *A. aegypti* strain cannot establish in the environment, due to the self-limiting genetic lethality, that is, the progeny will die, there are not likely to be any pathogenic and/or ecologically disruptive effects.

MRCU has a mandate from the Cayman Legislative Assembly to conduct research activities for mosquito control as enshrined in the Mosquito (Research and Control) Law (2007 Revision). Permission was obtained from the Department of Agriculture for the import and release of the mosquitoes, under the Animals Law (2003 Revision). The trial was also conducted in accordance with the provisions in the Cayman Islands draft National Conservation Law. Work conducted at Oxitec similarly complied with all relevent UK legislation. In addition to securing the necessary regulatory approval, relevant government departments (including ministry representatives from District Administration, Works and Gender Affairs (under which the MRCU operates); The Ministry of Health, Environment, Youth, Sports and Culture, Public Health; The Department of Agriculture; and The Department of the Environment) were briefed and consulted before and during release activities. Information about the project was provided more broadly through the media and by personal contact and briefing. Project personnel were present on-site ~5 d per week to provide information and conduct operations. All equipment, vehicles and personnel were clearly marked; equipment and written material included MRCU contact information. Permission was sought from individual householders before project activity on their property, for example, placement and servicing of BG-Sentinel traps. All such requests (100%) received a positive response and no negative comments regarding project operations were received.

**Release period.** Nov. 16, 2009 to Dec. 14, 2009 (first release devices placed Nov. 16; last release devices placed in the field Dec. 11, recovered Dec. 14). The release site was a central 10-ha region of East End (approximate latitude 19.2967 and longitude −81.1106). Importation and release of mosquitoes were conducted under permit from the Cayman Islands Dept of Agriculture; MRCU activities are also governed by the Mosquito (Research and Control) Law (2007 Revision). No formal *A. aegypti* control measures were implemented at East End in 2009, but aerial and vehicle-based spraying were occasionally used to control a salt-marsh mosquito, *A. taeniorhynchus*. No such spraying was conducted in East End during the experimental period.

**Source of released material.** OX513A-Aae, the OX513A insertion in *A. aegypti* (LA513A[7]) was introgressed into a Mexico-derived genetic background by five generations of backcrossing, then made homozygous. Homozygous OX513A eggs were transferred from Oxitec and reared to pupae at MRCU, then mechanically sorted to remove most of the females[35,36]. Microscopic examination of the resulting male population showed that this was 99.55% accurate (exact 95% CI: 99.46–99.63), that is, the sorted population contained only 0.5% females. These remaining females were removed by hand to avoid any possibility that transgenic eggs or larvae recovered from the field might have a RIDL mother rather than a RIDL father. Female pupae were rendered nonviable by chemical or temperature treatment.

**Release method.** Pupae were placed in pupal release devices (**Supplementary Fig. 1**). Three times per week (Monday, Wednesday and Friday), these devices were transferred to the field site, in each case (after the first release), the previous 'spent' device at the same release location was recovered and returned to the laboratory. Dead pupae, pupal exuviae and adults remaining in the device were then counted; adult release numbers were calculated as the difference between the number of pupae placed in the field and the number of

dead individuals in the returned devices. Release numbers varied considerably between releases (**Fig. 2b**) due to fluctuations in production and variable survival, including predation, of the pupae placed in the field. There were 40 release locations in the 10-ha release site. Approximately 25,500 pupae were placed in the field in release devices over a 4-week period and ~18,600 adults exited the release devices into the field environment.

**Monitoring.** Forty-three ovitraps and 15 BG-Sentinel adult traps (Biogents) were used in and around the release area; an equivalent untreated control site, Bodden Town, was also monitored (no mosquitoes were released in Bodden Town). Ovitraps were serviced weekly, BG Sentinels weekly or daily at different stages of the experiment. Adult trapping continued until Dec. 18 with nine traps, then at a reduced level (5–6 traps) into January 2010; ovitrapping continued into January 2010. The original intention was to trap until no fluorescent larvae were detected for two consecutive weeks (last positive ovipot recovered Dec. 21), however, ovitrap monitoring was extended to provide additional baseline data for any potential further trial at the same site. Eggs from ovitraps were hatched under vacuum; the resulting larvae were scored for red fluorescence indicating the presence of the DsRed2 marker[7,37,38] (Clontech Laboratories), using a Leica MZ10F epifluorescence microscope.

**PCR analysis.** For larval genotyping, genomic DNA was extracted from larvae using the Machery-Nagel Nucleospin kit according to the manufacturer's instructions. PCR was performed with Dreamtaq polymerase (Fermentas), using primers WT1 (GAAATCCCCTAGTAAAATTCGCGGAGAAATTC) and IRVI (CGTCATTTTGACTCACGCGGTCGTTATAGTTC) to detect the OX513A insertion and WT1 and WT2(CCAAGCGTTCTAACGATATTTTCAGCGTTC) or altWTF2(CTGCATTTGGTCCCTCGGTAGTG) and 513ins5R(CTTAGACCGATAAAGAAGTGTAAATAGAGCATG) to detect the WT alleles, with a Touchdown PCR program, annealing temperature from 60 °C to 55 °C. For adult genotyping, PCR reactions carried out in a volume of 25 µl with final concentrations of 2 mM $MgCl_2$, 0.2 mM each dNTPs, 0.5 µM each primers (DrosF - ATGAGCAATTAGCATGAACGTT and HspdiagR - GCAGATTGTTTAGCTTGTTCAGC) 2.5 units *Taq* polymerase (Kapa Biosystems) with a Touchdown PCR program, annealing temperature from 55 °C to 50 °C.

**Statistical analysis.** The proportion of adult males and larvae that were OX513A was estimated simply as the number of individuals found to be OX513A divided by the total number of individuals tested. The field competitiveness ($C$) was estimated by dividing the proportion of larvae found to be OX513A by the proportion of adult males found to be OX513A. Two bootstrap methods were examined for obtaining the 95% confidence intervals. The first was to resample individual mosquitoes with replacement and then report the 2.5 and 97.5 percentiles of the estimates obtained from 10,000 such bootstrap samples. The second was to resample traps (8 adult traps and 26 ovitraps caught at least one specimen) with replacement and similarly report the 2.5 and 97.5 percentiles of the estimates obtained from 10,000 such bootstrap samples. The latter would robustly reflect any extra-binomial variation in the data due to clustering of OX513A individuals within traps, as might be expected because female mosquitoes typically lay at one time batches of eggs of variable number. Fisher's exact tests were used to test for clustering of OX513A individuals within traps.


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doi:10.1038/nbt.2019