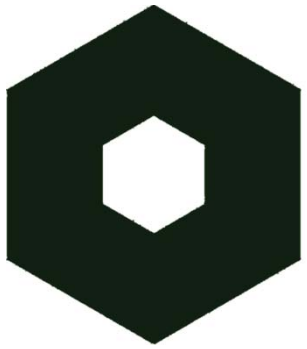


OXITEC
www.oxitec.com

RIDL; What Is It? How Does It Work? Does It Work? And What's In The Future…?

Dr Derric Nimmo

Background: Oxitec Limited

- Developing environment-friendly technology to control insect pests since 2002
  - Agriculture
  - Human Health
- Building on innovative research from Oxford University
- International recognition with a $ 5 million grant from the Gates Foundation Grand Challenges for Global Health Initiative

Male medfly

Aedes albopictus

What is RIDL?

RIDL® Release of Insects with Dominant Lethality

- Innovative and environment-friendly insect control technology
- Genetic enhancement of the Sterile Insect Technique (SIT)
- Targeting insect pests that are damaging to agriculture and human health worldwide

Pest control using GM insects

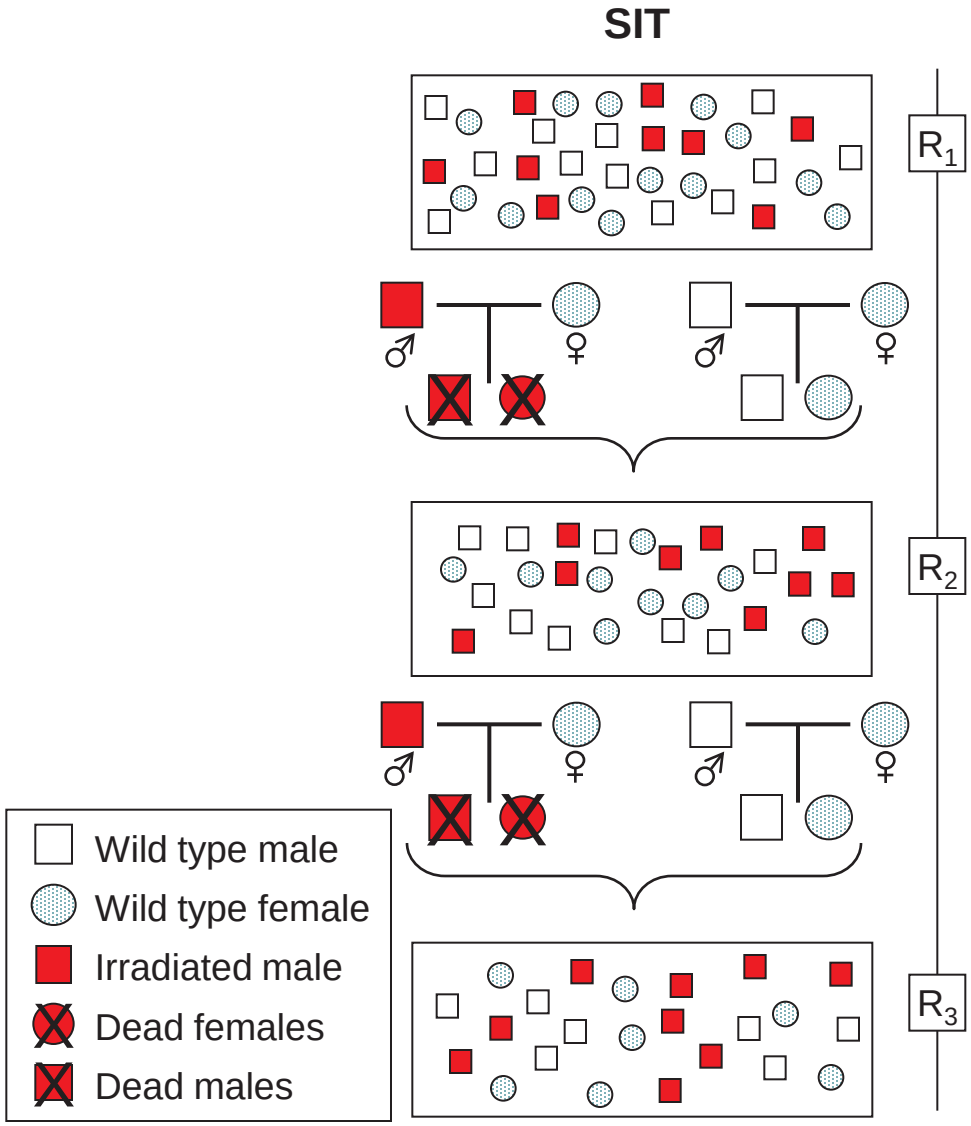
- Population suppression
  Goal: reduce numerical size of pest population

- “Population replacement” or
  “Refractory insect strategy”

  Goal: change pest population to less harmful form

Sterile Insect Technique (SIT)

- Rear millions of insects
- Sterilize (usually with irradiation)
- Release over wide area
- Native females that mate with sterile males produce non viable eggs
- ***target pest population declines***



Success of SIT

- SIT has been successfully used to eradicate/control;
  - New World Screwworm.
  - Mediterranean fruit fly.
  - Melon fly from Okinawa islands of Japan.
  - Queensland fruit fly in Australia.
  - Mexican fruit fly in Northern Mexico.
  - West Indian fruit fly in Northern Mexico.
  - Codling Moth in Canada.
  - Pink Bollworm in California.

Background: SIT and mosquitoes

- >20 trials in 1970's – 1980's

  [Reviewed by Mark Benedict and Alan Robinson (2003) *Trends Parasitol.* 19:349]

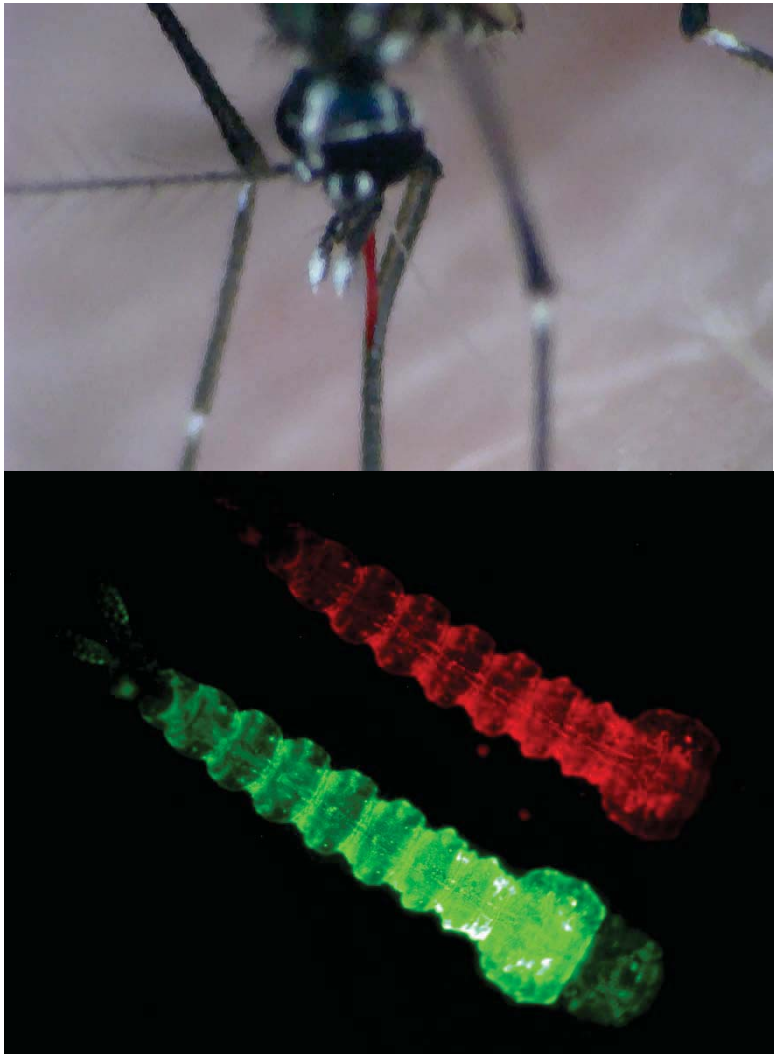
  - Variable success

  - Some problems revealed:

    - Immigration of pre-mated females
    - Poor competitiveness of irradiated/chemosterilised males
    - Breakdown and semi-sterility of strains using translocations or other chromosomal rearangments.

A Genetic Solution for mosquito control

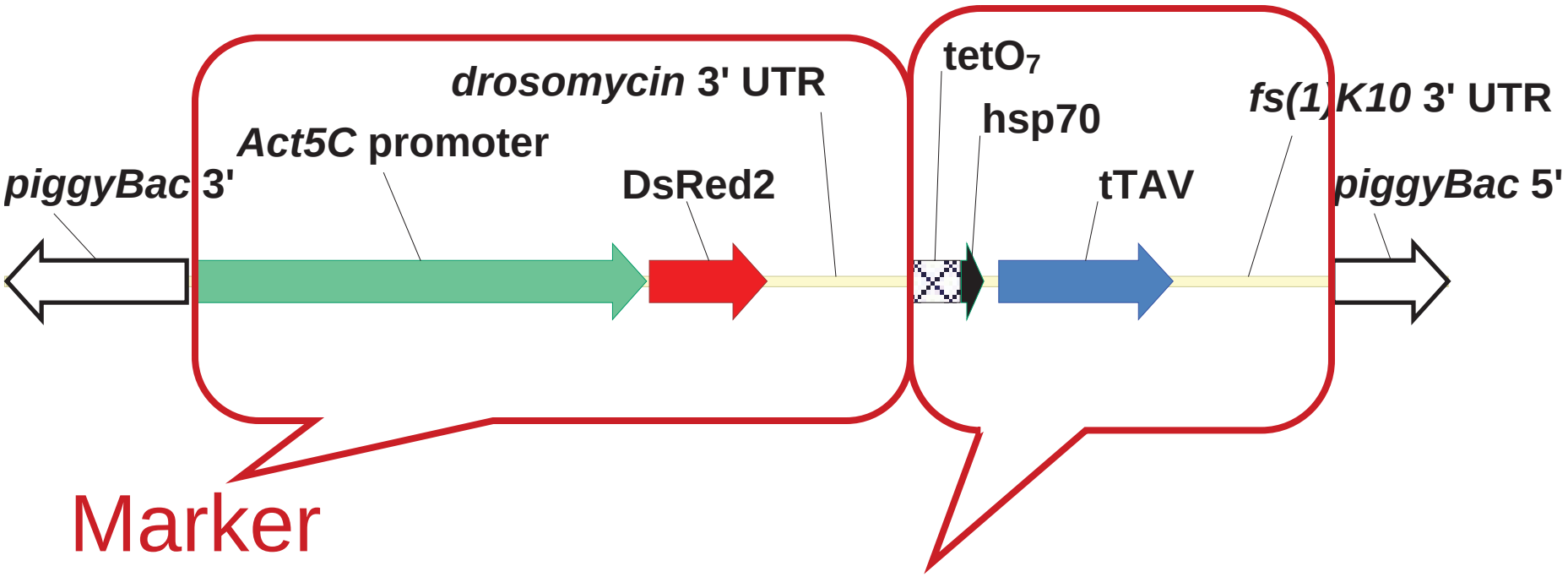
- Based on Sterile Insect Technique
- Uses modern molecular biology techniques to insert a lethal gene into insects
- Sterile transgenic insects can be used to control a range of pests.



"birth control for mosquitoes"

How does it work?

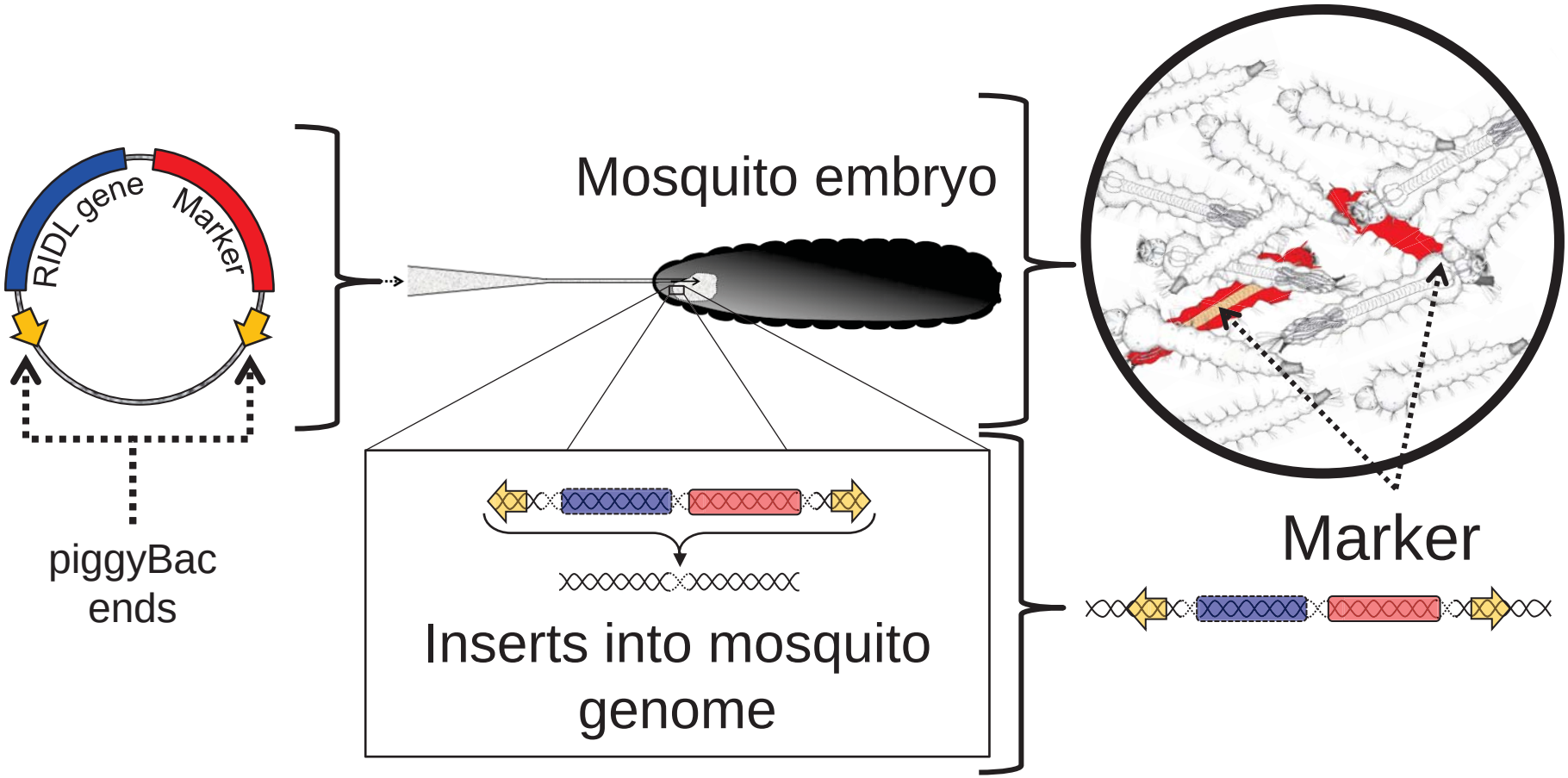
OX513 – structure



Phuc *et al.* 2007 BMC Biology

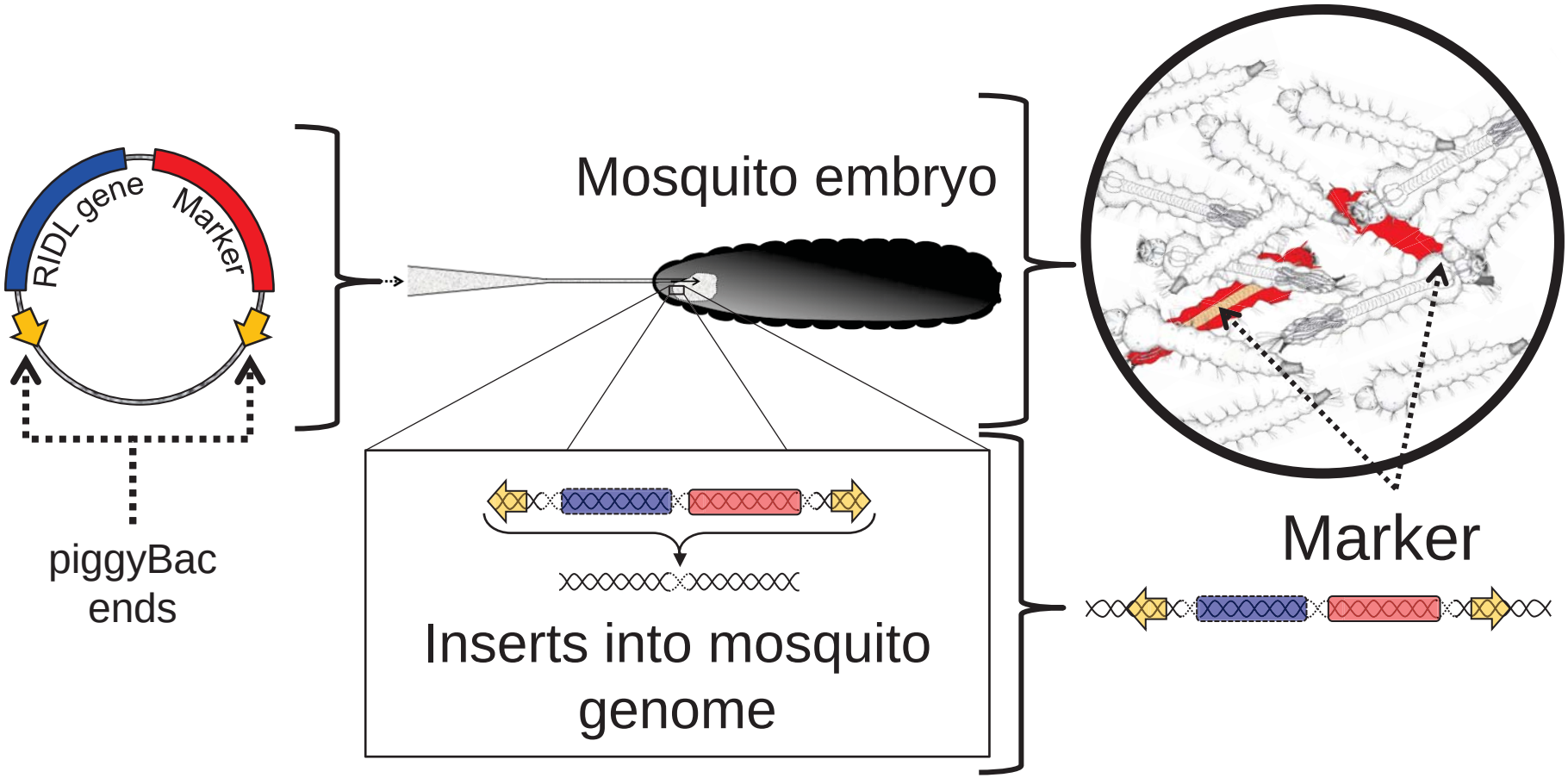
Microinjection

- Produce RIDL line

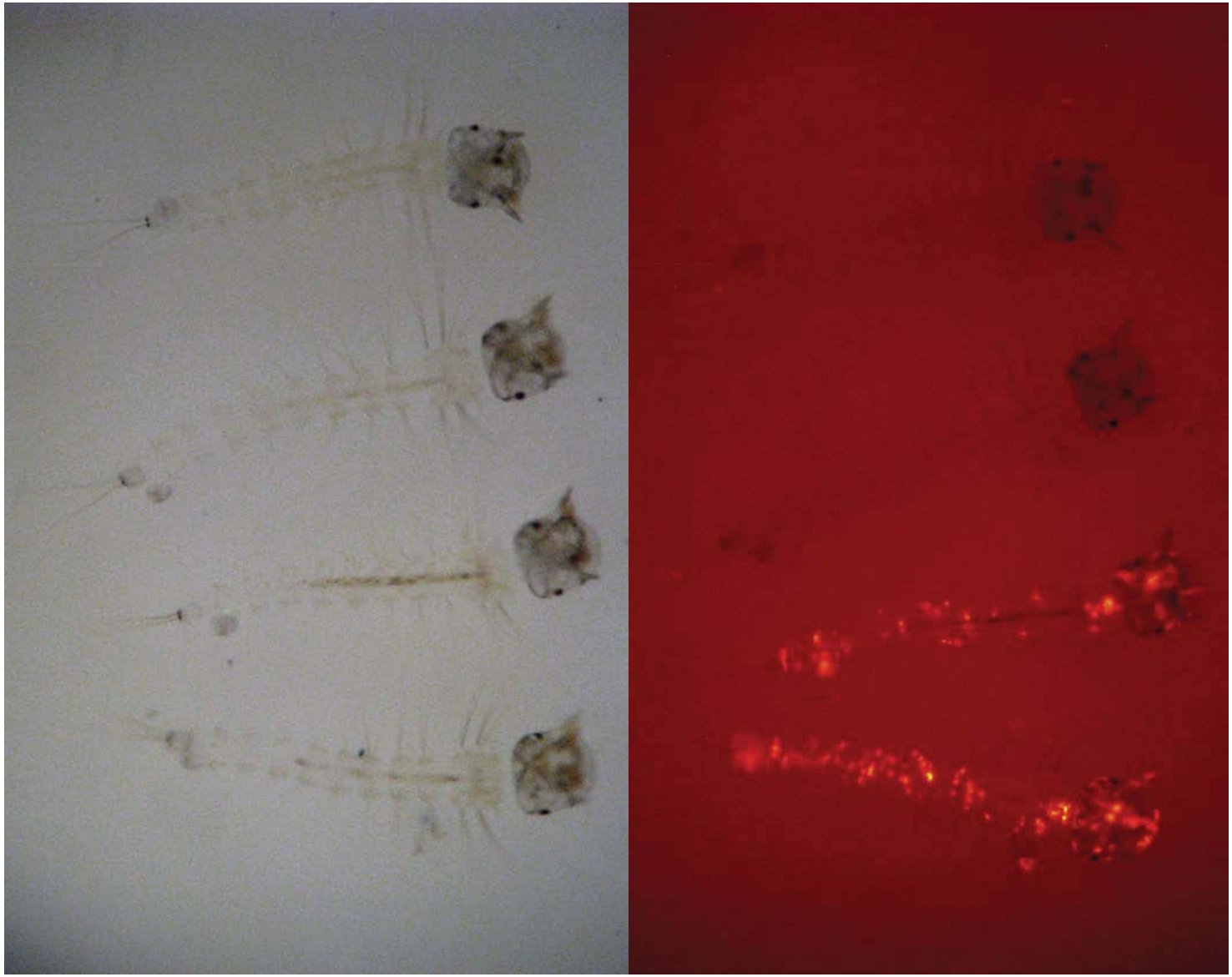


Microinjection

- Produce RIDL line

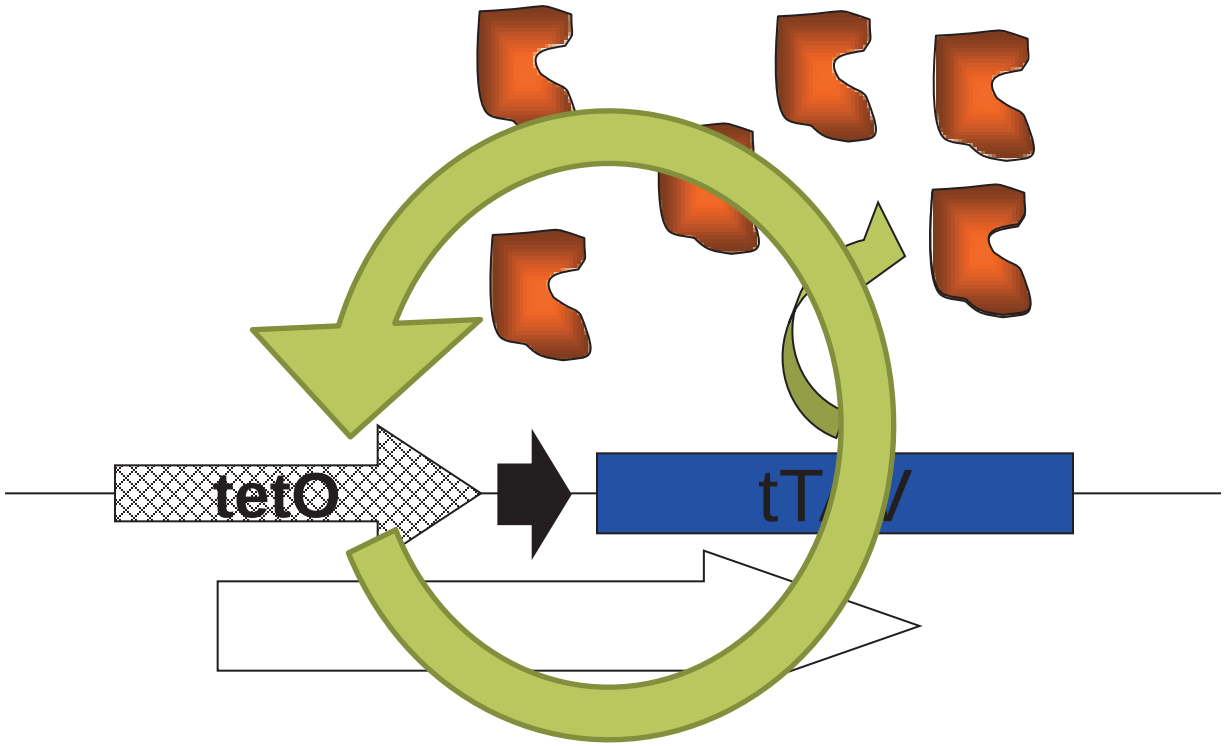


OX513A – Fluorescent marker



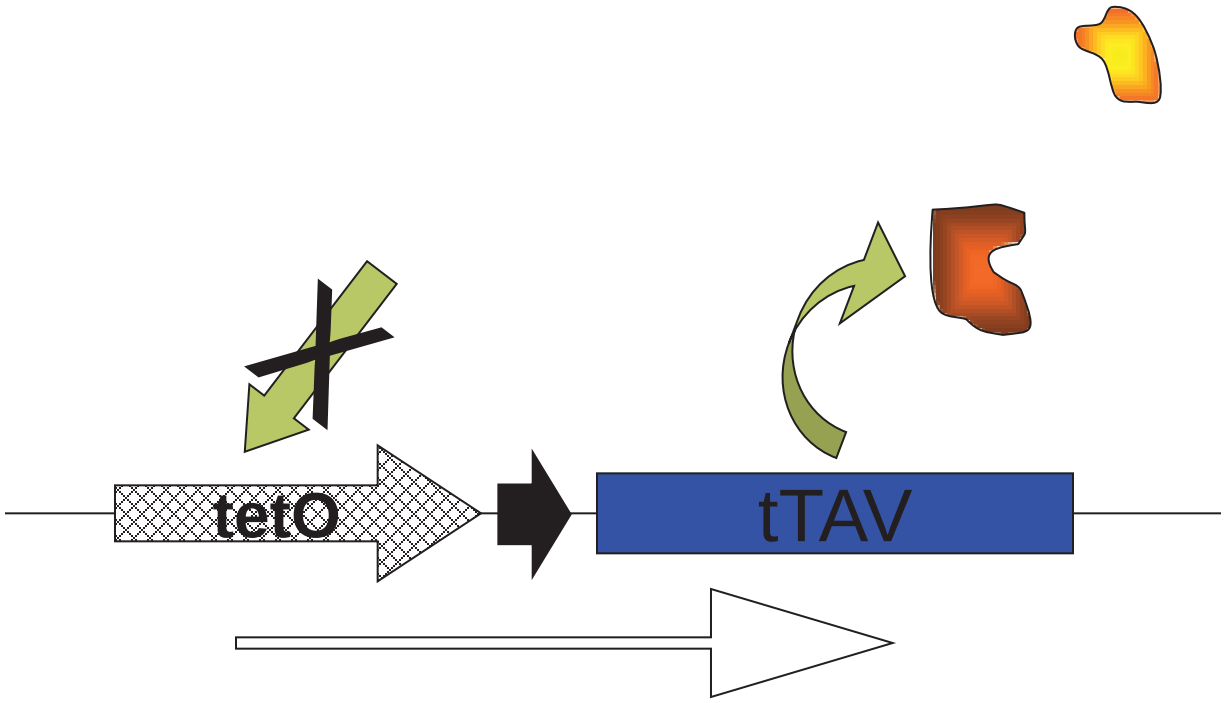
RIDL gene function

- Bi-sex RIDL is based on positive feedback system;



RIDL gene control

- Bi-sex RIDL is based on positive feedback system;



RIDL; summary of mode of action

- Accumulation of tTA in the cells causes insect death (transcriptional squelching): tTA is a **lethal** gene
- The accumulation of tTA is repressed by the presence of tetracycline: the lethality is **conditional**
- One copy of the tTA gene in the genome is enough to kill the insect: the lethal gene is **dominant**

Current tools for vector control

approach	required citizen compliance	advantages	disadvantages	best fit
Fogging/space spraying	high	quick kill	• contact required • human exposure • ineffective	• rapid response • small location
Larvicides	high	duration (weeks)	• impossible to treat all breeding sites • drinking water contamination	• programmatic activity • pre season
Lethal ovitraps	high	home treatment	• cost • replenishment • efficacy issues	• unproven
Aerosols, mats, coils, treated fabrics	high	in door use	• individual	• top up

High public compliance reliance + transient effect + 'contact cost' + insecticide resistance = global failure to arrest spread of dengue

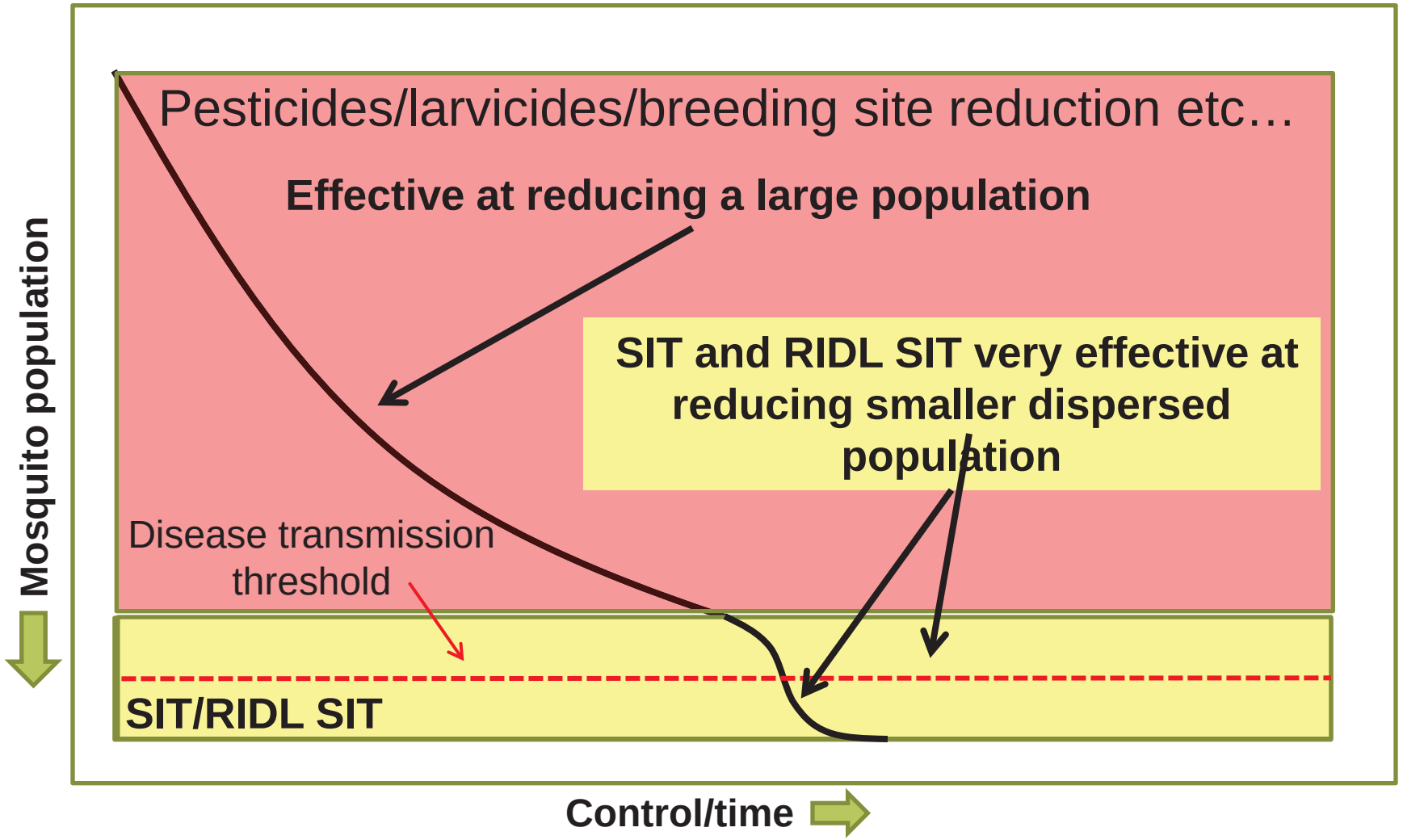
Traditional approaches

Fogging – areas Missed by vehicle

traditional approaches

Larviciding or placing lethal ovitraps by hand

Integrated Vector Control Strategy



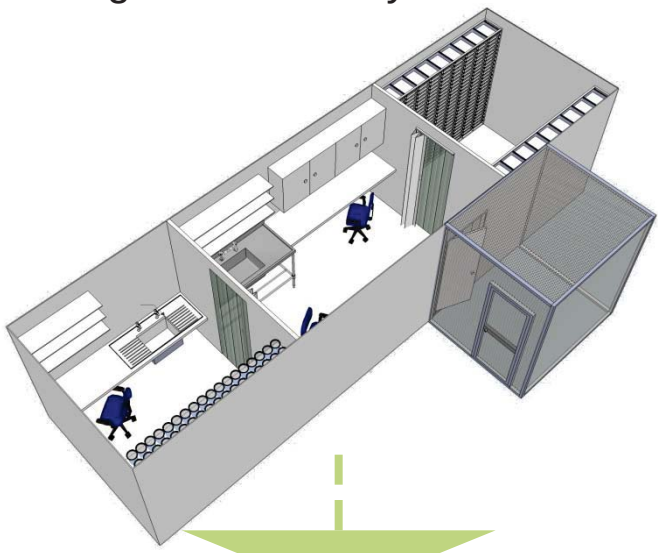
Trial process

Filter Colony (Oxitec UK)

- Hybrid strain
- High Quality control
- Fitness
- Genetic diversity
- Longevity

Eggs sent to field site

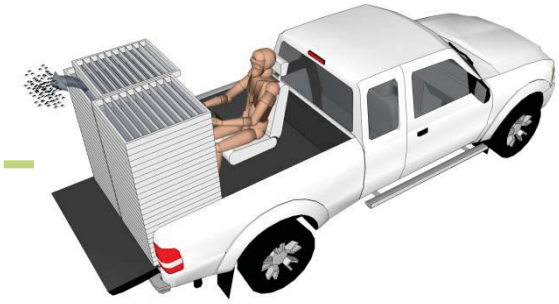
Rearing cabin / factory



Sex-sort pupae

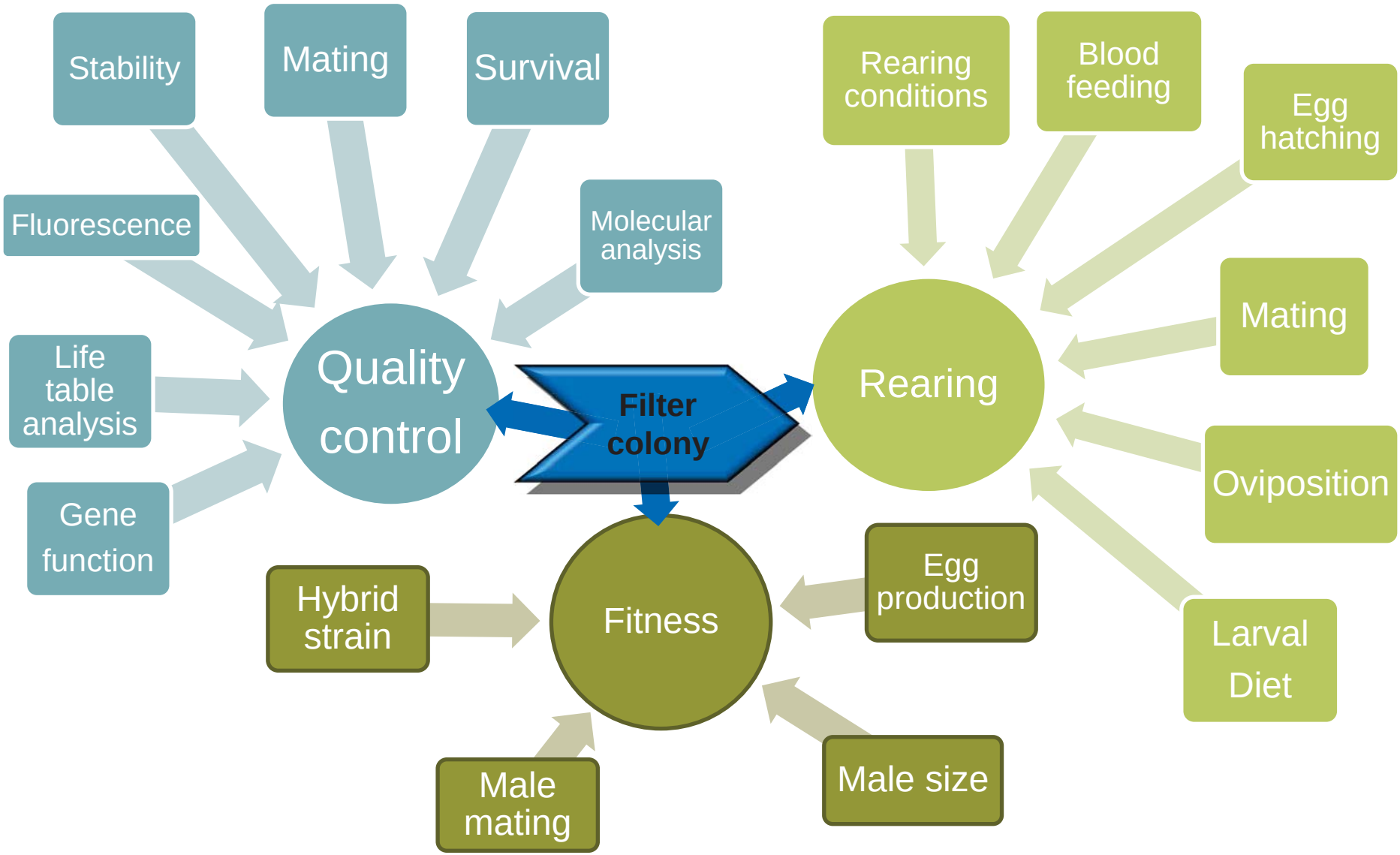
Males for release

Release by vehicle /hand

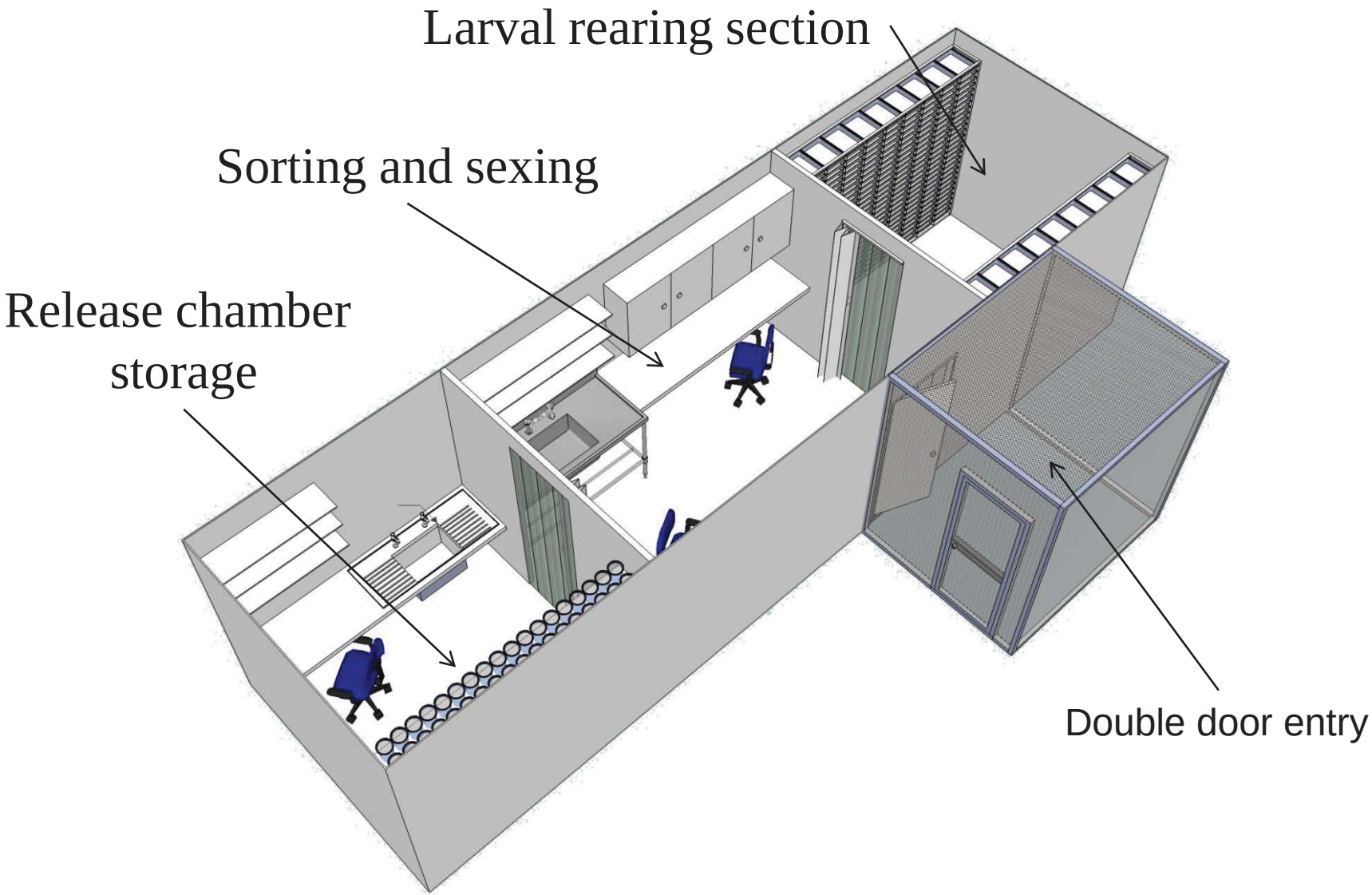


Monitoring

Filter colony UK - overview



Rearing facility



2010 suppression trial; methods

REARING

Mass rear RIDL strain

>2 million eggs a week

Rear release generation

240 trays a week

Separate male pupae

150,000 males a week

Place males in release devices

480 release devices a week

(3 people)

DISTRIBUTION

Release adult males 3x a week

3.3 million males released

MONITORING

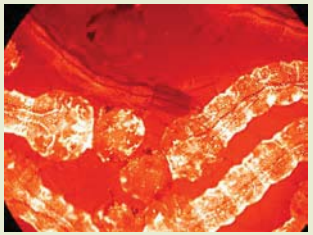
Eggs

Ovitraps

Adults

BG sentinels

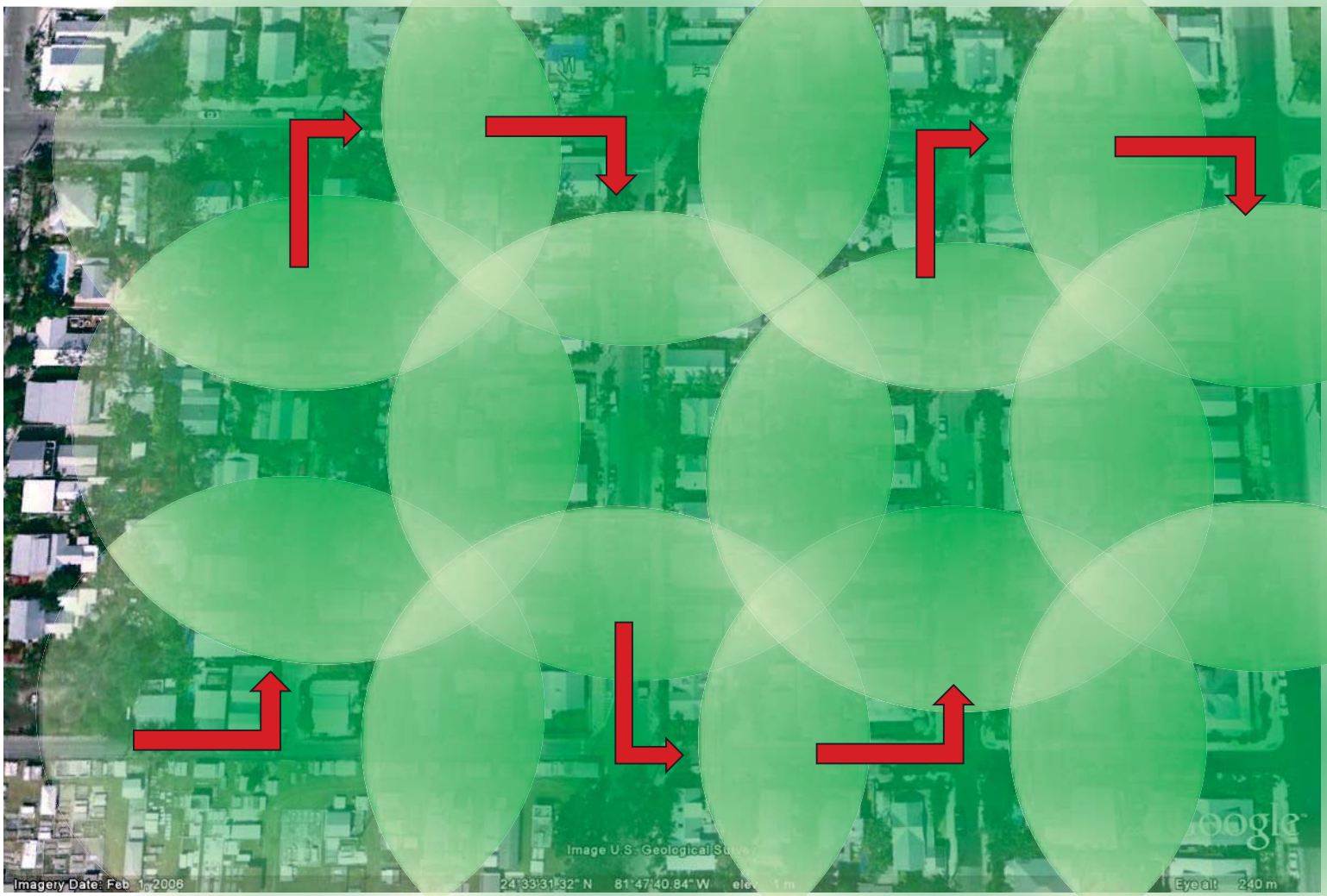
Mating



Fluorescence

(2 people)

Trial process: Distribution



RIDL points of release by vehicle

Trial process: Monitoring

- Monitoring
  - Monitor using ovitraps (200) and BG traps (40)

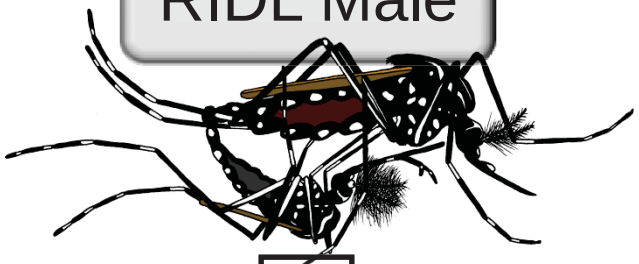
What happens after release?

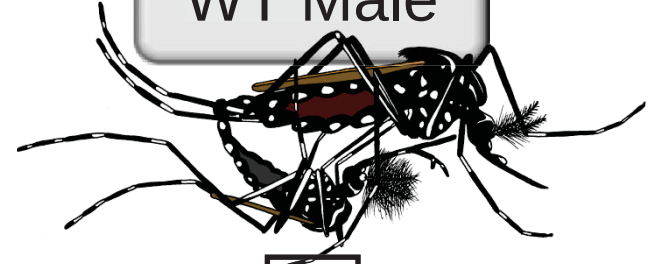
WT female mates:

RIDL Male

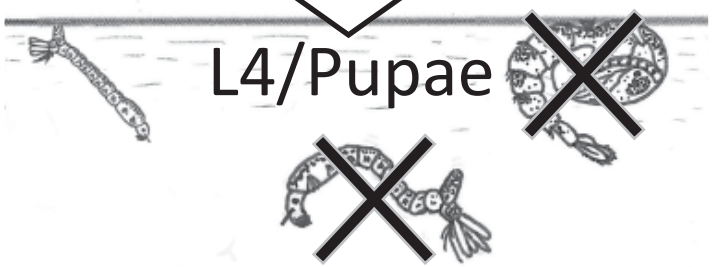
or

WT Male





L4/Pupae



(No Tetracycline)

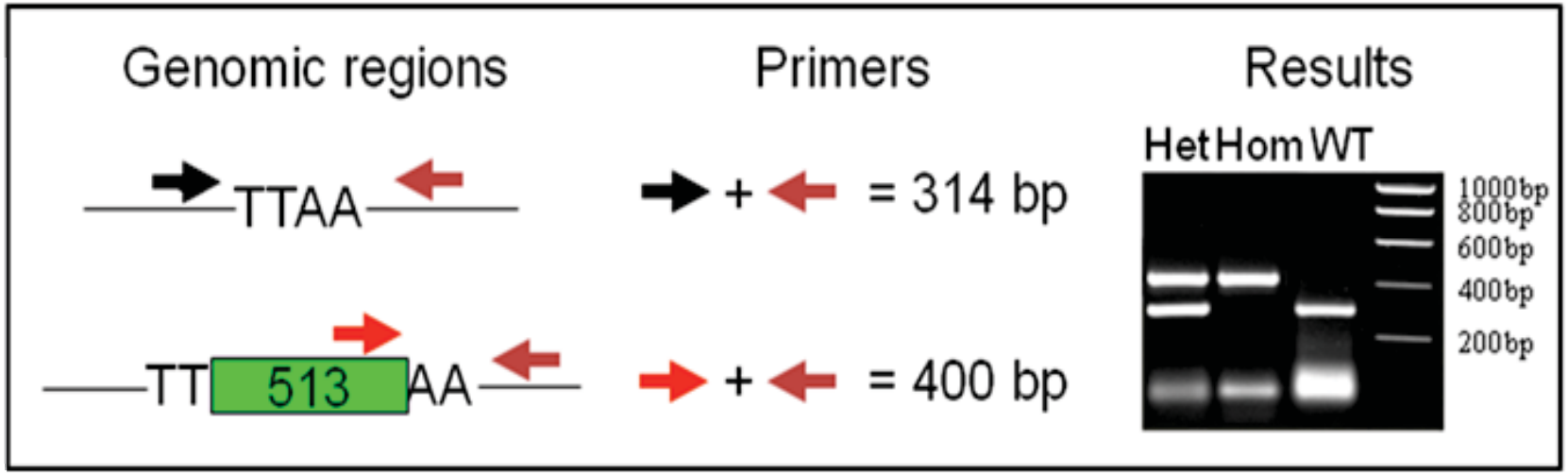
Does it work?

Strain testing

- Several strains tested for the following;
  - Molecular characterisation
  - Phenotype
  - Mating competitiveness
  - Life table parameters
  - Gene stability
  - Insecticide resistance
  - Effects on predators
- Field testing
  - Cayman
  - Brazil

OX513A molecular characterisation

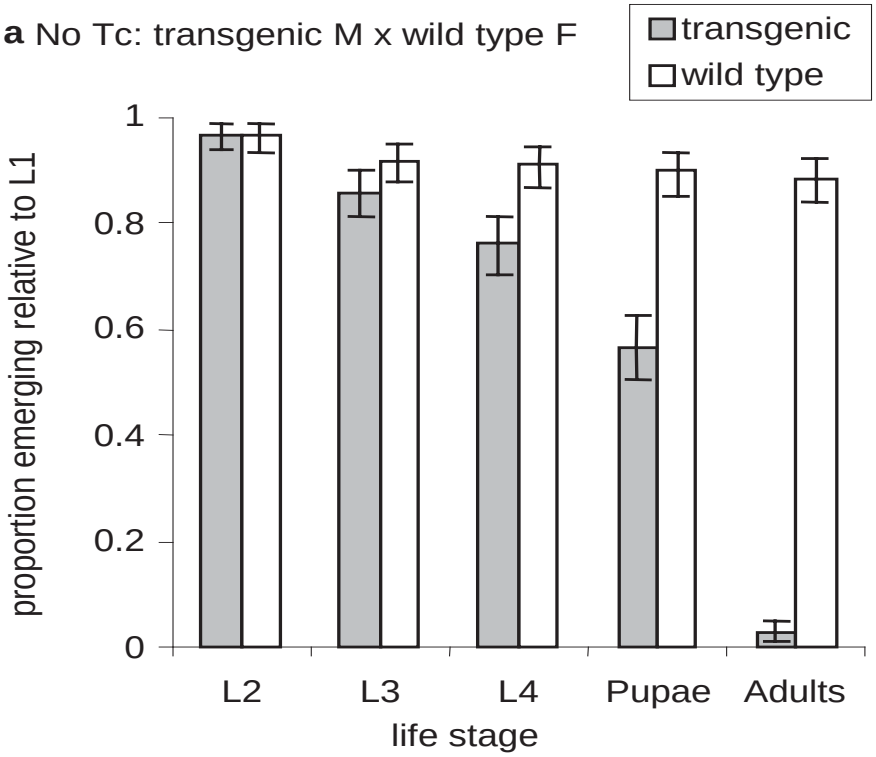
- OX513A was created in 2002, injected into a Rockefeller background
- Single integration event (Phenotypic and molecular analysis)

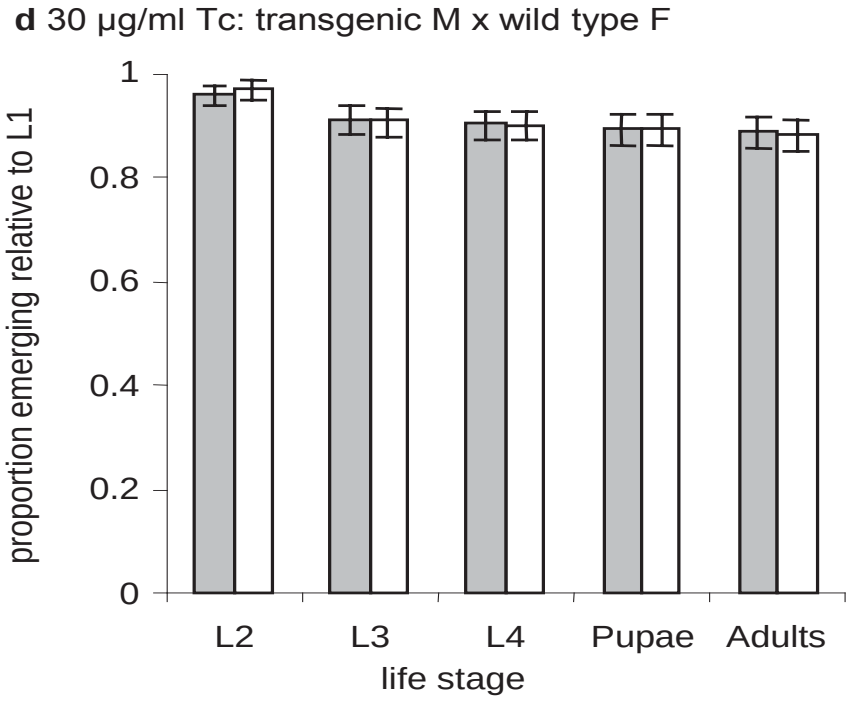


- Insertion has been sequenced

OX513A Aae phenotypic analysis

- Penetrance of RIDL in OX513A Aae

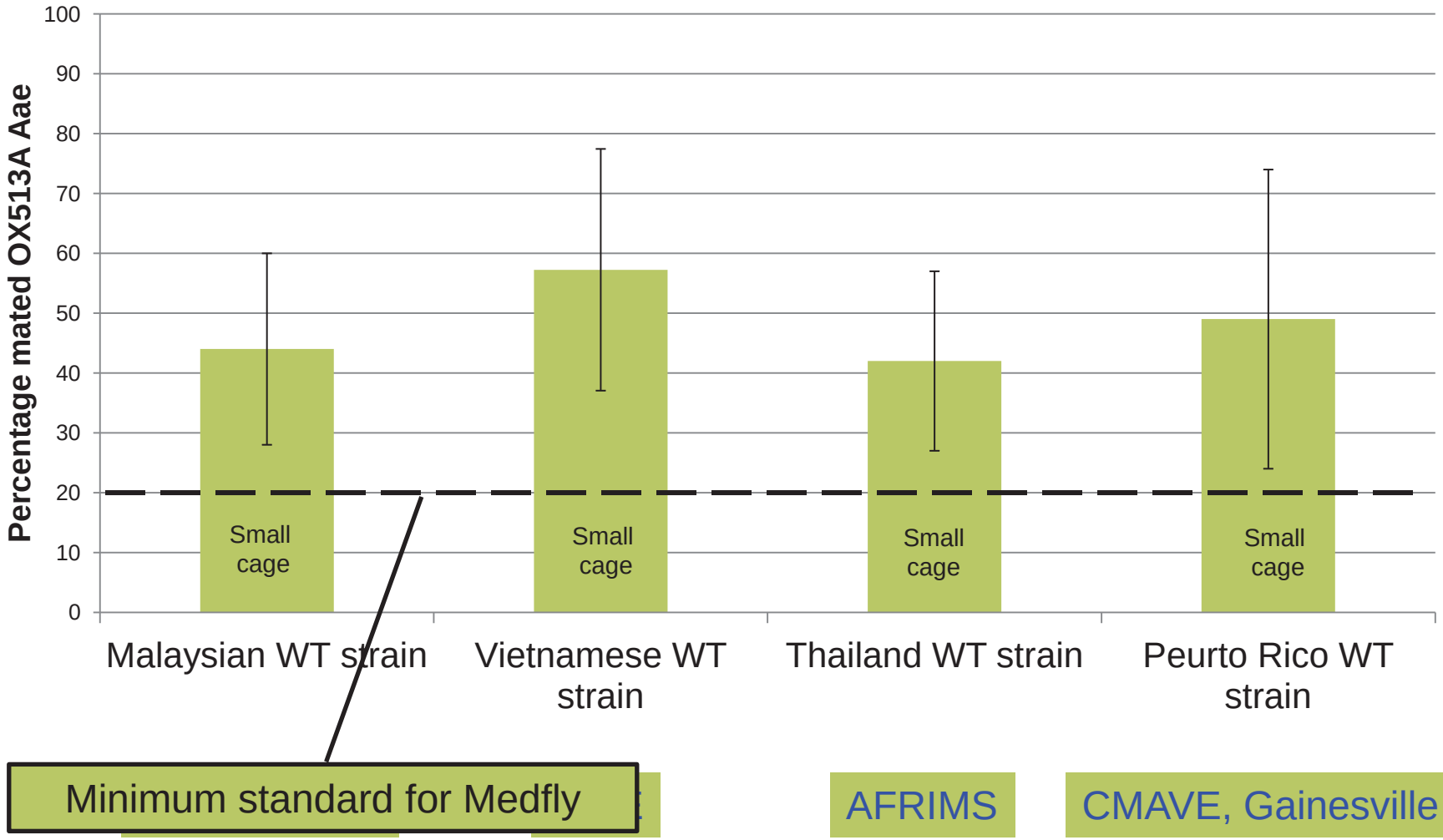




Phuc *et al.* 2007 BMC Biology

Contained mating trials results

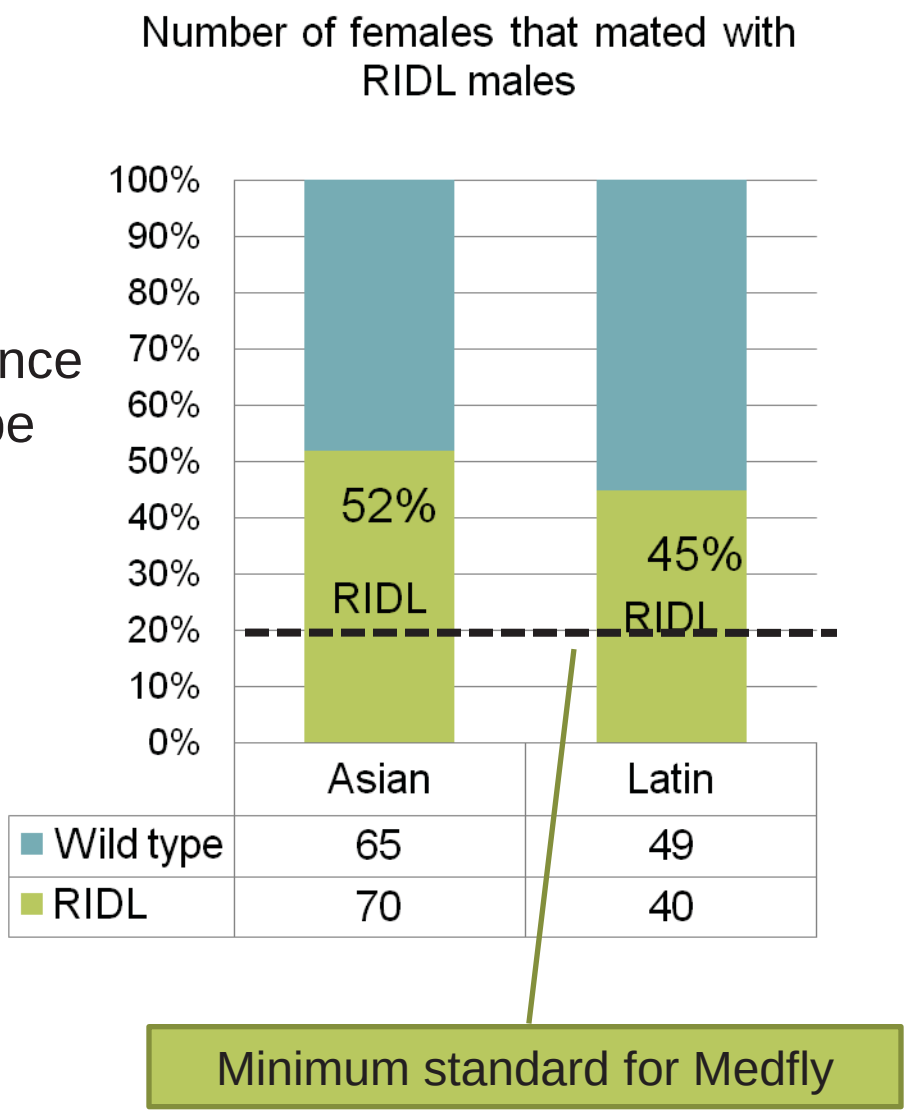
- Percentage mating of OX513A Aae and different WT strains



Lab & Field House (Contained) Testing Results

- **Laboratory;** No significant difference between OX513A and control wild type strain in all life history parameters

- **Field House;** No statistical difference in mate selection between wild type and RIDL of either background



OX513A Life Table comparison to WT

Parameter	Strain	Average	Mean Rank	p-Value
Eggs laid per female	RIDL	74.86 ± 16.94	16.03	**0.740**
	wild type	63.07 ± 6.855	14.97	
Larvae hatched per egg batch.	RIDL	49.86 ± 8.306	17.70	**0.171**
	wild type	31.60 ± 5.237	13.30	
Sterile eggs per egg batch	RIDL	25.00 ± 3.349	14.33	**0.468**
	wild type	31.47 ± 5.57	16.67	
Days spent in L4	RIDL	2.59 ± 0.06	142.84	**0.851**
	wild type	2.57 ± 0.58	141.20	
Days spent at pupal stage	RIDL	2.29 ± 0.042	133.52	**0.141**
	wild type	2.36 ± 0.40	144.99	
Days from hatching to adult	RIDL	7.85 ± 0.071	130.19	**0.200**
	wild type	7.49 ± 0.055	141.28	

Study performed by IMR

OX513A Gene stability

- Stable for over 60 generations as a homozygous line
  - Molecular and marker expression analysis
- Re-exposure to transposase does not cause movement at detectable levels
- Penetrance of phenotype unchanged

OX513A strain background

- Insecticide resistance of OX513A Aae Latin and Latin strain background has been tested

Insecticide	Latin background strain	OX513A Aae Latin strain	Resistant
Temephos	95%	100%	NO
Deltamethrin	99%	100%	NO
Permethrin	100%	100%	NO
Bendiocarb	73%	47%	YES
Malathion	100%	100%	NO

- Bendiocarb no longer used in US (too toxic)
- No kdr resistant alleles were found;
  - (Tetraplex (1534), Hola (Val1016Ile), F1534C and V1016G tested)

OX513A Aae effects on predators

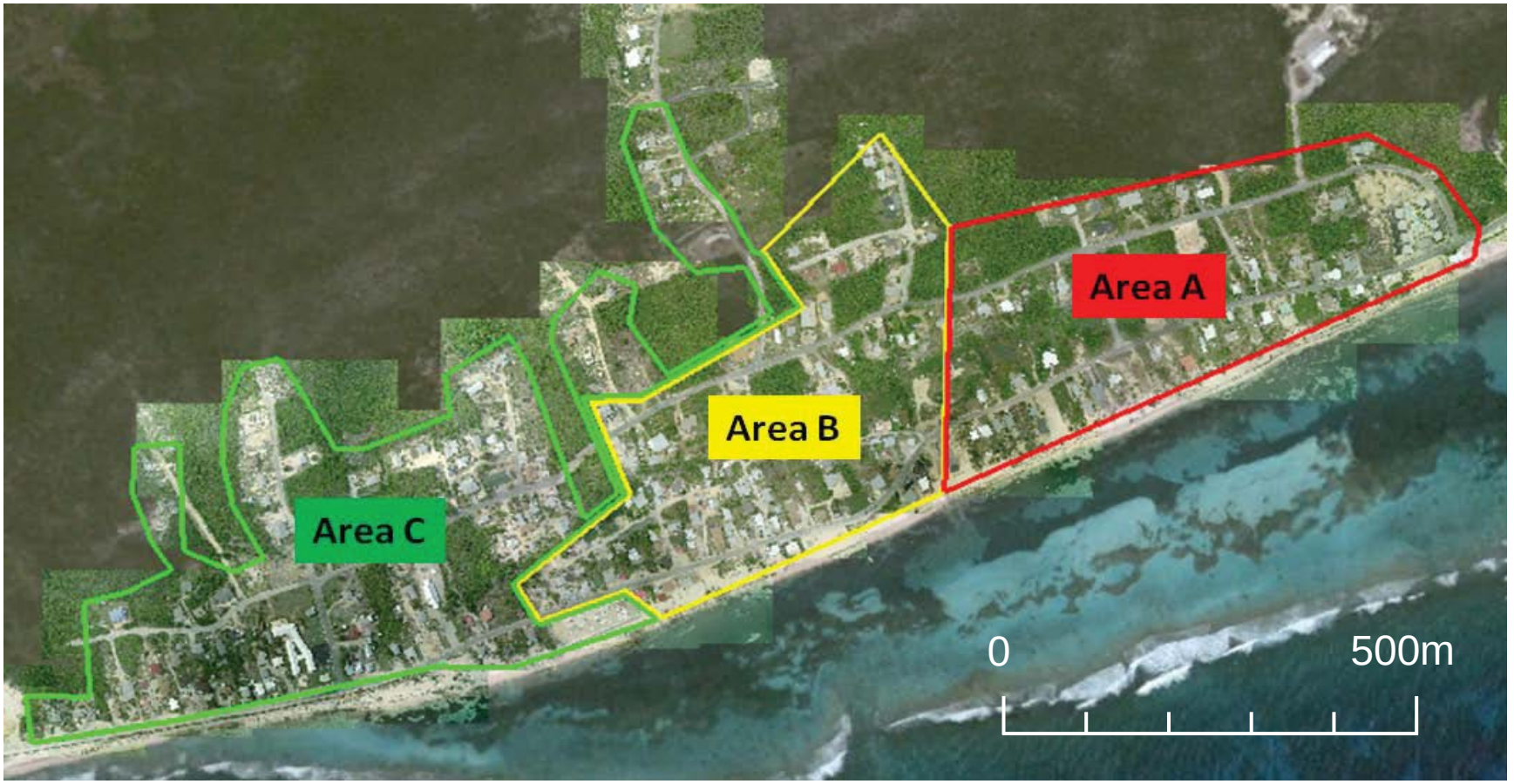
- Toxorhynchites; predatory species, larvae feed exclusively on other mosquito larvae
- Fed two different species of Toxorhynchites on OX513A Aae and WT larvae
- Found no significant difference in any of the following;
  - Larval development
  - Lifespan
  - Size
  - Fecundity
  - Survival of any life stage
- Study conducted by IMR, Malaysia

2010 Cayman Suppression Trial

Aims of the trial

- Increase in Male : Female ratio
  - Aimed for 10:1, based on SIT and models
- Mating competitiveness of RIDL males
  - Fluorescent genetic marker in larvae hatched from eggs collected in Ovitraps
  - >50%
- Suppression of target mosquito population
  - Ovitrap index
  - Significant and sustained difference between treated and control

Field site (East End)

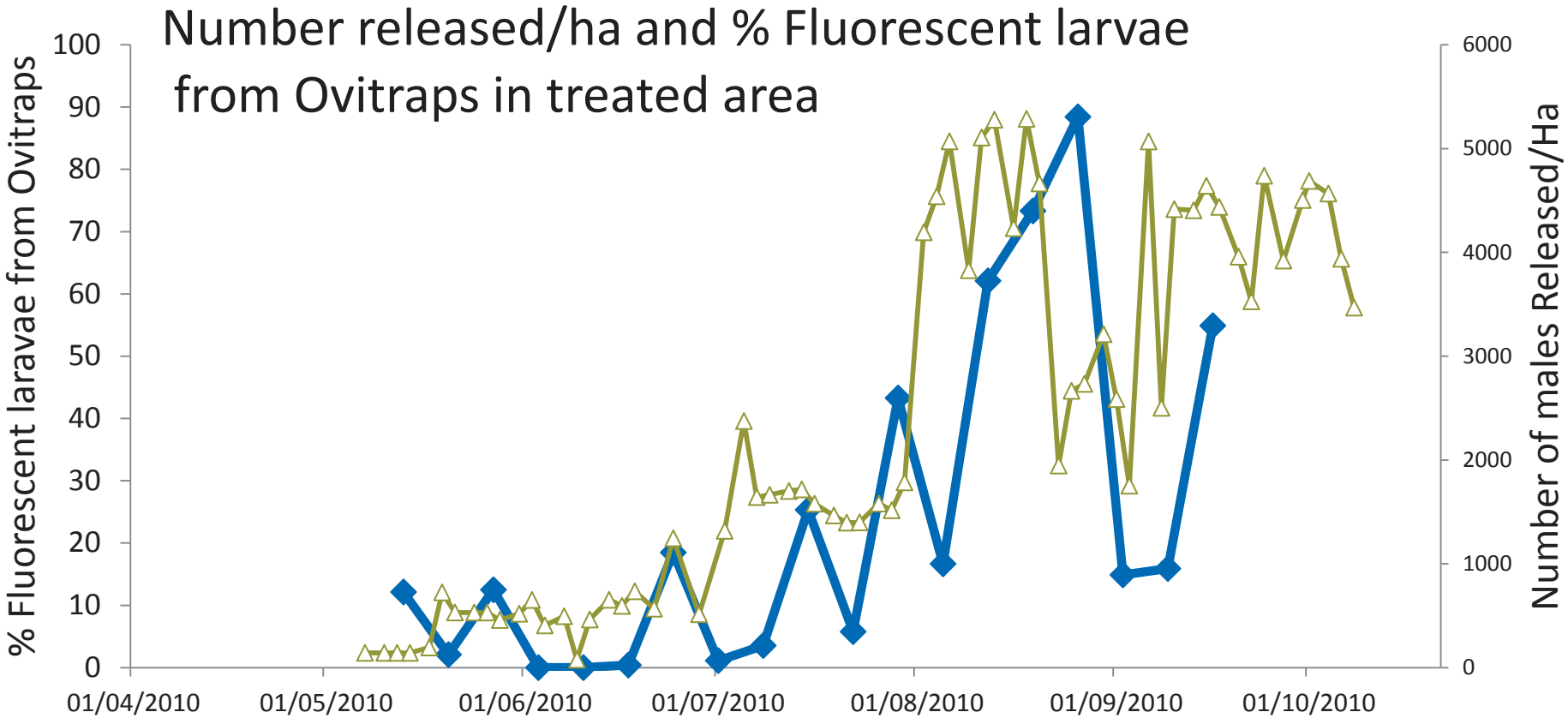


Each Area approx 16 Ha (40 acres)

No conventional control for *Aedes aegypti*

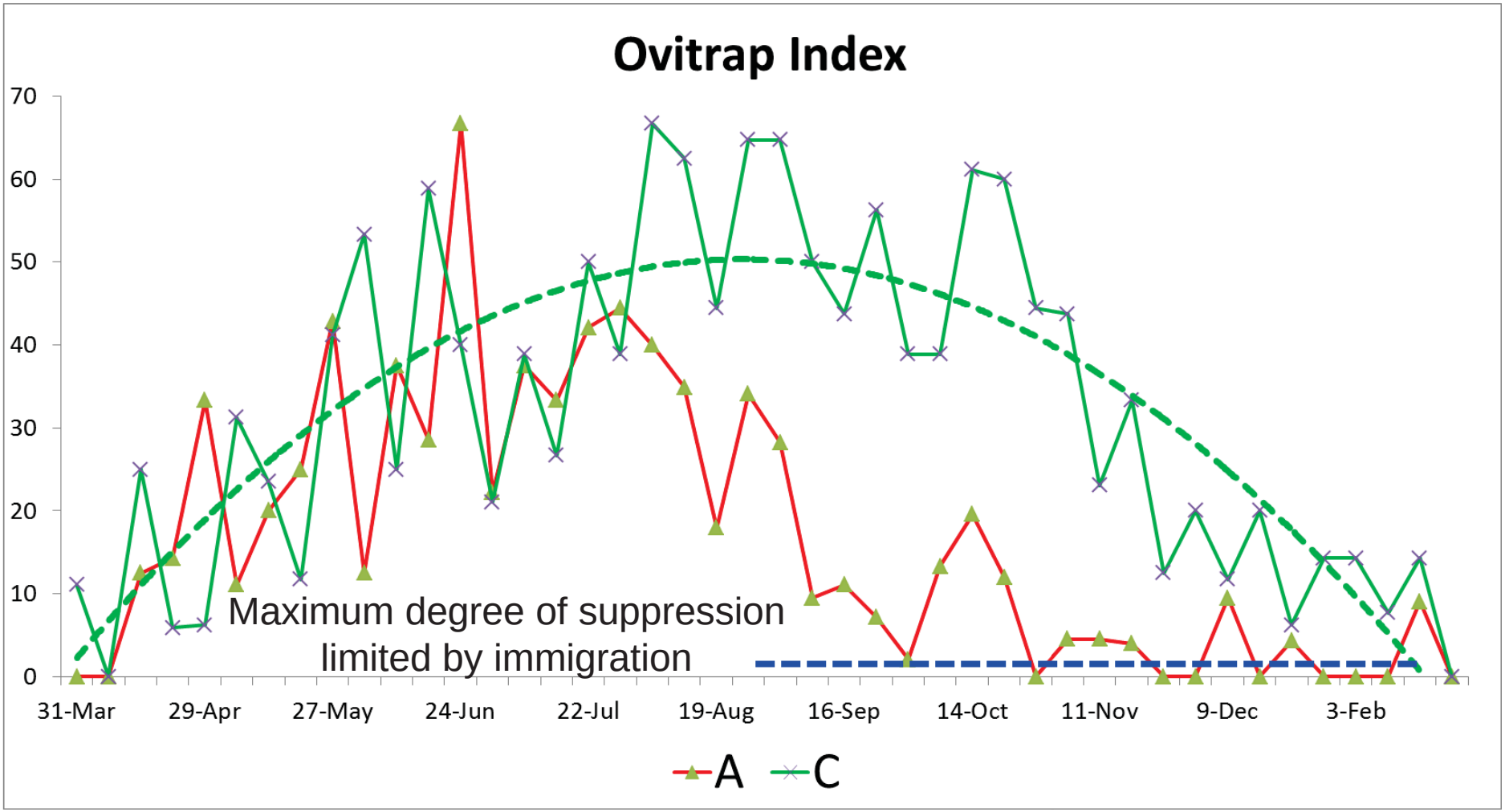
Release period May-October with pre- and post-release monitoring

Results - Fluorescent score



- Fluorescence increases in line with, but lagging, male releases
- Later dates had too few eggs to calculate ratio

Results: Ovitrap index



- Clear suppression from early August

Harris et al. Nat. Biotech. 30(9) 828-30

Cayman field trial summary

- Trial was successful
  - All endpoints successfully met
    - Sterile:wild male ratio in traps >10:1
    - Fluorescence ratio in progeny >50%
    - Population suppression
      - Sterile:wild male ratio 3-4:1 sufficient
- Sustained release of RIDL OX513A males can suppress a field population of *Aedes aegypti* mosquitoes
- GE mosquitoes can perform successfully in the field

2012; results from Brazil

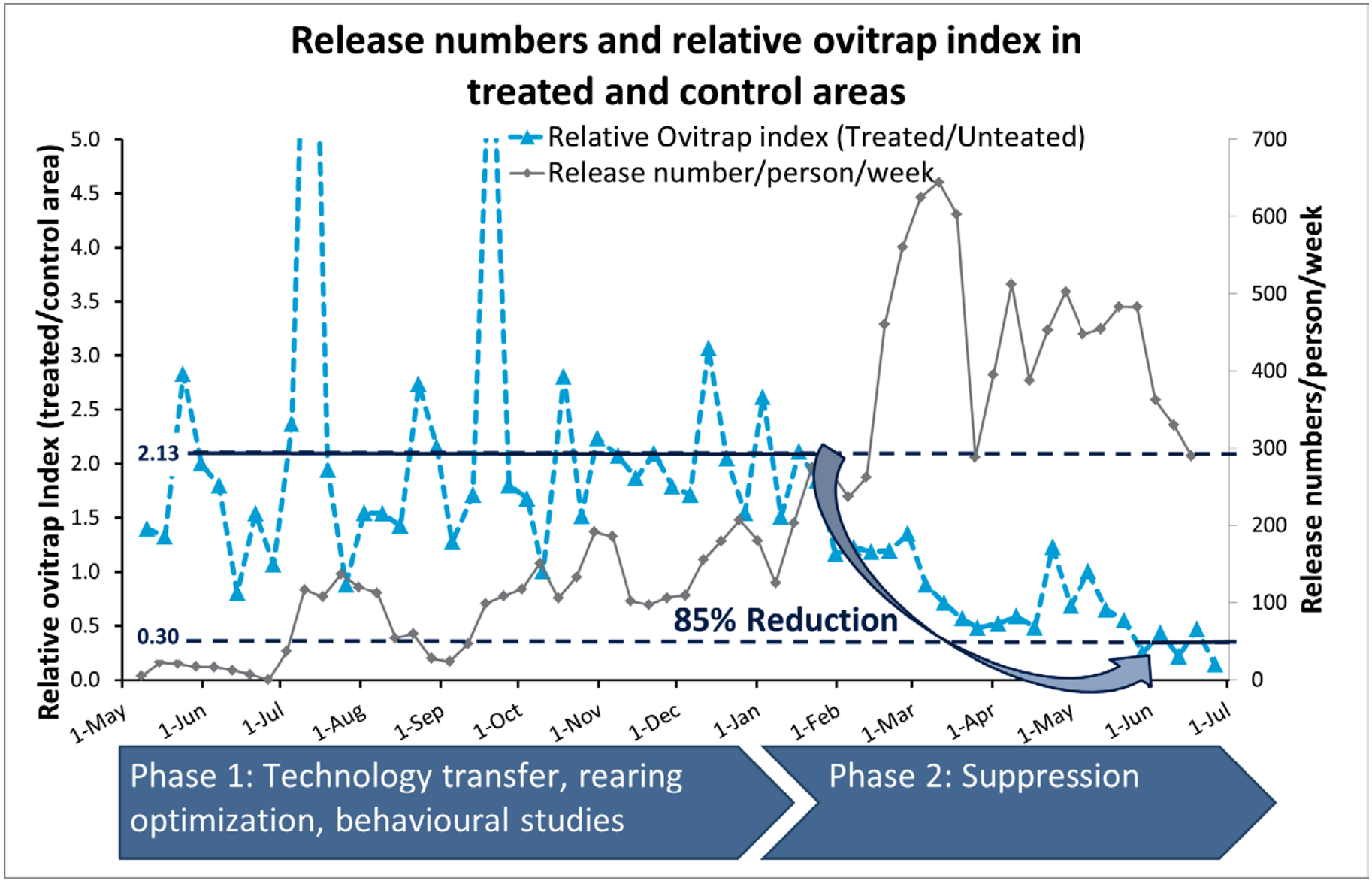
- Brazil is a collaboration with;

- Working in two trial sites for the past year
  - Iteberaba
  - Mandacaru

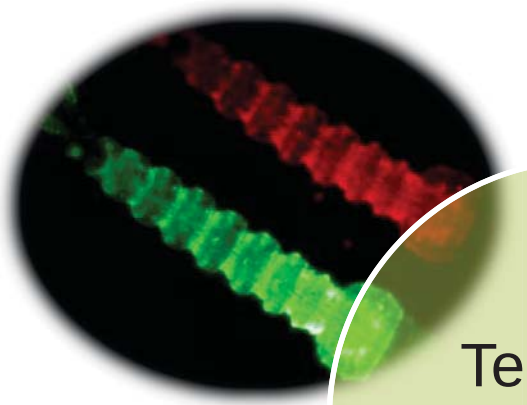
Itaberaba – Field site

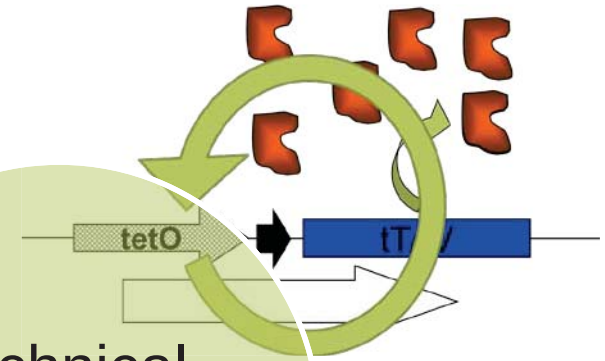
	Control (C)	Treated (T1)	Treated T(2)
Area (Ha)	40	5.5	5.5
Population (Est)	4300	900	900

Results Iteberaba



RIDL® bringing new technology to the field



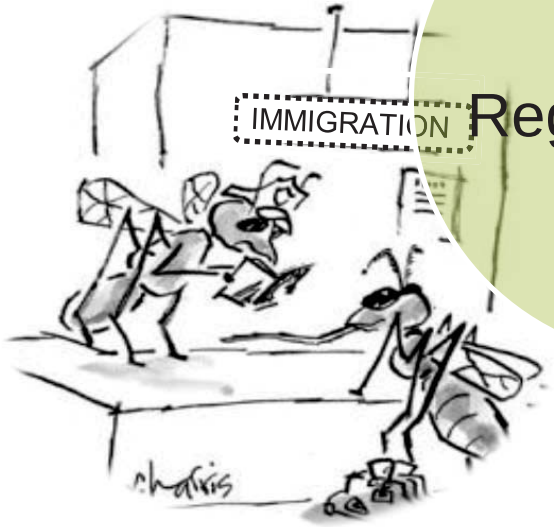


Technical

RIDL

Regulatory

Community



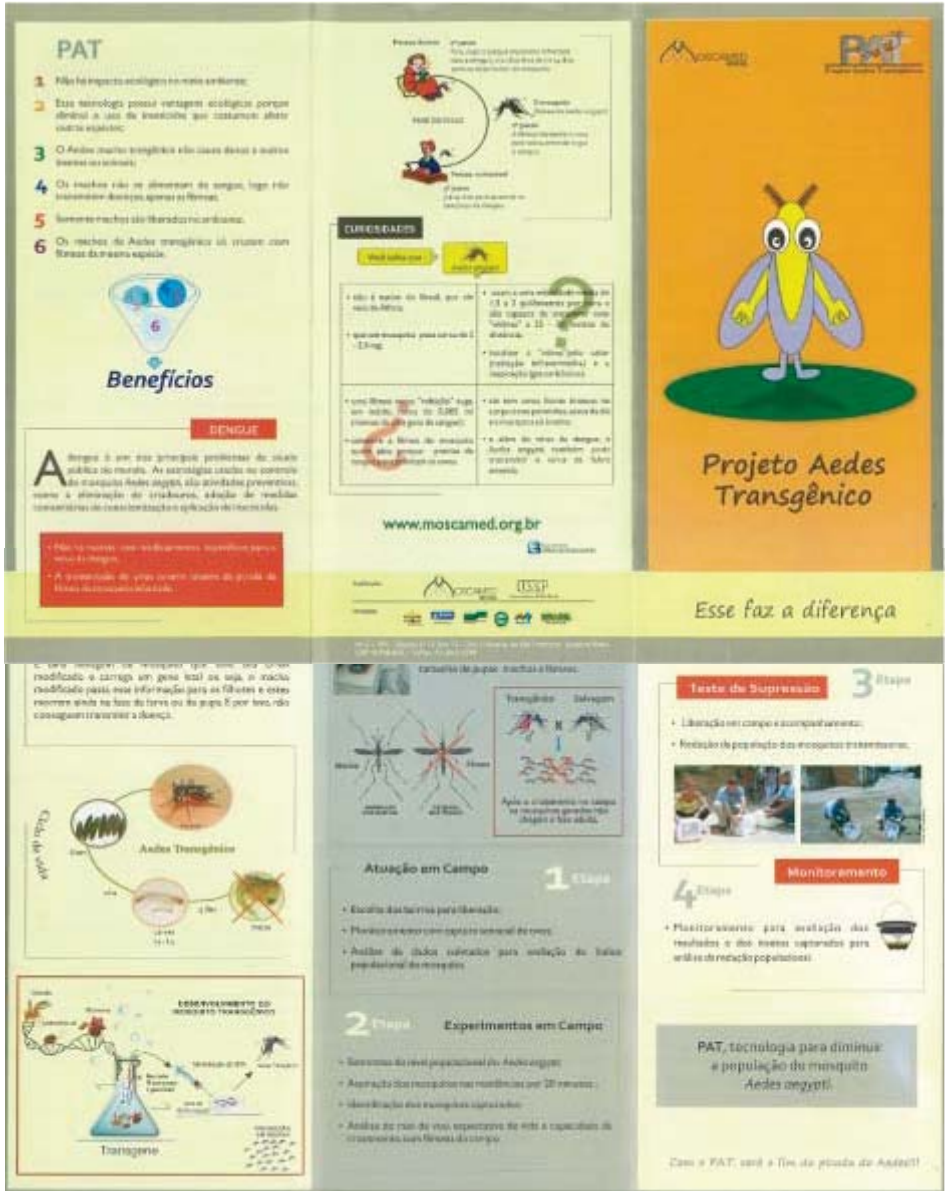
Regulatory process

- Regulatory framework is evolving, with trials approved and conducted in Malaysia, Brazil, Cayman.
- Even authorities with extensive experience in biotechnology regulation have to adapt frameworks for GE insect regulation
  - US process for a trial started in 2008
  - USDA-VS issued letter of no jurisdiction after 18 months deliberation
  - FDA and EPA still discussing who should regulate GE mosquitoes.

Community Engagement

- Community engagement plans are in place for each trial
- Main avenues of community engagement;
  - Radio interviews and local media
  - Public information events; for example public outreach to schools, guilds and hospitals, leaflets
  - Consultation with senators; through the regulatory process and communicating with local senators.
  - Web site information (local mosquito control district)

Community engagement



- Female mosquitoes mate only once
- If a wild female mates with a sterile male then she will not be able to produce offspring
- We are releasing sterile male mosquitoes as part of a new research project
- If many females mate with our sterile males they will produce no offspring and there will be a reduction in the Dengue Mosquito population
- **Male mosquitoes don't bite** – only the females blood feed in order to use the protein to make their eggs

Female Mosquitoes mate just once in their lifetime

Usually this means hundreds or thousands of offspring

For more information Contact.
The Mosquito Research & Control Unit
(345) 949-2837

...but if the Wild Female mates a Sterile Male

Then she has no children!

MOSQUITO

RESEARCH & CONTROL UNIT

We are currently testing a new technology to reduce numbers of the Dengue mosquito (*Aedes aegypti*

MRCU personnel are working in East End on a new project

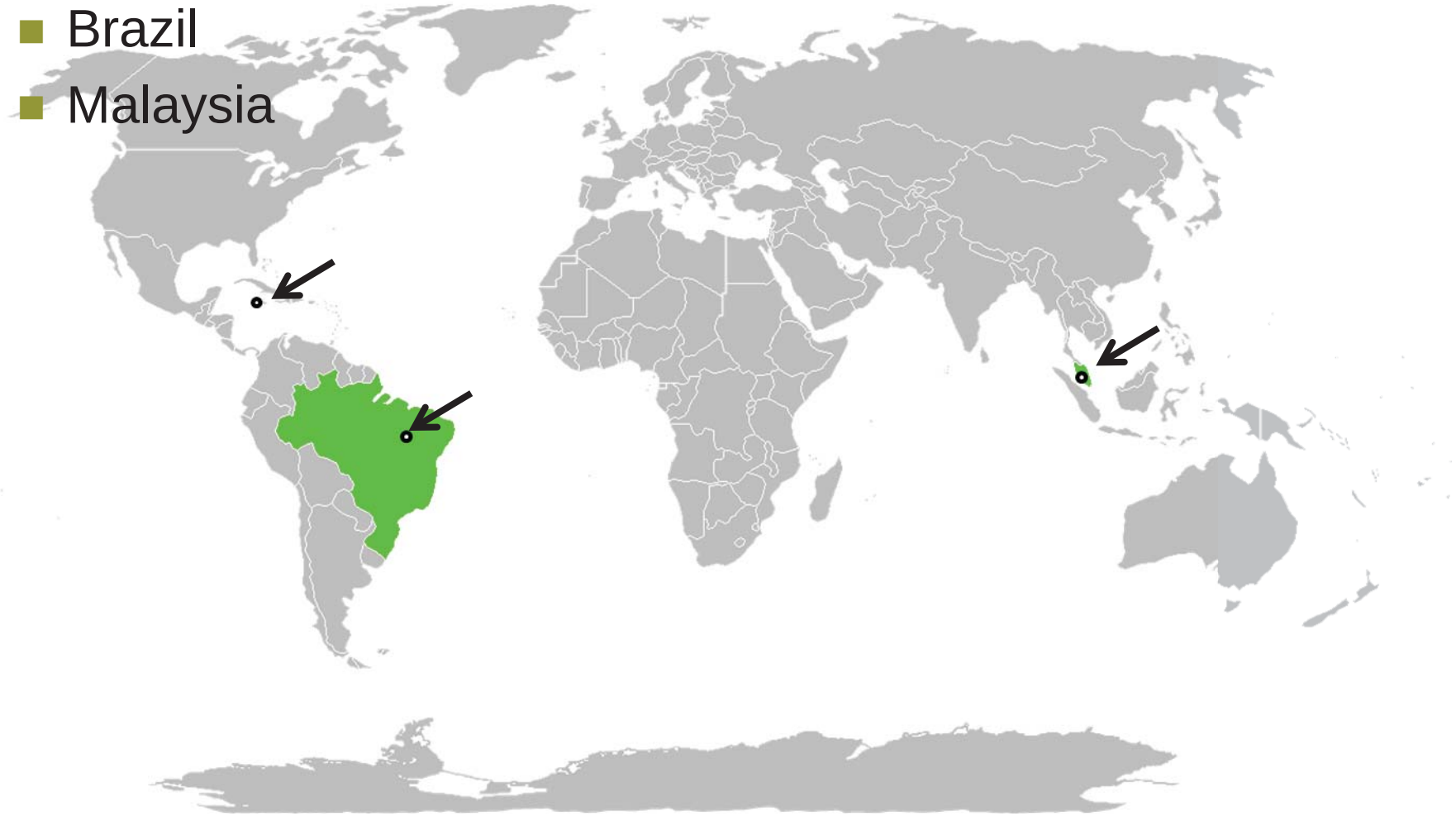
equipment around, we are using these traps to monitor our success with this new project

If you see this equipment please do not disturb it

What’s in the future?

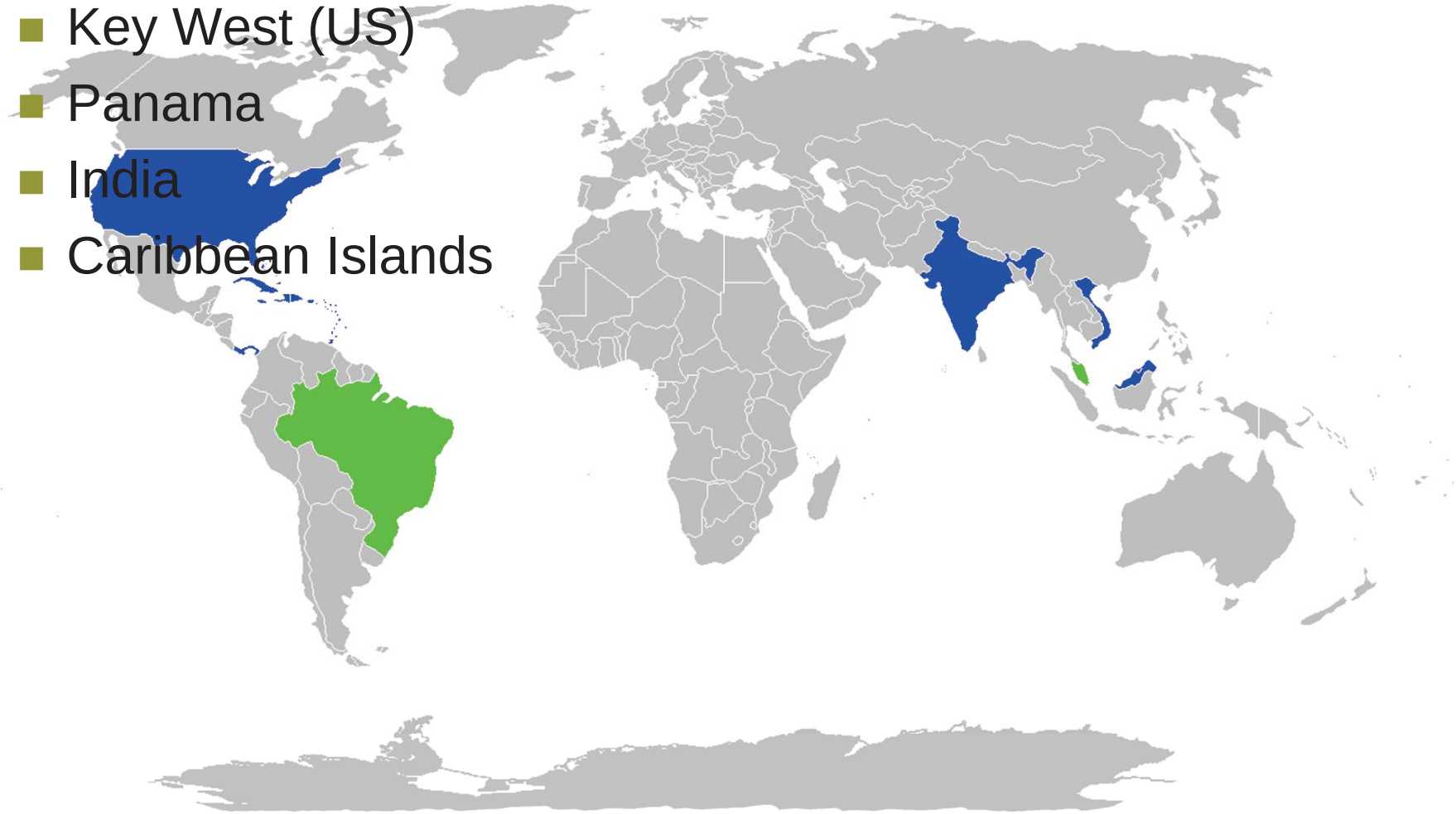
On-going control programmes

- Open field releases conducted so far;
  - Cayman Islands
  - Brazil
  - Malaysia



On-going control programmes

- Currently seeking regulatory approval to use RIDL;
  - Key West (US)
  - Panama
  - India
  - Caribbean Islands



New species (mosquitoes)

- *Aedes albopictus*

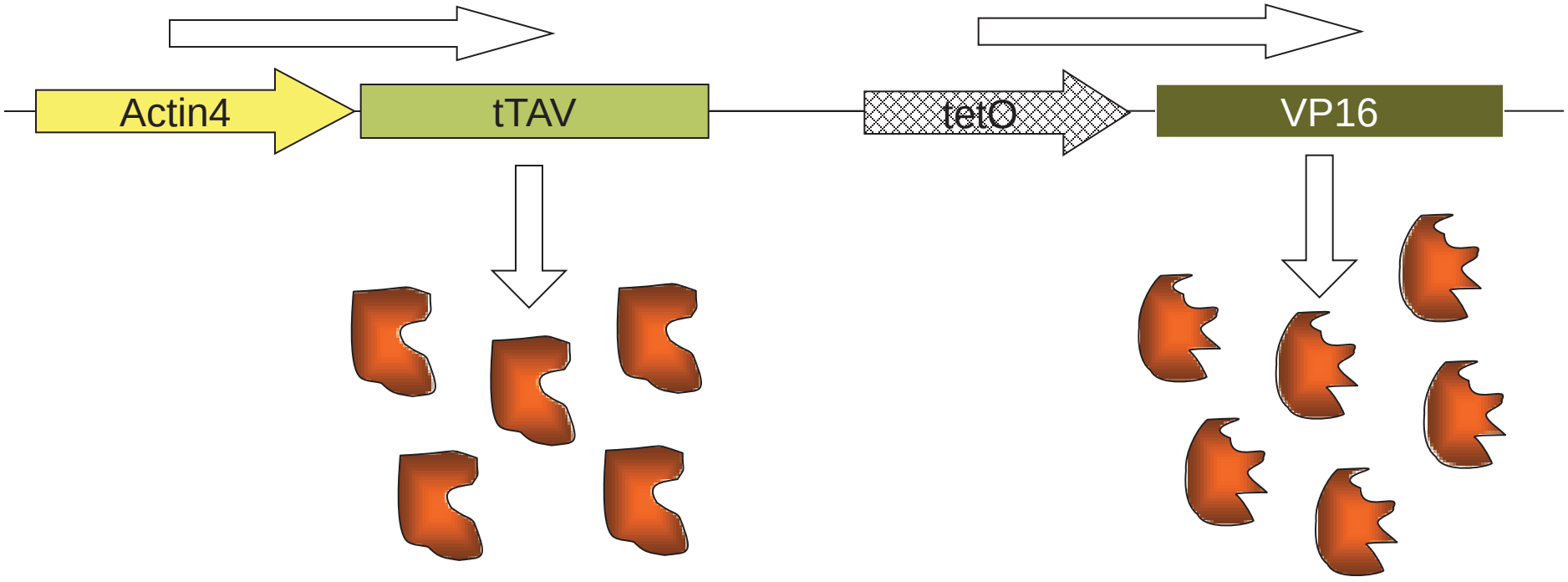
- Dengue and Chikungunya
- Have strains ready for field evaluation

New species (mosquitoes)

- Culex sp.
- SBIR grant to transform *Culex quinquefasciatus* (*C. pipiens* and *C. tarsalis*)
- Ultimate aim to develop RIDL strains for the control of WNV

Bi-sex RIDL technology

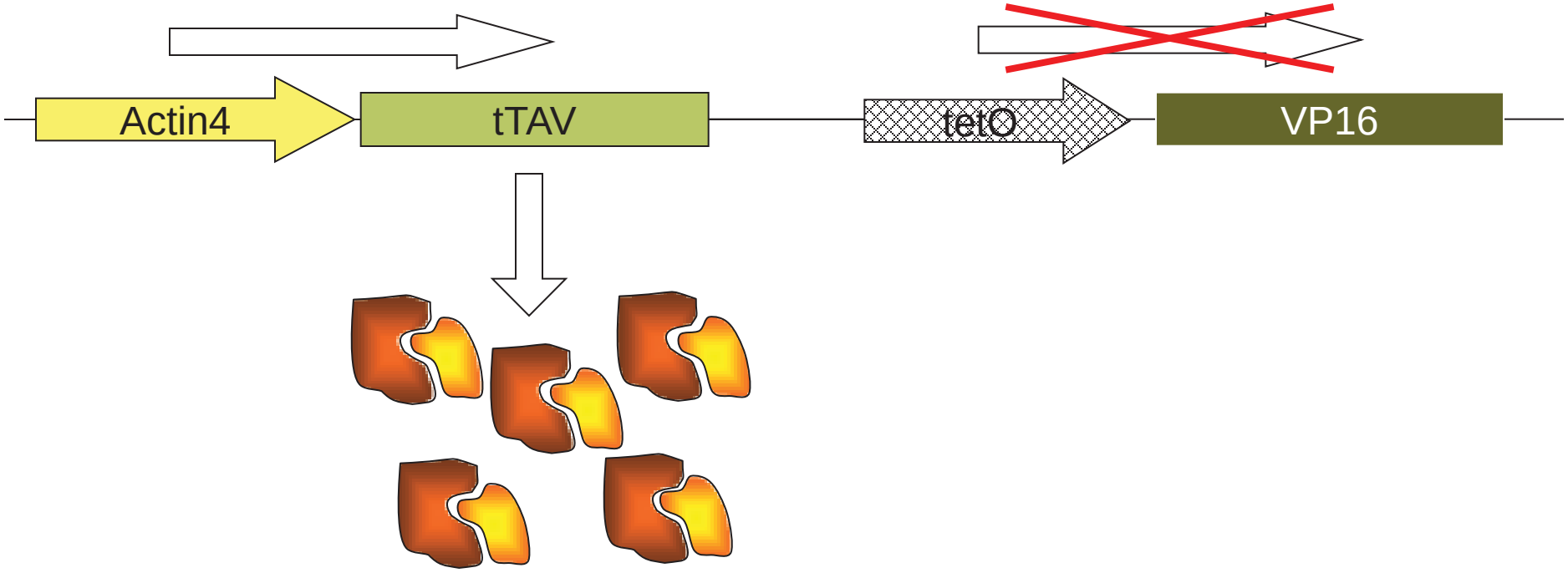
- Design of the sex-specific construct;



- This is not a positive feedback system!

Bi-sex RIDL technology

- Design of the sex-specific construct;



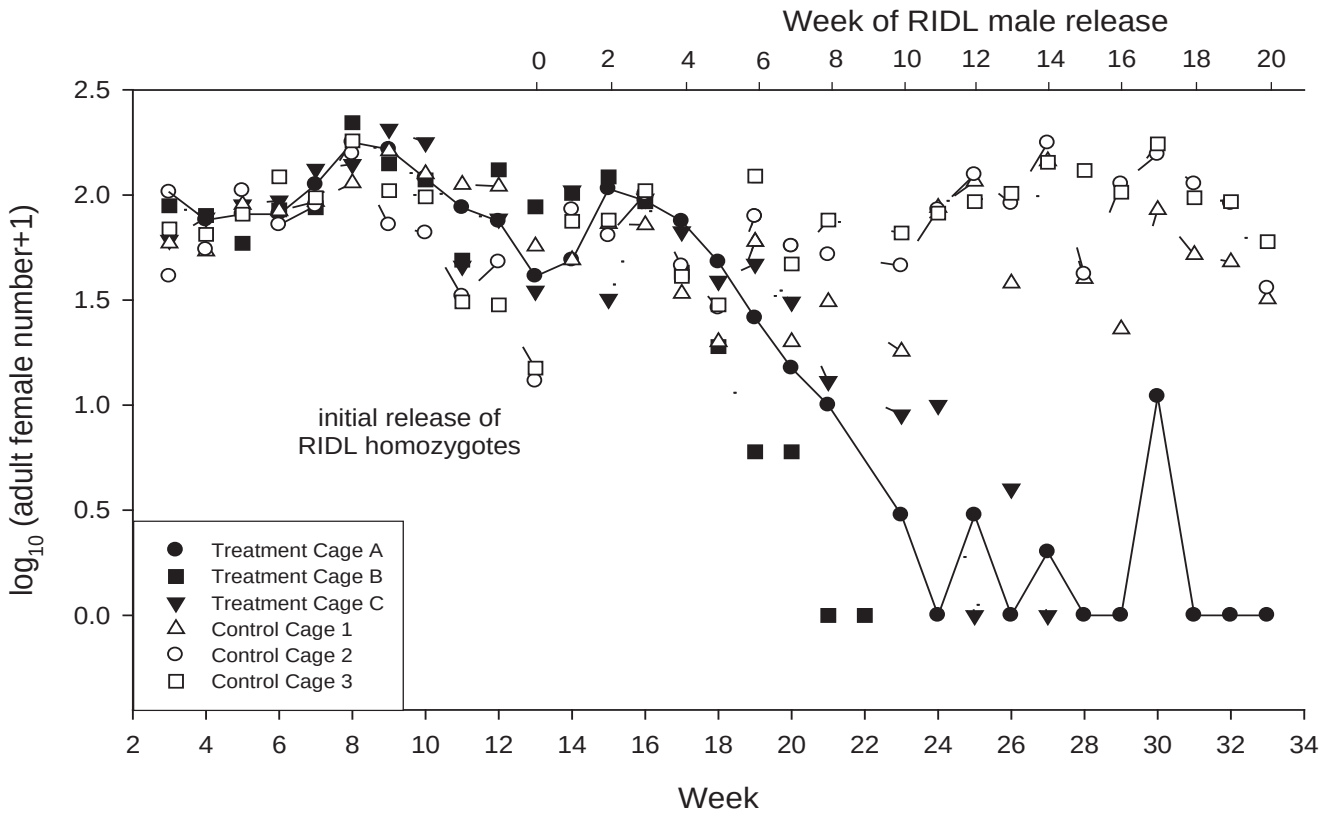
RIDL mosquitoes

Males

Females

Flightless mosquitoes cannot survive in wild (or find hosts). Unable to mate even in laboratory. Males have normal flight ability, as have females given antidote as larvae.

OX3604C Cage suppression trial results



- Colorado State University (Oxitec, Megan Wise de Valdez, Bill Black, Anthony James) experiment in indoor cages
- Control from RIDL was as predicted in simulation model
- Cage populations eradicated in under 20 weeks

Summary:

- RIDL is a safe and effective tool for mosquito control
- Species-specific, environmentally friendly technology
- Under regulatory review for use in the US (Key West trial, commercial licence)
- Applicable to a wide range of species

Acknowledgments

Thank you…