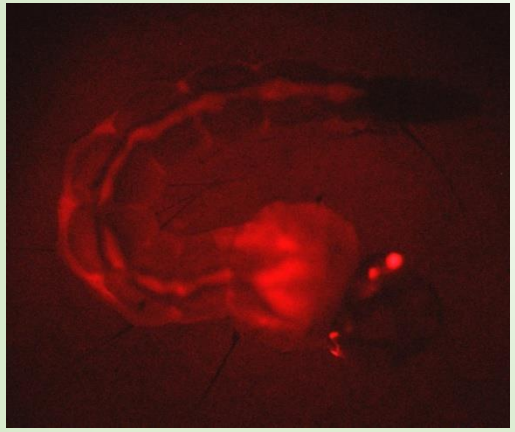
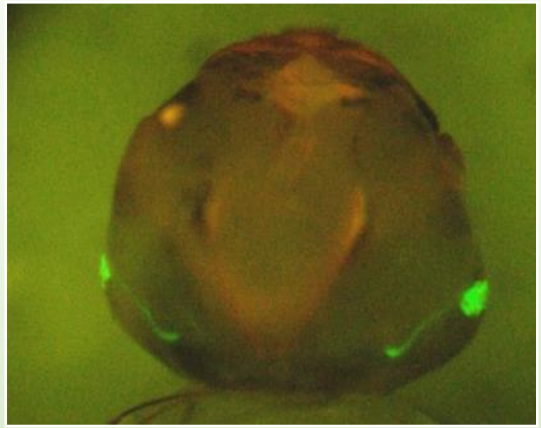
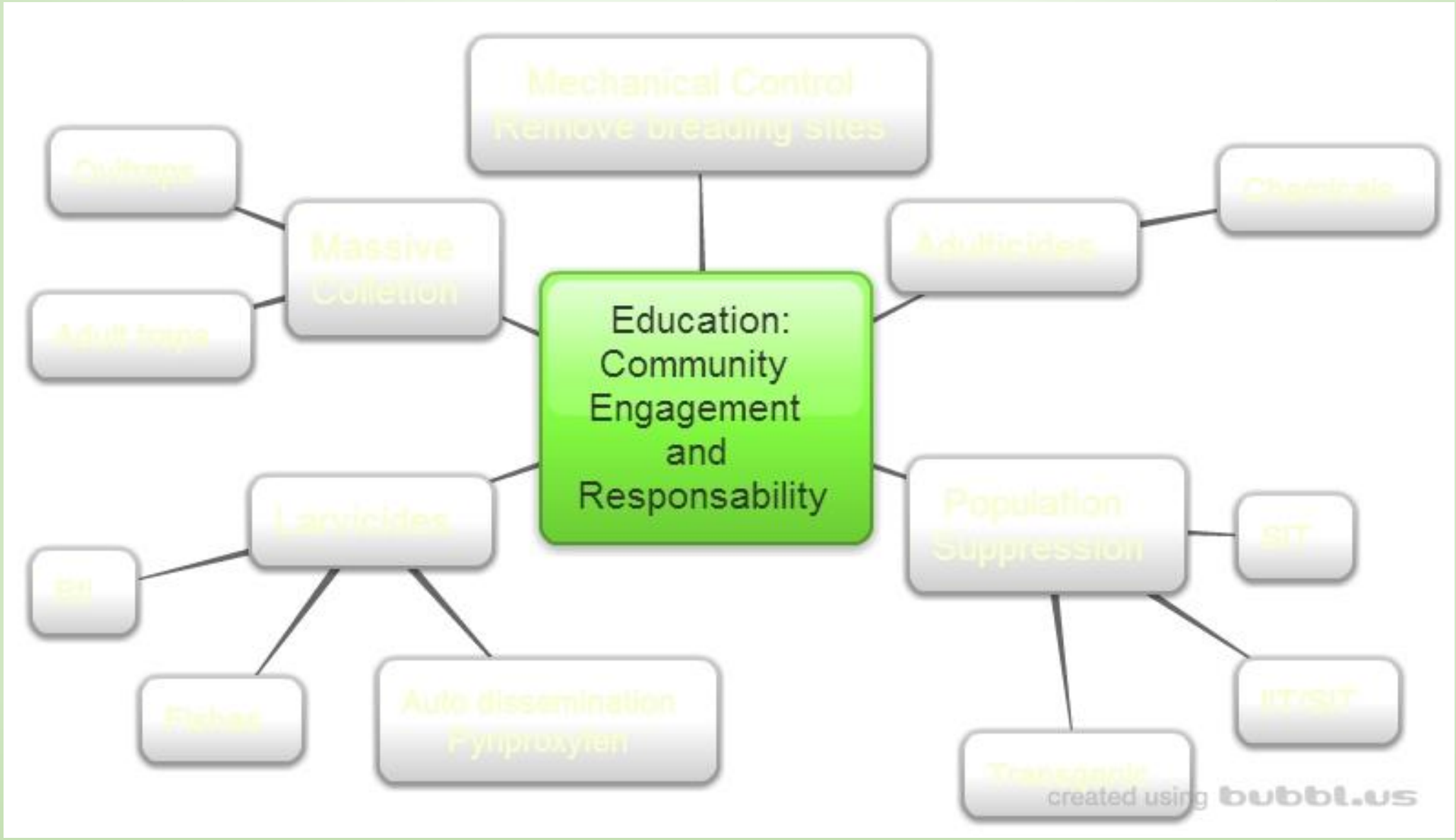
Improvement of transgenic strains of *Aedes aegypti* for the control of arbovirus transmission in Brazil

Margareth L. Capurro
mcapurro@icb.usp.br

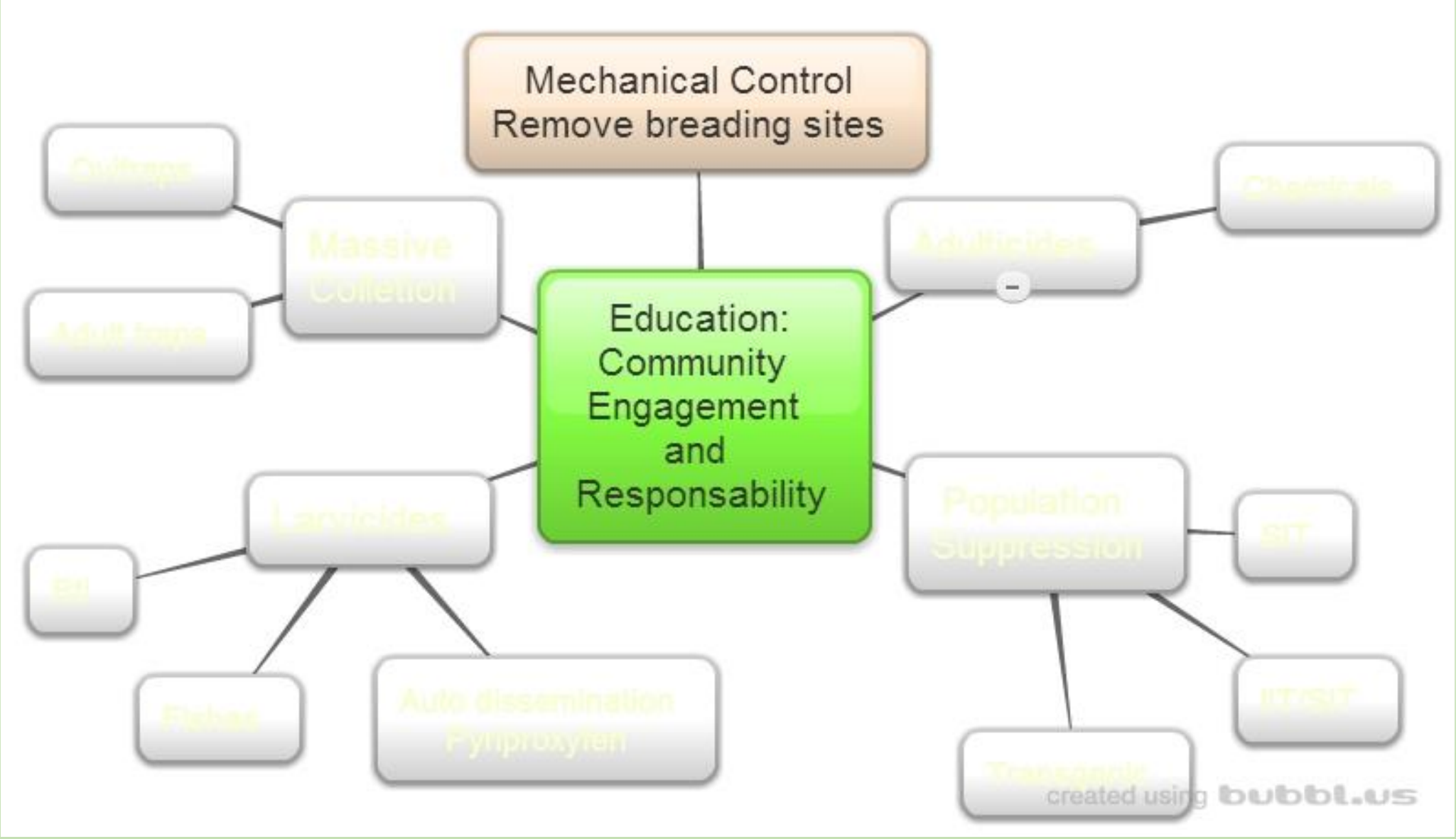




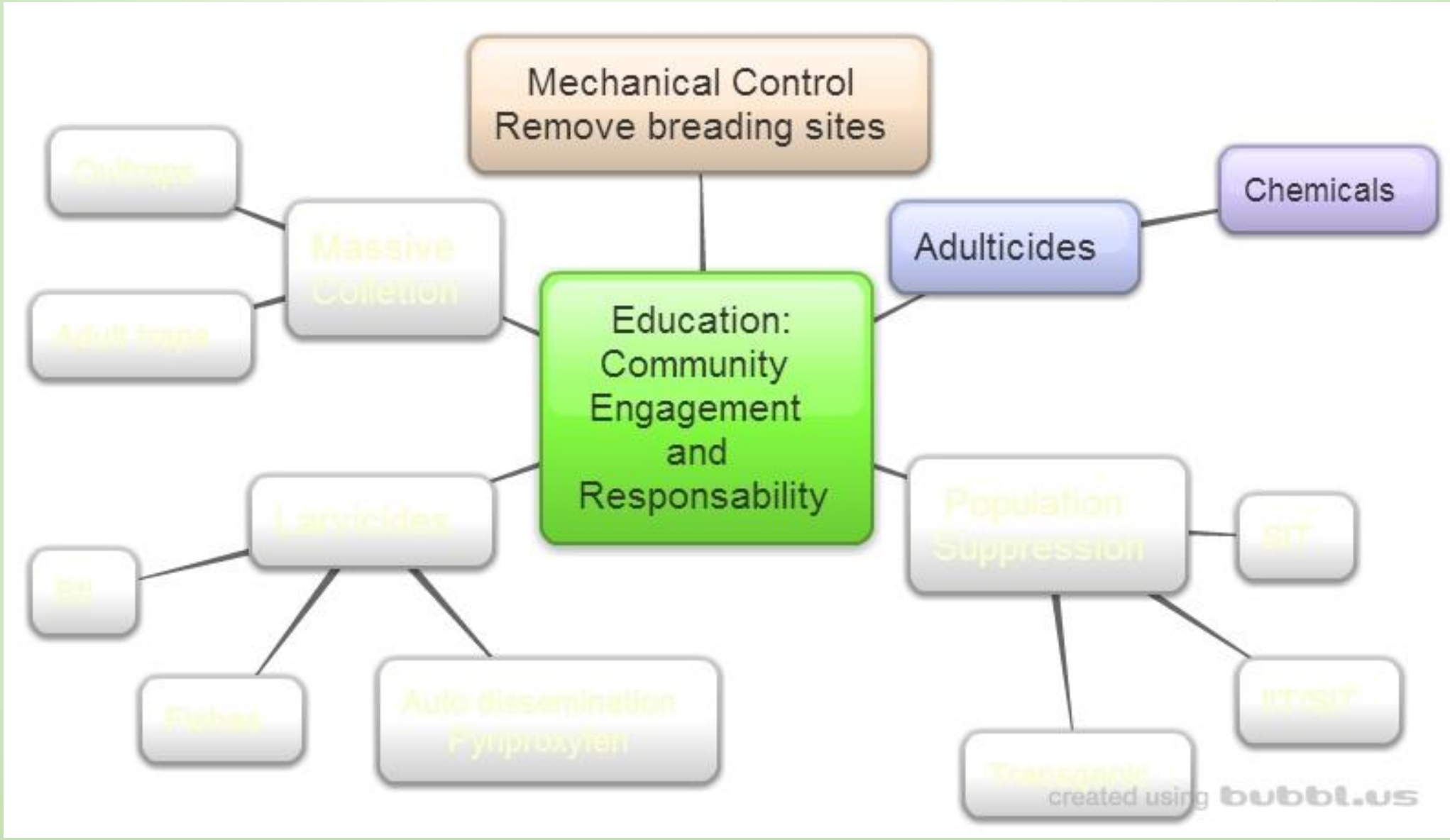
Integrate Control for *Aedes aegypti* Population Supression



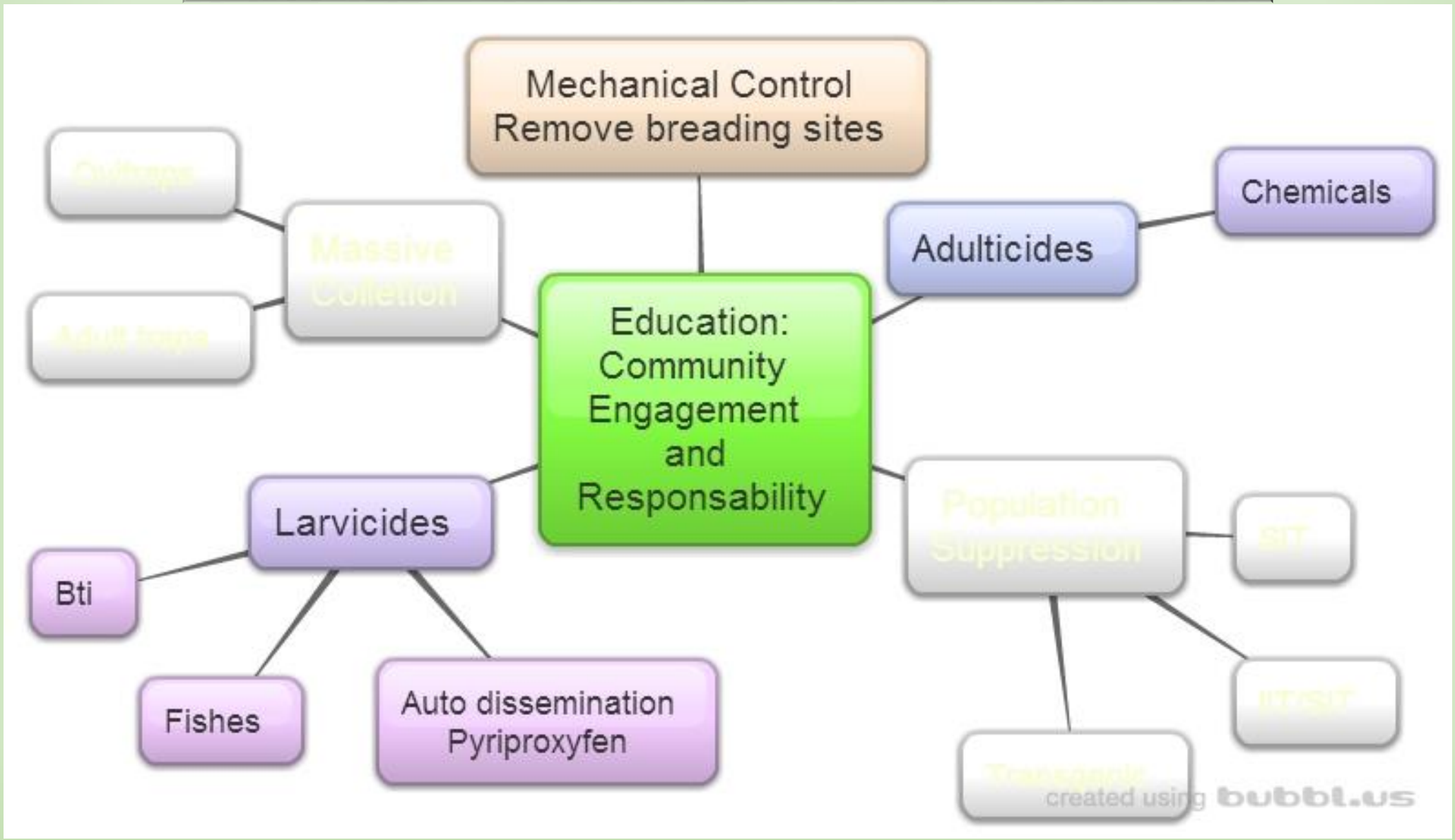
Integrate Control for *Aedes aegypti* Population Supression



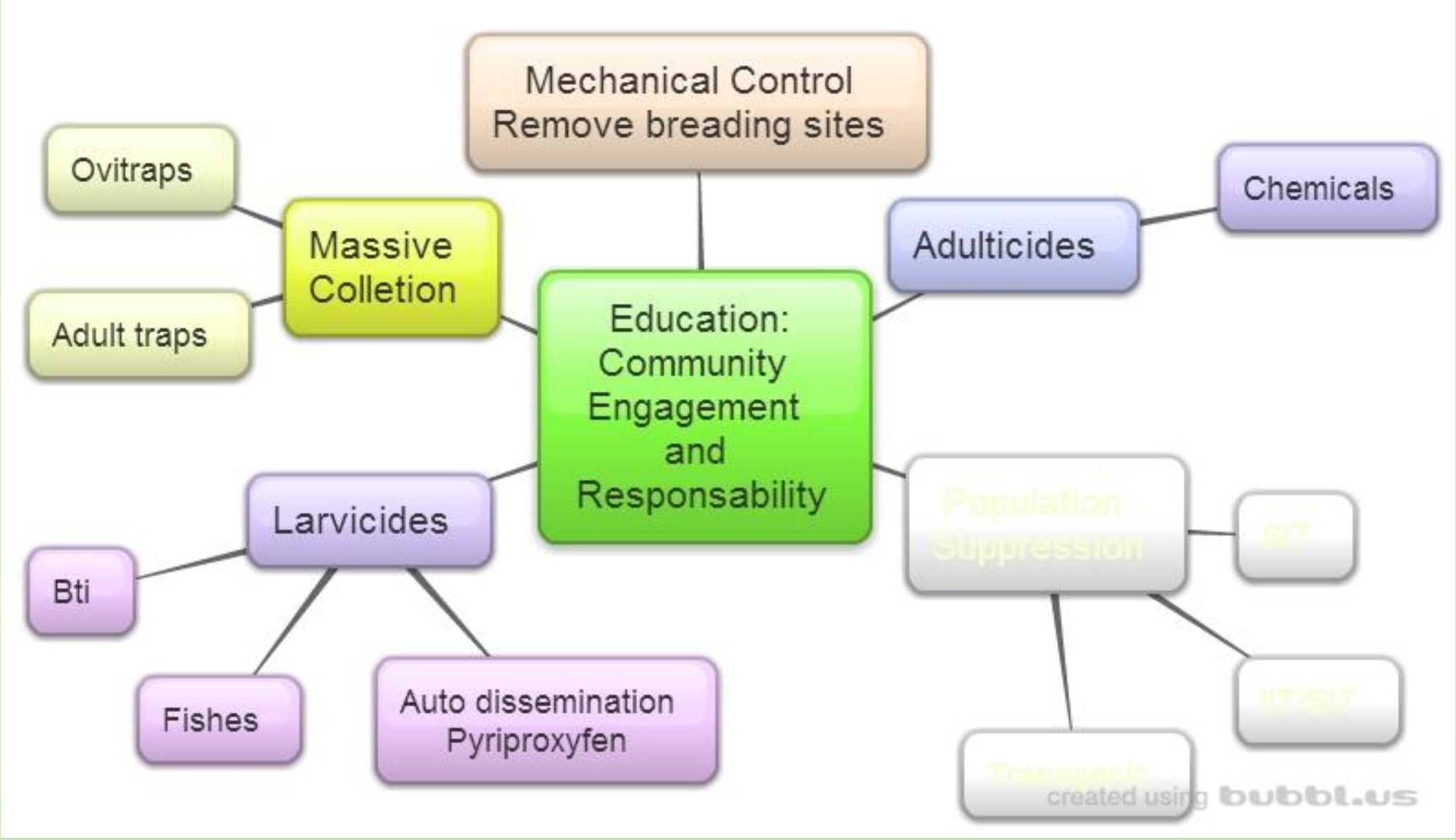
Integrate Control for *Aedes aegypti* Population Supression



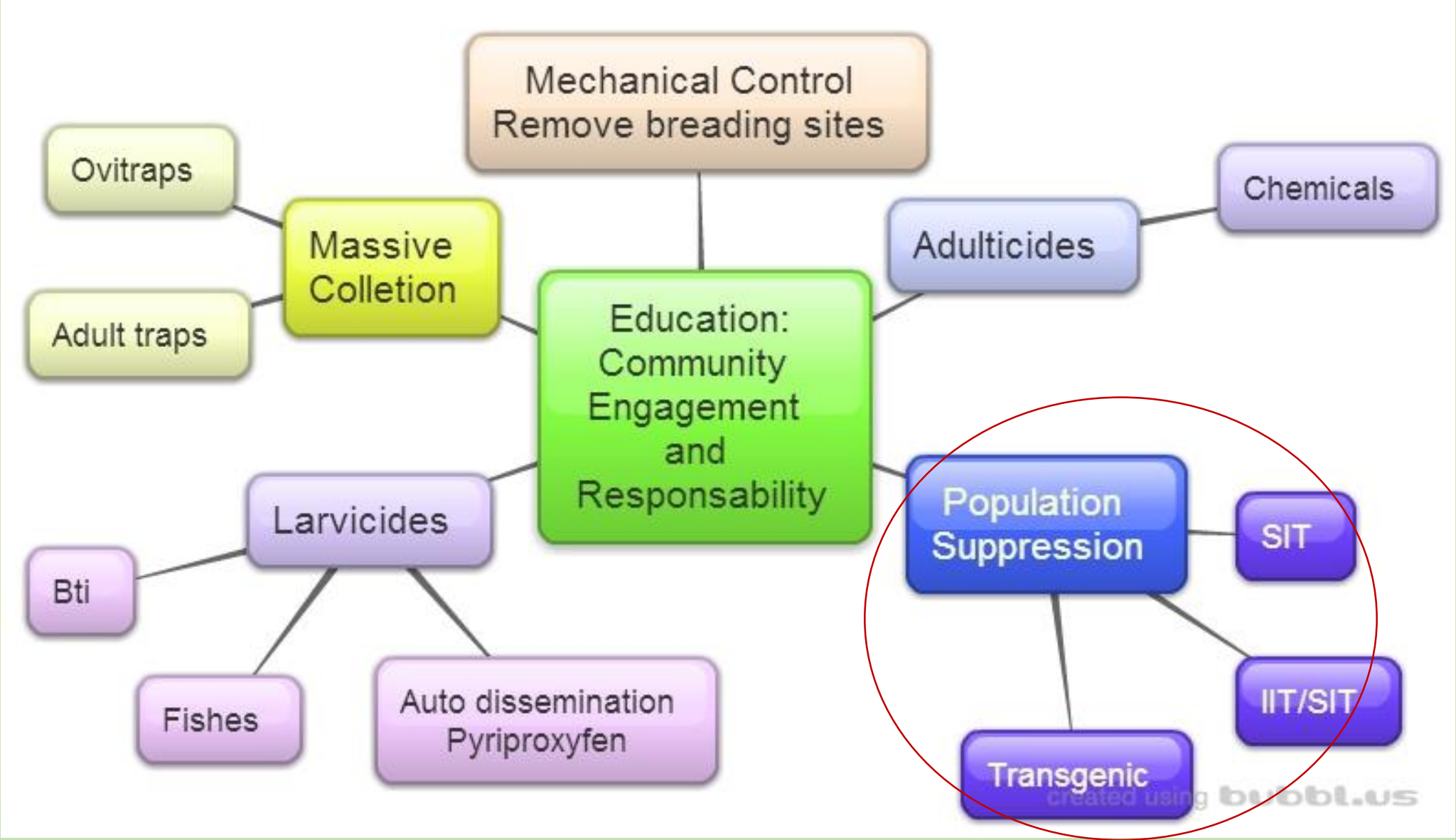
Integrate Control for *Aedes aegypti* Population Supression

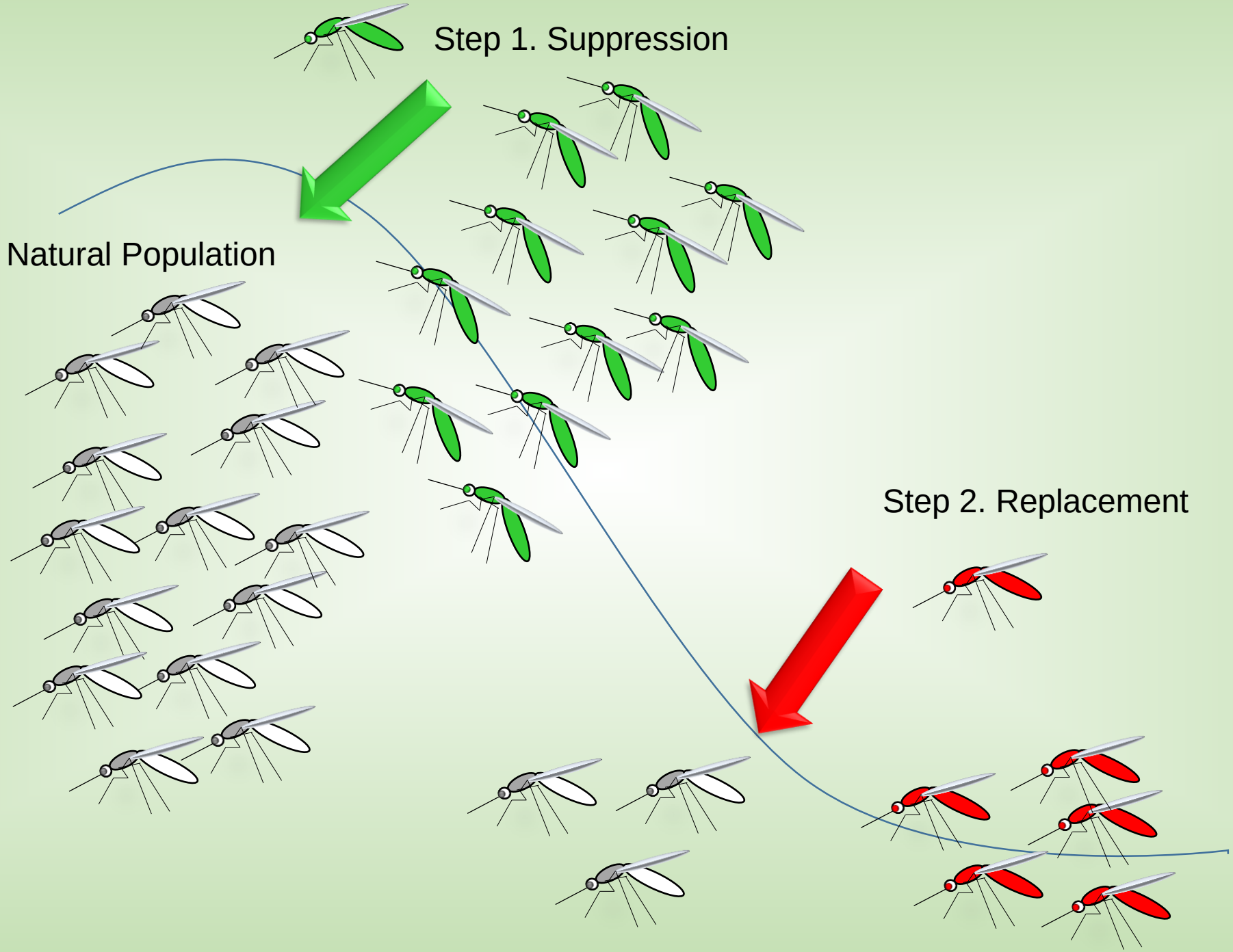


Integrate Control for *Aedes aegypti* Population Supression



Integrate Control for *Aedes aegypti* Population Supression

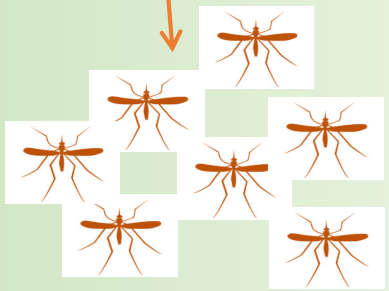



Step 1. Suppression
Natural Population
Step 2. Replacement

Sterile Insect Technique (SIT)

1. Male Sterilization

Irradiation



2. Releases

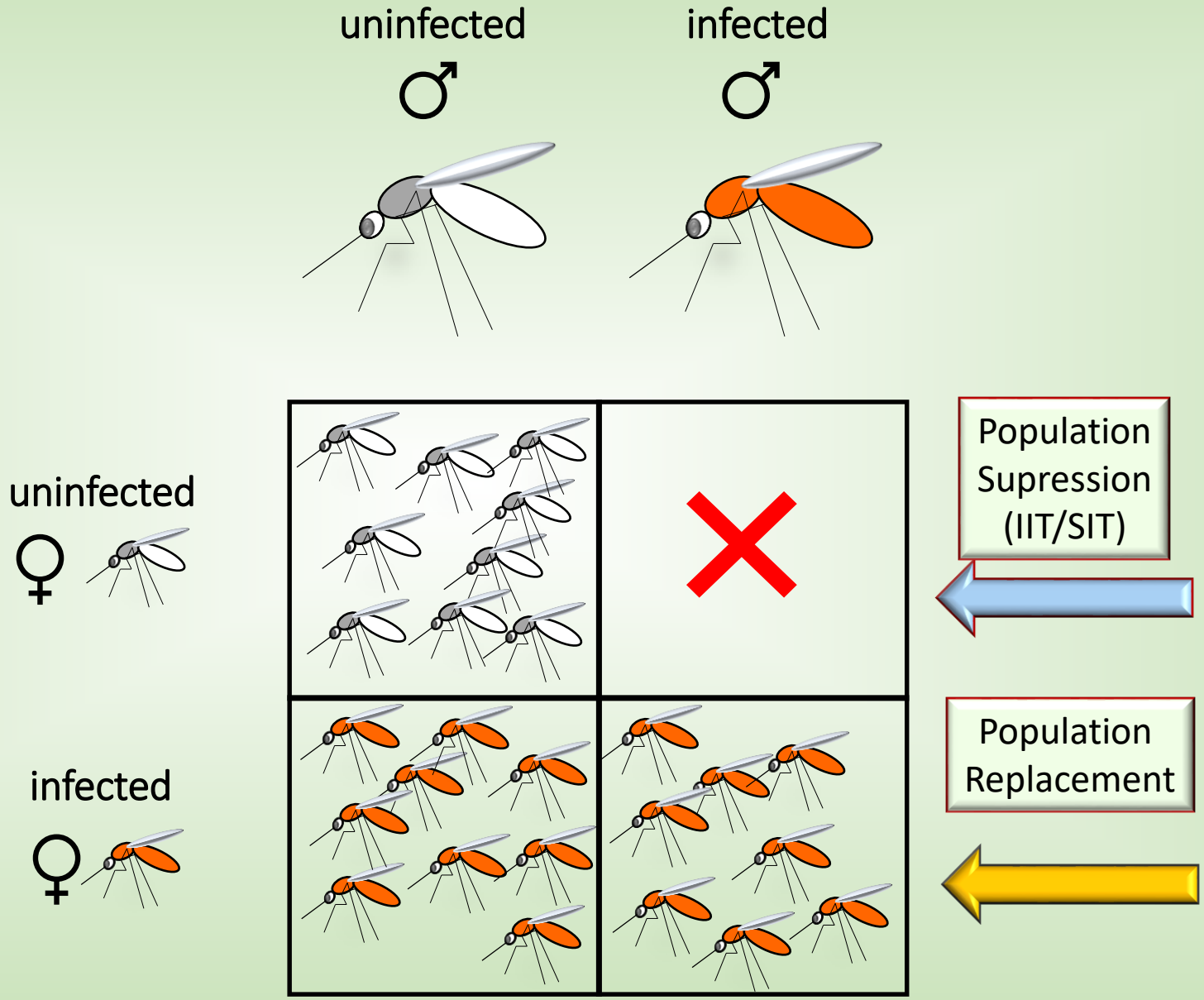
3. Supression

Birth Control Method:

- Mass rearing
- Sex separation
- Sterilization (irradiation)
- Packing, transport, release
- Sterile matings

= no offspring

The Wolbachia approach (Suppression) – IIT/SIT

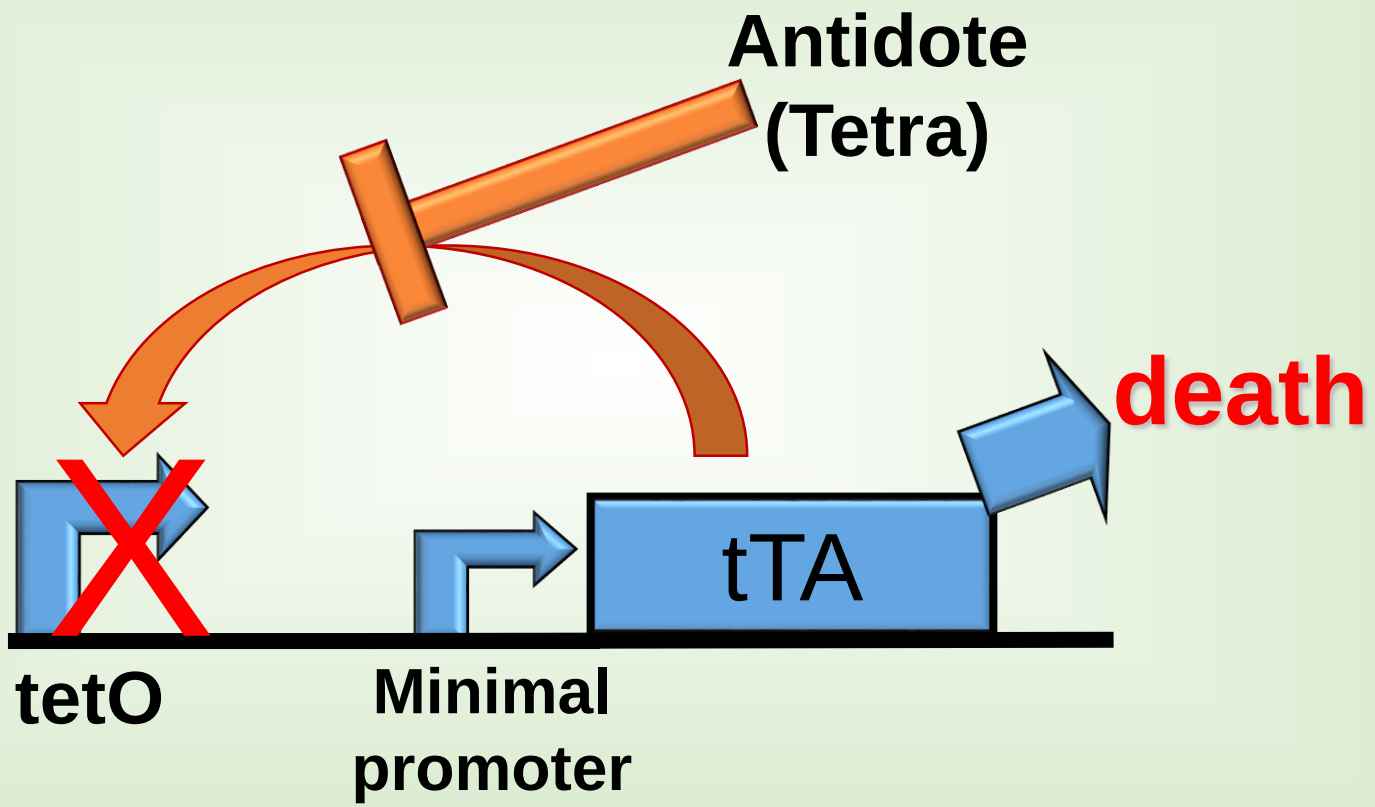


Open Field Release of **OX513A** *Aedes aegypti* Transgenic line evaluation

MOSCAMED BRASIL

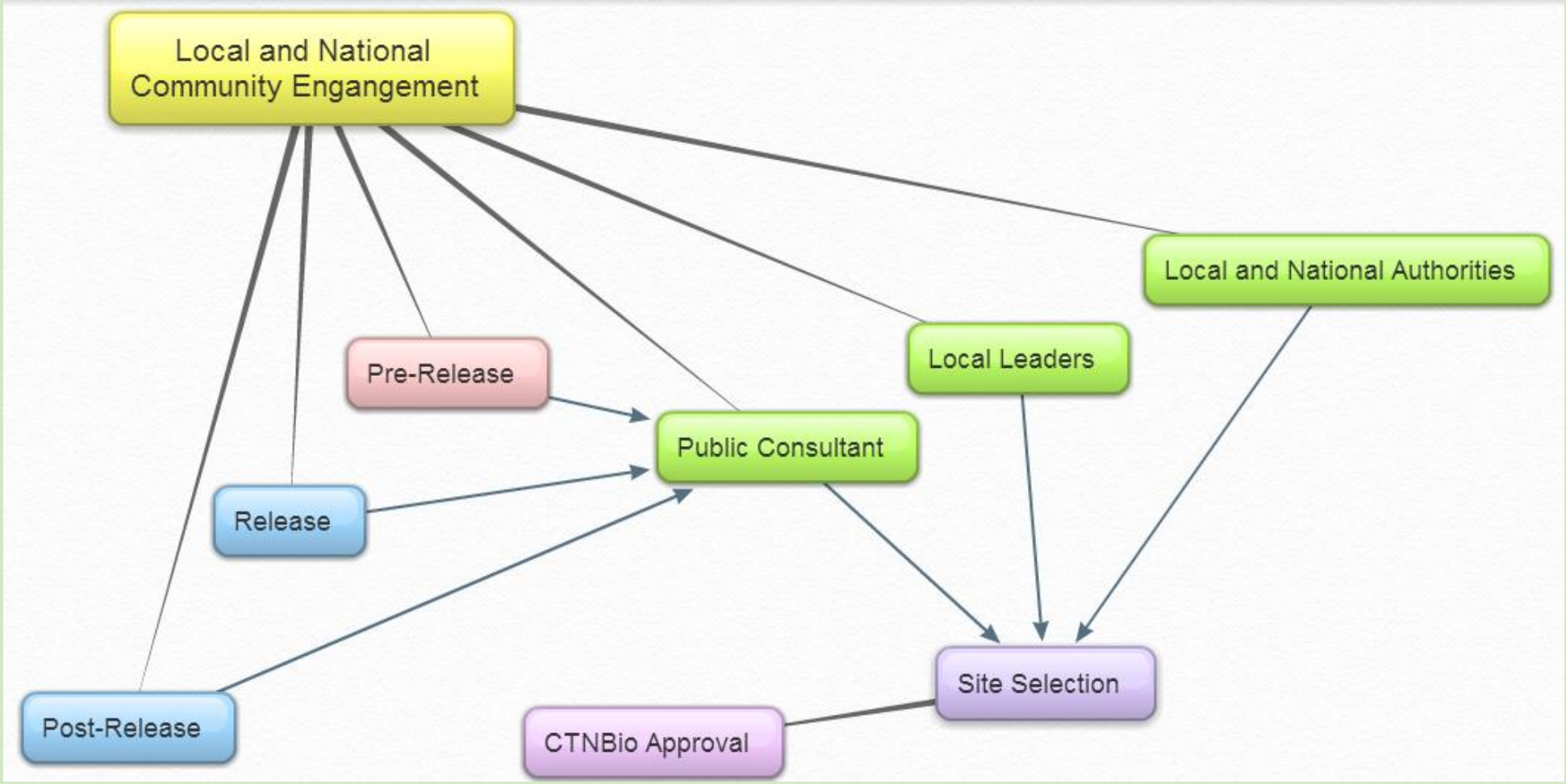
USP Universidade de São Paulo

Repressive of **I**nsects carrying a **D**ominant **L**ethal gene (RIDL)



Thomas *et al.* 2000 Science 287: 2474-6

Projeto Aedes Transgênico

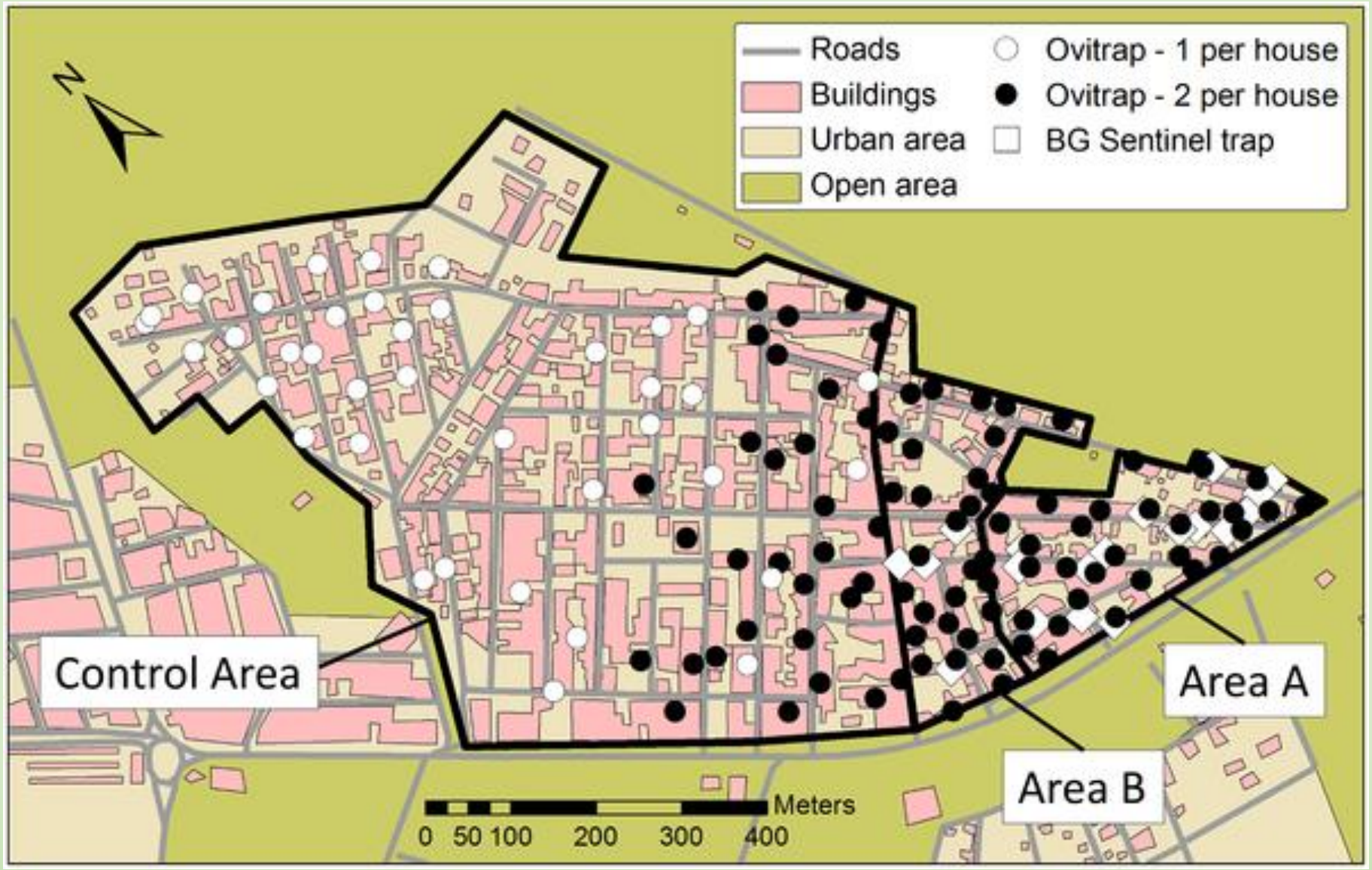


Aedes aegypti Production (UPAT)

COLONY
4 to 6 million eggs/week

Males for releases
1,5 million/week

Itaberaba – Field site



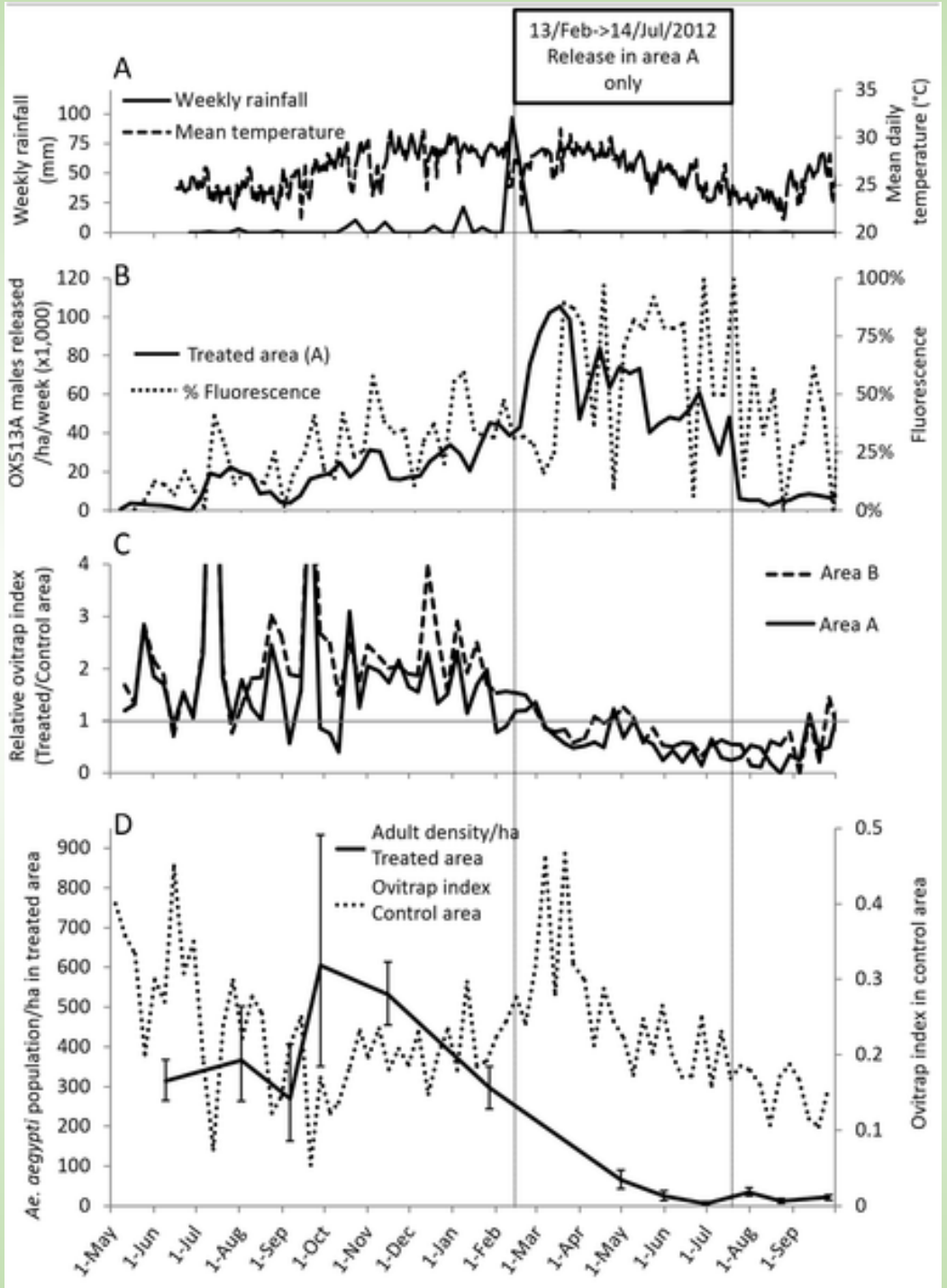
PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Suppression of a Field Population of *Aedes aegypti* in Brazil by Sustained Release of Transgenic Male Mosquitoes

Danilo O. Carvalho[1,2☯], Andrew R. McKemey[1☯*], Luiza Garziera[3], Renaud Lacroix[1], Christl A. Donnelly[4], Luke Alphey[1,5,6], Aldo Malavasi[3], Margareth L. Capurro[2,7]

PLOS Neglected Tropical Diseases | DOI:10.1371/journal.pntd.0003864 July 2, 2015



Projeto Aedes Transgênico

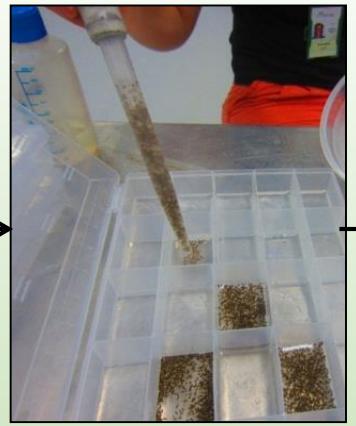
Project Phase 2 – Jacobina - Bahia

Pupa transportation (LEMI)

C25

BOD 16ºC ON

180,000 per container



Arriving at LEMI
Emergency , Monitoring and Information Lab

Preparation for release

How to implement Transgenic mosquitoes in Integrate Control Programs?

- Egg distribution is easy

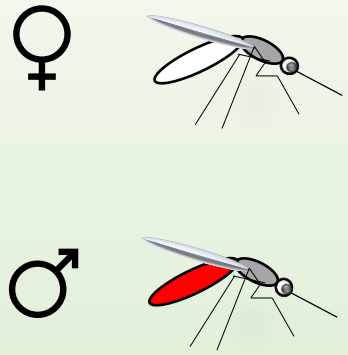
- Hatch centers – no larvae sorter
  - no tetracycline needs
  - after release no offspring

Improving transgenic lines *Aedes aegypti* and *Aedes albopictus*

- Producing GSS (Genetic Sexing Strain)
- Producing Sterile male strain (no Larvae)
- Use of tetracycline only in colonies

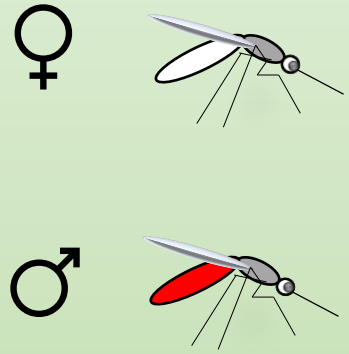
Sterility Conditional Construct - SCC

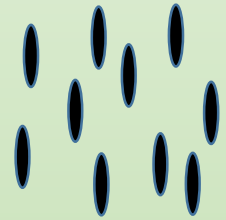
No Antidote



No Viable Eggs

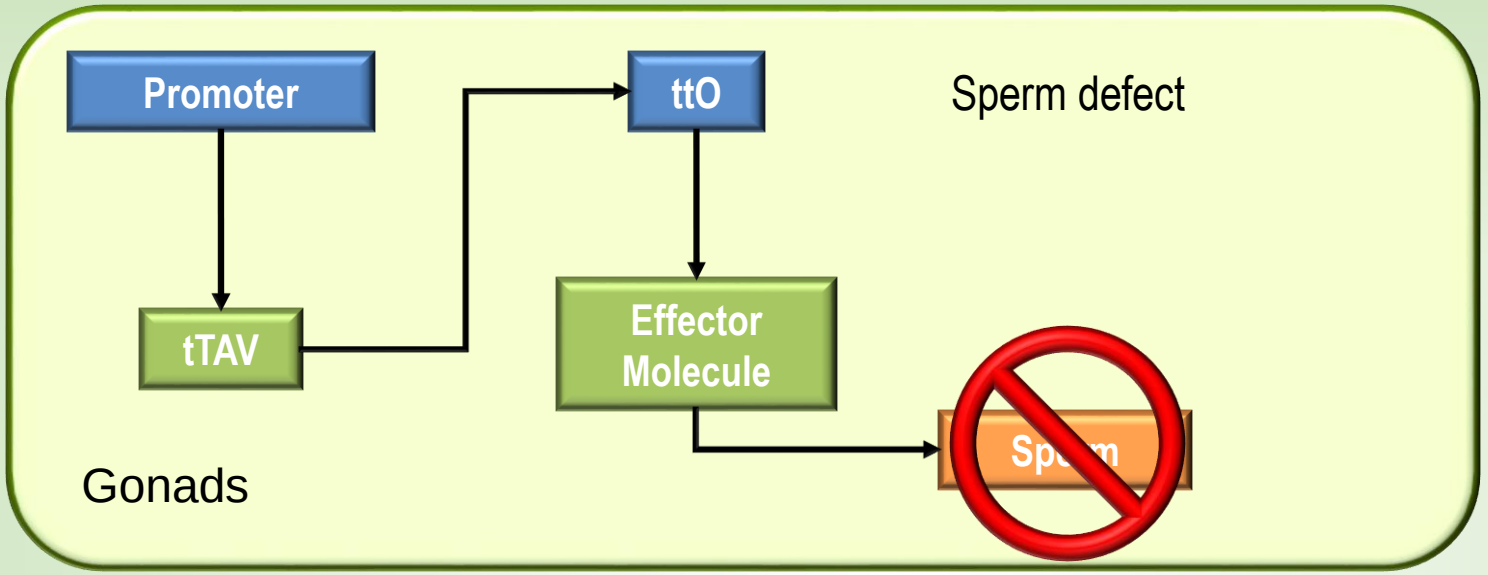
Antidote (Tet +)



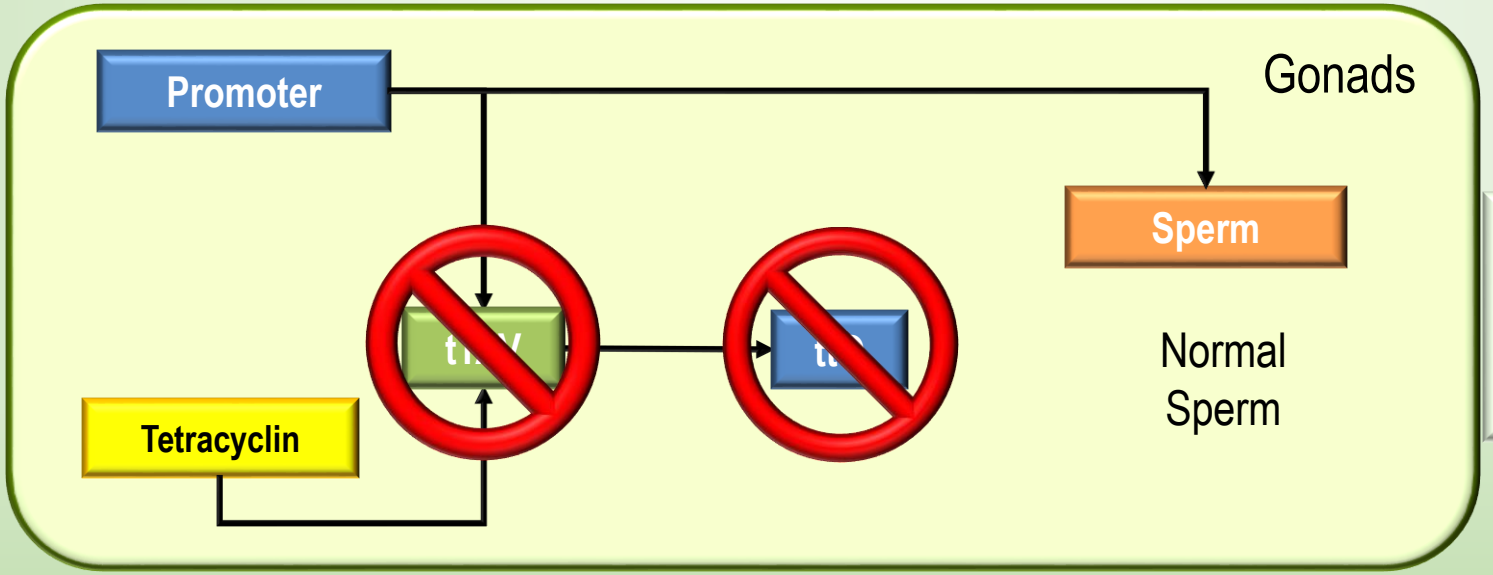


Viable Eggs

Sterility Conditional Construct - SCC



Males to Release

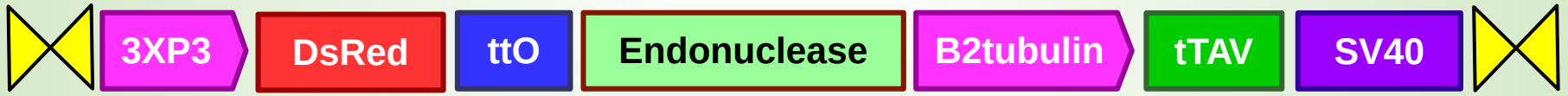


Egg Production

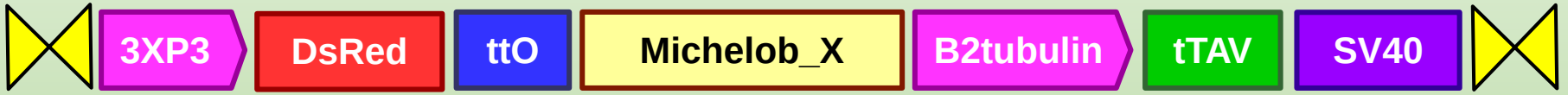
SCC Transgenes

- Two Effector molecules:

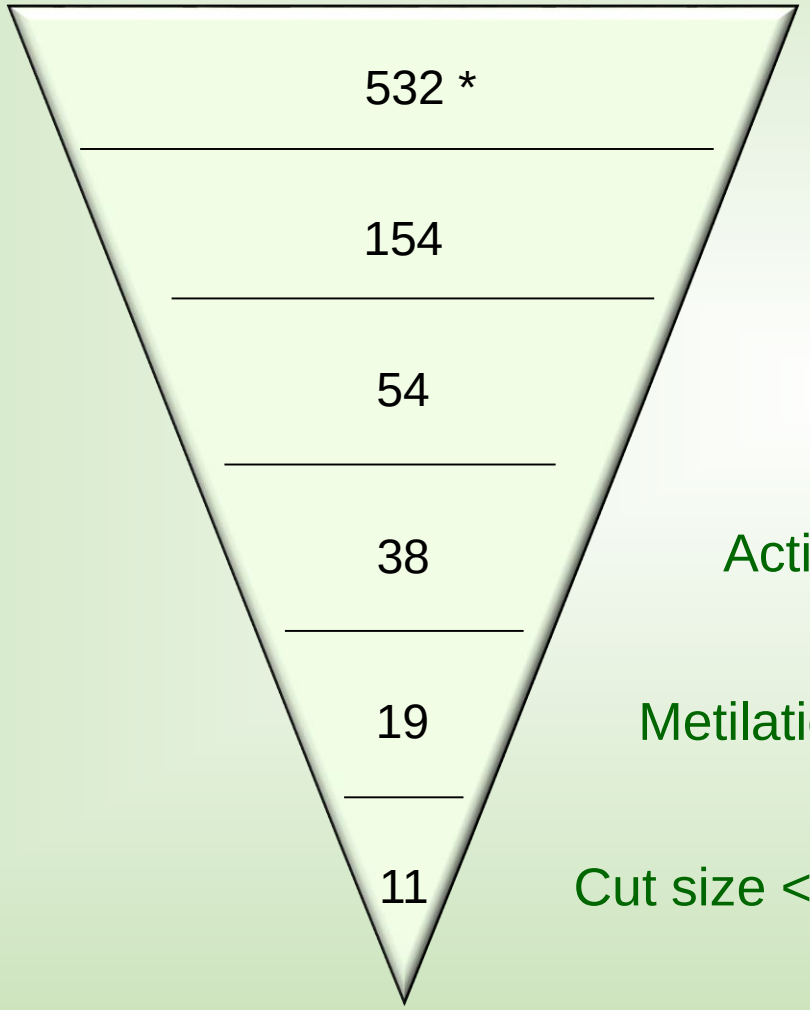
Endonuclease



IAP Antagonist



Endonucleases



Enzymes (NEB)

> 1 million cuts

Isoisomers

Activity temperature < 50 ºC

Metilation Sensitivity

Cut size < 5bp

Endonucleases

Position	Endonuclease	Cut Frequency	T °C	Type
1	HindI	30.032.972	37	I
2	CviAII	4.645.879	25	II
3	SelI	1.672.829	26	II
4	AluI	4.796.029	37	II
5	BfaI	2.962.089	37	II
6	CviRI	5.486.819	37	II
7	CviTI	12.095.603	37	II
8	HaeIII	1.980.700	37	II
9	MspI	2.225.385	37	II
10	Tru9I	8.779.417	37	II
11	TspEI	15.059.923	37	II

Endonucleases

Position	Endonuclease	Cut Frequency	T °C	Type
2	CviAII	4.645.879	25	II
3	SelI	1.672.829	26	II
4	AluI	4.796.029	37	II
5	BfaI	2.962.089	37	II
6	CviRI	5.486.819	37	II
7	CviTI	12.095.603	37	II
8	HaeIII	1.980.700	37	II
9	MspI	2.225.385	37	II
10	Tru9I	8.779.417	37	II
11	TspEI	15.059.923	37	II

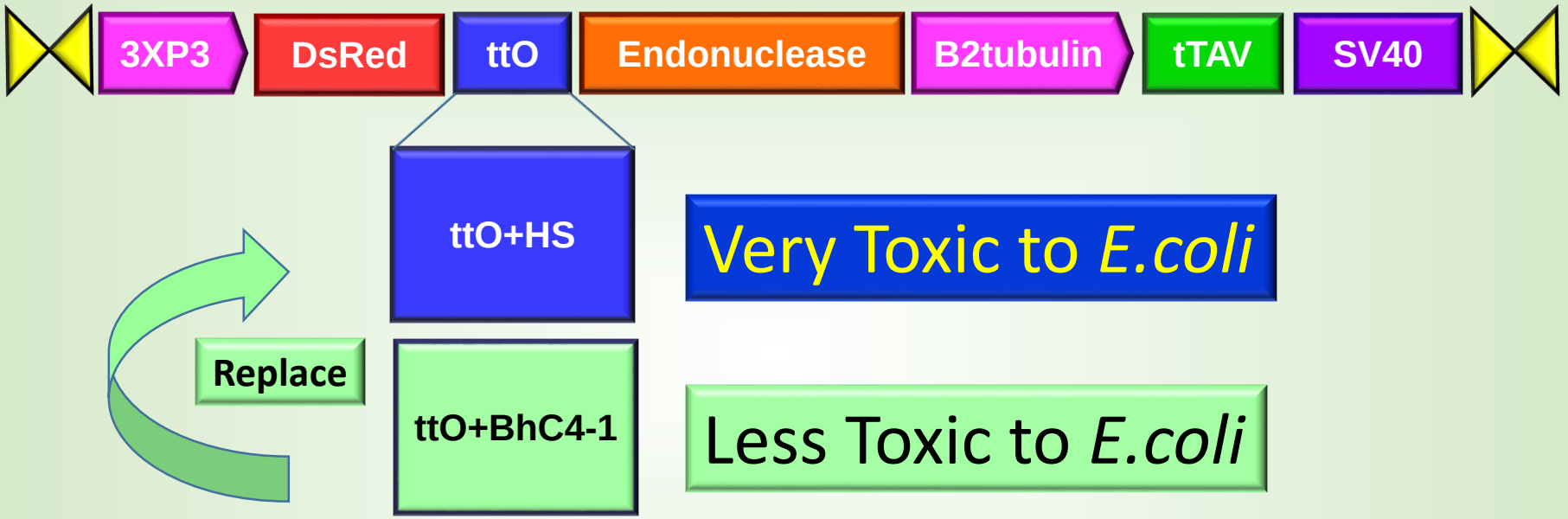
Endonucleases

Position	Endonuclease	Cut Frequency	T °C	Type
2	CviAII	4.645.879	25	II
3	SelI	1.672.829	26	II

Endonucleases

Position	Endonuclease	Cut Frequency	T °C	Type
2	CviAII	4.645.879	25	II

Transgene *CviAII*



Garcia *et al. BMC Molecular Biology* 2011, **12**:32
http://www.biomedcentral.com/1471-2199/12/32

BMC Molecular Biology

RESEARCH ARTICLE Open Access

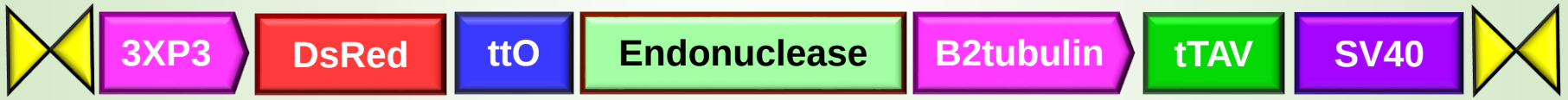
Functional characterization of the sciarid *BhC4-1* core promoter in transgenic *Drosophila*

Adriana C Garcia[1†], Daniel LG Gitaí[2,3†], Fernanda C Humann[2], Maria L Paçó-Larson[2] and Nadia Monesi[1*]

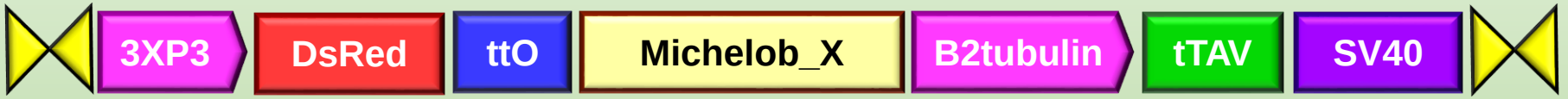
SCC Transgenes

- Two Effector molecules:

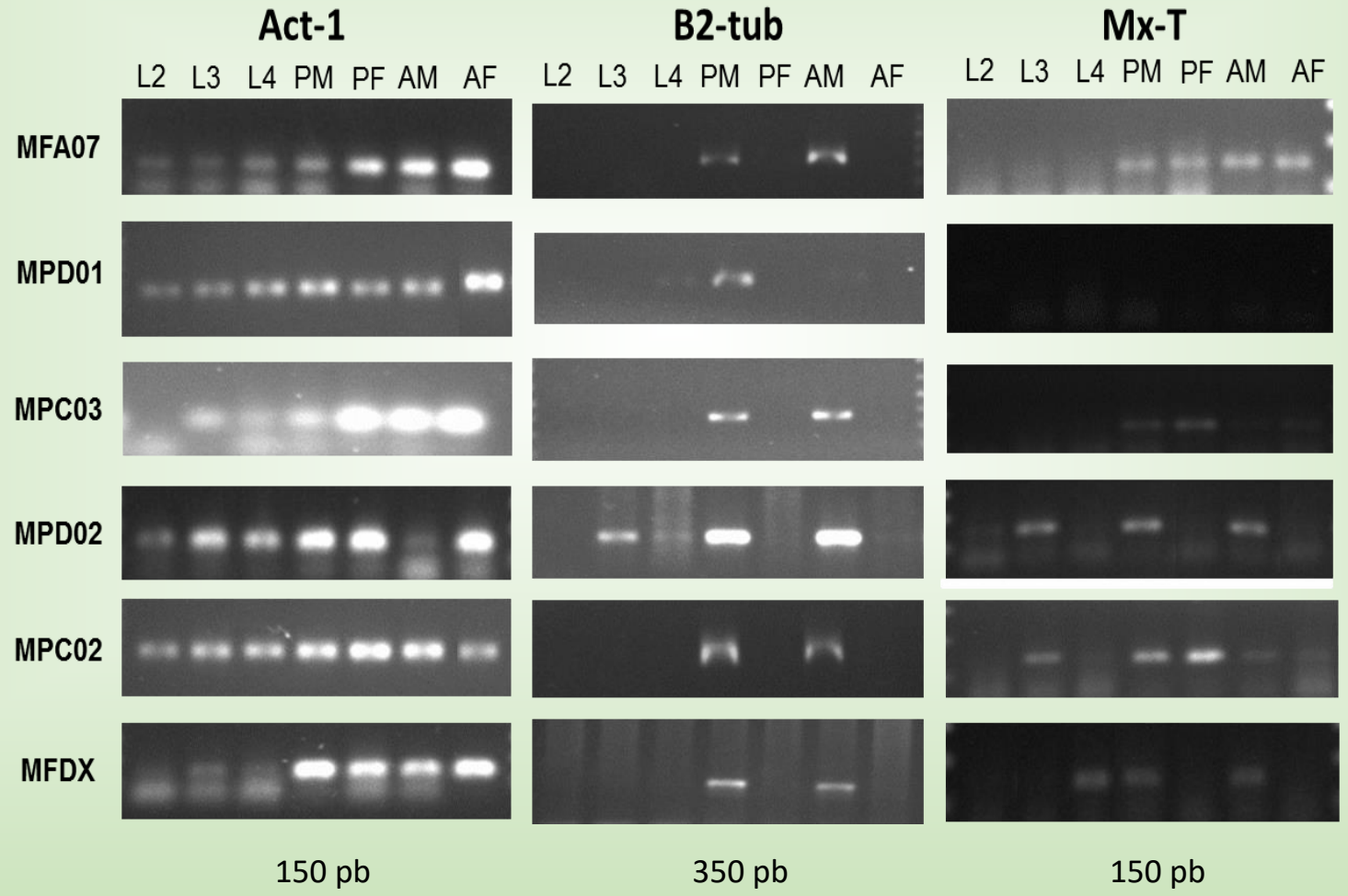
Endonuclease



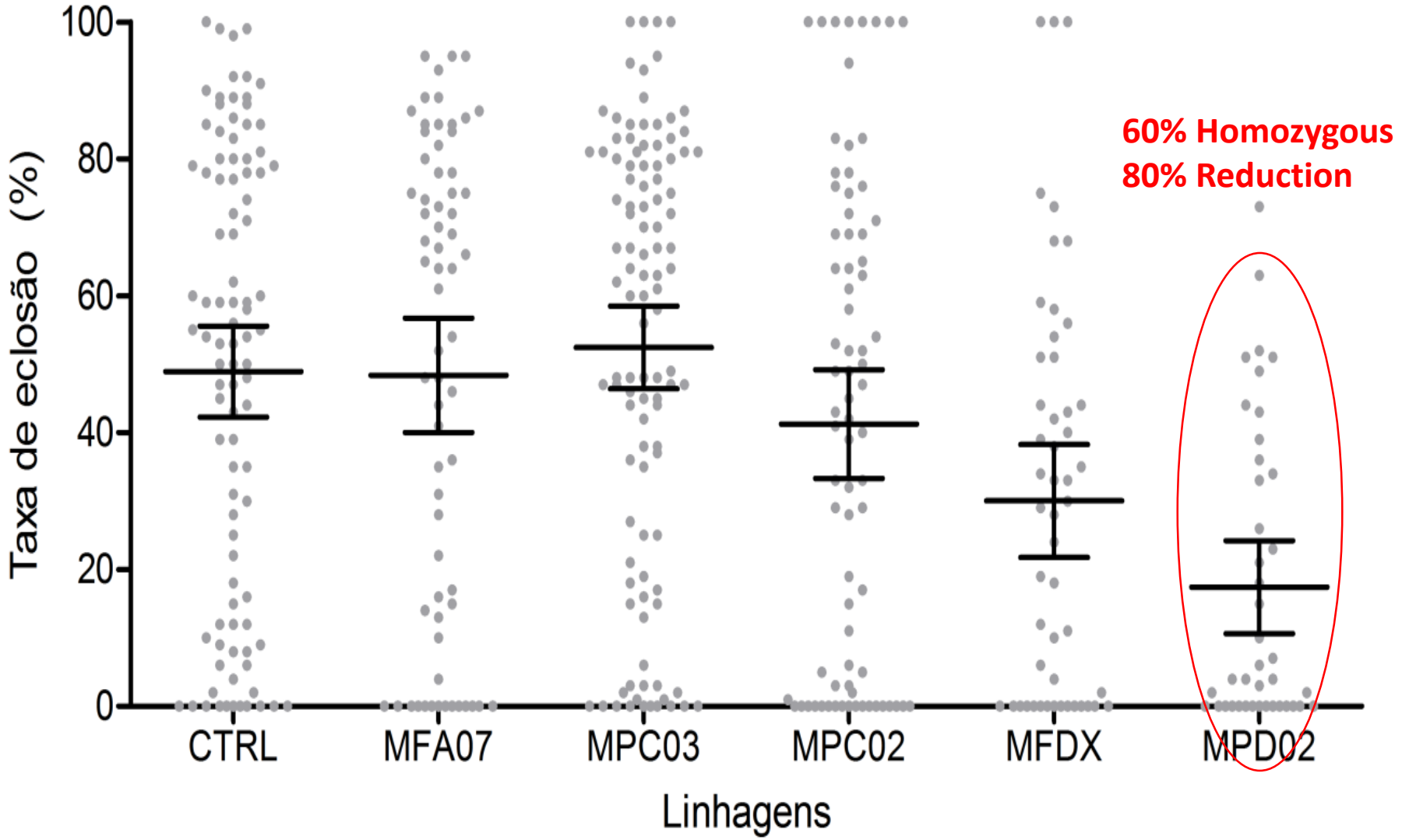
IAP Antagonist



Transgenic Lines

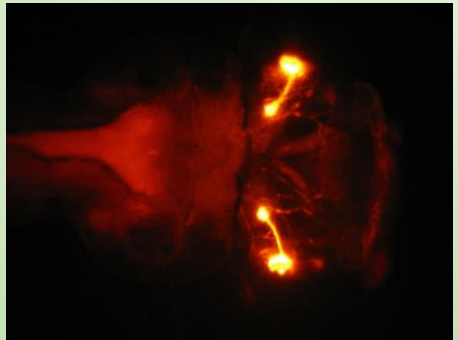


Sterile Conditional Construct (SCC)



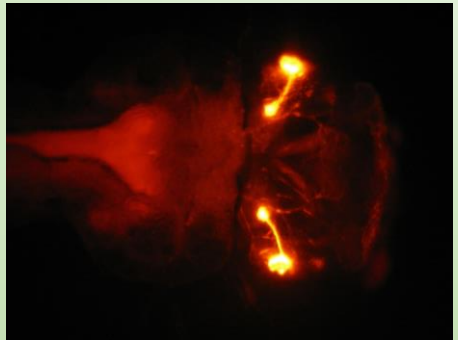
Improving transgenic lines *Aedes aegypti* and *Aedes albopictus*

- Producing GSS (Genetic Sexing Strain)
- Producing Sterile male strain (no Larvae) ✓
- Use of tetracycline only in egg production ✓

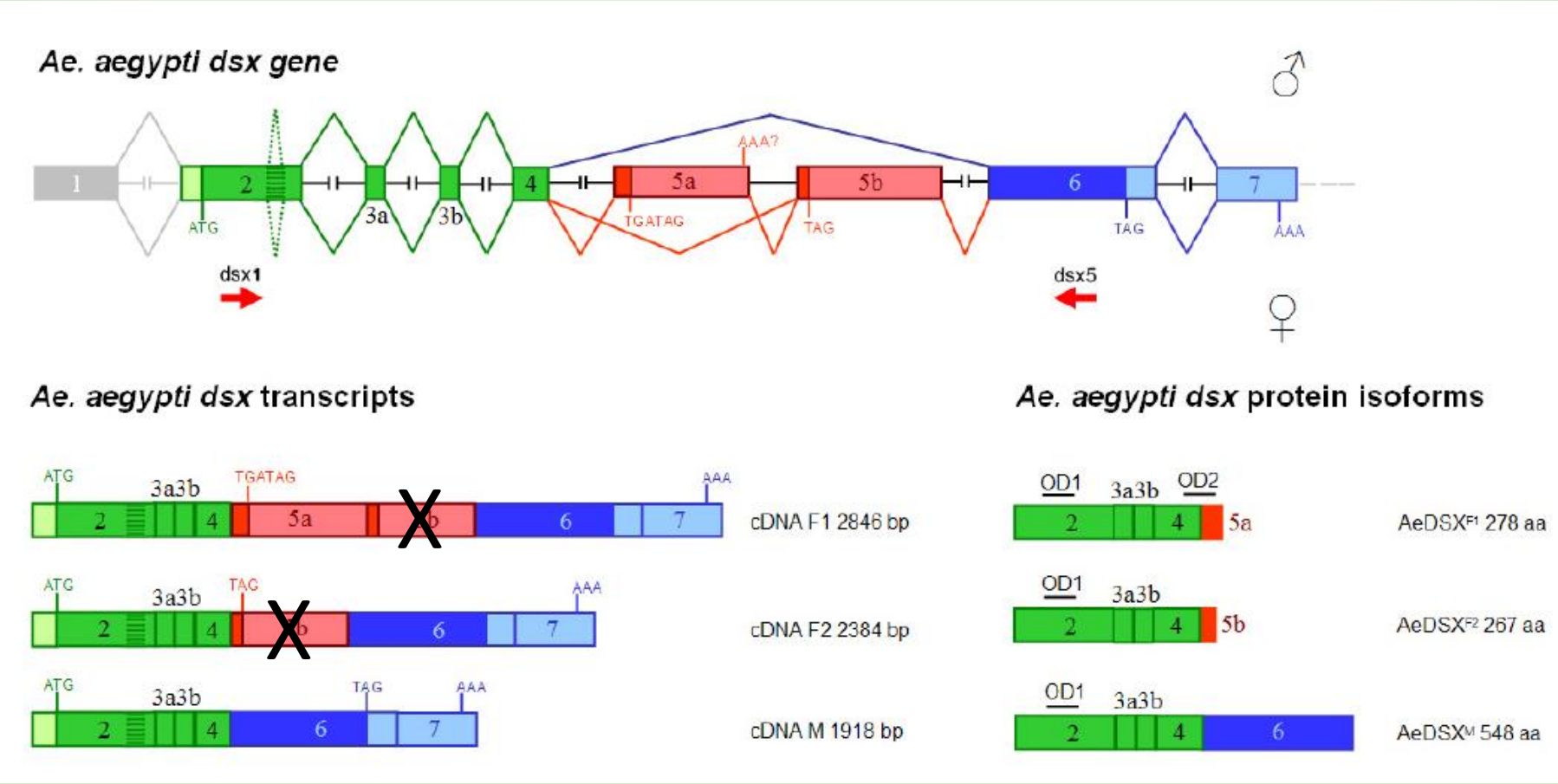


Improving transgenic lines *Aedes aegypti* and *Aedes albopictus*

- Producing GSS (Genetic Sexing Strain)
- Producing Sterile male strain (no Larvae) ✓
- Use of tetracycline only in egg production ✓



Salvemini *et al.*, 2011
BMC evol. biol. v. 11, n. 41, p. 1-19,



Producing GSS (Genetic Sexing Strain)

1) Mariner transposable element: Design of gene construction

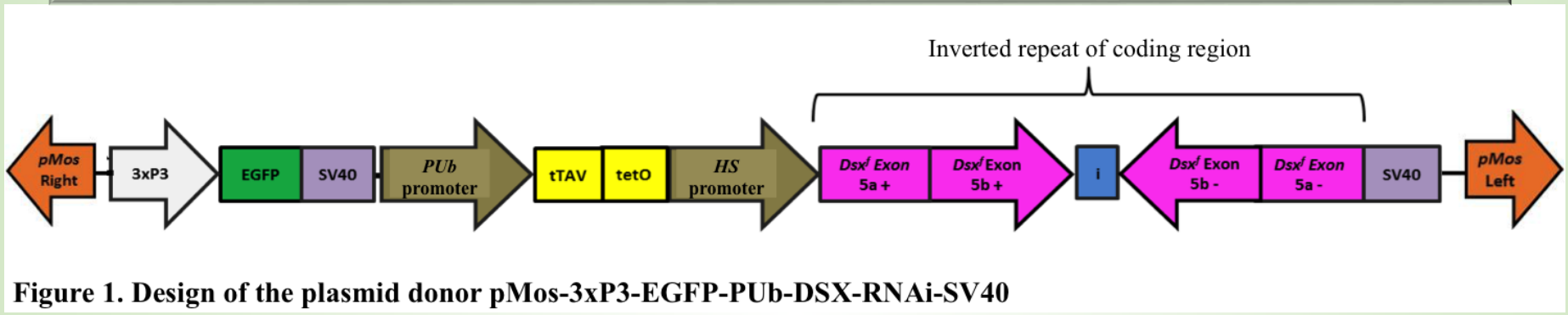
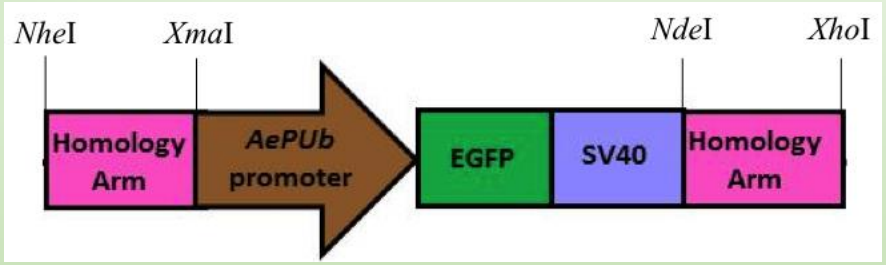
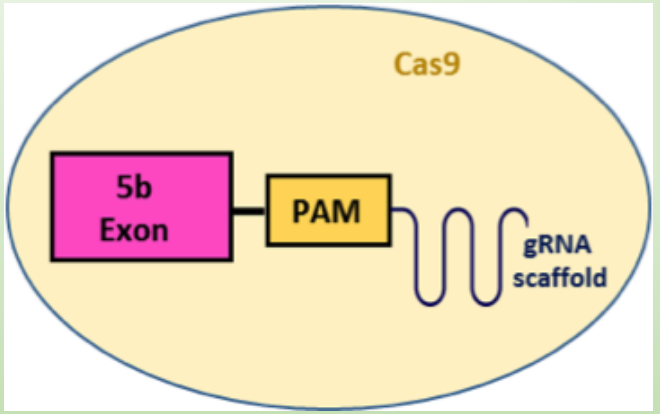


Figure 1. Design of the plasmid donor pMos-3xP3-EGFP-PUb-DSX-RNAi-SV40

2) CRISPR/Cas9: Design of gene construction

Dr. Chun-Hong Chen from National Health Research Institutes (NHRI, Taiwan)





SIT (IIT/SIT) X Transgenic

SIT – IIT/SIT	Transgenic
• Male or Female sterilization	• Sterile Male
• Damage (mutations)	• No damage
• Irradiator Source	• Male only production
• Logistics to send pupae after sterilization	• Eggs can be distribute (facilitate logistics)
• Public Engagement (easy)	• Public Engagement (difficult)

Dengue Prevention and 35 Years of Vector Control in Singapore

Eng-Eong Ooi,* Kee-Tai Goh,† and Duane J. Gubler‡

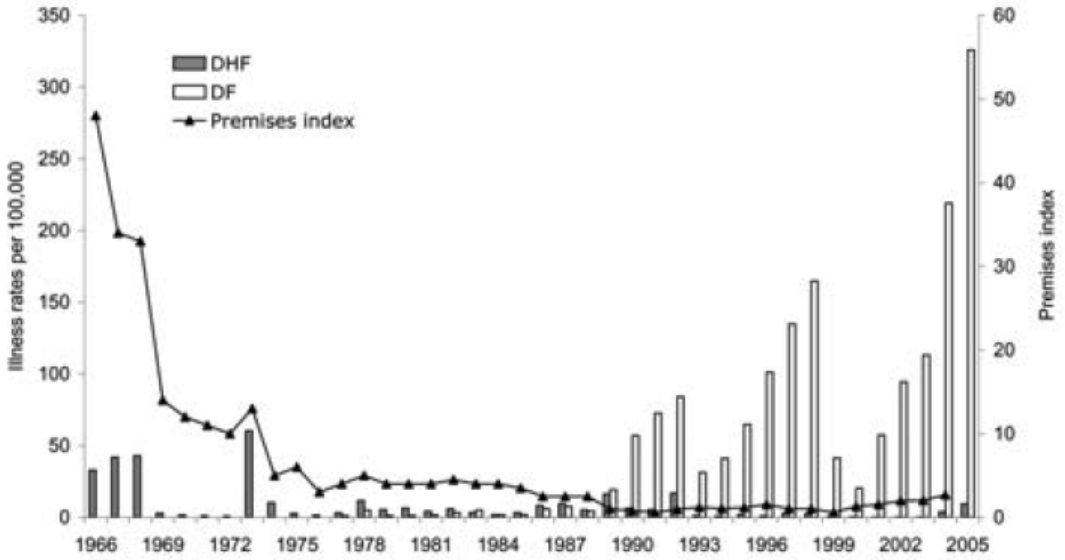
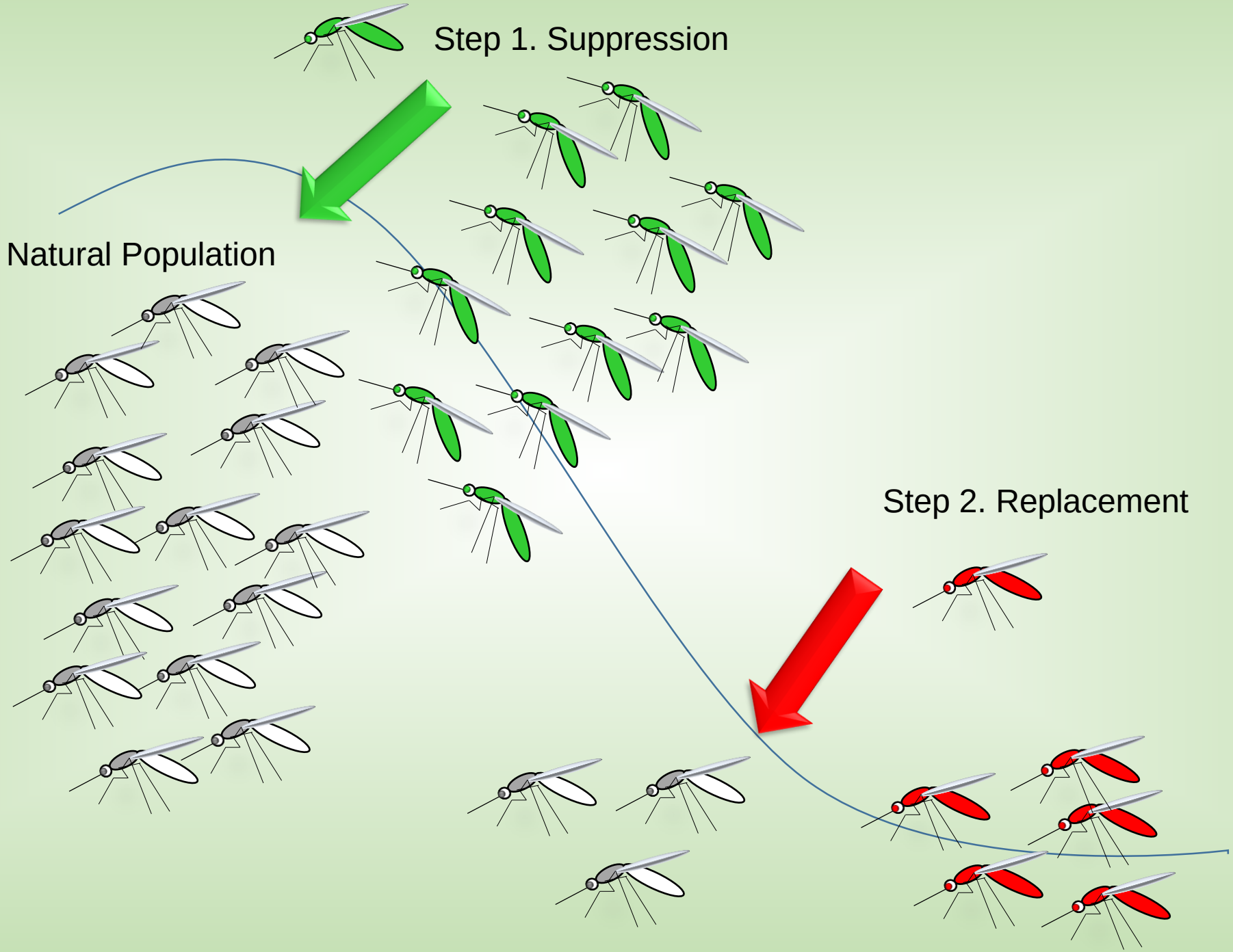


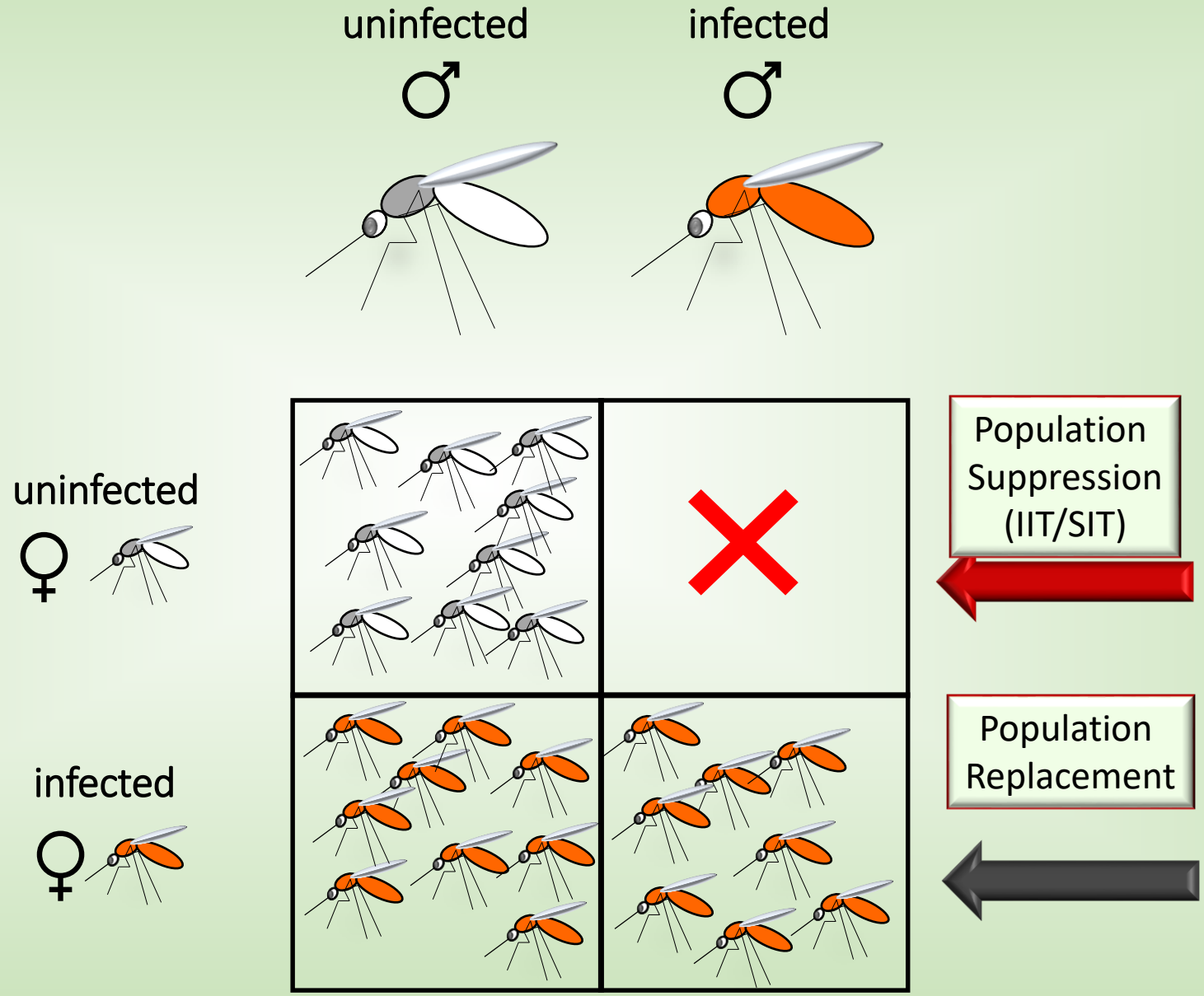
Figure 1. Annual incidence dengue fever (DF) and dengue hemorrhagic fever (DHF) and the premises index, Singapore, 1966–2005. DHF was made a notifiable disease in 1966, while DF became a notifiable disease in 1977. The annual incidences of DF and DHF reported in this figure were calculated from the number of reported cases each year from 1966 to 2004. The annual premises index is expressed as a percentage of the premises in which *Aedes aegypti* or *A. albopictus* larvae were found divided by the number of premises visited by environmental health officers.

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 12, No. 6, June 2006

After a 15-year period of low incidence, dengue has reemerged in Singapore in the past decade. We identify potential causes of this resurgence. A combination of lowered herd immunity, virus transmission outside the home, an increase in the age of infection, and the adoption of a case-reactive approach to vector control contribute to the increased dengue incidence. Singapore's experience with dengue indicates that prevention efforts may not be sustainable. For renewed success, Singapore needs to return to a vector control program that is based on carefully collected entomologic and epidemiologic data. Singapore's taking on a leadership role in strengthening disease surveillance and control in Southeast Asia may also be useful in reducing virus importation.

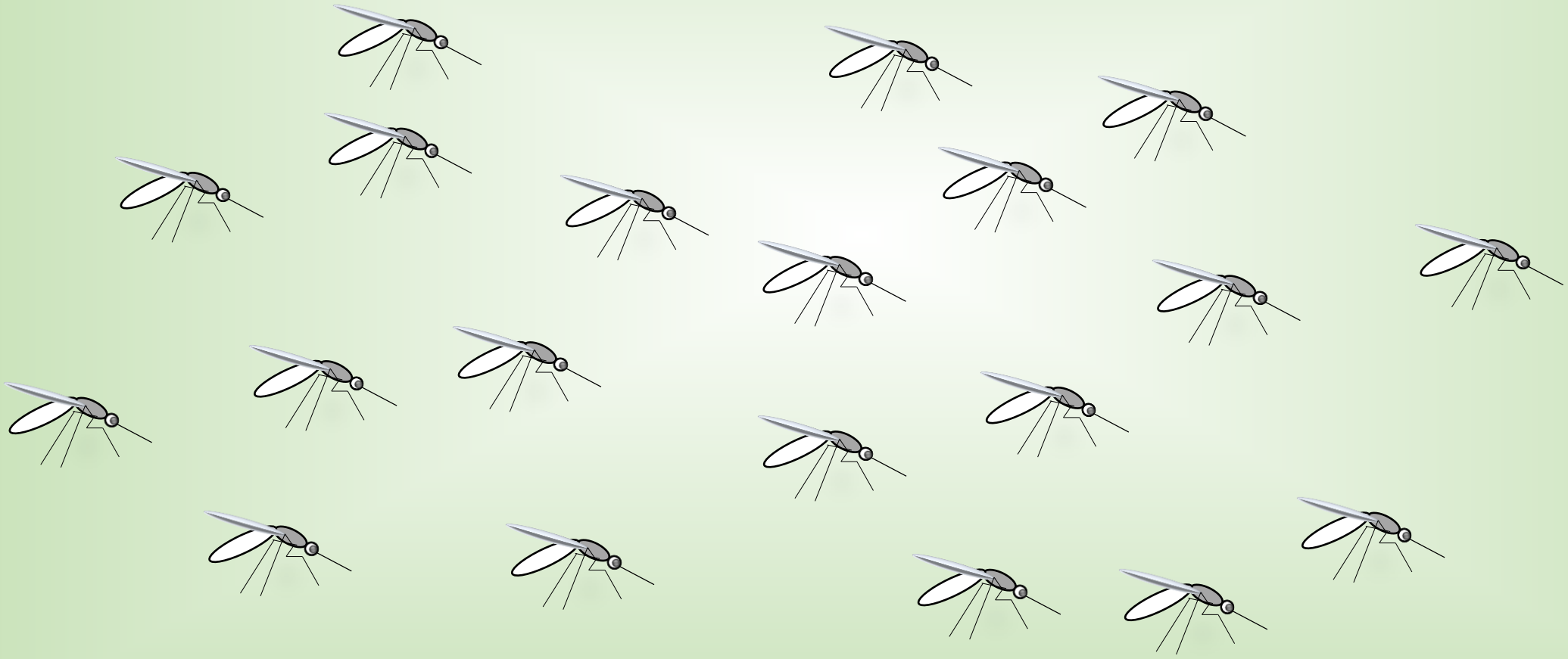

Step 1. Suppression
Natural Population
Step 2. Replacement

The Wolbachia approach (Replacement)



Gene Introduction
Virus-regulated mosquito gene
Suicidal Model (Double death model)

Natural Population

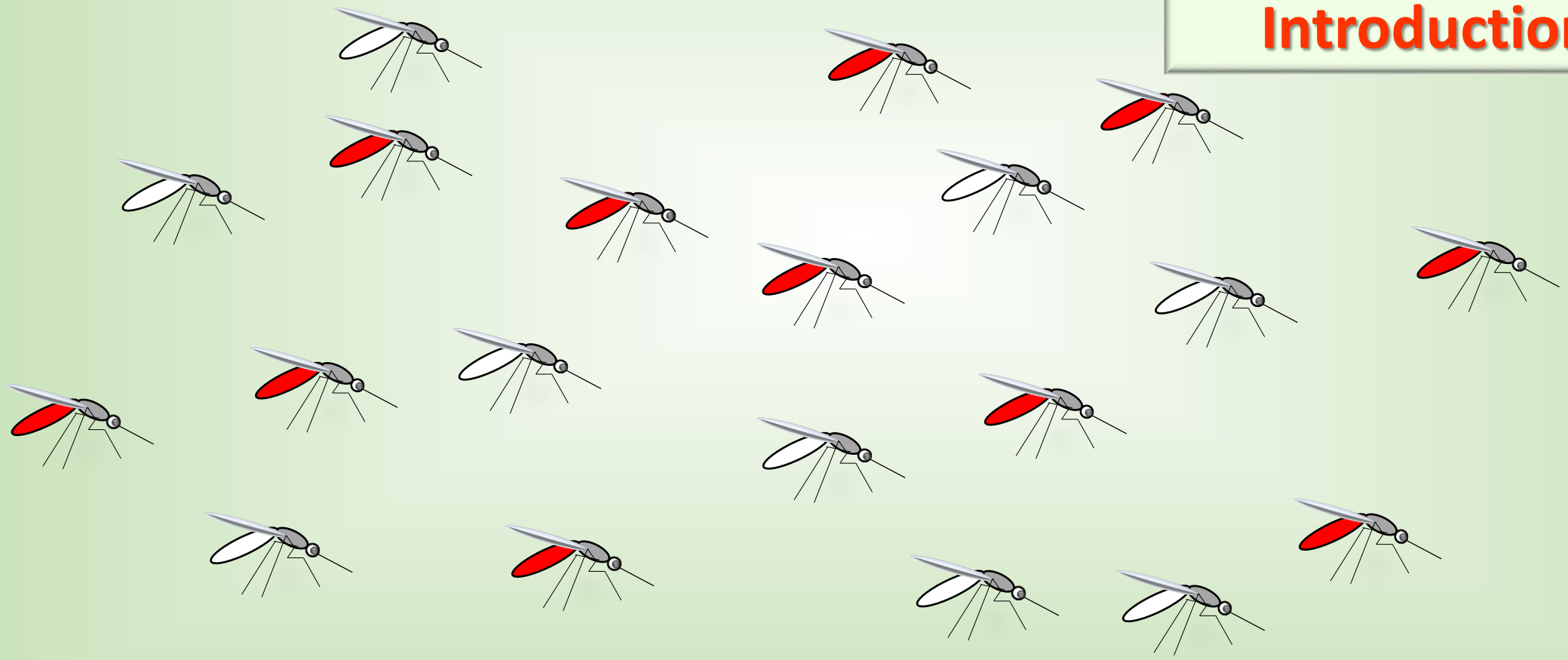


Gene Introduction
Virus-regulated mosquito gene
Suicidal Model (Double death model)

Natural Population

+

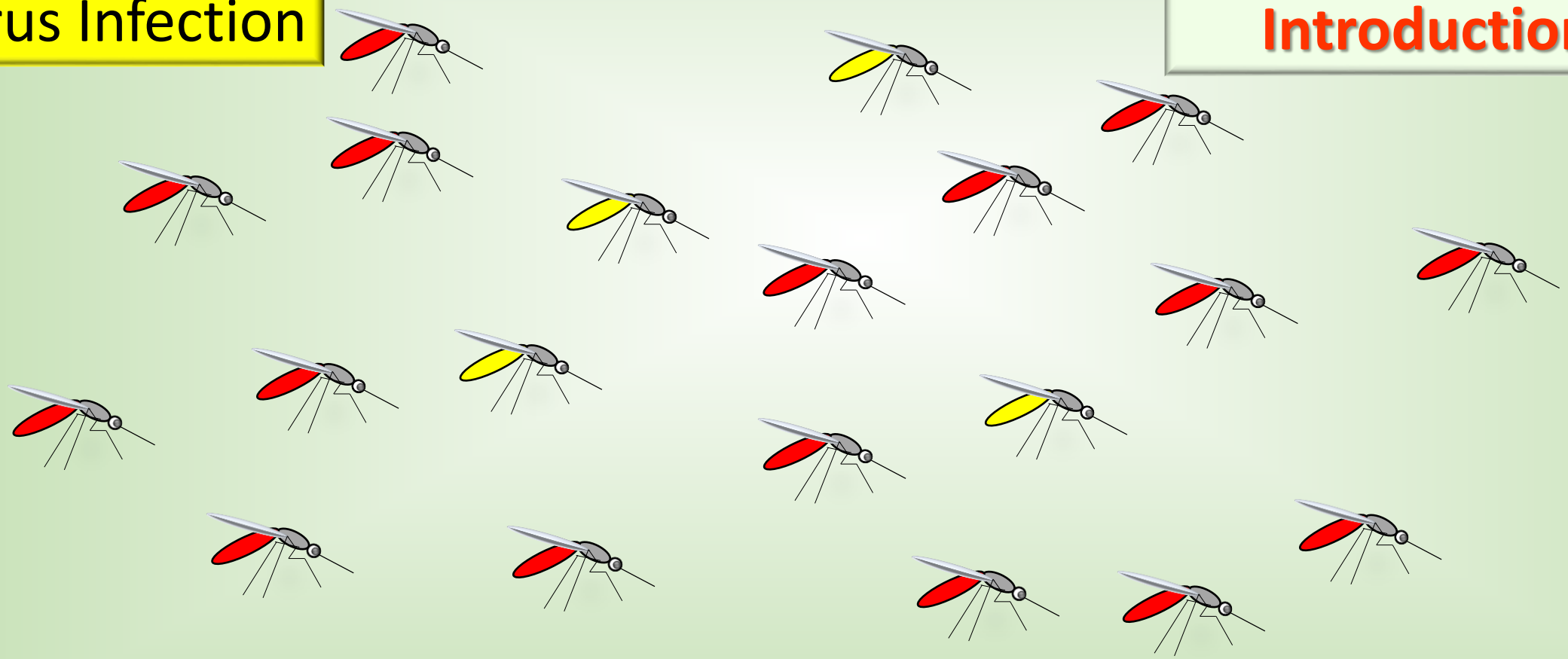
Introduction



Gene Introduction
Virus-regulated mosquito gene
Suicidal Model (Double death model)

Natural Population
+
Introduction

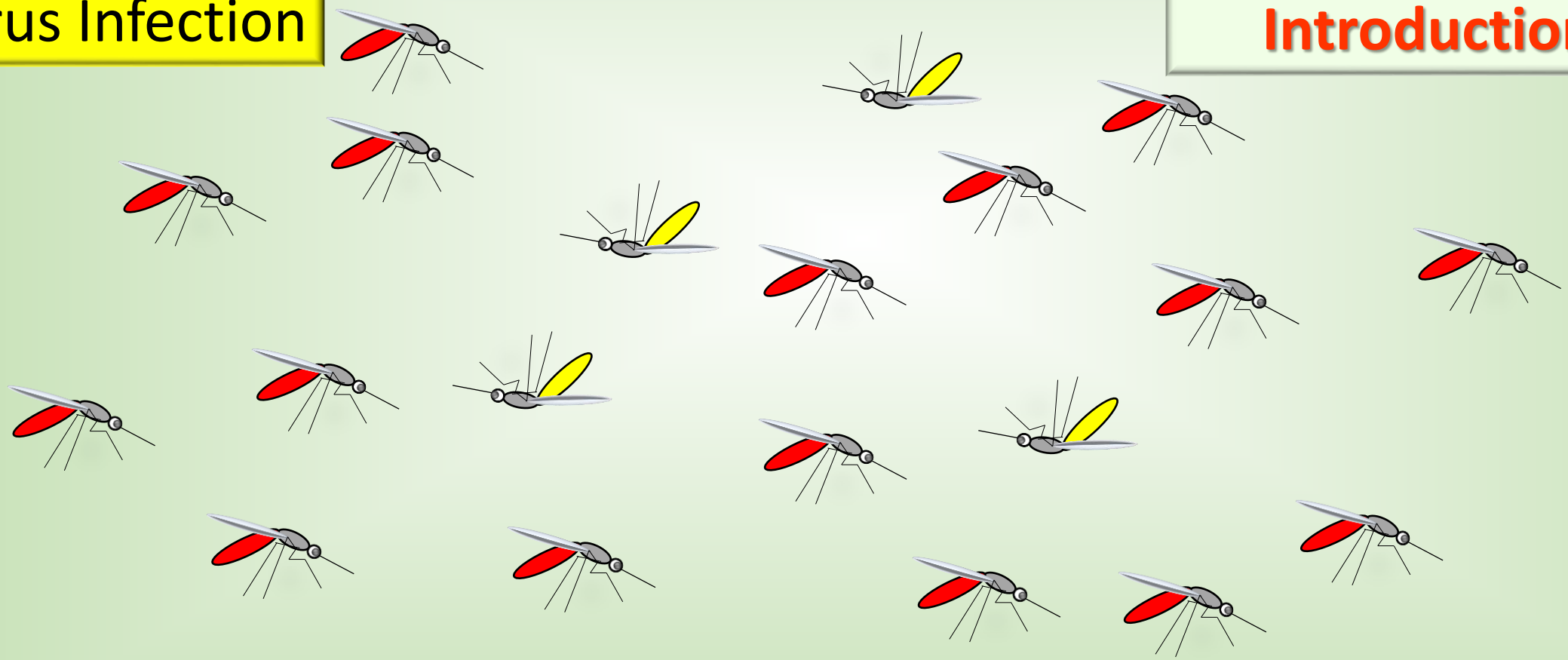
Virus Infection



Gene Introduction
Virus-regulated mosquito gene
Suicidal Model (Double death model)

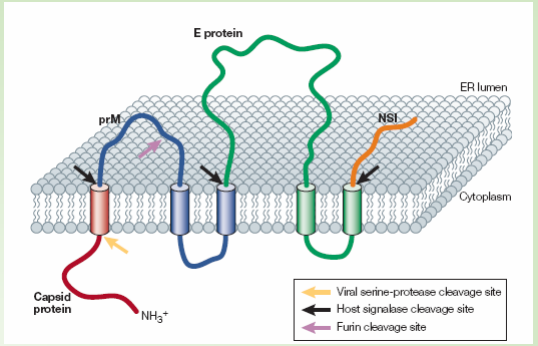
Natural Population
+
Introduction

Virus Infection



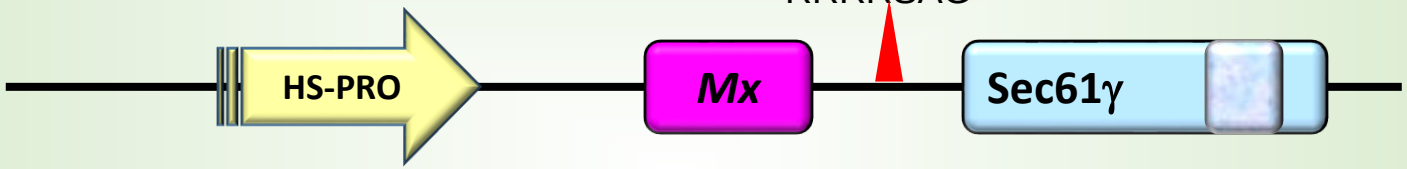
Gene Introduction
Virus-regulated mosquito gene
Suicidal Model (Double death model)

Step 2. Replacement



NS3 Cleavage site

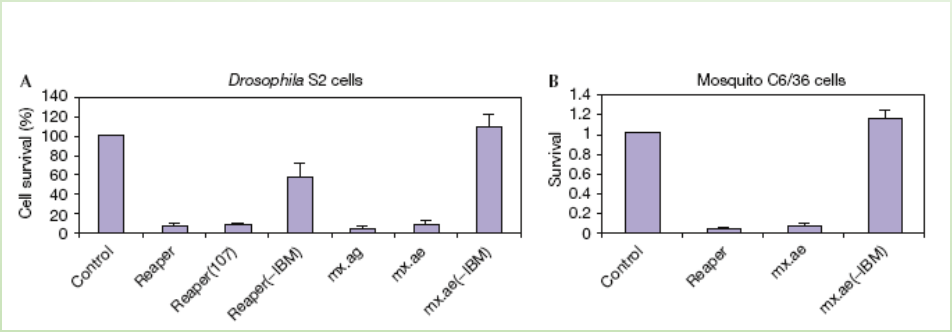
RRRRSAG

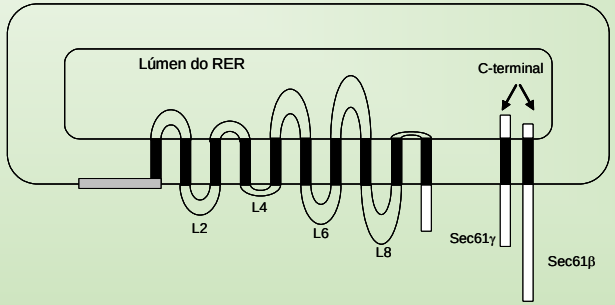


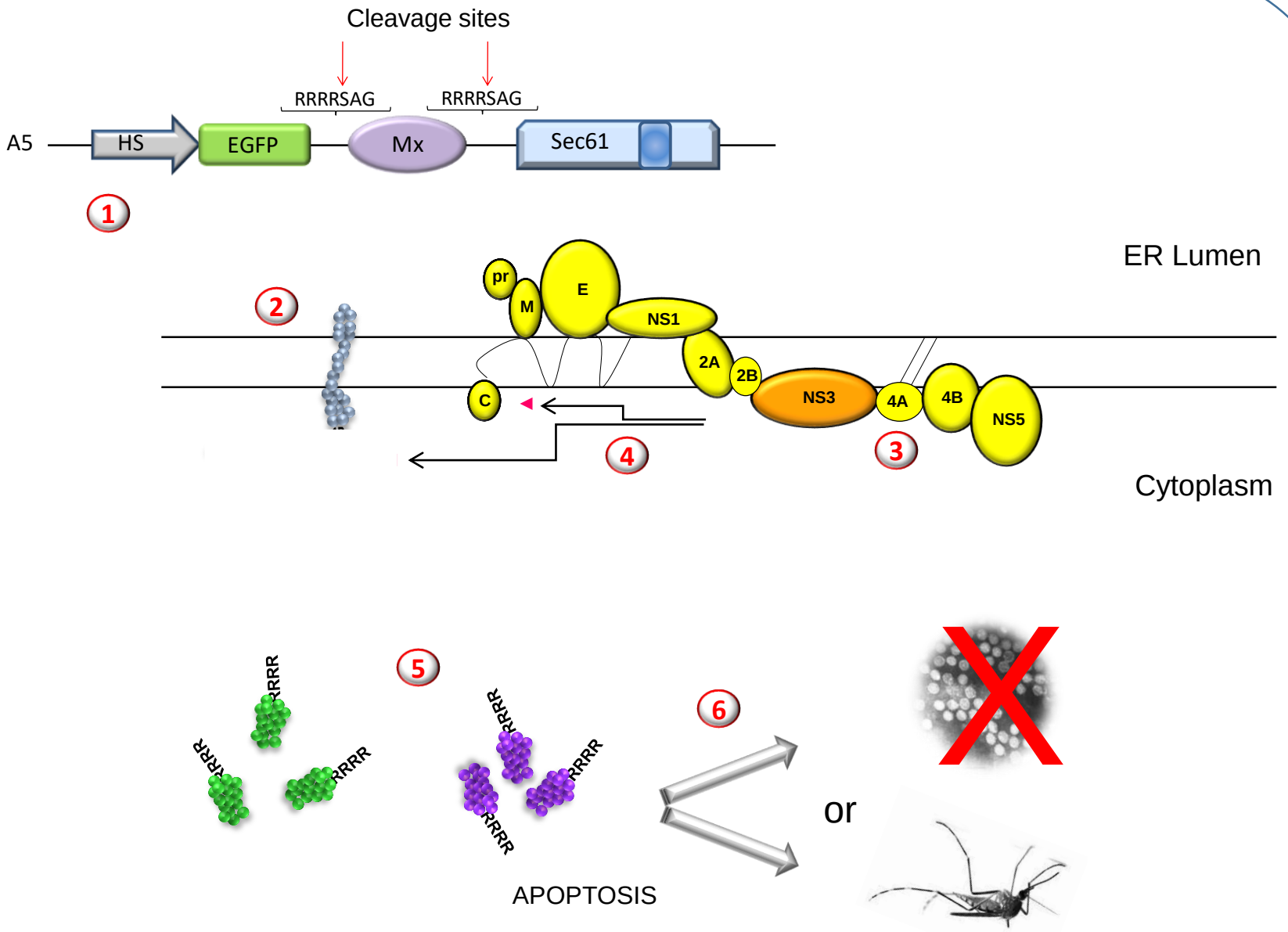
Constitutive Promoter

Lethal protein

ER localization

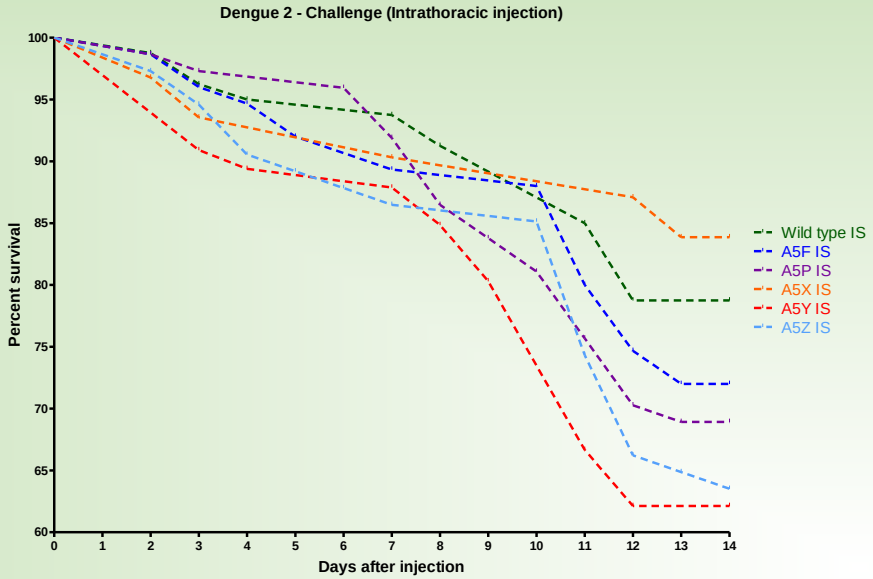


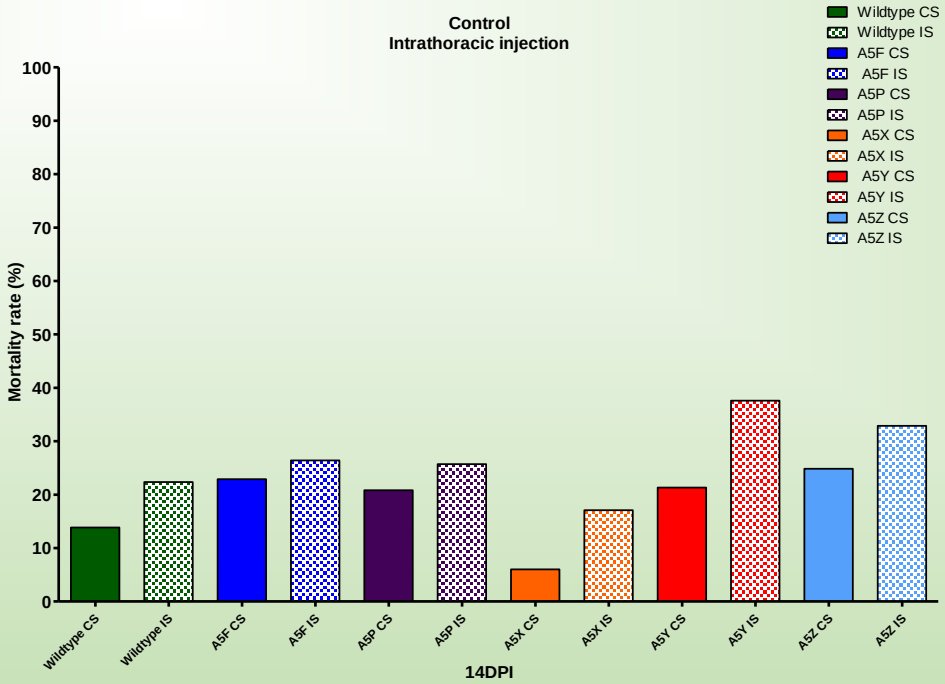




- **Double death model - Infection phenotypes in dengue challenged mosquito?**

- **Double death model - Infection phenotypes in dengue challenged mosquito?**





Wolbachia X Transgenic

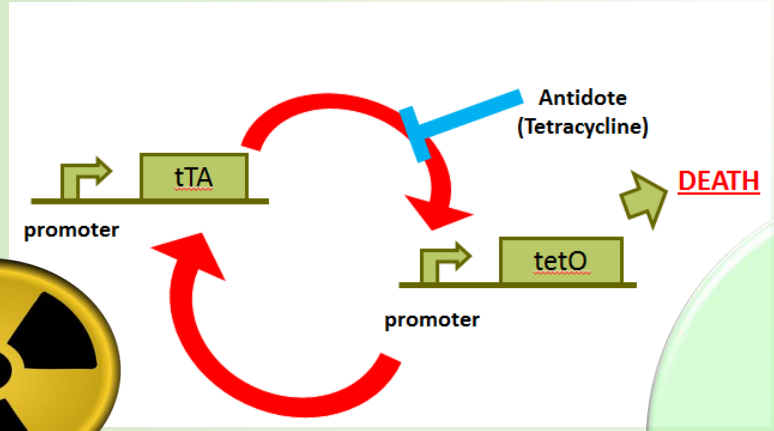
Wolbachia

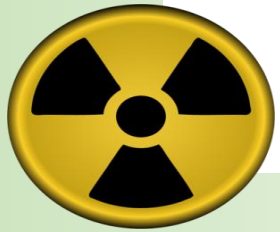
- Genome Microinjection
- Bacteria Introduction
- Female Release
- No Regulation
- Public Engagement (easy)

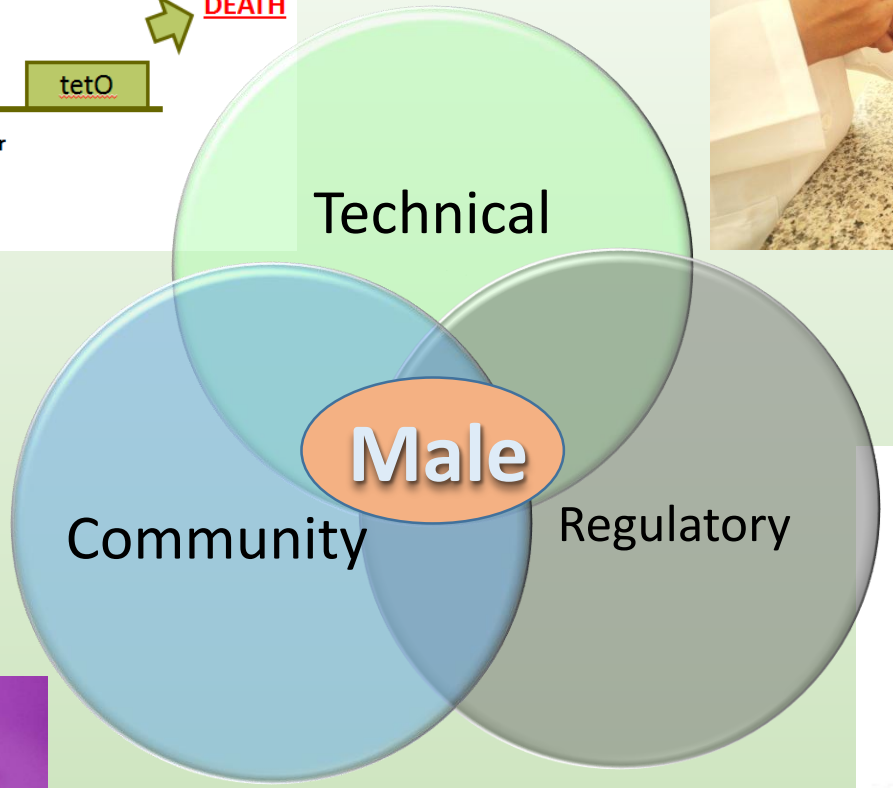
Transgenic

- Gene Microinjection
- Gene Drive
- Male Release
- Transgenic Law
- Public Engagement (difficult)

Bringing new technology to the field

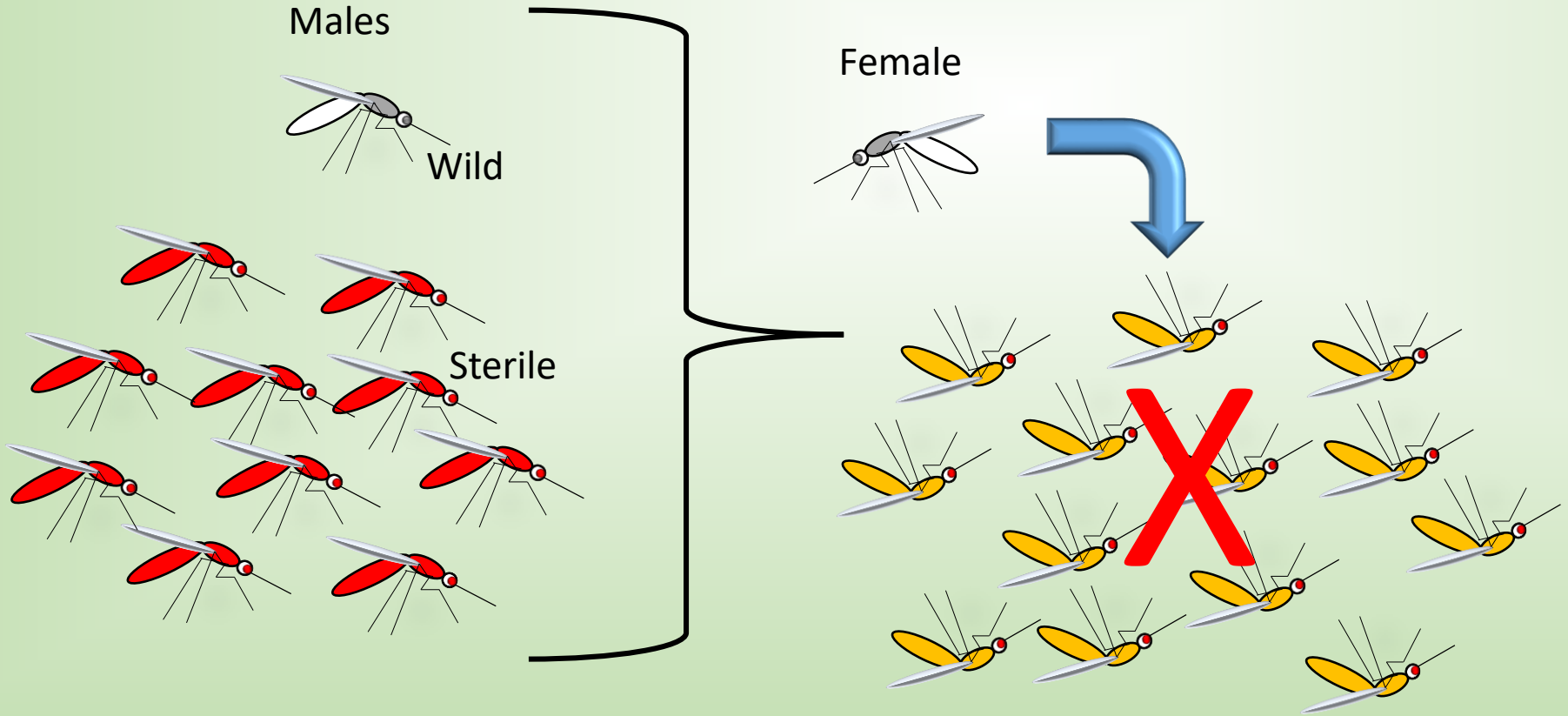






Nature

How the Sterility works?
How the Sterile Mosquito Works?
Why releasing male mosquitoes you kill mosquitoes?



Before releasing mosquitoes

- Site selection
  - Public Engagement:
    - Evolving Public Authorities (Government and agencies);
    - Local ones (community engagement);
    - Local people - explain what we are going to do in that area.




Community Engagement

Action		Period			
		Pre-release	Release		Post-release
		2010*	2011	2012	2013*
Domiciliary visit					
Internet	Social Network				
	Web site				
Interviews / appearances	TV				
	Radio				
	Newspaper				
	Magazines				
Jingle broadcast					
Leaflets distribution					
Meeting local leaders					
Questionnaires					
School presentations / lectures					
Monitoring system					
Truck loudspeakers					

* - In both years, the columns are representing the last two semesters and the first two respectively.

City Hall Public Hearing

Community Engagement

Total people 17,101,269 in Brazil – Based on the Brazilian Institute of Public Opinion and Statistics (IBOPE) data

Talks and Lectures

Leaflet distribution

Projeto Aedes Transgênico

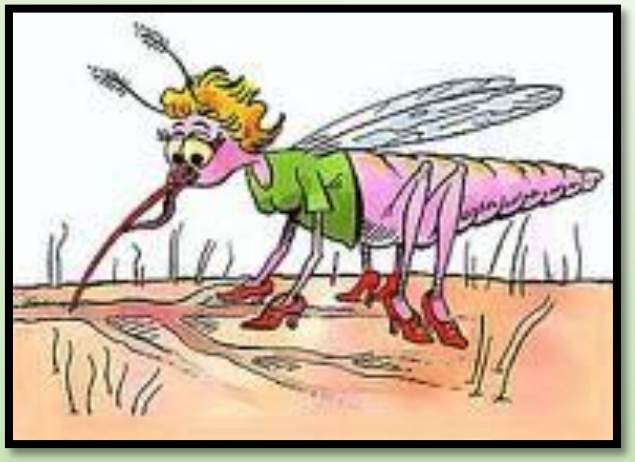
Mosquito Aedes \Dengue

Pica durante o dia (bite during the day)

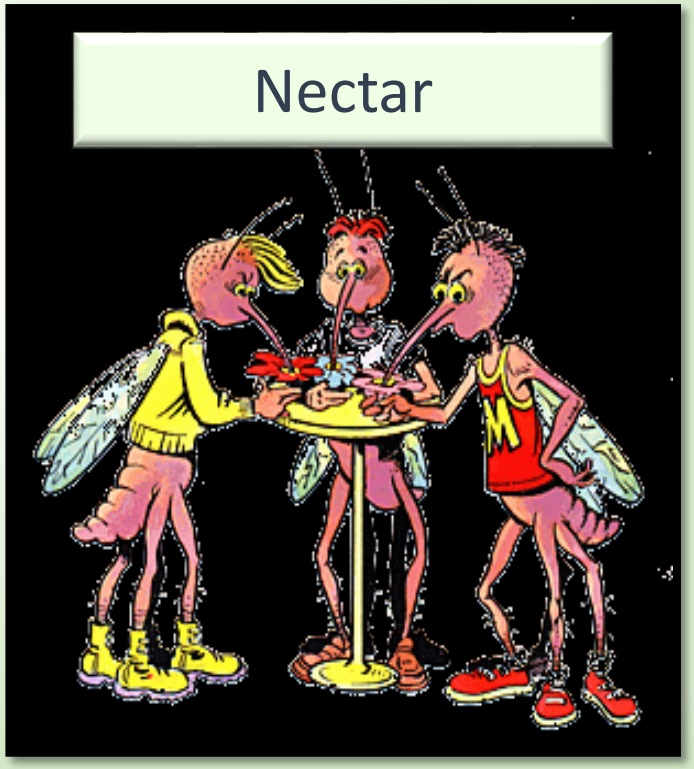
Muriçoca (*Culex)*

Pica durante a noite (bite during the night)

Bar – Blood for Sale!



Only females (girls) bite



Fonte: Google

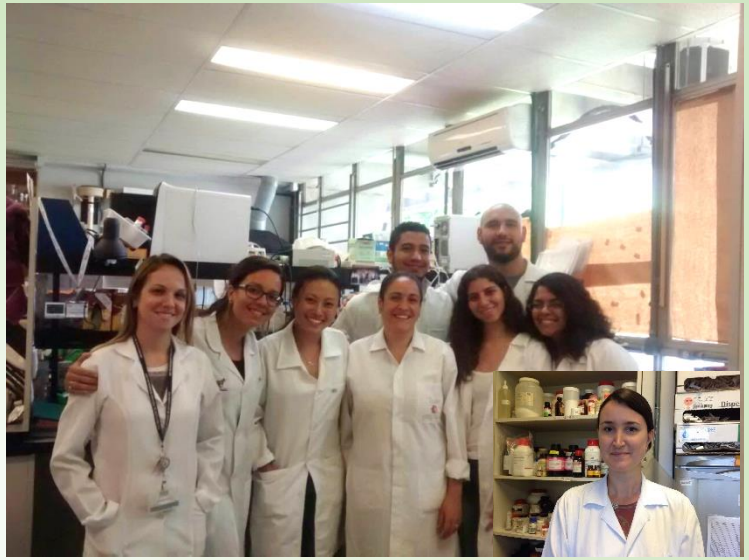
ACTION	TARGET POPULATION LEVEL	# EVENTS	# PEOPLE
Presentations/Lectures	Local/Regional	10	962
Leaflets [1]	Local	-	10,000
Jingle ([1])	Local	-	-
Meetings	National/ International	39	6,020
Interviews (radio)	Regional	15	1,500
Interviews (TV)	Regional/National	09	17,094,000 [2]
Interviews (newspaper/magazine)	Local/Regional/ National	13	-
Internet (website / social network)	Regional/National	24	-[3]
Houses visited/interviewed with residents	Local	581	2,341
Meetings with local leaders, health agents	Local	16	820
Presentations at elementary and middle school	Local	08	452
Presentation at community center/city hall/others	Local	06	456
Driving truck with loudspeakers in the releasing area	Local	-	500
Spots, jingles and short messages broadcasted in local radio station	Local	52	1,200 [4]
TOTAL			17,101,269

STRATEGIES		
Mandatory	Recommended	Suggested
- Visit/interview sample/every house in the target area	- Lectures at community centers/churches – targeting adults	- Action within a local event (parade, carnival, street fairs)
- Meetings with local leaders, school principals, district managers	- Radio spots, jingles and messages broadcasted	- Driving truck with loudspeakers in the targeting area – jingle and messages
- Lectures at schools – targeting kids/teens	- Press releases by Moscamed journalists	- Use of social media: Facebook and twitter
- Press coverage at local/regional level of PAT activities: production, releases	- PAT technical personnel interviewed by local/regional/(inter)national radio stations	- Press coverage at international level of PAT activities: releases
	- Press coverage at national level of PAT activities: production, releases	

Moscamed Brasil

UPAT

Universidade de São Paulo



LEMI

MOSCAMED BRASIL

USP Universidade de São Paulo

GOVERNO DA Bahia TERRA DE TODOS NÓS Secretaria de Ciência, Tecnologia e Inovação

FAPESP

CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico

IAEA International Atomic Energy Agency

INCT - EM
PRONEX/DECIT

SPOT

To control dengue Moscamed is releasing in this community

A large amount of TRANSGENIC MOSQUITOES .

We would like to recall that this mosquitoes are not the well known

CULEX

They are transgenic MALES and they DON'T BITE.

They are good fellows that will give you protection against dengue.

For more information call a health agent or get in touch with

MOSCAMED

By the phone

(74) 3612-5399

PAT –AEDES TRANSGENIC PROJECT

This one makes the difference.

Jingle Transgenic *Aedes*