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 Suppression

- **Mechanical Control**: Remove breeding sites
  - Quits ops
  - Adult traps
  - Massive Collection
- **Population Suppression**
  - Larycides
  - Adulticides
  - Biological Control
  - Auto dissemination: Pyriproxyfen
  - SIT
  - ITS/SIT
- **Education**: Community Engagement and Responsibility
Integrate Control for *Aedes aegypti* Population Suppression

- Mechanical Control: Remove breeding sites
  - Cilfapps
  - Massive Collection
  - Larvicides
  - Fishes
  - Auto-dissemination: Pyramidoxifen

- Adulticides
  - Chemicals
  - BIT
  - ECT

- Education: Community Engagement and Responsibility

- Population Suppression
  - Transgenic
Integrate Control for *Aedes aegypti* Population Suppression

- **Mechanical Control:** Remove breeding sites
  - Cultraps
  - Adult traps
- **Larvicides:**
  - Bti
  - Fishes
- **Auto dissemination Pyriproxyfen**
- **Education:** Community Engagement and Responsibility
- **Adulticides:**
- **Population Suppression:**
  - SIT
  - RTSI
Integrate Control for *Aedes aegypti* Population Suppression

- Mechanical Control: Remove breeding sites
- Adulticides
- Larvicides
  - Bti
  - Fishes
  - Auto dissemination Pyriproxyfen
- Education: Community Engagement and Responsibility
- Population Suppression
- Chemicals
- Transgenic created using
Integrate Control for \textit{Aedes aegypti} Population Supression
Step 1. Suppression

Natural Population

Step 2. Replacement
1. Male Sterilization

2. Releases

3. Suppression

Birth Control Method:

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

= no offspring
The Wolbachia approach (Suppression) – IIT/SIT

- **Population Suppression (IIT/SIT)**
- **Population Replacement**
Open Field Release of OX513A Aedes aegypti Transgenic line evaluation
Repressive of Insects carrying a Dominant Lethal gene (RIDL)

Antidote (Tetra)

dead

tetO Minimal promoter tTA

Thomas et al. 2000 Science 287: 2474-6
*Aedes aegypti* Production (UPAT)

**COLONY**
4 to 6 million eggs/week

**Males for releases**
1.5 million/week
Itaberaba – Field site
Project Phase 2 – Jacobina - Bahia
Pupa transportation (LEMI)

C25

BOD 16°C 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

Gonads

Promoter

Sperm defect

ttO

Effector Molecule

Sperm

Males to Release

Egg Production

ttO

Normal Sperm

Tetracyclin

Gonads

Promoter
SCC Transgenes

- Two Effector molecules:
  - Endonuclease
  - IAP Antagonist
Endonucleases

- Enzymes (NEB)
  - > 1 million cuts
- Isoisomers
- Activity temperature < 50 °C
- Metilation Sensitivity
- Cut size < 5bp

Bio Restriction Analysis
## Endonucleases

<table>
<thead>
<tr>
<th>Position</th>
<th>Endonuclease</th>
<th>Cut Frequency</th>
<th>T °C</th>
<th>Type</th>
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<tbody>
<tr>
<td>1</td>
<td>HindI</td>
<td>30.032.972</td>
<td>37</td>
<td>I</td>
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<tr>
<td>2</td>
<td>CviAII</td>
<td>4.645.879</td>
<td>25</td>
<td>II</td>
</tr>
<tr>
<td>3</td>
<td>Sell</td>
<td>1.672.829</td>
<td>26</td>
<td>II</td>
</tr>
<tr>
<td>4</td>
<td>AluI</td>
<td>4.796.029</td>
<td>37</td>
<td>II</td>
</tr>
<tr>
<td>5</td>
<td>Bfal</td>
<td>2.962.089</td>
<td>37</td>
<td>II</td>
</tr>
<tr>
<td>6</td>
<td>CviRI</td>
<td>5.486.819</td>
<td>37</td>
<td>II</td>
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<tr>
<td>7</td>
<td>CviTI</td>
<td>12.095.603</td>
<td>37</td>
<td>II</td>
</tr>
<tr>
<td>8</td>
<td>HaeIII</td>
<td>1.980.700</td>
<td>37</td>
<td>II</td>
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<tr>
<td>9</td>
<td>MspI</td>
<td>2.225.385</td>
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<td>II</td>
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<tr>
<td>10</td>
<td>Tru9I</td>
<td>8.779.417</td>
<td>37</td>
<td>II</td>
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<tr>
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<td>II</td>
</tr>
</tbody>
</table>
Transgene CviAll

3XP3  DsRed  ttO  Endonuclease  B2tubulin  tTAV  SV40

ttO+HS

Very Toxic to E. coli

Replace

ttO+BhC4-1

Less Toxic to E. coli

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

RESEARCH ARTICLE Open Access

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

Adriana C Garcia1, Daniel LG Giraldo1, Fernanda C Humann2, Maria L Paço-Larson2 and Nadia Monesi1
SCC Transgenes

- Two Effector molecules:
  - Endonuclease
  - IAP Antagonist
## Transgenic Lines

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<tr>
<th></th>
<th>Act-1</th>
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<th>B2-tub</th>
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<th>Mx-T</th>
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<td></td>
</tr>
<tr>
<td>L4</td>
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<tr>
<td>PM</td>
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<td></td>
<td></td>
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<tr>
<td>AF</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**MFA07**

**MPD01**

**MPC03**

**MPD02**

**MPC02**

**MFDX**

- **150 pb**
- **350 pb**
- **150 pb**
Sterile Conditional Construct (SCC)

60% Homozygous
80% Reduction
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 ✔
Producing GSS (Genetic Sexing Strain)

1) **Mariner transposable element: Design of gene construction**

![Diagram of plasmid donor pMos-3xP3-EGFP-PUb-DSX-RNAi-SV40](image)

**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
- Male or Female sterilization
- Damage (mutations)
- Irradiator Source
- Logistics to send pupae after sterilization
- Public Engagement (easy)

Transgenic
- Sterile Male
- No damage
- Male only production
- Eggs can be distribute (facilitate logistics)
- Public Engagement (difficult)
Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 12, No. 6, June 2006

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.

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)

- Uninfected males and females
- Infected males and females

Population Suppression (IIT/SIT)
Population Replacement
Gene Introduction
Virus-regulated mosquito gene
Suicidal Model (Double death model)
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)

Virus Infection

Natural Population +

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

Virus Infection

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

Step 2. Replacement

NS3 Cleavage site

Constitutive Promoter

Lethal protein

ER localization

- HS-PRO
- Mx
- Sec61γ

Graphs showing experimental results.
• 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
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

<table>
<thead>
<tr>
<th>Action</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-release</td>
</tr>
<tr>
<td></td>
<td>2010*</td>
</tr>
<tr>
<td>Domiciliary visit</td>
<td></td>
</tr>
<tr>
<td>Internet</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Social Network</td>
</tr>
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<td></td>
<td>Web site</td>
</tr>
<tr>
<td>Interviews / appearances</td>
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</tr>
<tr>
<td></td>
<td>TV</td>
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<tr>
<td></td>
<td>Radio</td>
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<tr>
<td></td>
<td>Newspaper</td>
</tr>
<tr>
<td></td>
<td>Magazines</td>
</tr>
<tr>
<td>Jingle broadcast</td>
<td></td>
</tr>
<tr>
<td>Leaflets distribution</td>
<td></td>
</tr>
<tr>
<td>Meeting local leaders</td>
<td></td>
</tr>
<tr>
<td>Questionnaires</td>
<td></td>
</tr>
<tr>
<td>School presentations / lectures</td>
<td></td>
</tr>
<tr>
<td>Monitoring system</td>
<td></td>
</tr>
<tr>
<td>Truck loudspeakers</td>
<td></td>
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</tbody>
</table>

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

Nectar
<table>
<thead>
<tr>
<th>ACTION</th>
<th>TARGET POPULATION LEVEL</th>
<th># EVENTS</th>
<th># PEOPLE</th>
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<tbody>
<tr>
<td>Presentations/Lectures</td>
<td>Local/Regional</td>
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<td>962</td>
</tr>
<tr>
<td>Leaflets (¹)</td>
<td>Local</td>
<td>-</td>
<td>10,000</td>
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<tr>
<td>Jingle (¹)</td>
<td>Local</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Meetings</td>
<td>National/International</td>
<td>39</td>
<td>6,020</td>
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<tr>
<td>Interviews (radio)</td>
<td>Regional</td>
<td>15</td>
<td>1,500</td>
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<td>Interviews (TV)</td>
<td>Regional/National</td>
<td>09</td>
<td>17,094,000 (²)</td>
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<tr>
<td>Interviews (newspaper/magazine)</td>
<td>Local/Regional/National</td>
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<td>-</td>
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<tr>
<td>Internet (website / social network)</td>
<td>Regional/National</td>
<td>24</td>
<td>- (³)</td>
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<tr>
<td>Houses visited/interviewed with residents</td>
<td>Local</td>
<td>581</td>
<td>2,341</td>
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<tr>
<td>Meetings with local leaders, health agents</td>
<td>Local</td>
<td>16</td>
<td>820</td>
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<tr>
<td>Presentations at elementary and middle school</td>
<td>Local</td>
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<td>452</td>
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<td>Presentation at community center/city hall/others</td>
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<td>06</td>
<td>456</td>
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<tr>
<td>Driving truck with loudspeakers in the releasing area</td>
<td>Local</td>
<td>-</td>
<td>500</td>
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<tr>
<td>Spots, jingles and short messages broadcasted in local radio station</td>
<td>Local</td>
<td>52</td>
<td>1,200 (⁴)</td>
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<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td>17,101,269</td>
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<td>STRATEGIES</td>
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<td>Recommended</td>
<td>Suggested</td>
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<tr>
<td>-----------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>- Visit/interview</td>
<td></td>
<td>- Lectures at community centers/churches – targeting adults</td>
<td>- Action within a local event (parade, carnival, street fairs)</td>
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<tr>
<td>sample/every house in the</td>
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<tr>
<td>target area</td>
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<td></td>
<td></td>
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<tr>
<td>- Meetings with local</td>
<td></td>
<td>- Radio spots, jingles and messages broadcasted</td>
<td>- Driving truck with loudspeakers in the targeting area – jingle and</td>
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<tr>
<td>leaders, school principals,</td>
<td></td>
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<td>messages</td>
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<td>district managers</td>
<td></td>
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<tr>
<td>- Lectures at schools –</td>
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<td>- Press releases by Moscamed journalists</td>
<td>- Use of social media: Facebook and twitter</td>
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<tr>
<td>targeting kids/teens</td>
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<tr>
<td>- Press coverage at</td>
<td></td>
<td>- PAT technical personnel interviewed by local/regional/(inter)national</td>
<td>- Press coverage at international level of PAT activities: releases</td>
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<tr>
<td>local/regional level of PAT</td>
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<td>radio stations</td>
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<tr>
<td>- Press coverage at</td>
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<td>- Press coverage at national level of PAT activities: production, releases</td>
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Moscamed Brasil
UPAT
Universidade de São Paulo
LEMI
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