Advances in *Wolbachia*-based biological control of mosquitoes: lessons learned from the South Pacific

Eric W. Chambers
Department of Biology
Valdosta State University, Valdosta GA
Outline of Presentation

• What’s the problem? - Review of lymphatic filariasis

• *Aedes polynesiensis* – A unique mosquito

• Wolbachia based control of *Aedes polynesiensis*

• Future research
Lymphatic filariasis (LF)

- Global Distribution - endemic in 83 countries
  - 120 million infected
  - 1 billion at risk
Lymphatic filariasis in the Pacific
<table>
<thead>
<tr>
<th>Vector</th>
<th>Countries Where Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aedes cooki</td>
<td>Niue</td>
</tr>
<tr>
<td>Aedes fijiensis</td>
<td>Fiji</td>
</tr>
<tr>
<td>Aedes horrensces</td>
<td>Fiji</td>
</tr>
<tr>
<td>Aedes kochi</td>
<td>Papua New Guinea</td>
</tr>
<tr>
<td>Aedes marshallensis</td>
<td>Kiribati</td>
</tr>
<tr>
<td>Aedes oceanicus</td>
<td>Tonga</td>
</tr>
<tr>
<td><strong>Aedes polynesiensis</strong></td>
<td>Am Samoa, Samoa, Cook Islands, Tokelau, Tuvalu, French Polynesia, Wallis and Futuna, Fiji</td>
</tr>
<tr>
<td>Aedes pseudoscutellaris</td>
<td>Fiji</td>
</tr>
<tr>
<td>Aedes rotumae</td>
<td>Rotuma Island in Fiji</td>
</tr>
<tr>
<td>Aedes samoanus</td>
<td>Samoa</td>
</tr>
<tr>
<td>Aedes tabu</td>
<td>Tonga</td>
</tr>
<tr>
<td>Aedes tutuilae</td>
<td>Samoa</td>
</tr>
<tr>
<td>Aedes upolensis</td>
<td>Samoa</td>
</tr>
<tr>
<td><strong>Ochlerotatus vigilax</strong></td>
<td>New Caledonia, Fiji</td>
</tr>
<tr>
<td><strong>An punctulatus complex</strong></td>
<td>Papua New Guinea, Solomon Islands, Vanuatu</td>
</tr>
<tr>
<td>Culex annulirostris</td>
<td>Irian Jaya</td>
</tr>
<tr>
<td><strong>Culex quinquefasciatus</strong></td>
<td>Kiribati, Pelau, Fed States Micronesia, PNG, Fiji, etc</td>
</tr>
<tr>
<td>Mansonia uniformis</td>
<td>Papua New Guinea</td>
</tr>
</tbody>
</table>
Aedes polynesiensis (Marks)

• Found only on the islands of the South Pacific
• Day-biting mosquito
• Exophilic
• Major vector of lymphatic filariasis (LF)
Is MDA enough in the South Pacific?

- Treatment with DEC since 1955
- Antigen prevalence of 4.6%
- Mosquito infection rate of 1.4%
Society islands – Marquesas = 12.3%

Tahiti = 11.5%

Australes-Tuamotu Gambier = 12.3%

Slide credit: Herve Bossin
What makes transmission of LF by Aedes polynesiensis in the South Pacific unique?

1. Multiple breeding sites - standard vector control is difficult

2. Efficiency as a vector increases as microfilarial load decreases
Representative breeding sites for *Aedes polynesiensis*

Photos courtesy Limb Hapairai
Additional *Aedes polynesiensis* breeding sites

Crab holes

Coconuts

Photos courtesy Herve Bossin
**Aedes polynesiensis**

**Limitation and facilitation in the vectors and other aspects of the dynamics of filarial transmission: the need for vector control against Anopheles-transmitted filariasis**

G. PICHON

*FIG. 1. A diagrammatic representation of limitation (L) and facilitation (F), here shown for the production of first-stage larvae (L₁) from ingested microfilariae. H, the inverse of the regression slope coefficient b, allows for measurement of the ‘reciprocal adaptation’ for a particular vector-parasite combination.*
FIG. 1. A diagrammatic representation of limitation (L) and facilitation (F), here shown for the production of first-stage larvae (L_1) from ingested microfilariae. H, the inverse of the regression slope coefficient b, allows for measurement of the ‘reciprocal adaptation’ for a particular vector-parasite combination.
Biological control using incompatible insect technique (IIT)

Incompatible male mosquito

*(Wolbachia CI)*
Wolbachia pipentis

- intracellular bacterium
- first observed in *Culex pipiens*
- may infect up to 60-70% insects
- maternally inherited
- does not infect vertebrates

Insect cell containing Wolbachia

Image courtesy Eliminate Dengue
Bidirectional cytoplasmic incompatibility

Abnormal heterochromatic paternal chromosome in developing embryo

Slide credits: Stephen Dobson
How to build a better mosquito!

- CP mosquito
  - AP genetic background
  - AR wolbachia type
  - >100 individuals/generation
# Crosses and Patterns of CI

<table>
<thead>
<tr>
<th>Female × Male</th>
<th>Percent egg hatch ± s.e.m.; no. replicate crosses</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR × APT</td>
<td>13.6 ± 17%; n=14</td>
</tr>
<tr>
<td>CP × AP</td>
<td>0.23 ± 0.11%; n=18</td>
</tr>
<tr>
<td>AP × CP</td>
<td>0.0 ± 0%; n=28</td>
</tr>
<tr>
<td>AP × AP</td>
<td>87.8 ± 9.7%; n=8</td>
</tr>
<tr>
<td>CP × CP</td>
<td>62.1 ± 4%; n=18</td>
</tr>
</tbody>
</table>

AR = *Aedes riversi*; AP = *Aedes polynesiensis*; APT = Aposymbiotic *Aedes polynesiensis*
CP = hybrid progeny from AR × APT cross

CP male competitiveness

Ae. polynesiensis population cage suppression with bidirectionally incompatible males

Slide Credit: Corey Brelsfoard
Moving to the field

Medical entomology Research station Paea, Tahiti

Slide credit: Herve Bossin
Why semi-field testing?

• laboratory strains may have lower relative fitness compared to wild type mosquitoes.

• fitness difference may not become apparent until lab strains are placed in natural conditions.
Experimental design

1) Allow males and females to mate - 24 hours
2) Remove mosquitoes/record mortality
3) Individualize females then bloodfeed
4) Hatch eggs and calculate % egg hatch
6) Determine proportion of females producing hatching broods

APA = Ae. polynesiensis mosquitoes collected in Atimaono, Tahiti (F1 used for experiments)
Experimental design

- Three treatments
  - 0% CP
  - 50% CP
  - 100% CP
- Six tent locations
- 2 reps
  - Each treatment appears twice in each rep

- Five treatments
  - 0% CP
  - 25% CP
  - 50% CP
  - 75% CP
  - 100% CP
- 5 tent locations
- 3 reps
Semi-Field Cage
Male mortality

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Ratio APA:CP</th>
<th>% mortality ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0:50</td>
<td>9.0±1.7</td>
</tr>
<tr>
<td></td>
<td>25:25</td>
<td>5.0±2.4</td>
</tr>
<tr>
<td></td>
<td>50:0</td>
<td>9.0±4.4</td>
</tr>
<tr>
<td>B</td>
<td>0:50</td>
<td>6.7±3.5</td>
</tr>
<tr>
<td></td>
<td>12:38</td>
<td>8.0±4.2</td>
</tr>
<tr>
<td></td>
<td>25:25</td>
<td>5.3±1.3</td>
</tr>
<tr>
<td></td>
<td>38:12</td>
<td>7.3±1.8</td>
</tr>
<tr>
<td></td>
<td>50:0</td>
<td>8.0±4.0</td>
</tr>
</tbody>
</table>

Assessment of *Aedes polynesiensis* CP male competitiveness in field cages

Distribution of egg hatch rates across treatment groups (pooled data from experimental designs A and B)

<table>
<thead>
<tr>
<th>% CP</th>
<th>≤10% Hatch</th>
<th>11-69% Hatch</th>
<th>≥70% Hatch</th>
<th>Total No. Broods</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7%</td>
<td>6%</td>
<td>86%</td>
<td>195</td>
</tr>
<tr>
<td>25</td>
<td>28%</td>
<td>13%</td>
<td>67%</td>
<td>72</td>
</tr>
<tr>
<td>50</td>
<td>45%</td>
<td>7%</td>
<td>48%</td>
<td>190</td>
</tr>
<tr>
<td>75</td>
<td>81%</td>
<td>5%</td>
<td>15%</td>
<td>62</td>
</tr>
<tr>
<td>100</td>
<td>97%</td>
<td>0%</td>
<td>3%</td>
<td>145</td>
</tr>
<tr>
<td>Number of spermathecae inseminated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>-----</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>11</td>
<td>586</td>
<td>8</td>
</tr>
<tr>
<td>Percent</td>
<td>0.8</td>
<td>1.8</td>
<td>96.1</td>
<td>1.3</td>
</tr>
</tbody>
</table>

doi:10.1371/journal.pntd.0001271.t002
Conclusions

• CP males are sexually compatible with APA wild type females

• CP males exhibit excellent mating competitiveness with APA wild type males in semi-field conditions

• *Aedes polynesiensis* females appear to only utilize sperm from one inseminated spermatheca
Mass rearing of CP mosquitoes

Sorting and shipping of mosquitoes for release
Field releases on uninhabited motu islands
Benefits of CI-based vector control strategy

- males only release
- no chemicals are required
- CP males are not transgenic - little risk of transfer of genetic material into the wild population
- CI is species-specific
- identification and elimination of breeding sites is not critical for success of control programs
- Active ongoing participation of community members is not essential (community engagement is still key)
- large-scale releases can target the entire vector population of each island
Future projects

• Small scale field releases – uninhabited islands
• Assessment of CP vector competence
  – Human filarial worms
  – Arboviruses (e.g. Dengue, Chikungunya)
• Development of genetic markers for monitoring CP, AP populations
• Improving mass rearing techniques
  – Genetic sexing
Mauruuru

Univ of Kentucky
Stephen Dobson
Dan Howe
Katie Garrity
Celeste Clevinger
Linda O’Connor
Mike Eskelson
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