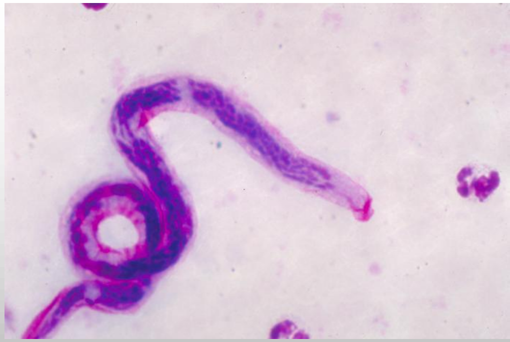
Advances in *Wolbachia*-based biological control of mosquitoes: lessons learned from the South Pacific

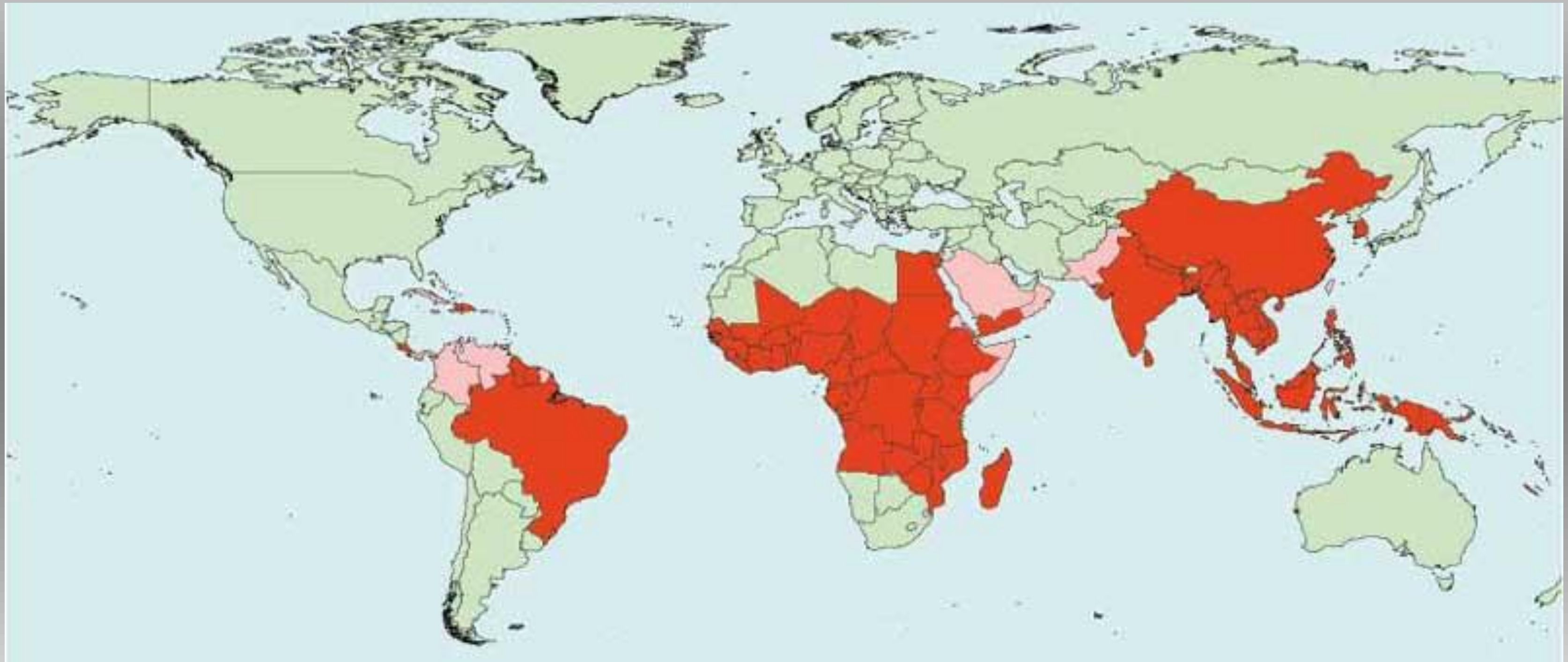
Eric W. Chambers
Department of Biology
Valdosta State University, Valdosta GA

Outline of Presentation

- What’s the problem? - Review of lymphatic filariasis
- *Aedes polynesiensis* – A unique mosquito
- Wolbachia based control of Aedes polynesiensis
- Future research

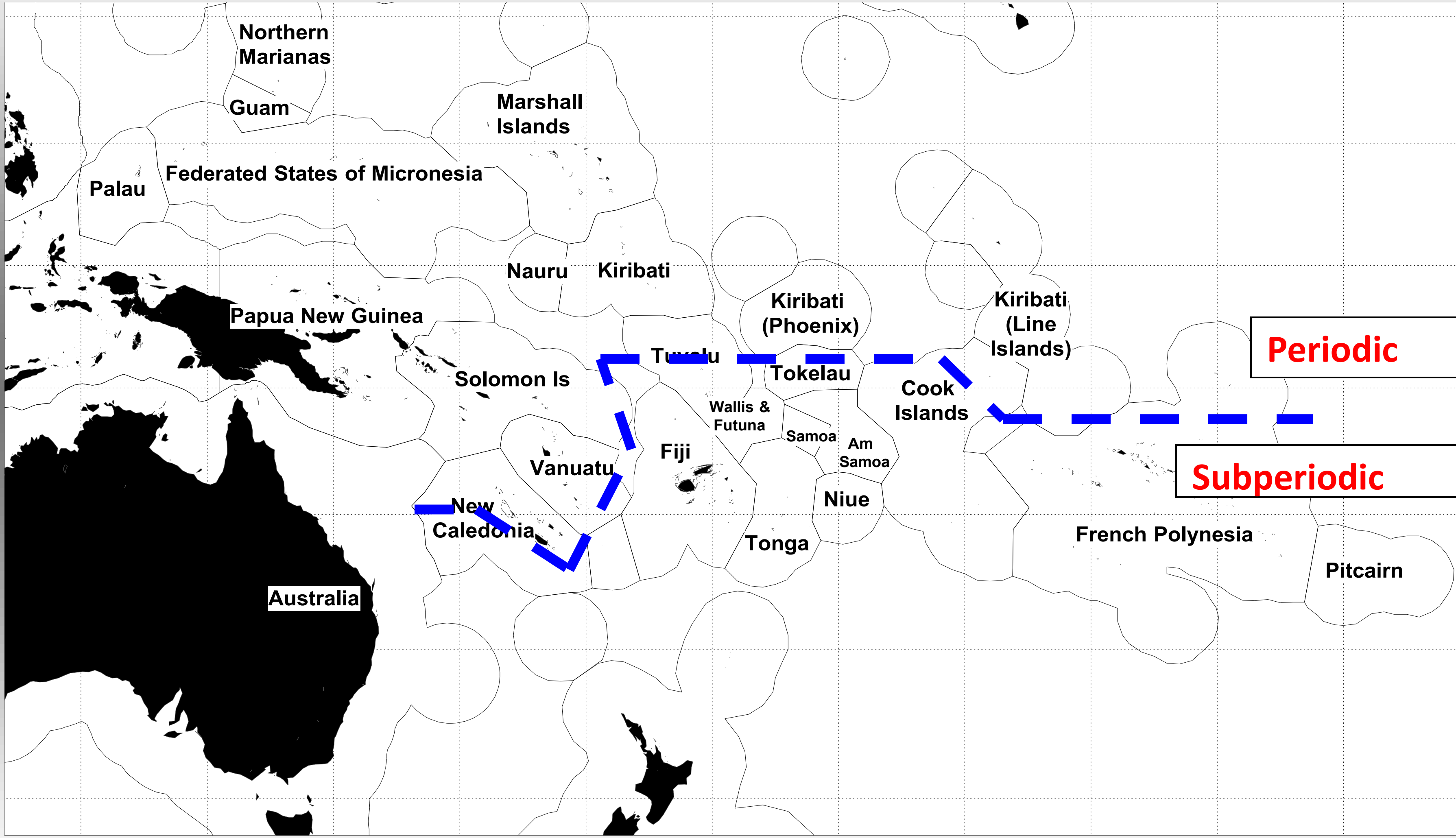


Lymphatic filariasis (LF)



- Global Distribution-endemic in 83 countries
  - 120 million infected
  - 1 billion at risk

Lymphatic filariasis in the Pacific

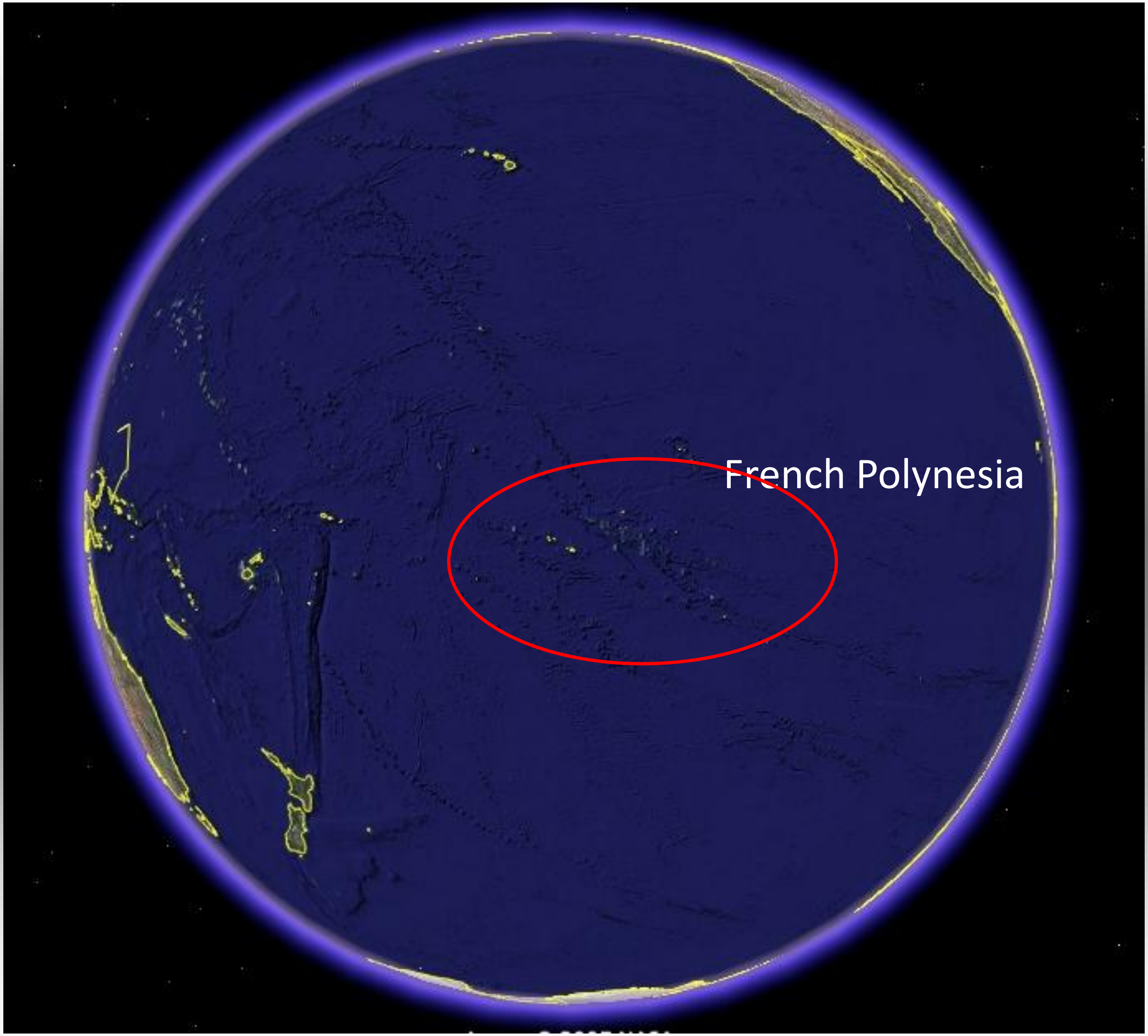


Lymphatic filariasis vectors in the Pacific

Vector	Countries Where Found
Aedes cooki	Niue
Aedes fijiensis	Fiji
Aedes horrensces	Fiji
Aedes kochi	Papua New Guinea
Aedes marshallensis	Kiribati
Aedes oceanicus	Tonga
Aedes polynesiensis	Am Samoa, Samoa, Cook Islands, Tokelau, Tuvalu, French Polynesia, Wallis and Futuna, Fiji
Aedes pseudoscutellaris	Fiji
Aedes rotumae	Rotuma Island in Fiji
Aedes samoanus	Samoa
Aedes tabu	Tonga
Aedes tutuilae	Samoa
Aedes upolenis	Samoa
Ochlerotatus vigilax	New Caledonia, Fiji
An punctulatus complex	Papua New Guinea, Solomon Islands, Vanuatu
Culex annulirostris	Irian Jaya
Culex quinquefasciatus	Kiribati, Pelau, Fed States Micronesia, PNG, Fiji, etc
Mansonia uniformis	Papua New Guinea

Aedes polynesiensis (Marks)

- Found only on the islands of the South Pacific
- Day-biting mosquito
- Exophilic
- Major vector of lymphatic filariasis (LF)


French Polynesia

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%

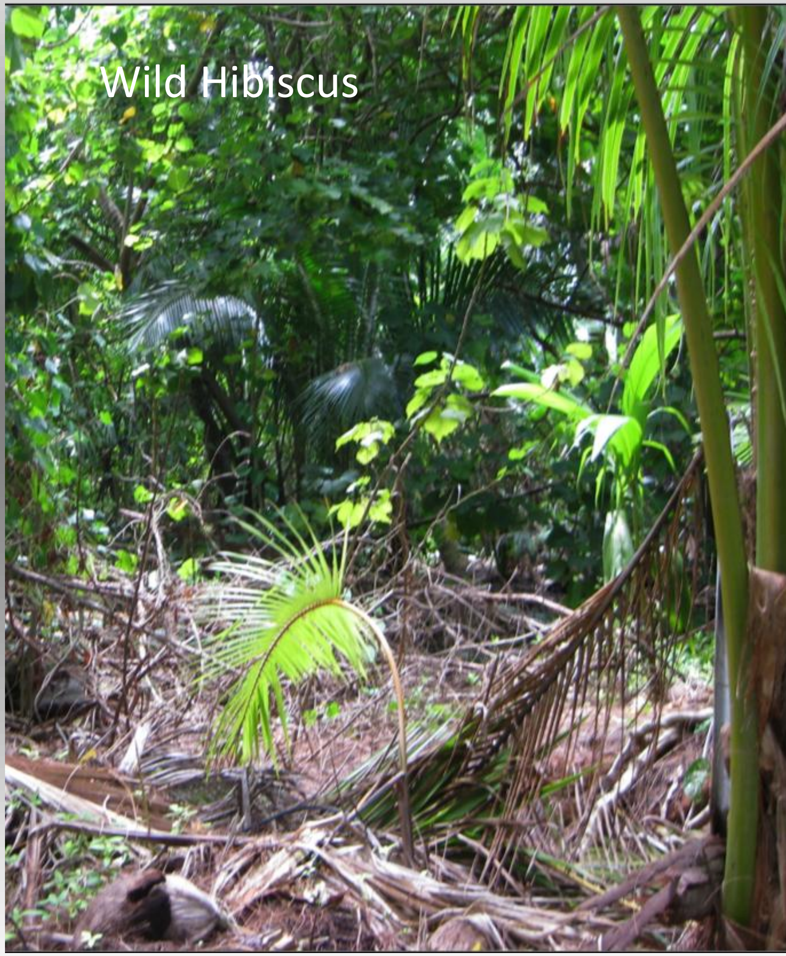
Slide credit: Herve Bossin

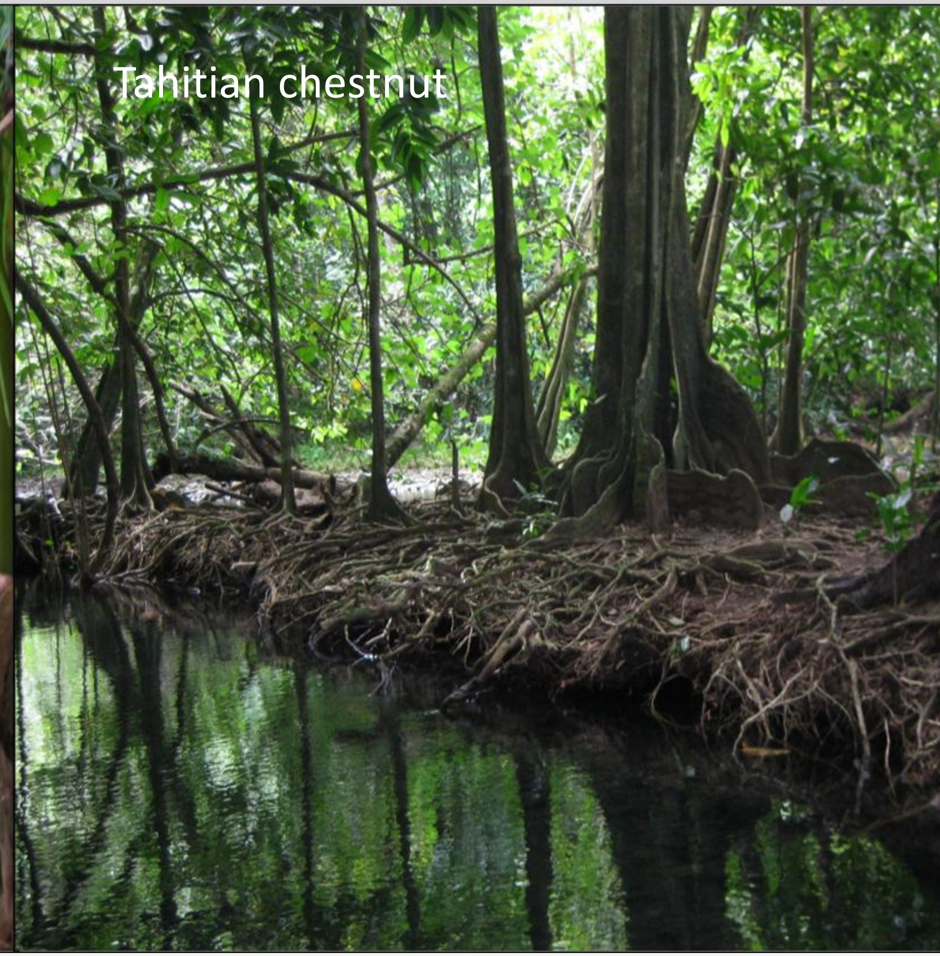
What makes transmission of LF by Aedes polynesiensis in the South Pacific unique?

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

Annals of Tropical Medicine & Parasitology, Vol. 96, Supplement No. 2, S143–S152 (2002)

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

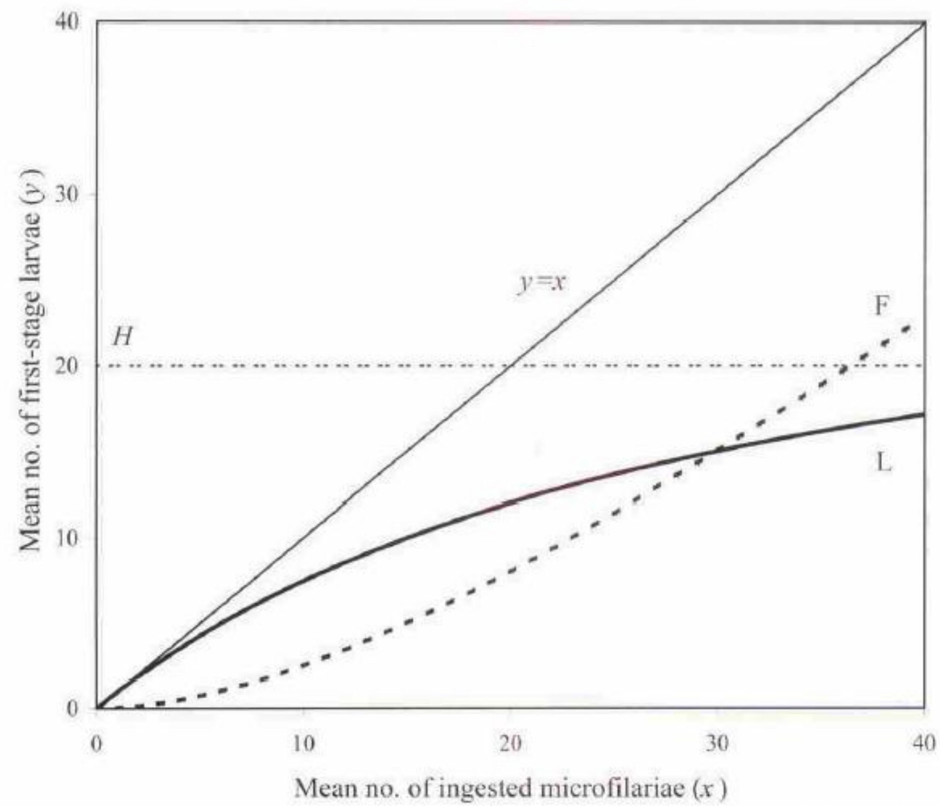
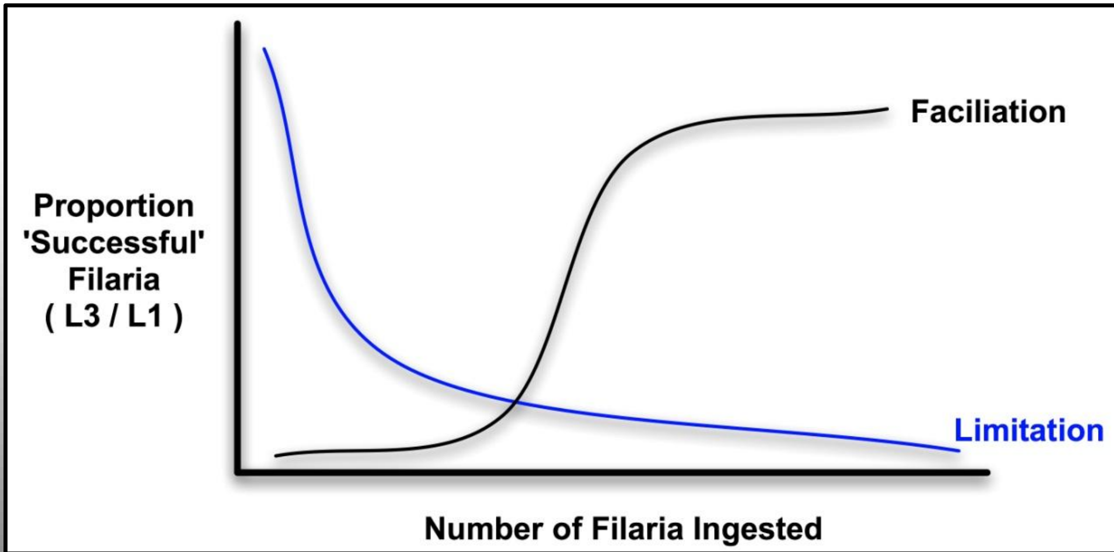


FIG. 1. A diagrammatic representation of limitation (L) and facilitation (F), here shown for the production of first-stage larvae (L_1) from ingested microfilariae. *H*, the inverse of the regression slope coefficient *b*, allows for measurement of the 'reciprocal adaptation' for a particular vector–parasite combination.



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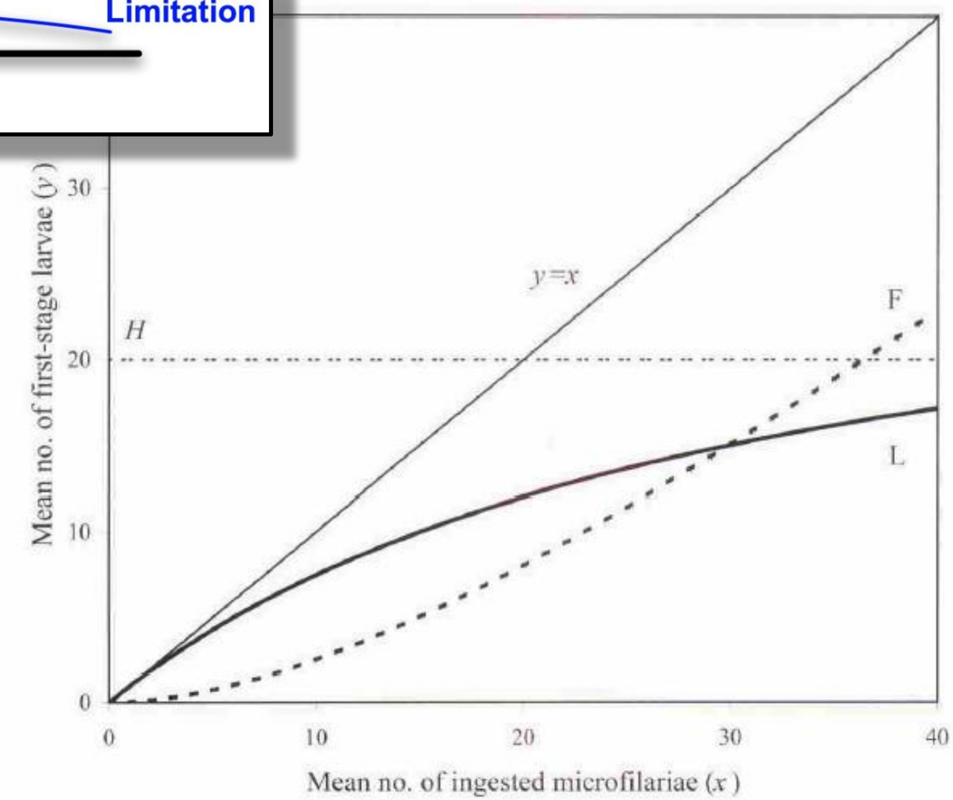

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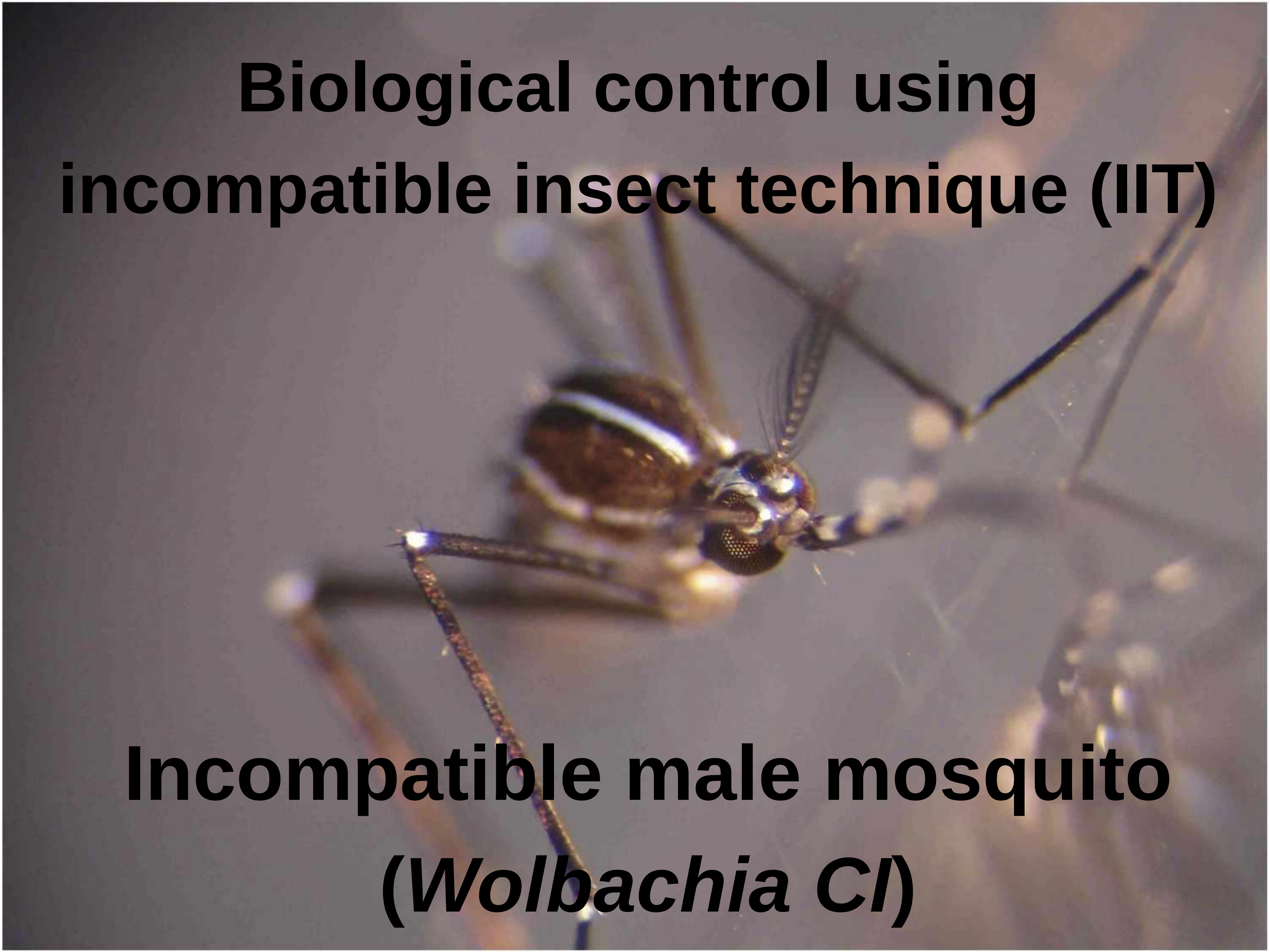
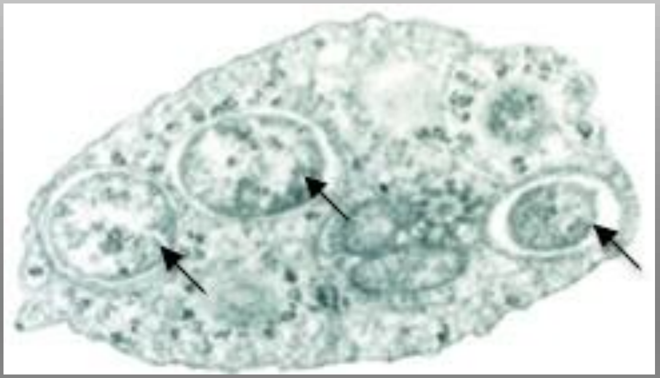


Biological control using incompatible insect technique (IIT)

Incompatible male mosquito (*Wolbachia CI*)

Wolbachia pipentis

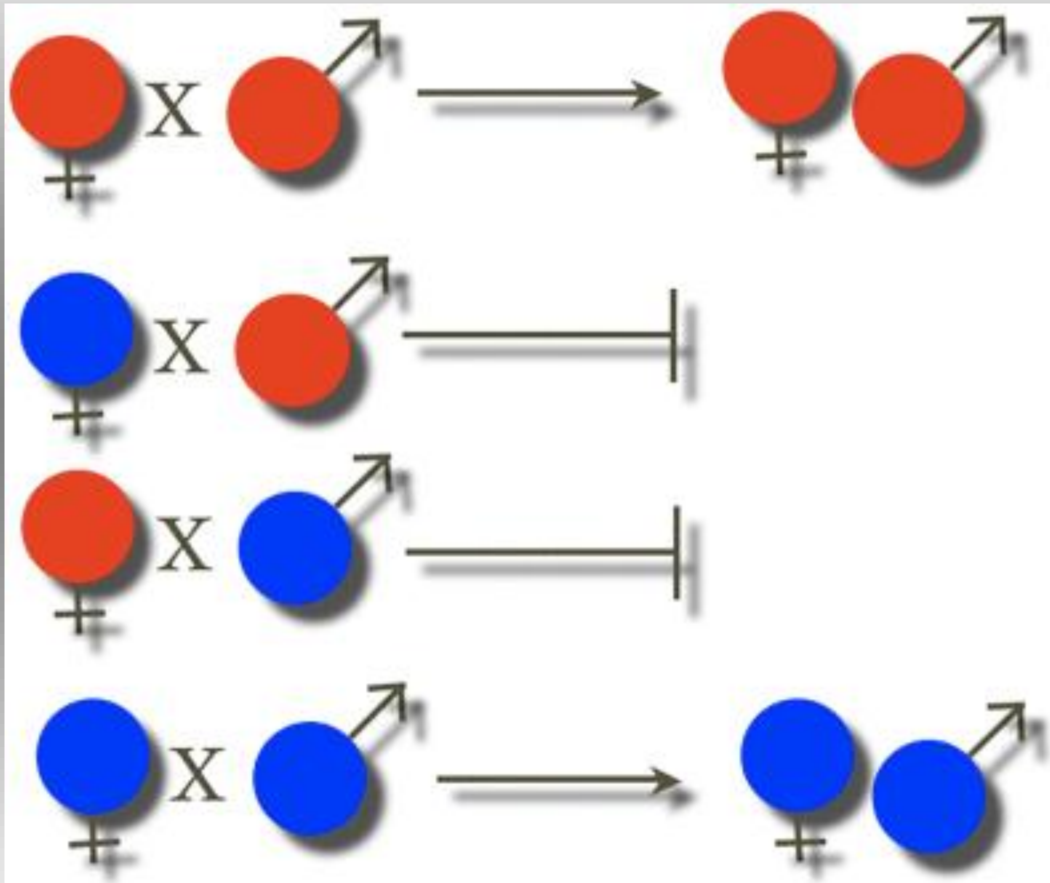
- intracellular bacterium
- first observed in *Culex pipiens*
- may infect up to 60- 70% insects
- maternally inherited
- does not infect vertebrates



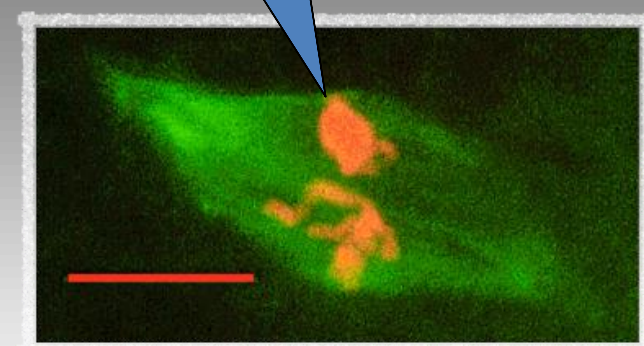
Insect cell containing Wolbachia

Image courtesy Eliminate Dengue

Bidirectional cytoplasmic incompatibility

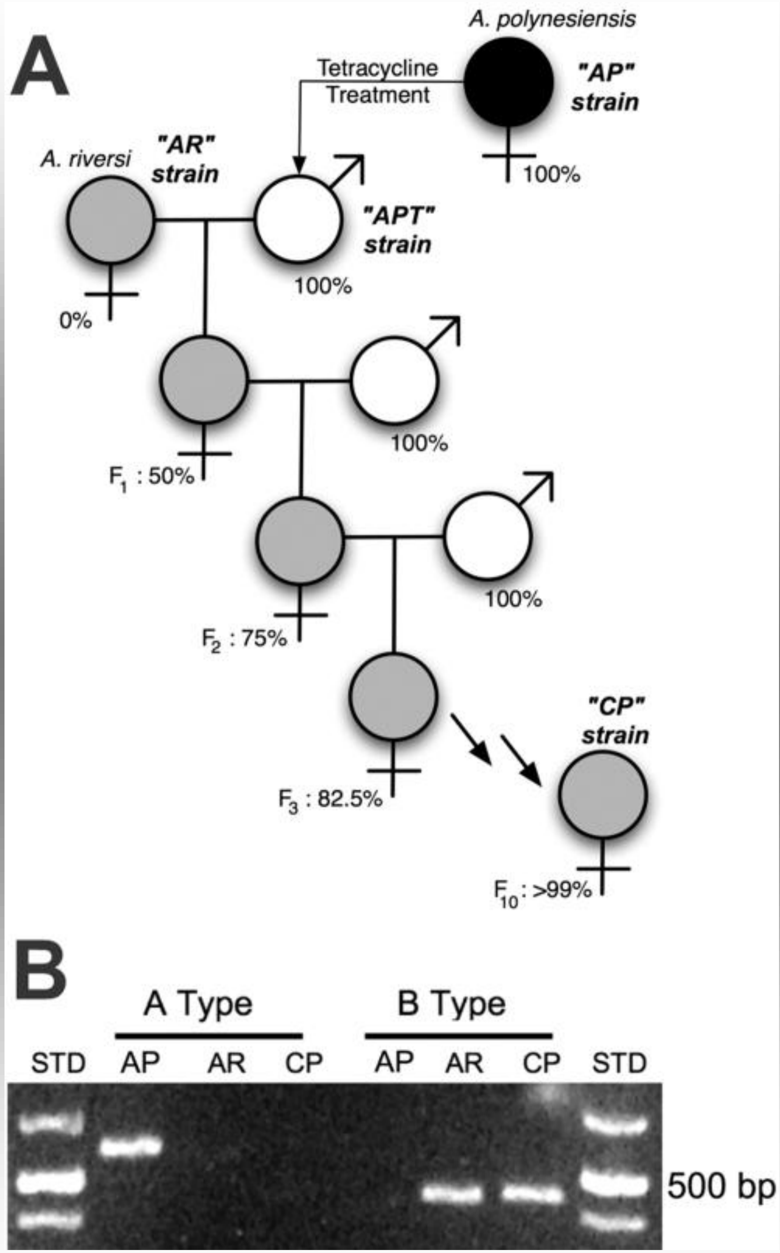


Abnormal heterochromatic paternal chromosome in developing embryo



Slide credits: Stephen Dobson

How to build a better mosquito!



- CP mosquito
  - AP genetic background
  - AR wolbachia type
  - >100 individuals/generation

Brelsfoard, et al. PLoS Negl Trop Dis. 2008 January;2(1):e129.

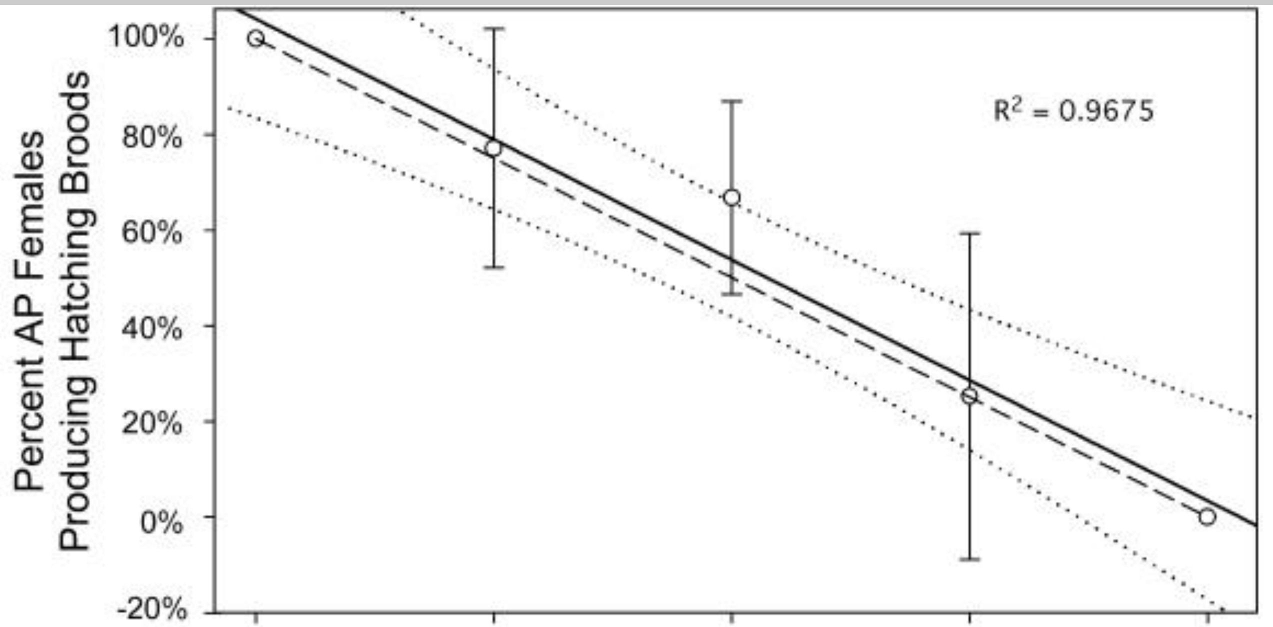
Crosses and Patterns of CI

Female x Male	Percent egg hatch ± s.e.m.; no. replicate crosses
AR x APT	13.6 ± 17%; n=14
CP x AP	0.23 ± 0.11%; n=18
AP x CP	0.0 ± 0%; n=28
AP x AP	87.8 ± 9.7%; n=8
CP x CP	62.1 ± 4%; n=18

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

Brelsfoard, et al. PLoS Negl Trop Dis. 2008 January;2(1):e129.

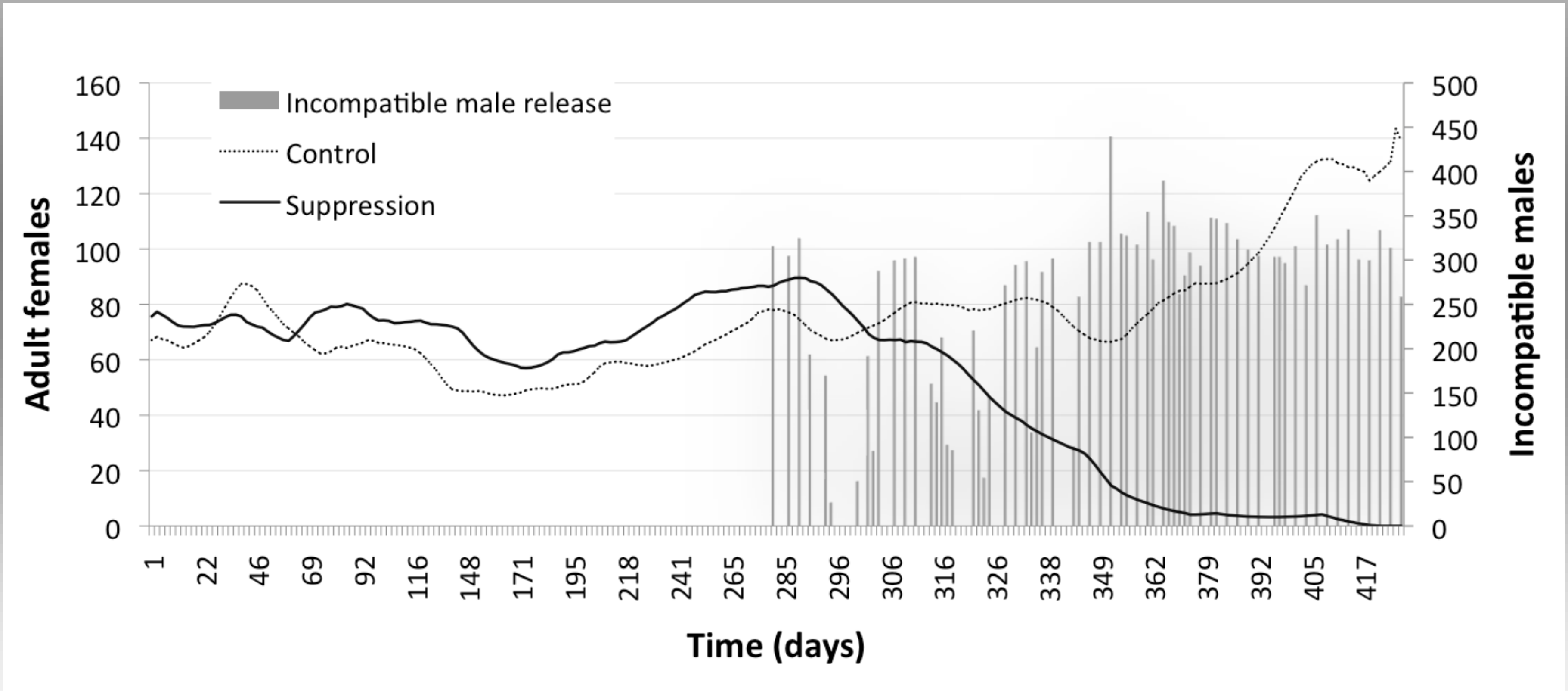
CP male competitiveness



Male Ratio (AP:CP):	20:0	15:5	10:10	5:15	0:20
Hatch (Mean±Std Err; n)					
All Broods:	75±3%; 36[a]	49±8%; 28[a]	53±7%; 28[a]	16±7%; 26[b]	0±0%; 28[b]
Compatible Broods:	75±3%; 36	77±7%; 18	74±5%; 20	84±10%; 5	NA; 0
Total Egg Number:	2743	1835	2008	2128	2231

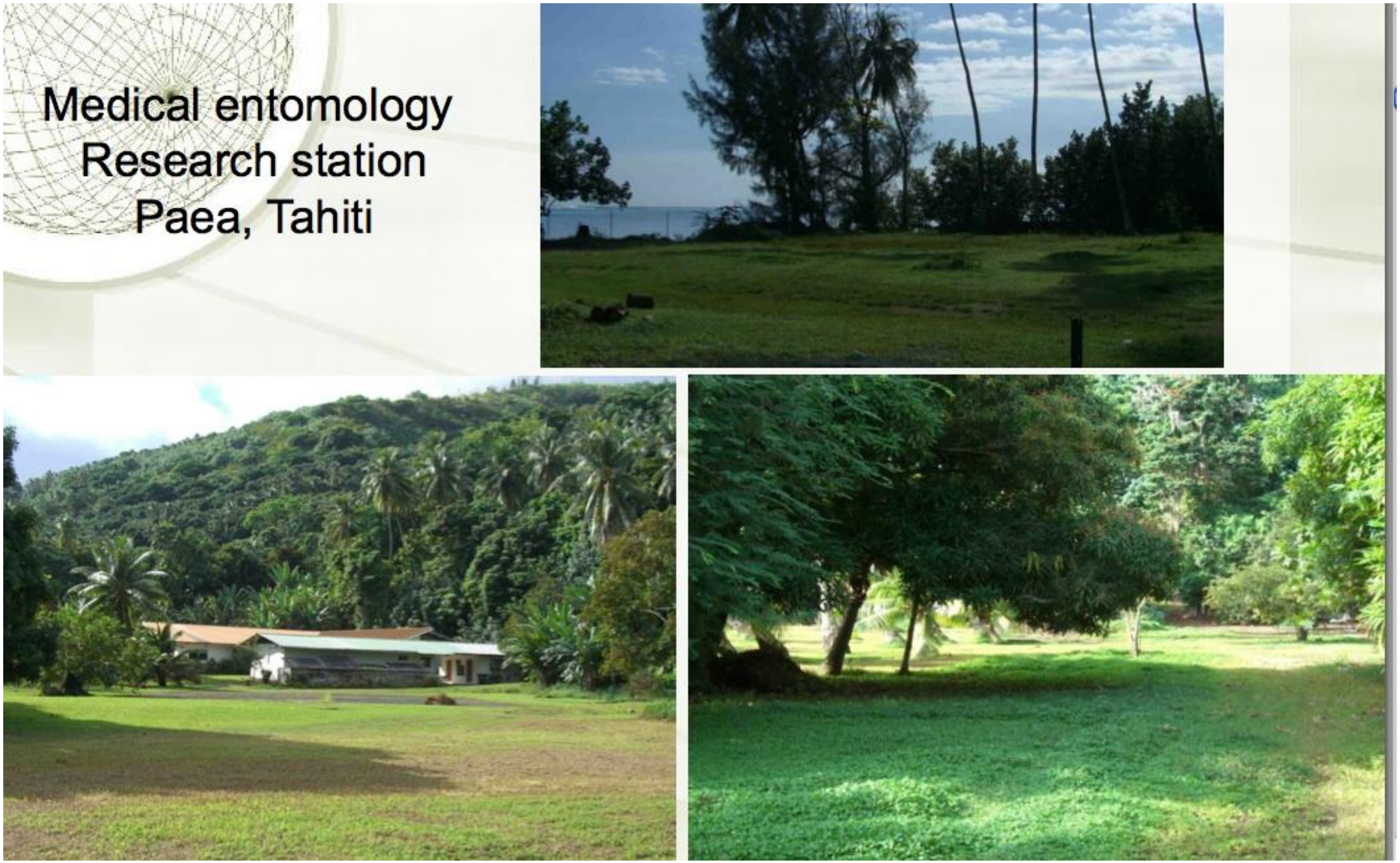
Brelsfoard, et al. PLoS Negl Trop Dis. 2008 January;2(1):e129.

Ae. polynesiensis population cage suppression with bidirectionally incompatible males



Slide Credit: Corey Brelsfoard

Moving to the field



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

APA:CP male mosquitoes
(50 mosquitoes)

50 female APA mosquitoes

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

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

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

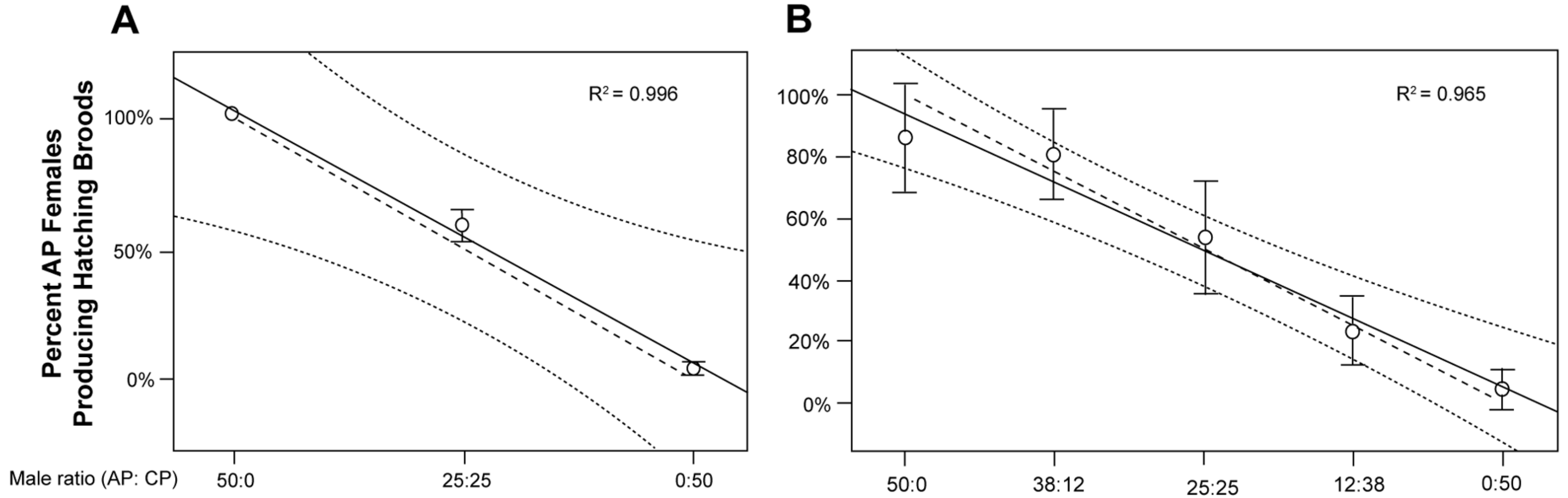
Semi-Field Cage

Male mortality

Experiment	Ratio APA:CP	% mortality ± SE
A	0:50	9.0±1.7
	25:25	5.0±2.4
	50:0	9.0±4.4
B	0:50	6.7±3.5
	12:38	8.0±4.2
	25:25	5.3±1.3
	38:12	7.3±1.8
	50:0	8.0±4.0

Chambers, et al. PLoS Negl Trop Dis. 2011 Aug;5(8):e1271.

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



Hatch (Mean ± Std Dev; n)	50:0 (A)	25:25 (A)	0:50 (A)	50:0 (B)	38:12 (B)	25:25 (B)	12:38 (B)	0:50 (B)
All Broods	91% ± 1;114	51% ± 3; 95	1% ± 1; 71	72% ± 2; 81	68% ± 5; 72	44% ± 4; 95	14% ± 4; 62	4% ± 2; 74
Compatible Broods	91% ± 1;114	88% ± 2; 55	75% ± 0; 1	86% ± 2; 67	85% ± 2; 57	81% ± 3; 51	71% ± 9; 12	90% ± 3; 3
Total Egg Number	12348	10720	8528	7660	7145	9691	6899	7620
Number of Replications	4	4	4	3	3	3	3	3

Chambers, et al. PLoS Negl Trop Dis. 2011 Aug;5(8):e1271.

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

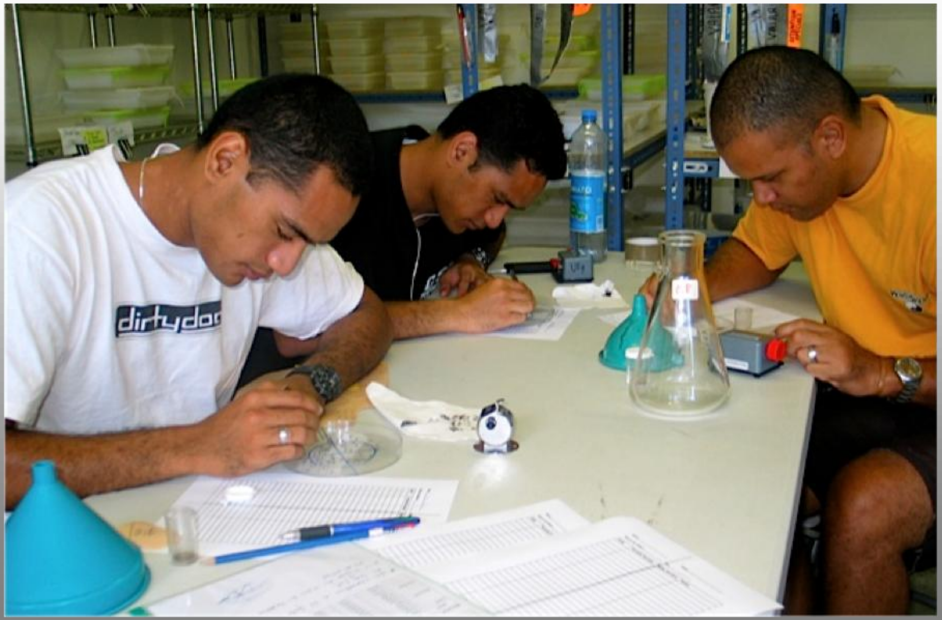
% CP	≤10% Hatch	11-69% Hatch	≥70% Hatch	Total No. Broods
0	7%	6%	86%	195
25	28%	13%	67%	72
50	45%	7%	48%	190
75	81%	5%	15%	62
100	97%	0%	3%	145

Number of spermathecae inseminated				
	0	1	2	3
Total	5	11	586	8
Percent	0.8	1.8	96.1	1.3

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



Sorting and shipping of mosquitoes for release

Mass rearing of CP mosquitoes

Field releases on uninhabited motus

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

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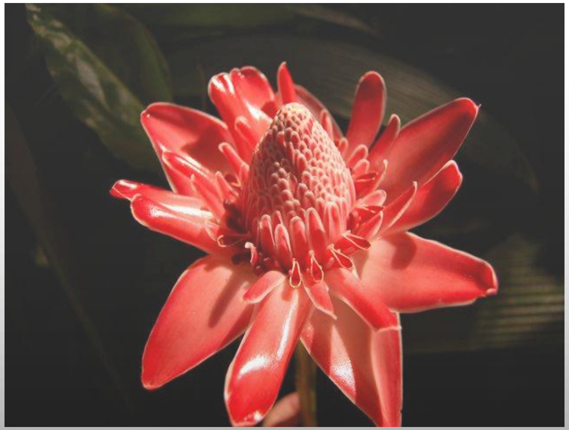

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Funding provided by the Bill and Melinda Gates Foundation and NIH

Benefits of living and working in the South Pacific


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