Professor Scott Ritchie
James Cook University

Wolbachia: how to optimise and sustain its promise of control of *Aedes* and *Aedes*-borne viruses

3 INTERNATIONAL CERTIFICATE OF VACCINATION OR REVACCINATION AGAINST YELLOW FEVER
CERTIFICAT INTERNATIONAL DE VACCINATION OU DE REVACCINATION CONTRE LA FIÈVRE JAUNE

This is to certify that
Je soussigné(e) certifie que Scott Ritchie

date of birth
né(e) le 2/4/1956 sex
sexe Male

whose signature follows
dont la signature suit

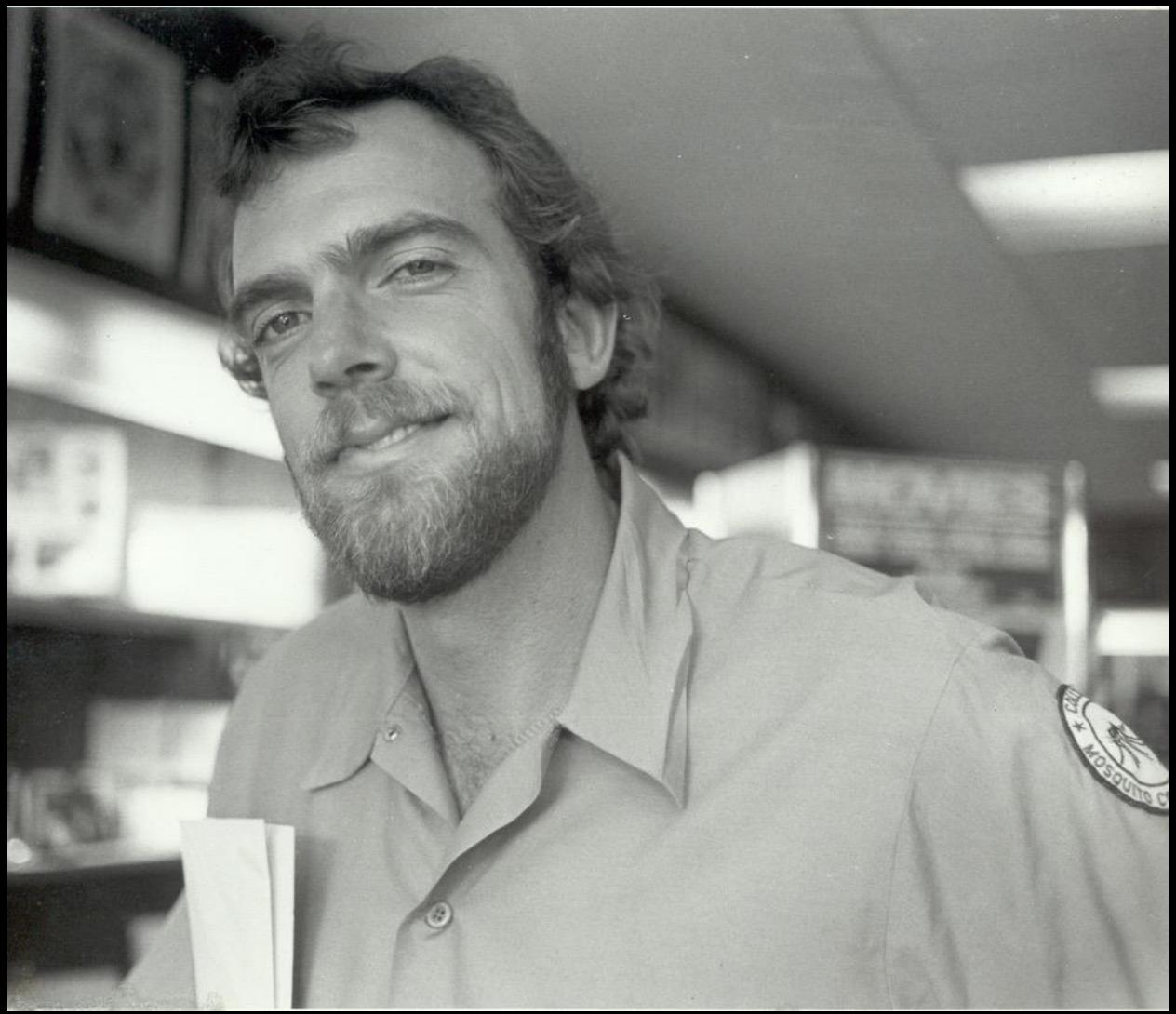
has on the date indicated been vaccinated or revaccinated against yellow fever
a été vacciné(e) ou revacciné(e) contre la fièvre jaune à la date indiquée.

Date	Signature and professional status of vaccinator Signature et titre du vaccinateur	Manufacturer and batch no. of vaccine Fabricant du vaccin et numéro du lot	Official stamp of vaccinating centre Cachet officiel de centre de vaccination
1 22/4/08	DR CAMPBELL CRILLY M.B. B.S. D.R.A.C.O.G. F.R.A.C.G.P. Grad.Dip. Rural G.P. PROVIDER No. 0633556H 2 CAIRNS CORPORATE TOWER 15 LAKE ST., CAIRNS 4870 PH 40411699 FAX 40411682	Stamaril V5444	GOVERNMENT OF AUSTRALIA APPROVED YELLOW FEVER VACCINATION CENTRE

This vaccination certificate is replicated as per the World Health Organisation model of the International Certificate of Vaccination or Revaccination against Yellow Fever. This is published in the WHO book: *International Travel and Health: Vaccination requirements and health advice, 2001, p12-13.*

- This certificate is valid only if the vaccine used has been approved by the World Health Organisation and if the vaccinating centre has been designated by the health administration for the territory in which that centre is situated.
- The validity of this certificate shall extend for a period of ten years, beginning ten days after the date of vaccination or, in the event of a revaccination within such period of ten years, from the date of that revaccination.
- This certificate must be signed in their own hand by a medical practitioner or other person authorized by the national health administration; an official stamp is not an accepted substitute for a signature.

[illegible] of this certificate, or erasure, or failure to complete any part of it, may



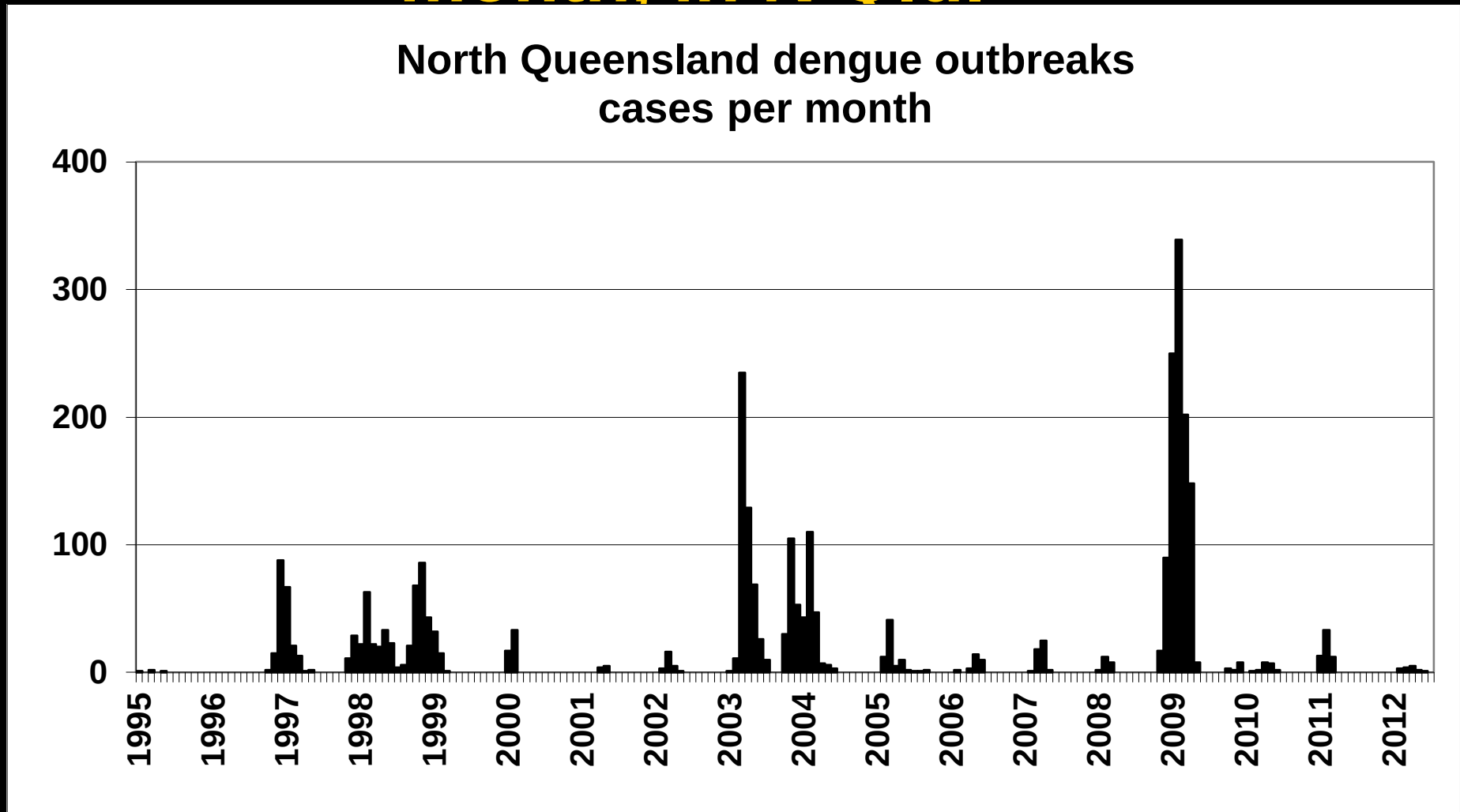
Collier Mosquito Control District, Naples Florida 1983

Spray ‘em and slay ‘em

DC3 spraying for mosquitoes, Naples Florida, ca. 1975


Indonesia
Cairns
Townsville
Australia
worldatlas

Locally-acquired dengue, by month, in N Qld.



Dengue fever KO'd after epic struggle

Roz Pulley

CAIRNS has finally rid itself of the dengue scourge that has plagued the city for the past year.

Yesterday health officials declared the city dengue-free after a prolonged battle with the deadly mosquito that has infected 79 people since January and resulted in a blitz on more than 4000 properties.

"The dengue fever outbreak is officially over," beamed Tropical Public Health Unit medical entomologist Dr Scott Ritchie, donning a mozzie-emblazoned shirt specially for the occasion.

He said the all-clear came after three months in which no new cases were reported, the time allowed to detect any lingering dengue transmission.

The Cairns outbreak was one of three simultaneous outbreaks the unit had to contend with during the past 12 months, others affecting 276 people in the Torres Strait and 58 people in Townsville.

In Cairns, the virus spread to Bentley Park, Bungalow, Babinda, Earlville, Edge Hill, Edmonton, Gordonvale, Manoora, Manunda, Parramatta Park, Portsmith and Westcourt.

"This year's outbreak in Cairns began when a person who contracted the virus overseas came into contact with local dengue mosquitoes, which then spread the virus to local residents," Dr Ritchie said.

He said the unit worked closely with Cairns City Council to get on top of the outbreak, conducting intensive house-to-house inspections and mosquito eradication.

"It is a difficult thing to get rid of. All it takes is one person with a lot of stuff lying around to breed enough mosquitoes to perpetuate it," he said.

Dr Ritchie warned against complaceny, saying dengue was on the increase overseas and could become entrenched in North Queensland. "If dengue became endemic here, the local population would be at constant risk," he said.

He said two new control officers had been employed to help prevent dengue outbreaks and research was continuing on trapping techniques.

"But the only way to beat it is for everyone to clean up their yards."

The most frequent breeding sites are pot plant bases, tyres, black plastic and discarded containers.

Hit 'em high, hit 'em low: Tropical Public Health Unit medical entomologist Dr Scott Ritchie has claimed victory in the ongoing battle to stop the spread of dengue fever in the Far North. Picture: MIKE WATT

Queensland Health Dengue Program

COURTESY CAIRNS POST
28/10/2004

Rear and release:
Let the mosquitoes go!

Talk Outline

1. The Wolbachia “rear and release” paradigm
   a. How Wolbachia works to KO mozzies and viruses
   b. Different Wolbachia strategies
2. Virus blocking (population replacement)
3. Population suppression (SIT by another name)
4. Relative merits of the 2 approaches
5. Challenges ahead

Ae. aegypti: container breeding mosquito

Ae. aegypti adults live in dwellings - the cockroach of mosquitoes!

Zika: a sense of urgency!

Space sprays: a lethal…but transient… cloud

Thermal fogging, New Dehli IPL match 2015 (courtesy Bruce Murphy, DFAT)

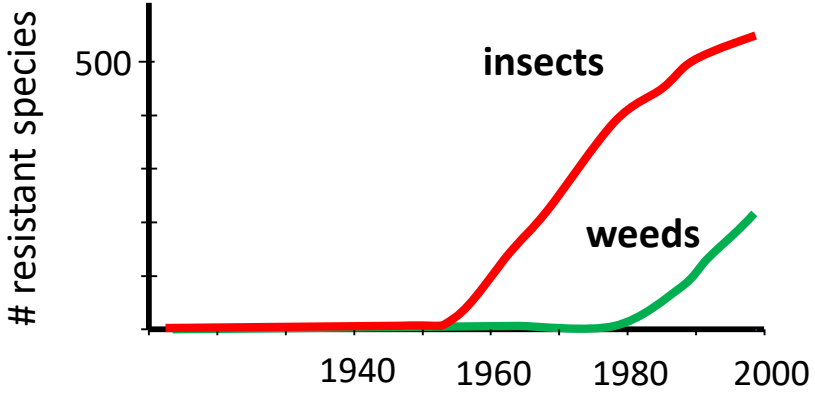
Current chemical pesticide issues

(Slide courtesy Steve Whyard, Un. Manitoba)

1. Increasing resistance to pesticides

More resistant species

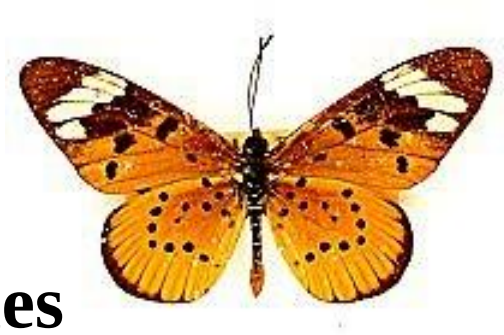
Higher levels of resistance



2. Off-target effects of pesticides

Broad-spectrum kill many non-target species

Wolbachia effects on reproduction:



Turns males into females

Male killing

Cytoplasmic incompatibility

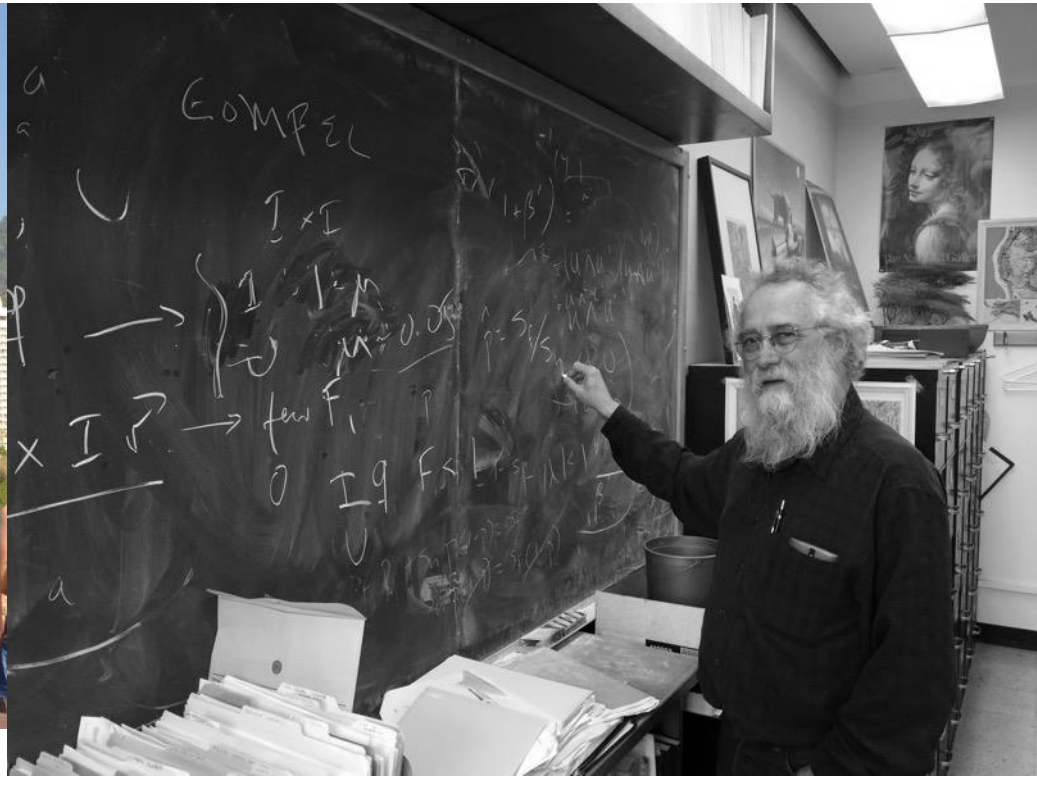
Parthenogenesis


Prof. Scott O’Neill,
Monash Un

Largely funded by Gates Foundation

Ary Hoffmann
(Melbourne Un.)

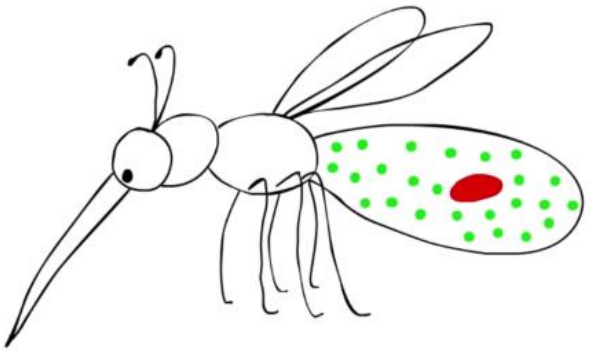
Michael Turelli
UC Davis



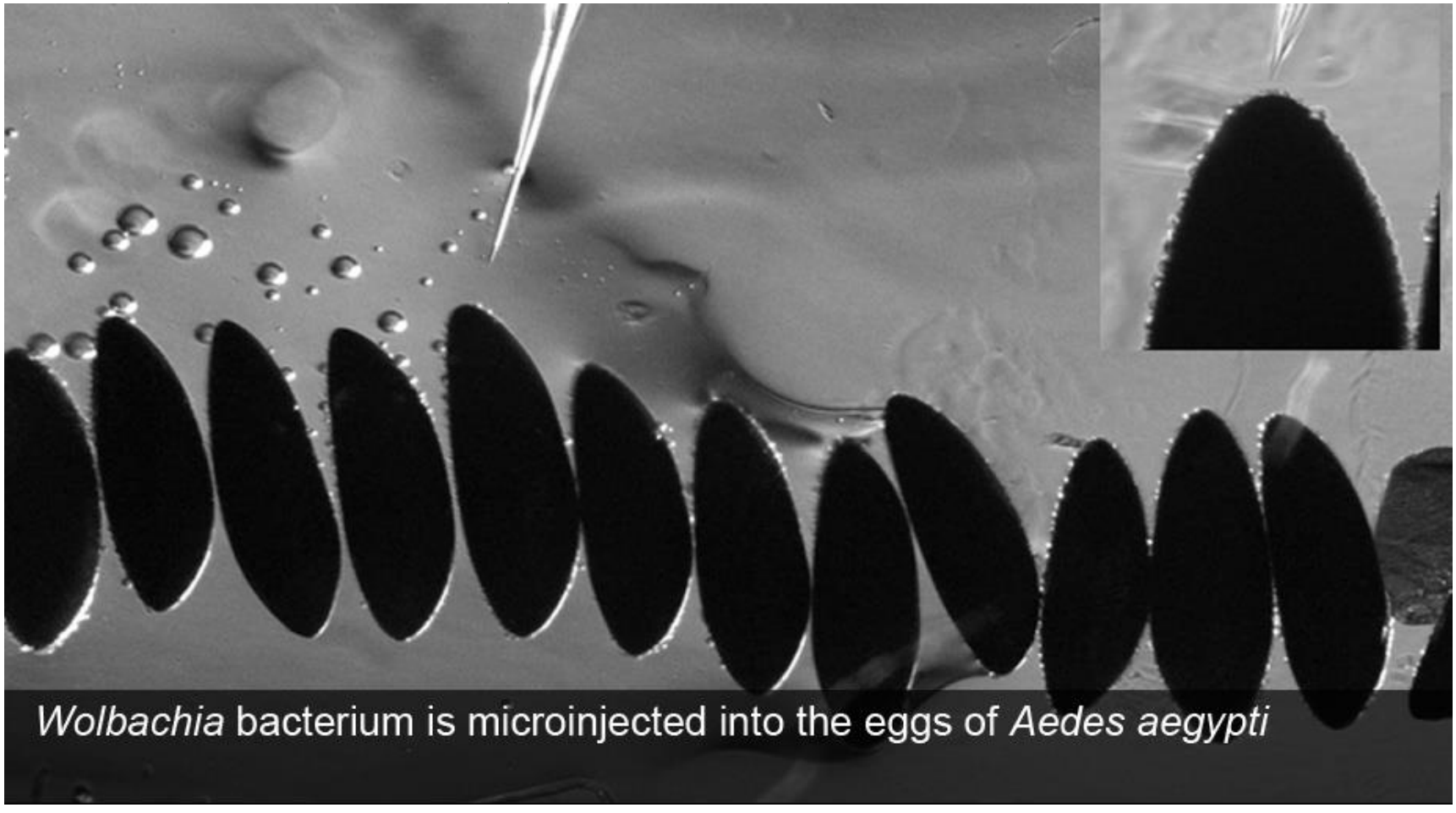
Formerly Eliminate Dengue

World Mosquito Program

Our control method

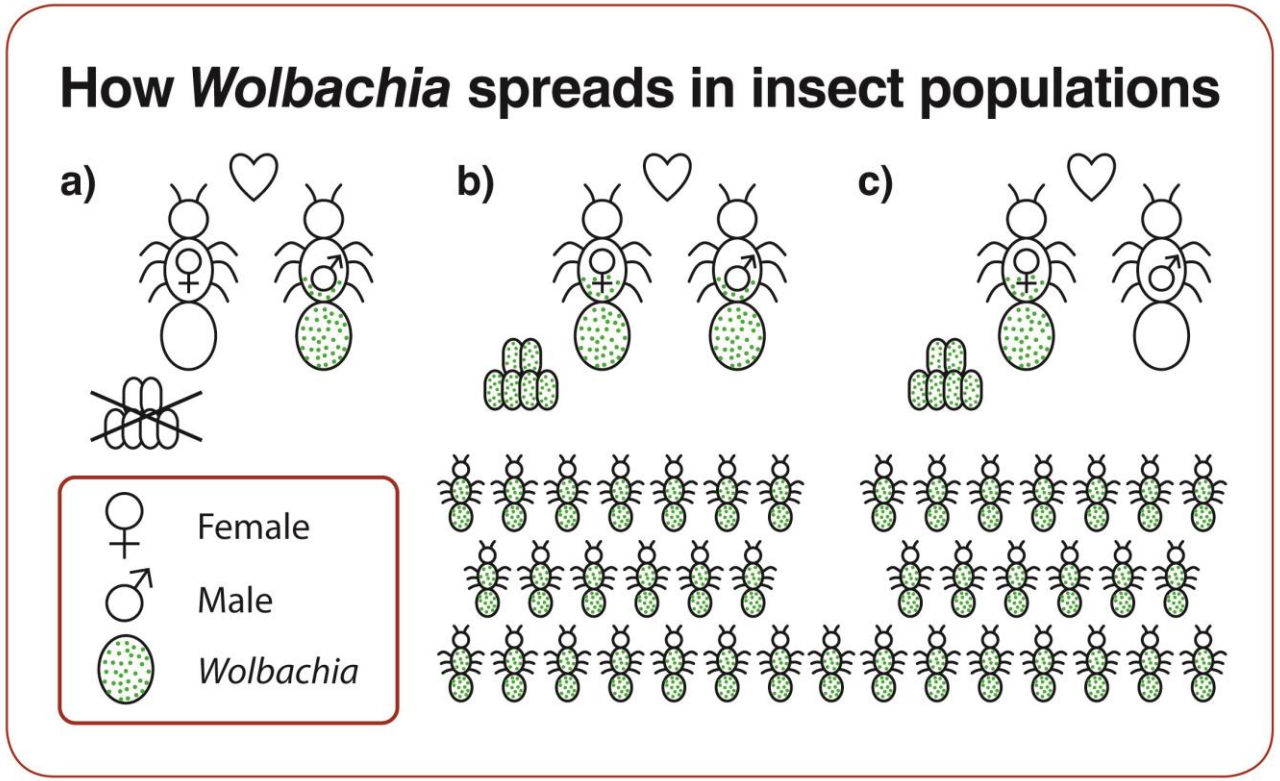


Getting Wolbachia into



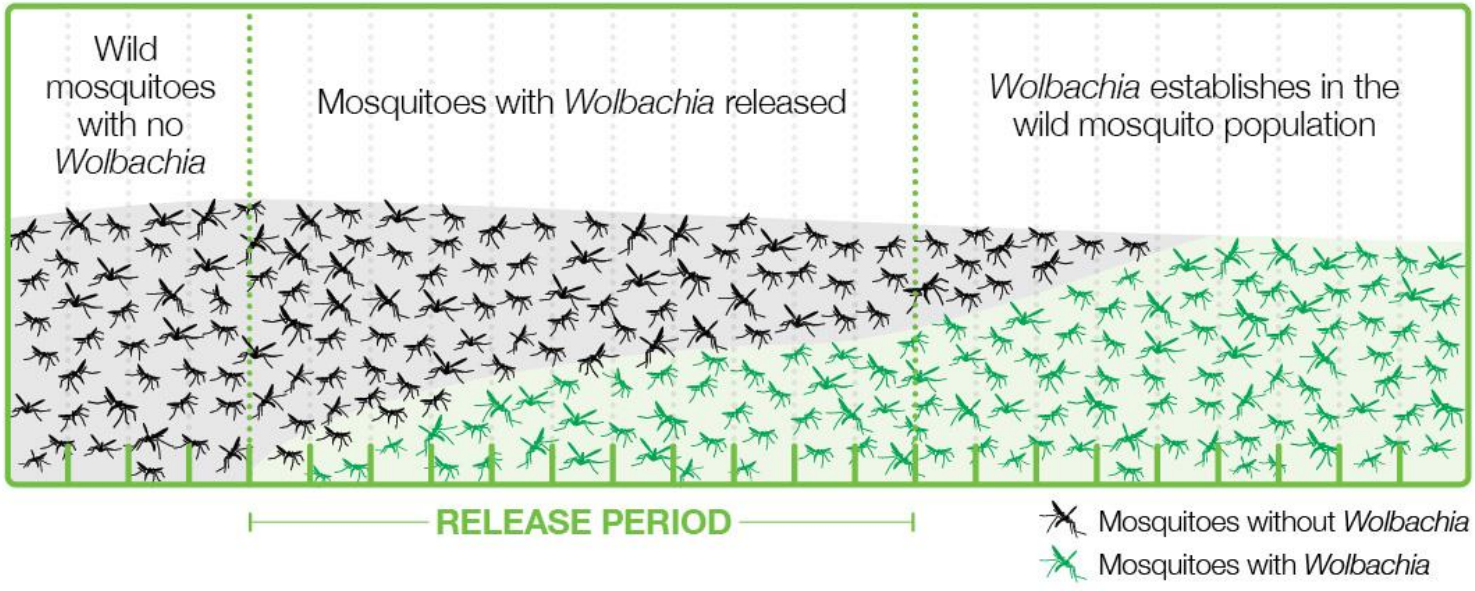
Wolbachia bacterium is microinjected into the eggs of *Aedes aegypti*

Cytoplasmic incompatibility



- If all *Aedes aegypti* mosquitoes have *Wolbachia* they wont be able to transmit the dengue virus between people.

Release *Wolbachia* mosquitoes



REAR AND RELEASE MOSQUITOES:
MAKING FRIENDS WITH INFLUENCERS

HELEN COOK
SINGAPORE JUNE 2018

"… RESIDENTS ARE ***ENABLED*** TO

PARTICIPATE, CRITIQUE, ASSESS & DETERMINE WHETHER THEY WANT THESE STRATEGIES TO BE TRIALED OR IMPLEMENTED IN THEIR BACKYARDS AND COMMUNITIES."

DARLENE MCNAUGHTON, 2012

TIME
&
PEOPLE

Field trials in far north Queensland

- **Community support**
- **Regulatory approval**
- **Communications**

Australian field trials

1. Rearing at JCU

2. Releasing once a week

3. Monitoring every 2 weeks

4. Sampling in the lab every 2 weeks

Rearing and deploying egg strips with *w*Mel

Mosquito release container

Water

Wolbachia mosquito eggs

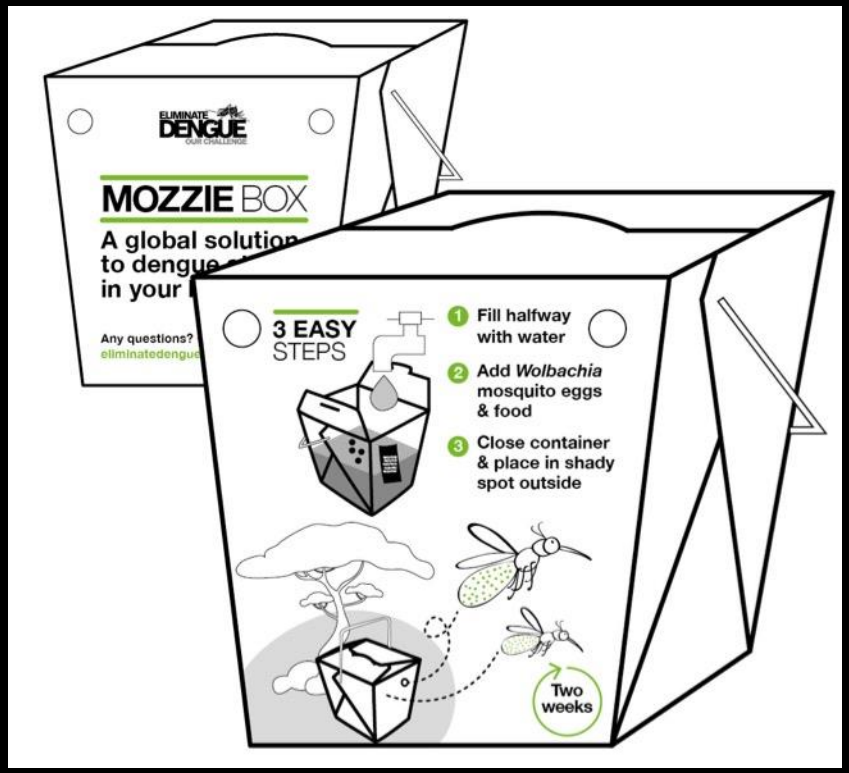
Mosquito food

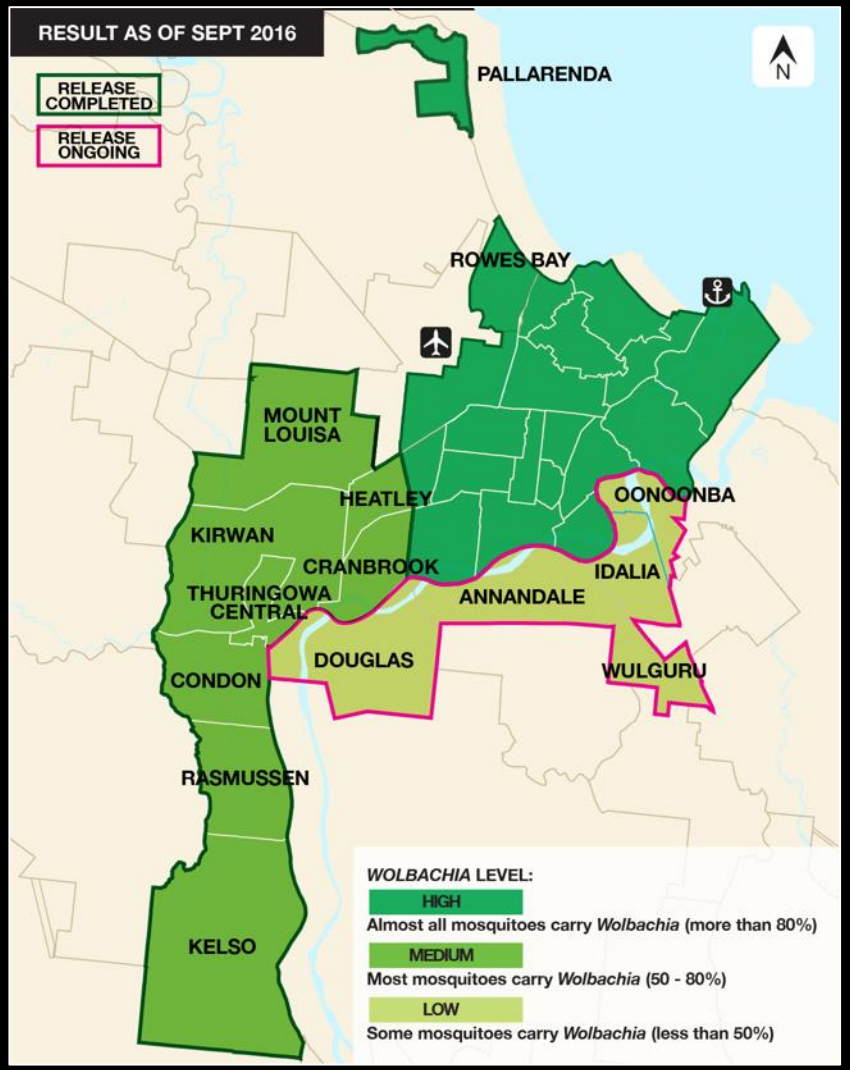
Egg strips easily produced

Low cost release containers

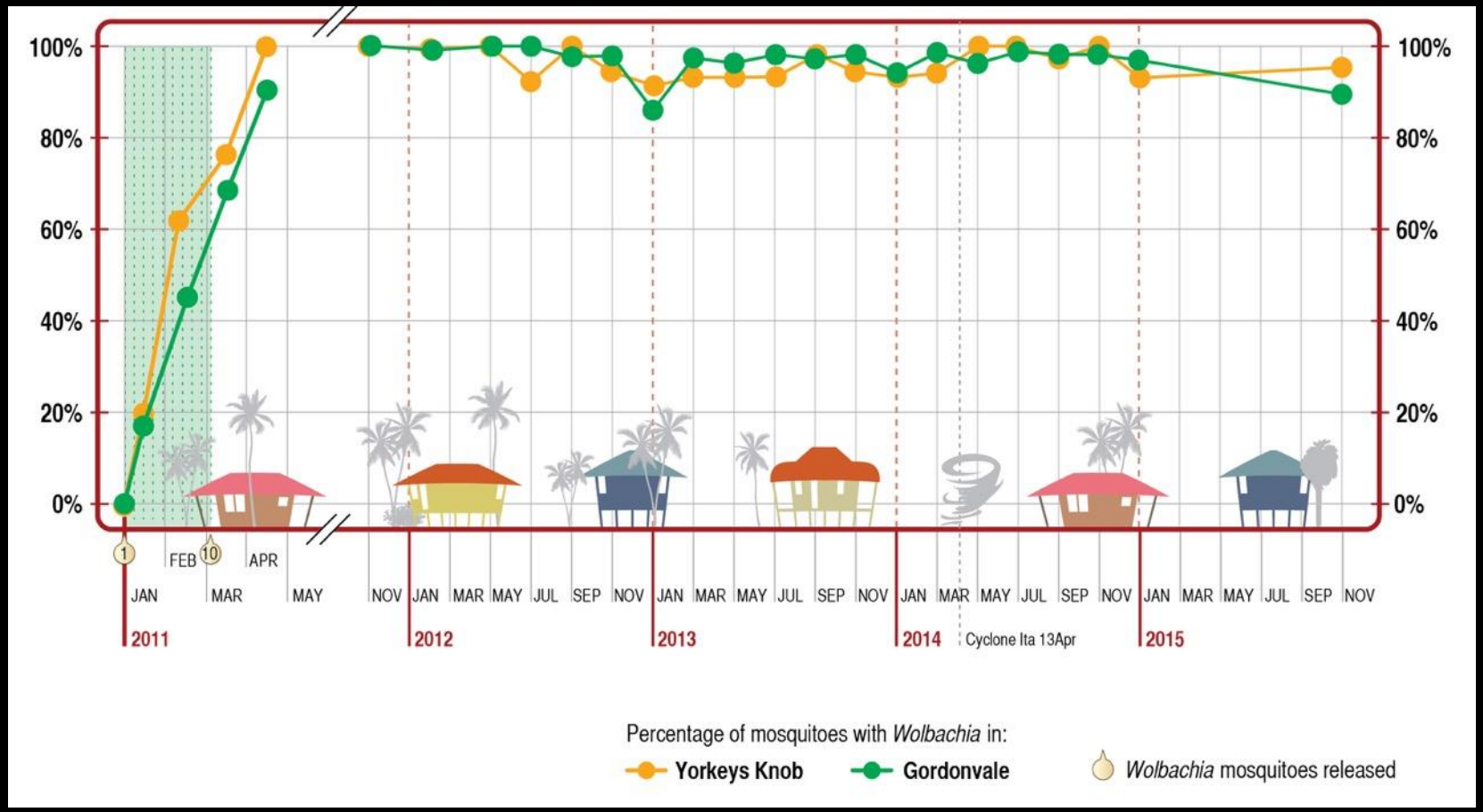
Streamline community deployment method

2016: Townsville, Australia



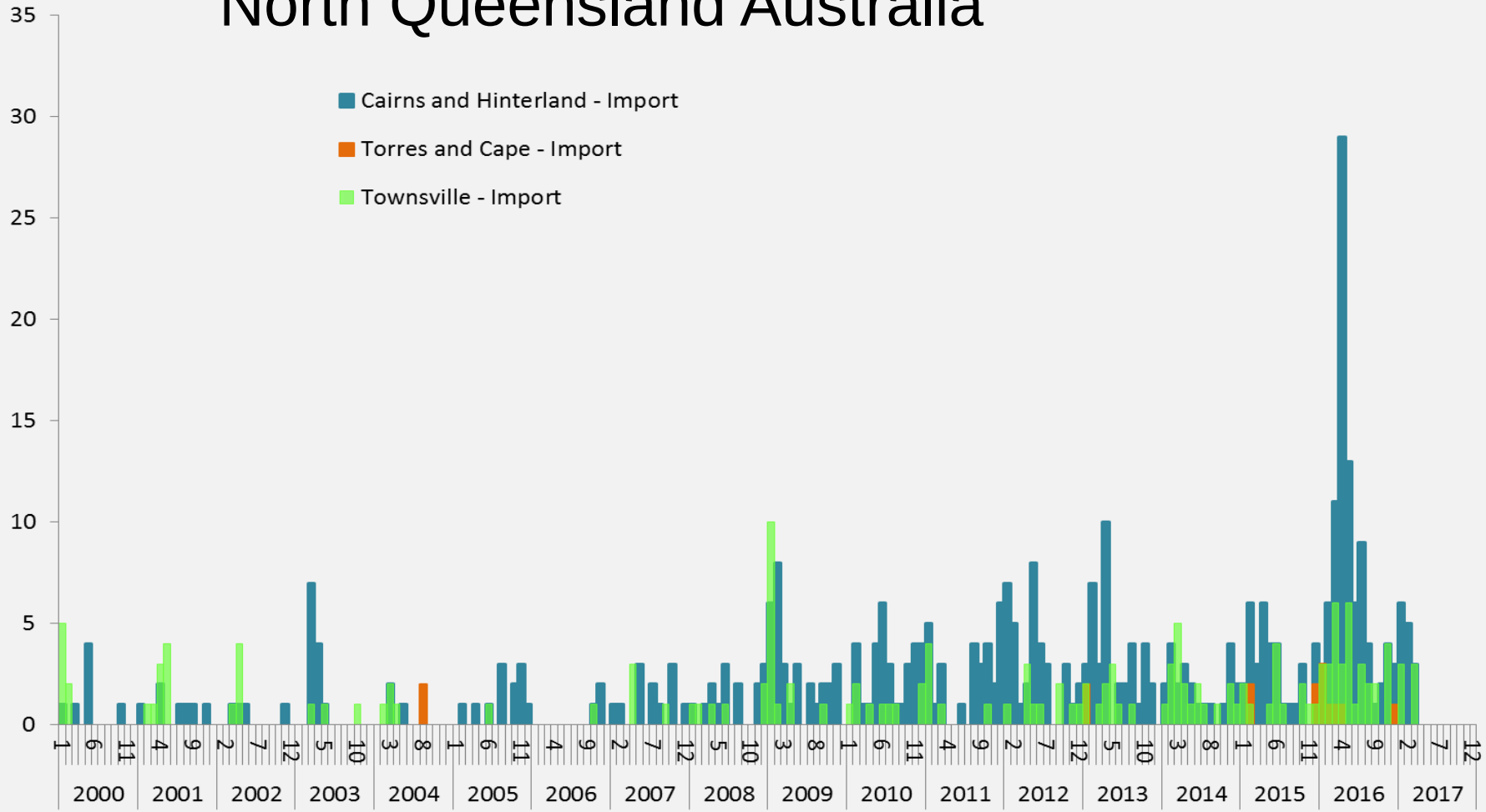


Since 2011 in Cairns

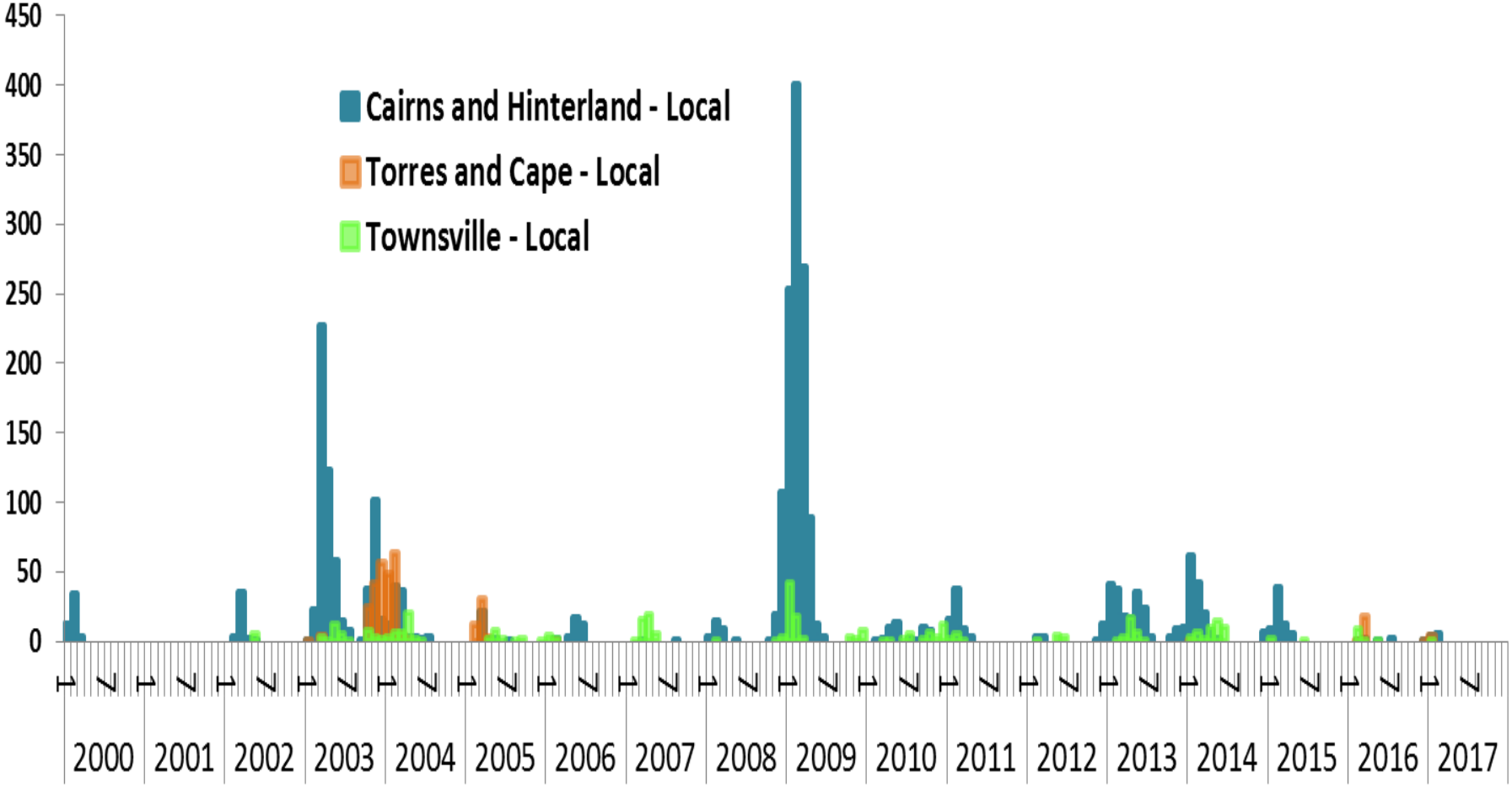


- *Wolbachia* still at high levels in mosquito populations
- No locally acquired dengue cases in release areas

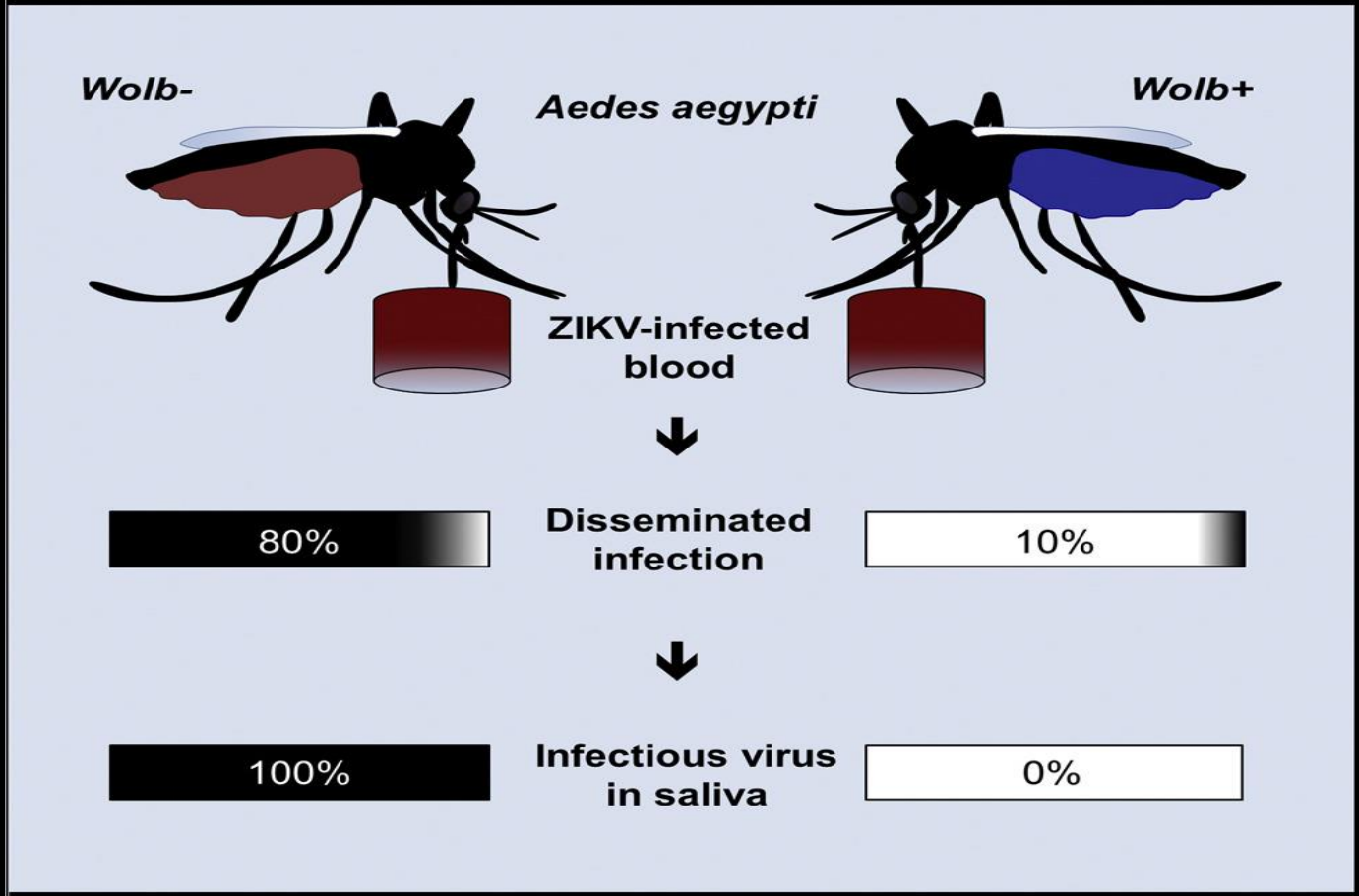
Rising dengue importations, North Queensland Australia



Decline in locally acquired dengue cases: Due to Wolbachia?



Wolbachia also blocks Zika virus

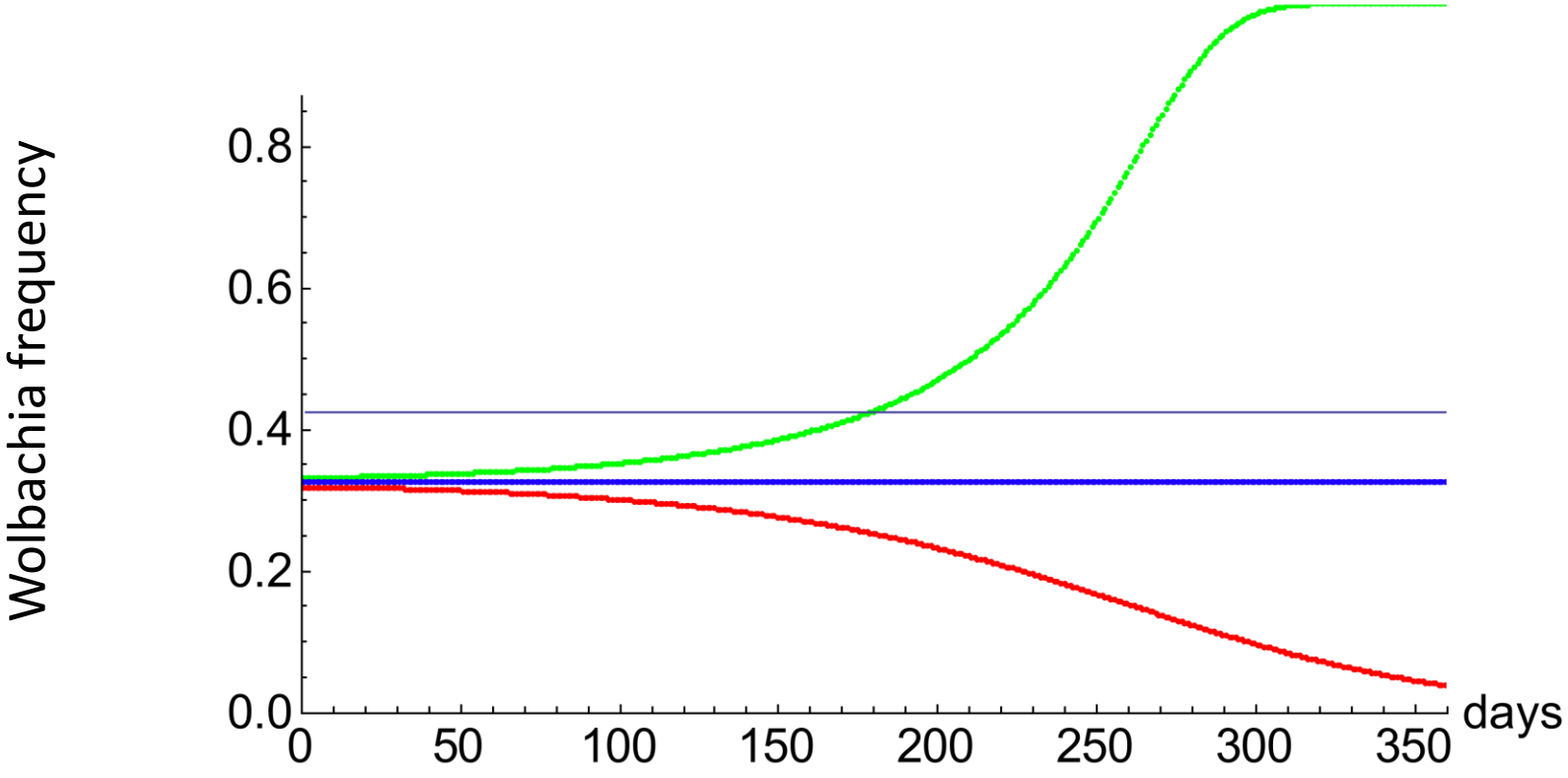


Cell Host & Microbe **DOI: (10.1016/j.chom.2016.04.021)**

World Mosquito Program
wMel *Ae. aegypti* rollouts 2018

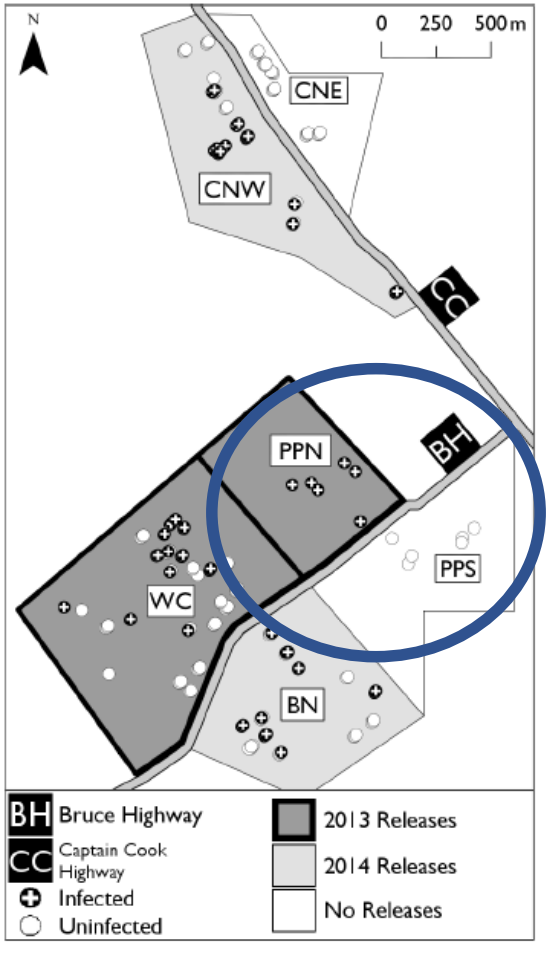
Barriers to establishment

Unstable equilibrium point; Wolbachia drops out at low frequencies



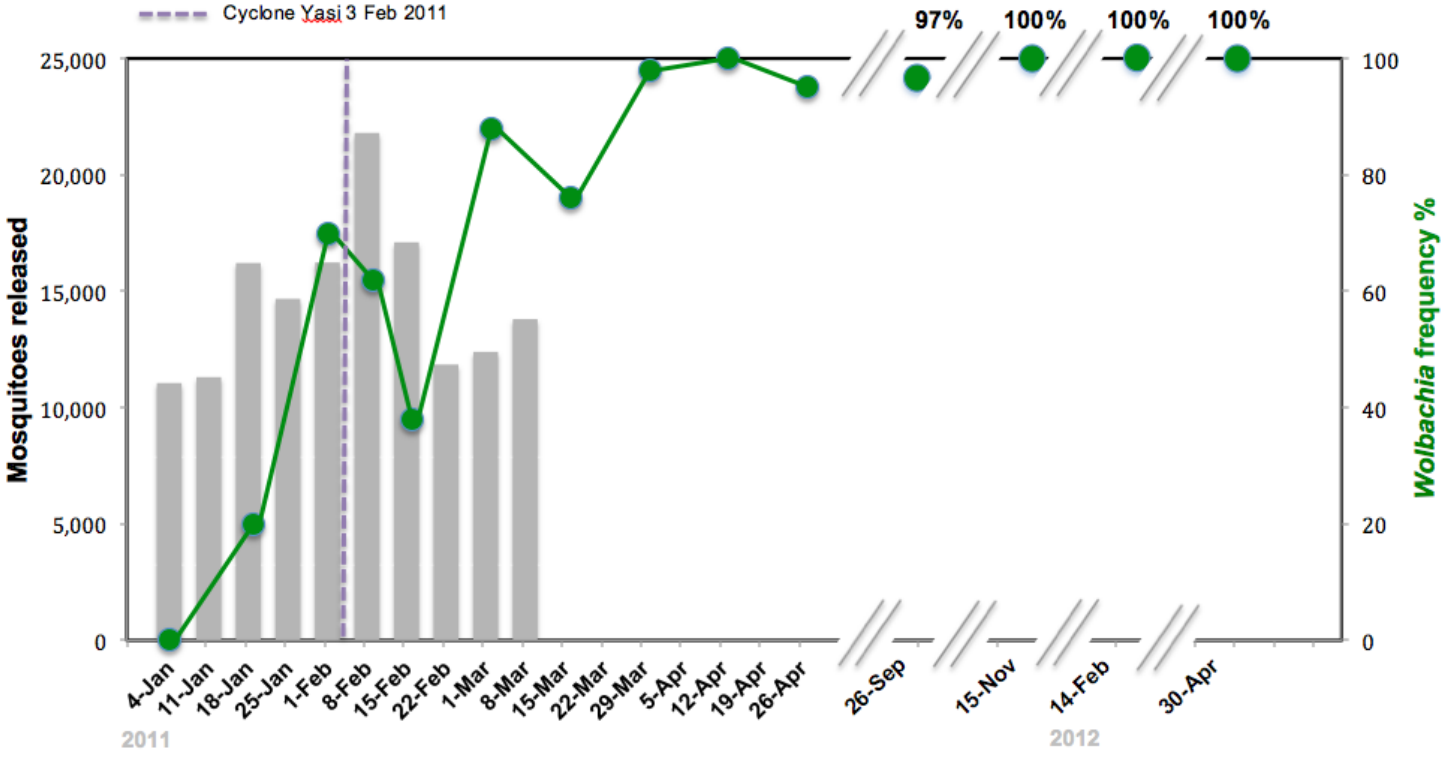
large roads, greenbelts as barriers to spread.

Schmidt et al. 2017 PLoS Biology. 2017 May 30;15(5):e2001894



2011 Field trial results

Yorkeys Knob



Hoffmann et al Nature 2011

Barriers to establishment and spread: hotspots of uninfected mosquitoes

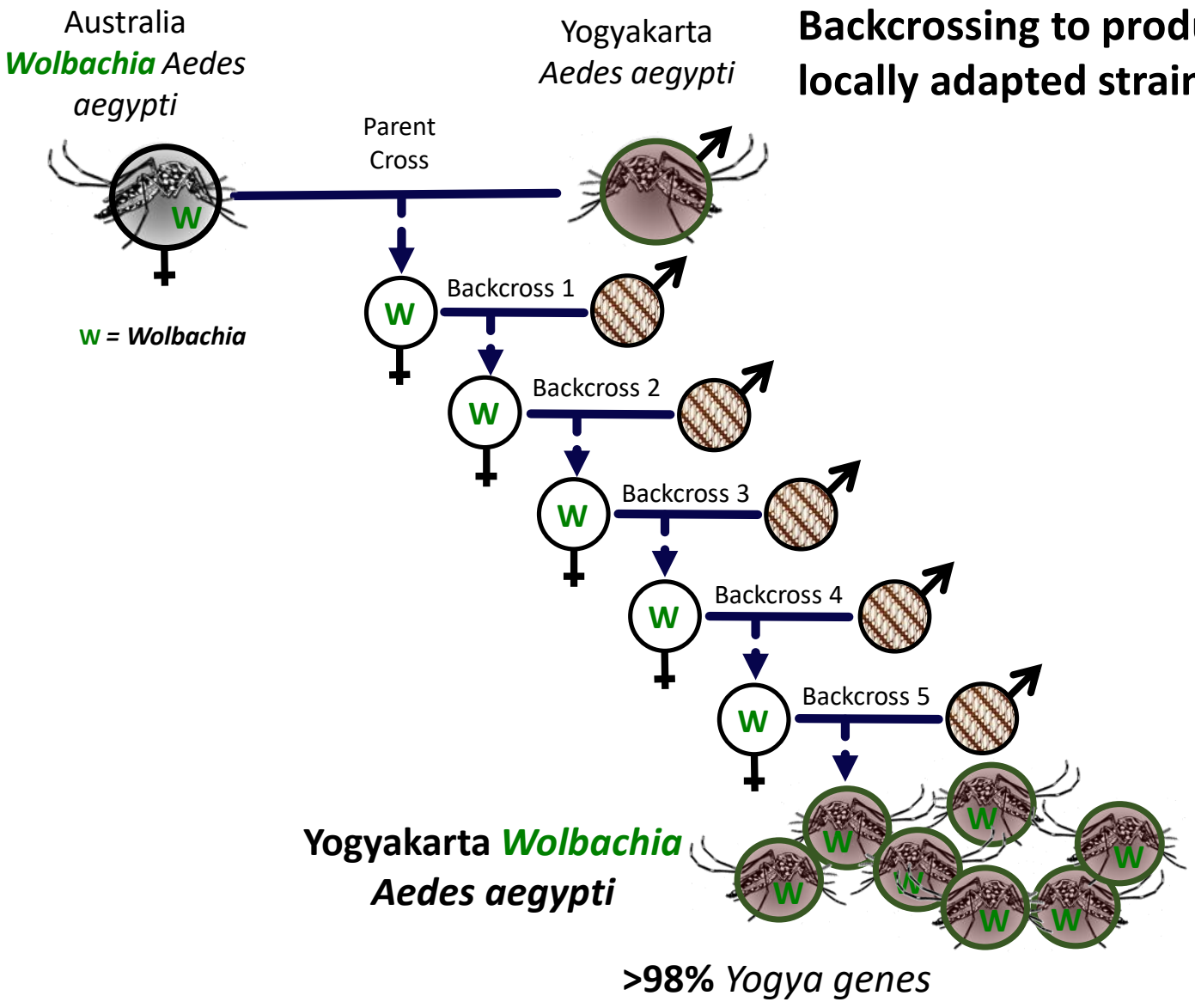
- Productive sites, especially cryptic subterranean sites and horders, can continue to pump out large numbers of uninfected mosquitoes for weeks.

- Identify high risk properties and treat beforehand.

- Increase release numbers in area

Barriers to establishment and spread:
pesticide resistance

Domestic use of pesticides can selectively kill released mosquitoes if wild population is resistant.

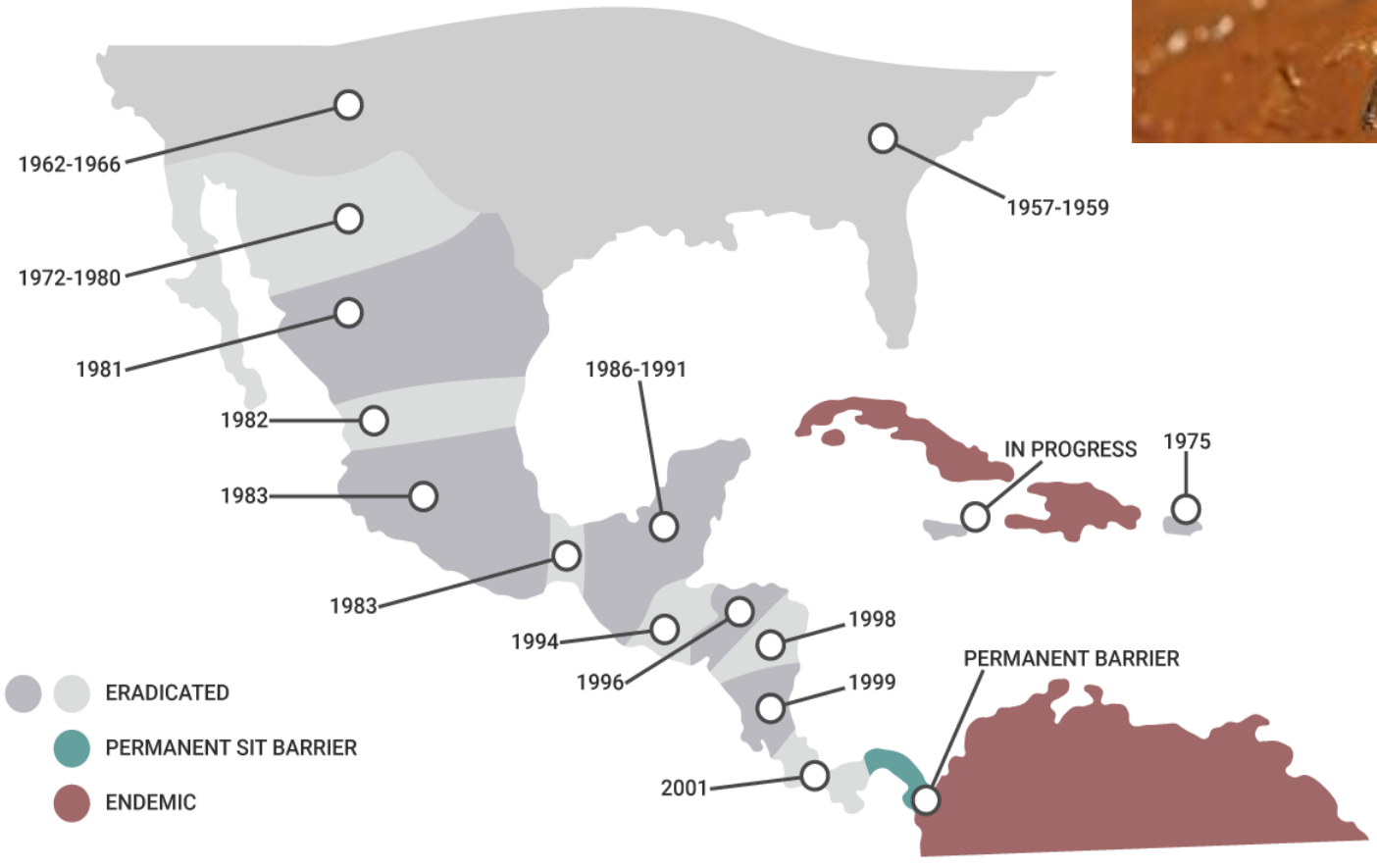

Backcrossing to produce locally adapted strain
Australia
Wolbachia Aedes aegypti
Yogyakarta
Aedes aegypti
Parent Cross
W
W = Wolbachia
Backcross 1
Backcross 2
Backcross 3
Backcross 4
Backcross 5
Yogyakarta Wolbachia
Aedes aegypti
>98% Yogya genes

Barriers to releases:
regulatory approval and public acceptance

How do we scale up for megacities?

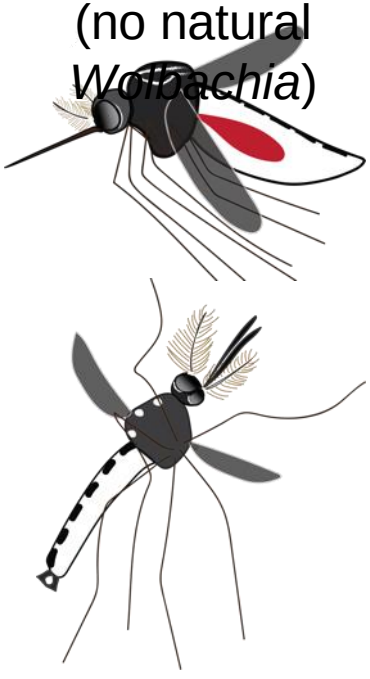
Wolbachia infected male releases for *Ae. aegypti*, *Ae. albopictus* and *Ae. polynesiensis*

Sterile Insect Technique

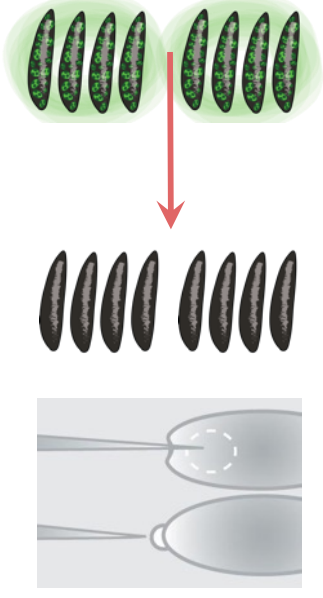

1962-1966
1957-1959
1972-1980
1981
1986-1991
1982
IN PROGRESS
1975
1983
1983
1998
1994
1996
1999
PERMANENT BARRIER
2001
ERADICATED
PERMANENT SIT BARRIER
ENDEMIC

How *Wolbachia* is used for SIT

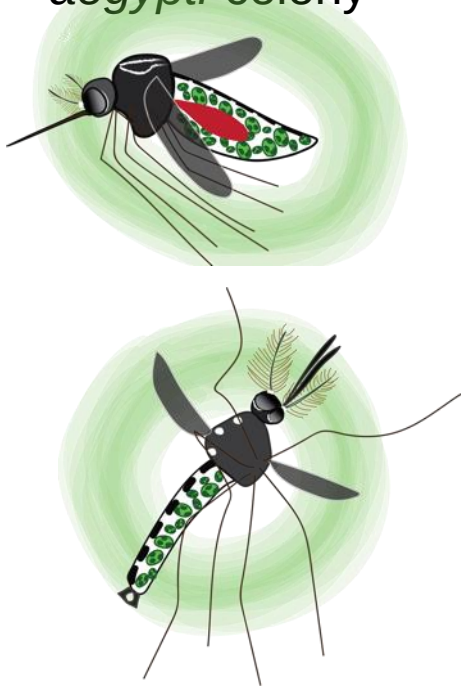
Wild *Aedes aegypti* (no natural *Wolbachia*)



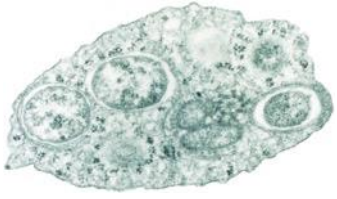
Transfect *Wolbachia* from a related species



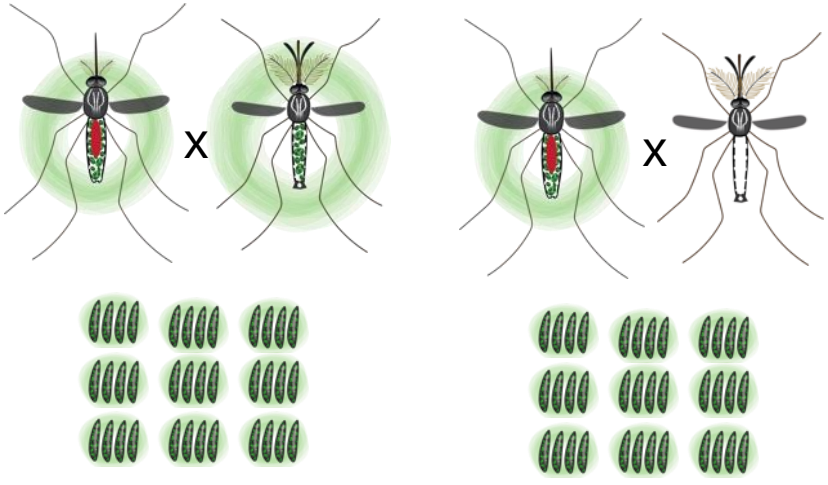
Wolbachia-infected *Aedes aegypti* colony



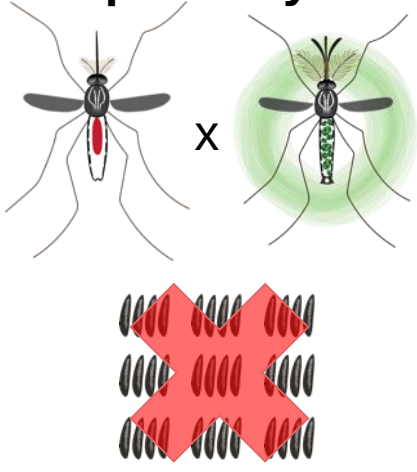
What is *Wolbachia*



Maternally Transmitted



Cytoplasmic Incompatibility



Wolbachia used for SIT a long time ago

NATURE, VOL. 216, OCTOBER 28, 1967

Eradication of *Culex pipiens fatigans* through Cytoplasmic Incompatibility

Culex pipiens fatigans is the chief vector of filariasis in south-east Asia. Urbanization has often caused the numbers of this mosquito—and with it the danger of filariasis infection—to increase alarmingly[1]. The natural vigour, tolerance and fast development of resistance to insecticides of this mosquito necessitate the development of other control methods, and cytoplasmic incompatibility[2] seems to be an ideal means.

Laven 1967

Crossing between members of allopatric populations of the *Culex pipiens* complex can produce four different results. Most populations will produce normal offspring in reciprocal crosses, while some give offspring in one direction and embryos which will not hatch in the opposite direction. Other crosses are infertile in both directions. This lack of offspring is due to cytoplasmic incompatibility[3], which is inherited cytoplasmically. It remains constant for indefinite numbers of generations in the female line. In an incompatible cross the sperm is blocked before it can fuse with the haploid egg nucleus, and if the embryos develop they do so from the haploid egg nucleus and die before hatching (unpublished work of H. Jost).

Burmese Population of *Cx fatigans* was wiped out

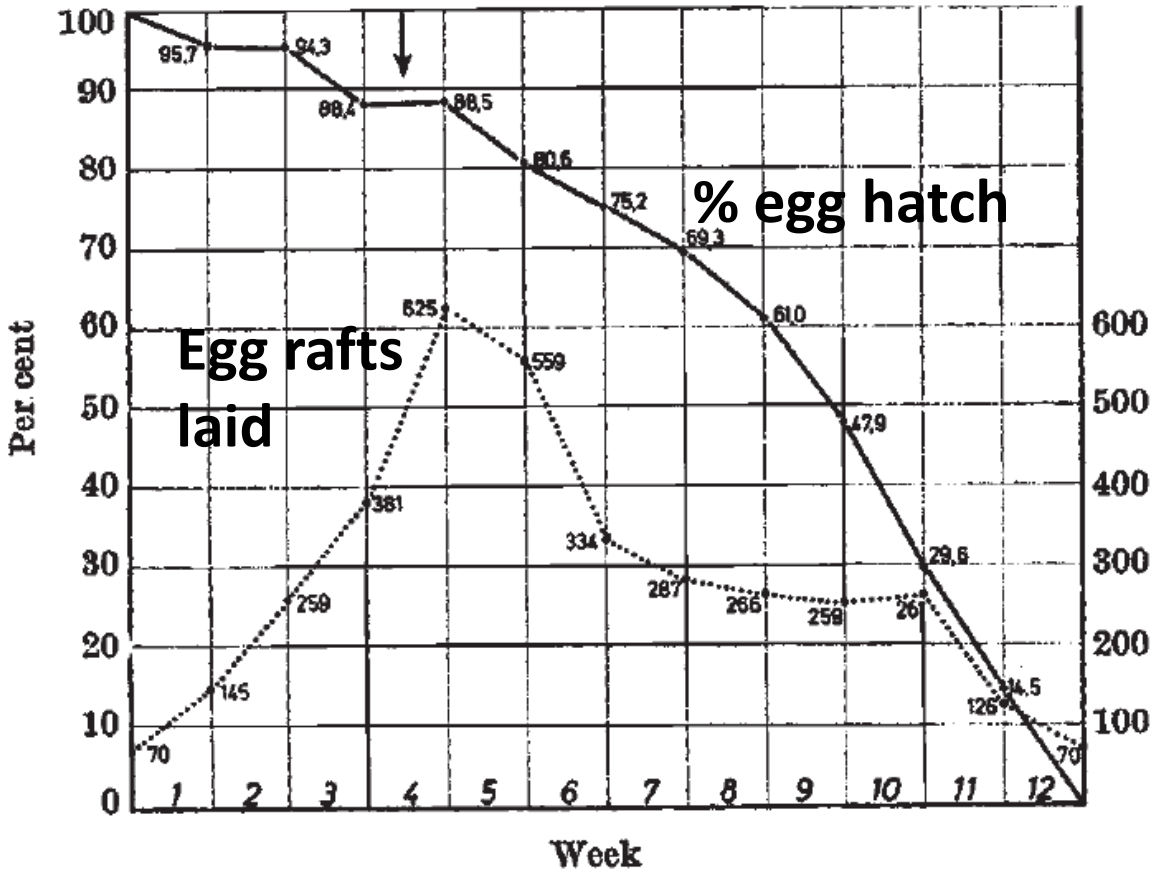


Fig. 1. Eradication of *C. fatigans* in the village of Okpo, Burma, February–May 1967. ——, Daily average percentage of hatching rafts per week 1–12; . . . . , daily average number of egg rafts per week 1–12.

Challenges with SIT in Mosquitoes

FIT STERILE MALES

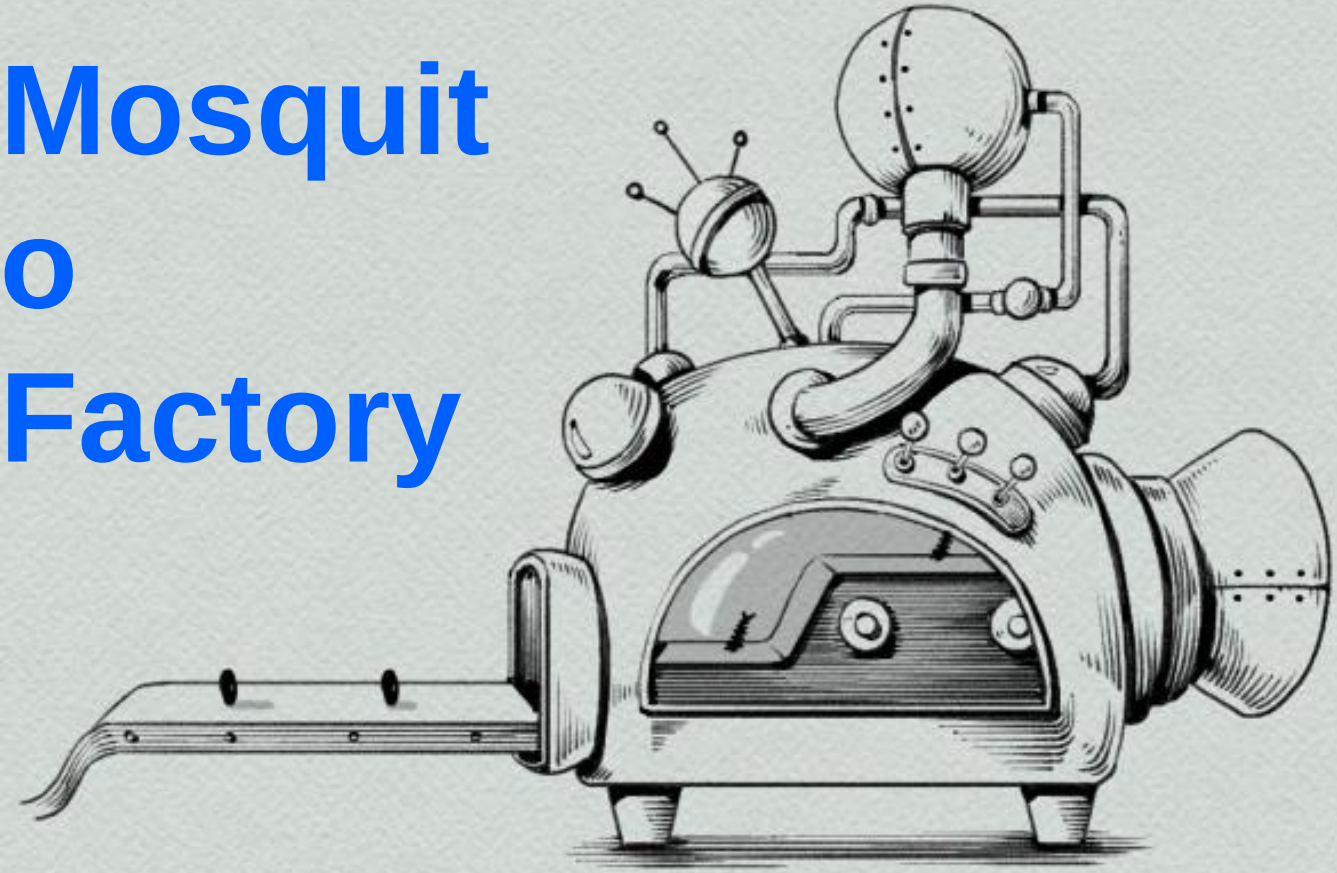
MOSQUITO FACTORIES

DEPLOYMENT

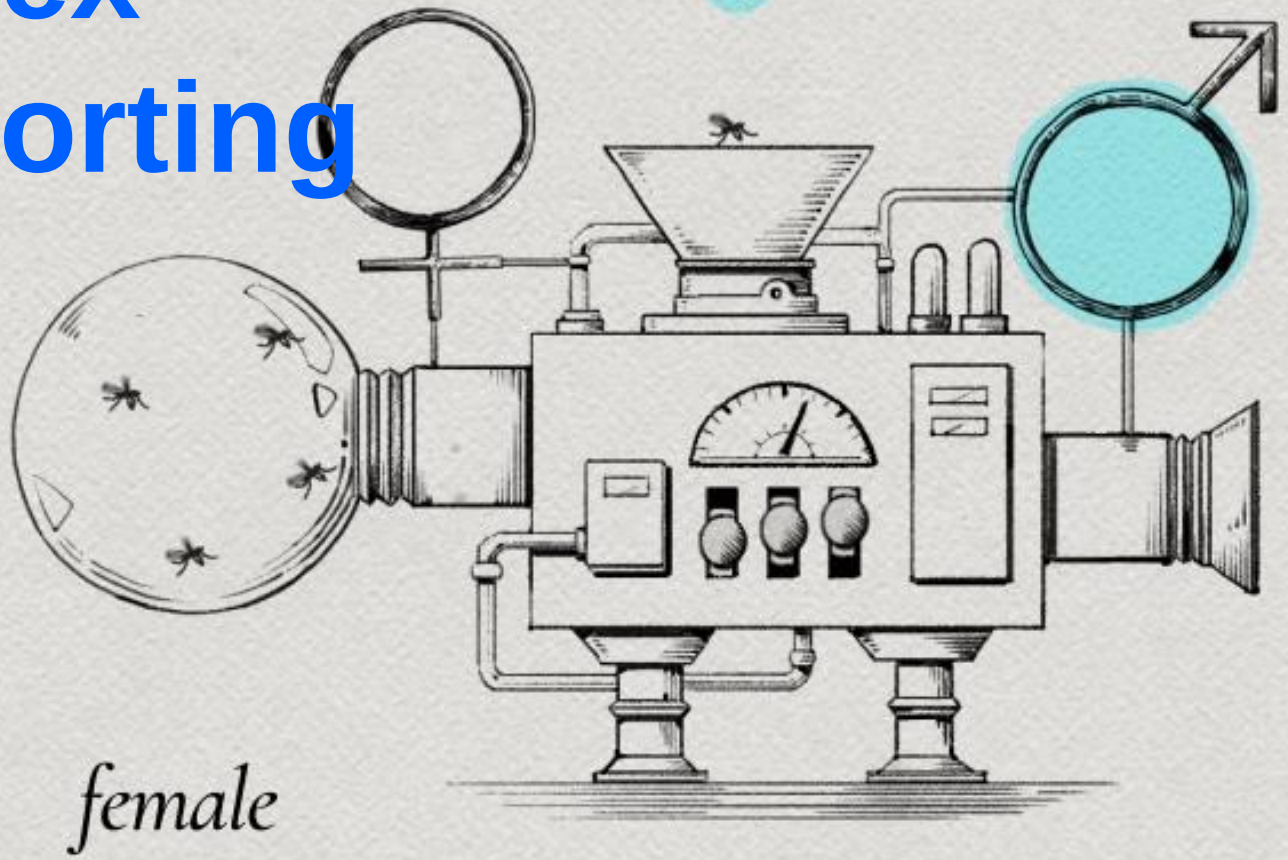
SENSORS

https://debug.com/

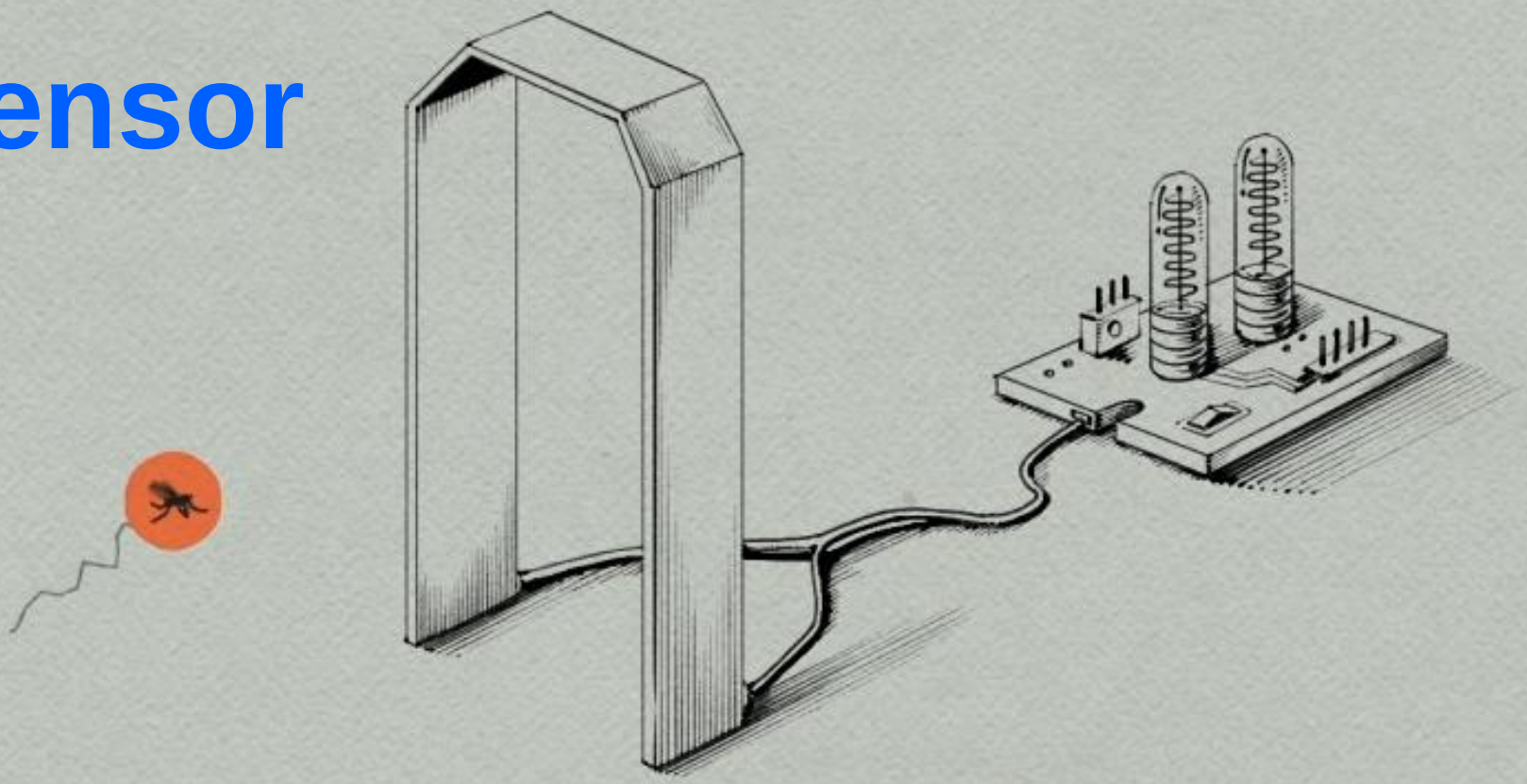
Mosquito Factory



Sex Sorting

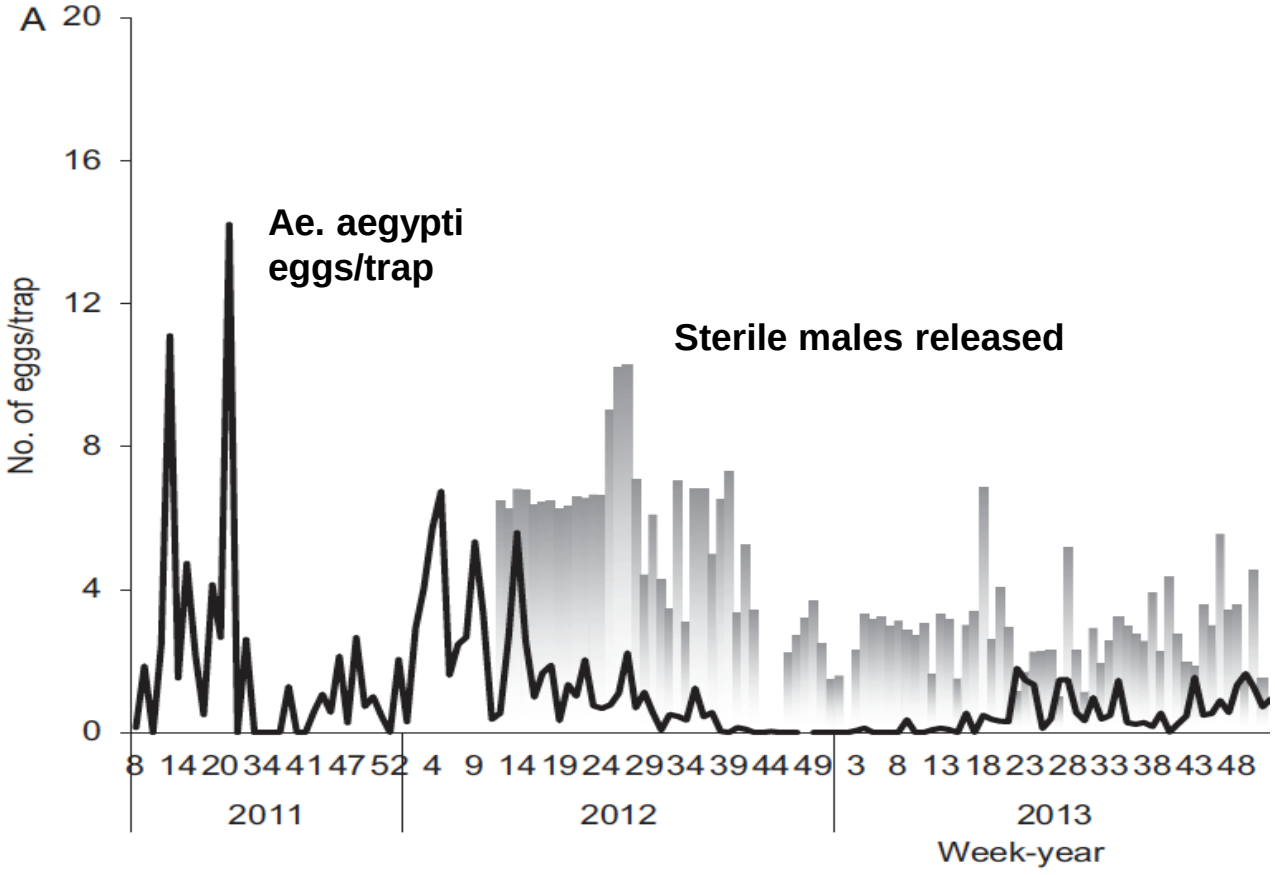


Sensor
s

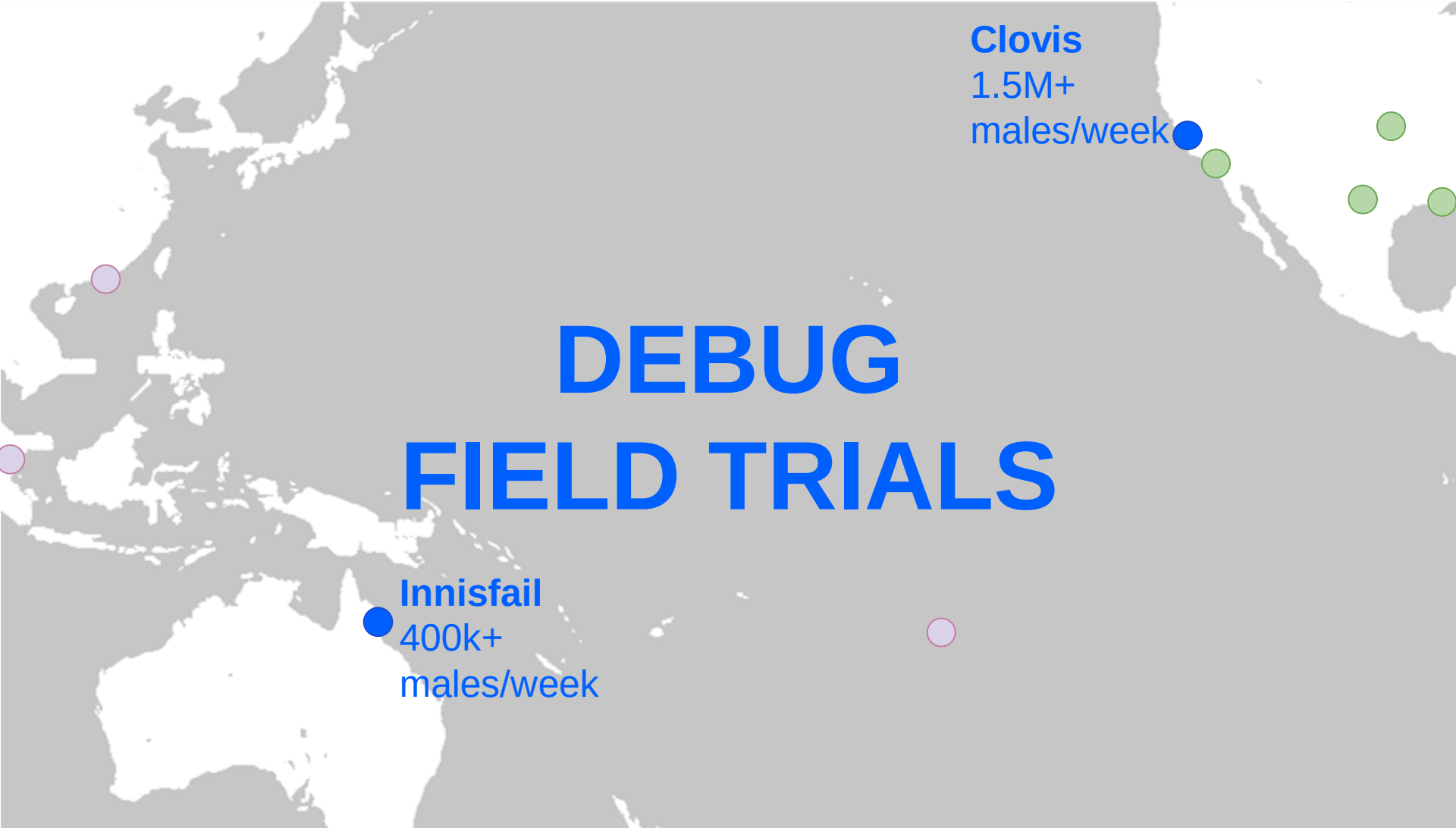


Verily mosquito release van, Fresno California

Male releases: Crash female population, RIDL



Entomologia Experimentalis et Applicata · November 2017
DOI: 10.1111/eea.12618


Clovis
1.5M+
males/week
DEBUG
FIELD TRIALS
Innisfail
400k+
males/week

MosquitoMATE:
Mosquito tubes posted

male *Aedes* releases for suppression

What next?

Trends in Parasitology

CellPress
REVIEWS

Opinion

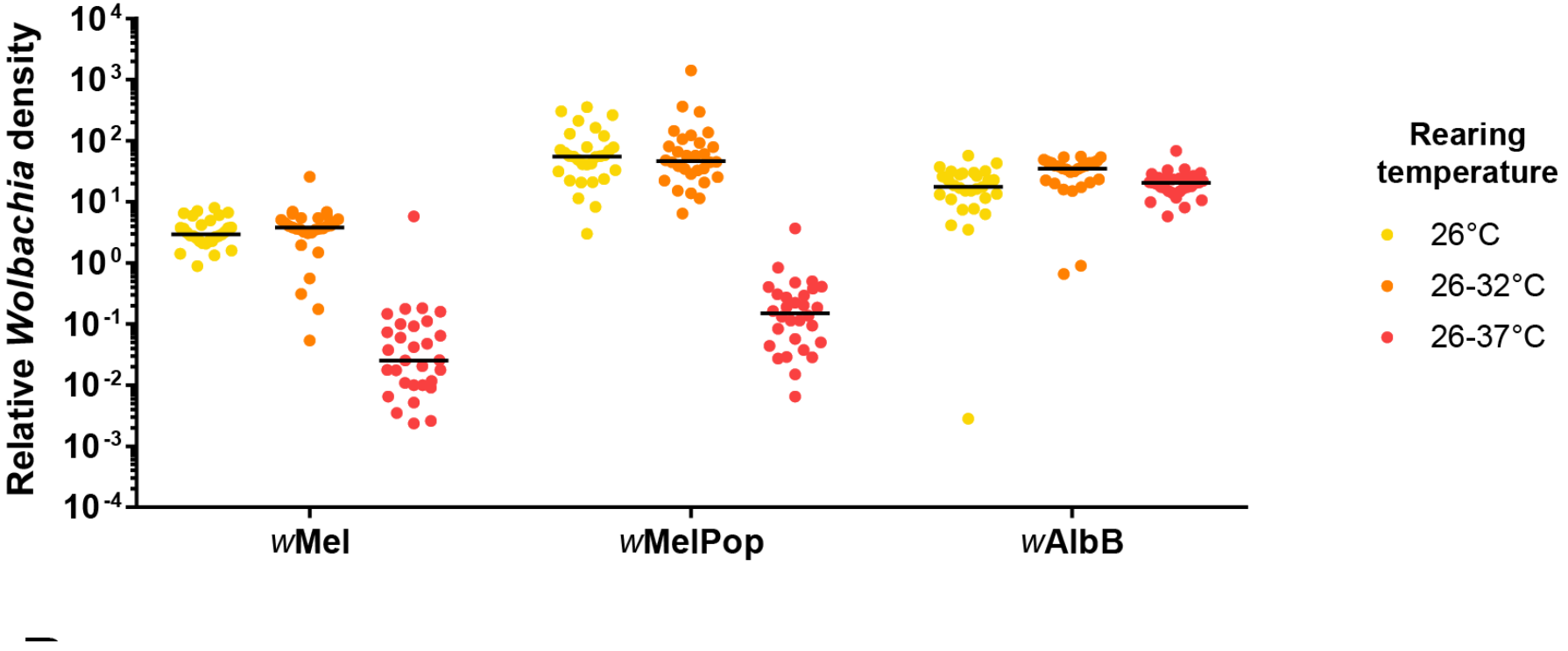
Mission Accomplished? We Need a Guide to the 'Post Release' World of *Wolbachia* for *Aedes*-borne Disease Control

Scott A. Ritchie,[1,2,*] Andrew F. van den Hurk,[3] Michael J. Smout,[2] Kyran M. Staunton,[1,2] and Ary A. Hoffmann[4]

Checklist of worries

- Could Wolbachia drop out of mosquitoes?
- Could a dengue strain become “resistant” to Wolbachia?
- Could another mosquito displace Wolbachia-infected *Ae. aegypti?*

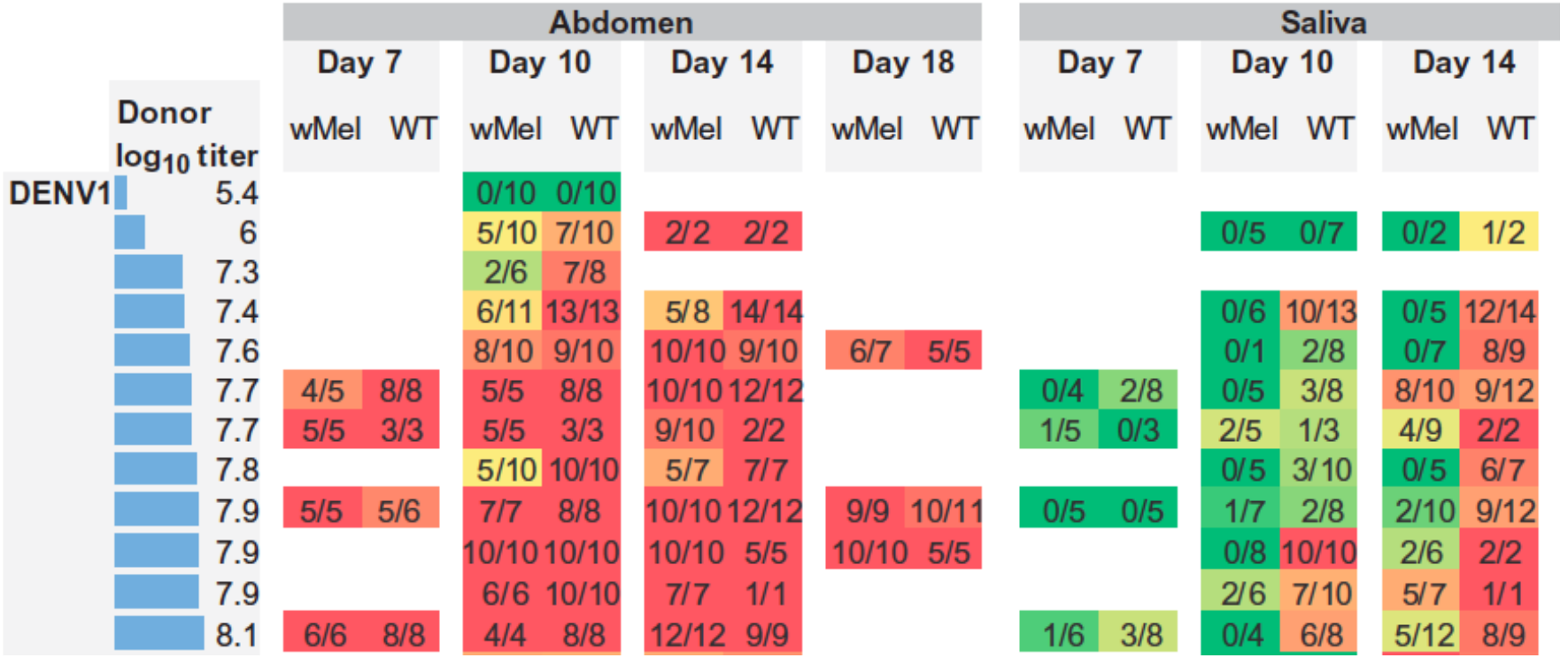
High temperatures can impact *Wolbachia* infection…but wMel persists in Cairns



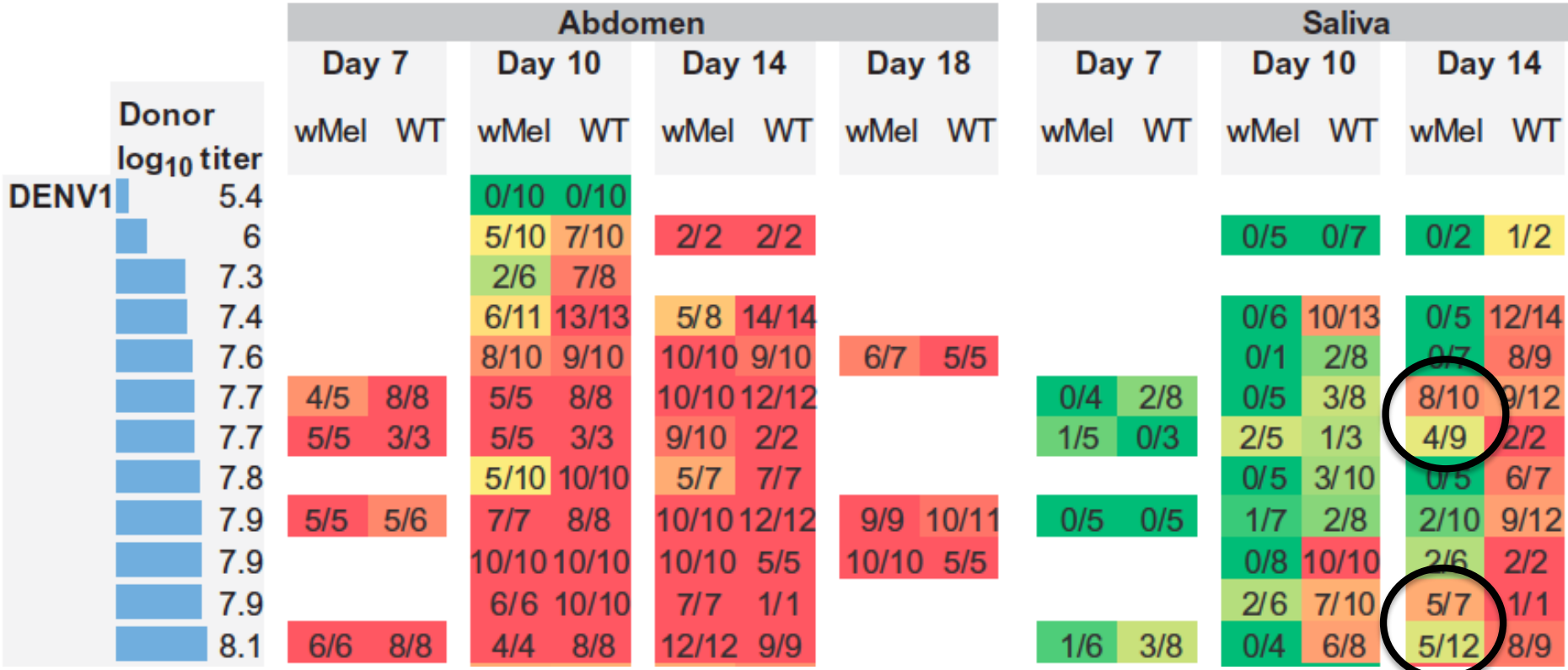
Ross et al. 2017. PLoS Pathog doi:10.1371/journal.ppat.1006006

Vector competence impact of wMel

Ferguson et al. 2015 Sci. Trans. Med. 279



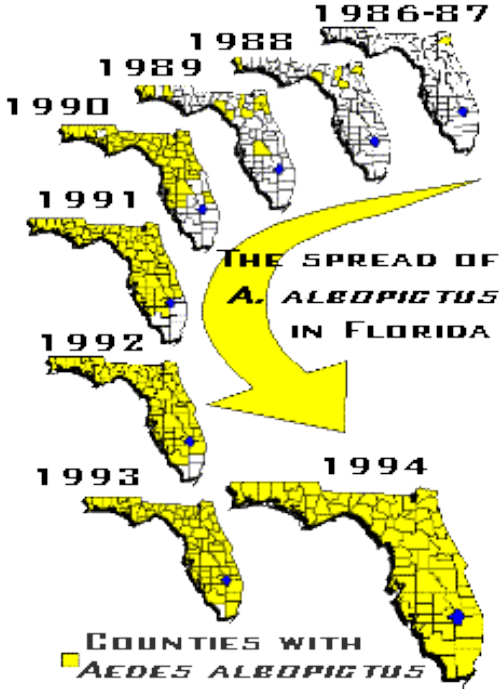
Could “break through” transmission select for virulent or resistant DENV?



	Donor log_{10} titer	Abdomen Day 7 wMel	Abdomen Day 7 WT	Abdomen Day 10 wMel	Abdomen Day 10 WT	Abdomen Day 14 wMel	Abdomen Day 14 WT	Abdomen Day 18 wMel	Abdomen Day 18 WT	Saliva Day 7 wMel	Saliva Day 7 WT	Saliva Day 10 wMel	Saliva Day 10 WT	Saliva Day 14 wMel	Saliva Day 14 WT
DENV1	5.4			0/10	0/10										
	6			5/10	7/10	2/2	2/2					0/5	0/7	0/2	1/2
	7.3			2/6	7/8										
	7.4			6/11	13/13	5/8	14/14					0/6	10/13	0/5	12/14
	7.6			8/10	9/10	10/10	9/10	6/7	5/5			0/1	2/8	0/7	8/9
	7.7	4/5	8/8	5/5	8/8	10/10	12/12			0/4	2/8	0/5	3/8	8/10	9/12
	7.7	5/5	3/3	5/5	3/3	9/10	2/2			1/5	0/3	2/5	1/3	4/9	2/2
	7.8			5/10	10/10	5/7	7/7					0/5	3/10	0/5	6/7
	7.9	5/5	5/6	7/7	8/8	10/10	12/12	9/9	10/11	0/5	0/5	1/7	2/8	2/10	9/12
	7.9			10/10	10/10	10/10	5/5	10/10	5/5			0/8	10/10	2/6	2/2
	7.9			6/6	10/10	7/7	1/1					2/6	7/10	5/7	1/1
	8.1	6/6	8/8	4/4	8/8	12/12	9/9			1/6	3/8	0/4	6/8	5/12	8/9

Ae. albopictus: The other vector
Could it displace *Wolbachia* infected *Ae. aegypti?*

Aedes albopictus: the great displacer!



http://fmel.ifas.ufl.edu/fmel---research-areas/invasion-biology-of-aedes-albopictus/

***Ae. aegypti* severely reduced in central and northern Florida**

Limited to urbanised areas

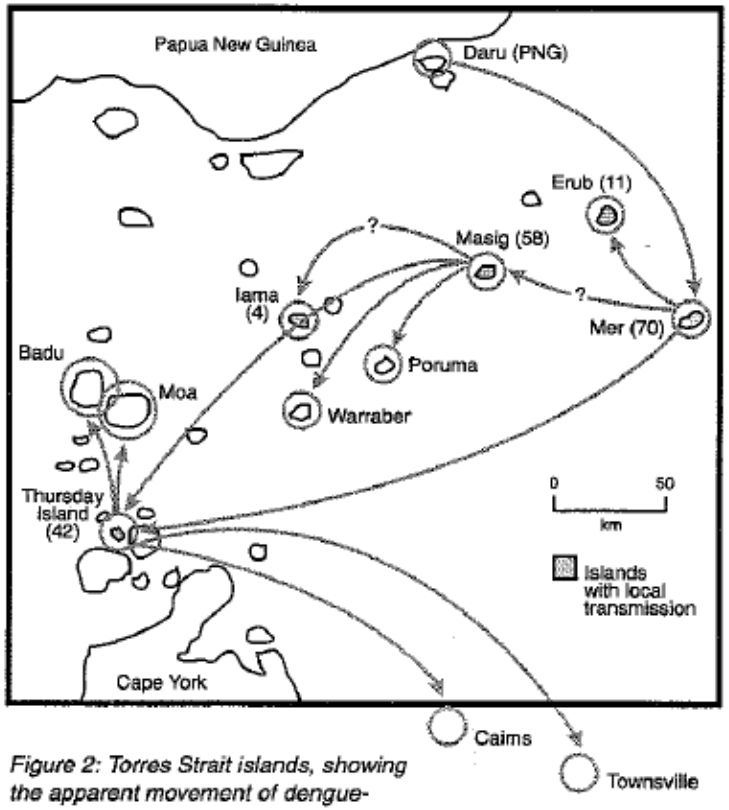


Figure 2: Torres Strait islands, showing the apparent movement of dengue-infected people within the region and the "dengue-receptive" region of mainland Australia. The total number of confirmed cases on the islands with local transmission is given in parentheses.

Hanna et al. 1998. Medical Journal of Australia 168: 221-225

1996-97: widespread dengue outbreak in Torres… Vectored by *Ae. aegypti*

And now only *Ae. albopictus* on Erub, Mer, Warraber, Iama, Moa, Horn, Hammond and Badu Is.

No *Ae. aegypti* detected in recent surveys!

What can we do?

Ritchie et al. 2018 Trends in Parasitology

- Research
  - Evolution of wolbachia in mosquito
  - Ability for mosquitoes to transmit virus (Vector competence)

What can we do?

- Monitor, monitor, monitor
  - Traps for mosquitoes,
  - PCR and LAMP for Wolbachia
  - Changes in species composition, pop dynamics

- If dengue occurs
  - Trap mosquitoes and see if infected … for DENV and Wolbachia
  - Eliminate the virus?; QH pros at this

Dengue fever KO'd after epic struggle

Roz Pulley

CAIRNS has finally rid itself of the dengue scourge that has plagued the city for the past year.

Yesterday health officials declared the city dengue-free after a prolonged battle with the deadly mosquito that has infected 79 people since January and resulted in a blitz on more than 4000 properties.

"The dengue fever outbreak is officially over," beamed Tropical Public Health Unit medical entomologist Dr Scott Ritchie, donning a mozzie-emblazoned shirt specially for the occasion.

He said the all-clear came after three months in which no new cases were reported, the time allowed to detect any lingering dengue transmission.

The Cairns outbreak was one of three simultaneous outbreaks the unit had to contend with during the past 12 months, others affecting 276 people in the Torres Strait and 58 people in Townsville.

In Cairns, the virus spread to Bentley Park, Bungalow, Babinda, Earlville, Edge Hill, Edmonton, Gordonvale, Manoora, Manunda, Parramatta Park, Portsmith and Westcourt.

"This year's outbreak in Cairns began when a person who contracted the virus overseas came into contact with local dengue mosquitoes, which then spread the virus to local residents," Dr Ritchie said.

He said the unit worked closely with Cairns City Council to get on top of the outbreak, conducting intensive house-to-house inspections and mosquito eradication.

"It is a difficult thing to get rid of. All it takes is one person with a lot of stuff lying around to breed enough mosquitoes to perpetuate it," he said.

Dr Ritchie warned against complacency, saying dengue was on the increase overseas and could become entrenched in North Queensland. "If dengue became endemic here, the local population would be at constant risk," he said.

He said two new control officers had been employed to help prevent dengue outbreaks and research was continuing on trapping techniques.

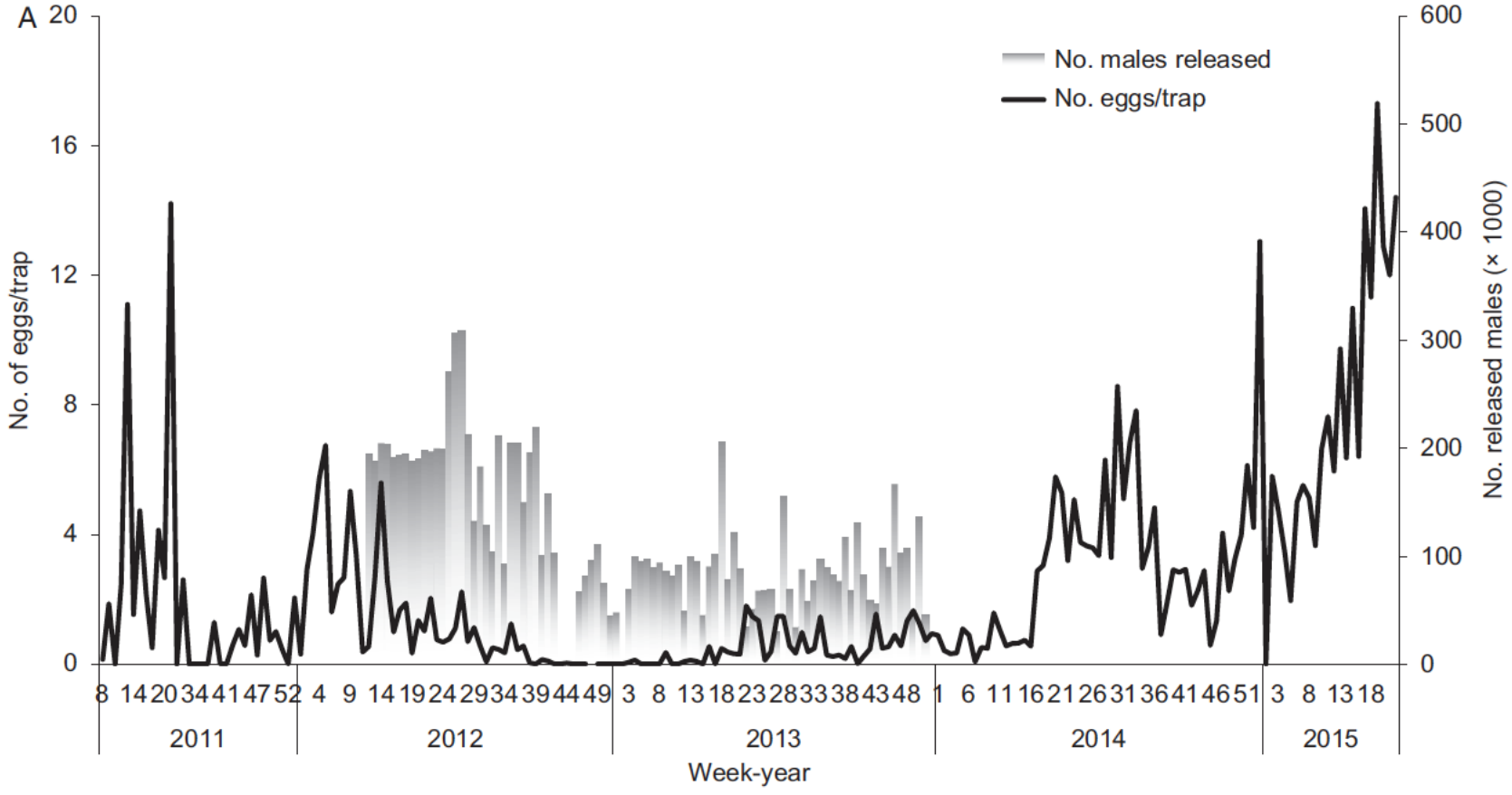
"But the only way to beat it is for everyone to clean up their yards."

The most frequent breeding sites are pot plant bases, tyres, black plastic and discarded containers.

Hit 'em high, hit 'em low: Tropical Public Health Unit medical entomologist Dr Scott Ritchie has claime victory in the ongoing battle to stop the spread of dengue fever in the Far North.

Picture: MIKE WAT

Male releases: Take your foot off the gas and…



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Soper: perifocal spraying DDT

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