

Urban Arboviral Emergence: Chikungunya, Zika, Dengue & Yellow Fever

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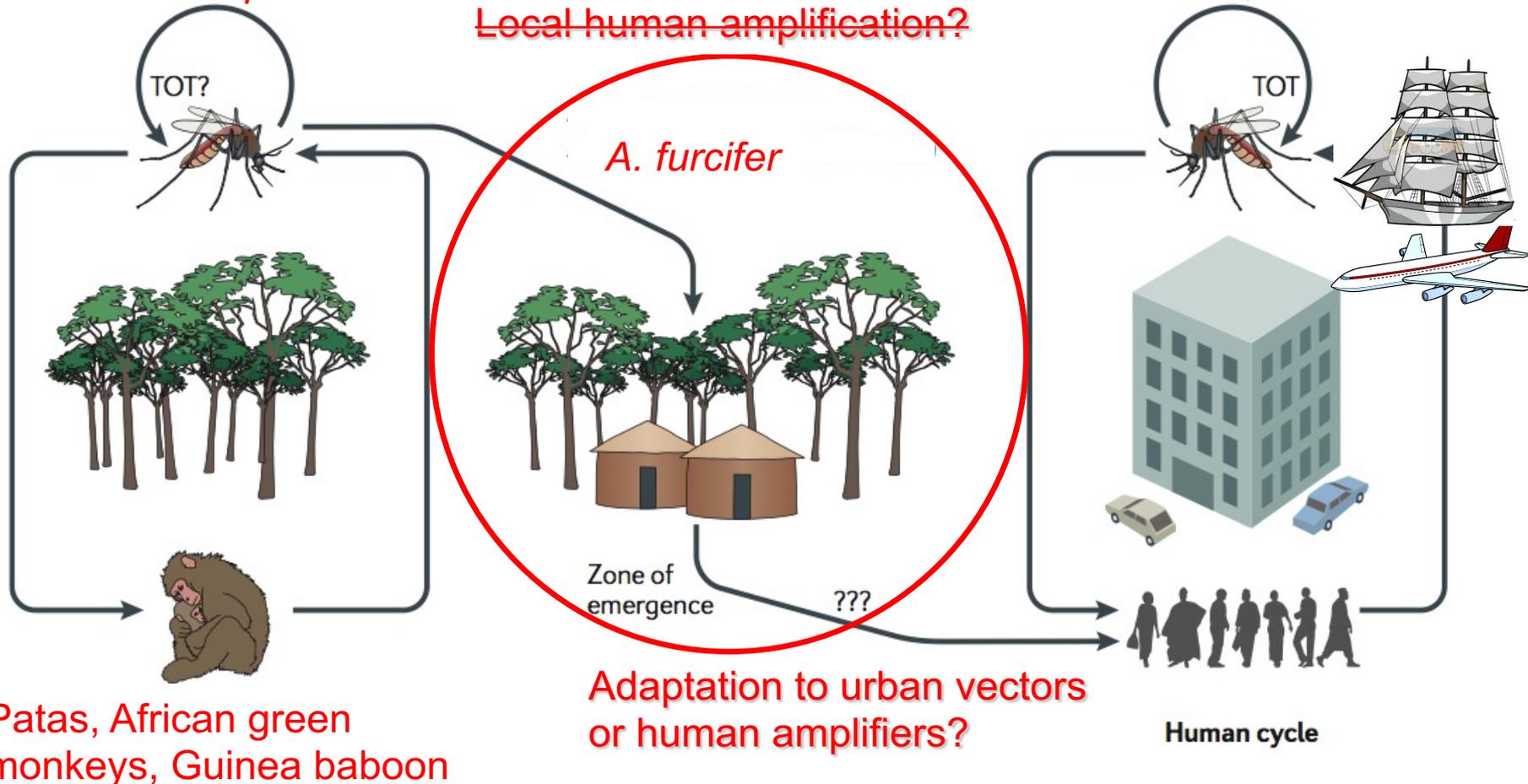
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African arboviruses with history of urban emergence via *Aedes* (*Stegomyia*) transmission and human amplification: **yellow fever, chikungunya, Zika**



A. furcifer
A. taylori
A. luteocephalus

A. aegypti
A. albopictus

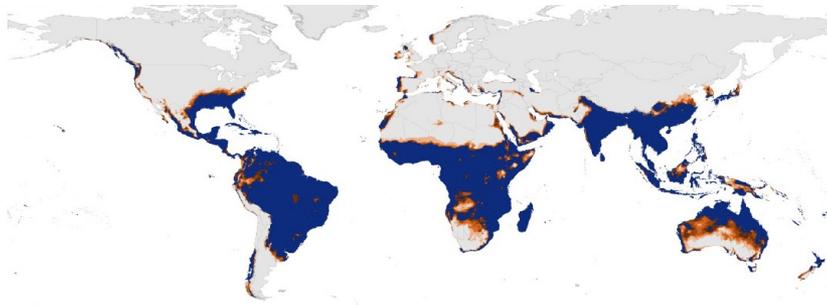


Urban *Aedes* Arbovirus Vectors

A. aegypti



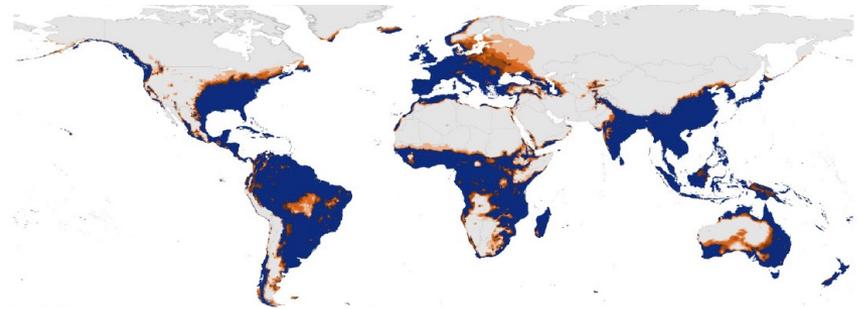
Originated in sub-Saharan Africa, spread throughout the tropics centuries ago after domestication



A. albopictus



Originated in Asia, spread to the Americas, Africa and Europe beginning in 1985; lesser level of domestication



Predicted extension of distributions due to climate change

Why is *Aedes aegypti* the principal vector of these urban arboviruses?

Highly evolved over hundreds-to-thousands of years in Africa to live in **close association with people**:

- Feeds almost exclusively on humans
- Takes multiple bloodmeals during a short time period
- Exploits artificial water containers as larval habitats
- Adult females enter houses and remain there
- “Skip oviposition”
- Exceptionally difficult to control using traditional methods

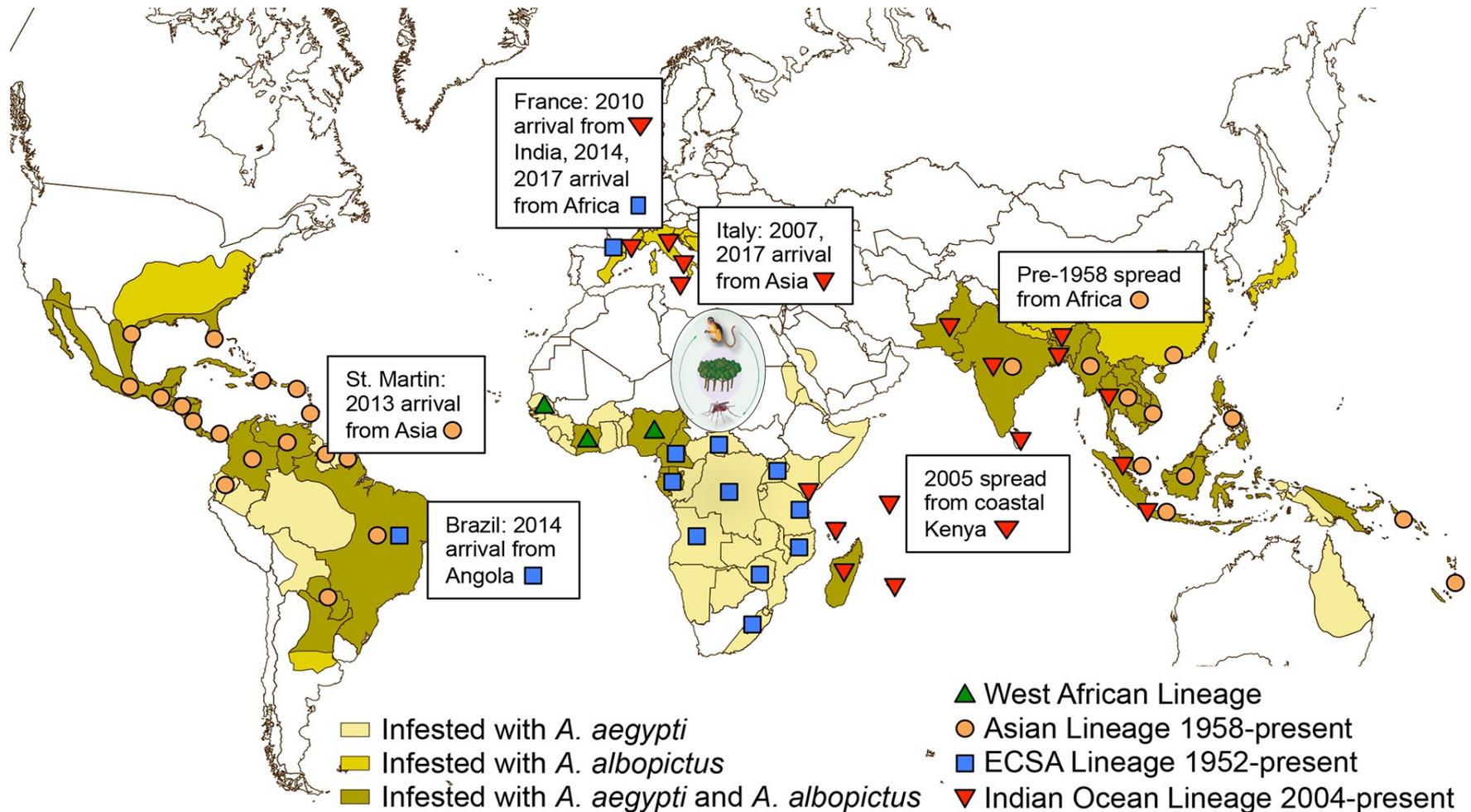


Chikungunya Virus

- Attack rates approach 50% in many regions, high apparent:inapparent ratio (unlike yellow fever, dengue, Zika)
- Fatal cases (ca. 0.1%) occur mainly in the elderly, perinatal and congenital infections (peripartum transmission), persons with underlying medical conditions
- Arthralgia is highly debilitating and often chronic, resulting in severe economic impacts
- DALY estimates can exceed 2/3 of the total population morbidity during outbreaks

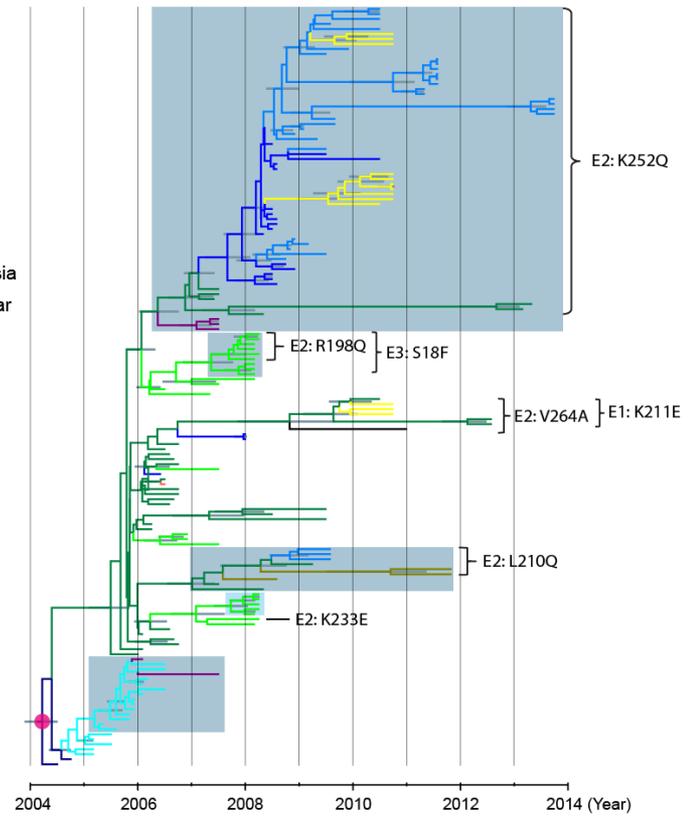


History of Chikungunya Virus Emergence and Spread

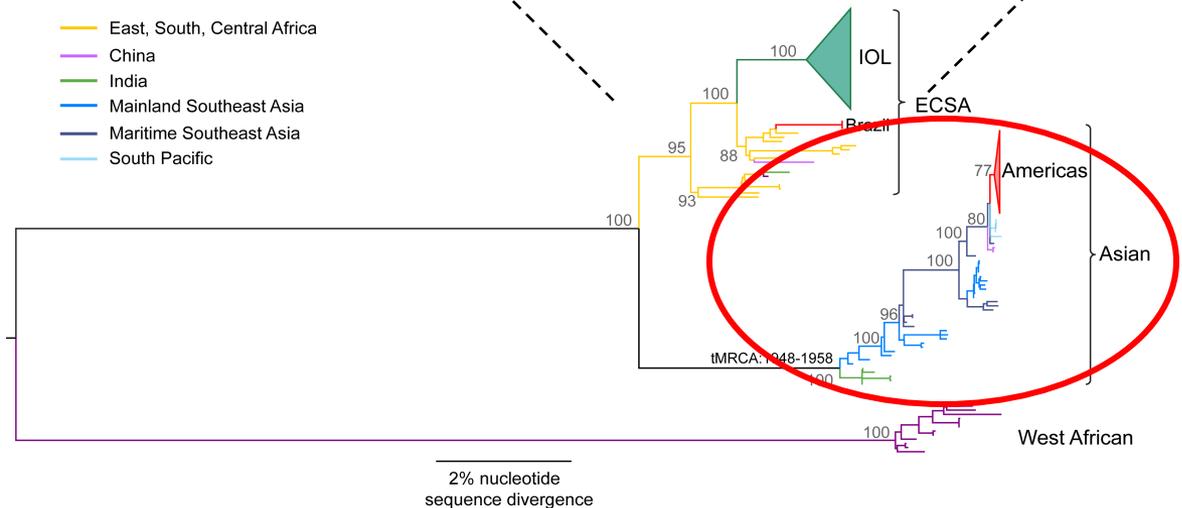


CHIKV Evolution

- India
- Sri Lanka
- Malaysia, Singapore, Indonesia
- Thailand, Cambodia, Myanmar
- China
- Italy, France, Germany
- United States
- Bangledash
- Indian Ocean Islands
- Yemen
- E1-A226V
- Root of IOL



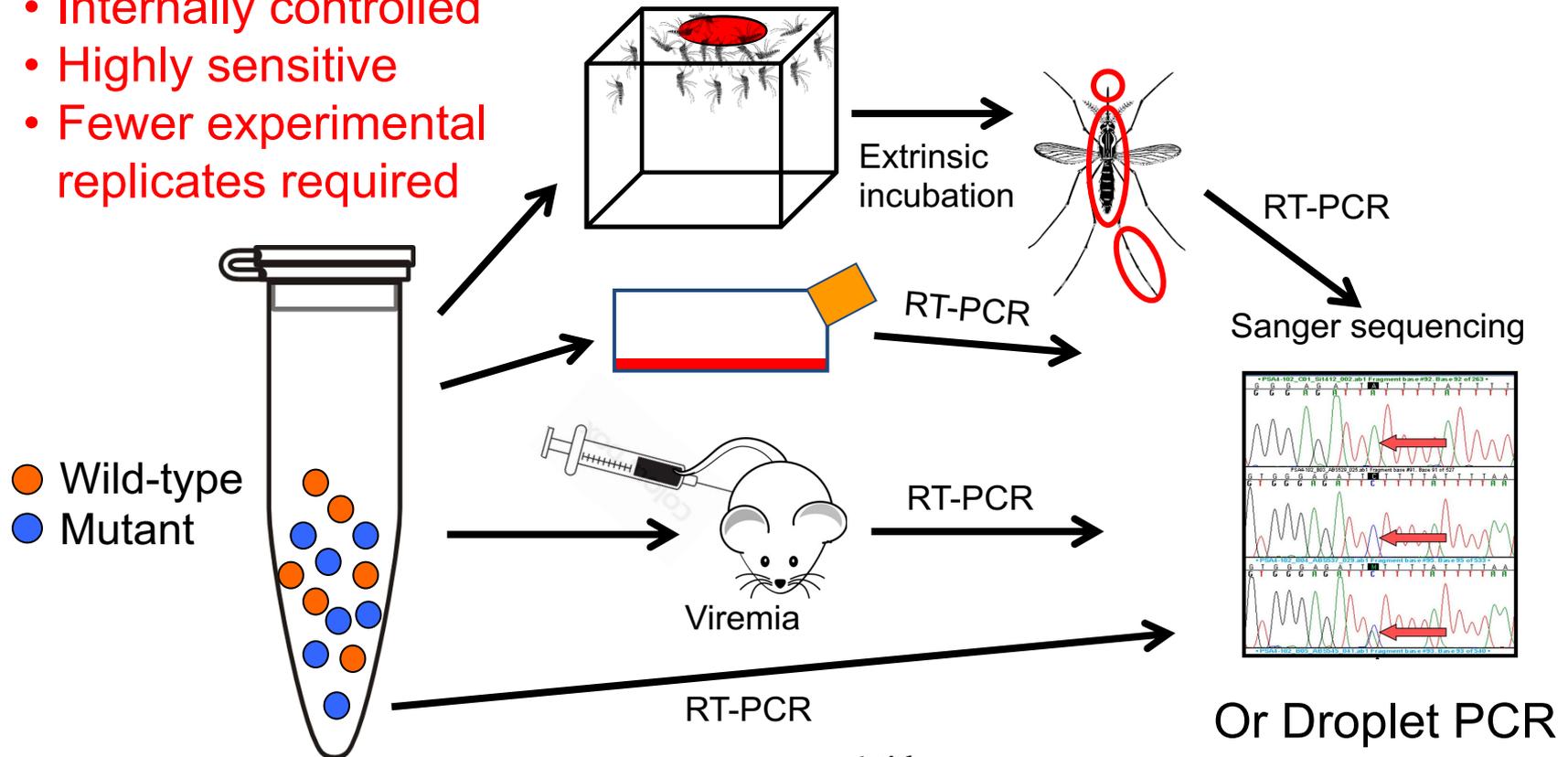
- East, South, Central Africa
- China
- India
- Mainland Southeast Asia
- Maritime Southeast Asia
- South Pacific



Competition Fitness Assay

Advantages:

- Internally controlled
- Highly sensitive
- Fewer experimental replicates required



H_0 : mutant:wt
final ratio/initial ratio=1 (i.e. no
effect of mutant on fitness)

Article

The N501Y spike substitution enhances SARS-CoV-2 infection and transmission

<https://doi.org/10.1038/s41586-021-04245-0>

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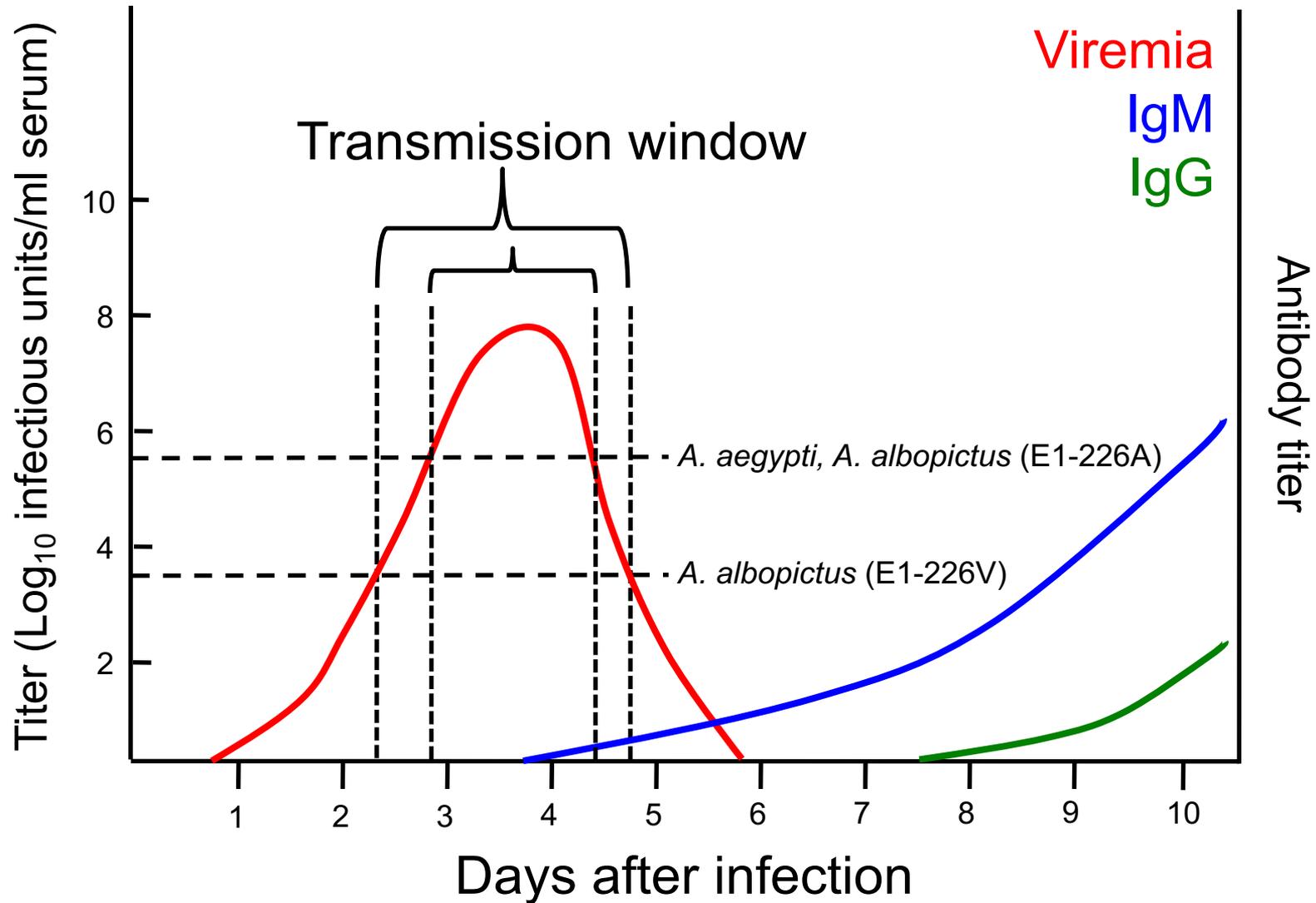
Yang Liu^{1,2,6}, Jianying Liu^{2,3,4,6}, Kenneth S. Plante^{2,3,4,6}, Jessica A. Plante^{2,3,4}, Xuping Xie¹, Xianwen Zhang¹, Zhiqiang Ku⁵, Zhiqiang An⁵, Dionna Scharton^{2,3,4}, Craig Schindewolf^{2,4}, Steven G. Widen¹, Vineet D. Menachery^{2,4}, Pei-Yong Shi^{1,2,5,6} & Scott C. Weaver^{2,3,4,5,6}

A. albopictus-adaptive CHIKV Darwinian Evolution

Lineage	First appearance	Protein	Substitution	Fitness for <i>A. albopictus</i> infection	Fitness for <i>A. aegypti</i> infection
IOL	2005	E1	A226V	40-fold increase	Slight decrease
IOL (SL1)	2007	E2	K252Q	8-fold increase	No effect
IOL (SL2 partial)	2008	E2	K233E	6-fold increase	No effect
IOL (SL3B)	2008	E2/E3	R198Q/S18F (synergistic)	16-fold increase	No effect
IOL (SL4)	2009	E2	L210Q	5-fold increase	No effect
Asian	Never	E1	A226V	No effect	Not done
Asian	Never	E2	K252Q	Little or no effect	Little or no effect
Asian	Never	E2	K233E	Little or no effect	Little or no effect
Asian	Never	E2/E3	R198Q/S18F (synergistic)	Little or no effect	Little or no effect
Asian	Never	E2	L210Q	Slight decrease	Not done

1. None of these mutations has a major effect on infection of *A. aegypti*.
2. All affect initial infection of the *A. albopitus* midgut
3. Structural studies allow us to predict additional adaptive mutations, confirmed experimentally

Human Infection Profile for CHIKV

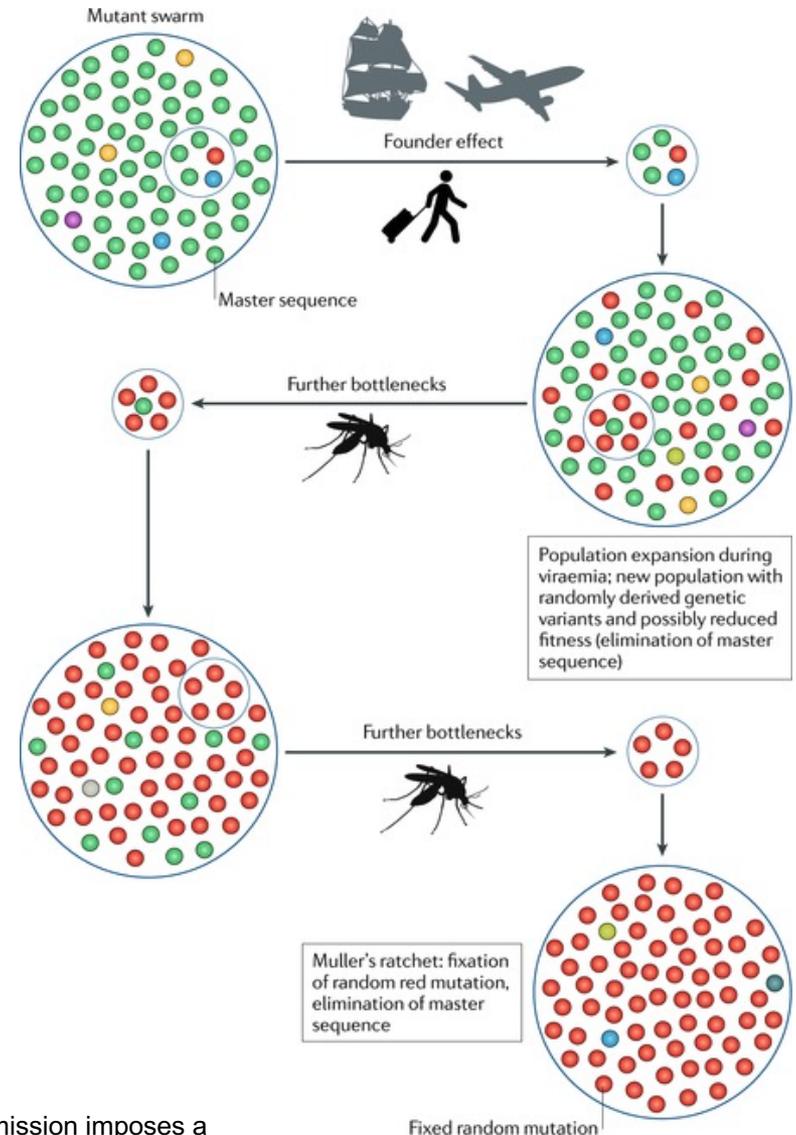
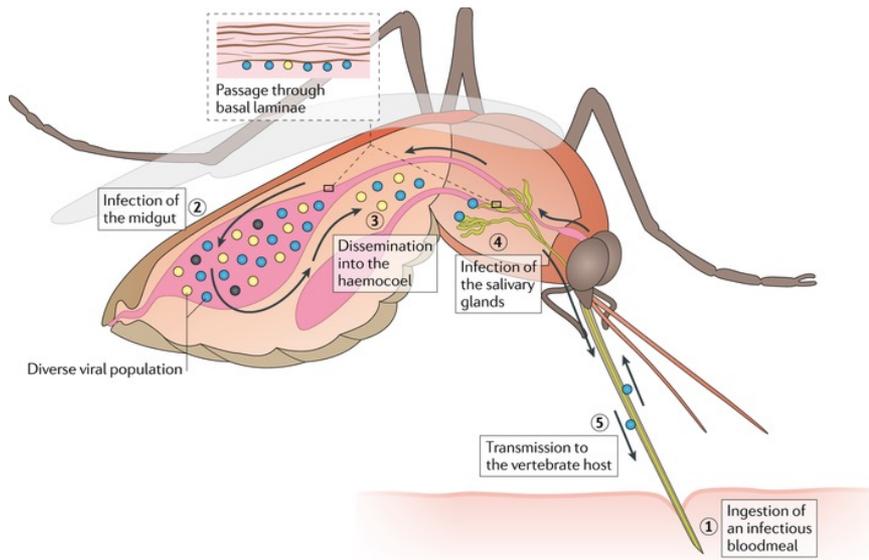


Stochastic nature of arboviral emergence due to **founder effects:**

Genetic drift: Random changes in the genetic make-up of a population due to chance, random sampling. Drift can dominate the evolution of a virus when population sizes remain small, reducing the efficiency of selection and genetic diversity.

Populations bottlenecks can result in fitness declines or epistatic constraints on adaptive evolution due to genetic drift

Arboviruses and Population Bottlenecks



Forrester NL, Guerbois M, Seymour RL, Spratt H, Weaver SC. Vector-borne transmission imposes a severe bottleneck on an RNA virus population. PLoS Pathog. 2012.

Amino acid substitutions that interact epistatically with *A. albopictus*-adaptive mutations

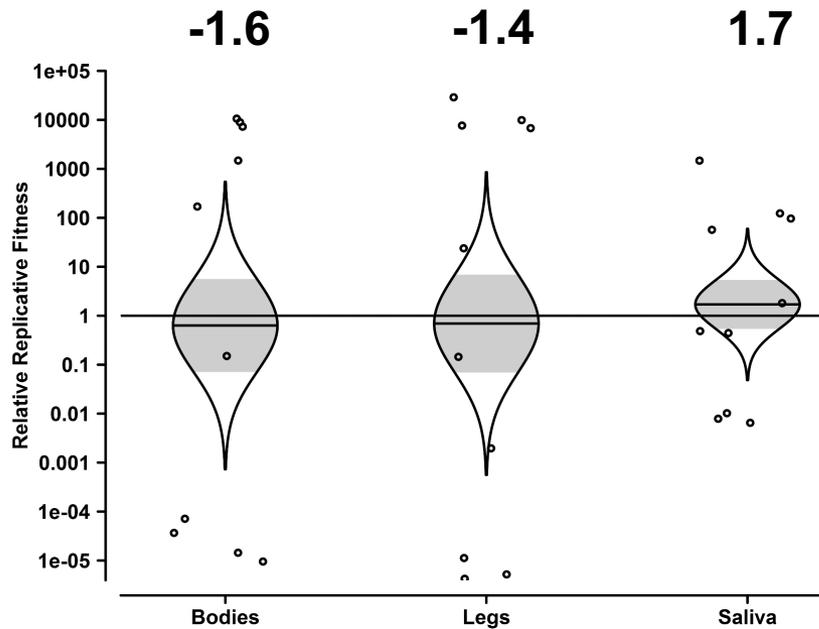
CHIKV Lineage	Year of first appearance	Protein	Amino acid substitution	Approximate infectivity increase or epistatic effect	Epistatic interaction
Asian	1958	E1	A98T All Asian and Asian/American strains have T	Completely prevents penetrance for <i>A. albopictus</i> infection	E1-226V
ECSA	1953	E2	I211T ECSA/Brazilian strain has I	Enables penetrance for <i>A. albopictus</i> infection	E1-226V

Tsetsarkin KA, McGee CE, Volk SM, Vanlandingham DL, Weaver SC, Higgs S. Epistatic roles of E2 glycoprotein mutations in adaptation of chikungunya virus to *Aedes albopictus* and *Ae. aegypti* mosquitoes. PLoS One 4:e6835.

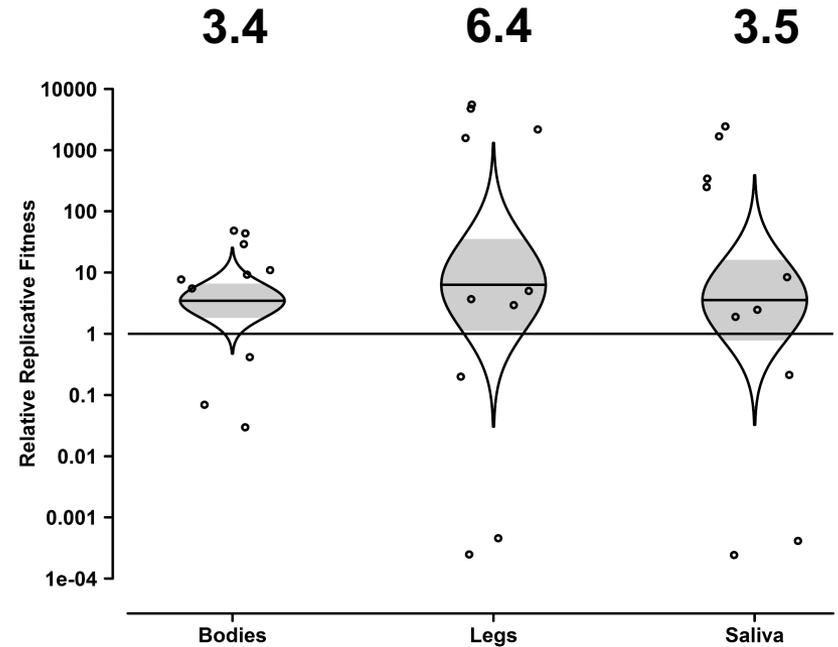
Tsetsarkin KA, Chen R, Leal G, Forrester N, Higgs S, Huang J, Weaver SC. Chikungunya virus emergence constrained in Asia by lineage-specific adaptive landscapes. Proc Natl Acad Sci U S A. 2011. 108:7872-

Competition of E1-A226V Against WT in Brazil-ECSA and Asian-American Backbones

Brazil-ECSA

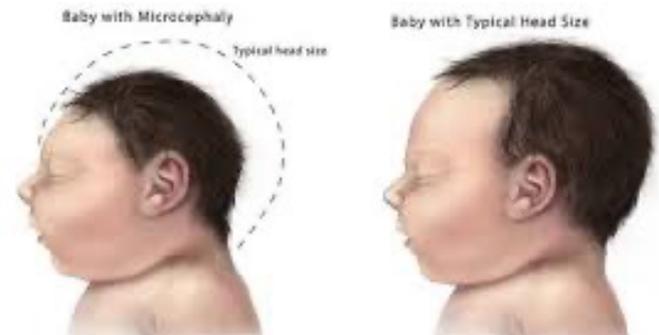
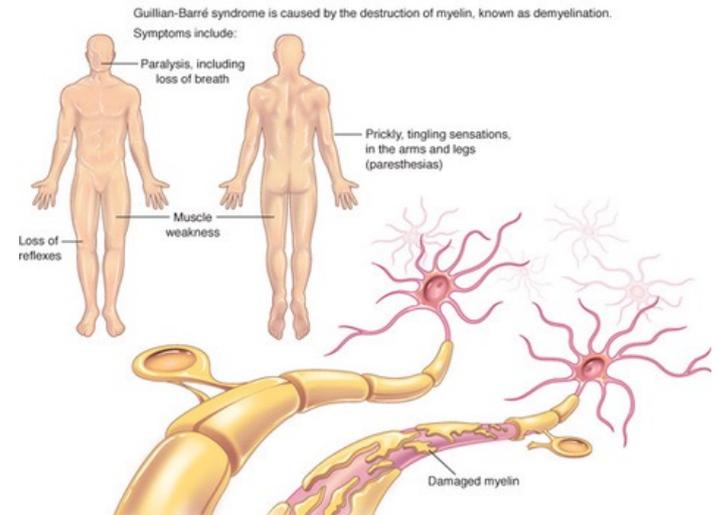


Asian-American

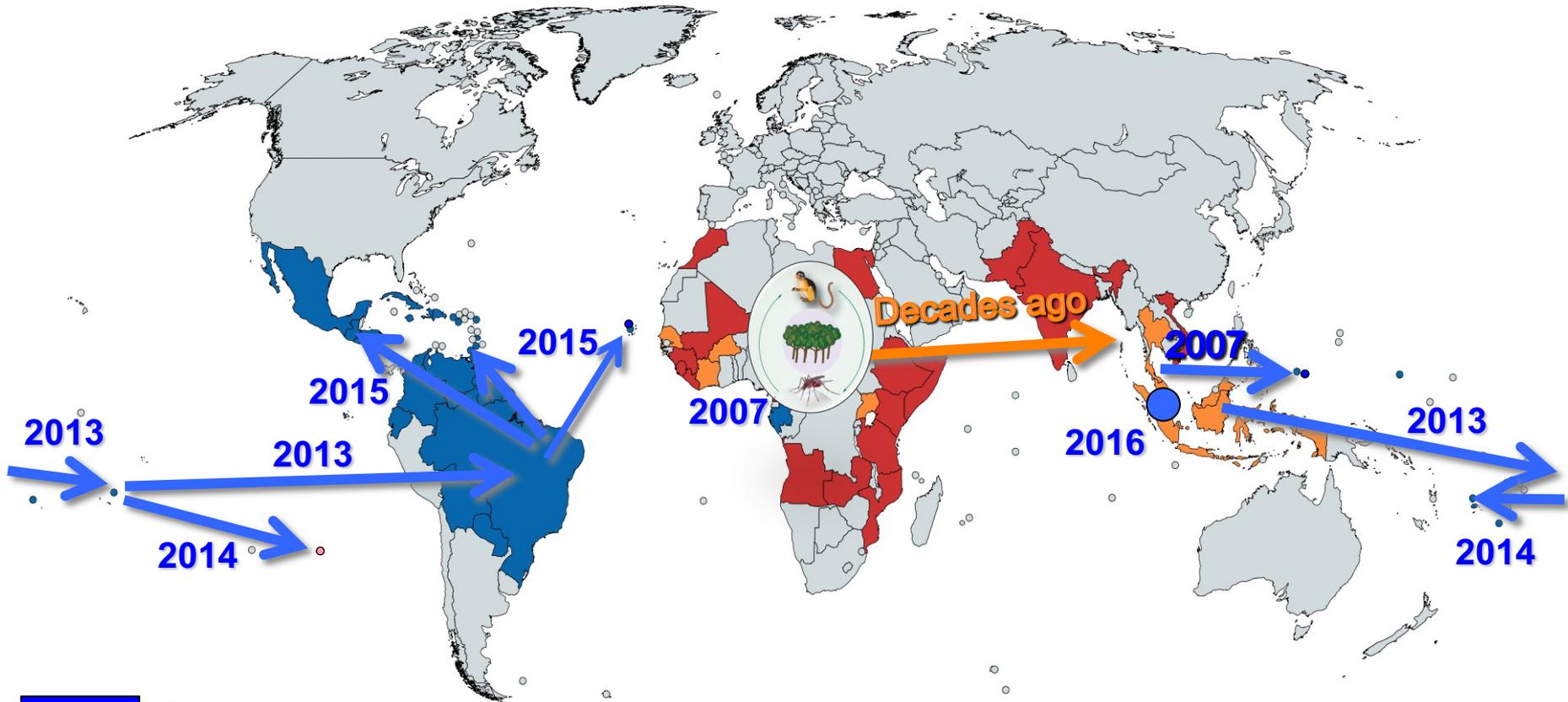


Association Between ZIKV and Microcephaly, Guillain–Barré syndrome

- ZIKV infection first associated in French Polynesia with a ca. 2-10-fold increase in **Guillain–Barré syndrome**
- **Microcephaly** first detected in northeastern Brazil in 2015 based on a 100-200-fold rise in incidence coincident with the ZIKV outbreak
- A wide range of congenital defects now termed **Congenital Zika Syndrome**



Historic Spread of Zika Virus



- Outbreaks
- Serologic evidence
- Virus detection/confirmed human case

OPEN ACCESS Freely available online



Genetic Characterization of Zika Virus Strains: Geographic Expansion of the Asian Lineage

Andrew D. Haddow^{1*}, Amy J. Schuh¹, Chadwick Y. Yasuda², Matthew R. Kasper^{2†}, Vireak Heang², Rekol Huy³, Hilda Guzman¹, Robert B. Tesh¹, Scott C. Weaver¹

¹ Institute for Human Infections and Immunity, Center for Tropical Diseases, Department of Pathology, University of Texas Medical Branch, Galveston, Texas, United States of America, ² United States Naval Medical Research Unit, No. 2, Phnom Penh, Cambodia, ³ National Dengue Control Program, Phnom Penh, Cambodia

Haddow AD, et al. PLoS Negl Trop Dis. 2012. 6:e1477.

Weaver SC, et al. Antiviral Res. 2016. 130:69-80.

ZIKV Adaptive Evolution for Urban Transmission?

LETTER

doi:10.1038/nature22365

Evolutionary enhancement of Zika virus infectivity in *Aedes aegypti* mosquitoes

Yang Liu^{1,2,3*}, Jianying Liu^{1,3*}, Senyan Du^{1*}, Chao Shan^{4*}, Kaixiao Nie¹, Rudian Zhang^{1,2}, Xiao-Feng Li⁵, Renli Zhang³, Tao Wang^{3,6}, Cheng-Feng Qin⁵, Penghua Wang⁷, Pei-Yong Shi⁴ & Gong Cheng^{1,3}

Regla-Nava JA, et al. Zika virus mutation enhances transmission potential and confers escape from protective dengue virus immunity. *Cell Rep.* 2022. 39:110655.

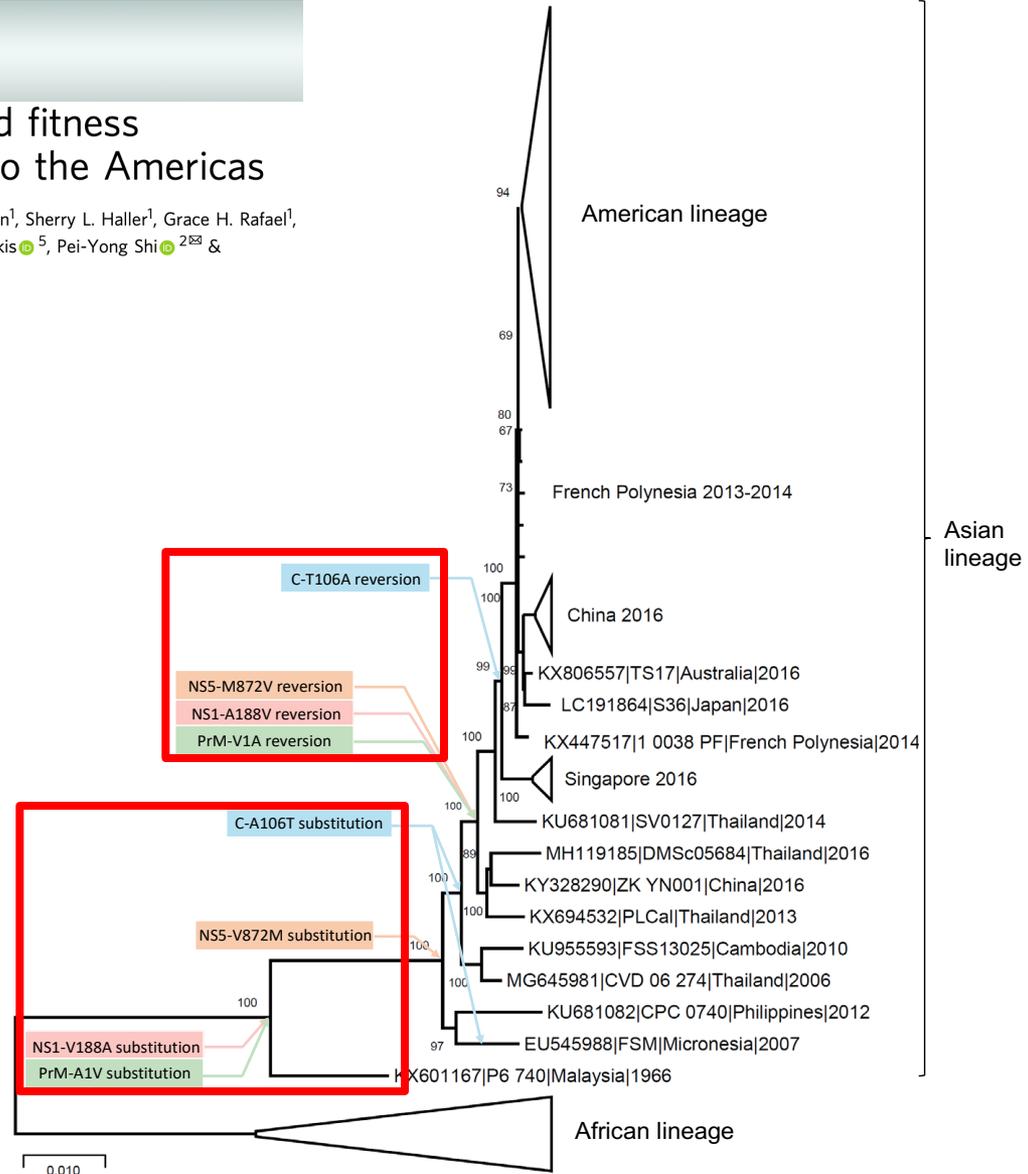
- NS1-188V substitution associated with more efficient infection of *A. aegypti*
- NS2B-I39V or -I39T mutation increases ZIKV replication in mosquitoes

Revertant ZIKV Amino Acid Substitutions

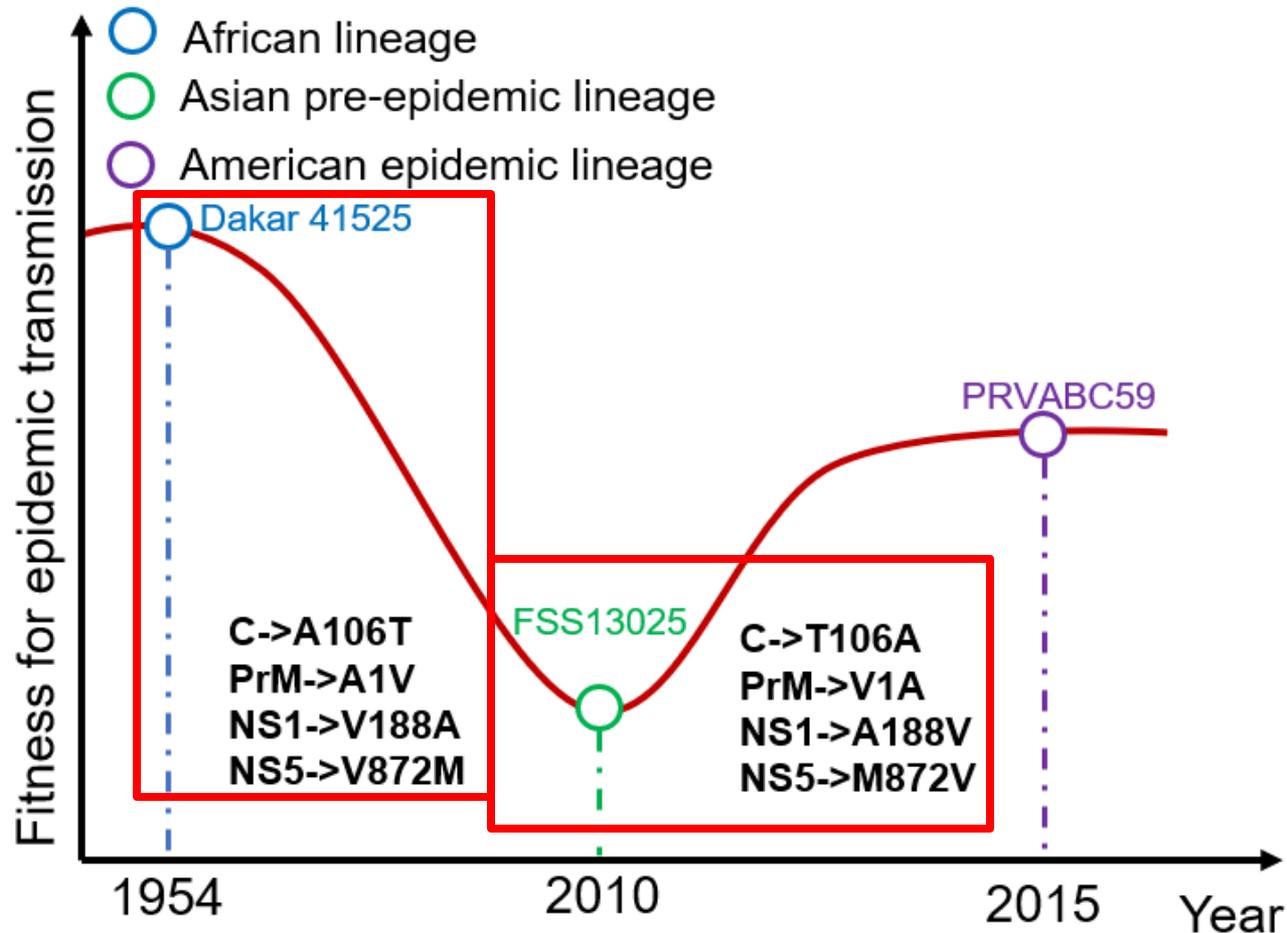


Role of mutational reversions and fitness restoration in Zika virus spread to the Americas

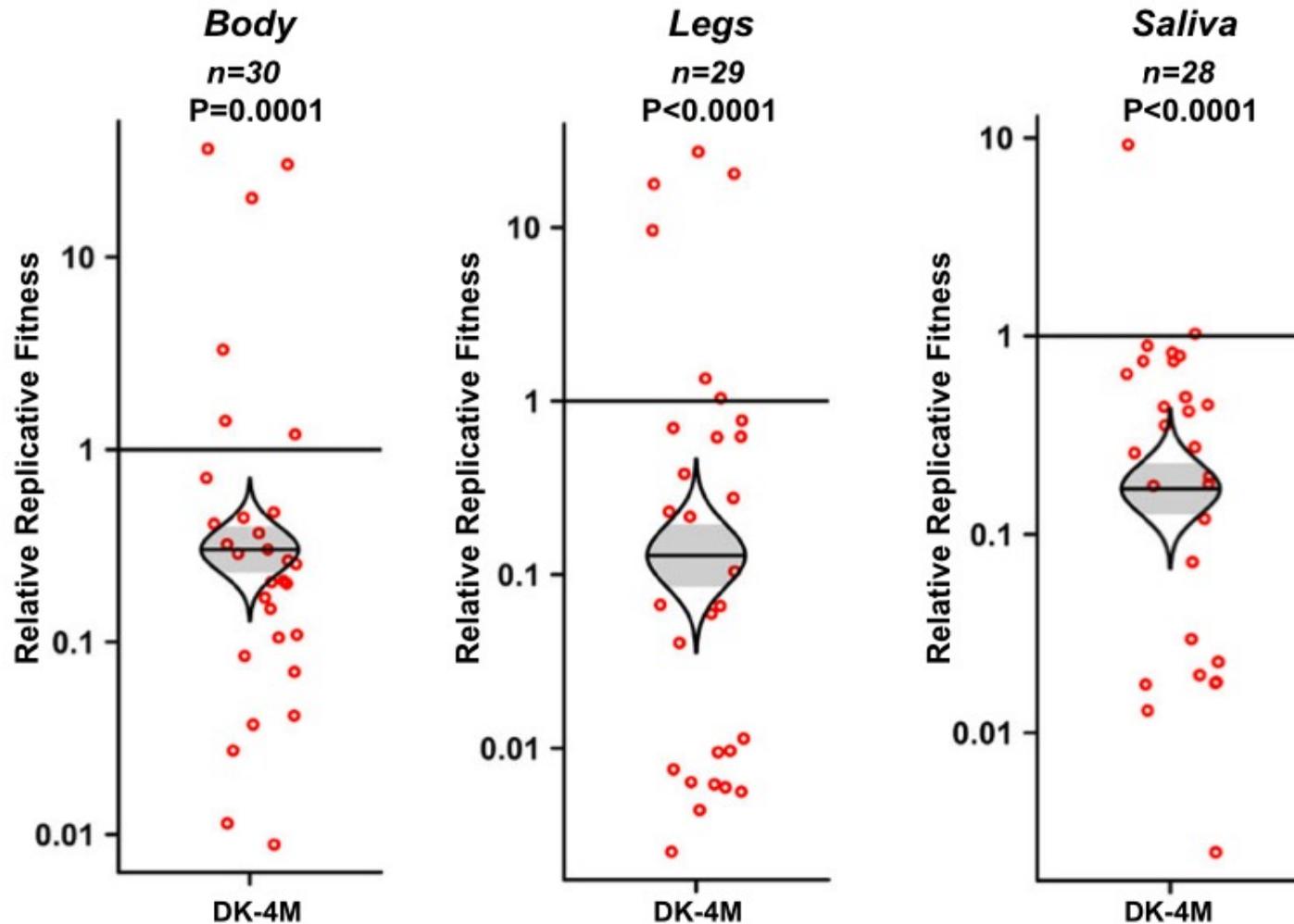
Jiaying Liu^{1,6}, Yang Liu^{2,6}, Chao Shan², Bruno T. D. Nunes³, Ruimei Yun¹, Sherry L. Haller¹, Grace H. Rafael¹, Sasha R. Azar¹, Clark R. Andersen⁴, Kenneth Plante¹, Nikos Vasilakis⁵, Pei-Yong Shi^{2,3} & Scott C. Weaver^{1,2,3}



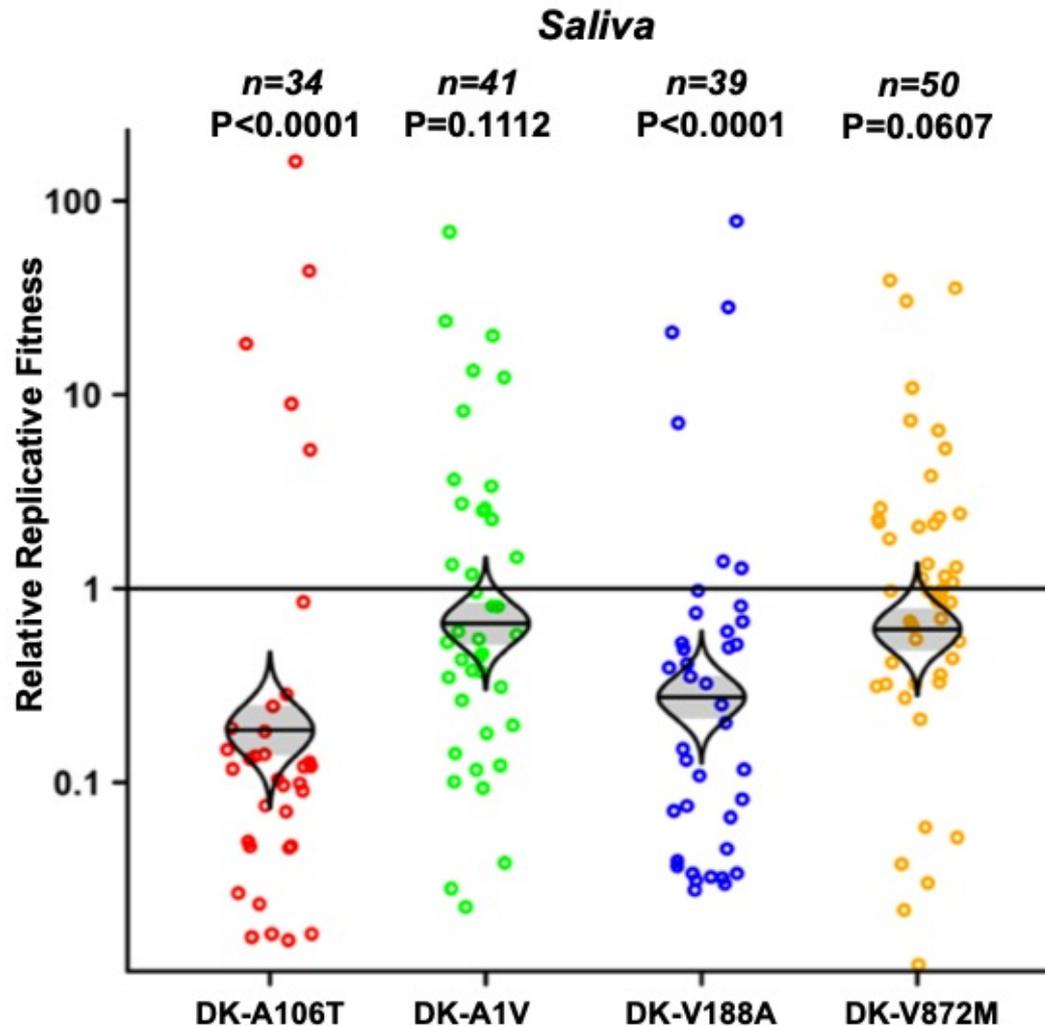
Revised ZIKV Adaptation Hypothesis: Fitness Losses Due to Founder Effects Followed by Partial Fitness Restoration



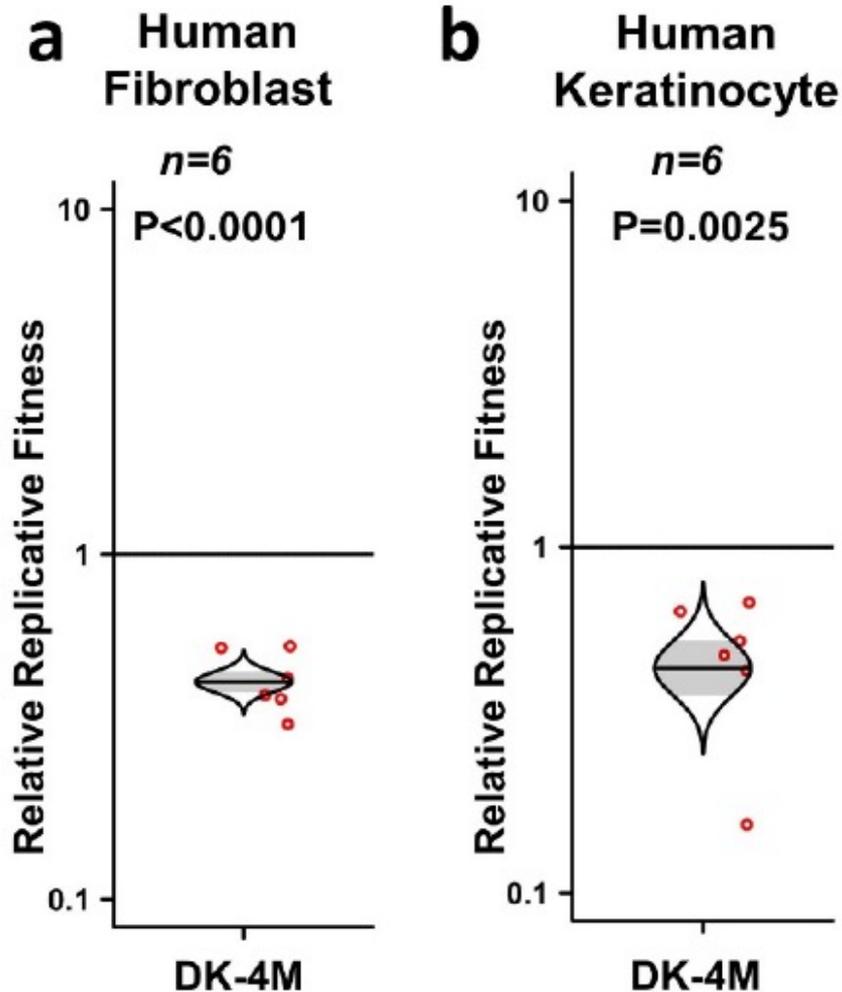
Effects of 4 Initial Mutations on African ZIKV Fitness in *A. aegypti* After Oral Infection



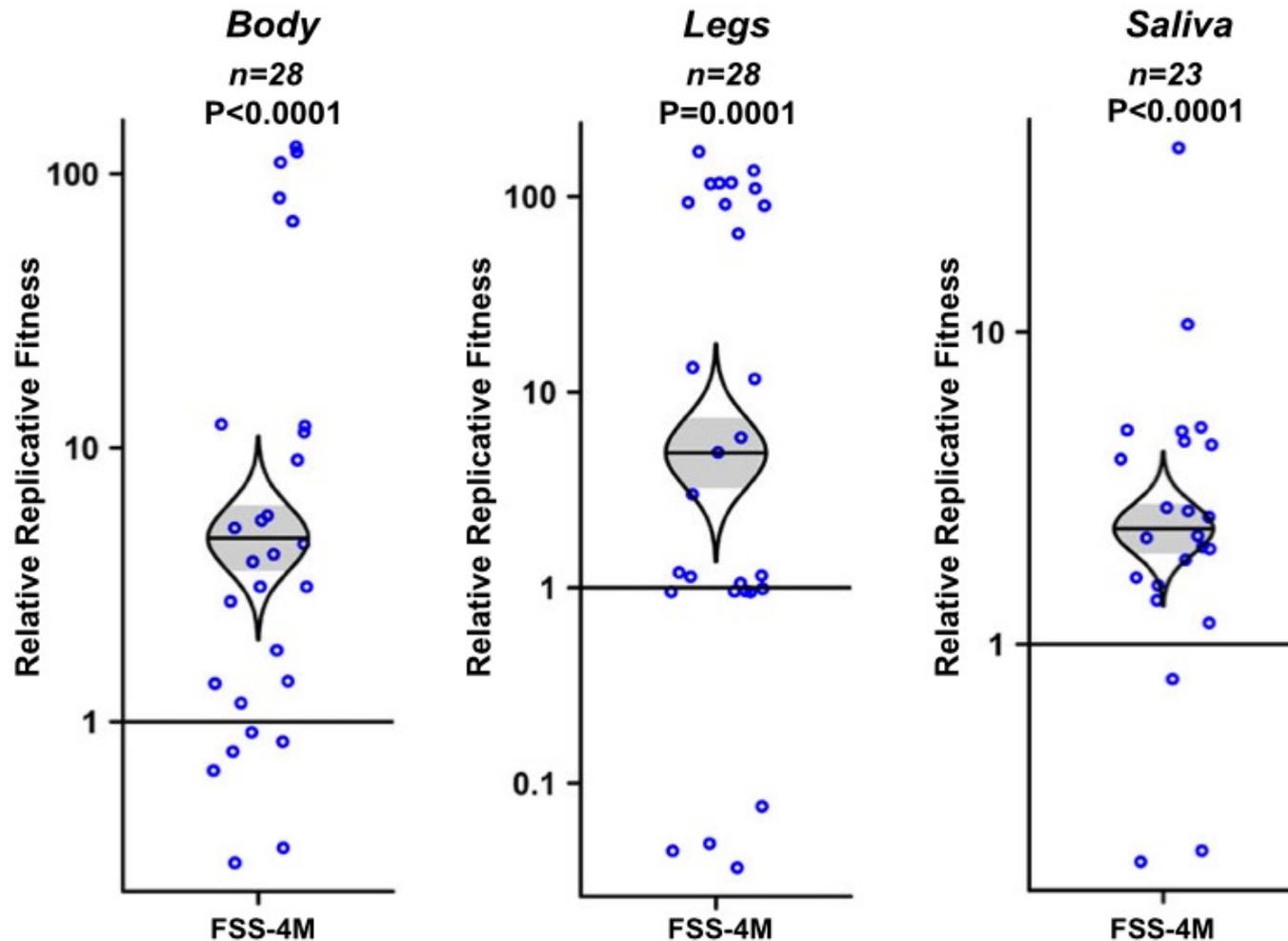
Effects of Individual Initial Mutations on African ZIKV Fitness for Mosquito Transmission



Effects of 4 Initial Mutations on African ZIKV Fitness in Primary Human Cells Believed to Seed Viremia



Effects of 4 Reversion Mutations on Asian ZIKV Fitness in *A. aegypti* After Oral Infection



Summary of Fitness Effects of 4 revertant ZIKV mutations

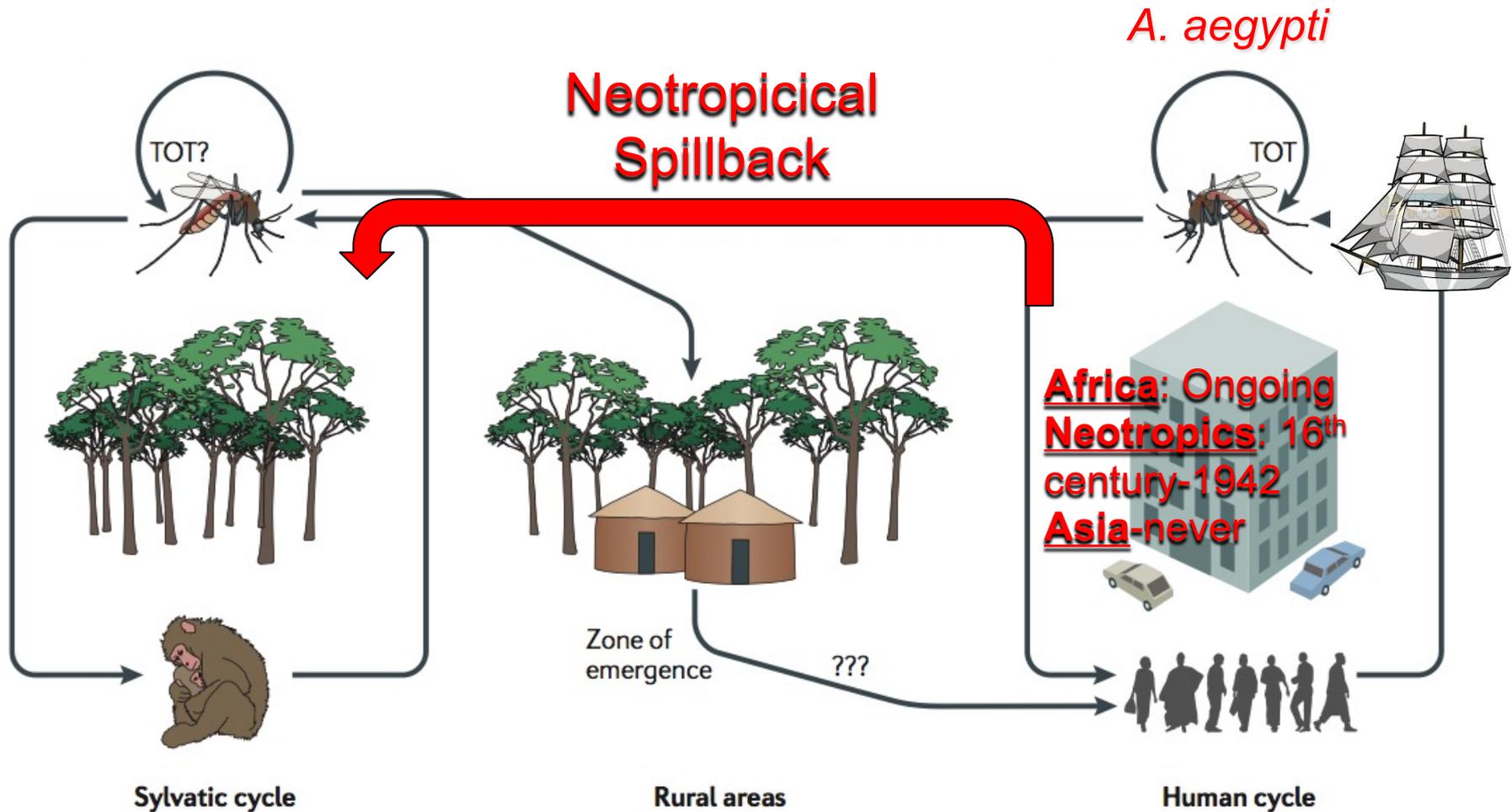
4 initial mutations:

- combination of 4 reduces infection by an African strain of *A. aegypti* saliva and human target cells for ZIKV viremia and amplification.
- Each individual mutation reduces infection by an African strain of at least one of these transmission targets.

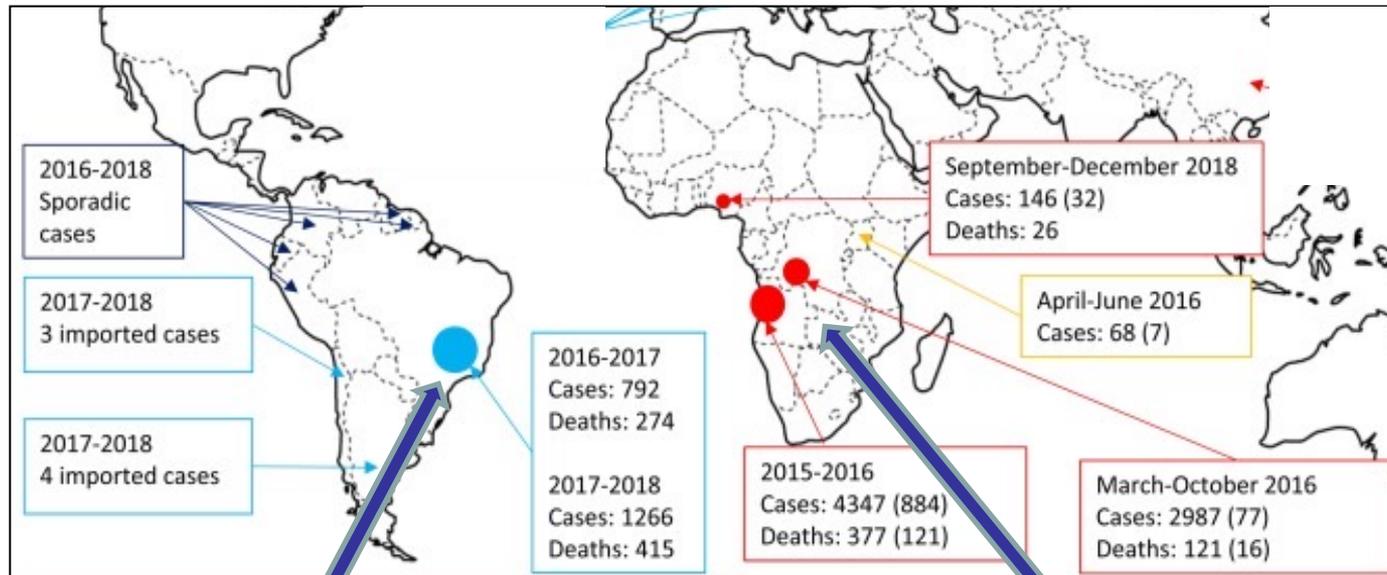
4 reversion mutations

- 3 of the 4 mutations independently enhance infection by an Asian ZIKV strain for at least one transmission phenotype.

Arboviruses **without** a **recent** history of urban emergence via *Aedes aegypti* transmission in some locations: **yellow fever**



Recent, Major Outbreaks of Yellow Fever: Angola and Brazil



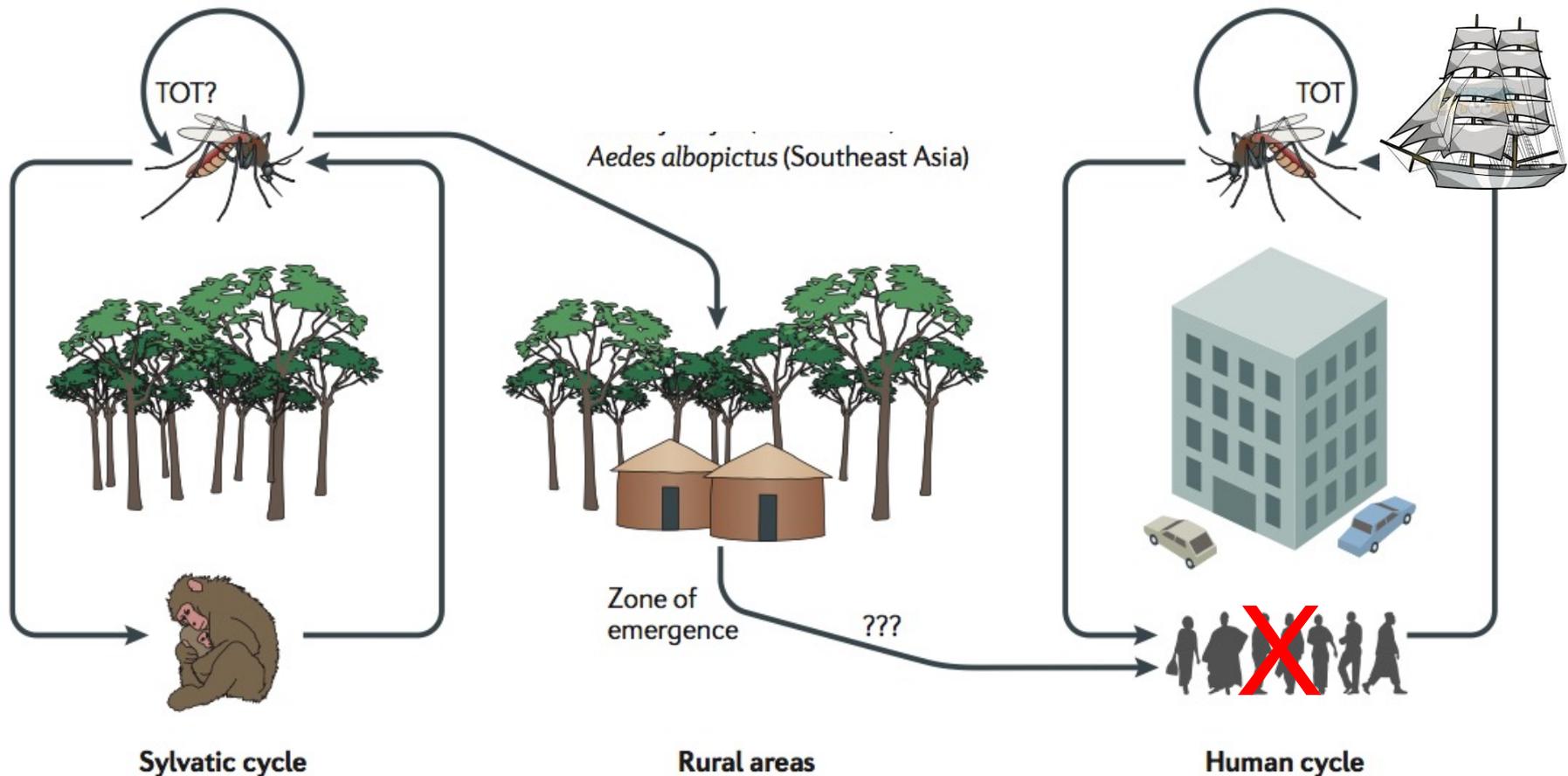
Spillover transmission by sylvatic mosquitoes

Human-amplified transmission by urban mosquitoes, *Aedes aegypti*

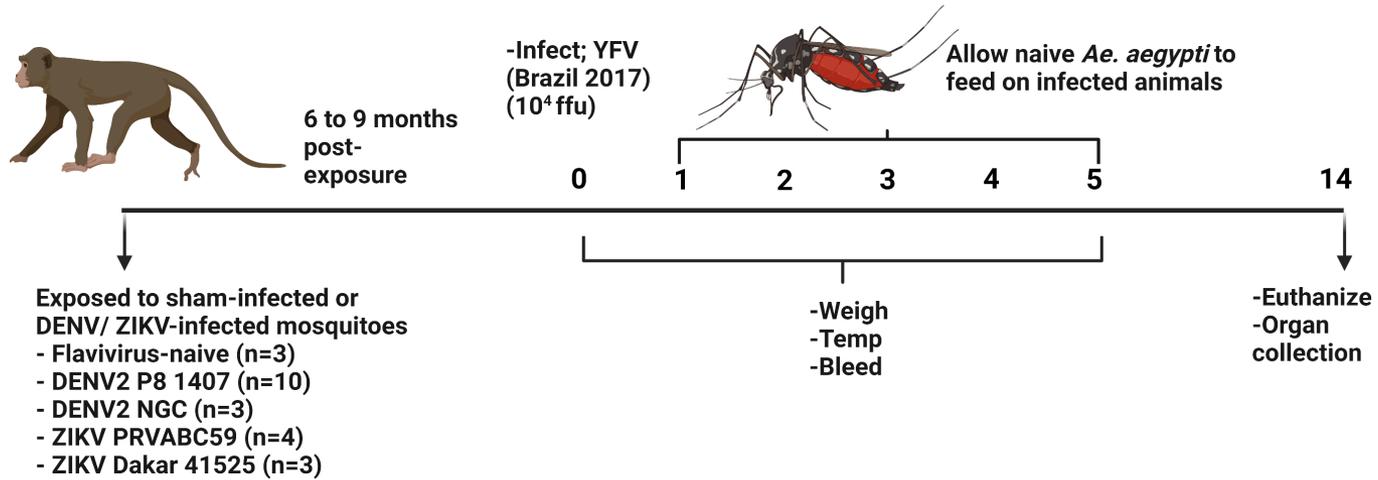
A Seminal Question in Arbovirology:

Despite the abundance of the principal urban vector, *Aedes aegypti*, susceptible humans, and opportunities for introduction, why is there no YFV transmission at all in Asia and no urban transmission in South America since 1942?

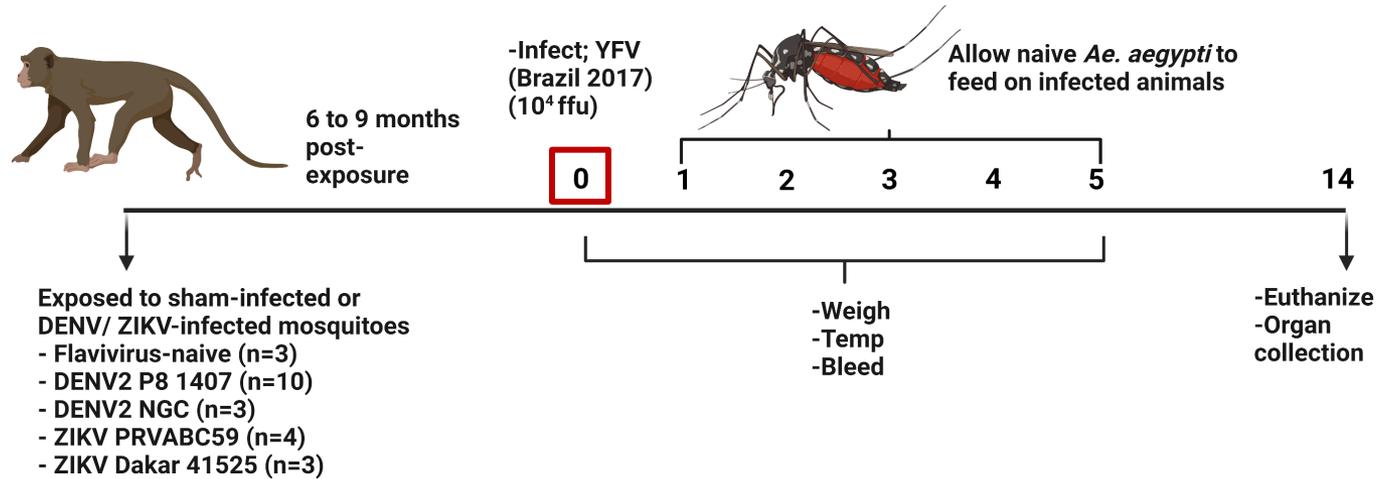
Hypothesis: Dengue immunity present for many decades in Asia and since the 1980s in the Americas, but less prevalent in Africam cross-protects against YFV viremia, suppressing human amplification potential; ZIKV present in Asia for decades and in Brazil since 2013 also cross-protects



Experimental Design: Cynomolgus Macaques

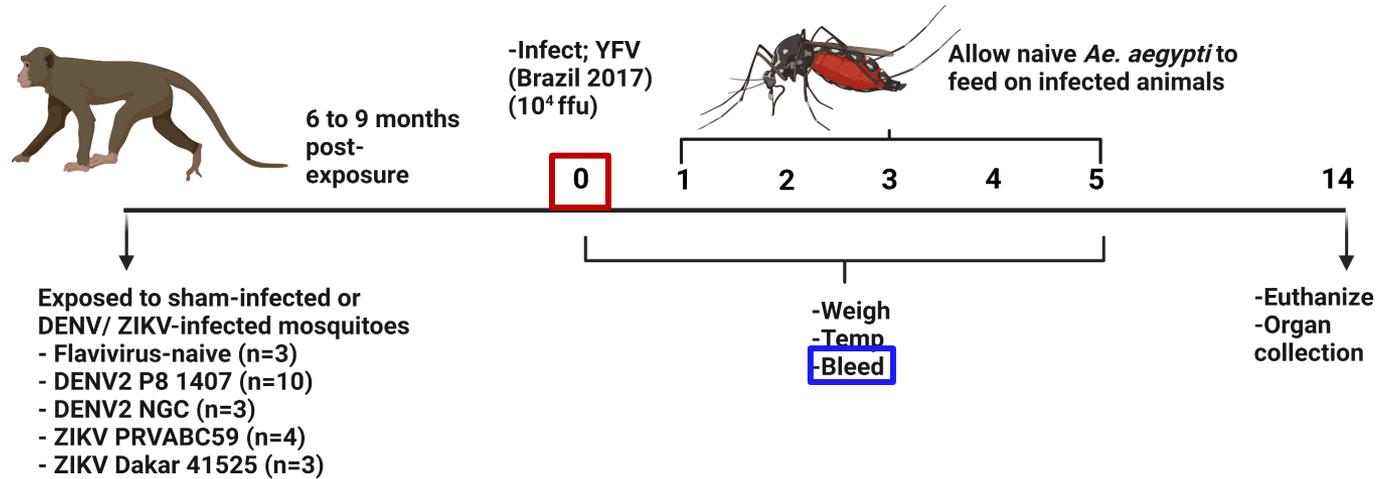


Experimental Design: Cynomolgus Macaques



Serum: PRNT, ELISA

Experimental Design: Cynomolgus Macaques



Serum: PRNT, ELISA

Blood:

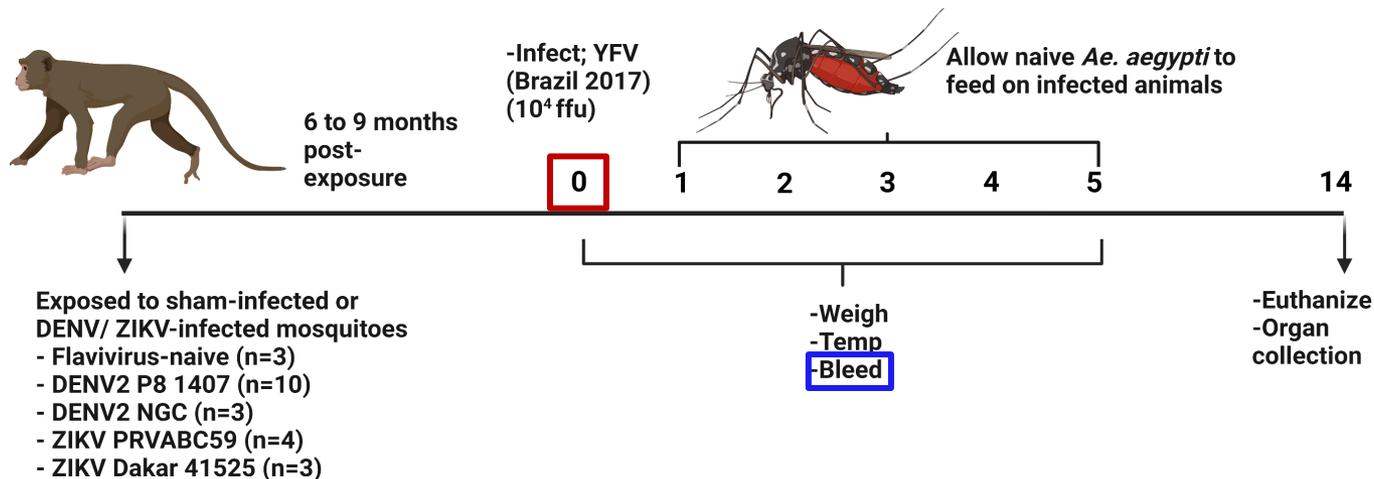
-Complete Blood Count

Serum:

-Viremia

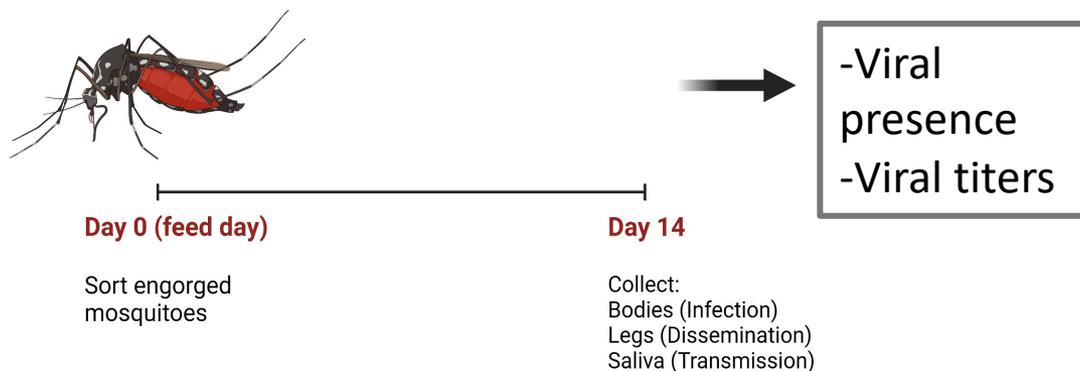
-Liver enzymes (ALT, AST)

Experimental Design: Cynomolgus Macaques

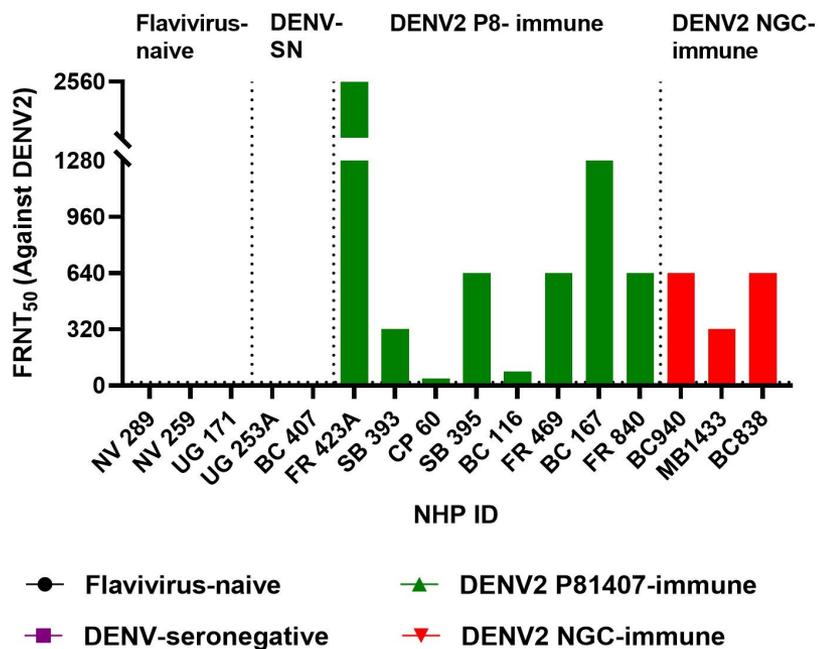


Serum: PRNT, ELISA

Blood:
-Complete Blood Count
Serum:
-Viremia
-Liver enzymes (ALT, AST)

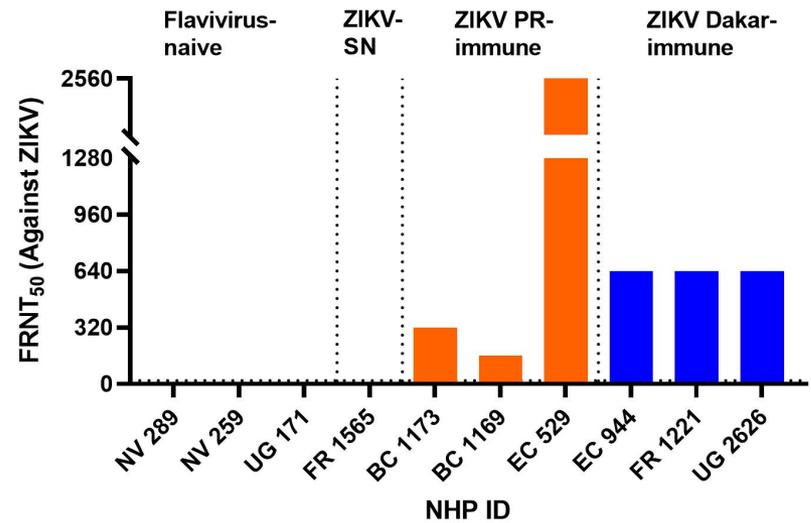
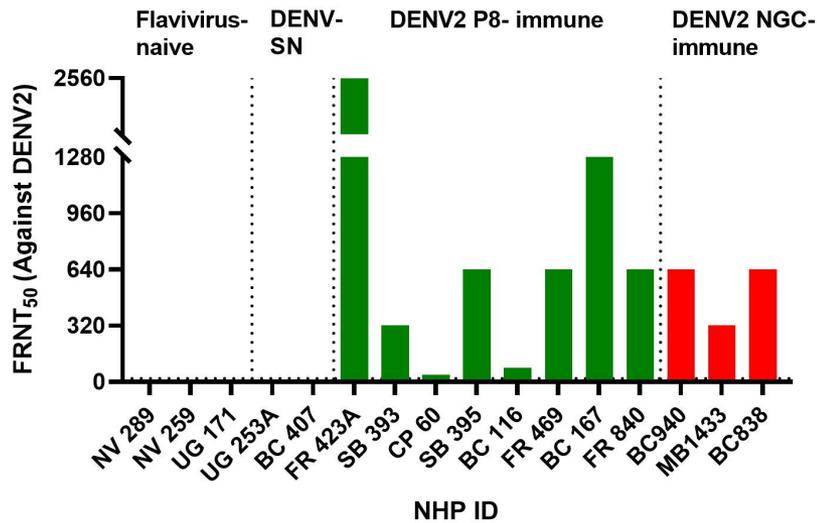


DENV-Neutralizing Antibody Titers



Group	Primary virus exposure	# of animals	# of animals with NAb (FRNT)	# of animals seronegative
Flavivirus-naïve	None	3	0	3
DENV2 P8	DENV2 P8 1407	10	8	2
DENV2 NGC	DENV2 NGC	3	3	0

ZIKV-Neutralizing Antibody Titers

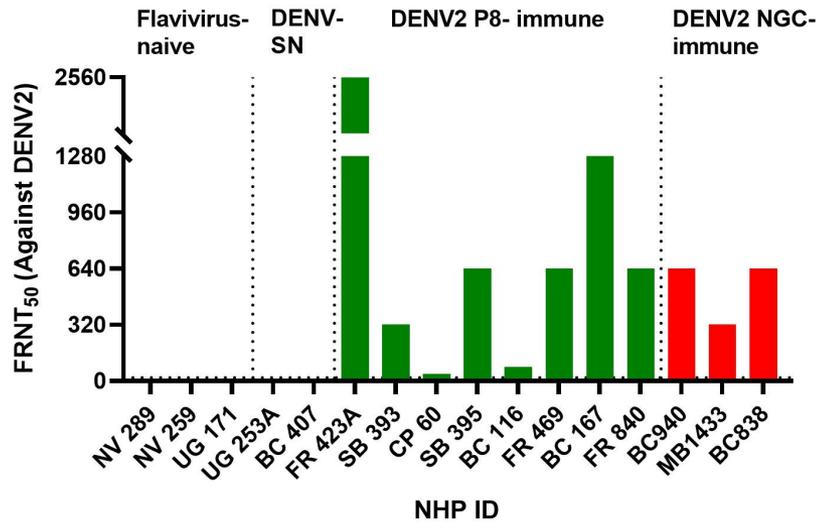


- Flavivirus-naive
- ▲ DENV2 P81407-immune
- DENV-seronegative
- ▼ DENV2 NGC-immune

- Flavivirus-naive
- ZIKV PR-immune
- ZIKV-seronegative
- ★ ZIKV Dakar-immune

Group	Primary virus exposure	# of animals	# of animals with NAb (FRNT)	# of animals seronegative
Flavivirus-naïve	None	3	0	0
DENV2 P8	DENV2 P8 1407	10	8	2
DENV2 NGC	DENV2 NGC	3	3	0
ZIKV PR	ZIKV PRVABC59	4	3	1
ZIKV Dakar	ZIKV Dakar 41525	3	3	0

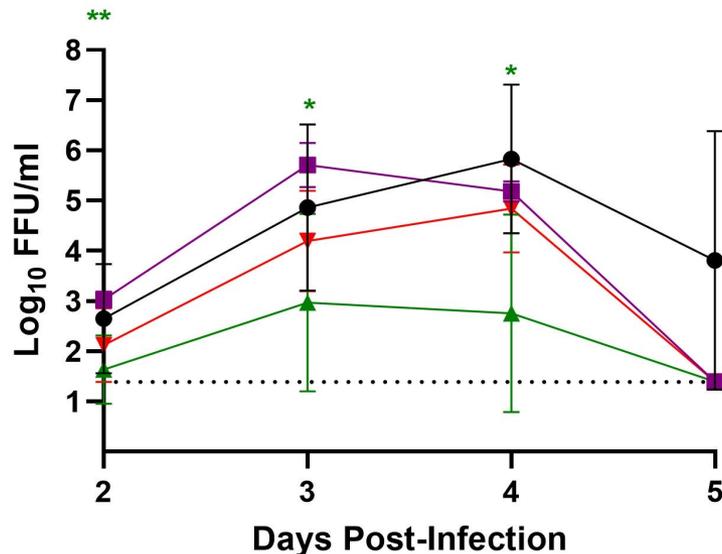
YFV Viremia in DENV2-exposed animals



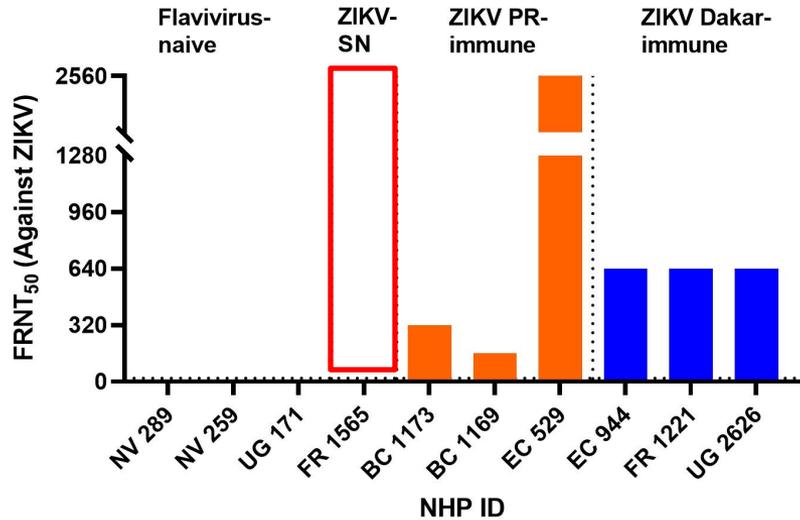
YFV Viremia

In comparison to flavivirus-naïve and DENV2-seronegative animals,

- DENV2 P8-immune animals had **much lower** viremia
- DENV2 NGC-immune animals had slightly lower viremia
- All DENV2-exposed animals had a **shorter duration of viremia**

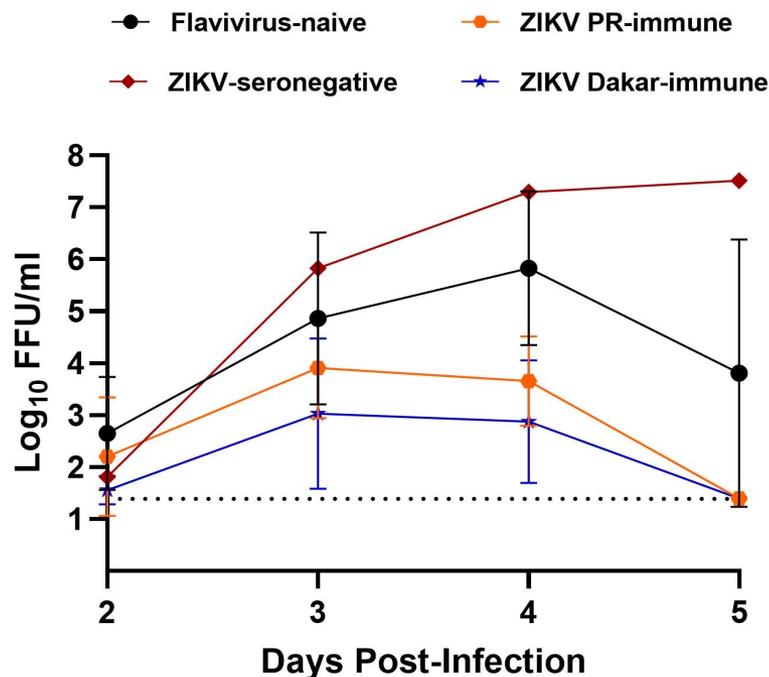


YFV Viremia in ZIKV-exposed animals

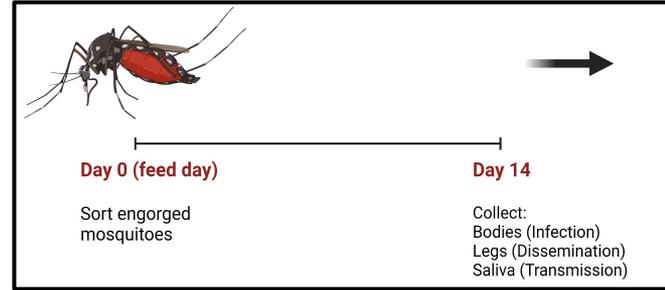
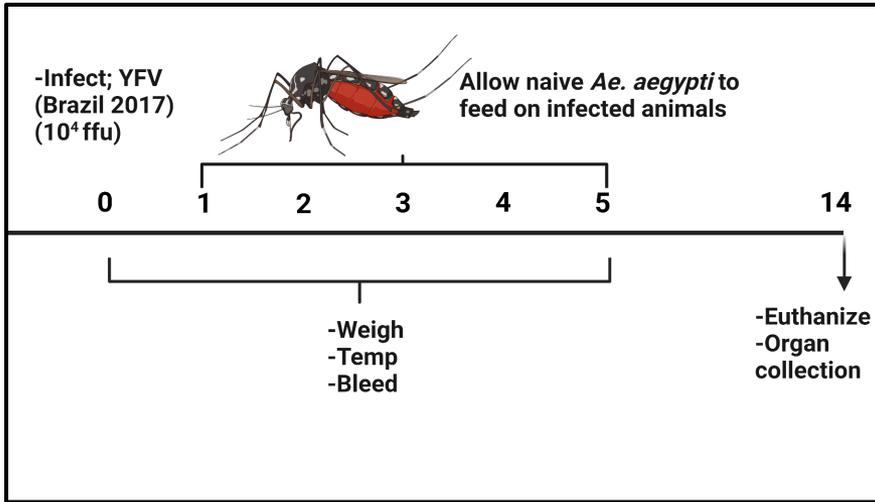


In comparison to flavivirus-naïve animals,

- ZIKV PR- and ZIKV Dakar-immune animals had **much lower** viremia
- All ZIKV-immune animals had a **shorter duration of viremia**
- **One ZIKV-SN (by PRNT) animal succumbed to illness on Day 9**
 - Did not have detectable Neutralizing Antibodies against ZIKV by PRNT
 - But, did have **binding antibodies by ELISA**
- Suggests the possibility of **immune enhancement**

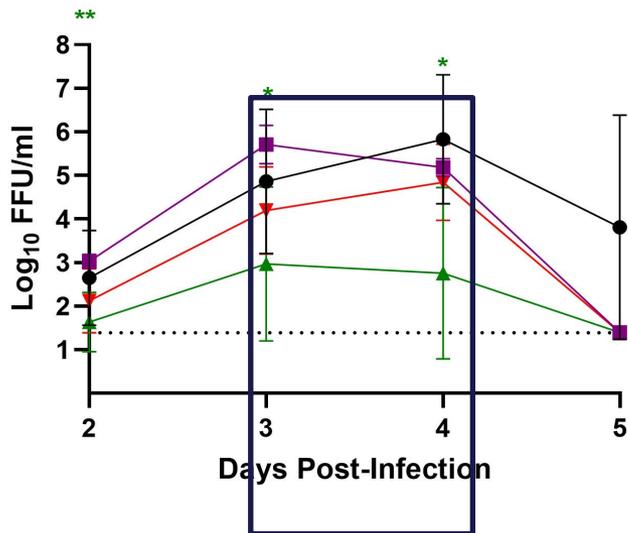


Aedes aegypti Infections

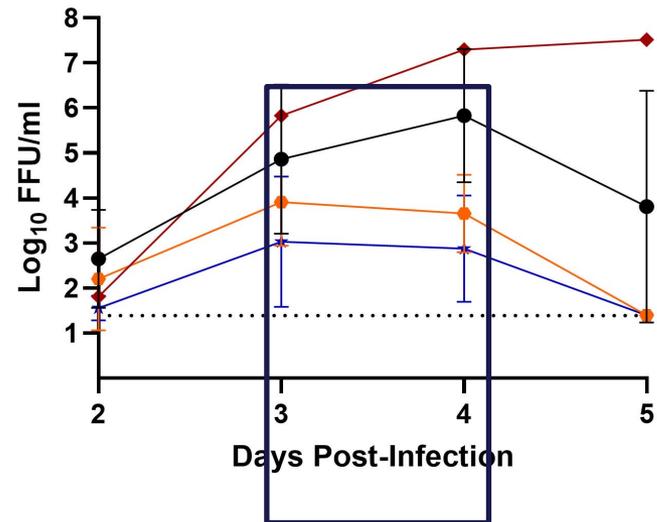


-Viral presence
-Viral titers

● Flavivirus-naive ▲ DENV2 P81407-immune
■ DENV-seronegative ▼ DENV2 NGC-immune

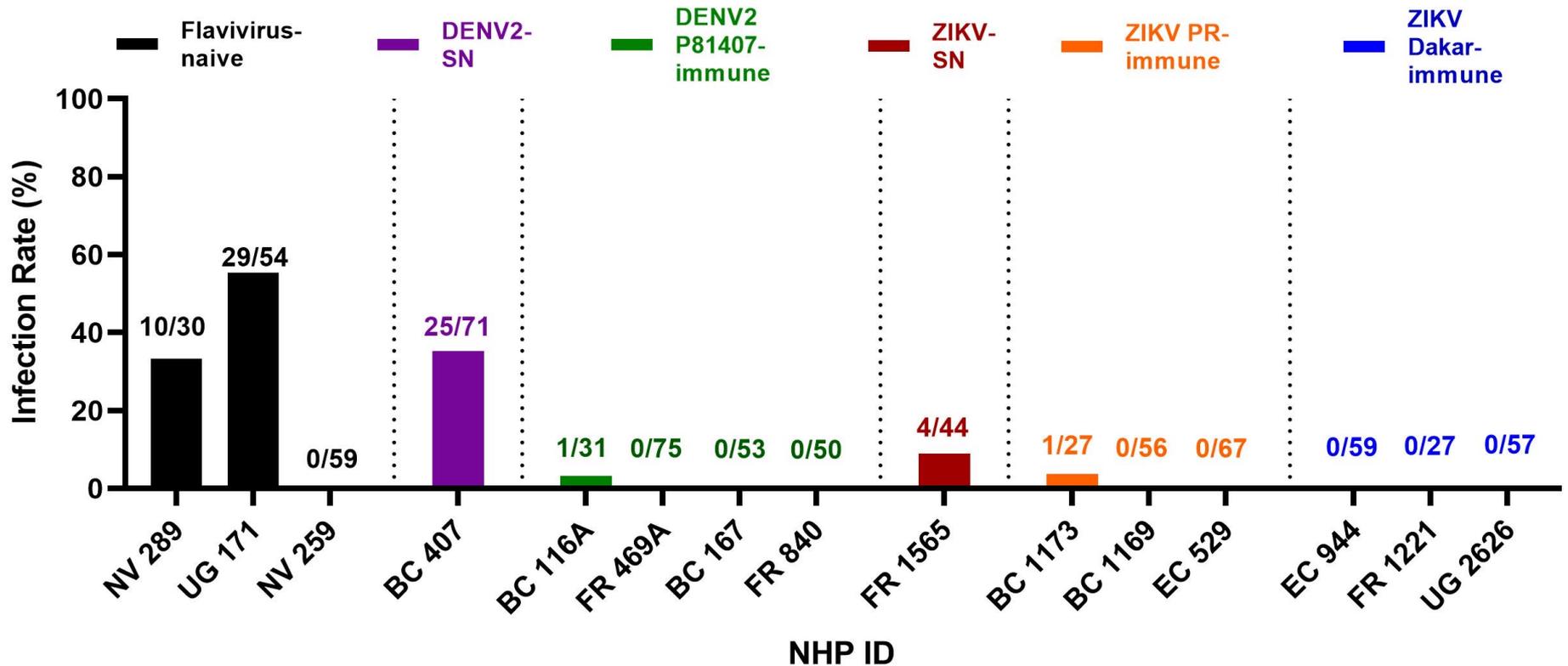


● Flavivirus-naive ● ZIKV PR-immune
◆ ZIKV-seronegative ★ ZIKV Dakar-immune



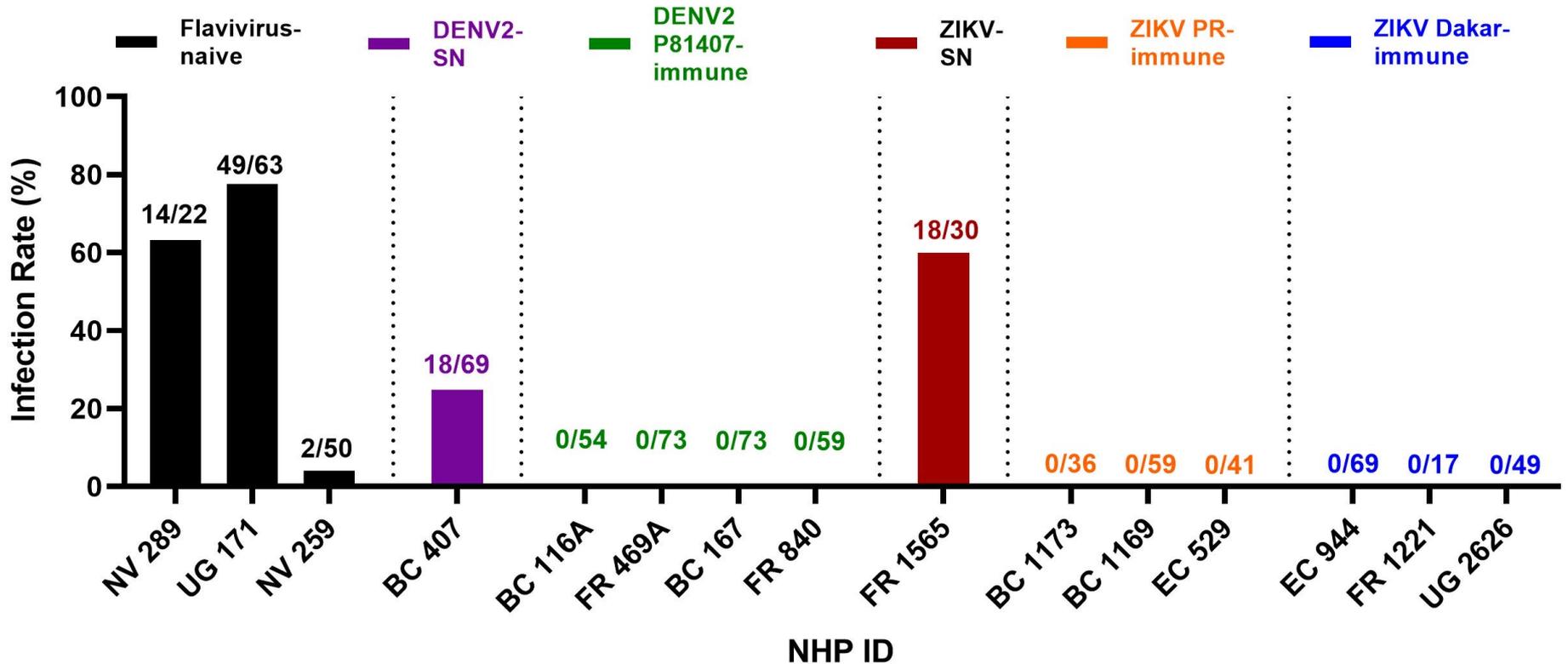
Peak Viremia: Days 3-4

Mosquito Infection Rate 3 Days Post-Infection



Majority of infected mosquitoes belong to the flavivirus-naïve or –seronegative groups

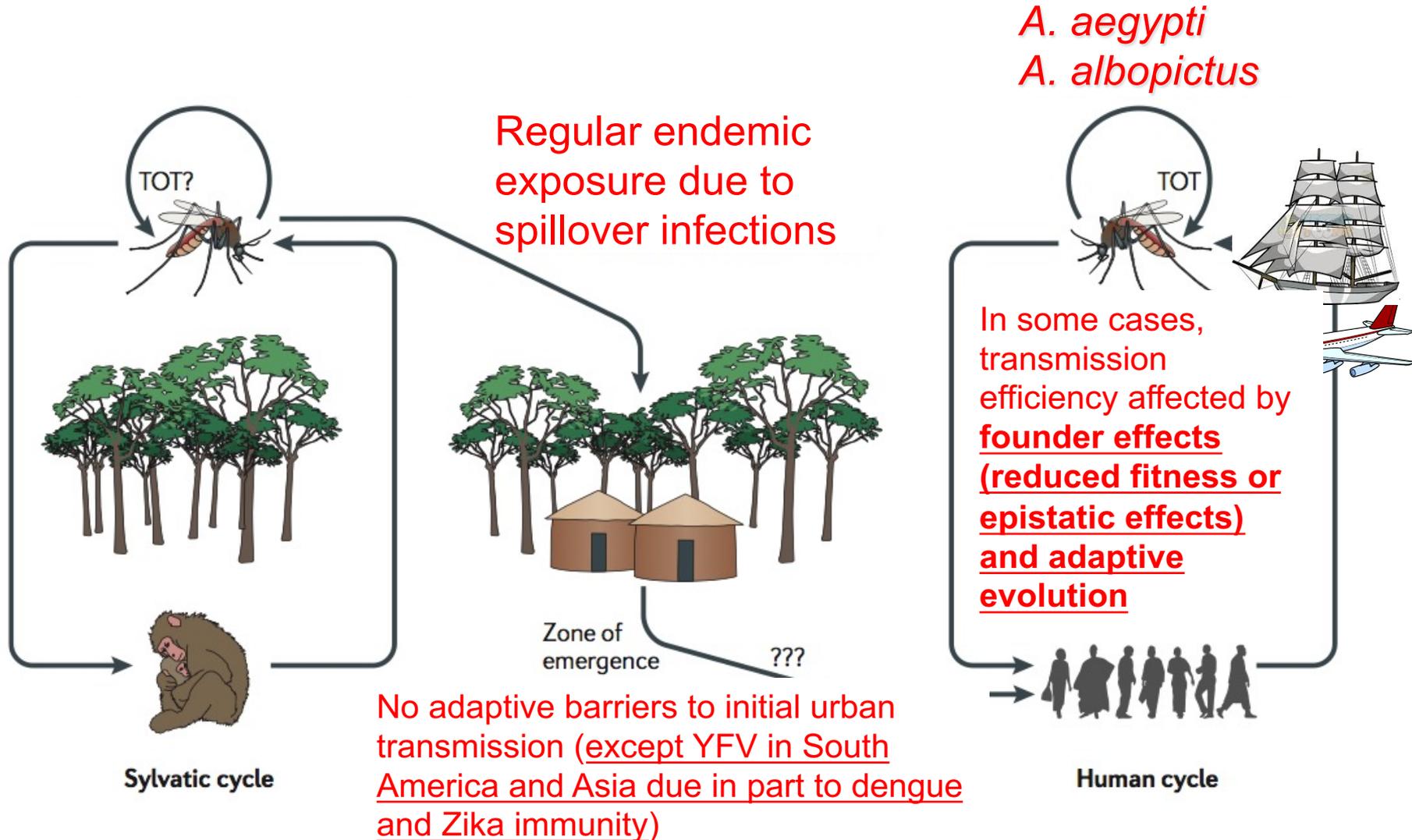
Mosquito Infection Rate 4 Days Post-Infection



Vast majority of infected mosquitoes belong to the flavivirus-naïve or –seronegative groups

Overall, DENV and ZIKV immunity also cross protected against yellow fever disease (with one possible enhancement)

African arboviruses with history of urban emergence via *Aedes aegypti* transmission: **yellow fever, chikungunya, Zika**



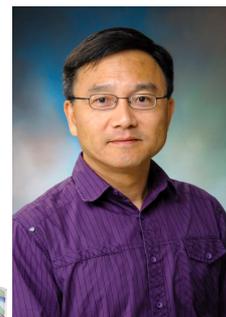
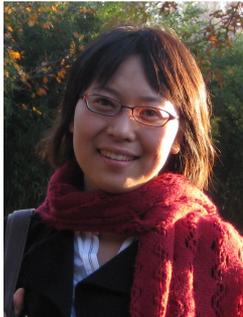
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N. Mexico State University
Kathy Hanley
UT El Paso
Doug Watts



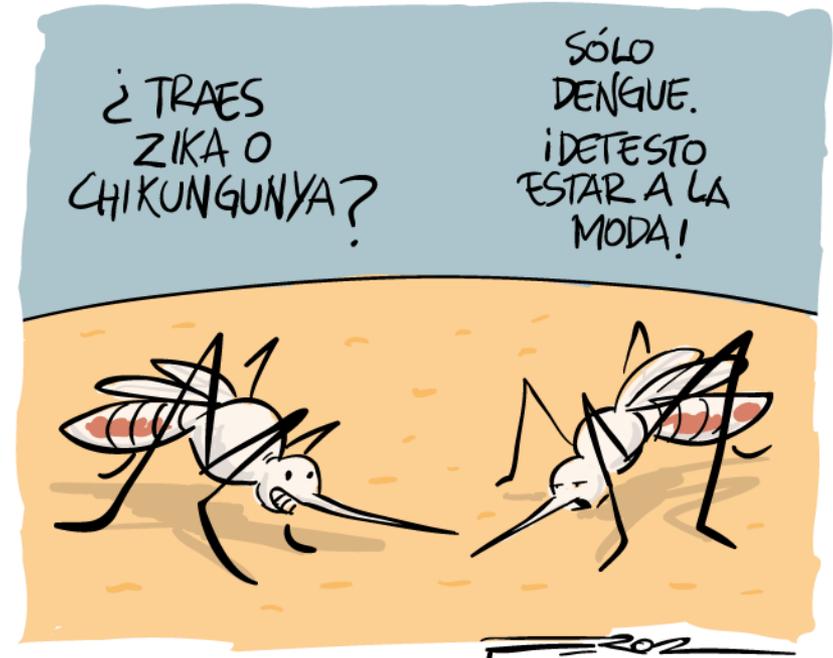
Funding: NIH-NIAID R01-AI069145, R01-AI071192, R01-AI48807

Questions?



A little clean water, please!

Clásico o moderno



Classic or Modern

- Are you carrying Zika or chikungunya?
- Only dengue. I detest being fashionable!

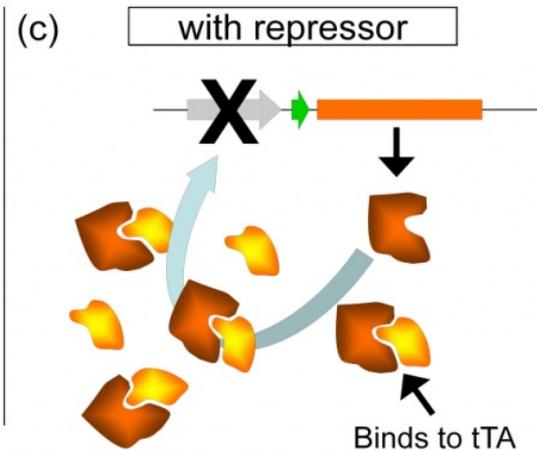
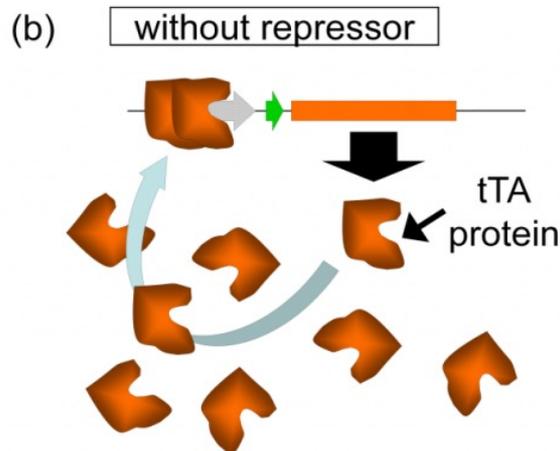
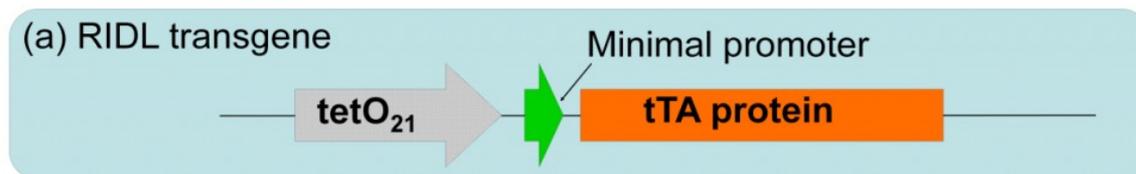
Failure of Traditional Mosquito Control to Control Dengue

- In the tropics adult female *Aedes aegypti* remain inside houses, limiting insecticide penetration
- Larval *A. aegypti* are found in low density in many artificial containers around homes, requiring entry into individual properties for inspection/control
- *A. aegypti* in many parts of the world are developing resistance to insecticides

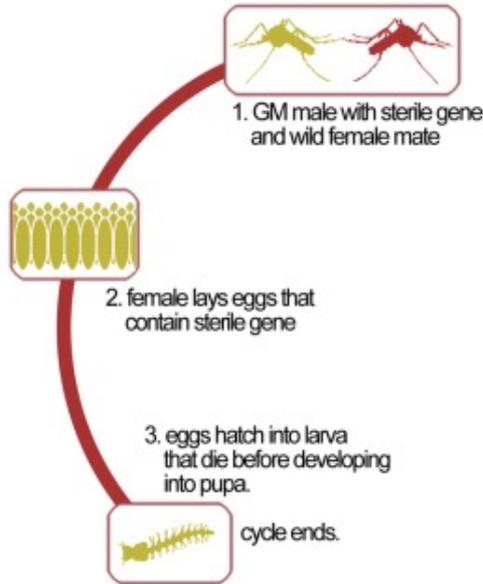


Novel Approach for Vector Control: Release of Insects with Dominant Lethality (RIDL)

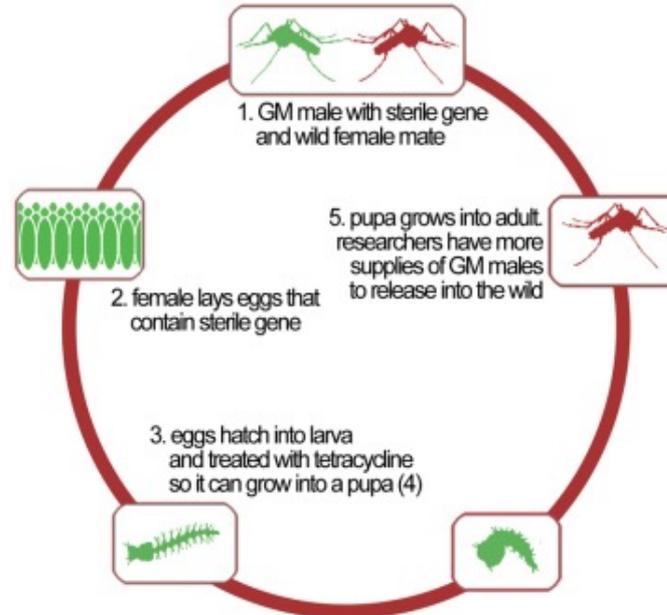
- Tetracycline repressible activator variant (tTAV): acts as a switch to control the activity of essential mosquito genes
- tTAV works only in insect cells; the non toxic protein ties up the cell's machinery so it's other genes aren't expressed and the insect dies in the larval stages
- Tetracycline binds to tTAV and disables it, allowing it to be added to laboratory larval water so that mosquitoes survive to the adult stage
- Because the tTAV gene is dominant, offspring of matings between released transgenic and wild mosquitoes die in the wild without tetracycline in their larval habitats



Implementation of RIDL: release of transgenic male mosquitoes



Introduction of GM males breaks this cycle as faulty gene causes offspring to prematurely die

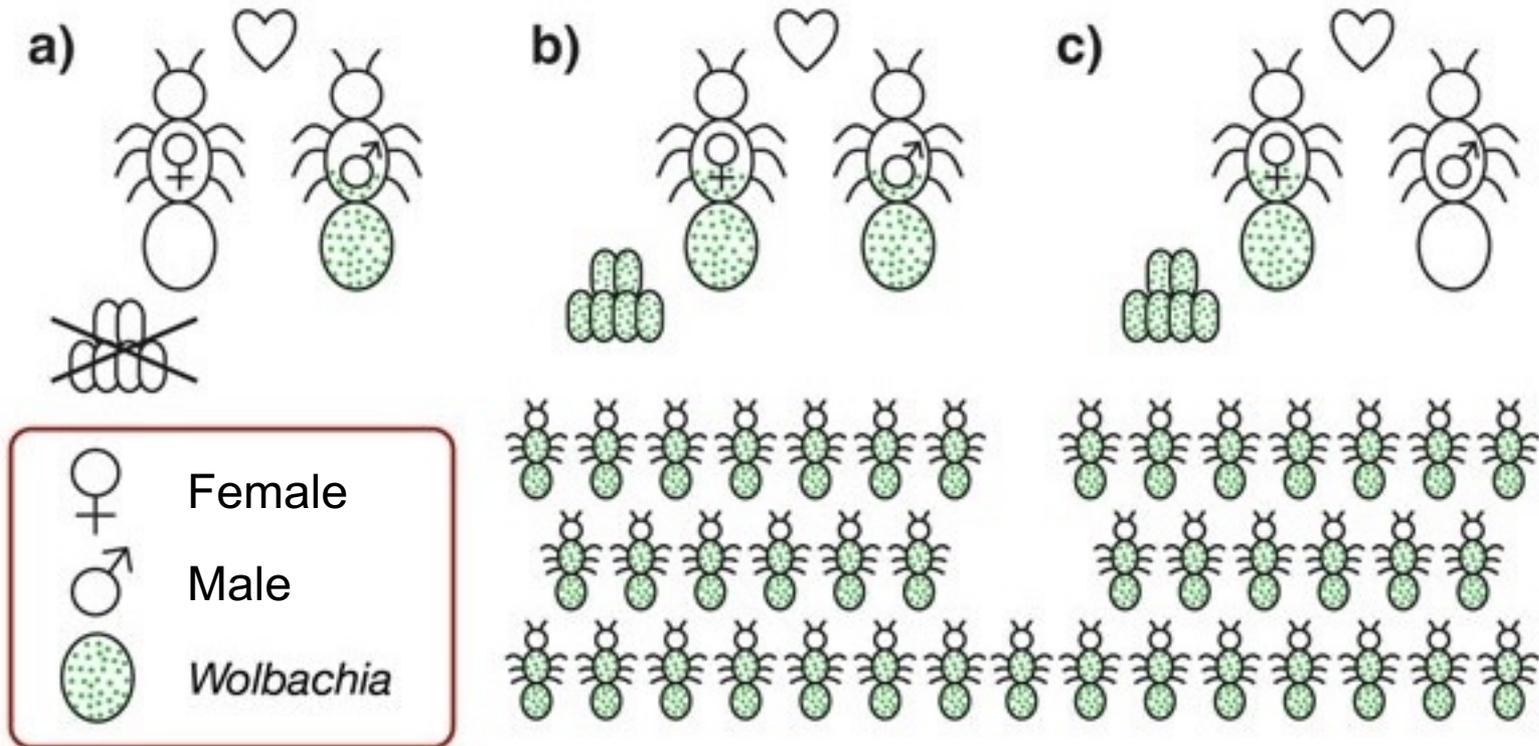


More GM males are created in the lab by adding tetracycline to larvae to allow development

Field trials: Cayman Islands, Malaysia, Panama and Brazil

- Safety advantage: transgene is “suicidal”
- Potential limitations: **Sustained release** of mosquitos is required, may be too **costly** for resource-limited regions endemic to urban arboviruses

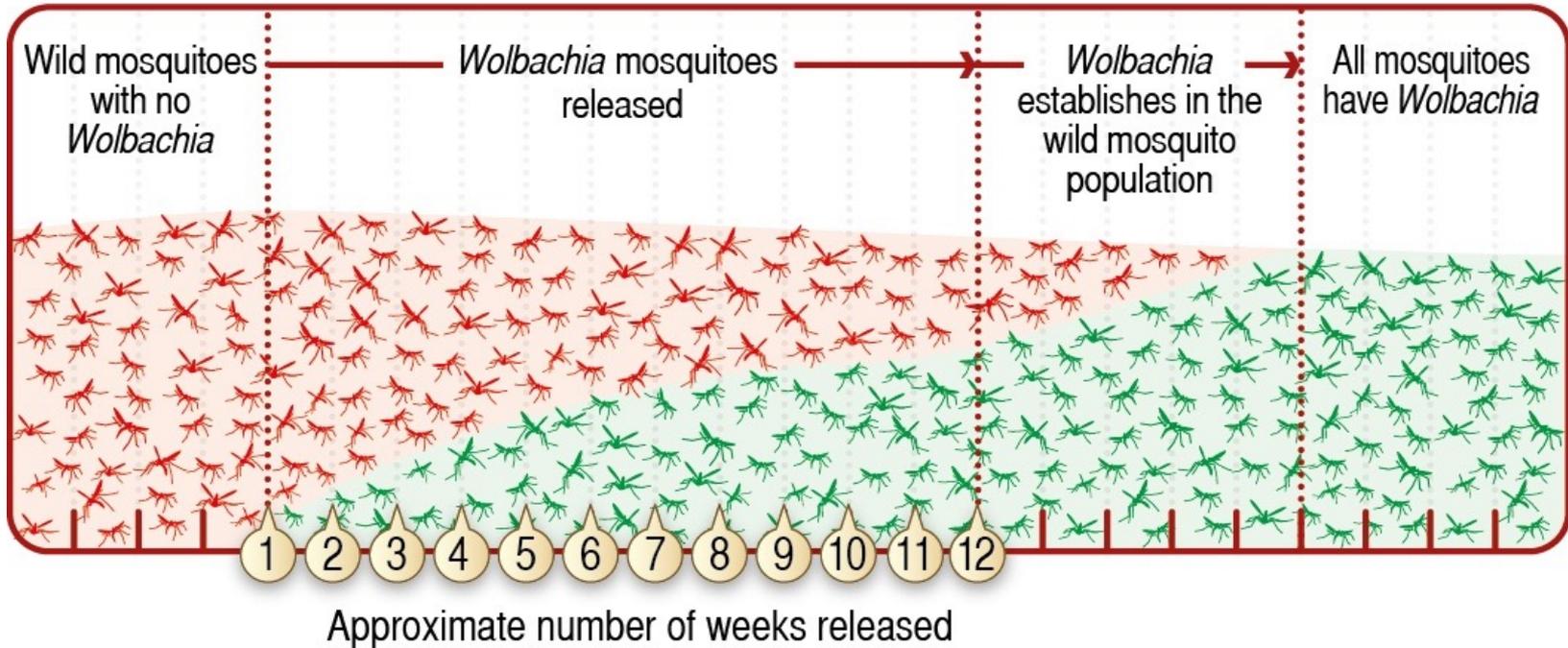
Wolbachia: Bacterial symbionts of many insects that can spread through populations through cytoplasmic incompatibility



- Infected males “sterilize” uninfected females
- When adapted to and introduced into *A. aegypti*, *Wolbachia* reduce their lifespan
- *Wolbachia* also reduce arbovirus replication and transmission

Field Trials: Australia (completed), Indonesia, Viet Nam, Brazil, Colombia

Wolbachia dengue control method



Potential limitations:

- Limited dispersal of *A. aegypti* will necessitate widespread release of female mosquitoes.
- Can arboviruses evolve resistance to *Wolbachia* suppression?

Genetically engineering refractoriness into mosquito vectors and dissemination via gene drive mechanisms

PNAS

Germline Cas9 expression yields highly efficient genome engineering in a major worldwide disease vector, *Aedes aegypti*

Ming Li^{a,b,1}, Michelle Bui^{a,b,1}, Ting Yang^{a,b,1}, Christian S. Bowman^{a,b}, Bradley J. White^{a,b,2}, and Omar S. Akbari^{a,b,1,3}

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Edited by Carolina Barillas-Mury, National Institutes of Health, Bethesda, MD, and approved October 23, 2017 (received for review June 27, 2017)

PNAS

Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*

Valentino M. Gantz^{a,1}, Nijole Jasinskiene^{b,1}, Olga Tatarenkova^b, Aniko Fazekas^b, Vanessa M. Macias^b, Ethan Bier^{a,2}, and Anthony A. James^{b,c,2}

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Contributed by Anthony A. James, October 26, 2015 (sent for review October 11, 2015; reviewed by Malcolm Fraser and Marcelo Jacobs-Lorena)

Lethal Mosquito Traps

- Autocidal gravid ovitrap
- Passive design, simple, inexpensive components
- 3 traps per house decreased *Aedes aegypti* mosquito abundance by 79% in southern Puerto Rico, reduced CHIKV infections

