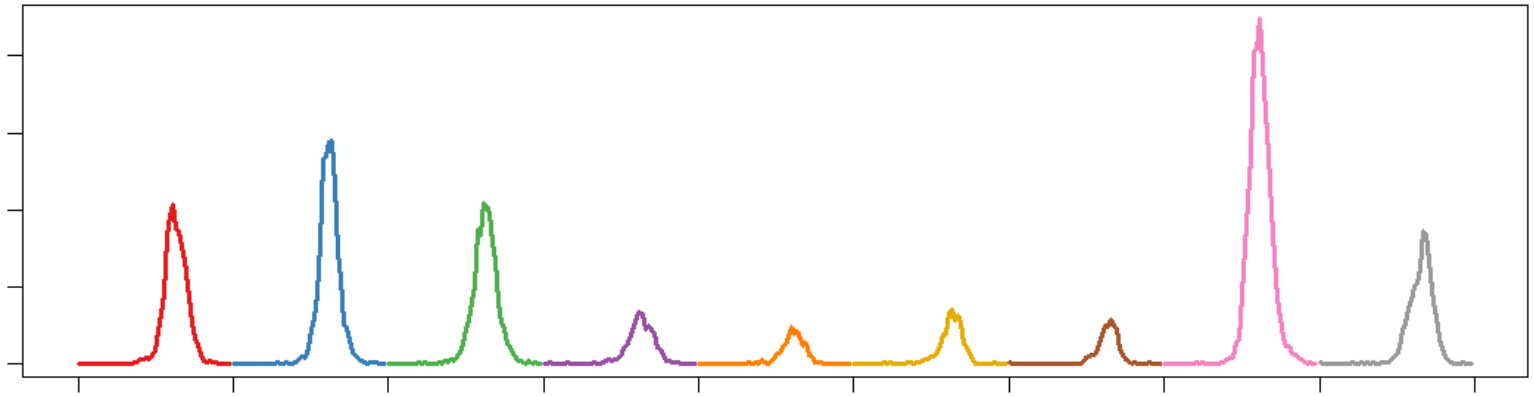


Translating data into evidence to support public-health decisions for mosquito-borne diseases

Christopher M. Barker

Department of Pathology, Microbiology & Immunology

School of Veterinary Medicine, UC Davis

UCDAVIS VETERINARY MEDICINE

PACIFIC SOUTHWEST CENTER OF EXCELLENCE IN VECTOR-BORNE DISEASES

Mosquito-borne diseases in California

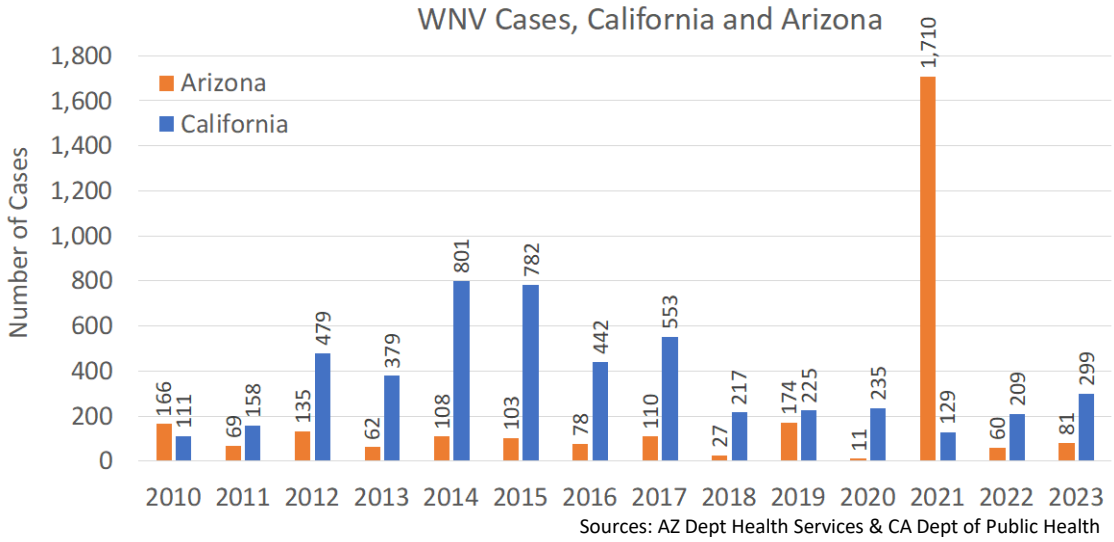
Zoonotic arboviruses

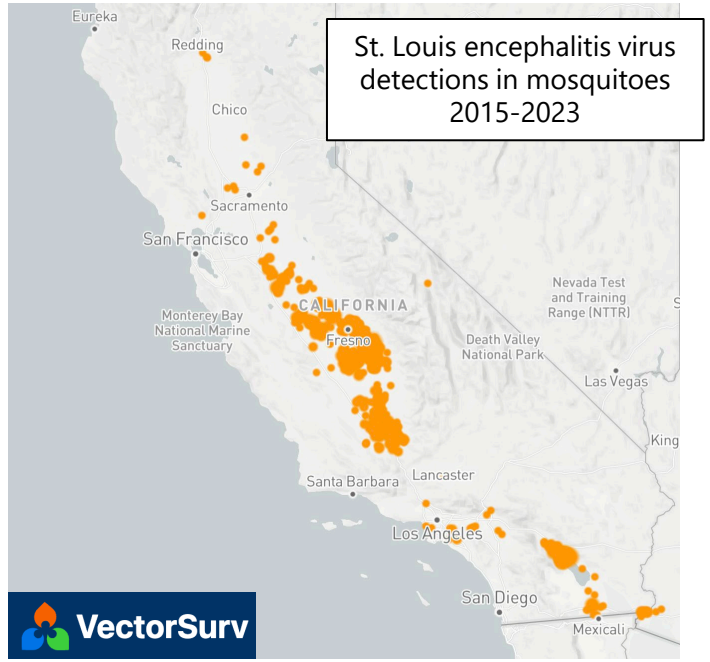
- West Nile virus - recurrent outbreaks
- St. Louis encephalitis virus – absent in CA following 2003, then re-emergent since 2015

Aedes-borne arboviruses

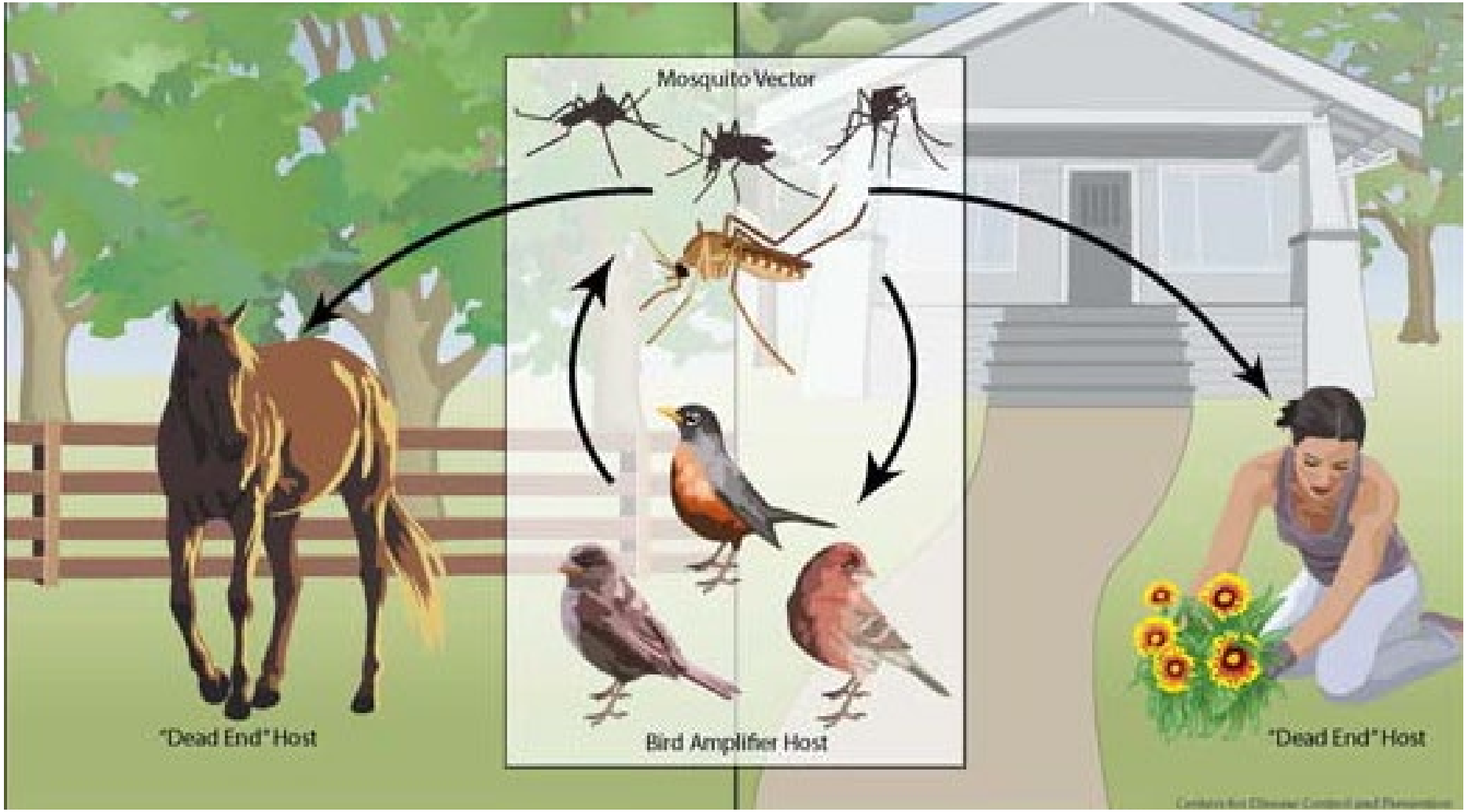
- Travelers often infected with *Aedes*-borne viruses
  - > 1,300 DENV/ZIKV infections detected since 2015
- Rapid spread of *Aedes aegypti* since 2013
  - > 370 cities & towns with *Ae. aegypti,*

    > 80 cities with *Ae. albopictus*
- 2 recent locally acquired infections in Pasadena, Long Beach



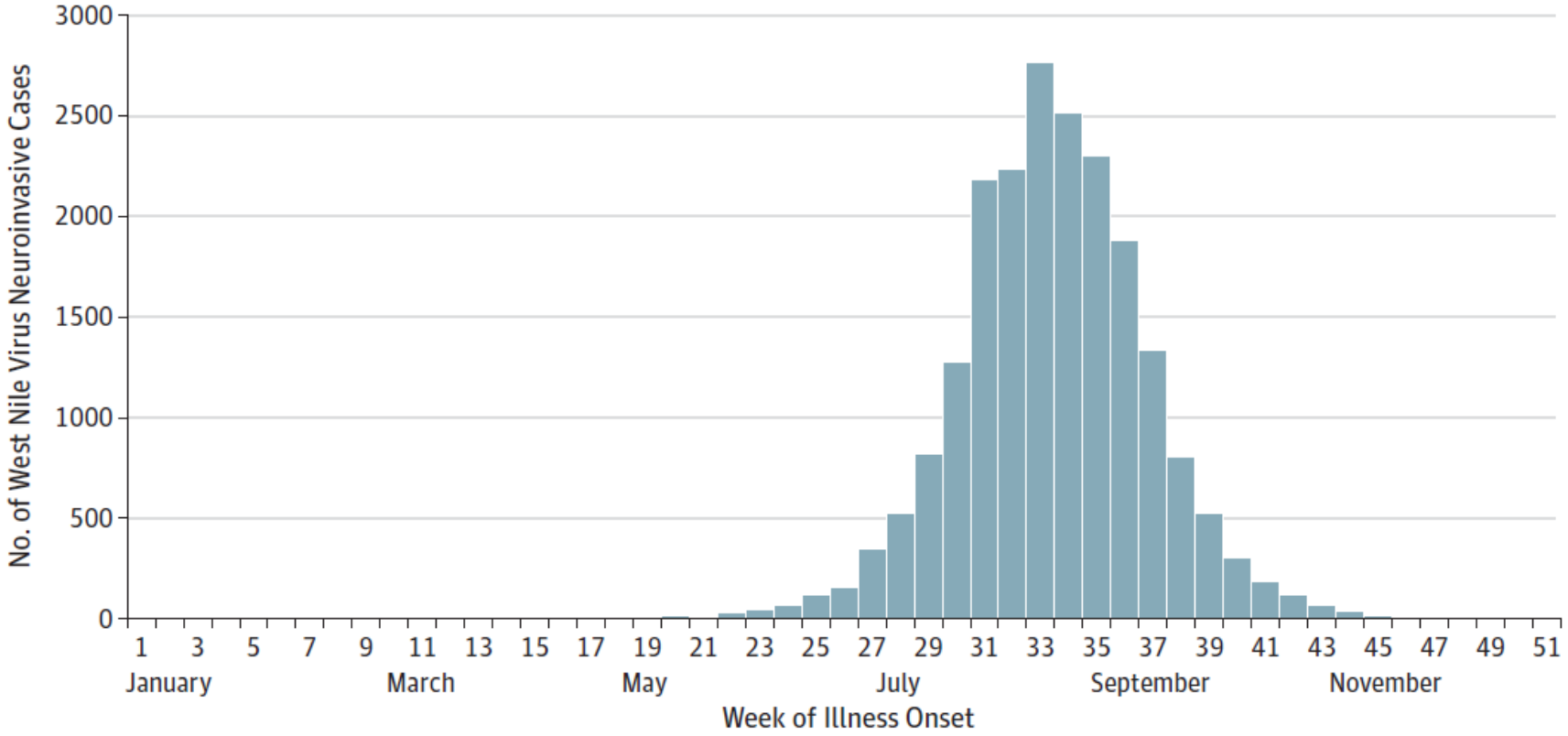


West Nile virus transmission



http://www.cdc.gov/westnile

Seasonal pattern of WNV neuroinvasive disease cases



Petersen et al. 2013. *JAMA* 310: 308-315.

THE SACRAMENTO BEE

Part of the McClatchy Media Network

HEALTH & MEDICINE

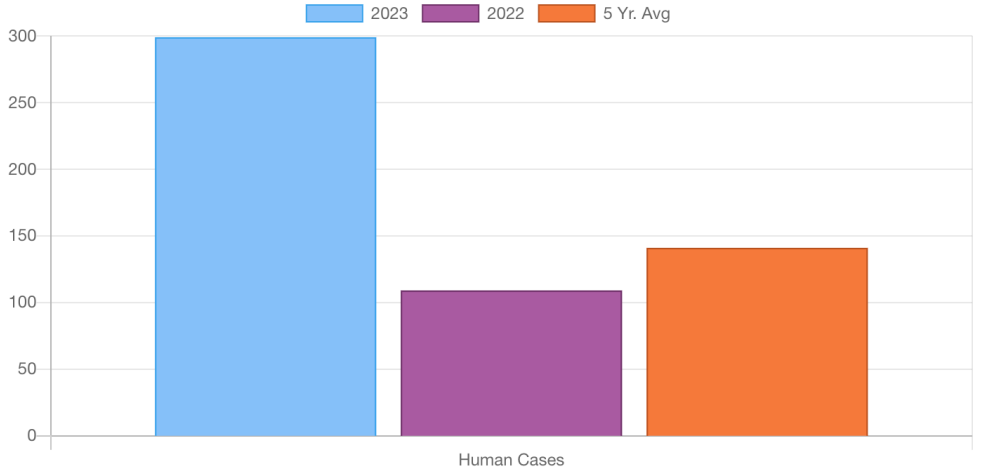
Is California's next health concern blood-sucking mosquitoes? All this water spells trouble

BY BRIANNA TAYLOR

UPDATED APRIL 03, 2023 10:11 AM

WEEK 1 - WEEK 44 AS OF NOV 03, 2023

Tip: Hover over or click on graph bars for details



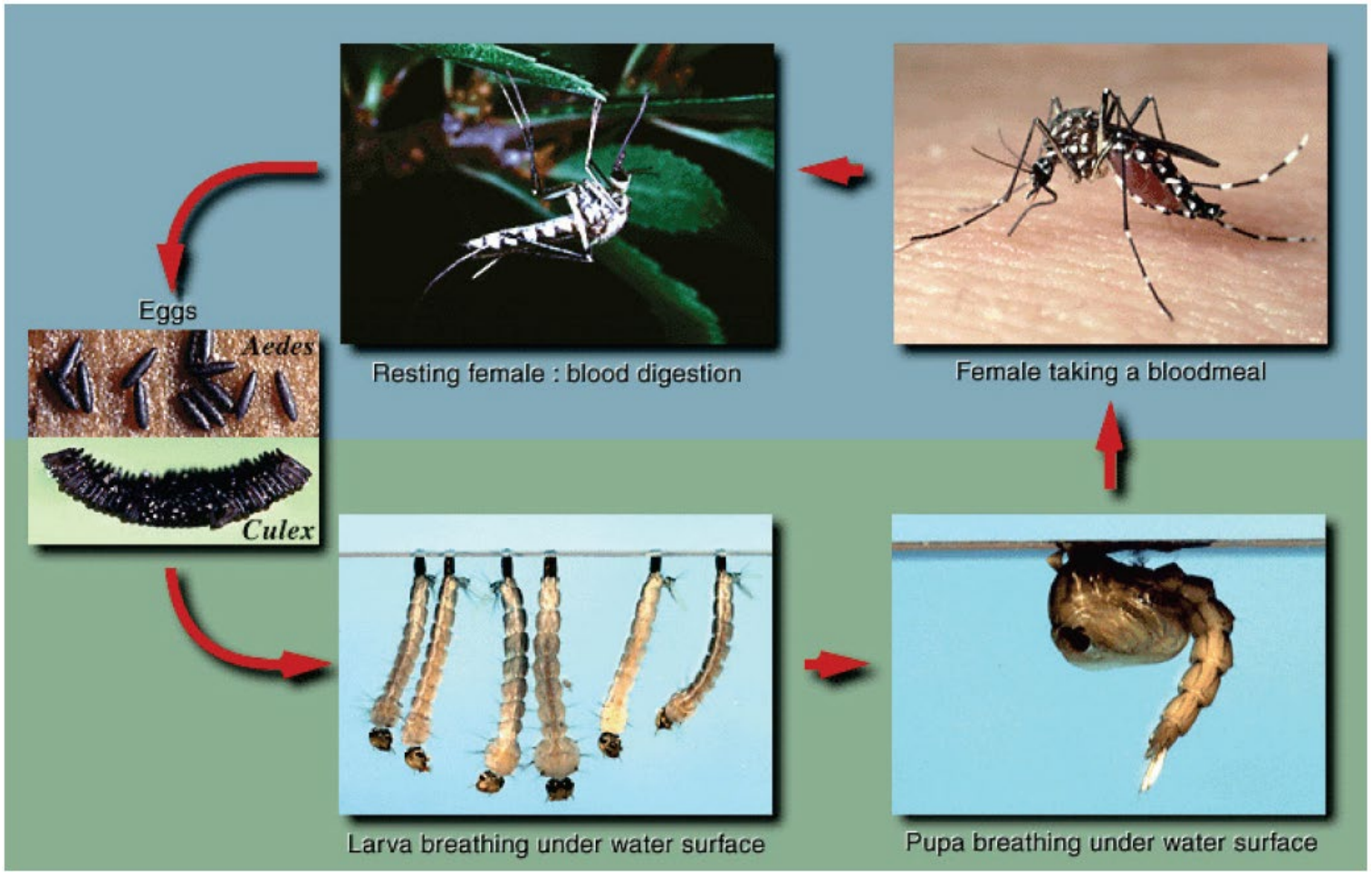
2023 **WEST NILE VIRUS ACTIVITY** IN CALIFORNIA

LAST UPDATED: NOV 03, 2023 3:56PM PST

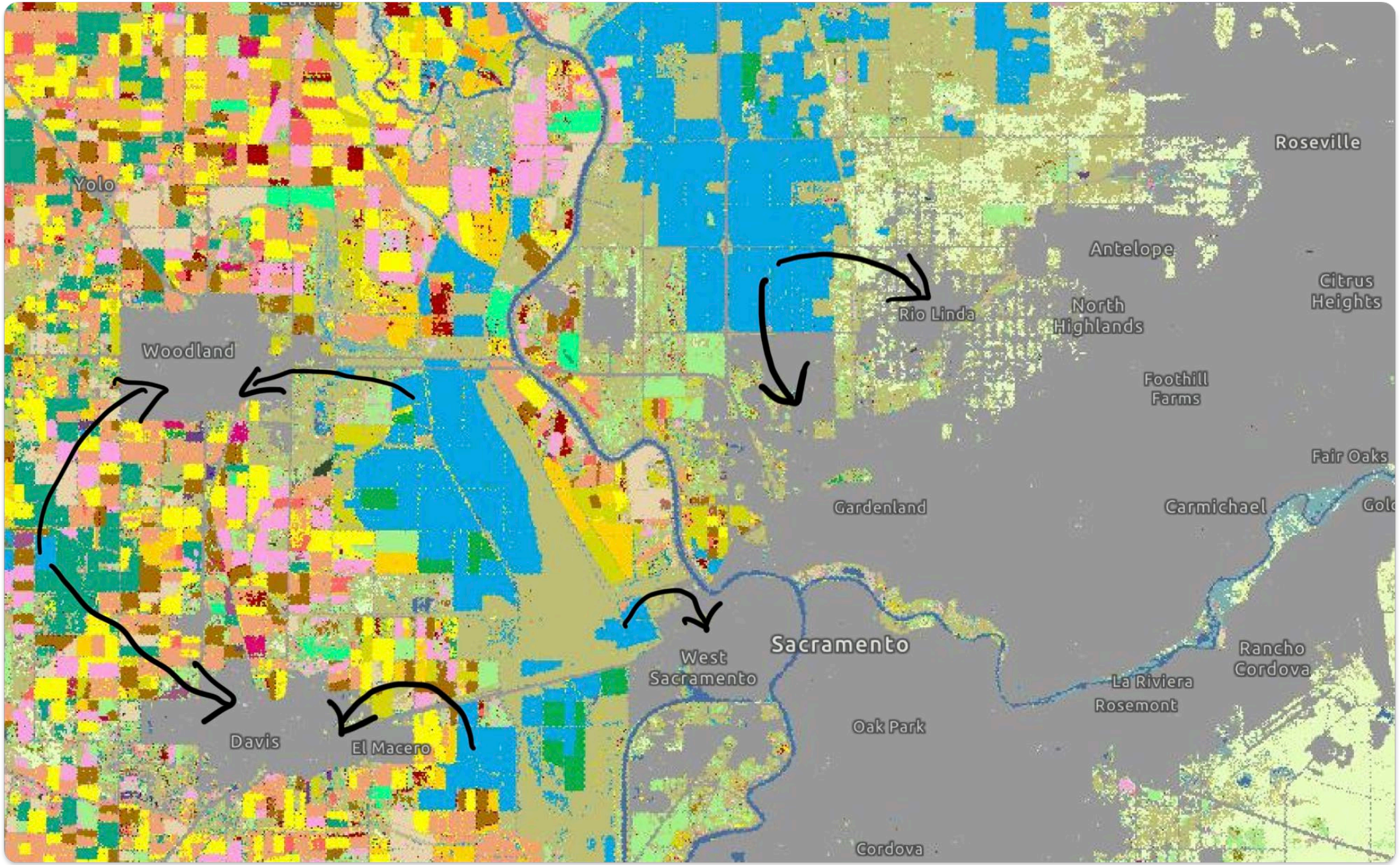
299	845	4,485	185	30
HUMAN CASES	DEAD BIRDS	MOSQUITO SAMPLES	SENTINEL CHICKENS	HORSES

source: https://westnile.ca.gov (CDPH)

Mosquito Life Cycle



ECDC, Guidelines for the surveillance of native mosquitoes, 2014



Source: USDA Cropland Data Layer

Urban mosquito sources





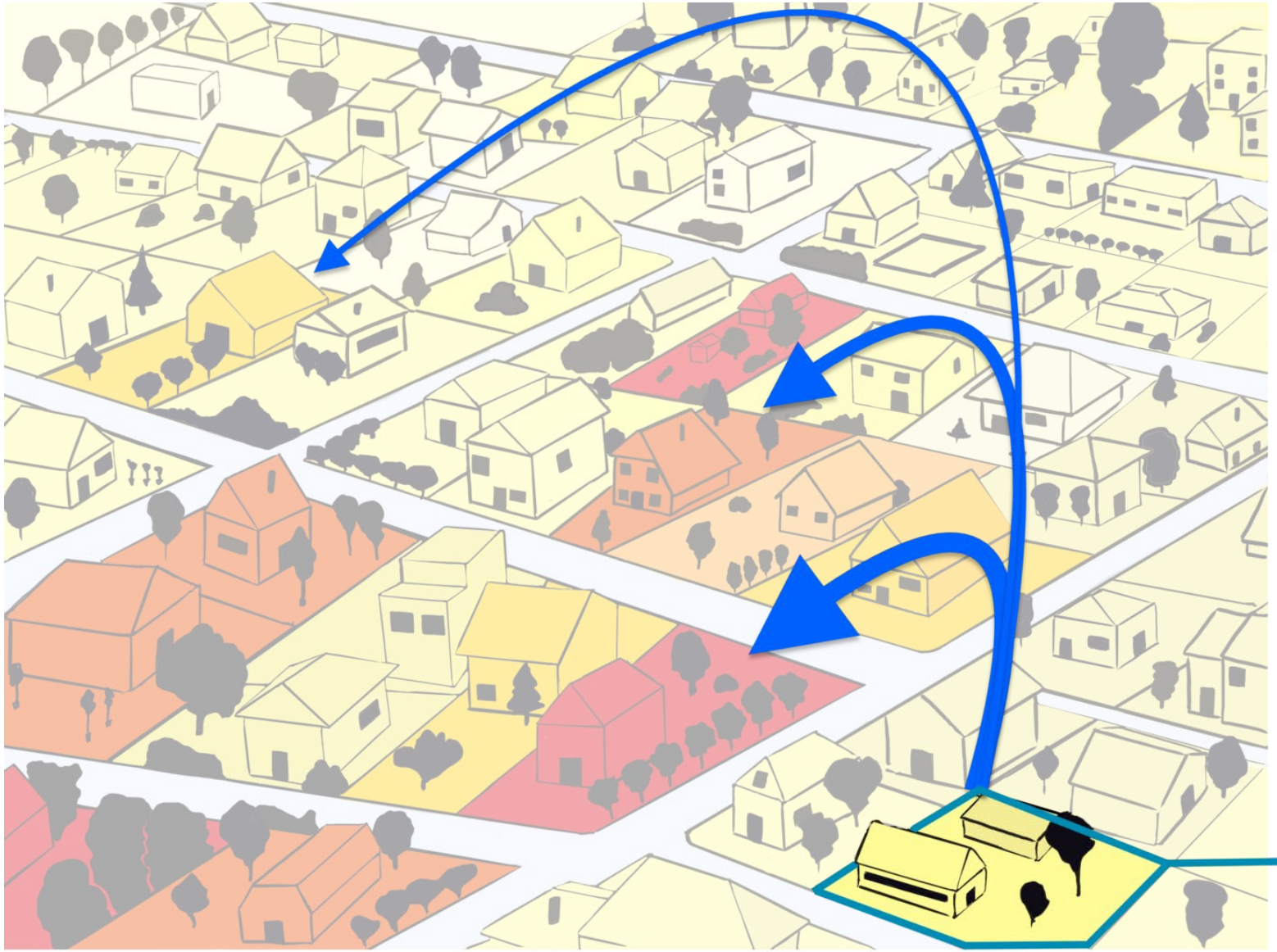







Photos: M Donnelly, C Barker, W Walton

Urban mosquito movement



Transmission of mosquito-borne viruses

What do you want to know?

Entomological Inoculation Rate

- Rate at which a person is bitten by infectious vectors per day

How many vectors are in the area?

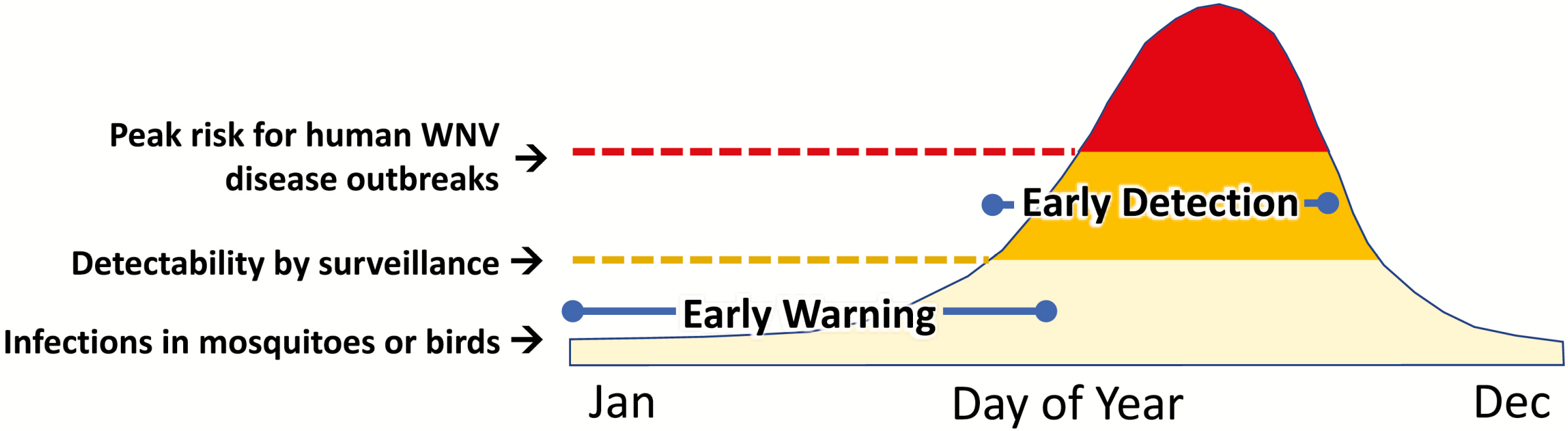
Which host(s) do they prefer?

$$EIR = \frac{vectors}{human} \times \frac{bites}{day} \times \frac{bites\ on\ humans}{all\ bites} \times \frac{infectious\ vectors}{all\ vectors}$$

How often do they bite?

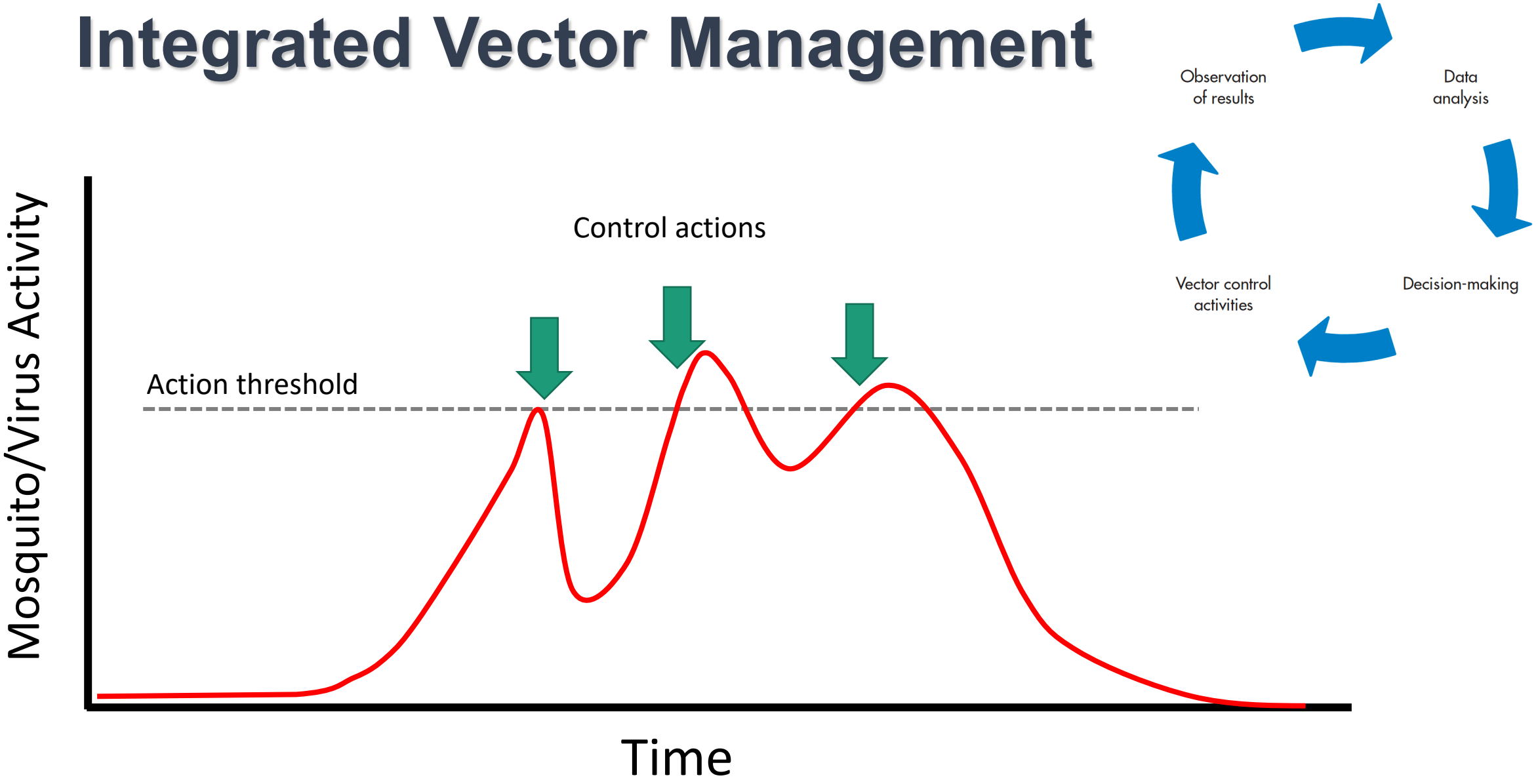
What fraction are capable of transmission?

Timeline for Surveillance Programs



Barker CM. 2019. Journal of Medical Entomology, 56: 1508–1515, https://doi.org/10.1093/jme/tjz150

Integrated Vector Management



Surveillance & Control: a ‘three-legged stool’

- CDPH: oversight, quality control, certification, planning
  - Response plans
  - Training and certification
  - Outbreak investigation
- Mosquito and Vector Control Association of California member agencies
  - surveillance, vector control, public education, emergency response
- UC Davis & partners: training, research, service
  - Training: Pacific Southwest Center of Excellence in VBDs
  - Research: vector biology, ecology, epidemiology of mosquitoes & pathogens
  - Service: decision support, laboratory diagnostics, data services, predictive models

CDPH California Department of PublicHealth

MVCAC Mosquito and Vector Control Association of California

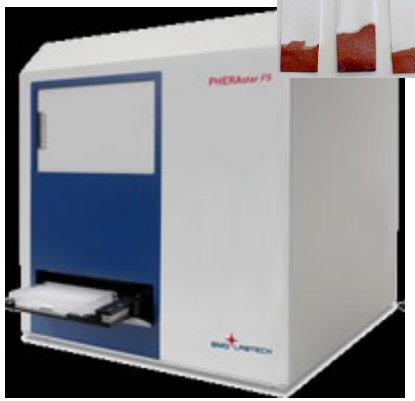
UCDAVIS UNIVERSITY OF CALIFORNIA

Surveillance Testing

Serology

Sentinel Chickens

Easier blood collection and shipment



Test blood for antibodies

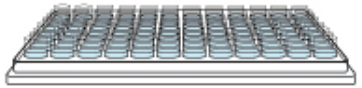
CDPH California Department of PublicHealth

Near real-time reporting

Viral detection

Mosquito pools

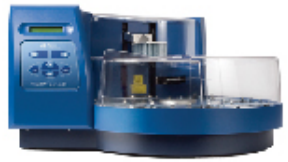
Dead birds



Homogenization
MagMAX™ Lysis/Binding Solution

UCDAVIS VETERINARY MEDICINE
DART DAVIS ARBOVIRUS RESEARCH & TRAINING Targeting Excellence

Rapid RNA extraction



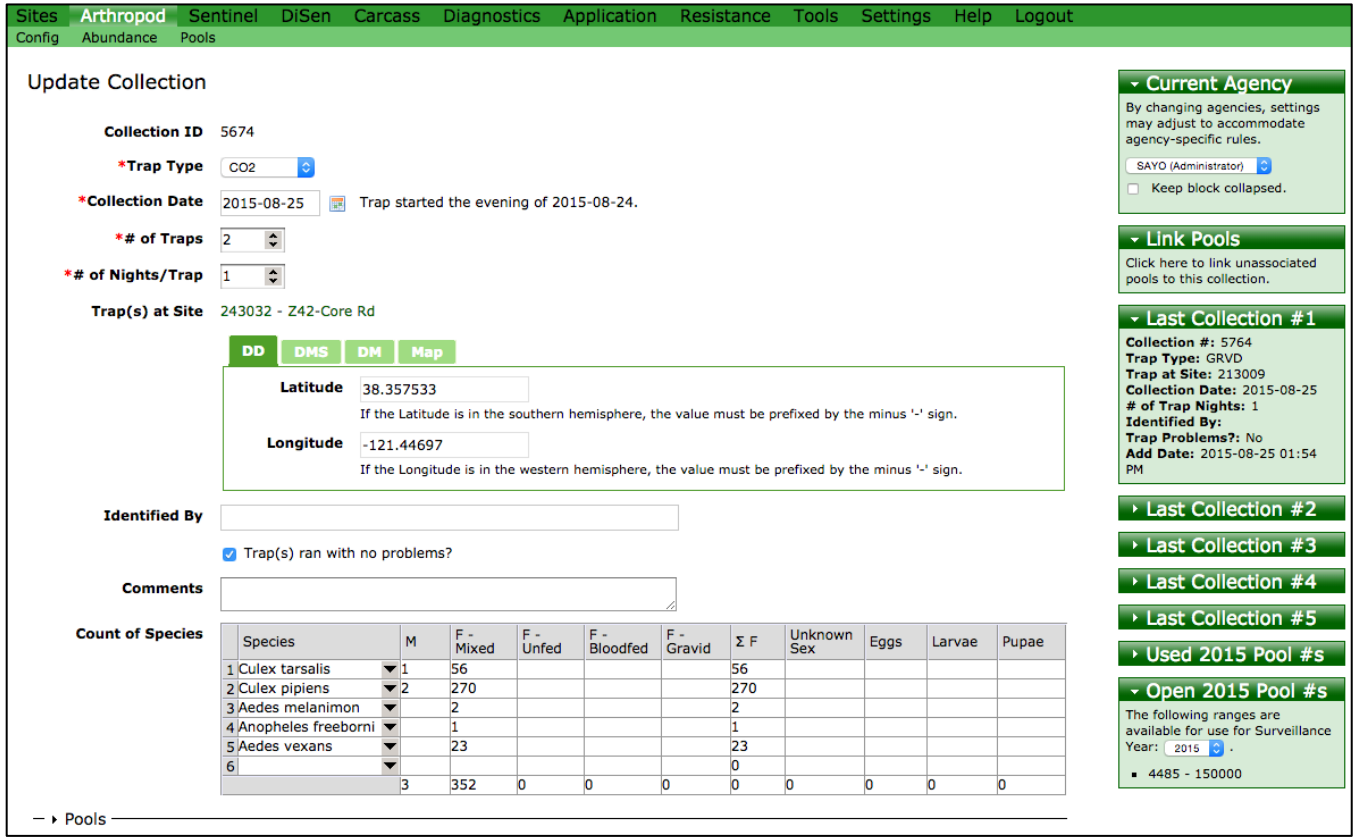
MagMAX™ Sample Preparation System
MagMAX™ Express-96 Magnetic Particle Processor
MagMAX™-96 Viral RNA Isolation Kit

Viruses
- WEEV
- SLEV
- WNV
- DENV
- CHIKV
- ZIKV

Real-Time RT-PCR
TaqMan® One-Step RT-PCR Kit
Applied Biosystems® 7900 HT
Fast Real-Time PCR System

VectorSurv

- **Online platform for management and analysis of "environmental" vector and pathogen surveillance data**
  - Surveillance Sites
  - Mosquito+Tick Collections
  - Mosquito+Tick Testing
  - Sentinel Chicken Testing
  - Pesticide Applications
  - Pesticide Resistance
- **Used by agencies large and small**
  - Direct data entry, analysis, and export
  - Web services for connection to in-house software



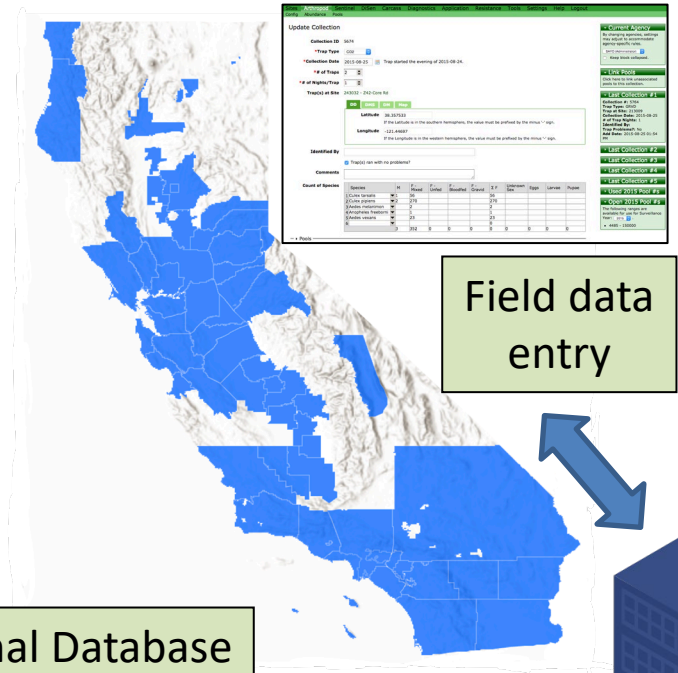
NASA CDPH California Department of PublicHealth MVCAC Mosquito and Vector Control Association of California CDC Centers for Disease Control and Prevention

CalSurv
powered by VectorSurv

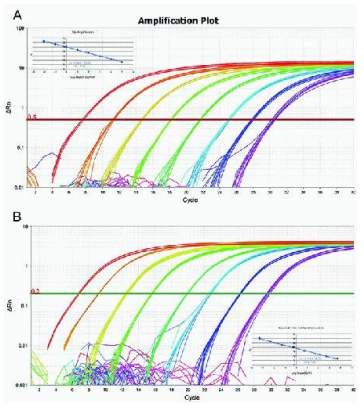
California Vectorborne Disease Surveillance Gateway

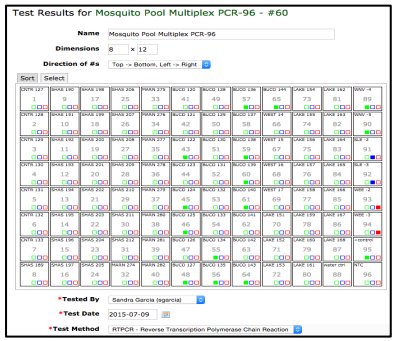
UC DAVIS VETERINARY MEDICINE
DART
DAVIS ARBOVIRUS RESEARCH & TRAINING

CDPH California Department of PublicHealth



Field data entry





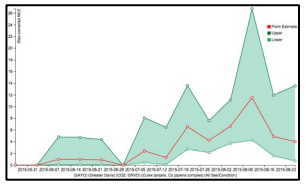
Laboratory data entry

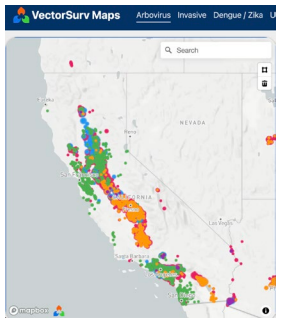
National Database

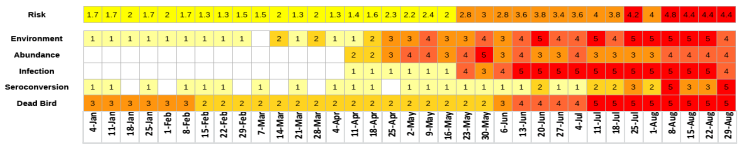
CDC

ArboNET

Interactive Maps and Tools







Weekly bulletins, Automated emails

CDPH California Department of PublicHealth

CALIFORNIA MOSQUITO-BORNE VIRUS SURVEILLANCE & RESPONSE PLAN

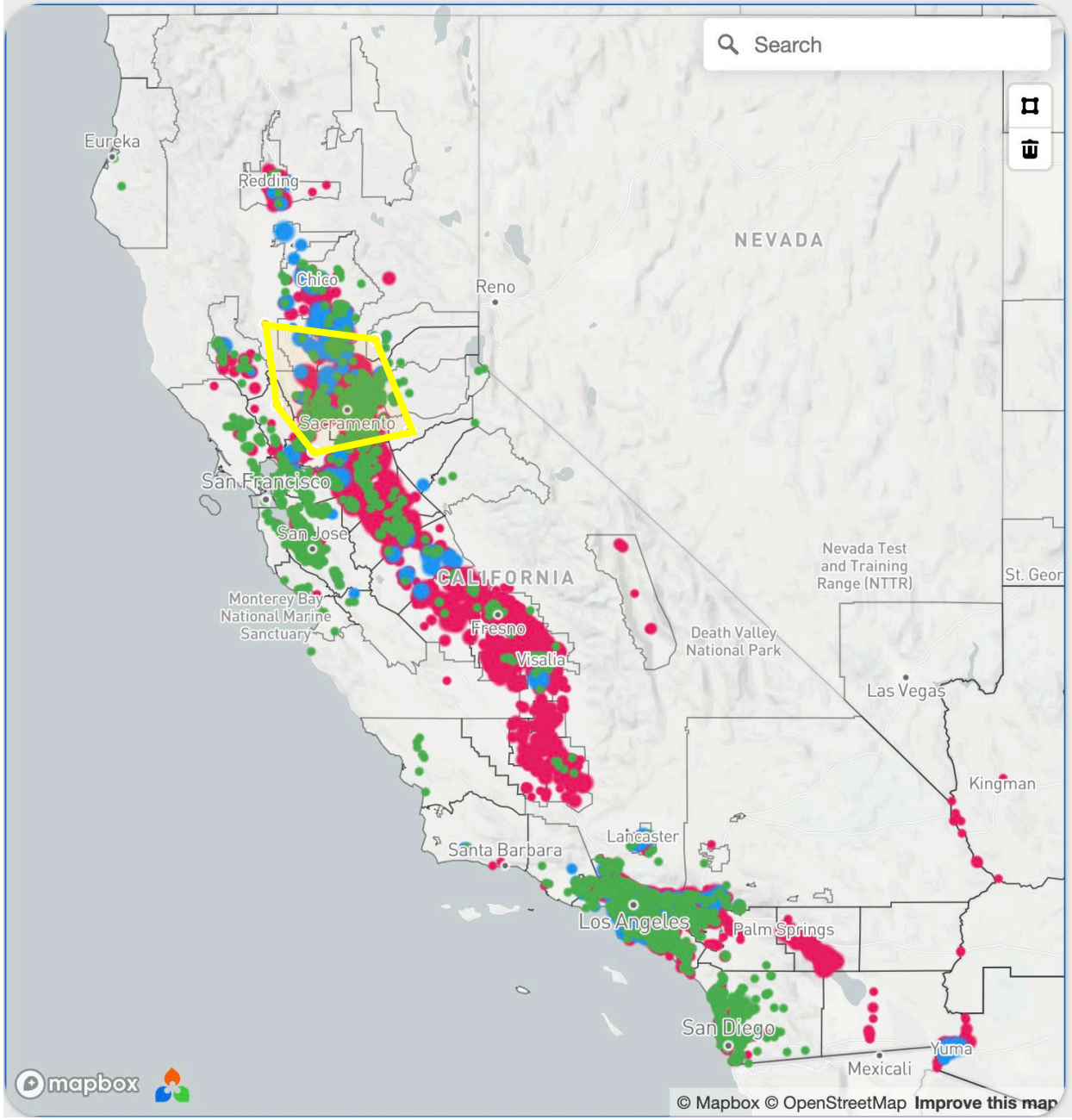
Gavin Newsom, Governor

California Department of Public Health
Mosquito & Vector Control Association of California
University of California

April 2019

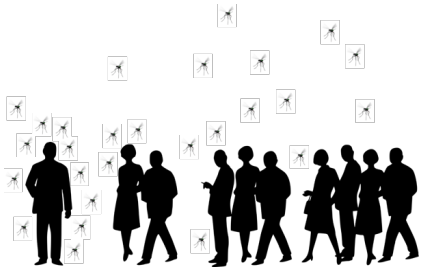
For further information contact:
Vector-Borne Disease Section
California Department of Public Health
(916) 552-9730
http://westnile.ca.gov

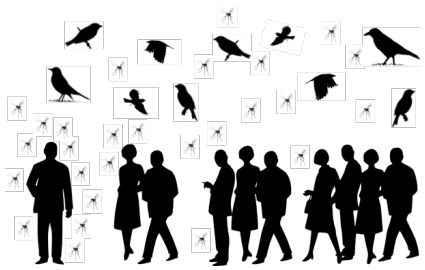
http://westnile.ca.gov/resources.php



maps.vectorsurv.org

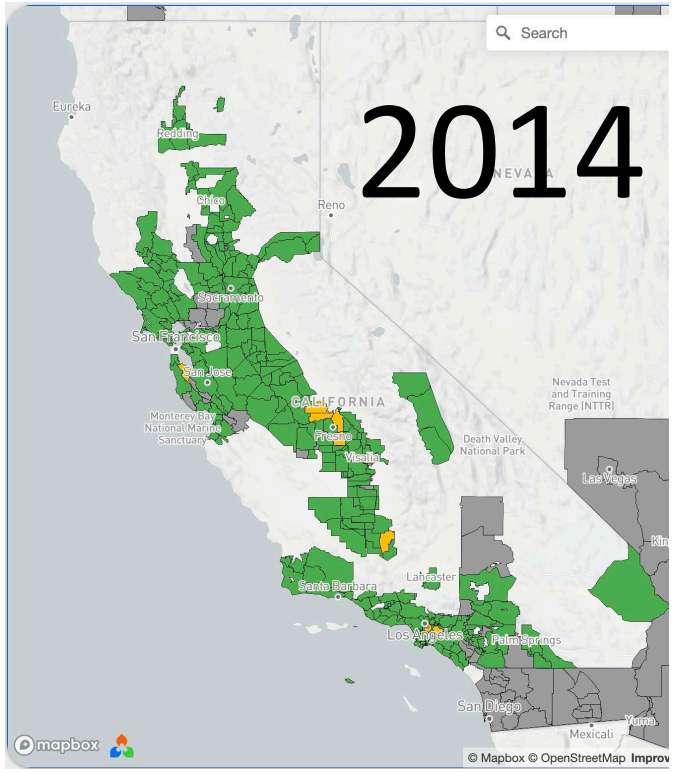
Relative Value of Surveillance Elements for Early Detection

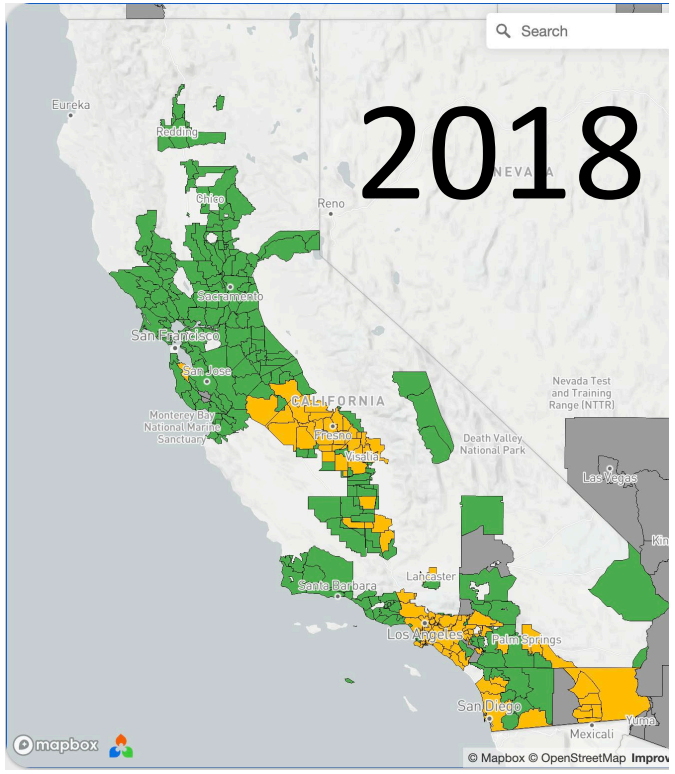


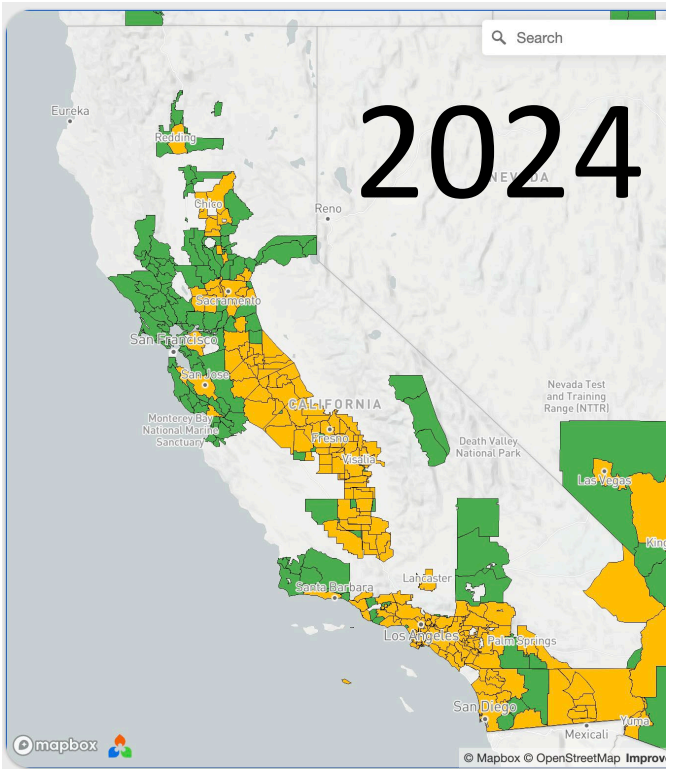


Dengue/Zika virus	Elements	West Nile virus
+++	Human disease	+
++	Weather/Climate	++
+	Vector abundance	+++
+	Vector infections	+++
N/A	Wild animals (Birds)	++

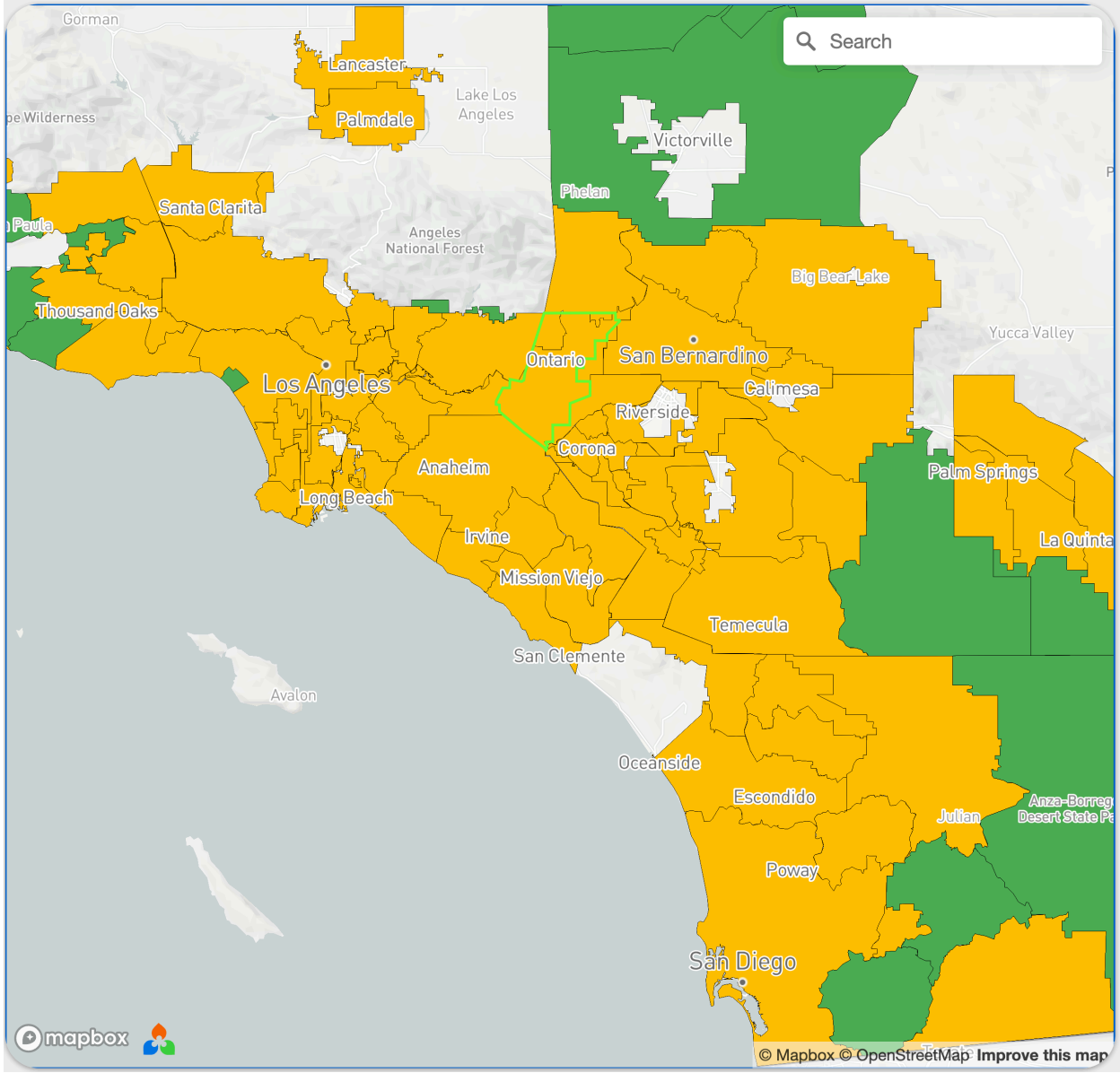
Spread of *Aedes aegypti*







VectorSurv Maps | Arbovirus | Invasive | Dengue / Zika | U.S. Pacific Islands | Abundance | Resistance | WNV Risk | Share

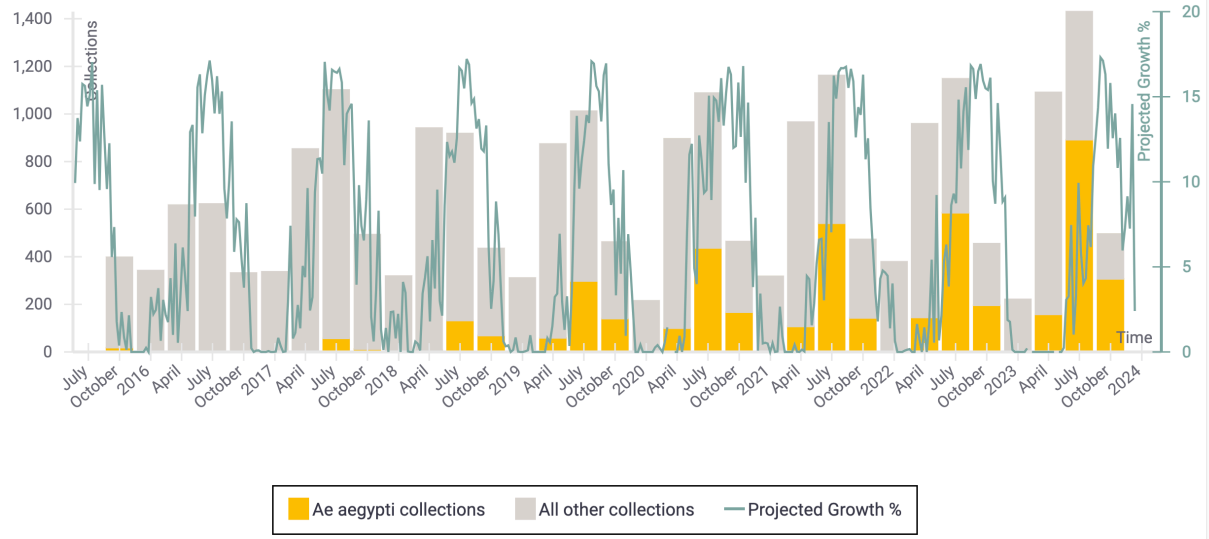


No Surveillance | Surveillance | Select species: Ae aegypti

Jul 2015 — Jan 2024

Collections in Ontario (Subdivision of SAN BERNARDINO County)

Show Help



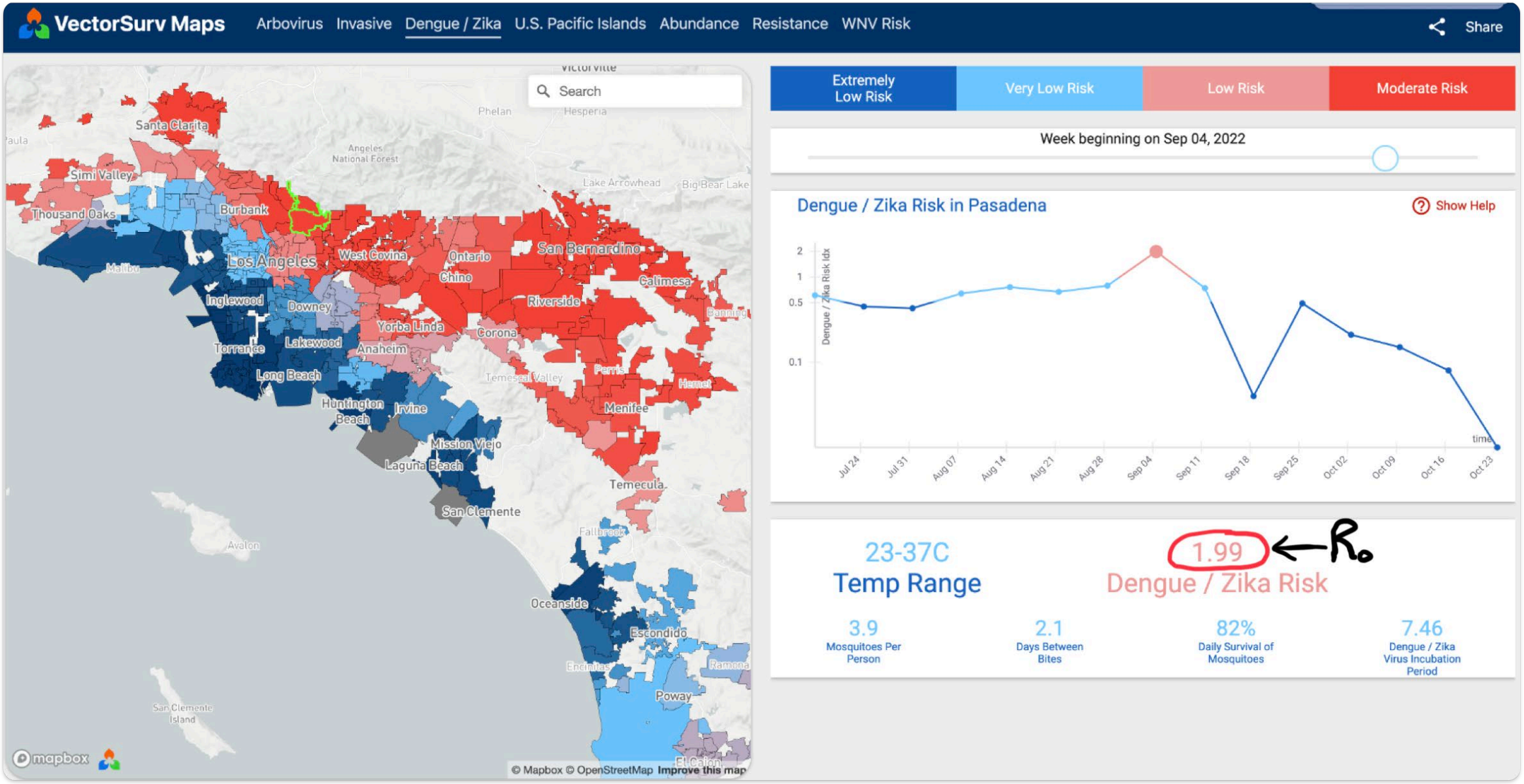
ONTARIO
West Valley MVCD

COUNTY: SAN BERNARDINO
http://www.wvmosquito.org/

	Ae aegypti	Ae albopictus	Ae japonicus	Ae notoscriptus
Total Detections (Selected Time)	4576	110	0	0
Total Detections (All Time)	4675	111	0	0
First Record	Sep 21, 2015	Oct 14, 2015	N/A	N/A
Last Record	Nov 01, 2023	Sep 12, 2022	N/A	N/A

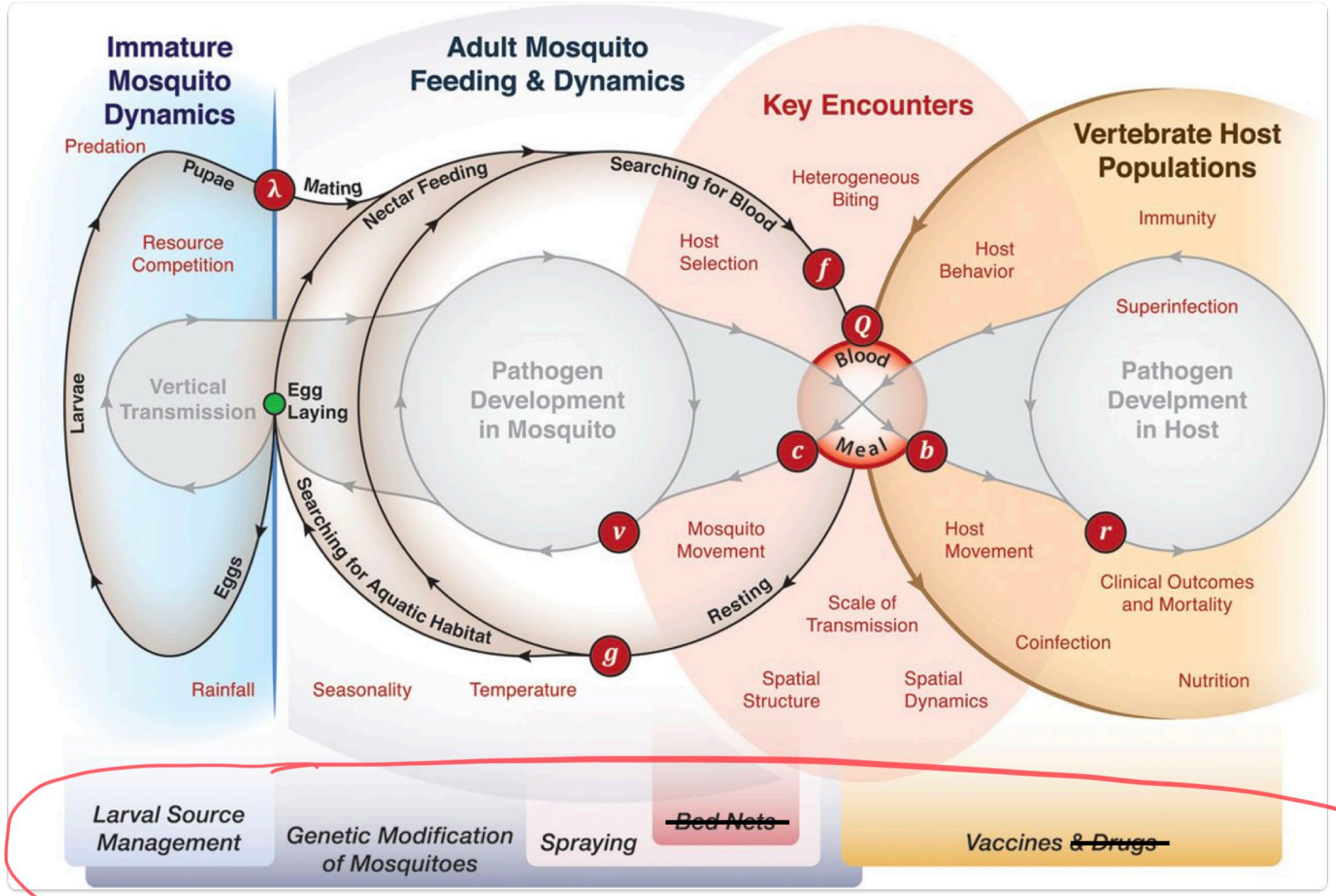
https://maps.vectorsurv.org → Invasive

Theory: Risk for dengue or Zika transmission in CA?



https://maps.vectorsurv.org → Dengue/Zika

Transmission of Mosquito-borne Pathogens



Smith DL et al. Recasting the theory of mosquito-borne pathogen transmission dynamics and control. *Trans R Soc Trop Med Hyg*. 2014;108(4):185-197.

Ways to connect: **Website: pacvec.us Twitter: @PacVecCenter**

Next-generation Sterile Insect Technique (ngSIT)

for safe and efficacious mosquito control in California.

Nikolay Kandul

November 8, 2023

UC San Diego
BIOLOGICAL SCIENCES

Synvect

The mosquito is the world's most deadly animal

Its consequences are vast and dire, and have been worsening due to the effects of climate change

Globally, per annum

700 million cases[1]

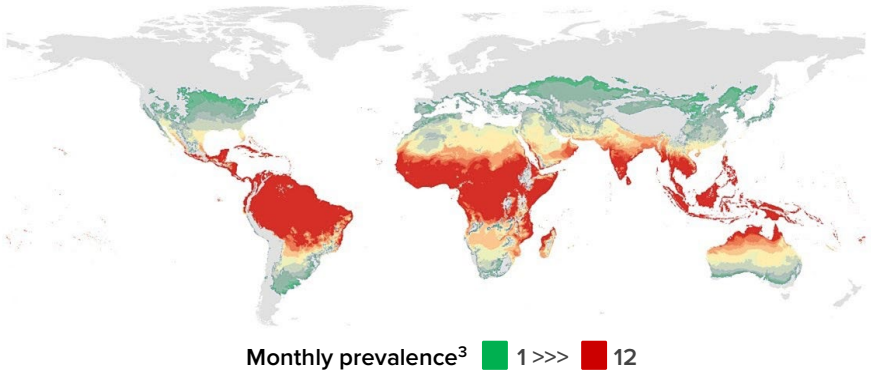
>1 million deaths[1]

The global trend of fewer cases has been plateauing since 2015[2]

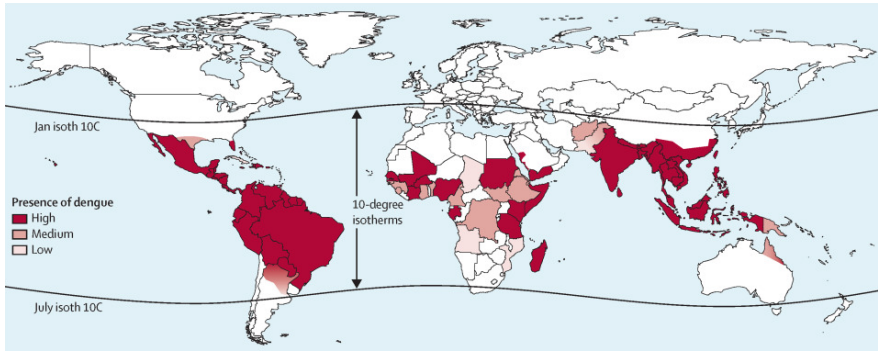
Aedes aegypti is an invasive mosquito, and 4 billion people are at risk of infection

Dengue Fever, Yellow Fever, Zika, Chikungunya, West Nile, etc.

Mosquito (aka. vector) burden



Dengue burden[4]



Global mosquito burden across all species is only expected to broaden due to the effects of climate change, globalization, and insecticides resistance – at a rate of 100 miles north per annum[2]

1. World Mosquito Program, Monash University 2021
2. WHO World Malaria Report 2020
3. Global expansion and redistribution of Aedes-borne virus transmission risk with climate change, PLoS NTD 2019
4. Dengue, Lancet 2014. & The global distribution and burden of dengue, Nature 2013.

Local transmission cases of mosquito-borne diseases are already rising in the US and Europe

LOCAL NEWS ›

First locally-acquired case of dengue virus discovered in Pasadena

KCAL NEWS

BY DANIELLE RADIN
OCTOBER 20, 2023 / 6:09 PM / KCAL NEWS

LOCAL WEATHER U.S. & WORLD THE SCENE ENTERTAINMENT NEWSL... 66°

TRENDING Wildfires Map Time Change Play the Challenge Watch: The Rundown I-Team Tips

LONG BEACH

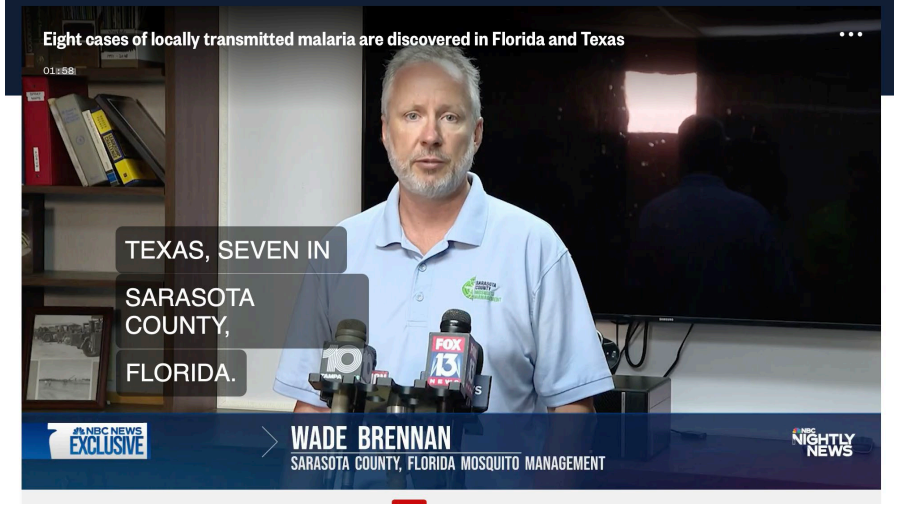
Second California case of locally acquired case of dengue virus reported in Long Beach

The virus is usually associated with people who have traveled outside the country; however, city officials say the resident infected had no history of travel.

By Elizabeth Chavolla • Published November 2, 2023 • Updated on November 2, 2023 at 6:52 pm

New malaria case in Florida brings national total to 8, the first U.S. acquired cases in 20 years

All seven of Florida's cases have been found in Sarasota County. A CDC official said the agency does not expect a nationwide outbreak.



'A first in Paris': city fumigates for tiger mosquitoes as tropical pests spread, bringing disease

Parisian health authorities treat French capital for the first time as Zika and dengue-carrying tiger mosquitoes advance through northeastern Europe

A Tiger Mosquito (Aedes Albopictus) bites through clothes in Paris, France on 20 August2023. This invasive mosquito brings tropical diseases such dengue, zika or chikungunya. Photograph: Geyres Christophe/ABACA/Shutterstock

Conventional approaches to control mosquito-borne diseases are vast and varied. But each suffers from its own set of challenges and limitations

Insecticides & Larvicides

- Growing resistance
- Environmentally unfriendly
- Labor intensive

Water drainage & Land control

- High cost at scale
- Challenging in temperate climates with high rainfall

Personal control

(e.g. bed nets)

- Variable rates of efficacy across species
- Behavioral change a limiting factor
- Non-compliance reduces efficacy

Vaccines and Drugs

- Growing drug resistance
- Uncertainty about vaccine development timelines

Biological Control

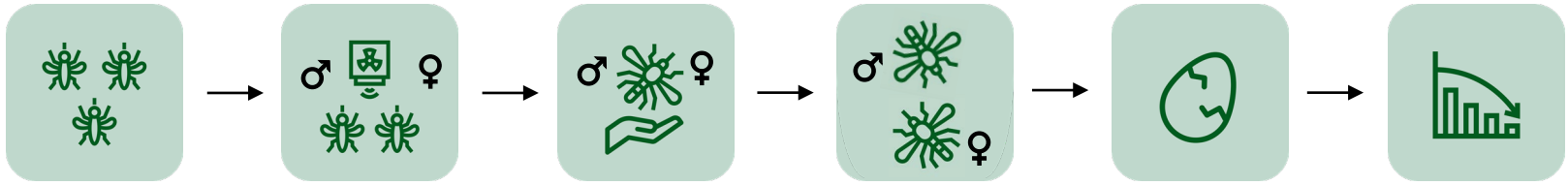
- Historically difficult to scale and deploy
- Non-economical for resource-poor settings

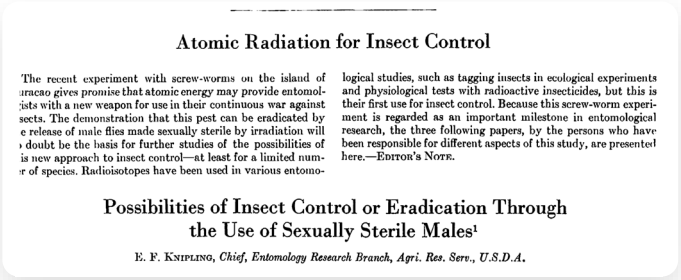
WHO World Malaria Report 2020

Sterile Insect Technique (SIT), an effective and sustainable biological approach. 2016 - "the only truly innovation in insect control in century"

STERILE INSECT TECHNIQUE (SIT)

New World Screwworm Fly



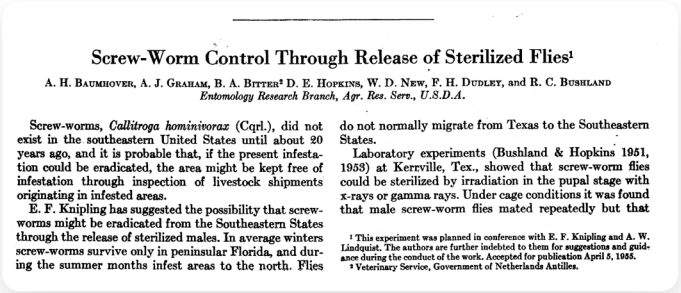


Atomic Radiation for Insect Control

The recent experiment with screw-worms on the island of Curacao gives promise that atomic energy may provide entomologists with a new weapon for use in their continuous war against insects. The demonstration that this pest can be eradicated by the release of male flies made sexually sterile by irradiation will no doubt be the basis for further studies of the possibilities of this new approach to insect control—at least for a limited number of species. Radioisotopes have been used in various entomological studies, such as tagging insects in ecological experiments and physiological tests with radioactive insecticides, but this is their first use for insect control. Because this screw-worm experiment is regarded as an important milestone in entomological research, the three following papers, by the persons who have been responsible for different aspects of this study, are presented here.—EDITOR'S NOTE.

Possibilities of Insect Control or Eradication Through the Use of Sexually Sterile Males[1]

E. F. KNIPLING, *Chief, Entomology Research Branch, Agri. Res. Serv., U.S.D.A.*



Screw-Worm Control Through Release of Sterilized Flies[1]

A. H. BAUMHOVER, A. J. GRAHAM, B. A. BITTER[2] D. E. HOPKINS, W. D. NEW, F. H. DUDLEY, and R. C. BUSHLAND
Entomology Research Branch, Agr. Res. Serv., U.S.D.A.

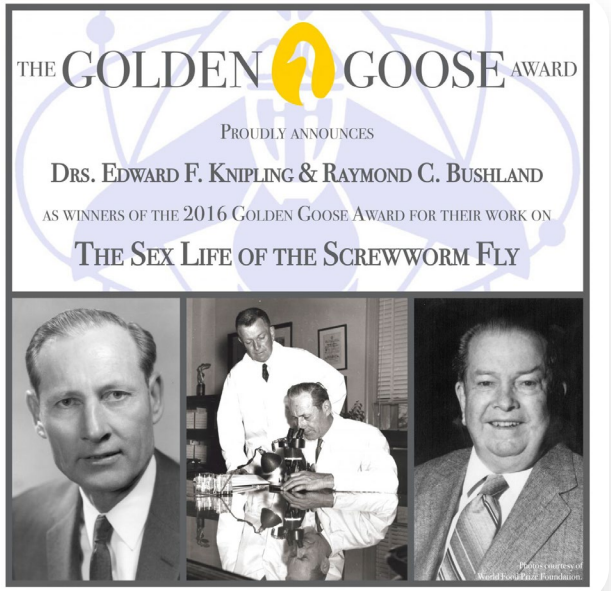
Screw-worms, *Callitroga hominivorax* (Coqrl.), did not exist in the southeastern United States until about 20 years ago, and it is probable that, if the present infestation could be eradicated, the area might be kept free of infestation through inspection of livestock shipments originating in infested areas.

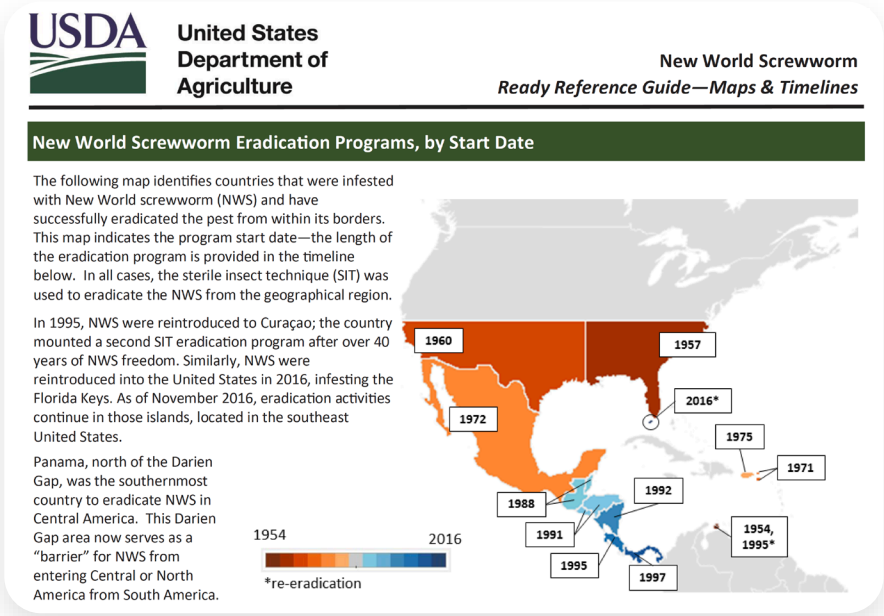
E. F. Knipling has suggested the possibility that screw-worms might be eradicated from the Southeastern States through the release of sterilized males. In average winters screw-worms survive only in peninsular Florida, and during the summer months infest areas to the north. Flies do not normally migrate from Texas to the Southeastern States.

Laboratory experiments (Bushland & Hopkins 1951, 1953) at Kerrville, Tex., showed that screw-worm flies could be sterilized by irradiation in the pupal stage with x-rays or gamma rays. Under cage conditions it was found that male screw-worm flies mated repeatedly but that

[1] This experiment was planned in conference with E. F. Knipling and A. W. Lindquist. The authors are further indebted to them for suggestions and guidance during the conduct of the work. Accepted for publication April 5, 1955.
[2] Veterinary Service, Government of Netherlands Antilles.

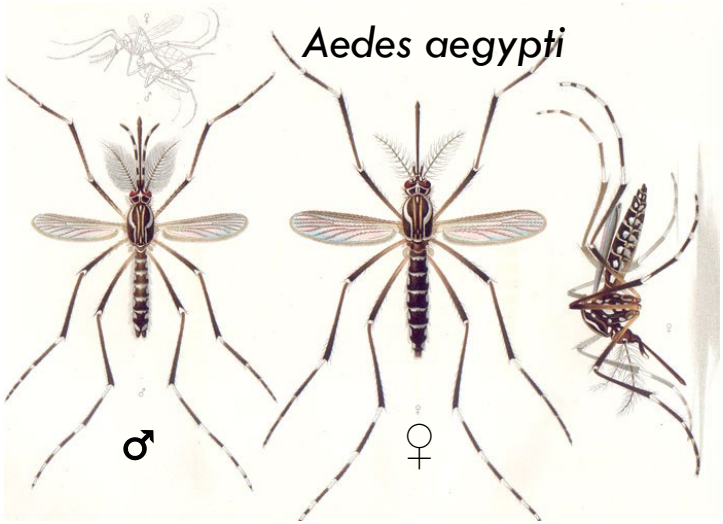
Journal of Economic Entomology 48:4 (1959)

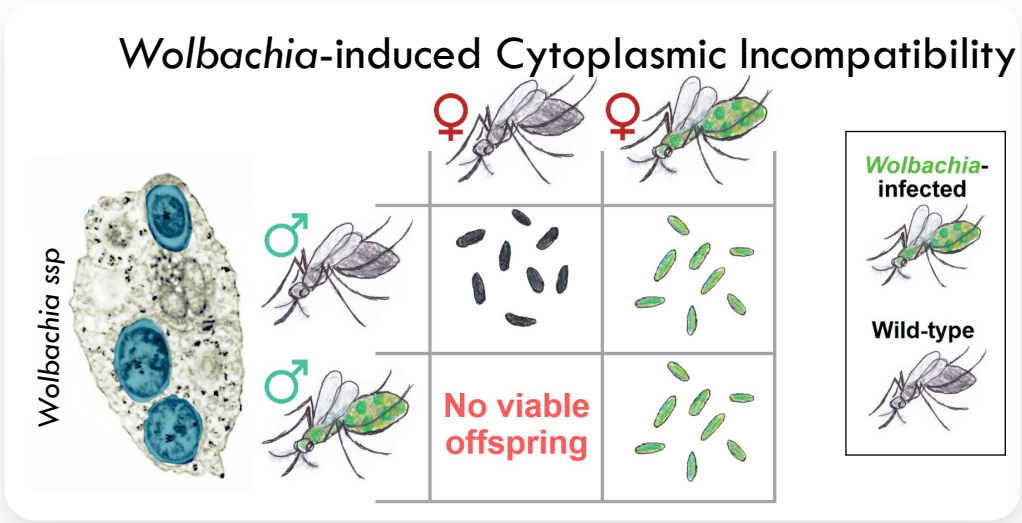




Female mosquitoes have to be removed (aka. sex-sorted) before sterilized only male mosquitoes are released over residential areas

(1) Only female mosquitoes bite and transmit diseases & (2) Female mosquito mate once in the lifetime





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.

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 E. Jost).

H. Lavin

STERILE INSECT TECHNIQUE (SIT) and/or *WOLBACHIA* INCOMPATIBLE INSECT TECHNIQUE (WIIT)

Major challenges of traditional SIT and *W*IIT for mosquito control

- **Efficient (aka. high-throughput and high-precision) sex-sorting**
  - Female mosquitoes have to be removed before massive releases of males
- **Economical efficiency**
  - Adult male mosquitoes are fragile and cannot be shipped globally
  - High capital costs of building local facilities
  - Continuous production is required for local releases, since adults cannot be stored
  - 50% of production capacity is wasted for rearing females: 0.5x scale
- **Suppression efficacy**
  - Handling male adults affect their fitness, competitiveness, and longevity
  - Deployment of uncompetitive adult males
  - Massive releases of sterilized males are required for effective suppression

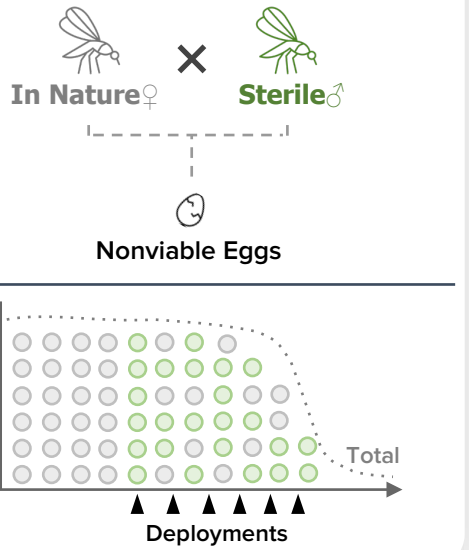
Biological approaches for controlling mosquito populations

Confined, Non Gene Drive

Suppression

CONFINED METHODS

SIT, *Wolbachia* IIT



MAJOR CHALLENGES

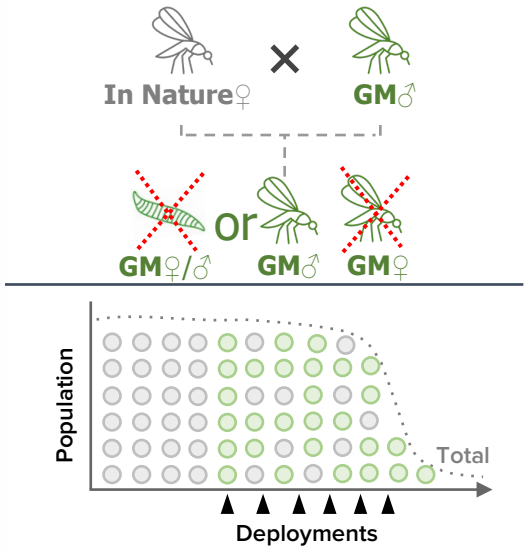
Low ♂ fitness

Costly to deploy

Suppression

CONFINED GM METHODS

RILD, fsRIDL, female Lethality



MAJOR CHALLENGES

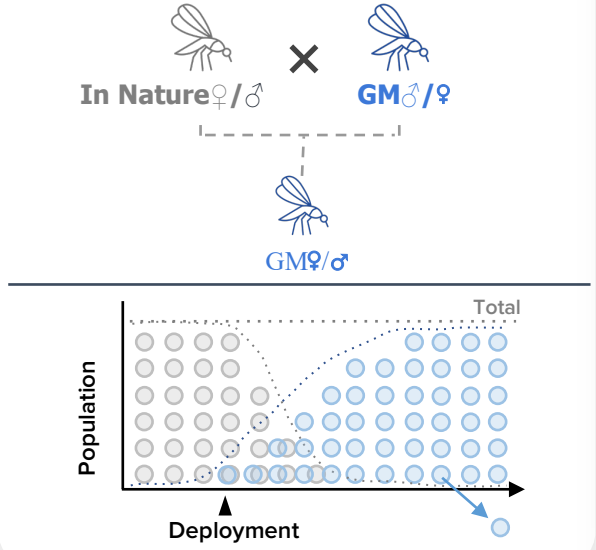
Titration of lethality is complex

Regulatory hurdles for passive GM

Spreading, Gene Drive

Modification

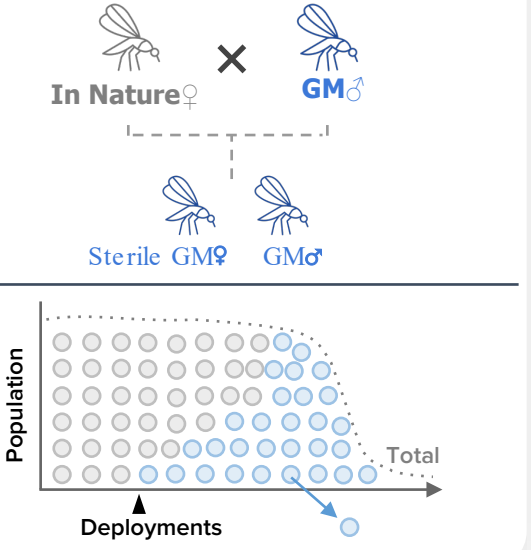
SELF-PROPAGATING METHODS



Suppression

SELF-PROPAGATING METHODS

Suppression Gene drive



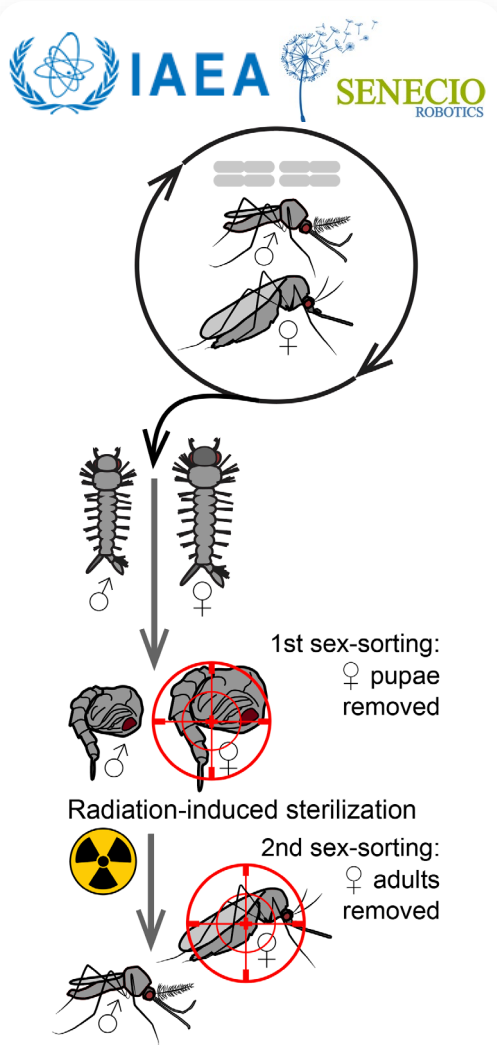
MAJOR CHALLENGES

Theoretically unstoppable spread, risk of resistance or adaptation

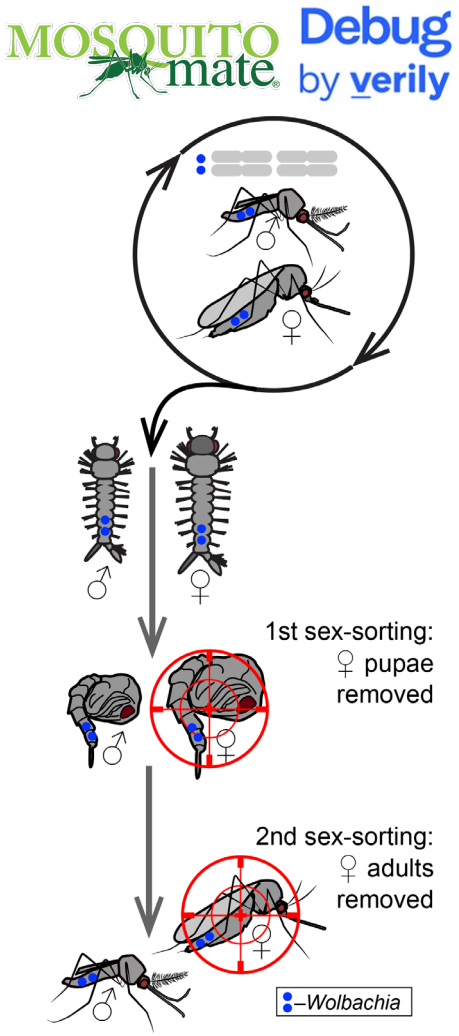
Severe regulatory hurdles for catalytic GM

Sex-sorting of mosquitoes was recently optimized by over-engineering

SIT

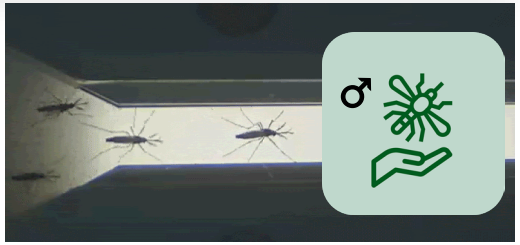


Wolbachia IIT



1st pupal size sex-sorting: 2-5% error
Pupal sex size dimorphism in *Culex* and *Aedes* species, not in *Anopheles* species

2nd optical sex-sorting of adults:[2] <0.001% error
Ai-assisted, proprietary, expensive, and not broadly available

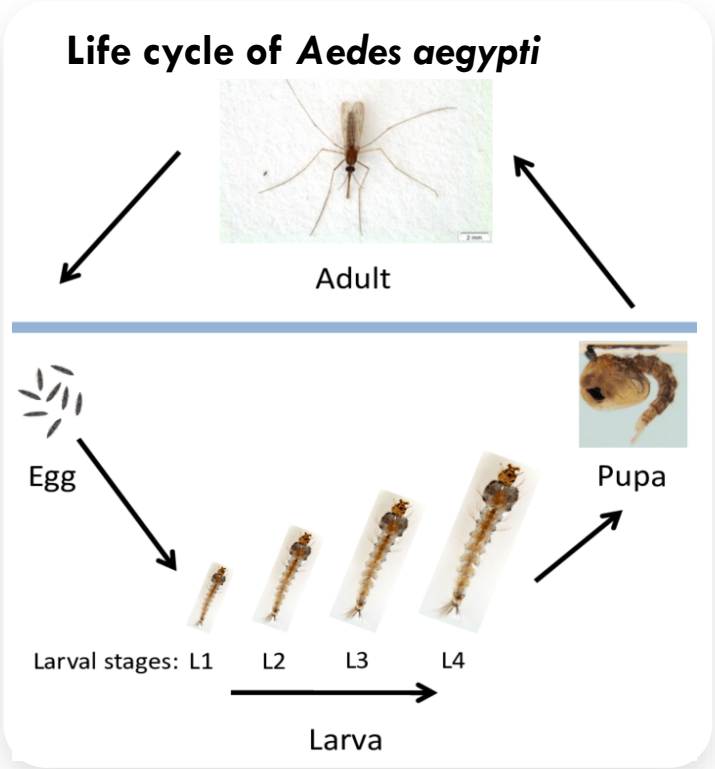


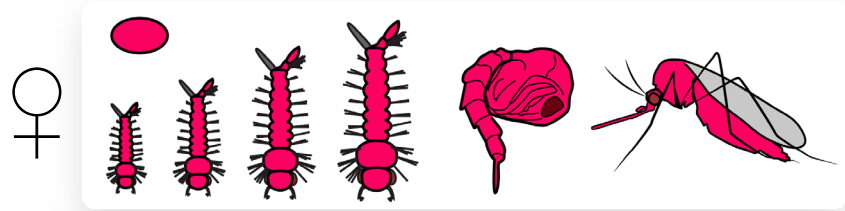
Sex-sorting approaches that can be applied in different mosquito species are required for the broad adaptation of SIT & *W* IIT for mosquito control

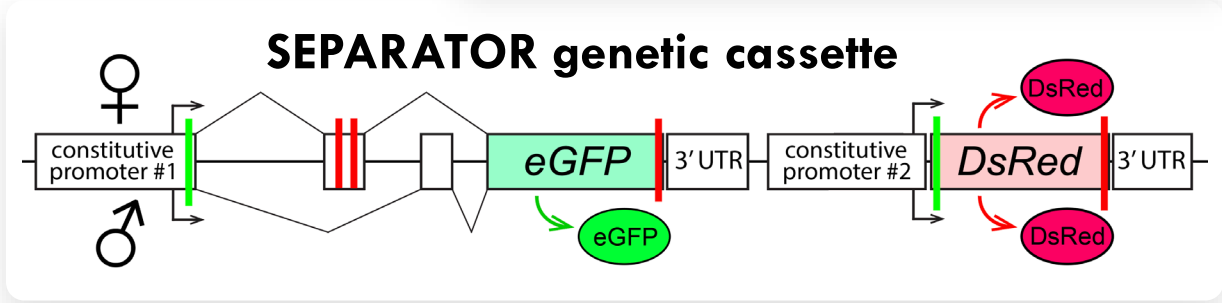
1. Moretti et al. Increased biting rate and decreased *Wolbachia* density in irradiated *Aedes* mosquitoes. *Parasites & Vectors* 2002. 15:67
2. Crawford et al. Efficient production of male Wolbachia-infected Aedes aegypti mosquitoes enables large-scale suppression of wild populations. *Nature Biotech* 2020. 38:482.

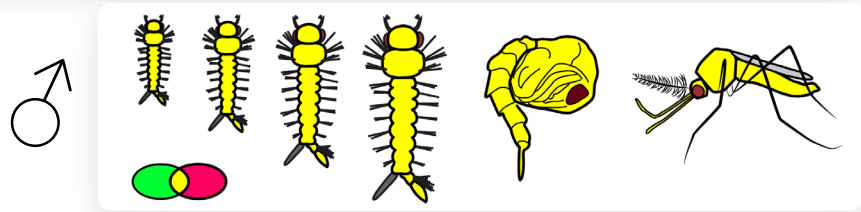
Sex-sorting of male mosquitoes by marker-assisted genetic approach:

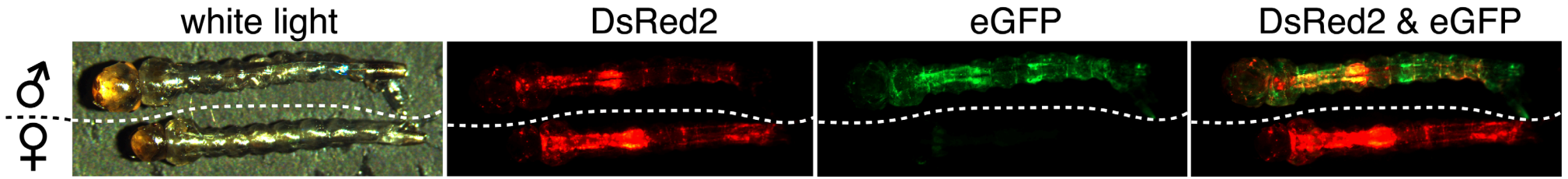
SEPARATOR (Sexing Element Produced by Alternative RNA-splicing of A Transgenic Observable Reporter) enables the fluorescence-assistant positive selection of early male larvae, at the L_1 larval stage[1]











1. Weng et al. Efficient sex separation by exploiting differential alternative slicing of a dominant marker in *Aedes aegypti*. 2023. *bioRxiv* pre-print at https://doi.org/10.1101/2023.06.16.545348

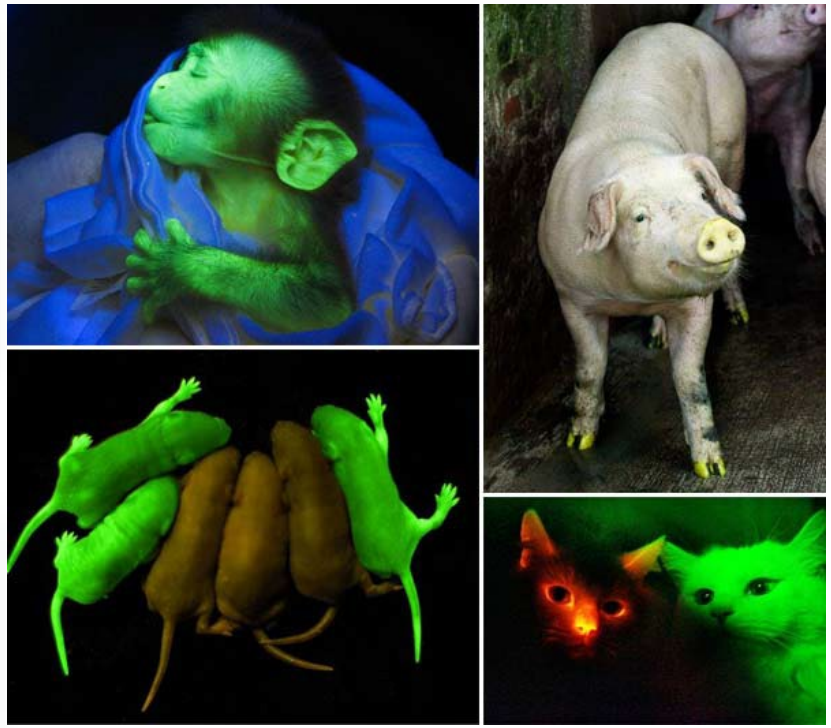
eGFP and DsRed are non-toxic, inert, innocuous, and safe markers

eGFP (**enhanced Green Fluorescent Protein**) is a basic green *constitutively fluorescent protein derived from Aequorea victoria in 1996.*
Ex λ = 488nm and Em λ = 507nm

DsRed (***Discosoma* Red**) is a basic constitutively fluorescent red protein derived from *Discosoma sp*. coral in 1999.
Ex λ = 558nm and Em λ = 583nm

eGFP and DsRed have been tagging the most sensitive cells
in vivo, such as dopaminergic and other types of neurons in mice, rats, and *Drosophila* without affecting normal physiology of the organism.[1-4]

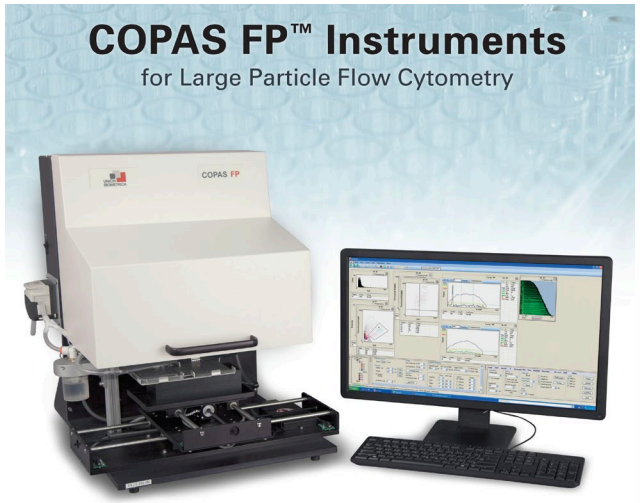
FDA Center for Food Safety and Applied Nutrition (CFSAN) raised no objections to the DsRed2 use in the corn plants for human safety.



1. Yi S *et al*. Dendritic peptide-conjugated polymeric nanovectors for non-toxic delivery of plasmid DNA and enhanced non-viral transfection of immune cells. *iScience* (2022) 25:104555
2. Lohr C *et al*. Using genetically encoded calcium Indicators to study astrocyte physiology: a field guide. *Front Cell Neurosci*. (2021) 15:690147
3. Liu R. *et al*. Biosensors of DsRed as FRET Partner with CFP or GFP for quantitatively imaging induced activation of Rac, Cdc42 in living cells. *Mol Imaging Biol* **13**, 424–431 (2011)
4. Richards HA *et al*. 2003. Safety assessment of recombinant green fluorescent protein orally administered to weaned rats. *The J. Nutr* ((2003) 133:1909

High-throughput and high-precision sex-sorting using the large particle flow cytometry.

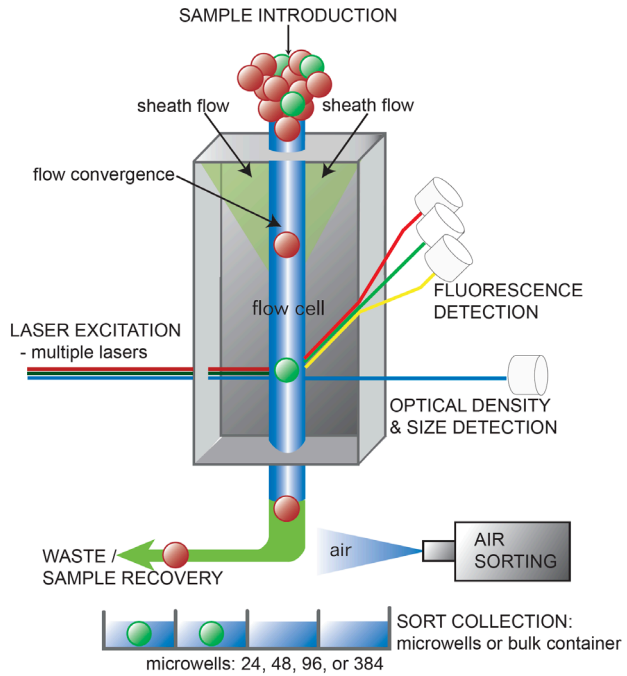
COPAS™ (Complex Object Parametric Analyzer and Sorter) "The worm sorter" was introduced in 1998 for high throughput manipulation of *C. elegans* in drug screening.



Some Examples of Application Areas

For more details you can see 200+ customer journal publications and posters at unionbio.com/publications.

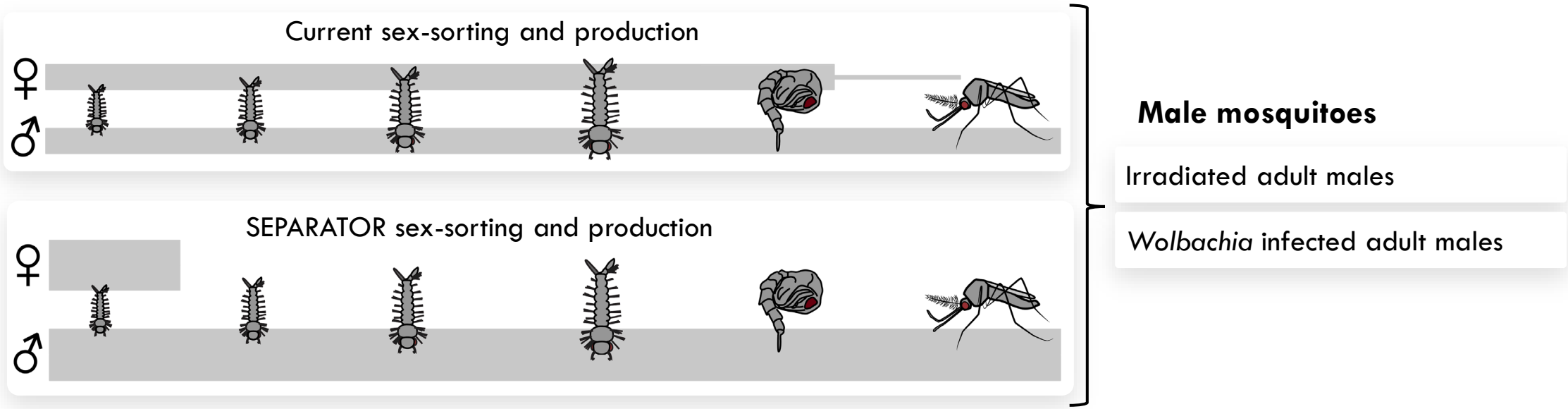
Large Cells/ Cell Clusters	Beads & Particles	Small Multi-Cellular Model Animals	Small Plant Models
• Adipocytes • Cardiomyocytes • Duct Cells (Kidney, Pancreatic, etc.) • Pancreatic Islets • Stem Cell Clusters / EBs • Spheroids & Organoids (mammary, neurospheres, intestinal, tumorspheres)	• Bead Based Assays • Cells in & on beads • Encapsulated samples • Microspheres	• *C. elegans* • *D. melanogaster* • Marine Plankton • *Medaka* • Mosquito • Zebrafish (*D. rerio*)	• *Arabidopsis & Nicotiana* seeds • Calli • Fungi • Pollen • Protoplasts



SEPARATOR enables the positive selection of L_1 male larvae at rates of 10-20 larvae per second.

1. Weng et al. Efficient sex separation by exploiting differential alternative slicing of a dominant marker in *Aedes aegypti*. 2023. *bioRxiv* pre-print at https://doi.org/10.1101/2023.06.16.545348

SEPARATOR enables the most efficient production male mosquitoes



Male mosquitoes

Irradiated adult males

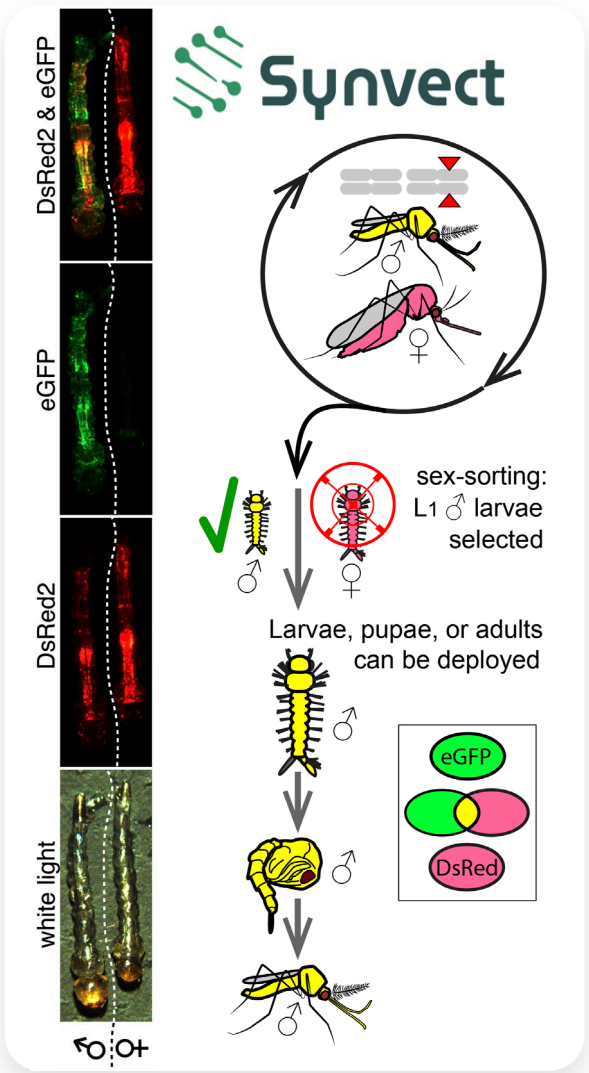
Wolbachia infected adult males

With SEPARATOR, the same production facility can produce two times male mosquitoes.

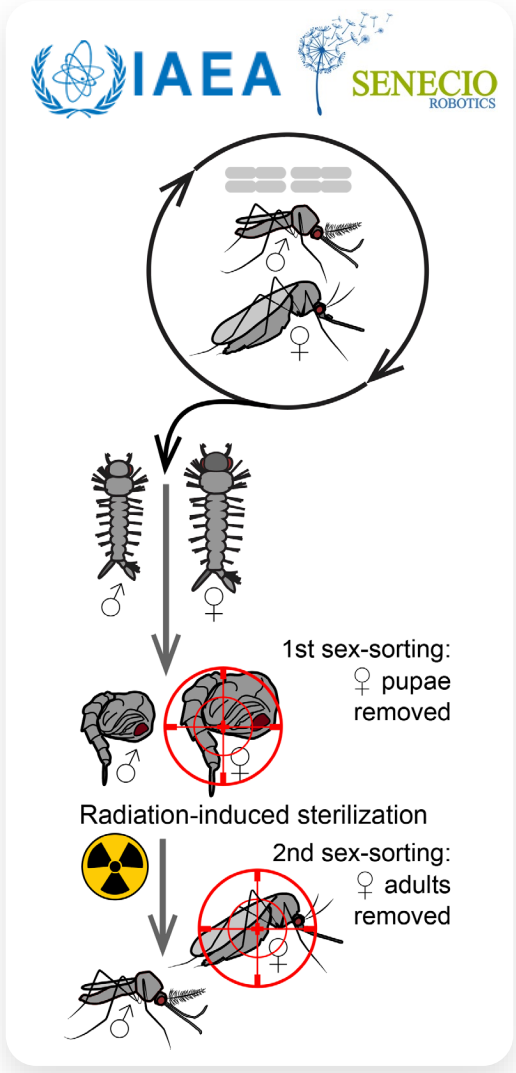
1. Weng et al. Efficient sex separation by exploiting differential alternative slicing of a dominant marker in *Aedes aegypti*. 2023. *bioRxiv* pre-print at https://doi.org/10.1101/2023.06.16.545348

Ae. aegypti SEPARATOR vs current two-step sex-sorting approaches

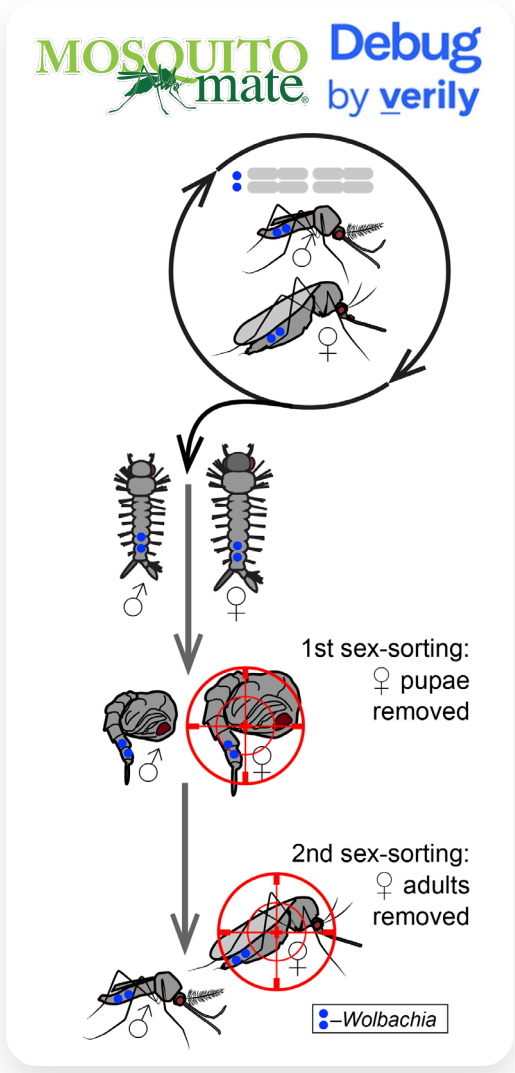
SEPARATOR



SIT



Wolbachia IIT



1. Weng et al. Efficient sex separation by exploiting differential alternative slicing of a dominant marker in *Aedes aegypti*. 2023. *bioRxiv* pre-print at https://doi.org/10.1101/2023.06.16.545348

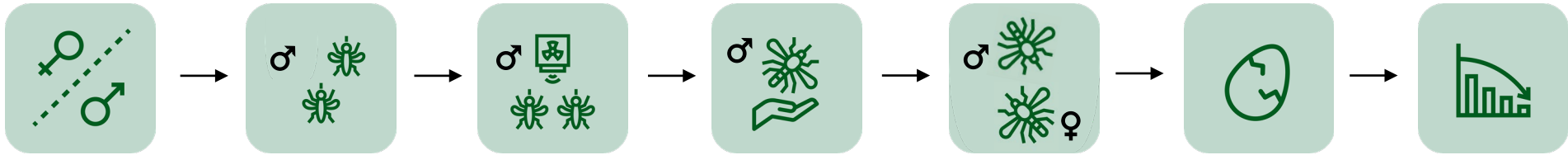
Aedes and *Anopheles* SEPARATOR strains can be utilized in SIT and *W*IIT

SEPARATOR enables effective mosquito male selection as early the L1 larvae

SEPARATOR + SIT and *W* IIT

Aedes aegypti

Anopheles stephensi



Costs effective

- High-throughput sex-sorting with large particle flow cytometry
- Sex sorting at the L_1 stage frees resources for rearing more males

Robust science

- Positive selection of male larvae with an innocuous marker
- Unbreakable genetic sex-sorting system

Not bio-pesticidal ingredient

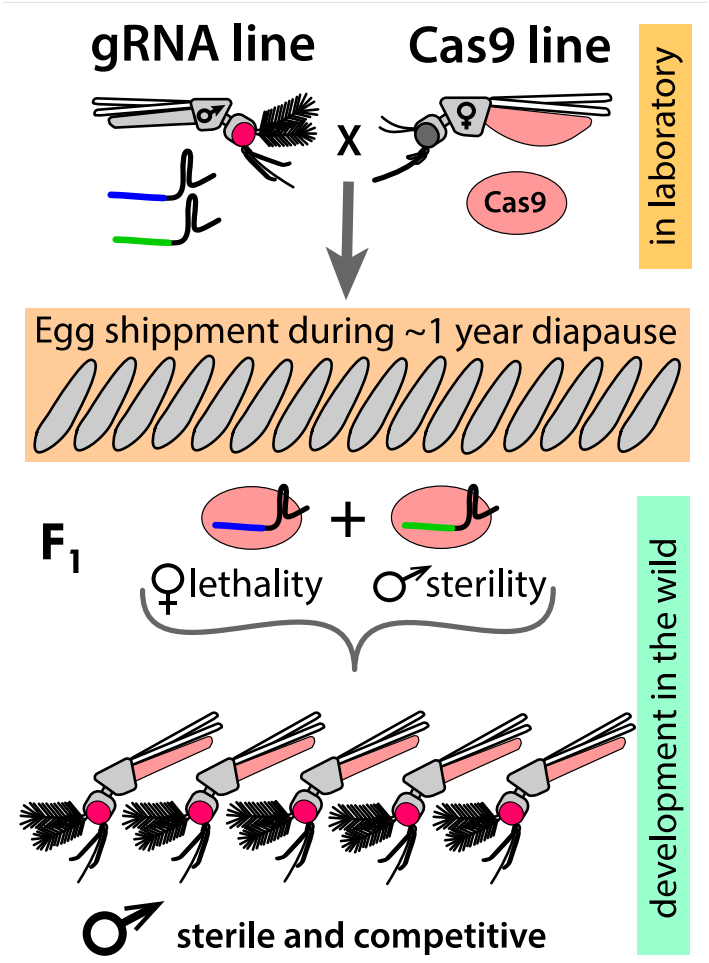
- Not regulated as bio-pesticide by EPA
- Considered as change to production process

1. Weng et al. Efficient sex separation by exploiting differential alternative slicing of a dominant marker in *Aedes aegypti*. 2023. *bioRxiv* pre-print at https://doi.org/10.1101/2023.06.16.545348

Major challenges of traditional SIT and *W*IIT for mosquito control

- **Effective (aka. high-throughput and high-precision) sex-sorting**
  - Female mosquitoes have to be removed before massive releases of males
- **Economical efficiency**
  - Adult male mosquitoes are fragile and cannot be shipped globally
  - High capital costs of building local facilities
  - Continuous production is required for local releases, since adults cannot be stored
  - 50% of production capacity is wasted for rearing females: 0.5x scale
- **Suppression efficacy**
  - Handling male adults affect their fitness, competitiveness, and longevity
  - Deployment of uncompetitive adult males
  - Massive releases of sterilized males are required for effective suppression

CRISPR-mediated confined technology for effective insect control, precision-guided SIT (pgSIT)



- Cas9 and gRNA components of CRISPR are separated into two mosquito strains: Cas9 and gRNA strains
- gRNA strain expresses multiple gRNAs targeting two genes essential for female viability and male fertility
- Cas9 or gRNA strains are maintain as homozygous strains
- The genetic cross between Cas9 and gRNA strains bring CRISPR components together in F_1 embryos
- Both female removal (aka. sex-sorting) and male sterilization happen autonomously during the development of F_1 embryos resulting in emergence of sex-sorted and sterilized males
- Only two specific genes are knocked out in the pgSIT males resulting in high fitness, competitiveness, and longevity

pgSIT offers a safe, economical, scalable, and effective solution for vector control

Thoroughly Understood and Safe Technology

Our genetic approach is thoroughly understood at the molecular level and consistently leads to male sterility and female removal.

Robustly Efficient

Precision-guided knockouts of specific genes lead to male sterility without sacrificing male fitness, longevity, and competitiveness; all while remaining self-limiting.

Low-cost Production and Deployment of Eggs

Egg production is at >> **100x** per reared mosquito.
Eggs can be stored and deployed globally.
Egg deployment results in higher efficacy of suppression.

Platform Scalability

Our technical approach can be easily ported to new species and strains – reducing the cost of new product development

As a platform biotechnology, we have optimized a pipeline for development of Cas9 and gRNA strains in major mosquito vectors

SPECIES	PRIMARY DISEASE(S)	ADDITIONAL DISEASE(S)	MAJOR REGIONS
 *Aedes aegypti*	Dengue, Zika, Yellow Fever, Chikungunya	Dog heartworm	USA, Australia, SSA, South America, India, China
 *Aedes albopictus*	Dengue, Zika, Yellow Fever, Chikungunya	Dog heartworm	Southern Europe, India, South America, China
 *Culex quinquefasciatus*	Lymphatic filariasis, St Louis encephalitis	West Nile	USA, Central America, Australia, West Africa, Southeast Asia
 *Anopheles gambiae*	Malaria	Lymphatic filariasis, Dog heartworm	SSA
 *Anopheles stephensi*	Malaria	Dog heartworm	India, SSA, Middle East, Southeast Asia, China

The next-generation SIT (ngSIT): SEPARATOR + pgSIT

An all-in-one genetically integrated technology for confined and efficacious control

NEXT-GENERATION STERILE INSECT TECHNIQUE

Engineer Cas9 & gRNA strains → **High-throughput sex-sort strains** → **Strains are crossed for egg production** → **Store and ship ngSIT eggs** → **ngSIT eggs deployed & develop in the wild** → **Only sterile males hatch in the wild** → **Population dwindles**

Cas9 and gRNA strains also contain SEPARATOR enabling high-throughput sex-sorting for massive genetic crosses.

Female removal and male sterilization happen then Cas9 and gRNAs are brought together by the genetic cross.

Mated females produce many batches of ngSIT eggs. The eggs can be stored and shipped globally for deployment.

Precision-guided knockouts of two highly specializes genes preserves fitness of ngSIT sterile males.

Next-generation SIT overcomes *every* technical and economic barrier of alternative confined approaches for mosquito control

	Synvect	oxitec	MOSQUITO mate / Debug by verily	IAEA / SENECIO ROBOTICS / Diptera.ai
Technology	Next-generation SIT	Tetracycline repressible female lethality (fsRIDL)	*Wolbachia*-induced IIT	Radiation-induced SIT
Released into the environment	Eggs developing into sterile males	Eggs developing into fertile males	*Wolbachia* infected sex-sorted adult males	Irradiated sex-sorted adult males
Deployment	Adult males emerge from egg-to-adult boxes	Adult males emerge from egg-to-adult boxes	Adult males are released	Adult males are released
Sex-sorting	Genetically integrated	Unnecessary	Very high-cost automation	High-cost imaging and semi-automation
Efficacy	High	Moderate – High	Low – Moderate	Low
Production scale factor	>> 100x	100x	0.5x	0.5x
Deployment logistics	Global	Global	Local	Local
Overall cost	Lowest	Low	High – Moderate	High – Moderate
Platform pipeline	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5

Tech platform legend

1. *Aedes aegypti*
2. *Anopheles gambiae*
3. *Aedes albopictus*
4. *Anopheles stephensi*
5. *Culex quinquefasciatus*

- Ready for deployment
- Engineering under way
- Technical challenges
- Insurmountable tech challenges

The regulatory path for *Ae. aegypti* SEPARATOR and next-generation SIT is now straightforward at the federal and state levels

GM mosquitos are classified as bio-pesticides and are under the jurisdiction of EPA and CDPR

United States Environmental Protection Agency

California Department of Pesticide Regulation

Regulatory process:

- Pilots to validate safety and efficacy of bio-pesticide under the FIFRA act
- Experimental Use Permit for open-air pilots by EPA (7 months)
- Research Authorization for open-air pilots by CDPR (? months)
- Pilot results submitted to EPA for Commercial Registration by EPA
- Commercial Registration by CDPR

Synvect

SEPARATOR strain for SIT & *W* IIT applications

Regulations

- In active discussion with EPA about feasibility of SEPARATOR classification as non-pesticidal ingredient

Commercial Partnerships and Pilots

- Establish an ACL2 facility
- Showcase SEPARATOR to potential customers and conduct pilots

Next-generation SIT

Regulations

- Cas9/gRNA is an active pesticidal ingredient in ngSIT males
- Pilots to validate safety and efficacy
- Partnerships with Mosquito Control Districts in California and Florida
- Develop production and deployment protocols
- EPA Experimental Use Permit Application

Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

BILL & MELINDA GATES foundation

EPA and CDPR are indecisive about confined GM solutions for mosquito control since 2018. We are starting to lose the fight with mosquito-borne diseases.

Novel, Non-Chemical Technologies for Pest and Vector Management - Engineered and Sterile Insects, and Related Technologies - WORKSHOP -

PUBLIC WELCOME

DATE: 10/5/2023 DOORS OPEN: 8:30 a.m.

LOCATION: Coastal Hearing Room (2nd Floor)
CalEPA Building, 1001 I Street, Sacramento

ONLINE: https://us02web.zoom.us/j/81064330998?pwd=ajFNYUY2VUhrZGRueUIreDNoTzdXZz09

The goal for workshop participants is to learn about existing and developing technologies and current research on pest management tools that are alternative to chemical tools. Scientists from public sector, private industry and universities will present on past and current projects and re-search on new and emerging techniques so attendees can learn about these alternative pest management technologies and the science behind them.

UC San Diego

BIOLOGICAL SCIENCES

Prof. Omar Akbari
Junru Liu
Ming Li
Shih-Che Weng
Andrea Smidler
Anna Buchman
Robyn Raban
Michele Bui
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Elena Benetta

USDA

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

CALIFORNIA CHERRIES

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NIH National Institutes of Health
Turning Discovery Into Health

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