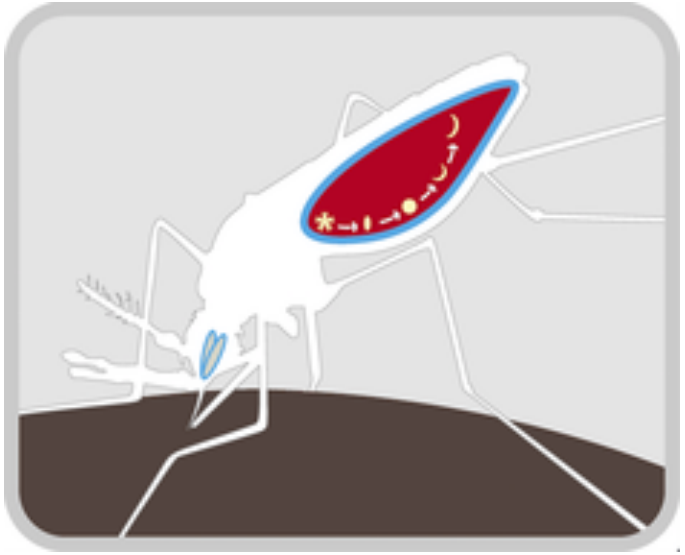
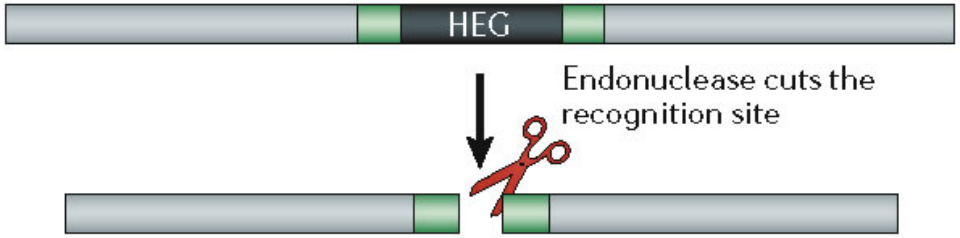
Can malaria, dengue & Zika be controlled by a CRISPR-based gene drive?





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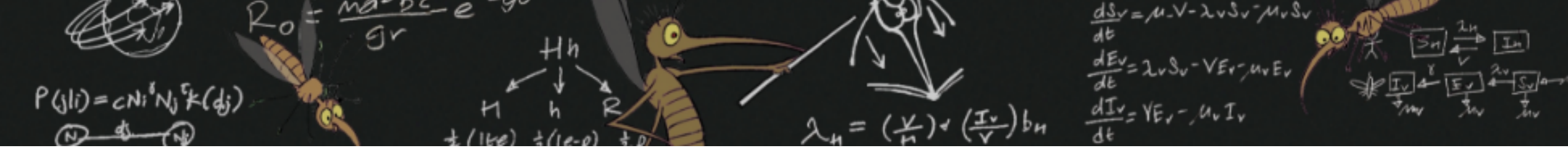


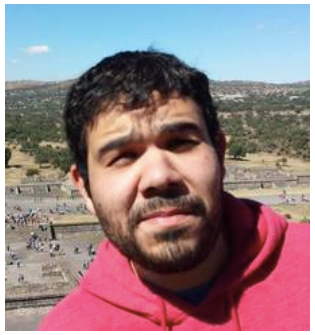
John Marshall Samson Kiware

Héctor M. Sánchez C.

Gordana Rašić

Yogita Sharma

Tomás León



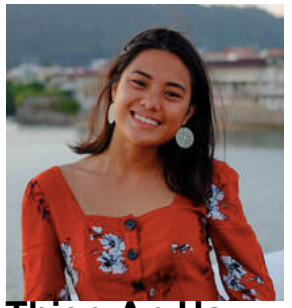
Victor Ferman

Sean Wu

Jared Bennett

Valeri Vasquez

Francois Rerolle



Thien-An Ha

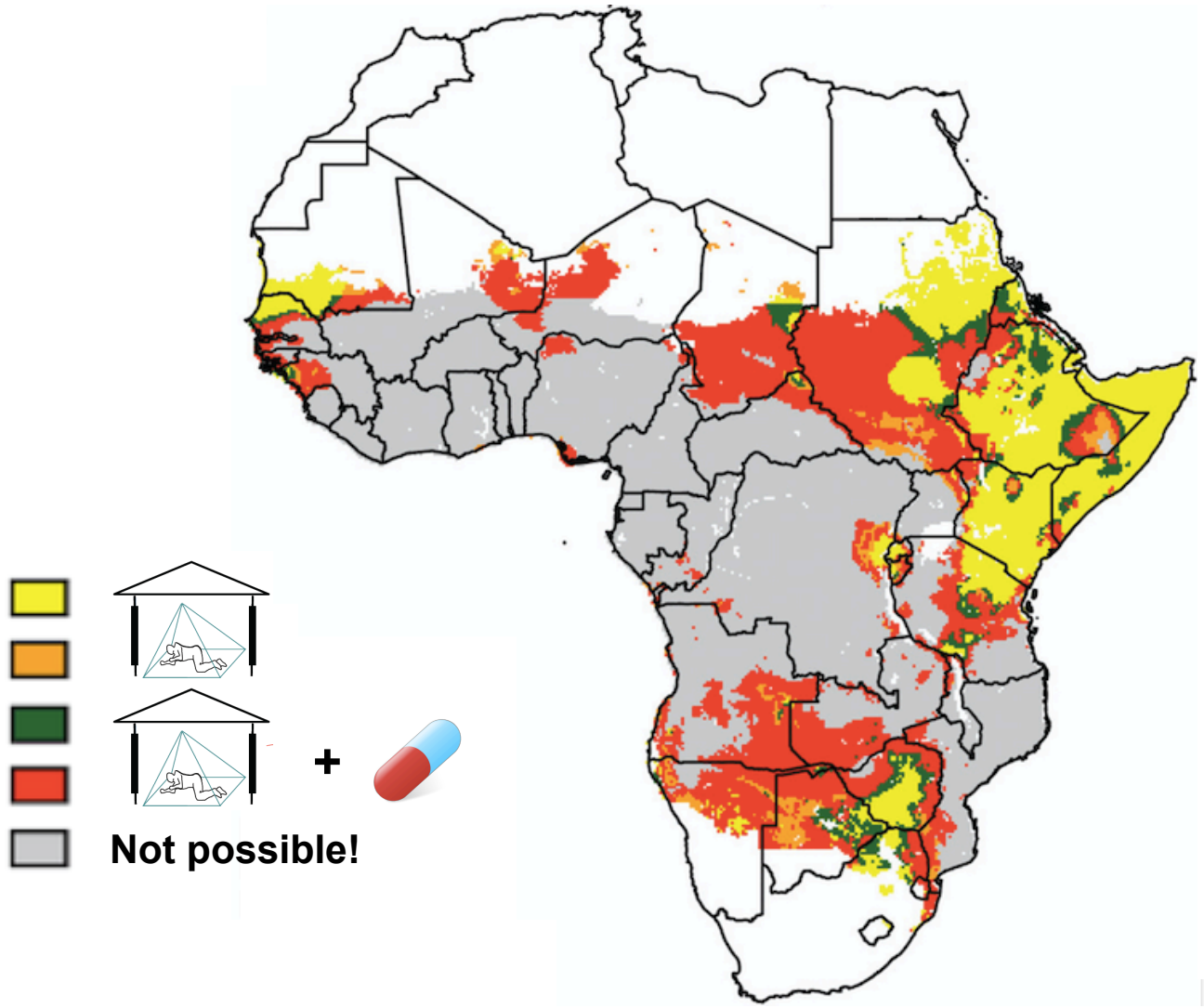
Biyonka Liang

Sarafina Smith

Gillian Chu

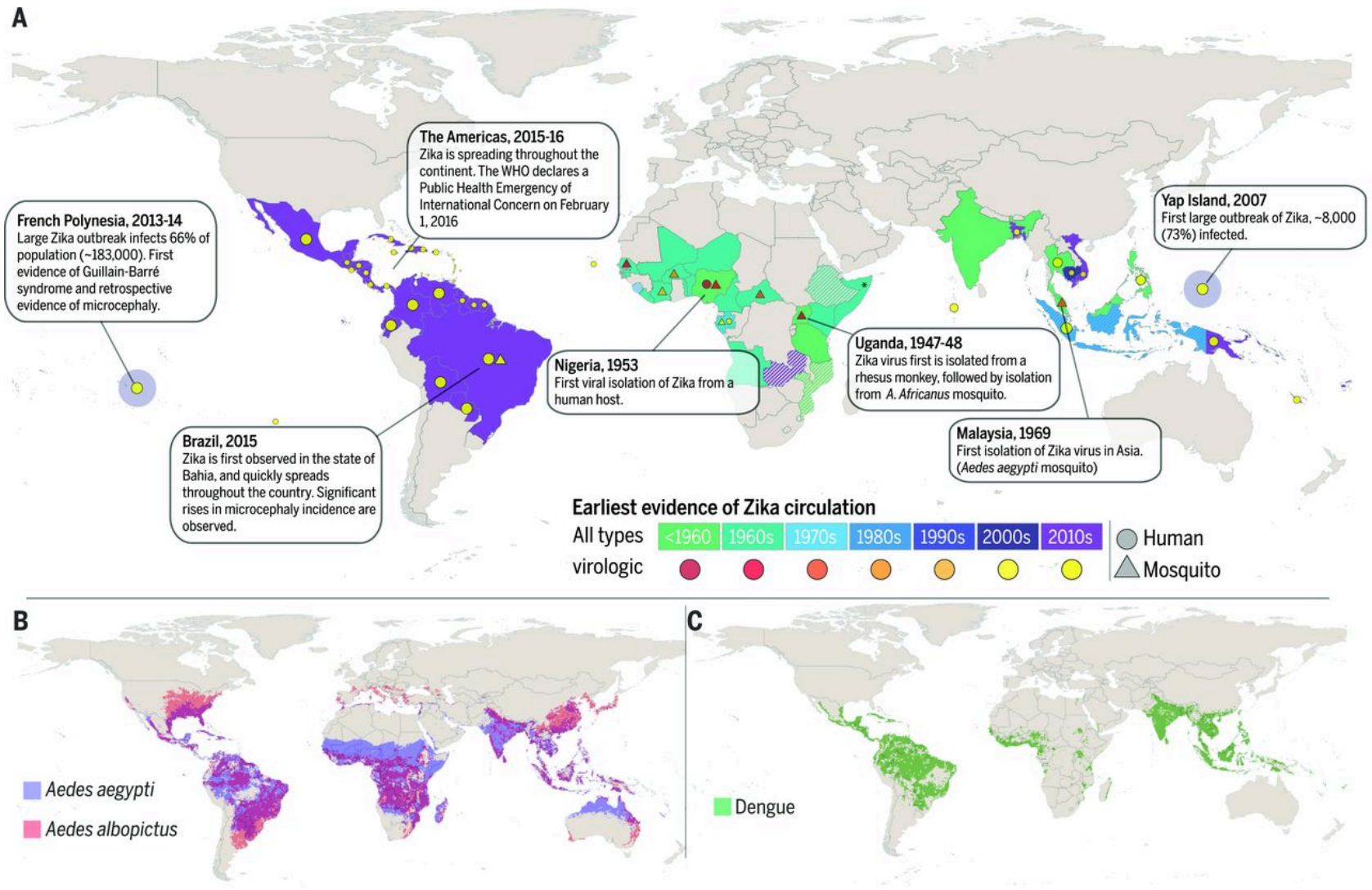
Yi Li

Optimal interventions to eliminate malaria



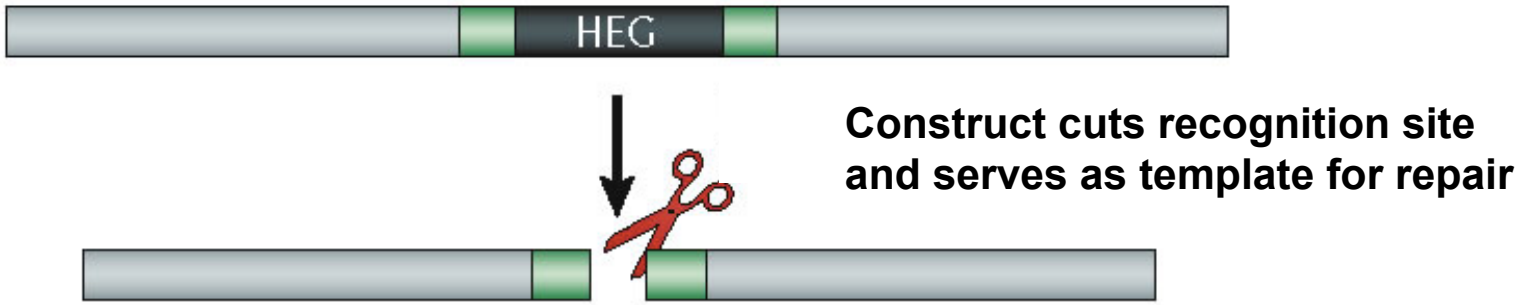
- Walker PGT, Griffin JT, Ferguson NM, Ghani AC (2016) Lancet Global Health

Arboviruses are on the rise worldwide

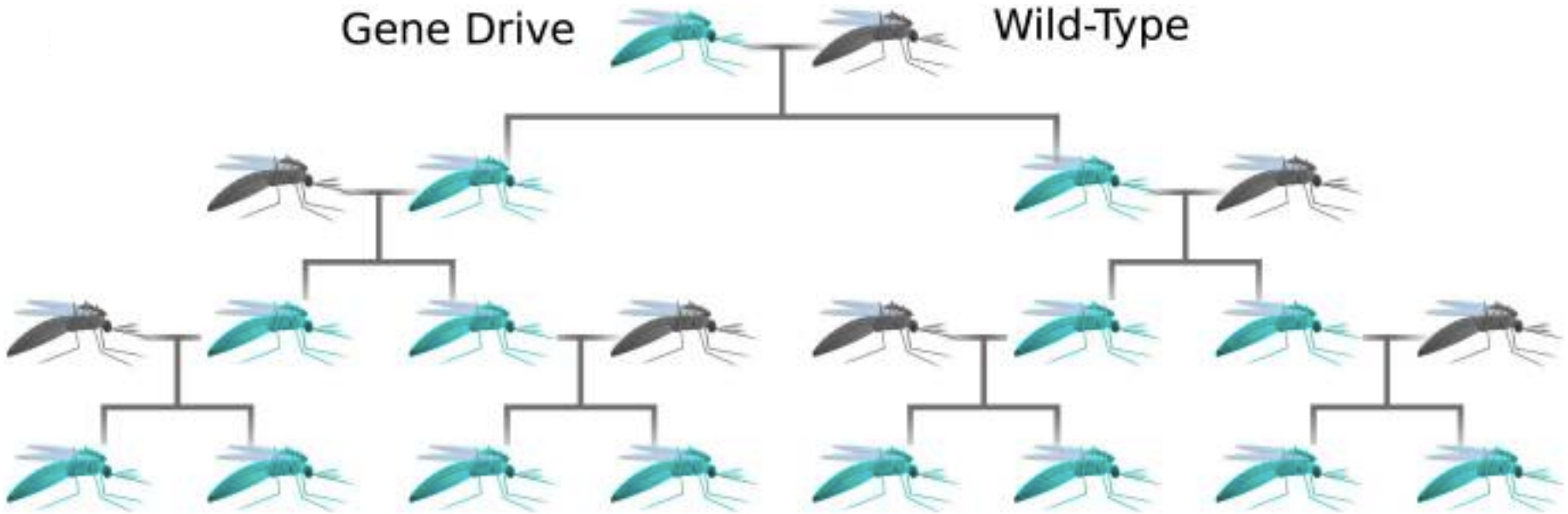


- Lessler J, Chaisson LH, Kucirka LM, Bi Q, Grantz K *et al.* (2016) Science 353: aaf8160

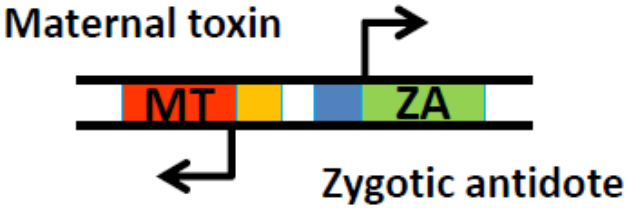
Homing-based CRISPR-mediated gene drive



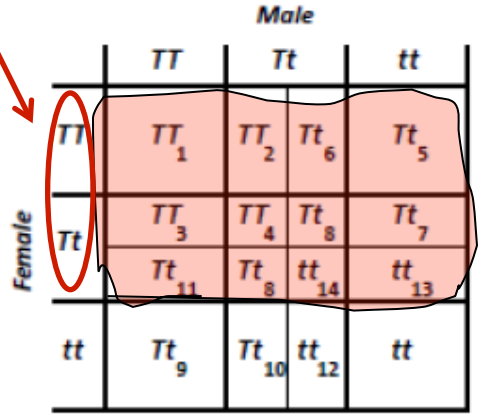
Construct cuts recognition site and serves as template for repair



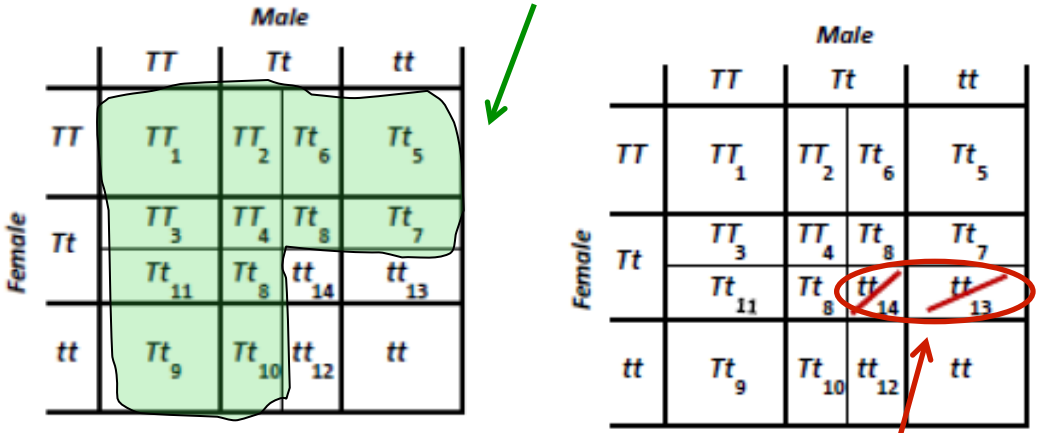
Medea & toxin-antidote based gene drive



Transgenic mothers produce **toxin**



Transgenic offspring produce **antidote**



This causes the **death** of all **offspring** of **heterozygous mothers** that **do not inherit** the ***Medea* allele** (*tt*).

Public attitudes to gene editing for malaria control in Mali

"You have to start somewhere. From this, people will know whether it's good or bad… I would like you to conduct a trial in my village because I would like to be an example for another community."

Elder, Koporo-na, Mali

"I would have to see an example of modified mosquitoes reducing malaria in another village before I believe this claim"

Elder, Tienfala, Mali

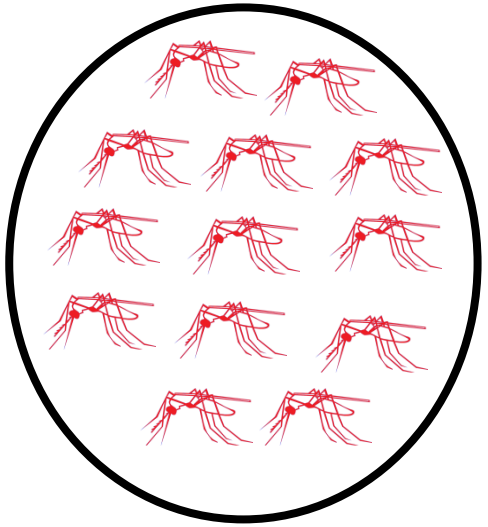
- Marshal JM, Toure MB, Traore MM, Famenini S, Taylor CE (2010) Malaria Journal 9: 128

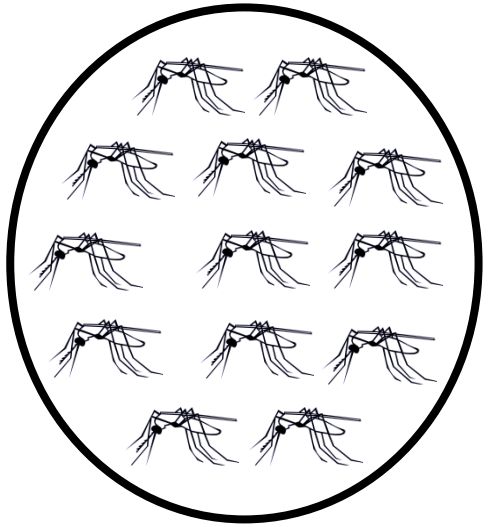
Can we conduct a confined field trial of gene-edited mosquitoes?

7 km

Banambani, Mali

Doneguebougou, Mali

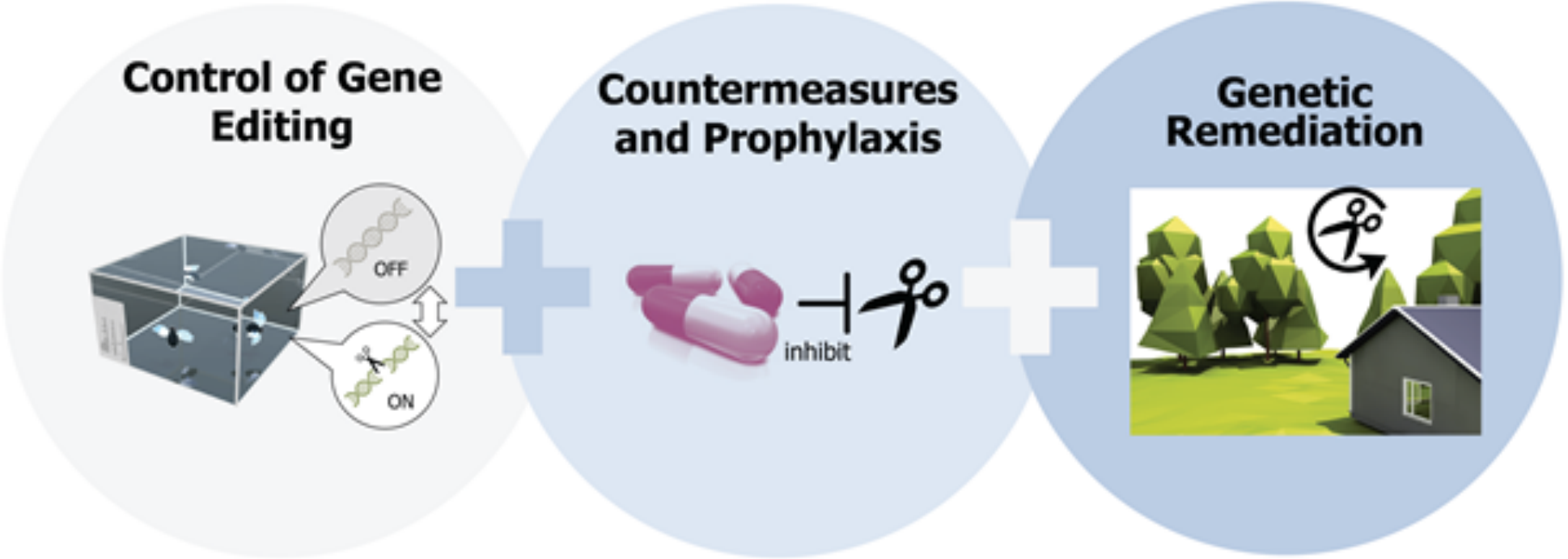




Gene-edited mosquitoes

Wild-type mosquitoes

DARPA Safe Genes Program

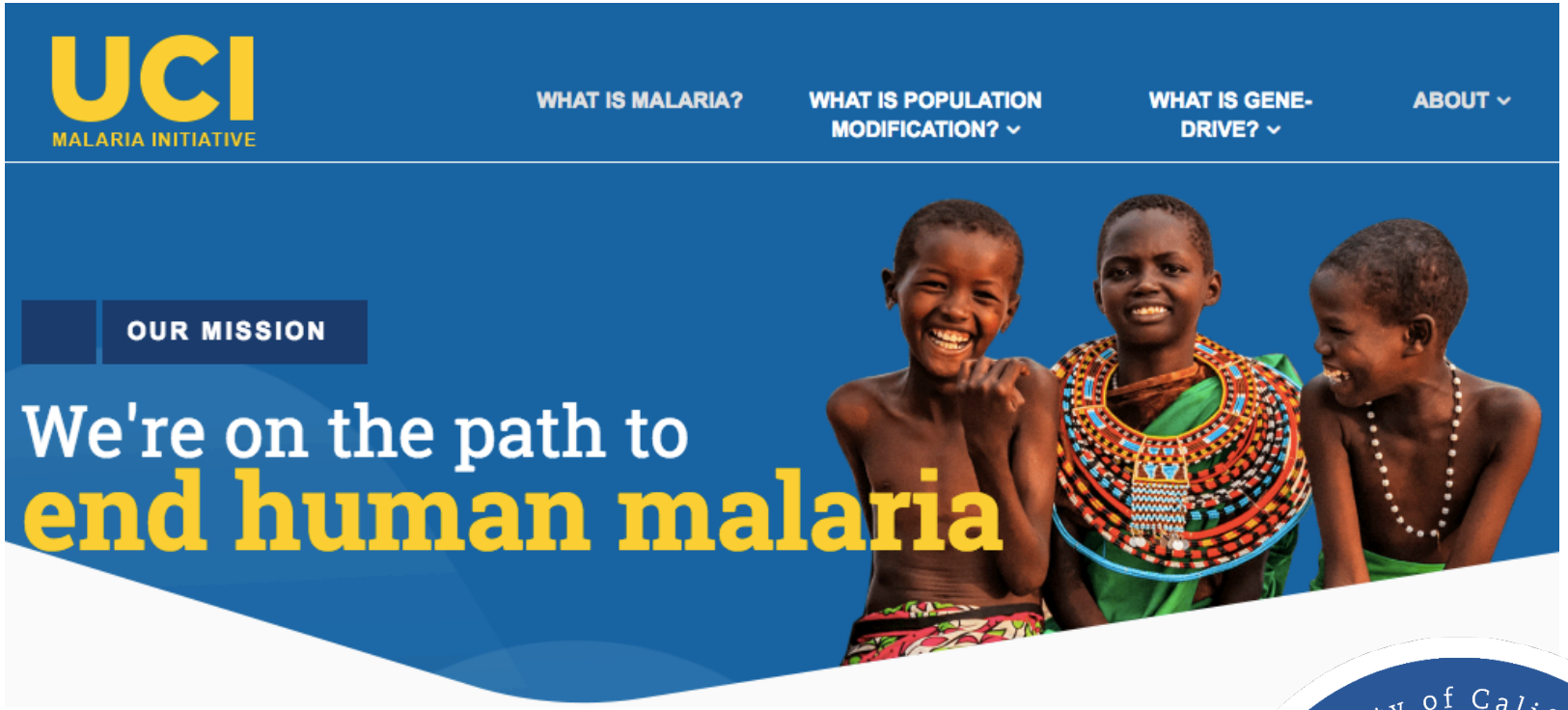


Enable temporal, spatial, and reversible control of gene editors

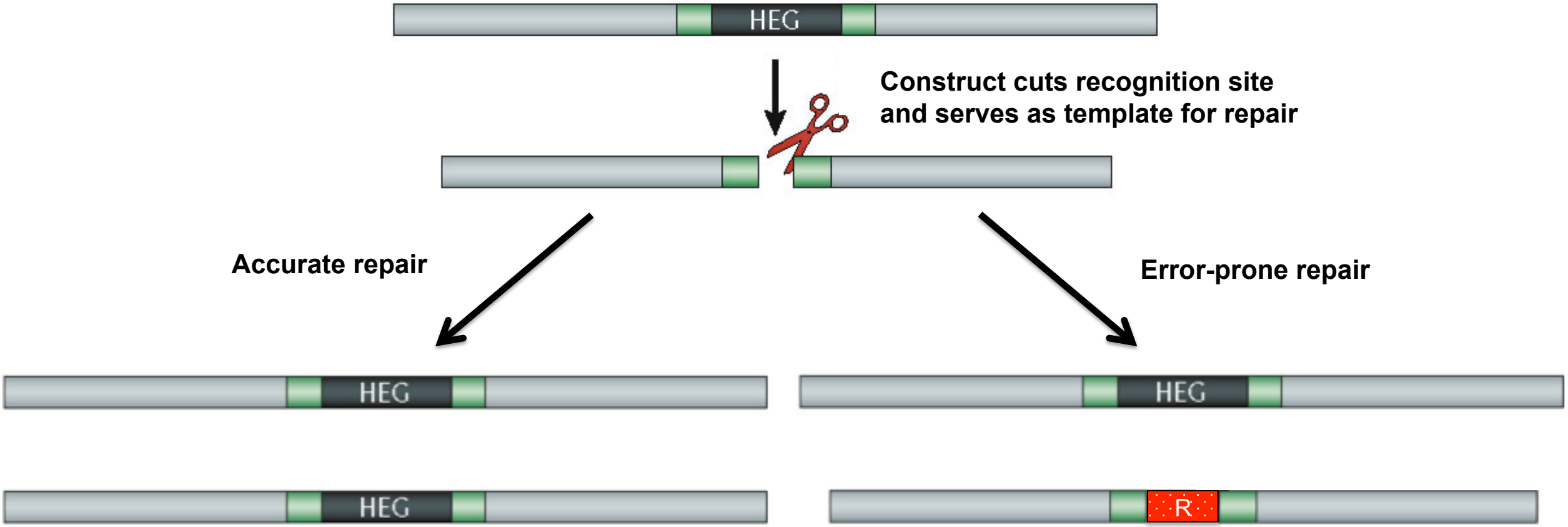
Inhibit unwanted gene editing activity

Remove engineered genes from environments to return to baseline

UC Irvine Malaria Initiative

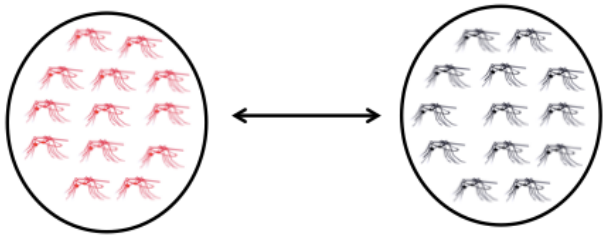


Can CRISPR-based gene drive be effective at controlling disease on a wide scale?

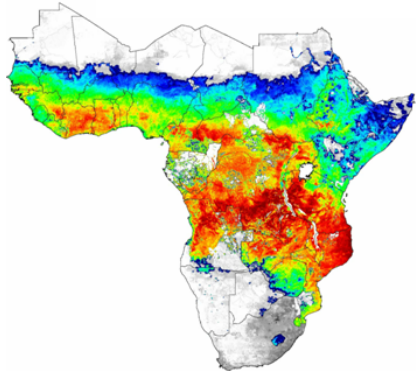


Talk outline

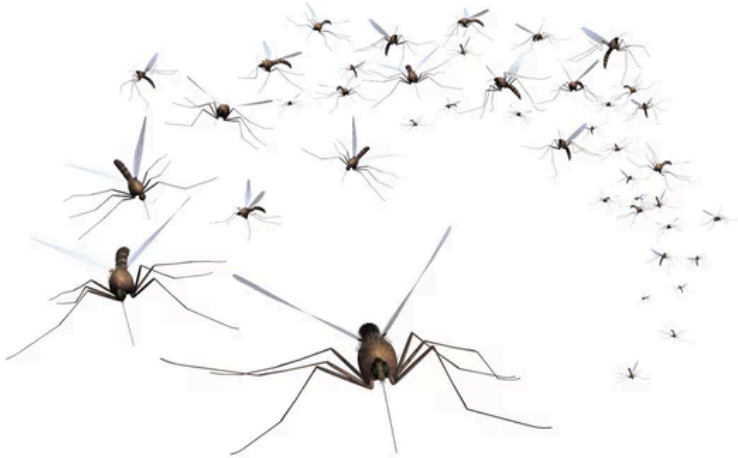
Q1. Can we conduct a confined field trial of gene-edited mosquitoes?



Q2. Can CRISPR-based gene drive be effective at controlling disease on a wide scale?



Q3. What are the best approaches for quantifying mosquito movement patterns of relevance to both questions?

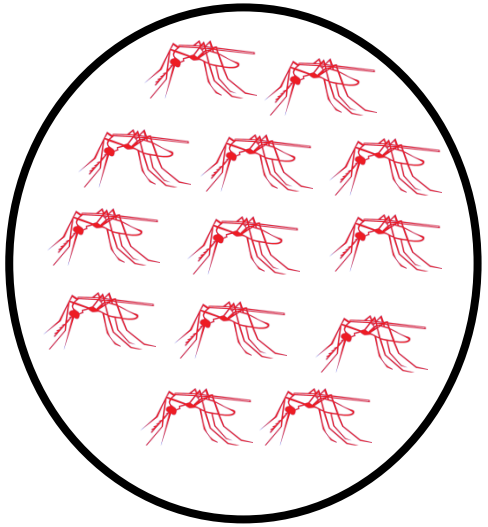


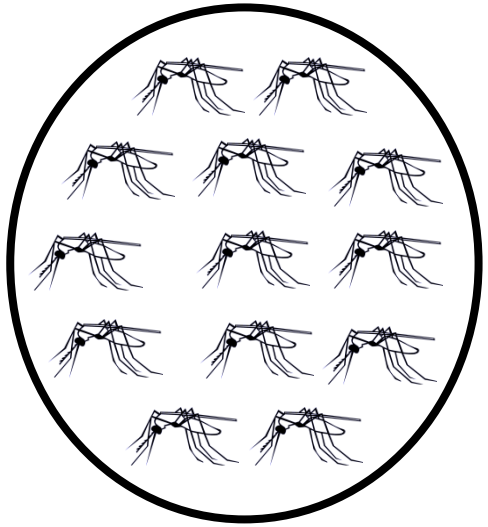
Q1. Can we conduct a confined field trial of gene-edited mosquitoes?

7 km

Banambani, Mali

Doneguebougou, Mali

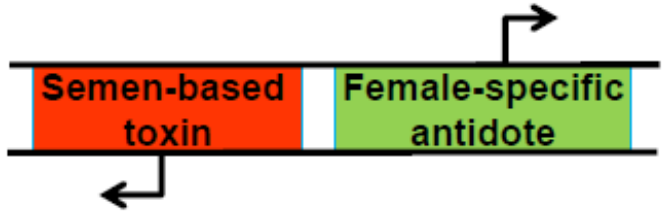




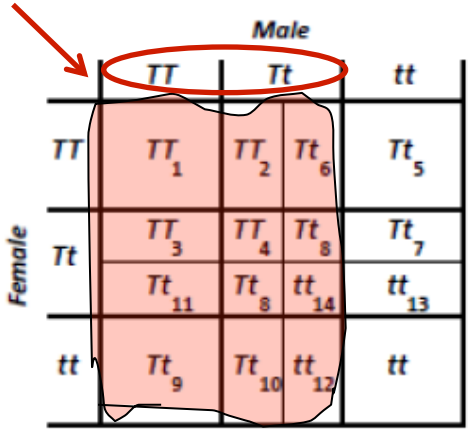
Gene-edited mosquitoes

Wild-type mosquitoes

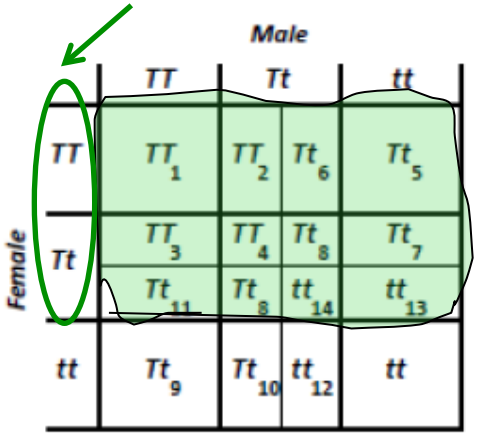
Semele & threshold-dependent gene drive

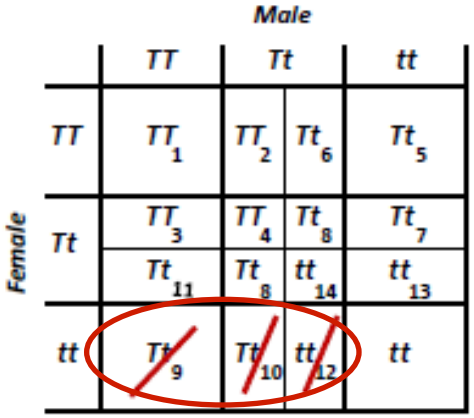


Transgenic males produce **toxic semen**

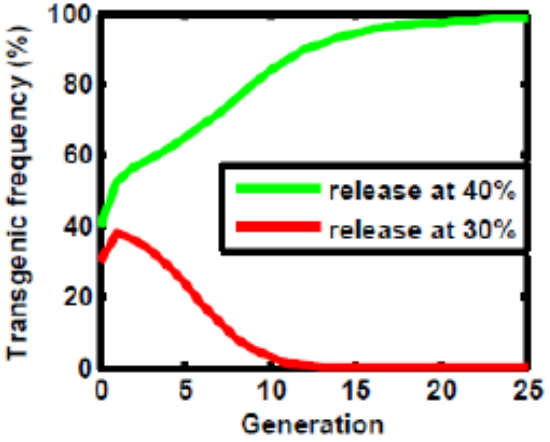


Transgenic females produce **antidote**





This causes crosses between **transgenic males** and **wild females** to produce **no viable offspring**.



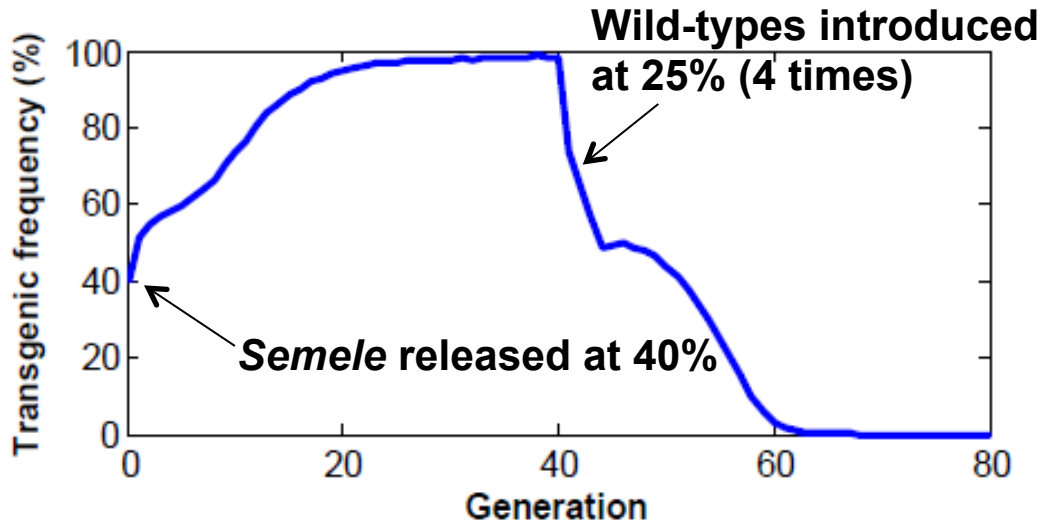
- Marshall JM, Pittman GW, Buchman A, Hay BA (2011) Genetics 187: 535-551.

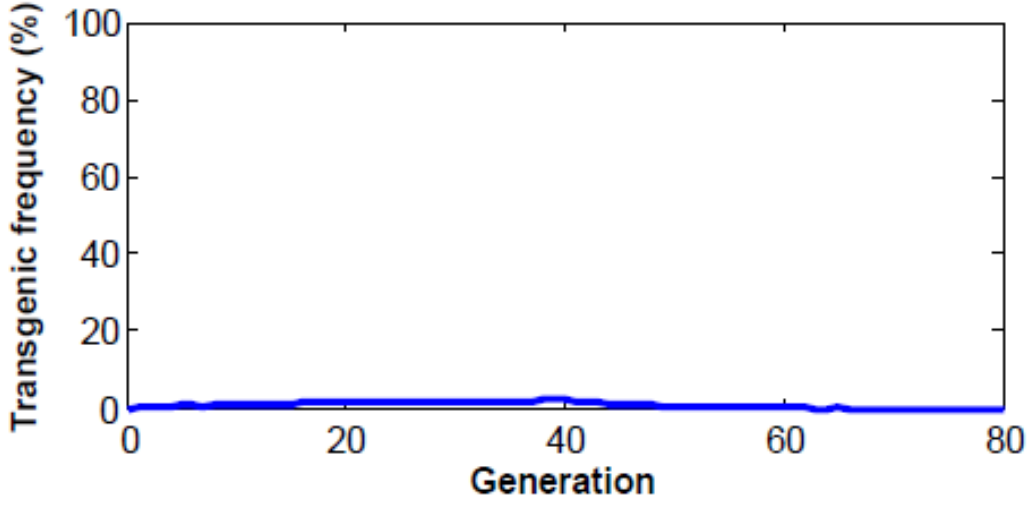
Releases of *Semele* are confineable and reversible

Banambani, Mali

Migration rate = ~1% / generation

Doneguebougou, Mali

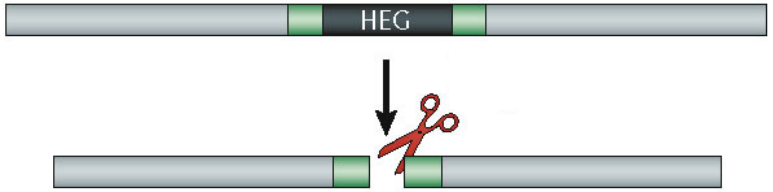




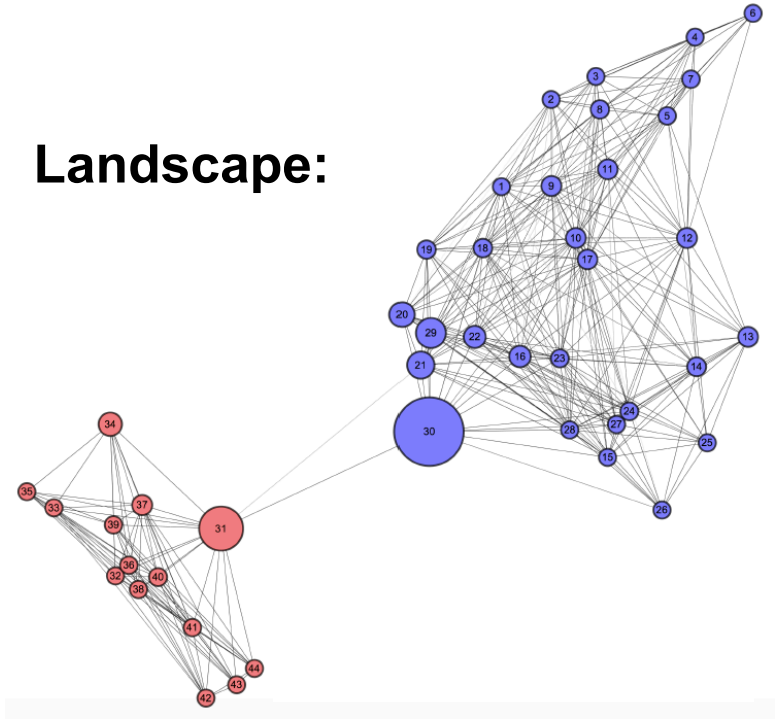
- Marshall JM, Pittman GW, Buchman A, Hay BA (2011) Genetics 187: 535-551.

Integrated spatially-explicit, ecological model

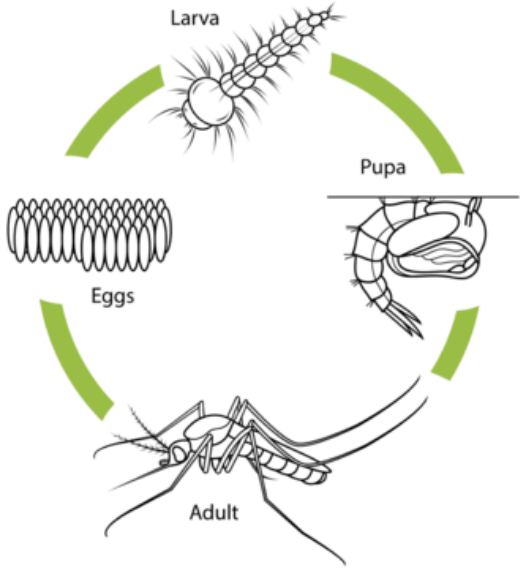
Inheritance pattern:



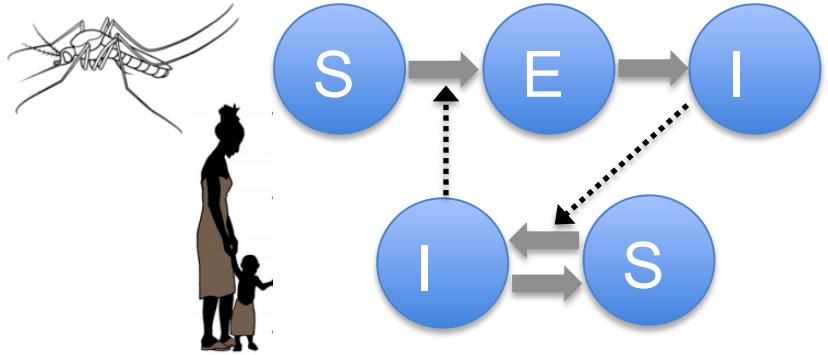
Landscape:



Mosquito life cycle:



Disease epidemiology:



Mosquito Gene Drive Explorer (MGDrivE)

MGDrivE

Mosquitos + Tensors + Genetics + CS + Networks + Math + Coffee

View **Releases List**

Browse **Documentation**

View on **Youtube**

Fork on **GitHub**

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Download **TAR Ball**

Developed in John Marshall's Lab by:

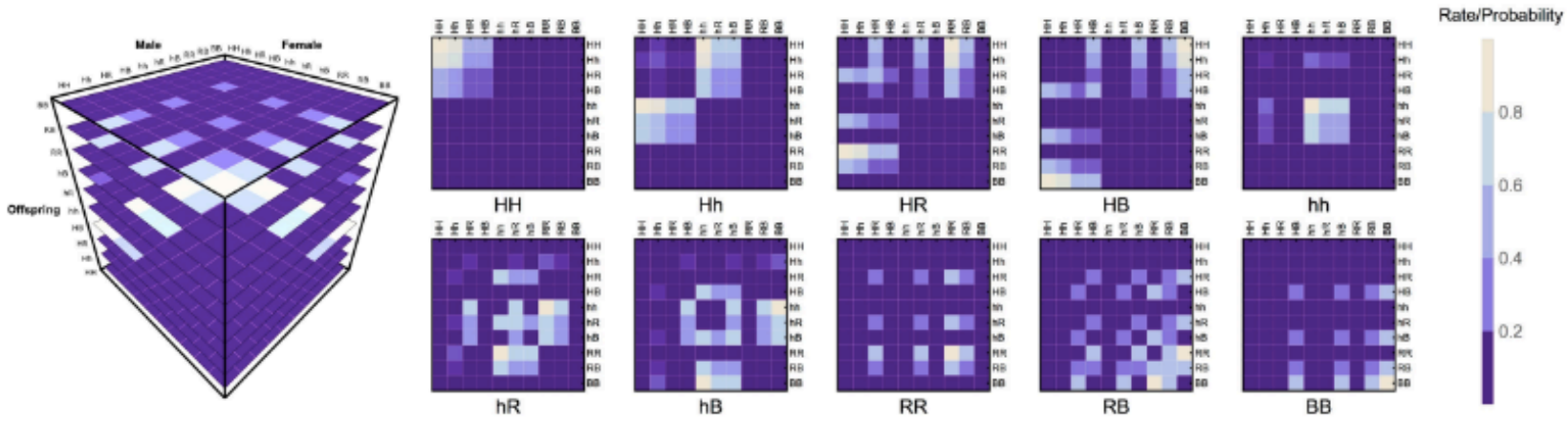
-Lead: Héctor M. Sánchez C.
-Core Dev: Sean L. Wu,Jared Bennett
-Spatial Analysis: Biyonka Liang, Sarafina Smith, Sabrina Wong
-Movement Kernels: Partow Imani

...and, of course, our PI: John M. Marshall!

Mosquito Gene Drive Explorer

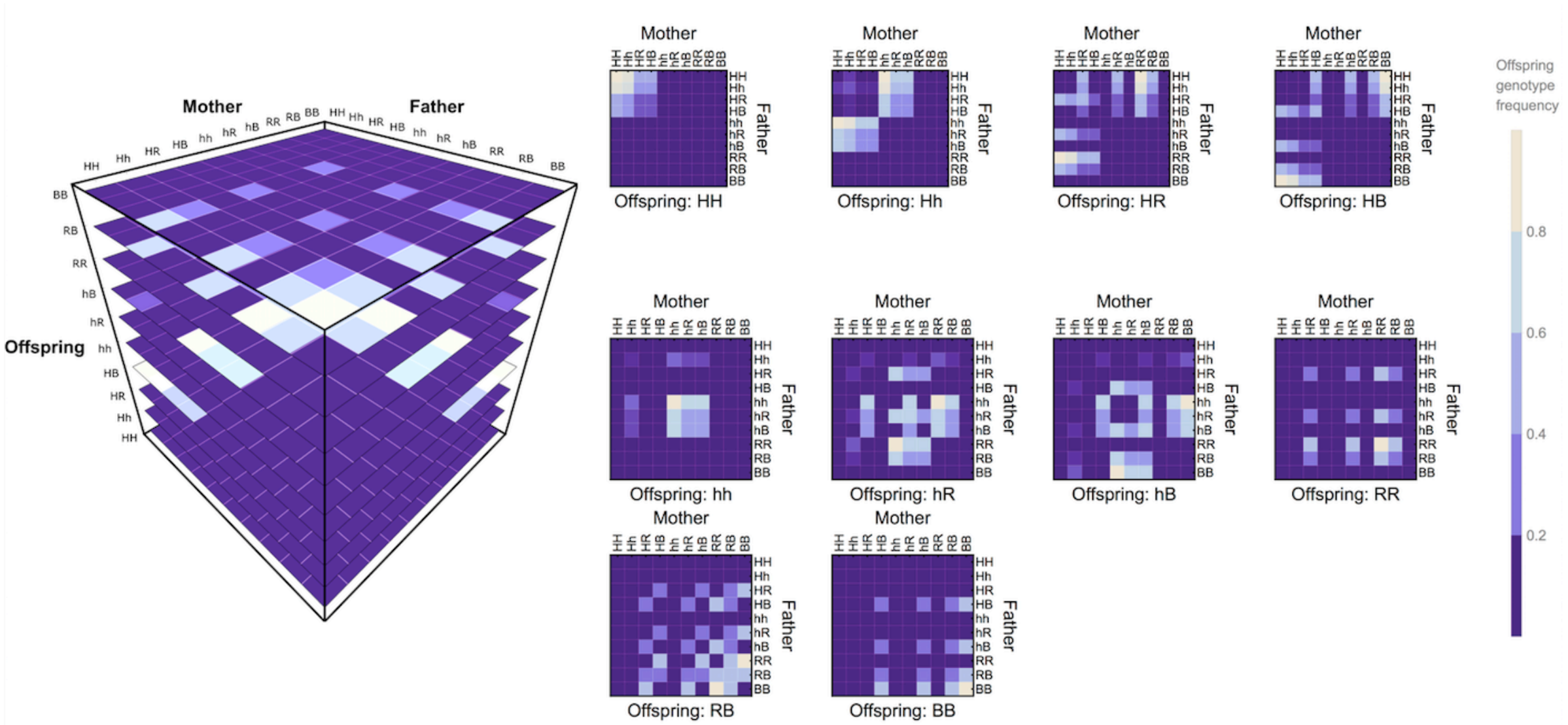
Brief Description

MGDrivE is a framework designed to serve as a testbed in which gene-drive releases for mosquito-borne diseases control can be tested. It is being developed to accommodate various mosquito-specific gene drive systems within a population dynamics model that allows migration of individuals between nodes in a spatial landscape.



- Sánchez HM, Wu SL, Bennett JB, Marshall JM (2018) bioRxiv doi: http://dx.doi.org/10.1101/350488

MGDrivE: Inheritance module



- Sánchez HM, Wu SL, Bennett JB, Marshall JM (2018) bioRxiv doi: http://dx.doi.org/10.1101/350488

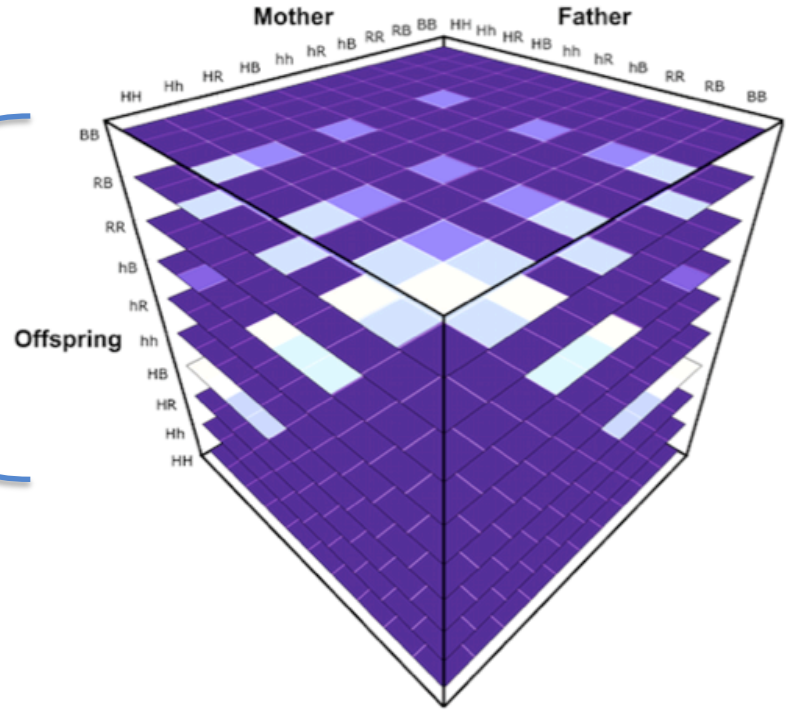
Mathematics of inheritance module

- The number of eggs having genotype *i* that are laid at time *t*, $E_{i,[t]}$, is given by:

$$E_{i,[t]} = \overline{1_{1\times n}} \times \left(\beta \cdot (\overline{s_{n\times 1}} \otimes \overline{1_{n\times 1}}) \circ \overline{\overline{F_{[t]}}} \circ \overline{\overline{Ih(,,i)}} \circ \overline{\overline{\Lambda(,,i)}} \right) \times \overline{1_{n\times 1}}$$

β = number of eggs produced per day
s_i = female genotype-specific multiplier

$$\overline{M} = \begin{pmatrix} M_1 \\ M_2 \\ M_3 \\ \vdots \\ M_g \end{pmatrix} \qquad \overline{\overline{F}} = \begin{pmatrix} F_{11} & F_{12} & F_{13} & \cdots & F_{1g} \\ F_{21} & F_{22} & F_{23} & \cdots & F_{2g} \\ F_{31} & F_{32} & F_{33} & \cdots & F_{3g} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ F_{g1} & F_{g2} & F_{g3} & \cdots & F_{gg} \end{pmatrix}$$

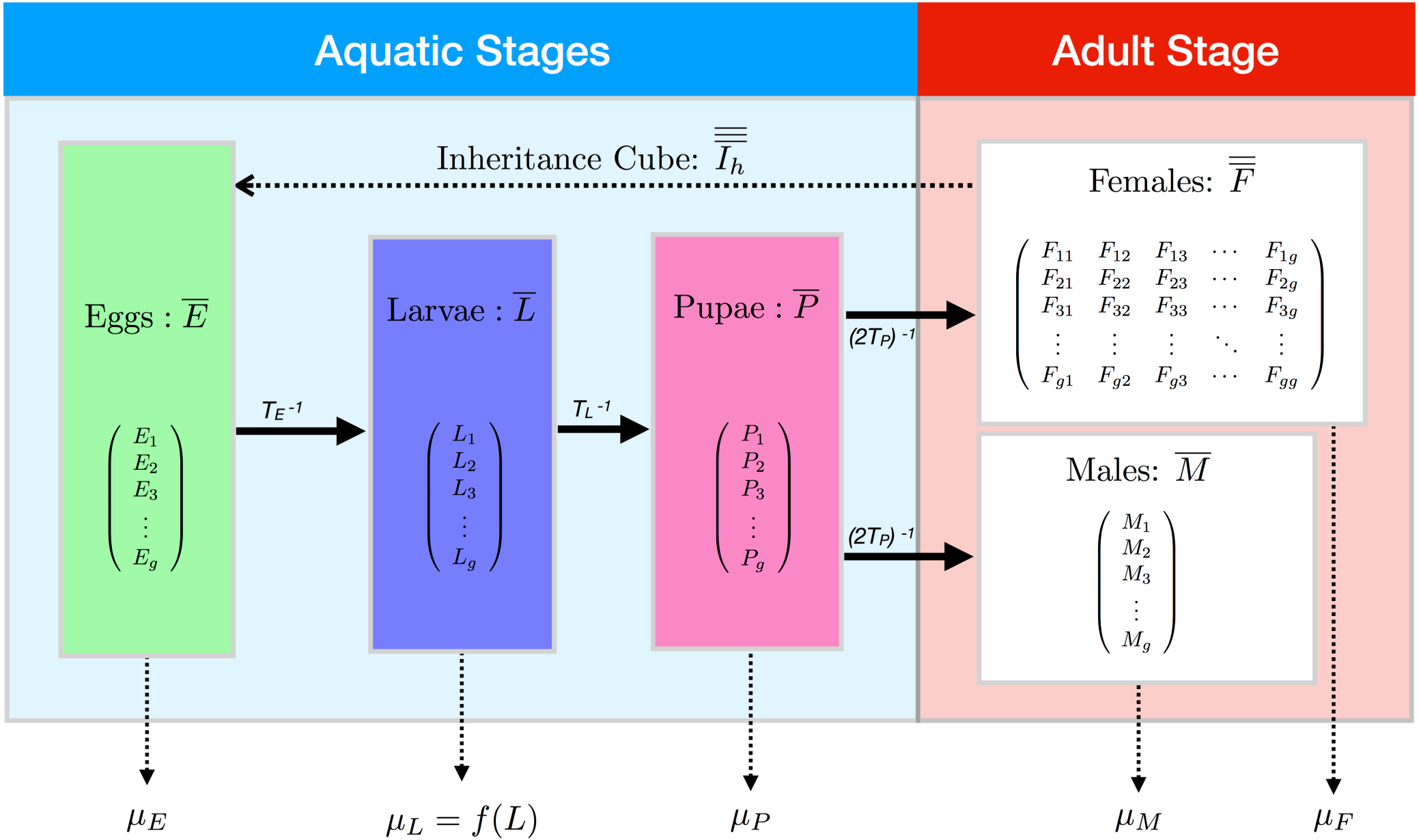


Number of adult males having each genotype, M_i

Number of adult females having each mated genotype, F_{ij}, *i* = own genotype, *j* = genotype of father

For each pairing, the vertical columns represent the proportion of offspring having each genotype

MGDrivE: Ecology module



Mosquito vector species of interest

Aedes aegypti

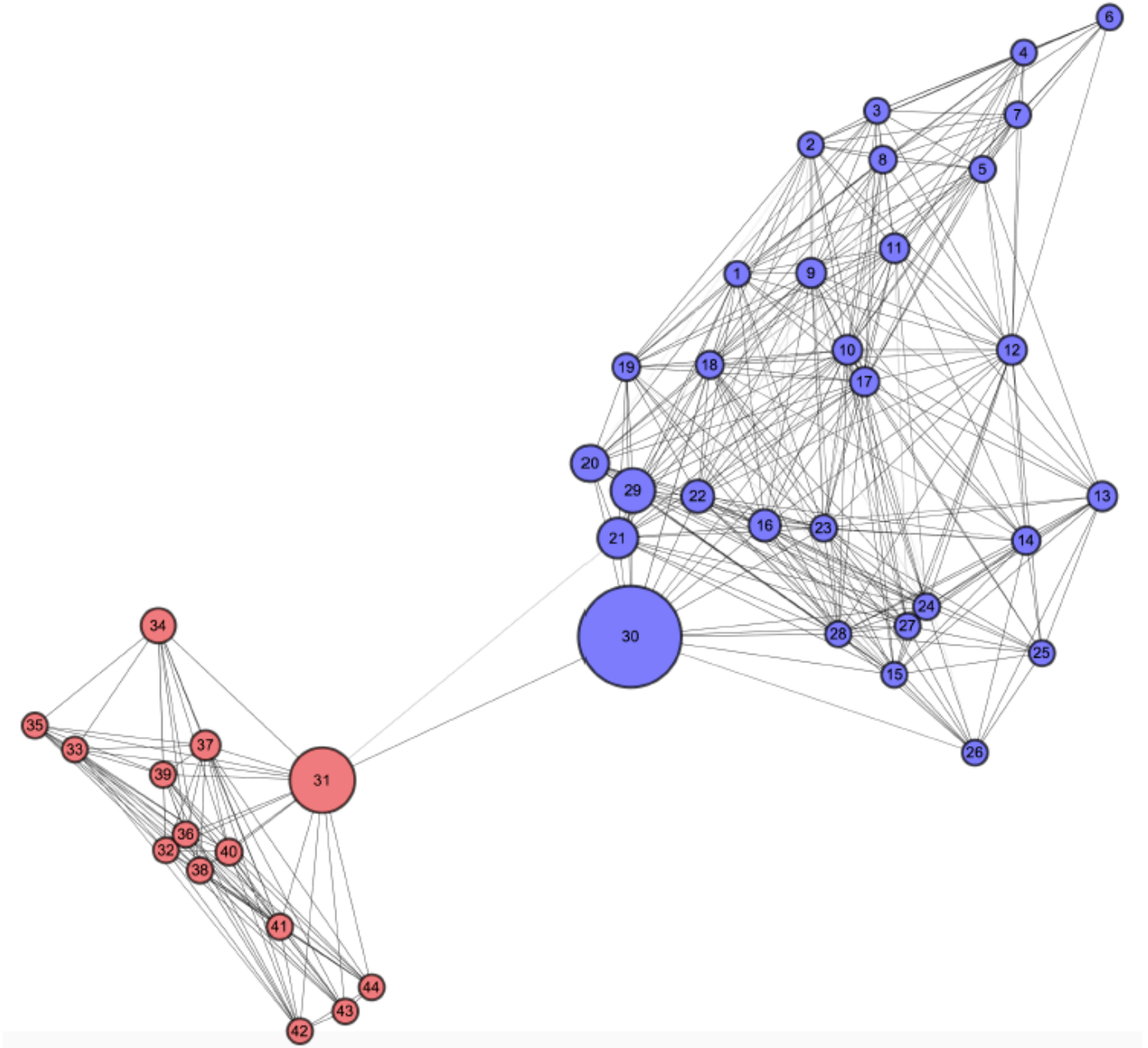
Anopheles gambiae

Anopheles stephensi

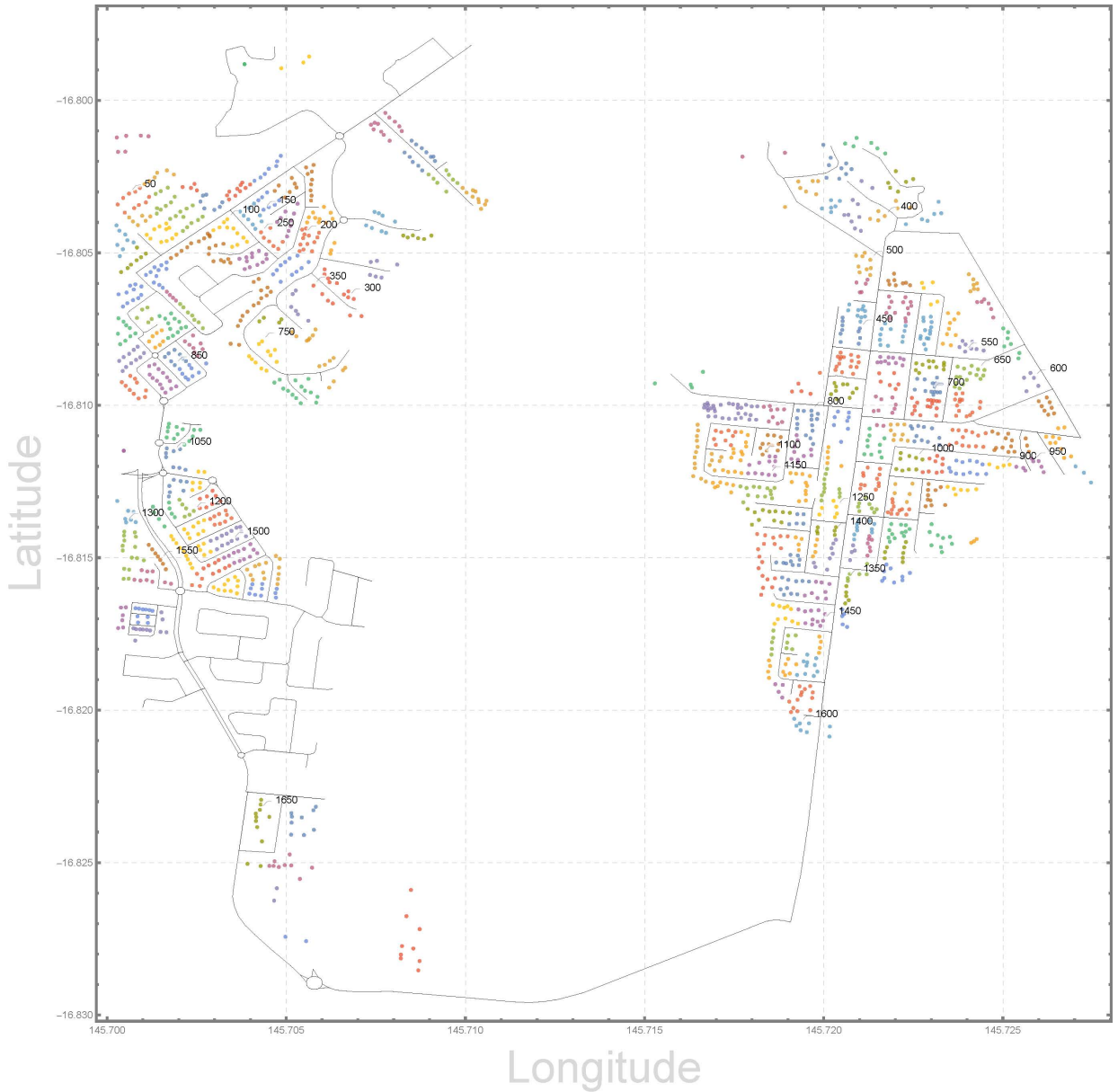
Parameter	Symbol	*Ae. aegypti*	*An. gambiae*	*C. capitata*
Egg production per female (day^{-1})	β	20 [36]	32 [37]	20 [38]
Duration of egg stage (days)	T_E	5 [39]	1 [37]	2 [38]
Duration of larval stage (days)	T_L	6 [39]	13 [37]	6 [38]
Duration of pupa stage (days)	T_P	4 [39]	1 [37]	10 [38]
Daily population growth rate (day^{-1})	r_M	1.175 [40]	1.096 [41]	1.031 [42]
Daily mortality risk of adult stage (day^{-1})	μ_M, μ_F	0.090 [43–45]	0.123 [41]	0.100 [46]

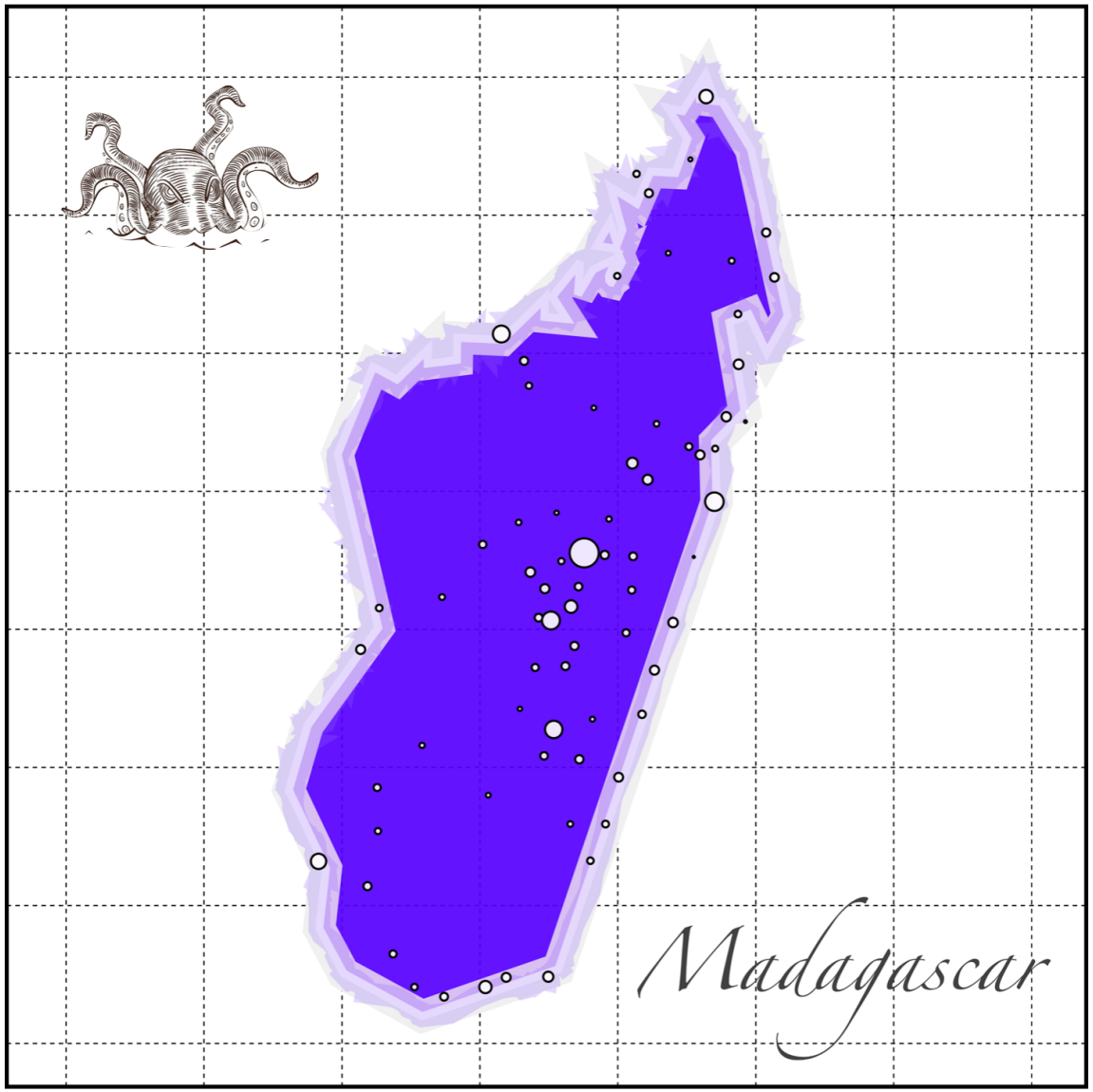
Table 2. Life history module parameter values for three species of interest (at a temperature of 25 Celsius).

MGDrivE: Landscape module

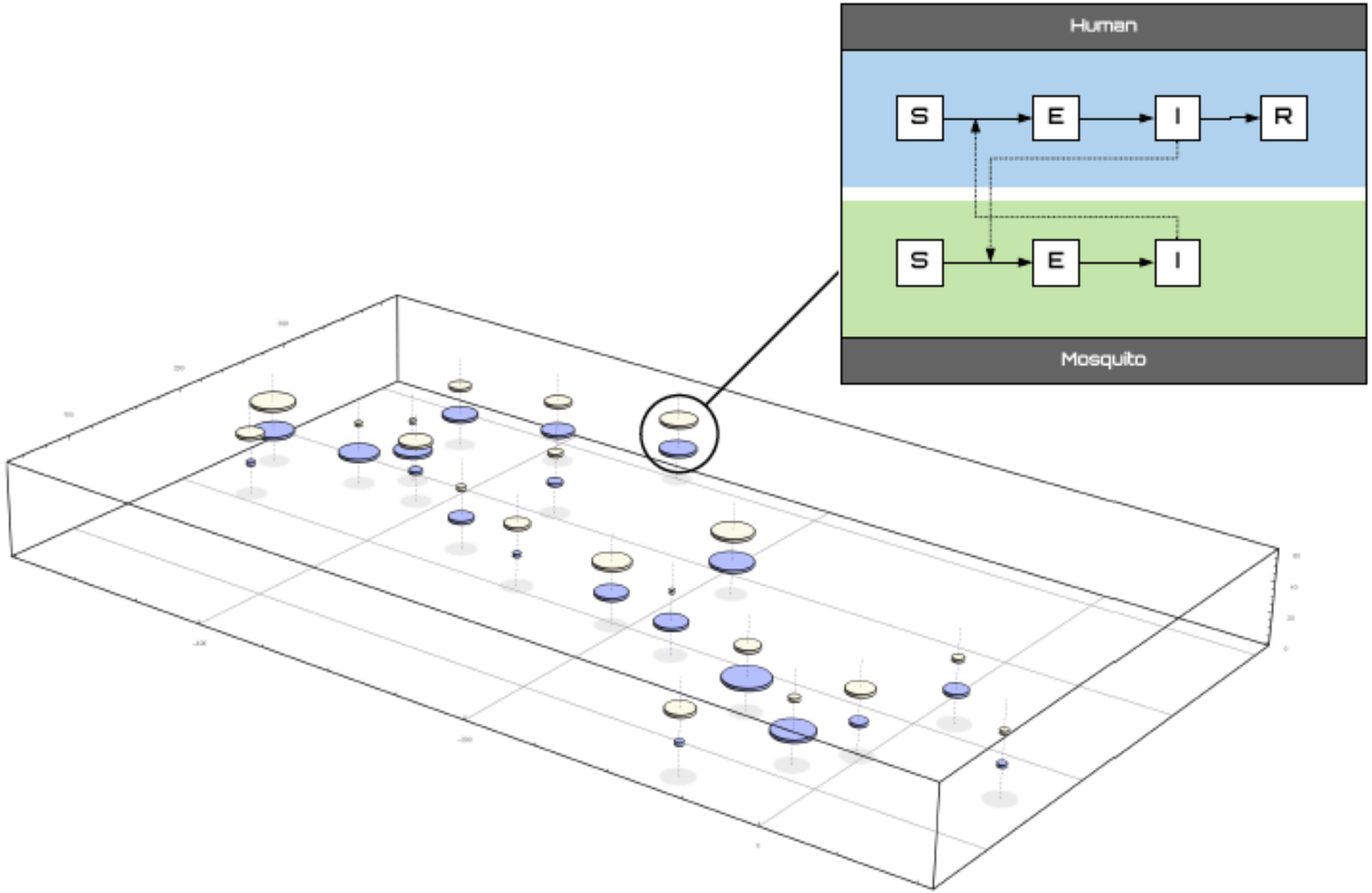


Landscapes of interest for mosquito vectors

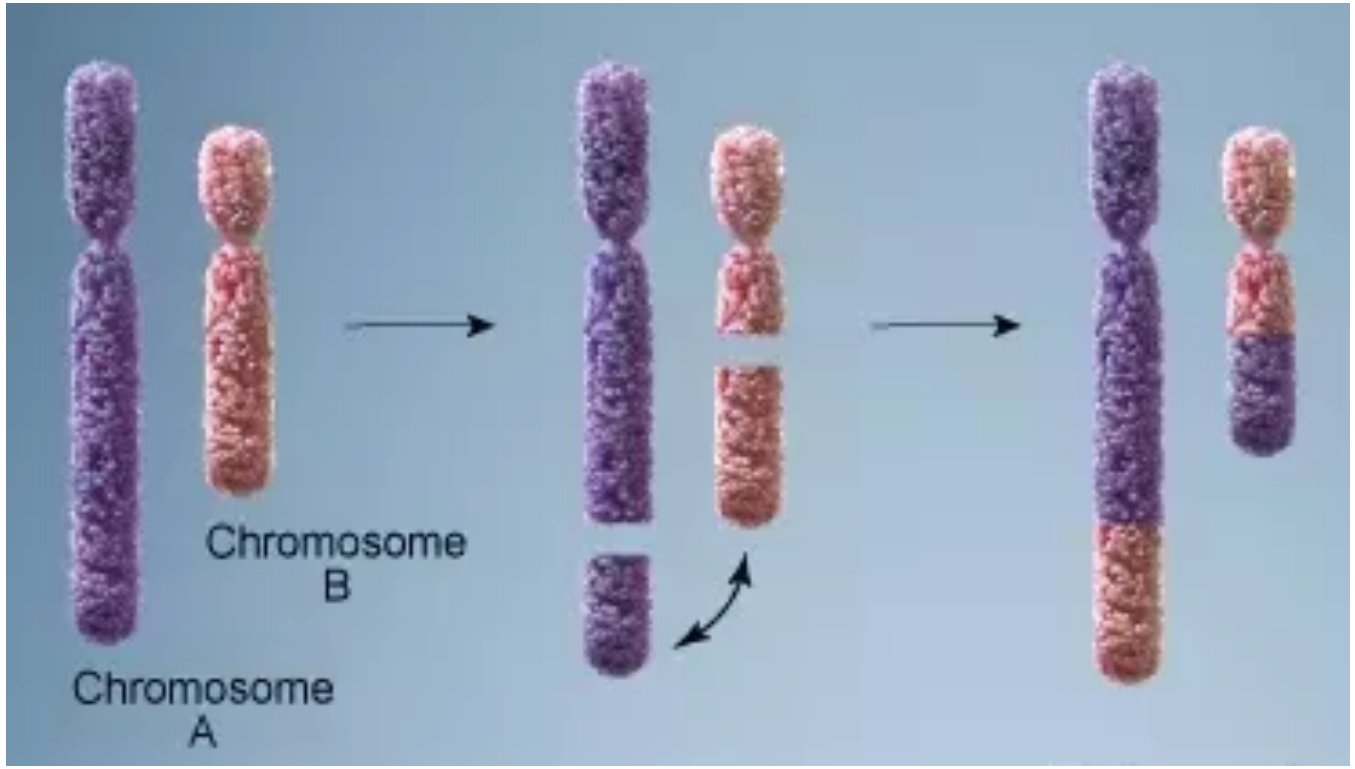


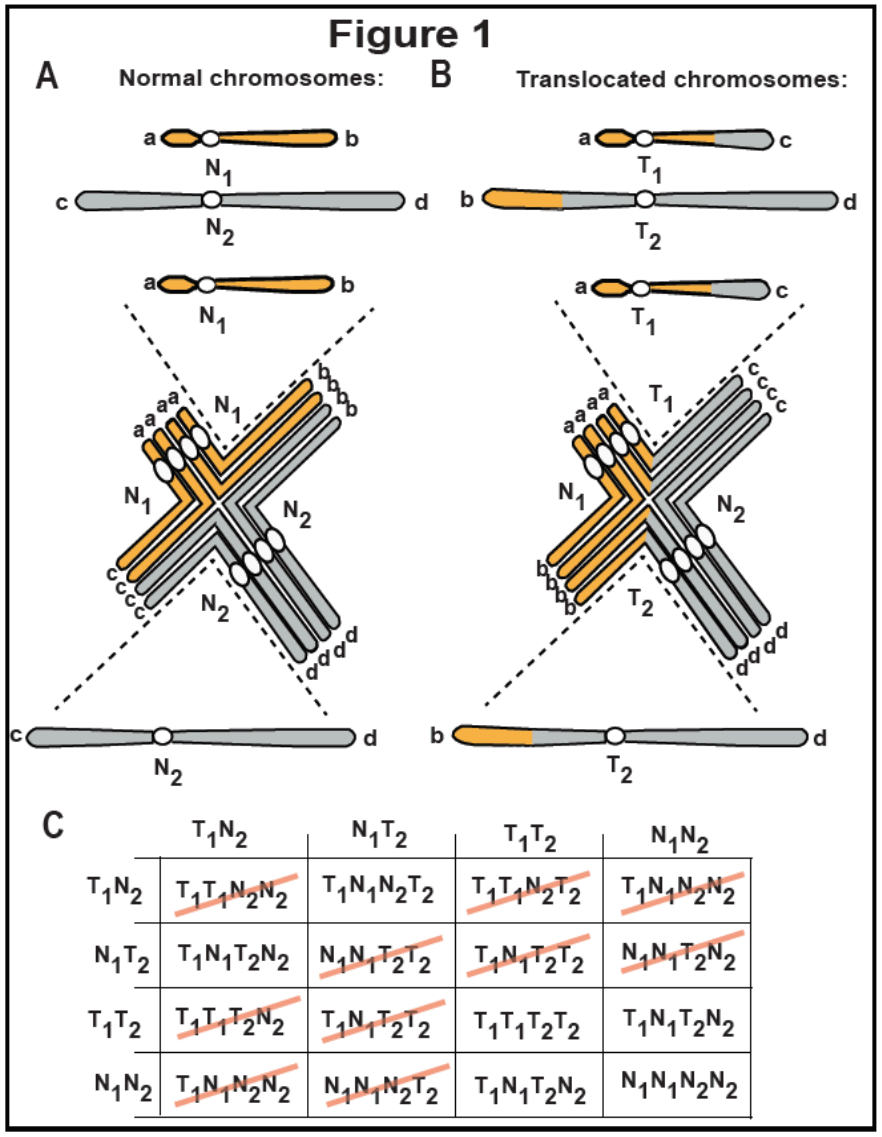


Epidemiological extension for mosquito-borne diseases



Application: Chromosomal translocations

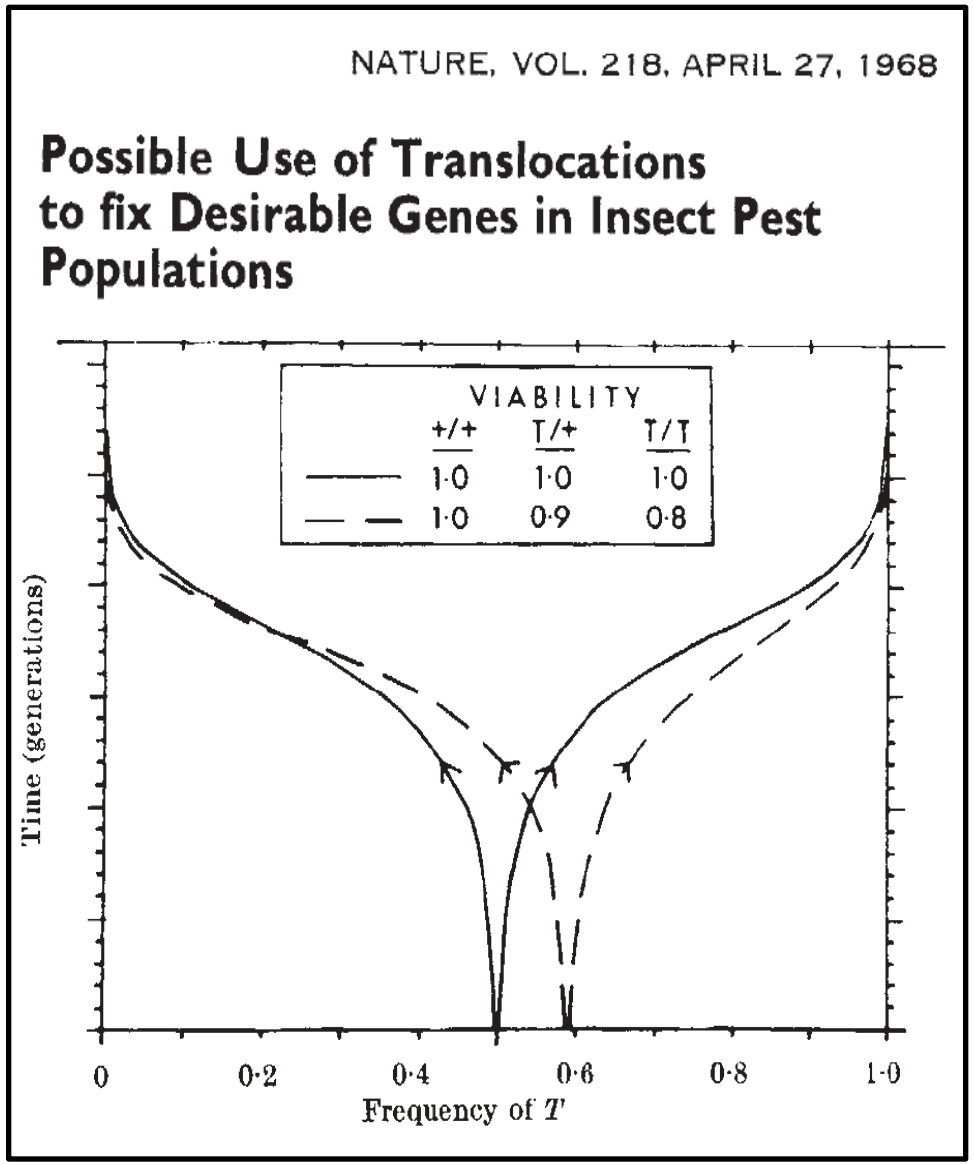




	T_1N_2	N_1T_2	T_1T_2	N_1N_2
T_1N_2	~~$T_1T_1N_2N_2$~~	$T_1N_1N_2T_2$	~~$T_1T_1N_2T_2$~~	~~$T_1N_1N_2N_2$~~
N_1T_2	$T_1N_1T_2N_2$	~~$N_1N_1T_2T_2$~~	~~$T_1N_1T_2T_2$~~	~~$N_1N_1T_2N_2$~~
T_1T_2	~~$T_1T_1T_2N_2$~~	~~$T_1N_1T_2T_2$~~	$T_1T_1T_2T_2$	$T_1N_1T_2N_2$
N_1N_2	~~$T_1N_1N_2N_2$~~	~~$N_1N_1N_2T_2$~~	$T_1N_1T_2N_2$	$N_1N_1N_2N_2$

- Buchman A, Ivy T, Marshall JM, Akbari OS, Hay BA (2018) ACS Synthetic Biology

Application: Chromosomal translocations



NATURE, VOL. 218, APRIL 27, 1968

Possible Use of Translocations to fix Desirable Genes in Insect Pest Populations

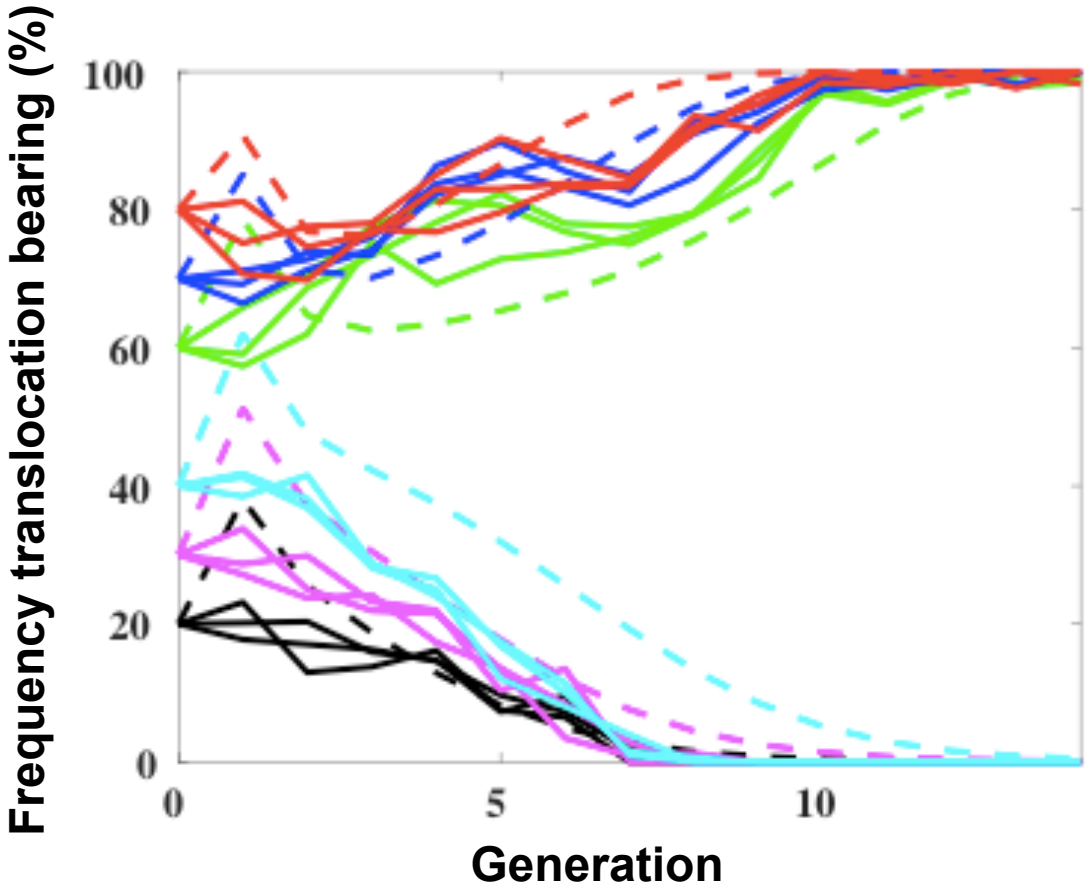
VIABILITY

	+/+	T/+	T/T
———	1·0	1·0	1·0
— —	1·0	0·9	0·8

Time (generations)

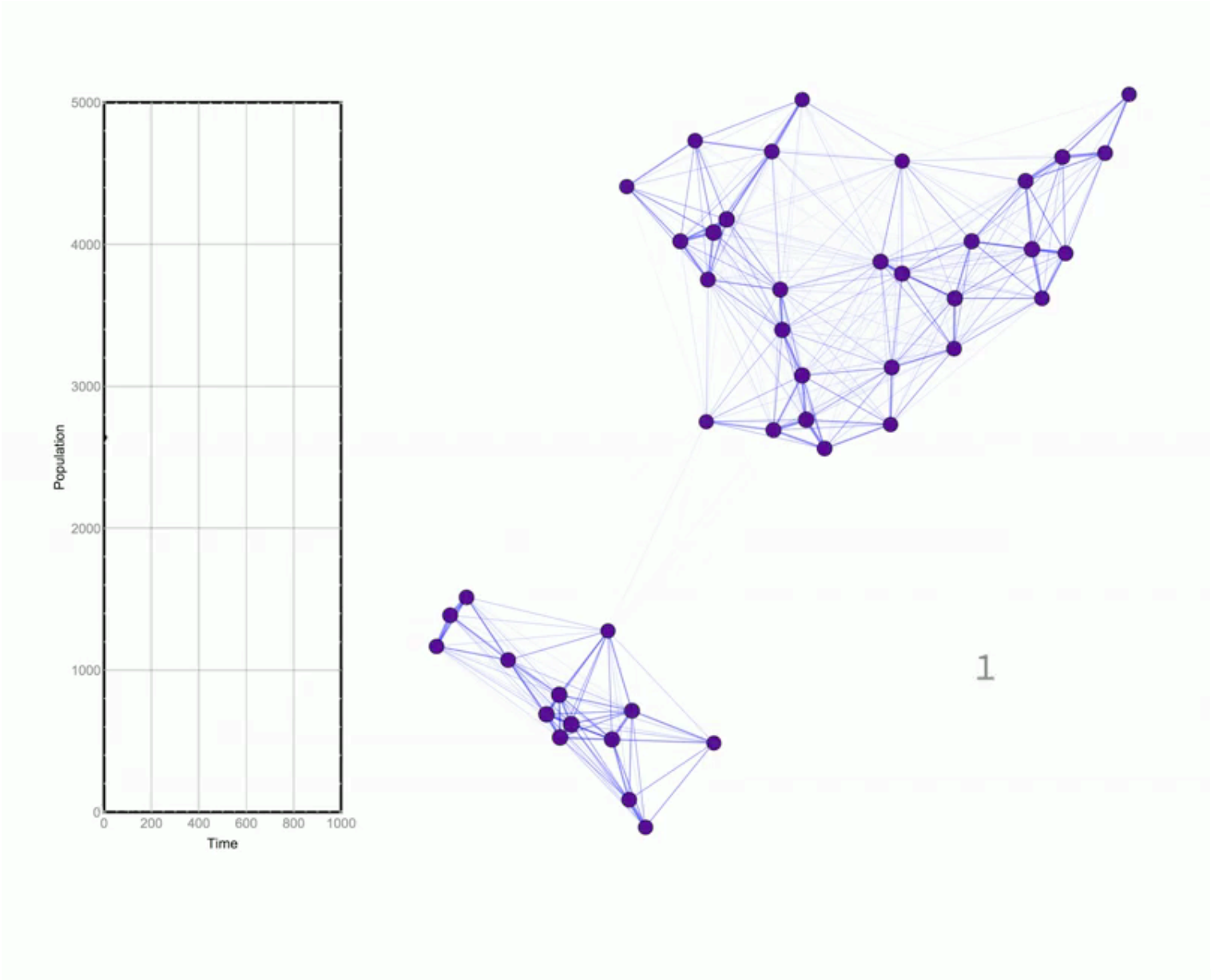
0 0·2 0·4 0·6 0·8 1·0

Frequency of *T*

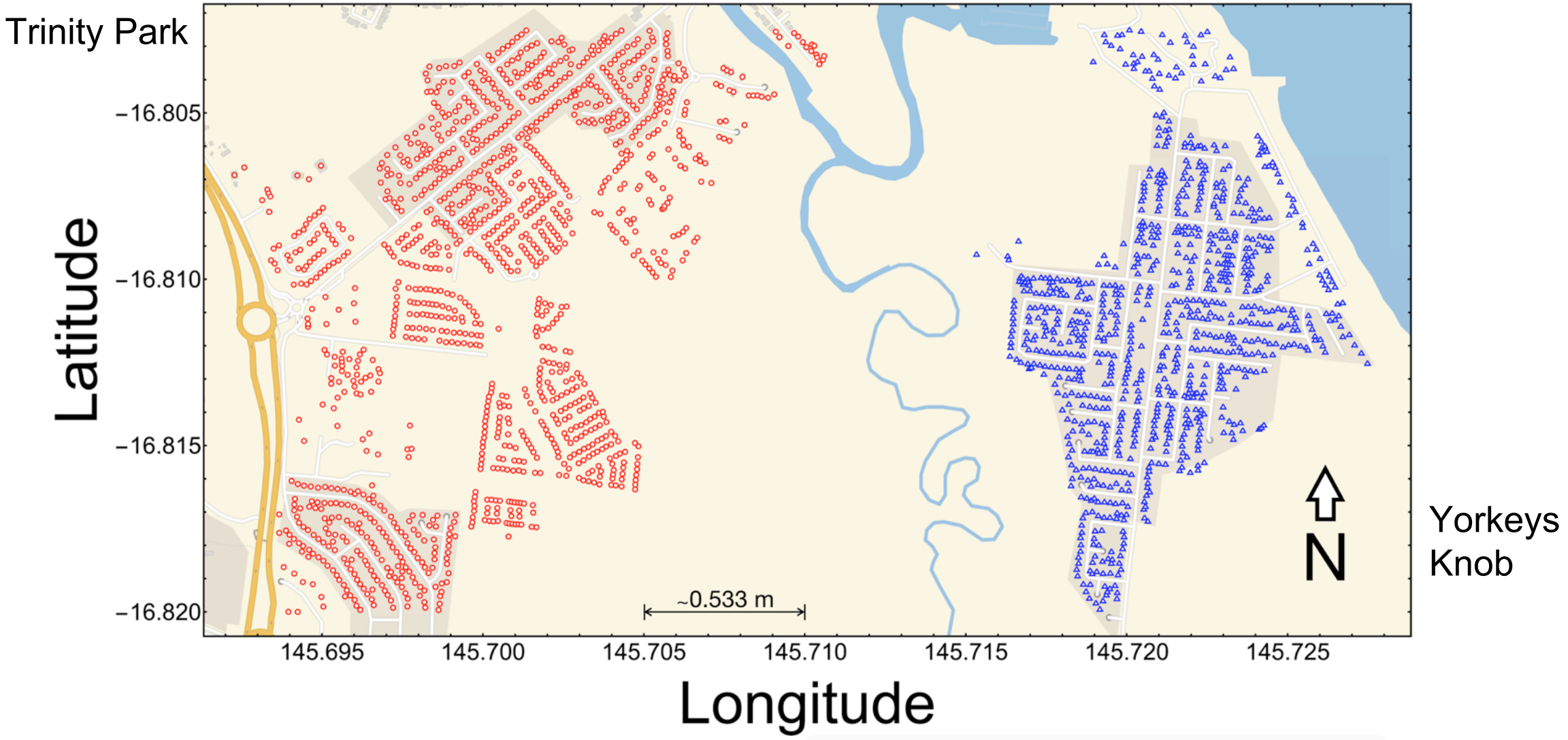


- Curtis CF (1968) Nature 218: 368-369
- Buchman A, Ivy T, Marshall JM, Akbari OS, Hay BA (2018) ACS Synthetic Biology

Application: Translocations with remediation



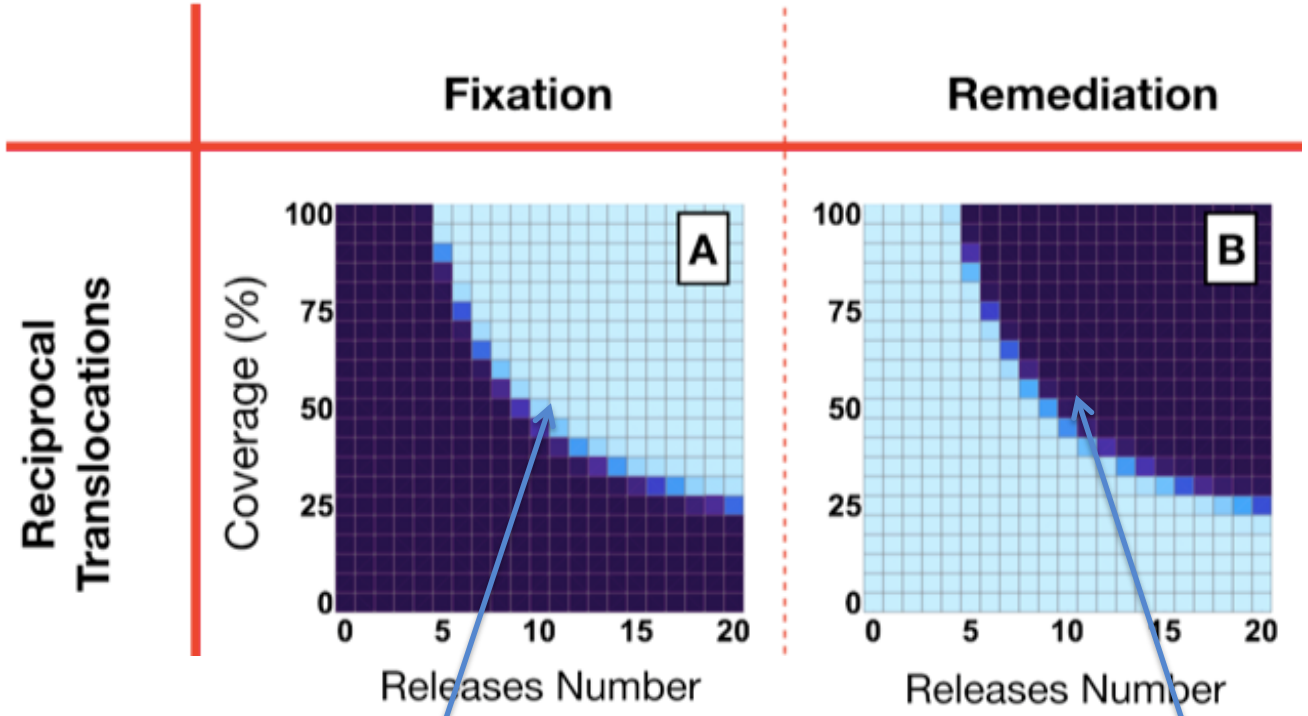
Landscapes of interest for *Aedes aegypti*

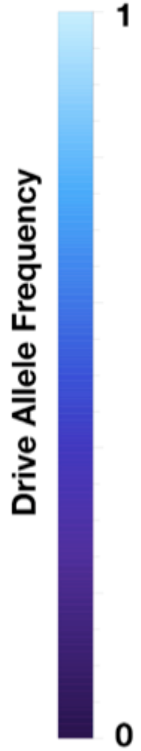


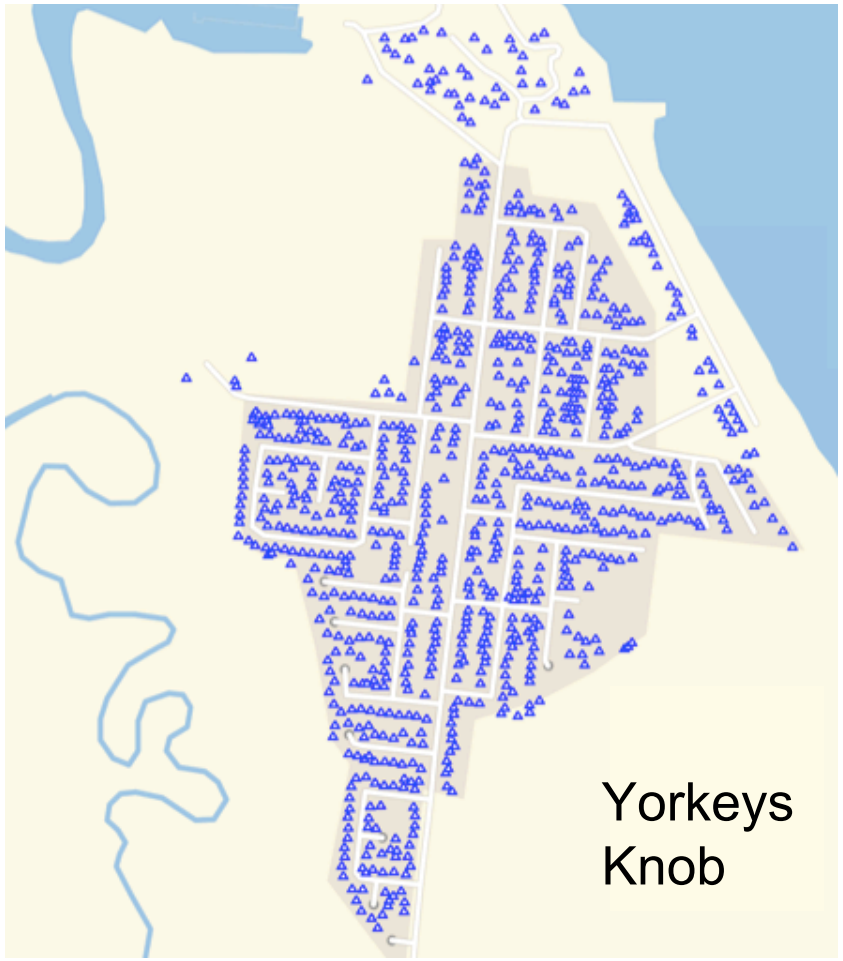
YORKEYS KNOB

Application: Replacement & remediation of translocations

- Data suggests there are ~15 adult *Ae. aegypti* per household
- Weekly releases of 20 adult males having the translocation
- Vary household coverage & number of releases







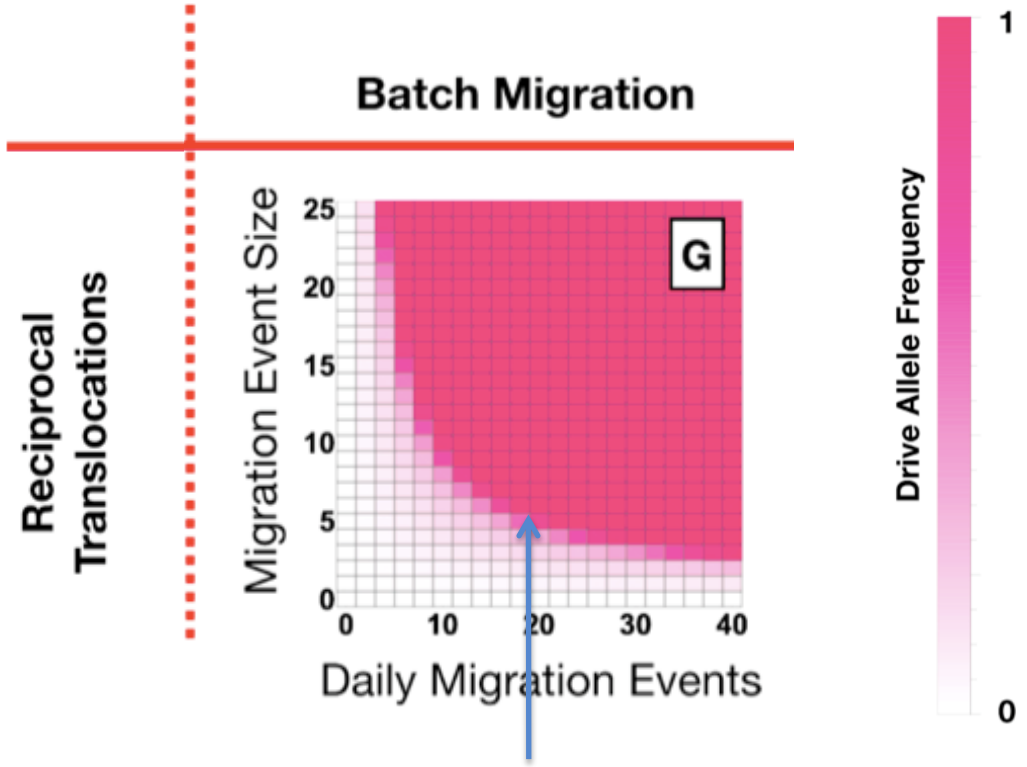
At a coverage level of 50%,
≥10 releases result in fixation

At a coverage level of 50%,
≥10 releases result in remediation

- Sánchez HM, Bennett JB, Wu SL, Rasic GL, Akbari OS, Marshall JM (2019) bioRxiv

Application: Confinement of translocations

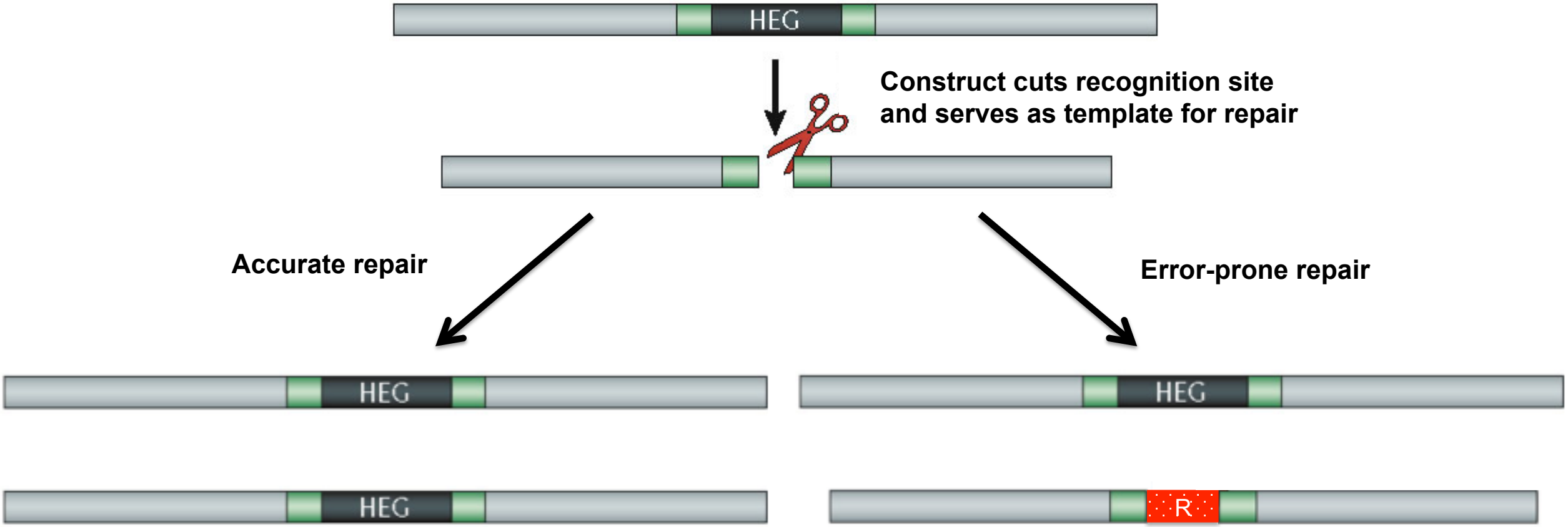
- Consider translocations fixing in Yorkeys Knob
- Consider batches of *Ae. aegypti* being transported from Yorkeys Knob to Trinity Park by human transport
- Vary size & number of daily migration events



For a batch size of 5 adults,
≥20 daily batch migration events required

- Sánchez HM, Bennett JB, Wu SL, Rasic GL, Akbari OS, Marshall JM (2019) bioRxiv

Q2. Can CRISPR-based gene drive be effective at controlling disease on a wide scale?

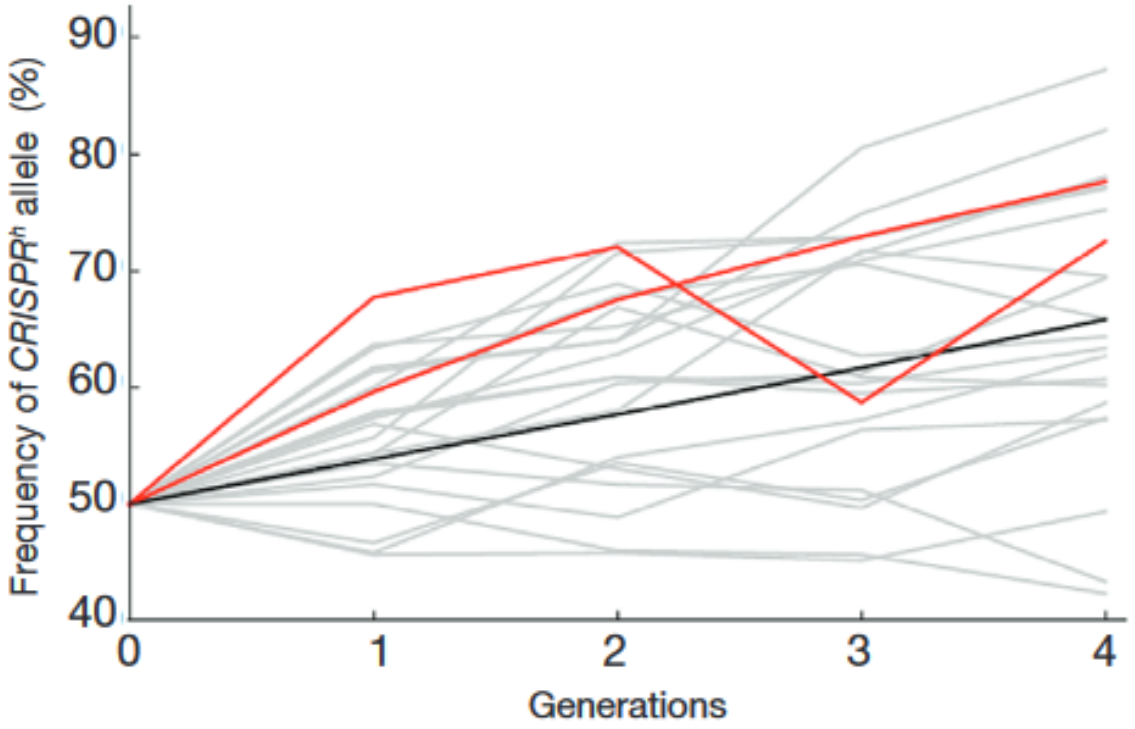


Application: Homing drive targeting female fertility gene

LETTERS

nature biotechnology

A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*



Parental genotype: HW

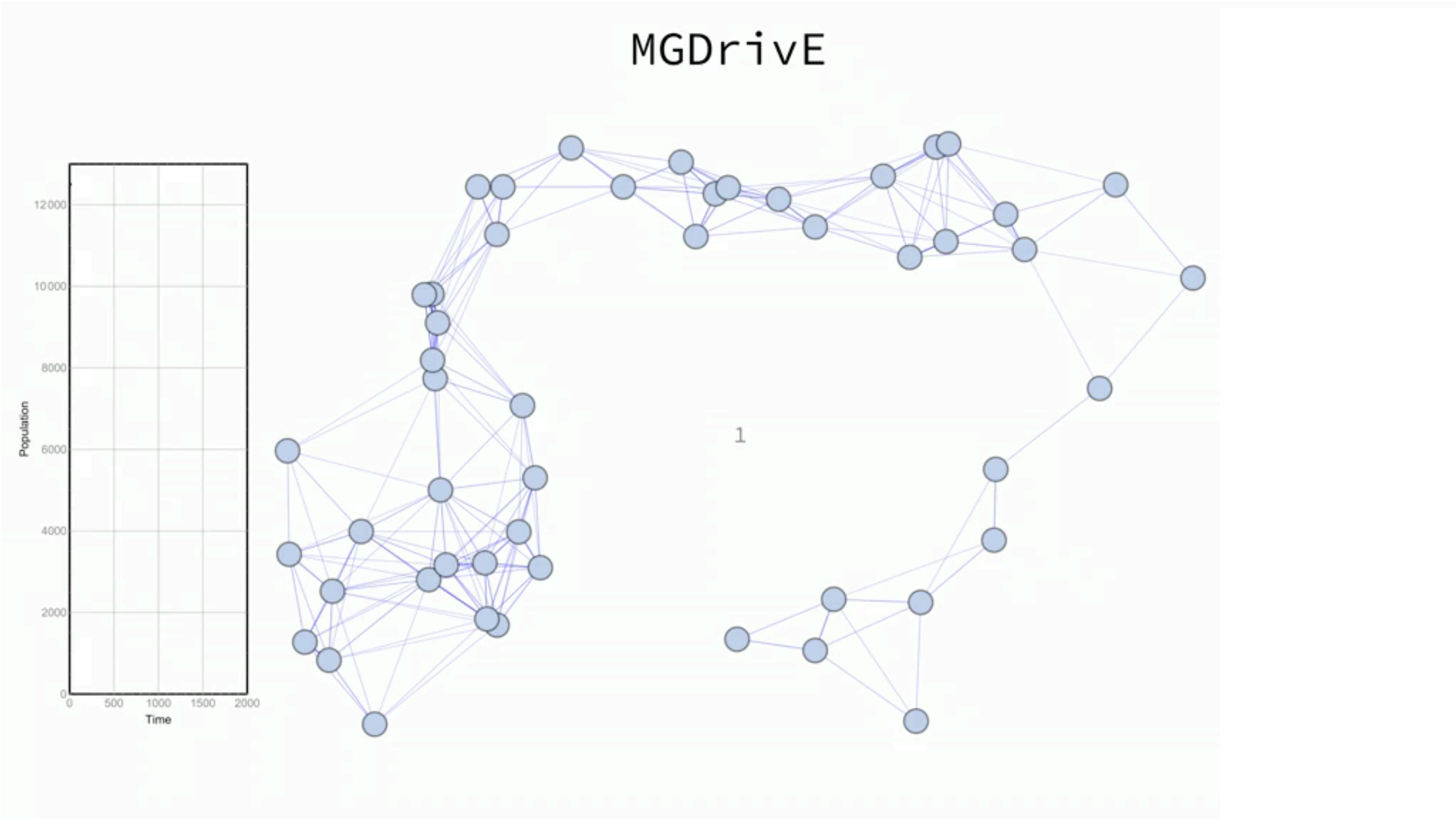
Gamete distribution:

H	R	W
99%	½ x 0.13%	0.094%

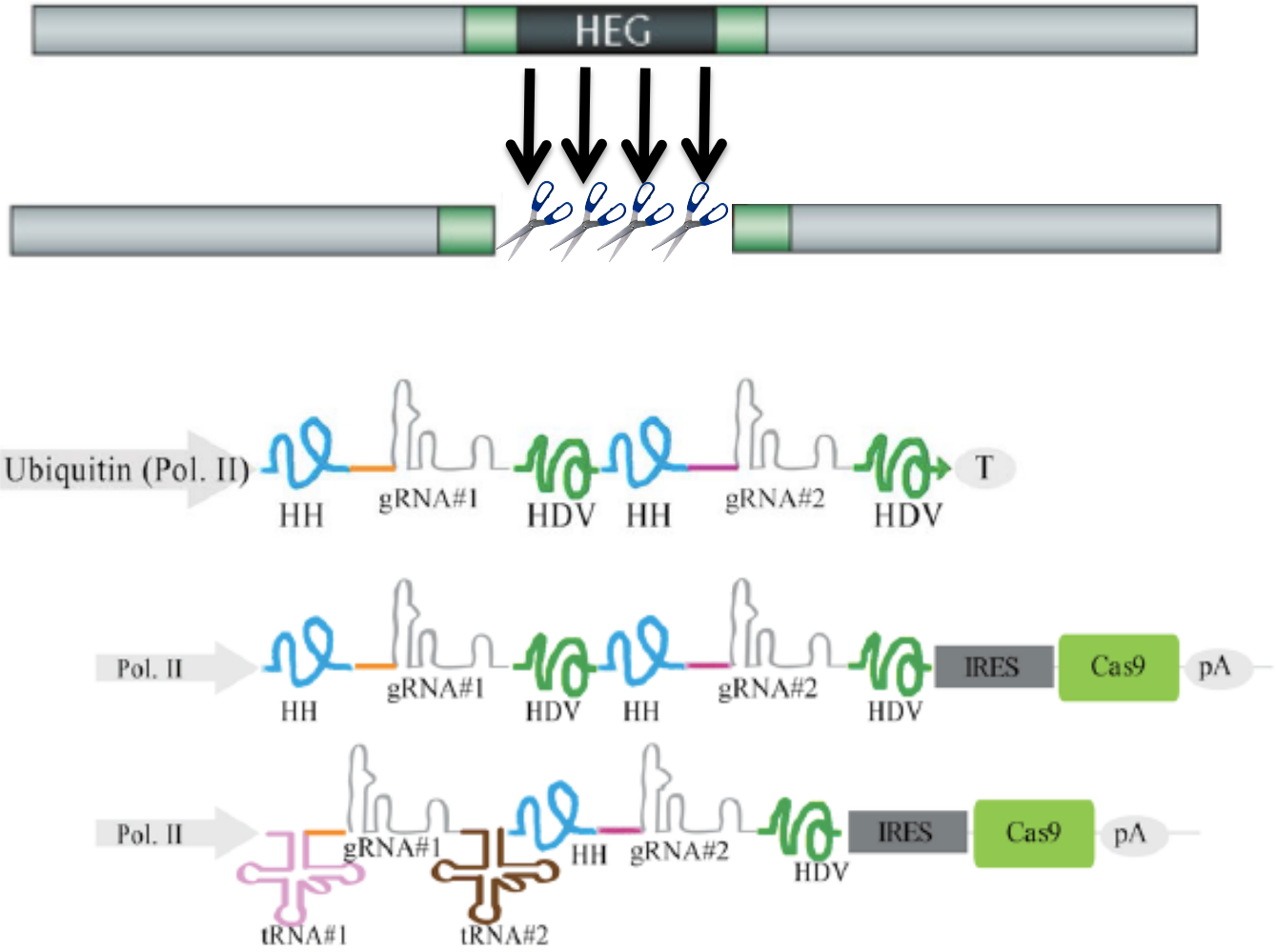
In-frame resistant allele generation rate

- Hammond A, Galizi R, Kyrou K, Simoni A, …, Windbichler N, Crisanti A, Nolan T (2016) Nature Biotechnology 34: 78-83

Application: Homing drive targeting female fertility gene



Application: Homing drive targeting female fertility gene



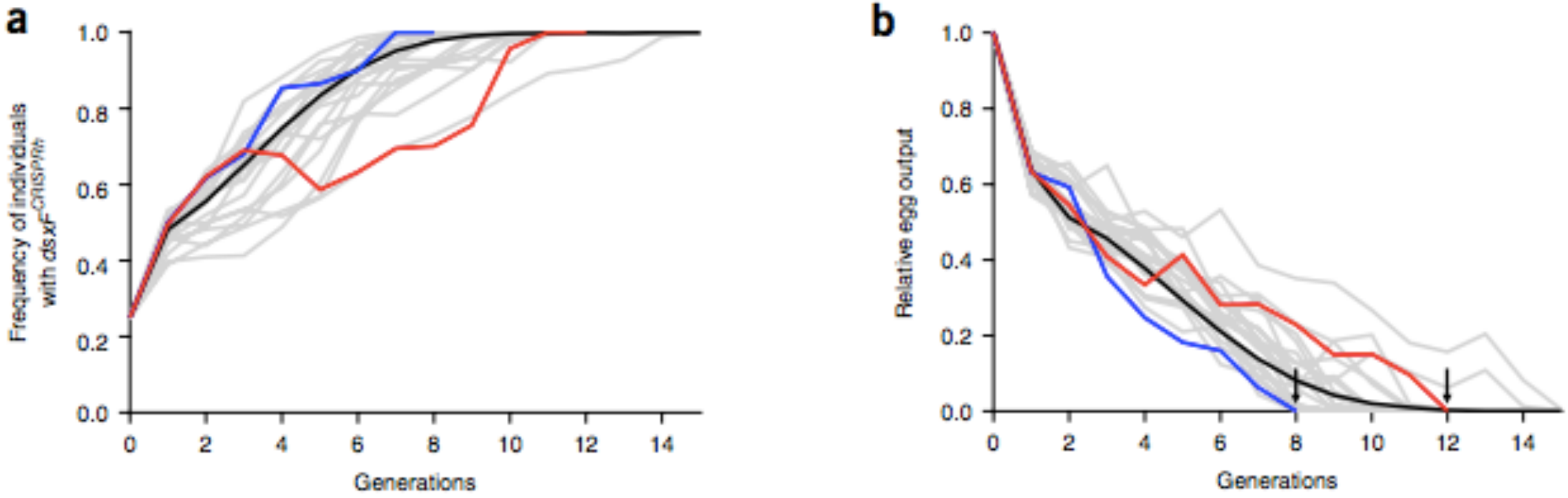
Multiplex number:	In-frame resistant allele generation rate:	Population size capable of eliminating (90% of simulations):
1	0.13%	32
2	0.00017%	24 thousand
3	2.2×10^{-9}	19 million
4	2.9×10^{-12}	14 billion

- Marshall JM, Buchman A, Sanchez HM, Akbari OS (2017) Nature Sci Rep 7: 3776

A CRISPR–Cas9 gene drive targeting *doublesex* causes complete population suppression in caged *Anopheles gambiae* mosquitoes

nature biotechnology

Kyros Kyrou[1,2], Andrew M Hammond[1,2], Roberto Galizi[1], Nace Kranjc[1], Austin Burt[1], Andrea K Beaghton[1], Tony Nolan[1] & Andrea Crisanti[1]

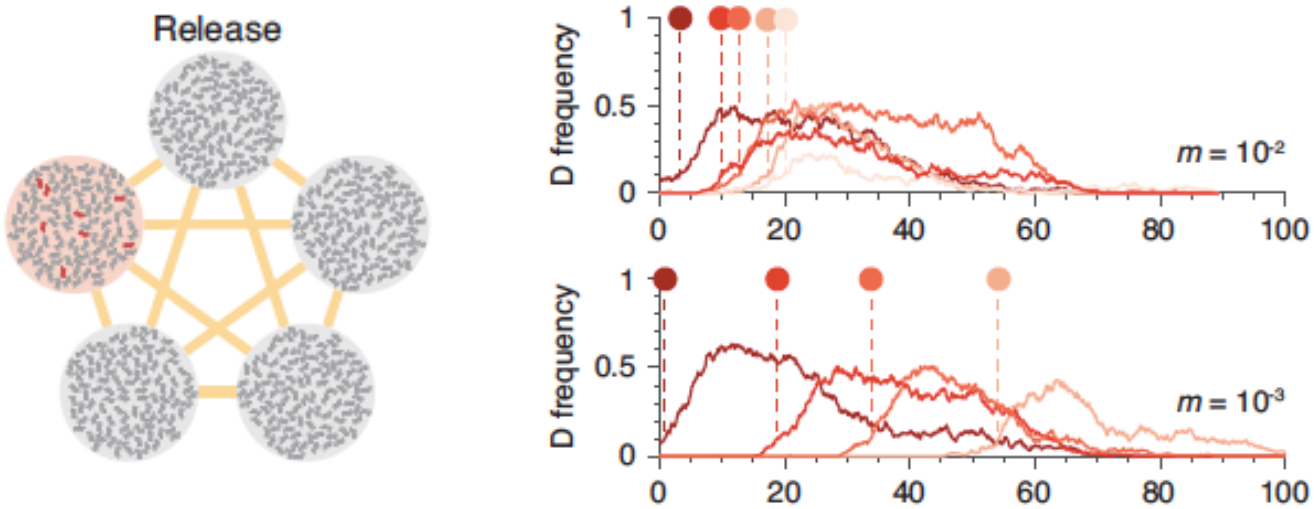


- Kyros K, Hammond AM, Galizi R, …, Nolan T, Crisanti A (2018) Nature Biotechnol 36, 1062–1066

eLIFE

Current CRISPR gene drive systems are likely to be highly invasive in wild populations

Charleston Noble[1,2,3†], Ben Adlam[1,4†], George M Church[2,3], Kevin M Esvelt[5*], Martin A Nowak[1,6,7*]



The New York Times

MATTER

'Gene Drives' Are Too Risky for Field Trials, Scientists Say

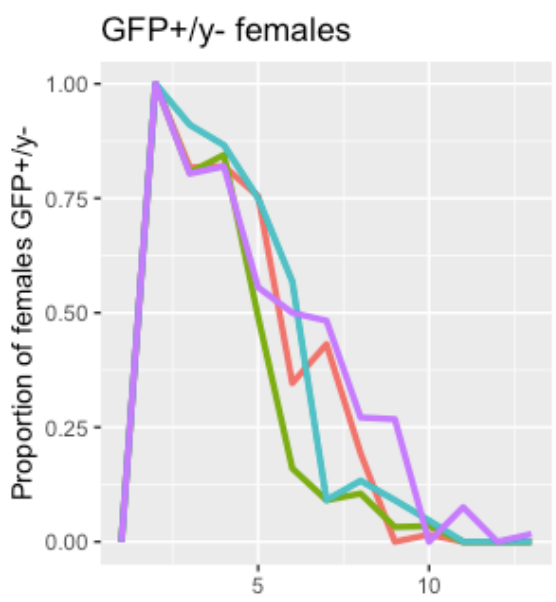
That may well mean that experiments in the real world are just too risky right now.

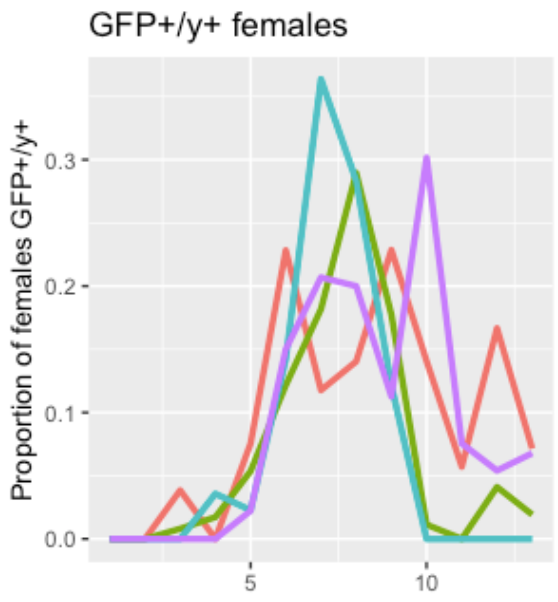
"The very idea of a field trial is that it's a trial that's confined to an area," Dr. Esvelt said. "Our model indicates that this is not the case."

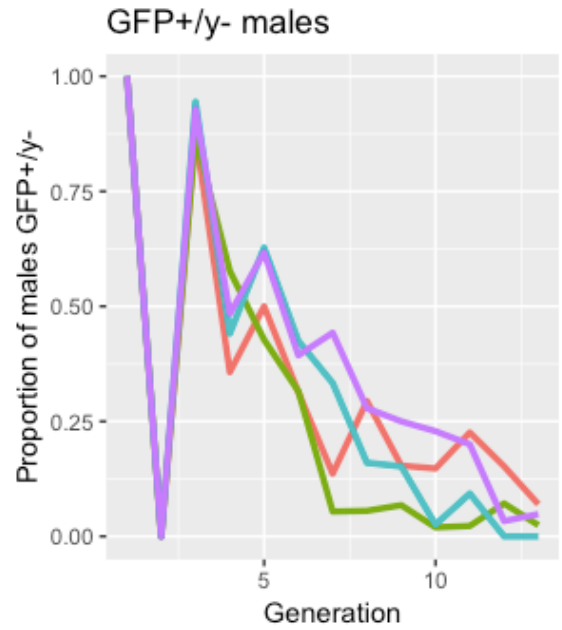
"It's an important contribution," said John M. Marshall, a mathematical biologist at the University of California, Berkeley, said of the new research. "A study like this is the beginning of a formal analysis we need."

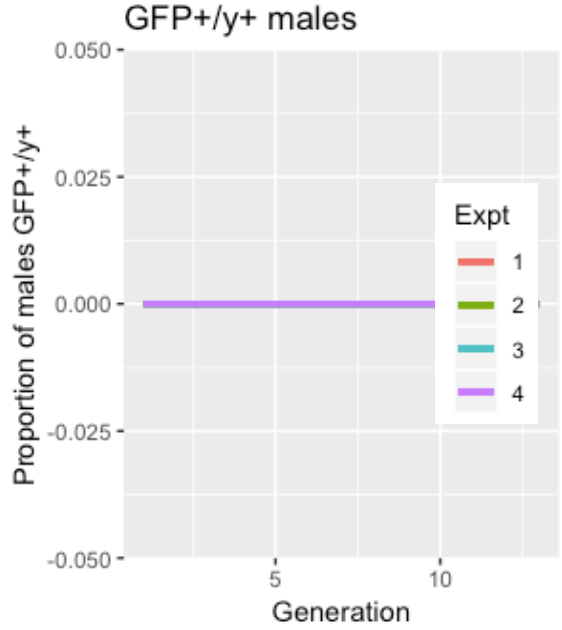
- Noble C, Adlam B, Church GM, Esvelt KM *et al.* (2018) eLife doi: 10.7554/eLife.33423

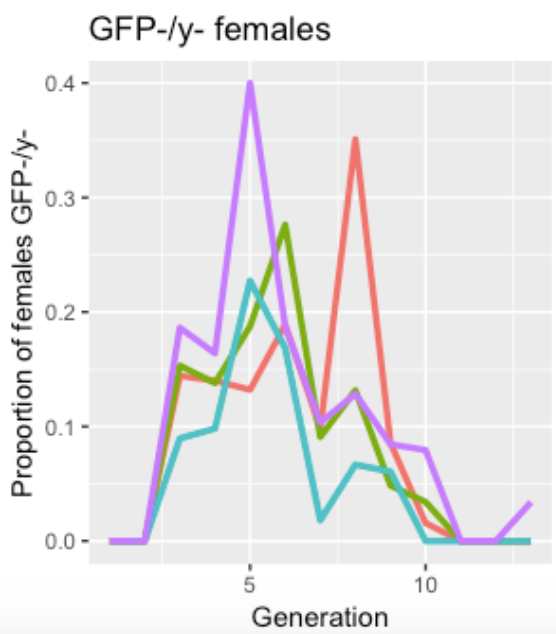
MCR homing drive construct from Bier Lab, UCSD

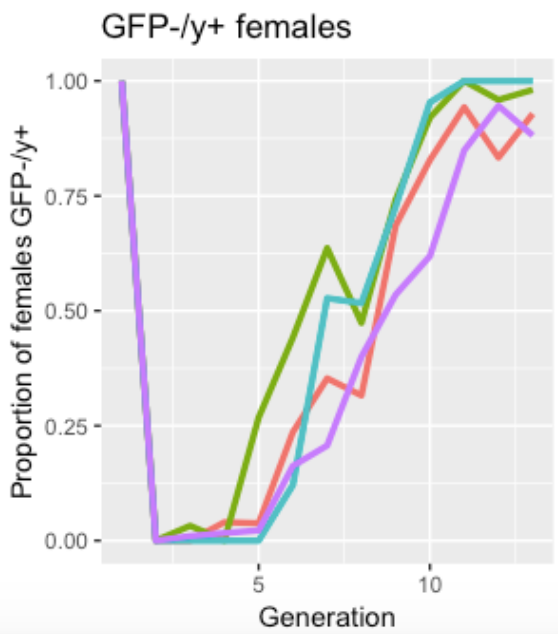


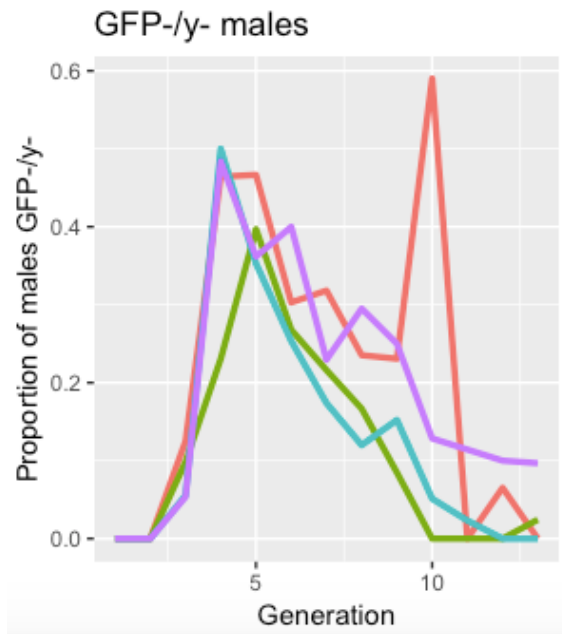


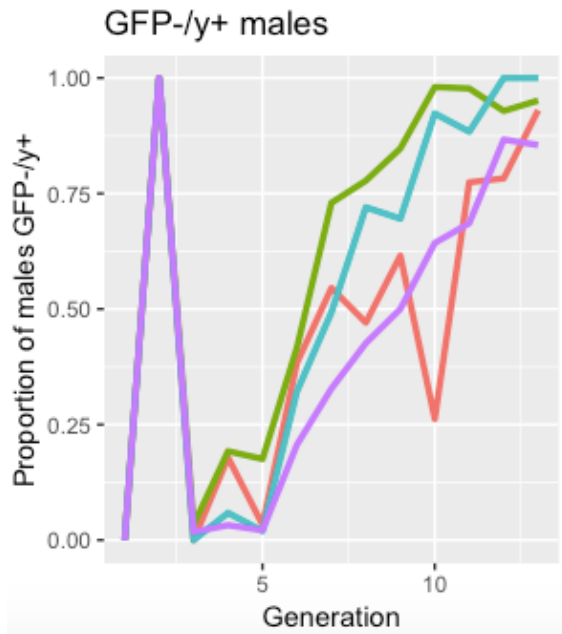




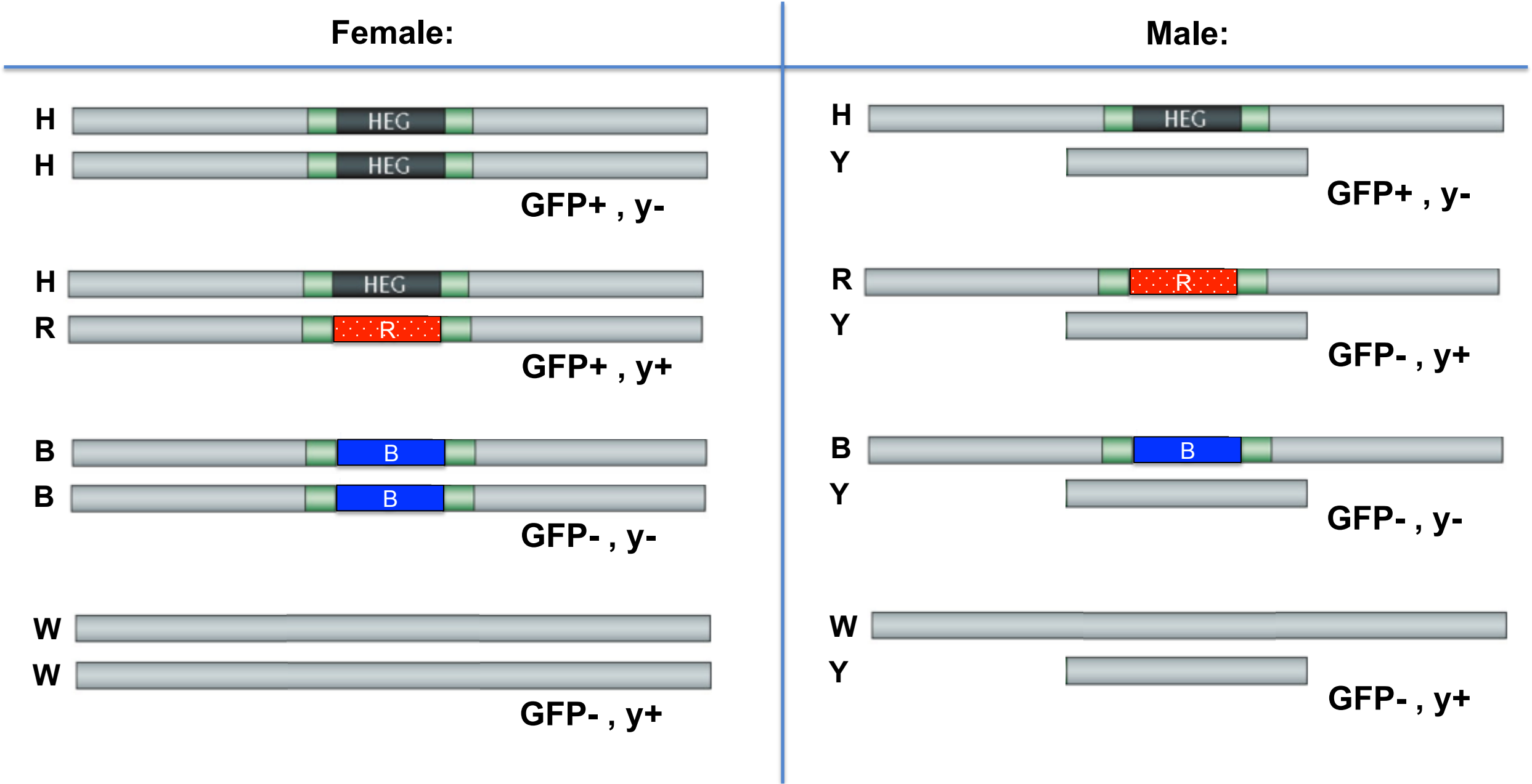








Model fitting: Current homing-based drive system



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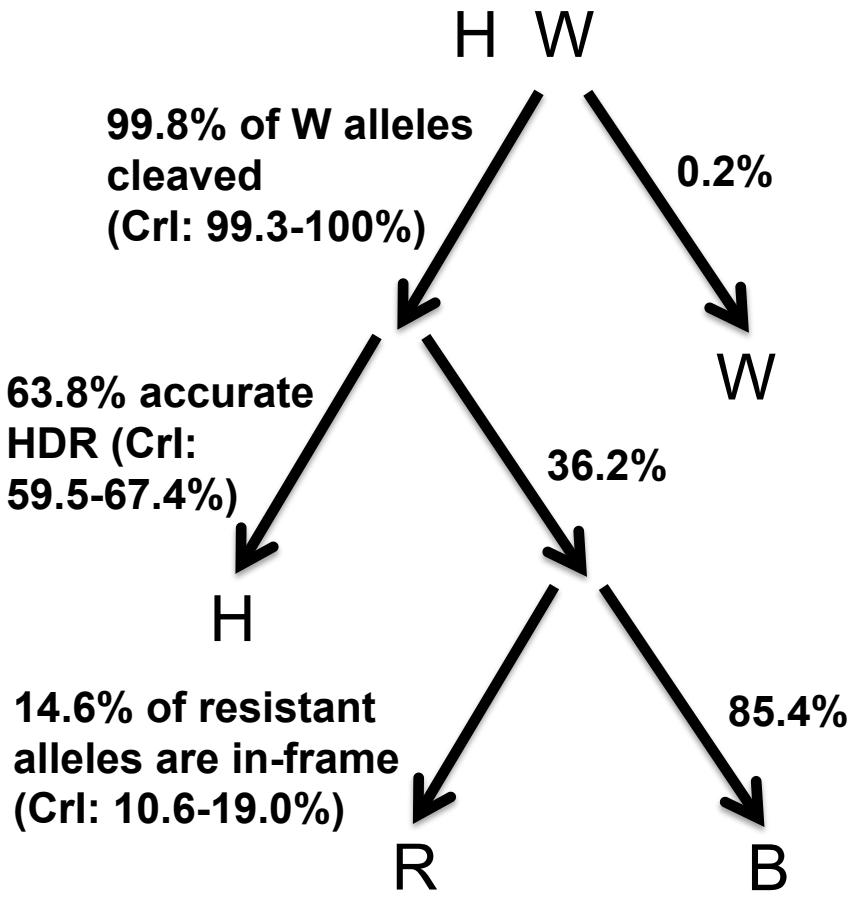
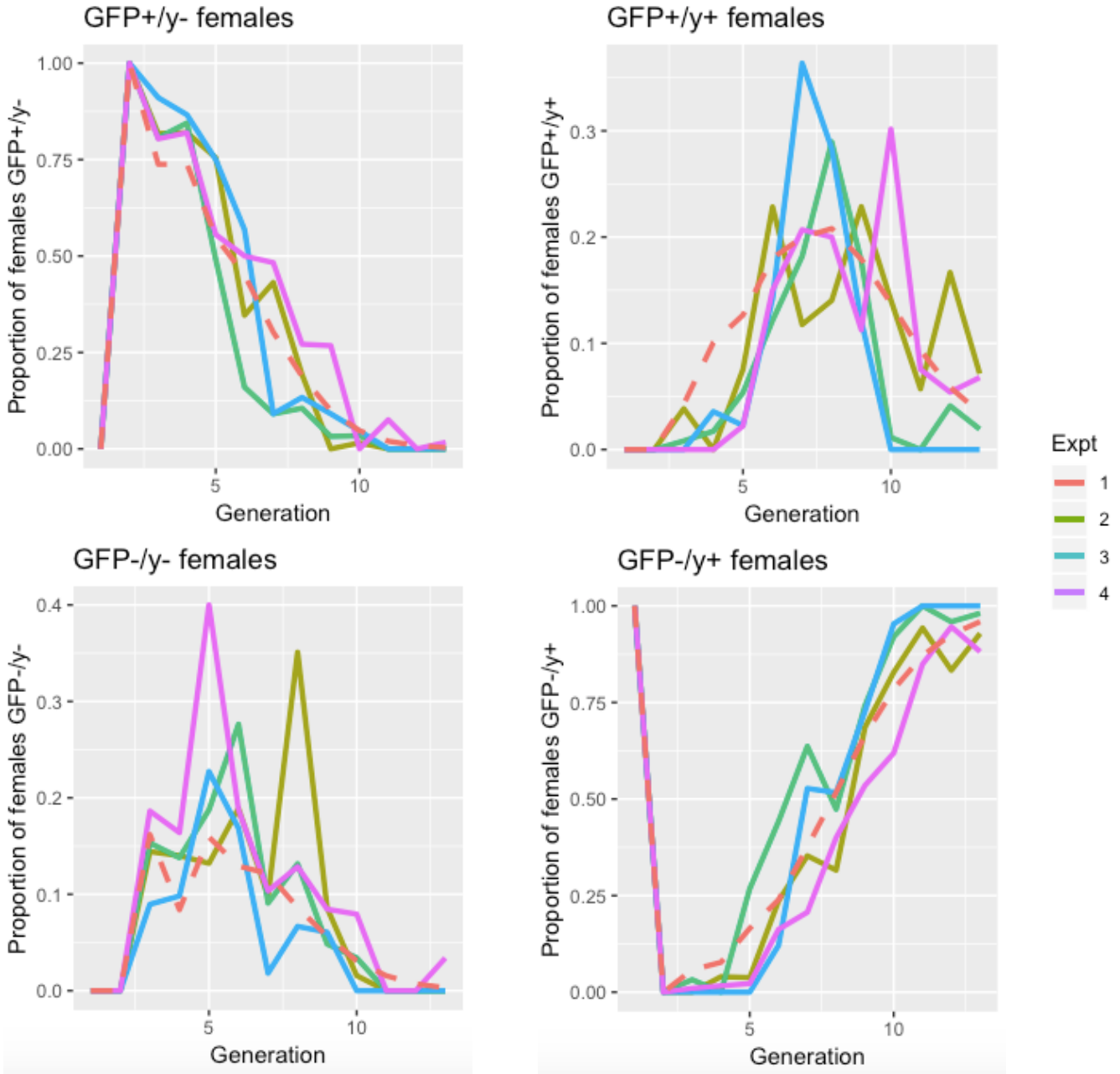
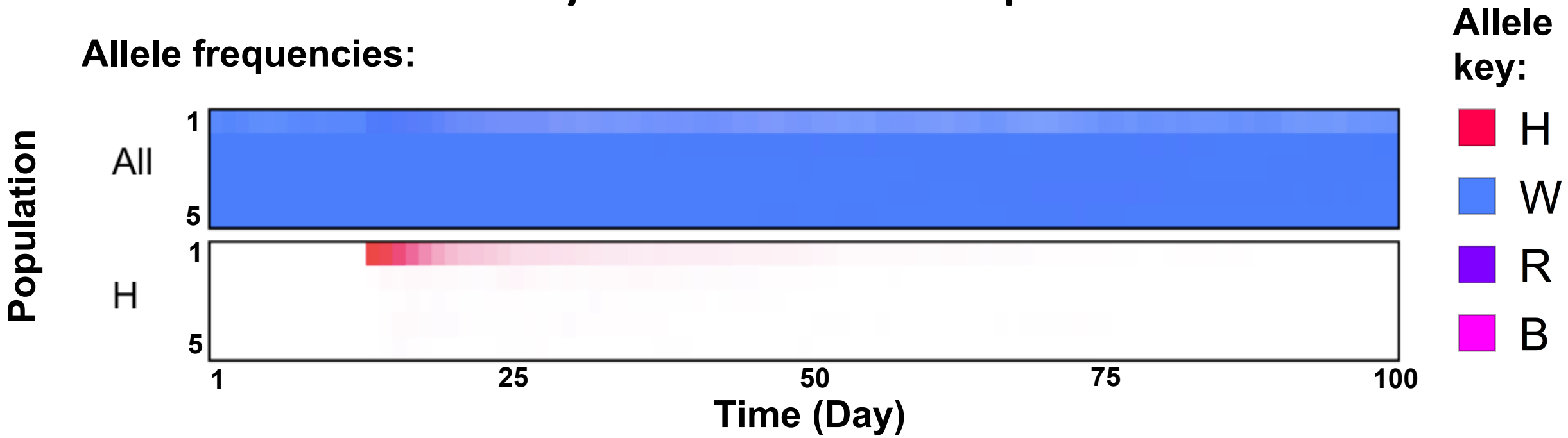


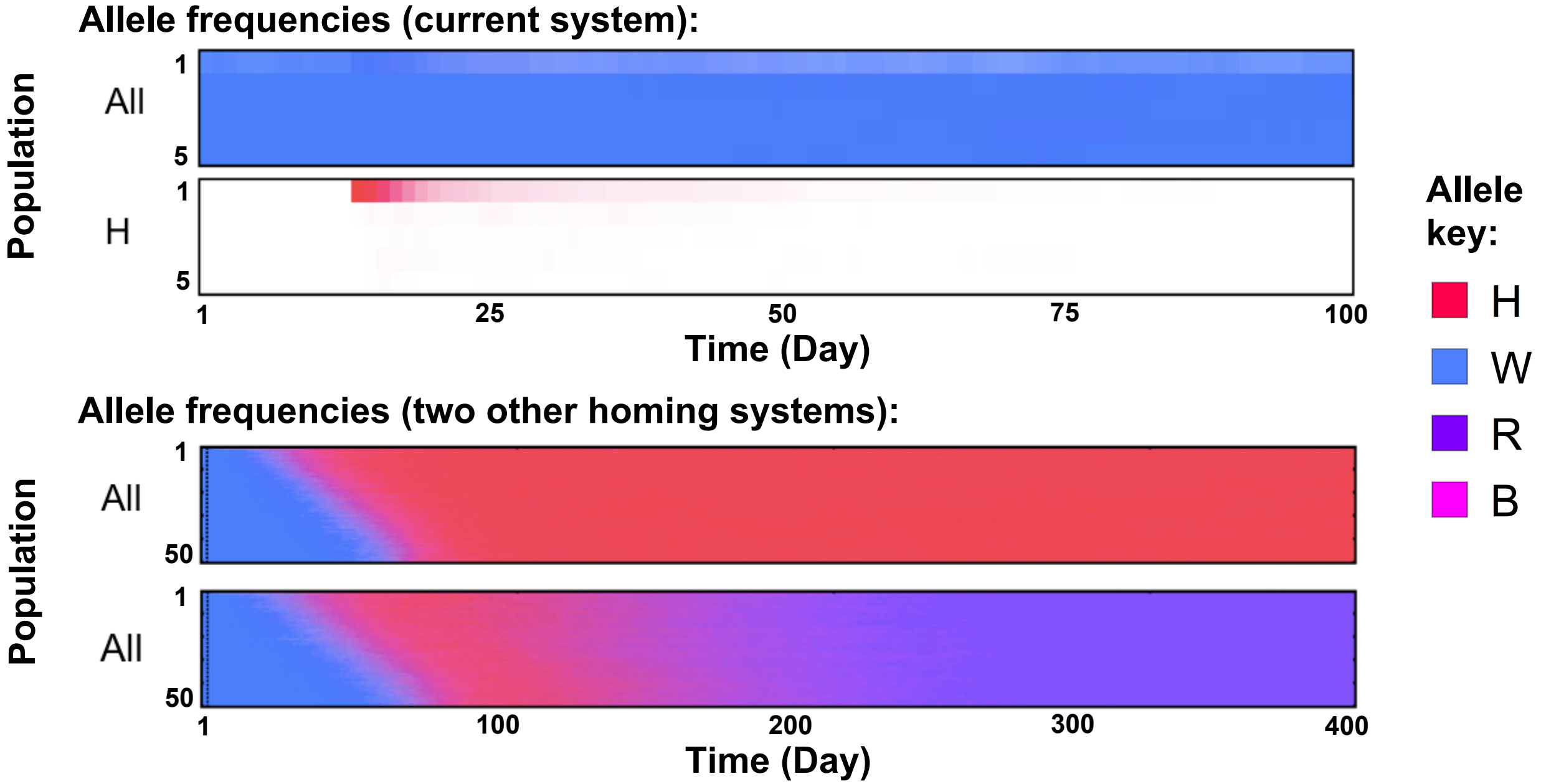
- Fitness cost of H allele = 46.6% (CrI: 45.1-47.9%)
- Fitness cost of B allele = 37.9% (CrI: 36.3-39.4%)



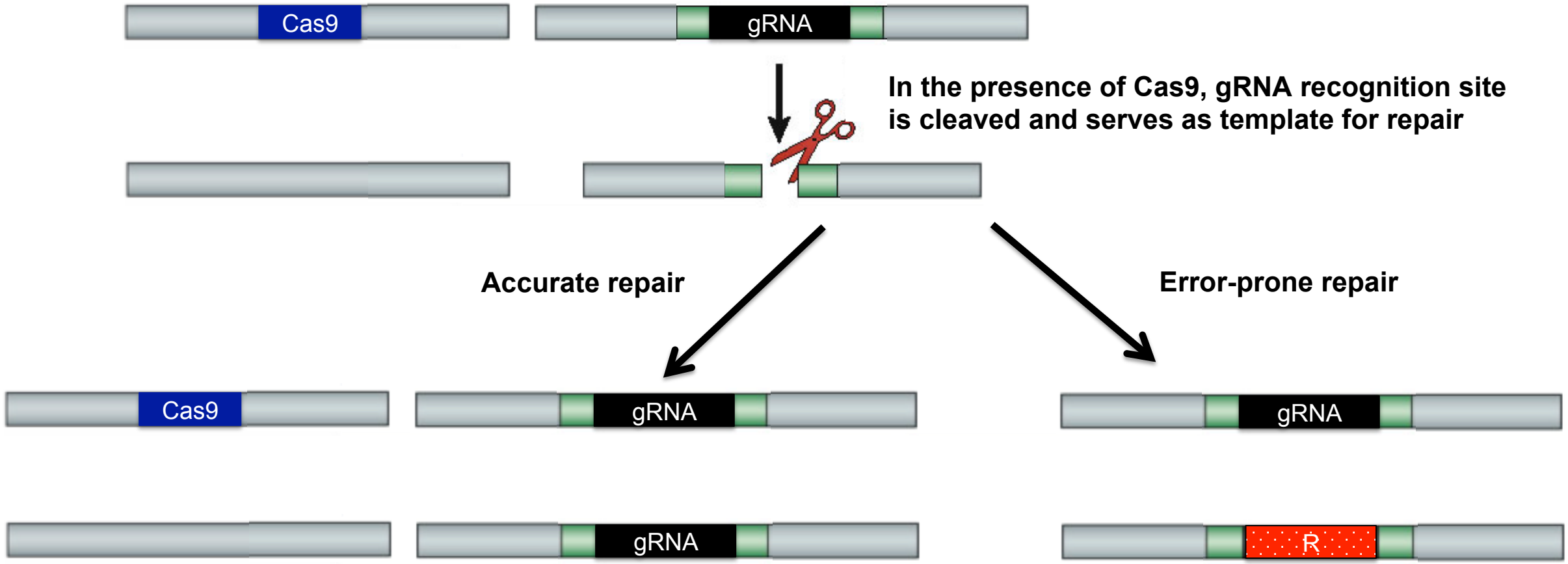
Current systems don’t spread far



Current systems don’t spread far… but others might



Application: CRISPR-based split drive



- Li M, Yang T, Kandul N, Biu M, Gamez S, Bennett JB, Sánchez HM, …, Marshall JM, Akbari OS (2019) bioRxiv

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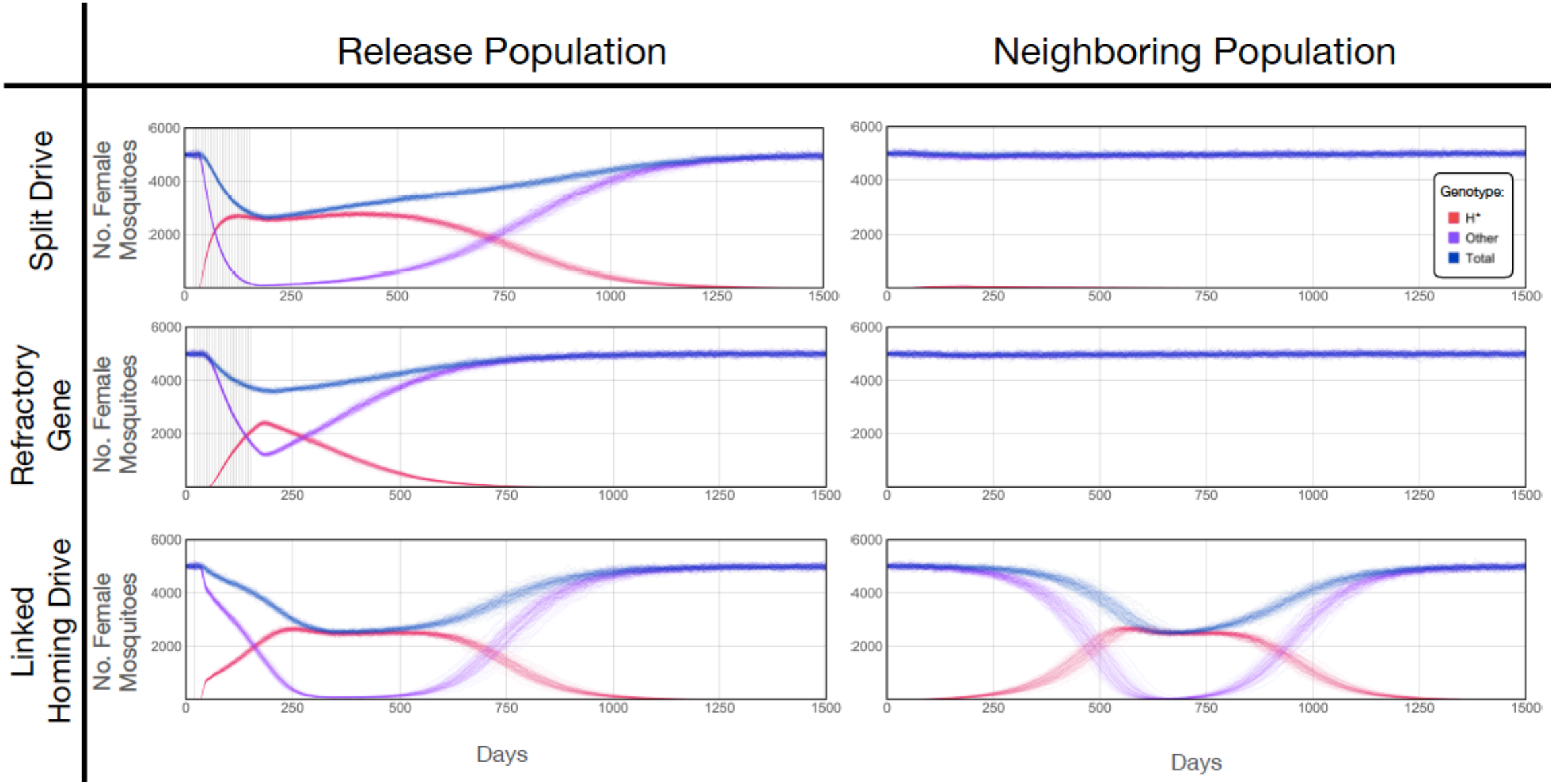
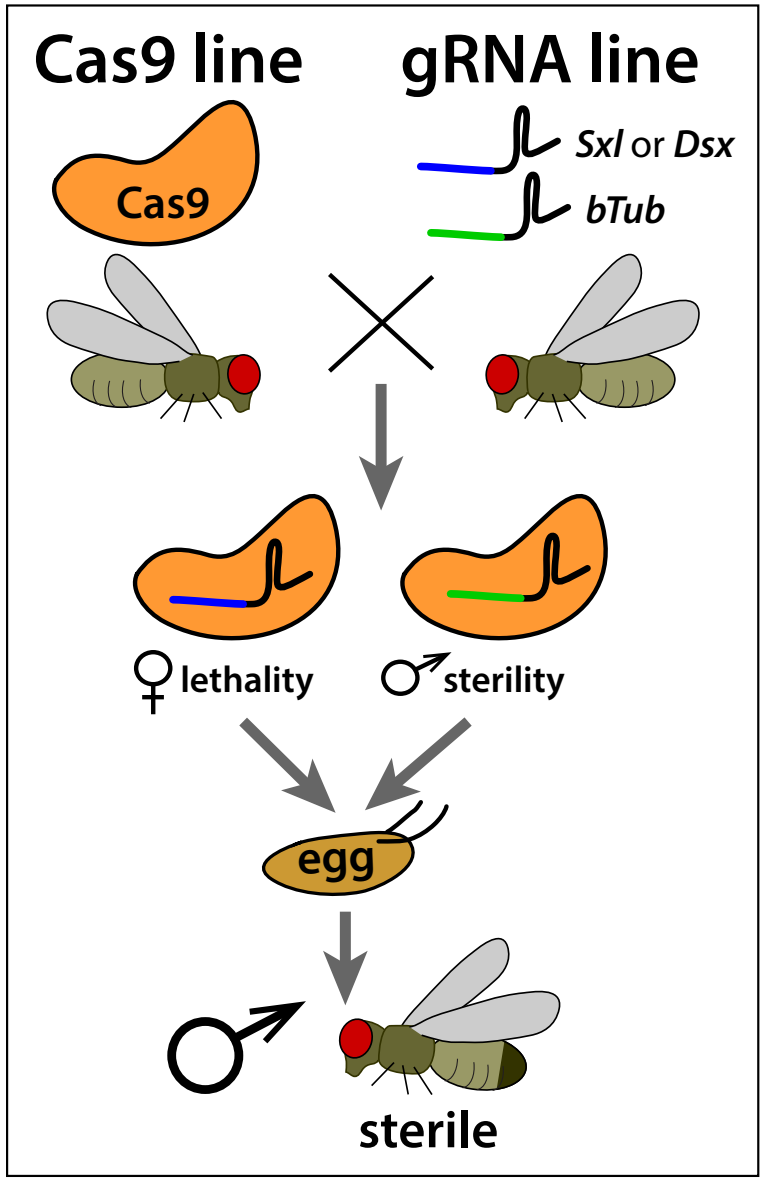
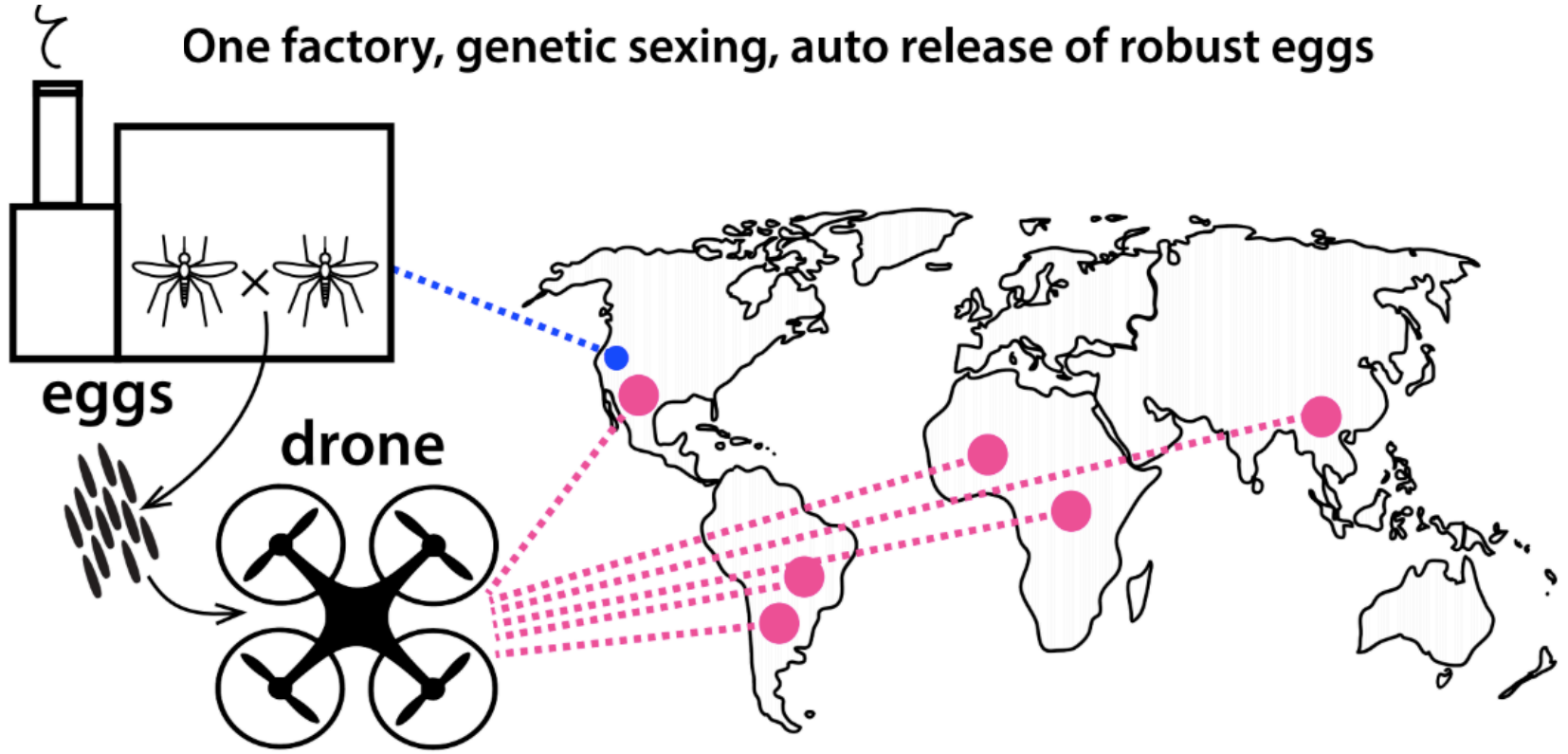


- Li M, Yang T, Kandul N, Biu M, Gamez S, Bennett JB, Sánchez HM, …, Marshall JM, Akbari OS (2019) bioRxiv

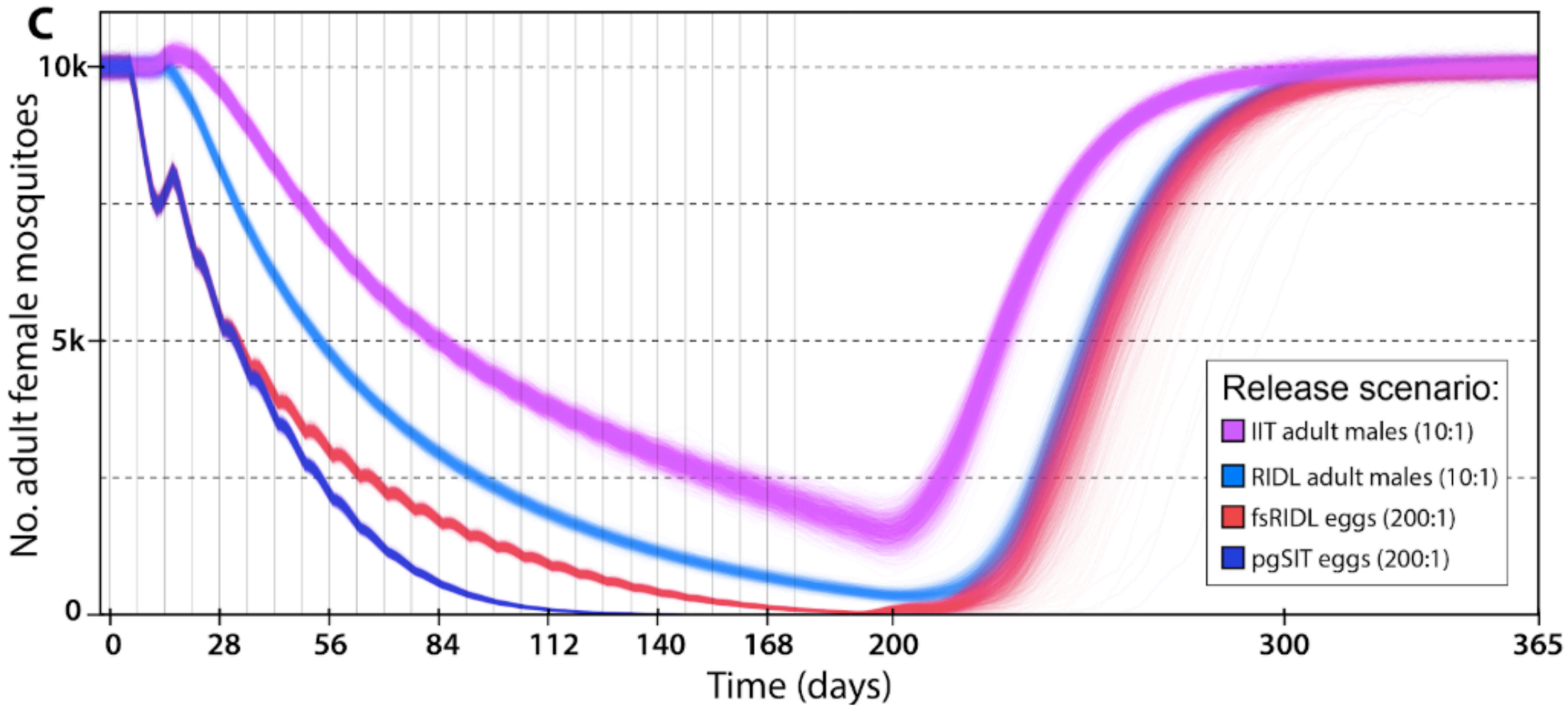
Application: Precision-guided sterile insect technique





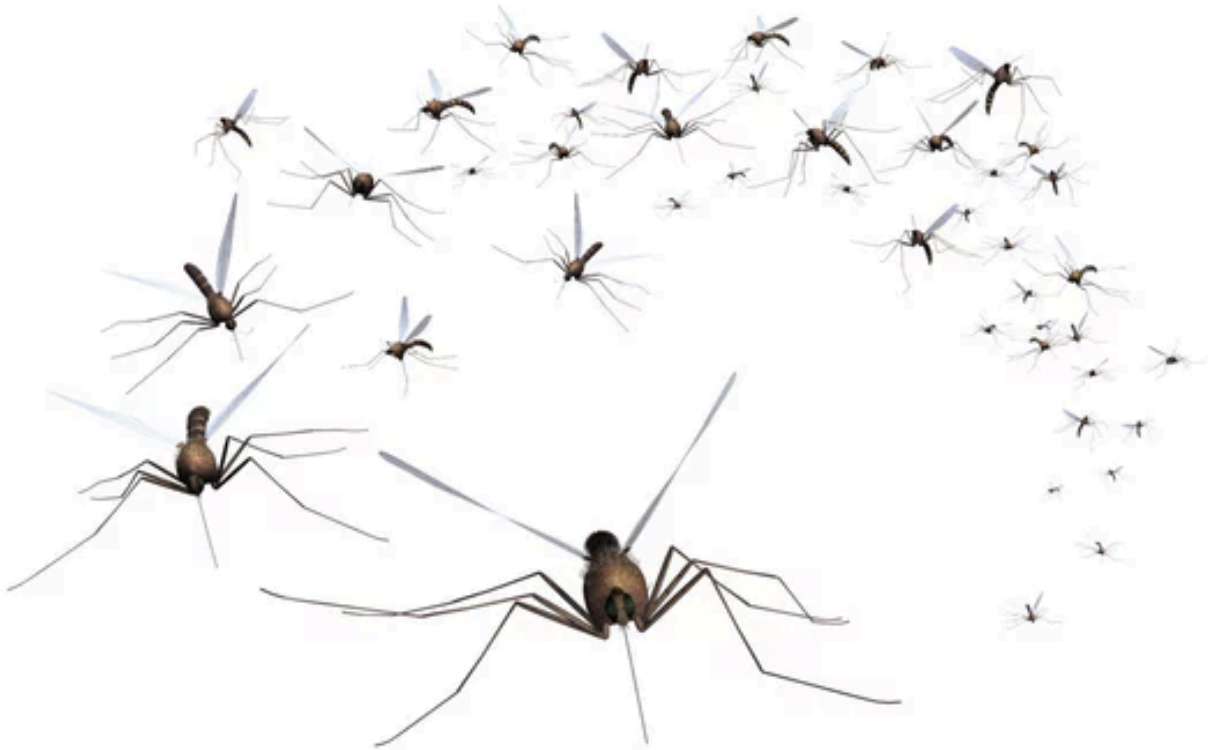
- Kandul NP, Liu J, Sánchez HM, Wu SL, Marshall JM, Akbari OS (2019) Nature Communications 10: 84

Application: Precision-guided sterile insect technique

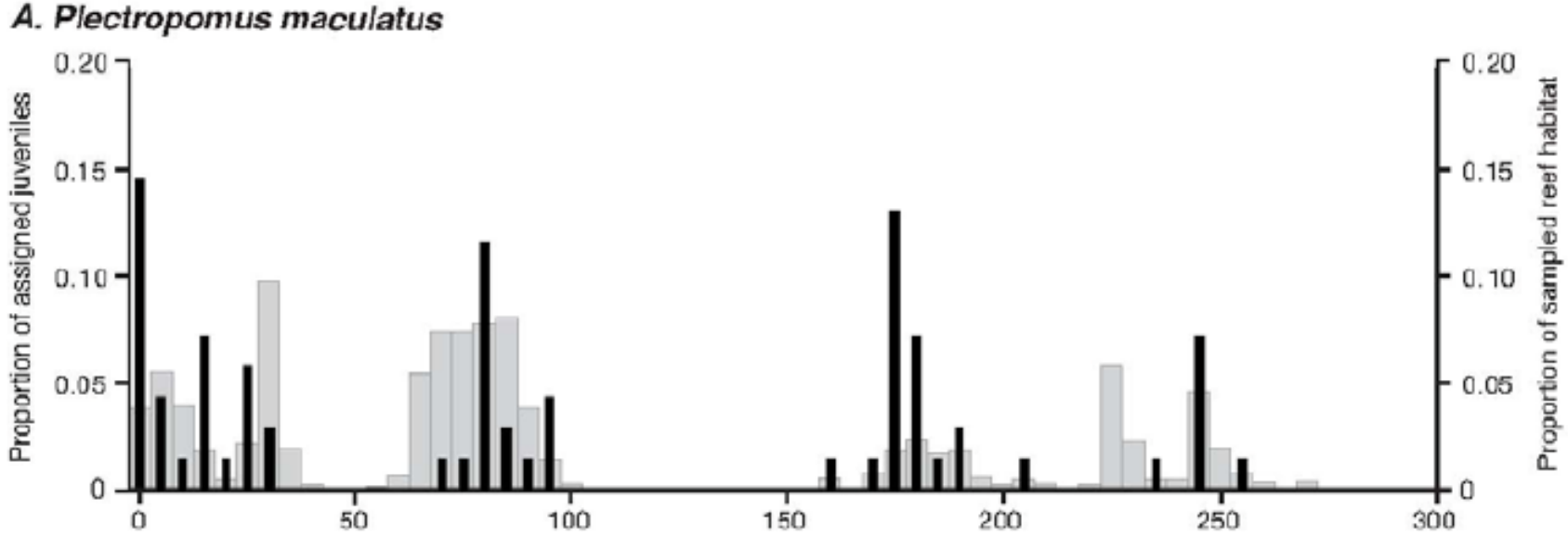


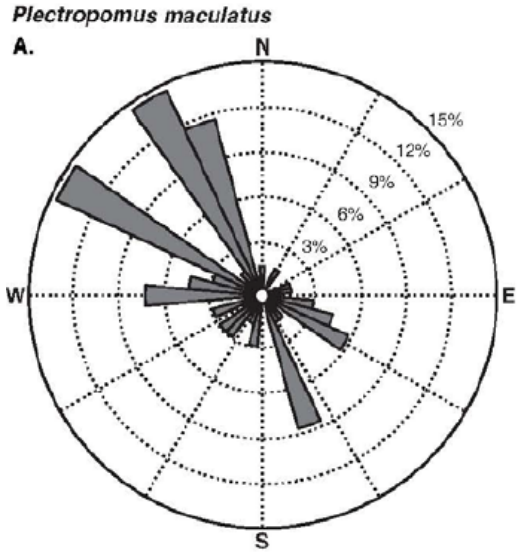
- Kandul NP, Liu J, Sánchez HM, Wu SL, Marshall JM, Akbari OS (2019) Nature Communications 10: 84

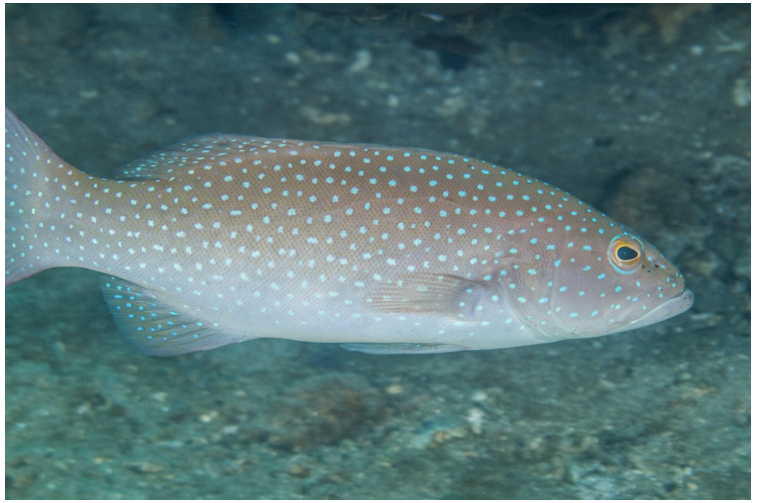
Q3. What are the best approaches for quantifying mosquito movement patterns of relevance to both questions?



Inspiration from coral trout

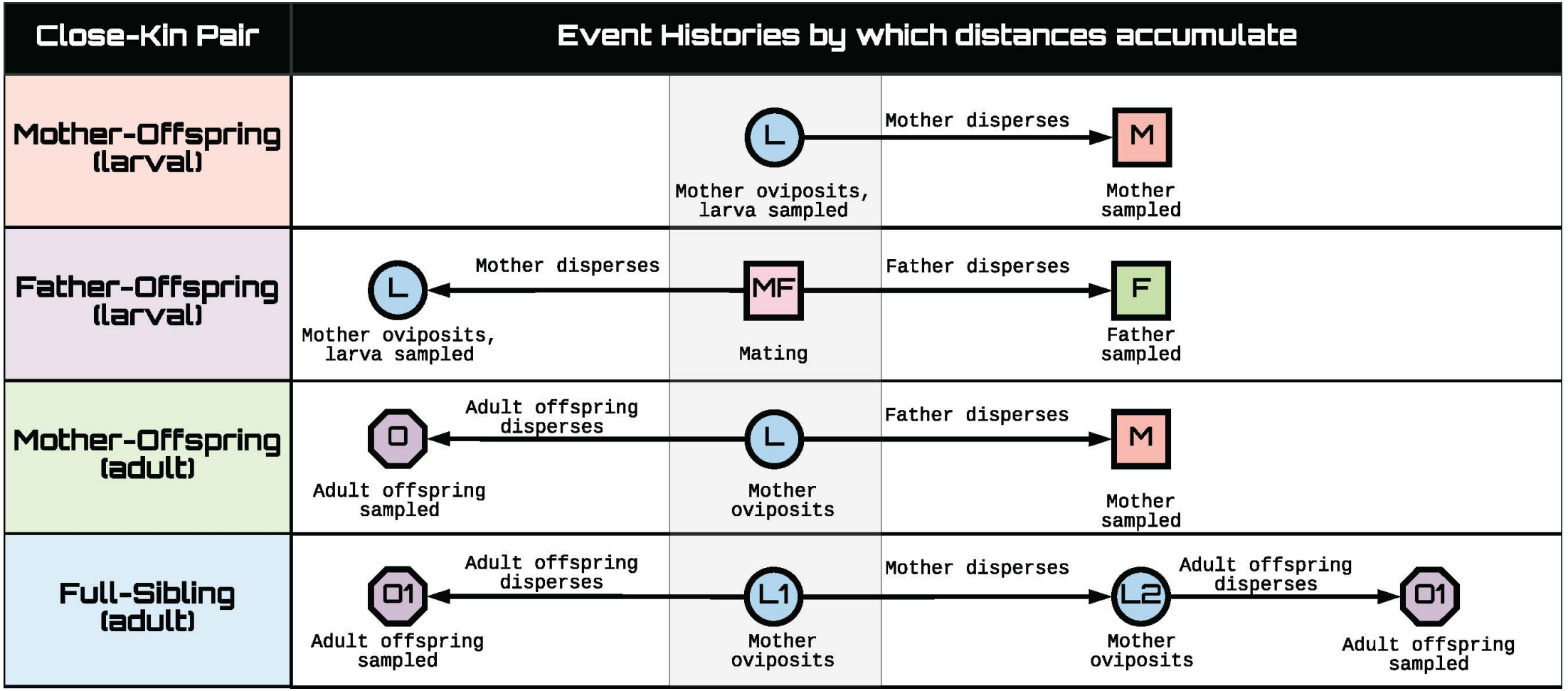




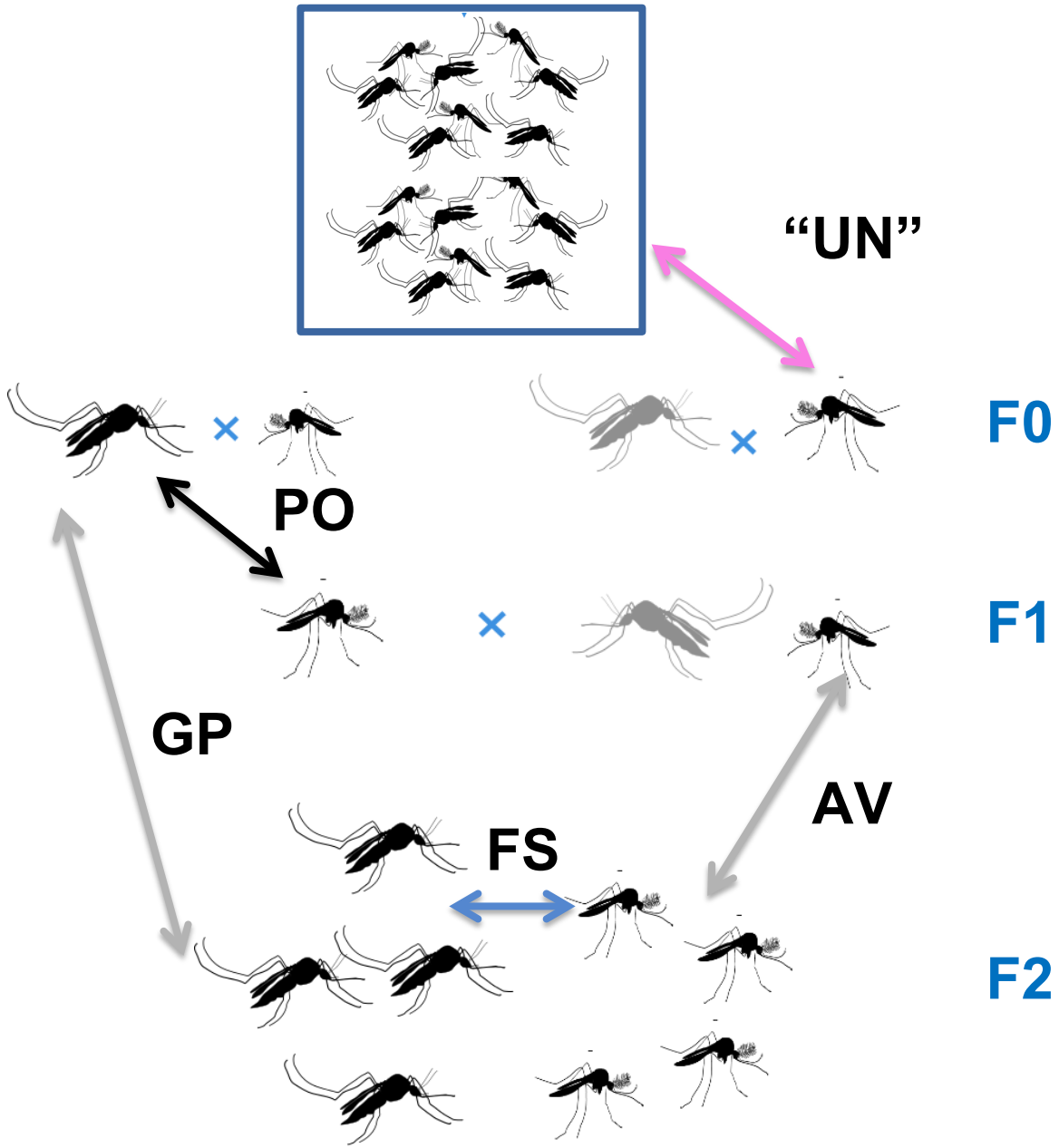


- Williamson DH, Harrison HB, Almany GR, Berumen ML *et al.* (2016) Mol. Ecol. 25L 6039-6054

Close-kin genetic methods to infer mosquito dispersal



Determining familial relationships from lab colonies



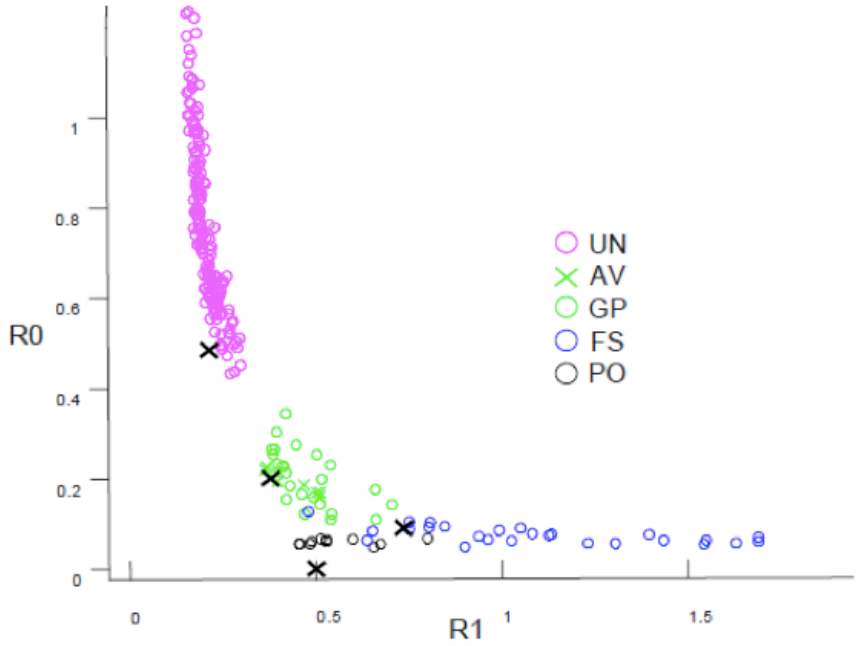
MOLECULAR ECOLOGY

ORIGINAL ARTICLE | Open Access

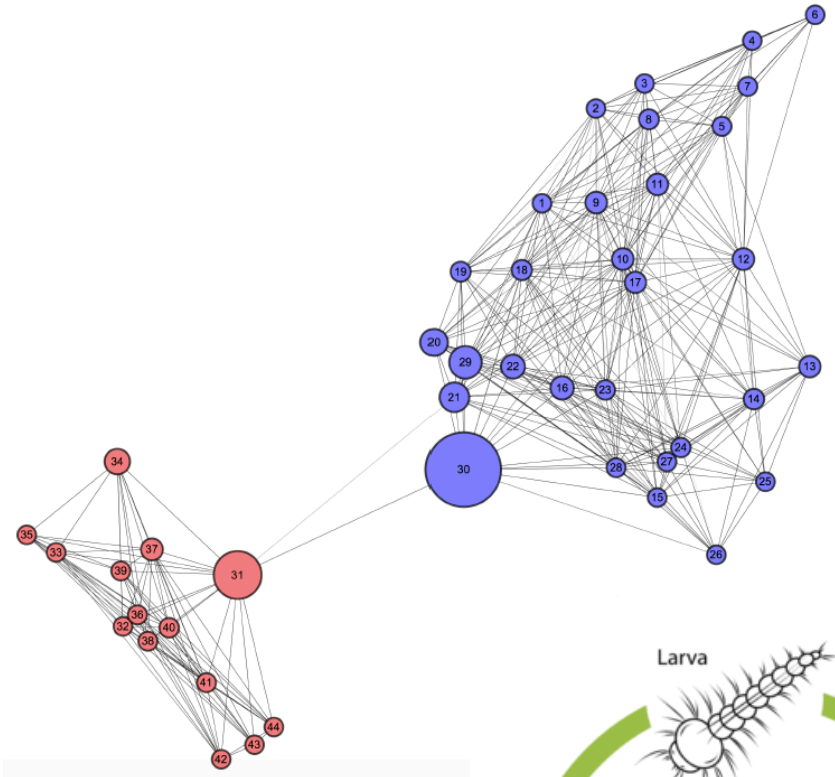
Allele frequency-free inference of close familial relationships from genotypes or low-depth sequencing data

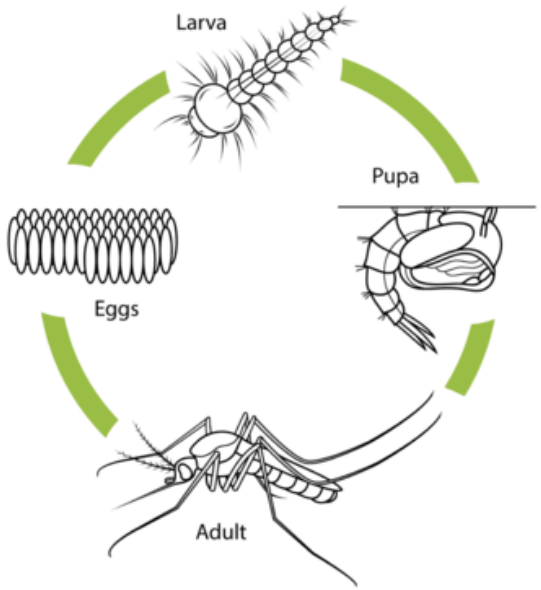
Ryan K. Waples, Anders Albrechtsen, Ida Moltke

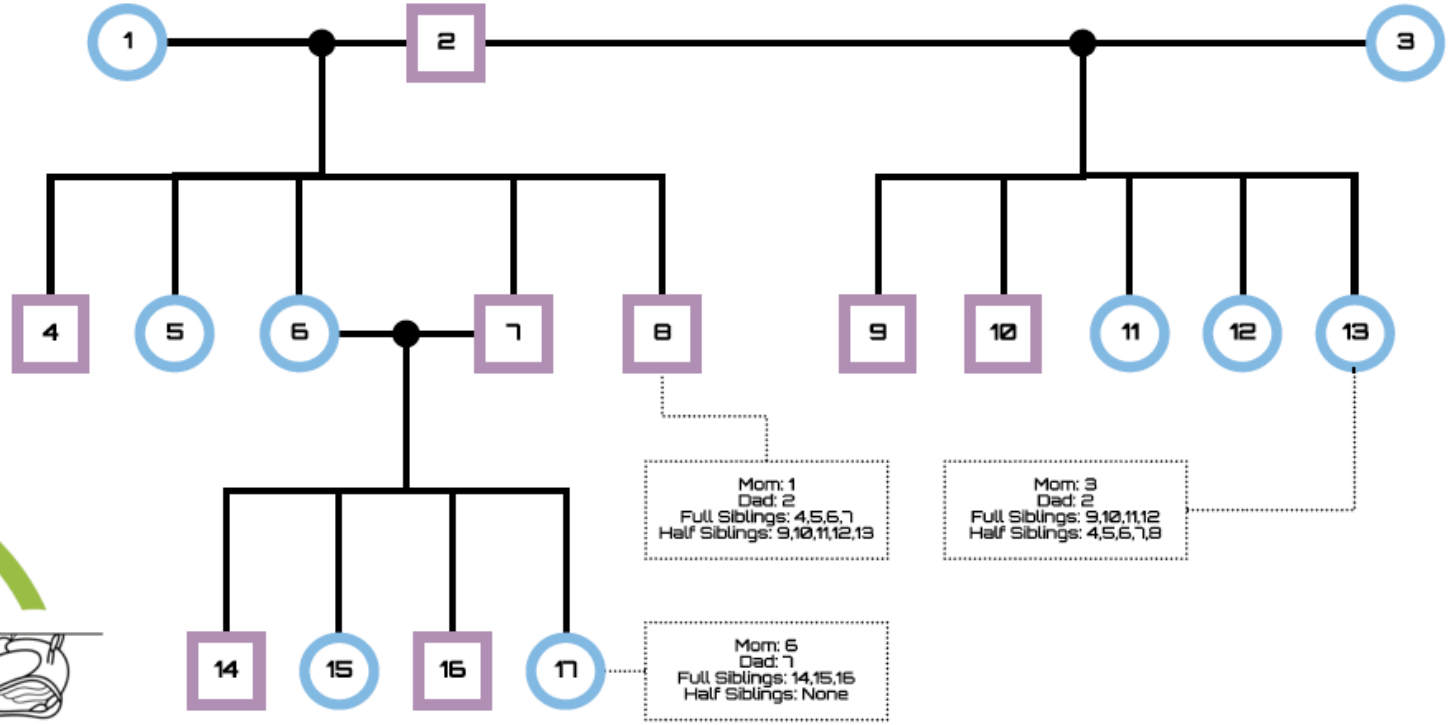
First published: 21 November 2018 | https://doi.org/10.1111/mec.14954



Determining power of close-kin methods *in silico*



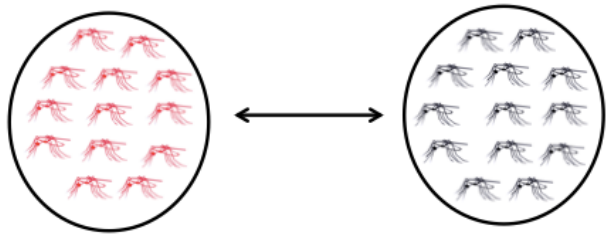




Summary

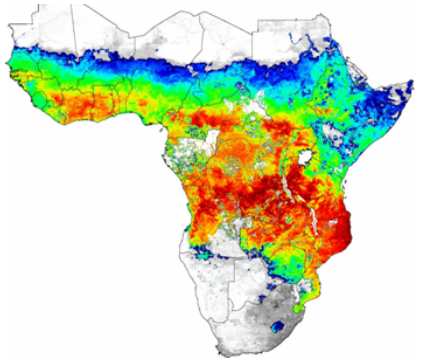
Q1. Can we conduct a confined field trial of gene-edited mosquitoes?

- Threshold-dependent systems may be confineable to partially isolated populations
- More study is needed on population structure & batch migration



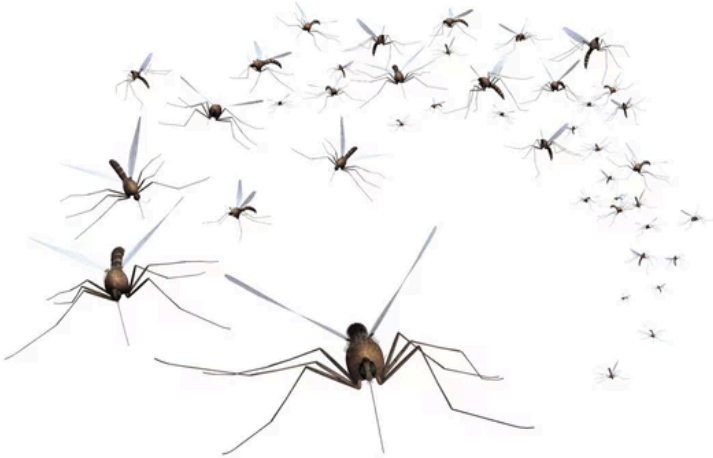
Q2. Can CRISPR-based gene drive be effective at controlling disease on a wide scale?

- Multiplexing guide RNAs could sufficiently reduce resistant allele generation rates
- More study is needed of the molecular mechanisms
- Current homing-based drive systems are not as invasive as hyped; but future ones could be



Q3. What are the best approaches for quantifying mosquito movement patterns?

- Close-kin capture methods are promising for inferring details of the fine-scale movement patterns of mosquitoes



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