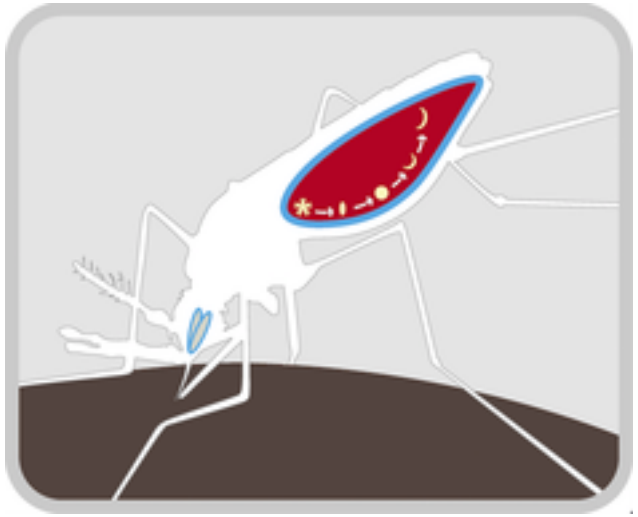
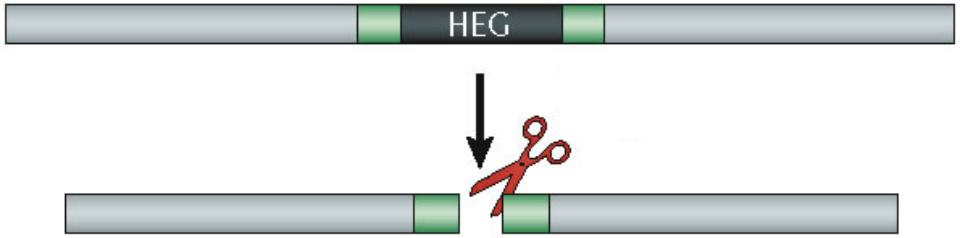
Can malaria, dengue and Zika be controlled by CRISPR-based gene drive?





John M. Marshall
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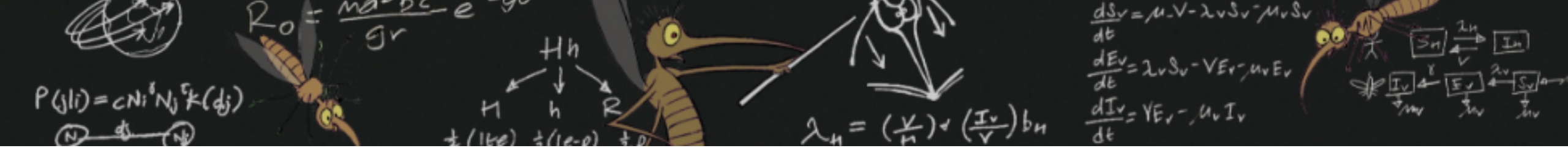
Division of Biostatistics and Epidemiology
University of California, Berkeley

Berkeley School of Public Health



John Marshall Samson Kiware

Hector Sanchez

Gordana Rašić

Yogita Sharma

Valeri Vasquez

Sean Wu

Jared Bennett

Partow Imani

Francois Rerolle

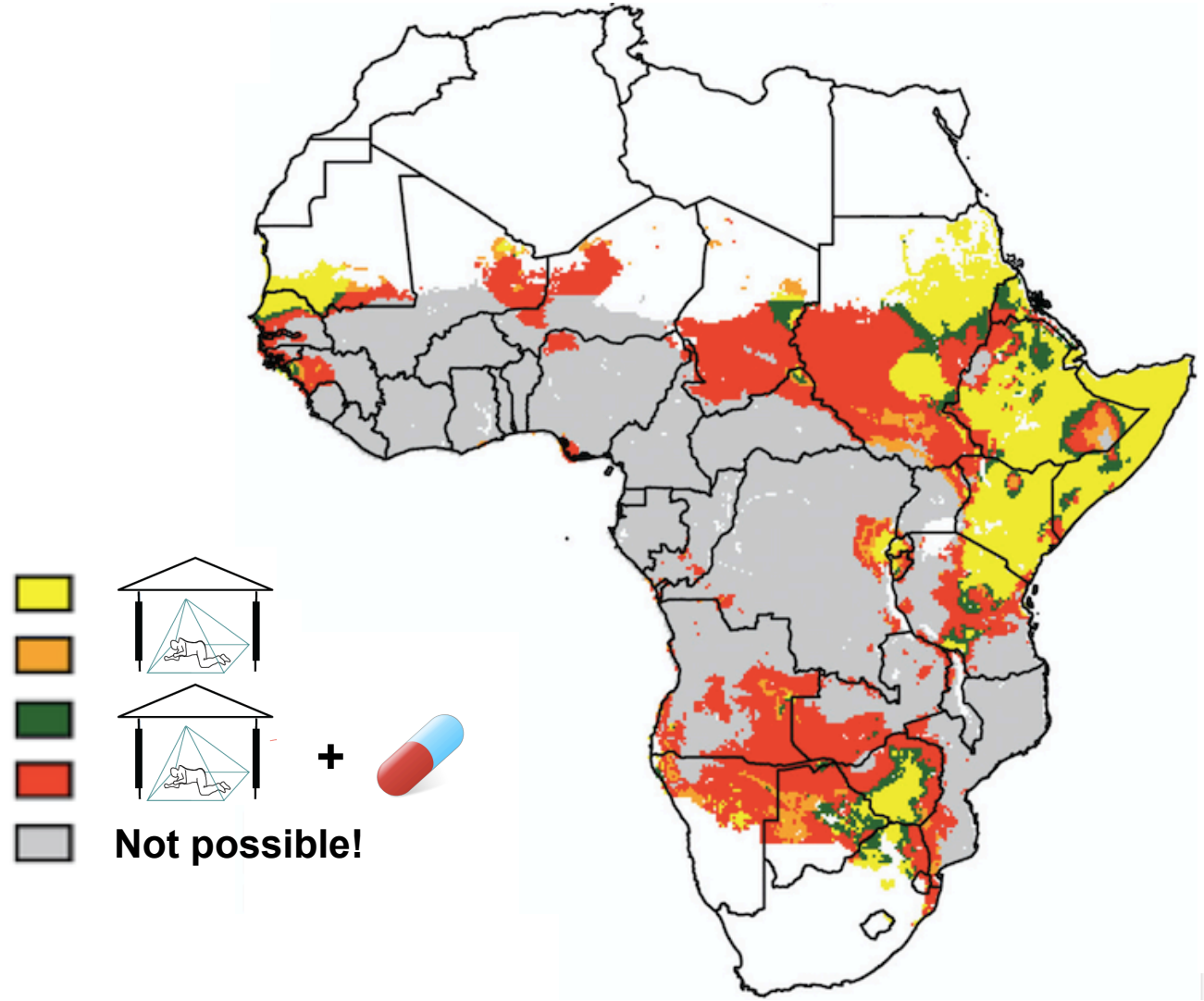
Tomas Leon

Sarafina Smith

Biyonka Liang

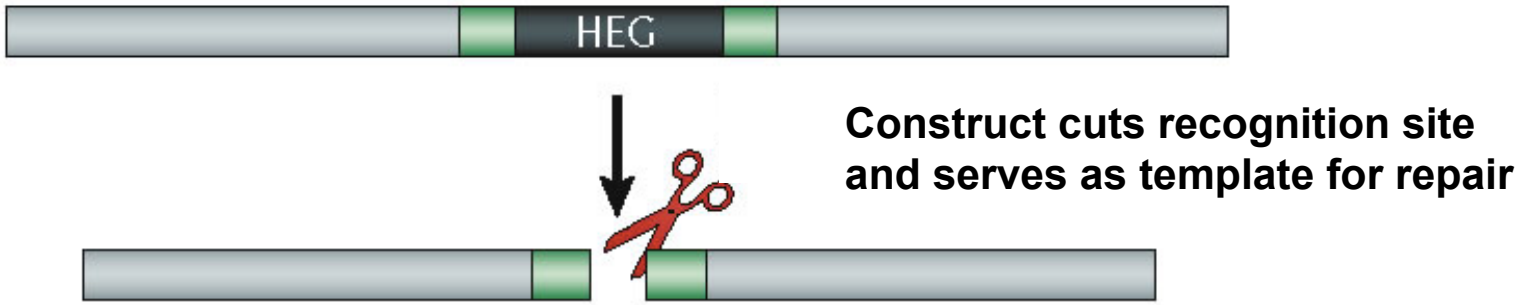
Yi Li

Optimal interventions to eliminate malaria

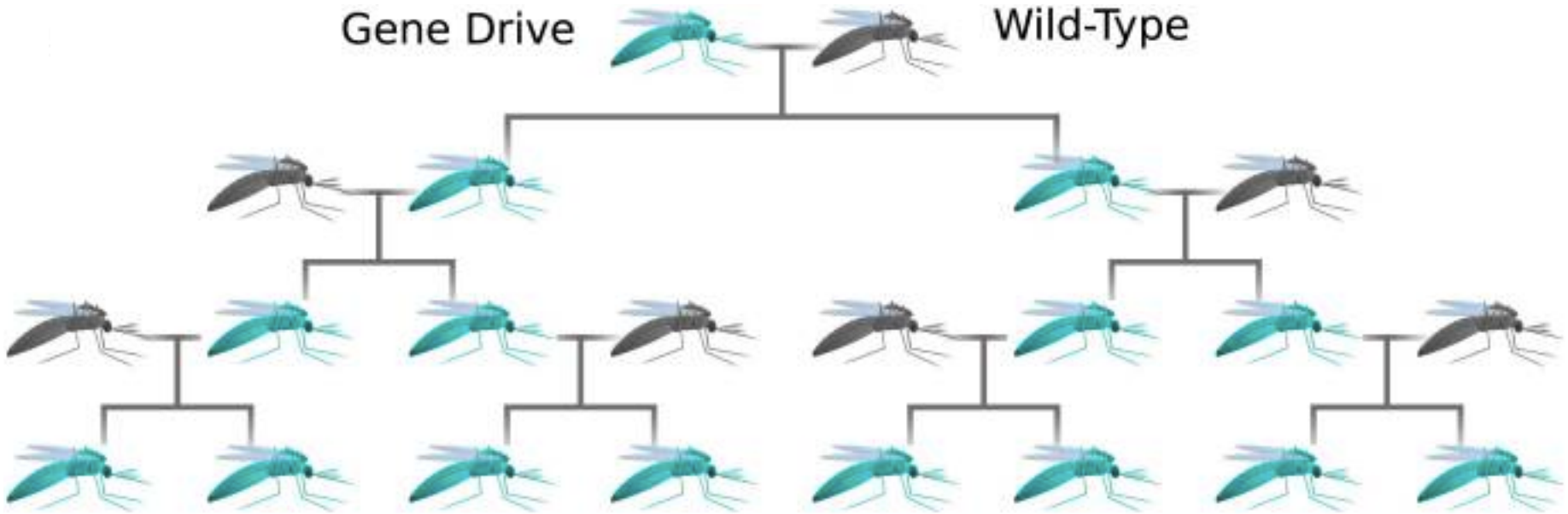


- Walker PGT, Griffin JT, Ferguson NM, Ghani AC (2016) Lancet Global Health

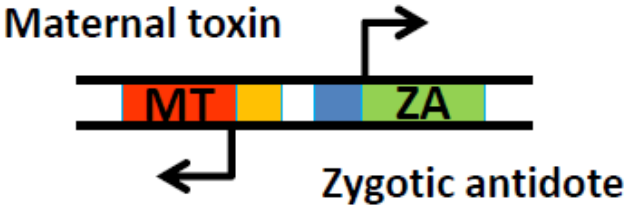
Homing-based CRISPR-mediated gene drive



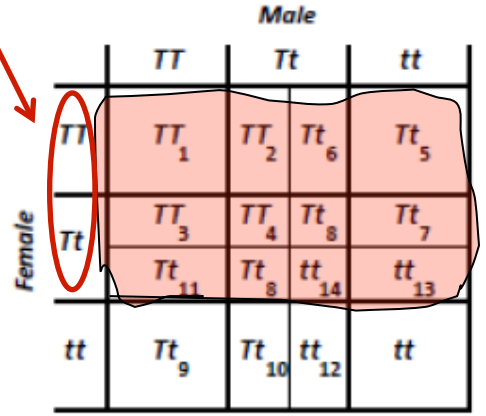
Construct cuts recognition site and serves as template for repair



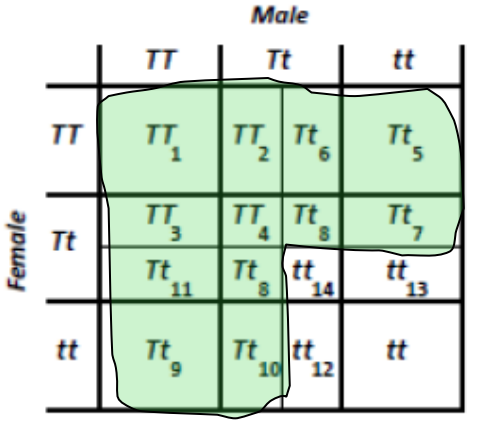
Medea & toxin-antidote based gene drive

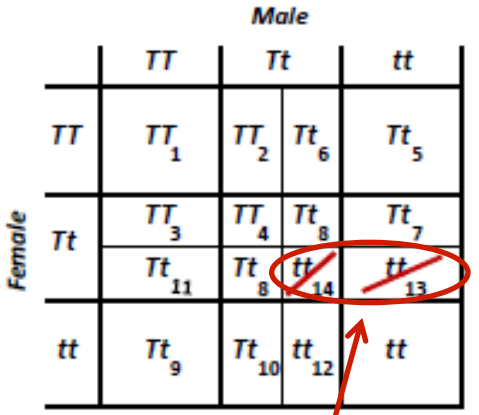


Transgenic mothers produce **toxin**



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

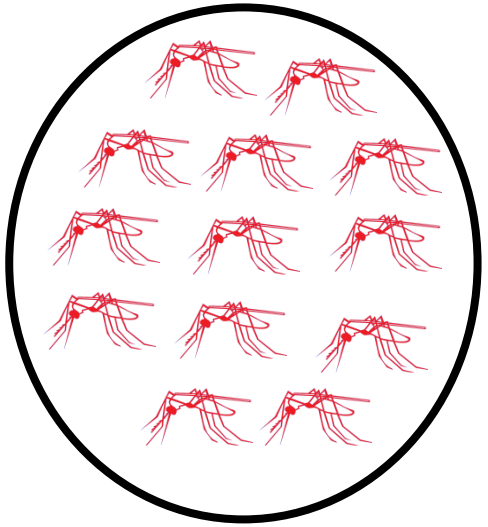
- Marshal JM, Toure MB, Traore MM, Famenini S, Taylor CE (2010) Malaria Journal 9: 128

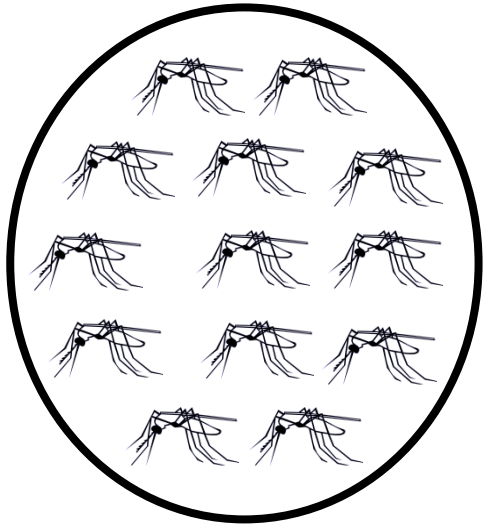
Q1. Can we conduct a confined field trial of gene-edited mosquitoes?

7 km

Banambani, Mali

Doneguebougou, Mali

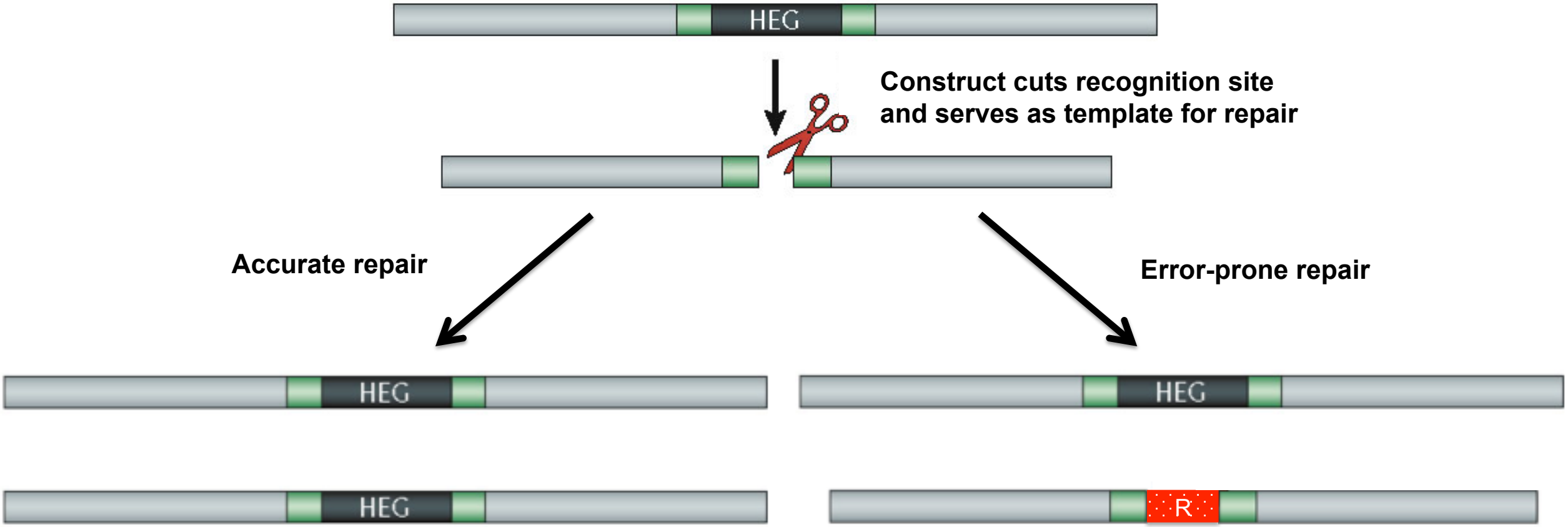




Gene-edited mosquitoes

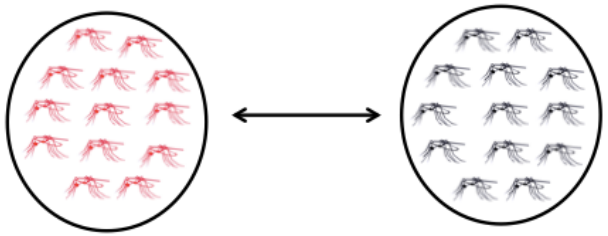
Wild-type mosquitoes

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

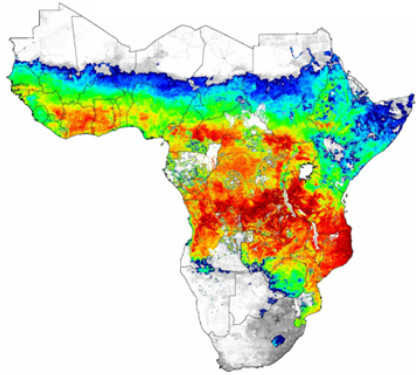


Talk outline

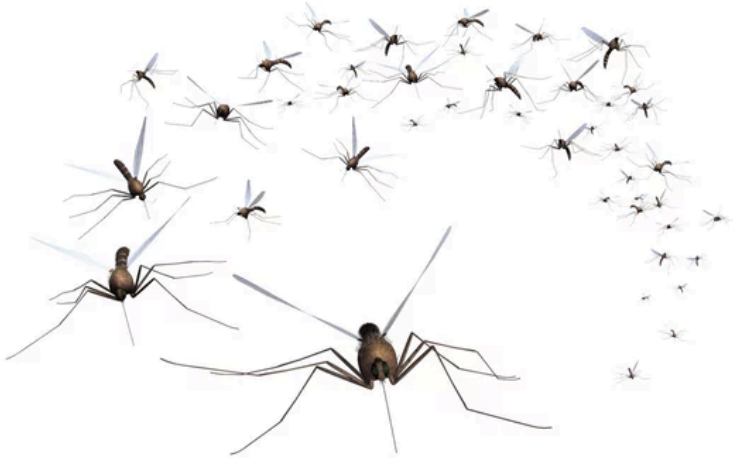
Q1. Can we conduct a confined field trial of gene-edited mosquitoes?



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?

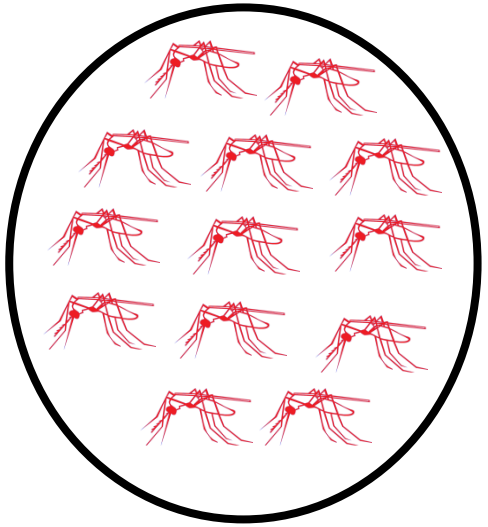


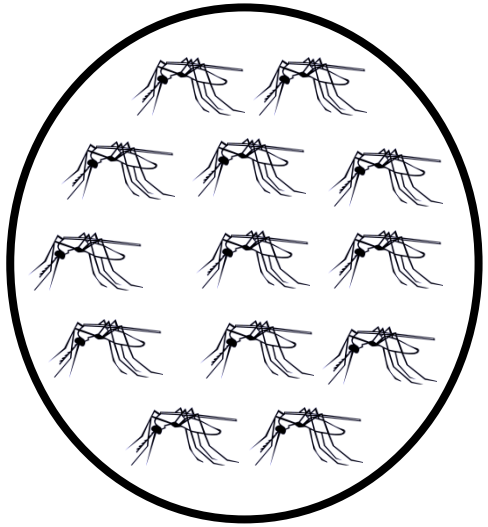
Q1. Can we conduct a confined field trial of gene-edited mosquitoes?

7 km

Banambani, Mali

Doneguebougou, Mali

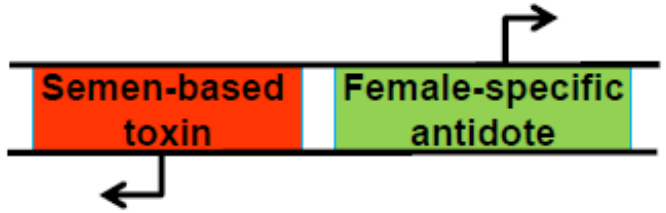




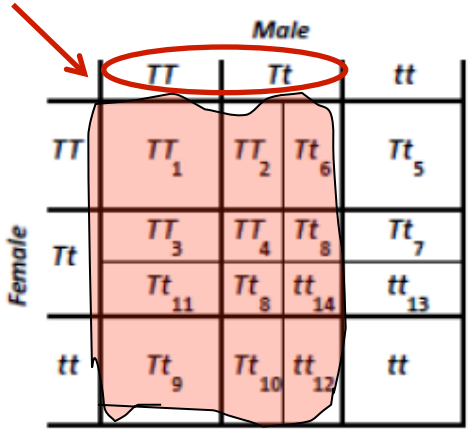
Gene-edited mosquitoes

Wild-type mosquitoes

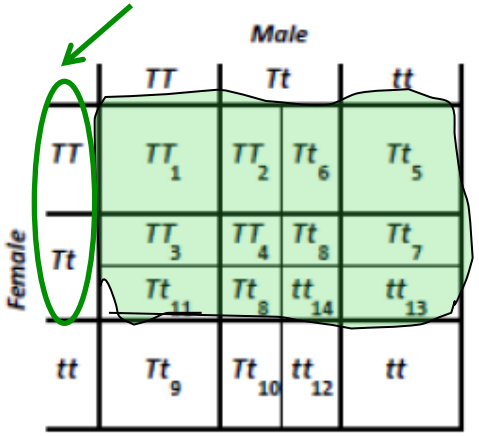
Semele & threshold-dependent gene drive

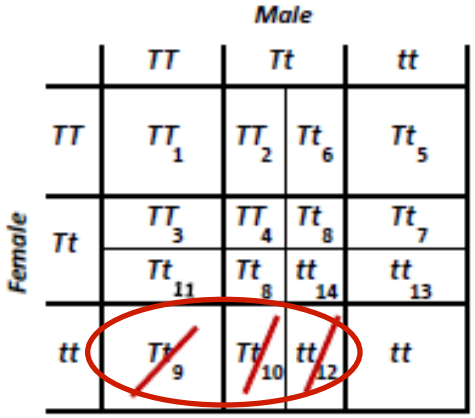


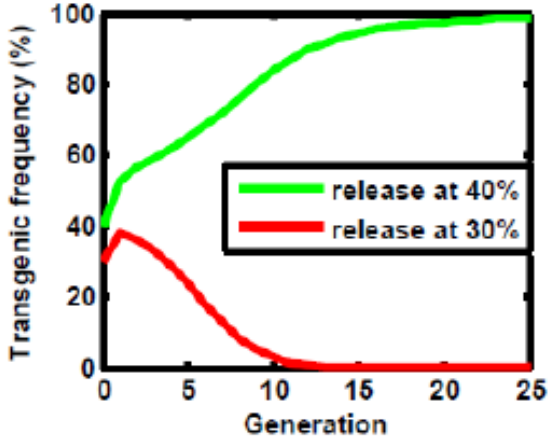
Transgenic males produce **toxic semen**



Transgenic females produce **antidote**







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

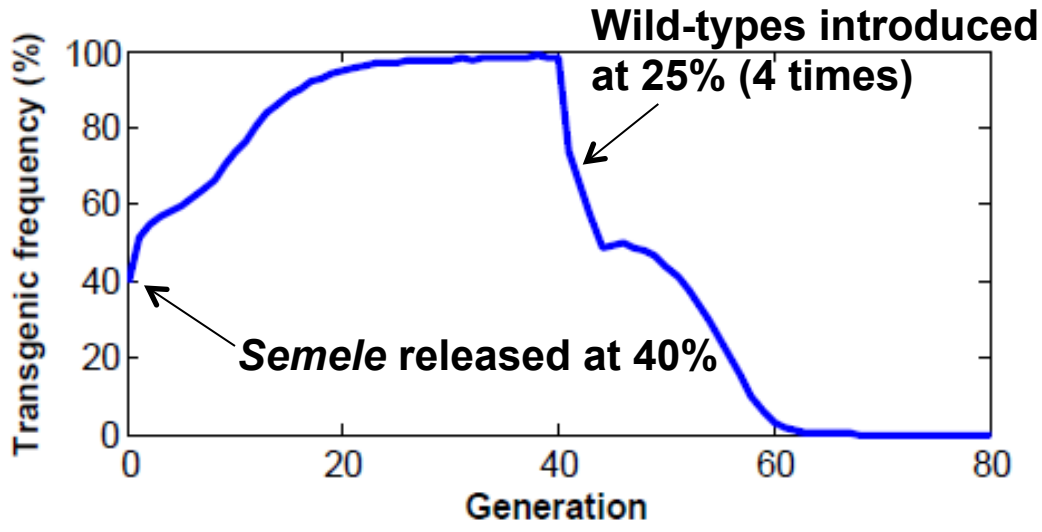
- Marshall JM, Pittman GW, Buchman A, Hay BA (2011) Genetics 187: 535-551.

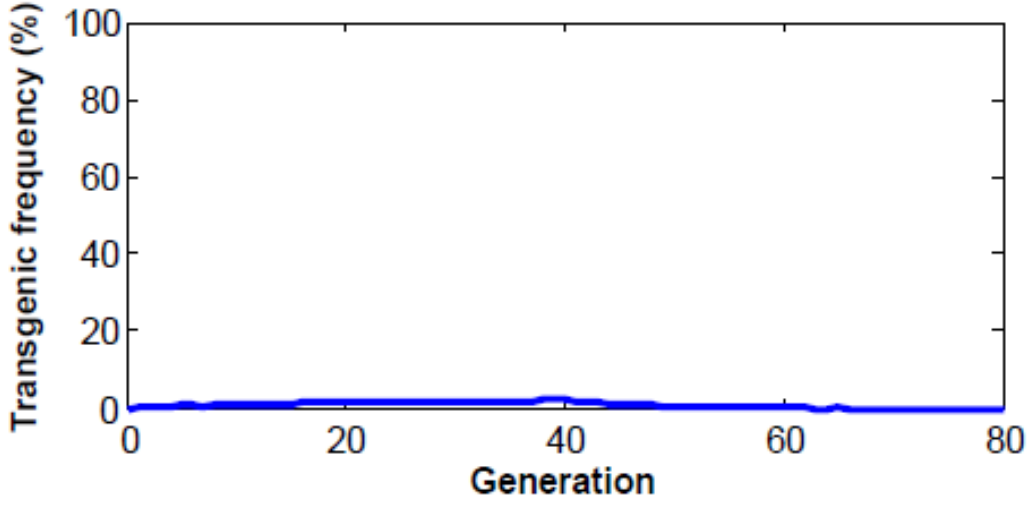
Releases of *Semele* are confineable and reversible

Banambani, Mali

Migration rate = ~1% / generation

Doneguebougou, Mali

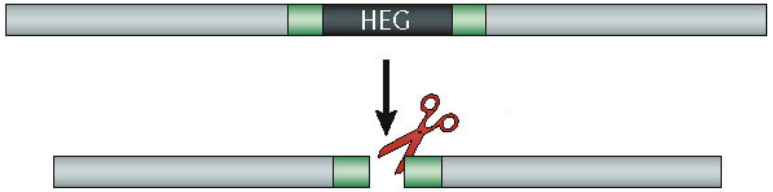




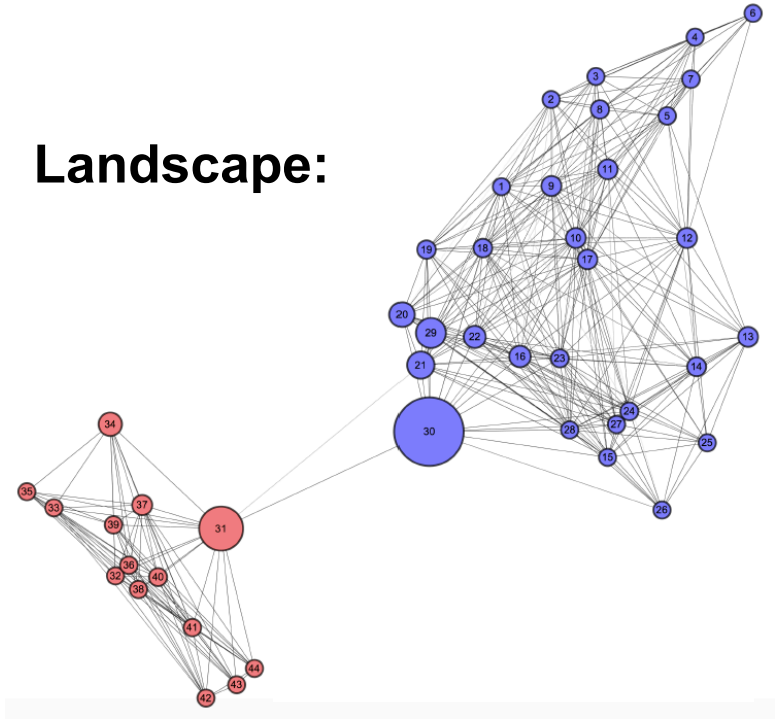
- Marshall JM, Pittman GW, Buchman A, Hay BA (2011) Genetics 187: 535-551.

Integrated spatially-explicit, ecological model

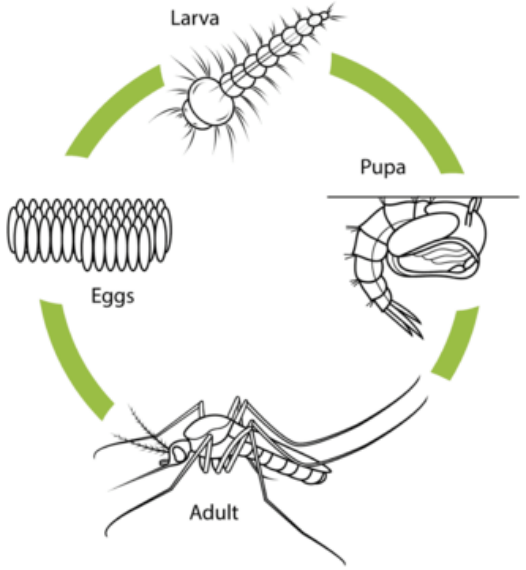
Inheritance pattern:



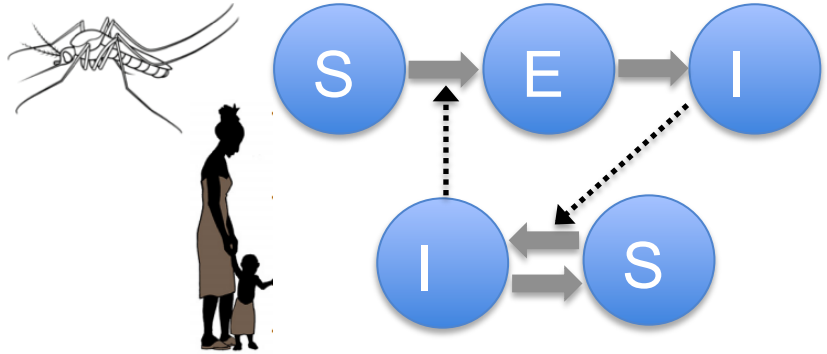
Landscape:



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**

Download **ZIP File**

Download **TAR Ball**

Developed in John Marshall's Lab by:

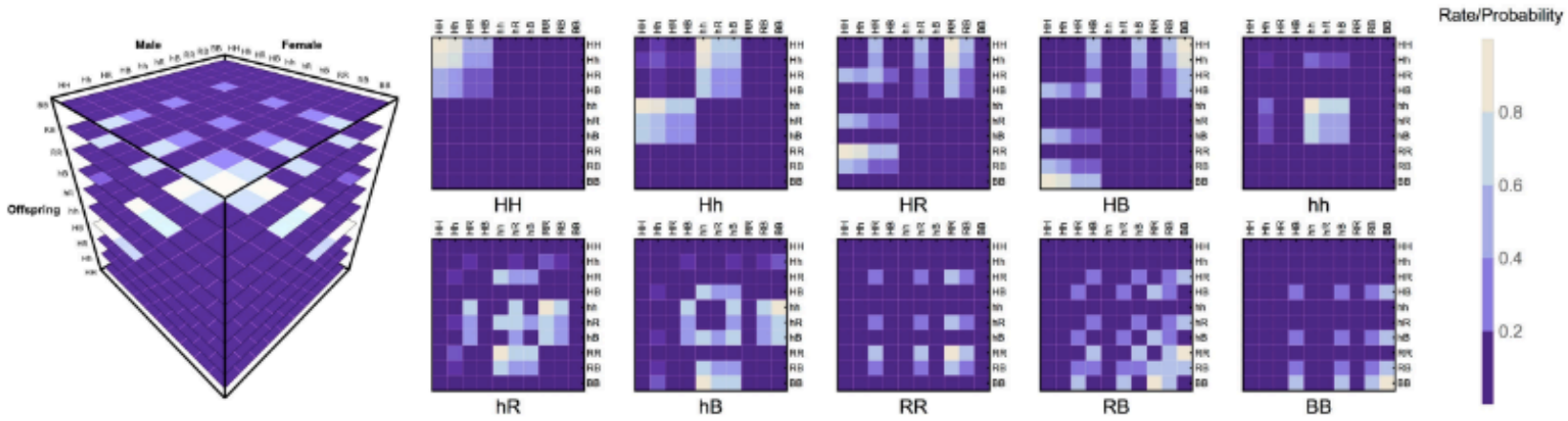
-Lead: Héctor M. Sánchez C.
-Core Dev: Sean L. Wu,Jared Bennett
-Spatial Analysis: Biyonka Liang, Sarafina Smith, Sabrina Wong
-Movement Kernels: Partow Imani

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

Mosquito Gene Drive Explorer

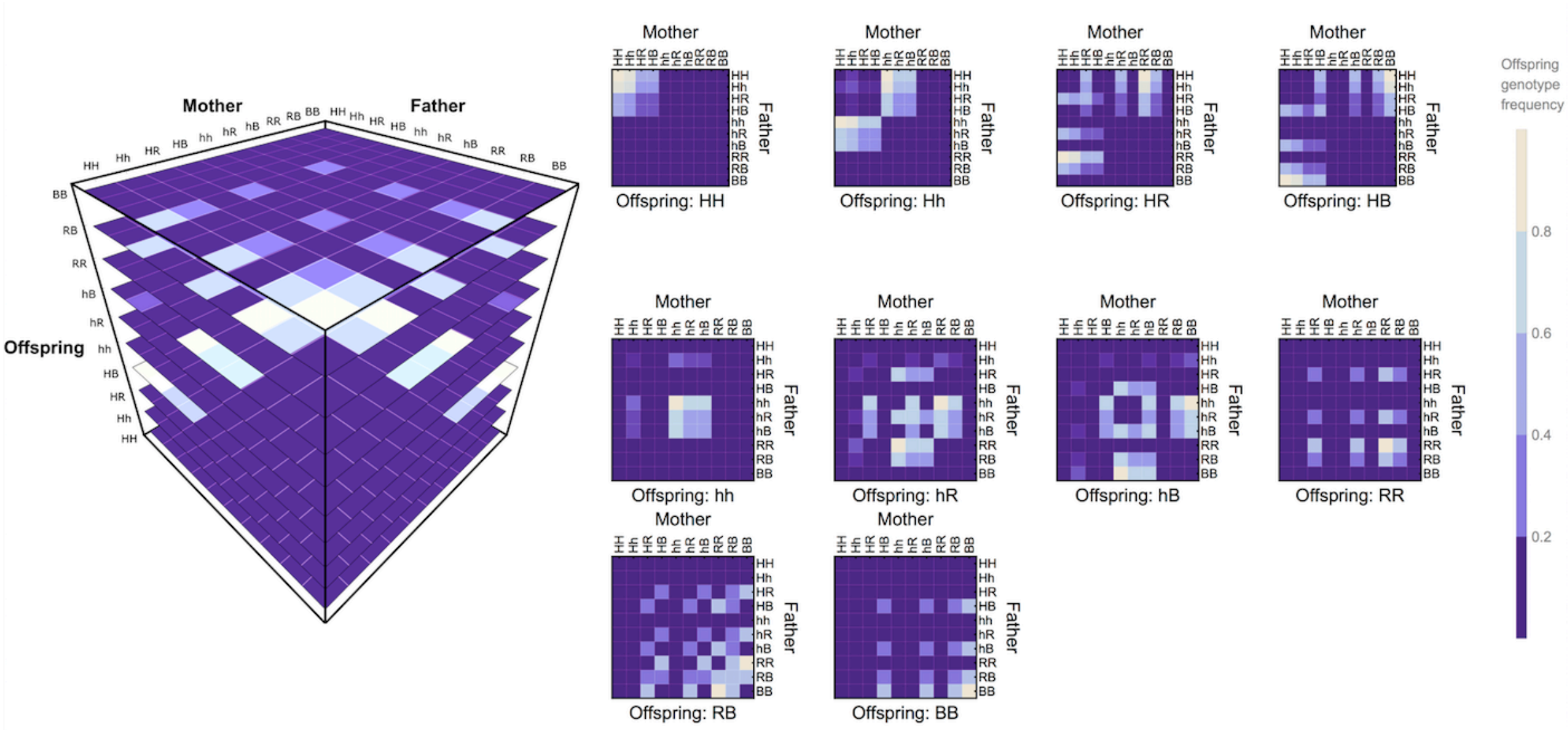
Brief Description

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.

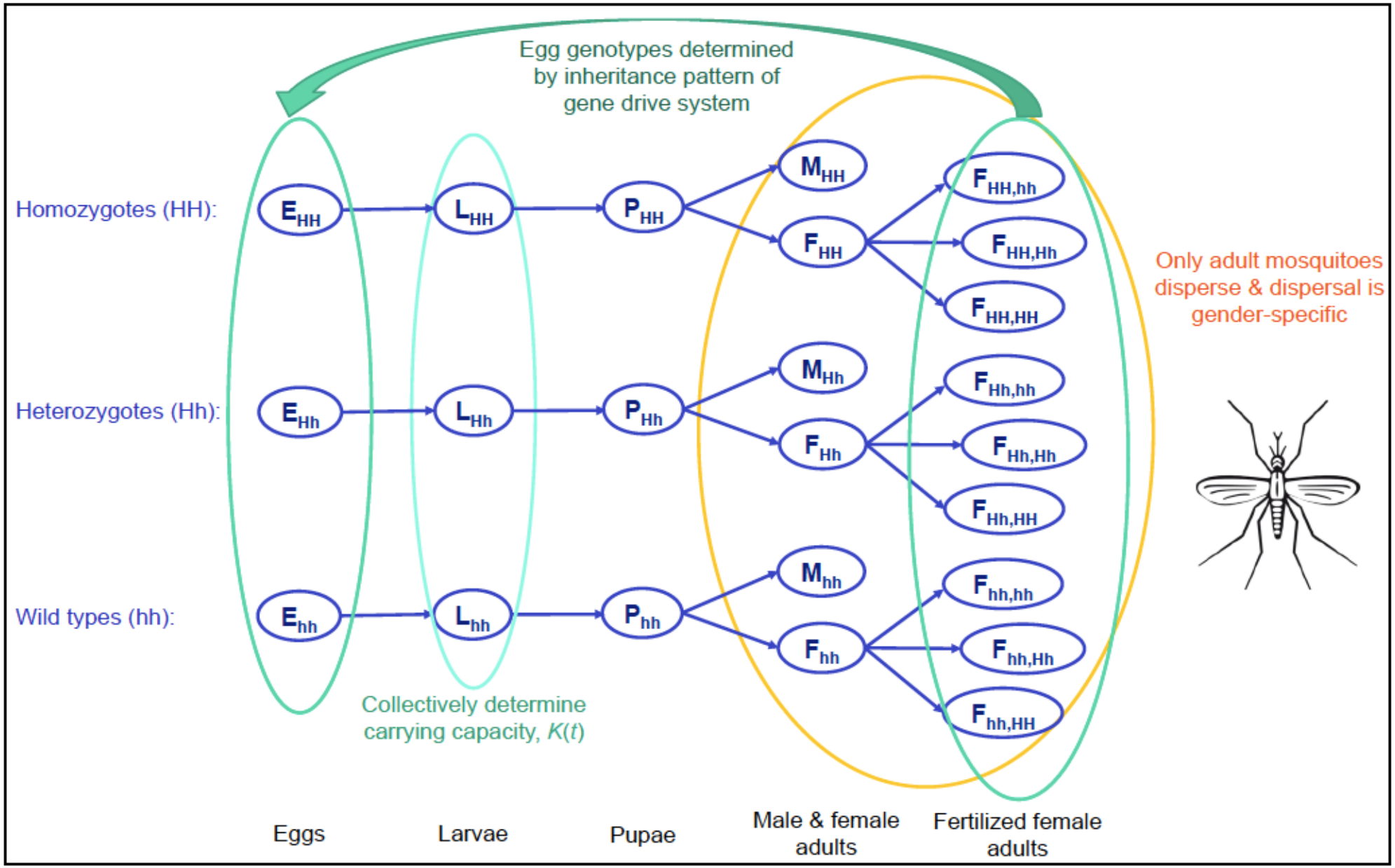


Demonstration

MGDrivE: Inheritance module



MGDrivE: Ecology module



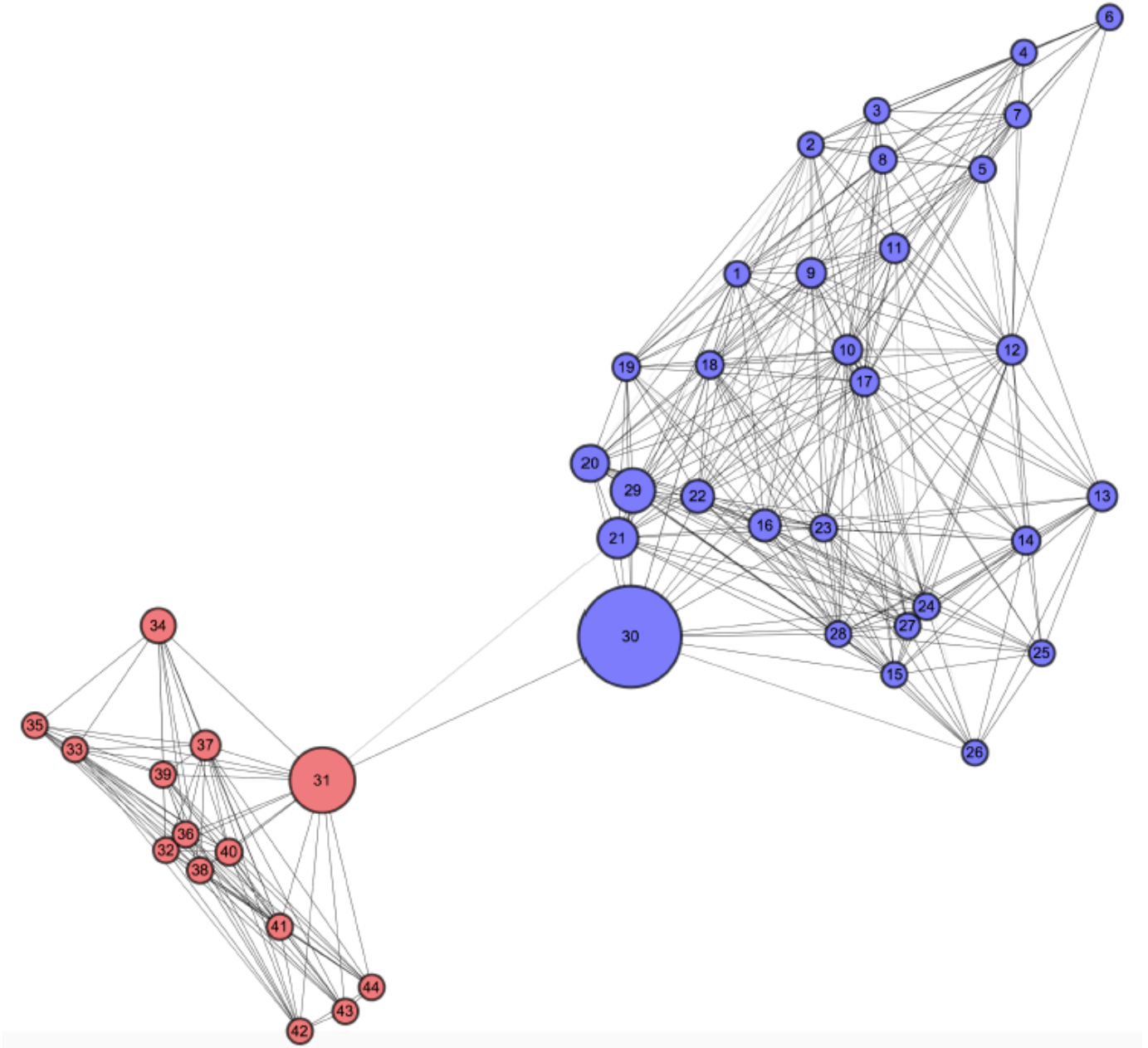
Mosquito vector species of interest

Aedes aegypti

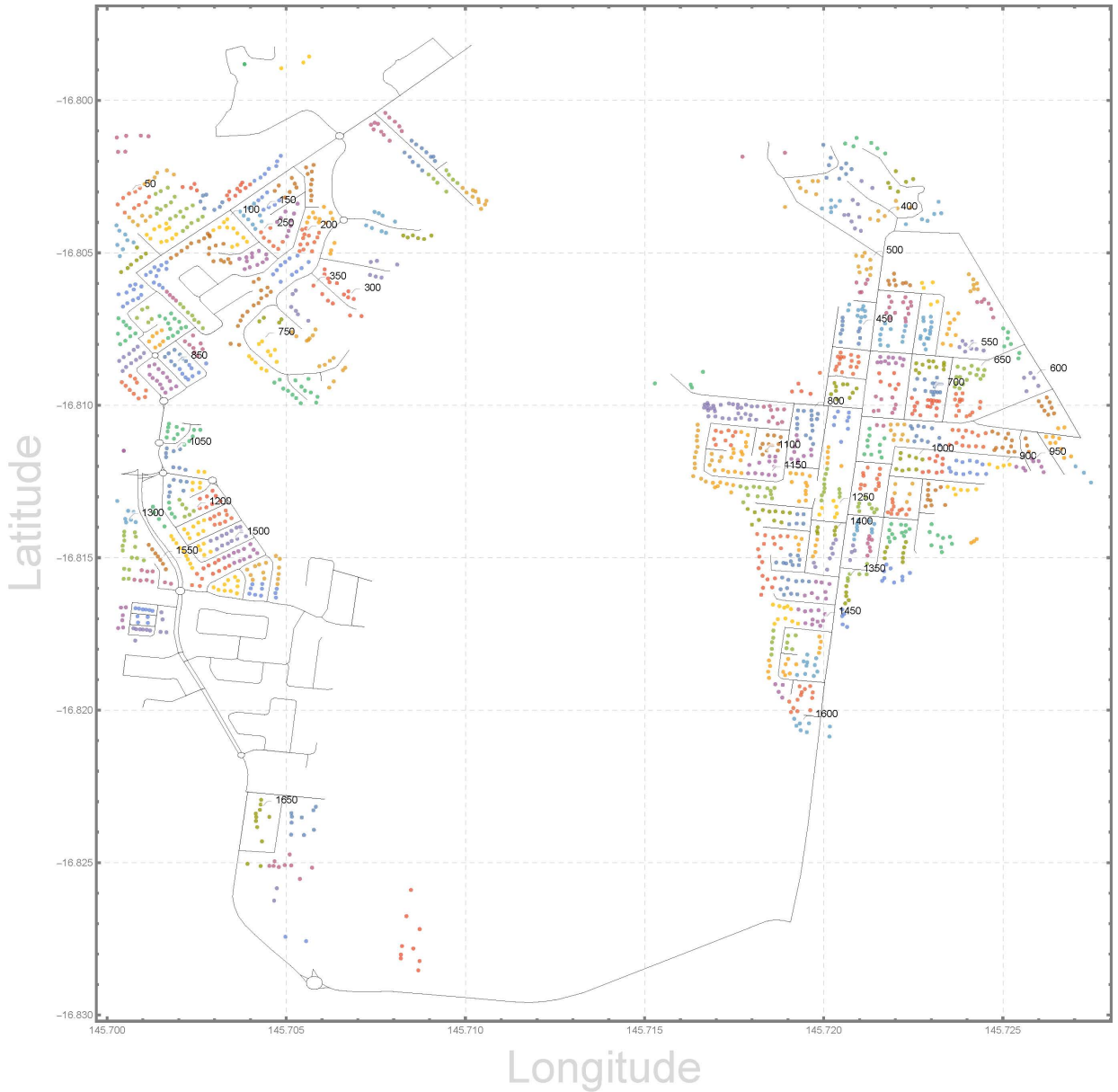
Anopheles gambiae

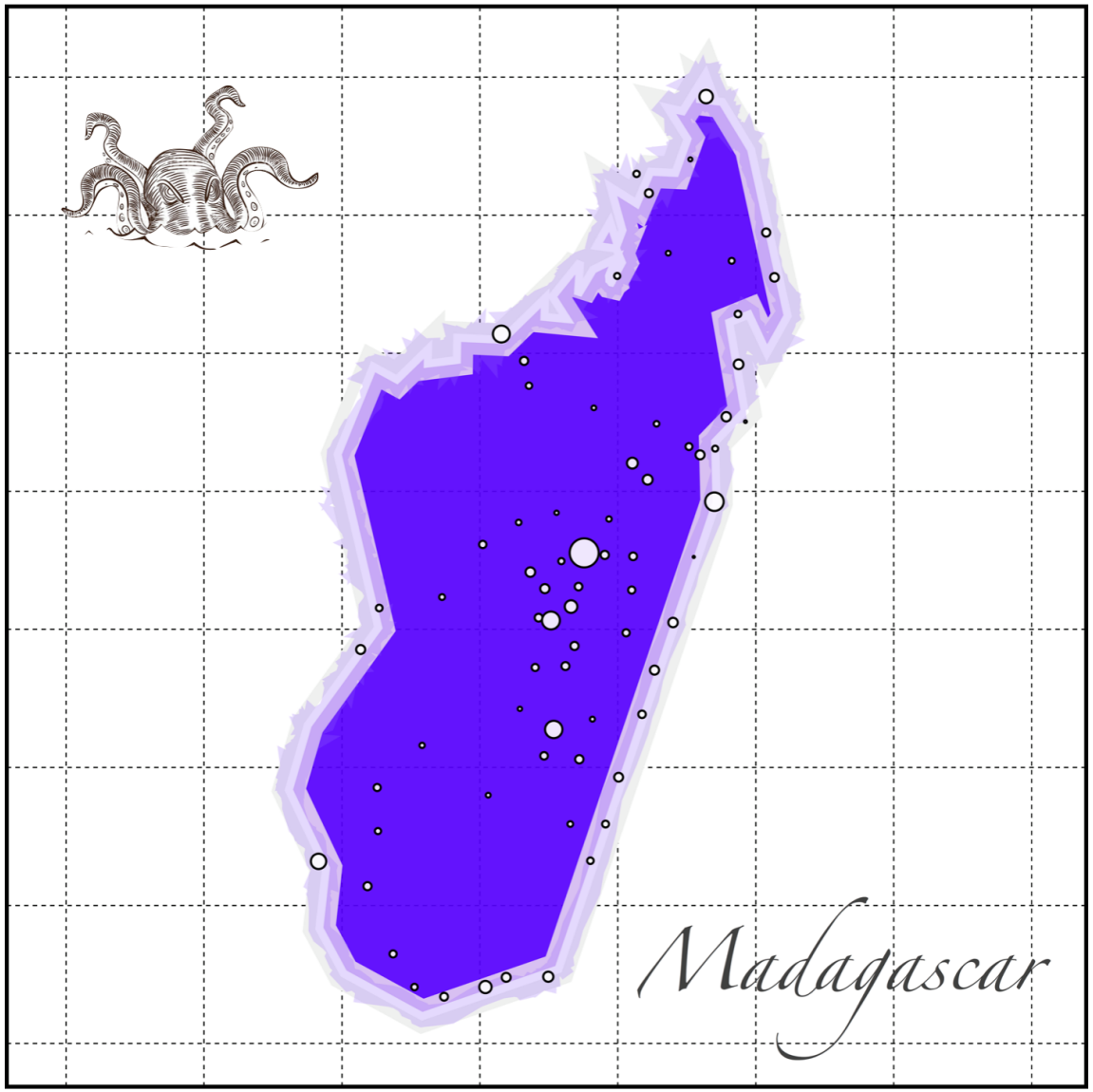
Anopheles stephensi

MGDrivE: Landscape module

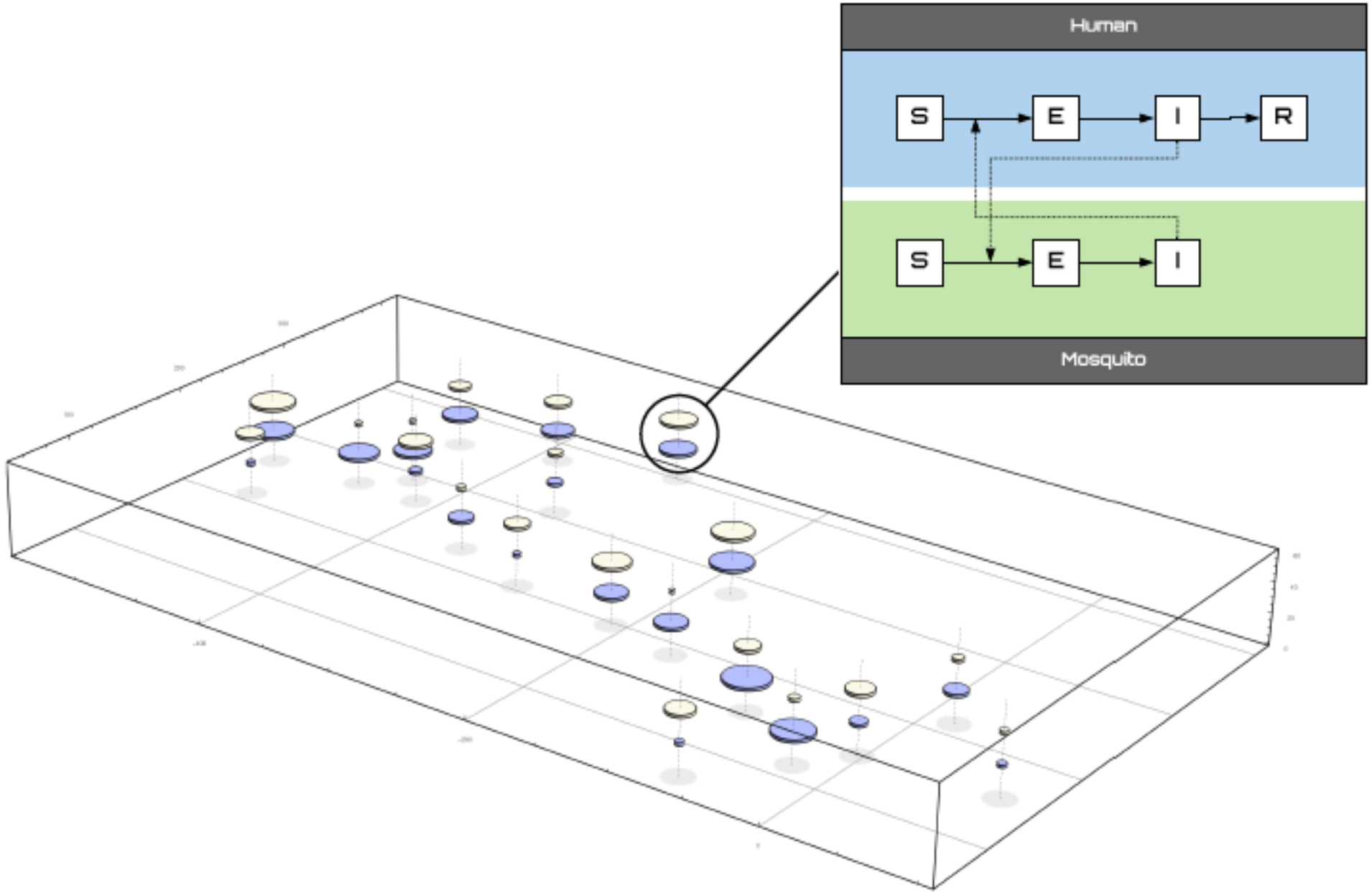


Landscapes of interest for mosquito vectors

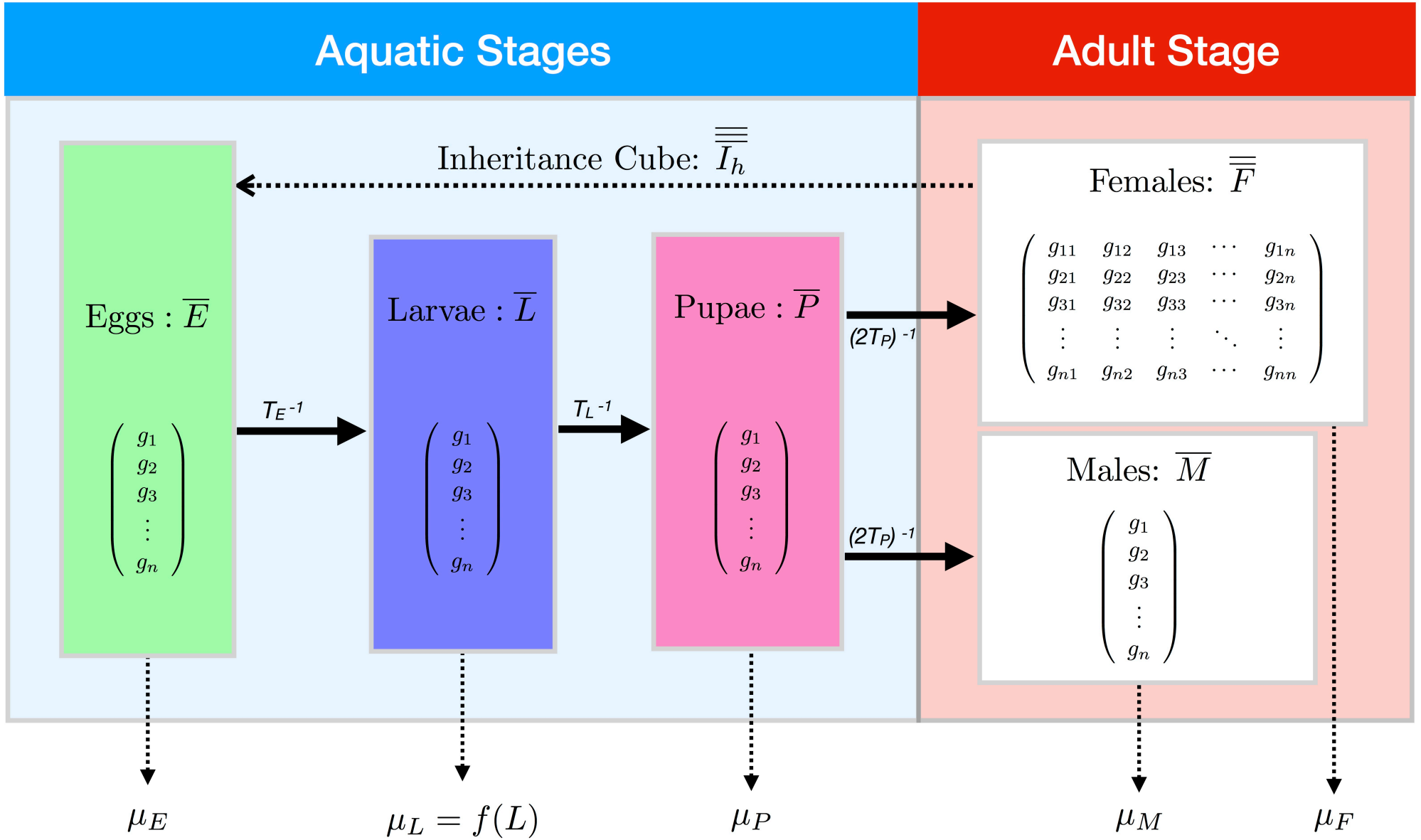




Epidemiological extension for mosquito-borne diseases



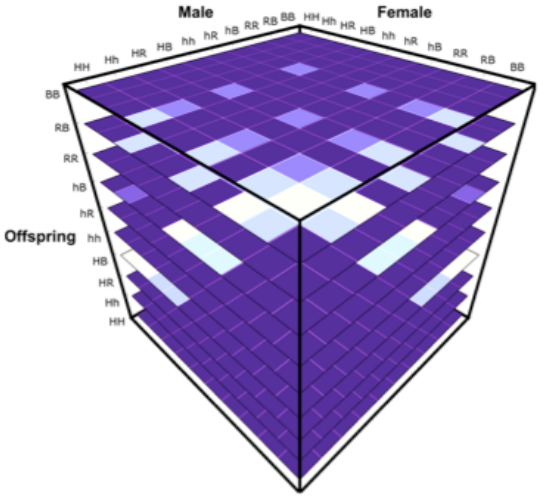
MGDrivE: Tensor modeling framework



MGDrivE: Tensor modeling framework

Inheritance & oviposition:

$$\overline{O(T_x)} = \sum_{j=1}^{n} \left(\left((\beta * \overline{s} * \overline{\overline{Af_{[t-T_x]}}}) * \overline{\overline{Ih}} \right) * \Lambda \right)^{\top}_{ij}$$

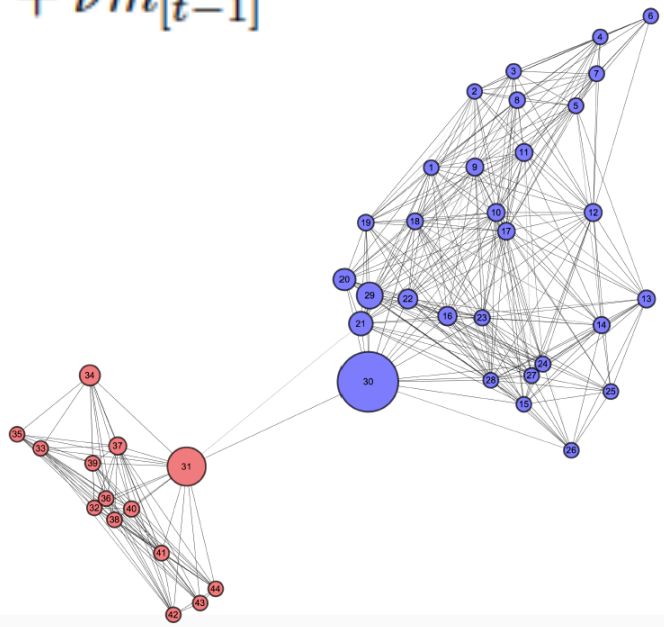


Adult male survival & development:

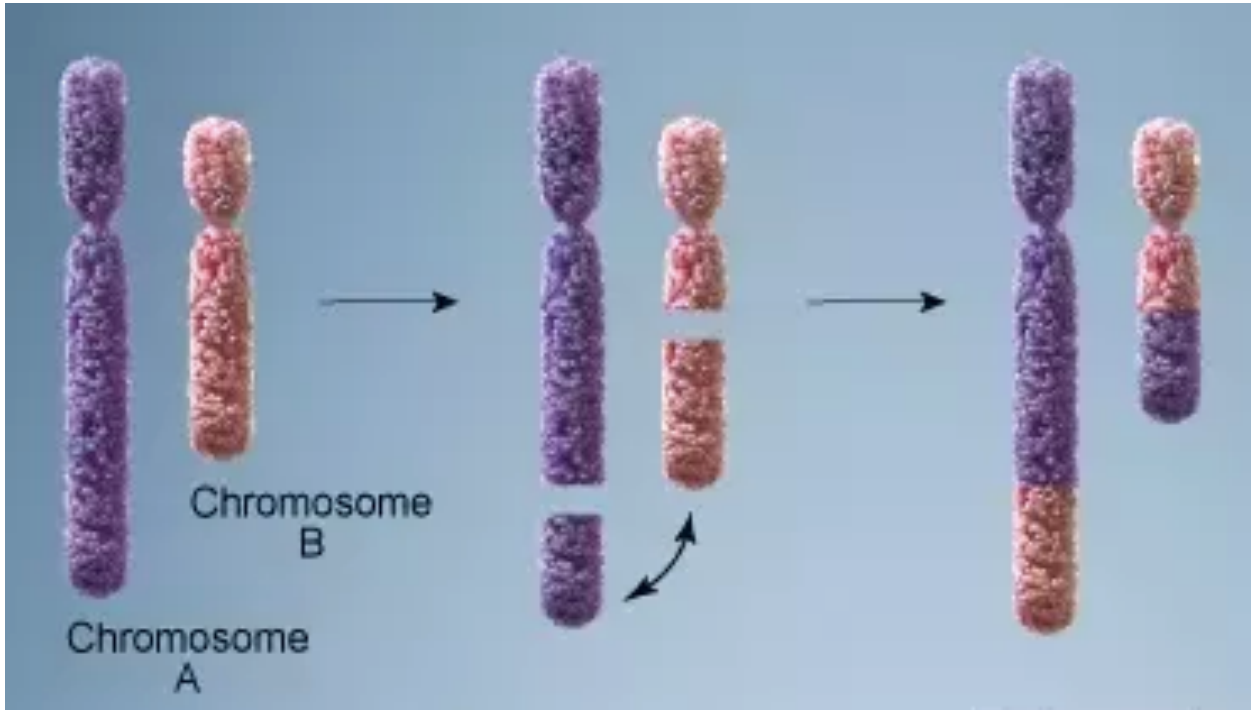
$$\overline{Am_{[t]}} = \overline{Am_{[t-1]}} * (1 - \mu_{ad}) * \overline{\omega_m} + (1 - \overline{\phi}) * \overline{E'} + \overline{\nu m_{[t-1]}}$$

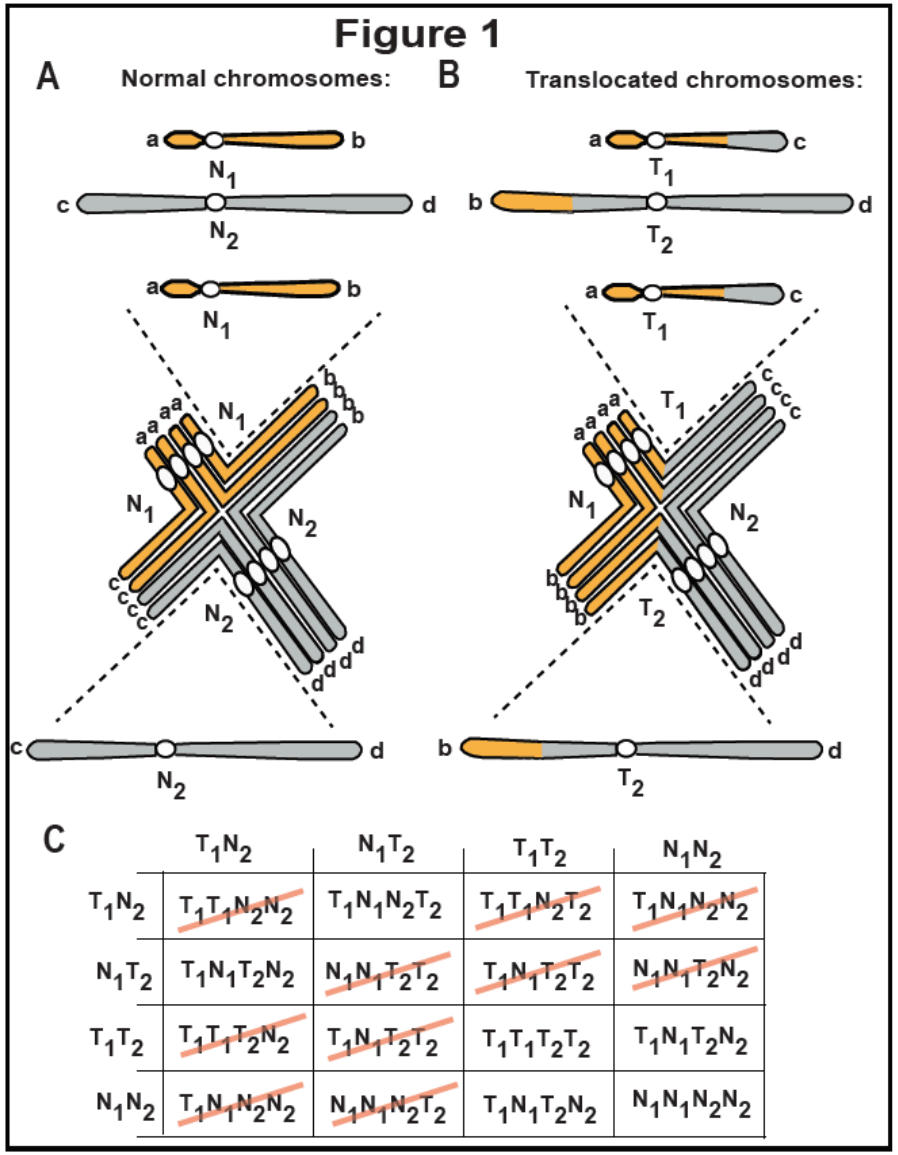
Adult male migration:

$$\overline{Am^i_{(t)}} = \sum \overline{A^j_m} \otimes \overline{\overline{\tau m_{[t-1]}}}$$



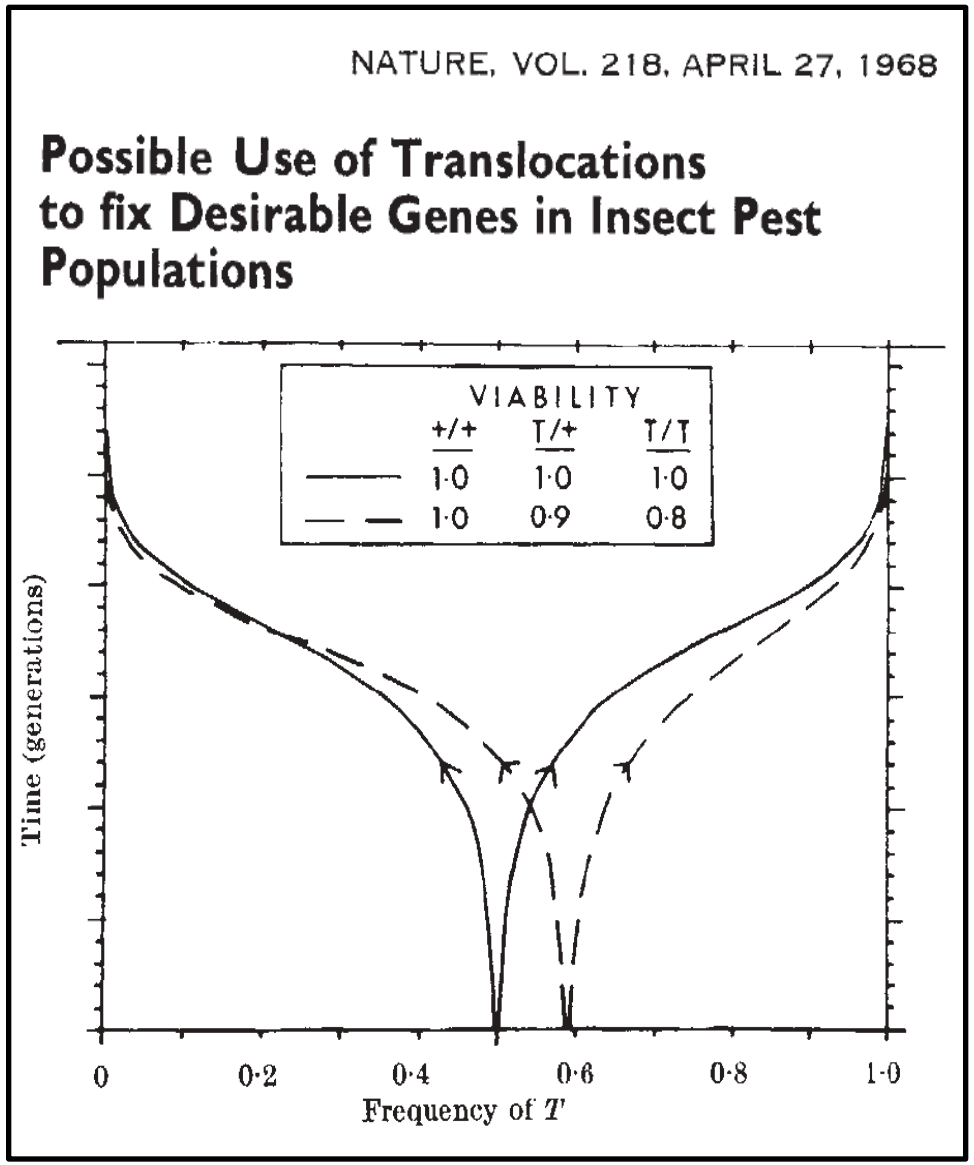
Reciprocal chromosomal translocations





	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	~~$T_1N_1N_2N_2$~~	~~$N_1N_1N_2T_2$~~	$T_1N_1T_2N_2$	$N_1N_1N_2N_2$

Reciprocal chromosomal translocations



NATURE, VOL. 218, APRIL 27, 1968

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

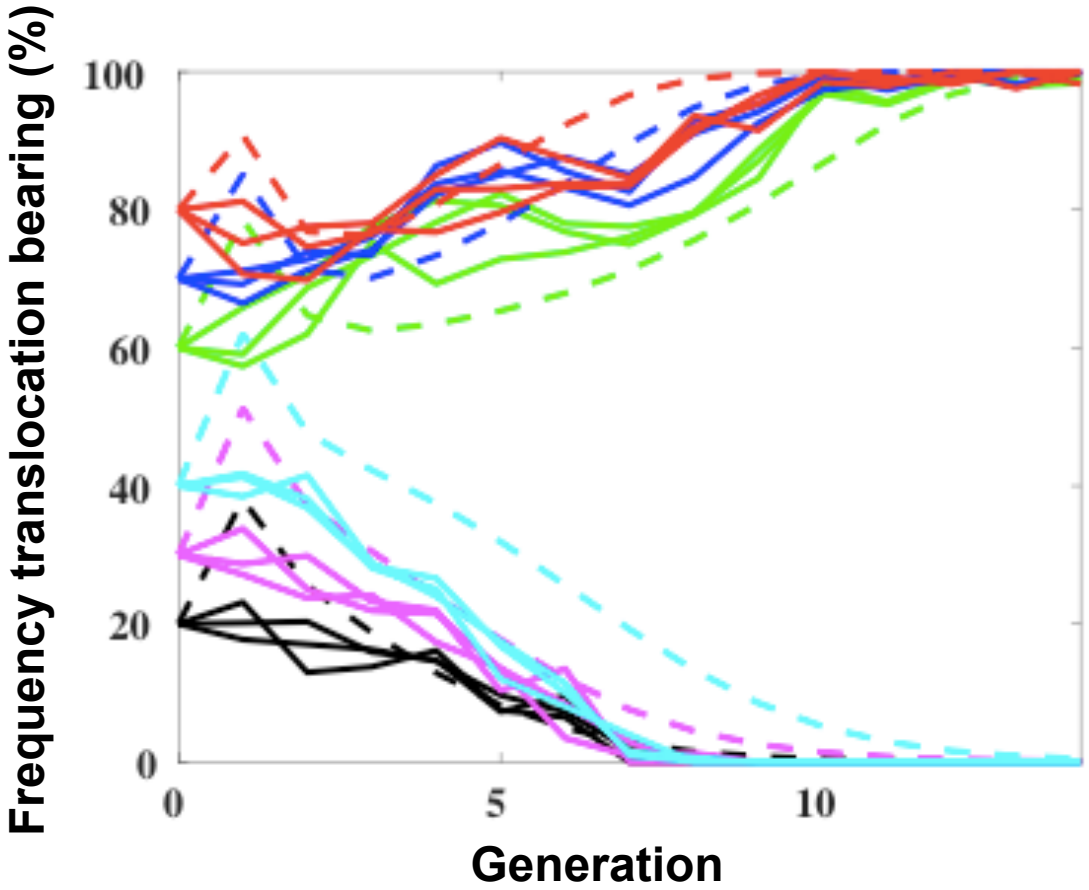
VIABILITY

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

Time (generations)

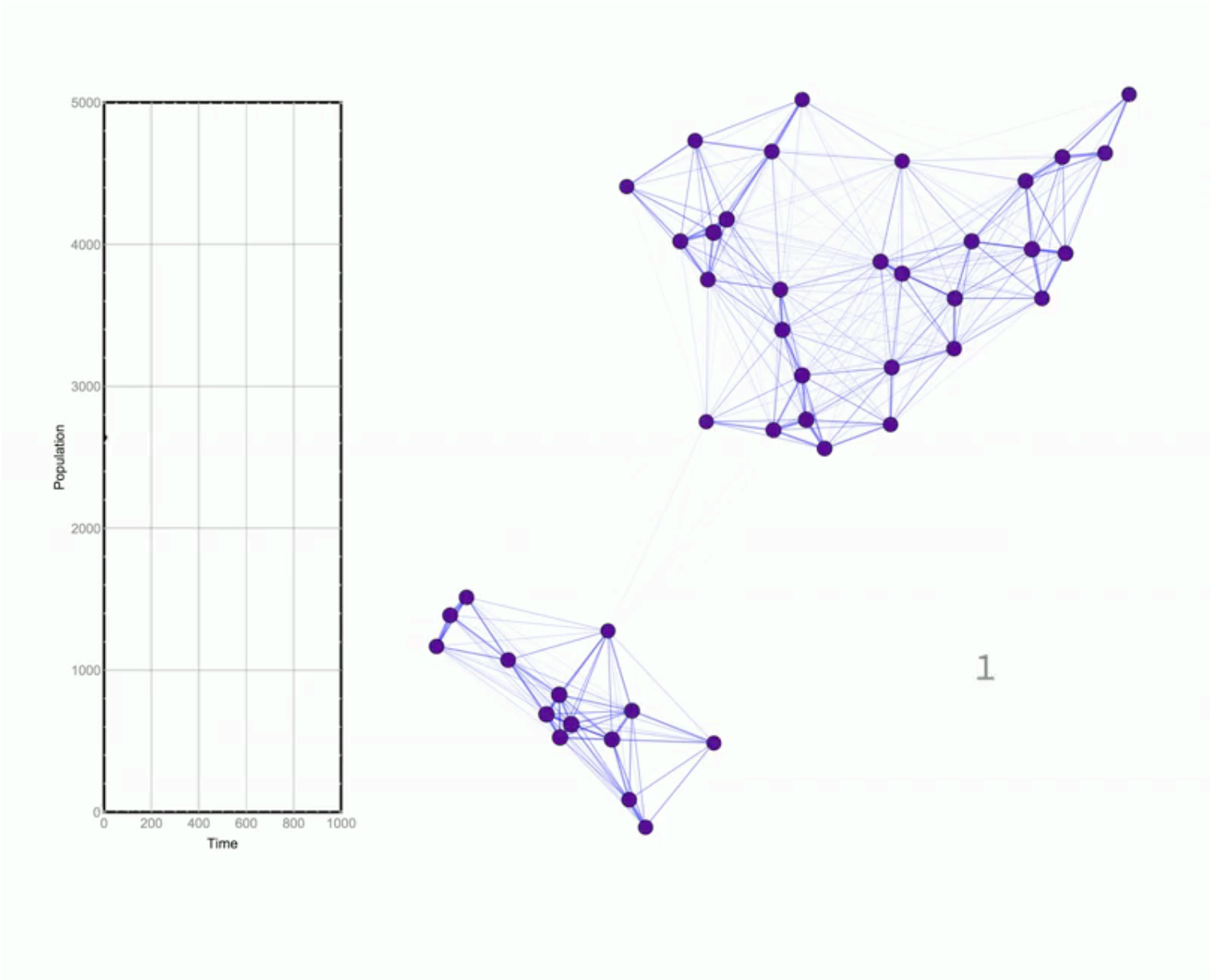
0 0·2 0·4 0·6 0·8 1·0

Frequency of *T*

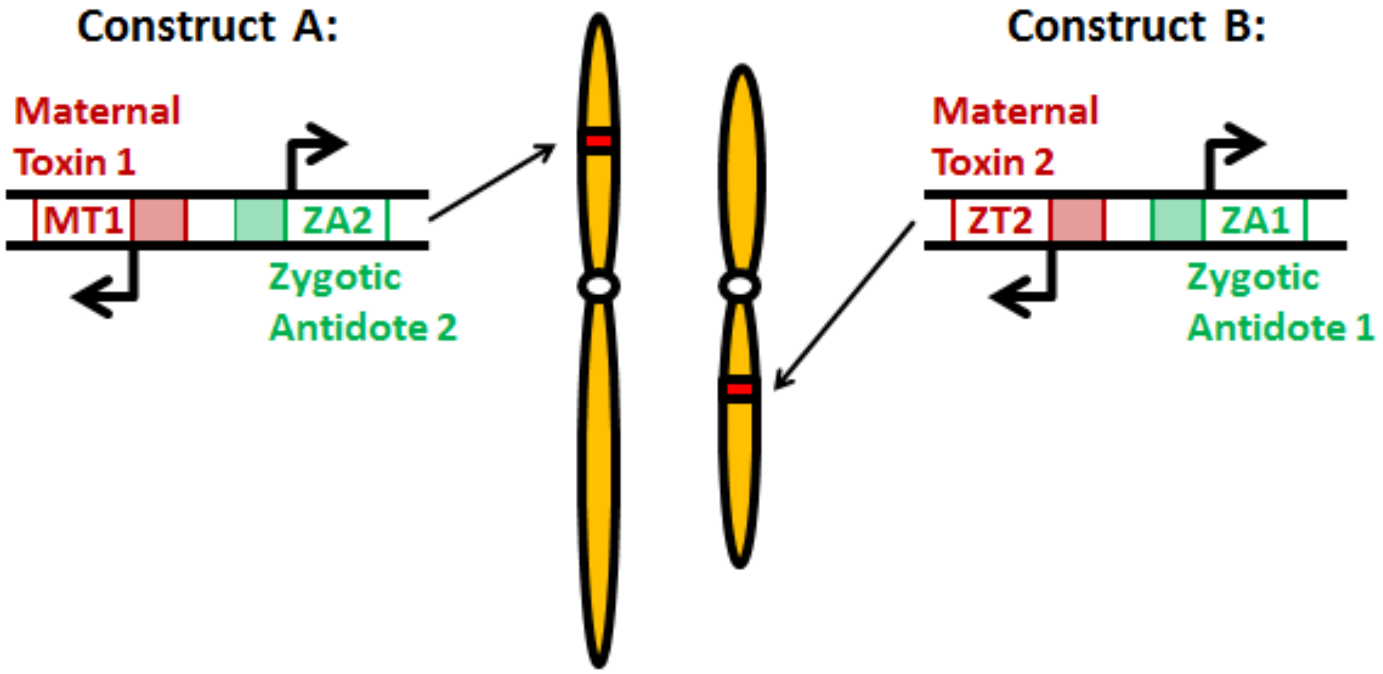


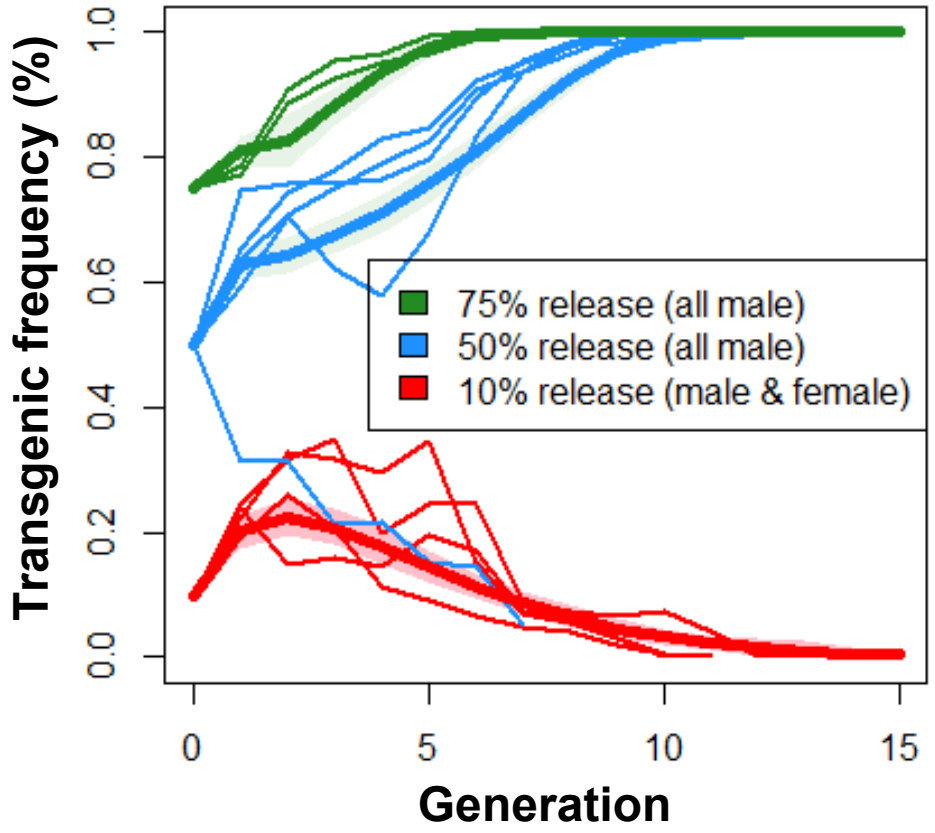
- Curtis CF (1968) Nature 218: 368-369
- Buchman A, Ivy T, Marshall JM, Akbari OS, Hay BA (2018) ACS Synthetic Biology

MGDrivE: Translocations with remediation



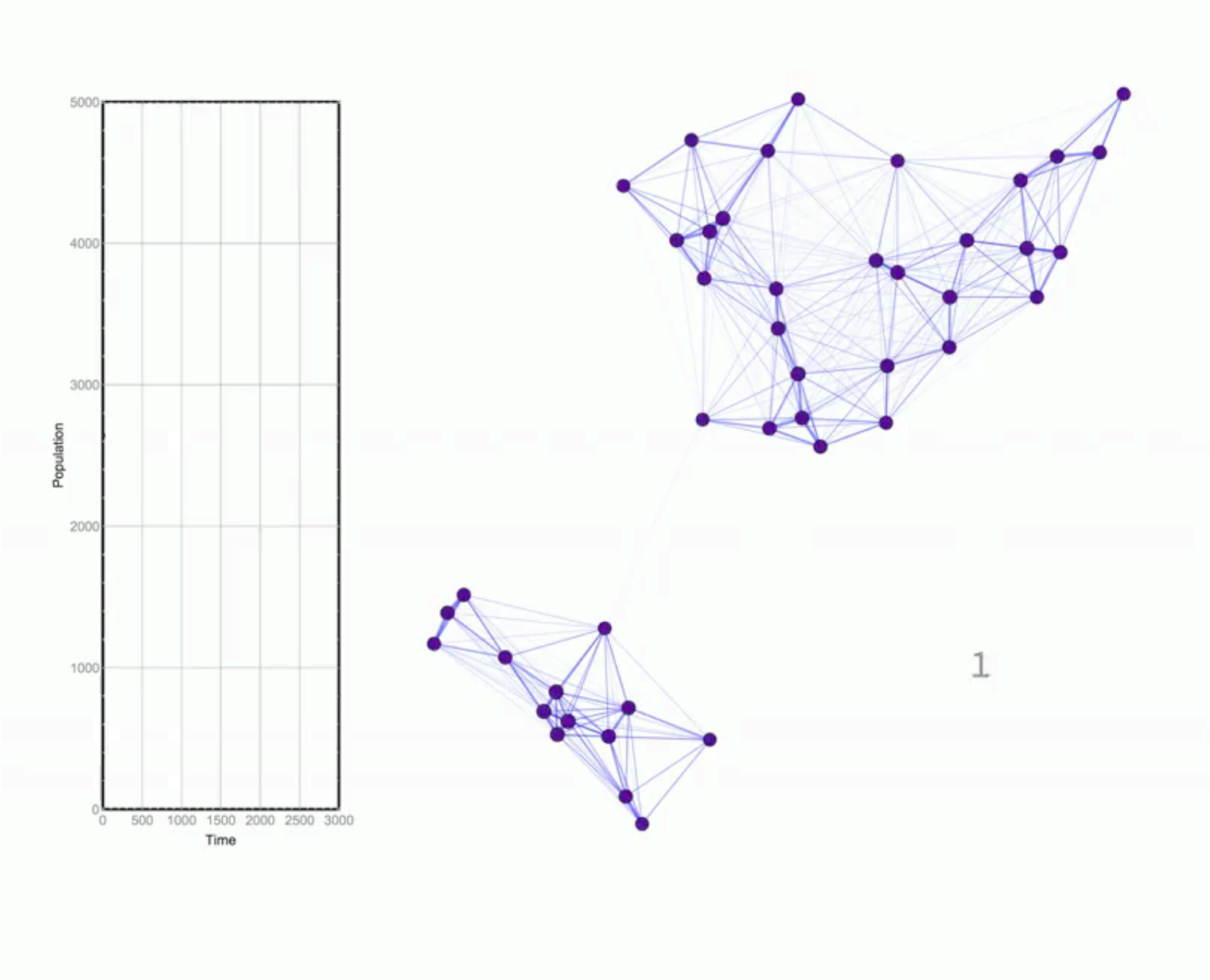
Toxin-antidote-based underdominance



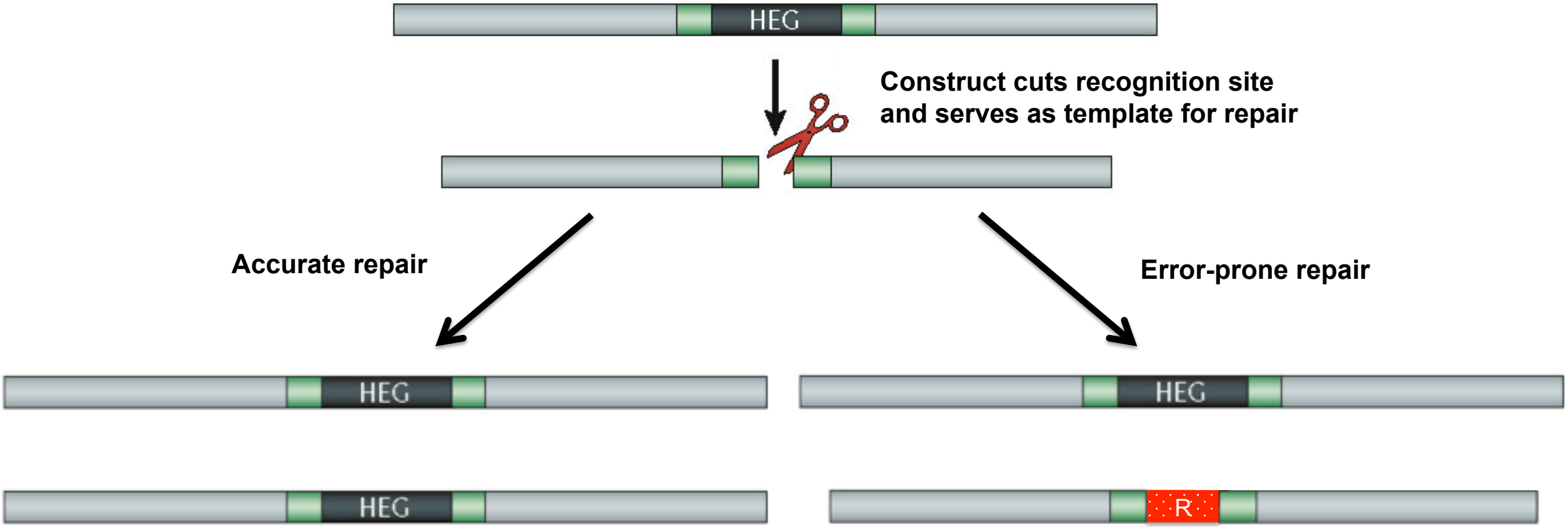


- Akbari OS, Matzen KD, Marshall JM, Huang H *et al.* (2013) Current Biology

MGDrivE: Toxin-antidote-based underdominance



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

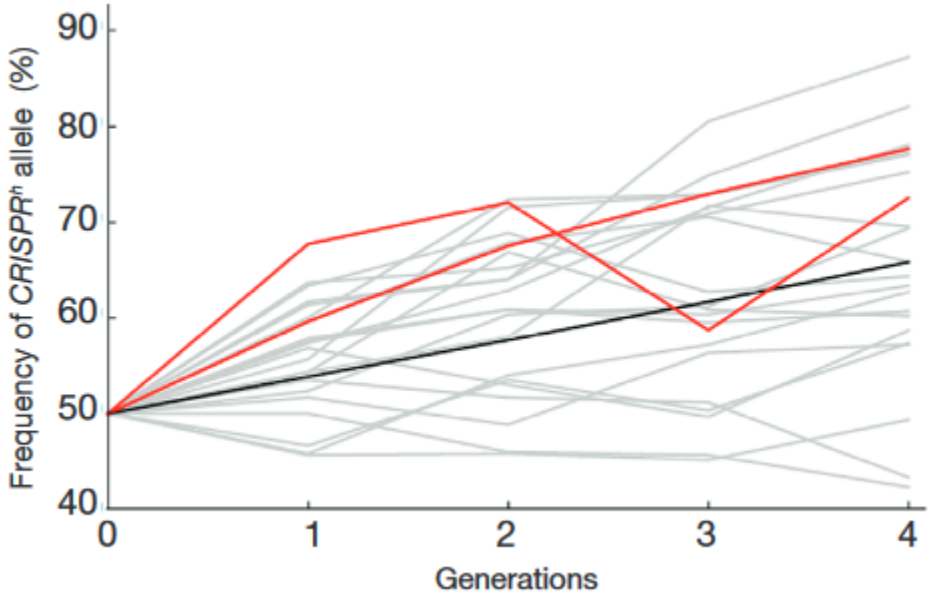


Homing-based gene drive targeting a female fertility gene & resistant allele generation

LETTERS

nature biotechnology

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



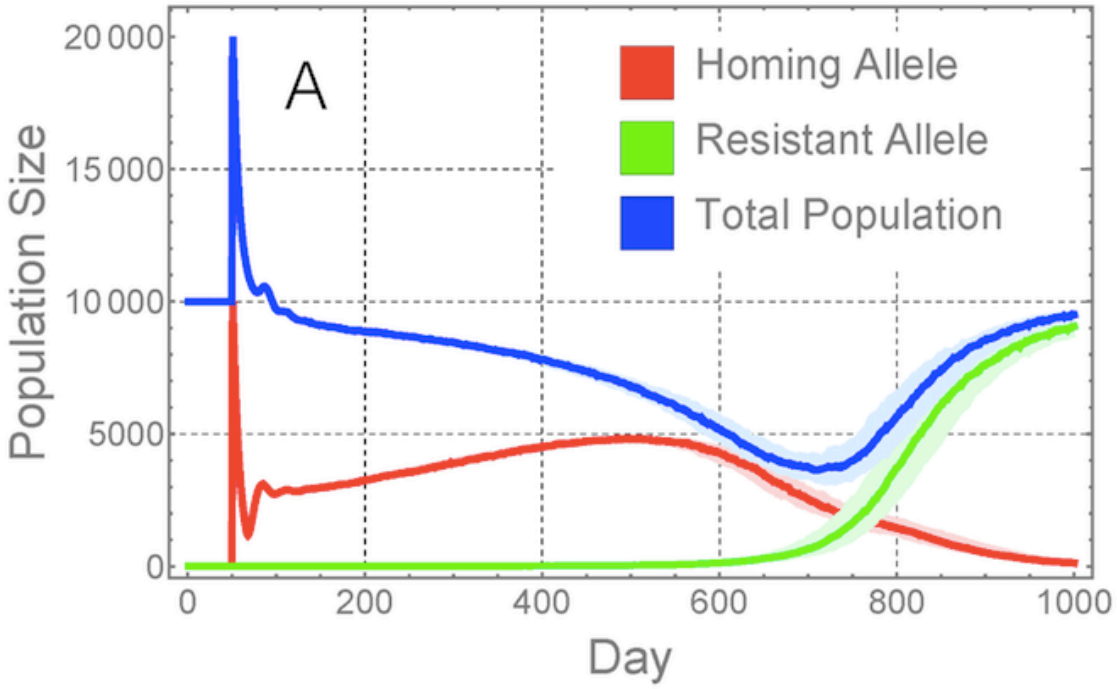
- Homing rate = 98%
- Non-cleavage rate = 1%
- Resistant allele generation rate = 0.13% (in-frame indels)
- Fertility of heterozygous females reduced by 90.7%

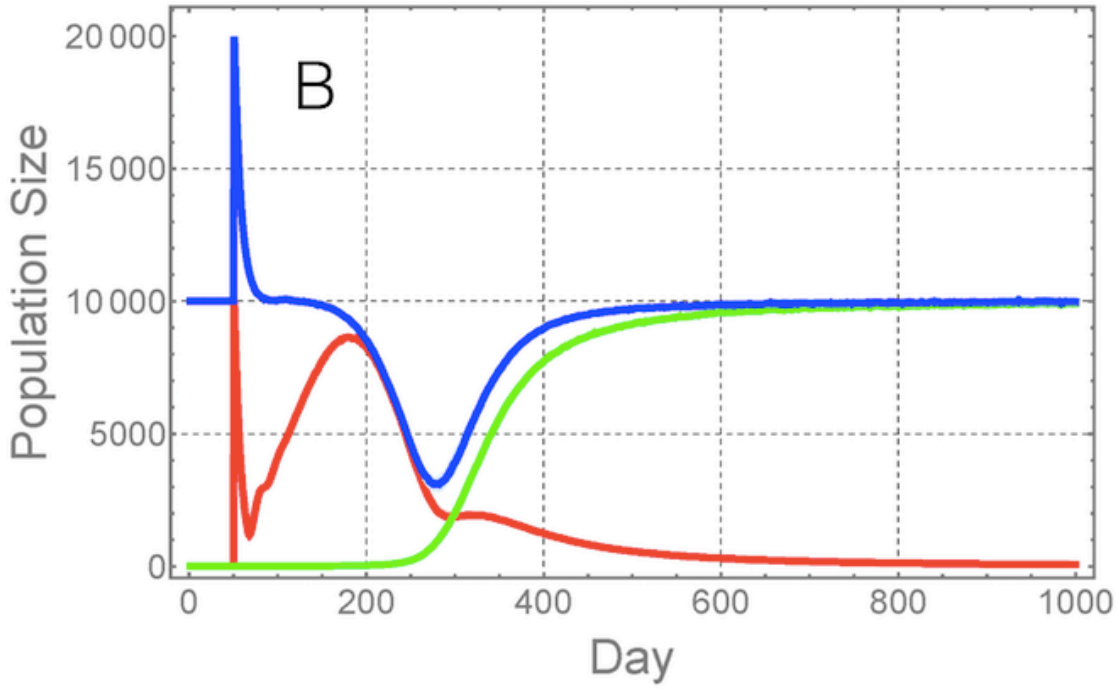
- Hammond *et al.* (2016) Nature Biotechnology

Expected dynamics of Hammond *et al.* (2016) construct

- Homing rate = 98%
- Non-cleavage rate = 1%
- Resistant allele generation rate = 0.13% (in-frame indels)
- Fertility of heterozygous females reduced by 90.7%

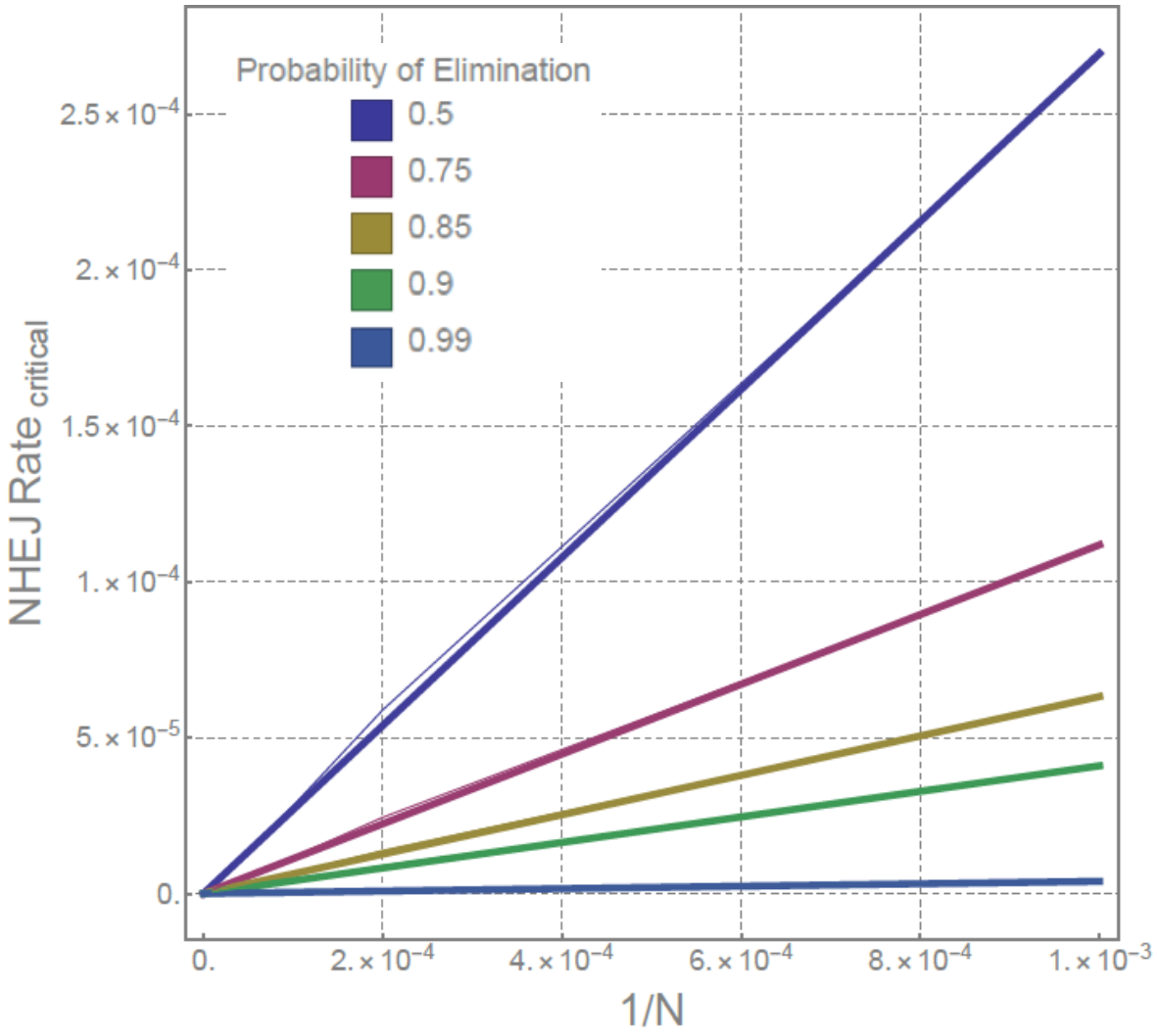
- Homing rate = 98%
- Non-cleavage rate = 1%
- Resistant allele generation rate = 0.13% (in-frame indels)
- *Fertility of heterozygous females same as wild-type*





- Marshall JM, Buchman A, Sanchez HM *et al.* (2017) Nature Sci Rep 7: 3776

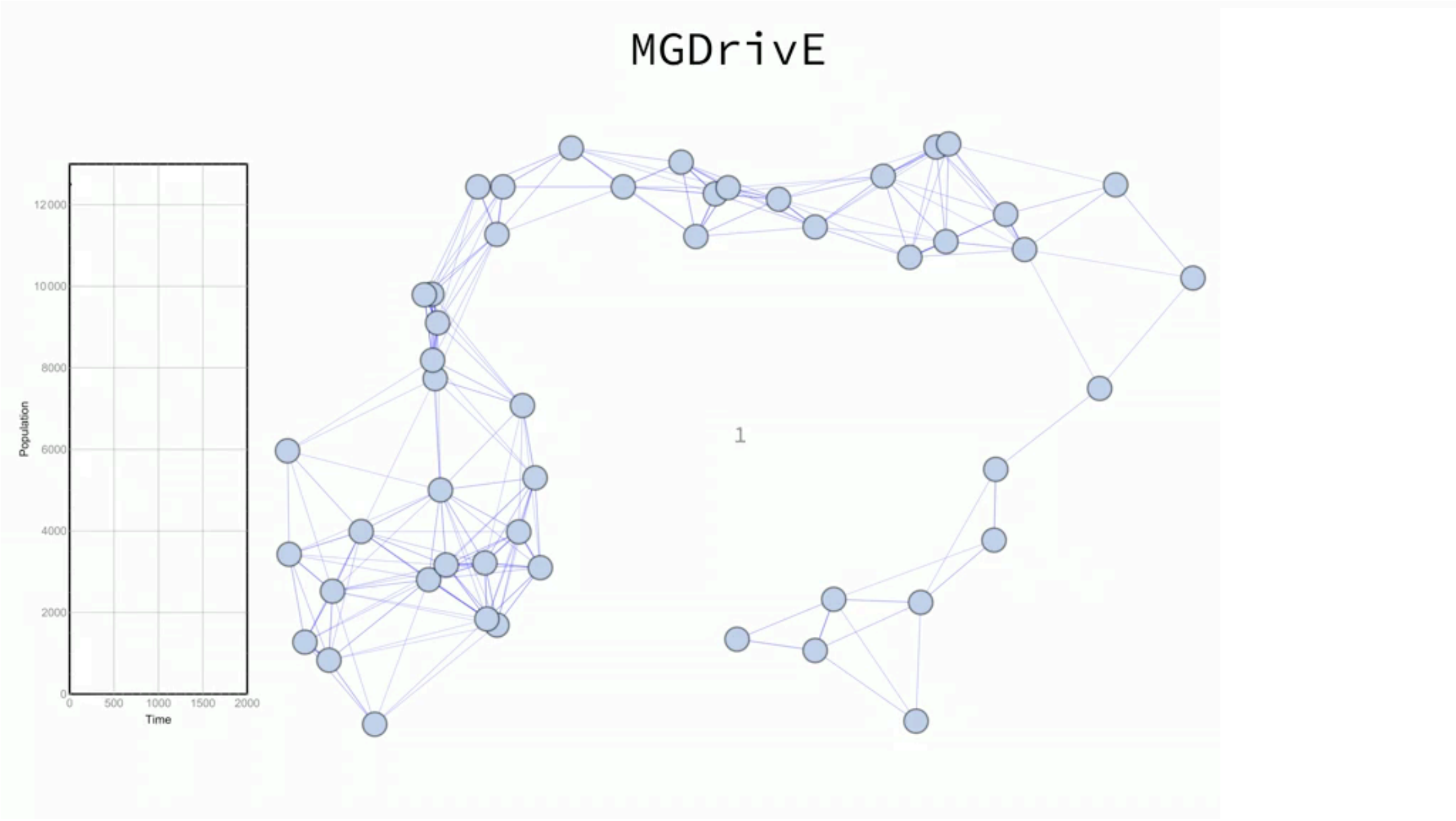
Multiplexing gRNAs as a solution to resistant alleles



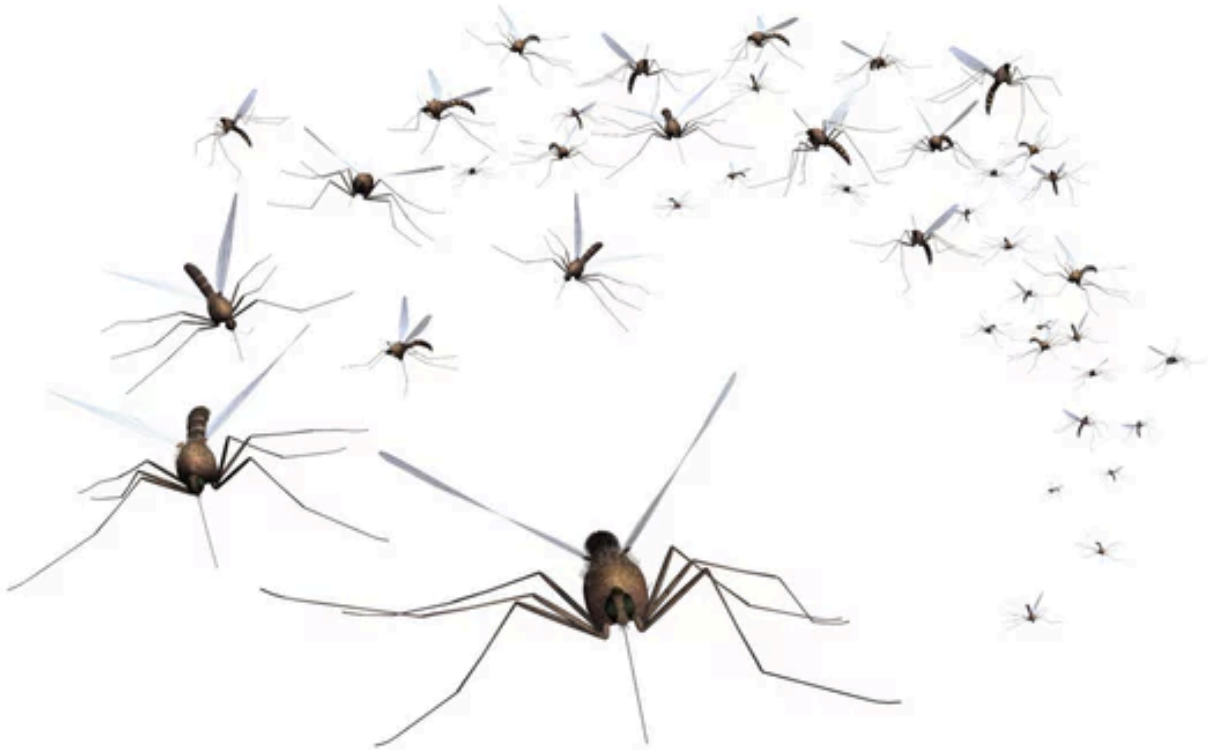
Multiplex number:	Resistance allele generation rate:	Population size capable of eliminating (90% of sims):
1	1.3×10^{-3}	32
2	1.7×10^{-6}	24 thousand
3	2.2×10^{-9}	19 million
4	2.9×10^{-12}	14 billion

- Marshall JM, Buchman A, Sanchez HM *et al.* (2017) Nature Sci Rep 7: 3776

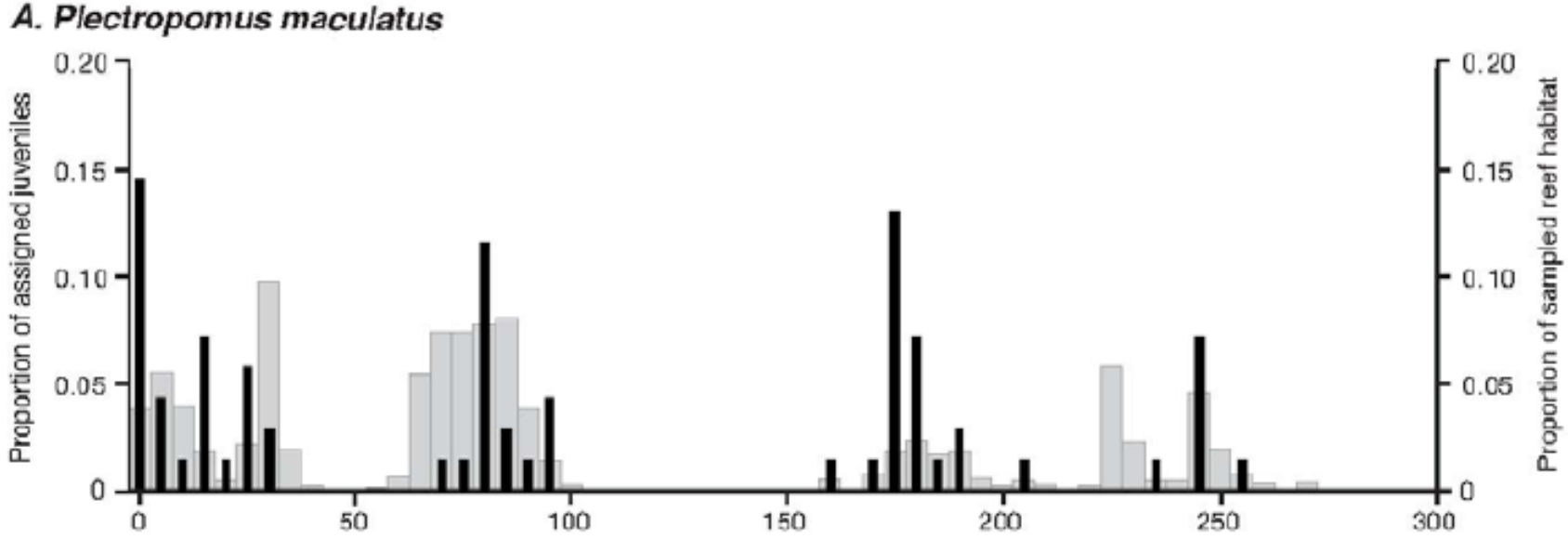
MGDrivE: Homing-based gene drive with resistance

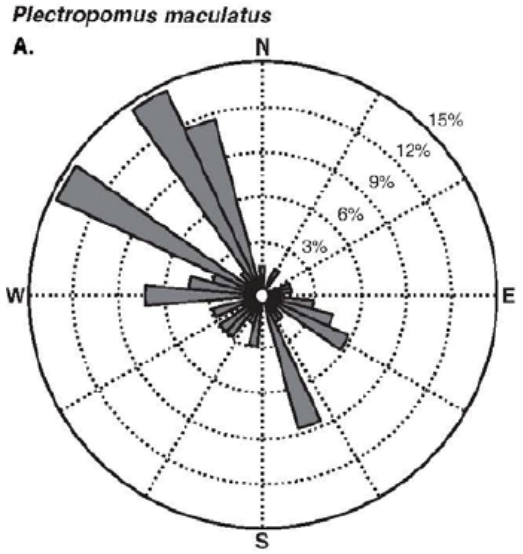


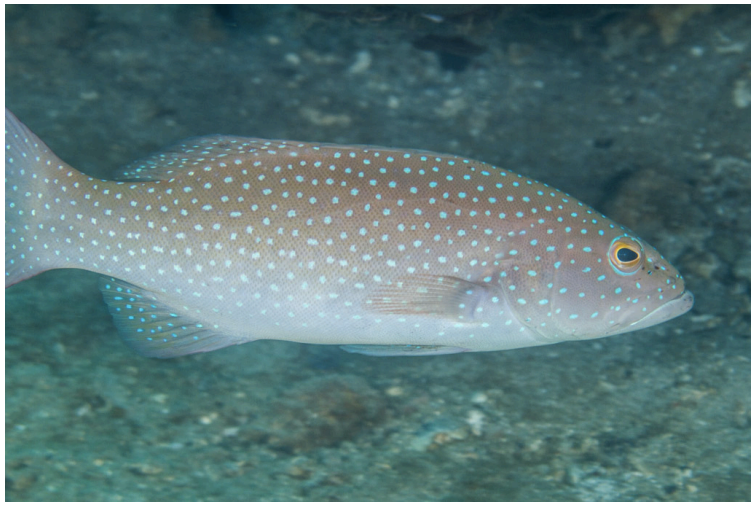
Q3. What are the best approaches for quantifying mosquito movement patterns of relevance to both questions?



Fine-scale movement patterns from parentage analysis

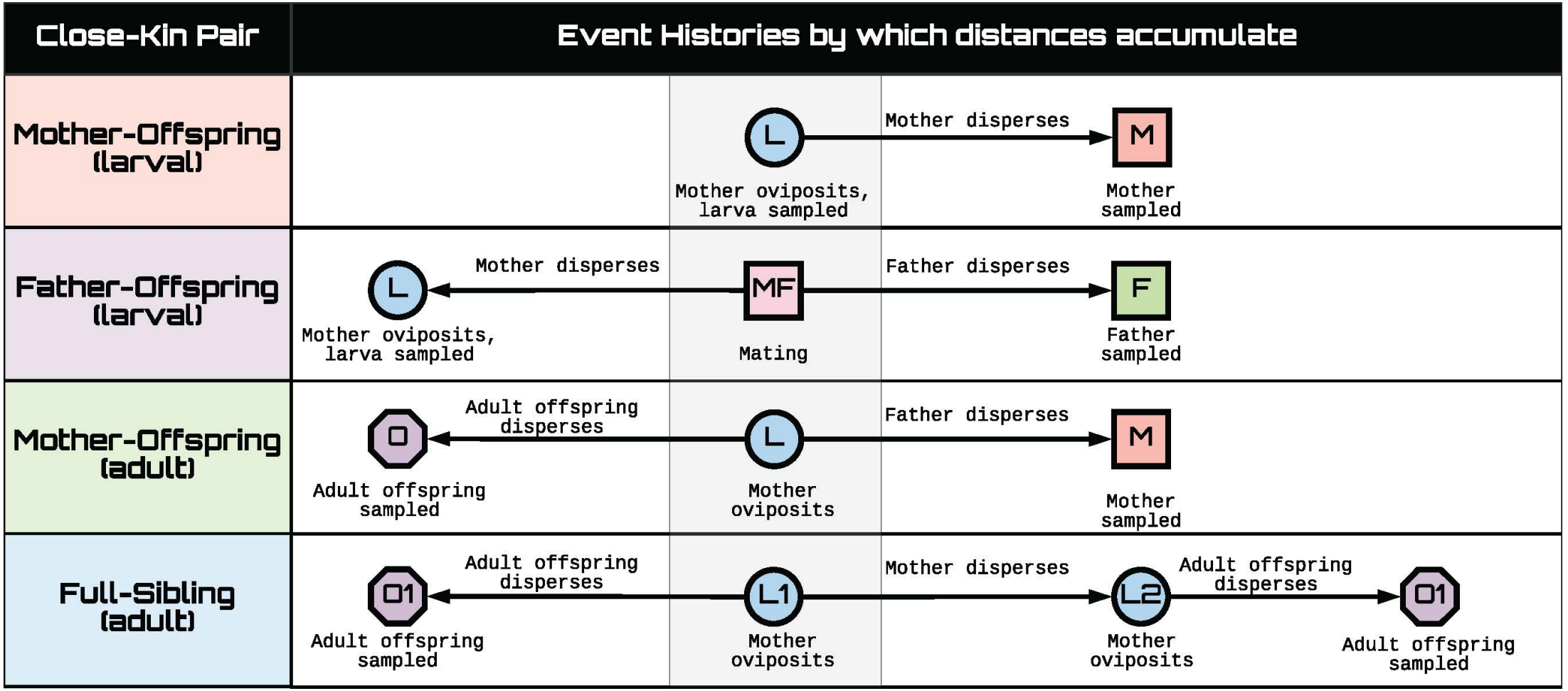




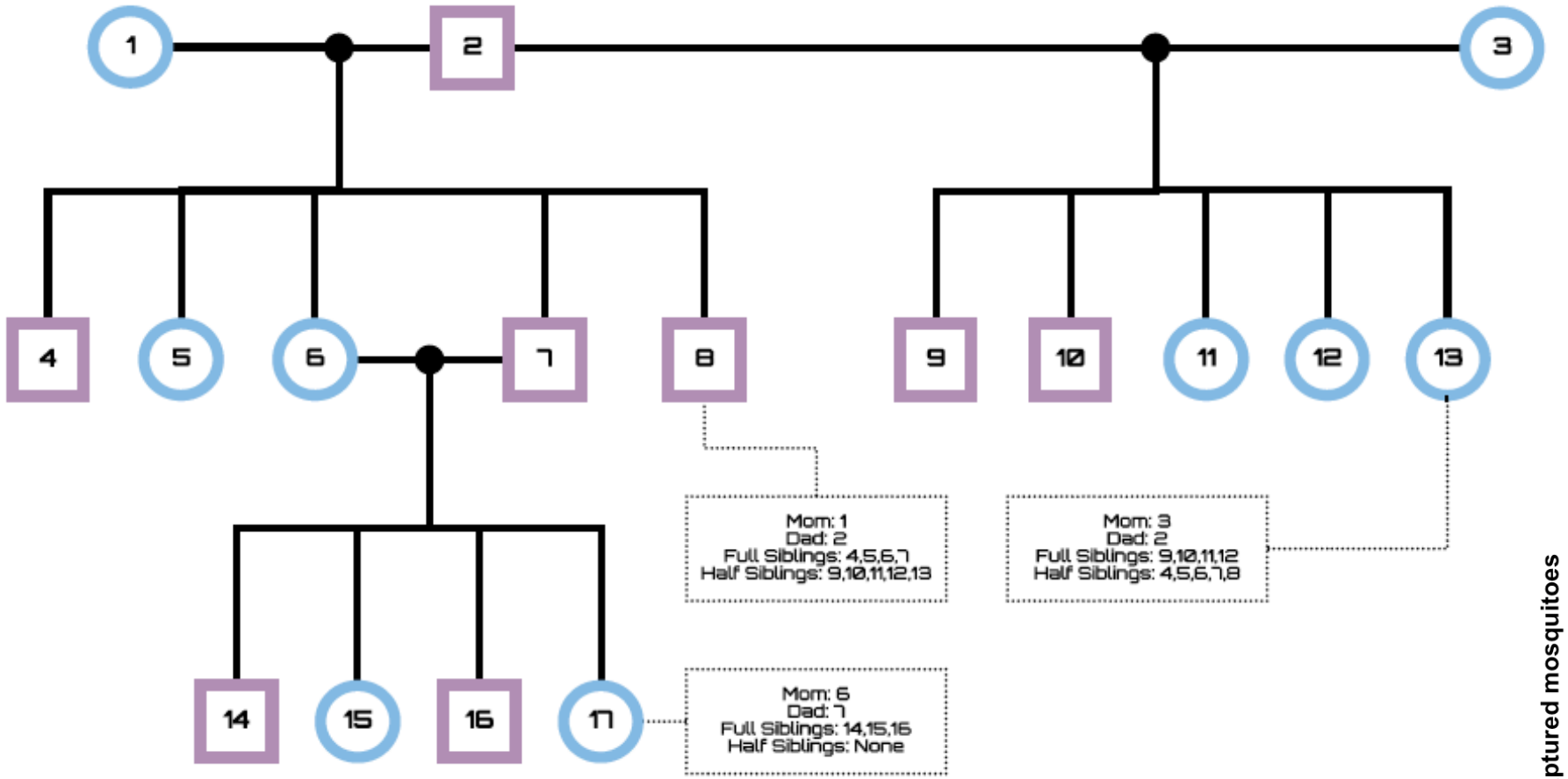


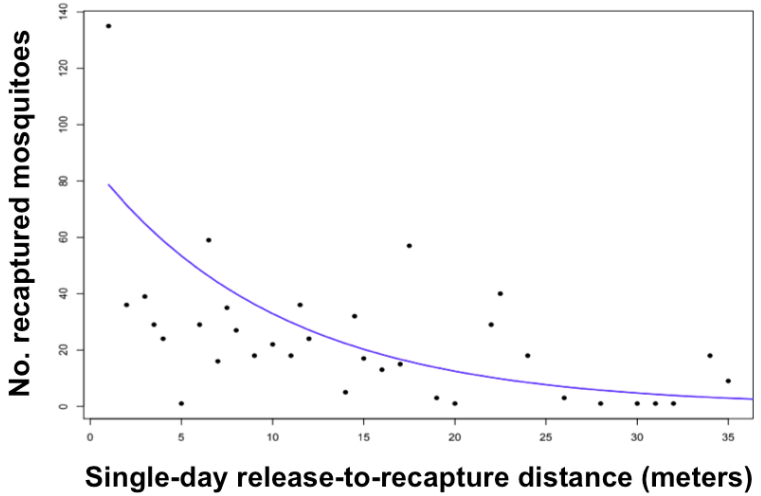
- Williamson DH, Harrison HB, Almany GR, Berumen ML *et al.* (2016) Mol. Ecol. 25L 6039-6054

Close-kin capture methods to infer mosquito dispersal



Incorporating parental IDs into MGDrivE





Importance of environmental barriers for *Ae. aegypti*

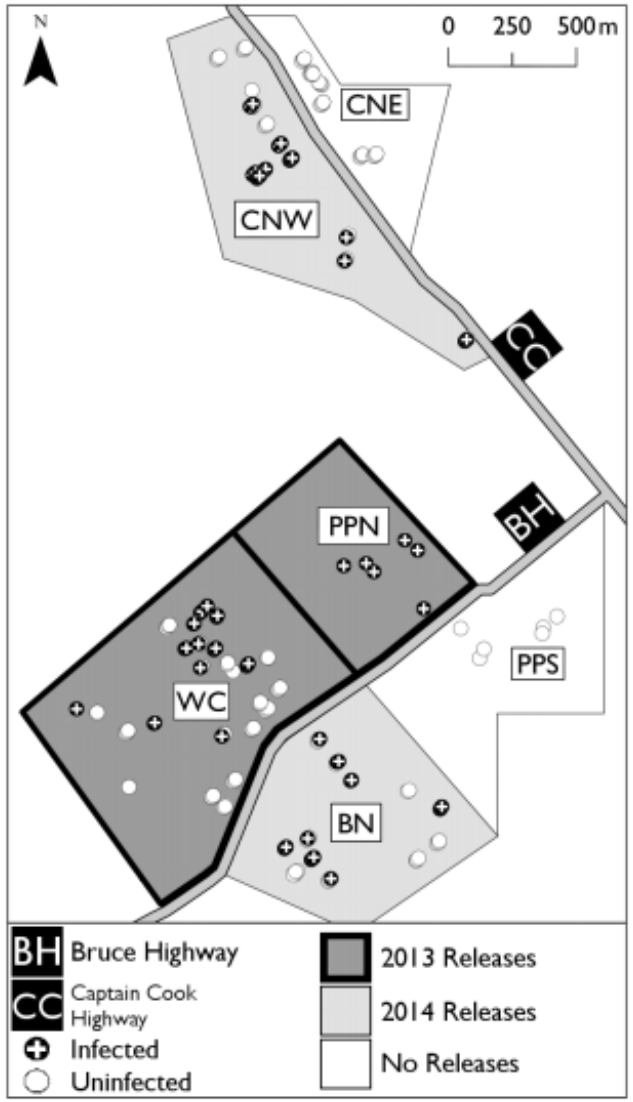
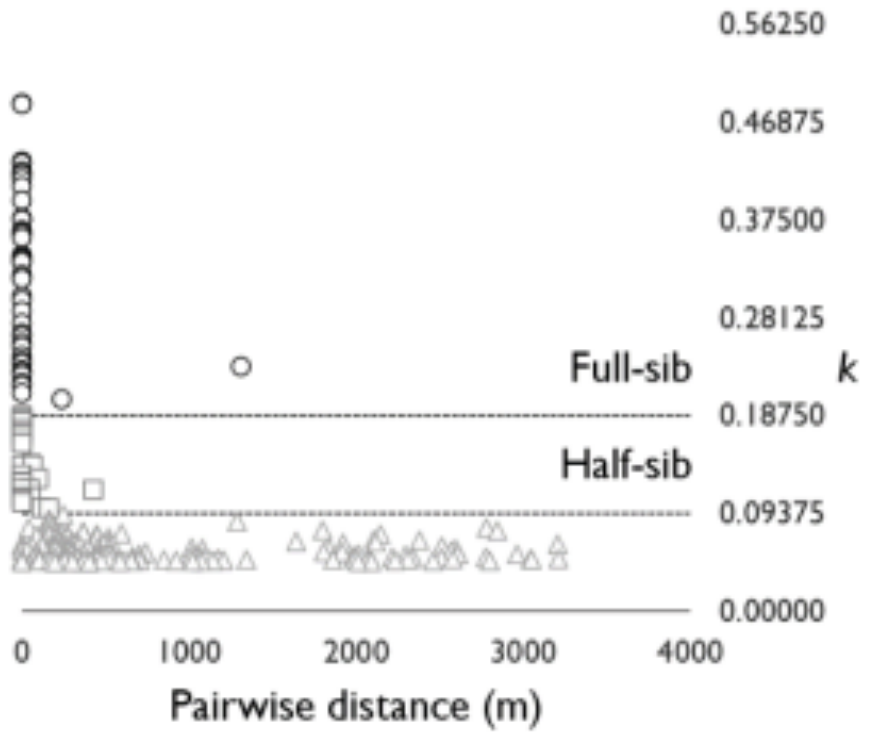
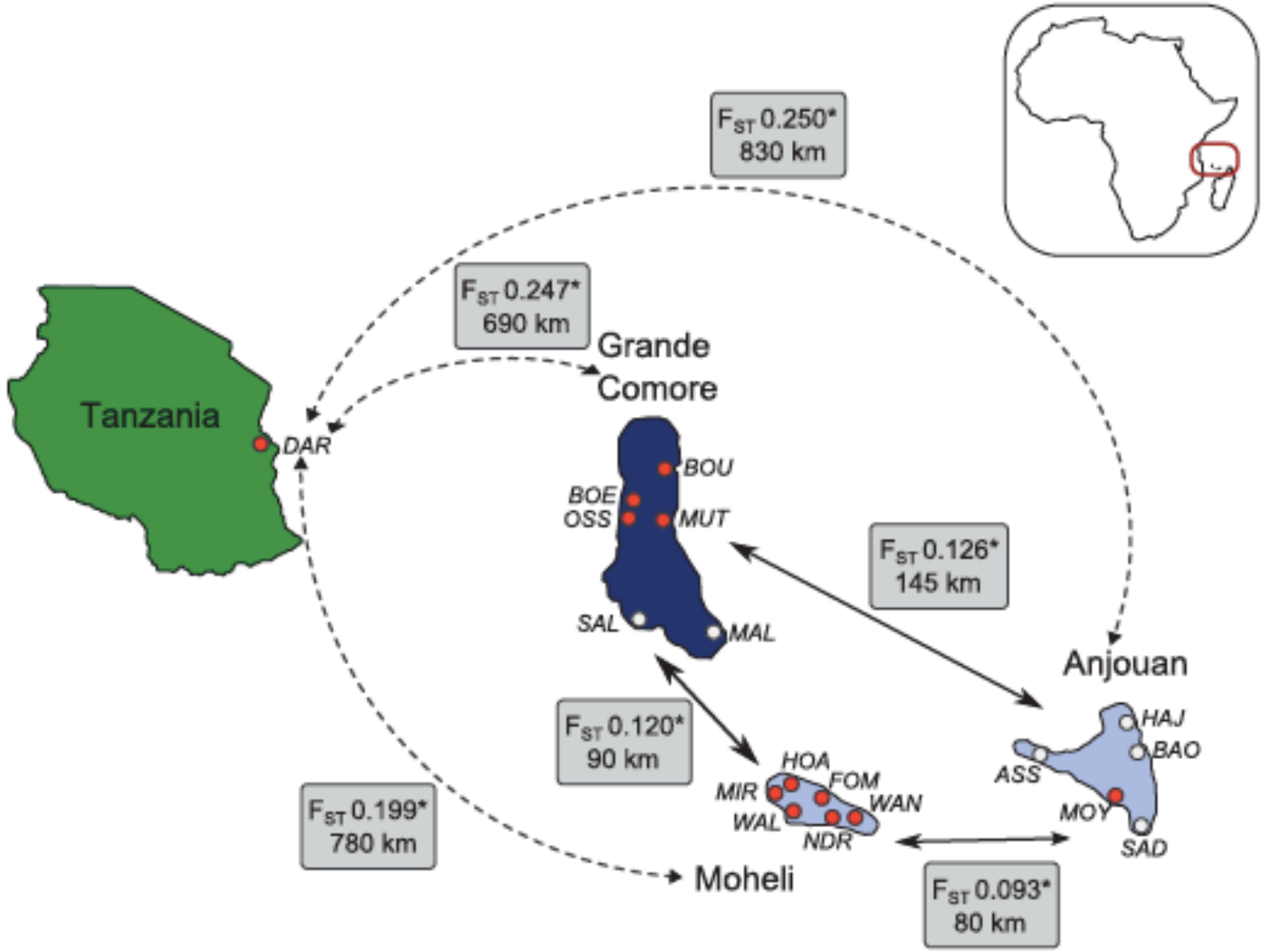


Figure 3: Loiselle's *k* estimates for sample pairs of relatedness $k > 0.046875$. Pairs of $0.09375 < k < 0.1875$ are most likely half-sibs, those of $k < 0.1875$ are most likely full-sibs. Most related pairs were found within the same trap, but separation distances of up to 1312m were observed.



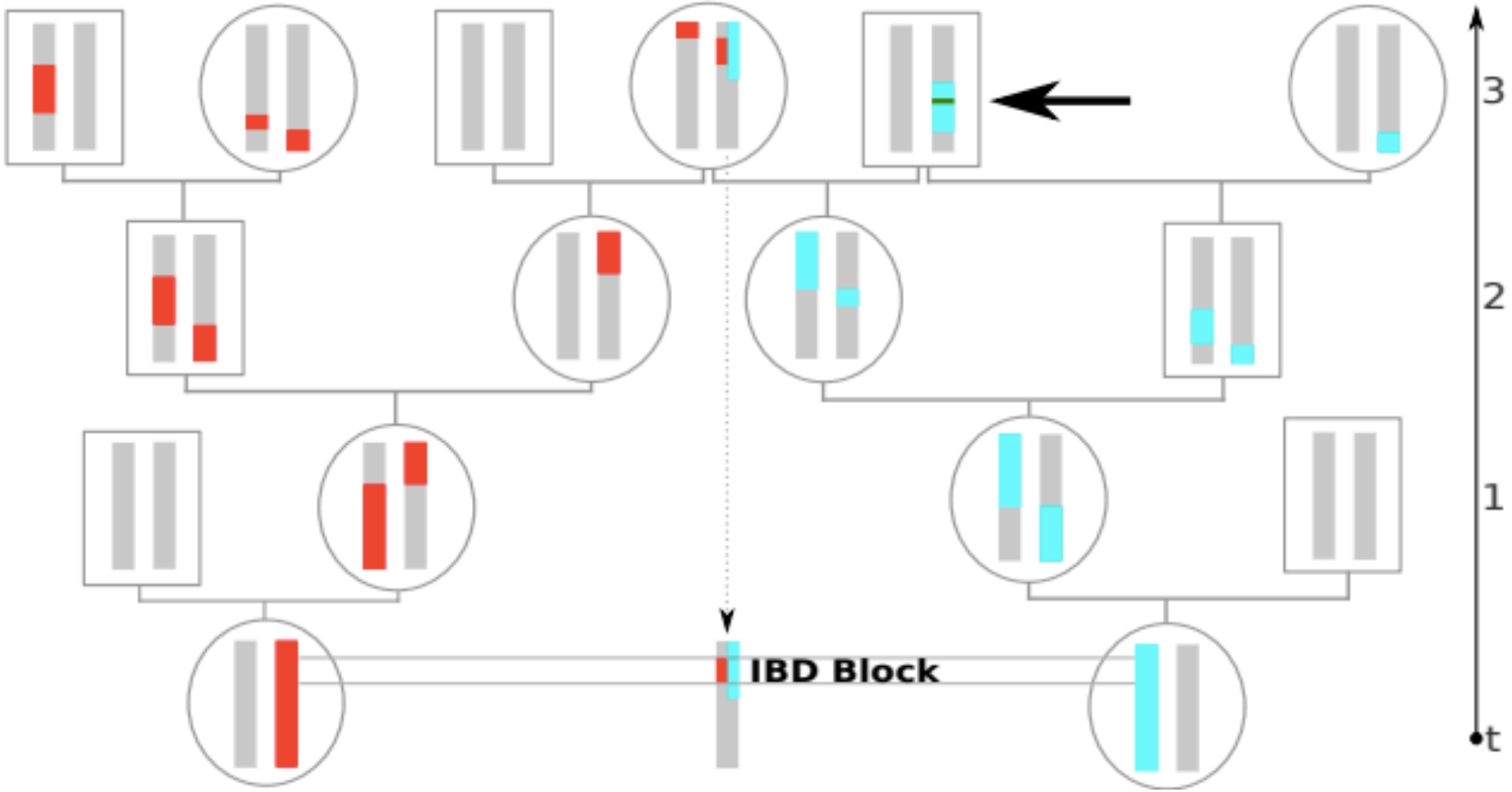
- Schmidt TL, Filipovic I, Hoffmann AA, Rasic G (2018) Heredity 120: 386–395

Movement rates inferred from F_{ST} values for *An. gambiae*



- Marsden CD, Cornel A, Lee Y, Sanford MR *et al.* (2013) Evol. App. 6: 706-720

Intermediate movement from identity by descent (IBD)

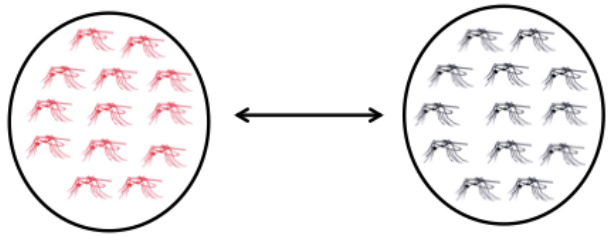


- Ringbauer H, Coop G, Barton NH (2017) Genetics doi: 10.1534/genetics.116.196220.

Summary

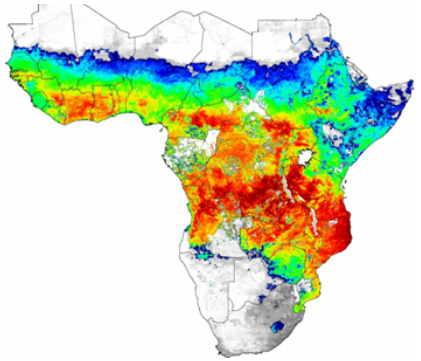
Q1. Can we conduct a confined field trial of gene-edited mosquitoes?

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



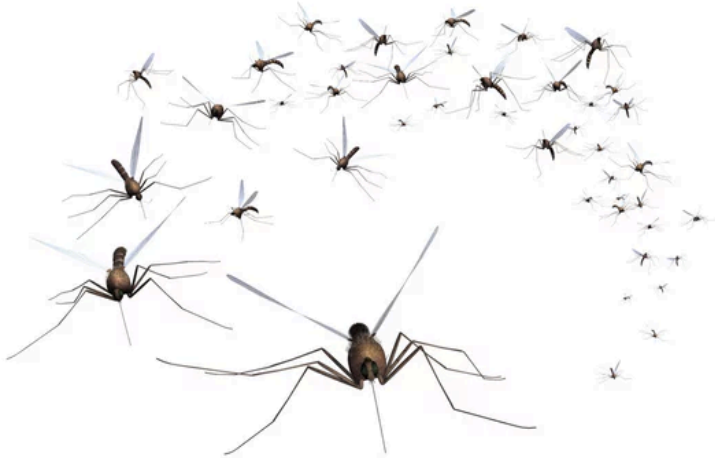
Q2. Can CRISPR-based gene drive be effective at controlling disease on a wide scale?

- Multiplexing guide RNAs could sufficiently reduce resistant allele generation rates
- More study is needed of the molecular mechanisms



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

- Close-kin capture methods are promising for inferring details of the fine-scale movement patterns of mosquitoes
- IBD methods may be appropriate for inferring intermediate-to-long distance dispersal



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- **Lanzaro Lab** @ UC Davis
- **James Lab** @ UC Irvine
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