International Conference on Entomology, 26th September 2016

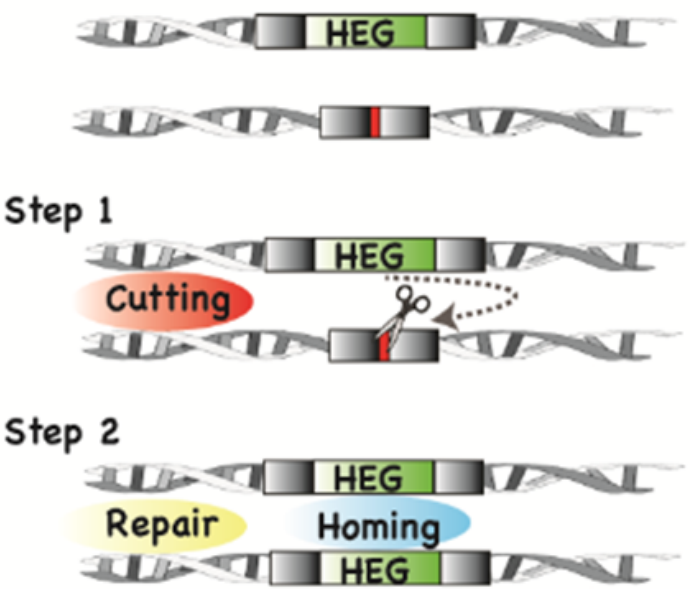
Gene drive: What is possible at the population level with currently-available molecular components?

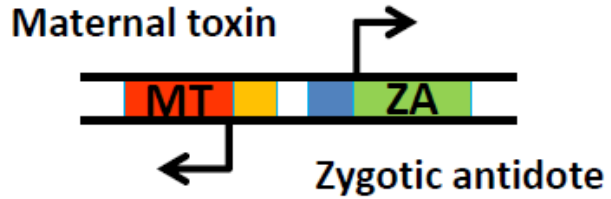
John Marshall[1], Anna Buchman[2], Hector Sanchez[1,3], Omar Akbari[2]

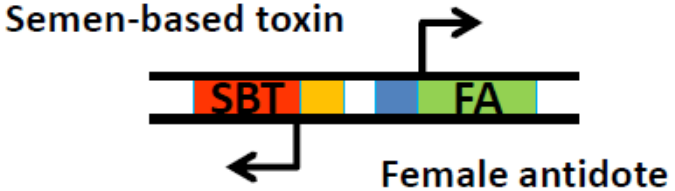
[1] Divisions of Biostatistics & Epidemiology, School of Public Health, University of California, Berkeley

[2] Department of Entomology, University of California, Riverside

[3] School of Medicine, Tecnologico de Monterrey, Estado de Mexico, Mexico





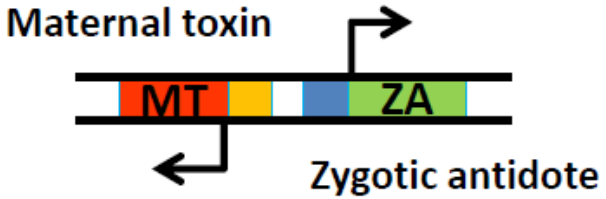


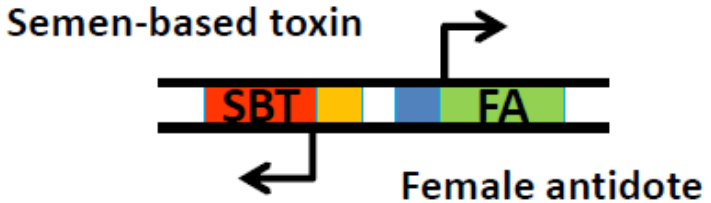
Berkeley School of Public Health

Talk outline

Toxin-antidote-based systems:

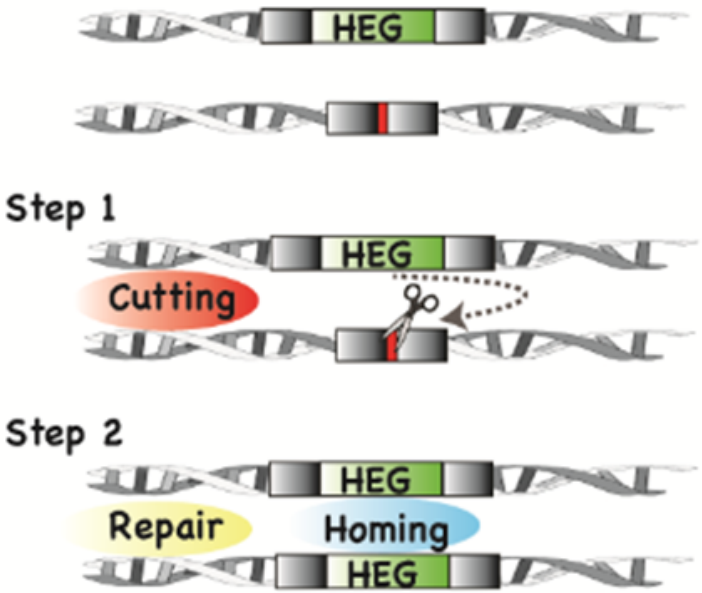
1. ***Medea*, *Semele*, underdominance**
2. **Thresholds, confinement & reversibility**
3. **The need for spatially-structured modeling frameworks**



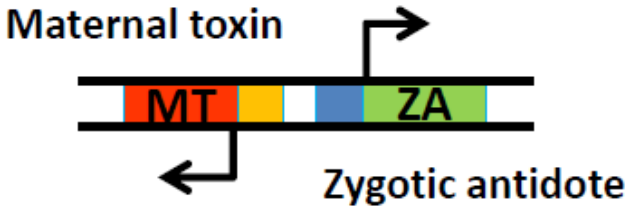


Homing-based systems:

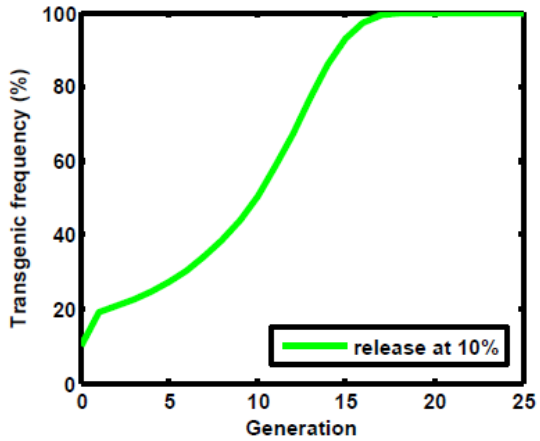
1. **Multiplexing to overcome resistant allele generation**
2. **Persistence following an accidental release**
3. **Reversal drive systems & remediation strategies**



Medea



Male \ Female	M/M	M/+		+/+
M/M	M/M	M/M	M/+	M/+
M/+	M/M	M/M	M/+	M/+
	M/+	M/+	+/+	+/+
+/+	M/+	M/+	+/+	+/+



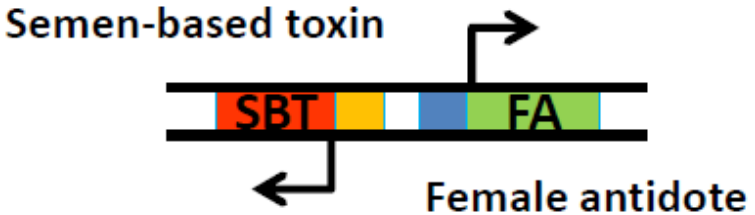
- Named after ***Medea*** from **Greek mythology** and **Maternal Effect Dominant Embryonic Arrest.**

- Causes **death of offspring of heterozygous mothers** that **do not inherit the *Medea* allele.**

- Spreads to **transgene fixation** from **very low initial frequencies.**

Chen *et al.* (2007) Science

Are there other toxin-antidote gene drive systems that could spread transgenes in a confined manner?



GENERAL PRINCIPLES OF SINGLE-CONSTRUCT CHROMOSOMAL GENE DRIVE

John M. Marshall[1,2,3] and Bruce A. Hay[1]

Male \ Female	S/S	S/+	+/+
S/S	S/S	S/S, S/+	S/+
S/+	S/S	S/S, S/+	S/+
	S/+	S/+, +/+	+/+
+/+	S/+	S/+, +/+	+/+

Marshall (2011) Bioeng. Bugs

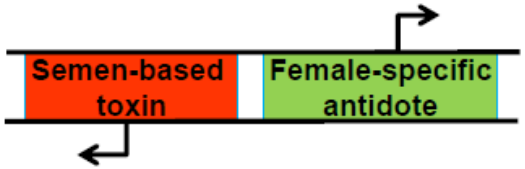
Marshall & Hay (2012) J. Theor. Biol.

Marshall & Hay (2012) Evolution

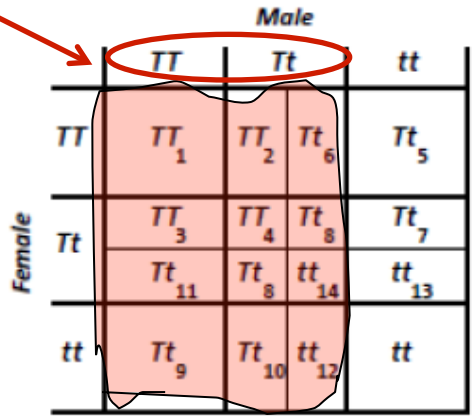
Marshall & Hay (2011) J. Hered.

Marshall *et al.* (2011) Genetics

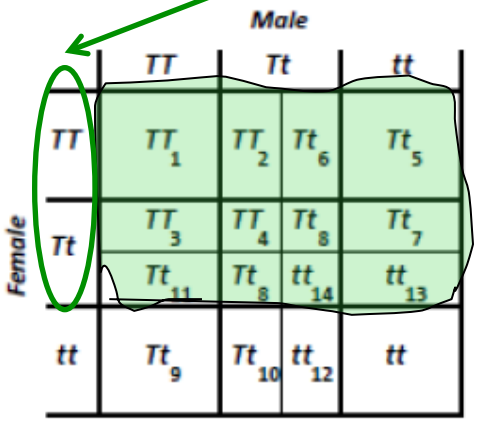
Semele

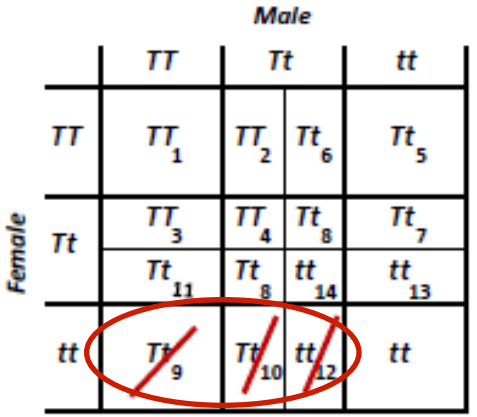


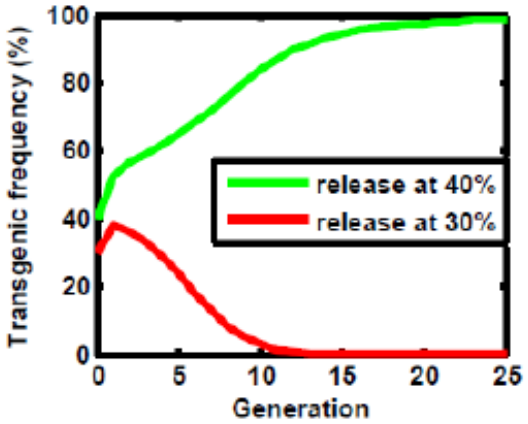
Transgenic males
produce **toxic semen**



Transgenic females
produce **antidote**



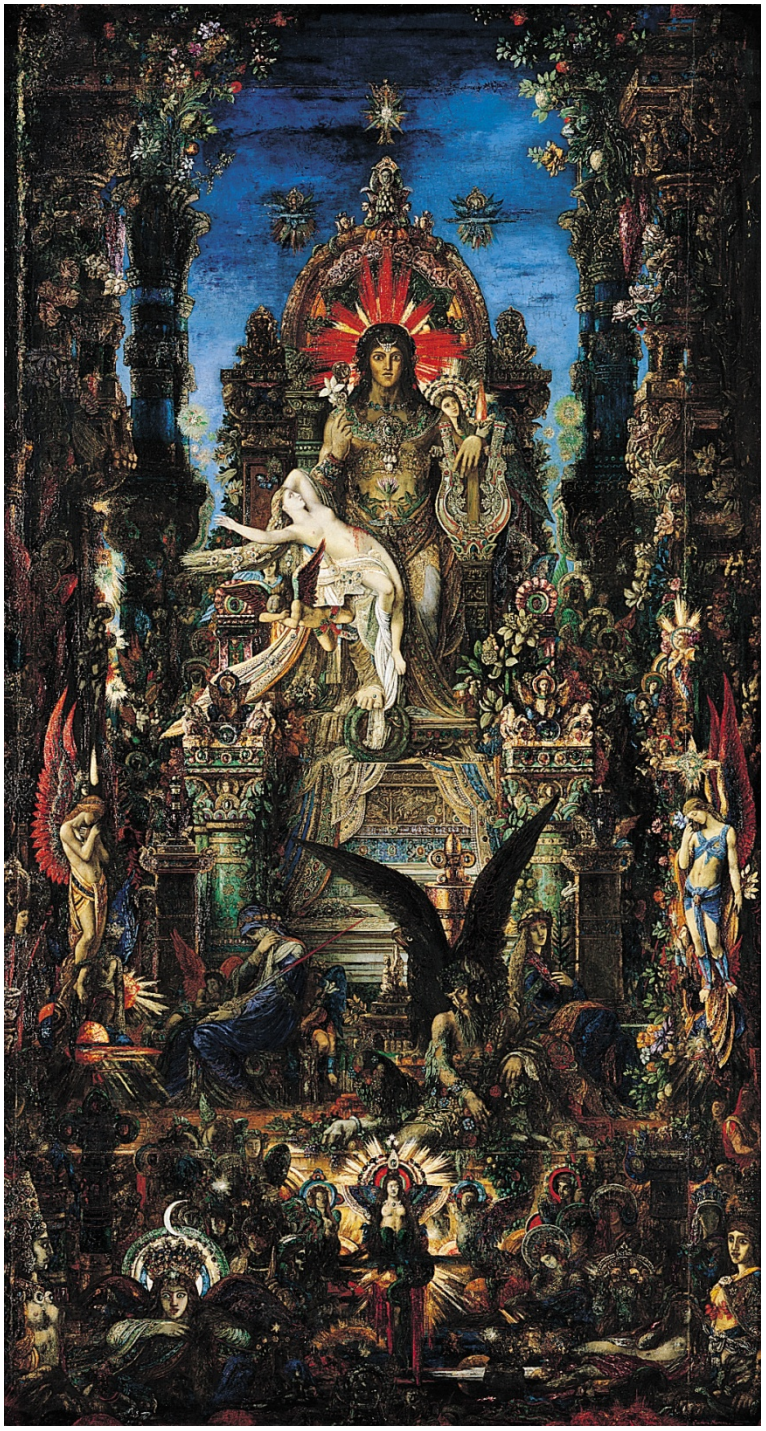




- A **release including females** results in **gene drive** (GM females are favored at high population frequencies).

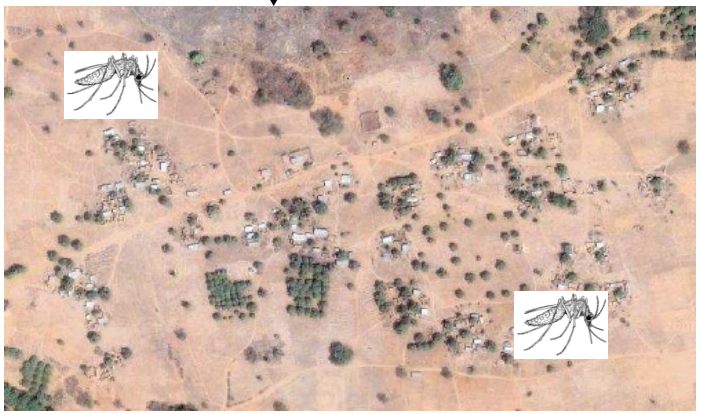
- **Release threshold = 36.4%**:

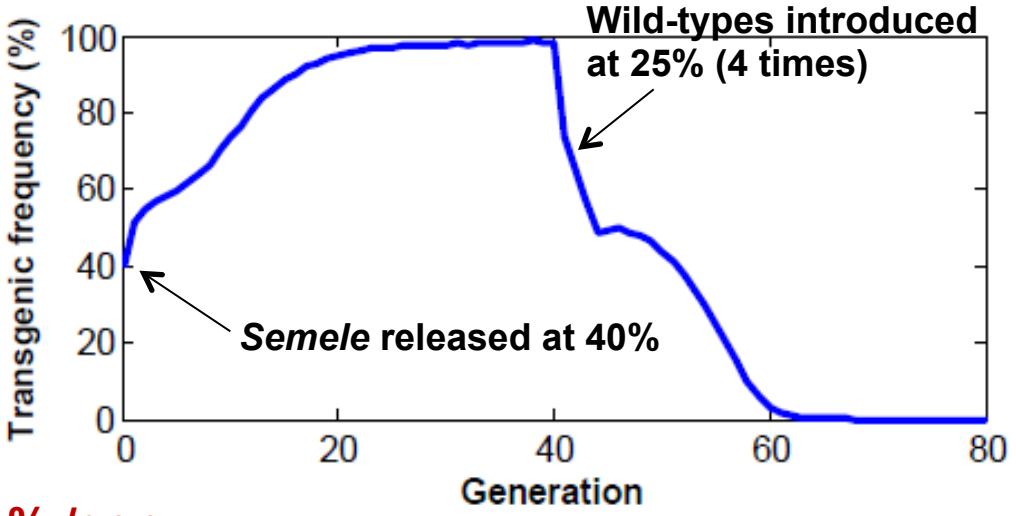
Marshall *et al.* (2011) Genetics

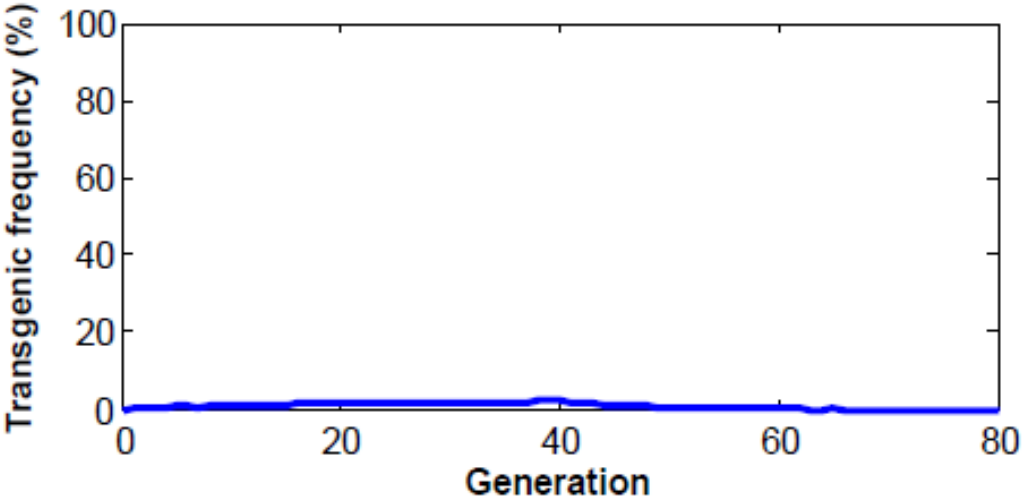


Introduction of *Semele* is predicted to be confineable and reversible

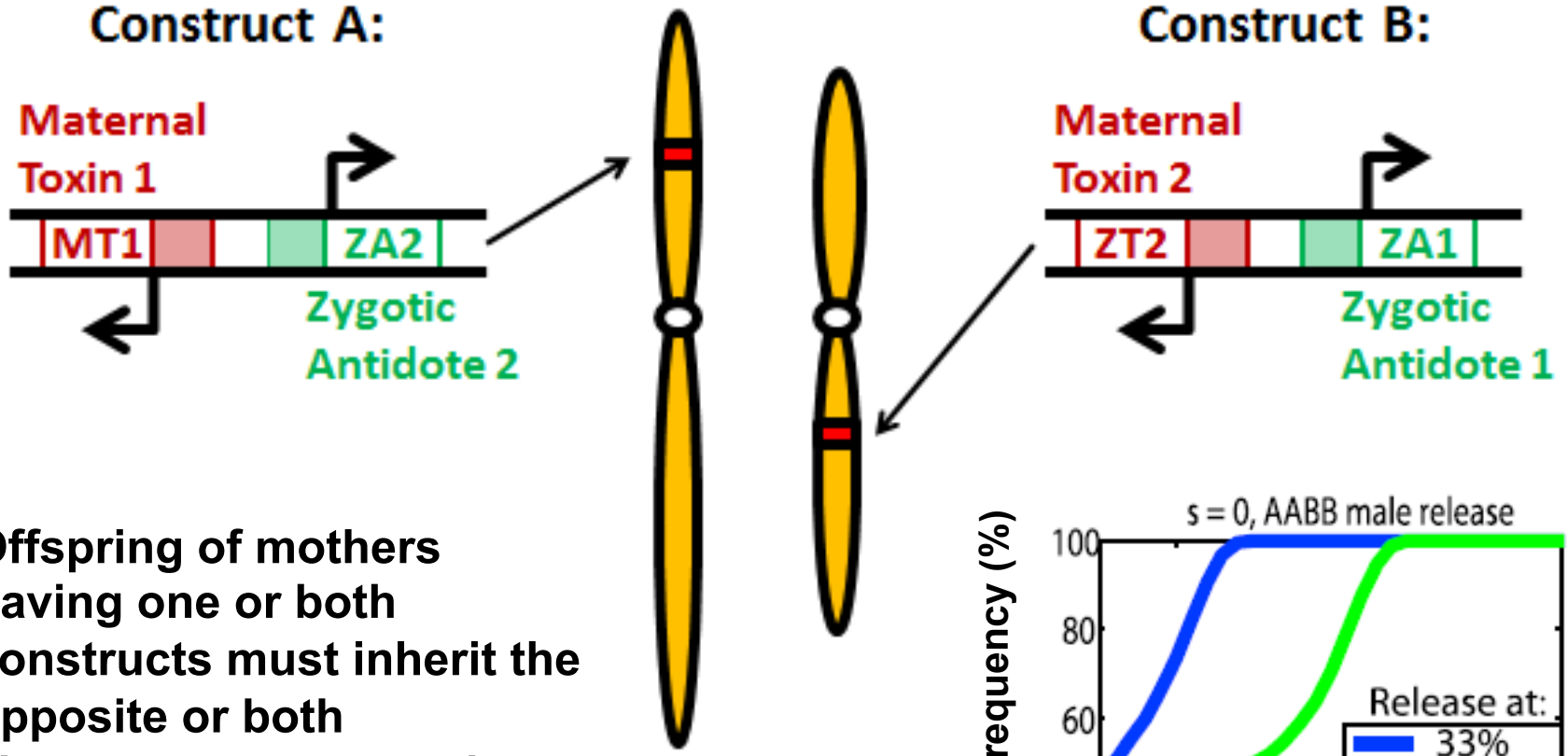
Migration rate = 1% /gen



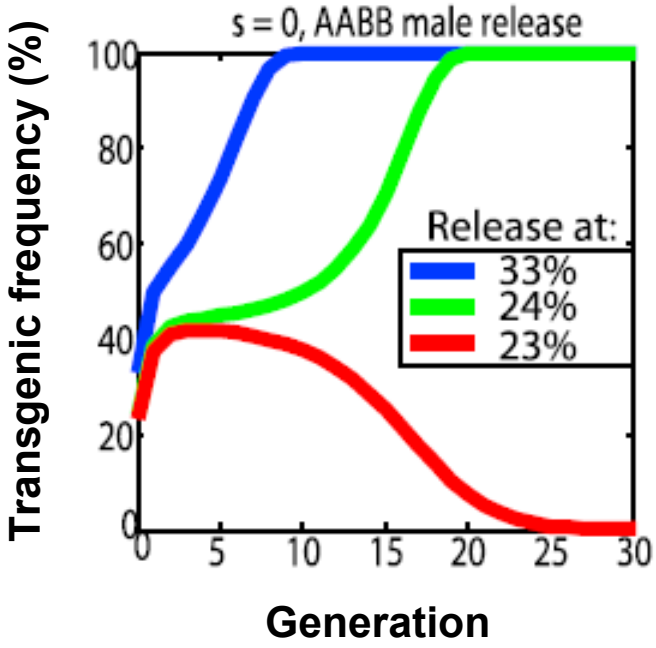




UD^{MEL}

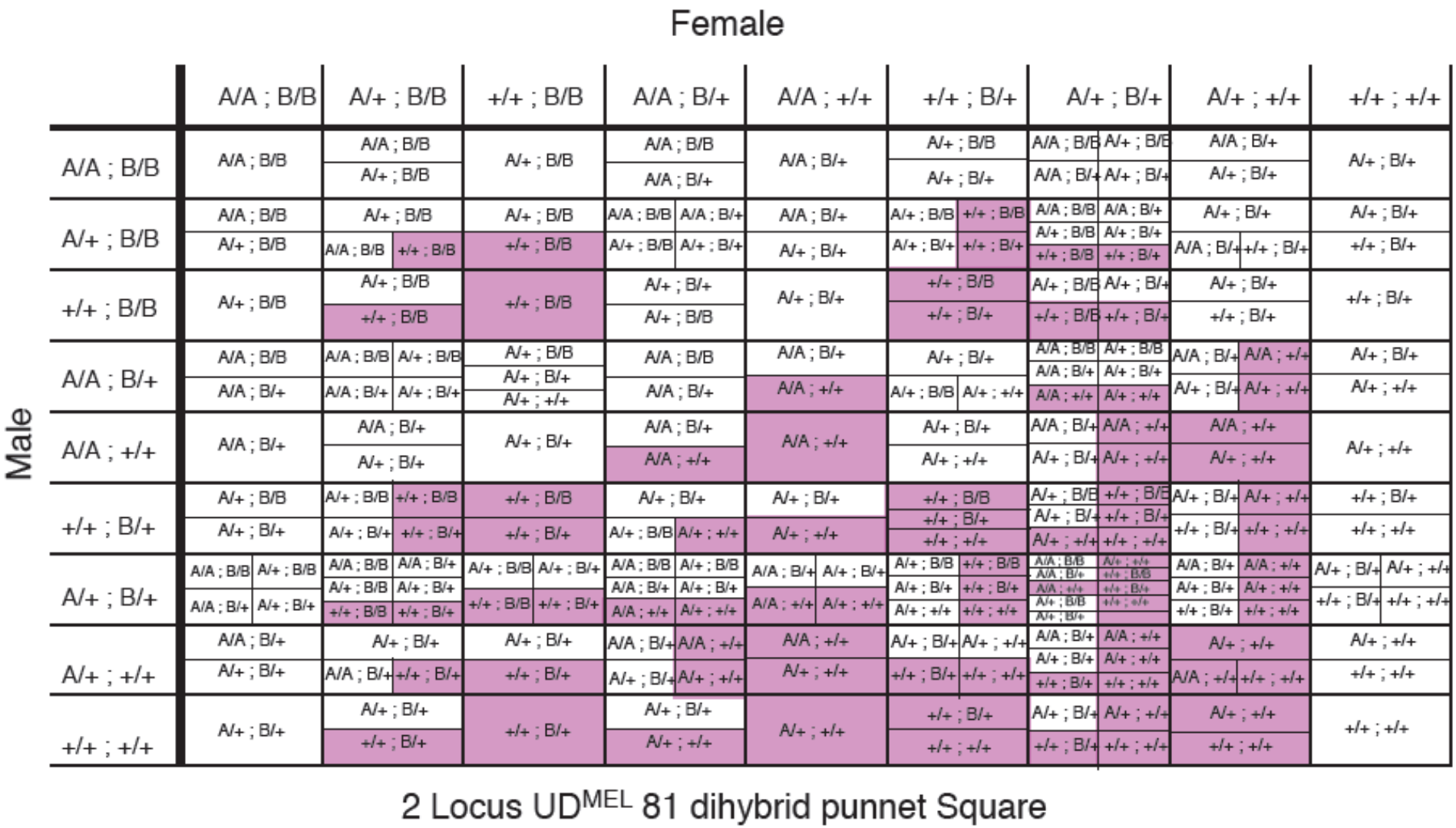


- **Offspring of mothers having one or both constructs must inherit the opposite or both chromosomes to survive.**
- This is **more likely** at **higher population frequencies (>24%)** leading to **frequency-dependent drive**.



Akbari*, Matzen*, Marshall* *et al.* (2013) Curr. Biol.

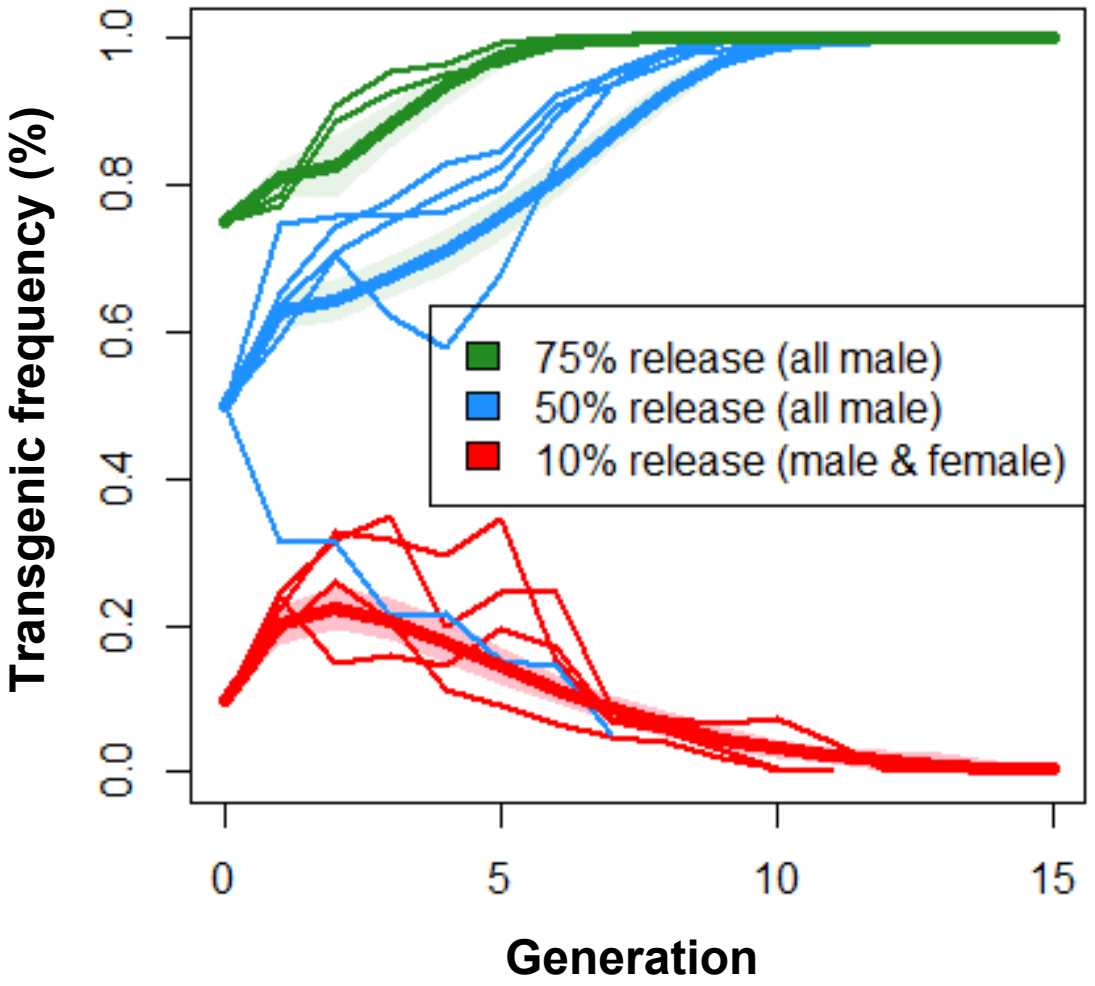
Inheritance pattern of UD^{MEL}



2 Locus UD^{MEL} 81 dihybrid punnet Square

Akbari*, Matzen*, Marshall* *et al.* (2013) Curr. Biol.

UD^{MEL} model fitted to laboratory drive experiments



Construct has frequency-dependent fitness cost:

- **6% fitness benefit in fully-wild-type pop.**
- **21% fitness cost in fully transgenic pop.**

Akbari*, Matzen*, Marshall* *et al.* (2013) Curr. Biol.

Introduction of UD^{MEL} is predicted to be confineable & reversible

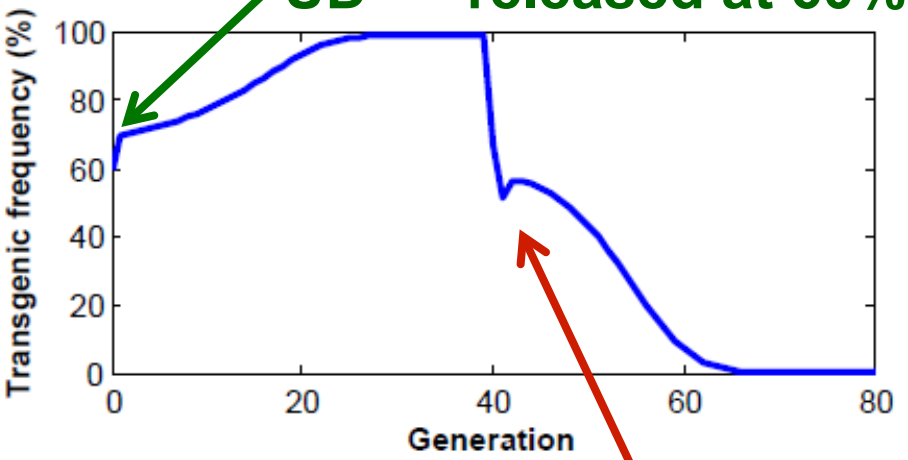
Banambani, Mali

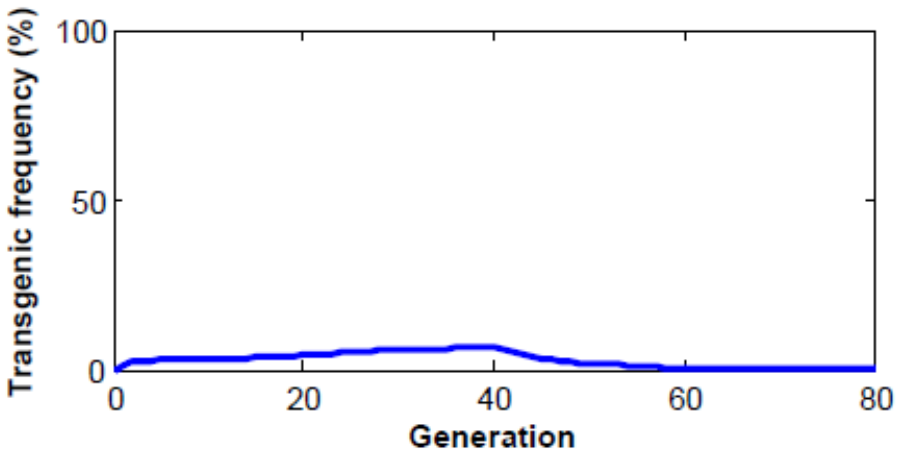
7 km

~1% / gen

Doneguebougou, Mali

UD^{MEL} released at 60%





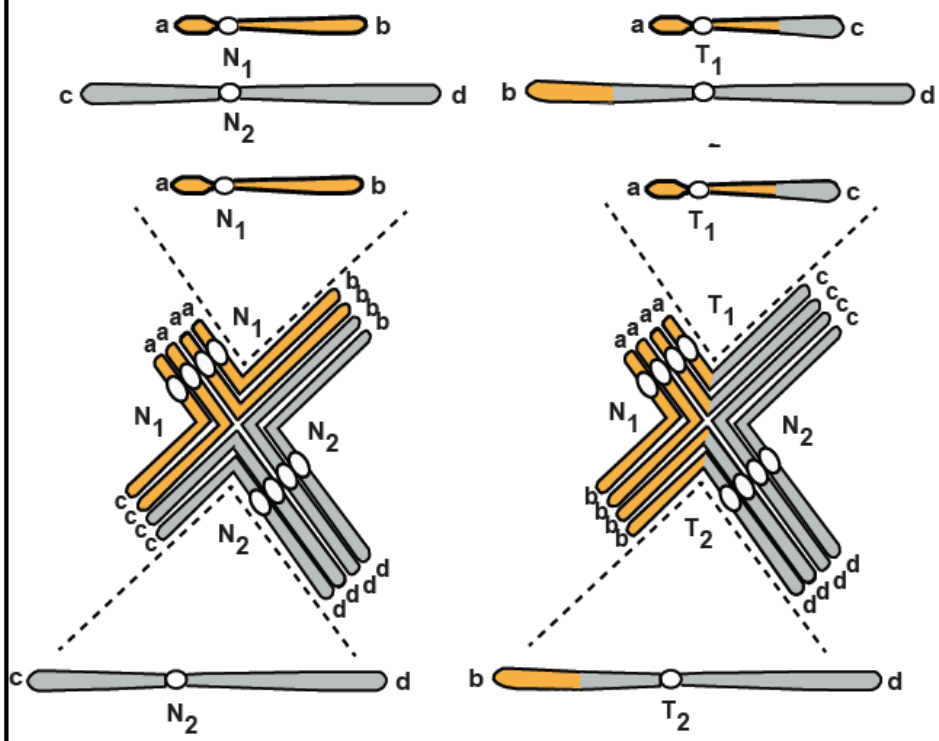
Wild-types introduced at 33% (2 times)

Akbari*, Matzen*, Marshall* *et al.* (2013) Curr. Biol.

Translocations display threshold-dependent dynamics

Figure 1

A Normal chromosomes: B Translocated chromosomes:

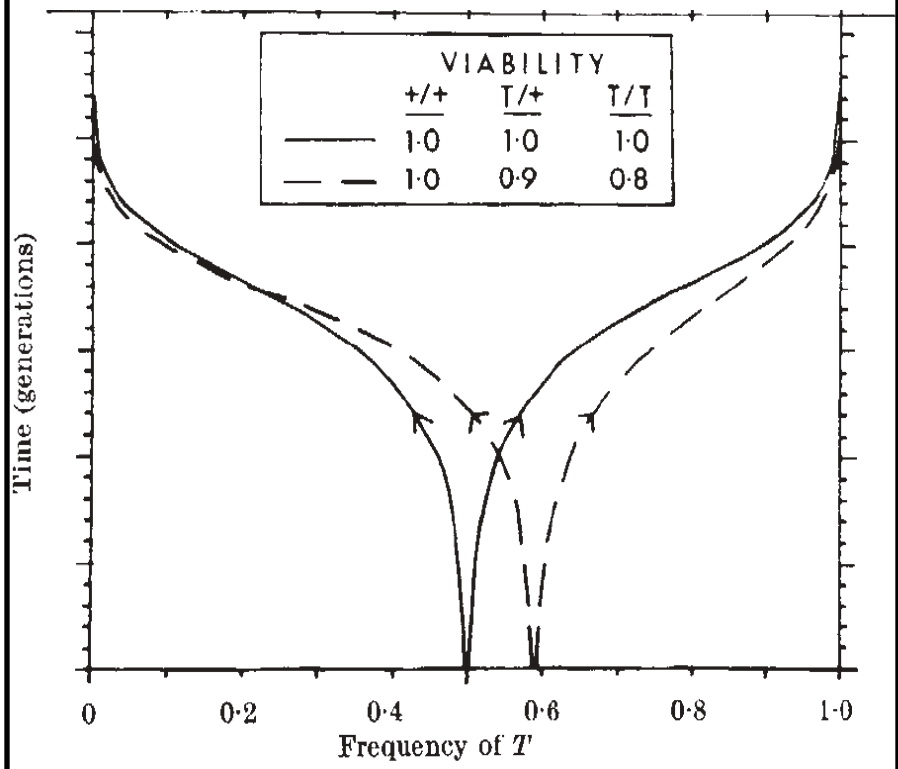


C

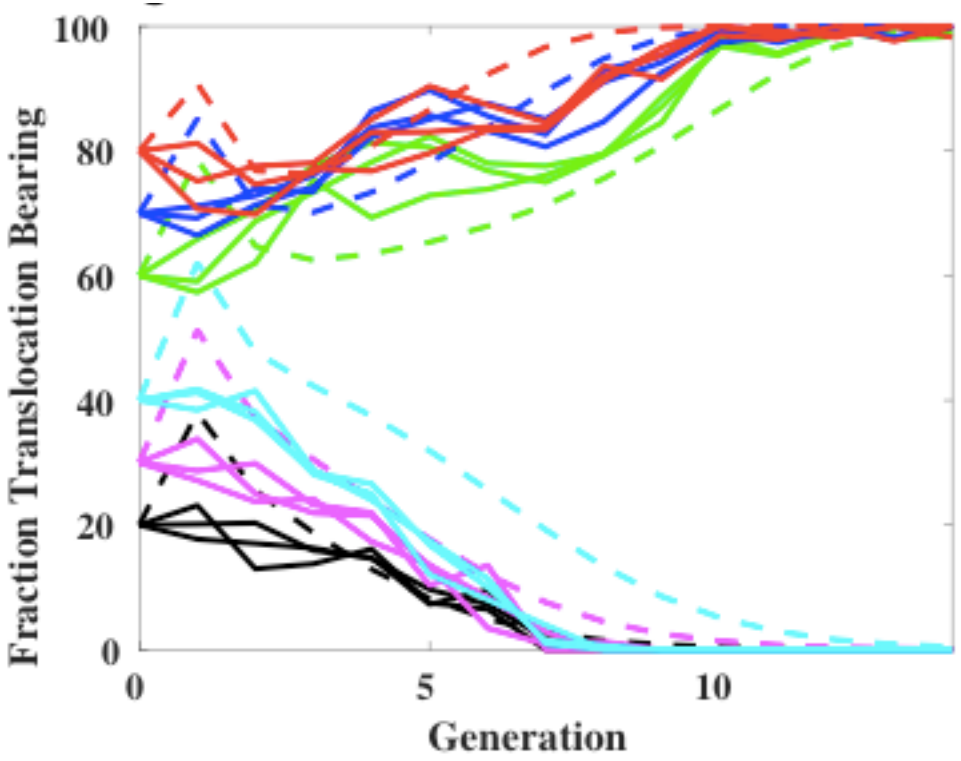
	T_1N_2	N_1T_2	T_1T_2	N_1N_2
T_1N_2	~~$T_1T_1N_2N_2$~~	$T_1N_1N_2T_2$	~~$T_1T_1N_2T_2$~~	~~$T_1N_1N_2N_2$~~
N_1T_2	$T_1N_1T_2N_2$	~~$N_1N_1T_2T_2$~~	~~$T_1N_1T_2T_2$~~	~~$N_1N_1T_2N_2$~~
T_1T_2	~~$T_1T_1T_2N_2$~~	~~$T_1N_1T_2T_2$~~	$T_1T_1T_2T_2$	$T_1N_1T_2N_2$
N_1N_2	~~$T_1N_1N_2N_2$~~	~~$N_1N_1N_2T_2$~~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

NATURE, VOL. 218, APRIL 27, 1968

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



Translocation model fitted to laboratory drive experiments



Translocation has time-dependent fitness costs:

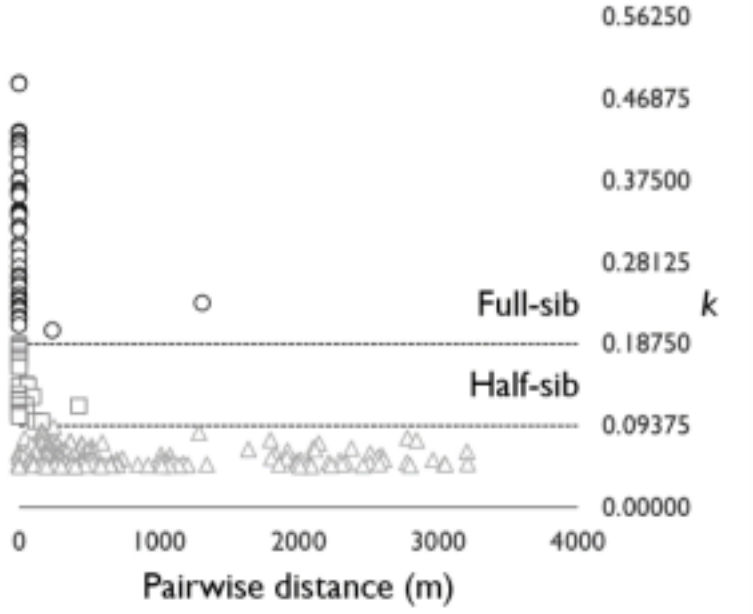
- **Translocation homozygotes are initially very unfit; but rapidly increase in fitness in a couple of generations.**
- **Translocation heterozygotes have relatively consistent, near wild-type fitness.**

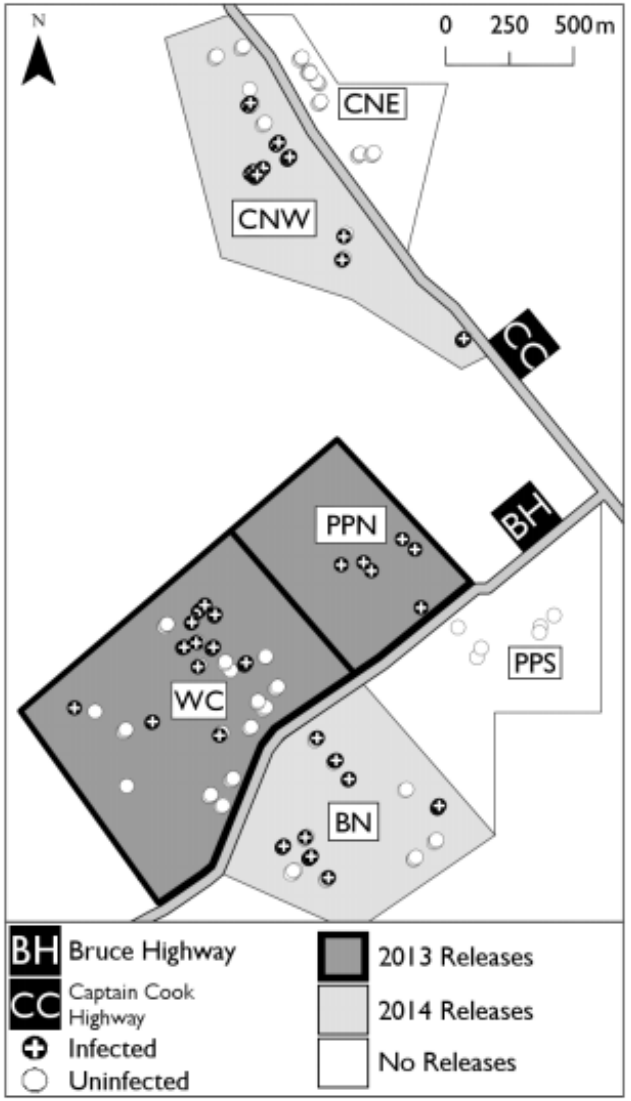
Buchman, Ivy, Marshall, Akbari & Hay (2016) bioRxiv

Fine-scale landscape genomics of Aedes aegypti reveals loss of Wolbachia transinfection, dispersal barrier and potential for occasional long distance movement

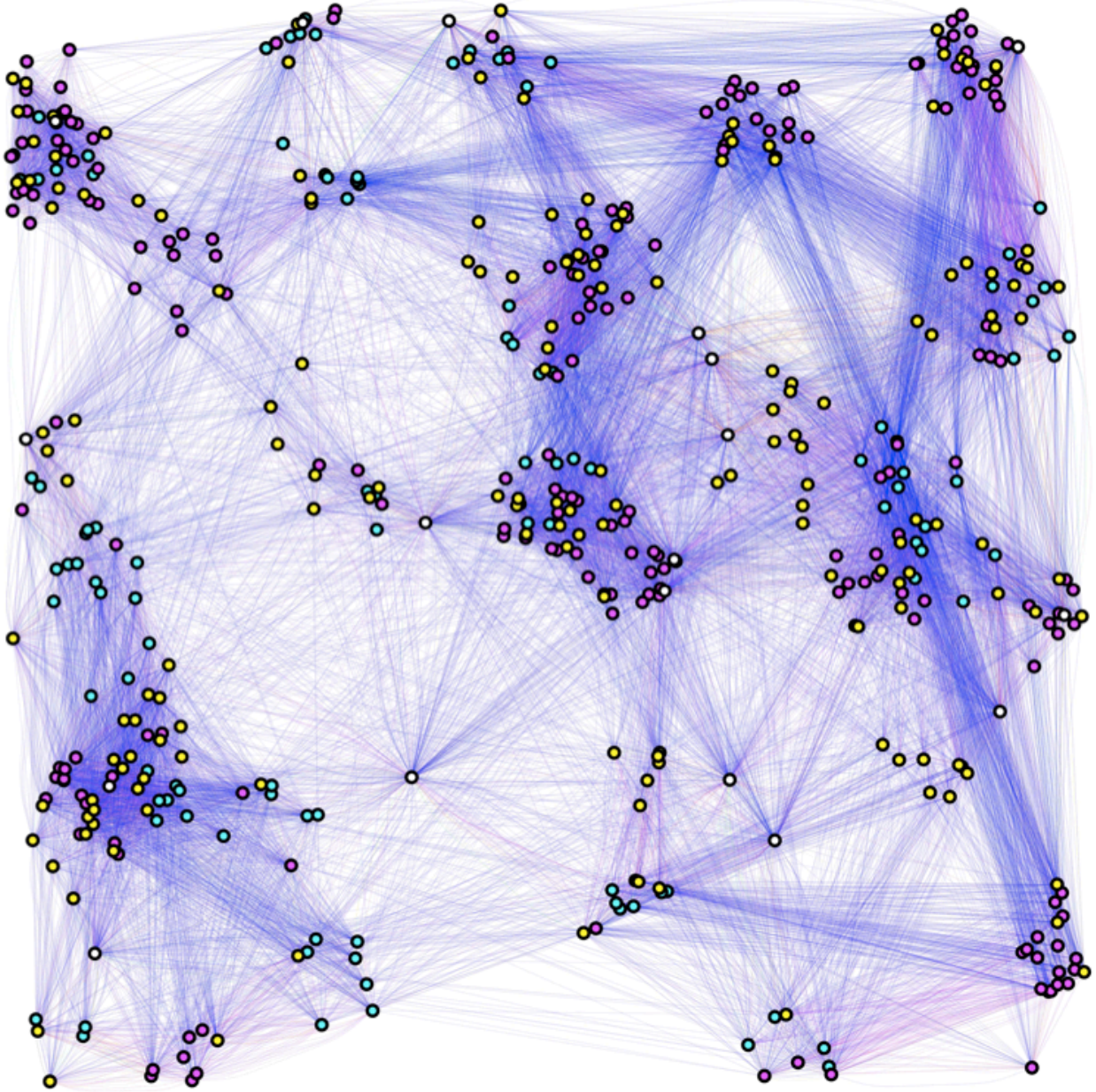
Thomas L Schmidt, Igor Filipovic, Ary A Hoffmann, Gordana Rasic

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.



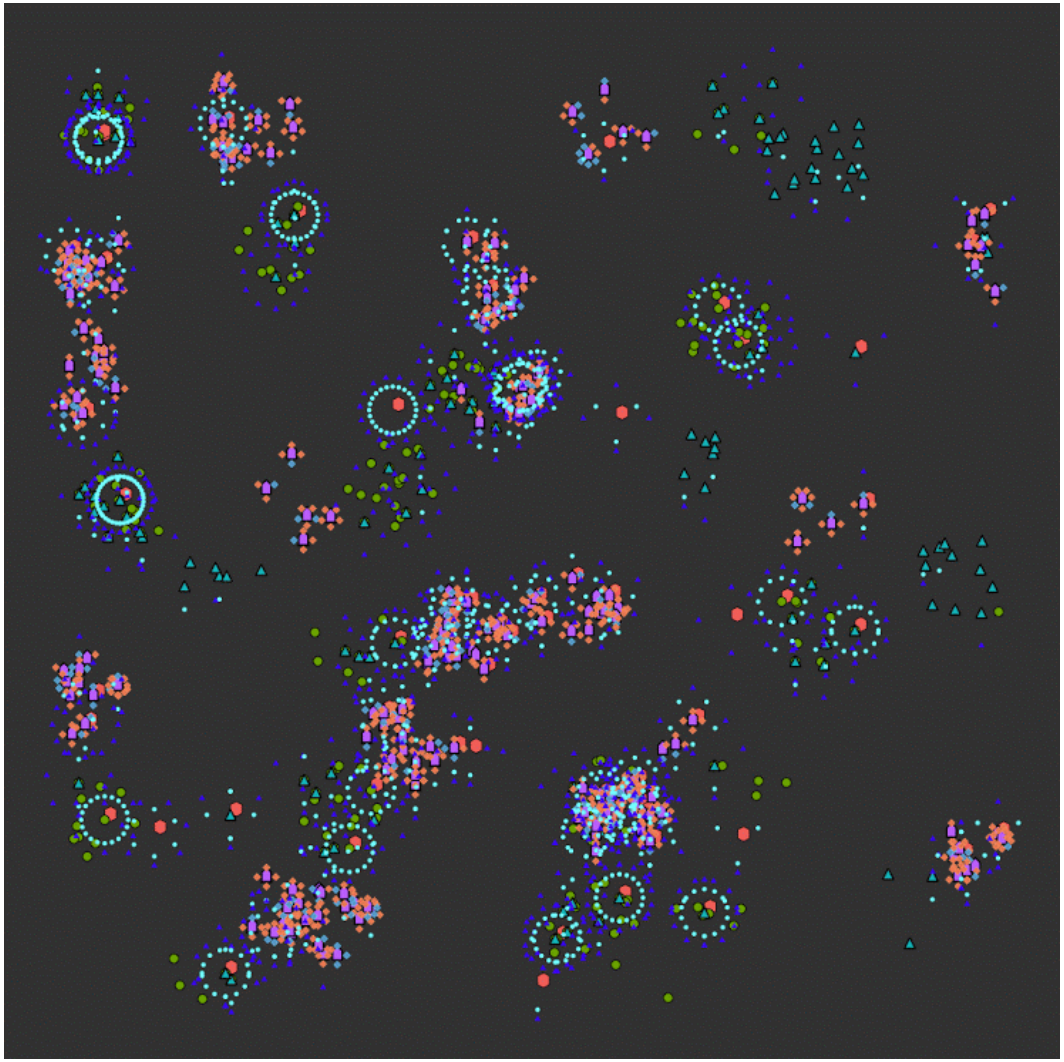


MASH (Modular Analysis and Simulation for human Health)



MASH (Modular Analysis and Simulation for human Health)

- Moving pink triangles are female mosquitoes
- Moving blue triangles are male mosquitoes
- Lines represent the trace of their movement
- Purple pentagons are blood-feeding sites
- Green circles are sugar-feeding sites
- Blue triangles are breeding sites/aquatic habitats
- Salmon hexagons are swarming/mating sites
- Blue/salmon diamonds around houses are susceptible and infectious humans



Homing-based gene drive systems

The mutagenic chain reaction: A method for converting heterozygous to homozygous mutations

Valentino M. Gantz* and Ethan Bier*

Sciencexpress

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

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

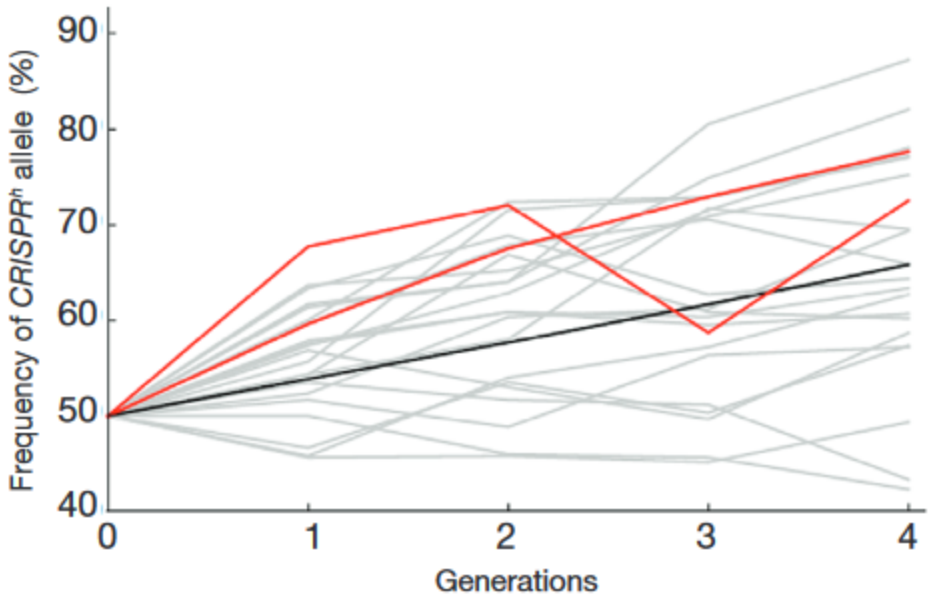
PNAS

LETTERS

nature biotechnology

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

Rate of resistant allele generation for Hammond *et al.* (2016) constructs



	Sequenced	Wild-type	Indel (independent)	Incomplete homing (independent)
AGAP011377	14	10	4 (3)	0
AGAP005958	13	5	8 (2)	0
AGAP007280	5	2	1 (1)	2 (1)
	32	**17**	**15**	

Hammond *et al.* (2016) Nature Biotech

- Homing rate = 98%
- Fertility of heterozygotes reduced by 90.7%

- Incomplete homing or indel events were observed in 15/32 screened organisms in which an error-free homing event was not observed.
- 1/7 of the homing events that originated these may be considered resistant alleles.
- Resistant allele generation rate = 0.13%

NHEJ is the most important source of homing-resistant alleles

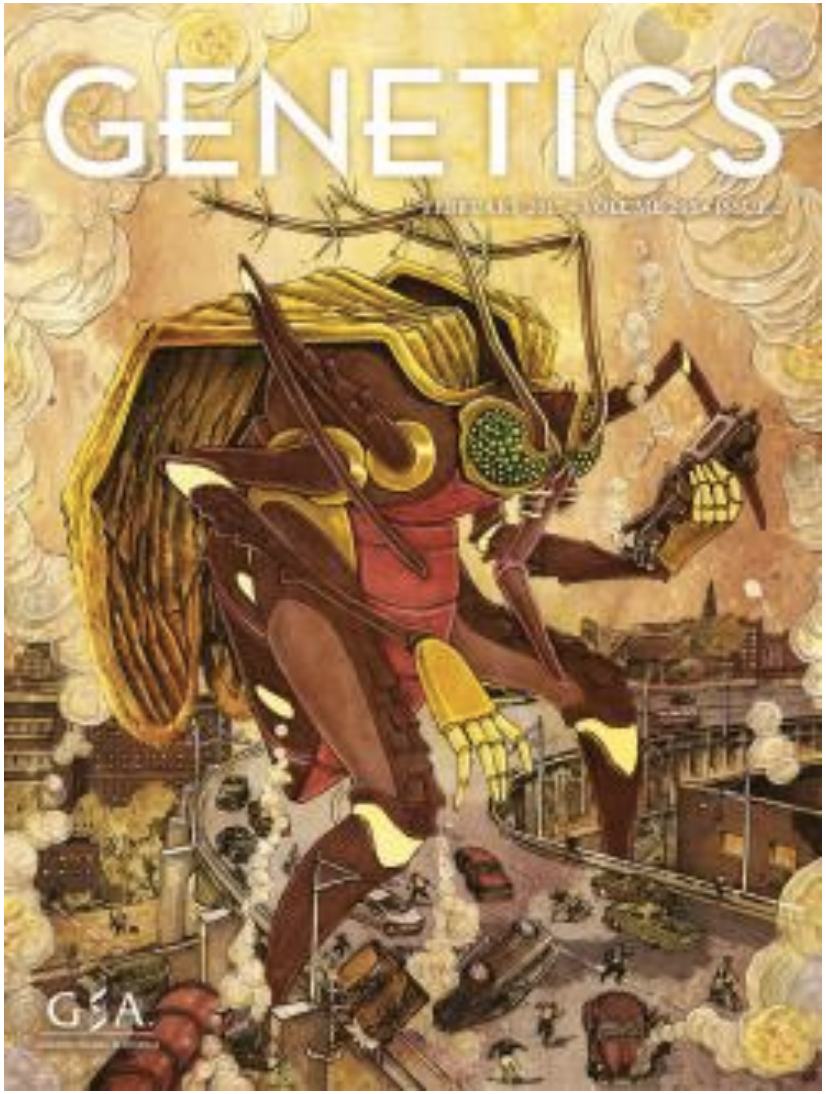
Evolution of resistance against CRISPR/Cas9 gene drive

Robert L. Unckless[1], Andrew G. Clark[2,3], Philipp W. Messer[3,*]

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS
[2]Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY
[3]Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY
[*]Corresponding author. Email: messer@cornell.edu

ABSTRACT

CRISPR/Cas9 gene drive (CGD) promises a highly adaptable approach for spreading genetically engineered alleles throughout a species, even if those alleles impair reproductive success. CGD has been shown to be effective in laboratory crosses of insects, yet it remains unclear to what extent potential resistance mechanisms will affect the dynamics of this process in large natural populations. Here we develop a comprehensive population genetic framework for modeling CGD dynamics, which incorporates potential resistance mechanisms as well as random genetic drift. Using this framework, we calculate the probability that resistance against CGD evolves from standing genetic variation, *de novo* mutation of wildtype alleles, or cleavage-repair by nonhomologous end joining (NHEJ) – a likely byproduct of CGD itself. We show that resistance to standard CGD approaches should evolve almost inevitably in most natural populations, unless repair of CGD-induced cleavage via NHEJ can be effectively suppressed, or resistance costs are on par with those of the driver. The key factor determining the probability that resistance evolves is the overall rate at which resistance alleles arise at the population level by mutation or NHEJ. By contrast, the conversion efficiency of the driver, its fitness cost, and its introduction frequency have only minor impact. Our results shed light on strategies that could facilitate the engineering of drivers with lower resistance potential, and motivate the possibility to embrace resistance as a possible mechanism for controlling a CGD approach. This study highlights the need for careful modeling of the population dynamics of CGD prior to the actual release of a driver construct into the wild.



Modeling error-prone homing-based gene drive

Male

Female

	HH	Hh	HR	hh	hR	RR
HH	1 HH	((1+e)/2) HH ((1-e-ρ)/2) Hh (ρ/2) HR	(1/2) HH (1/2) HR	(1) Hh	(1/2) Hh (1/2) HR	(1) HR
Hh	((1+e)/2ρ) HH ((1-e-ρ)/2) Hh (ρ/2) HR	Cross A	Cross B	((1+e)/2) Hh ((1-e-ρ)/2) hh (ρ/2) hR	Cross D	((1+e)/2) HR ((1-e-ρ)/2) hR (ρ/2) RR
HR	(1/2) HH (1/2) HR	Cross C	(1/4) HH (1/2) HR (1/4) RR	(1/2) Hh (1/2) hR	(1/4) Hh (1/4) HR (1/4) hR (1/4) RR	(1/2) HR (1/2) RR
hh	(1) Hh	((1+e)/2) Hh ((1-e-ρ)/2) hh (ρ/2) HR	(1/2) Hh (1/2) hR	(1) hh	(1/2) hR (1/2) hh	(1) hR
hR	(1/2) Hh (1/2) HR	Cross E	(1/4) Hh (1/4) HR (1/4) hR (1/4) RR	(1/2) hR (1/2) hh	(1/4) hh (1/2) hR (1/4) RR	(1/2) hR (1/2) RR
RR	(1) HR	((1+e)/2) HR ((1-e-ρ)/2) hR (ρ/2) RR	(1/2) HR (1/2) RR	(1) hR	(1/2) hR (1/2) RR	(1) RR

Cross A

$(((1+e)^2)/4)$ HH
$(((1-e-ρ)^2)/4)$ hh
$(((1+e)(1-e-ρ))/2)$ Hh
$(((1-e-ρ)ρ)/2)$ hR
$(((1+e)ρ)/2)$ HR
$((ρ^2)/4)$ HR

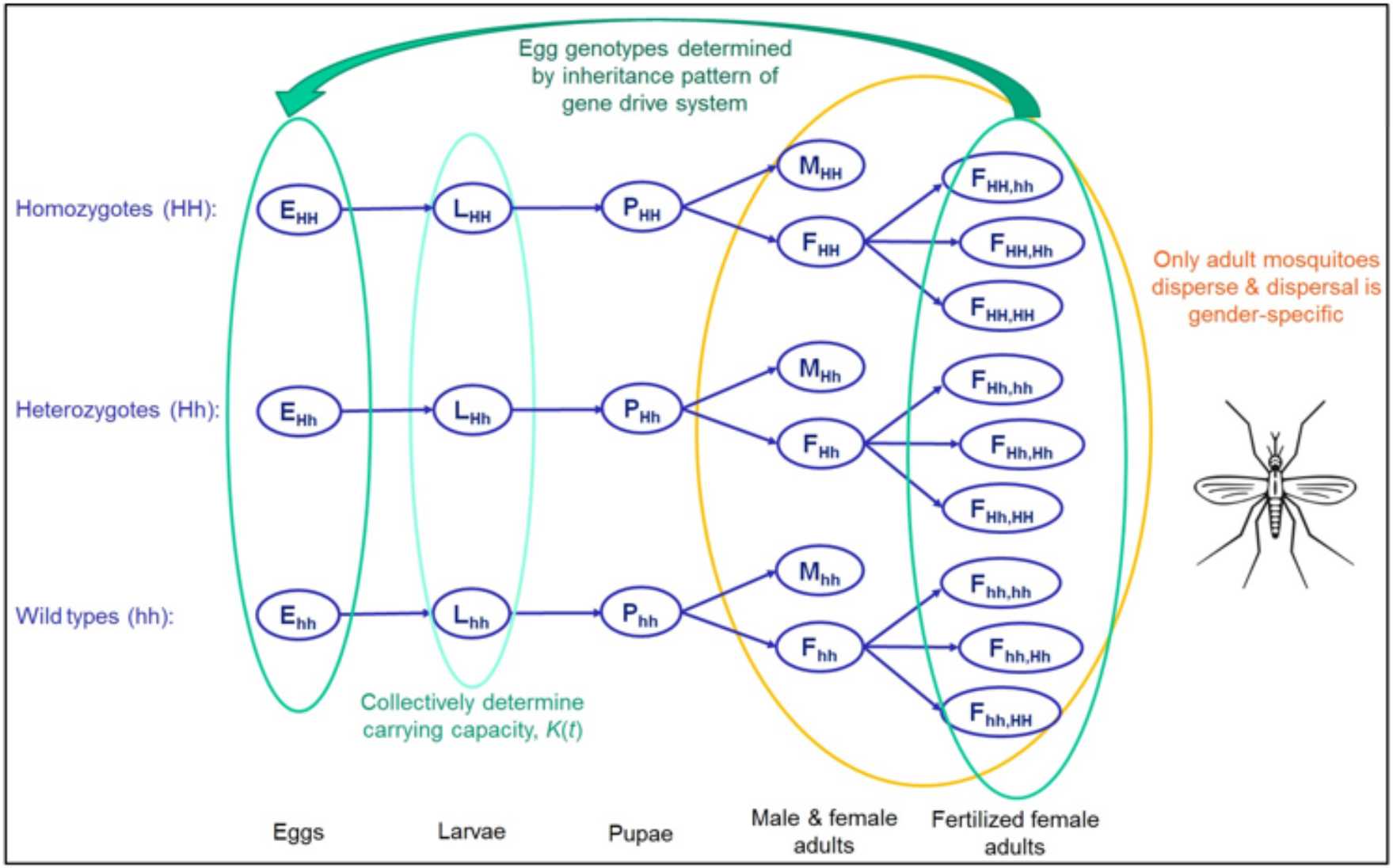
Cross B/C

((1+e)/4) HH
((1-e-ρ)/4) hR
((1-e-ρ)/4) Hh
((ρ)/4) RR
((1+e+ρ)/4) HR

Cross D/E

((1+e)/4) Hh
((1-e-ρ)/4) hh
((1+e)/4) HR
((1-e)/4) hR
((ρ)/4) RR

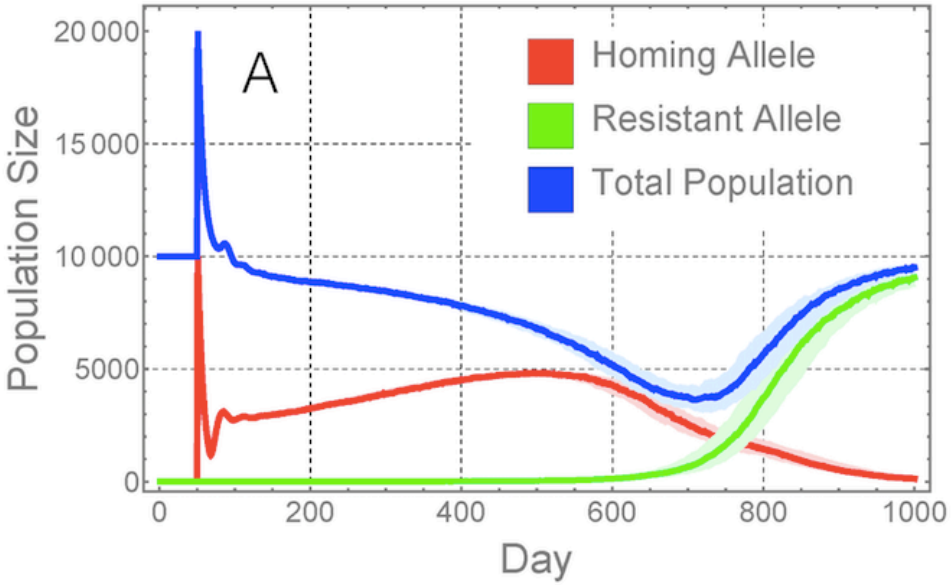
Population dynamic model (with overlapping generations, density-dependence, stochasticity)

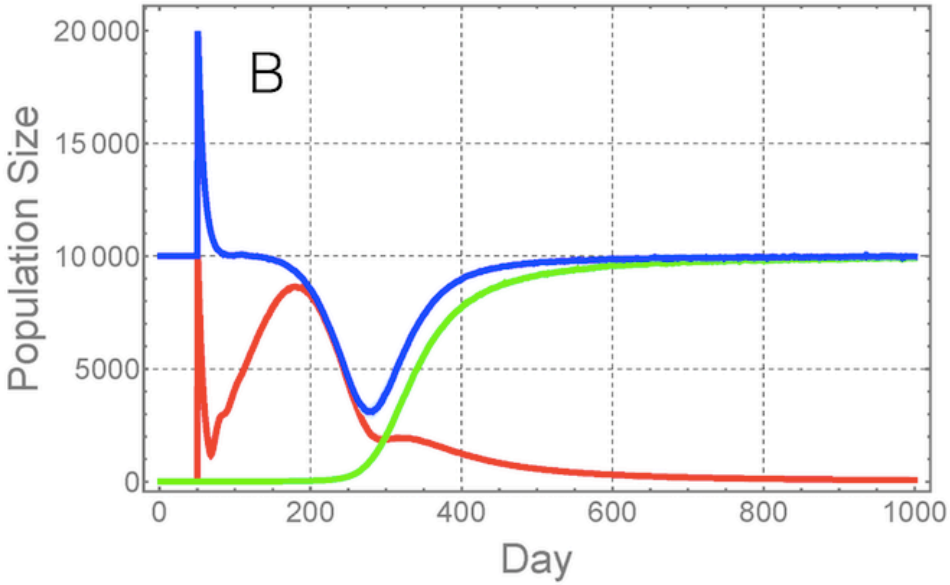


Dynamics of current constructs

a) Hammond *et al.* (2016) construct:
- Homing rate = 98%
- NHEJ rate = 0.13%
- Fertility of heterozygotes reduced by 90.7%

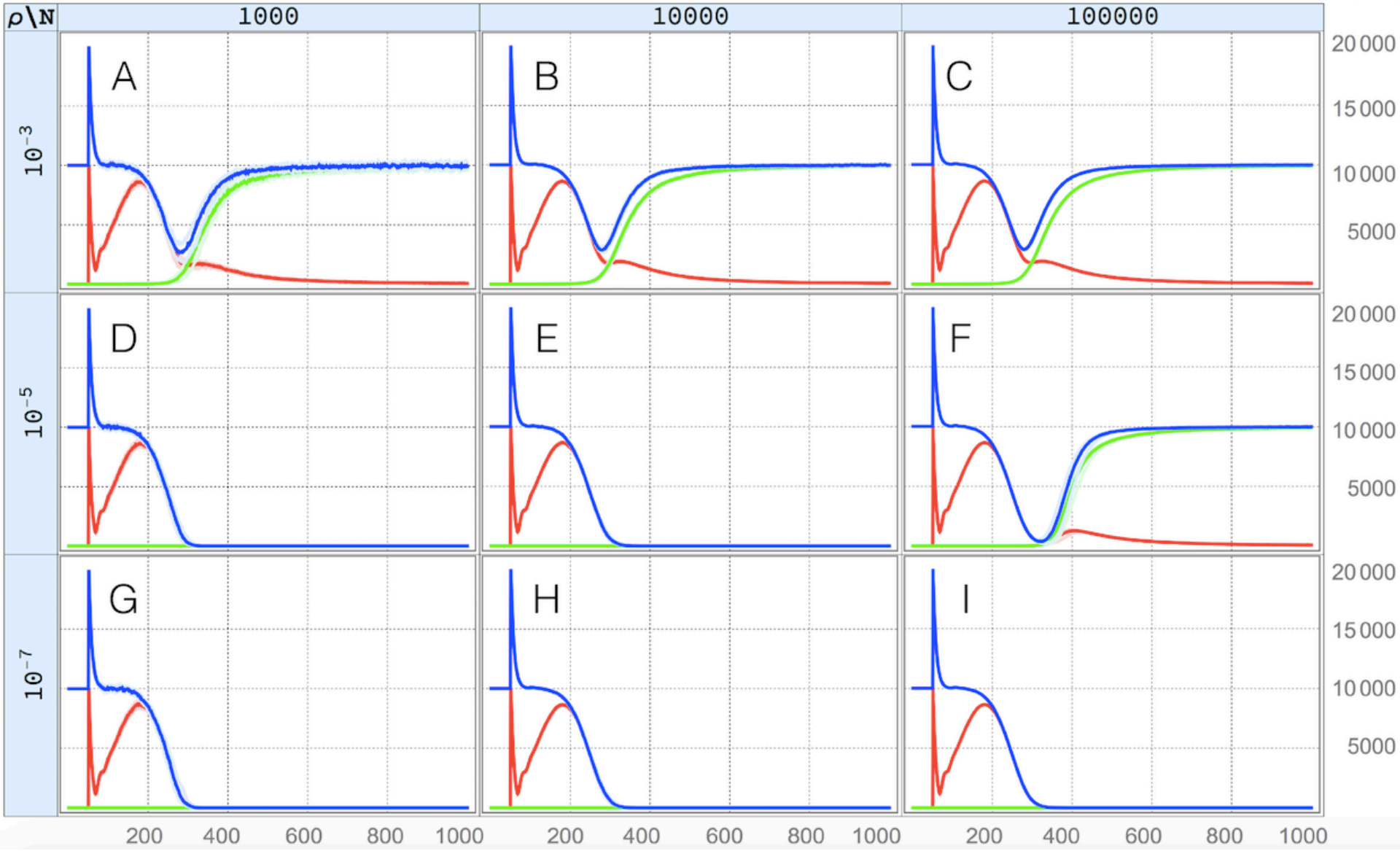
b) Hammond *et al.* (2016) construct:
- Homing rate = 98%
- NHEJ rate = 0.13%
- **Fertility of heterozygotes same as wild-type**



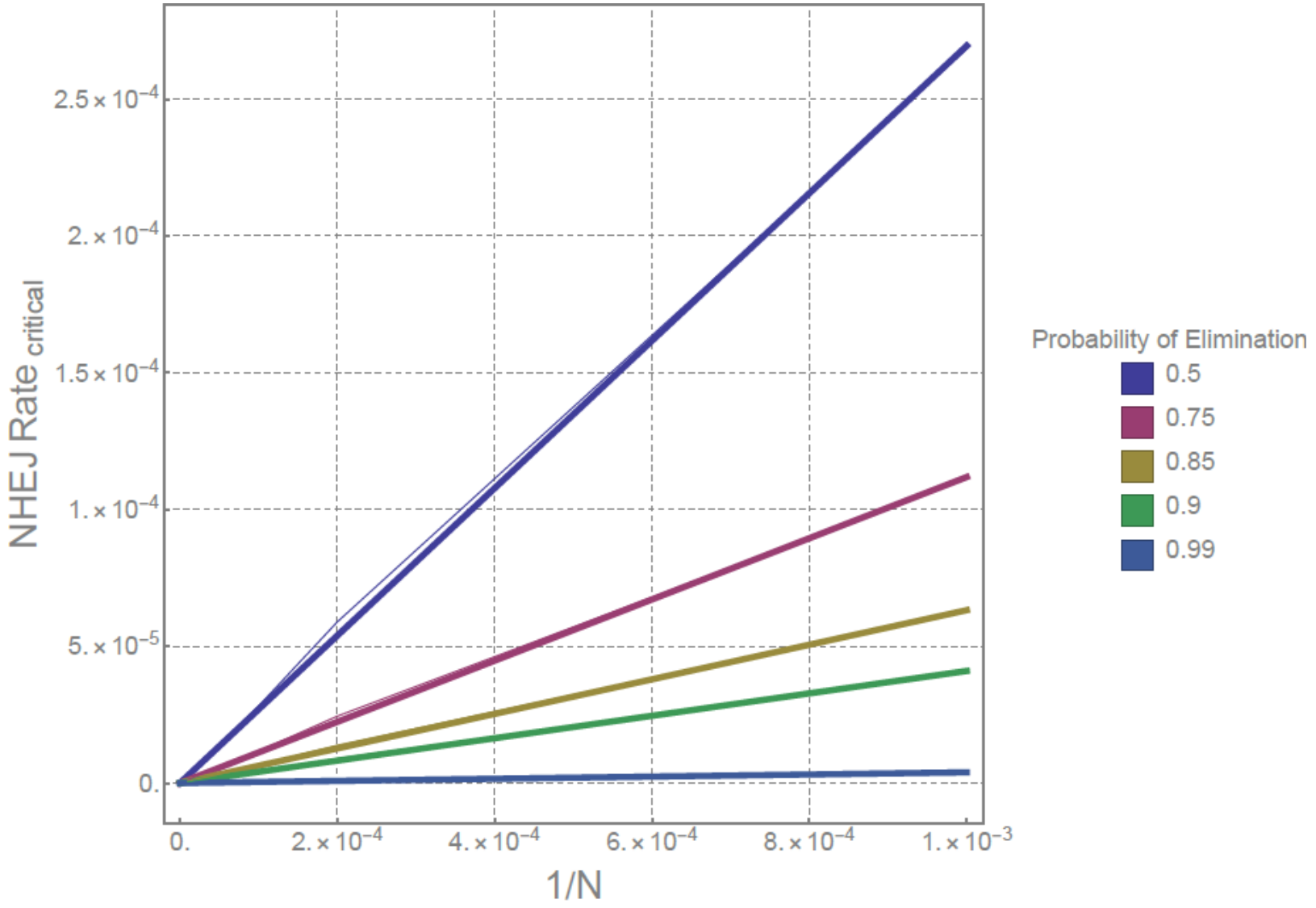


Marshall, Buchman, Sanchez & Akbari (2016) bioRxiv

Population elimination depends on the resistant allele generation rate & population size

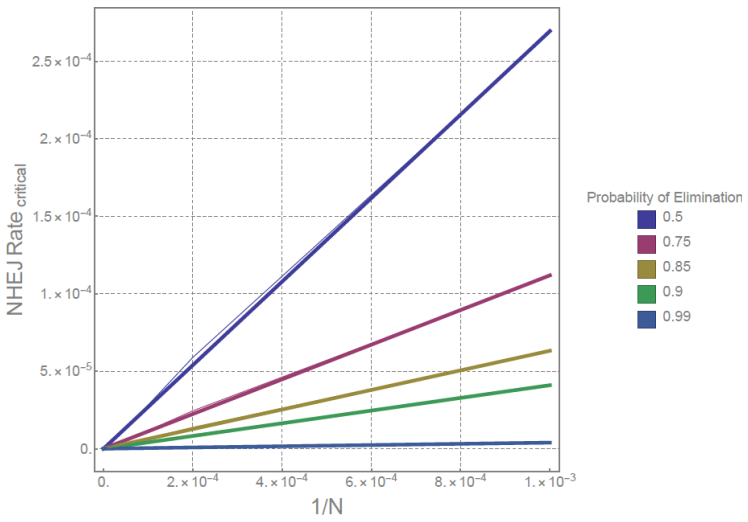


Tolerable rates of resistant allele generation are inversely proportional to the population size



Marshall, Buchman, Sanchez & Akbari (2016) bioRxiv

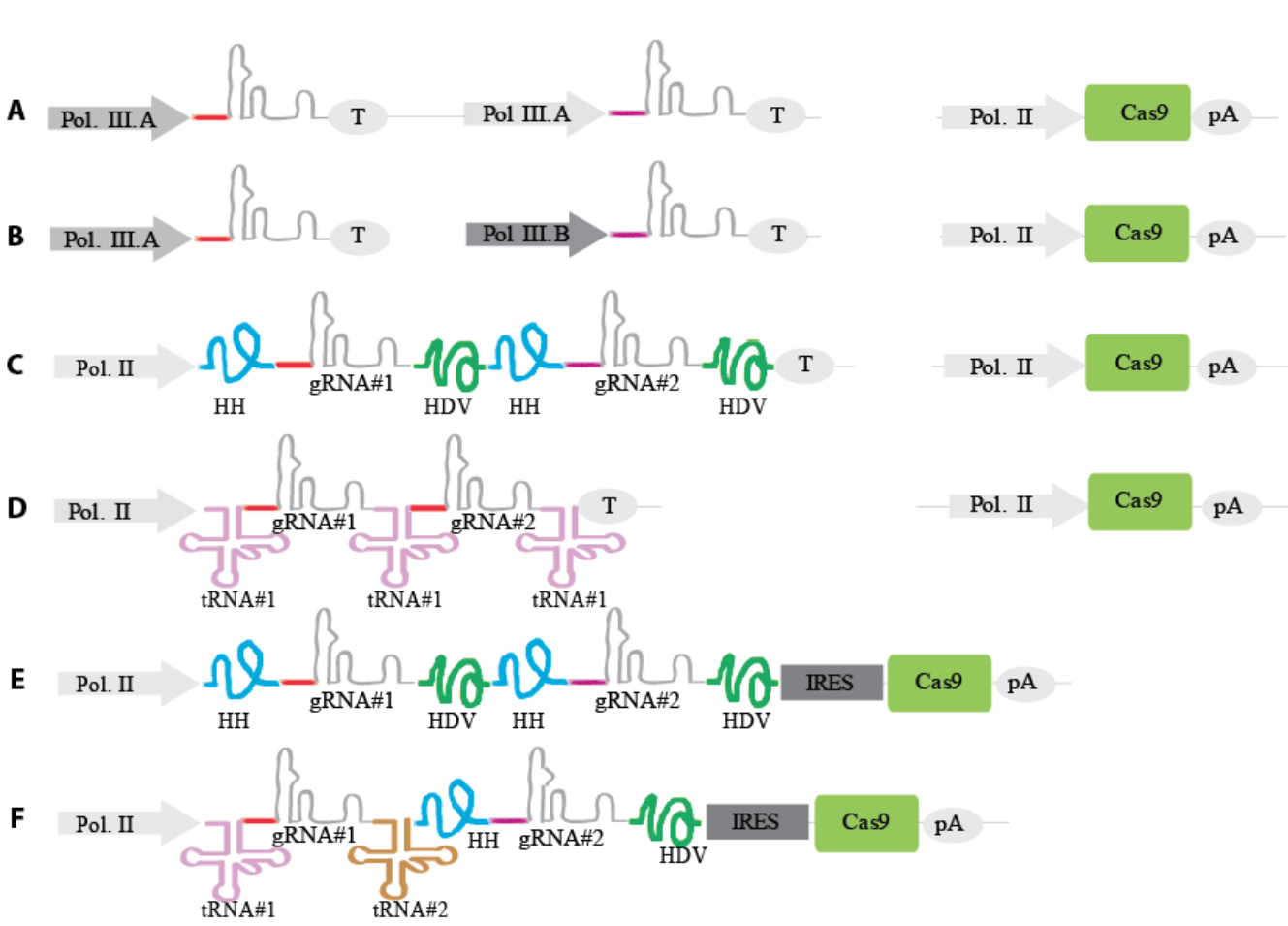
So the resistant-allele generation rate we need to achieve is…



Population size (*N*):	Homing-resistant allele generation rate for 90% probability of elimination:
1 thousand	4×10^{-5}
1 million	4×10^{-8}
1 billion	4×10^{-11}
10 billion	4×10^{-12}

Marshall, Buchman, Sanchez & Akbari (2016) bioRxiv

Multiplexing gRNAs



Marshall, Buchman, Sanchez & Akbari (2016) bioRxiv

Population elimination possible through multiplexing

$$\rho_{m=2} = \frac{P\{hh\}}{P\{hh\}+P\{hR\}}\rho^2 + \frac{P\{hR\}}{P\{hh\}+P\{hR\}}\rho \qquad \rho_{m=2} \approx \rho^2$$

$$\rho_{m=3} = \frac{P\{hhh\}\rho^3 + P\{hhR\}\rho^2 + P\{hRR\}\rho}{P\{hhh\}+P\{hhR\}+P\{hRR\}} \qquad \rho_{m=3} \approx \rho^3$$

Multiplex number:	Homing-resistant allele generation rate:	Population size capable of eliminating:
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

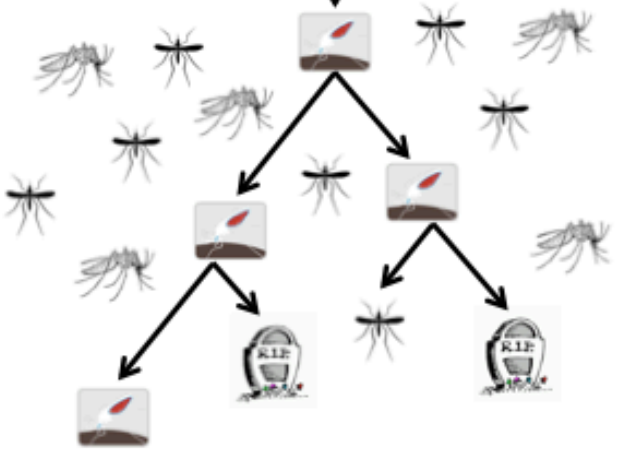
Marshall, Buchman, Sanchez & Akbari (2016) bioRxiv

The effect of gene drive on containment of transgenic mosquitoes

John M. Marshall *

Department of Biomathematics, UCLA School of Medicine, University of California at Los Angeles, Los Angeles, CA 90095-1766, USA

- Prior to a release, **trials** are required in **outdoor field cages**.
- Some **breaches of containment** are difficult to protect against.
- Gene drive systems **enhance** the **invasiveness** of introduced genes.
- Question #1: **"How likely is it that gene drive systems will persist following an accidental release?"**



***Branching process*:**

- A small number of GM mosquitoes escape into an **infinite population** of **wild-types**.
- **Mating** and **death** of GM mosquitoes is modeled in **continuous time**.
- **Patterns of inheritance** are **specific** to each **gene drive system**.

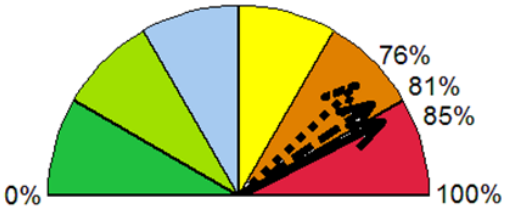
Marshall (2009) J Theor Biol

Homing-based systems are highly invasive following an accidental release

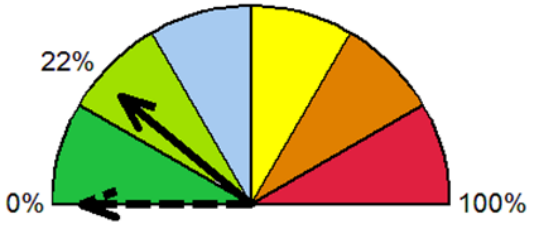
- **Homing-based systems** are more likely to spread than not for releases > 5
- **Toxin-antidote systems like *Medea*** are unlikely to spread because their drive only becomes significant at higher population frequencies
- **Threshold-dependent systems** are unlikely to spread following an accidental release

Persistence probability following an accidental release of 10 GM mosquitoes:

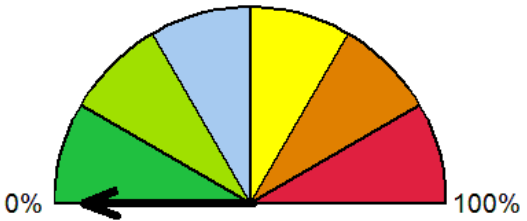
HEGs / homing systems:

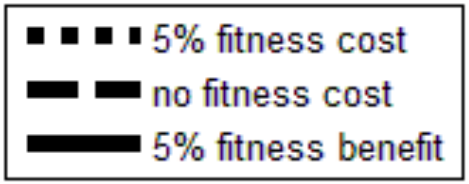


***Medea*:**



Underdominance:



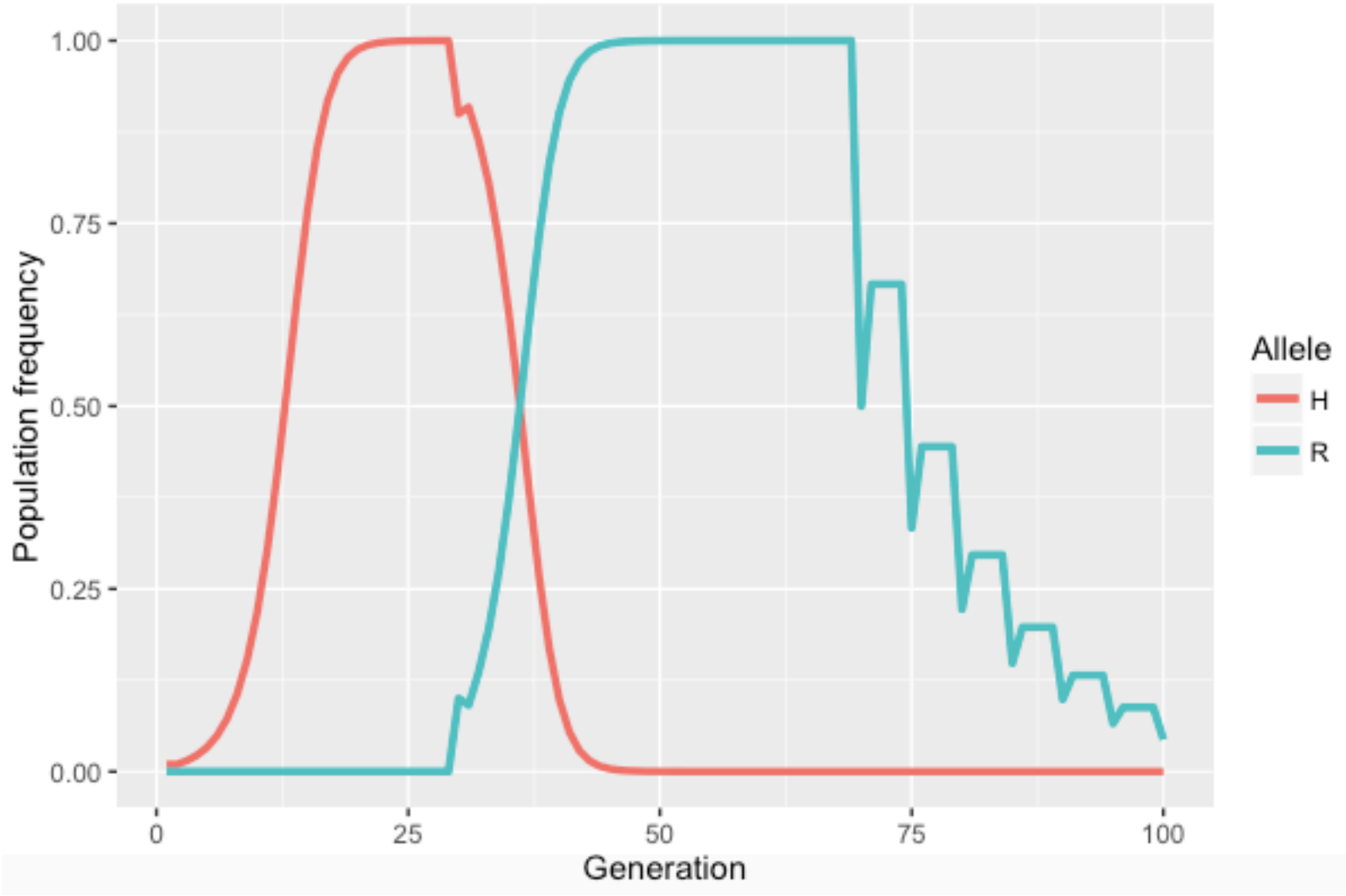


Marshall (2009) J Theor Biol

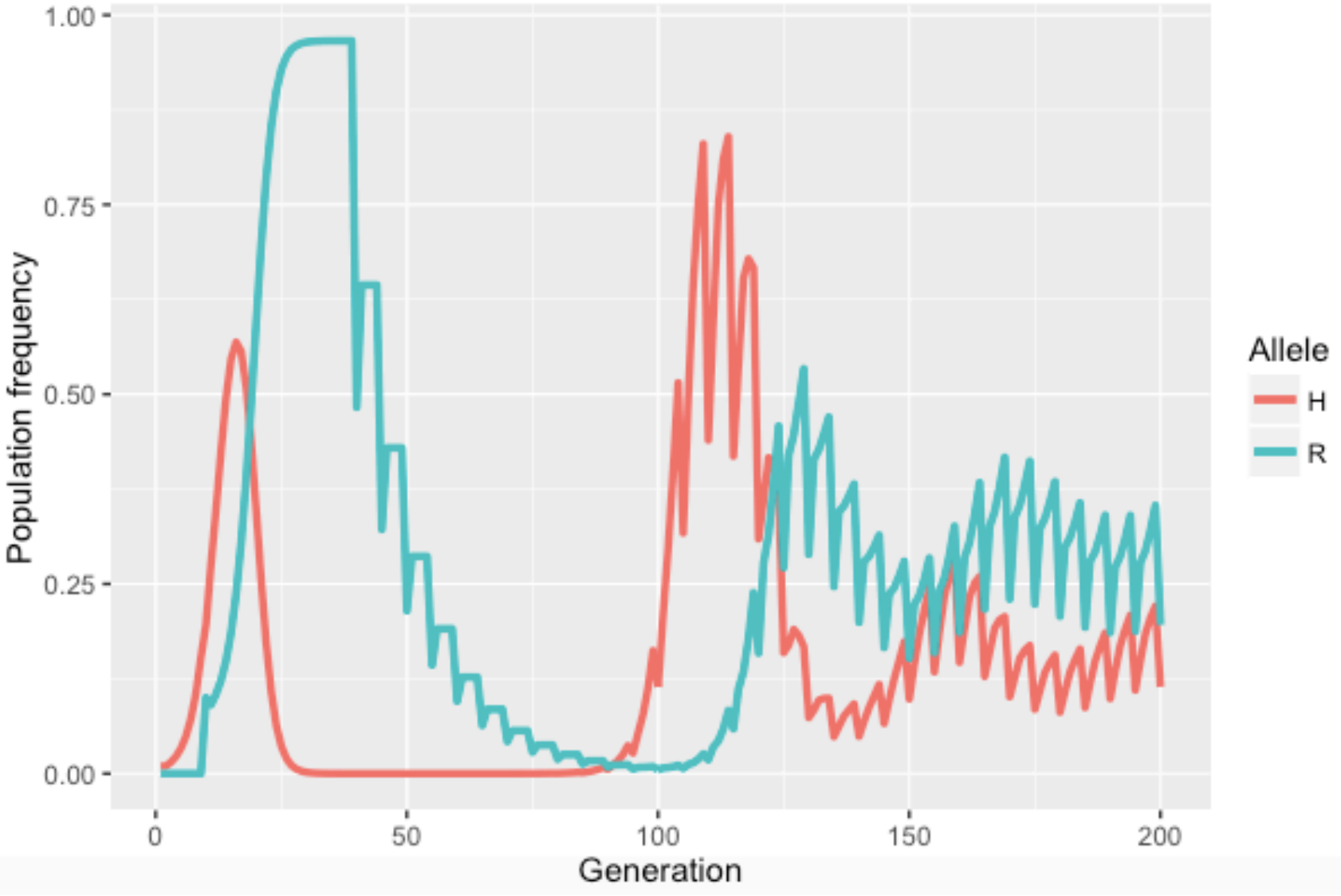
Remediation systems for homing-based gene drive

Note: After reversal, over time these will be lost from population by dilution with wildtype alleles

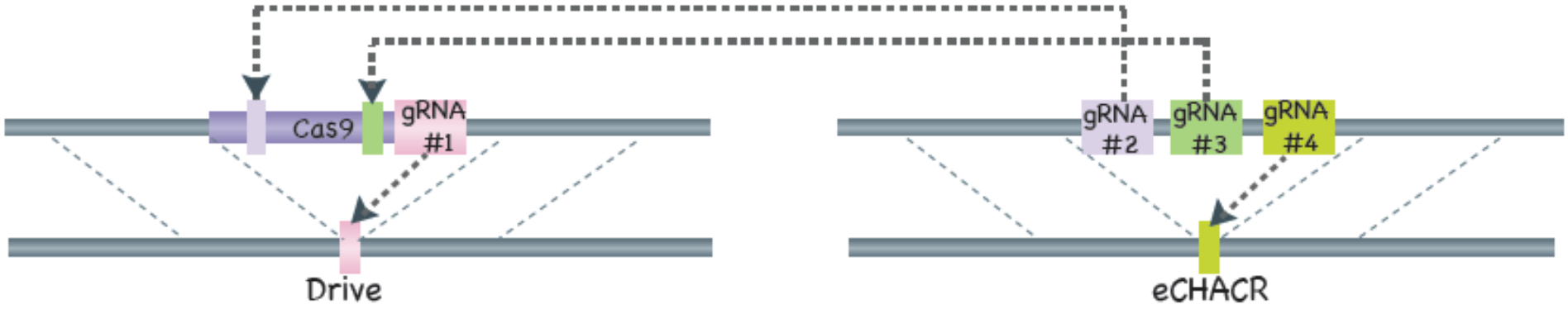
Drive (red) + Reversal drive or ERACR (turquoise)



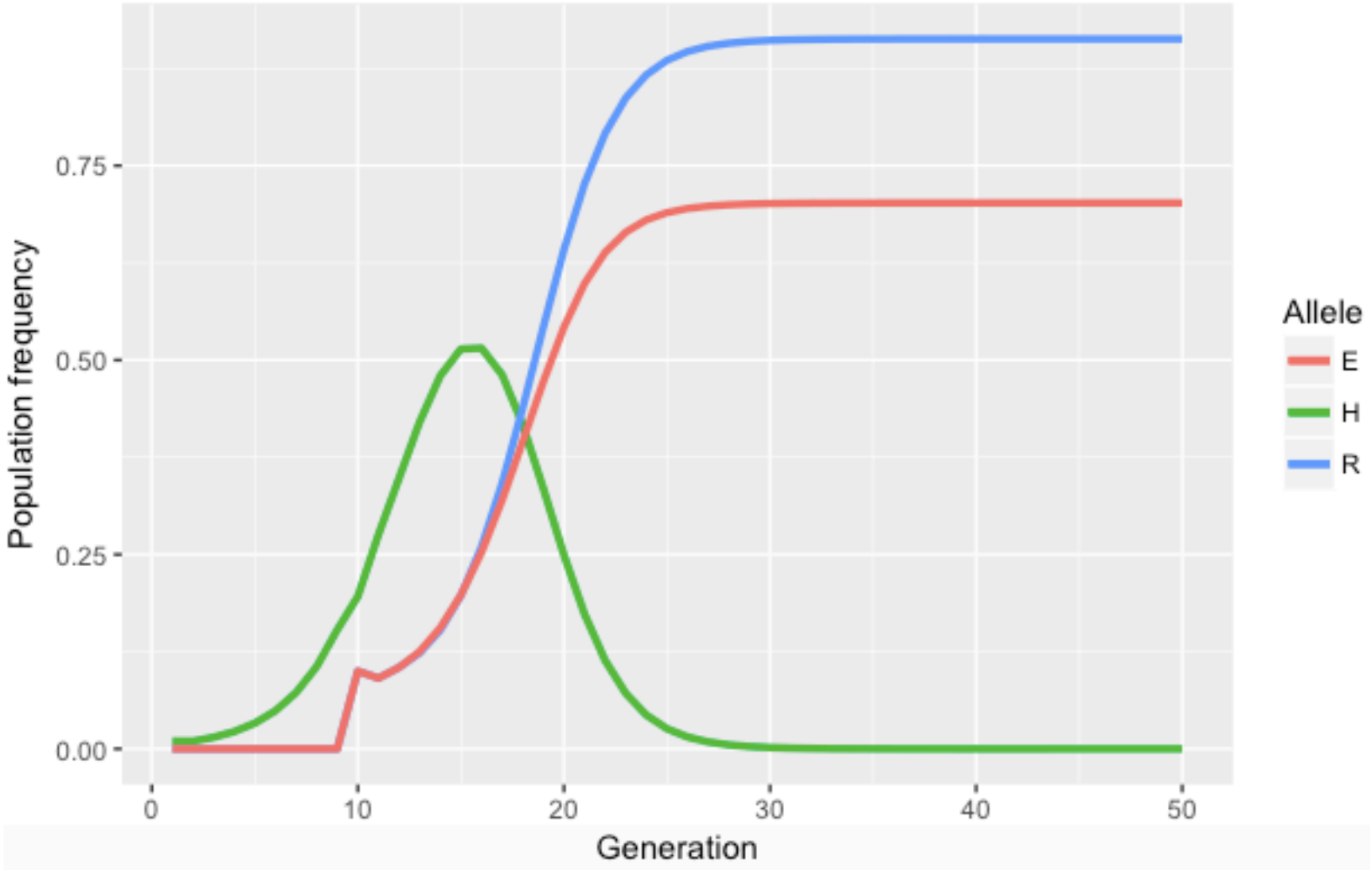
Longer-term reversal & dilution dynamics



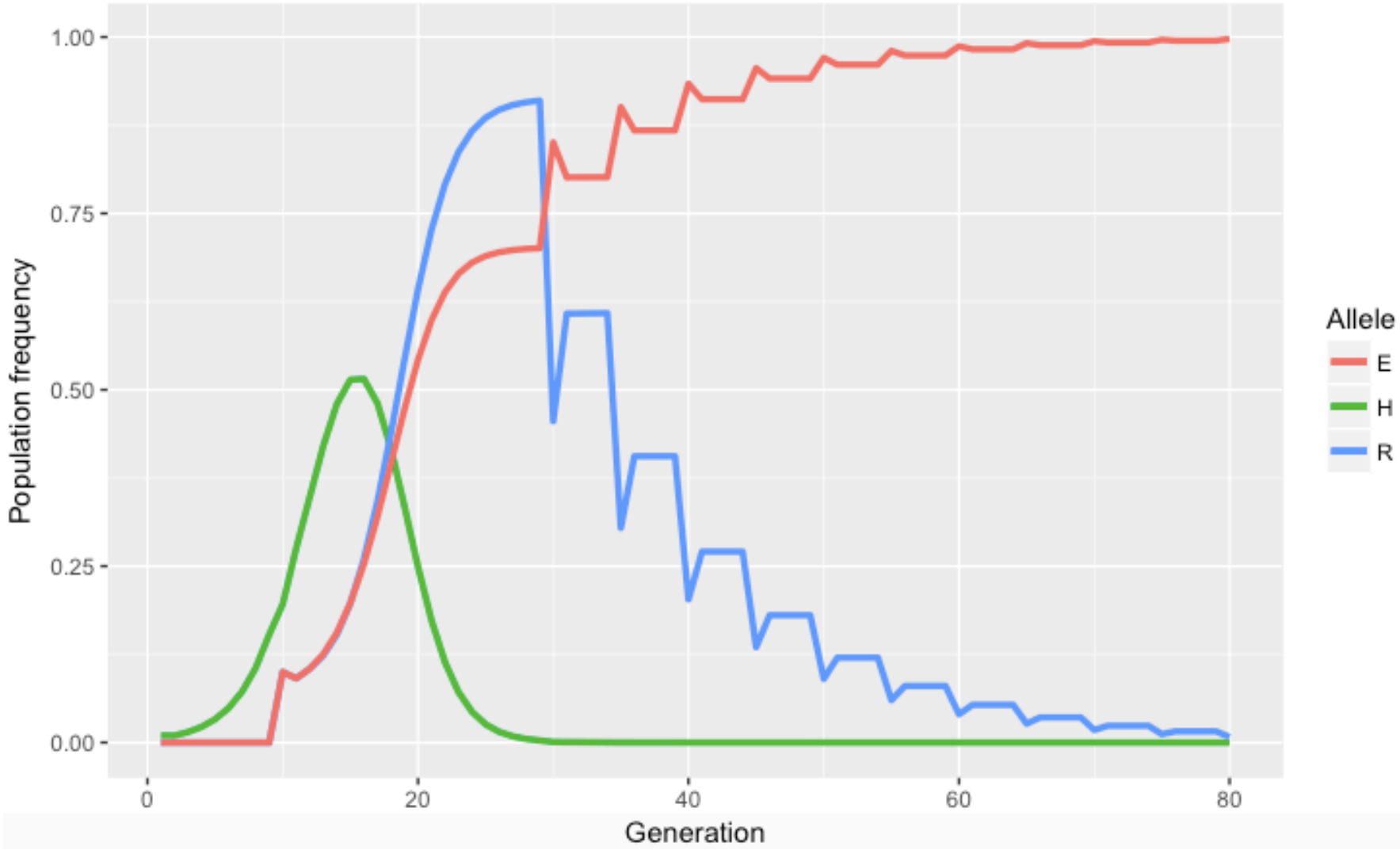
eCHACR & other reversal systems



Drive (red), Resistant allele (blue) + eCHACR (green)

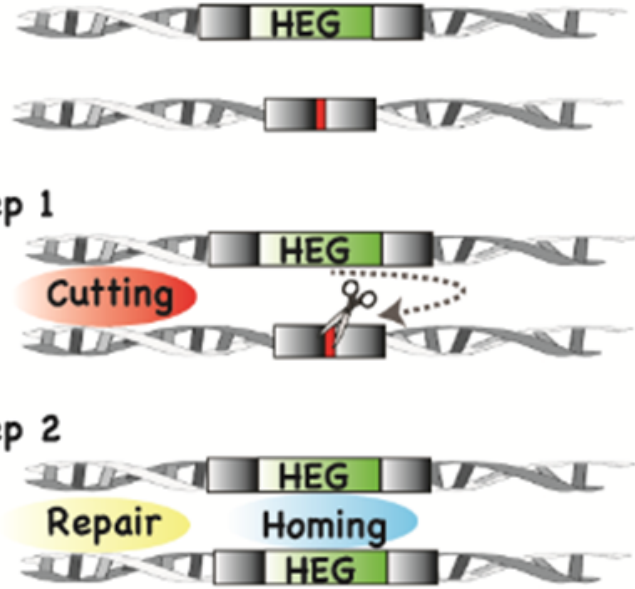


Dilution dynamics retaining eCHACR allele



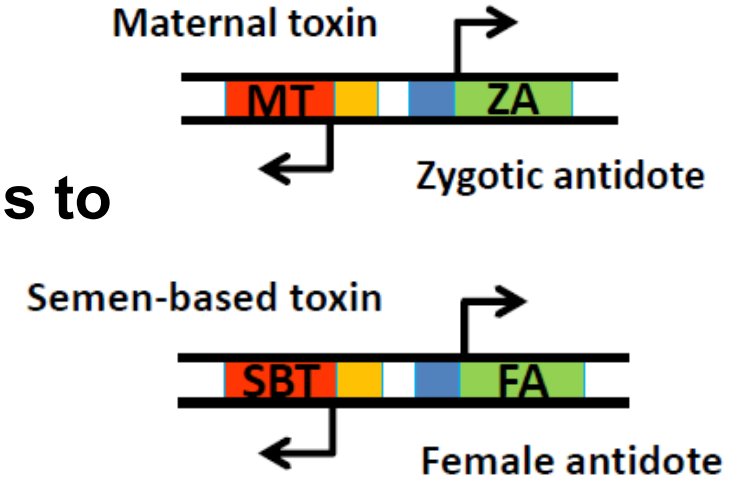
Conclusions

Homing-based systems:



1. Multiplexing provides a solution to resistant allele generation
2. Homing systems are highly invasive
3. Remediation strategies need to be explored in structured populations

Toxin-antidote-based systems:



1. Recent success in engineering threshold-dependent systems
2. Impact of population structure needs to be explored
3. Strange fitness effects, etc. need to be explored in wild populations

Acknowledgements

COLLABORATORS:

- Akbari Lab @ UC Riverside
- Hay Lab @ Caltech
- Lanzaro Lab @ UC Davis
- Malaria Elimination Initiative @ UC San Francisco
- David Smith @ University of Washington
- Malaria Modeling Group @ Imperial College London
- School of Public Health @ UC Berkeley

FUNDERS:

THE PARKER FOUNDATION

BILL & MELINDA GATES foundation

UC MEXUS ESTABLISHED 1980

RESEARCH Open Access

Perspectives of people in Mali toward genetically-modified mosquitoes for malaria control

John M Marshall*[1], Mahamoudou B Touré[2], Mohamed M Traore[2], Shannon Famenini[3,4] and Charles E Taylor[3,4]

- **Public attitude surveys** in **Mali** suggest that people would like to see a **successful confined trial** before accepting a release:

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

72-year-old man, Tienfala, Mali

Marshall *et al.* (2009) Malaria Journal