

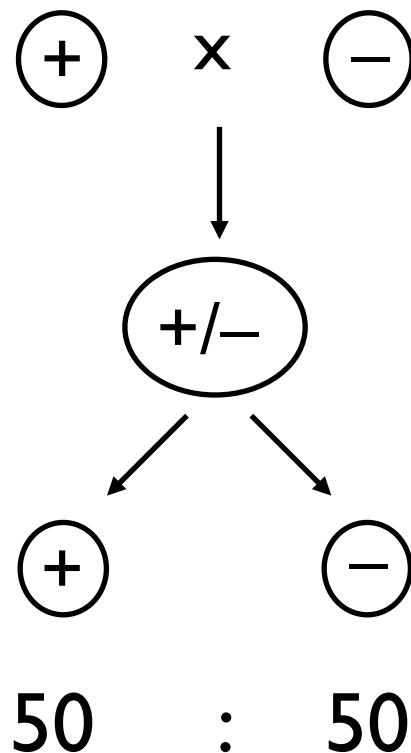
Gene drive and its potential use for malaria control

Austin Burt

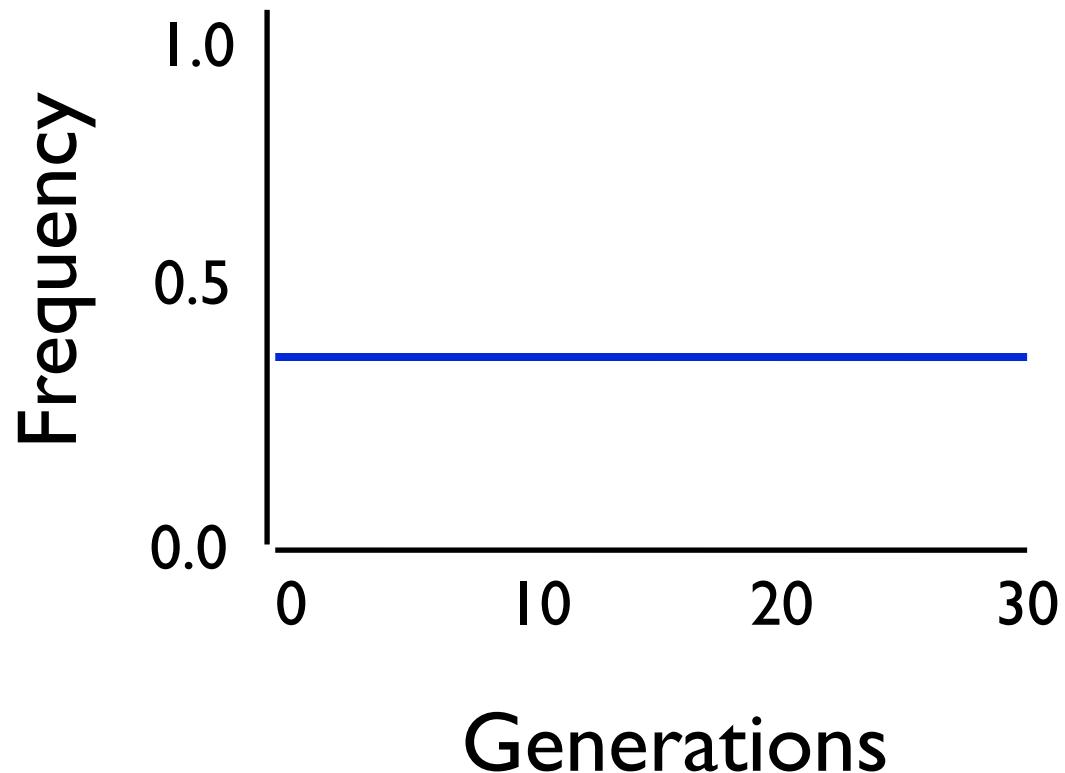
CMEE, Feb 2021

Mendelian transmission

Equal, unbiased inheritance

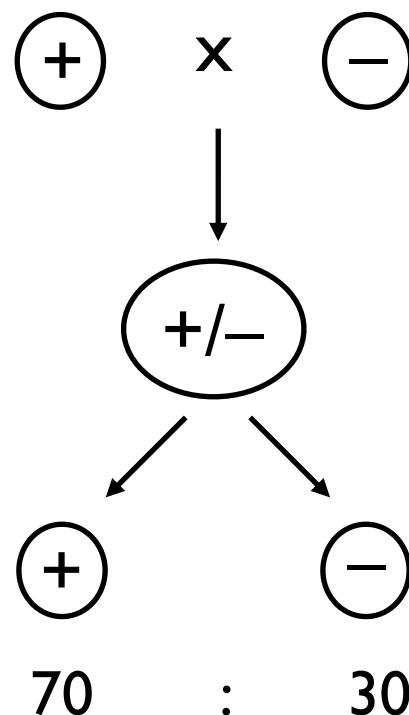


All else equal,
no change in gene frequency

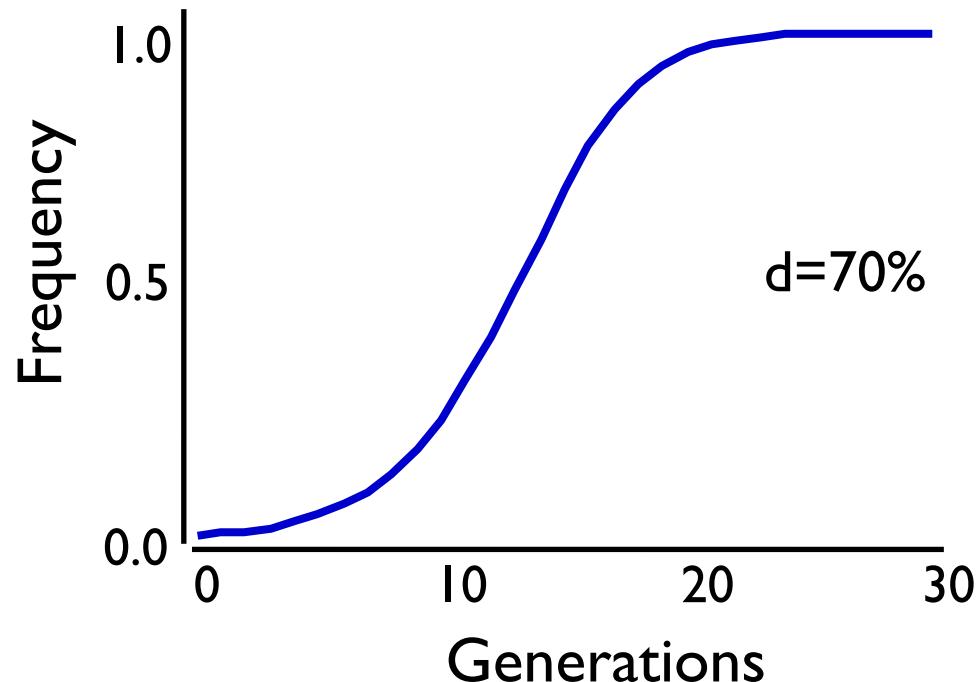


Gene drive

Preferential inheritance



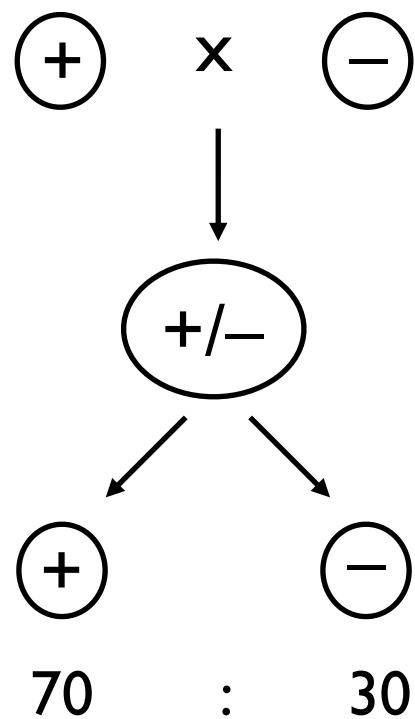
All else equal,
will lead to spread in population



Drive can even lead to spread of genes that
cause some harm to the carriers

Gene drive

Preferential inheritance



A "5th force" in population genetics, in addition to

- mutation
- migration
- drift
- selection

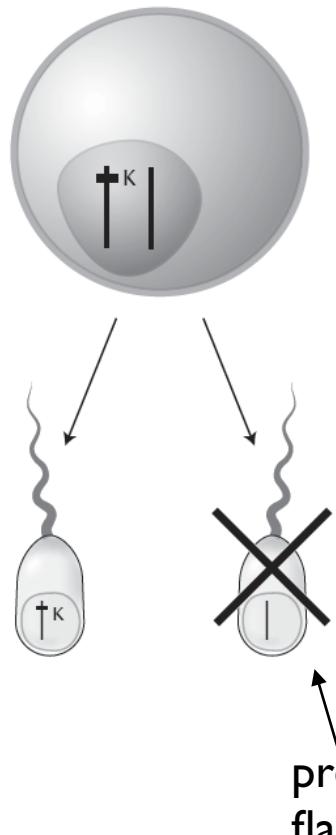
3 ways to drive

- Killing the competition
- Over-replication
- Directed movement towards the germline

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t-haplotype in mice



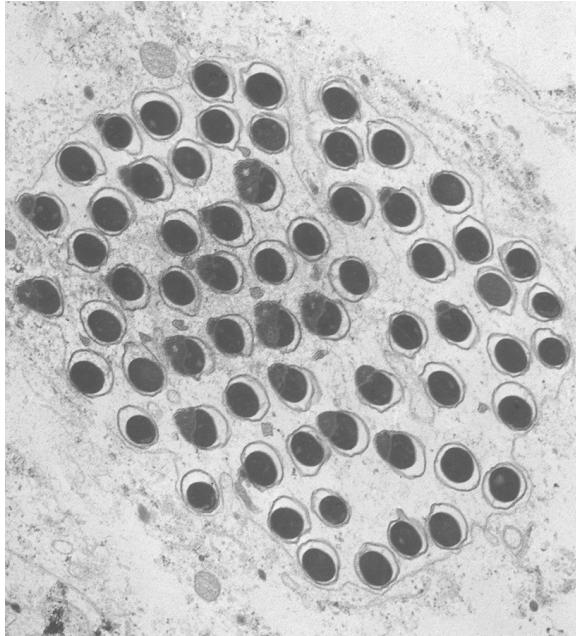
- 30-40Mb on 17th chromosome (1.2% of genome)
- transmitted 90% through males
- inherited as a unit: 4 inversions that prevent recombination
- homozygous sterile and/or lethal
- ~5% in natural populations
- sequence analysis suggests ~3MY old

premature acrosome reaction and/or
flagellar dysfunction

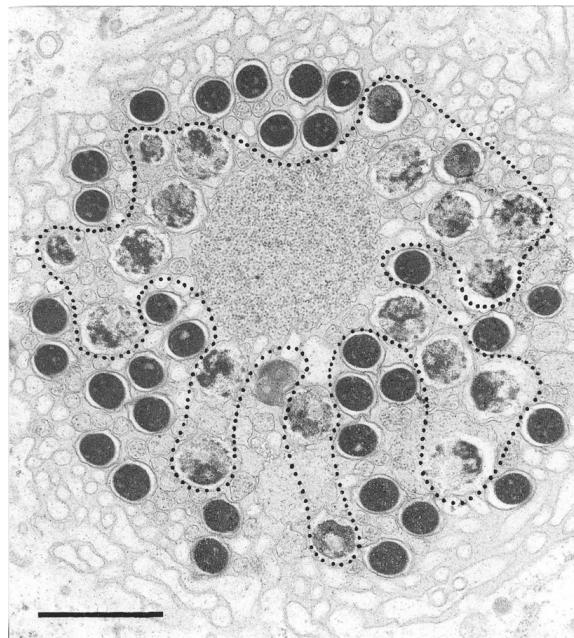
Segregation Distorter in *Drosophila*



Normal (−/−)



SD/−



- transmitted 95-99% through males
- failures in chromatin condensation
- 1-5% in natural populations
- sequence analysis suggests recent origin

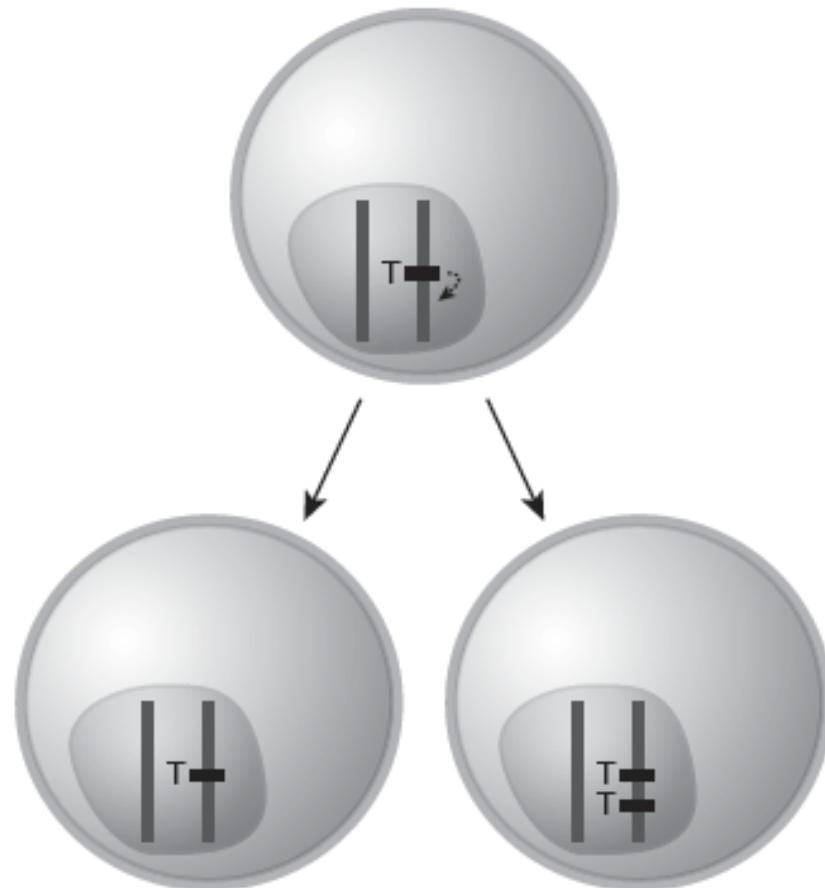
Electron micrographs of developing spermatids in *Drosophila*

3 ways to drive

- Killing the competition
- Over-replication
- Directed movement towards the germline

Over-replicating elements

- Homing endonuclease genes
- Transposable elements — “jumping genes”

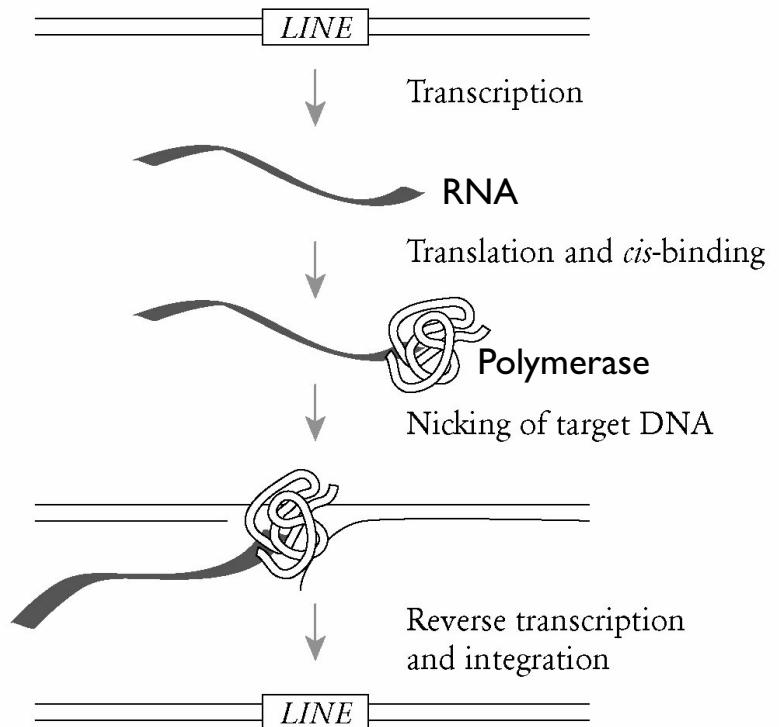


Transposable elements — “jumping genes”

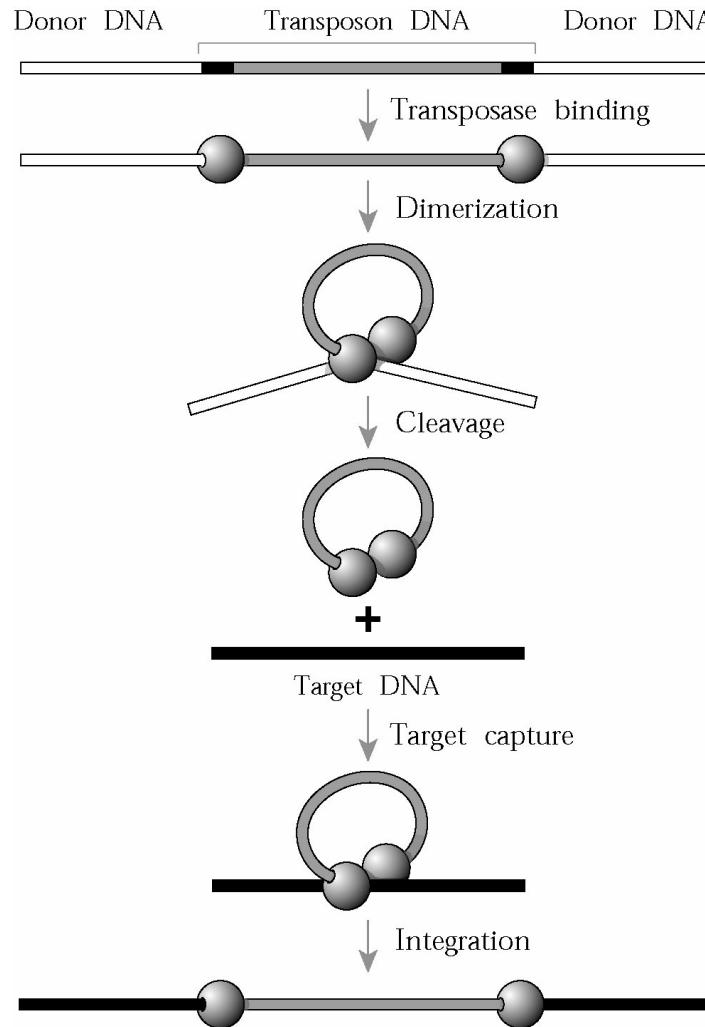
Many different mechanisms

e.g., retroelements encode
their own DNA polymerase:

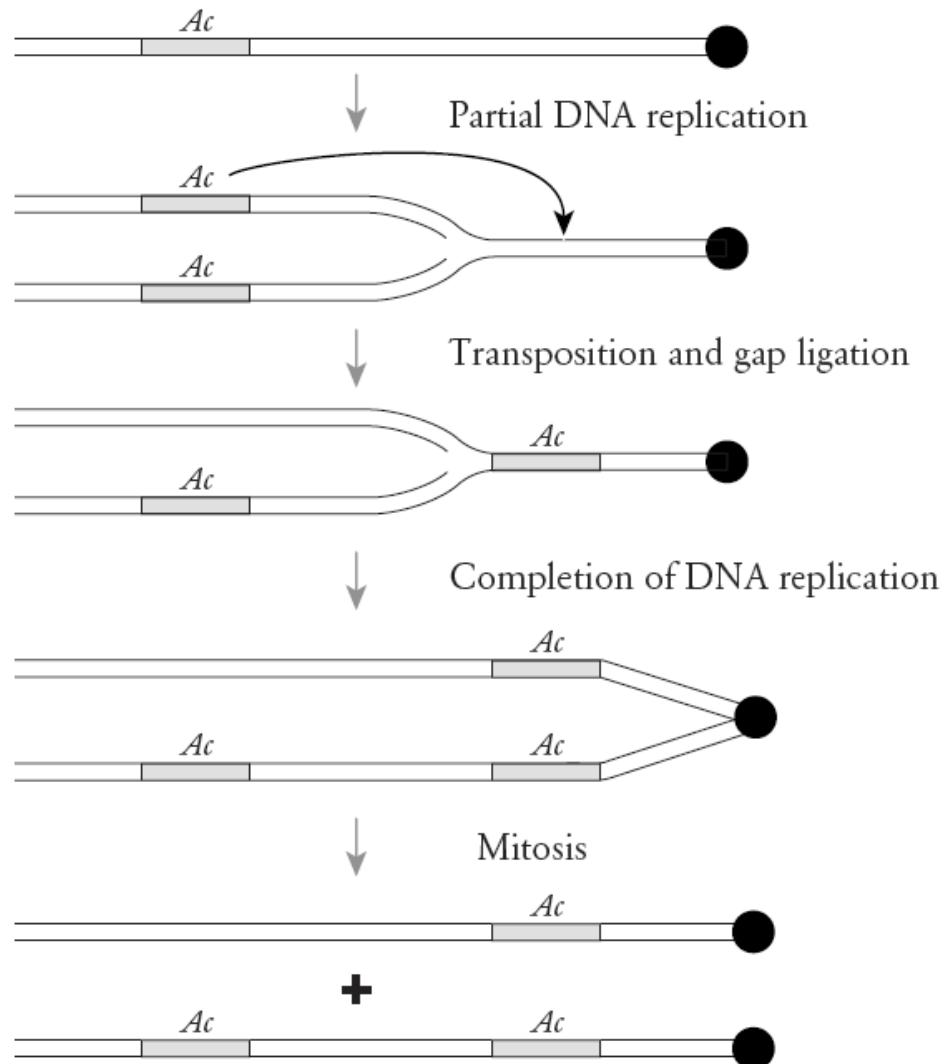
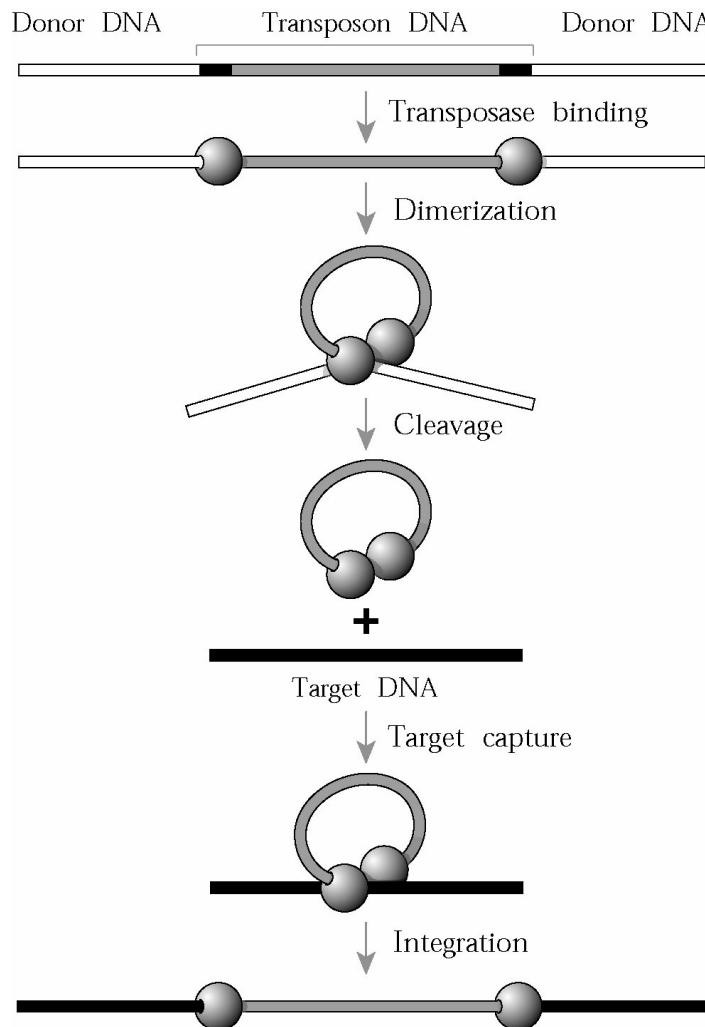
DNA → RNA → DNA



DNA transposons



DNA transposons

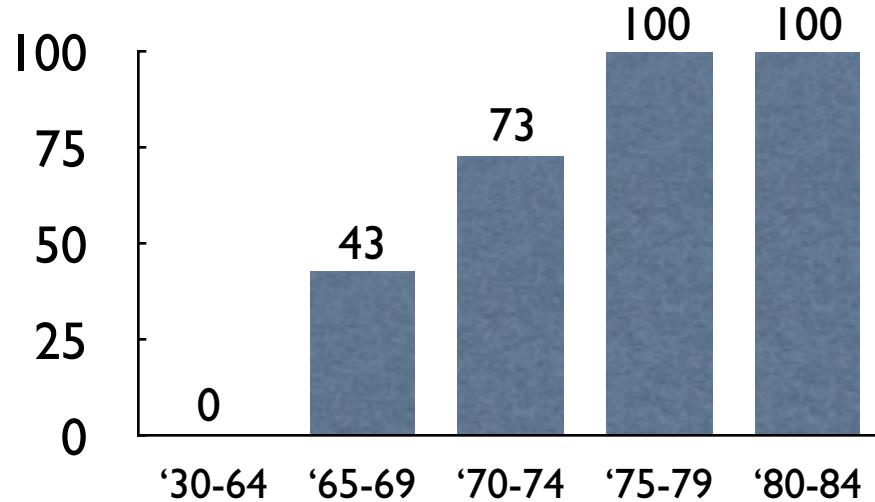


Rapid spread of P elements in *Drosophila*



In nature

Frequency of strains
with P-elements (%)



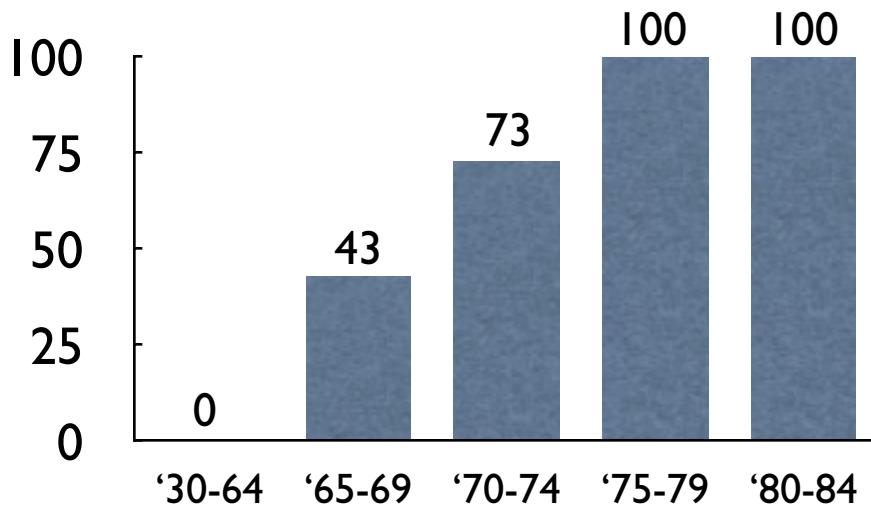
Year collected

Rapid spread of P elements in *Drosophila*



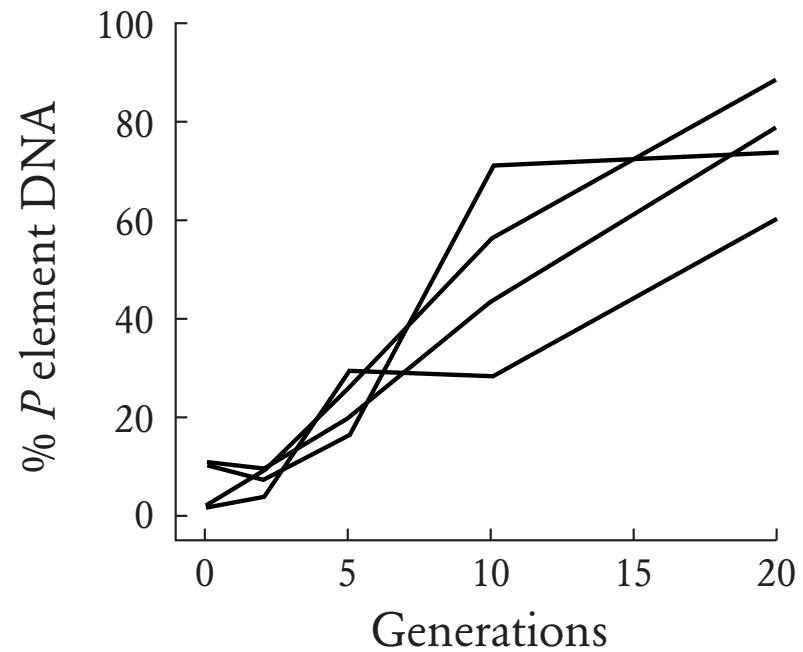
In nature

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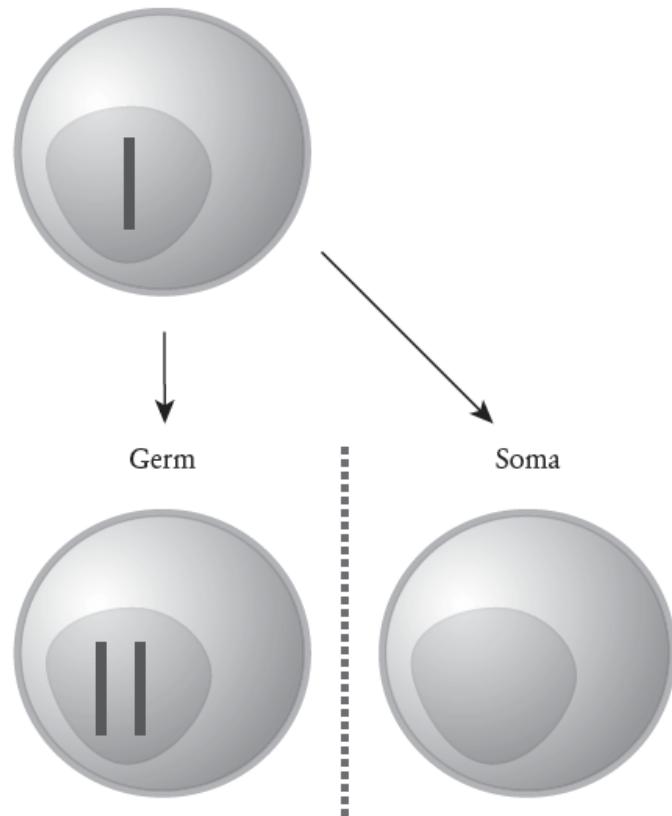
In the lab



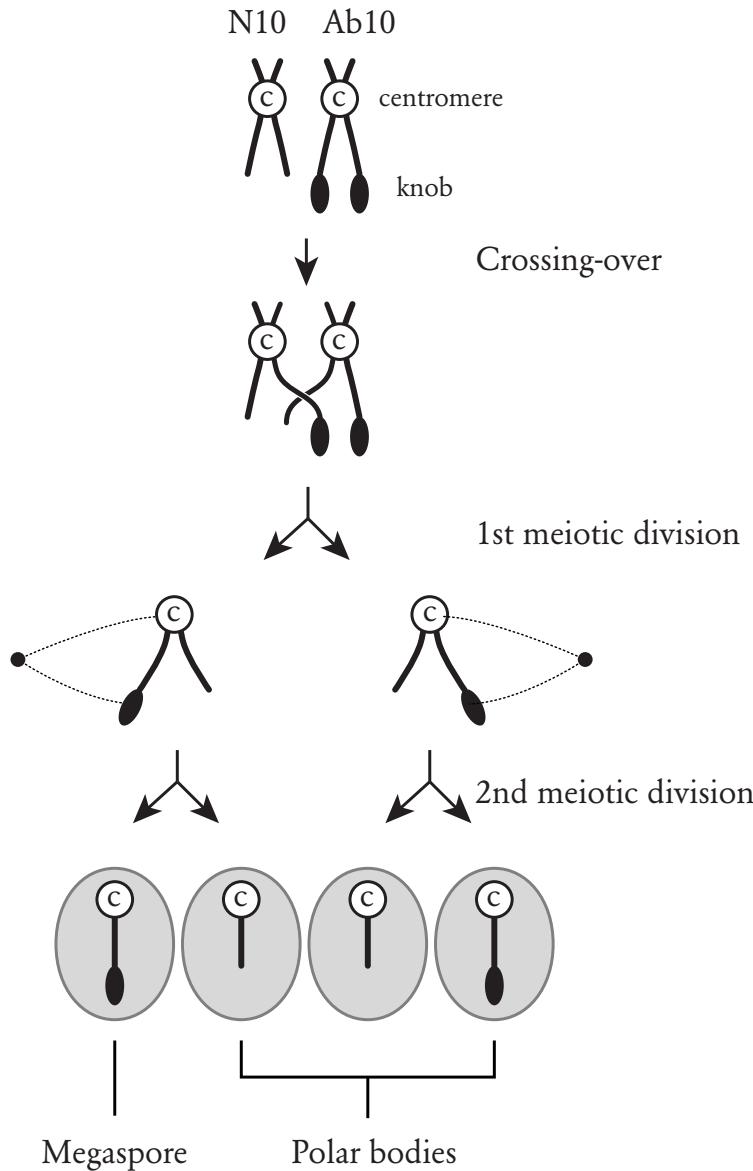
3 ways to drive

- Killing the competition
- Over-replication
- Directed movement towards the germline

3. Directed movement towards the germ line — “gonotaxis”



The knobs of maize



Meiotic drive



Mendelian inheritance



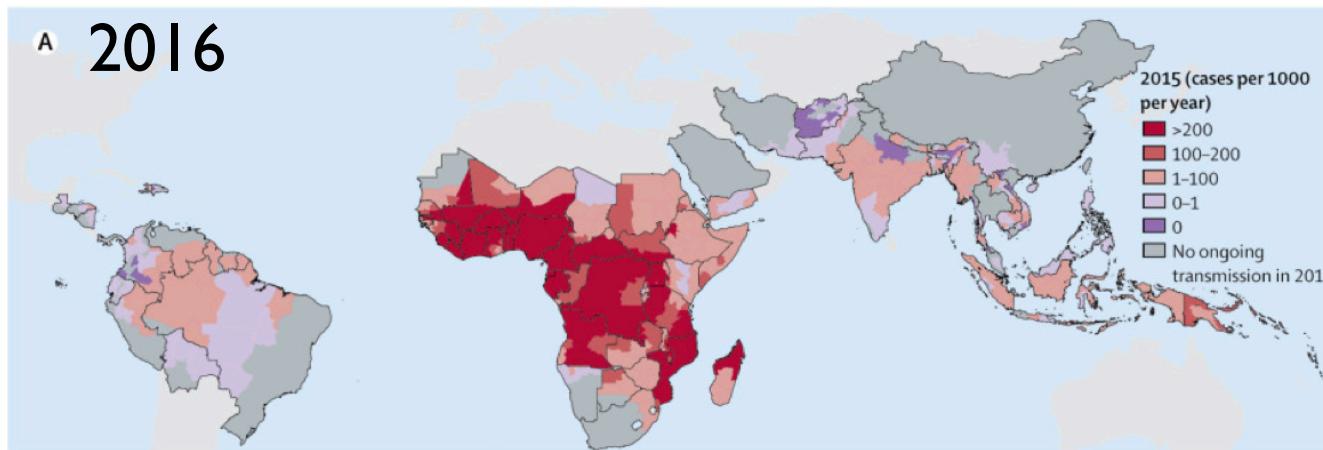
Potential use of gene drive for malaria control

Rationale

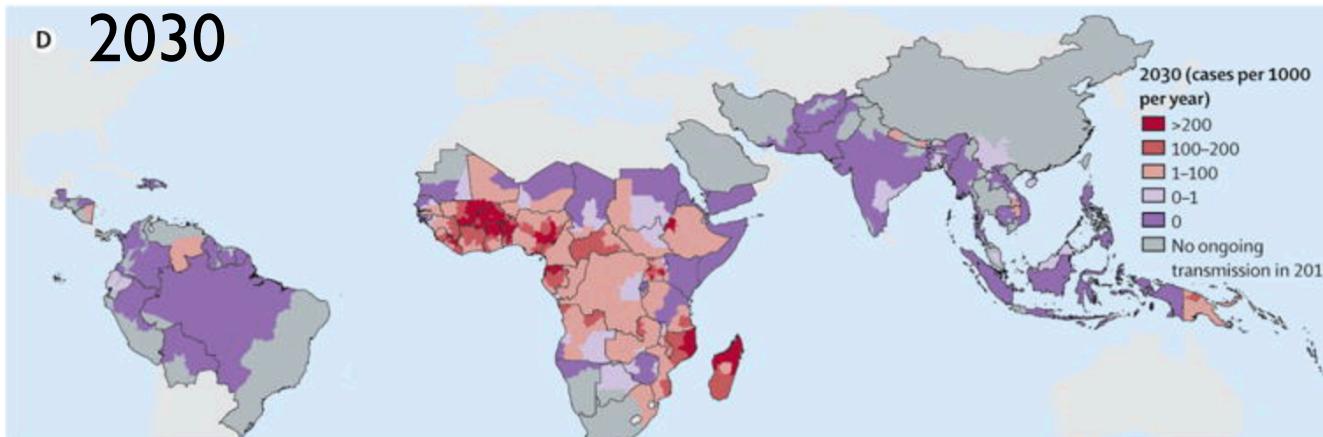
- Malaria continues to impose a huge burden
 - 100s of millions of infections every year
 - 100s of thousands of deaths every year
 - Mostly infants and children
 - Mostly in Africa
- Current interventions (nets, spraying, drugs) have saved millions but:
 - Not enough to eliminate
 - Drug- and insecticide-resistance may lead to reversal
 - Cost more than there is funding available
 - Most optimistic scaling-up scenario (cost up to \$9B/yr) still leaves malaria in 62 countries in 2030
- Millions more set to die over coming years

Extrapolating use of current interventions

Projected geographical distribution of *Plasmodium falciparum* malaria under the Accelerate 2 scenario between 2015 and 2030 (Griffin *et al.*, 2016).



- The Accelerate 2 scenario shows that we require 5 billion USD/year and up to an estimated 9 billion USD/year in 2030.



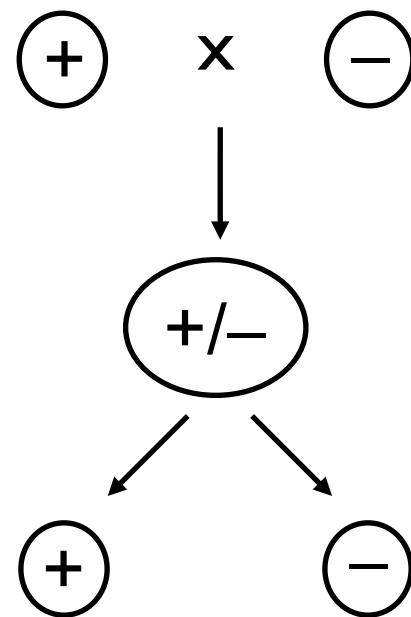
- We currently have 2.7 billion USD/year dedicated to malaria control.

Biological background

- Malaria in Africa is largely rural
- There are 4 species of *Plasmodium* causing malaria in Africa
 - *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*
- In Africa, most transmission is by 3 closely related *Anopheles* species (*An. gambiae*, *An. coluzzii*, *An. arabiensis*), plus *An. funestus*
 - Other species can be important in specific locations
 - These mosquito species can also transmit filariasis and O'nyong'nyong virus
 - Worldwide, there are ~3500 species of mosquitoes
 - The vast majority do not transmit disease
- Only female mosquitoes bite people and transmit disease

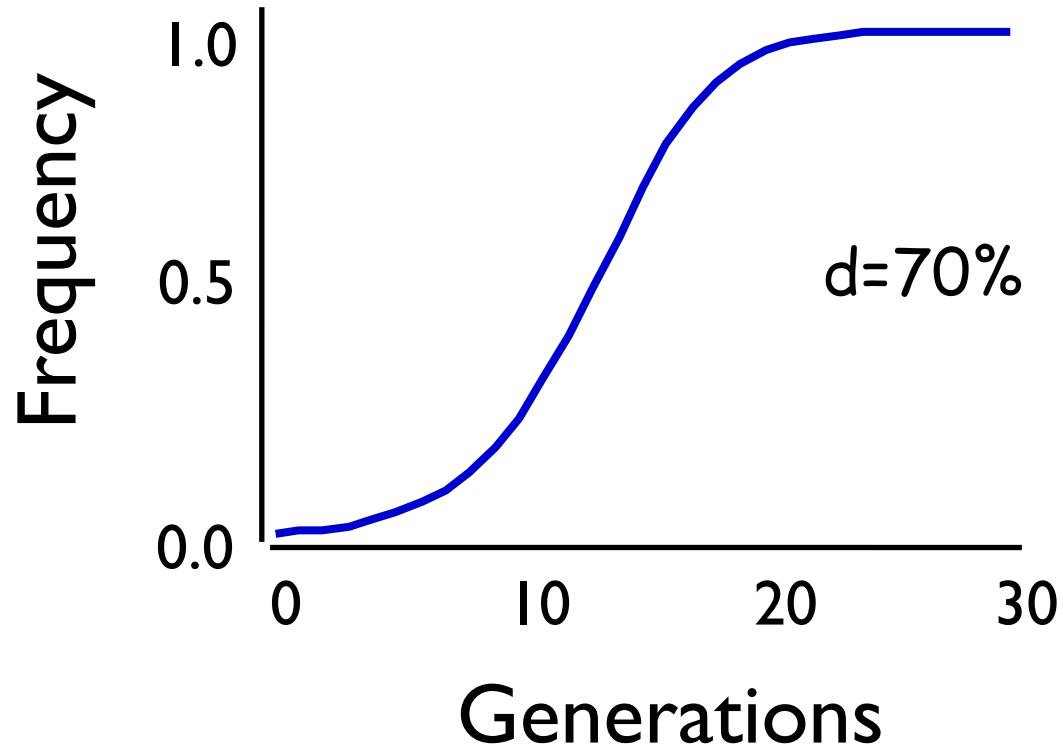
What is gene drive?

Preferential inheritance



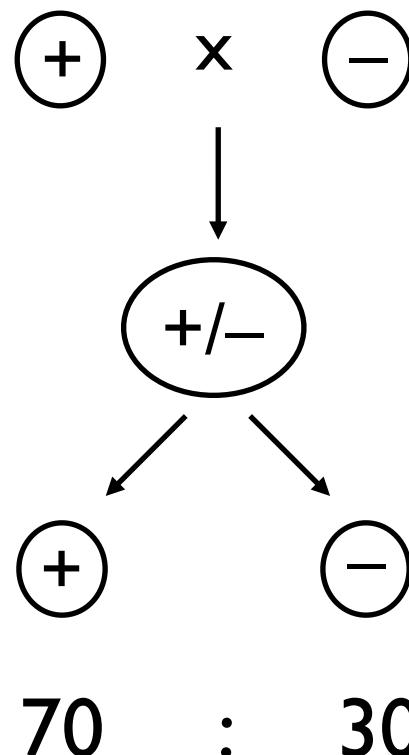
70 : 30

Spread in population



What is gene drive?

Preferential inheritance



Key points:

- Drive is a natural process which we are learning to mimic
- Drive can lead to the spread of genes that cause harm to the individuals carrying them
- Makes for a potentially attractive new platform to control pests & vector-borne diseases

What can be targeted?

Goal to reduce malaria transmission

$$R_0 = \frac{a^2 b c \theta}{\mu_A r} E A$$

Diagram illustrating the Ross-McDonald model for vector-borne pathogens, showing the components of the basic reproduction number (R_0) and their targets for reduction:

- Human biting rate**: Targets a .
- Per-bite infectivity rates**: Targets b and c .
- Number of female mosquitoes**: Targets E and A .
- Intrinsic rate of increase of the parasite**: Targets θ .
- Adult mosquito survival**: Targets μ_A and r .

Ross-McDonald model for vector-borne pathogens

What can be targeted?

Goal to reduce malaria transmission

$$R_0 = \frac{a^2 b c \theta}{\mu_A r} E A$$

Diagram illustrating the Ross-McDonald model for vector-borne pathogens, showing factors that can be targeted to reduce malaria transmission:

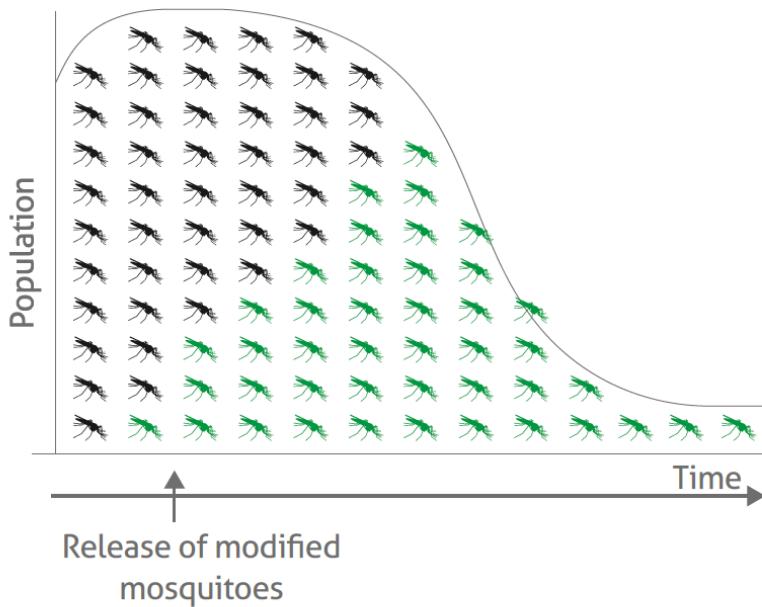
- Human biting rate (Host preference)
- Per-bite infectivity rates (Vector competence)
- Intrinsic rate of increase of the parasite
- Number of female mosquitoes
- Survival, reproduction, sex ratio
- Adult mosquito survival (targeted)
- Adult mosquito survival (targeted)

Ross-McDonald model for vector-borne pathogens

Gene drive: two basic strategies

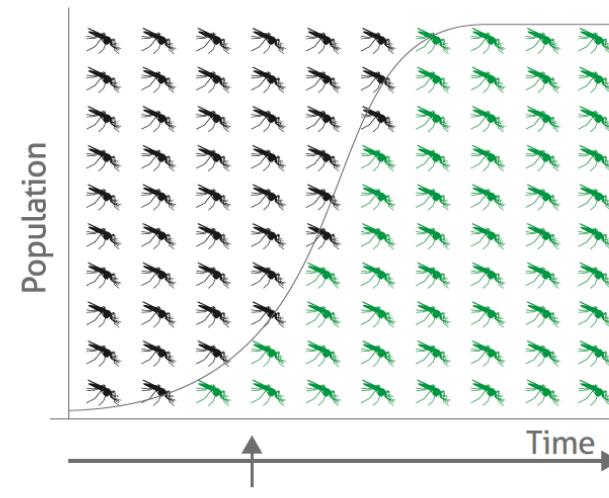
Population suppression

Releasing modified mosquitoes into the population can cause transient or permanent population suppression



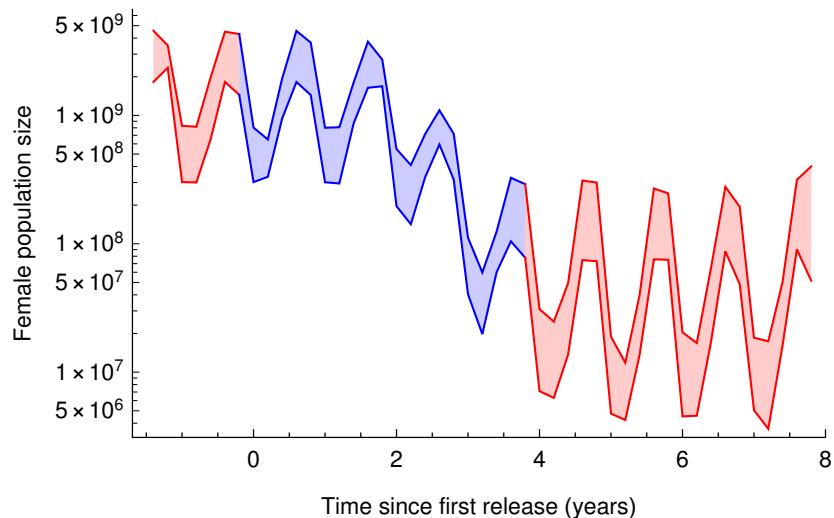
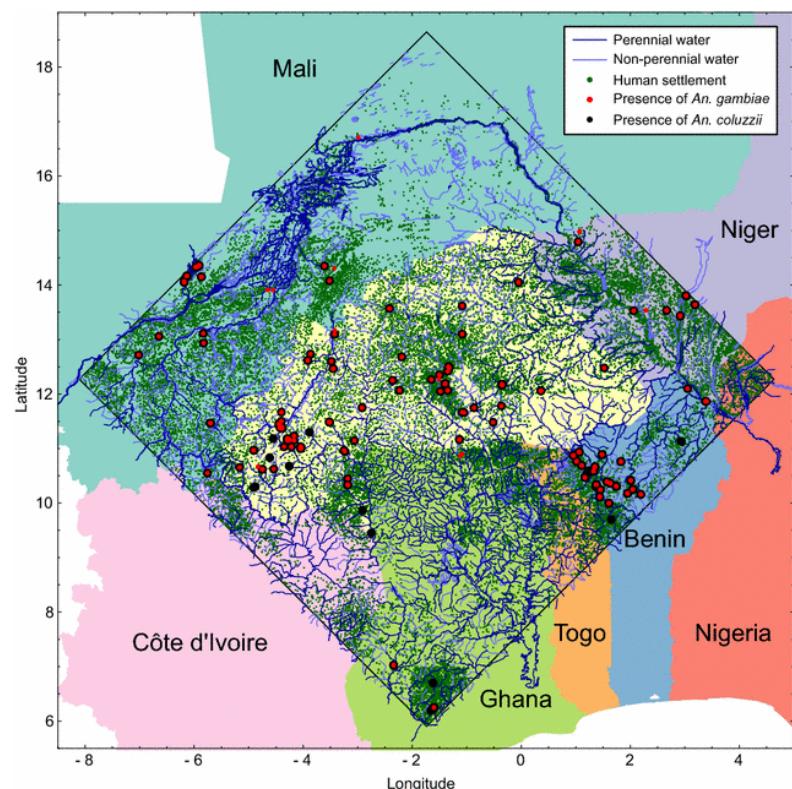
Population replacement

Releasing modified mosquitoes into the population can lead to the spread of a gene that blocks malaria transmission



In principle the two approaches could be combined

Some modelling...

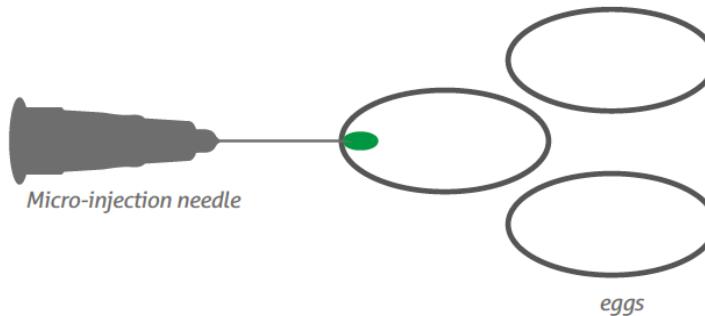


- 1000 x 1000 km area modelled
- 42,000 settlements
- Releases into 1% (420) settlements / year
- 10 'ideal' males per release
- 95% suppression after 4 years
- Robust to assumptions about dispersal, dry season ecology



How do we “make” modified mosquitos?

*Plasmid & helper
RNA microinjected
into eggs*



*Injected adults
are crossed into
wild type*



*Larvae are screened
for green fluorescence
to identify transgenics*



*Adult modified
mosquitoes*



Requirements for success (up to 1st release)

We need:

1. Genetic construct(s) with the required characteristics
2. Regulatory authorisation to release them
3. Public support / political will to release them
4. Sufficient local capacity to release them

Technical progress

What works
best in the
computer?

Can we make
it in the lab?

Does it work
in the field?

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Two gene drive strategies identified and confirmed as potentially useful:

Driving Y chromosome

Gene knock-out by homing

Technical progress

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Driving Y chromosome

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Technical progress

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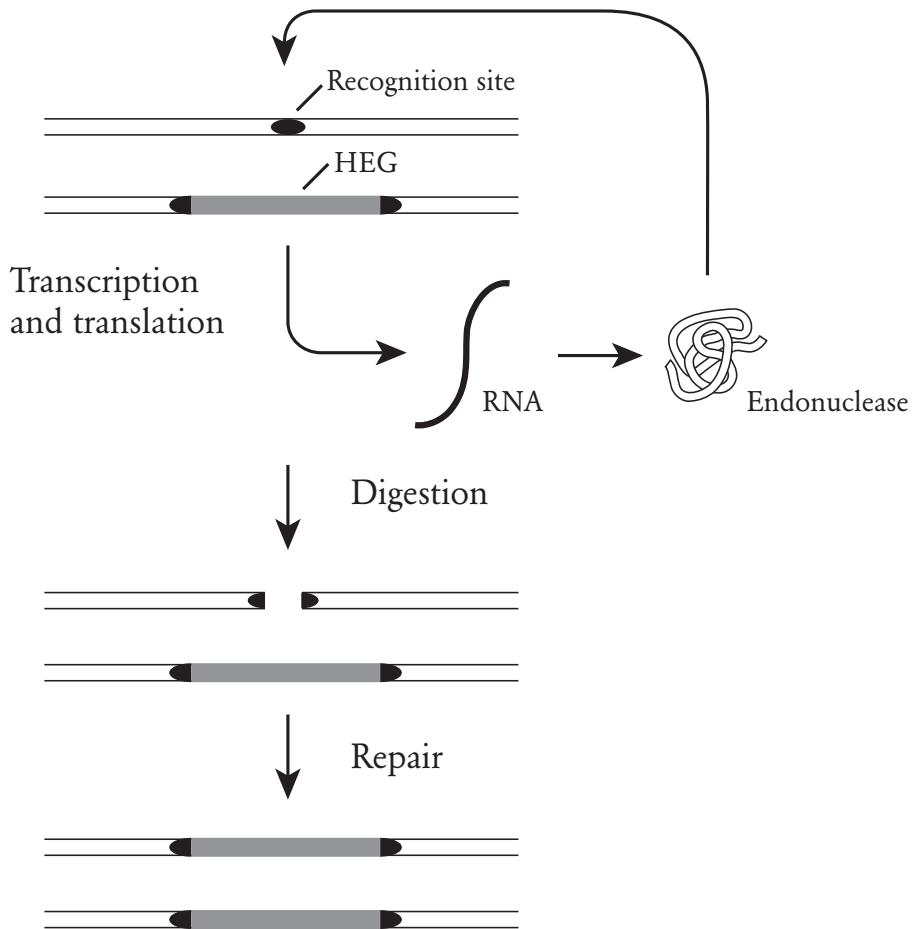
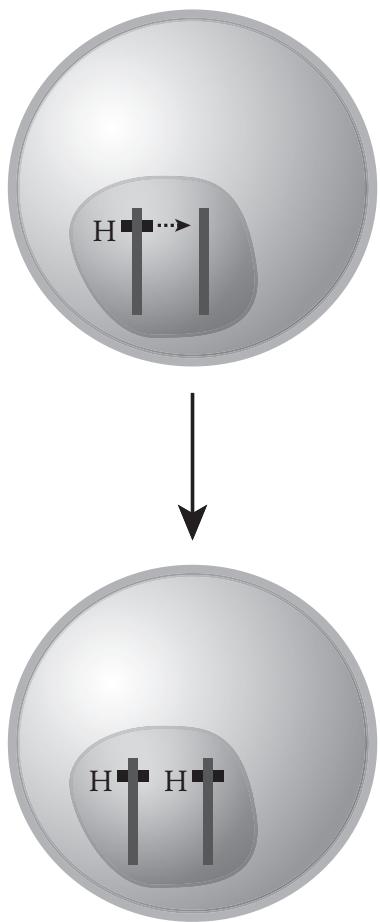
Can we make
it in the lab?

Does it work
in the field?

All molecular entomology work
done in lab of Andrea Crisanti

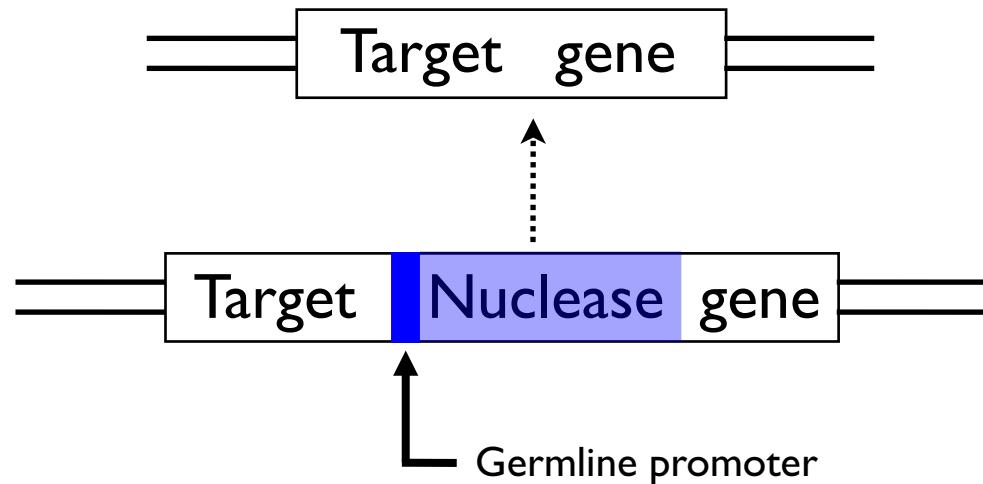
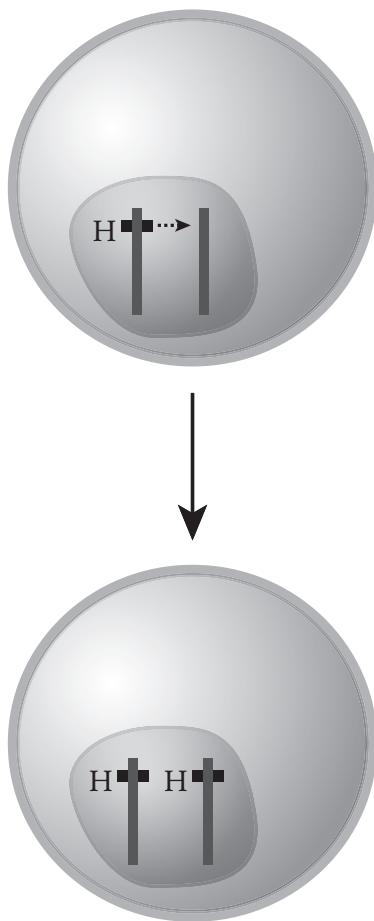


The homing reaction



Natural process used by homing endonuclease genes
in many microbes

Gene knock-out by homing



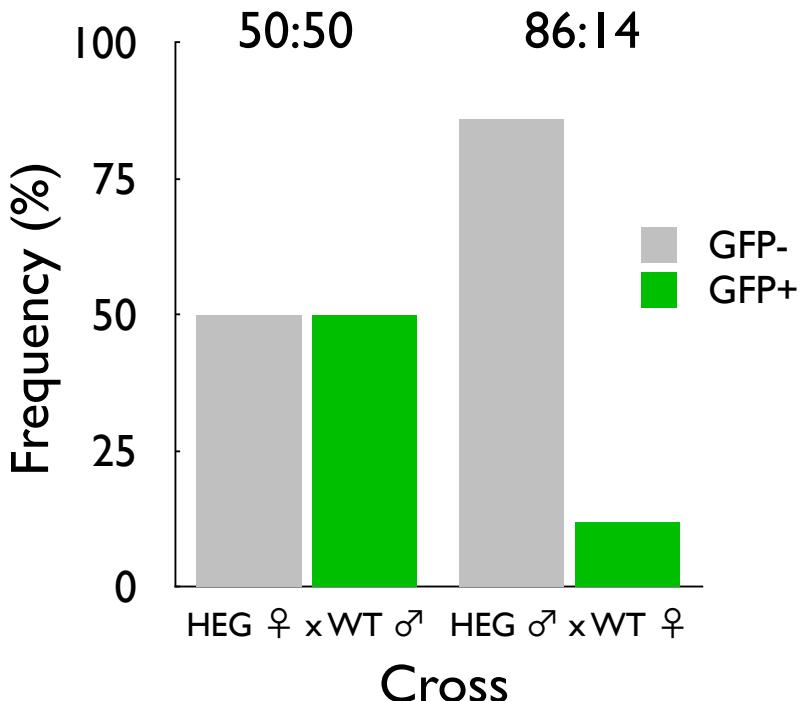
- Idea is to put nuclease gene in middle of target gene
- Homing can lead to population-wide knock-out of target gene
- Impact will depend on gene targeted
- Female fertility → Population reduction

Requires enzymes that recognise and cut specific DNA sequences.
Over years have tried many types of enzymes (meganucleases,
ZFNs, TALENs. Now using CRISPR, as much easier to use.

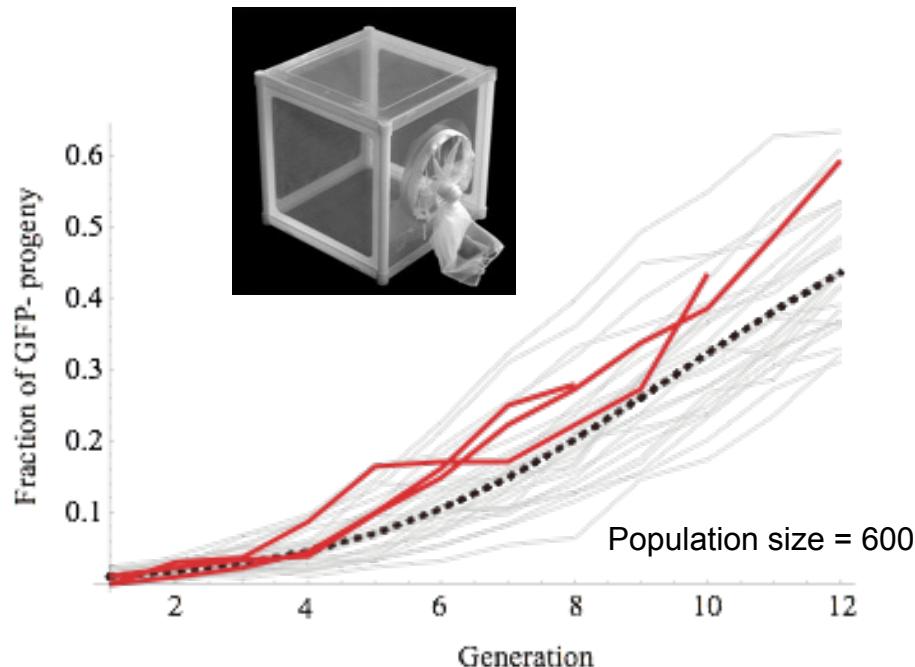
Does the homing reaction occur in mosquitoes?

Experiments using a nuclease and its recognition sequence from yeast

Preferential inheritance



Spread through populations



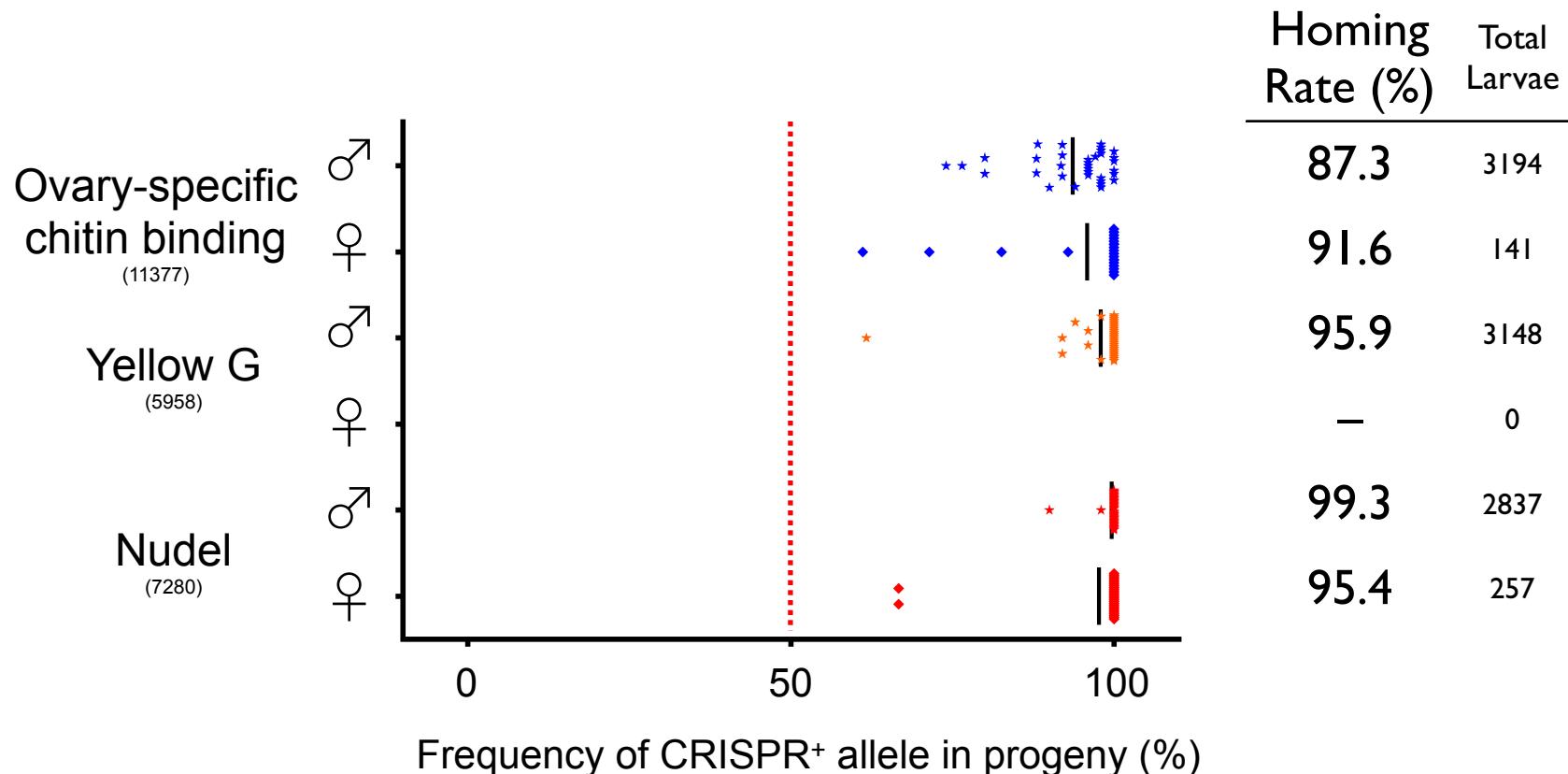
Together with *Drosophila* (Russell lab),
1st demonstrations of homing in animals



A Vector Control Research Alliance

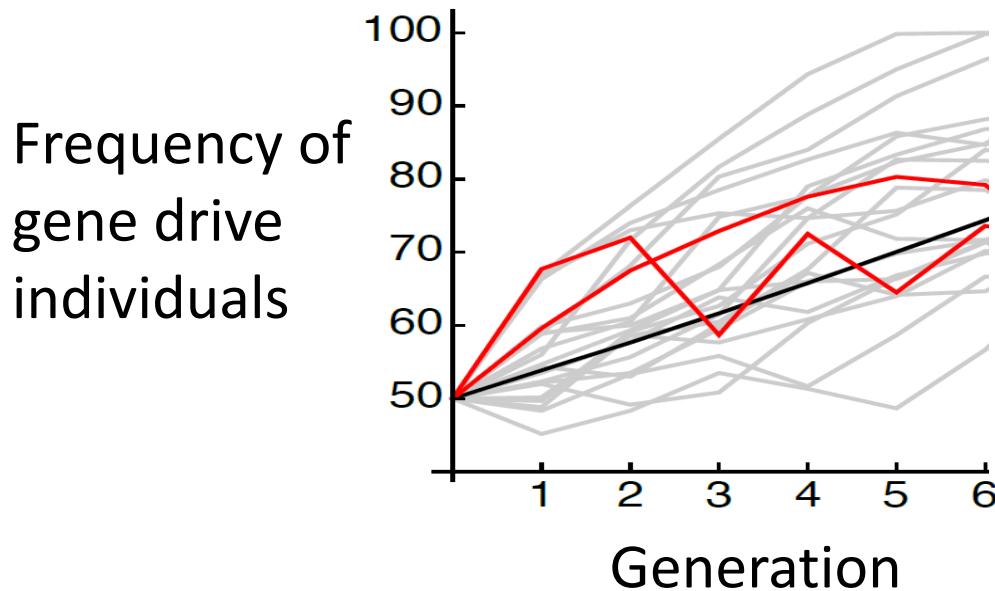
Can we design nucleases to home into endogenous mosquito genes?

Identified 3 female fertility genes and designed CRISPR constructs against them



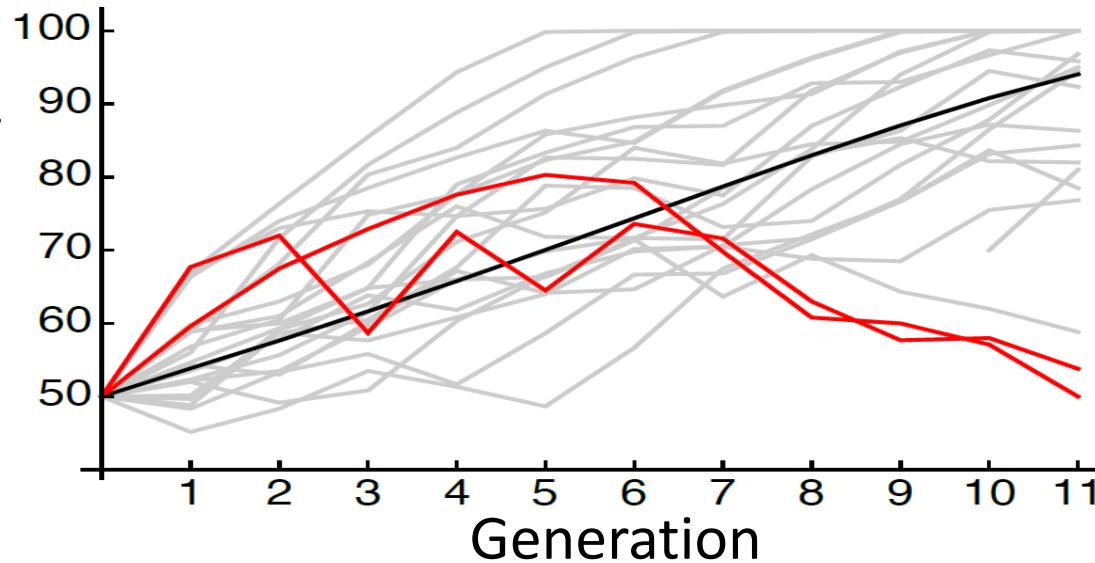
Average homing rate across 3 genes is 94%

Will they spread in a cage?



Will they spread in a cage?

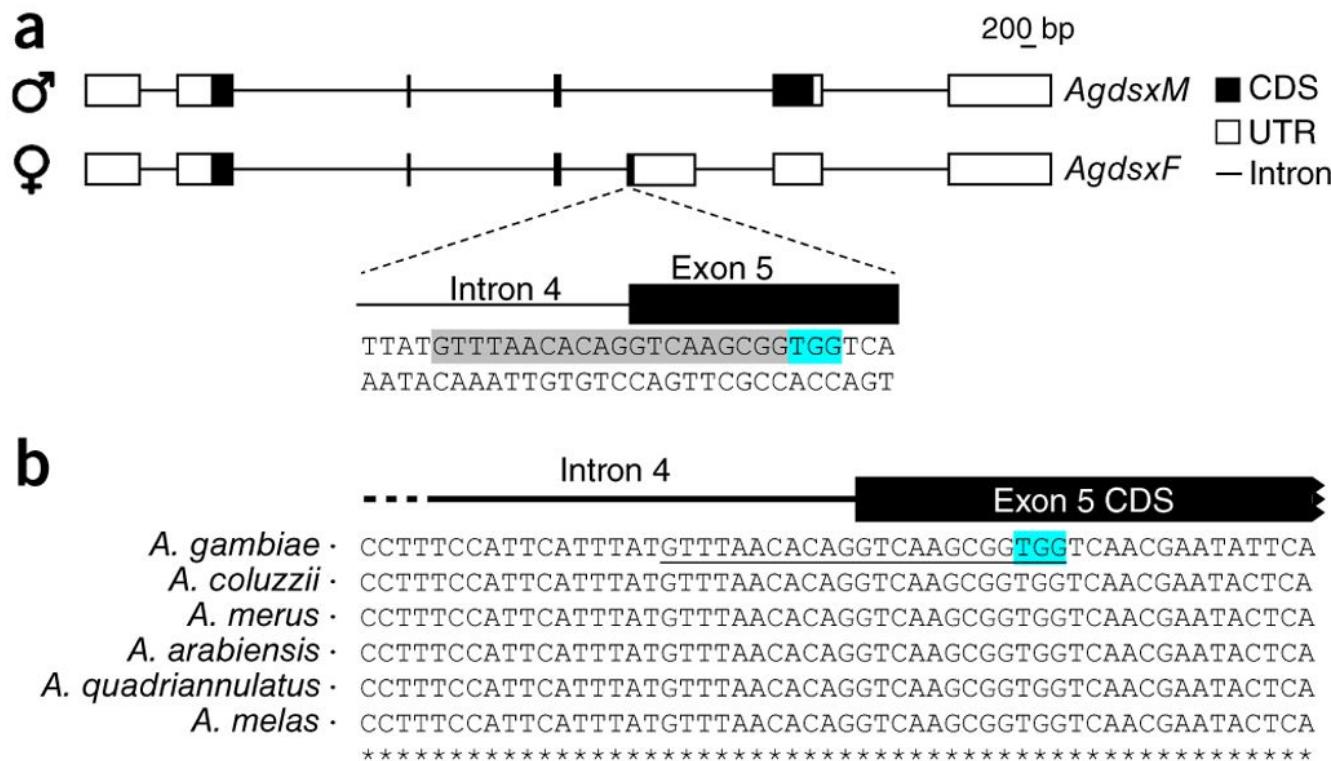
Frequency of gene drive individuals



- As with any form of pest & pathogen control, need to worry about resistance
- Molecular & genetic analyses showed:
 - Resistance due to changes at target site that prevented cleavage and restored gene function
 - All detected resistant alleles were in-frame insertions / deletions (no SNPs) from end-joining repair
 - Changes due to nuclease activity, not pre-existing

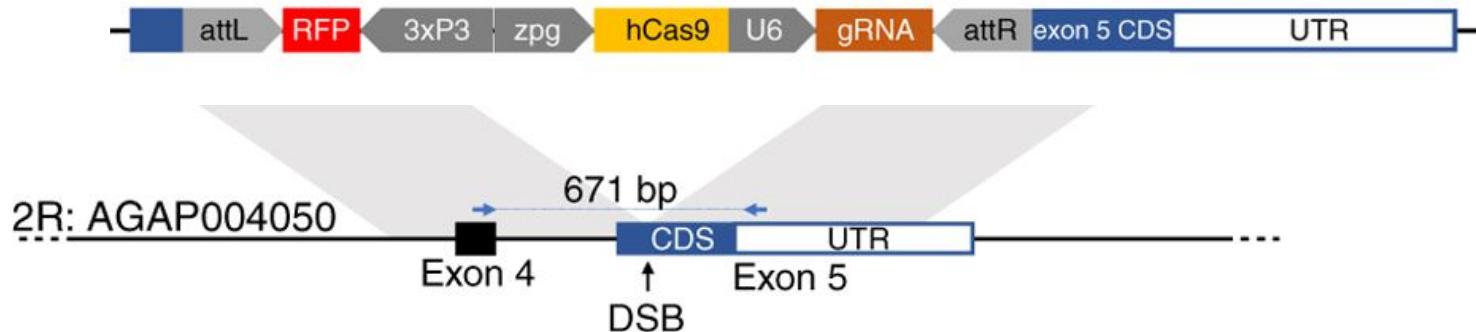
Can we avoid resistance?

- Target sequences less able to tolerate changes while maintaining function
 - The *doublesex* gene, involved in insect sex determination

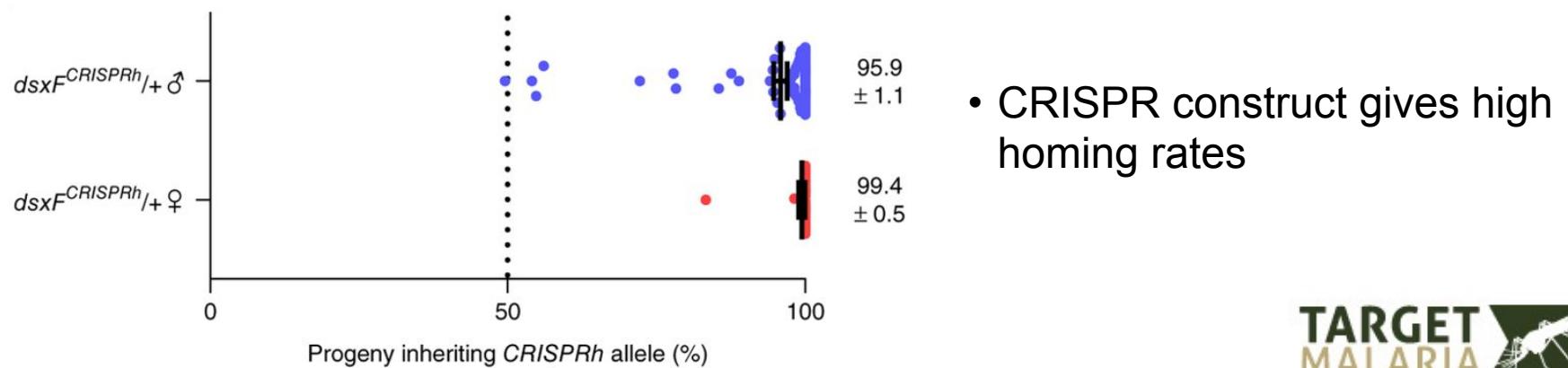


Highly conserved across species, suggests changes not easily tolerated

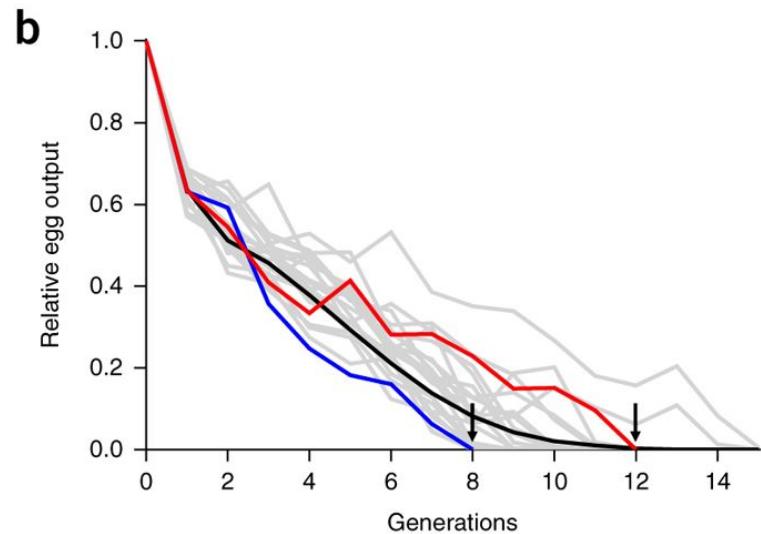
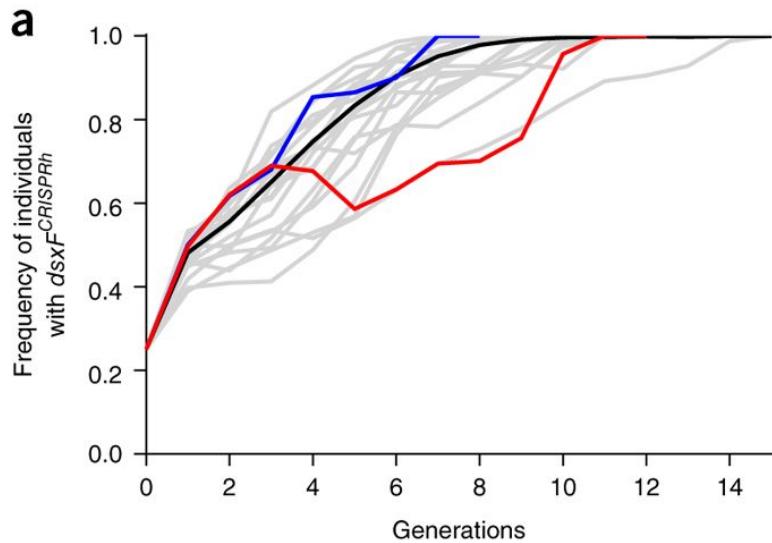
Can we avoid resistance?



- Homozygous knock-out females are sterile (and cannot blood feed)
- No obvious effect on males

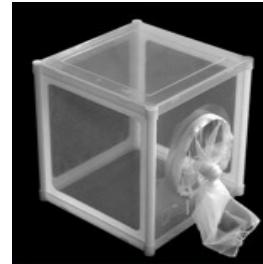


Can we avoid resistance?



- Construct spread to fixation in two cage populations
- Led to population crash
- No sign of resistance — mutations produced, but not selected

Scaling up from



to:

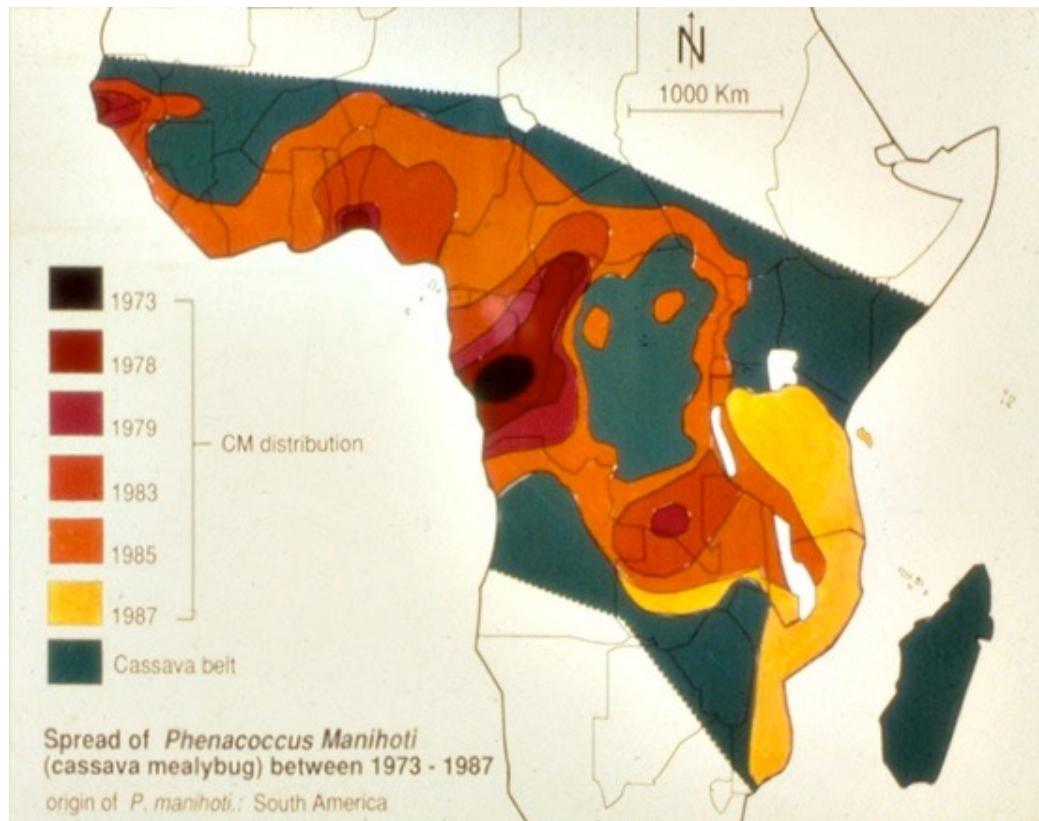
- Can mimic tropical light, temperature and humidity dynamics
- Allows lower, more realistic densities and mating environment (swarming)



A precedent

Biological control of the cassava mealybug

The problem



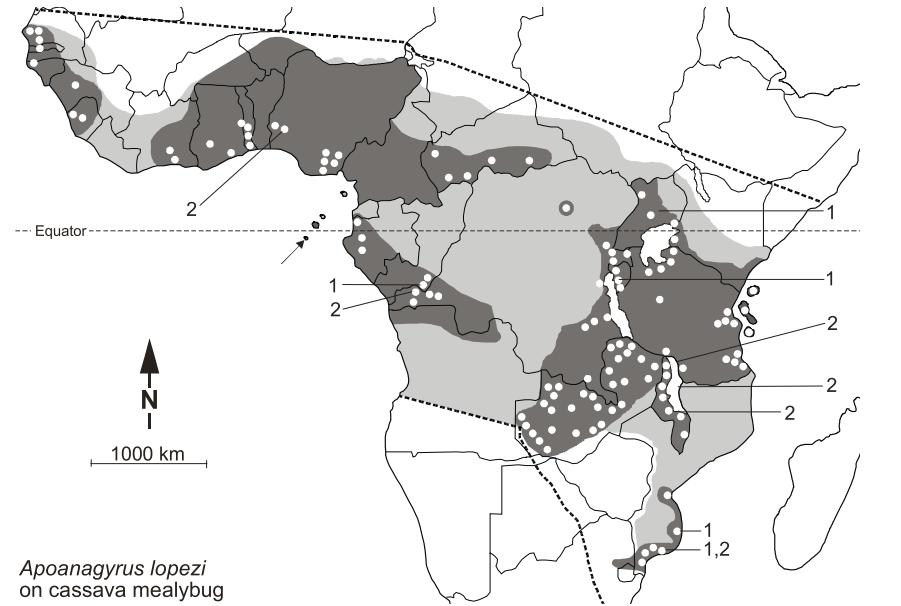
A precedent

Biological control of the cassava mealybug

The product



Apoanagyrus lopezi from
Paraguay & Brazil



1981 – 1995: N=150 releases throughout tropical Africa

Impacts

10x reduction of cassava mealybug...

- within 2-4 years
- on 95% of all fields
- in all countries
- no resurgence in the next 15 years

Benefits US\$ 9-20 billion **Costs:** ~US\$ 34 million over 35 years

For more information:
www.targetmalaria.org

Projects

**Designing and assessing alternative 2-
and 3-locus strategies**

**Demographic inference from
population genomic data**