

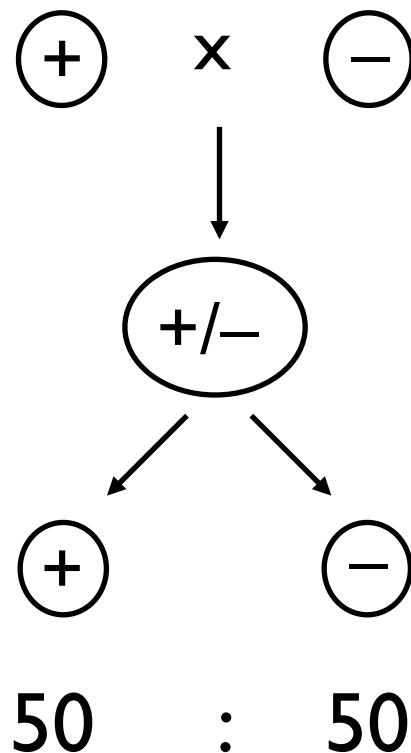
# Gene drive and its potential use for malaria control

Austin Burt

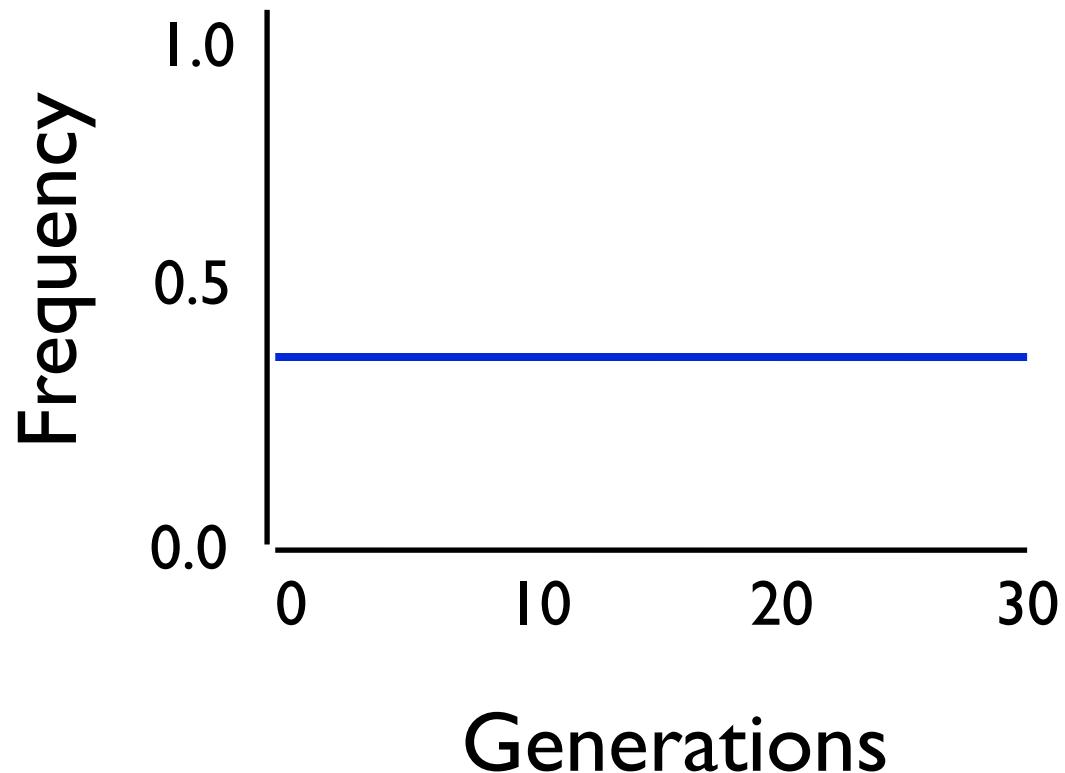
CMEE, Feb 2021

# Mendelian transmission

Equal, unbiased inheritance

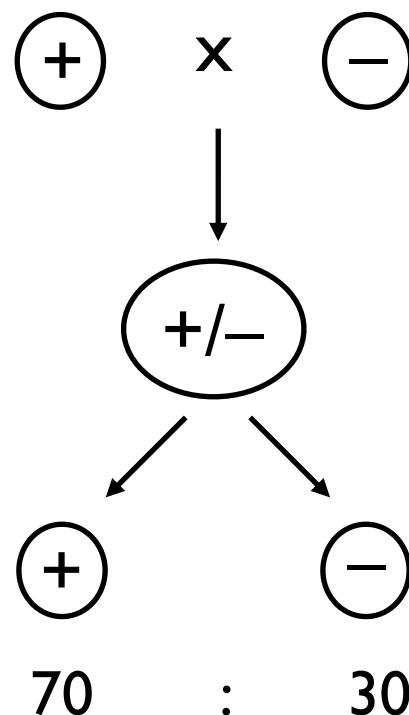


All else equal,  
no change in gene frequency

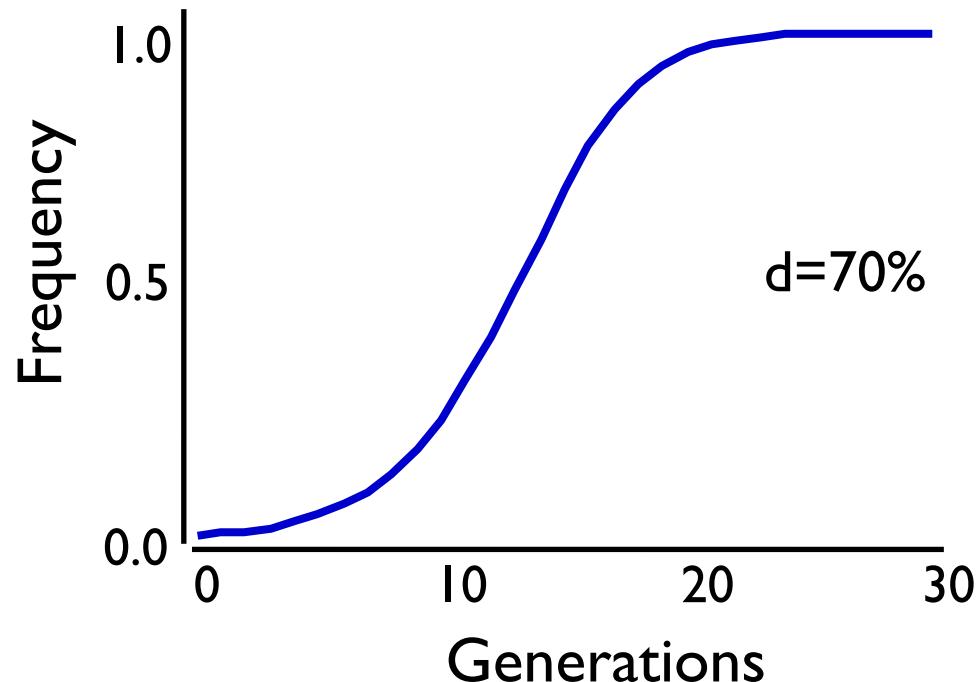


# Gene drive

Preferential inheritance



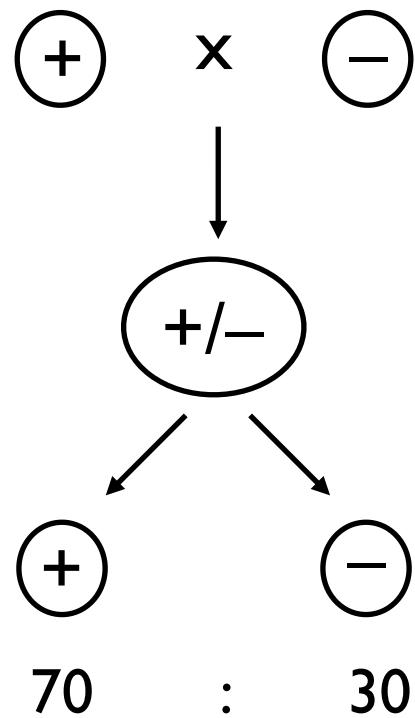
All else equal,  
will lead to spread in population



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

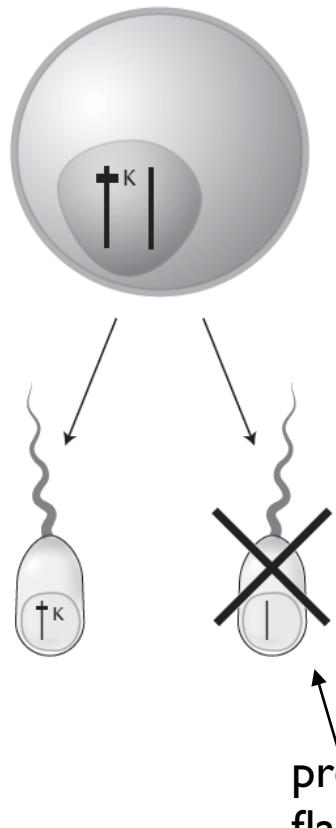
# 3 ways to drive

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

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# *t*-haplotype in mice



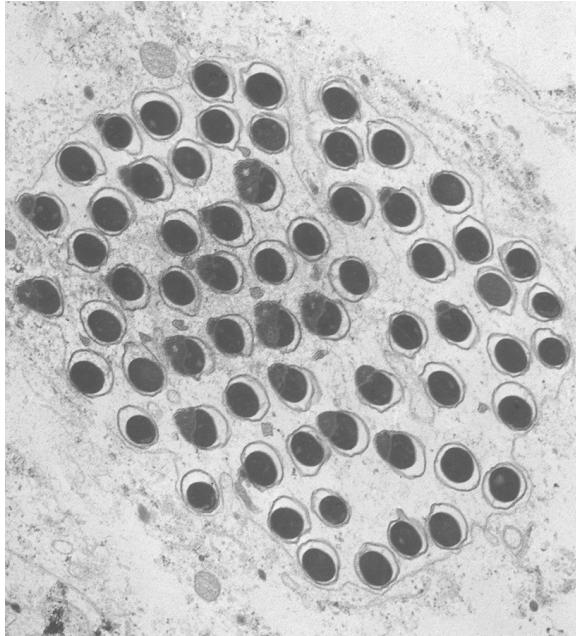
- 30-40Mb on 17th chromosome (1.2% of genome)
- transmitted 90% through males
- inherited as a unit: 4 inversions that prevent recombination
- homozygous sterile and/or lethal
- ~5% in natural populations
- sequence analysis suggests ~3MY old

premature acrosome reaction and/or  
flagellar dysfunction

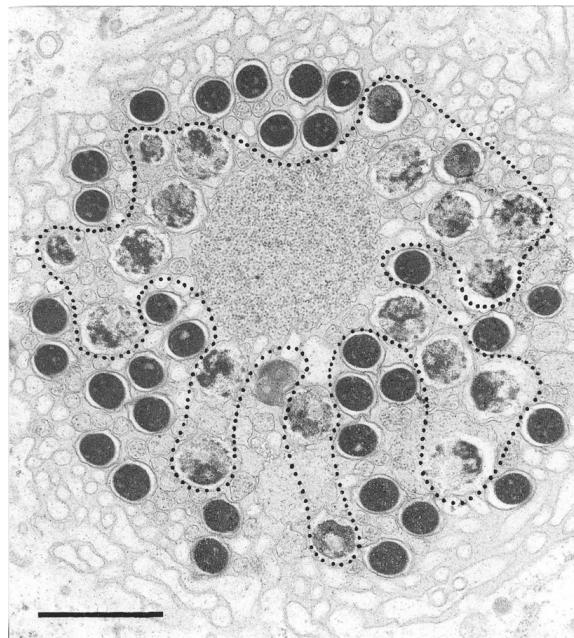
# Segregation Distorter in *Drosophila*



Normal (−/−)



SD/−



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

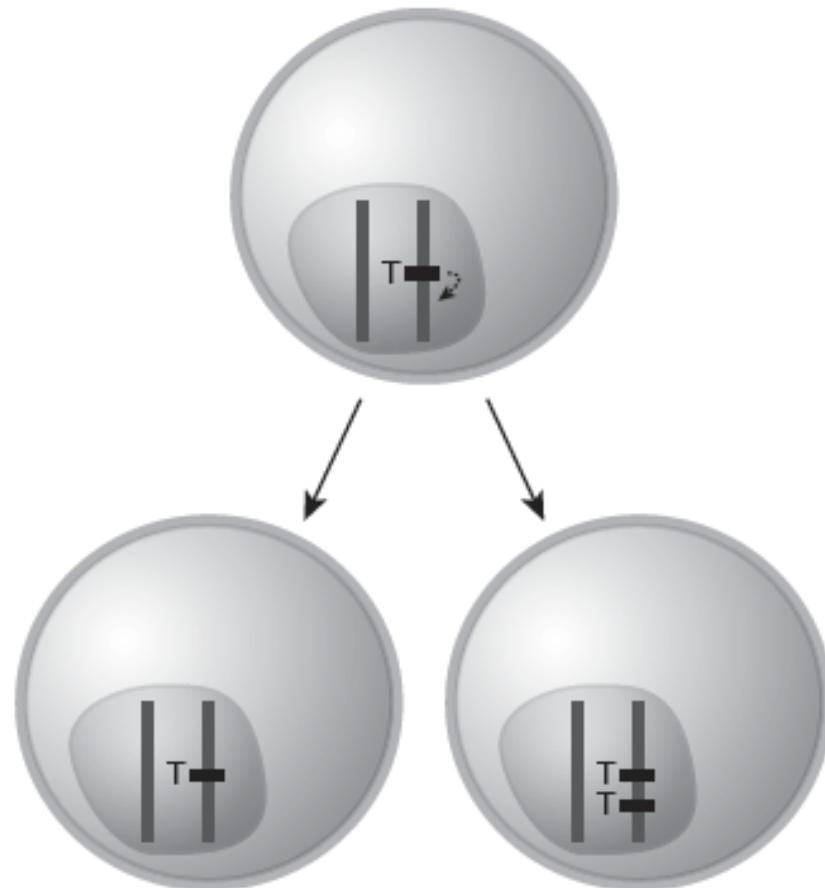
Electron micrographs of developing spermatids in *Drosophila*

# 3 ways to drive

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

# Over-replicating elements

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

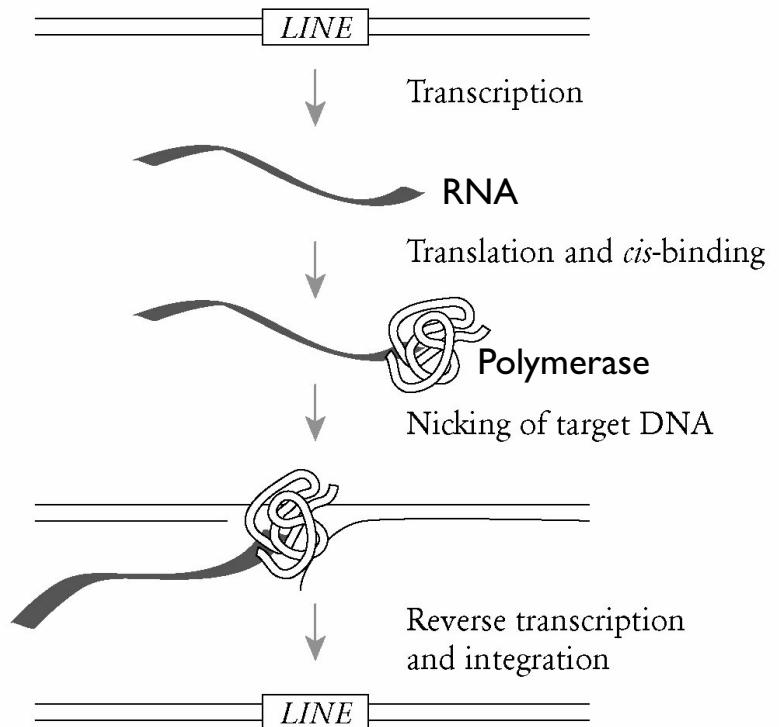


# Transposable elements — “jumping genes”

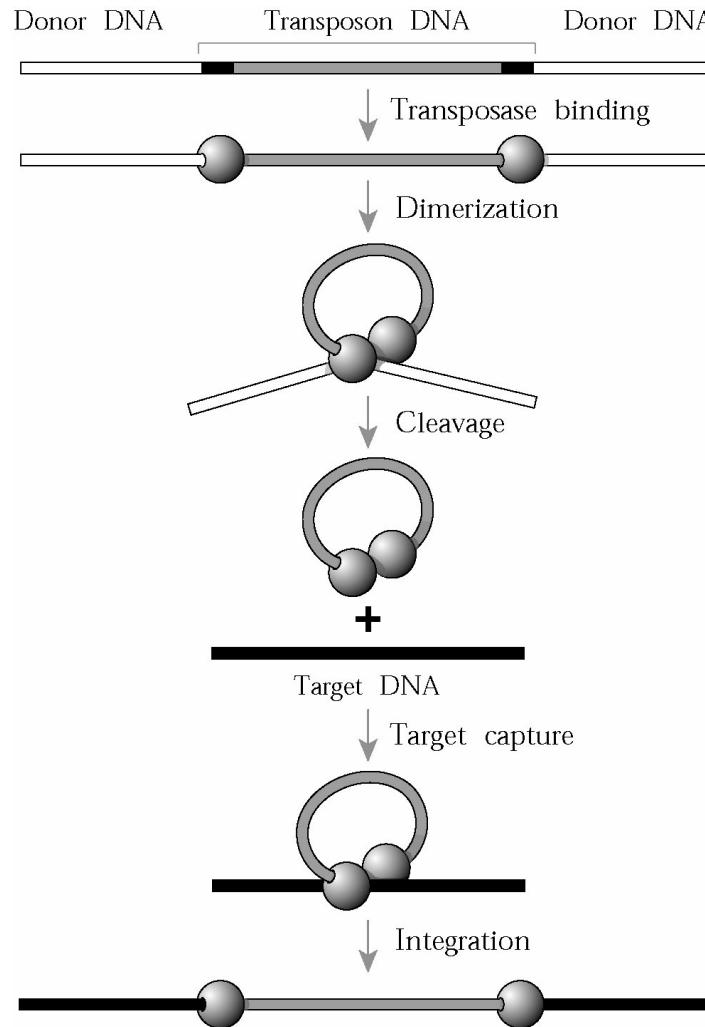
Many different mechanisms

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

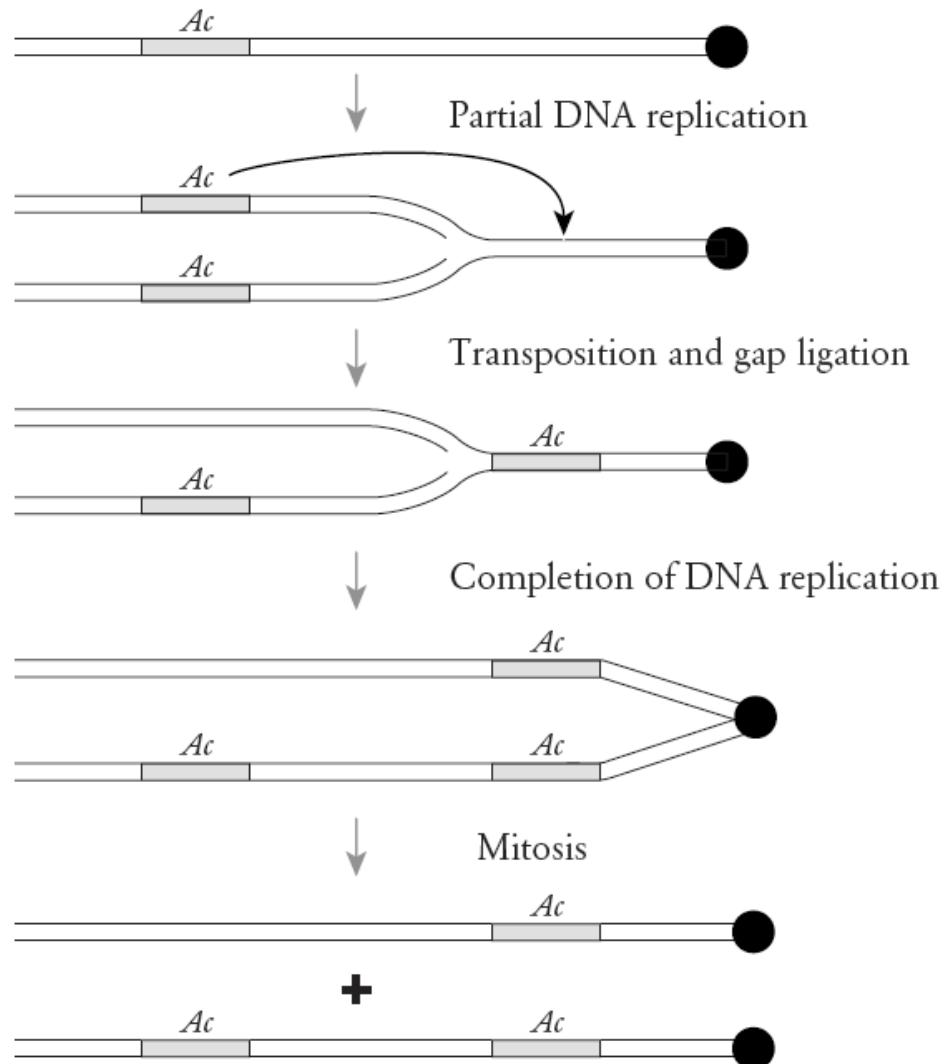
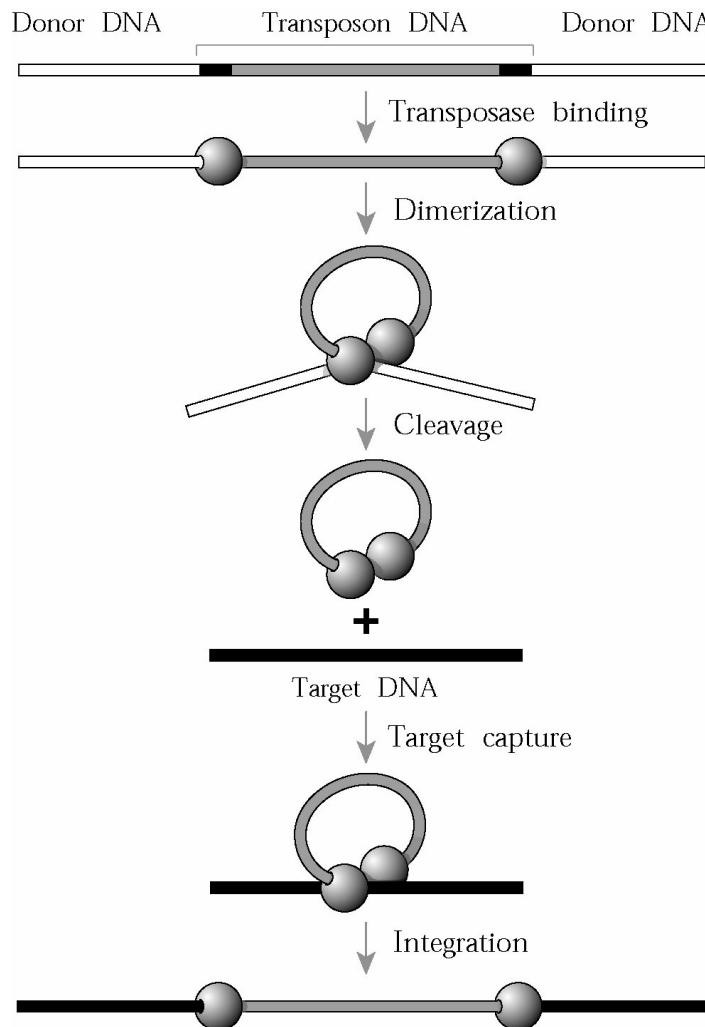
DNA → RNA → DNA



# DNA transposons



# DNA transposons

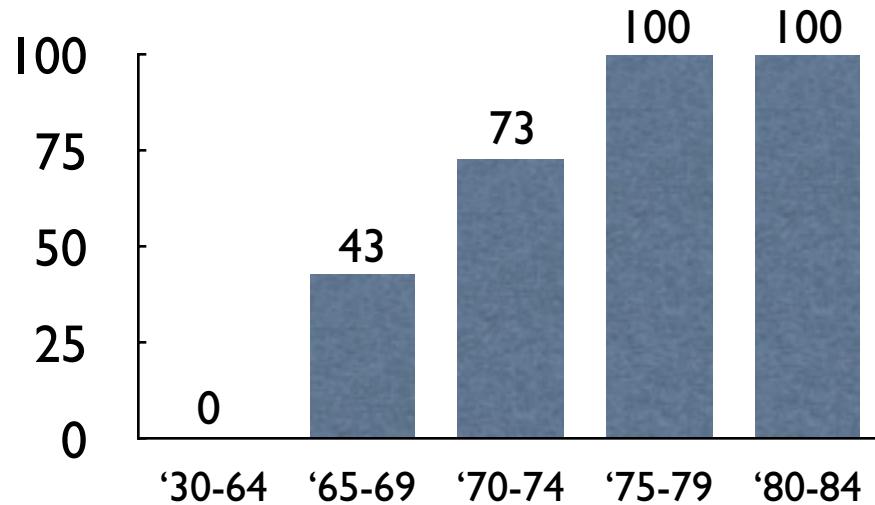


# Rapid spread of P elements in *Drosophila*



In nature

Frequency of strains  
with P-elements (%)



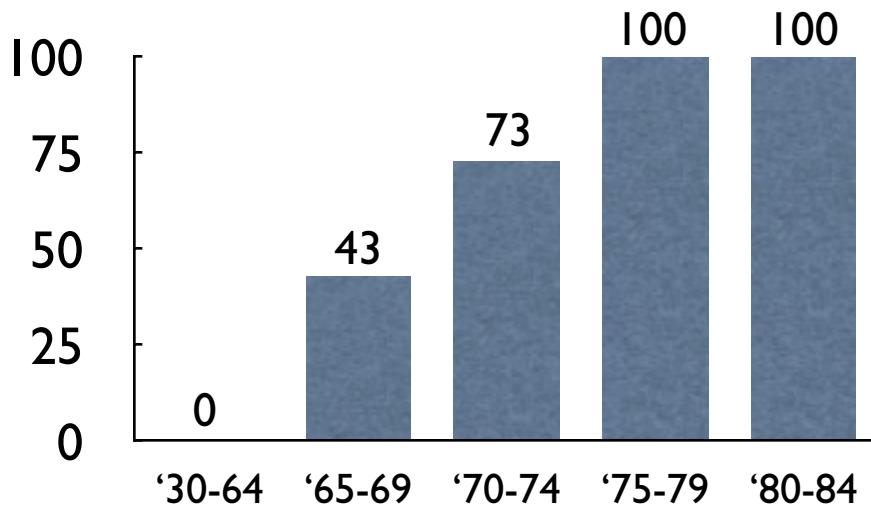
Year collected

# Rapid spread of P elements in *Drosophila*



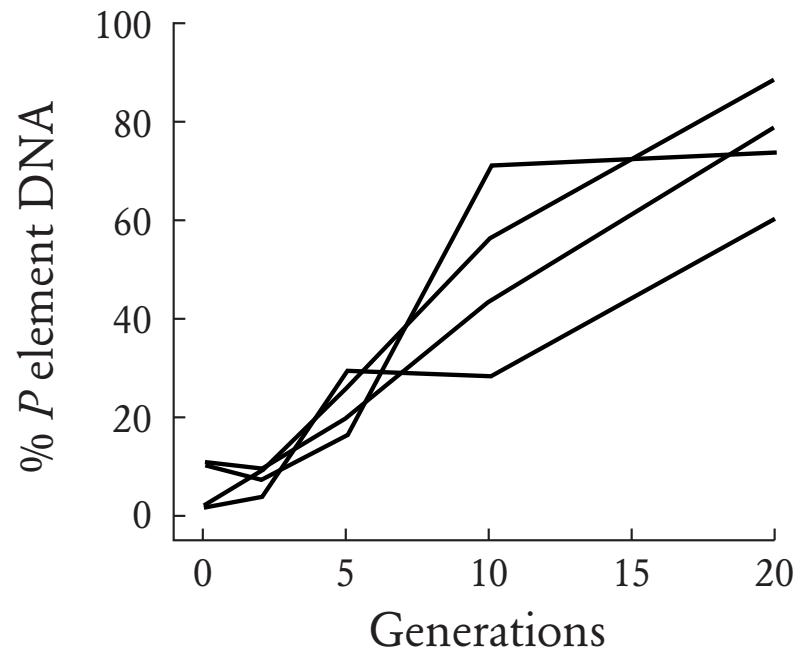
In nature

Frequency of strains  
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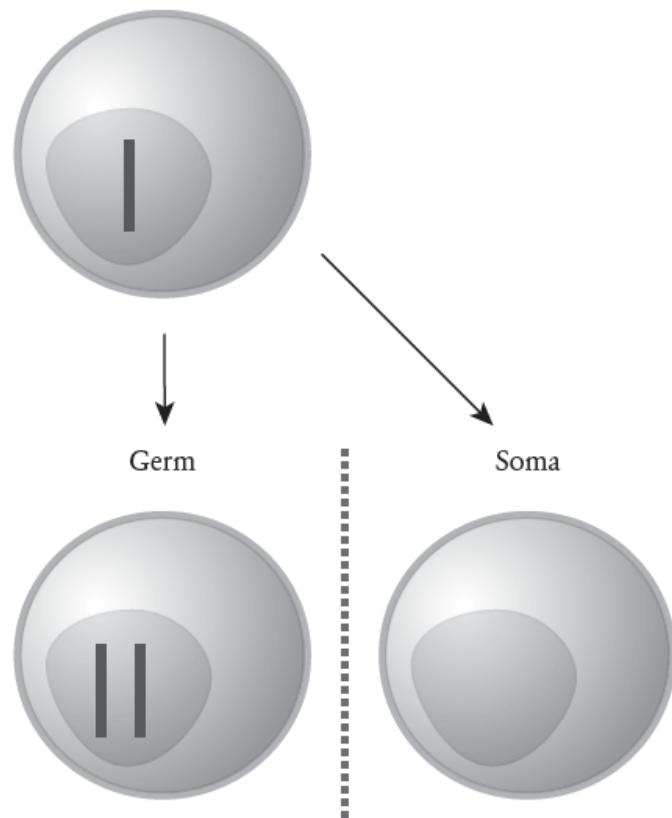
In the lab



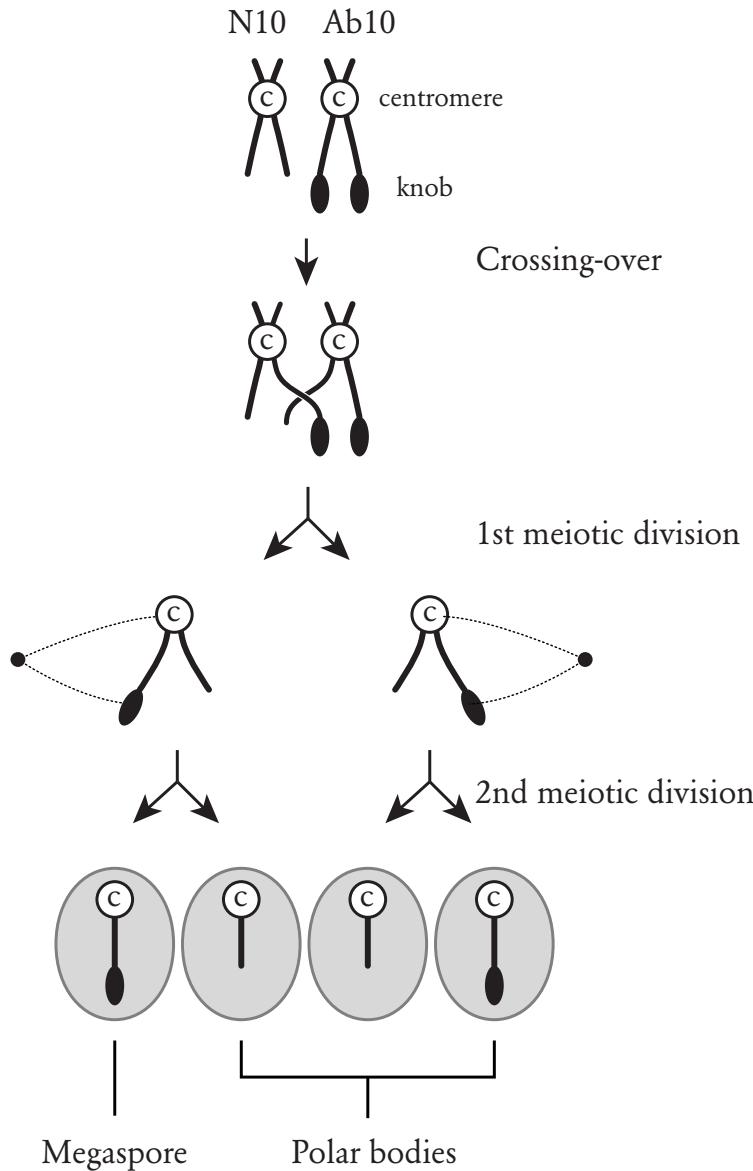
# 3 ways to drive

- 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



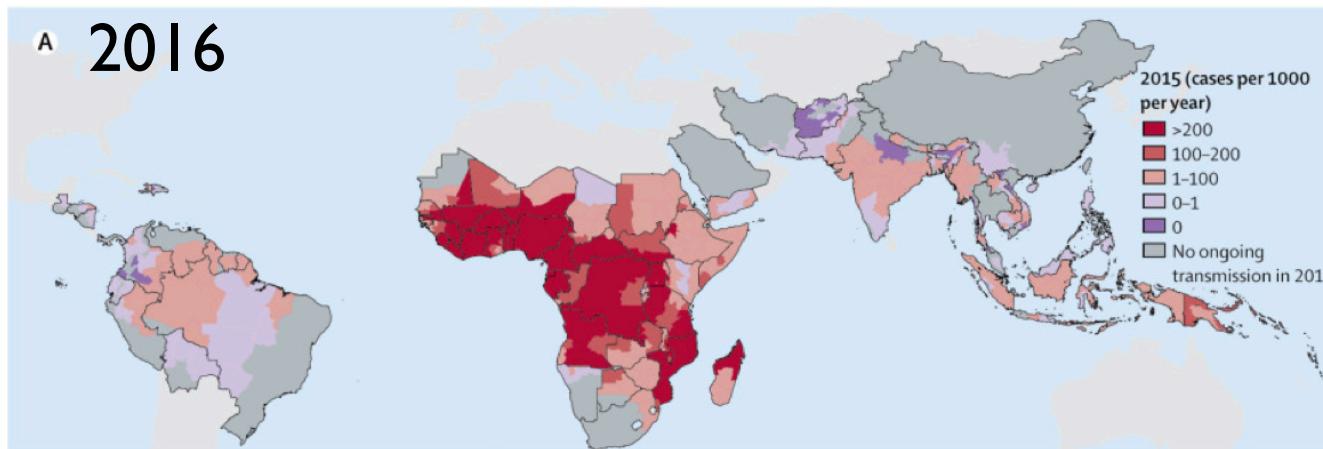
# Potential use of gene drive for malaria control

# Rationale

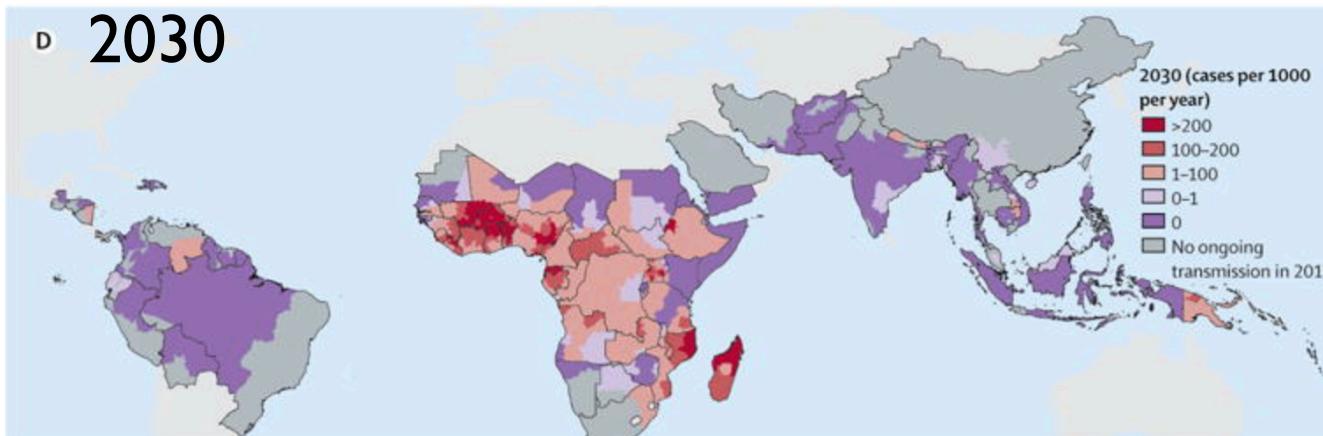
- Malaria continues to impose a huge burden
  - 100s of millions of infections every year
  - 100s of thousands of deaths every year
  - Mostly infants and children
  - Mostly in Africa
- Current interventions (nets, spraying, drugs) have saved millions but:
  - Not enough to eliminate
  - Drug- and 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.



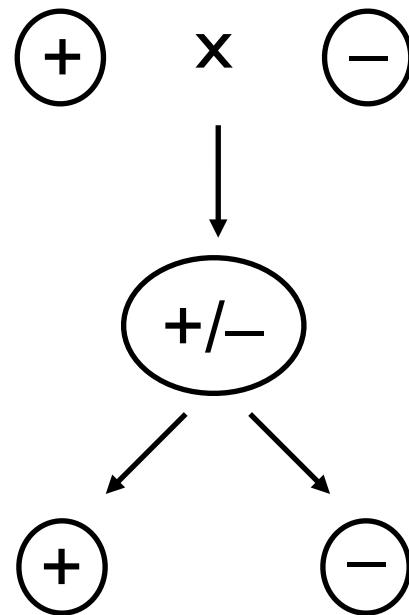
- We currently have 2.7 billion USD/year dedicated to malaria control.

# 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

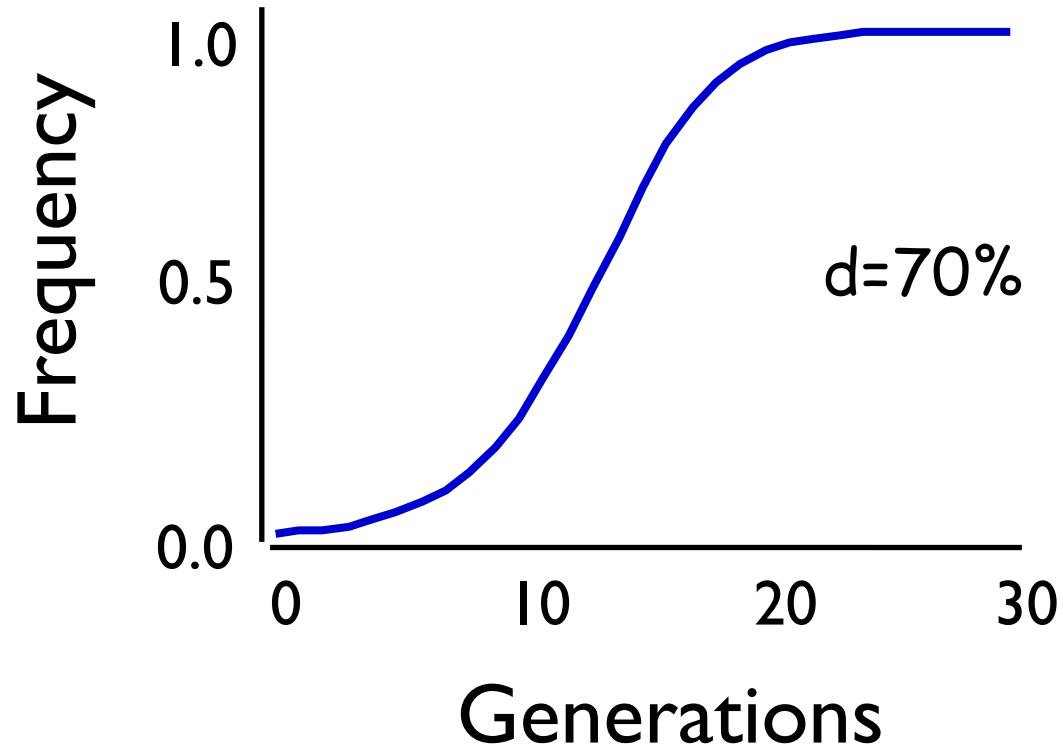
# What is gene drive?

Preferential inheritance



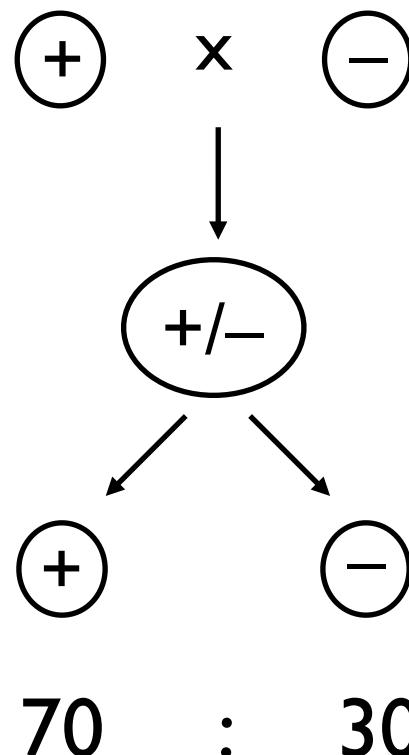
70 : 30

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  $\theta$ .
- Adult mosquito survival**: Targets  $\mu_A$  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:

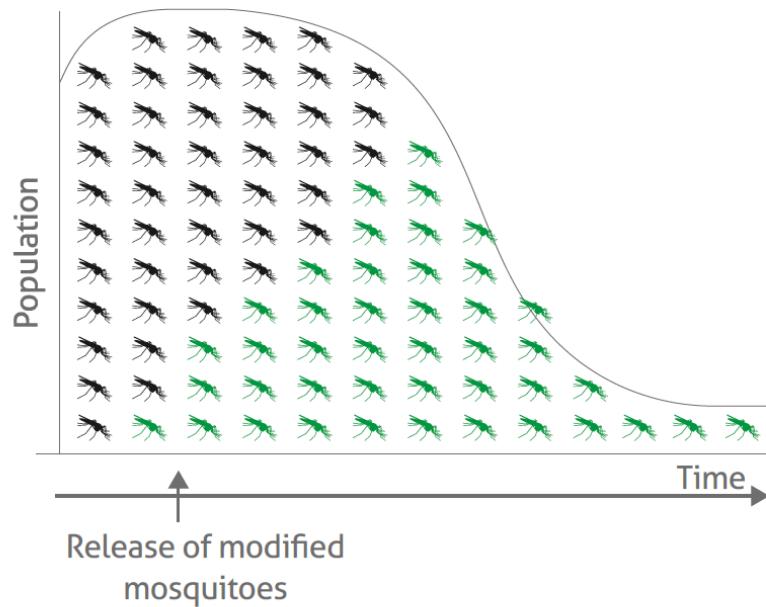
- Human biting rate (Host preference)
- Per-bite infectivity rates (Vector competence)
- Intrinsic rate of increase of the parasite
- Number of female mosquitoes
- Survival, reproduction, sex ratio
- Adult mosquito survival (targeted)
- Adult mosquito survival (targeted)

Ross-McDonald model for vector-borne pathogens

# Gene drive: two basic strategies

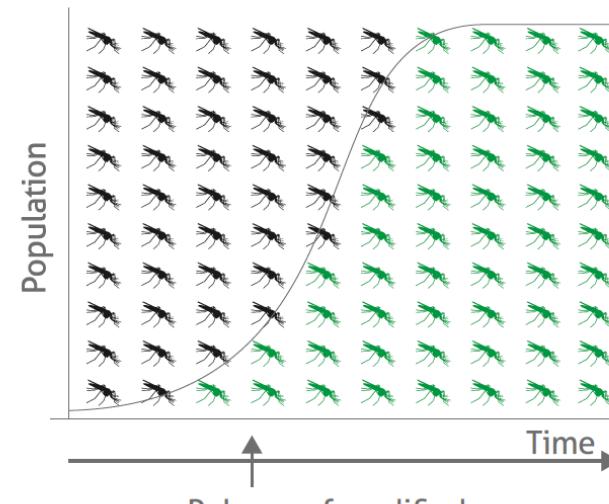
## Population suppression

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



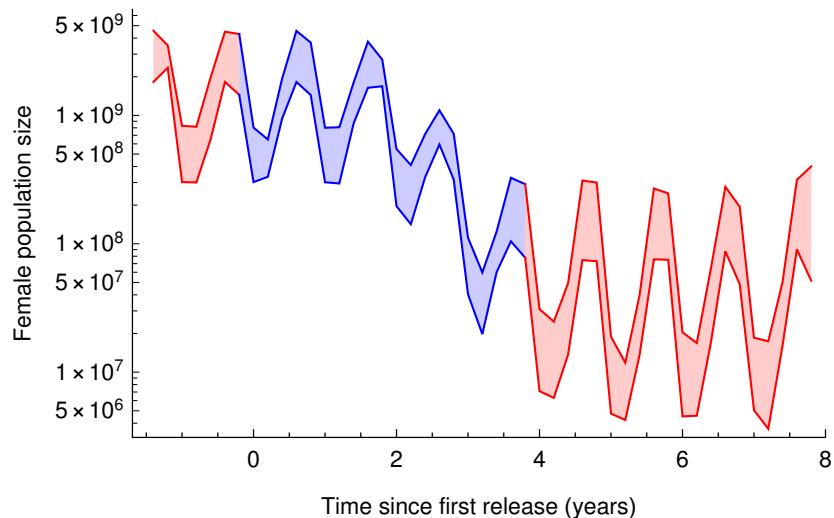
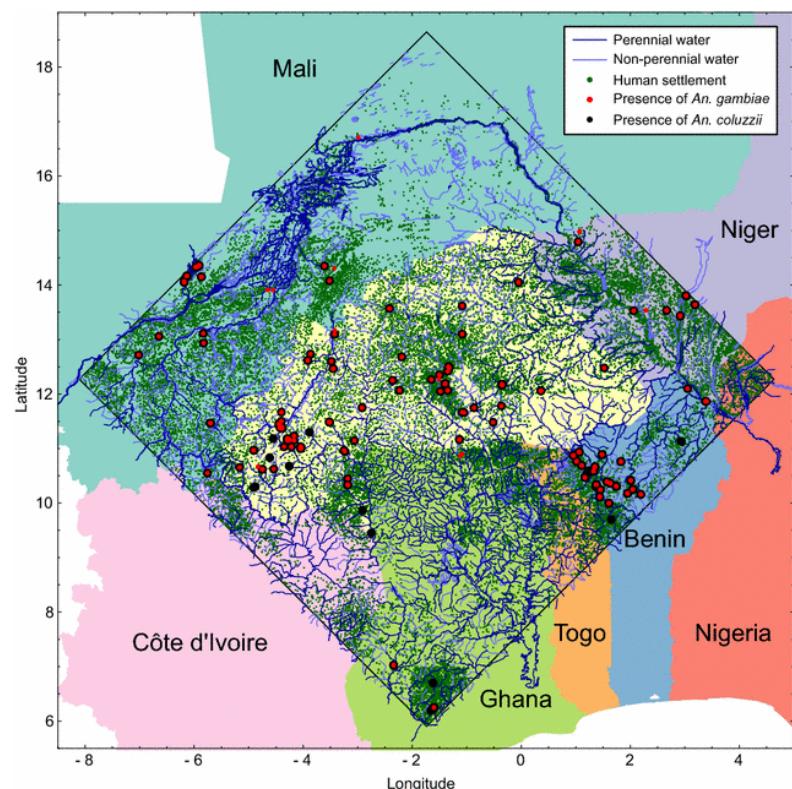
## Population replacement

*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...

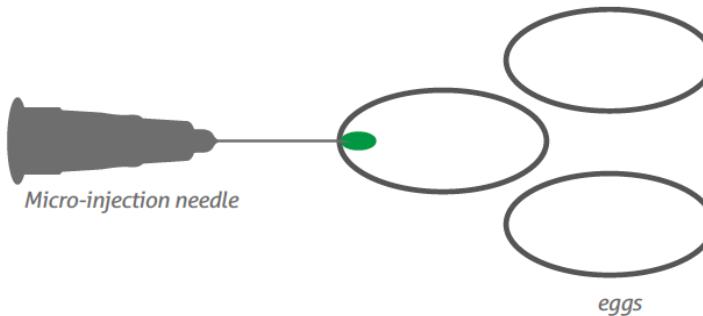


- 1000 x 1000 km area modelled
- 42,000 settlements
- Releases into 1% (420) settlements / year
- 10 'ideal' males per release
- 95% suppression after 4 years
- Robust to assumptions about dispersal, dry season ecology



# 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?

# Technical progress

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Does it work  
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**Two gene drive strategies identified and confirmed as potentially useful:**

Driving Y chromosome

Gene knock-out by homing

# Technical progress

What works  
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Two gene drive strategies identified and confirmed as potentially useful:

Driving Y chromosome

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# Technical progress

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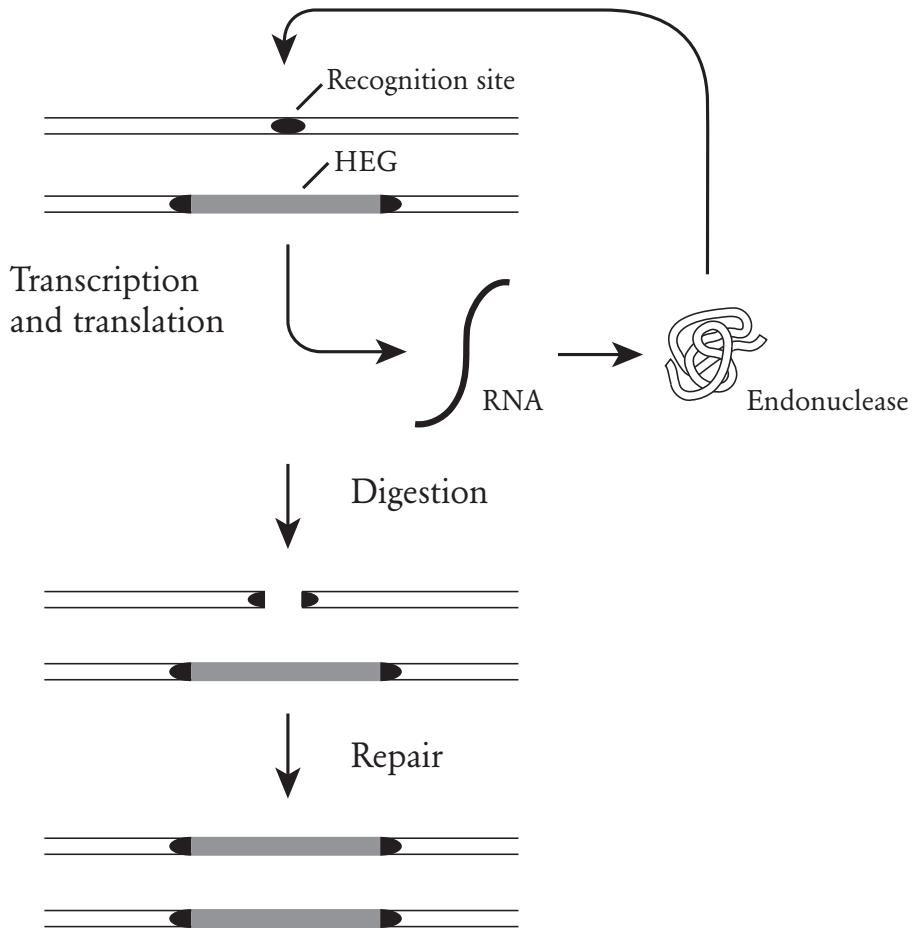
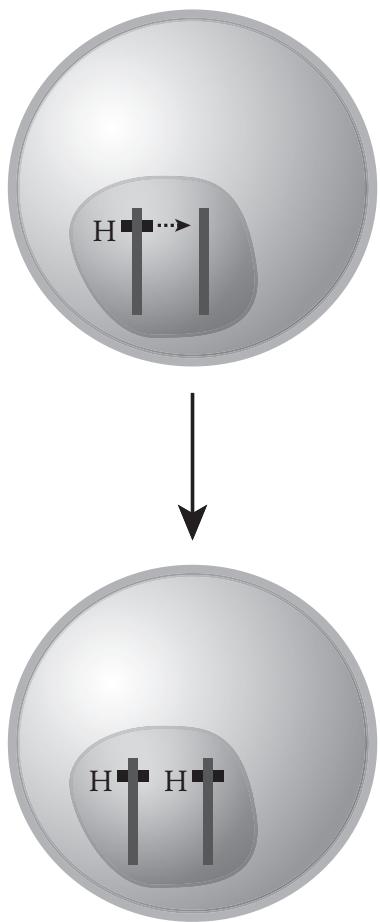
Can we make  
it in the lab?

Does it work  
in the field?

All molecular entomology work  
done in lab of Andrea Crisanti

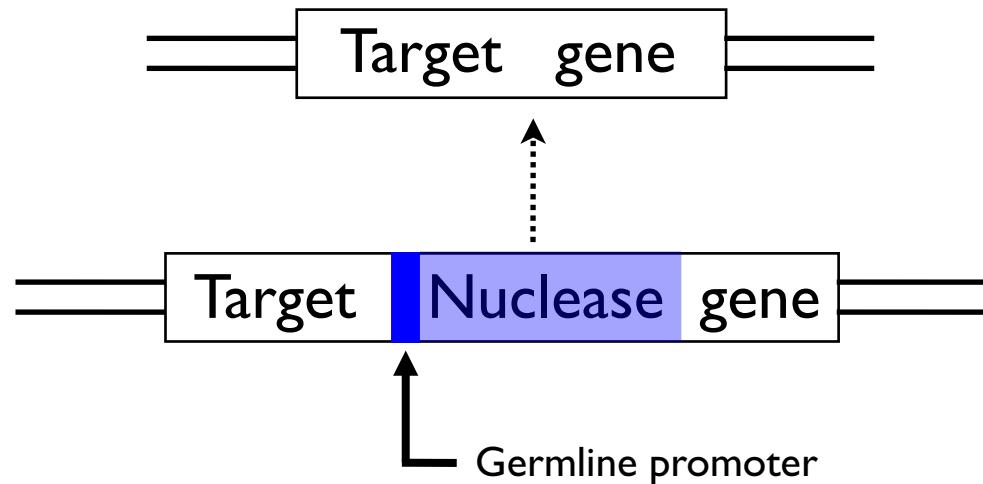
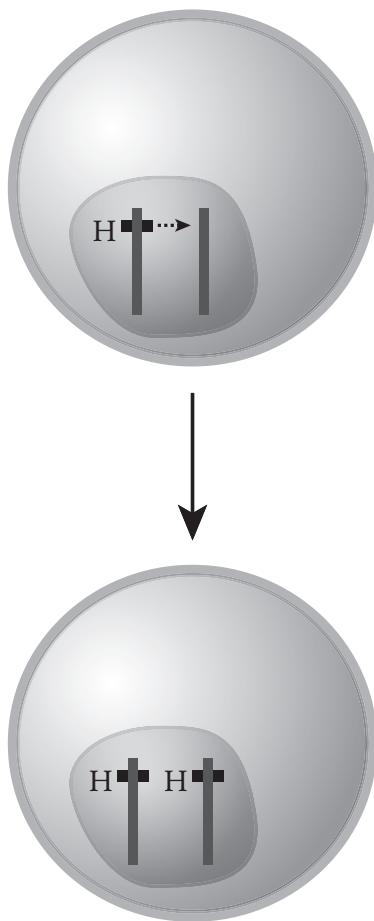


# The homing reaction



Natural process used by homing endonuclease genes  
in many microbes

# Gene knock-out by homing



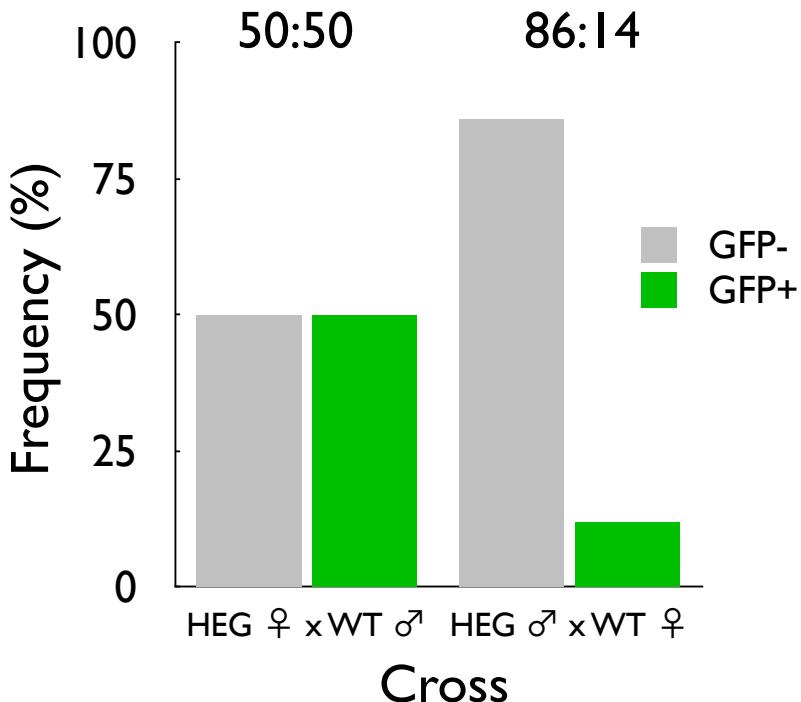
- Idea is to put nuclease gene in middle of target gene
- Homing can lead to population-wide knock-out of target gene
- Impact will depend on gene targeted
- Female fertility → Population reduction

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.

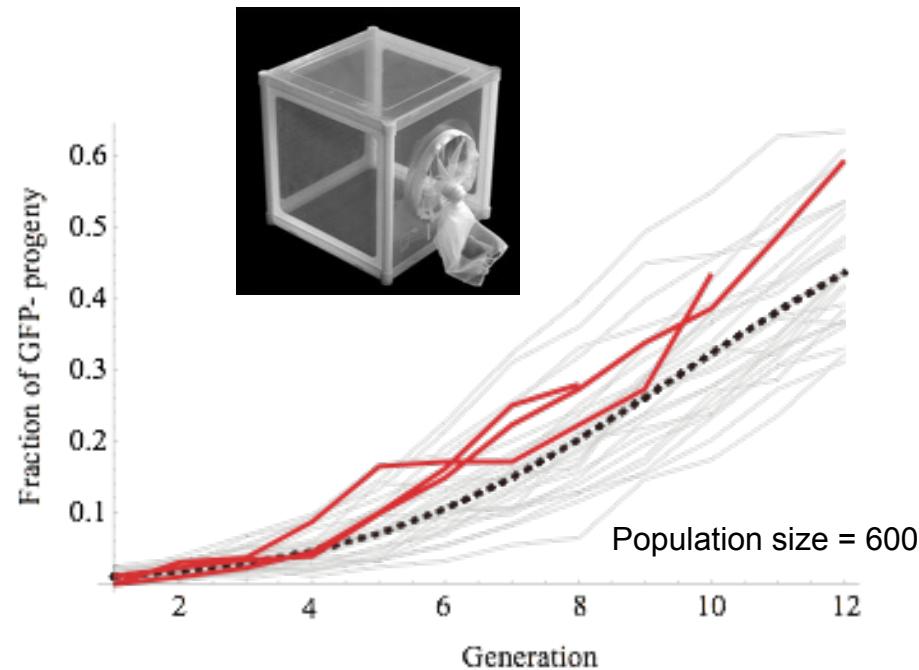
# Does the homing reaction occur in mosquitoes?

Experiments using a nuclease and its recognition sequence from yeast

## Preferential inheritance



## Spread through populations



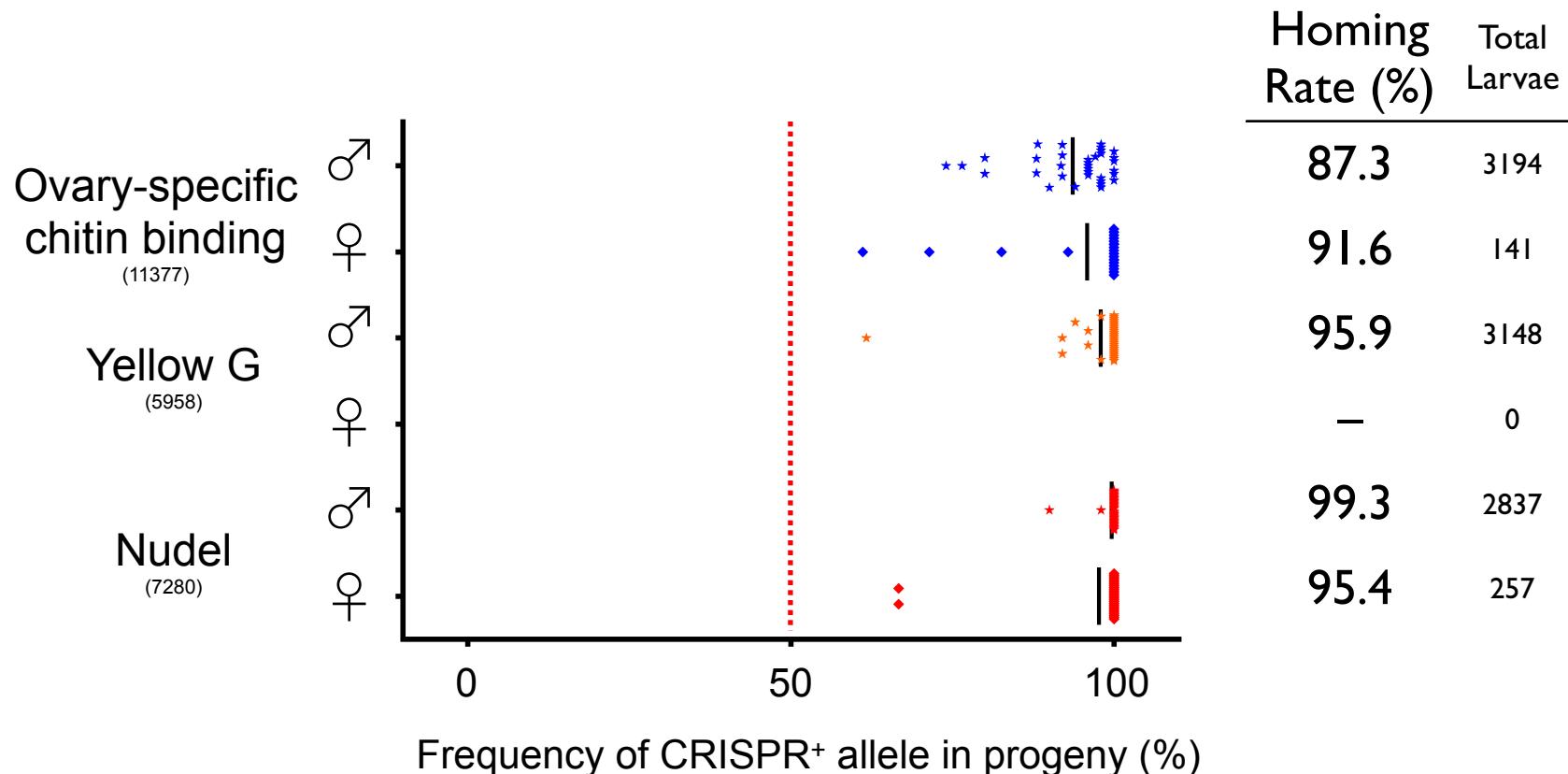
Together with *Drosophila* (Russell lab),  
1st demonstrations of homing in animals



A Vector Control Research Alliance

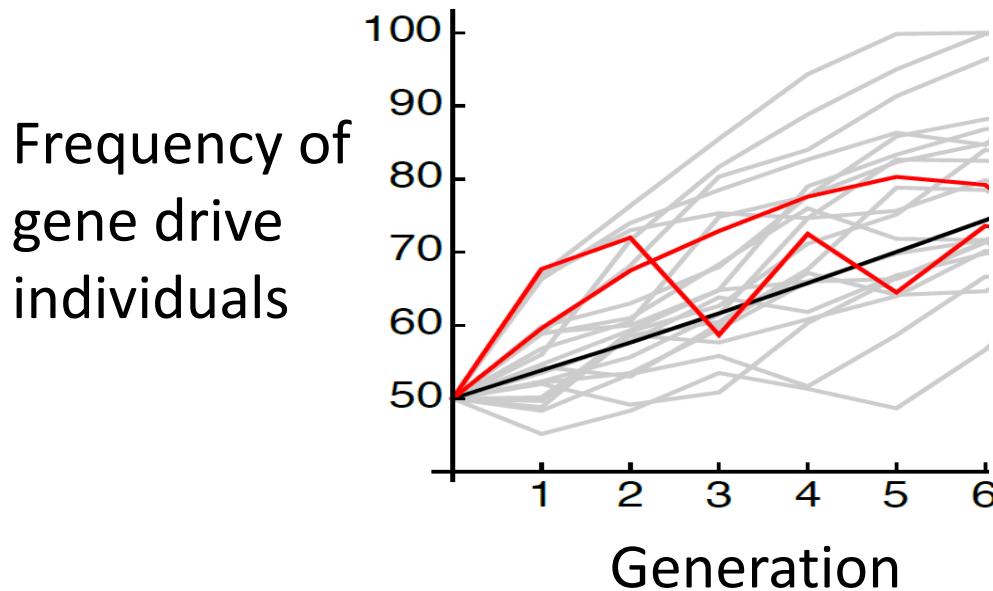
# Can we design nucleases to home into endogenous mosquito genes?

Identified 3 female fertility genes and designed CRISPR constructs against them



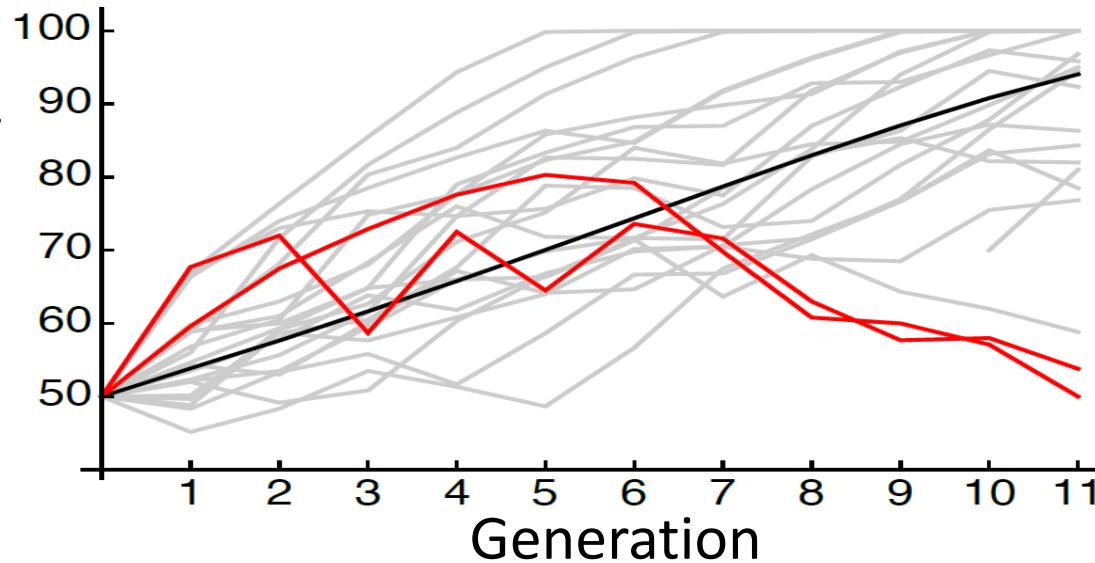
Average homing rate across 3 genes is 94%

# Will they spread in a cage?



# Will they spread in a cage?

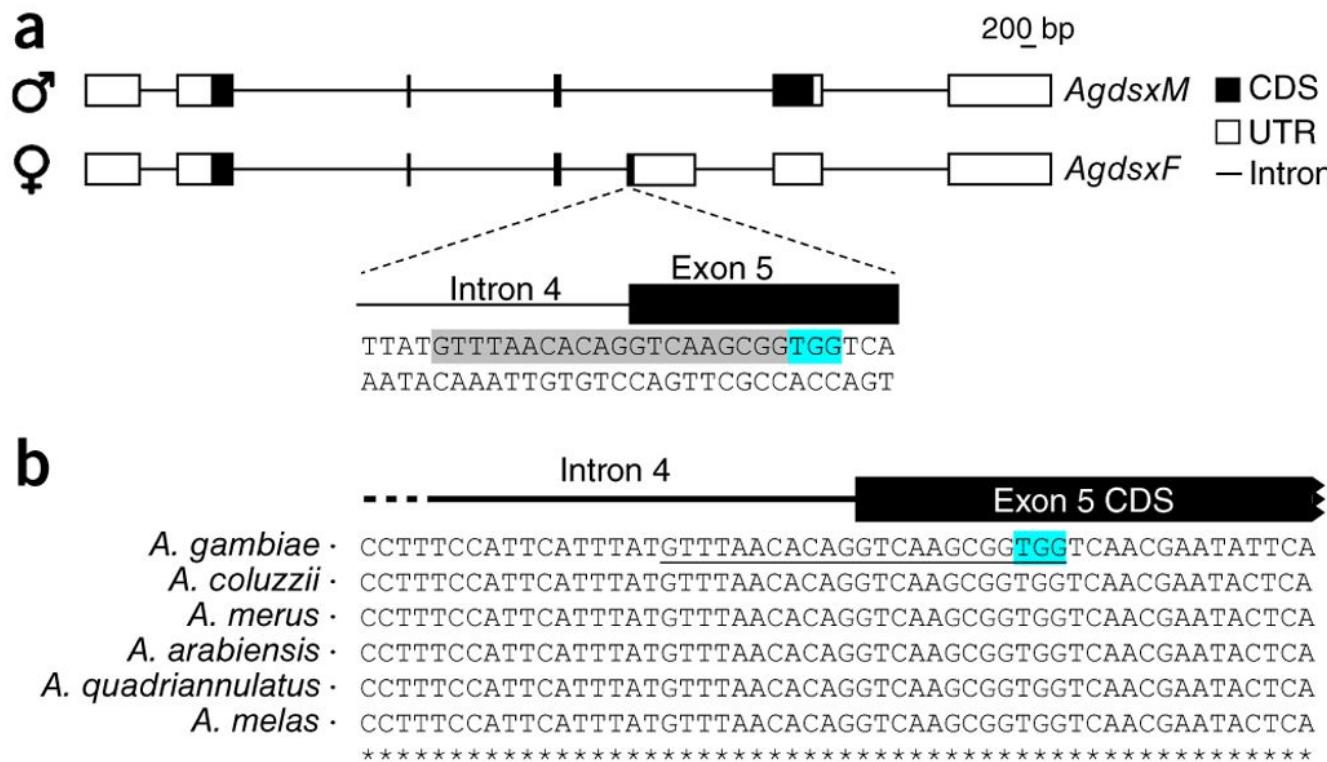
Frequency of gene drive individuals



- 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

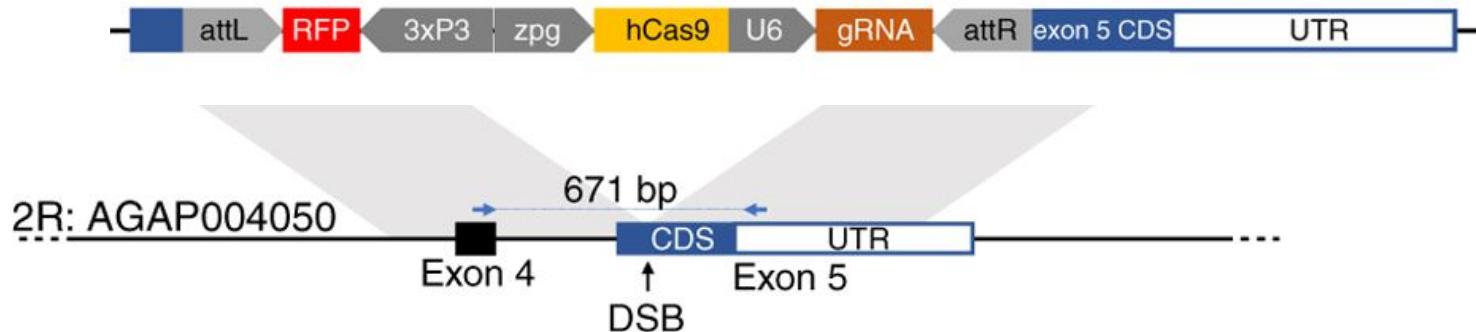
# Can we avoid resistance?

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

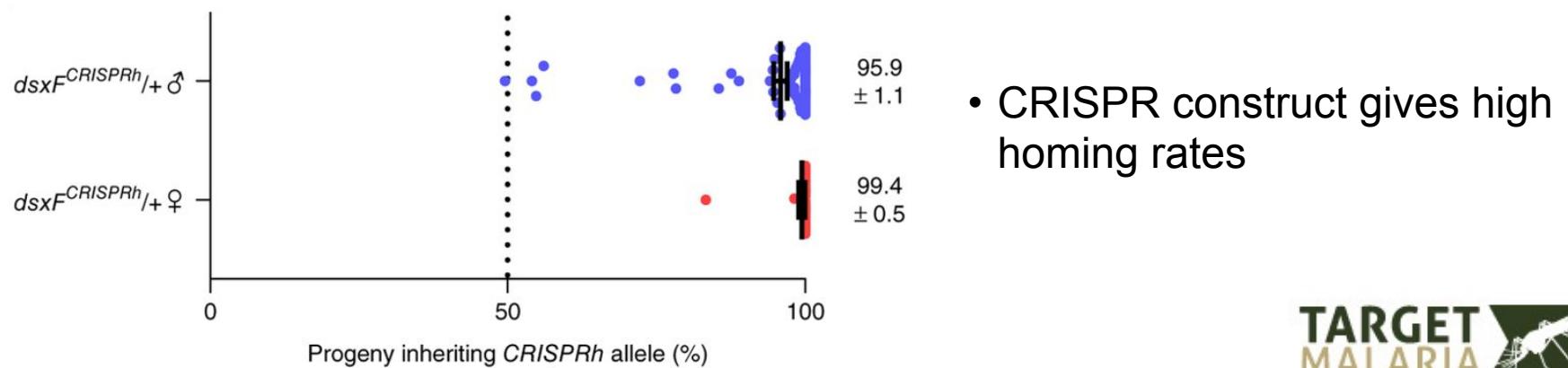


**Highly conserved across species, suggests changes not easily tolerated**

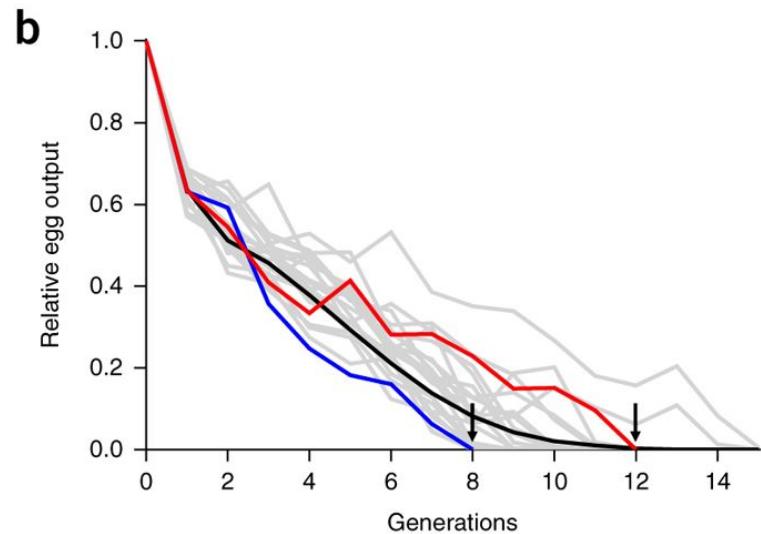
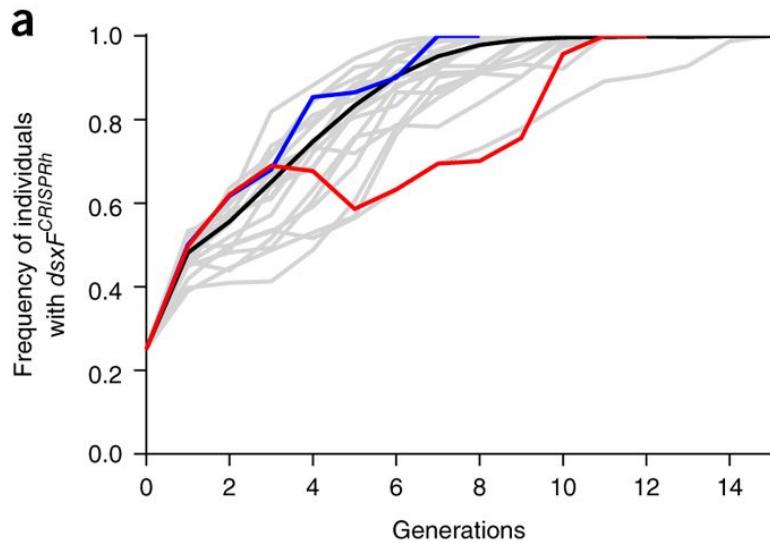
# Can we avoid resistance?



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

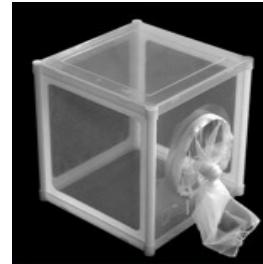


# Can we avoid resistance?



- 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:

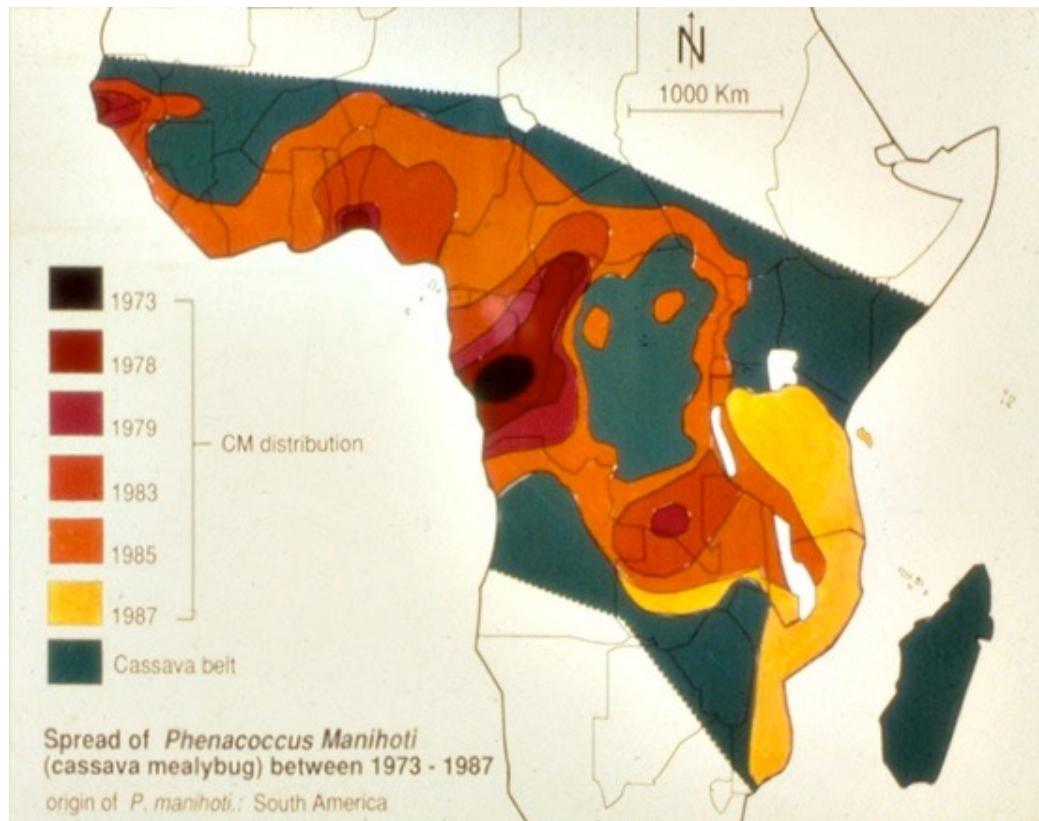
- Can mimic tropical light, temperature and humidity dynamics
- Allows lower, more realistic densities and mating environment (swarming)



# A precedent

## Biological control of the cassava mealybug

### The problem



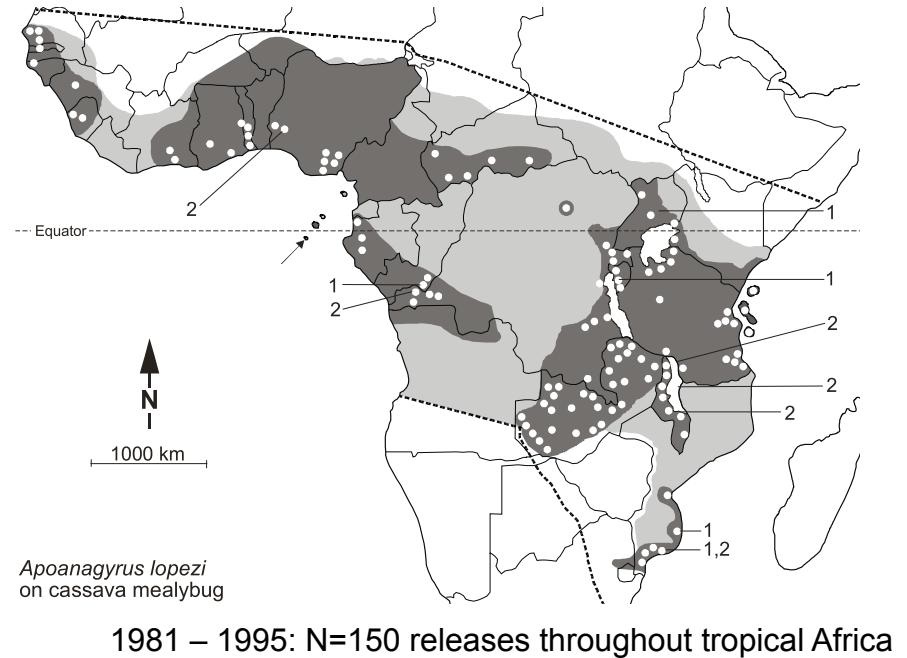
# A precedent

## Biological control of the cassava mealybug

### The product



*Apoanagyrus lopezi* from  
Paraguay & Brazil



### 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](http://www.targetmalaria.org)**

# **Projects**

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

**Demographic inference from  
population genomic data**