

# Manipulating mosquitoes for malaria control

Austin Burt

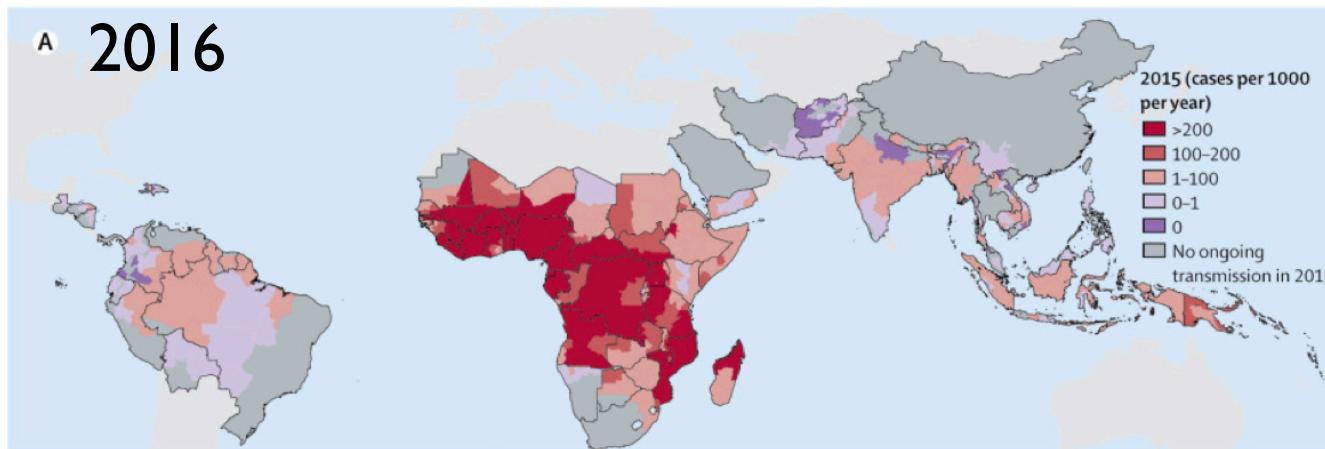
CMEE 2025

# Rationale (1)

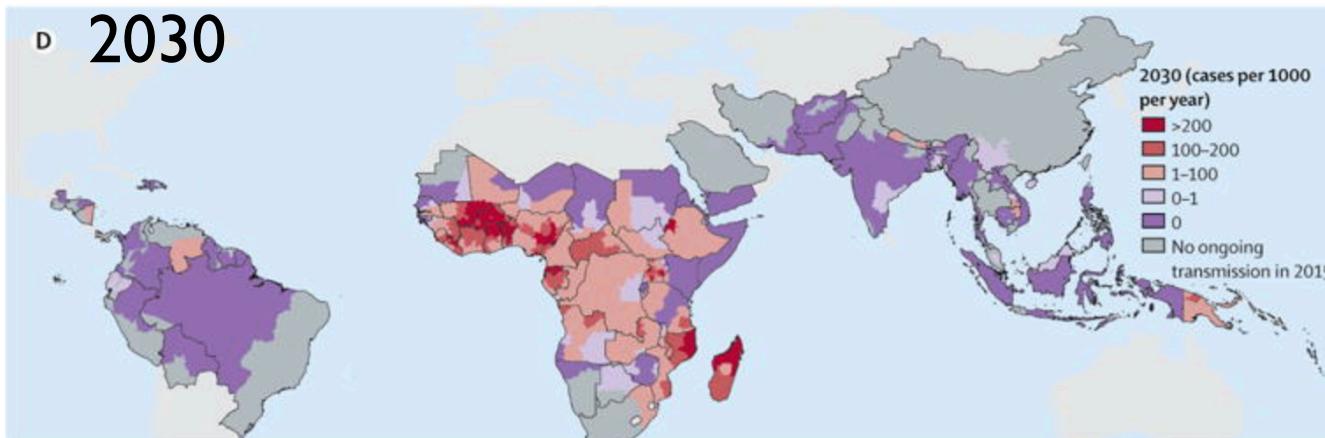
- Malaria continues to impose a huge burden
  - 100s of millions of infections every year
  - 100s of thousands of deaths every year
  - Mostly infants and children
  - Mostly in Africa
- Current interventions (nets, spraying, drugs) have saved millions but:
  - Not enough to eliminate
  - Drug- and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

# Rationale (2)

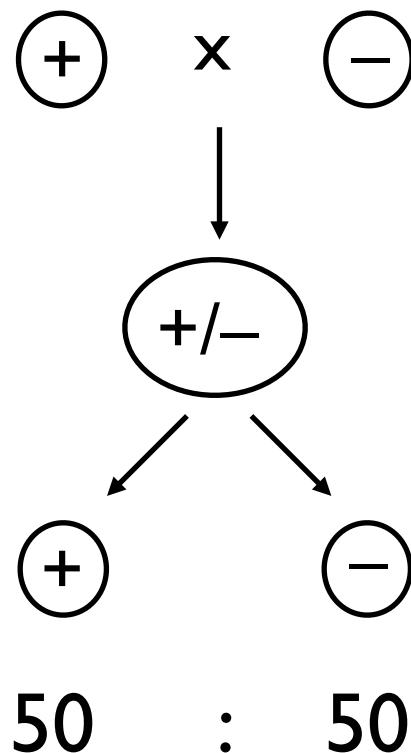
- Recent advances in molecular biology potentially allow for a transformational new intervention platform using ***genetic approaches***, including ***gene drive***, to control the mosquitoes that transmit malaria.
- By leveraging normal biological processes (e.g., DNA error repair, reproduction, dispersal), gene drive could potentially be the basis for ***highly efficient*** interventions
  - Analogous to vaccines leveraging the adaptive immune system
  - Efficiency particularly important in context of large geography and few resources.

# Biological background

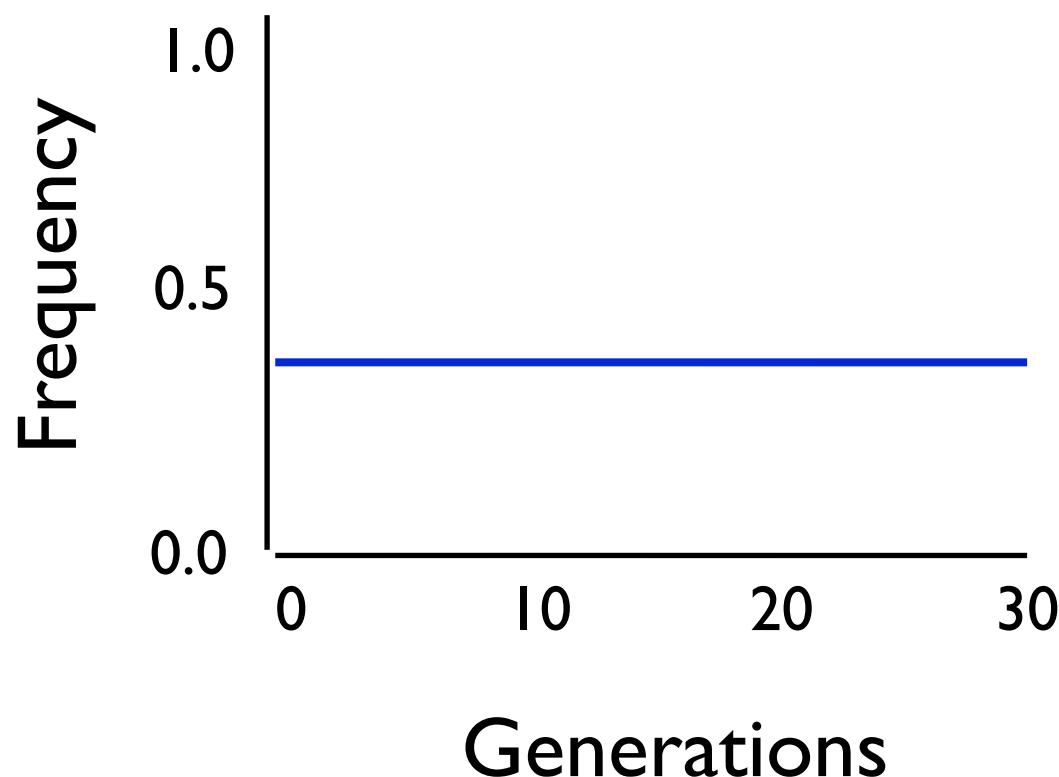
- Malaria in Africa is largely rural
- There are 4 species of *Plasmodium* causing malaria in Africa
  - *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*
- In Africa, most transmission is by 3 closely related *Anopheles* species (*An. gambiae*, *An. coluzzii*, *An. arabiensis*), plus *An. funestus*
  - 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

# Mendelian transmission

Equal, unbiased inheritance

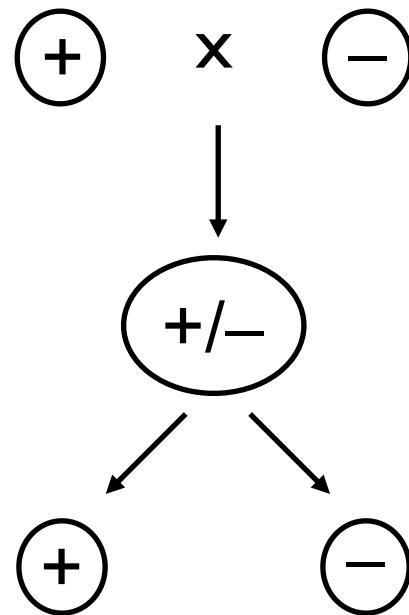


All else equal,  
no change in gene frequency



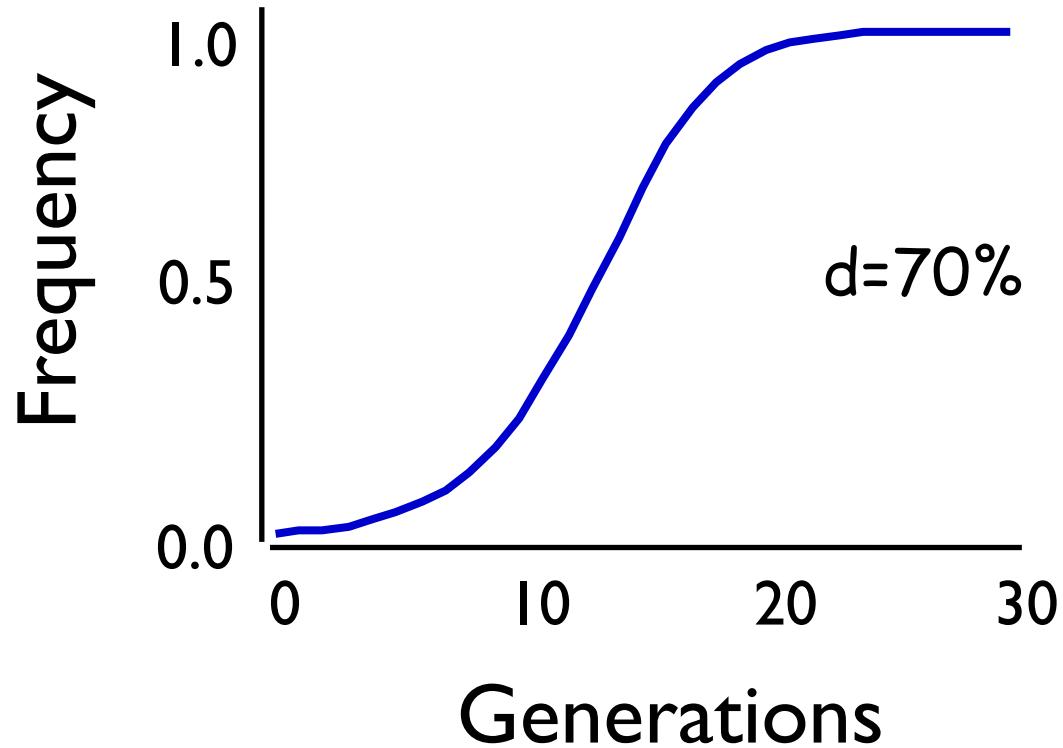
# What is gene drive?

Preferential inheritance



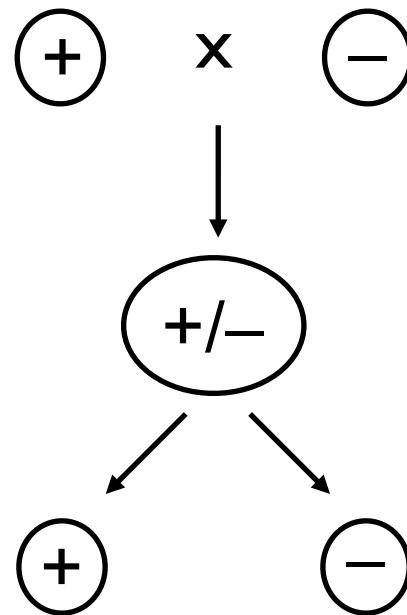
70 : 30

Spread in population



# What is gene drive?

Preferential inheritance



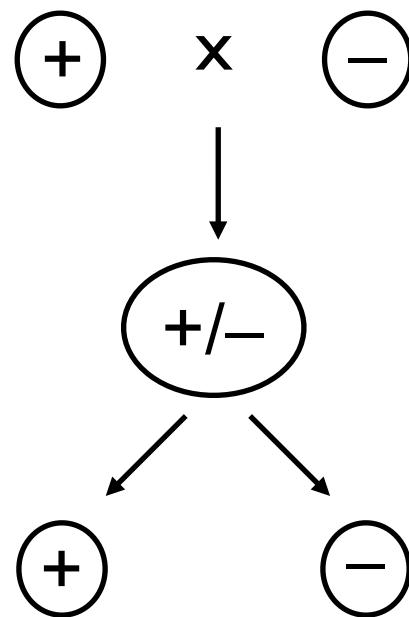
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A "5th force" in population genetics, in addition to

- mutation
- migration
- drift
- selection

# What is gene drive?

Preferential inheritance



70 : 30

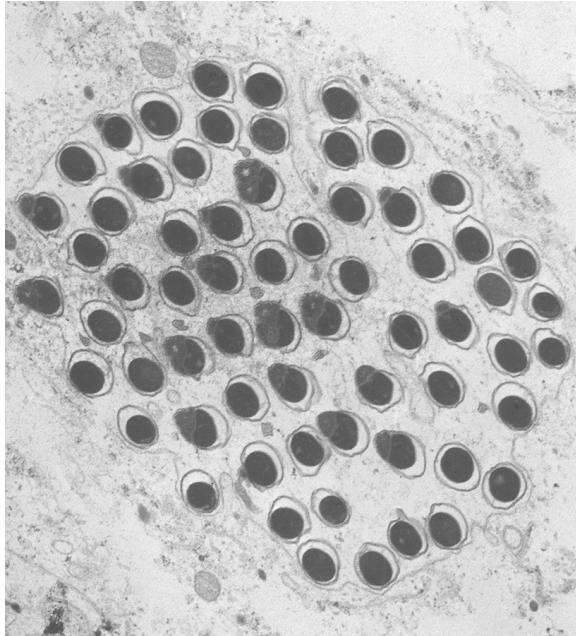
Key points:

- 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

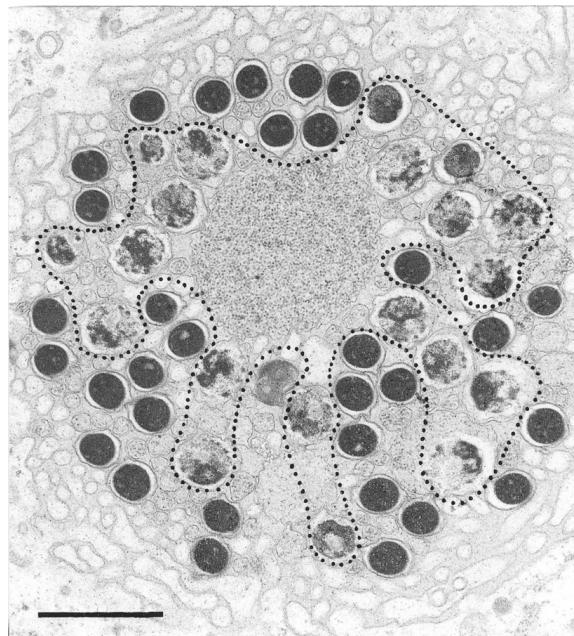
# Segregation Distorter in *Drosophila*



Normal (−/−)



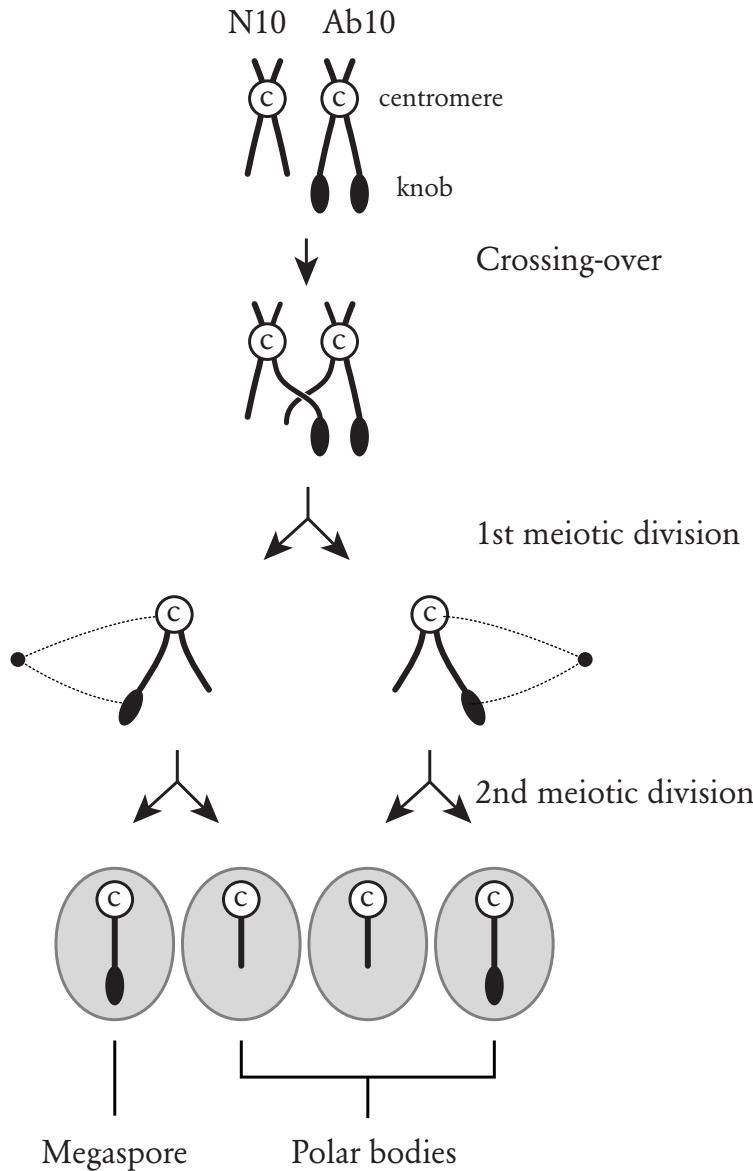
SD/−



- 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*

# The knobs of maize



Meiotic drive



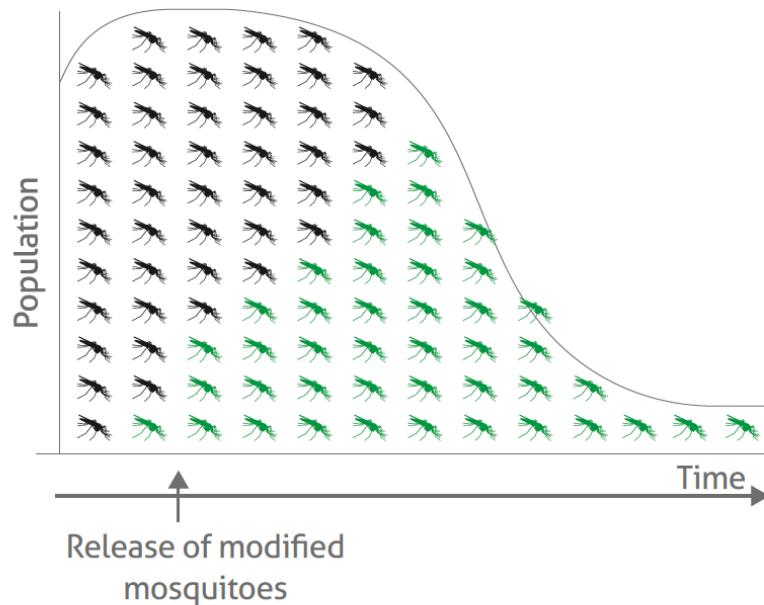
Mendelian inheritance



# Gene drive: two basic strategies

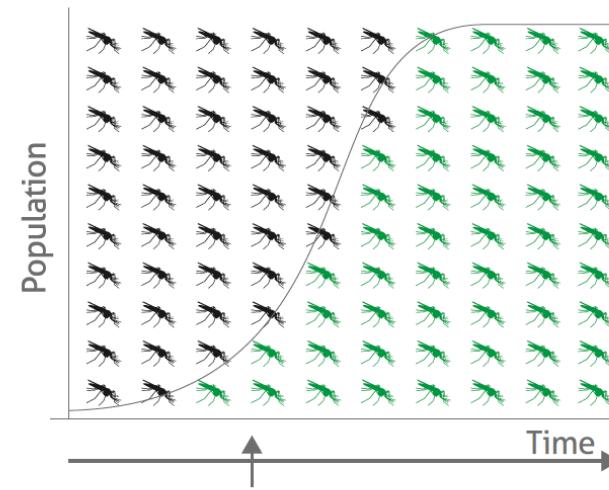
## Population suppression

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



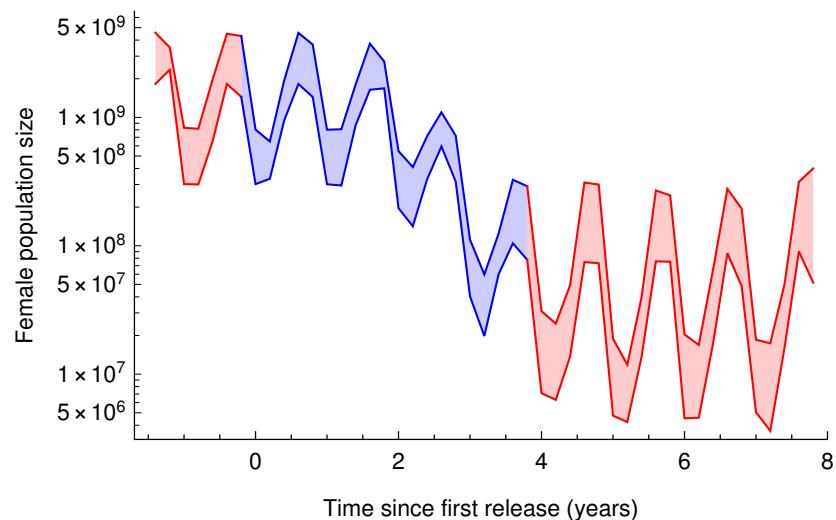
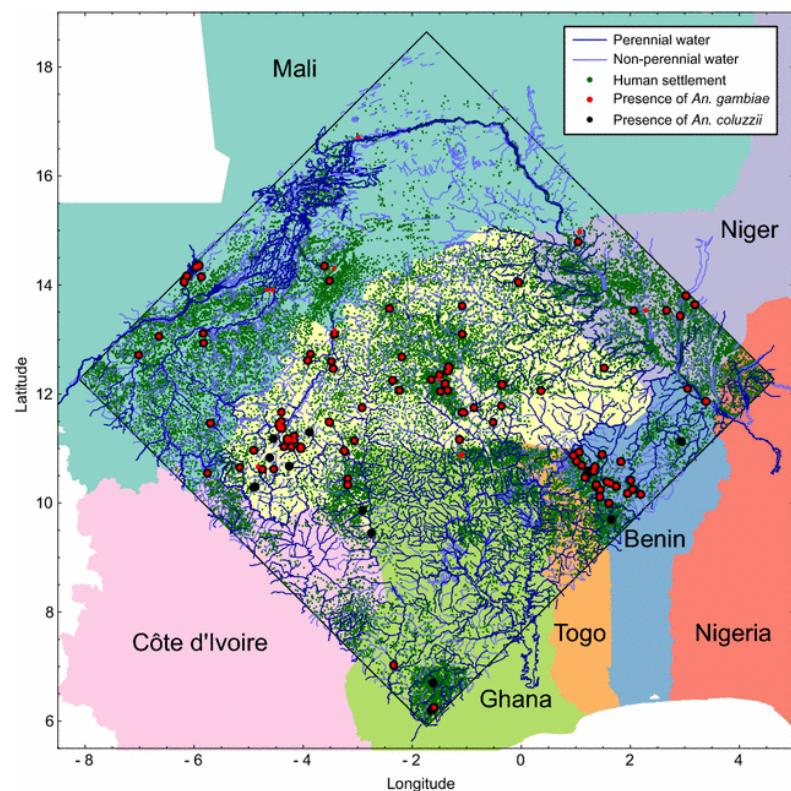
## Population replacement

*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



# 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

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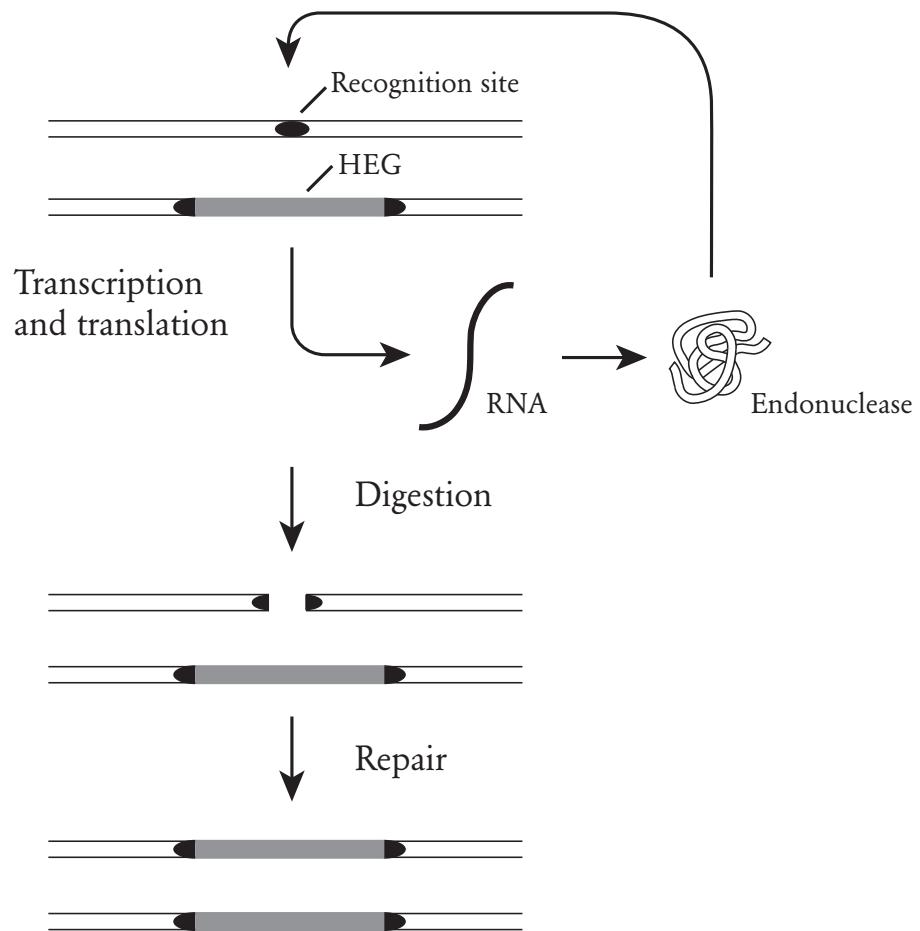
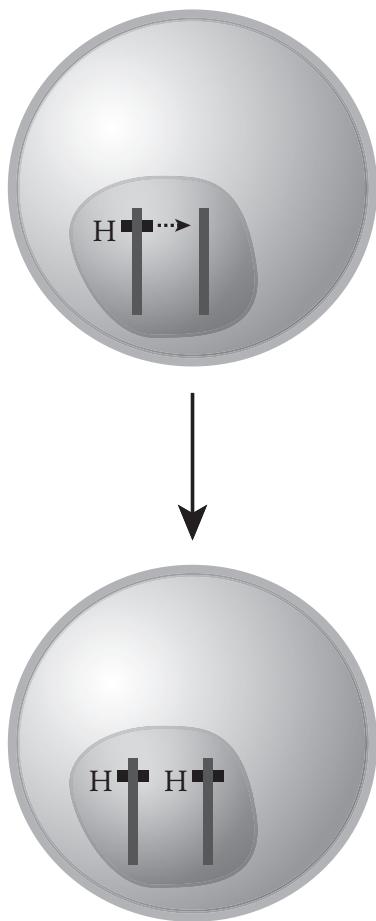


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Driving Y chromosome

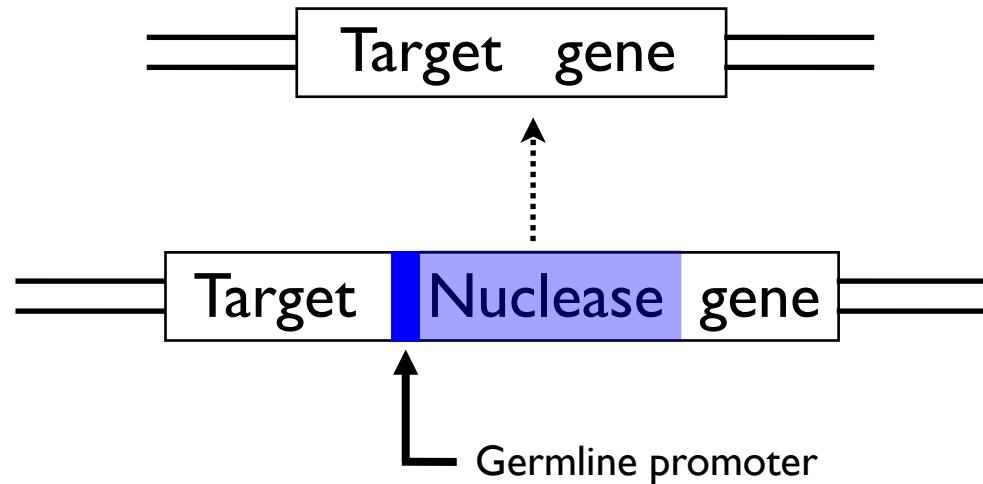
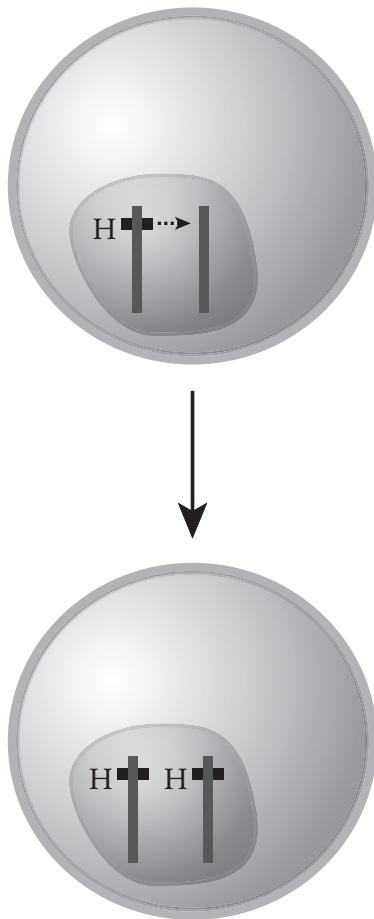
Gene knock-out by homing

# Gene knock-out by homing



**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.

# Technical progress

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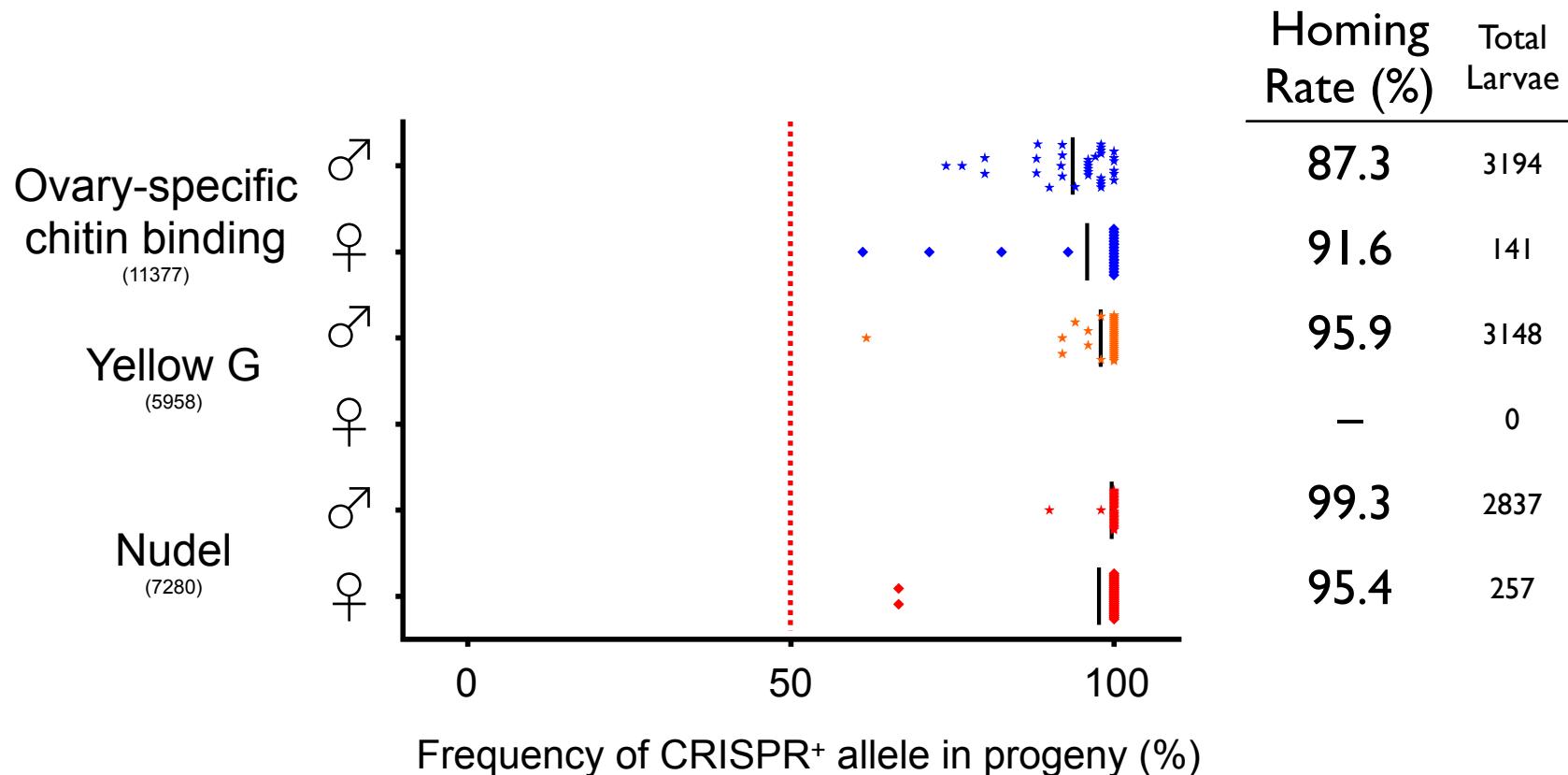
Does it work  
in the field?

All molecular entomology work  
done in lab of Andrea Crisanti



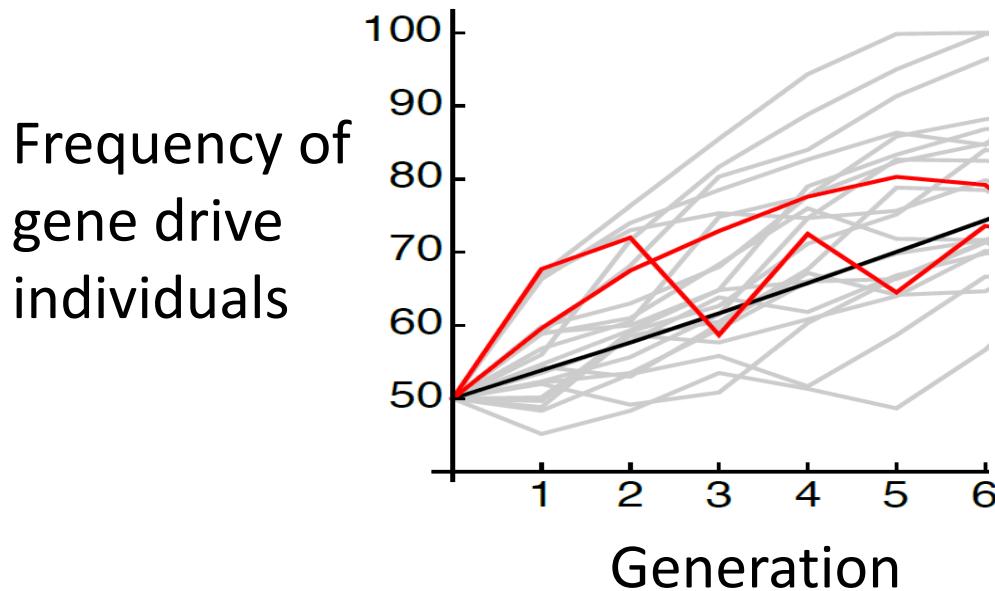
# Can we design nucleases to home into endogenous mosquito genes?

Identified 3 female fertility genes and designed CRISPR constructs against them



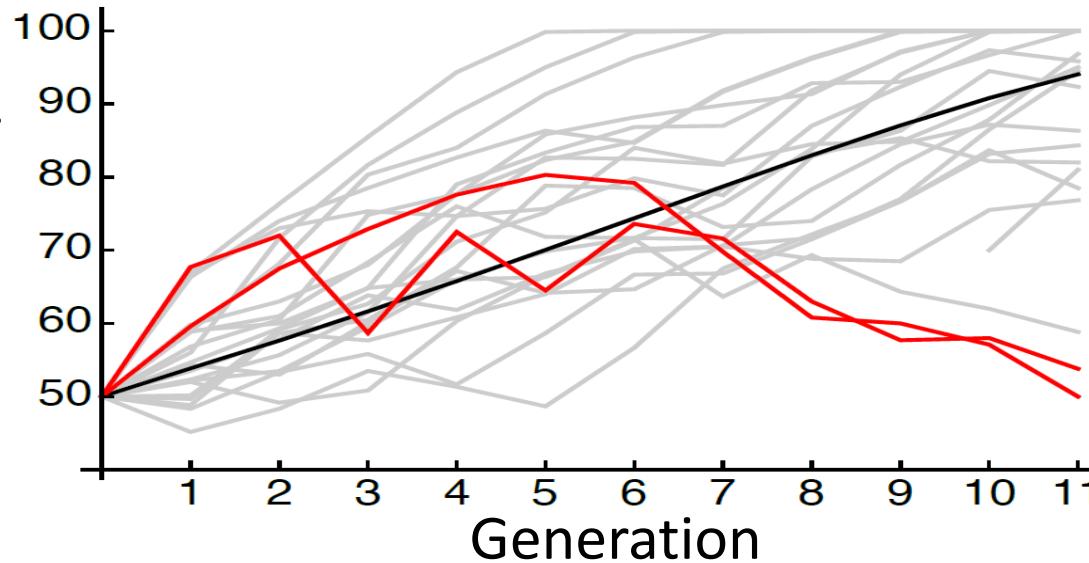
Average homing rate across 3 genes is 94%

# Will they spread in a cage?



# Will they spread in a cage?

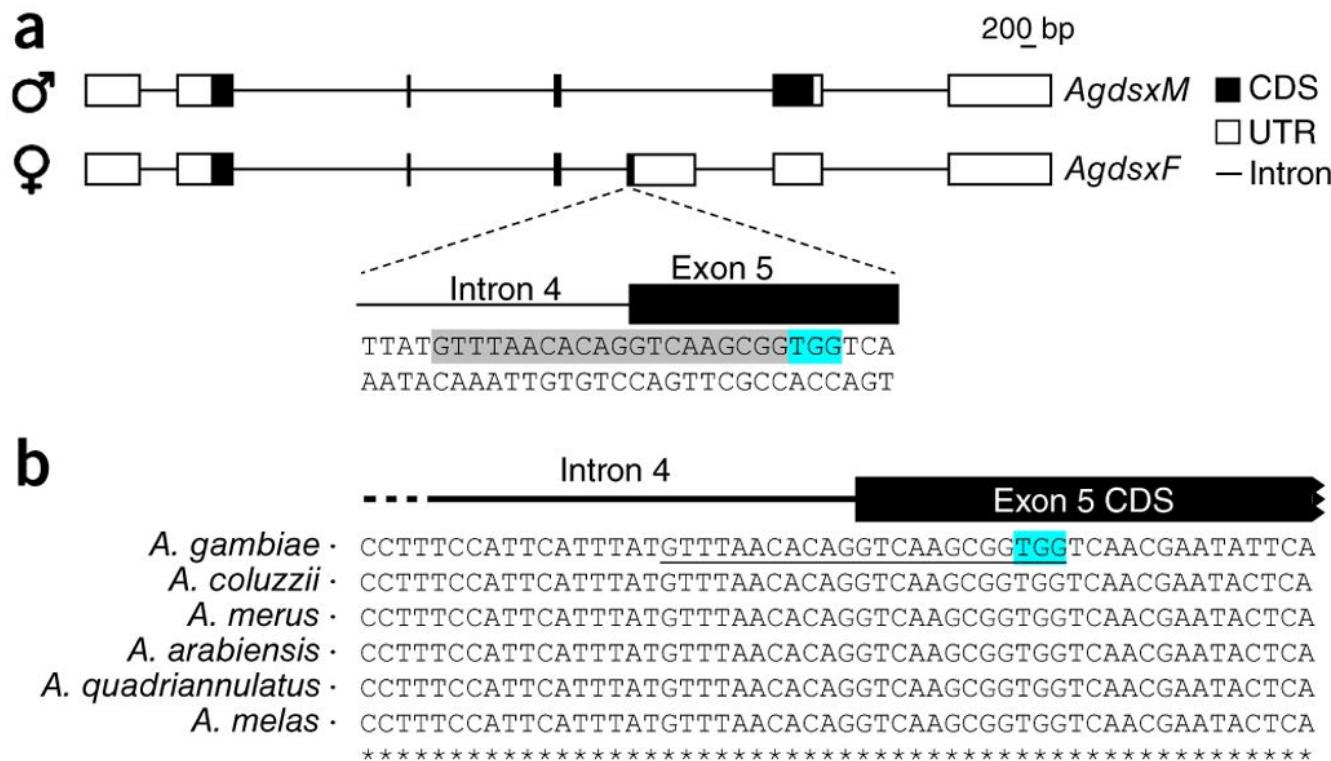
Frequency of gene drive individuals



- 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

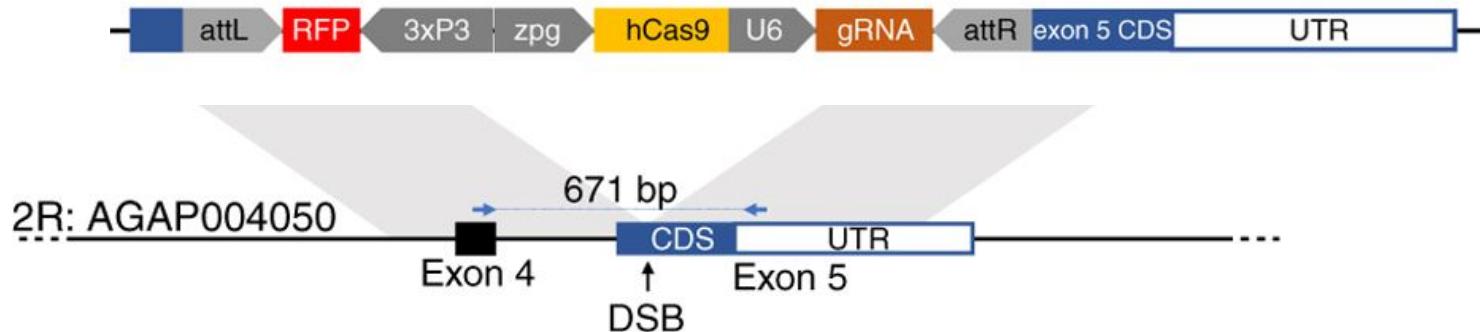
# Can we avoid resistance?

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

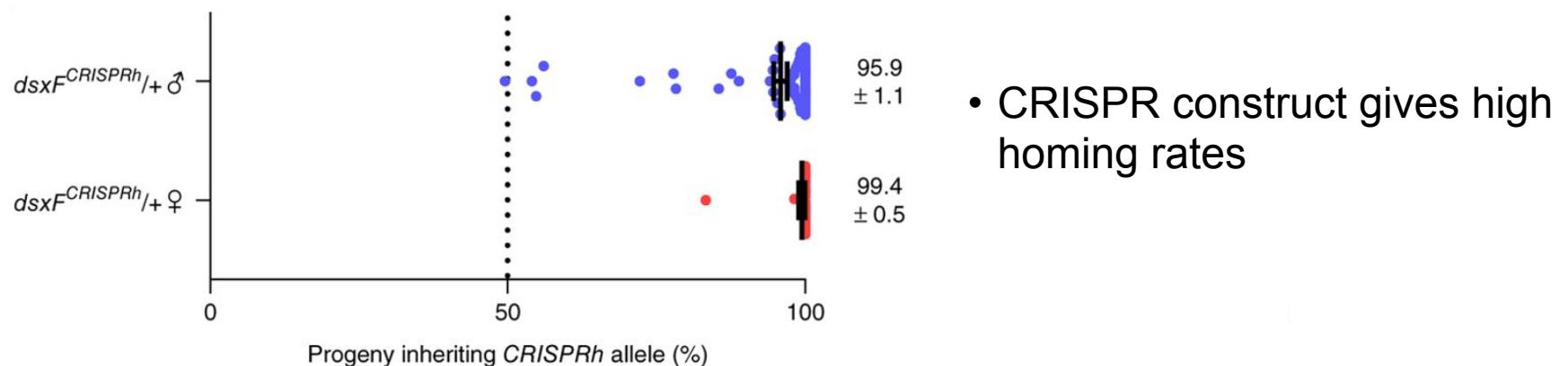


**Highly conserved across species, suggests changes not easily tolerated**

# Can we avoid resistance?

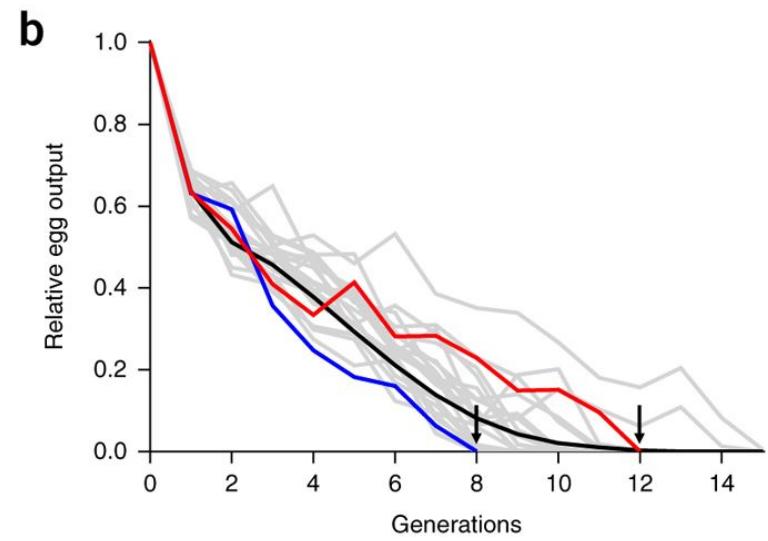
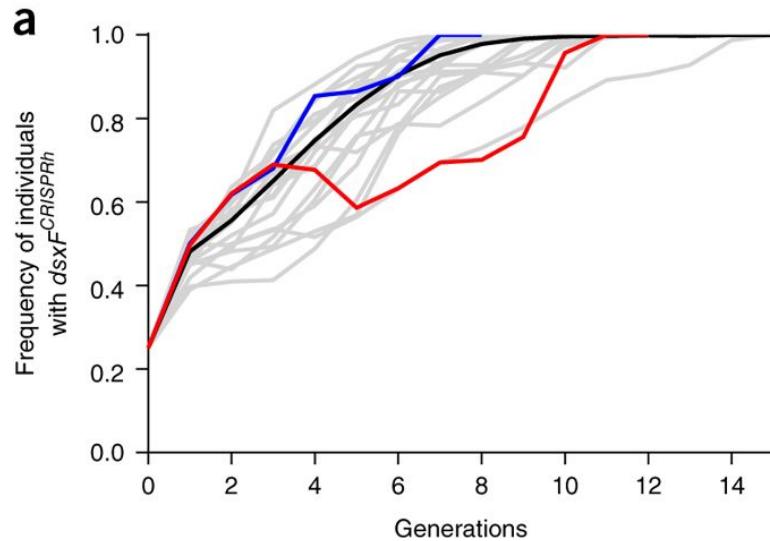


- Homozygous knock-out females are sterile (and cannot blood feed)
- No obvious effect on males



# Can we avoid resistance?

- Targeting a different gene, at a site that is not so tolerant of change

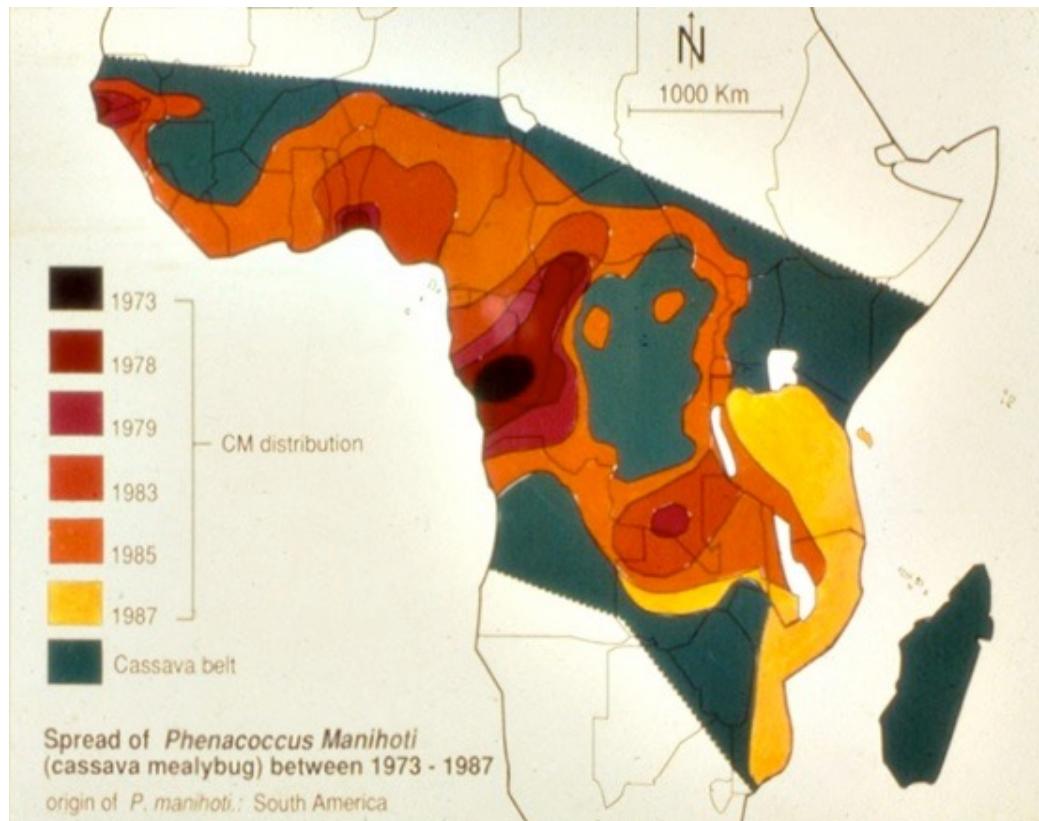


- Construct spread to fixation in two cage populations
- Led to population crash
- No sign of resistance — mutations produced, but not selected
- Now trying 2 target sites — like combination therapy

# A precedent

## Biological control of the cassava mealybug

### The problem



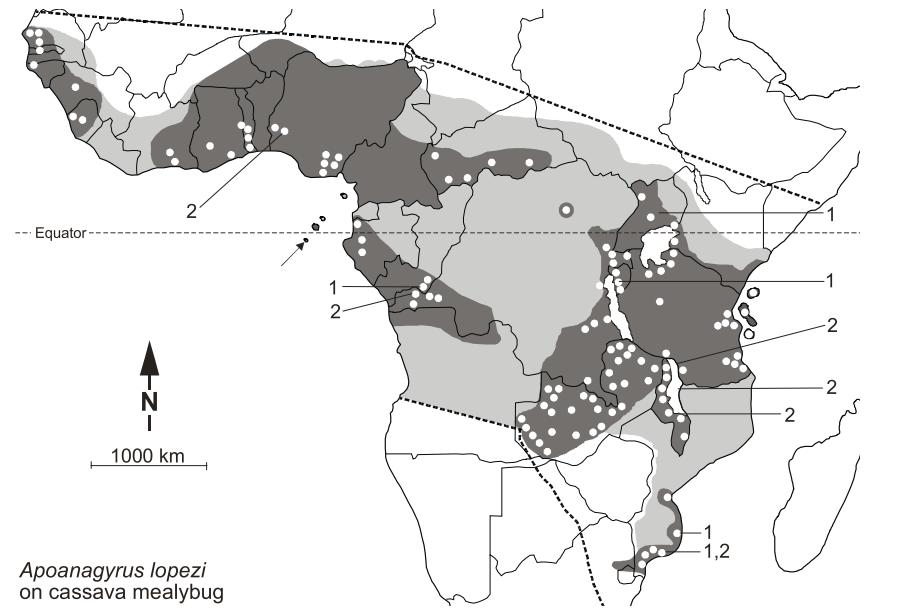
# A precedent

## 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

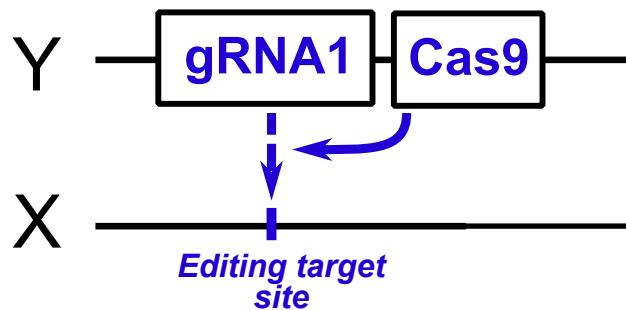
**Benefits** US\$ 9-20 billion **Costs:** ~US\$ 34 million over 35 years

# Other genetic approaches

- Gene drive constructs discussed thus far will spread throughout species range if there is almost any movement
- For other potential use cases for genetic biocontrol, may not want to have the impact spread much beyond the release area — e.g., invasive species
  - Sterile Insect Technique (SIT)
    - Sterility based on irradiation, bacteria, or genetic modification
    - Used for screwworm, medfly, *Aedes*, etc.
    - Need to release a lot: impractical for very large populations or species that are difficult to rear

# Other genetic approaches

## Y-linked editors (YLEs)



- Insert on the Y chromosome an editor that causes female descendants to die or be sterile — dominant effect
- Y chromosome is not transmitted to those females anyway, so it is not harmed by the damage it causes — it persists
- Could be 10-100-fold more efficient than SIT and still remain localised
- Still at the stage of trying to show proof-of-principle in the lab

**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