

Selfish genes as new tools in the fight against malaria

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A Vector Control Research Alliance

Outline

- The problem and a potential new approach
- Technical activities
- Co-development activities

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Malaria

The burden

- More than 200 million infections & half million deaths each year, ~90% in Africa, mostly infants & children, mostly the poor.
- Economic losses in Africa ~\$12 billion a year

The biology

- Malaria is caused by a parasite called *Plasmodium*
- *Plasmodium* is spread to people through the bites of infected mosquitoes
- There are ~3500 species of mosquito, but in Africa most transmission is by a single species complex, the *Anopheles gambiae* complex
- Only female mosquitoes bite and transmit the parasite



Photo: John R. Clayton

Malaria

Current methods of control are good but not enough

- ITNs, IRS, ACTs have reduced mortality rates, saving millions of lives
- But they are not enough to eliminate the disease
- Drug- and insecticide-resistance mean recent progress could be reversed
- \$5.1B/yr required for malaria control, more than the amount available
- Additional cost-effective, sustainable vector control methods are needed

Target Malaria is developing new methods using 'gene drive' to spread nucleases that will disrupt mosquito reproduction

Malaria

Genetic approaches that could contribute to vector control

- Swamp population with sterile males or equivalent (SIT, RIDL)
Technically easiest to develop in the lab; unclear it can be cost-effective against a continent-wide rural disease
- Change mosquito populations to be unable to transmit malaria
Technically most challenging
- Release mosquitoes with selfish gene that can drive through population while disrupting mosquito reproduction
Intermediate technical difficulty; should be cost effective

Malaria

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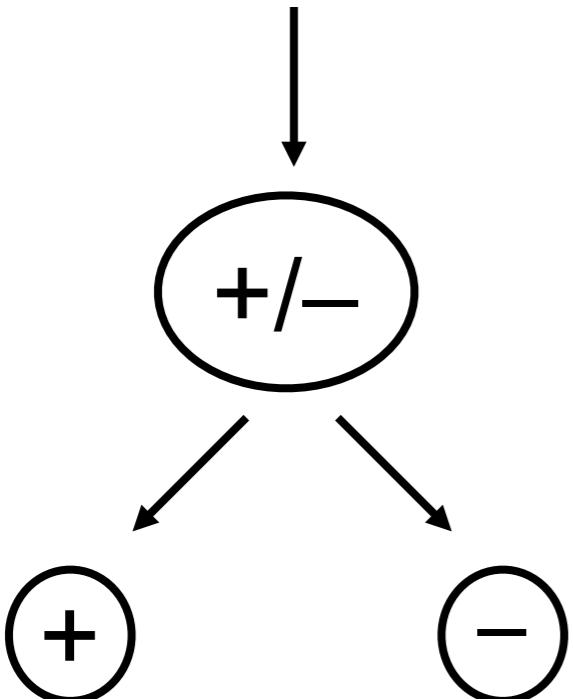
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Gene drive systems

Preferential inheritance

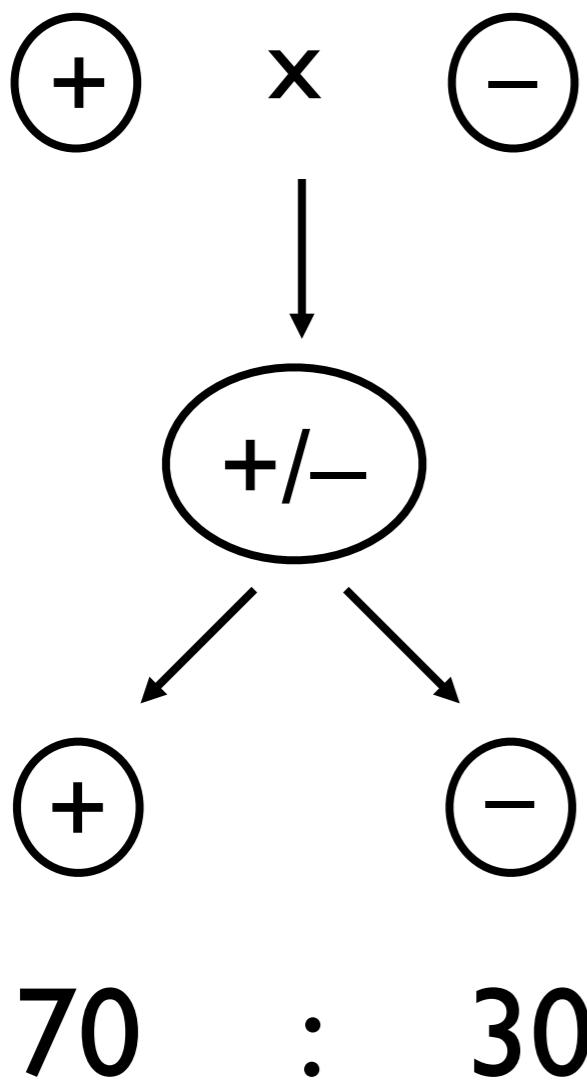
(+) x (-)



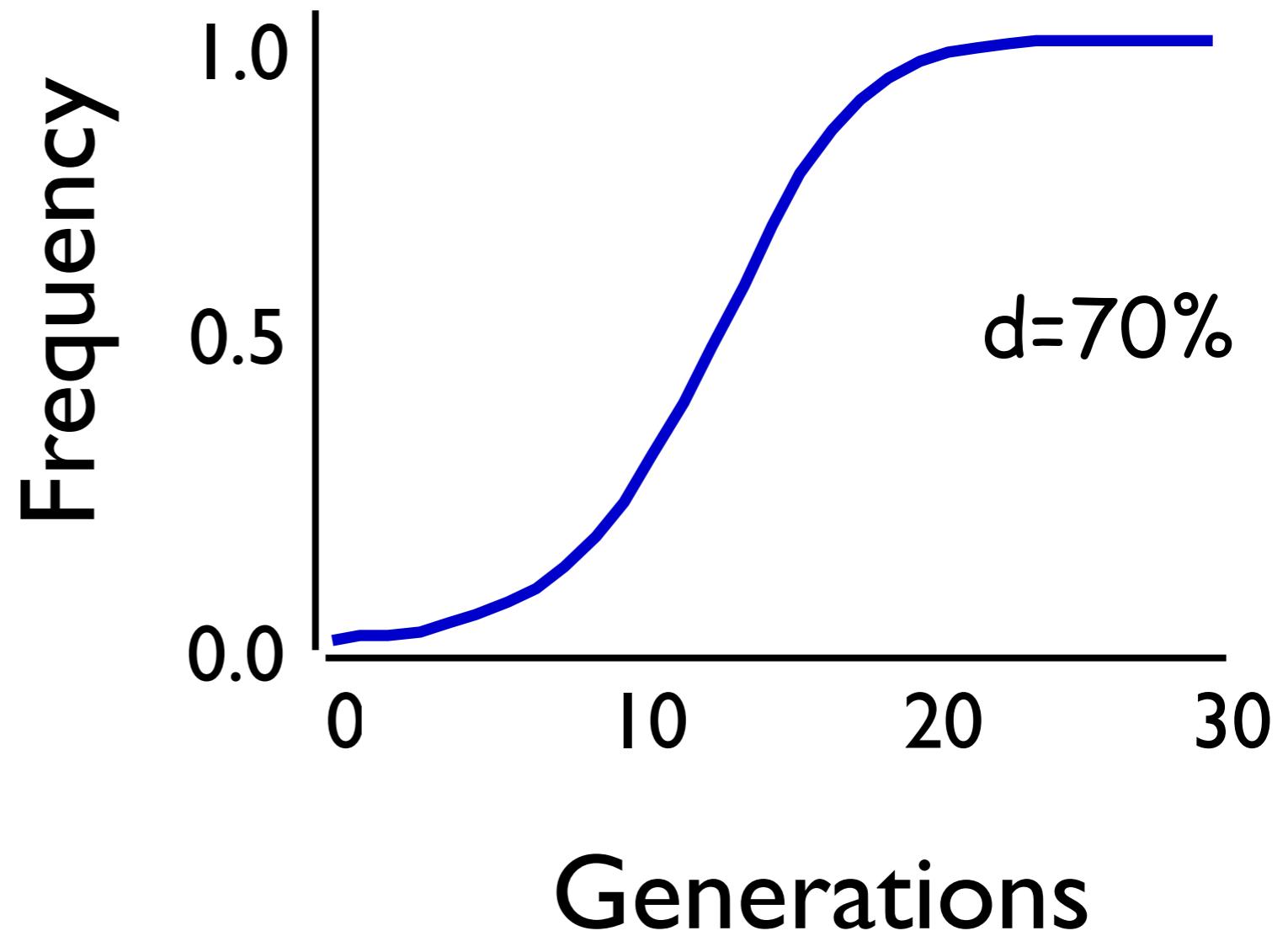
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Gene drive systems

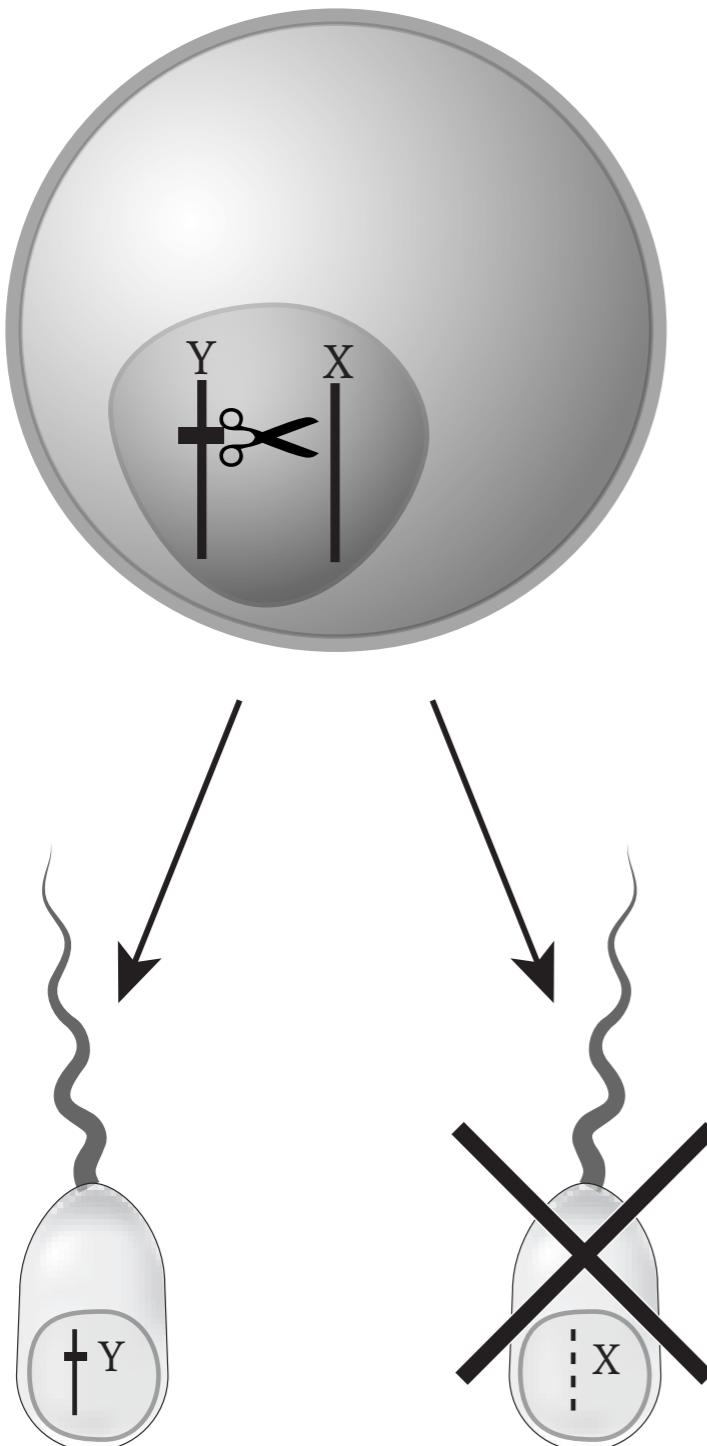
Preferential inheritance



Spread in population

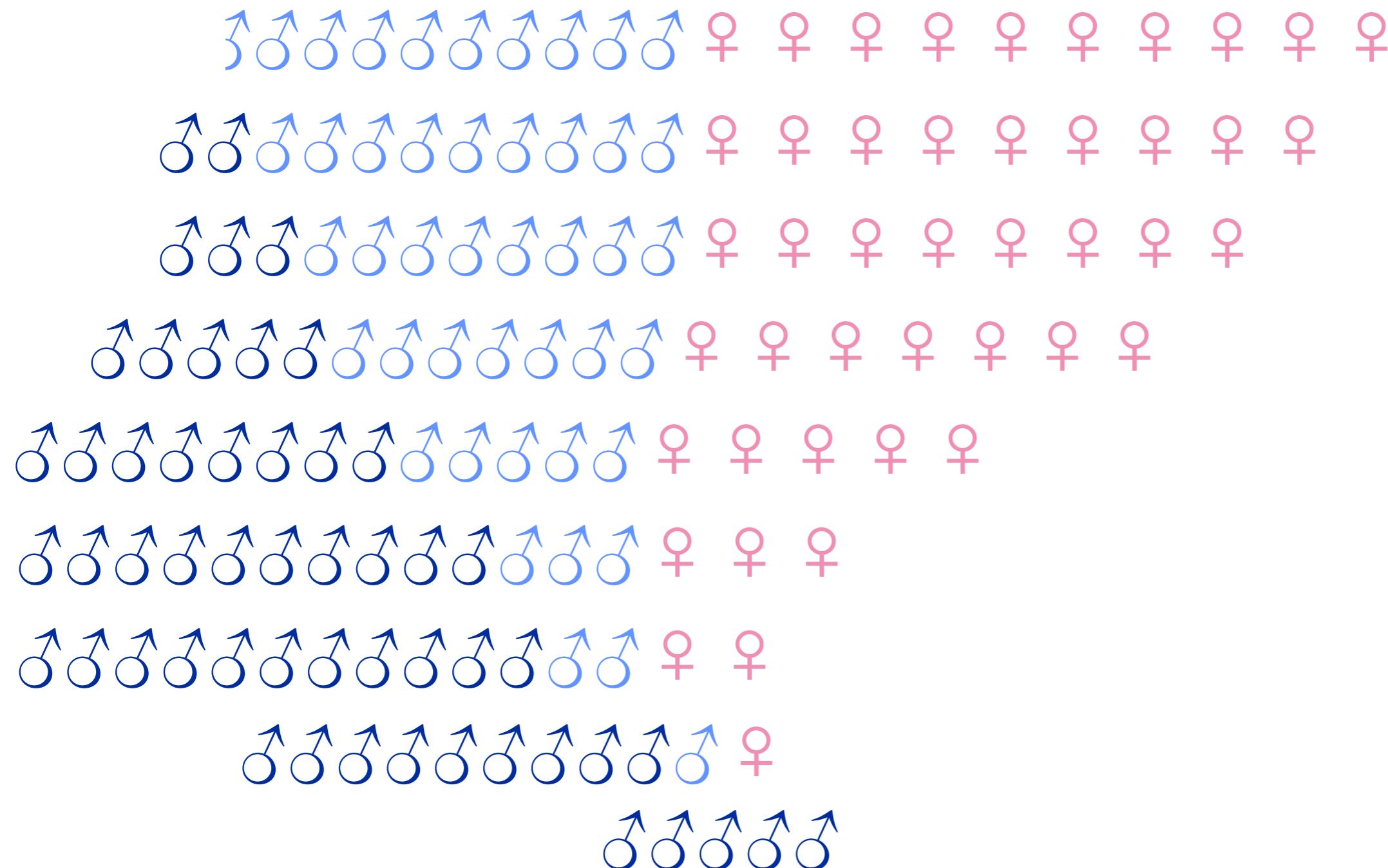


Driving Y chromosome



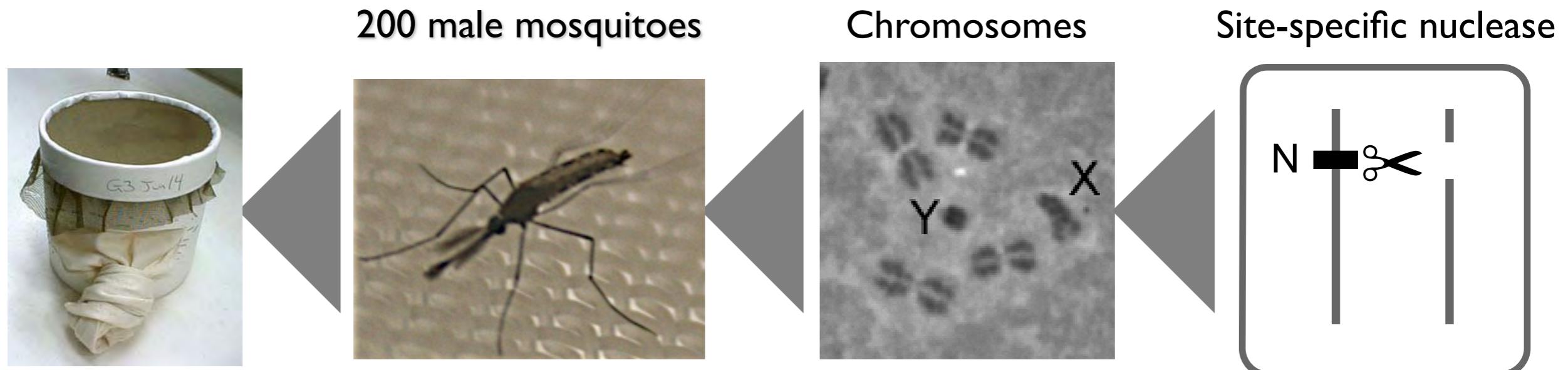
- Put gene on Y that makes enzyme that cleaves X
- Design it so gene only active during spermatogenesis
- Will gradually convert a population so it is almost all males

Gradually convert a population so it is almost all males



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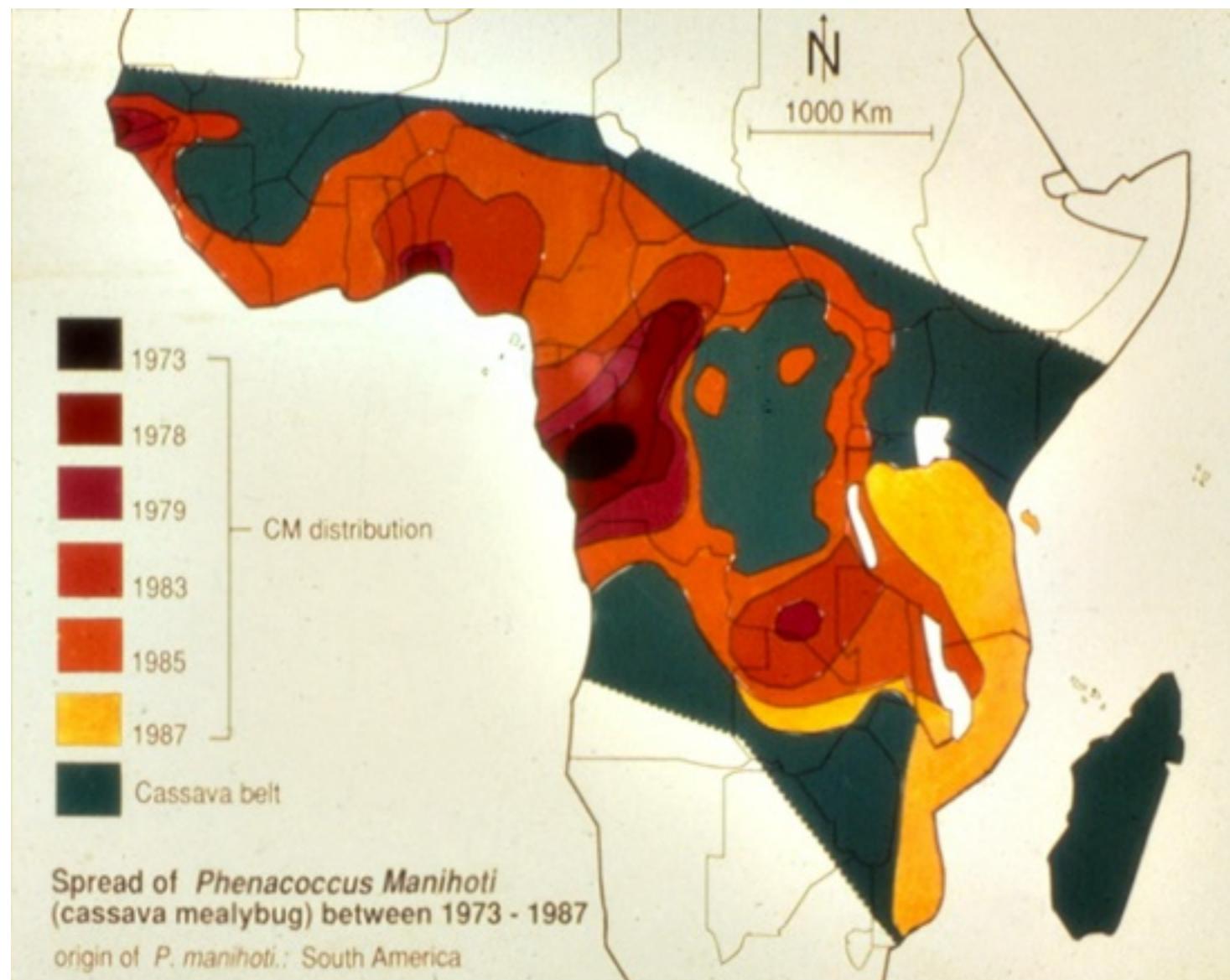
The product



Mosquito populations to be controlled by
releasing into them a small number of
modified males

A precedent: biological control of the cassava mealybug

The problem

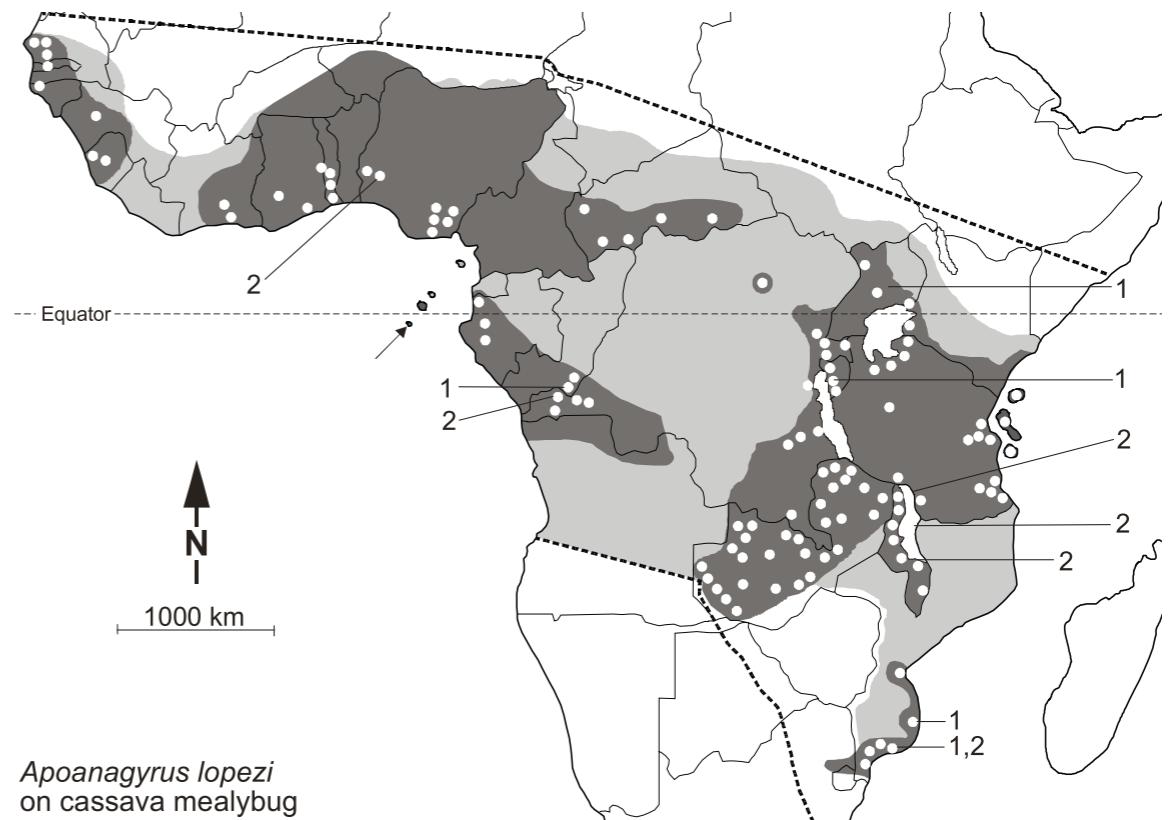


A precedent: biological control of the cassava mealybug

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

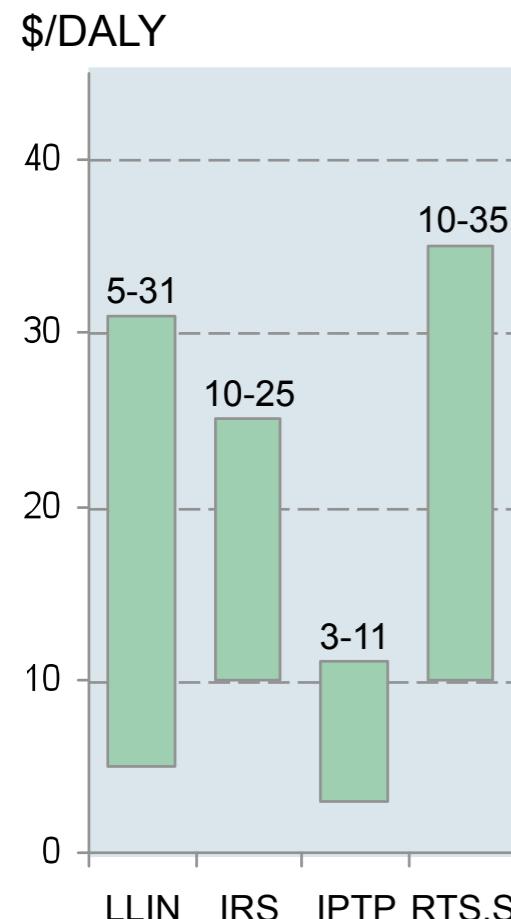
Benefits US\$ 9-20 billion **Costs:** ~US\$ 34 million over 35 years

Intended key features of HEG-based approaches to malaria control

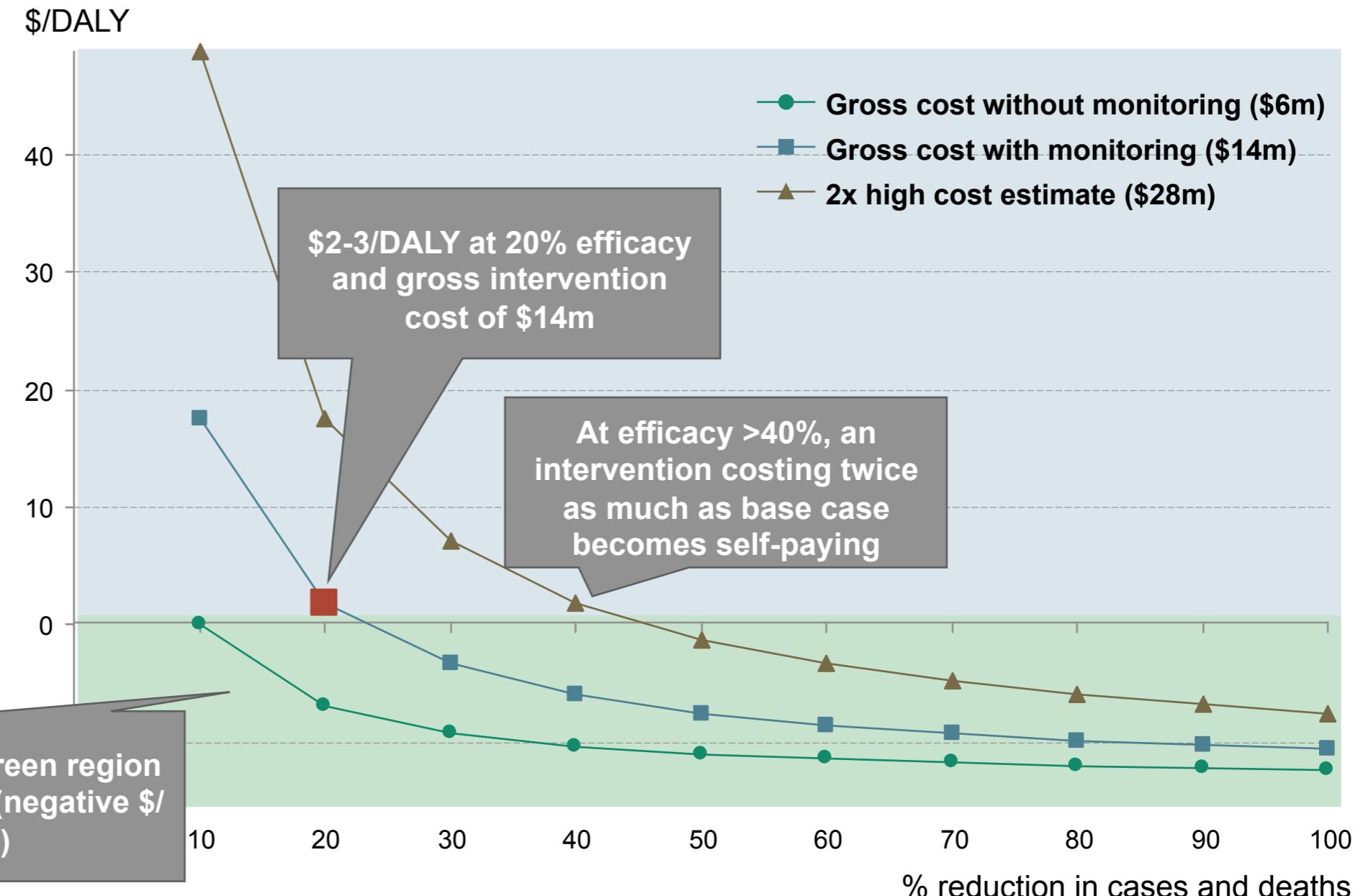
- Reduces transmission
- Widely applicable
 - hypo- & holo-endemic
 - urban & rural
 - indoor & outdoor biters
 - strong & weak health systems
 - stable & unstable states
 - communities easy & difficult to access
 - 1st mile, last mile & preventing reintroductions after local elimination
- Area-wide & regional control; egalitarian
- Long-lasting
- Compatible with and helpful to other interventions; no cross-resistance
 - ITNs, IRS, MDA, vaccines
- Safe
- Inexpensive; easy to deliver; no need for behavioral change

MM cost-effective at a range of efficacies and gross costs, compared with existing and potential new interventions

Comparable interventions



MM \$/DALY by gross intervention cost and effect



Note: All costs and DALYs discounted at 3% annually to year 0
Source: BCG analysis

Cost Effectiveness 19 October 2010 v4.ppt

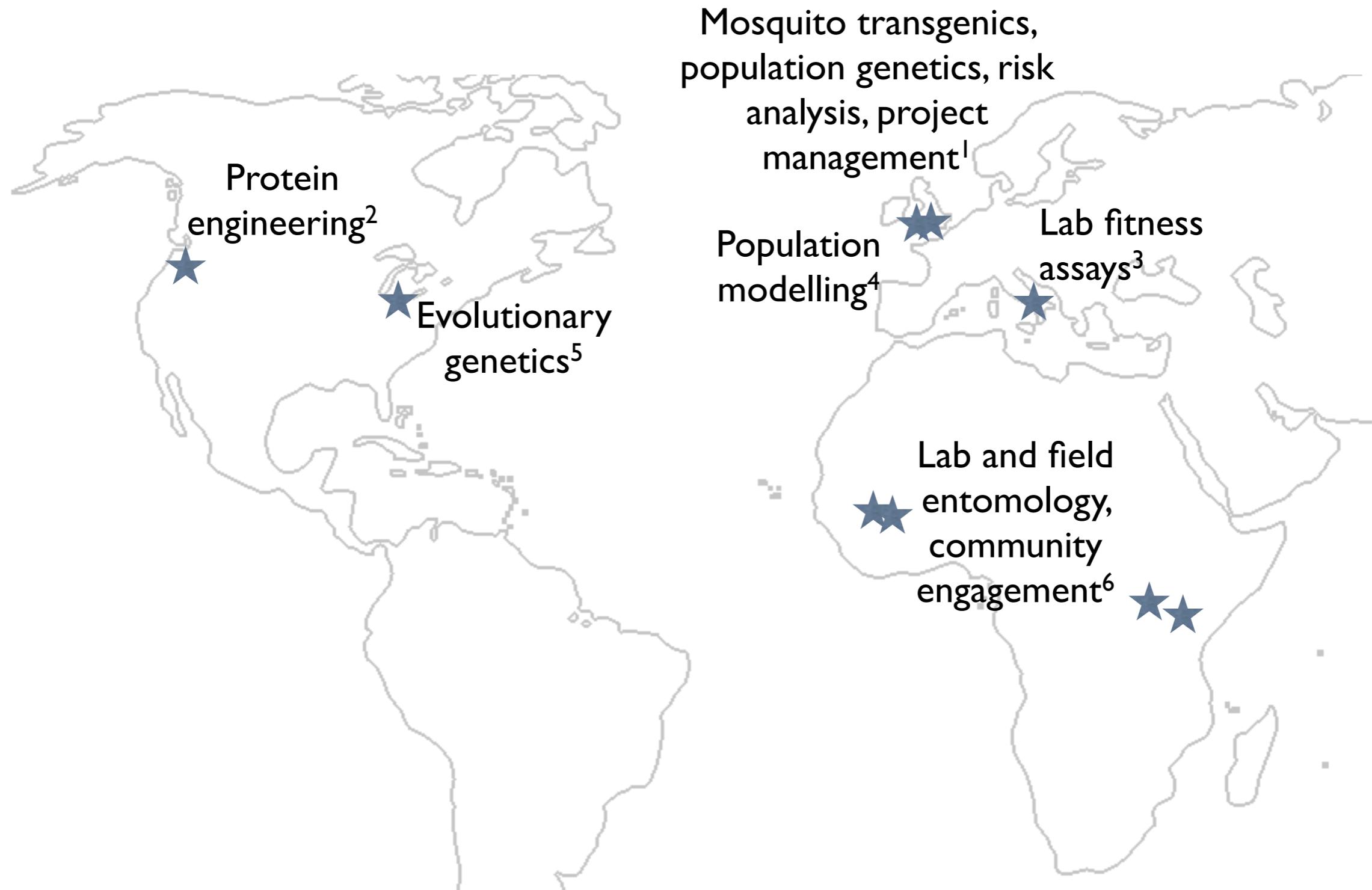


A Vector Control Research Alliance

Target Malaria is:

- A not-for-profit network of researchers
- Funded by the Foundation for the National Institutes of Health (FNIH) through a program of the Bill & Melinda Gates Foundation (BMGF)
- Working to develop new methods to suppress the mosquito vectors of malaria using enzymes that cleave specific DNA sequences

Target Malaria: a diverse international research team



¹ Imperial College London

² FHCRC, Seattle Children's Hospital, Pregnen, University of Washington

³ University of Perugia, Polo d'Innovazione di Genomica, Gentica e Biologia

⁴ University of Oxford

⁵ University of Notre Dame

⁶ University of Bamako, IRSS (Burkina Faso), ICIPE (Kenya), UVRI (Uganda)

¹ Austin Burt, Andrea Crisanti, John Mumford

² Barry Stoddard, Andy Scharenberg, Jordan Jarjour

³ Mark Benedict

⁴ Charles Godfray

⁵ Nora Besansky

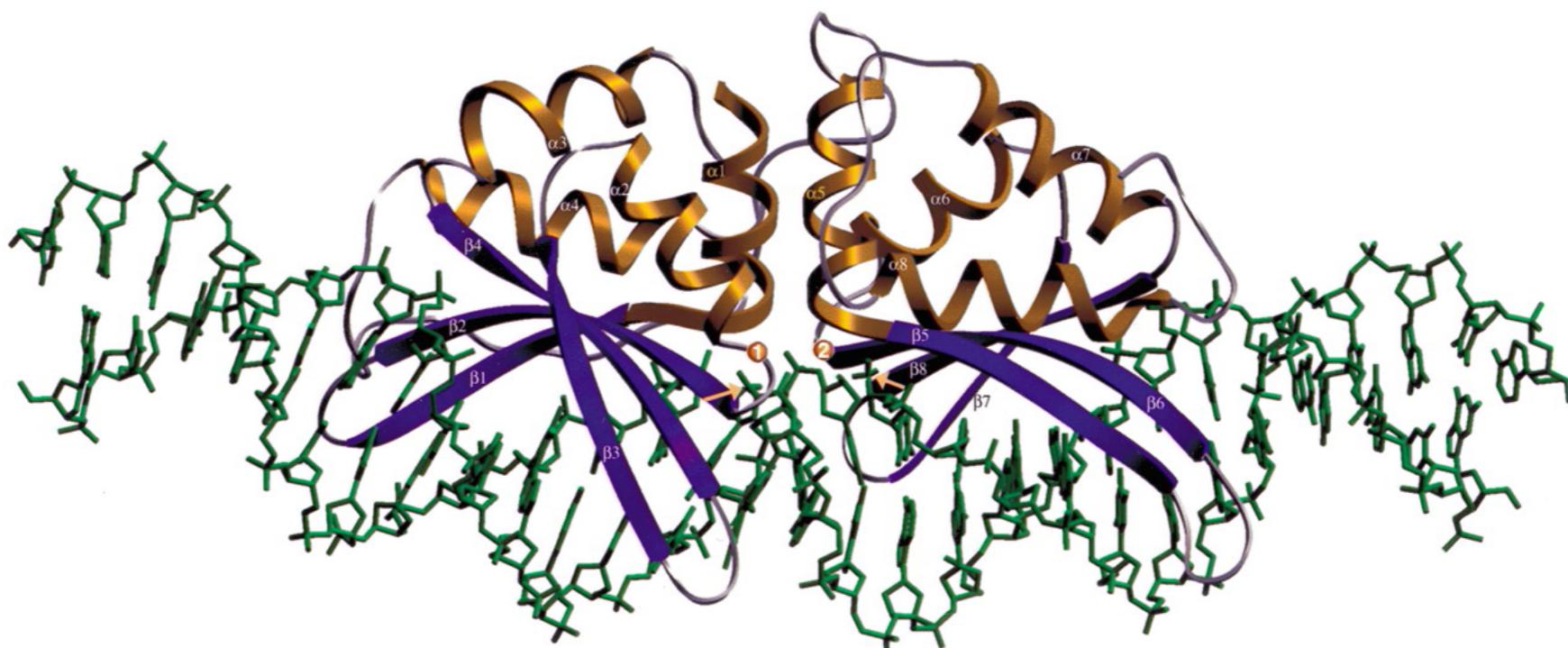
⁶ Mamadou Coulibaly, Abdoulaye Diabate, Richard Mukabana, Daniel Masiga, Jonathan Kayondo

Outline

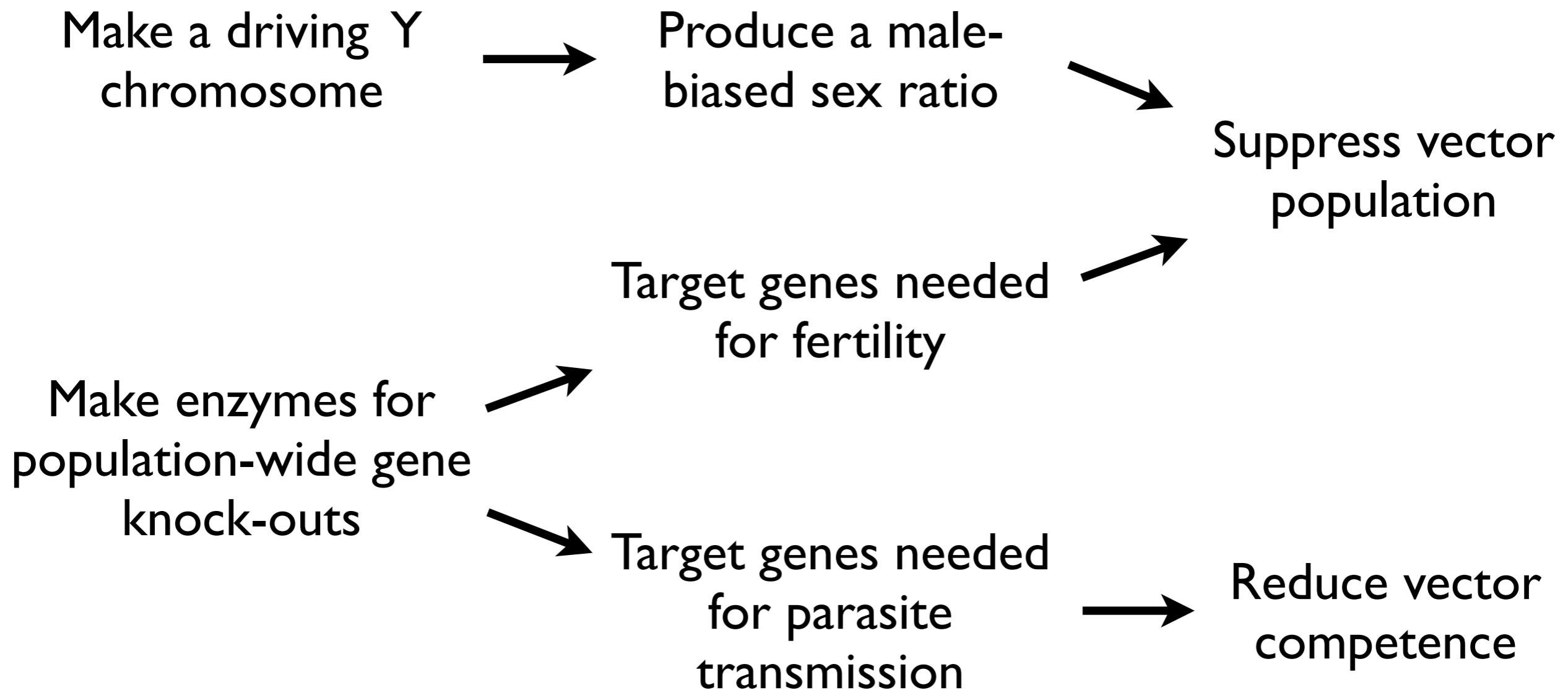
- The problem and a potential new approach
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Our approach uses homing endonuclease genes (HEGs)

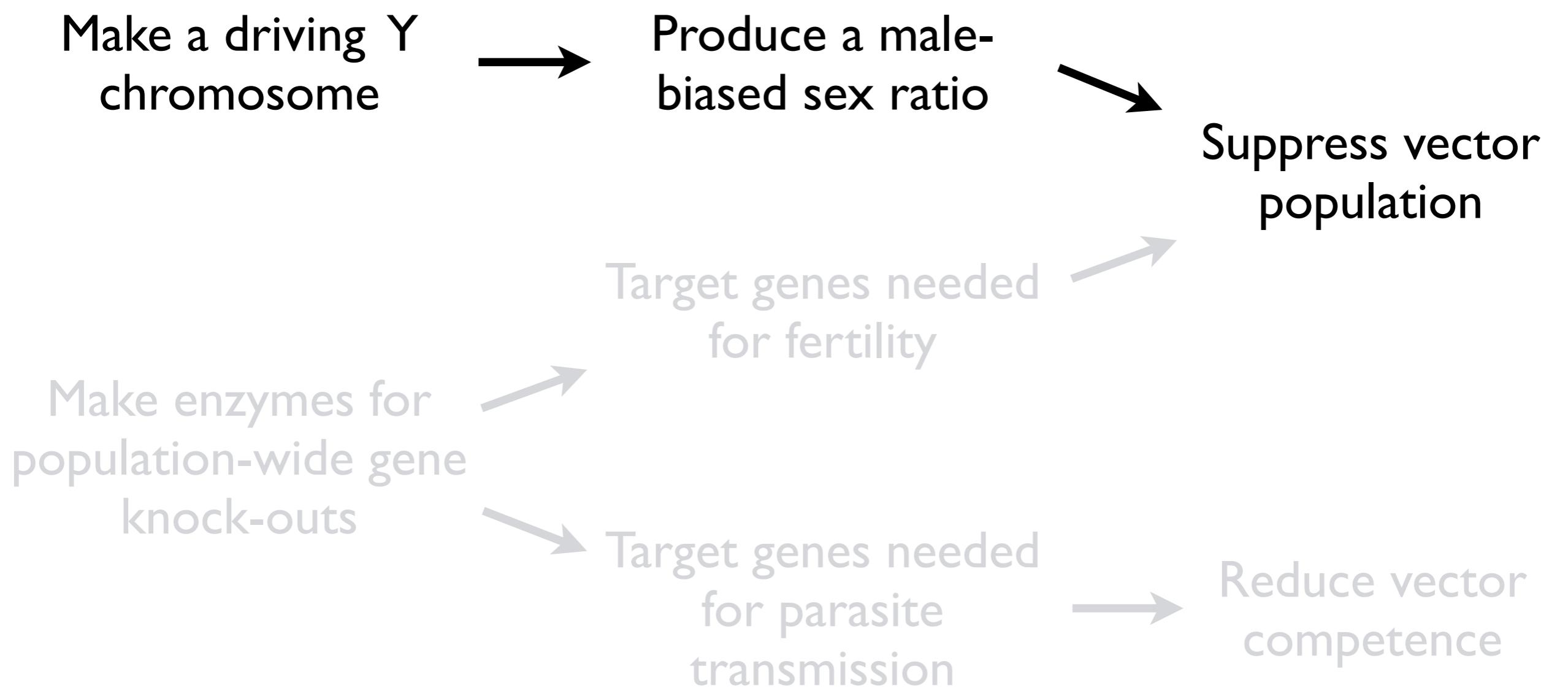
- Gene drive systems found naturally in fungi, algae, protists, viruses; not animals
- Encode enzymes that recognise and cleave a specific ~20bp DNA sequence
- Selfish/parasitic genes of no benefit to the host organism



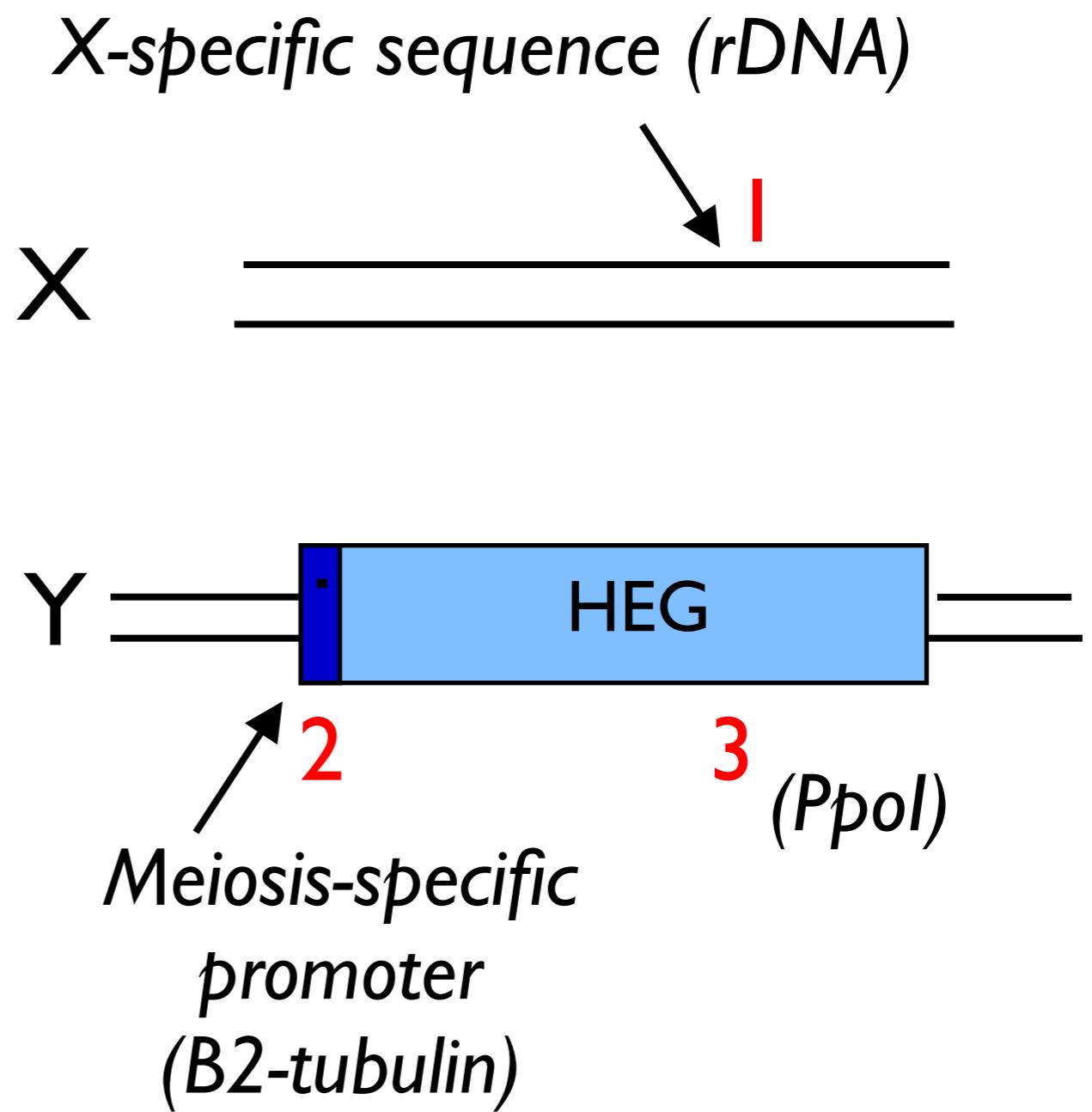
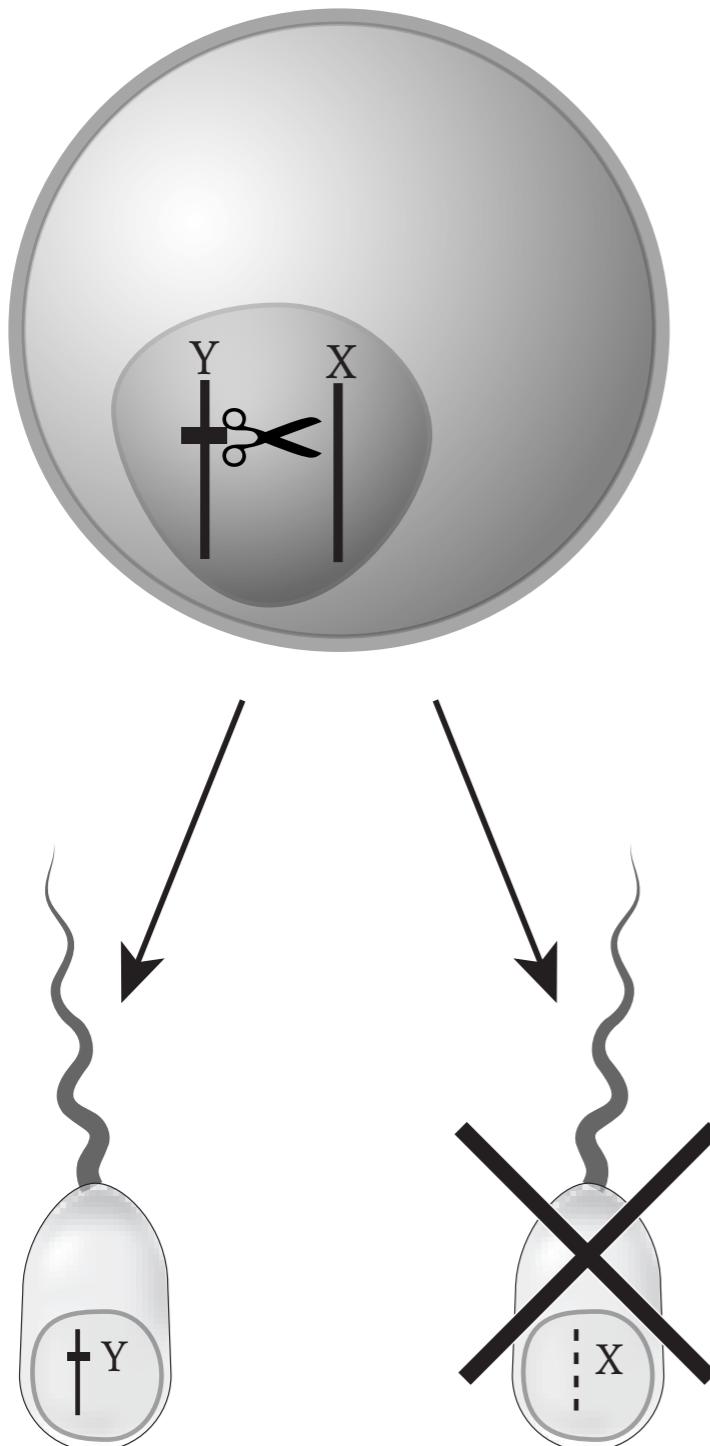
Multiple ways to use nucleases for malaria control



Driving Y

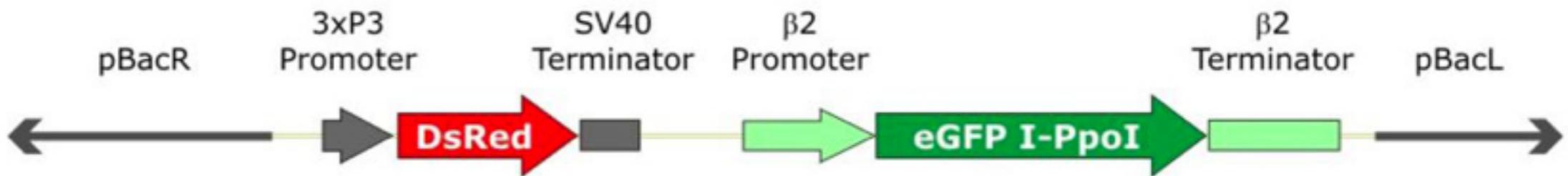


Driving Y chromosome



β 2-Ppol

construct:



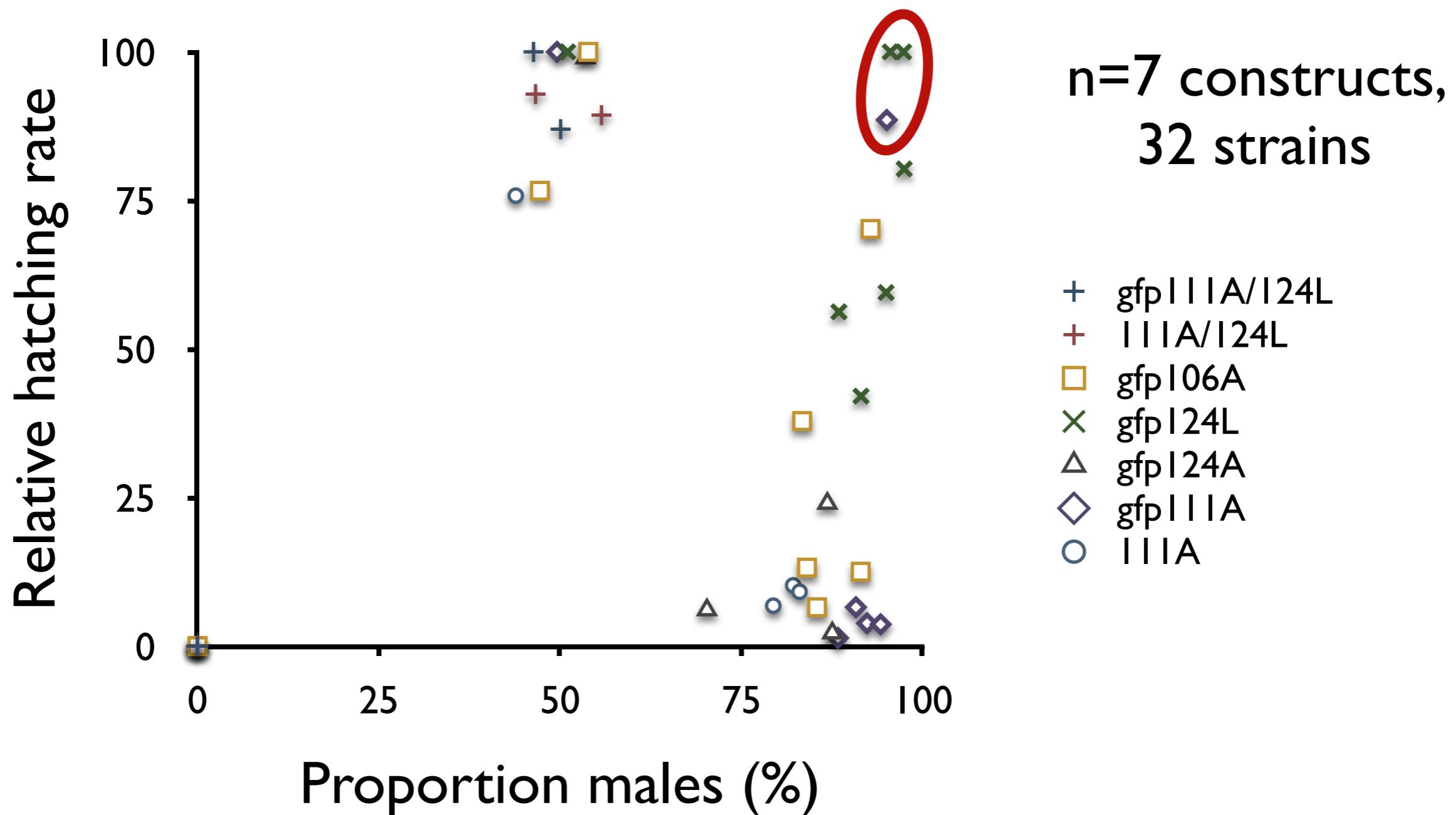
1st time we tried it: all zygotes die

Cross (25 x 25)	Eggs	Larvae
Ppol ♀ x wt ♂	2542	1747
Ppol ♂ x wt ♀	2640	0

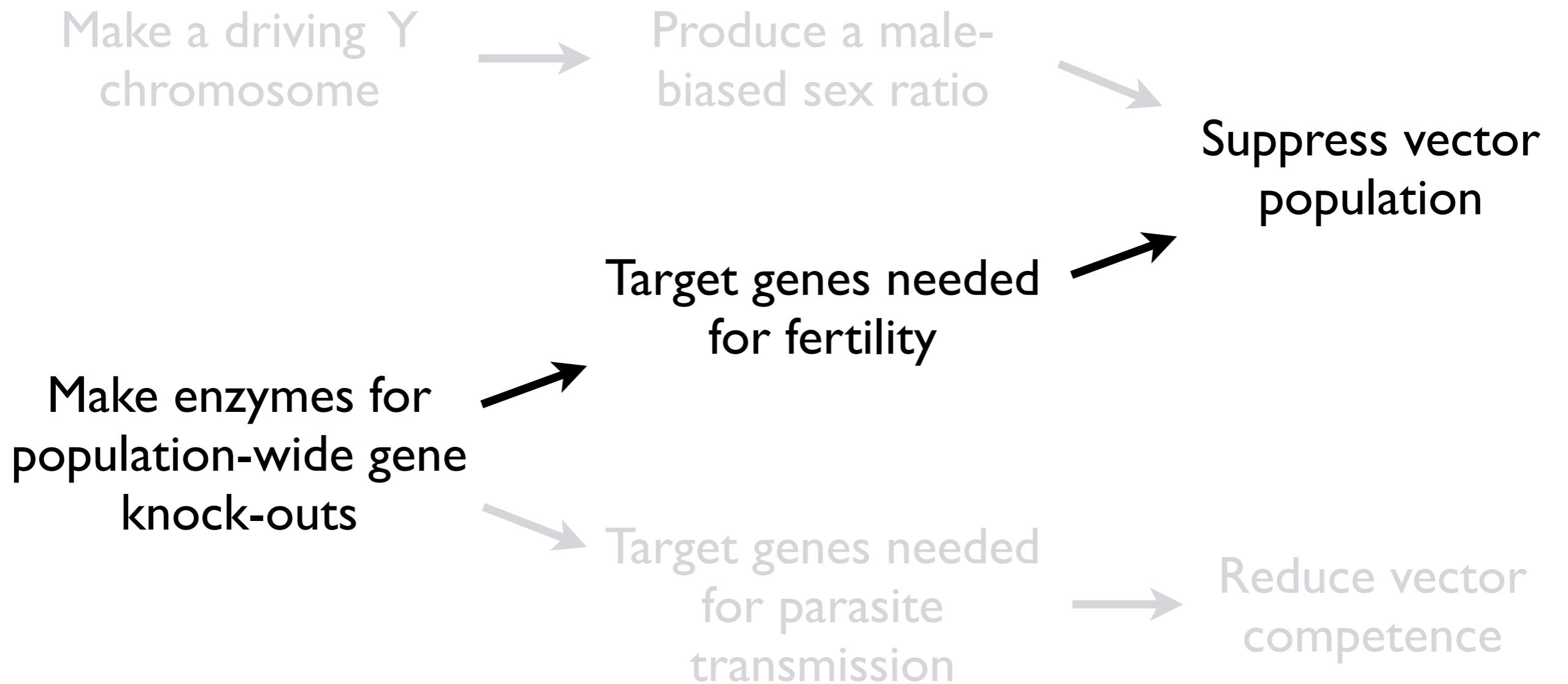
Male-sterile line

Proof-of-principle: sex-ratio distortion in *Anopheles*

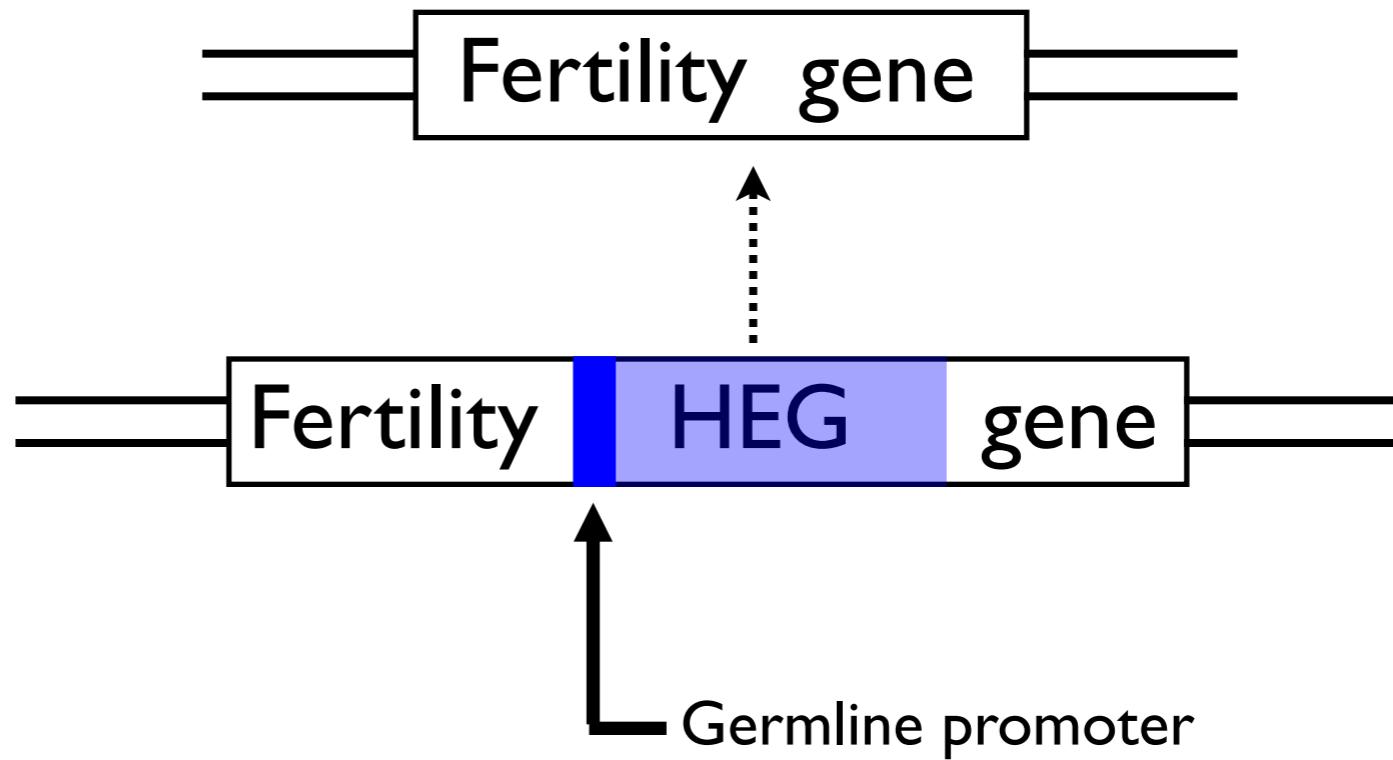
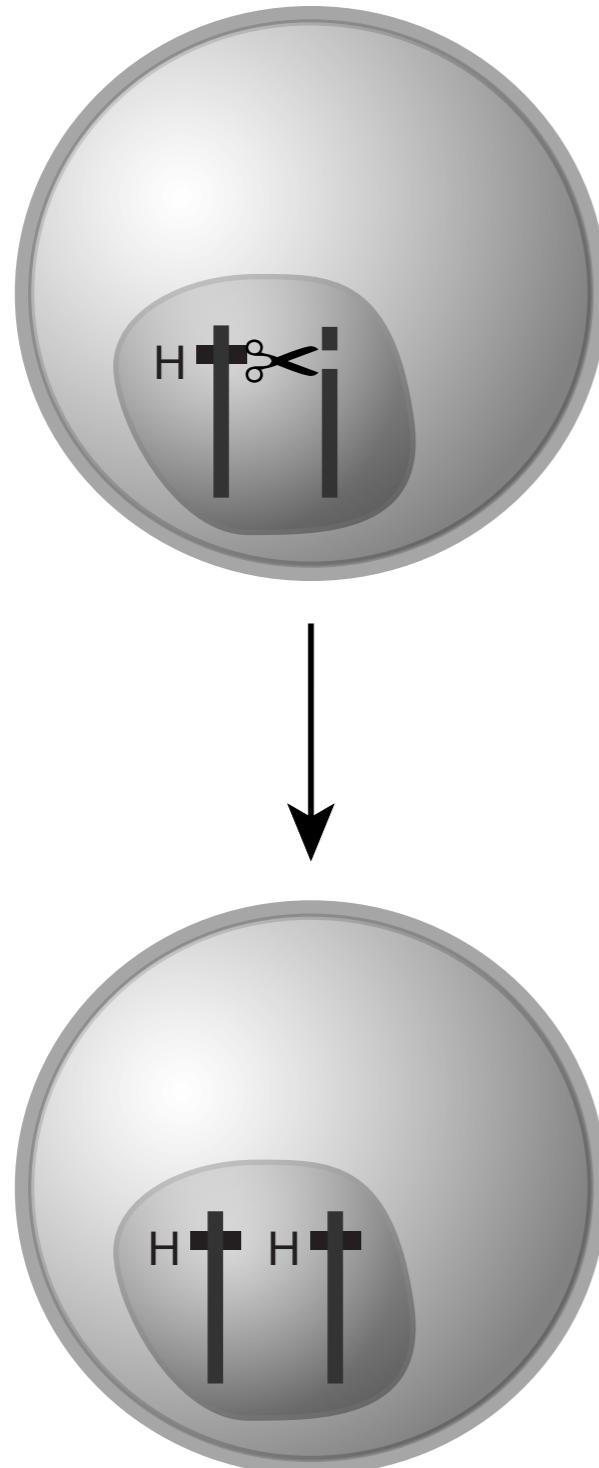
After 7 more tries:



Targeting female fertility



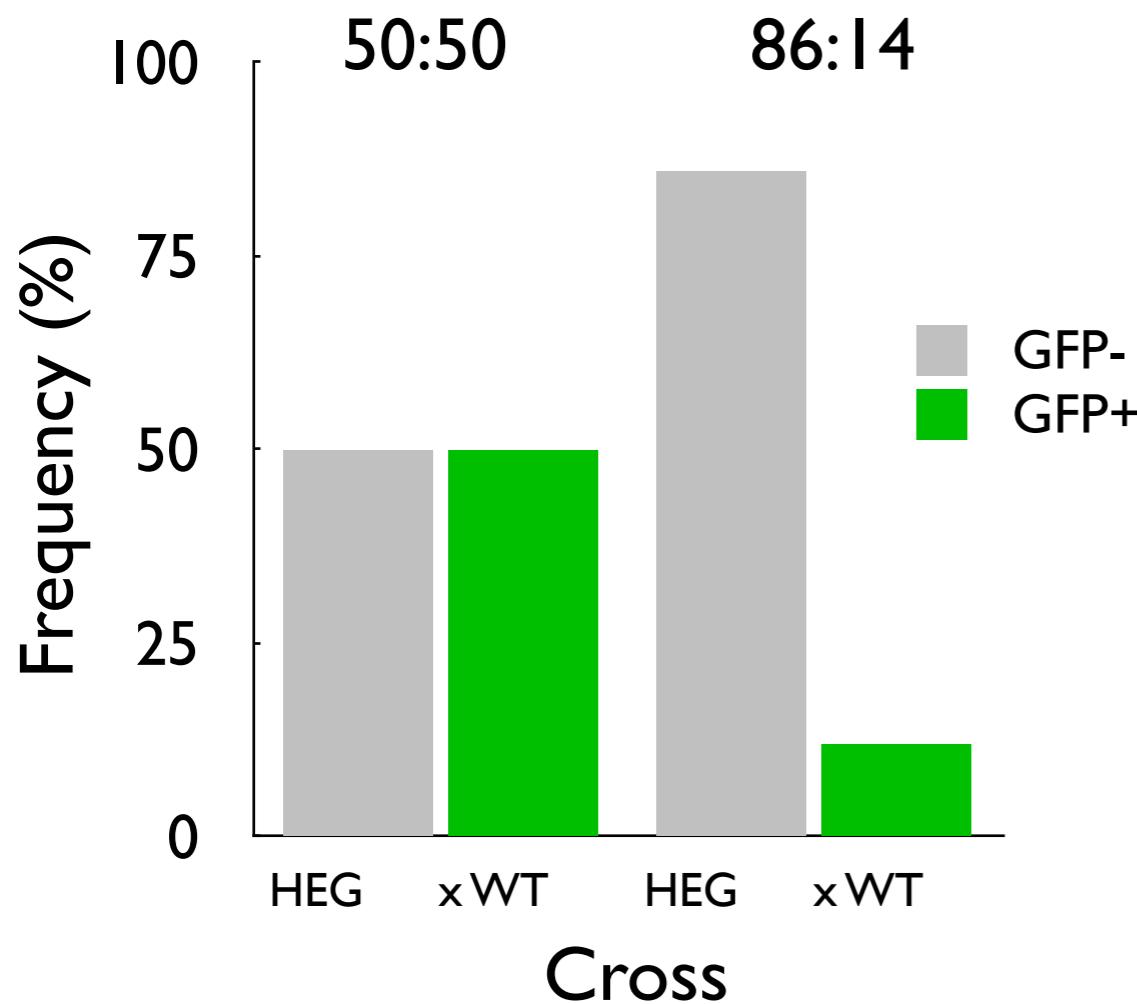
Enzymes for population-wide gene knock-outs



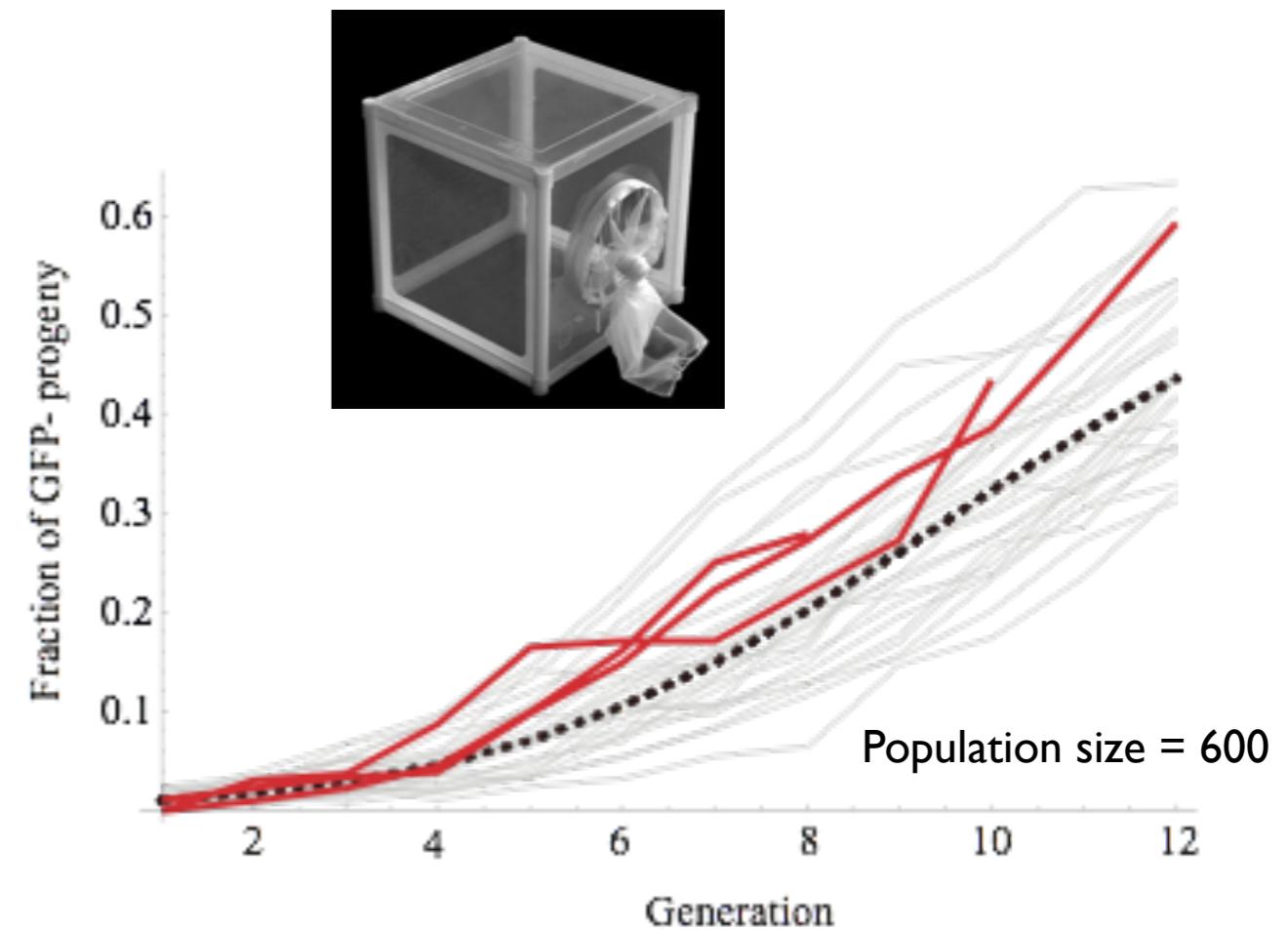
HEG will cause population-wide knock-out of target gene

Proof-of-principle: Homing in *Anopheles*

Preferential inheritance

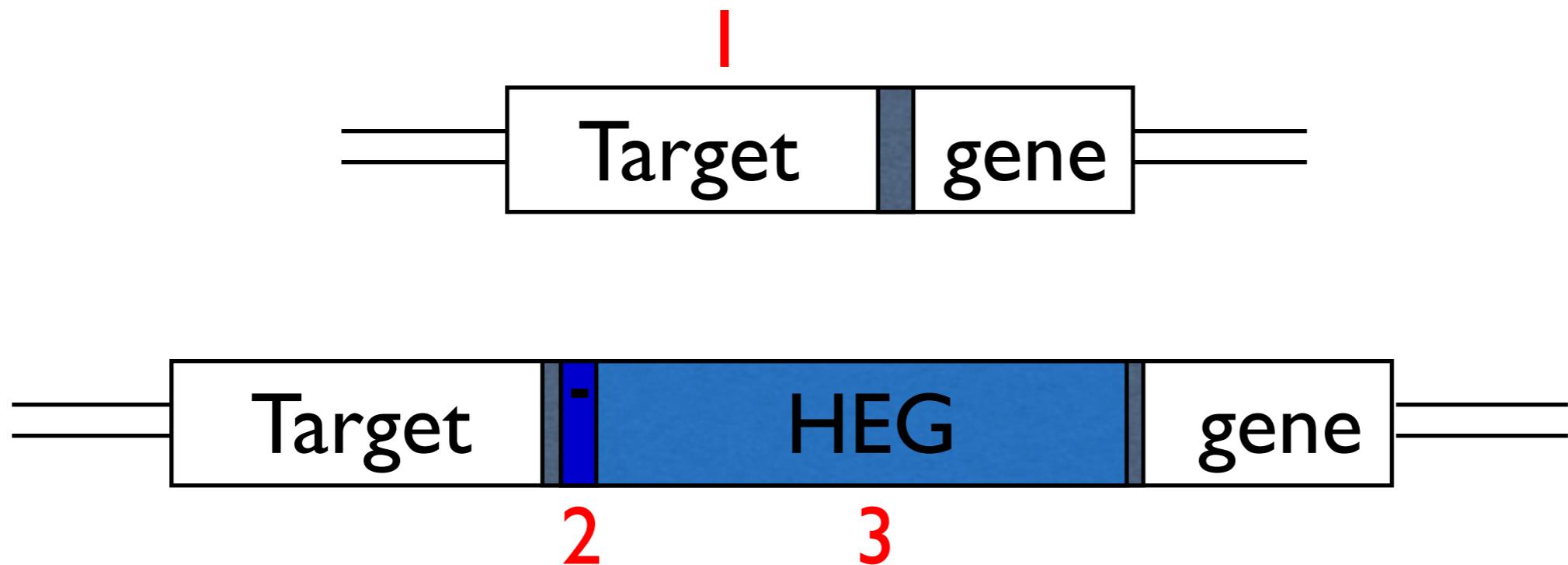


Spread through populations



Experiments using a HEG and its recognition sequence from yeast
Together with *Drosophila*, 1st demonstrations of
homing in any animal

Population-wide gene knockouts



Summary & conclusions (I)

- New methods of malaria control are needed
- We developing self-sustaining constructs that will suppress vector populations after small inoculative releases, using enzymes that target specific DNA sequences
- We have demonstrated important proofs-of-principle in the lab:
 - Males that produce ~95% male progeny
 - HEG transmission rates of ~80% (compared to Mendelian 50%).
- Progress sufficiently promising to begin process of co-development with African collaborators and stakeholders.

Outline

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Bringing modified mosquitoes to Africa

Background and overview

- First transgenic *Anopheles* in 2000 (Imperial College)
- Transgenic mosquitoes have not yet been transferred to nor created in an African country
- Transgenics are typically made using lab strains far removed from nature
- We do not want to wait till we have a driving construct to bring modified mosquitoes to African DECs, nor do we want that to be the 1st construct transferred
- Have designed a ‘first generation’ modified mosquito for co-learning exercises
 - Has fluorescent markers to allow monitoring of transgenics
 - Causes male mosquitoes to be sterile, so will not persist in the environment
 - Not intended to be a final product for malaria control
 - Goals are to transfer knowledge and build capacity in Africa, and gain relevant regulatory, social, and biological knowledge to guide future developments

DNA construct inserted onto mosquito chromosome

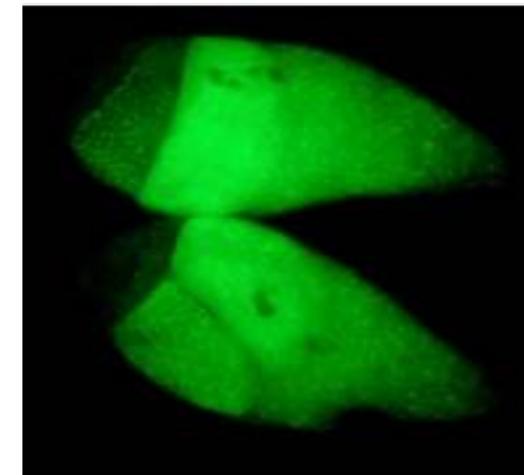


- Inclusion of fluorescent markers allows transgenic males and females to be visually distinguished from wild-type individuals

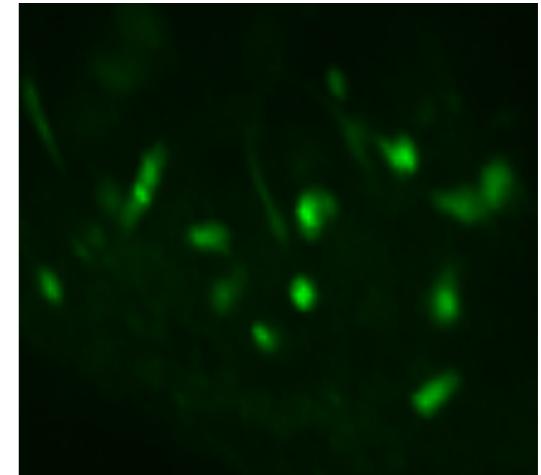
Larval & adult eyes
glow red



Adult testes glow
green



Sperm
glow green



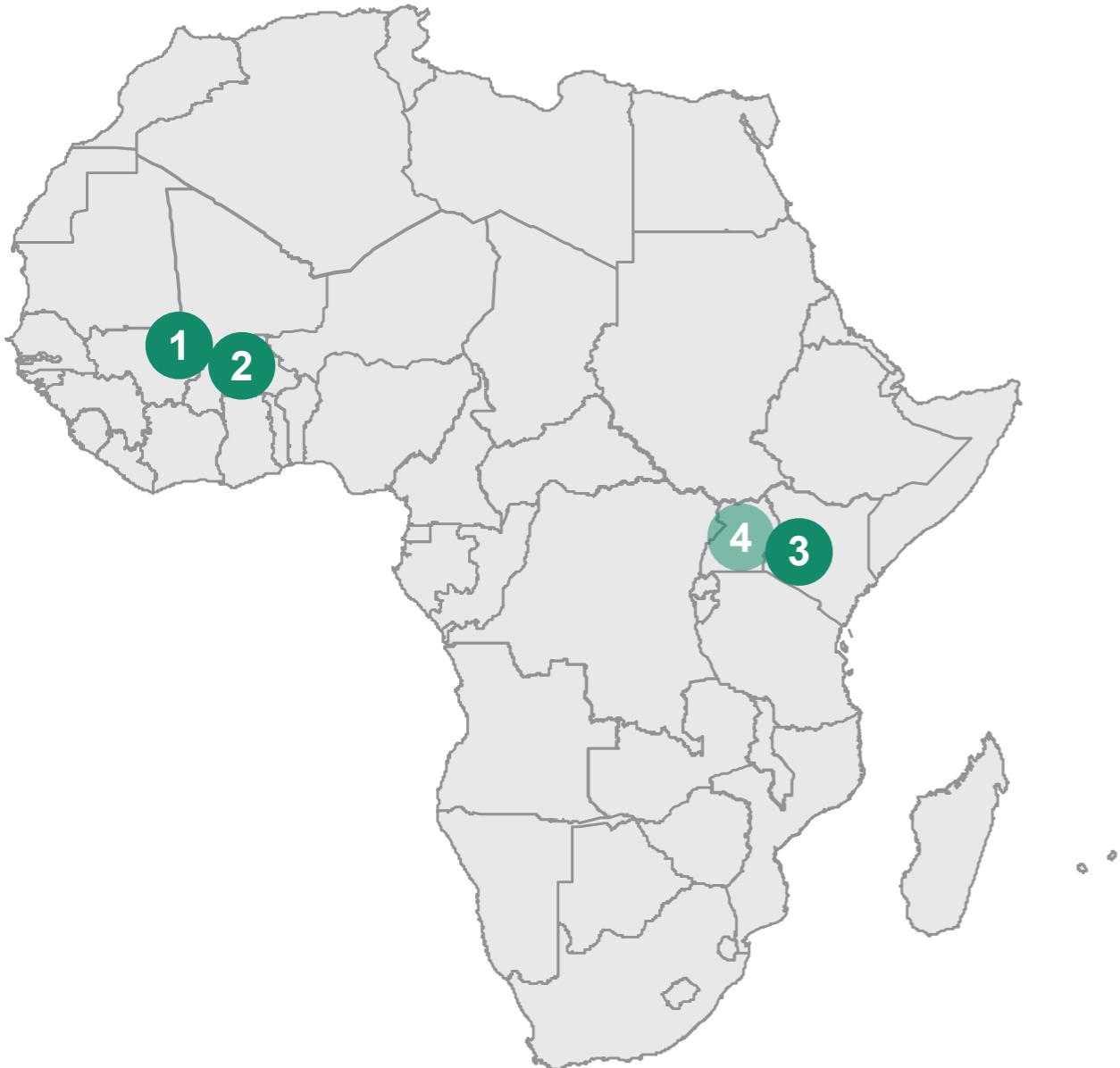
Enzyme used for creation of a sterile male line: I-Ppol

- I-Ppol is a naturally-occurring enzyme from the slime mold *Physarum polycephalum*
- It recognises and cuts a DNA sequence that is repeated hundreds of times on the mosquito X chromosome (in the rDNA repeat), destroying or “shredding” the X chromosome
- Because enzyme is transmitted by sperm to embryo, males are effectively sterile (have no surviving progeny)

Slime-mold *Physarum polycephalum*



Collaborating institutions in Africa



1 Malaria Research and Training Center Entomology
• Bamako, Mali



2 Institut de Recherche en Sciences de la Santé
• Ouagadougou, Burkina Faso



3 International Centre of Insect Physiology and Ecology (icipe)
• Nairobi, Kenya



4 Mosquito Research Programme, Virus Research Institute
• Entebbe, Uganda



All countries have:

- Significant malaria burdens
- Strong in-country entomological expertise that wants to develop the technology
- A legal framework for GMMs
- Experience running regulated clinical trials

1st generation modified mosquitoes in Africa

Preparatory activities in progress

- Laying biosafety groundwork
 - Establishing facilities for physical containment
 - Developing SOPs for risk management
 - Training personnel
 - Independent risk assessment
- Regulatory engagement
- Community engagement, communications

Field sites also being identified, and baseline data collected, for future open releases (subject to regulatory permission and stakeholder acceptability)

Insectaries under construction / renovation



Burkina Faso



Mali



Kenya

Laboratory experiments in Africa

Experiments to be performed once strains are imported into DECs include:

- Transfer transgene into local genetic background
- Verification of fluorescent markers
- Verification of male mosquito sterility
- Measure effect of transgene on larval development and male mating competitiveness

Summary & conclusions (2)

- We have developed '1st generation' strain to begin bringing modified mosquito technology to disease-endemic countries
 - Has fluorescent markers to aid monitoring
 - Makes male mosquitoes sterile, so will not persist in environment
- We are using this strain to drive investment in infrastructure, SOP development and training suitable for GEMs
- Also participating in independent risk assessment by CSIRO
- Separately, we are participating in co-learning activities with regulators
- We hope to be in a position to submit applications for importation of 1st generation GEM in Q2 2015



HEG Consortium-PTM- 2nd to 9th november 2013 BURKINA FASO