genetic control of the malaria mosquito using gene drive

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Malaria: the problem

The burden:
More than 200 million infections & half million deaths each year, ~90% in Africa, mostly the poor, mostly infants & children
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
In Africa most transmission is by 3 closely related species (*An. gambiae, An. coluzzii* and *An. arabiensis*), plus *An. funestus*
There are ~3500 species of mosquito, the vast majority of which do not transmit malaria
Other species can be important in specific locations
Only female mosquitoes bite and transmit the parasite
Current methods of control are good but not sufficient

Insecticide Treated Nets, Indoor Residual Spraying, Artemisinin-based Combined Treatments have reduced mortality rates, saving millions of lives, but not enough to eliminate the disease. Drug- and insecticide-resistance mean recent progress could be reversed.

$5.1B/yr currently required for malaria control, more than the amount available.

Additional cost-effective & sustainable vector control methods are needed.
what is genetic control?

Population suppression

Population replacement

gene drive can be used for both approaches
what is gene drive?

INDIVIDUAL LEVEL

Inheritance of allele (+)

<table>
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<tr>
<th>+</th>
<th>×</th>
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<tbody>
<tr>
<td>+</td>
<td>50%</td>
<td>50%</td>
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<tr>
<td>−</td>
<td>sperm/eggs</td>
<td>organism</td>
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<tr>
<td>+</td>
<td>70%</td>
<td>30%</td>
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<td>−</td>
<td>sperm/eggs</td>
<td>gene drive</td>
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mendelian inheritance

gene drive
what is gene drive?

**INDIVIDUAL LEVEL**

Inheritance of allele (+)

<table>
<thead>
<tr>
<th>Mendelian Inheritance</th>
<th>Gene Drive</th>
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<tr>
<td>50% × 50% = 25%</td>
<td>70% × 30% = 21%</td>
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**POPULATION LEVEL**

Frequency of a rare allele (+) in a population

- Mendelian Inheritance: Frequency remains constant over generations.
- Gene Drive: Frequency increases over generations, even if the allele decreases fitness by 70%.

A driving gene can spread in a population (even if it decreases fitness).
what is gene drive?

Mendelian inheritance

Mosquito with 1 copy of altered gene without gene drive  
Wild type mosquito

Offspring have a 50% chance of inheriting the altered gene.

Gene drive inheritance

Mosquito with 1 copy of altered gene with gene drive  
Wild type mosquito

Gene drive converts the wild type gene to the altered gene. Offspring will almost always inherit the altered gene.

gene drive allows to spread a genetic modification into a population in an efficient way
gene drive of endonucleases

- naturally occurring “homing” endonucleases
- designed endonucleases (e.g. CRISPR/Cas9)
Homing Endonuclease Genes

Highly specific DNA endonucleases (cut DNA only at unique target sites)

DNA breaks are repaired using the HEG allele as template. Thus the HEG is copied from one chromosome to another.

Commonly found in microorganisms (introns of yeasts, fungi, protists). Do not occur in nuclear genomes of animals (animals have a segregated germline).
Homing Endonuclease Genes

DNA breaks are repaired using the HEG allele as template. Thus the HEG is copied from one chromosome to another.
HEG transmitted to a high proportion of the progeny

DNA breaks are repaired using the HEG allele as template. Thus the HEG is copied from one chromosome to another.
generating new endonuclease genes – CRISPR is a game changer

classic homing endonuclease

original target site
difficult to modify
months of work,
for a large team

mosquito target gene AGAP007280
generating new endonuclease genes – CRISPR is a game changer

Classic homing endonuclease

CRISPR/Cas9 endonuclease

very easy to modify!

original target site

mosquito target gene AGAP007280

TTTCCACTTATTCAACCTTTTA

difficult to modify

months of work, for a large team

CCTCCCTCACTTTCTTTCTCACC

CCTCCCTCACTTTCTTTCTCACC
generating new endonuclease genes – CRISPR is a game changer
gene drive to achieve population replacement
gene drive to achieve population replacement

an anti-parasite effector gene can be placed inside the gene drive construct

the anti-parasite gene will be active in a different tissue (e.g. the salivary glands) and will prevent the mosquito from transmitting the malaria parasite

the gene drive locus will over several generations drive itself and the anti-parasite gene into the population

the gene drive locus will spread to fixation until every individual in the population is a carrier of the anti-parasite gene
gene drive to achieve population suppression

gene drive targeting an essential recessive female fertility gene
(homozygote females are sterile)
gene drive in two population cages (targeting a single female fertility gene)
● to counter resistance many genes need to be targeted simultaneously
  ● the fear of the elimination of species is unfounded

gene drive in two population cages (targeting a single female fertility gene)
suppressive gene drive designed to target 3 major vectors

Worldwide, there are over 3,500 species of mosquitoes.

837 of those species are in Africa.

Targeting these mosquitoes can help save many of the 395,000 people who die from malaria in Africa every year, the vast majority of which are children.
summary

gene drive technology has been significantly boosted by the rise of CRISPR/Cas9 (but is not identical to it)

gene drive can be used to suppress mosquito populations or to render them unable to transmit disease

proof of principle implementations for both approaches have been demonstrated

not a silver bullet, must work alongside other interventions that are already having an impact (e.g. bednets, drugs)

working out legal/ethical/societal issues is currently lagging behind the scientific possibilities
Questions?
classic homing endonuclease

CRISPR/Cas9 endonuclease

DNA double strand break

NHEJ mutations

HOMING! (what we want)
HEG invades a new species by horizontal gene transfer.

HEG is homing in the new species.

HEG is increasing in frequency.

HEG has reached fixation – is now a gene without a function!

HEG pseudogene degeneration.
HEGs can recognize and cut sequence variants

**EcoRI**
(Restriction enzyme)

*HEG I-AniI* (bound to DNA)

it's experimentally established specificity profile

it's target site in the mitochondrial cobA gene

TGAGGAGGTTTCTCTGTAAA
HEGs can recognize and cut sequence variants

The specificity profile of some HEGs is significantly correlated to the reading frame of the host gene down to the position of individual basepairs at wobble vs. non-wobble positions in individual codons!
gene drive to achieve population suppression

targeting female fertility gene

inject into embryos

target gene disrupted

endonuclease transgene is in place!

targeting female fertility gene
gene drive to achieve population suppression

soma

germline (ovaries/testes)

cleavage & repair

progeny

expectation if endonuclease inactive

observed (AGAP007280 target gene)

gene drive!

50% GFP + 50% GFP-
mendelian inheritance

% GFP+ progeny