genetic control of the malaria mosquito using gene drive

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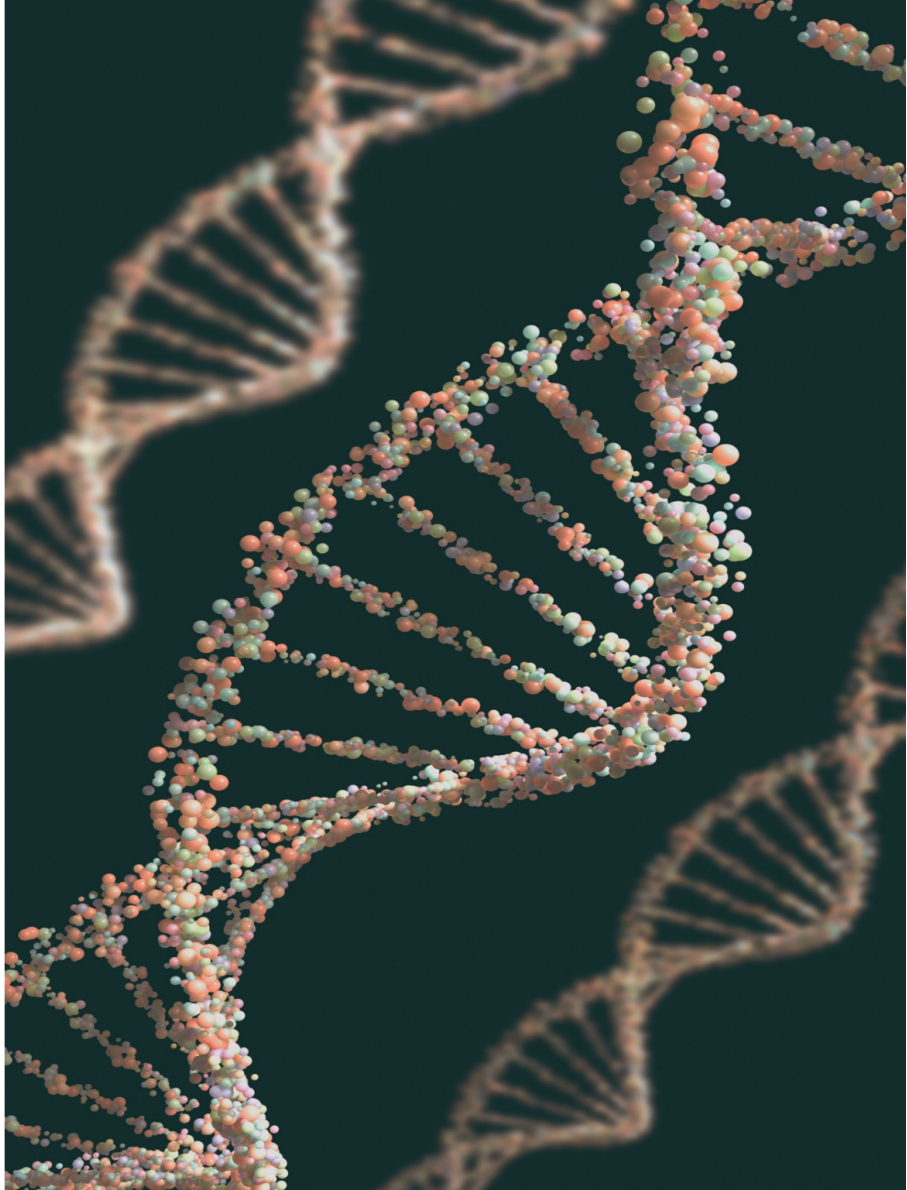


Malaria: the problem

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

Malaria: current interventions

Current methods of control are good but not sufficient

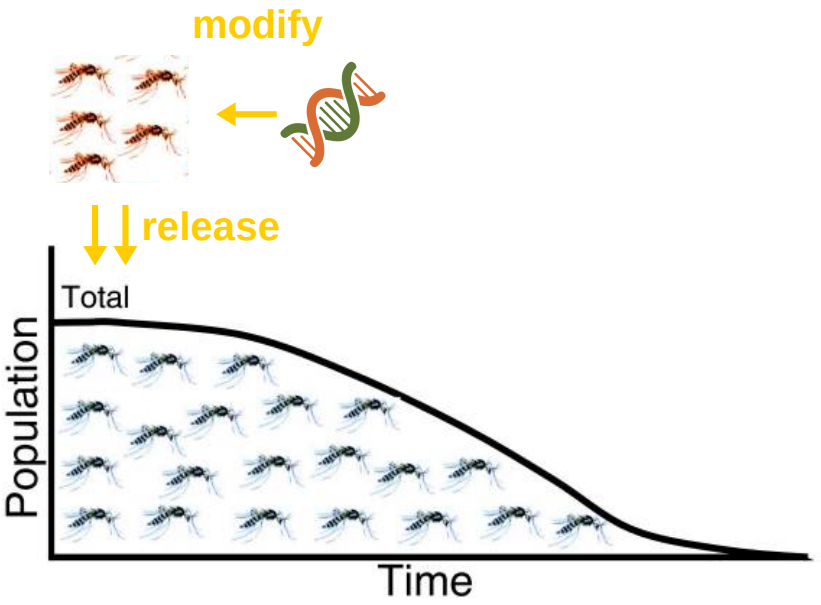
Insecticide Treated Nets, Indoor Residual Spraying, Artemisinin-based Combined Treatments have reduced mortality rates, saving millions of lives, but not enough to eliminate the disease

Drug- and insecticide-resistance mean recent progress could be reversed

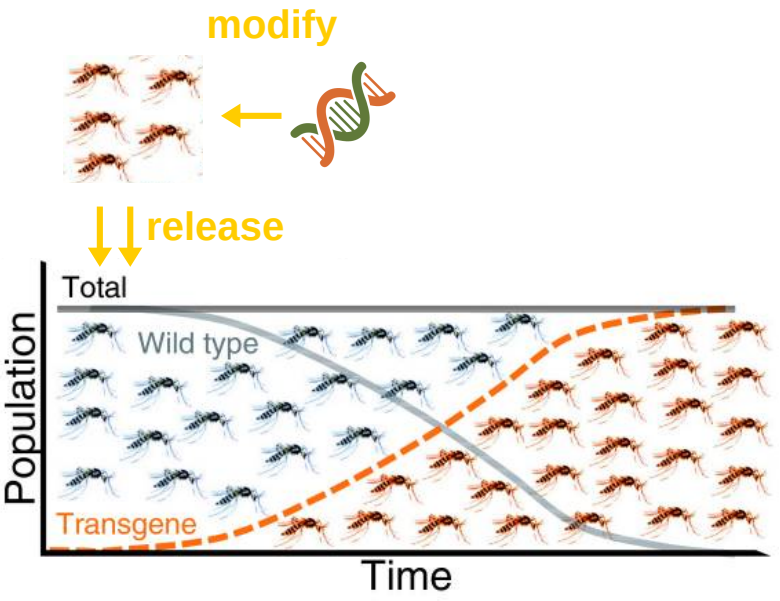
$5.1B/yr currently required for malaria control, more than the amount available

Additional cost-effective & sustainable vector control methods are needed

what is genetic control?



Population suppression



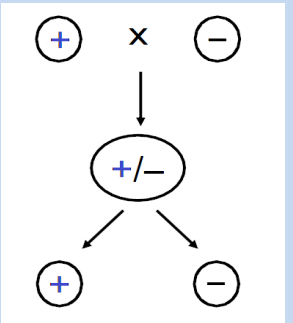
Population replacement

gene drive can be used for both approaches

what is gene drive?

INDIVIDUAL LEVEL

Inheritance of allele (+)



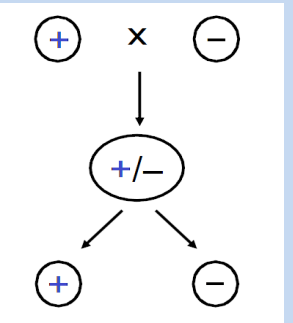
50% 50%

mendelian inheritance

sperm/eggs

organism

sperm/eggs



70% 30%

gene drive

what is gene drive?

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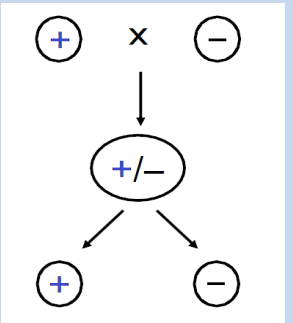

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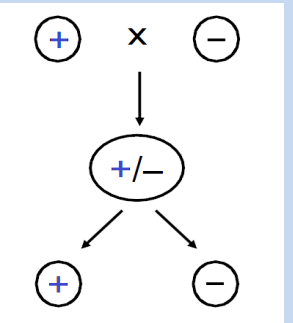
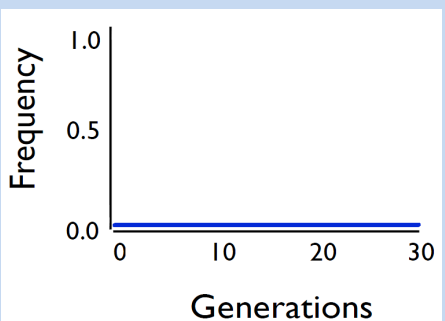


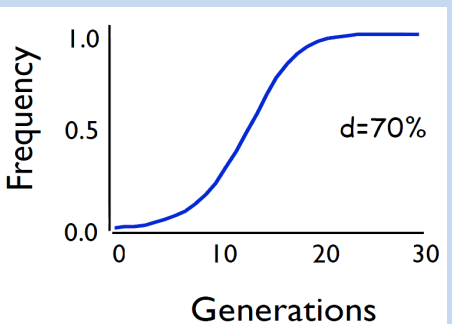
70% 30%

gene drive

POPULATION LEVEL

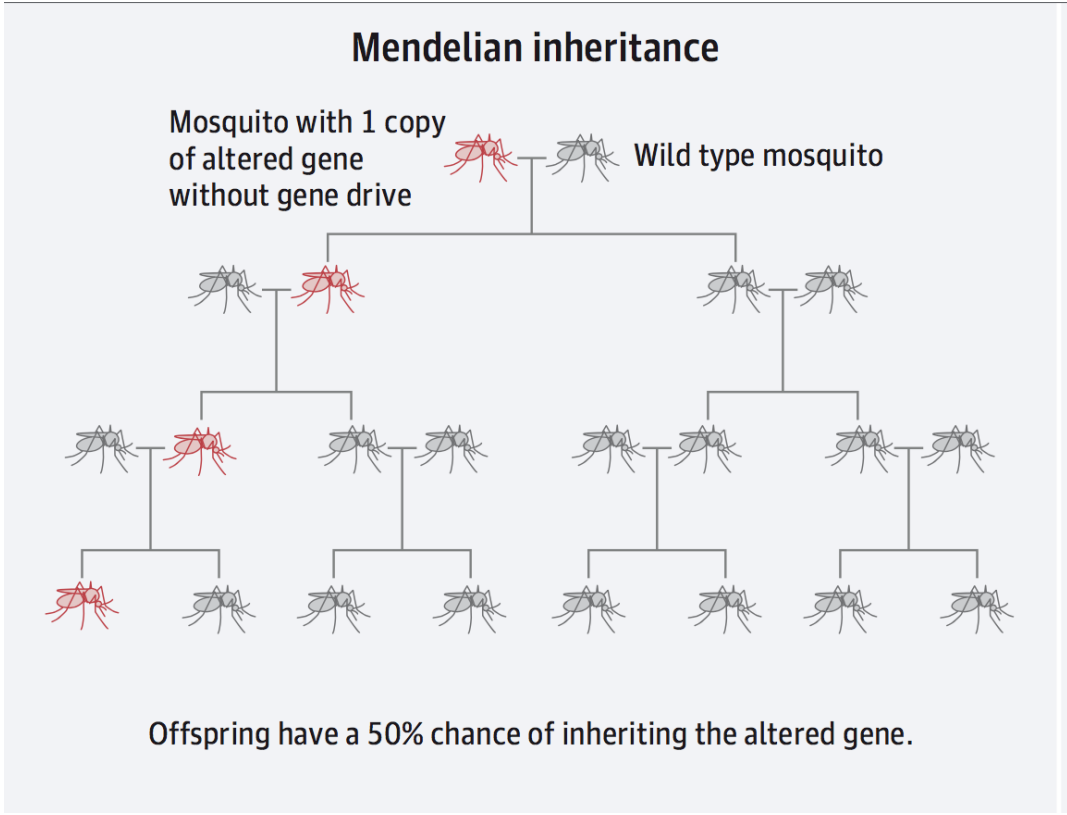
Frequency of a rare allele (+) in a population

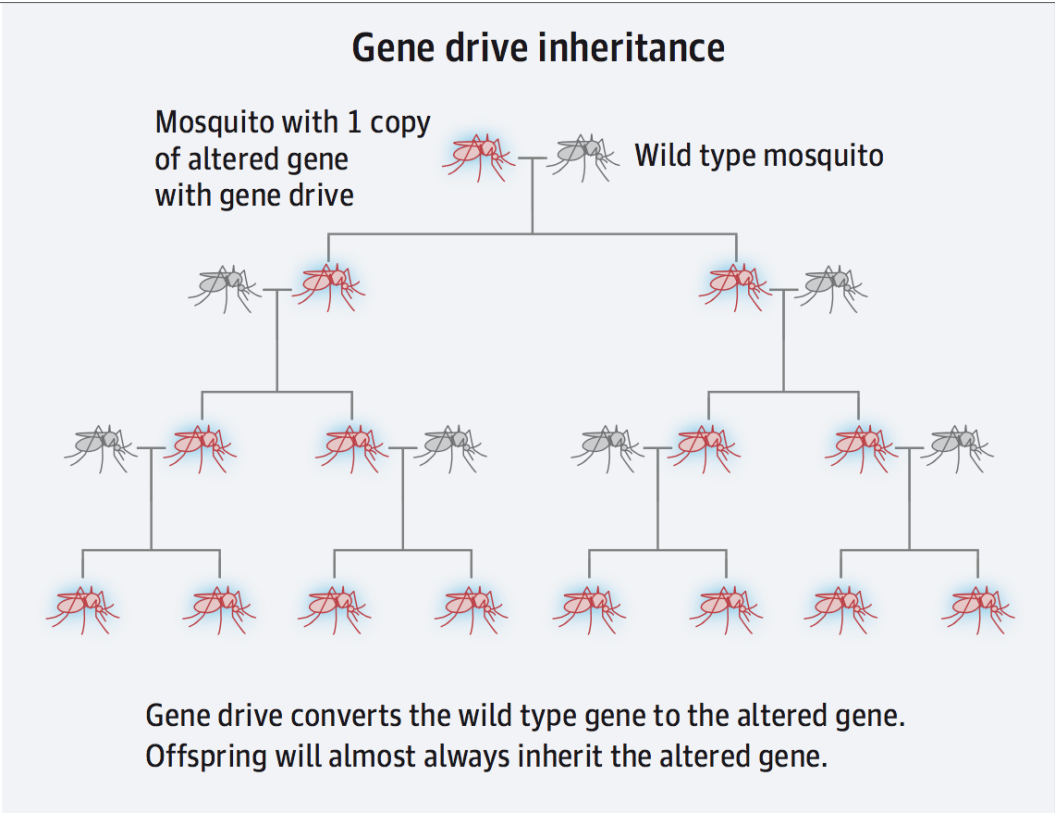




a driving gene can spread in a population (even if it decreases fitness)

what is gene drive?





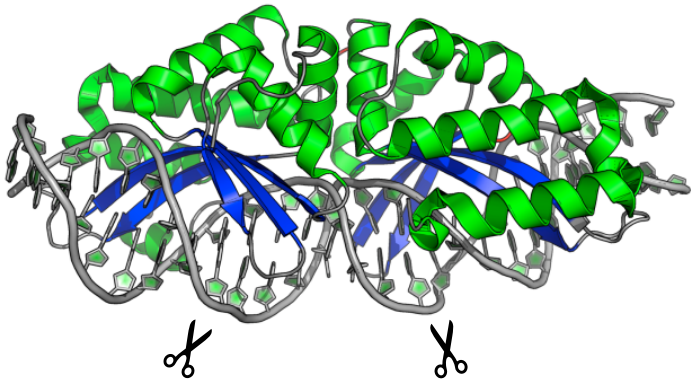
gene drive allows to spread a genetic modification into a population in an efficient way

gene drive of endonucleases

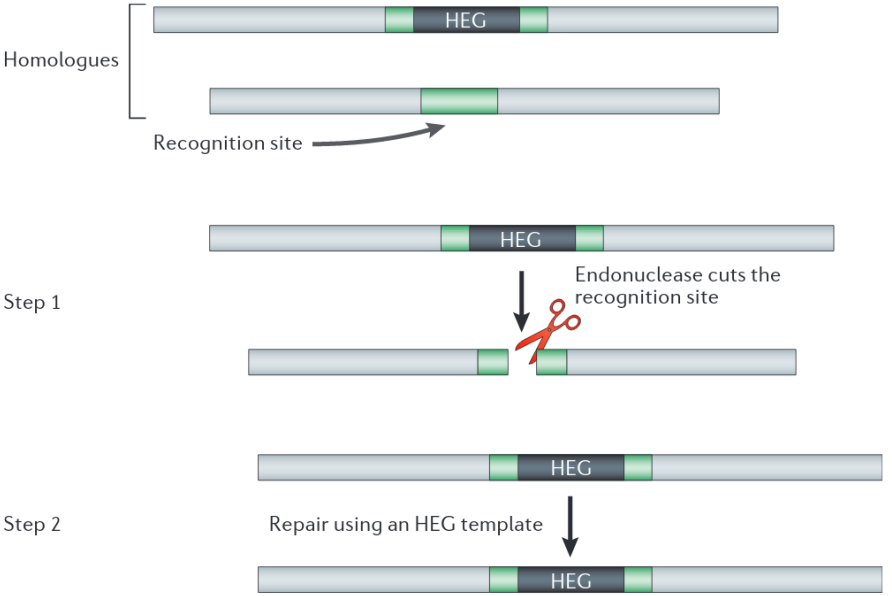
- naturally occurring “homing” endonucleases

- designed endonucleases (e.g. CRISPR/Cas9)

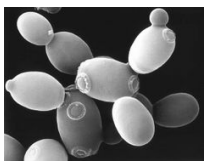
Homing Endonuclease Genes



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

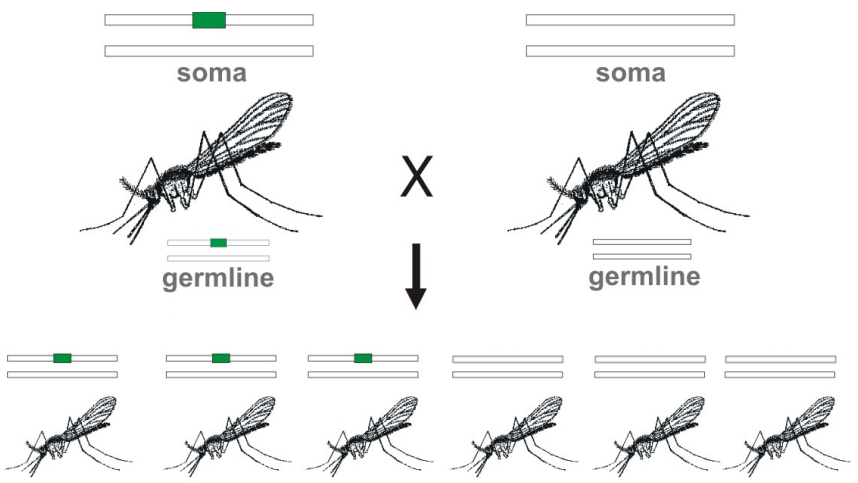


DNA breaks are repaired using the HEG allele as template
Thus the HEG is copied from one chromosome to another

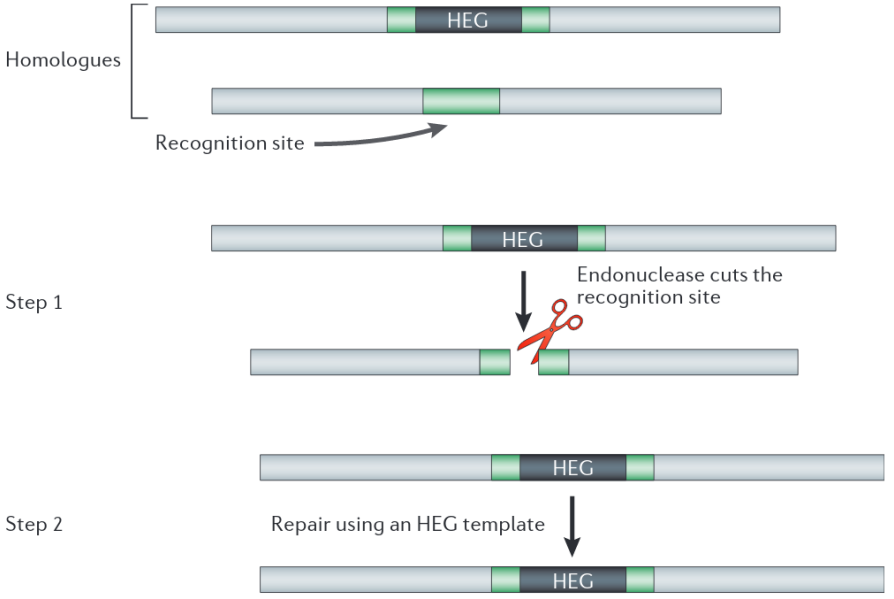


Commonly found in microogranisms (introns of yeasts, fungi, protists)
Do not occur in nuclear genomes of animals (animals have a segregated germline)

Homing Endonuclease Genes



Mendelian inheritance



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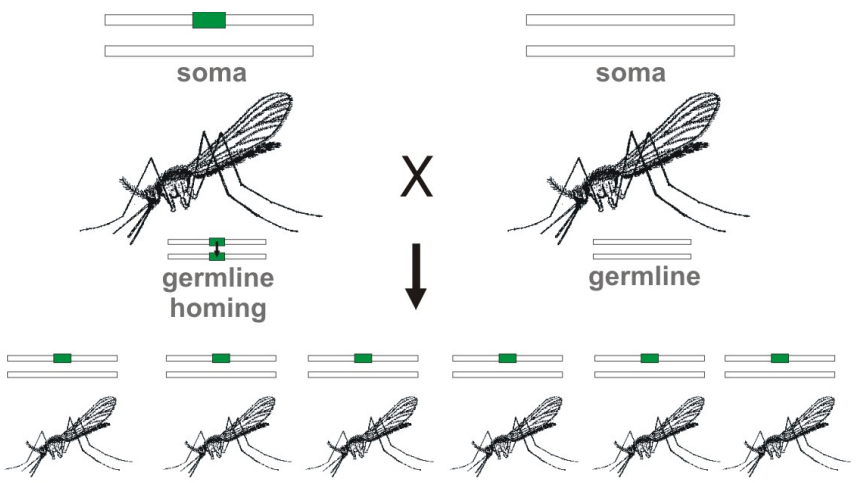
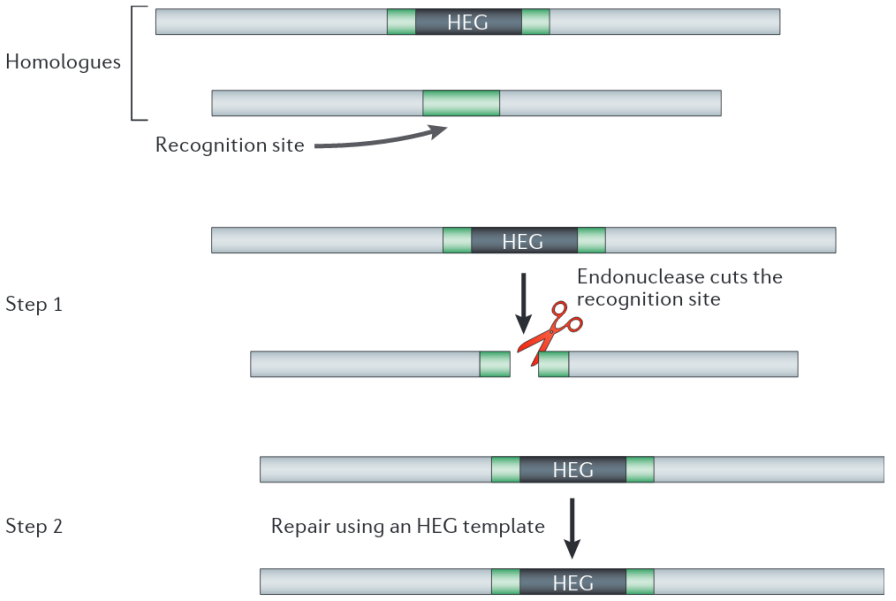


HEG transmitted to a high proportion of the progeny



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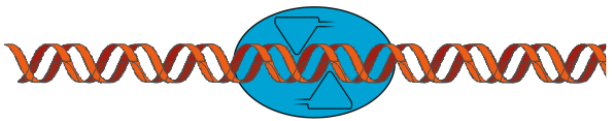
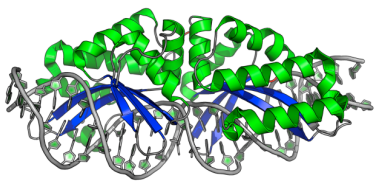
generating new endonuclease genes – CRISPR is a game changer

classic homing endonuclease

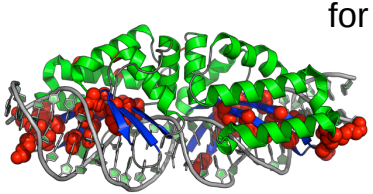




TTTCCACTTATTCAACCTTTTA

original target site

difficult to modify
months of work,
for a large team

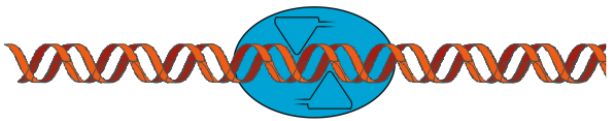


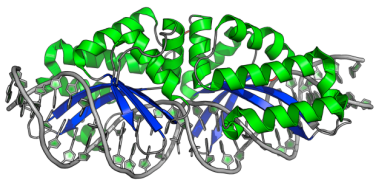
CCTCCTCACTTTCTTCCTCACC

mosquito target gene AGAP007280

generating new endonuclease genes – CRISPR is a game changer

classic homing endonuclease





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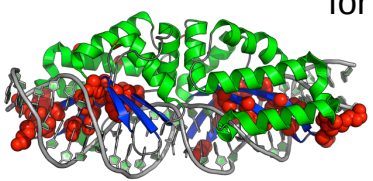

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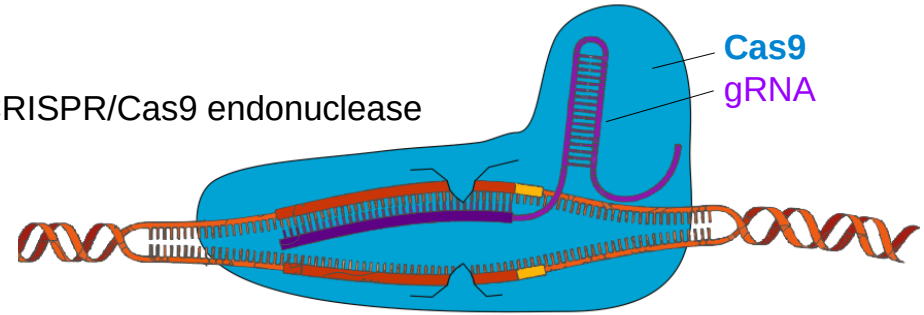
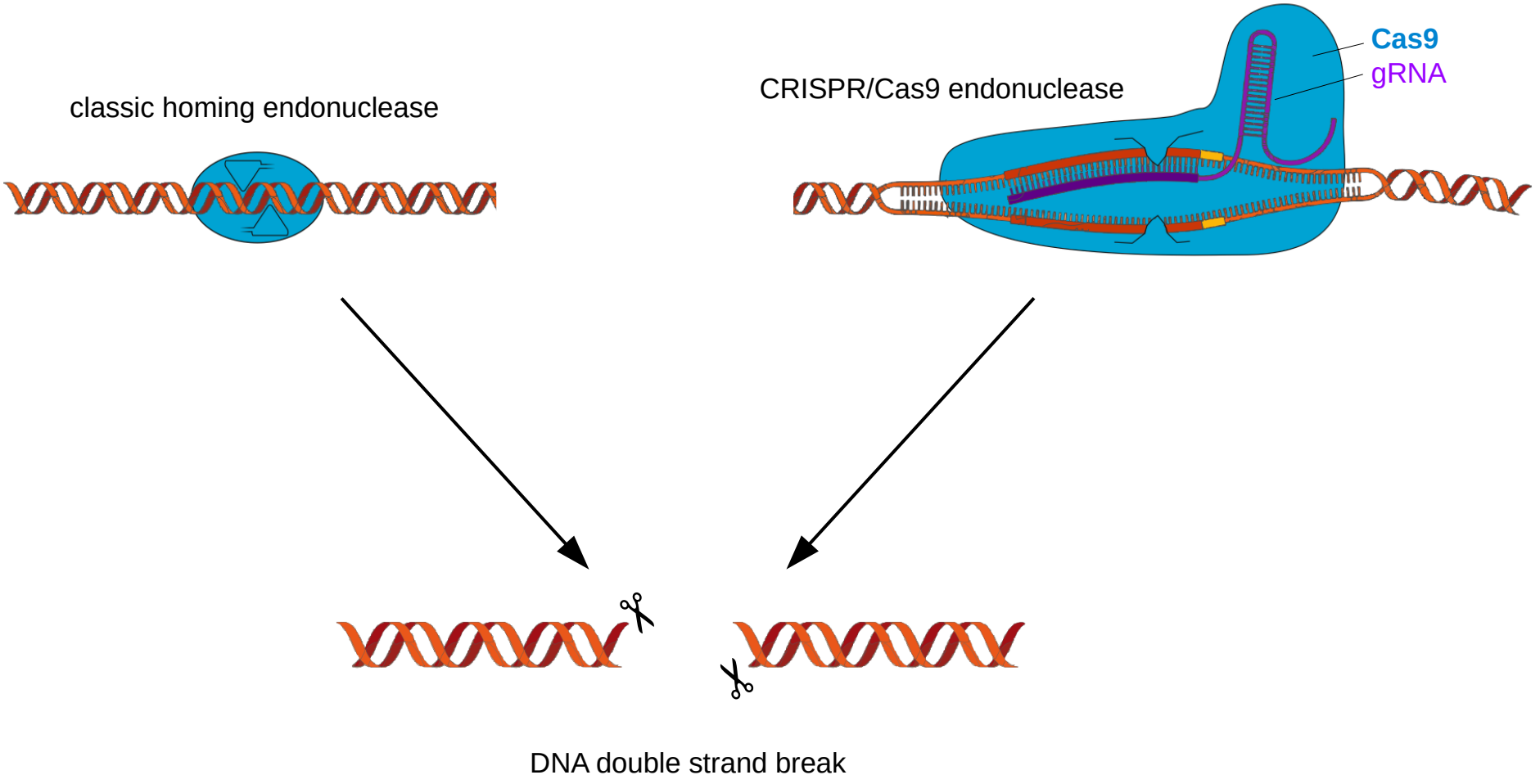
CRISPR/Cas9 endonuclease



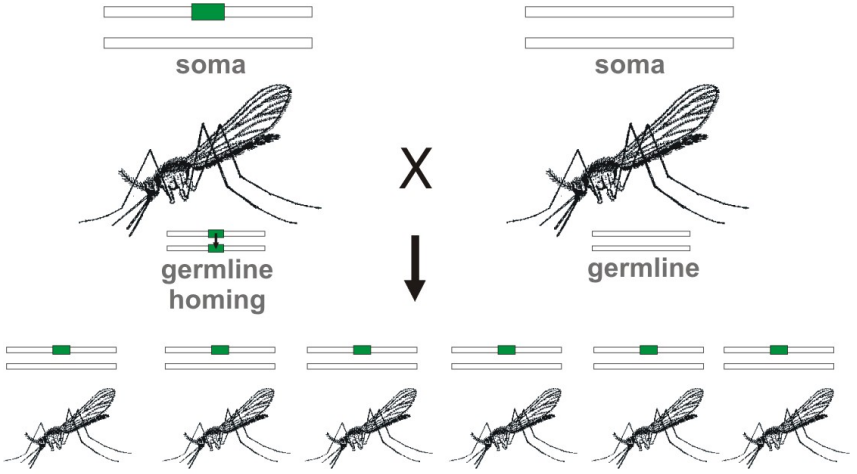
very easy to modify!

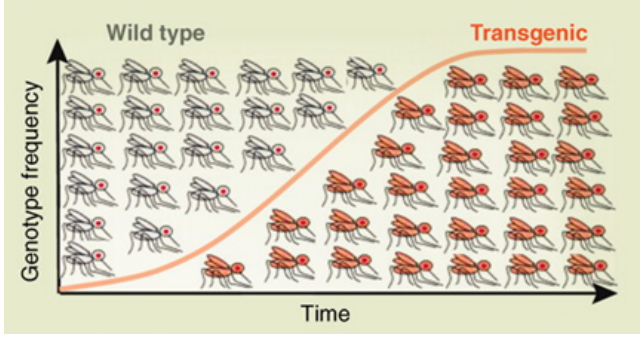
CCTCCTCACTTTCTTCCTCACC

generating new endonuclease genes – CRISPR is a game changer



gene drive to achieve population replacement





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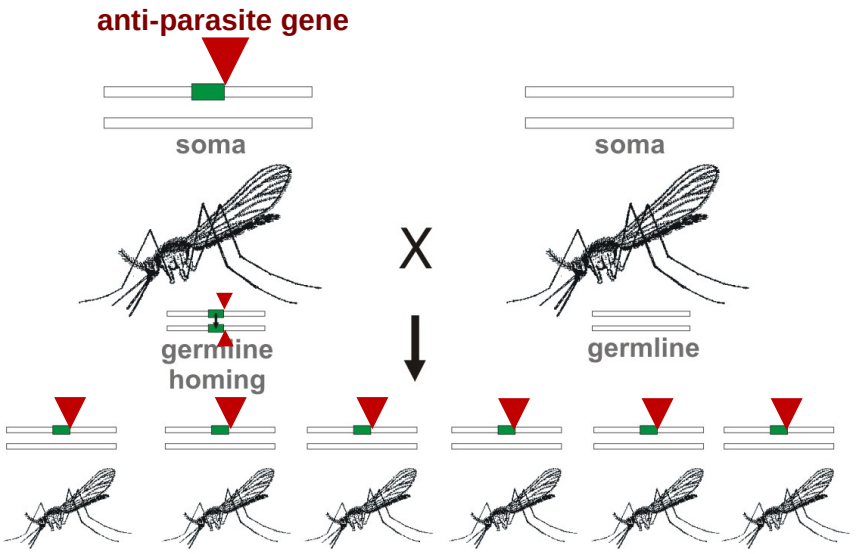
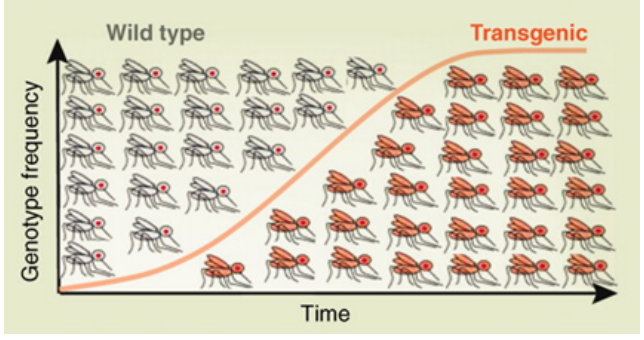




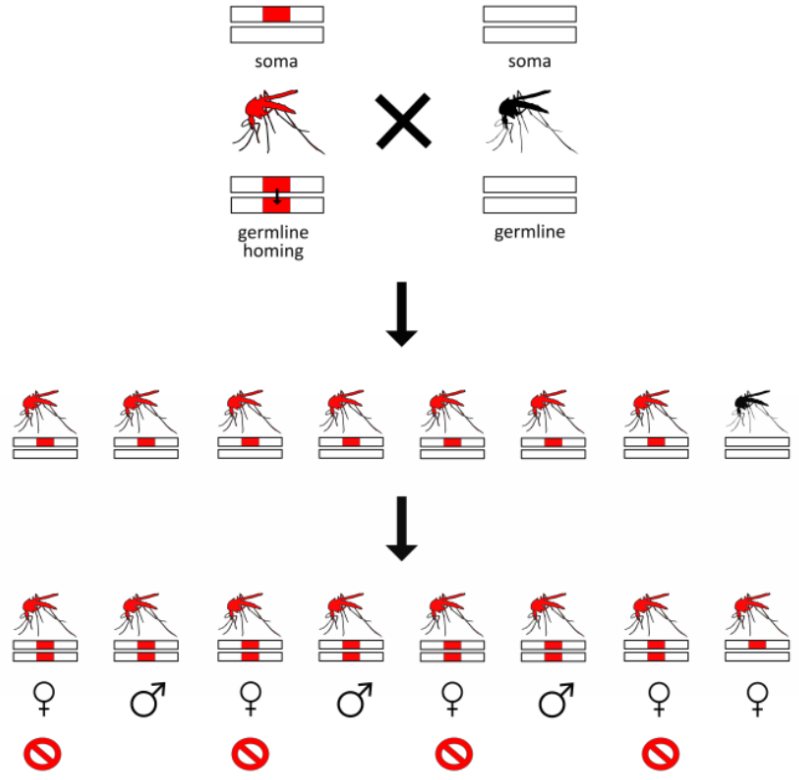
an anti-parasite effector gene can be placed inside the gene drive construct

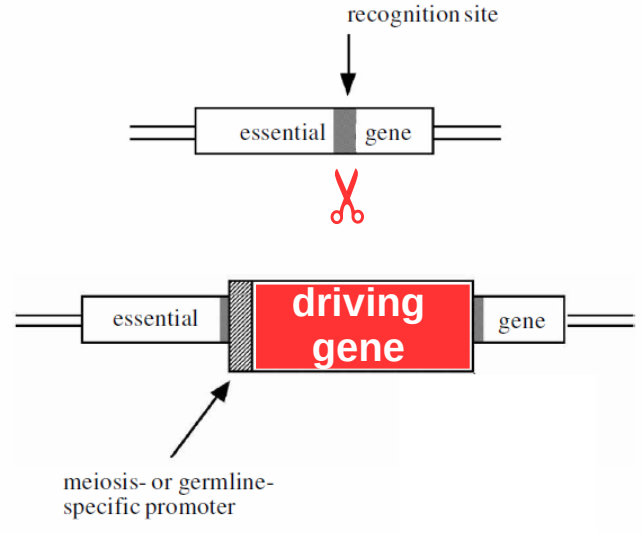
the anti-parasite gene will be active in a different tissue (e.g. the salivary glands) and will prevent the mosquito from transmitting the malaria parasite

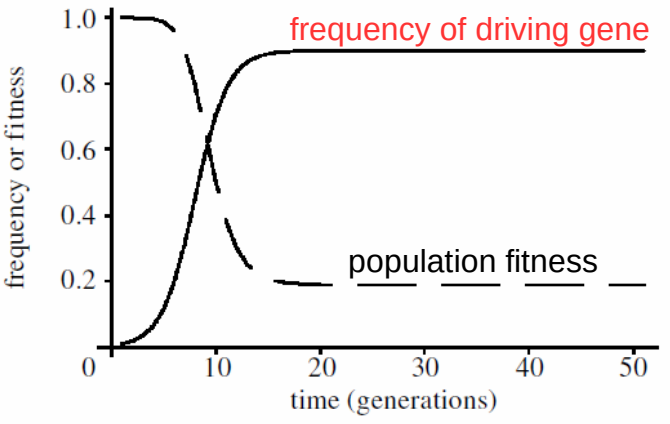
the gene drive locus will over several generations drive itself and the anti-parasite gene into the population

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

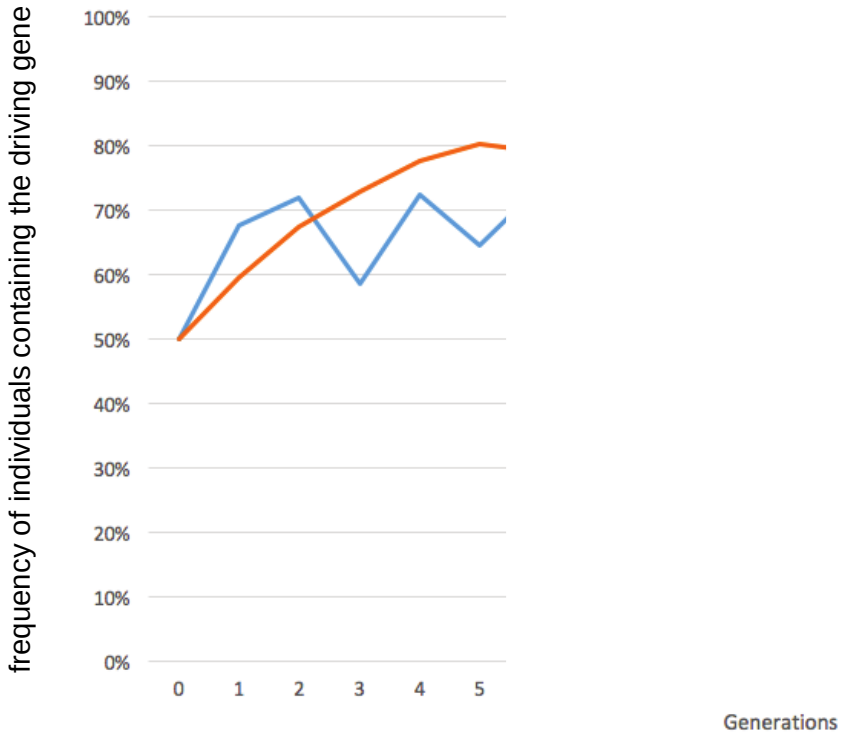






gene drive targeting an essential recessive female fertility gene
(homozygote females are sterile)

gene drive in two population cages (targeting a single female fertility gene)



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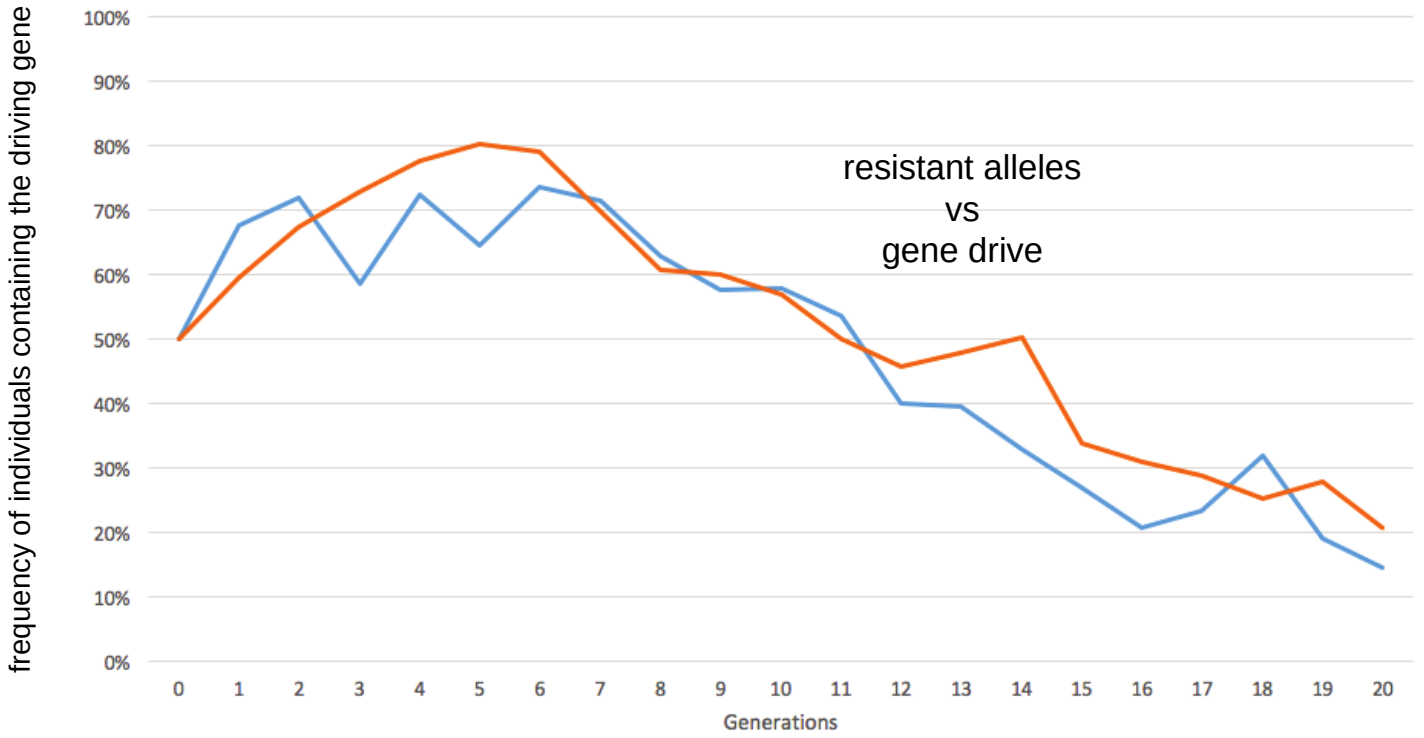
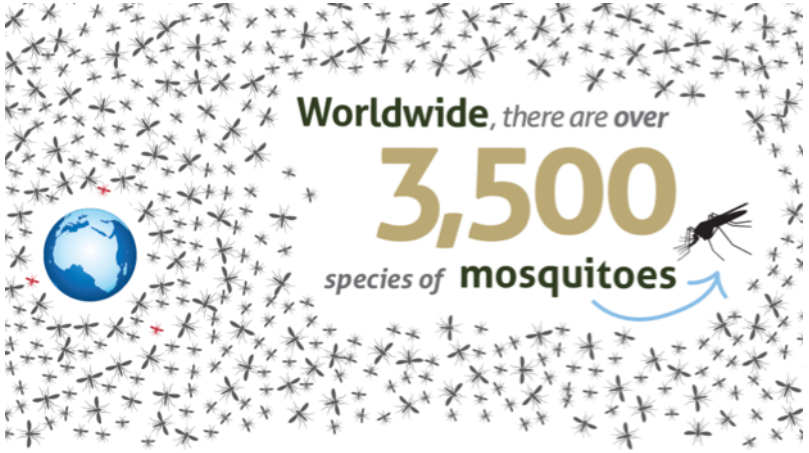
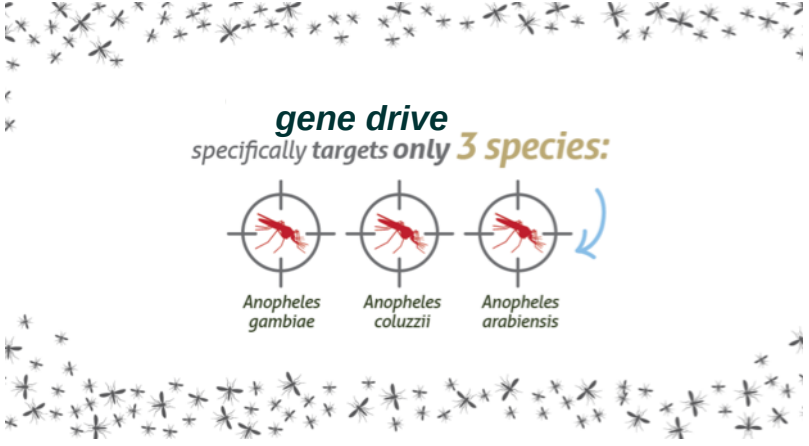
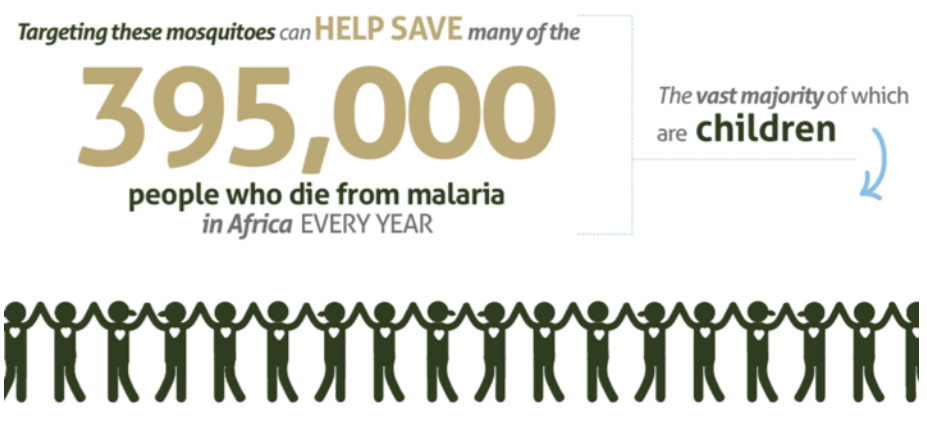


- to counter resistance many genes need to be targeted simultaneously
- the fear of the elimination of species is unfounded

suppressive gene drive designed to target 3 major vectors







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

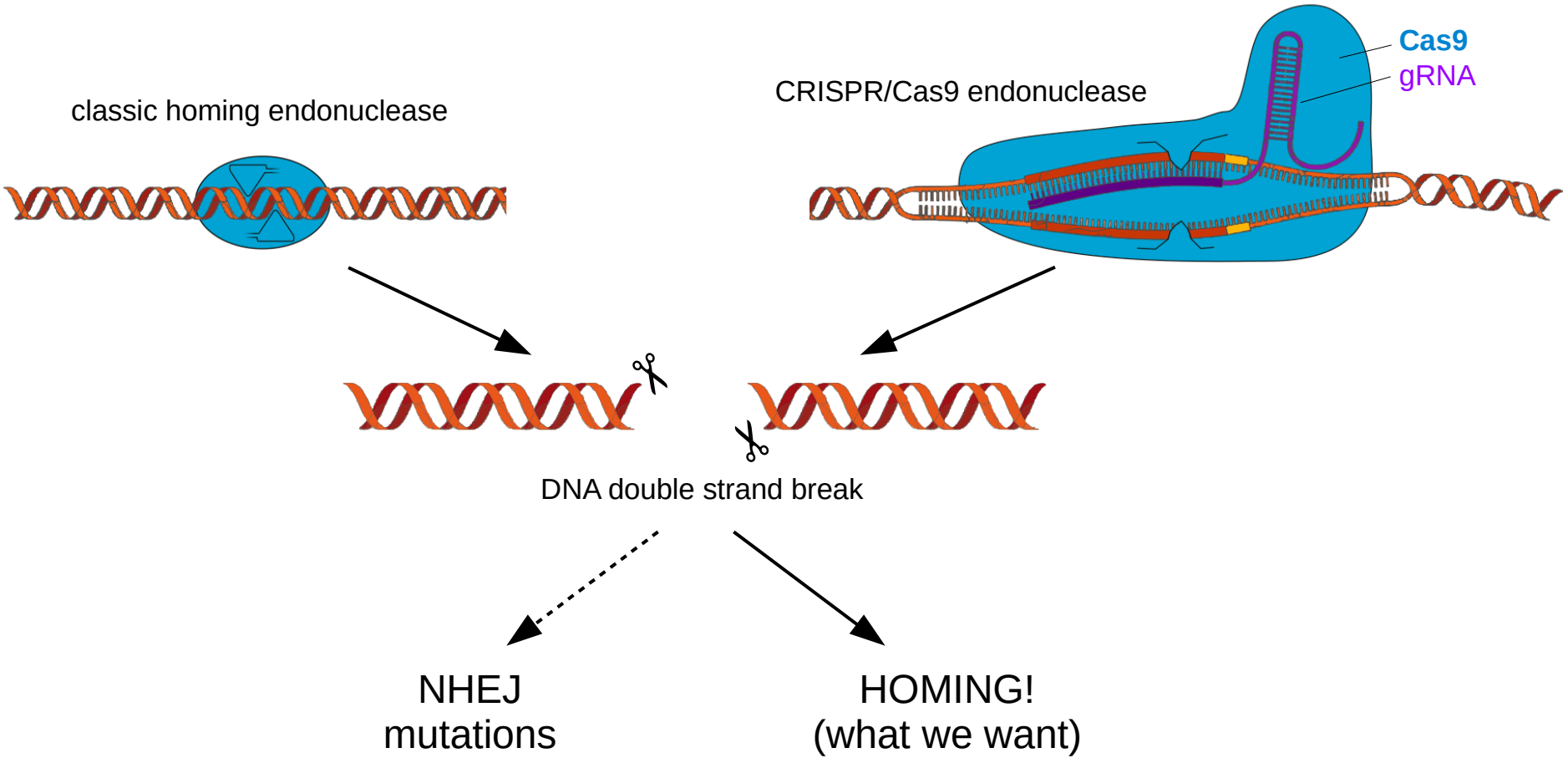
Questions?

FNIH
Foundation for the National Institutes of Health

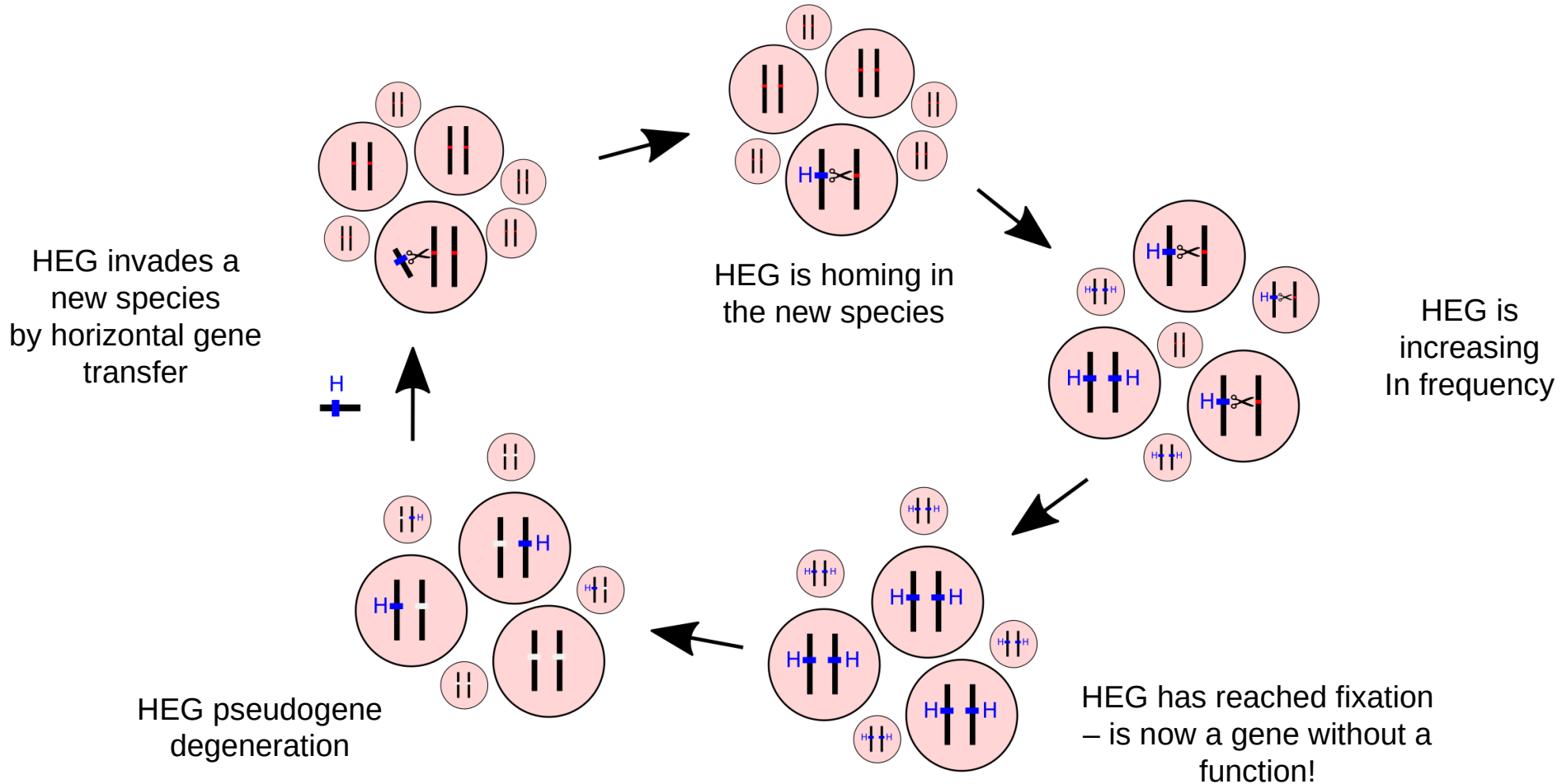
erc

Grand Challenges in Global Health

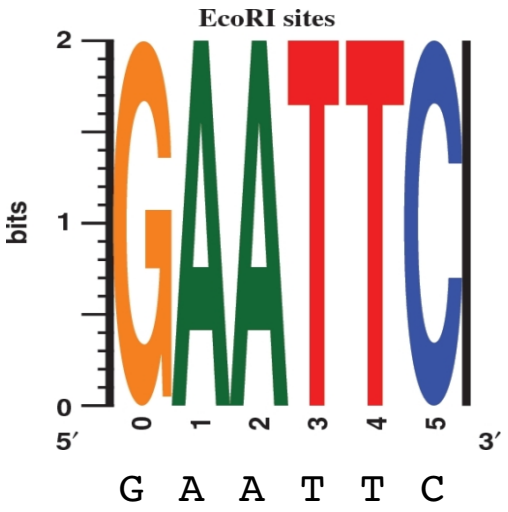
BBSRC
bioscience for the future


classic homing endonuclease
CRISPR/Cas9 endonuclease
Cas9
gRNA
DNA double strand break
NHEJ
mutations
HOMING!
(what we want)

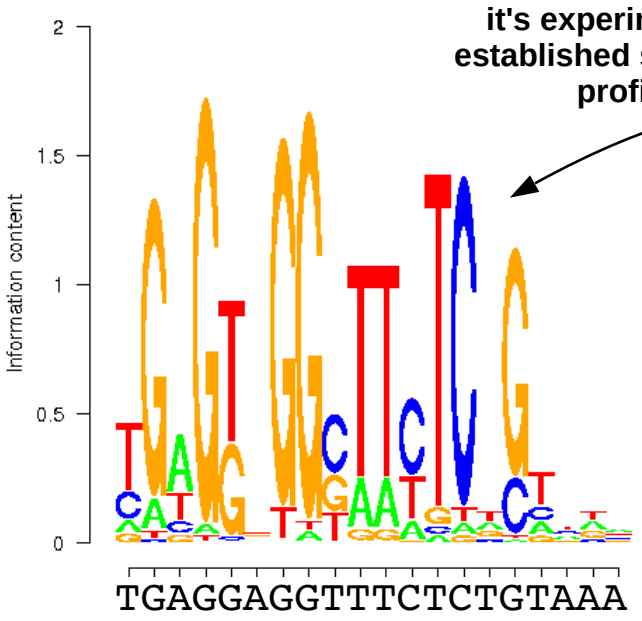
homing endonucleases - evolutionary dynamic



HEGs can recognize and cut sequence variants

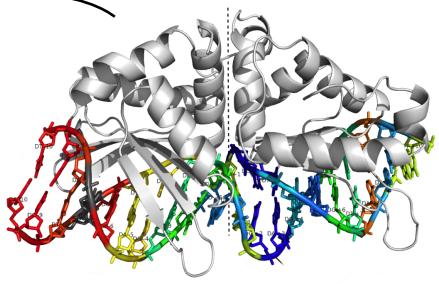


EcoRI
(Restriction enzyme)



it's experimentally established specificity profile

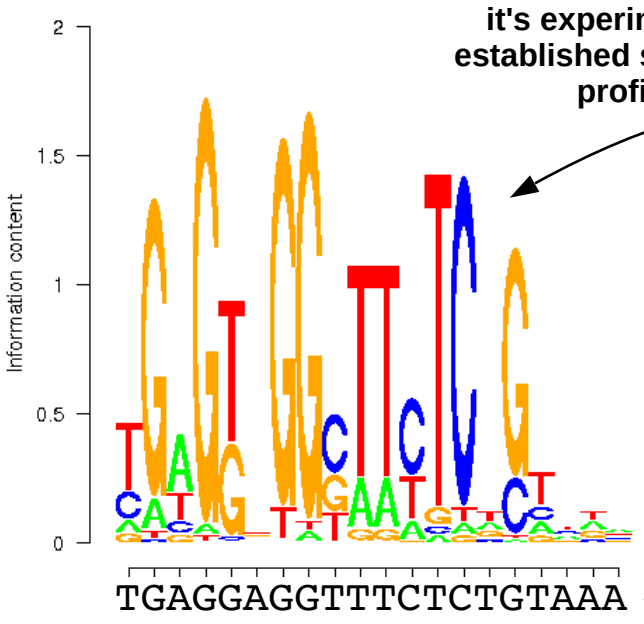
HEG I-AniI
(bound to DNA)



it's target site in the mitochondrial cobA gene

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 !



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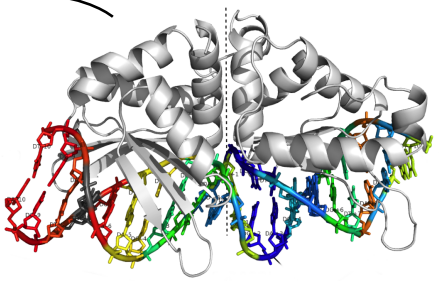

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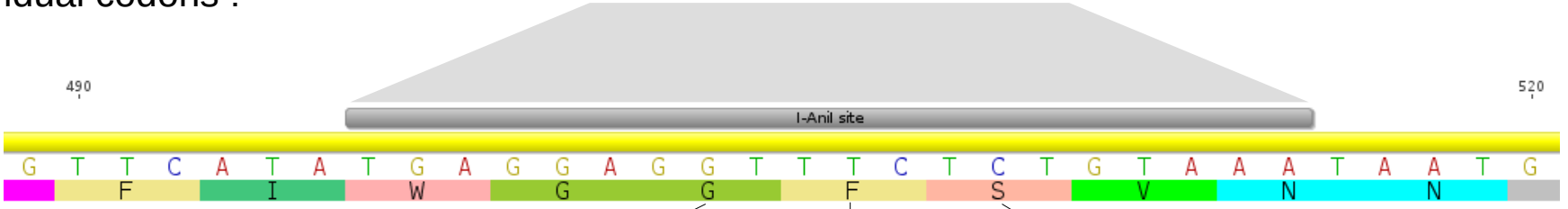
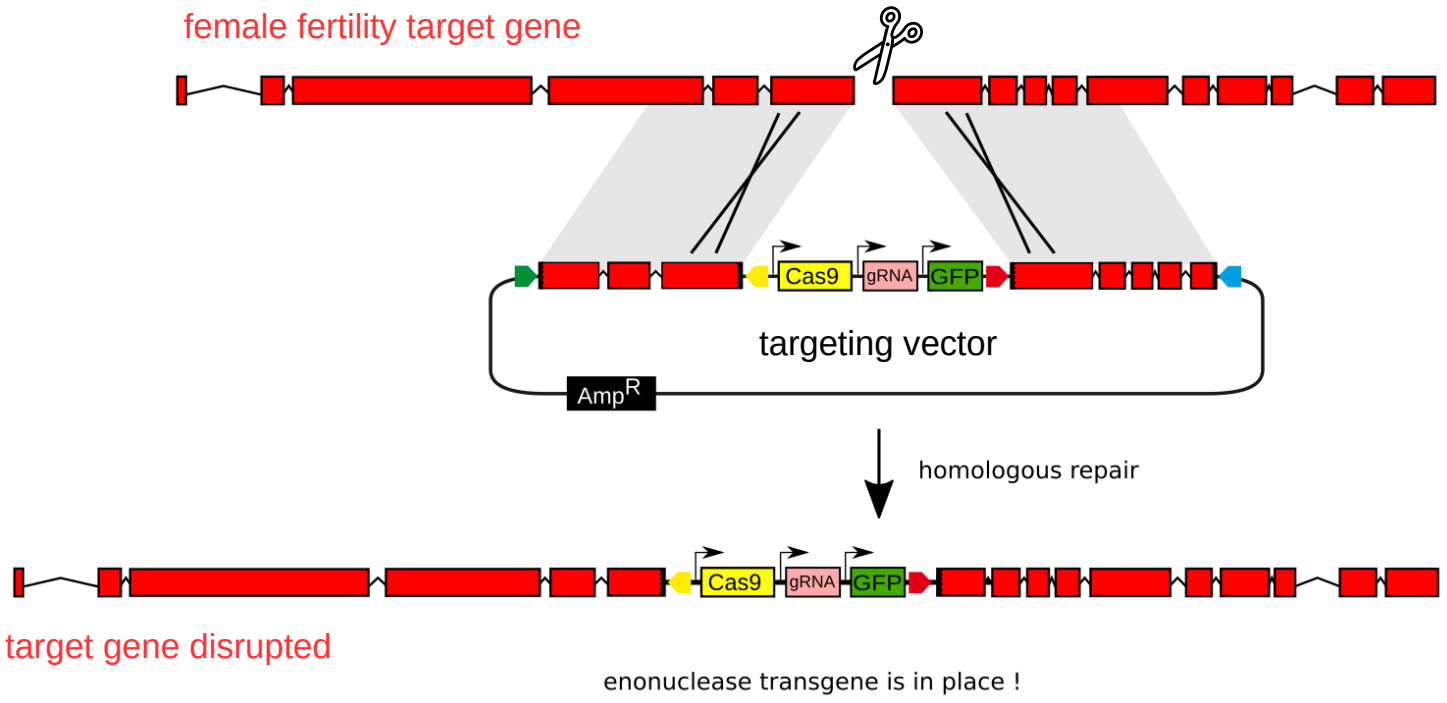


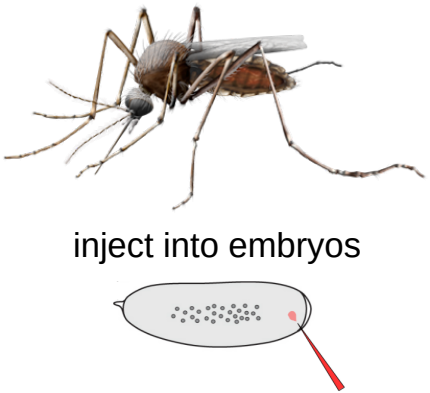
cobA open reading frame

(mold mitochondrial genetic code)

```
GGT G Gly      TTT F Phe      TCT S Ser
GGC G Gly      TTC F Phe      TCC S Ser
GGA G Gly                     TCA S Ser
GGG G Gly                     TCG S Ser
```

gene drive to achieve population suppression

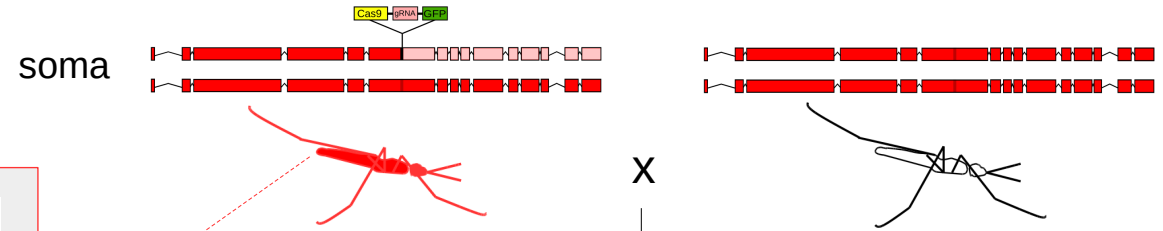




targeting female fertility gene

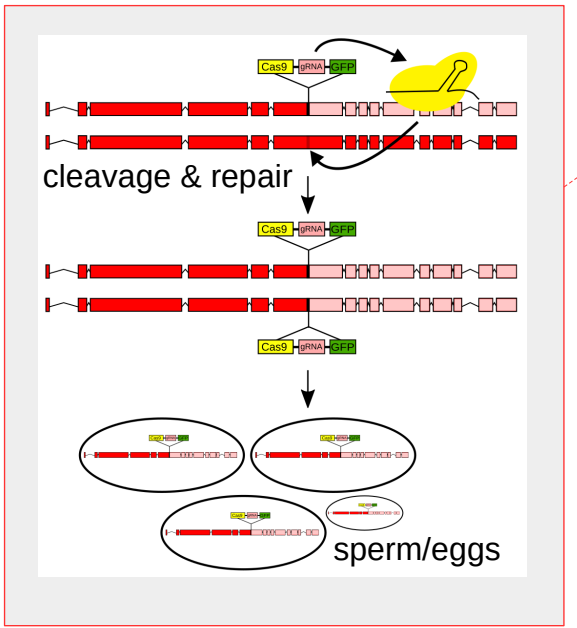
gene drive to achieve population suppression

soma



x

germline (ovaries/testes)



progeny

expectation if endonuclease inactive

50% GFP +
50% GFP-

mendelian inheritance

observed (AGAP007280 target gene)

gene drive!