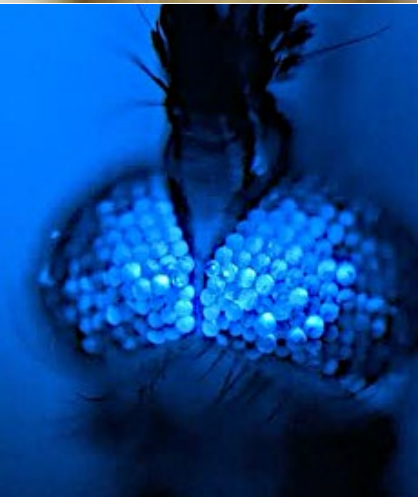
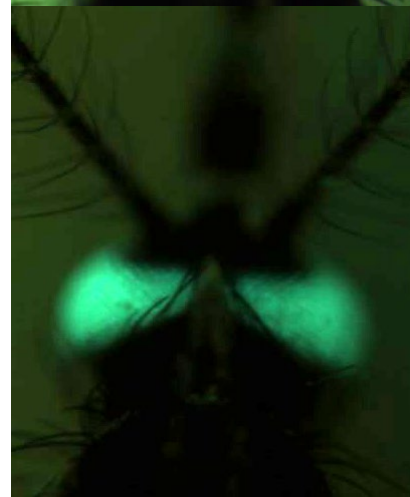




# Overview of Gene Drive Technology and Applications

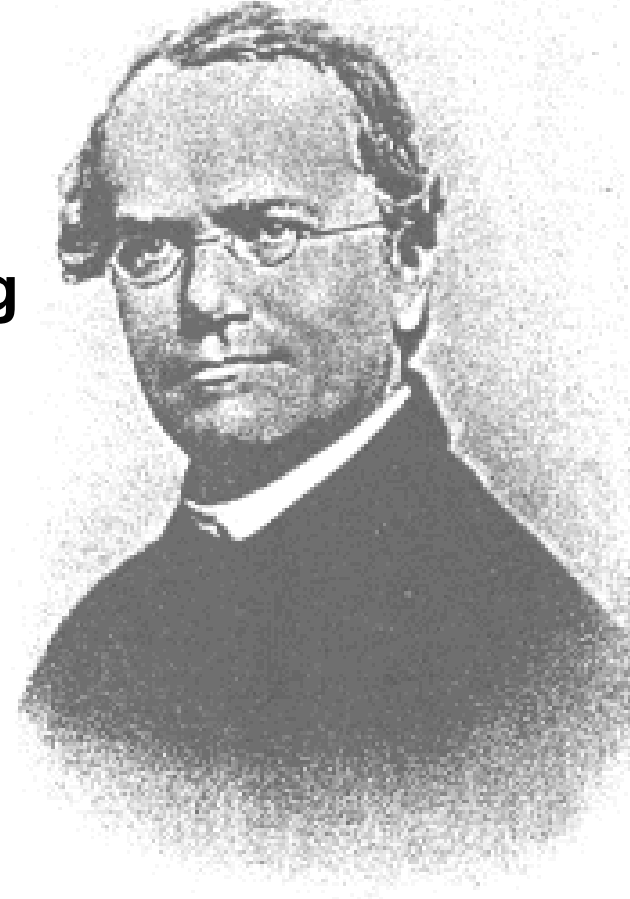
Anthony James, PhD, UC Irvine



# What is gene drive?

**Gene drive:** inheritance bias for  
a specific genotype  
(most common in diploid organisms during  
sexual reproduction)

**circumvention of Mendelian  
patterns of inheritance**  
(random segregation)



Gregor Mendel  
1822-1884



# *What is gene drive?*

***Drive mechanism***: underlying biological drive feature

***Drive system***: final synthetic product that achieves inheritance bias

# What are possible gene-drive mechanisms?

## Genetic phenomena

‘chromosome mechanics’

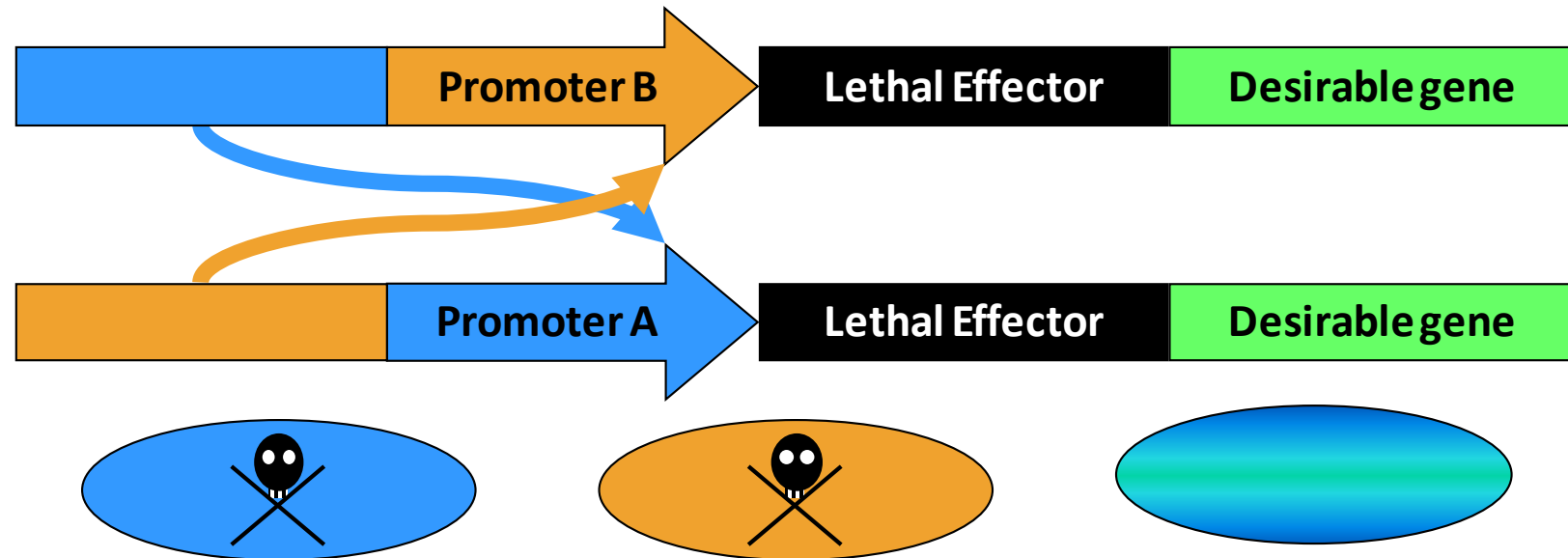
competitive displacement

reduced heterozygous fitness

under-dominance

hybrid sterility

## Underdominant system (Adapted from Davis *et al* 2001)



# ***What are gene-drive mechanisms?***

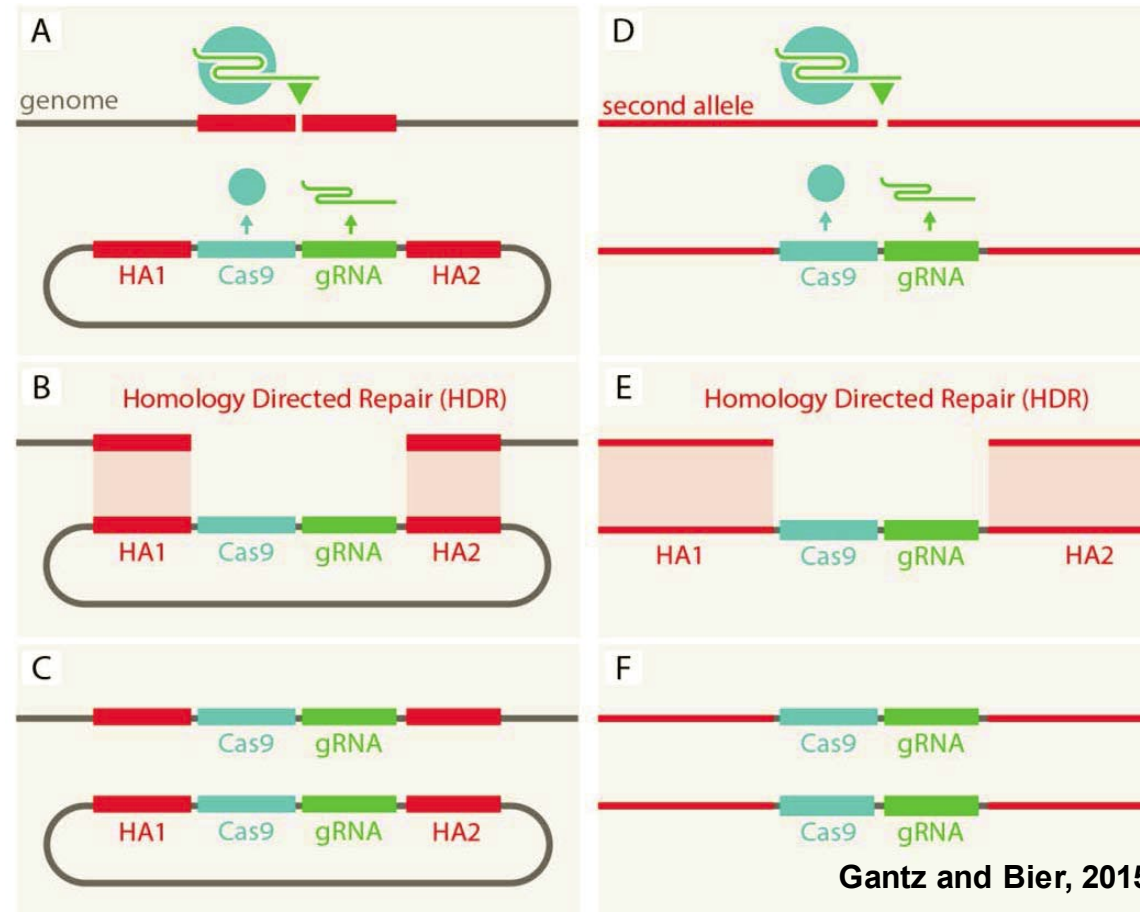
meiotic drive

segregation distorters (SD)

gene conversion

DNA-break induced repair

nuclease-mediated



# What are gene-drive mechanisms?

## Infectious and infectious-like agents

extracellular and intracellular symbiotic microorganisms

viruses

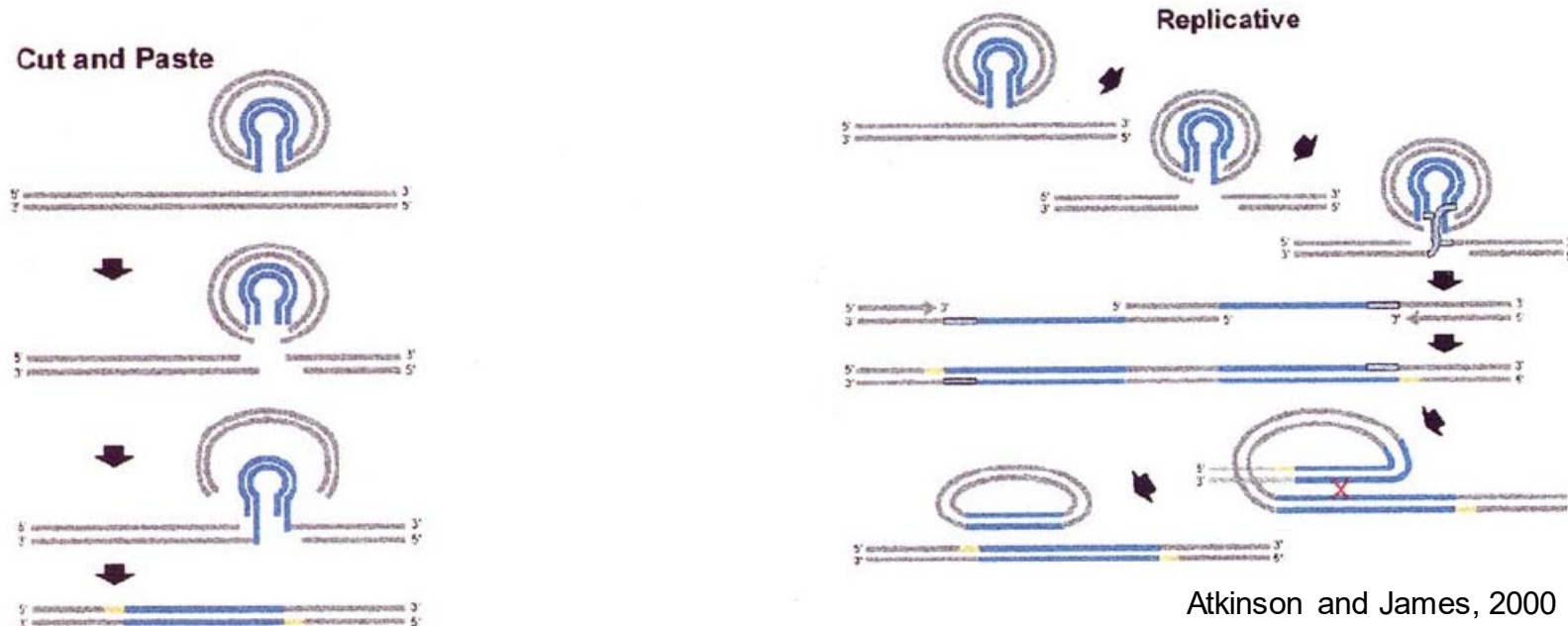
cytoplasmic incompatibility (*Wolbachia* species)

paratransgenesis

Transposons

conservative

replicative



Atkinson and James, 2000

# ***What are some useful concepts?***

## **Endogenous:**

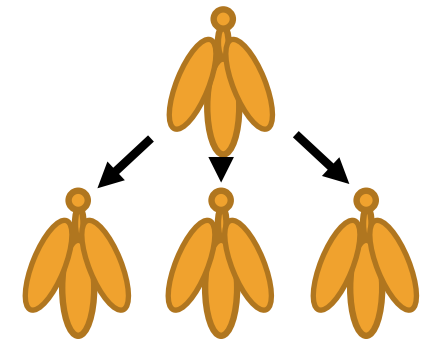
Genetic or epigenetic element originating from or common to the wild-type of the species of interest

## **Exogenous:**

Genetic or epigenetic element *not* originating from or common to the wild-type of the species of interest

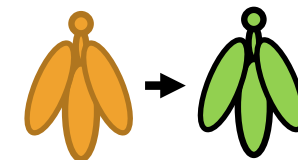
## **Vertical transmission:**

Genetic or epigenetic element passed from parent to progeny (germ cells, *fomites*)



## **Horizontal transmission (transfer):**

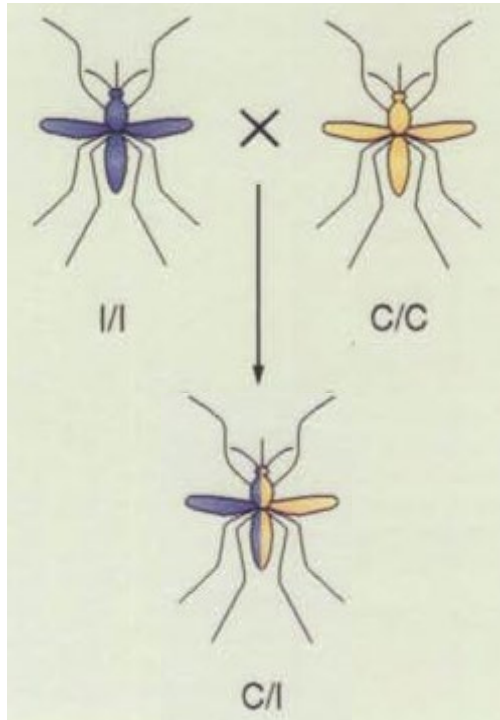
Genetic or epigenetic element passed from one organism to another (same or different species)



# What are the genetics of 'conversion-like' drive?

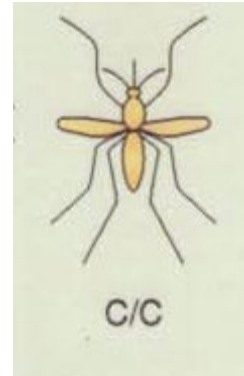
## Genotypic and phenotypic consequences:

Mendelian inheritance

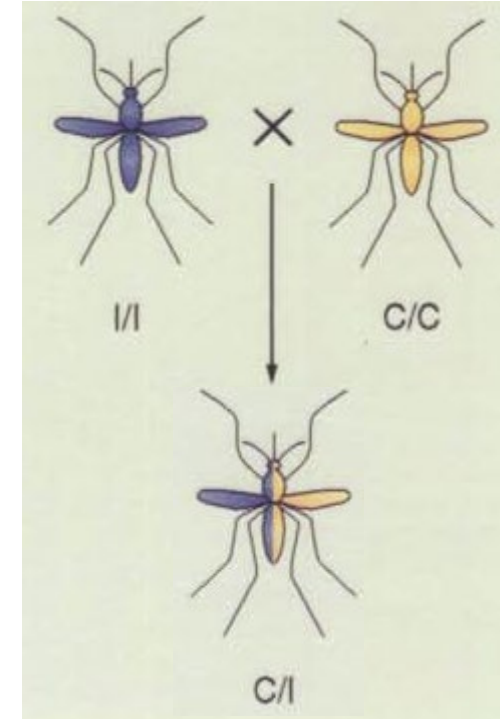


I, C: alternate alleles  
I: dominant  
C: recessive

Test cross



Gene drive



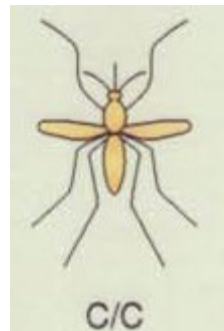
!

?

50%



50%



All I/I



# What are the genetics of gene drives?

Different types of crosses need to see evidence of other mechanisms:

competitive displacement

reduced heterozygous fitness

under-dominance

hybrid sterility

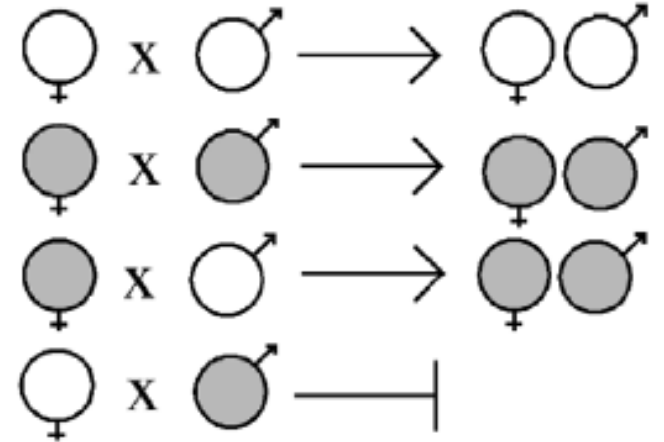
extracellular and intracellular symbiotic microorganisms

viruses

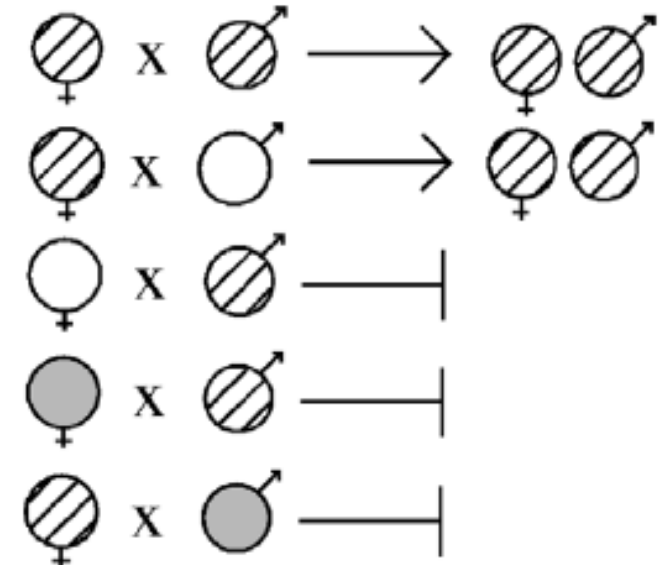
*Wolbachia* species

paratransgenesis

Cytoplasmic incompatibility



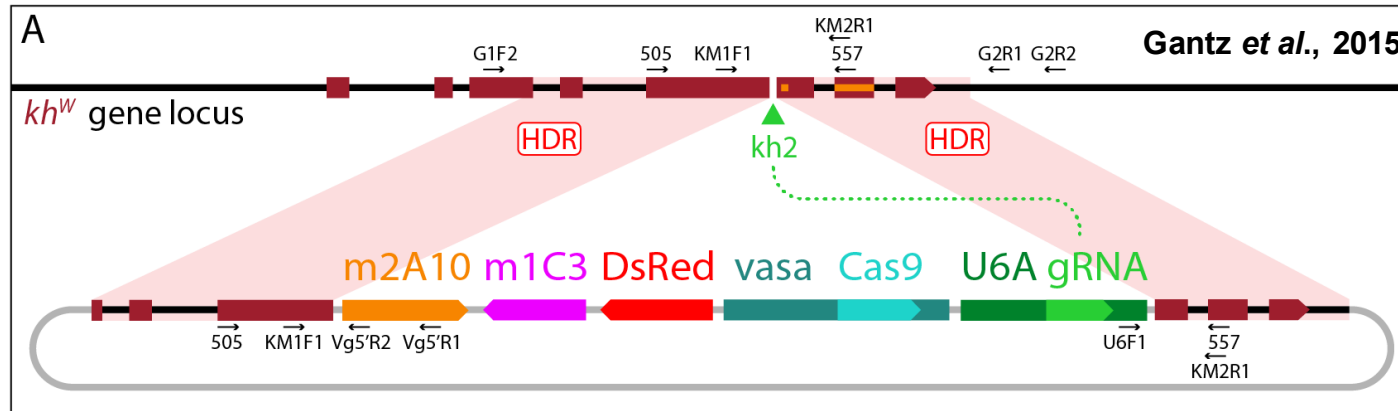
Uni-directional



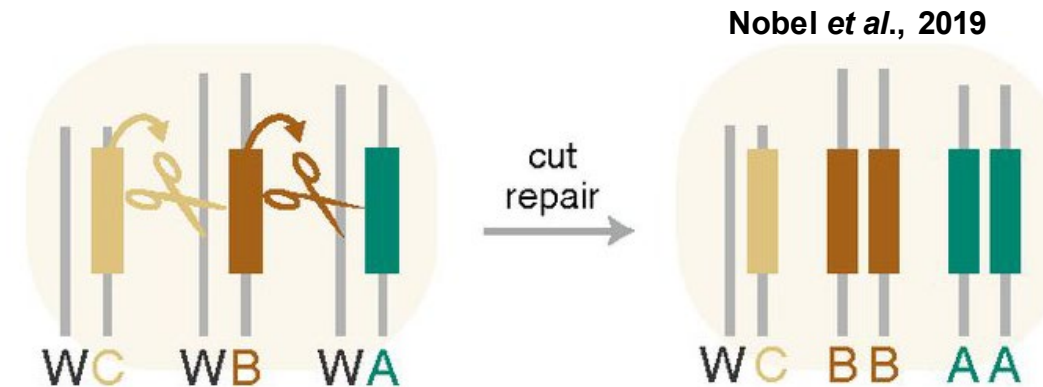
Bi-directional

# What are some gene-drive system features?

**Autonomous** systems (also known as ‘autocatalytic’):  
carry all the genetic information needed to self-mobilize or cause an inheritance bias tightly-linked in a *cis* configuration as part of a single construct



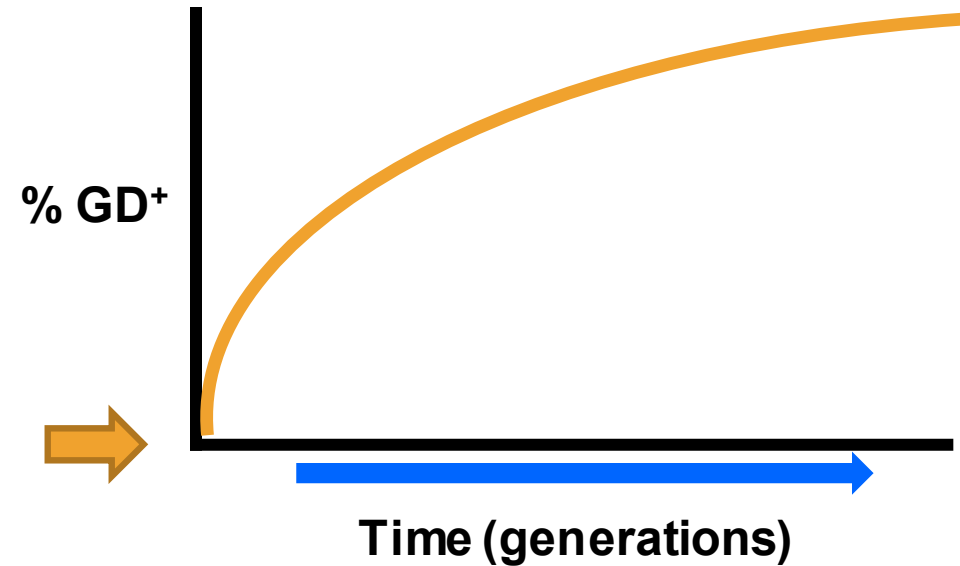
**‘Split’** systems (physical, temporal separation):  
components are at separate loci on homologous or heterologous chromosomes, only function when all components are in the same cell



# ***What are some gene-drive system features?***

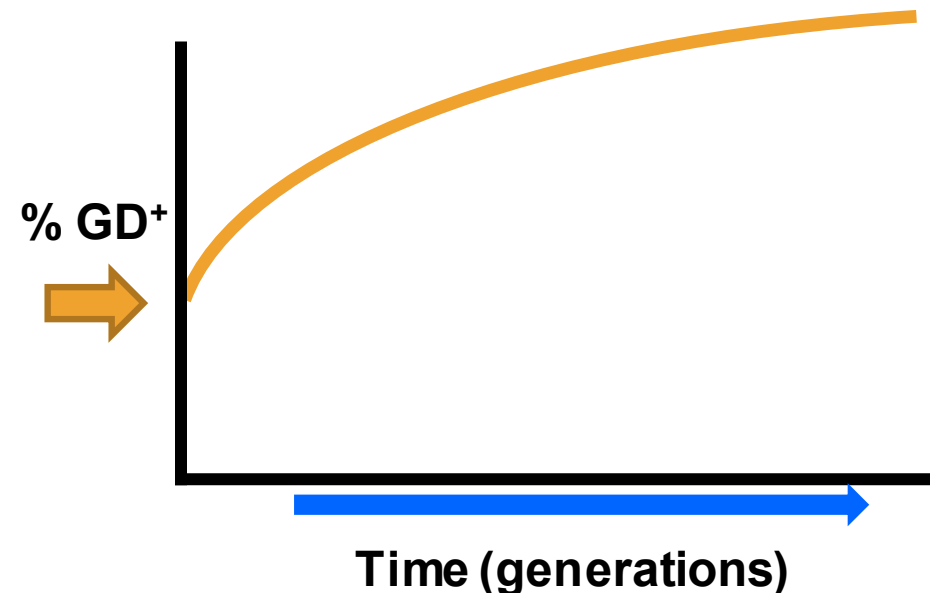
## **Low (no) threshold dynamics:**

single releases of small numbers of gene drive organisms result in every organism in the population carrying the drive system



## **High threshold dynamics:**

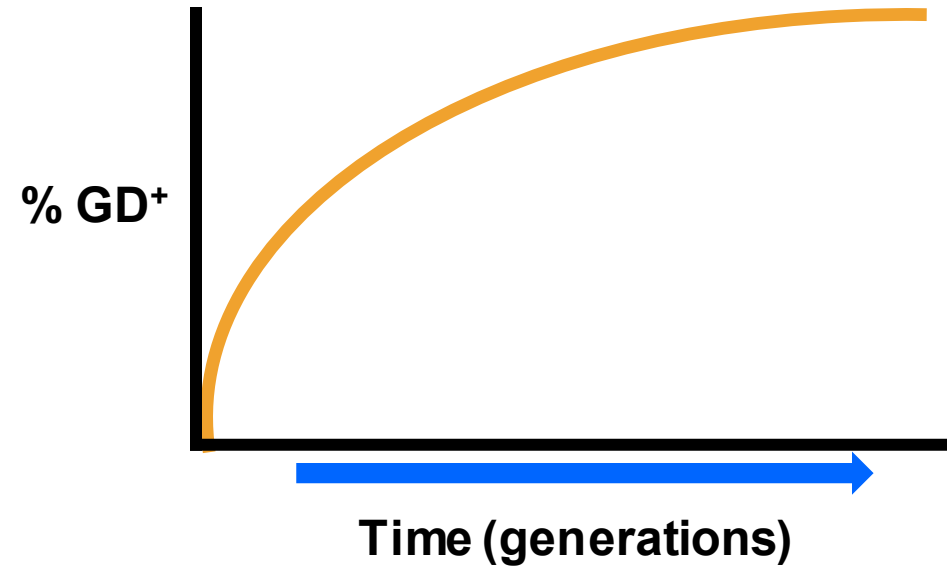
gene-drive organisms must be released above a minimal frequency in relation to the target population (either by one-time releases of larger numbers of mosquitoes or a succession of serial releases)



# ***What are some gene-drive system features?***

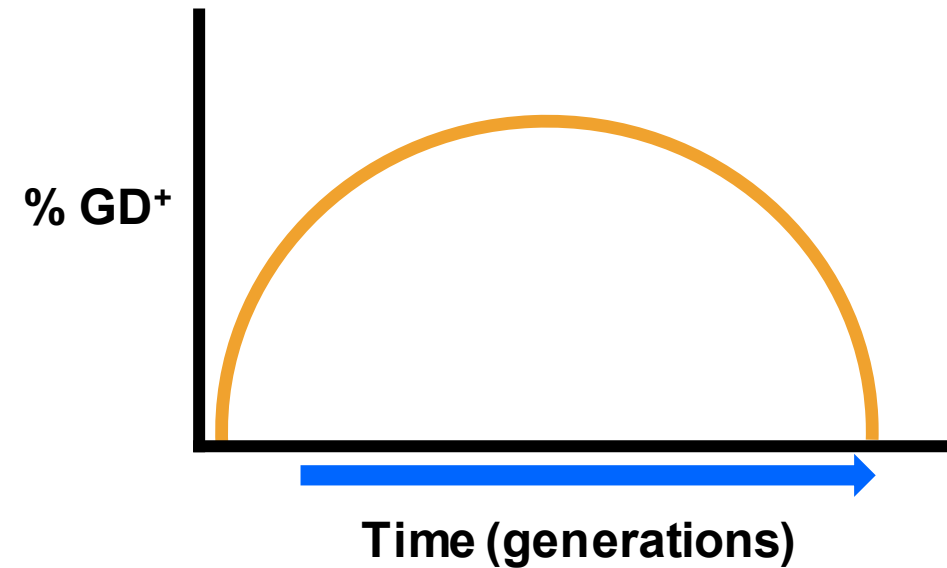
## **Non-limiting:**

Gene drive organisms intended to persist in the environment



## **Self-limiting:**

Gene drive design features cause it to be lost from the population

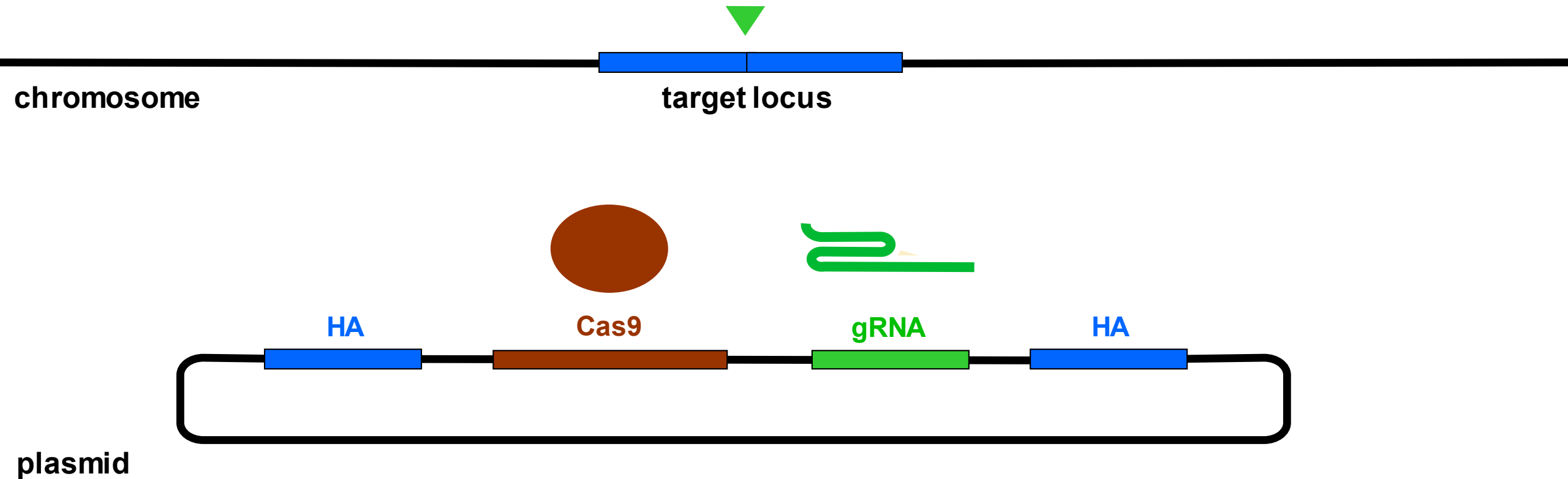


# How do you make an autonomous Cas9 drive system?

**Cas9 nuclease**  
endonuclease

**'guide' RNA**  
23 nucleotides in length

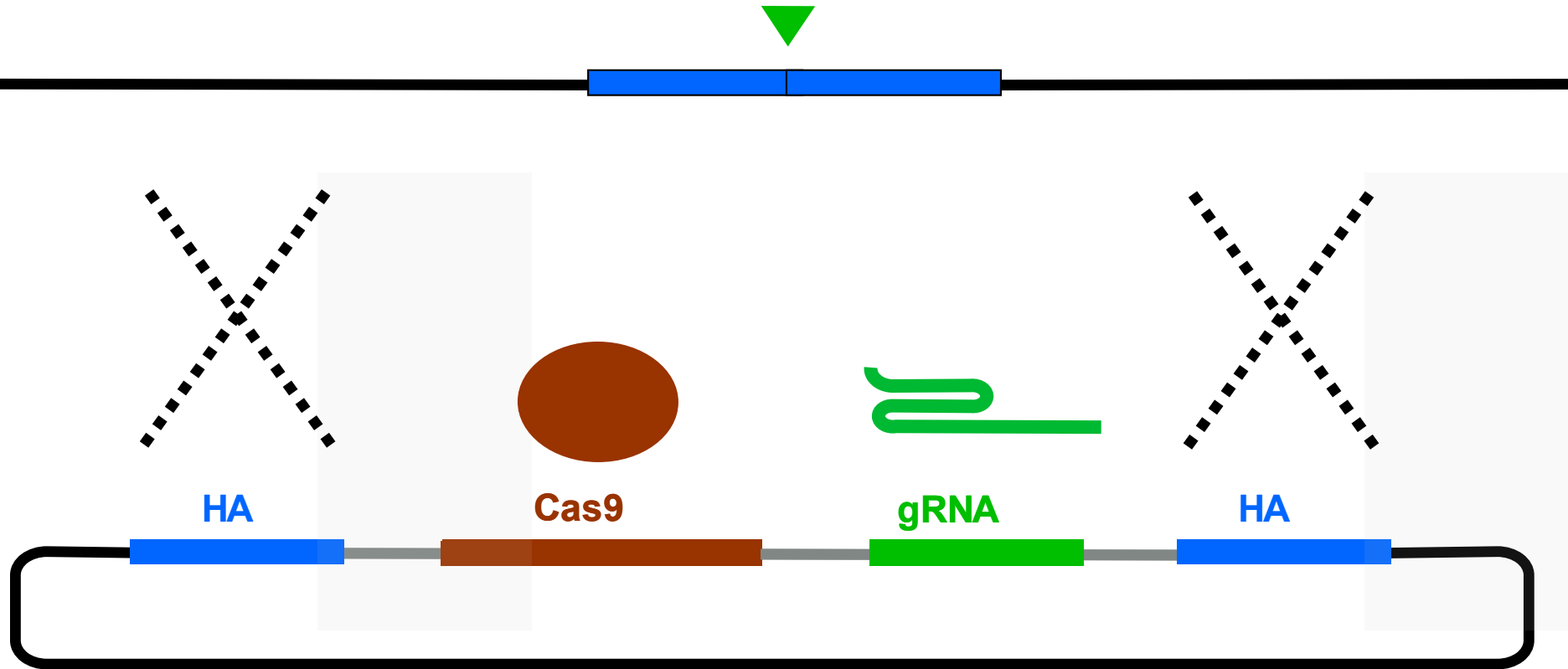
**Homology Arms**  
1-2 kilobases in length\*



\*Homology arms as short as 100bp have worked with cargoes ( $\leq 5\text{kb}$ ) in *Drosophila melanogaster* if the primary construct is linearized *in vivo* (Kanca et al., 2019)

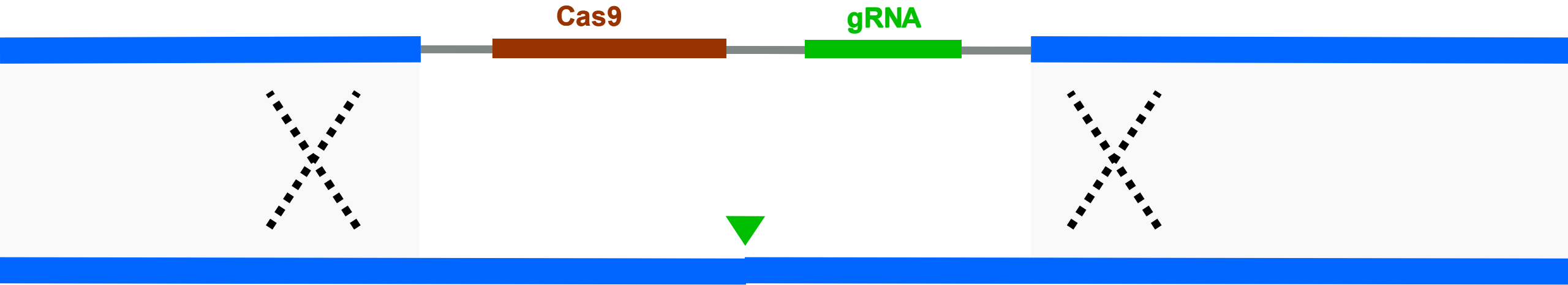
# *How does an autonomous Cas9 drive system work?*

Primary integration into chromosome



# *How does an autonomous Cas9 drive system work?*

Gene drive (interchromosomal)



# *What can gene drive be used for?*

Introduce **favorable** traits into populations



Photo: Scott Bauer

**E.F. Knipling**

**Population suppression**

**Population replacement  
(modification/alteration)**



**C. F. Curtis**

*Likely to work best in organisms with short life cycles*

# ***What are possible Environmental/Ecological merits?***

## **Invasive species**

### **Mosquitoes in Hawai'i**



### **Rats on islands**



### **Fish in lakes**



### **Plants**



# ***What are possible Agricultural merits?***

## **Favorable traits**



**Disease resistance**



**Yield increases**



**Disease resistance**



**Nutrient/habitat diversity**



**Citrus: Mexican fruit fly**

*Anastrepha ludens*

## **Pest species**



**Cotton: Pink Bollworm**

*Pectinophora gossypiella*



**Many:**

*Drosophila suzukii*



**Cattle: Screw worm**

*Cochliomyia hominivorax*

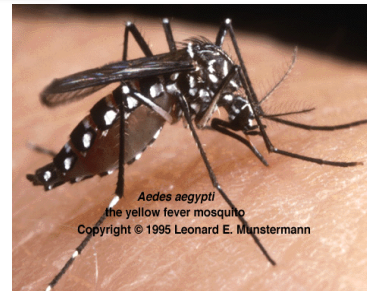
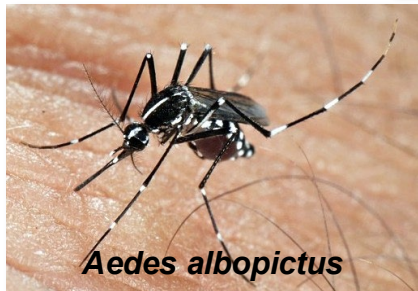
# Control/alter:

## Vectors

**Malaria**



**Dengue  
Chikungunya  
Zika**

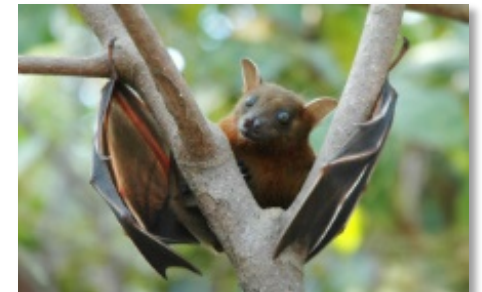


**Lyme's Disease**

## Pathogens



## Reservoirs



**'humanize' experimental and donor animals**

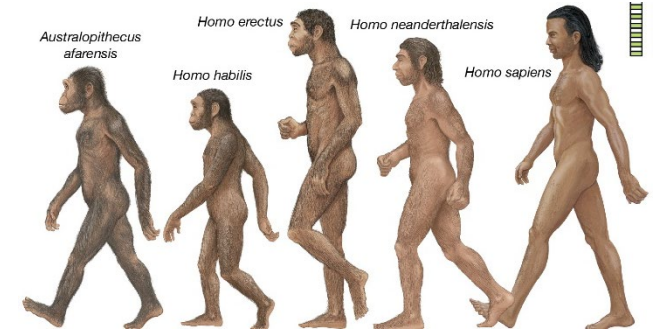
# ***What are some challenges?***



## **Space and time**

**Regional vs global impacts**

**Human vs evolutionary time scales**



## **Safety and efficacy**

**Consequences of target and non-target effects**

**Consequences of drive or cargo failures**

## **Science and society**

**National and international regulatory realms**

**Individual vs community consent**

# What are some mitigating measures adopted by the research community?

## Potentially stringent confinement strategies for gene drive research

Multiple stringent confinement strategies should be used whenever possible.

| TYPE         | STRINGENT CONFINEMENT STRATEGY                                                                                                                                                                                    | EXAMPLES                                                                                                                                                                                             |
|--------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Molecular    | Separate components required for genetic drive<br>Target synthetic sequences absent from wild organisms                                                                                                           | sgRNA and Cas9 in separate loci (8)<br>Drive targets a sequence unique to laboratory organisms (3,4,8)                                                                                               |
| Ecological   | Perform experiments outside the habitable range of the organism<br>Perform experiments in areas without potential wild mates                                                                                      | <i>Anopheles</i> mosquitoes in Boston<br><i>Anopheles</i> mosquitoes in Los Angeles                                                                                                                  |
| Reproductive | Use a laboratory strain that cannot reproduce with wild organisms                                                                                                                                                 | <i>Drosophila</i> with compound autosomes*                                                                                                                                                           |
| Barrier      | Physical barriers between organisms and the environment<br>• Remove barriers only when organisms are inactive<br>• Impose environmental constraints<br>• Take precautions to minimize breaches due to human error | Triply nested containers, >3 doors (6)<br>Anesthetize before opening (6)<br>Low-temperature room, air-blast fans<br>Keep careful records of organisms, one investigator performs all experiments (6) |

\*An example of reproductive confinement would be *Drosophila* laboratory strains with a compound autosome, where both copies of a large autosome are conjoined at a single centromere. These strains are fertile when crossed inter se but are sterile when outcrossed to any normal or wild-type strain because all progeny are monosomic or trisomic and die early in development.

## Containment

# ***A few thoughts:***

**Review and reconcile past efforts:** many discussion/publications available already

**Strive for consensus:** adopt unified language; facilitates adoption of guidelines

**No 'one-size-fits all' solutions:** genetic plasticity, dispersal, reproductive capacity

**Consider biology, not labels:** avoid simplistic classifications

**Be precise in language:** avoid jargon and catch-phrases

**Do not over-regulate, better to amend than revise**

***Lack of knowledge never an answer to solving complex problems***



***Thank you!***

***Questions and discussion!***