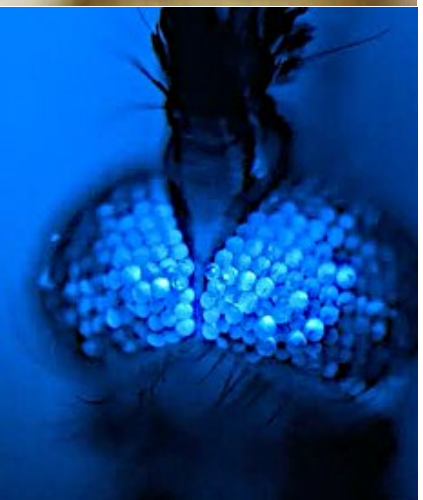
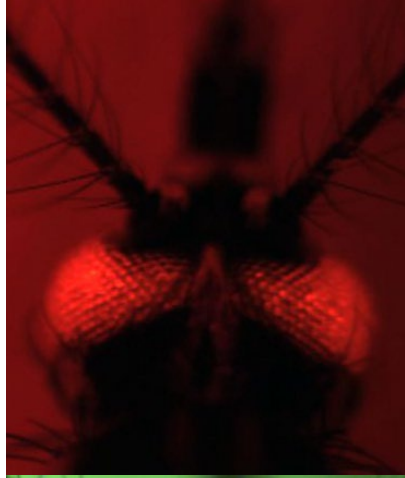
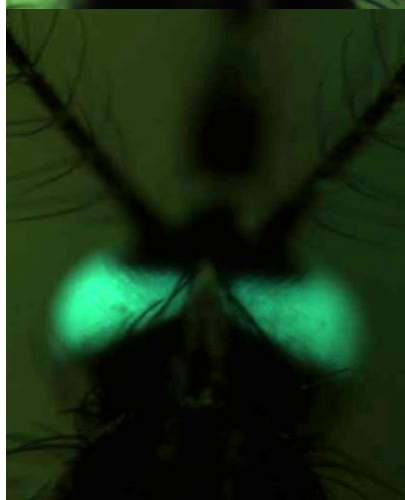




Overview of Gene Drive Technology and Applications

Anthony James, PhD, UC Irvine

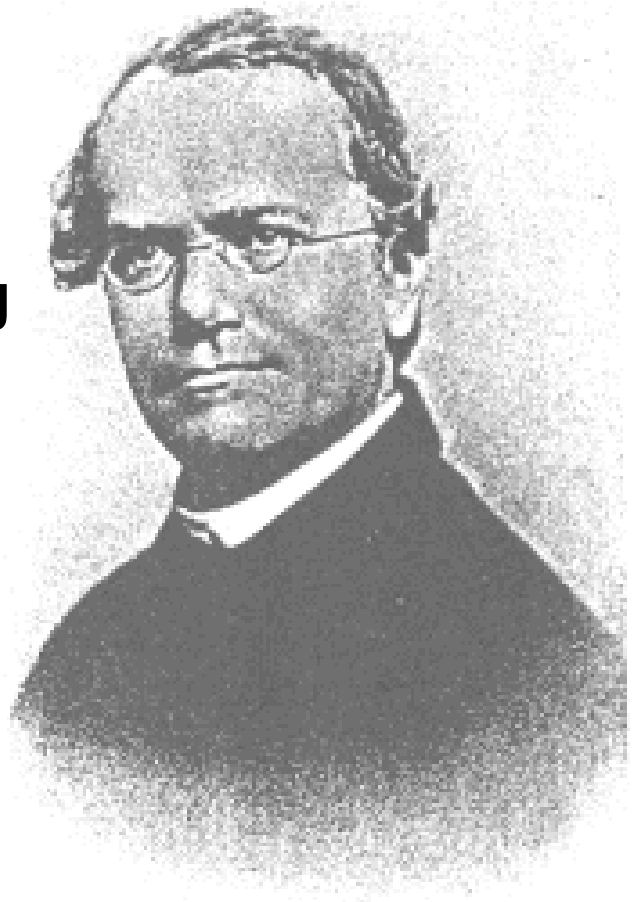
NExTRAC: Gene Drives in Biomedical Research Working Group, November 9-10, 2020

What is gene drive?

Prof. Dr. Otto Wilhelm Thomé
Flora von Deutschland Österreich und der Schweiz.

***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**

Opinion TRENDS in Parasitology Vol.21 No.2 February 2005

Gene drive systems in mosquitoes: rules of the road

Anthony A. James

Departments of Microbiology & Molecular Genetics and Molecular Biology & Biochemistry, University of California, Irvine, CA 92697-3900, USA

What are possible gene-drive mechanisms?

Genetic phenomena

'chromosome mechanics'

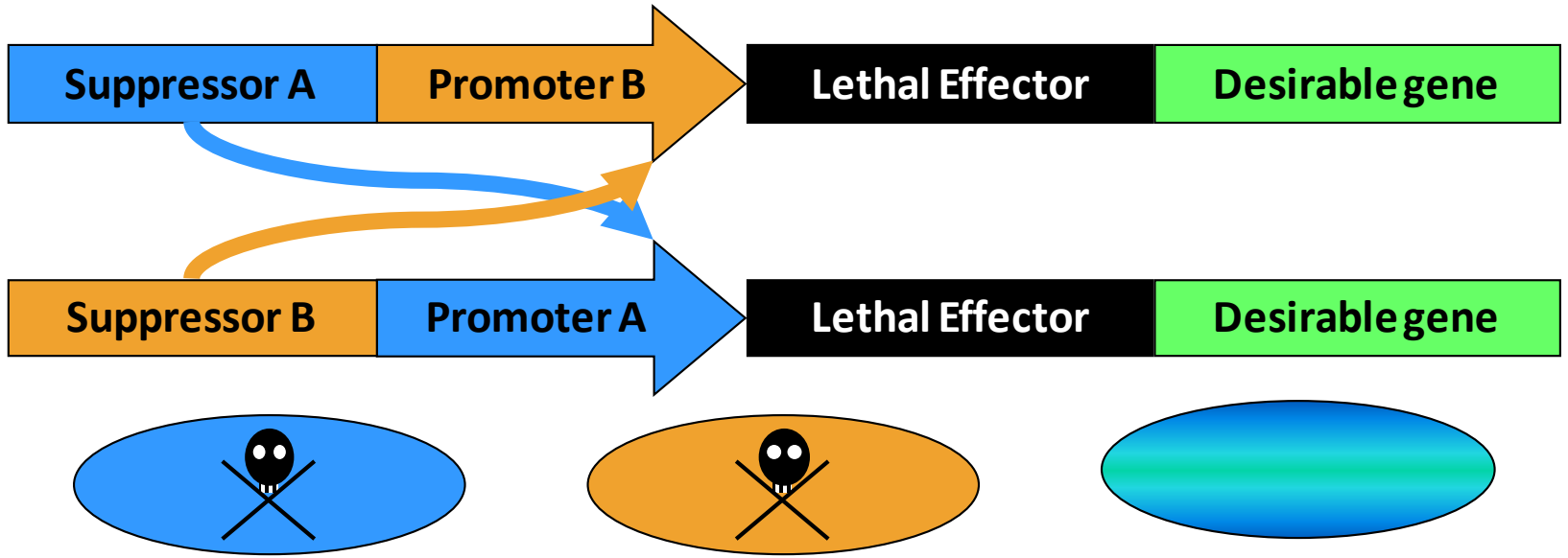
competitive displacement

reduced heterozygous fitness

under-dominance

hybrid sterility

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



What are gene-drive mechanisms?

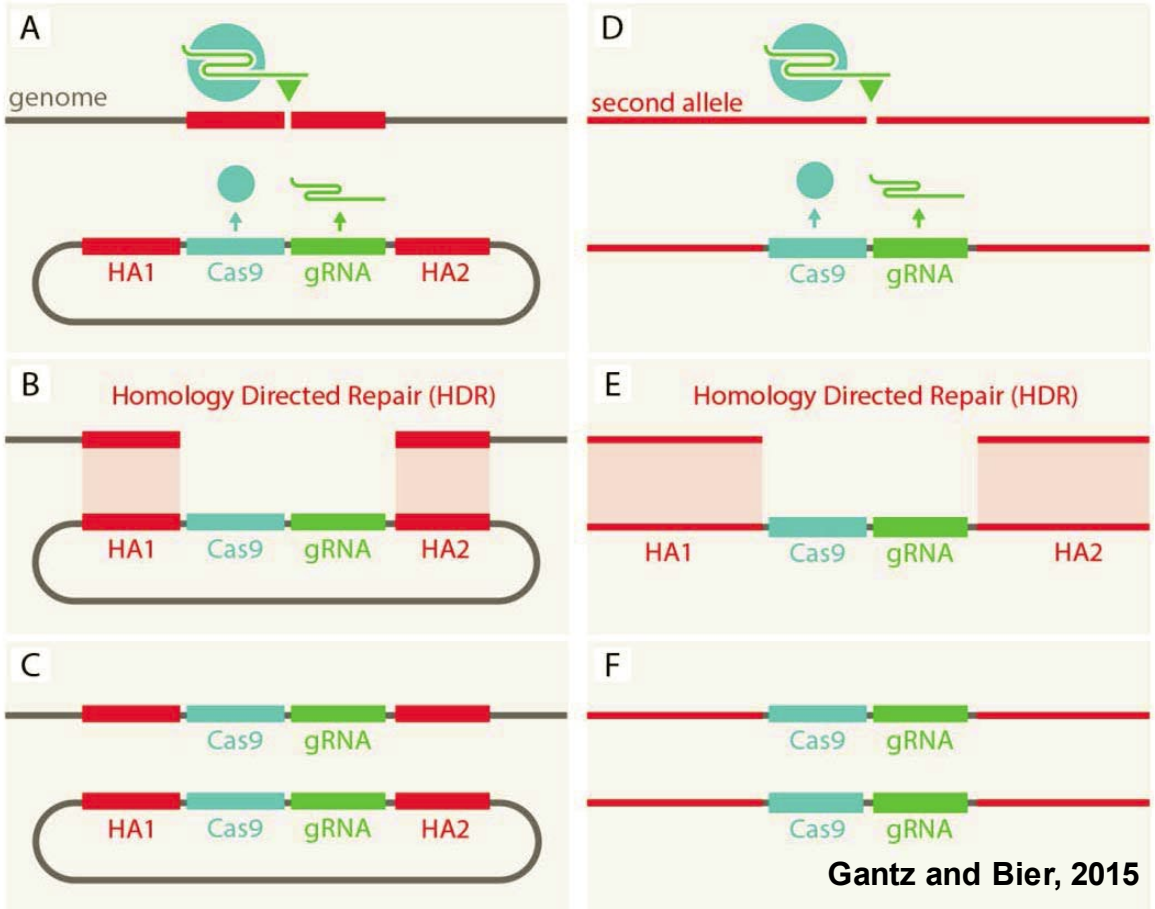
meiotic drive

segregation distorters (SD)

gene conversion

DNA-break induced repair

nuclease-mediated

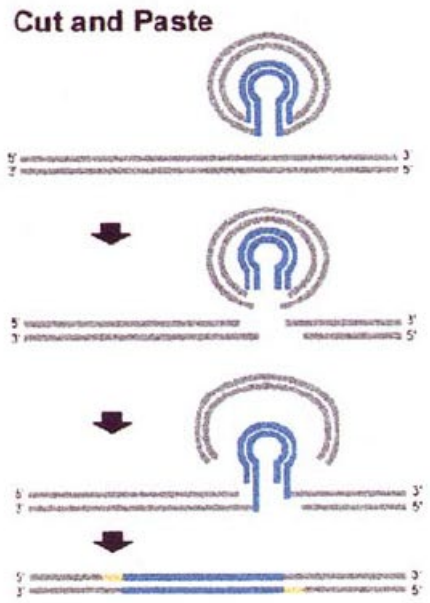


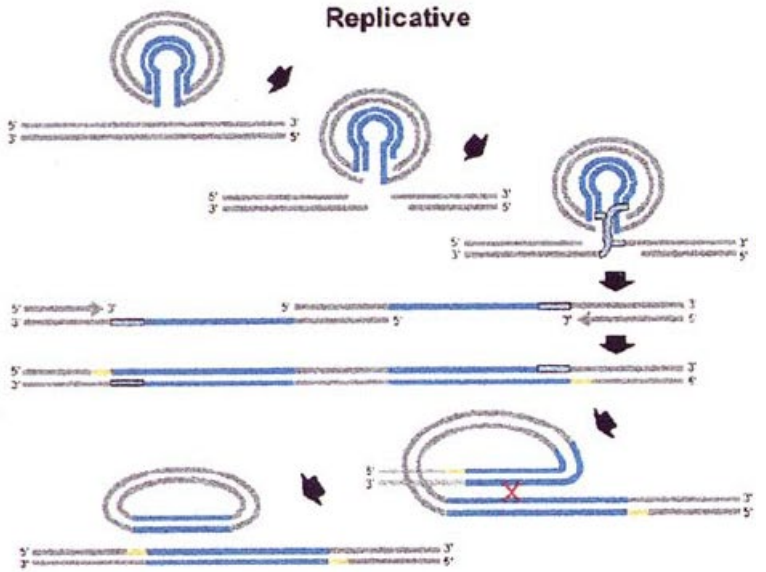
Gantz and Bier, 2015

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:

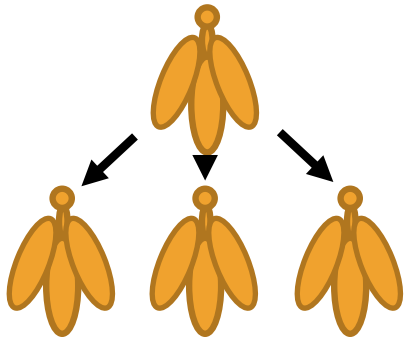
Genetic or epigenetic element originating from or common to the wild-type of the species of interest

Exogenous:

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

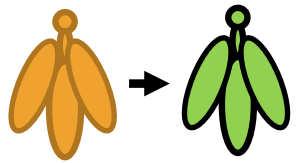
Vertical transmission:

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



Horizontal transmission (transfer):

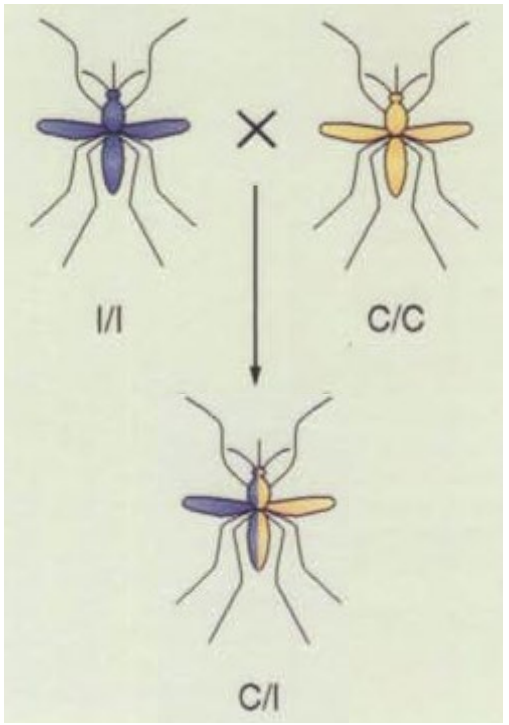
Genetic or epigenetic element passed from one organism to another (same or different species)



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

Genotypic and phenotypic consequences:

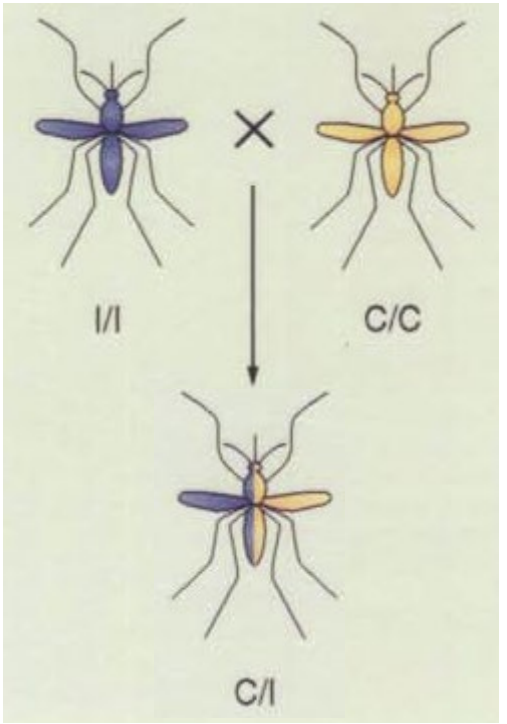
Mendelian inheritance



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

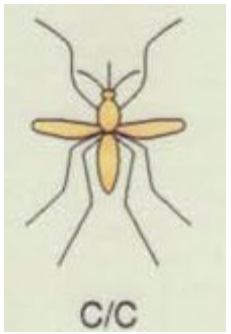
Test cross

Gene drive



!

50%



50%

All I/I

?

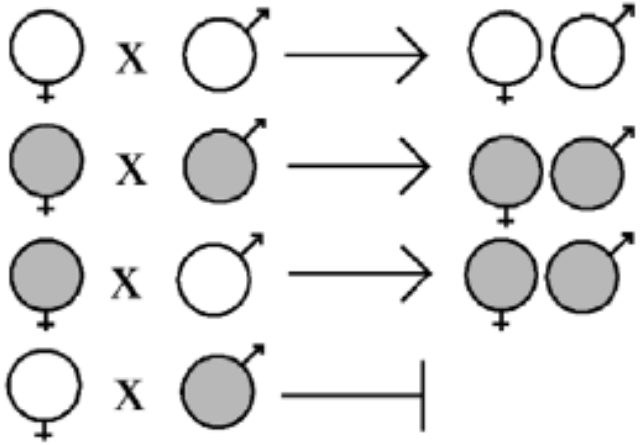
What are the genetics of gene drives?

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

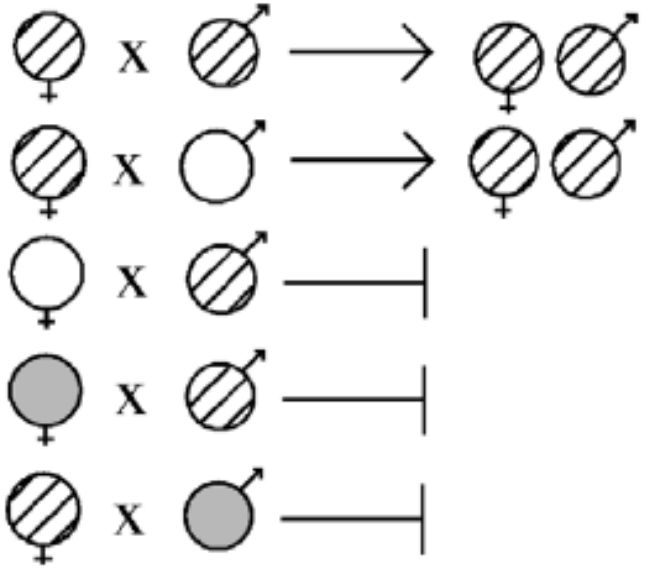
competitive displacement
- **reduced heterozygous fitness**
  - **under-dominance**
  - **hybrid sterility**

extracellular and intracellular symbiotic microorganisms
- **viruses**
- ***Wolbachia* species**
- **paratransgenesis**

Cytoplasmic incompatibility



Uni-directional

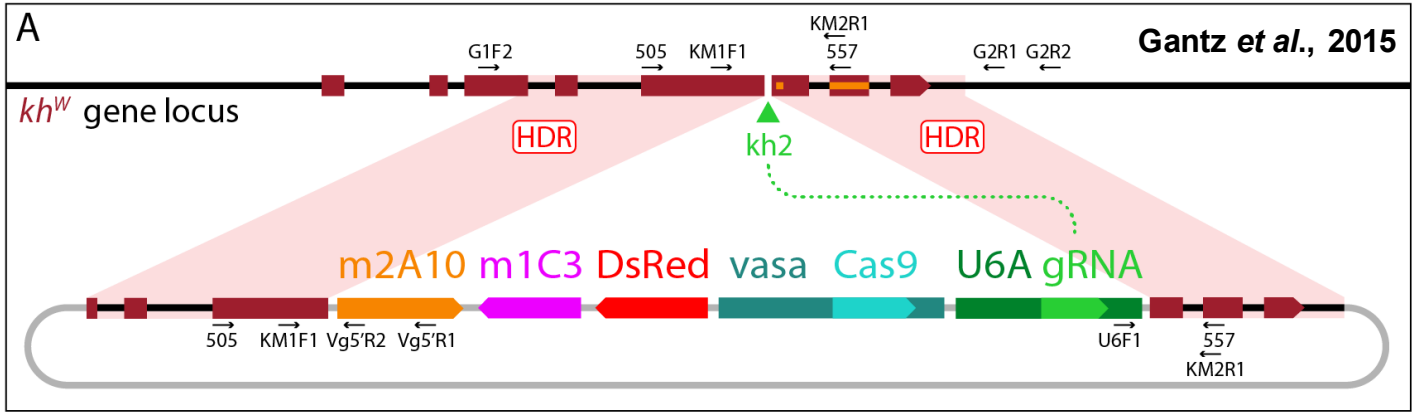


Bi-directional

What are some gene-drive system features?

Autonomous systems (also known as 'autocatalytic'):

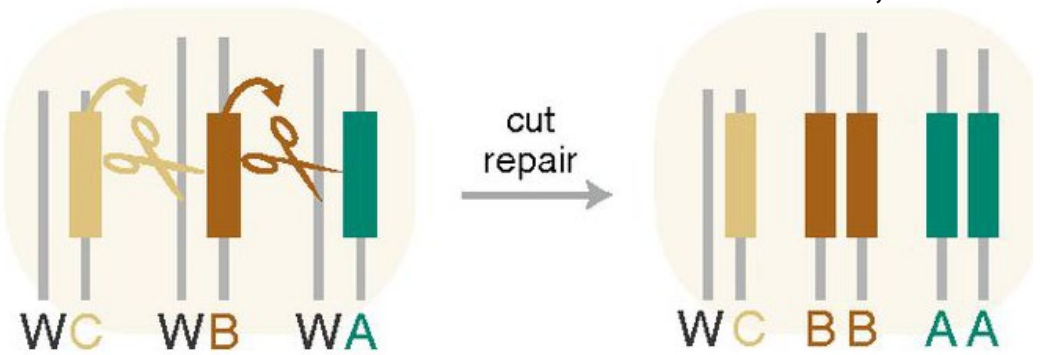
carry all the genetic information needed to self-mobilize or cause an inheritance bias tightly-linked in a *cis* configuration as part of a single construct



'**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

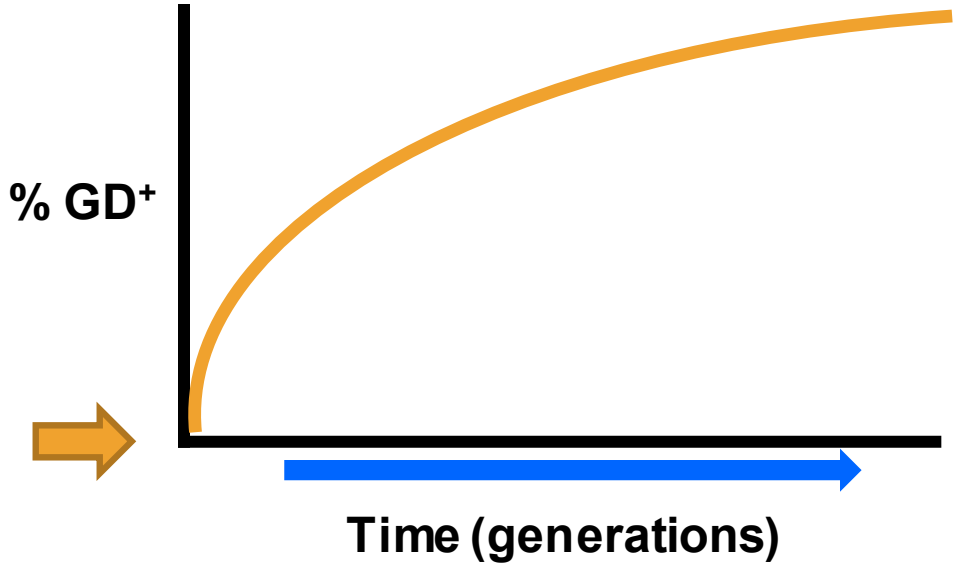
Nobel *et al.*, 2019



What are some gene-drive system features?

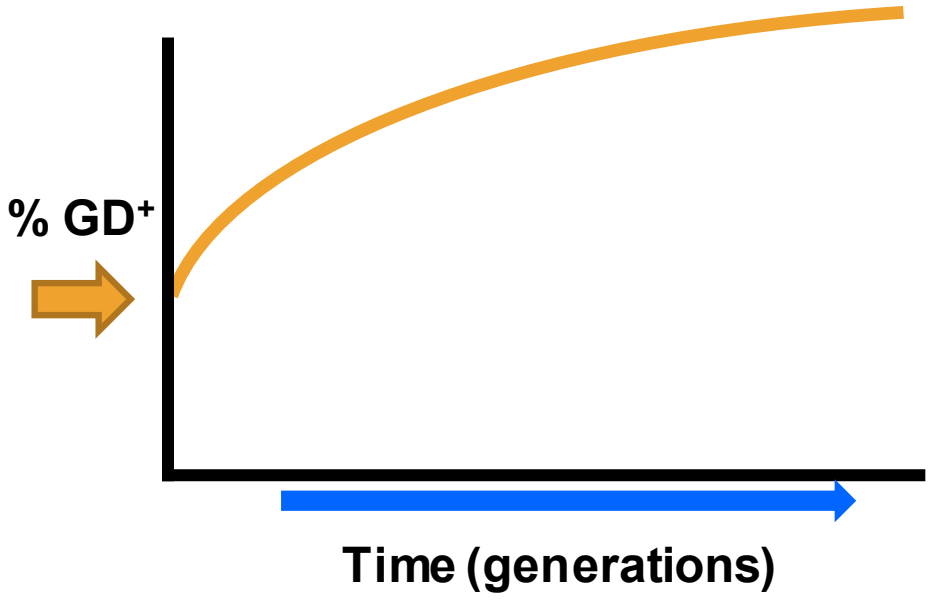
Low (no) threshold dynamics:

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



High threshold dynamics:

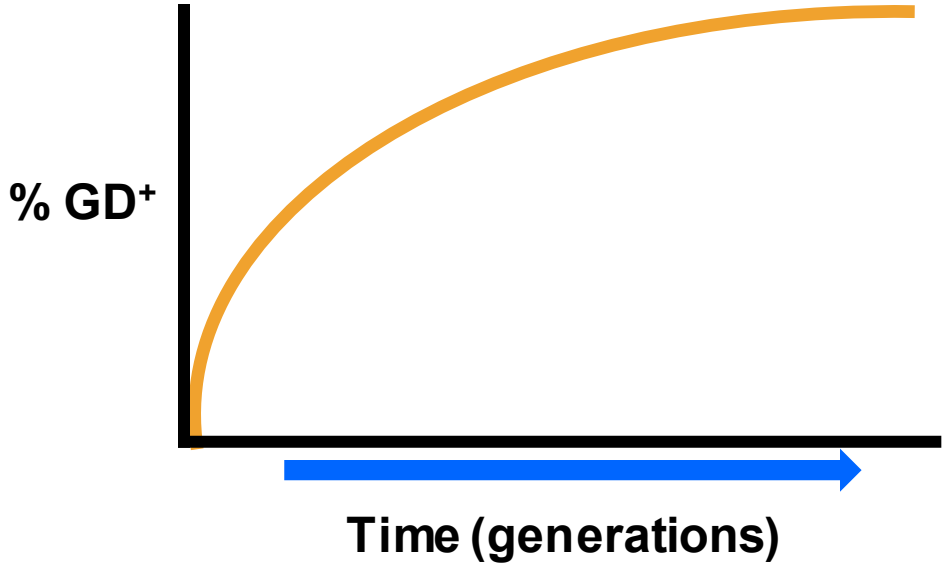
gene-drive organisms must be released above a minimal frequency in relation to the target population (either by one-time releases of larger numbers of mosquitoes or a by succession of serial releases)



What are some gene-drive system features?

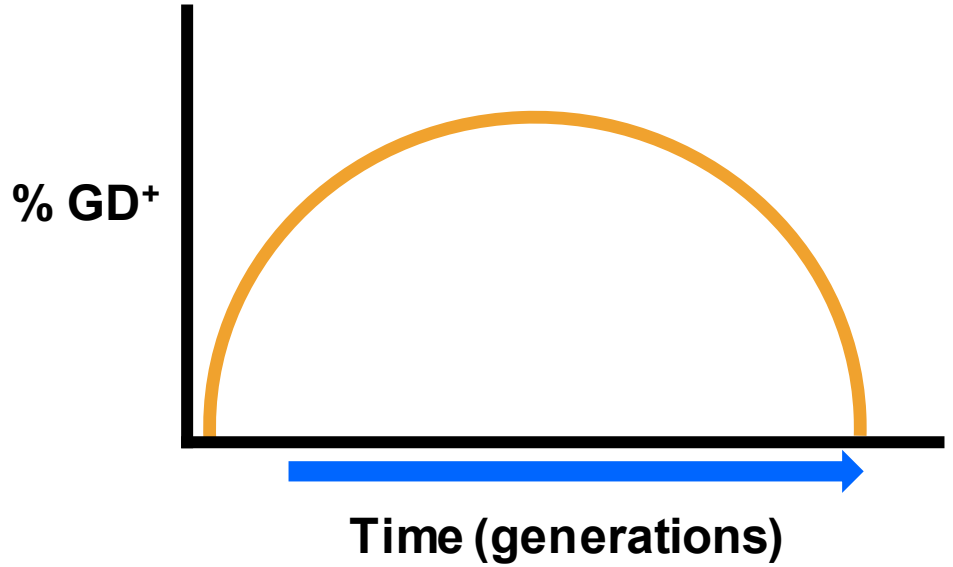
Non-limiting:

Gene drive organisms intended to persist in the environment



Self-limiting:

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

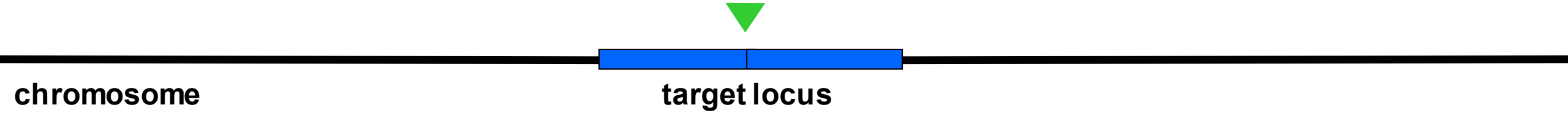


How do you make an autonomous Cas9 drive system?

Cas9 nuclease
endonuclease

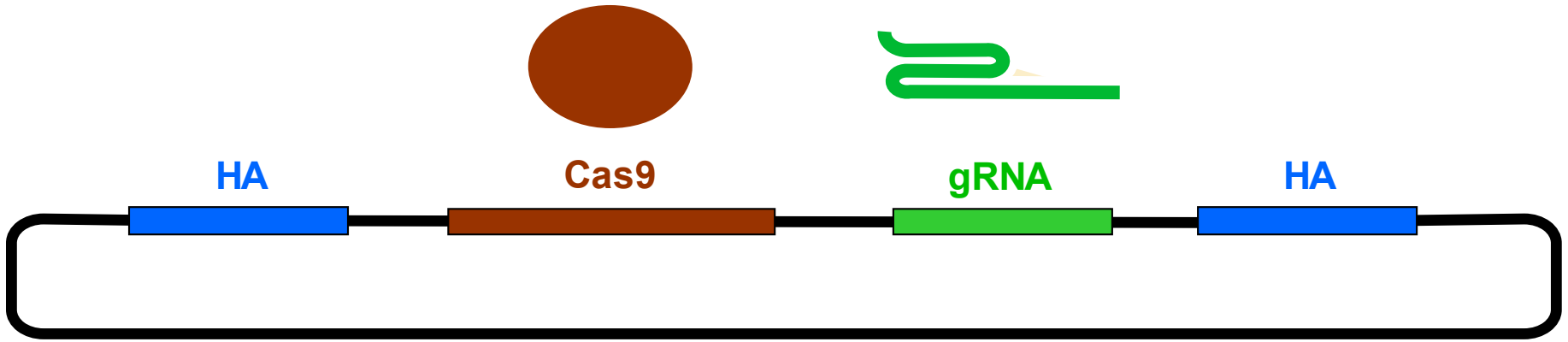
'guide' RNA
23 nucleotides in length

Homology Arms
1-2 kilobases in length*



chromosome

target locus



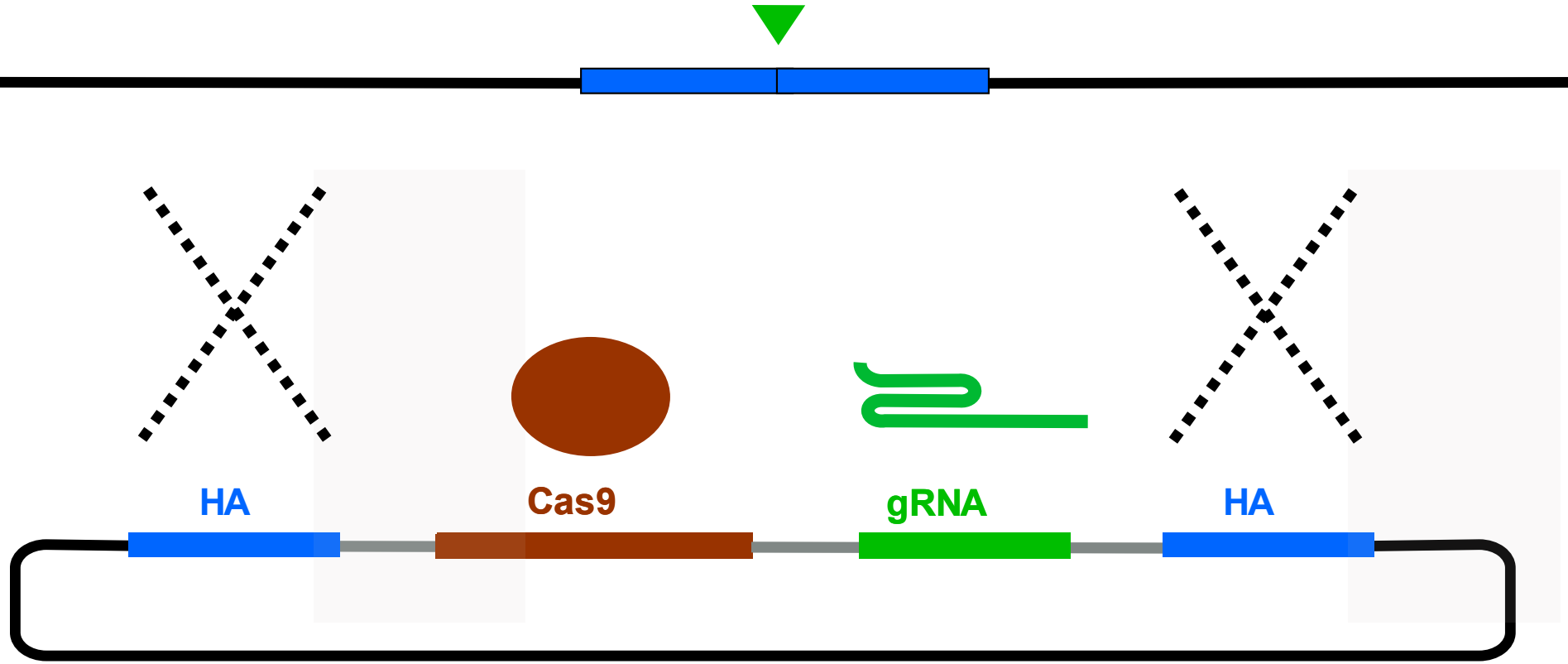
plasmid

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

Images courtesy of V. Gantz and E. Bier

How does an autonomous Cas9 drive system work?

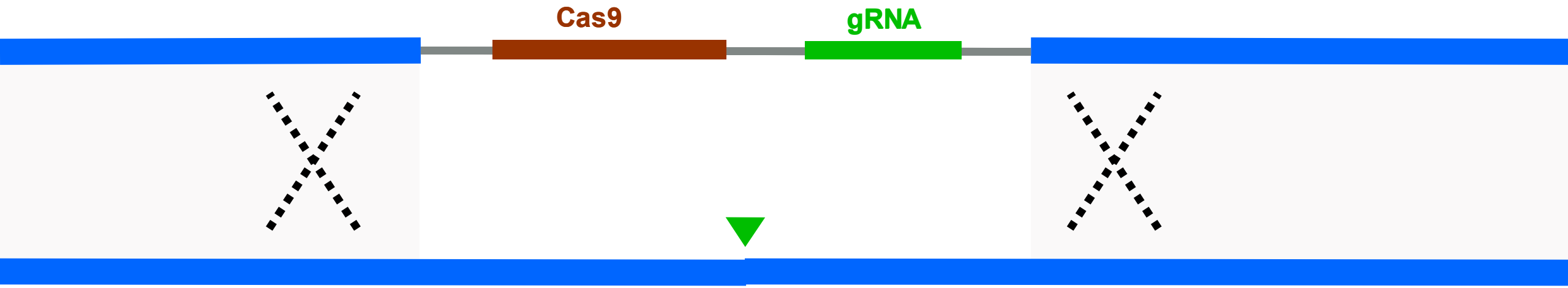
Primary integration into chromosome



Images courtesy of V. Gantz and E. Bier

How does an autonomous Cas9 drive system work?

Gene drive (interchromosomal)



Images courtesy of V. Gantz and E. Bier

What can gene drive be used for?

Introduce **favorable** traits into populations

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

Pest species

Cotton: Pink Bollworm

Pectinophora gossypiella

Citrus: Mexican fruit fly

Anastrepha ludens

Many:

Drosophila suzukii

Cattle: Screw worm

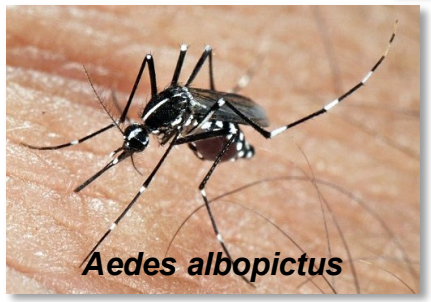
Cochliomyia hominivorax

What are possible public health merits?

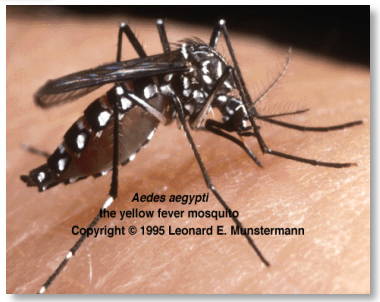
Control/alter:

Vectors

Malaria



Dengue
Chikungunya
Zika



Lyme's Disease

Pathogens

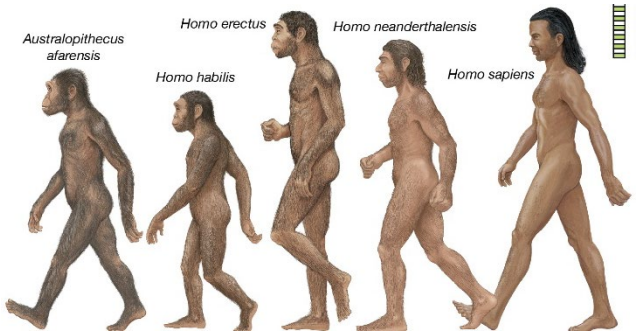
Reservoirs

'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?

Containment

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	sgRNA and Cas9 in separate loci (*8*)
	Target synthetic sequences absent from wild organisms	Drive targets a sequence unique to laboratory organisms (*3*,*4*,*8*)
Ecological	Perform experiments outside the habitable range of the organism	*Anopheles* mosquitoes in Boston
	Perform experiments in areas without potential wild mates	*Anopheles* mosquitoes in Los Angeles
Reproductive	Use a laboratory strain that cannot reproduce with wild organisms	*Drosophila* with compound autosomes*
Barrier	Physical barriers between organisms and the environment	Triply nested containers, >3 doors (*6*)
	•Remove barriers only when organisms are inactive	Anesthetize before opening (*6*)
	•Impose environmental constraints	Low-temperature room, air-blast fans
	•Take precautions to minimize breaches due to human error	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.

Akbari *et al.*, (2015) Safeguarding gene drive experiments in the laboratory. *Science* 349, 927-929. PMID: 26229113

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!