Overview of Gene Drive Technology and Applications

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**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

Gantz and Bier, 2015
Infectious and infectious-like agents
extracellular and intracellular symbiotic microorganisms
viruses
cytoplasmic incompatibility (*Wolbachia* species)
paratransgenesis
Transposons
conservative
replicative

What are gene-drive mechanisms?

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

**Gene drive**

- Test cross

50% 50%

All I/I
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

What are the genetics of gene drives?

- Uni-directional
- Bi-directional
- Cytoplasmic incompatibility
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
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 by succession of serial releases).
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 (≤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
Introduce favorable traits into populations

What can gene drive be used for?

Population suppression

Population replacement (modification/alteration)

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
- 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
  - *Anopheles*
- Dengue
  - *Aedes albopictus*
- Chikungunya
- Zika

**Pathogens**
- *Schistosoma mansoni*

**Reservoirs**
- *Biomphalaria glabrata*

‘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?

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

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!