Ecological Engineering with CRISPR and Gene Drive

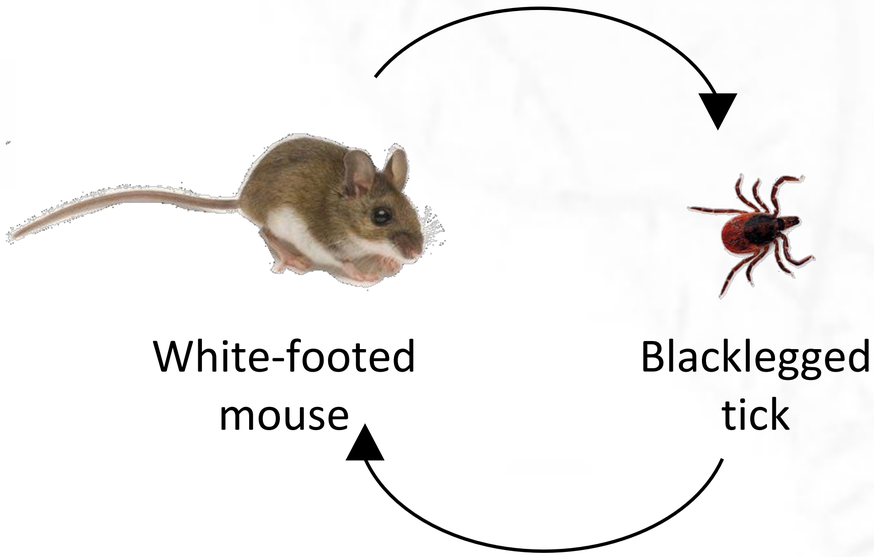
Kevin M. Esvelt, PhD
Assistant Professor, MIT Media Lab

MIT Industrial Liaison Program, 2017

Lyme Disease Incidence, 2015

Tick-borne disease is an ecological problem

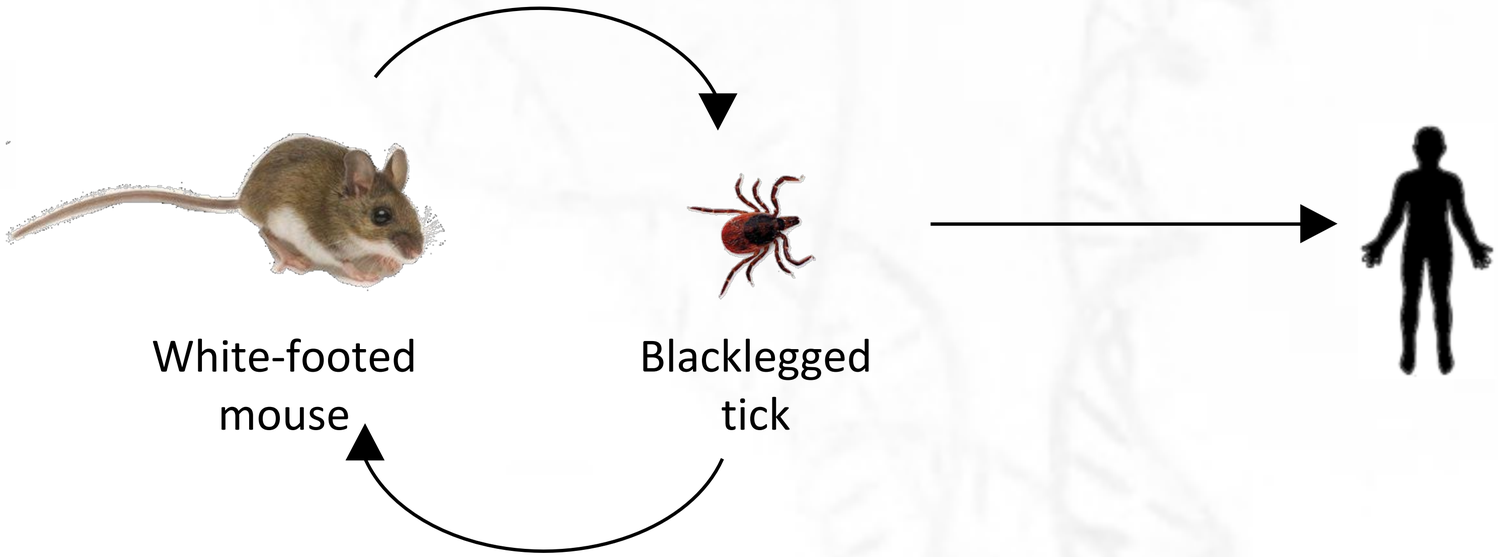
Borrelia
Babesia
Anaplasma
Ehrlichia



The pathogens that cause tick-borne disease persist by moving between mice and ticks

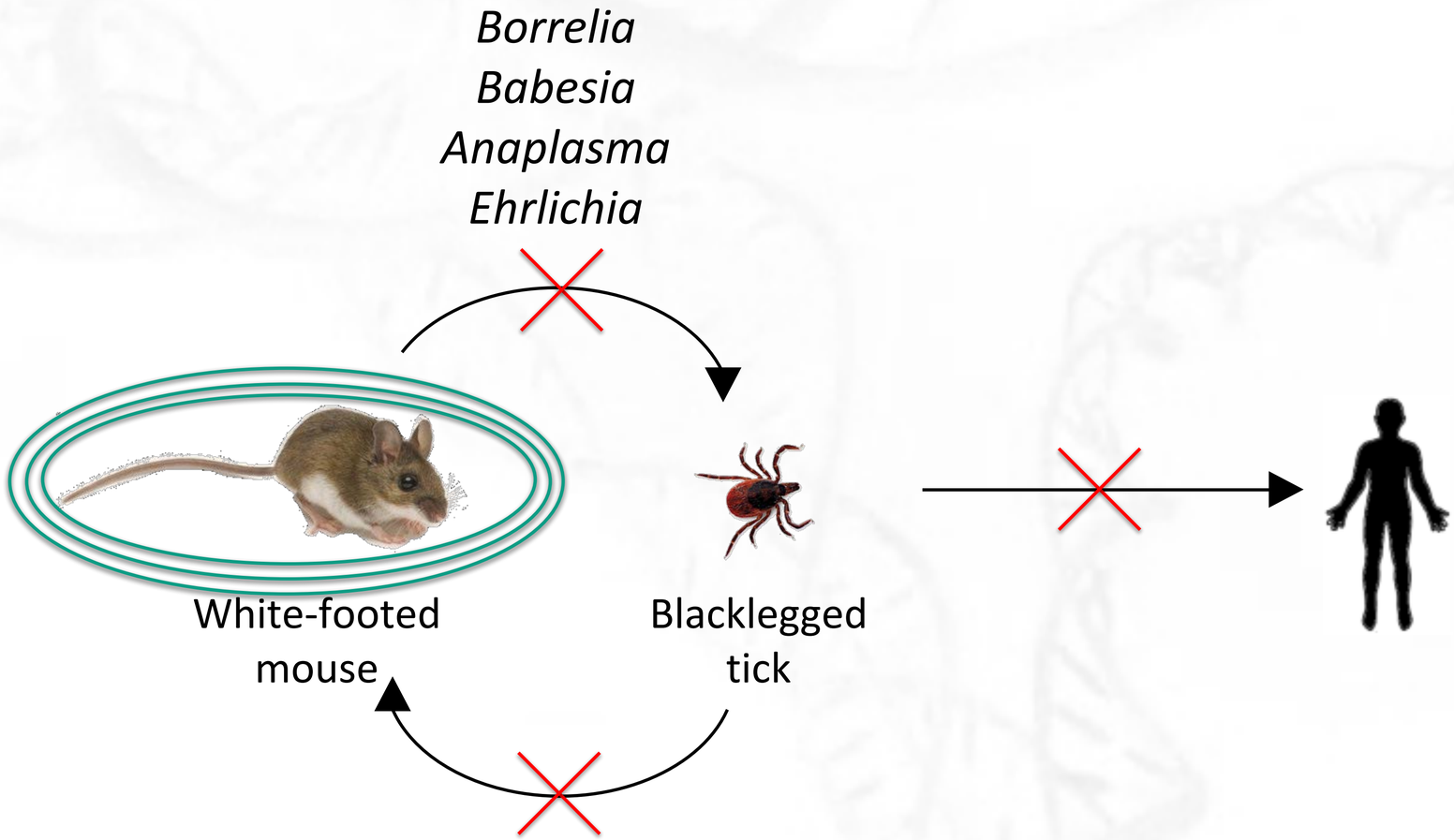
Tick-borne disease is an ecological problem

Borrelia
Babesia
Anaplasma
Ehrlichia



Ticks pass the pathogens to humans, causing disease

Tick-borne disease is an ecological problem



What if white-footed mice could not become infected?

Mice Against Ticks

sculpting evolution

mit media lab

A Community Effort to Prevent Tick-Borne Disease

photo: Yousur Al-Hlou/ The New York Times

How do you lastingly engineer a wild population (of white-footed mice)?

Release LOTS of engineered mice in spring

Moral Challenges Posed By Ecotechnologies

People who aren't informed of experiments **are denied a voice**
in decisions intended to affect them

Local, Open, Responsive Science

- Address problems obvious to all
- Openly share proposals before experiments begin
- Actively invite concerns & community guidance
- Arrange for independent assessment

Nantucket & Vineyard Community Meetings

~30 citizens on Nantucket (+ Board of Health)

~120 citizens on Martha's Vineyard (+ health agents of 6 towns)

Community decisions:

- **Research should begin immediately**
- **Avoid introducing any non-mouse DNA**
- **Project should be community-governed**

Rules for Engineering Complex Systems

Be humble!

1. **Make the smallest change that might solve the problem**

2. **Start local and scale up if warranted**

Two types of antibodies for different problems

Anti-Lyme Antibody

Protects mice from the Lyme spirochete only

Antibody target: *OspA*
an outer surface protein on
Borrelia burgdorferi

Anti-tick Antibody

Protects mice from ***all*** pathogens carried by black-legged ticks

Antibody target: *subolesin*,
a tick salivary protein

Communities: "Do everything! But be sure to eliminate ticks"

Preventing tick-borne disease by ecological vaccination

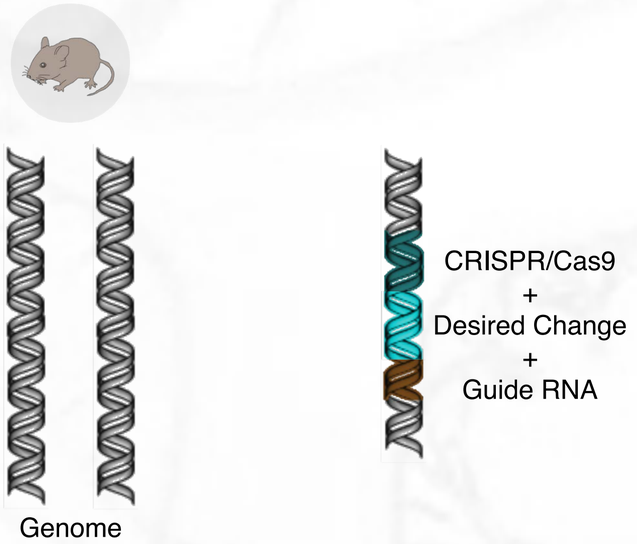
White-footed mice

Steering Committees are meeting this winter to begin applying for regulatory approval

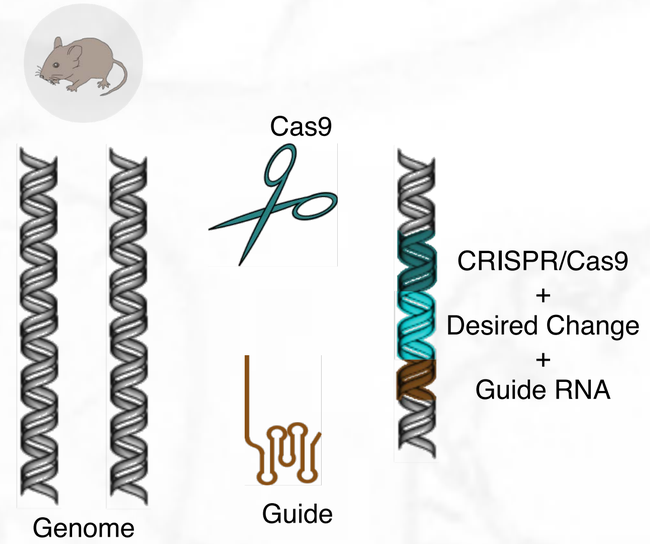
Earliest possible release on Nantucket/Vineyard = 2024

But what about the mainland?

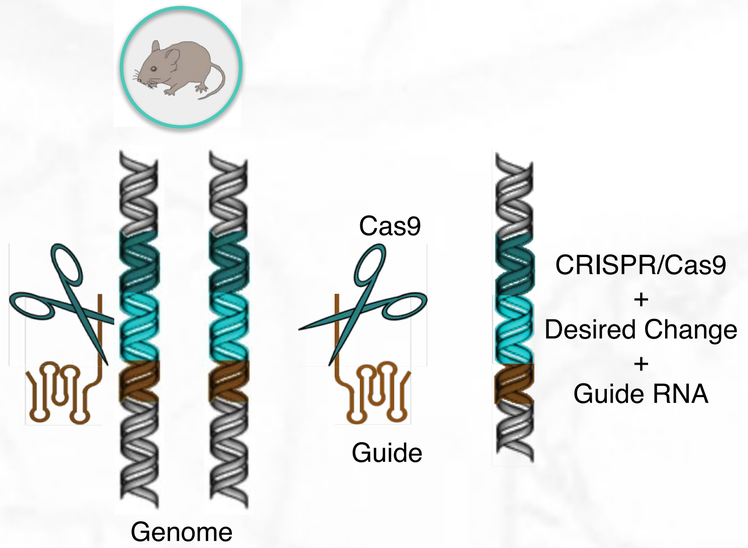
Gene Drive



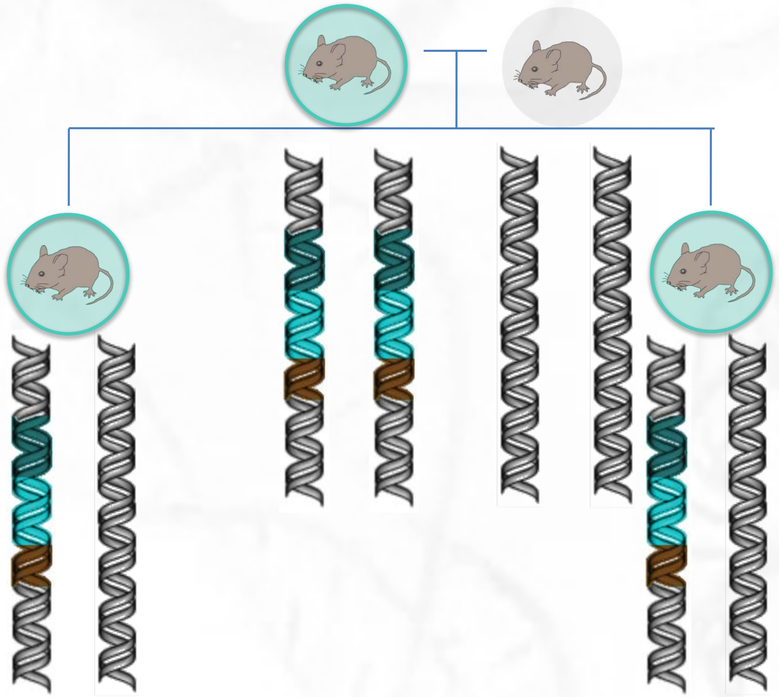
Gene Drive



Gene Drive

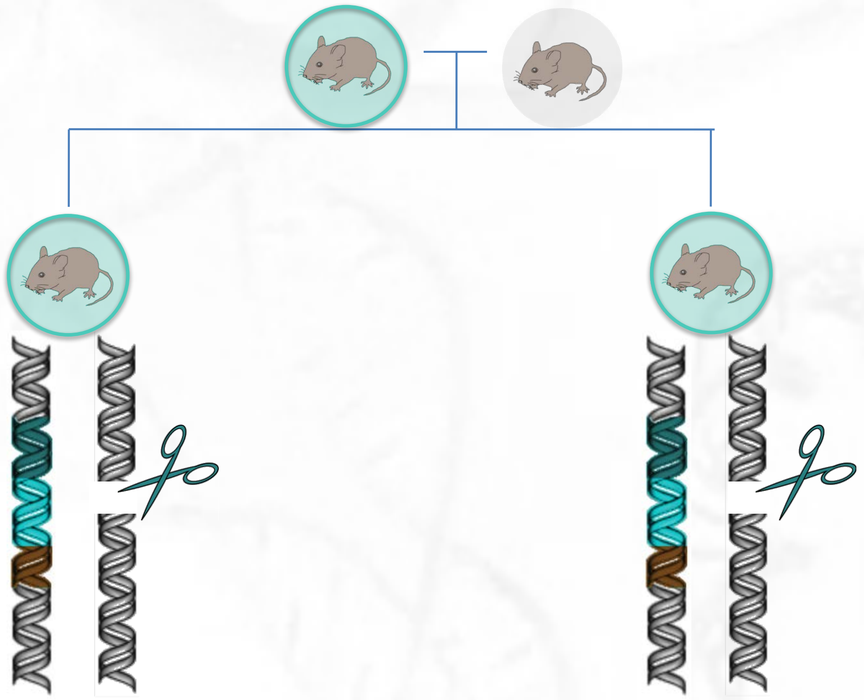


Gene Drive



Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*
Oye K, Esvelt K et al. (2014) *Science*

Gene Drive



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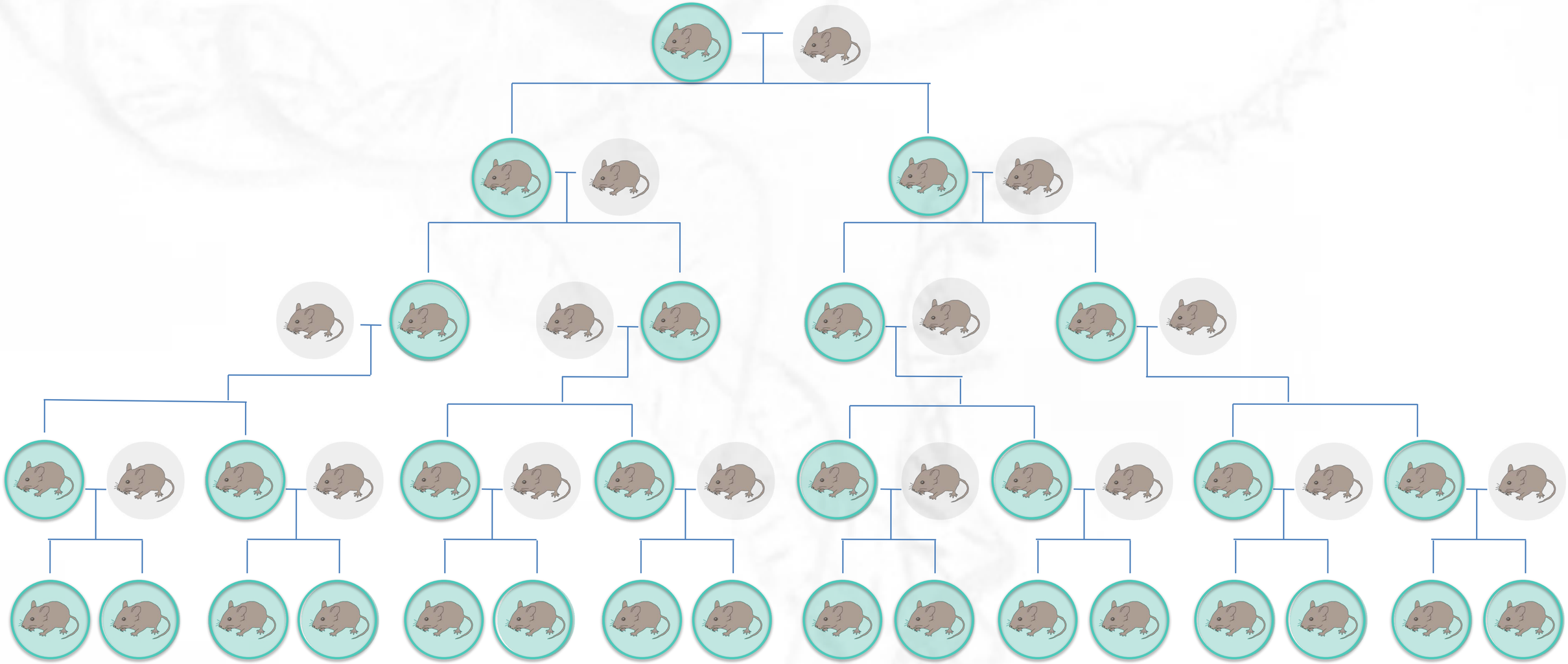

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CRISPR-based gene drive could save millions of human lives….

… but faces major hurdles

Should we be conducting gene drive experiments in secret?

eLIFE
elifesciences.org

Concerning RNA-guided gene drives for the alteration of wild populations

KEVIN M ESVELT*, ANDREA L SMIDLER, FLAMINIA CATTERUCCIA* AND GEORGE M CHURCH*

Sciencexpress

BIOTECHNOLOGY

Regulating gene drives

Regulatory gaps must be filled before gene drives could be used in the wild

By **Kenneth A. Oye**,[1,2*†] **Kevin Esvelt**,[3*] **Evan Appleton**,[4] **Flaminia Catteruccia**,[5,6] **George Church**,[3] **Todd Kuiken**,[7] **Shlomiya Bar-Yam Lightfoot**,[2] **Julie McNamara**,[2] **Andrea Smidler**,[5,8] ***and*** **James P. Collins**[9]

cannot be used to engineer populations of viruses or bacteria. Second, a newly released drive will typically take dozens of generations to affect a substantial proportion of a target population, unless drive-containing organisms are released in numbers consti-

We pre-register all of our gene drive experiments and make all grant proposals publicly avilable

New Results

Daisy-chain gene drives for the alteration of local populations

Charleston Noble, John Min, Jason Olejarz, Joanna Buchthal, Alejandro Chavez, Andrea L Smidler, Erika A DeBenedictis, George M Church, Martin A Nowak, Kevin M Esvelt

doi: https://doi.org/10.1101/057307

This article is a preprint and has not been peer-reviewed [what does this mean?].

New Results

Daisy quorum drives for the genetic restoration of wild populations

John Min, Charleston Noble, Devora Najjar, Kevin Esvelt

doi: https://doi.org/10.1101/115618

This article is a preprint and has not been peer-reviewed [what does this mean?].

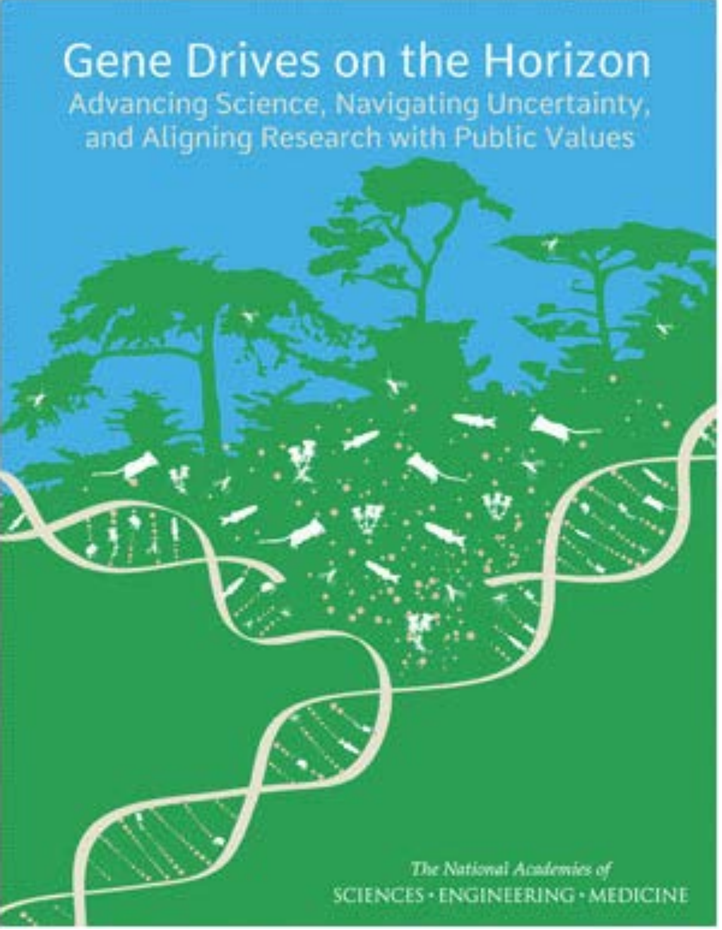
New Results

Daisyfield gene drive systems harness repeated genomic elements as a generational clock to limit spread

John Min, Charleston Noble, Devora Najjar, Kevin M Esvelt

doi: https://doi.org/10.1101/104877

This article is a preprint and has not been peer-reviewed [what does this mean?].

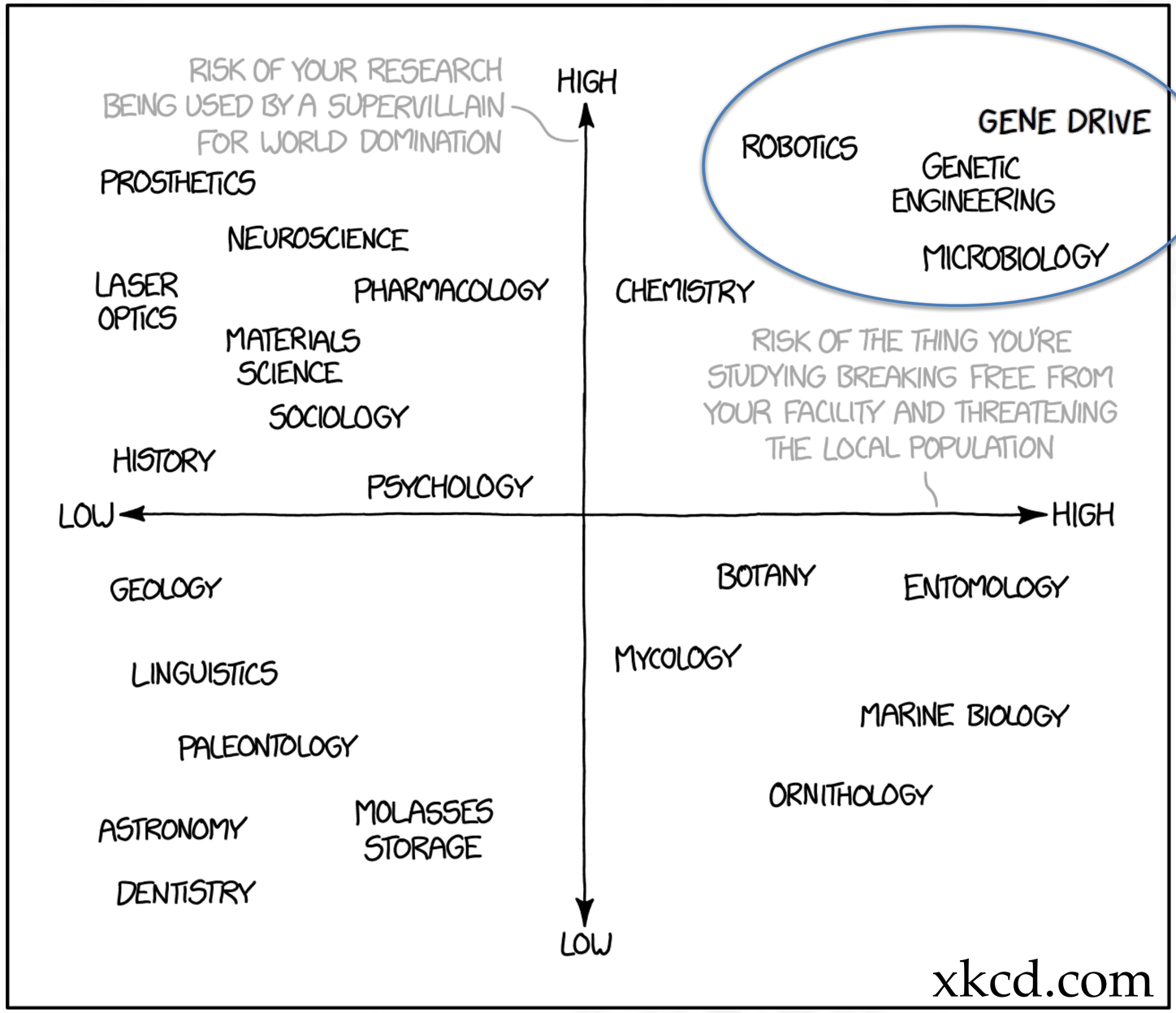


"The best course of action is to ensure that those who would be affected by a proposed project or policy have an opportunity to have a voice in decisions about it."

- U.S. National Academies report on gene drive

But almost no one with leverage is doing anything to make it happen

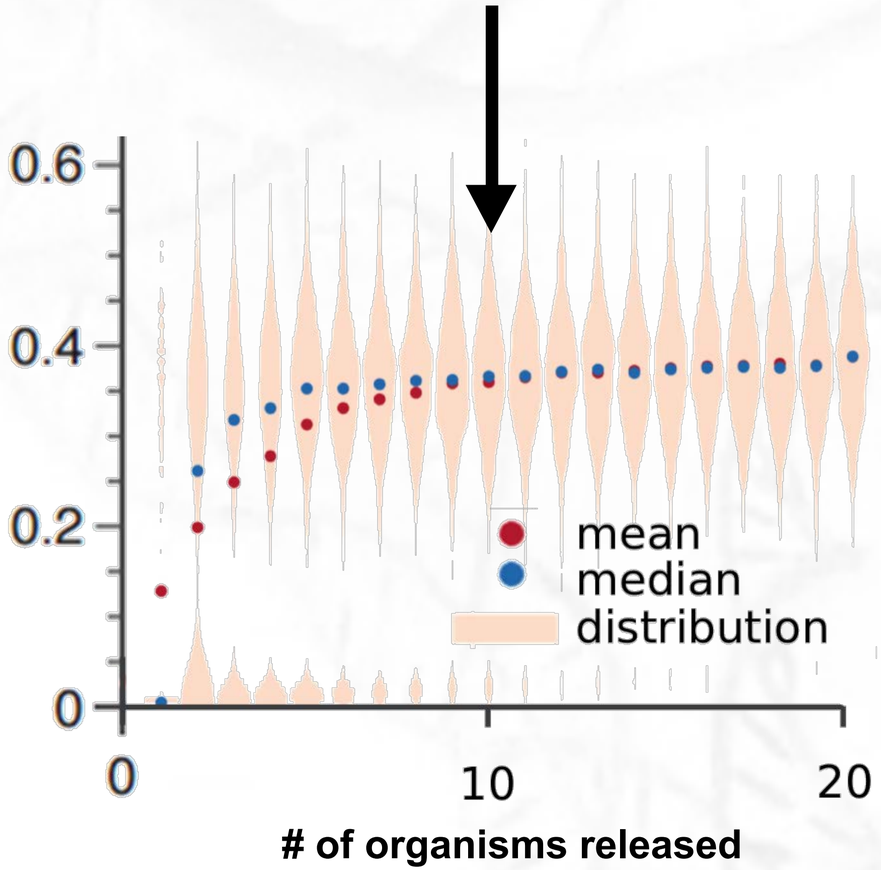
We are working to change scientific incentives via IP, journals, funders, policy


RISK OF YOUR RESEARCH BEING USED BY A SUPERVILLAIN FOR WORLD DOMINATION
HIGH
ROBOTICS
GENE DRIVE
GENETIC ENGINEERING
MICROBIOLOGY
PROSTHETICS
NEUROSCIENCE
LASER OPTICS
PHARMACOLOGY
CHEMISTRY
MATERIALS SCIENCE
SOCIOLOGY
HISTORY
PSYCHOLOGY
RISK OF THE THING YOU'RE STUDYING BREAKING FREE FROM YOUR FACILITY AND THREATENING THE LOCAL POPULATION
LOW
HIGH
GEOLOGY
BOTANY
ENTOMOLOGY
LINGUISTICS
MYCOLOGY
MARINE BIOLOGY
PALEONTOLOGY
ORNITHOLOGY
ASTRONOMY
MOLASSES STORAGE
DENTISTRY
LOW
xkcd.com

Another problem: invasiveness

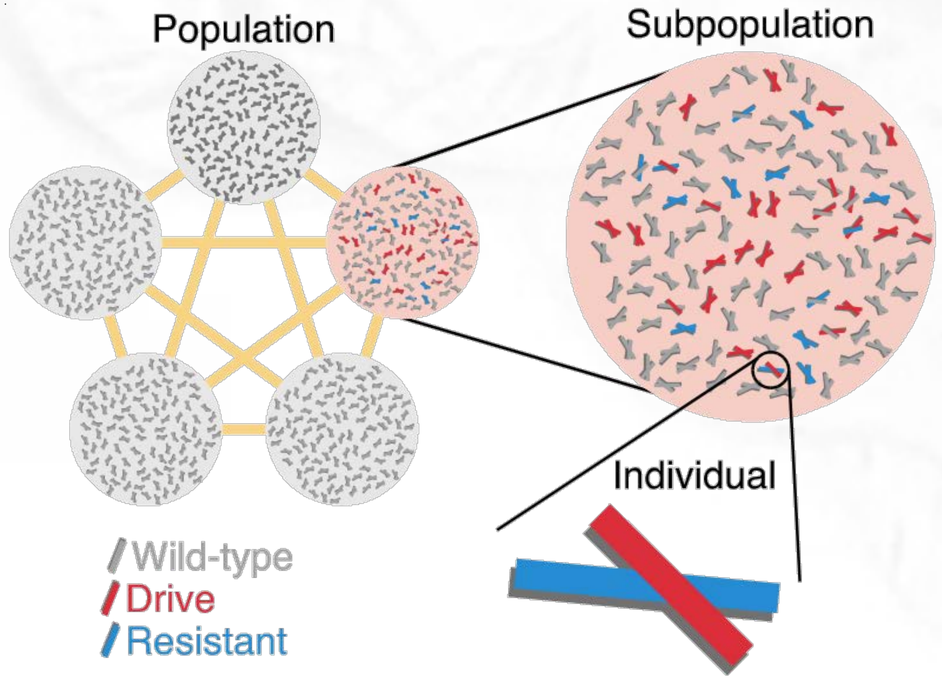
10 organisms = highly likely to invade

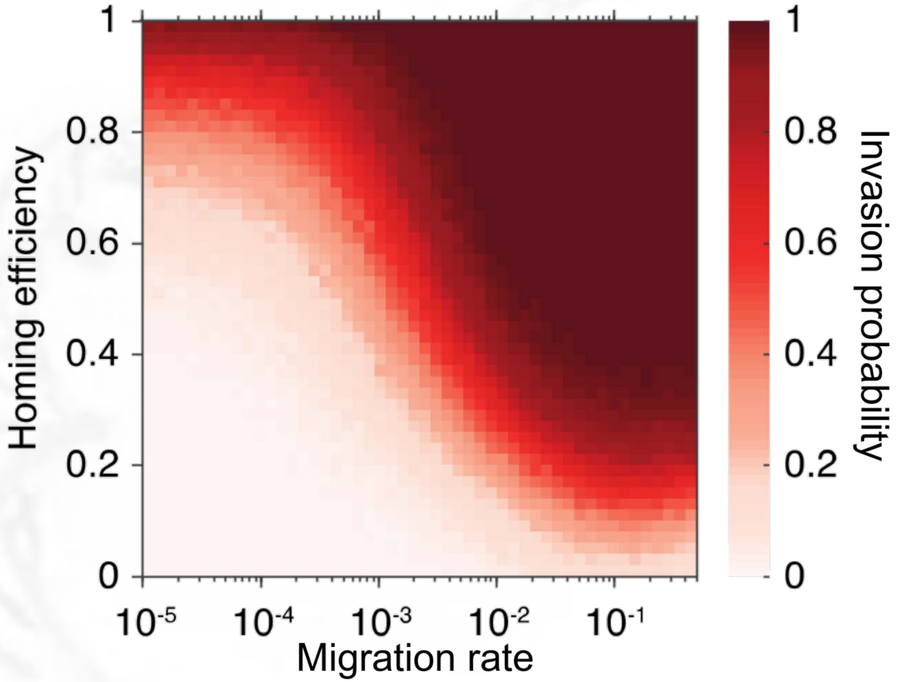
Weakest reported CRISPR gene drive system (fruit fly)



Charleston Noble

Noble C, Adlam B, Church GM, Esvelt KM, Nowak MA *submitted*





Charleston Noble

Noble C, Adlam B, Church GM, Esvelt KM, Nowak MA *submitted*

A single accident could be devastating for public trust

Sciencexpress | Policy Forum

Safeguarding gene drive experiments in the laboratory

By **Omar S. Akbari,[1,2] Hugo J. Bellen,[3,4] Ethan Bier,[5*] Simon L. Bullock,[6] Austin Burt,[7] George M. Church,[8,9] Kevin R. Cook,[10] Peter Duchek,[11] Owain R. Edwards,[12] Kevin M. Esvelt,[8*] Valentino M. Gantz,[5] Kent G. Golic,[13] Scott J. Gratz,[14] Melissa M. Harrison,[15] Keith R. Hayes,[16] Anthony A. James,[17] Thomas C. Kaufman,[10] Juergen Knoblich,[11] Harmit S. Malik,[18,19] Kathy A. Matthews,[10] Kate M. O'Connor-Giles,[14,20] Annette L. Parks,[10] Norbert Perrimon,[9,21] Fillip Port,[6] Steven Russell,[22] Ryu Ueda,[23,24] Jill Wildonger[25]**

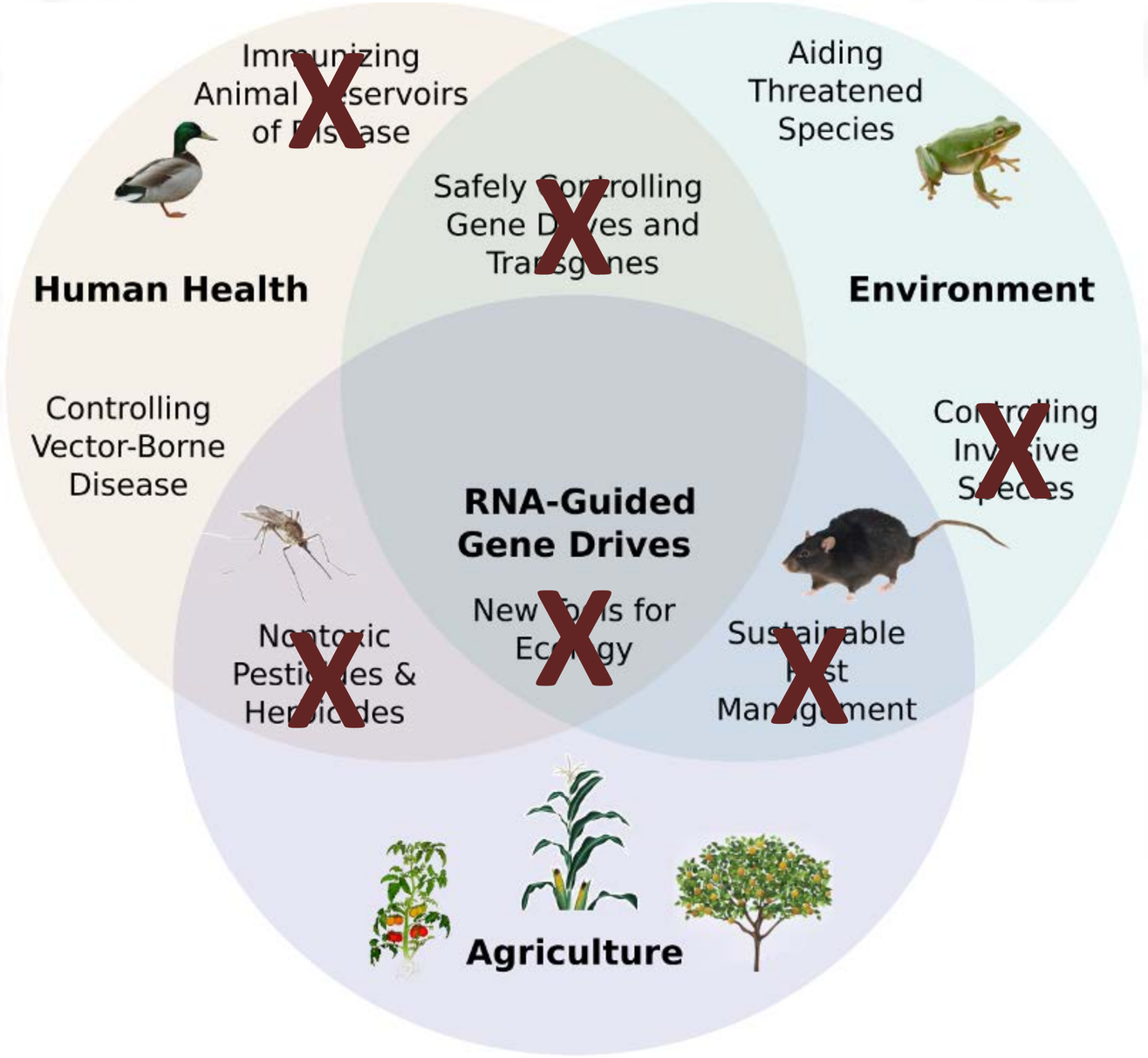
self-propagating pathogens must ensure that these agents do not escape to the outside world, scientists working in the laboratory with gene drive constructs are responsible for keeping them confined (*4*, *6*, *7*).

Two of us recently used a CRISPR/Cas9-based gene drive system to generate a *Drosophila* strain homozygous for a loss-of-function mutation [the mutagenic chain reaction (*6*)] (see the figure). Even though *D. melanogaster* ordinarily poses no threat to human health or agriculture,

Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*
Oye K, Esvelt K et al. (2014) *Science*
DiCarlo*, Chavez*, Dietz, Esvelt^, Church^ (2015) *Nature Biotechnology*

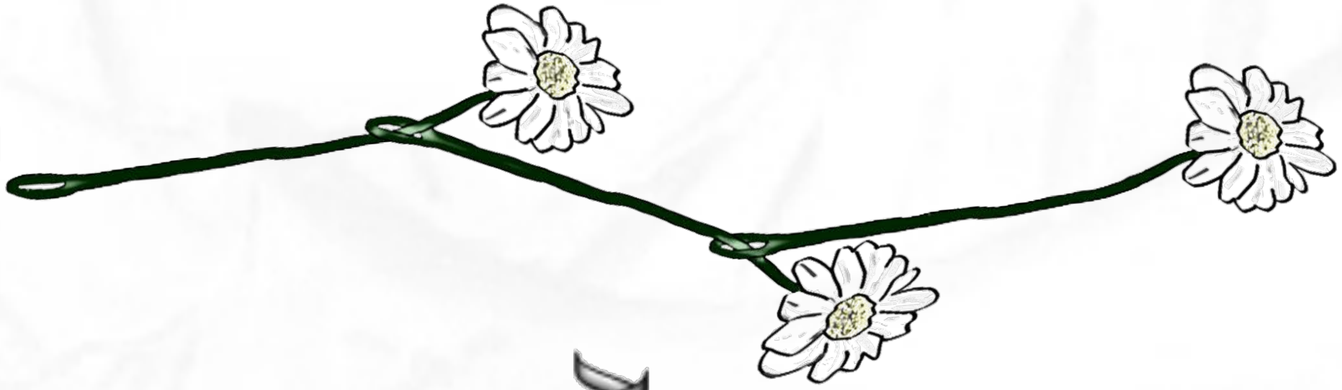
There is no such thing as a safe field trial

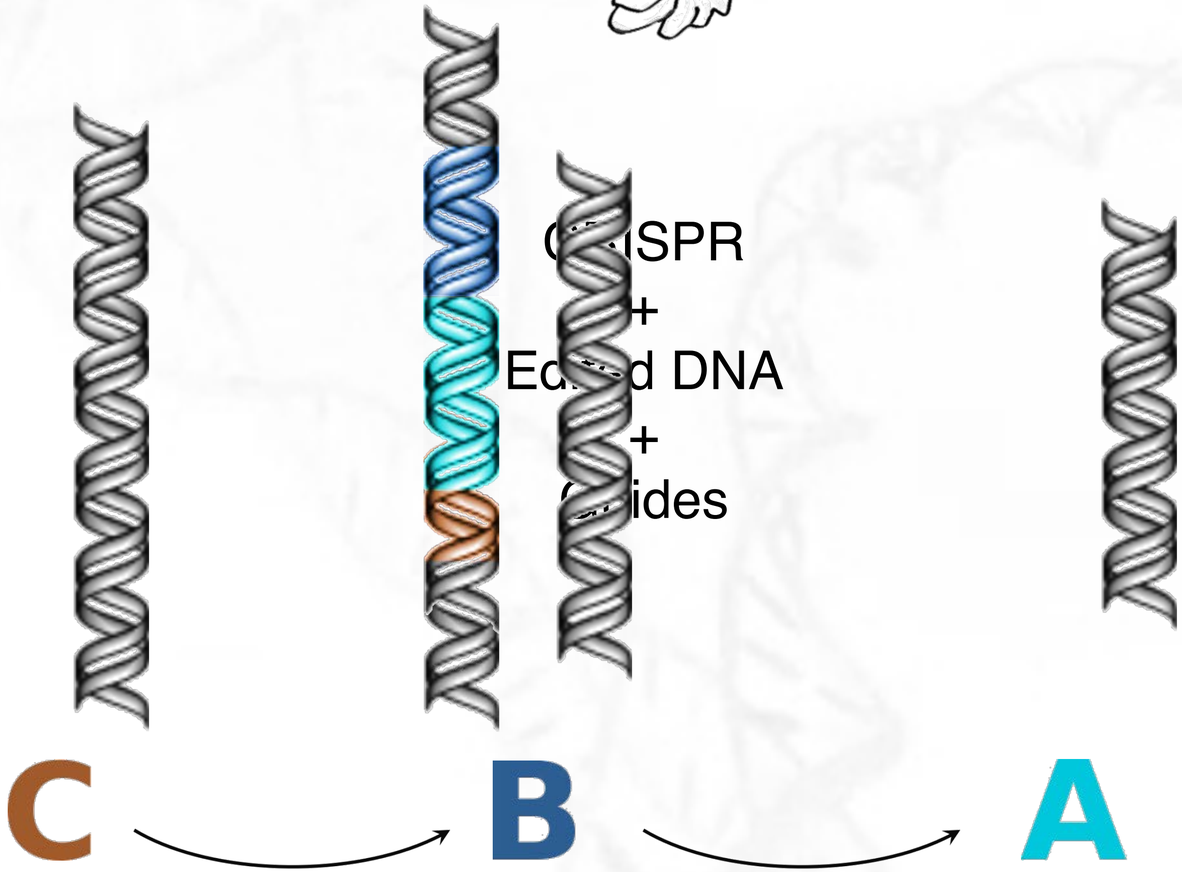
Every nation harboring the target species must agree *in advance*



Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*

Daisy-Chain Drive





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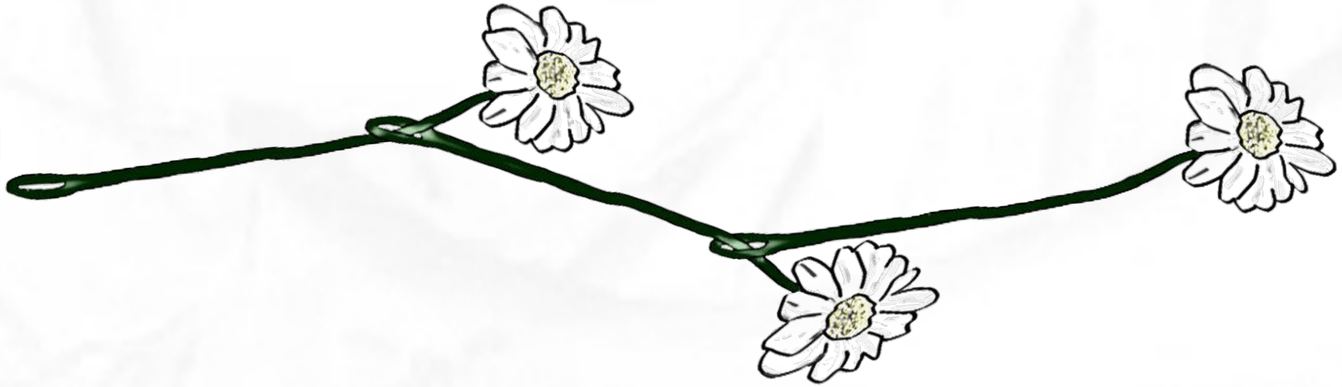
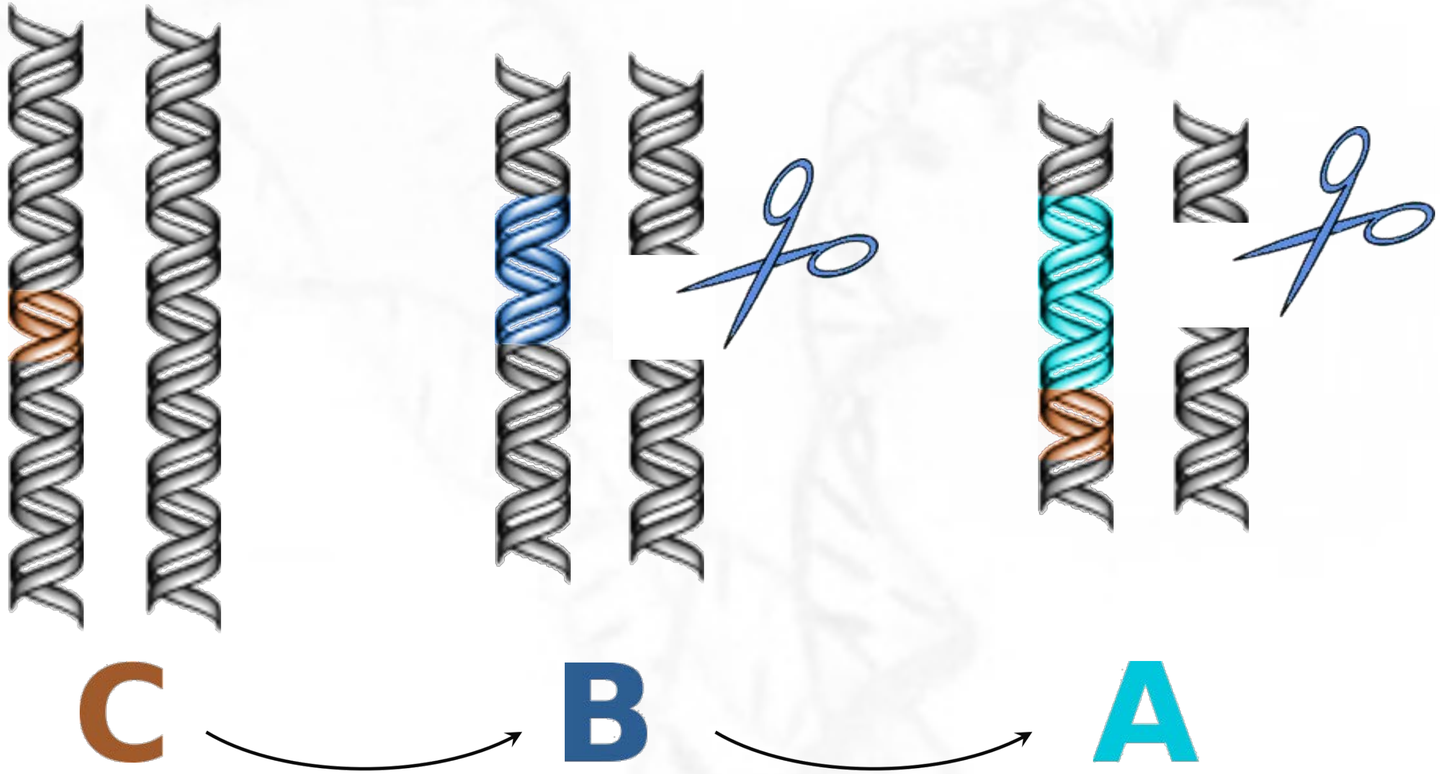
Daisy-Chain Drive





Daisy-Chain Drive

mit media lab

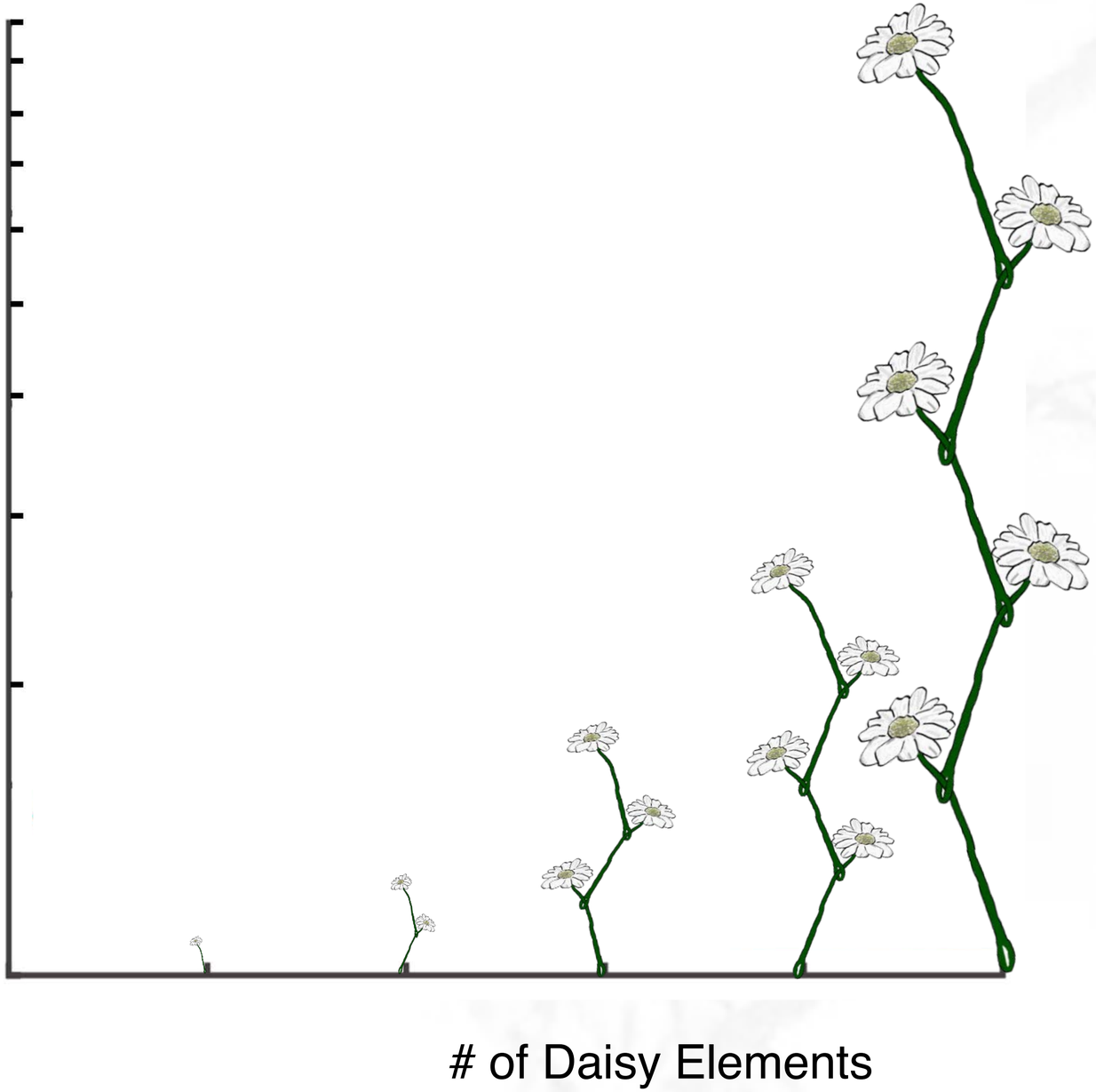
Noble C*, Min J*, Olejarz J, Buchthal J, Chavez A, Smidler AL, DeBenedictis EA, Church GM, Nowak M, Esvelt KM (2016) *bioRxiv*

CRISPR & Gene Drive

K. Esvelt

sculpting evolution

Daisy-Chain Drive



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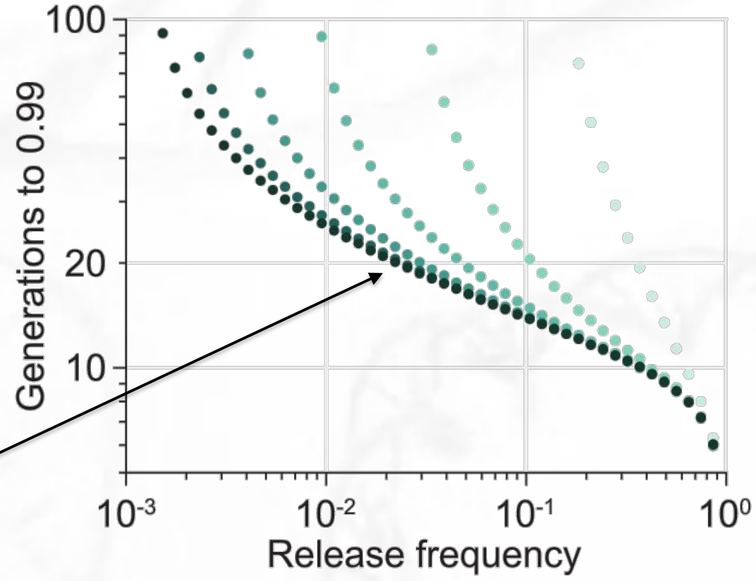
Daisy-Chain Drive

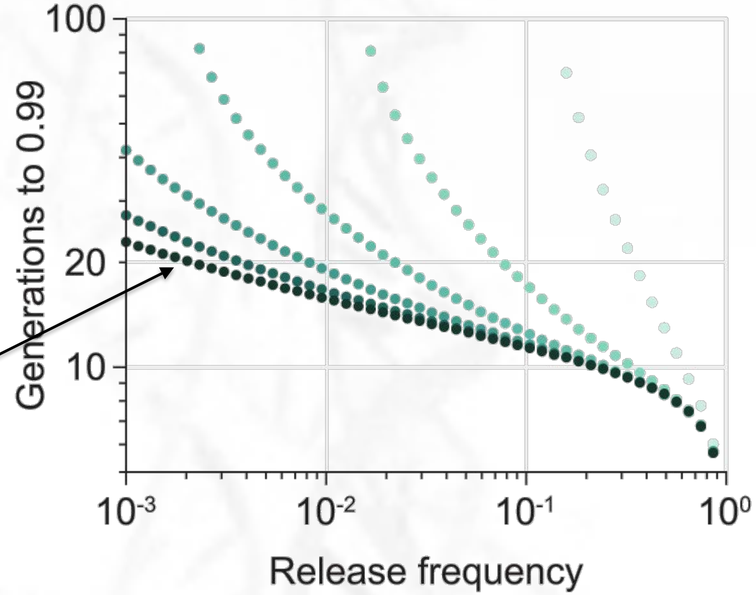
90%
homing

Release 1 daisy drive organism per 50 wild ones



98%
homing

Release 1 daisy drive organism per 500 wild ones



Parameters:

- cargo cost 10%
- cargo resistance lethal
- daisy cost 1% each
- daisy resistance neutral
- 1% release

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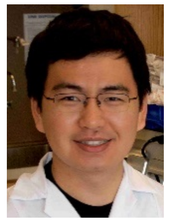
Min J, Noble C, Najjar D, Esvelt KM (2017) *bioRxiv*

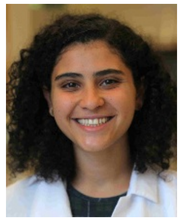
How do you stop gene flow across political boundaries?

Let the genes vote!

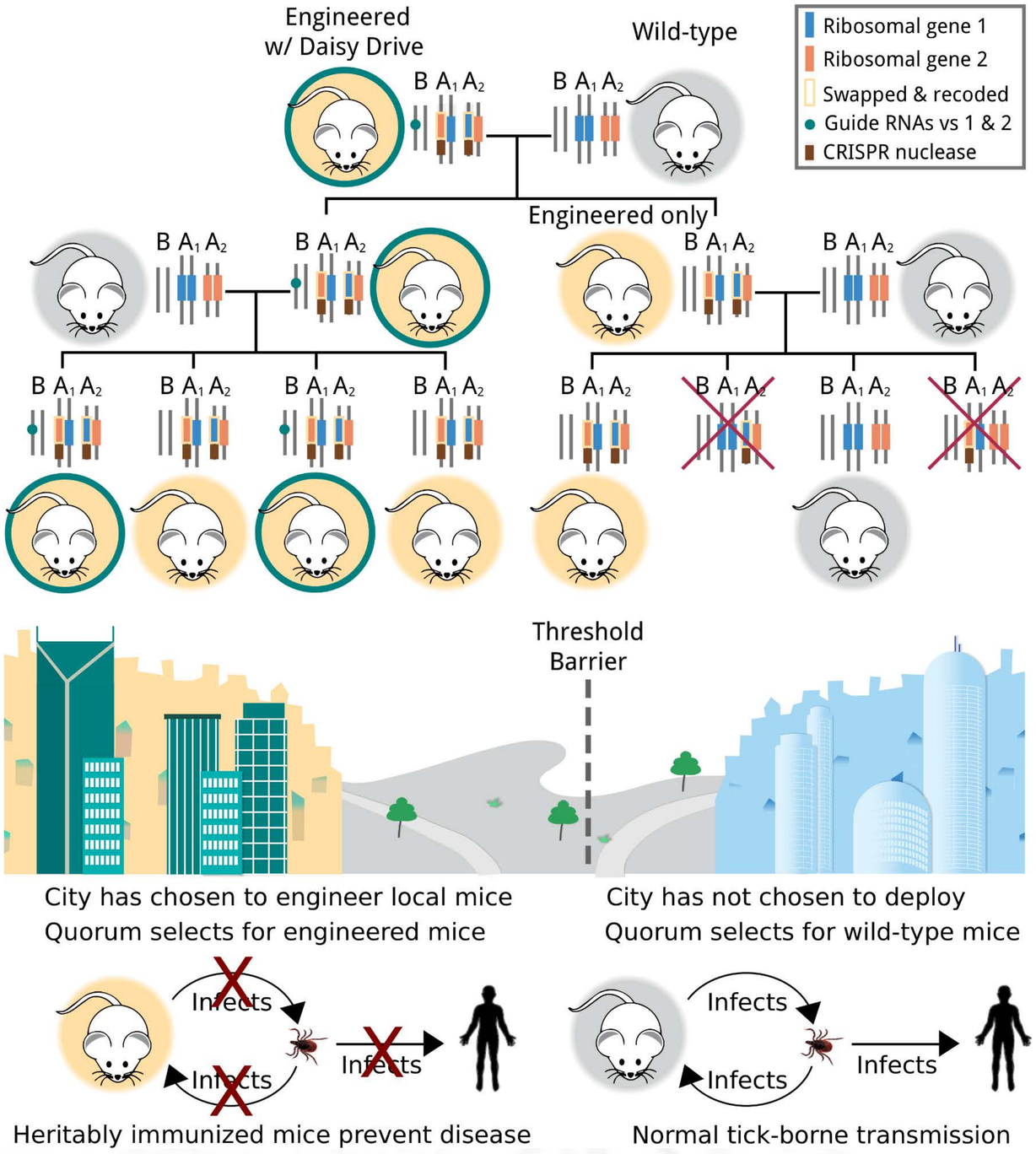
Daisy Quorum



John Min



Devora Najjar



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Mice Against Ticks

sculpting evolution

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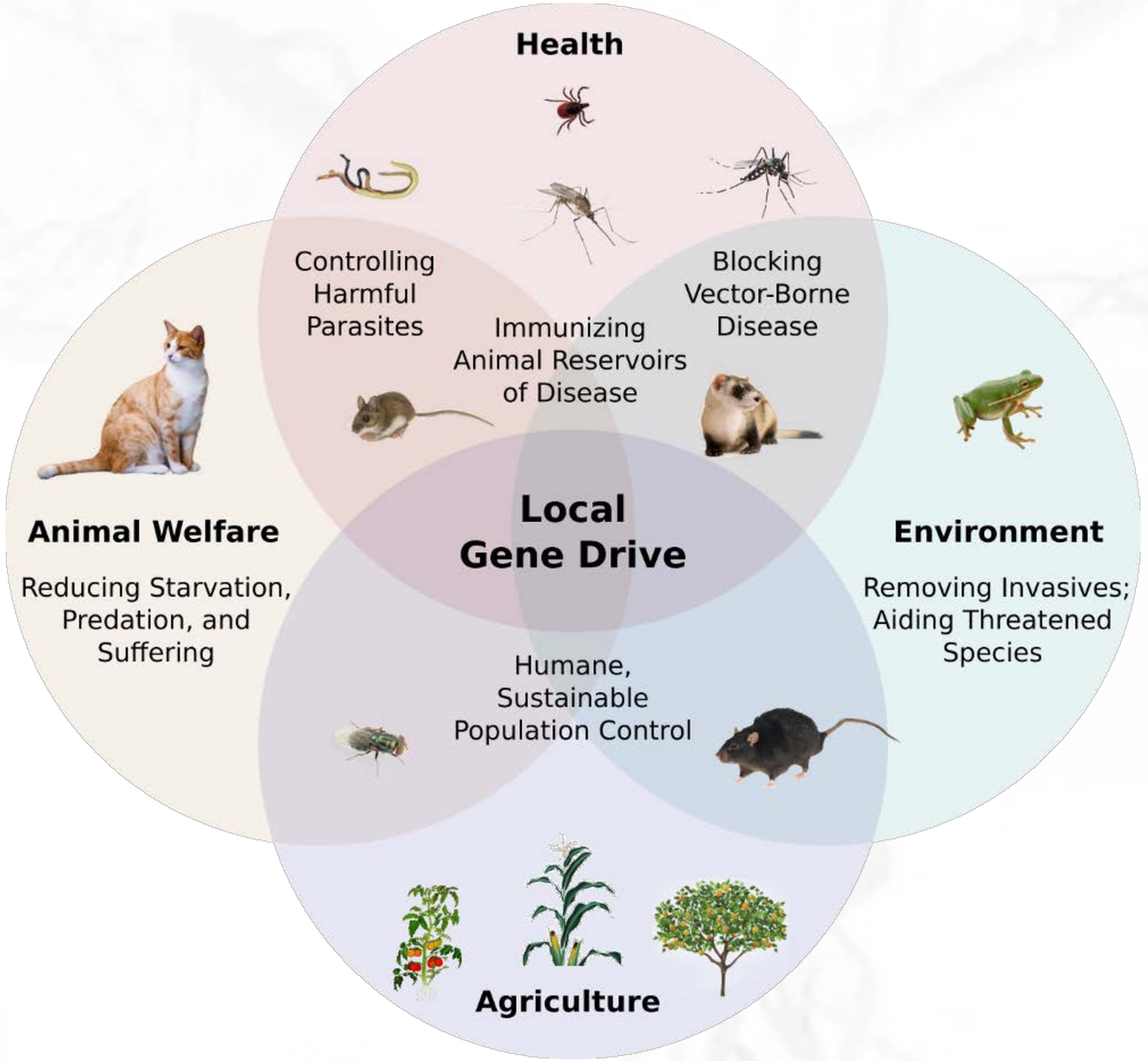
A Community Effort to Prevent Tick-Borne Disease

photo: Yousur Al-Hlou/ The New York Times


Health
Controlling Harmful Parasites
Blocking Vector-Borne Disease
Immunizing Animal Reservoirs of Disease
Animal Welfare
Reducing Starvation, Predation, and Suffering
Local Gene Drive
Environment
Removing Invasives; Aiding Threatened Species
Humane, Sustainable Population Control
Agriculture

Acknowledgements

Daisy-Chain Drive (Noble, Min et al 2016)

John Min Charleston Noble Alex Chavez

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Daisyfield Drive (Min et al 2017)

John Min Charleston Noble **Devora Najjar**

Daisy Quorum (Min et al 2017)

John Min Charleston Noble **Devora Najjar**

Daisy Drive Aotearoa

John Min **Stephen Von Stetina** **Ashton Strait** **Devora Najjar** Neil Gemmell Dan Tompkins The Kahui Maori

Responsive Science

Avery Normandin **Devora Najjar** **Dana Gretton** **Joanna Buchthal** Shlomiya Lightfoot Jeantine Lunshof Travis Rich Sam Weiss Evans

Mice Against Ticks

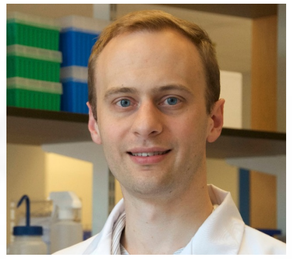
Joanna Buchthal **John Min** **Devora Najjar** Sam Telford Linden Hu Duane Wesemann The communities of Nantucket and Martha's Vineyard

Robotic PACE

Erika DeBenedictis

sculpting evolution

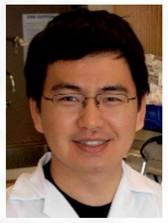
exploring evolutionary and ecological engineering



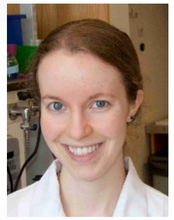
Kevin Esvelt

Stephen Von Stetina

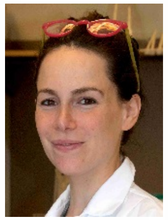
Kristina Bonikowski



John Min

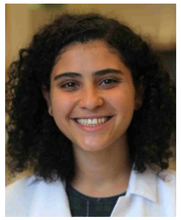


Erika DeBenedictis



Joanna Buchthal

Cody Gilleland



Devora Najjar

Avery Normandin

Dana Gretton

Ashton Strait

Lily Fitzgerald

Massachusetts Institute of Technology

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Questions? esvelt@mit.edu

CDMRP

NATIONAL INSTITUTES OF HEALTH

RAINWATER Charitable Foundation

MAXMIND

BURROUGHS WELLCOME FUND

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NATURE | NEWS

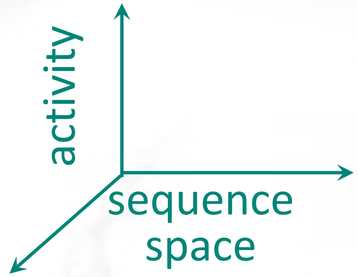
Gene drives thwarted by emergence of resistant organisms

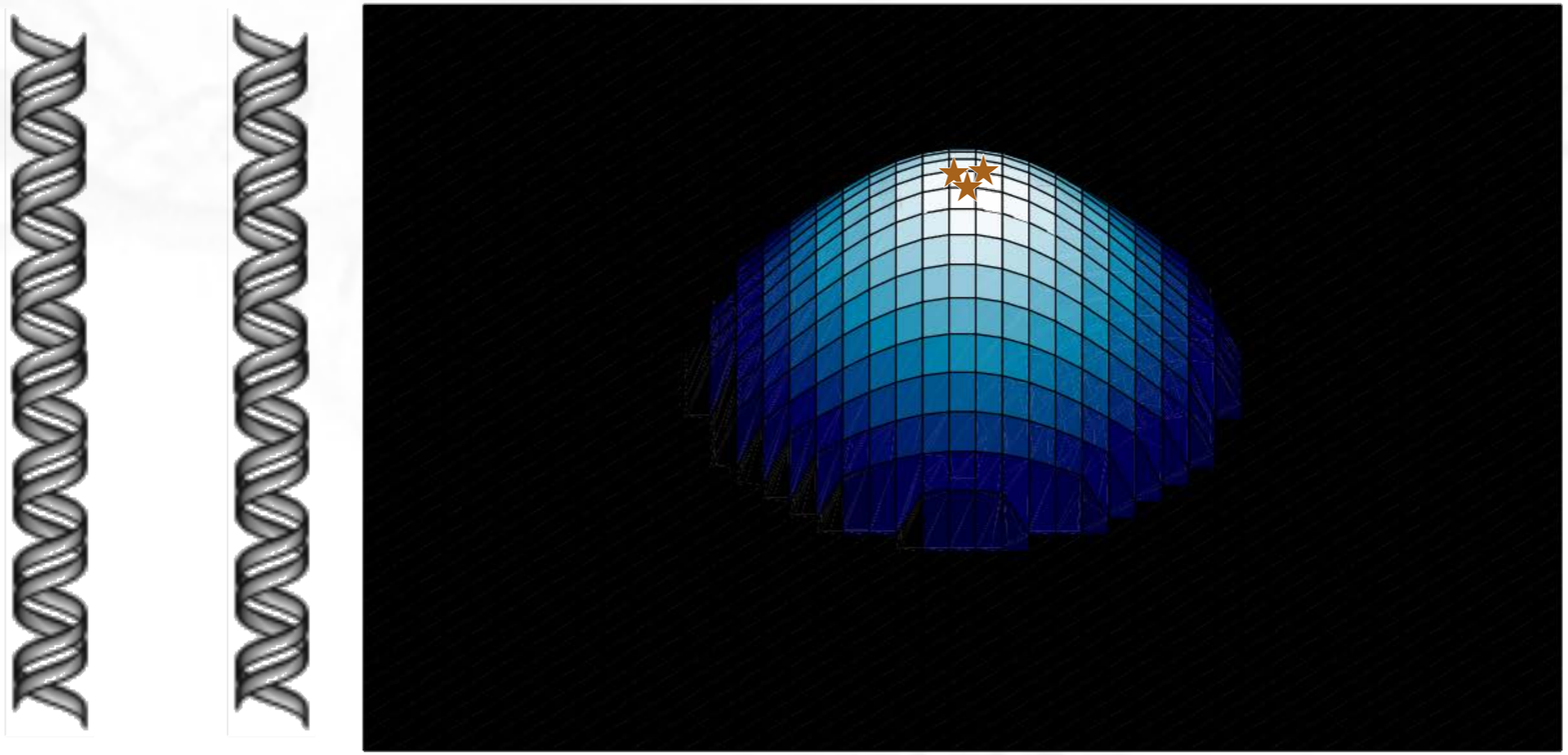
Until this obstacle is overcome, the technology is unlikely to succeed in the wild.

Ewen Callaway

31 January 2017

Evolutionary Stability

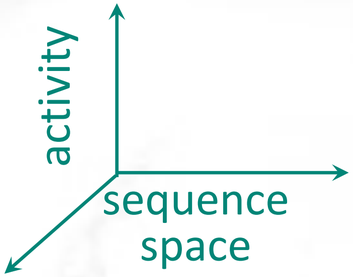


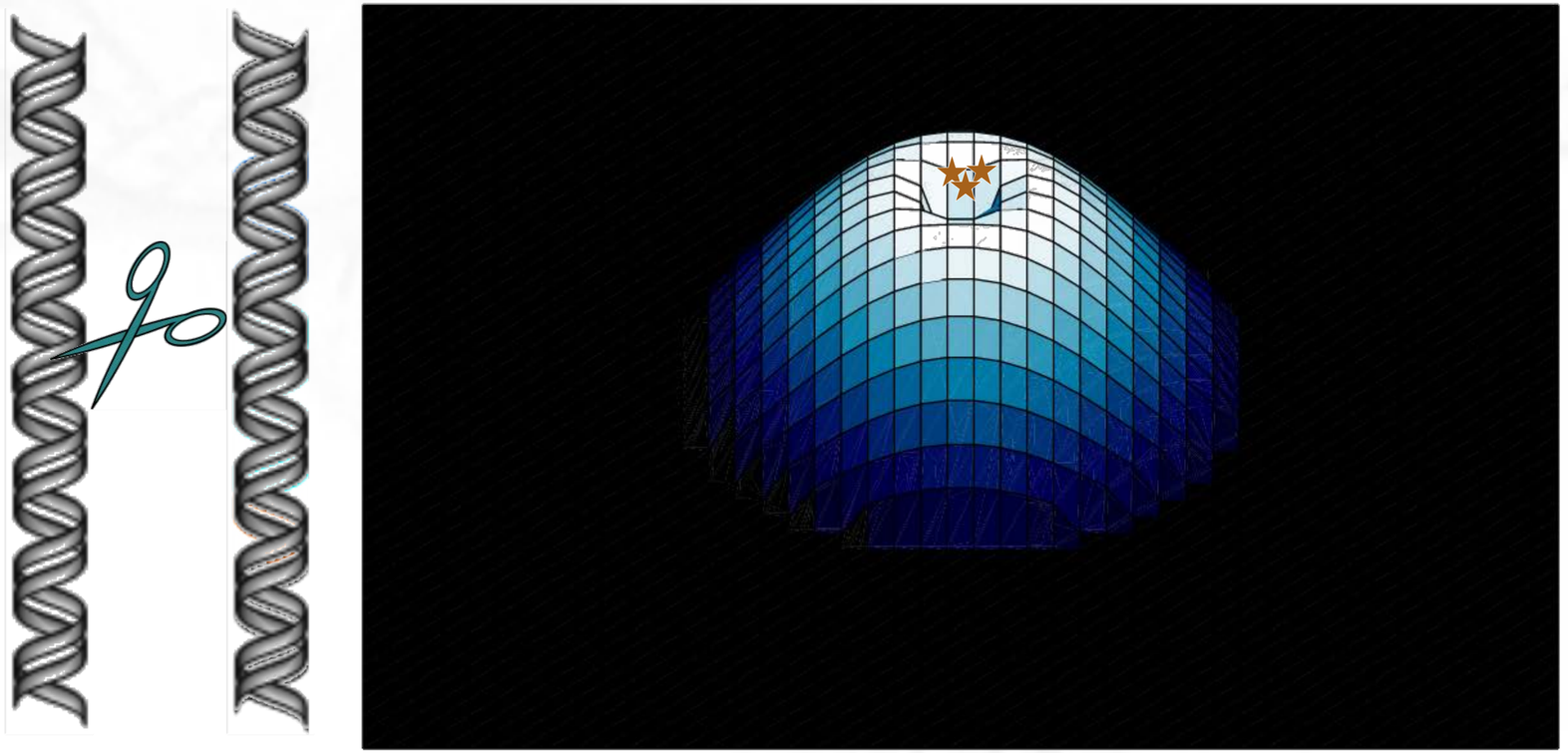


Fitness landscape of the target gene without the drive

Noble C, Olejarz J, Church GM, Esvelt KM, Nowak MA (2016) *bioRxiv*

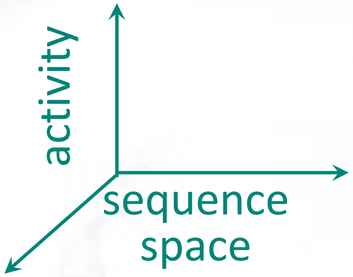
Evolutionary Stability

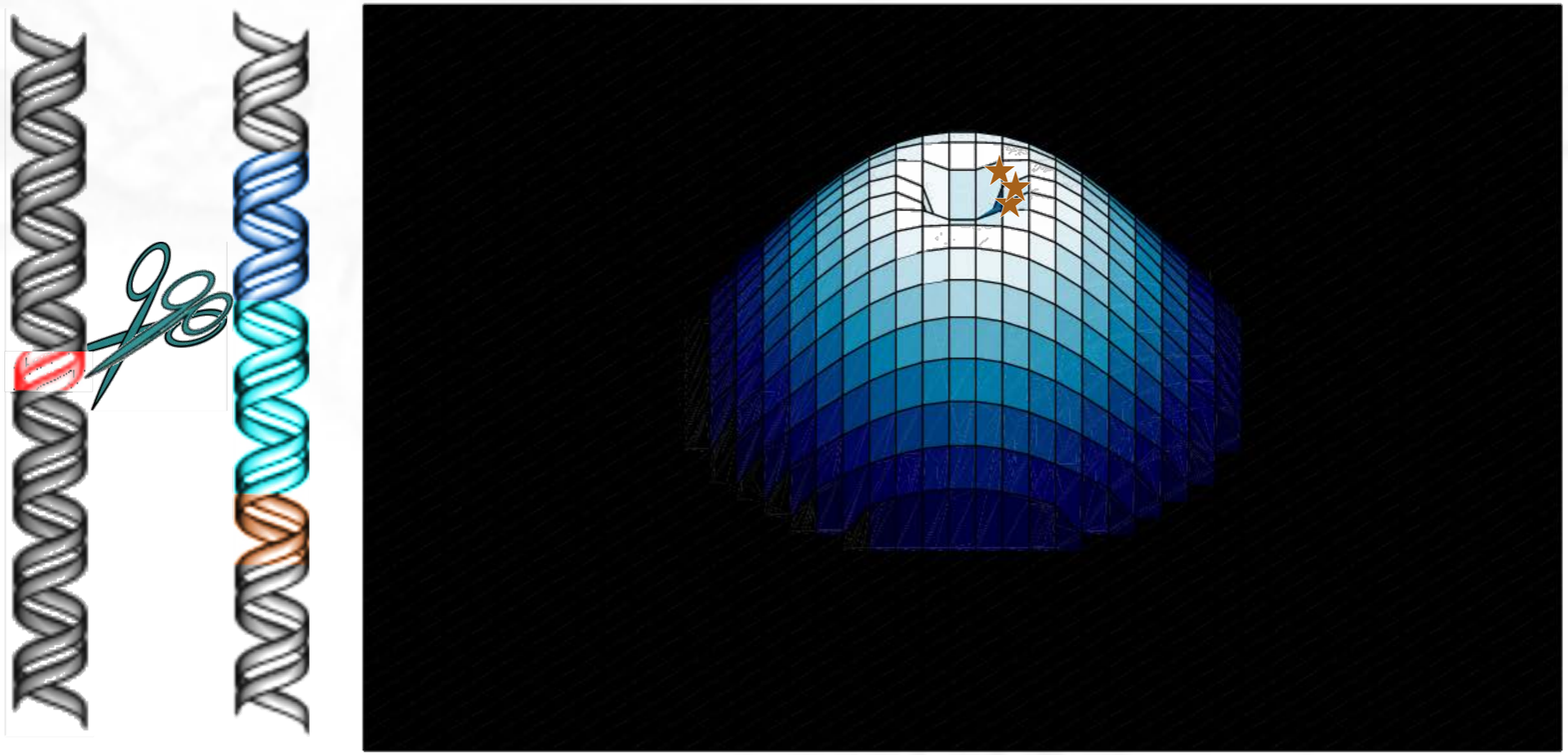




Fitness landscape of the target gene with the drive

Evolutionary Stability





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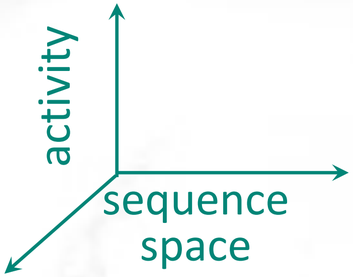
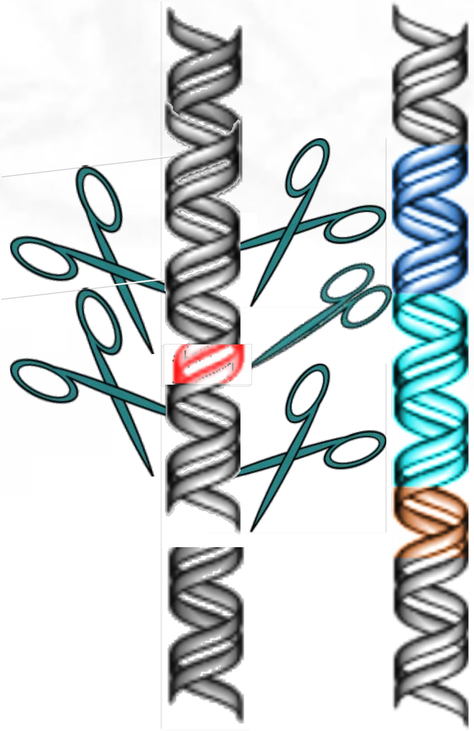
Noble C, Olejarz J, Church GM, Esvelt KM, Nowak MA (2016) *bioRxiv*

Evolutionary Stability

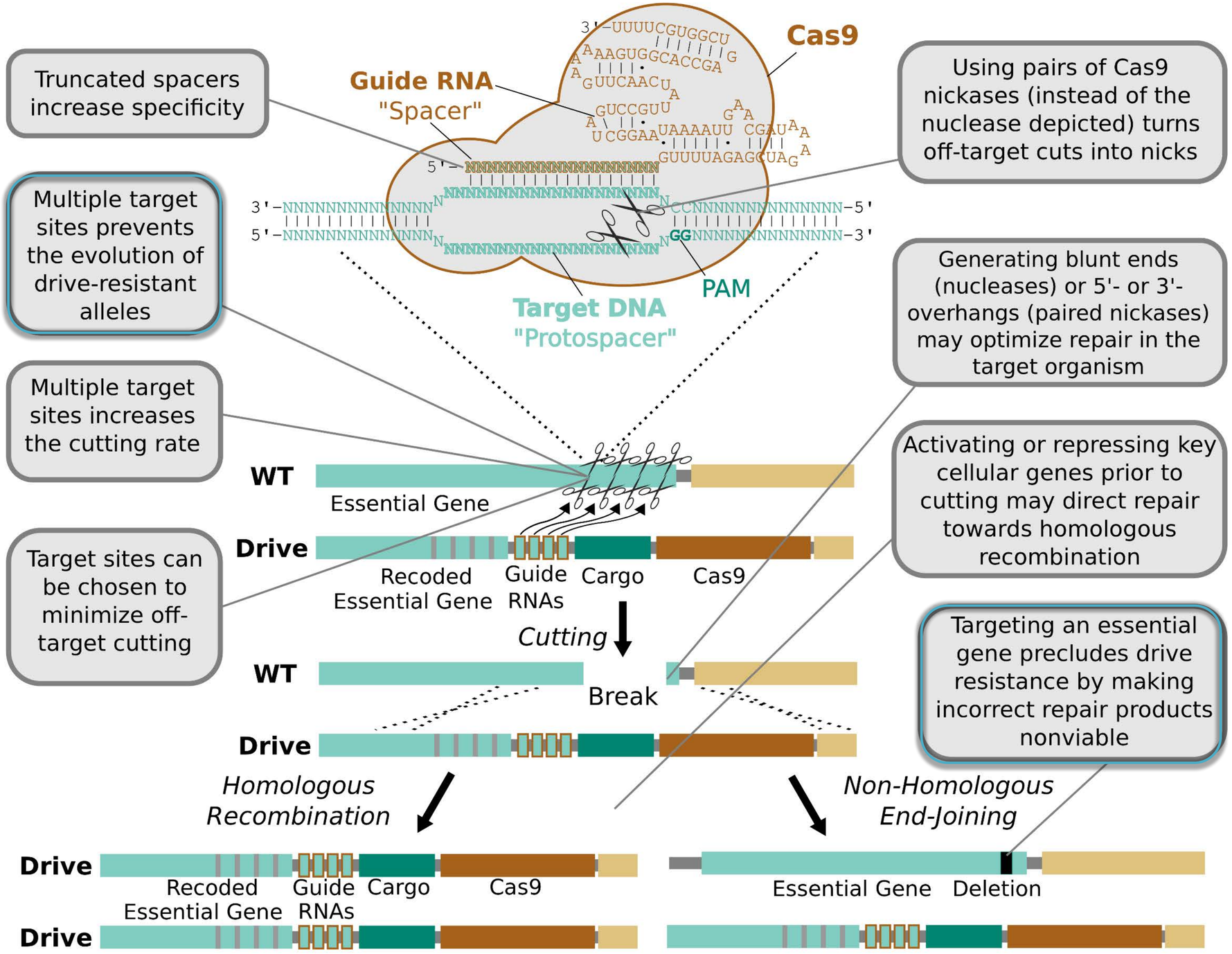




We can build evolutionarily stable gene drives by targeting *multiple sites…*

… within genes that are *important for fitness*

Noble C, Olejarz J, Church GM, Esvelt KM, Nowak MA (2016) *bioRxiv*

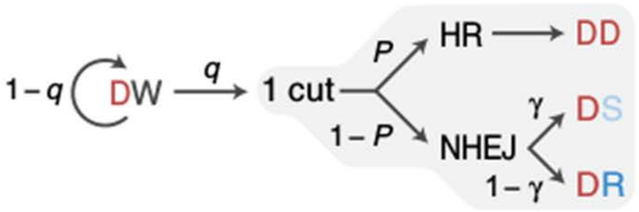


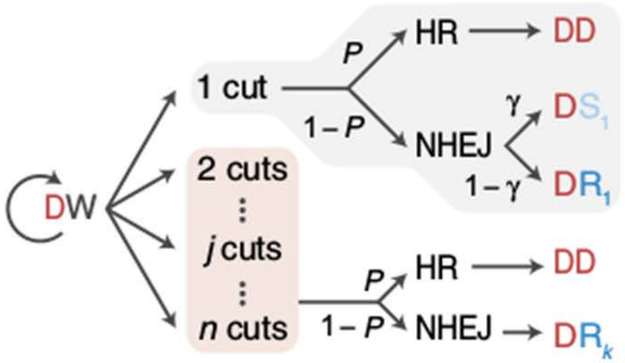
Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*

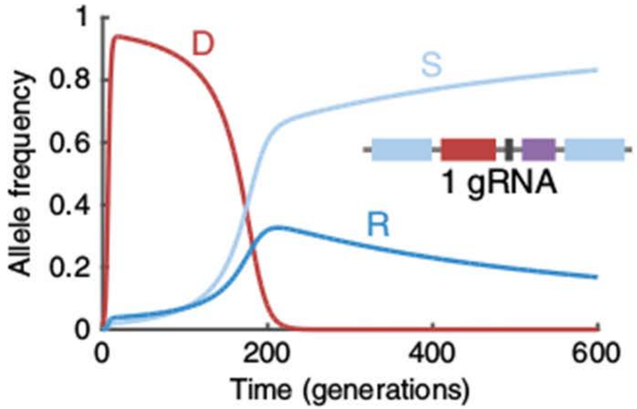
Modeling Multiplex Cutting and Gene Drive Spread

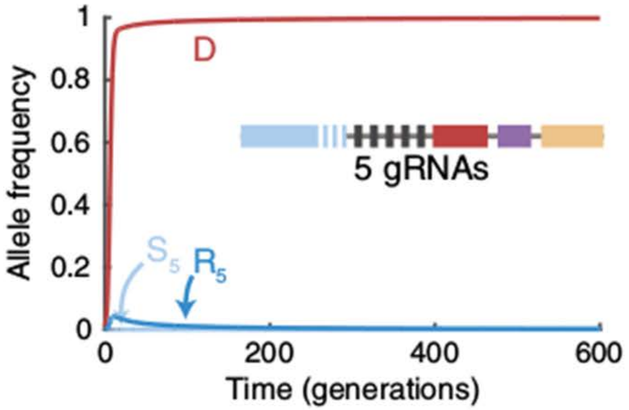
Assume very favorable drive parameters:

- high cutting + copying rate (95%)
- low drive fitness cost (5%)
- low cost of resistance (1%)
- high cost of target gene disruption (lethal; 2/3 probability if copying fails)









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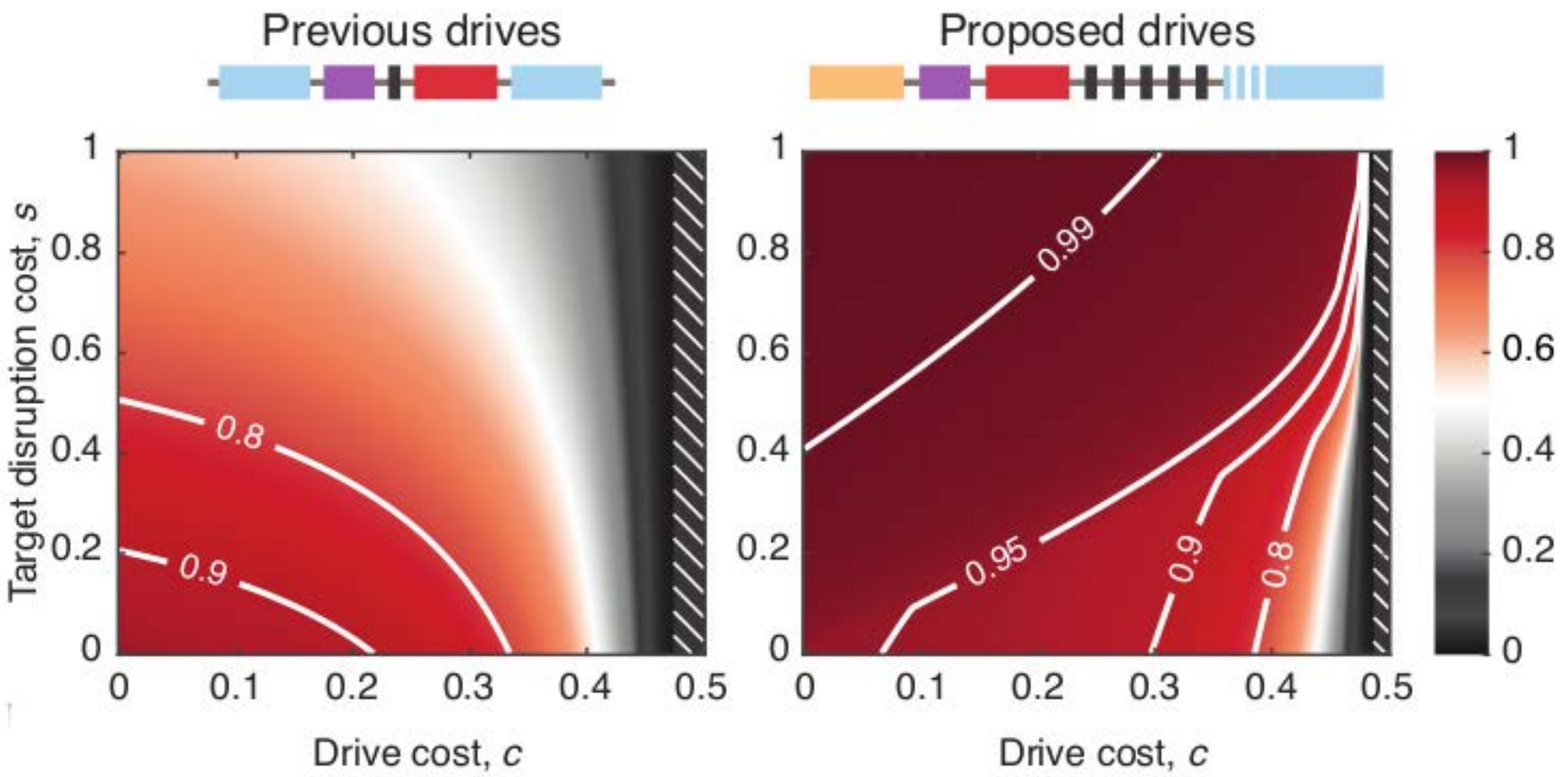
Noble C, Olejarz J, Church GM, Esvelt KM^, Nowak MA^ (20167 *Science Advances*

What Fraction of the Population is Affected?

Maximum frequency of the drive system in the population



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John Marshall/Omar Akbari and coworkers & Paul Thomas and coworkers independently obtained similar results for suppression drive systems

Noble C, Olejarz J, Church GM, Esvelt KM^, Nowak MA^ (2017) *Science Advances*
Marhsall et al.(2017) *Sci. Reports*
Prowse et al.(2017) *Proc. Roy. Soc. B*