Genetic manipulation of entire populations with CRISPR gene drives

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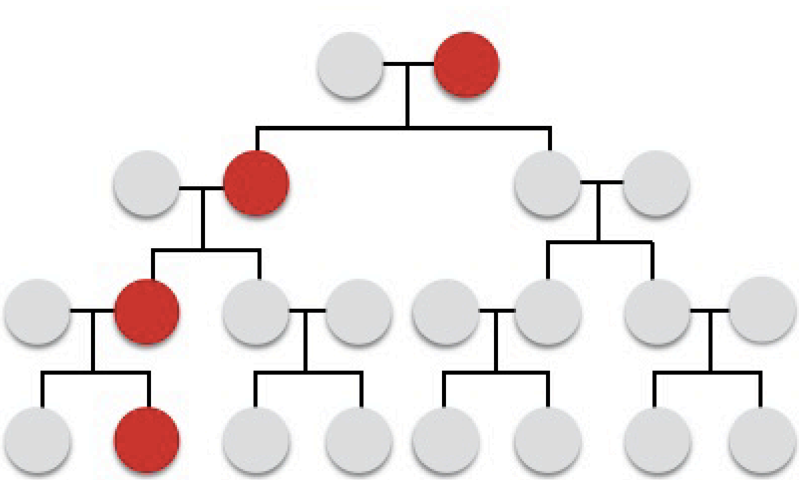
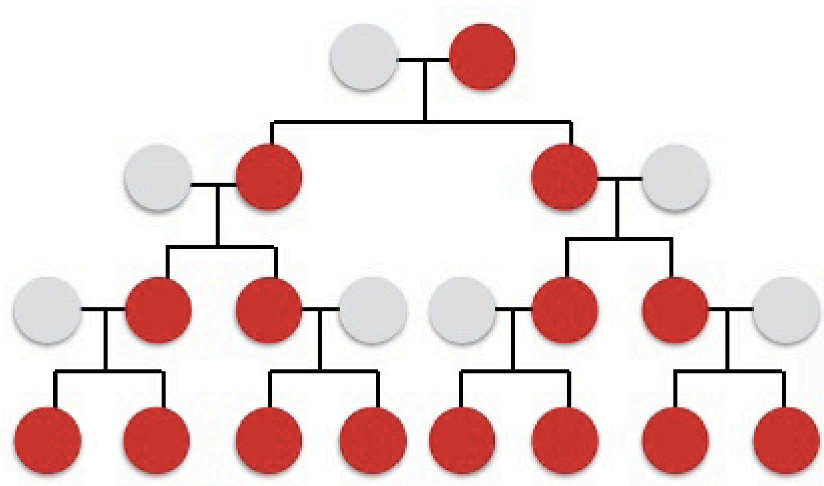
Gene drives

Mendelian inheritance:

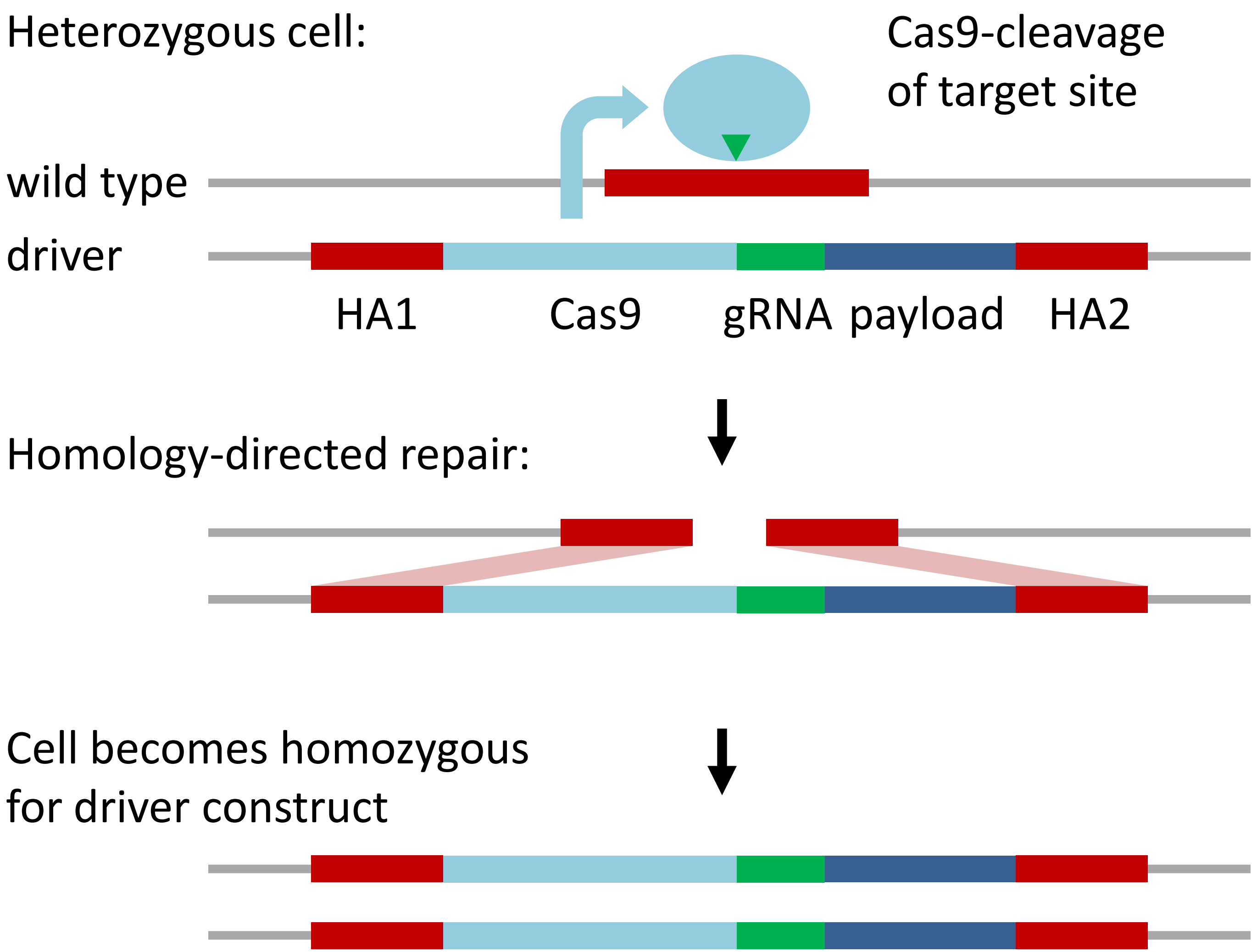


Super-Mendelian inheritance:



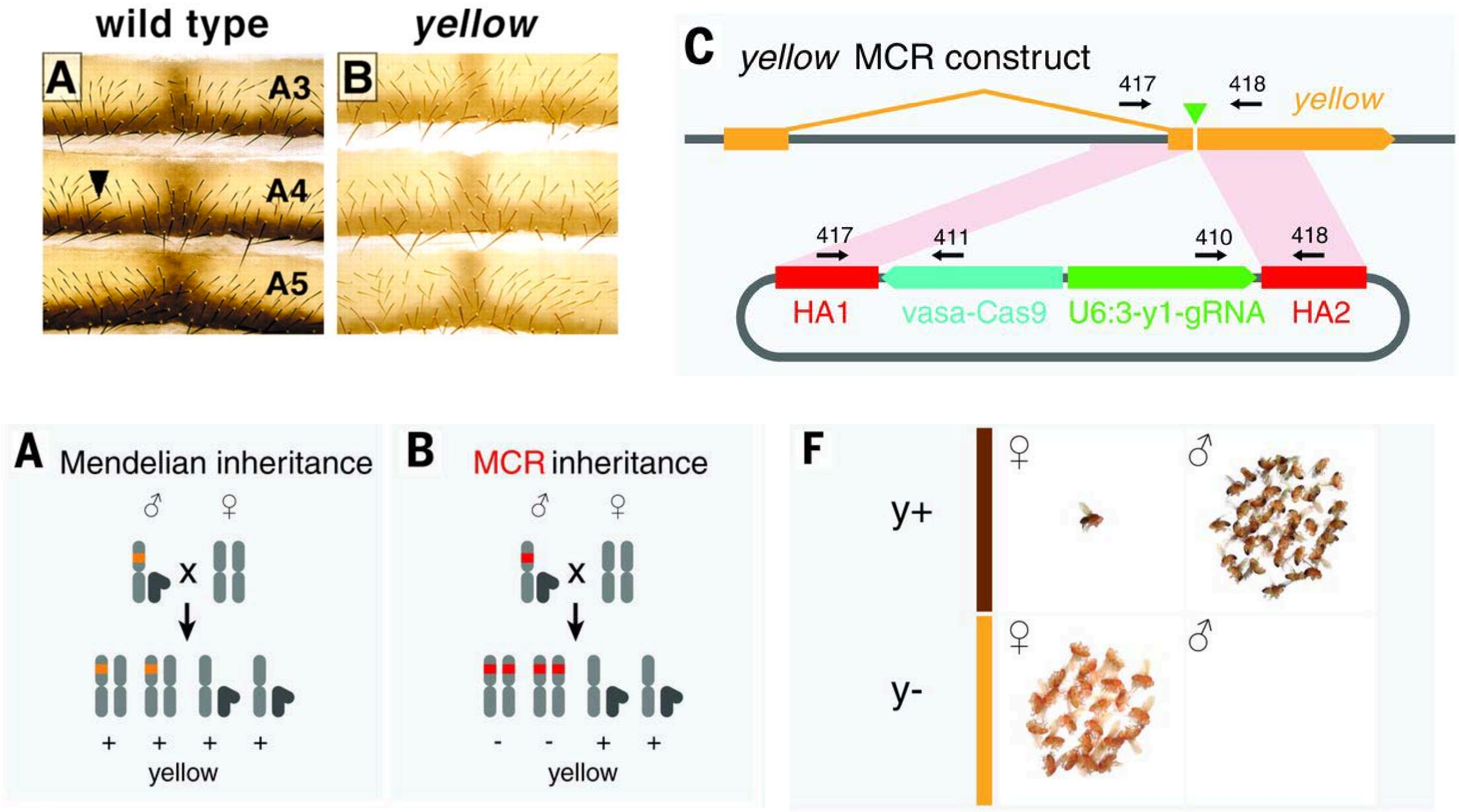
- Gene drives could in principle even spread deleterious alleles
- Natural examples: P element in flies, t haplotype in mice

CRISPR gene drives



Gantz and Bier, *Science* 2015

Gantz and Bier experiment



[Gantz and Bier, *Science* 2015]

Proposed applications

Altering vector species so it can no longer transmit disease:

Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi* (2015)

Valentino M. Gantz[a,1], Nijole Jasinskiene[b,1], Olga Tatarenkova[b], Aniko Fazekas[b], Vanessa M. Macias[b], Ethan Bier[a,2], and Anthony A. James[b,c,2]

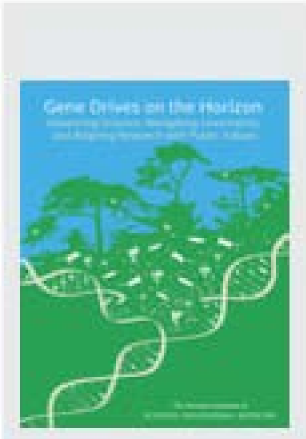
Direct suppression of vector population:

nature biotechnology

(2016)

Tony Nolan[1]

THE NATIONAL ACADEMIES PRESS



Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values

DETAILS

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BIOTECHNOLOGY

US agencies tackle gene drives

National-security community studies risks of method to quickly spread DNA modifications.

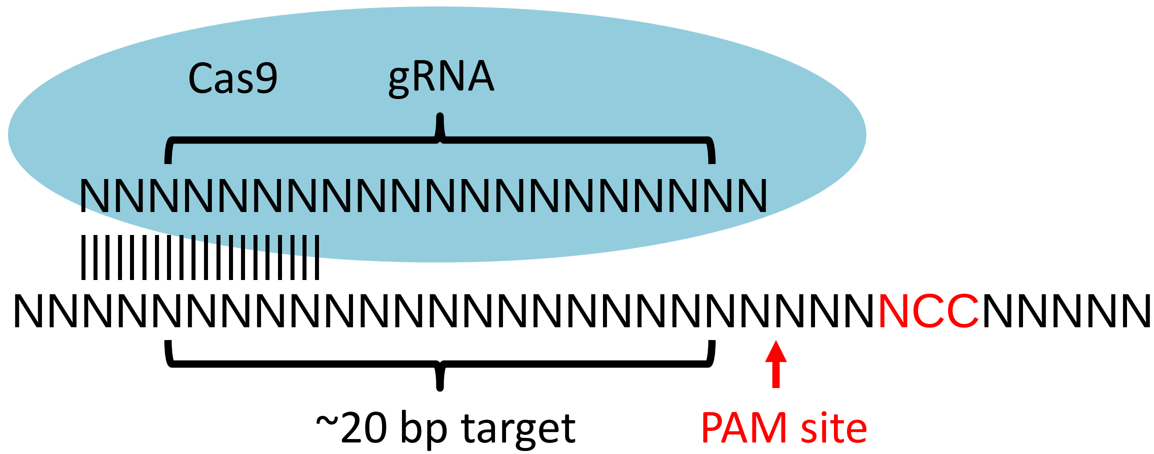
BY EWEN CALLAWAY

The JASONs, a group of elite scientists that advises the US government on national security, has weighed in on issues ranging from cybersecurity to renewing country's nuclear arsenal. But at a meeting in June, the secretive group took stock of a new threat: gene drives, a genetic-engineering technology that can swiftly spread modifications through entire populations and could help vanquish malaria-spreading mosquitoes.

That meeting forms part of a broader US national security effort this year to grapple with the possible risks and benefits of a technology that could drive species extinct and alter whole ecosystems. On 19 July, the US Defense Advanced Research Projects Agency (DARPA) announced US$65 million in funding to scientists studying gene-editing technologies; most of the money will be for work on gene drives. And a US intelligence counterpart to DARPA is planning to fund research into detecting organisms containing gene drives and other modifications.

"Every powerful technology is a national security issue," says Kevin Esvelt, an evolutionary engineer at the Massachusetts Institute of Technology in Cambridge, who won DARPA funding to limit the spread of gene drives.

Evolution of resistance?

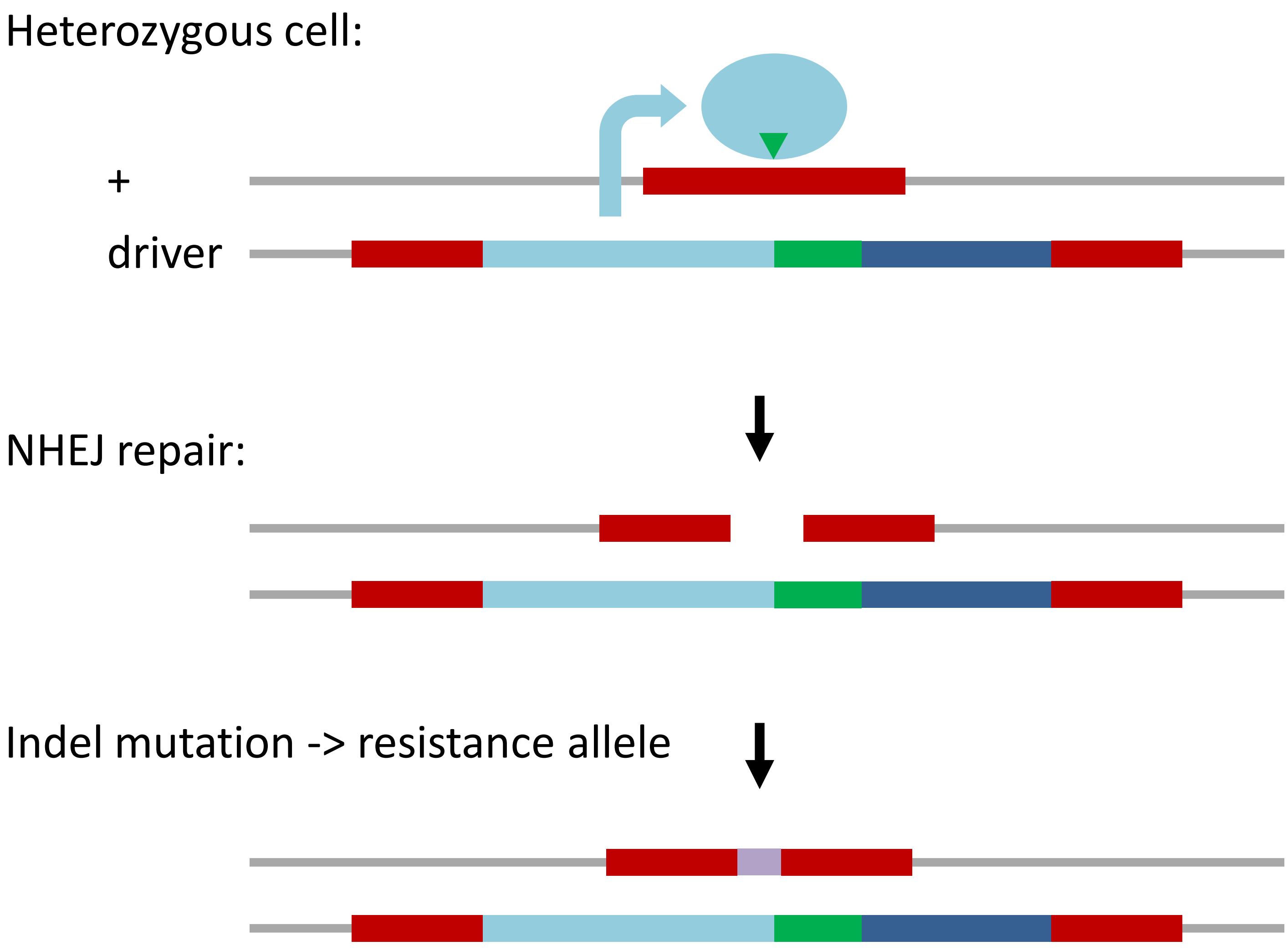


Genetic variation at target can prevent gRNA binding:

- >2 nucleotide differences in target
- Single mutation in the PAM site
- Most indels in target

Origin of resistance alleles:

- Standing genetic variation
- Cleavage repair by NHEJ


Heterozygous cell:
+
driver
NHEJ repair:
Indel mutation -> resistance allele

Population genetic model

Wright-Fisher model, single locus, 3 alleles:

- Wild type (0)
- Driver (*d*)
- Resistance (*r*)

$$E[x'_{\mathrm{d}}] = \frac{x_{\mathrm{d}}x_0[(1-c)\omega_{\mathrm{d}0} + 2c\omega_{\mathrm{dd}}] + x_{\mathrm{d}}x_{\mathrm{r}}\omega_{\mathrm{dr}} + x_{\mathrm{d}}^2\omega_{\mathrm{dd}}}{\overline{\omega}(t)}, \quad (1)$$

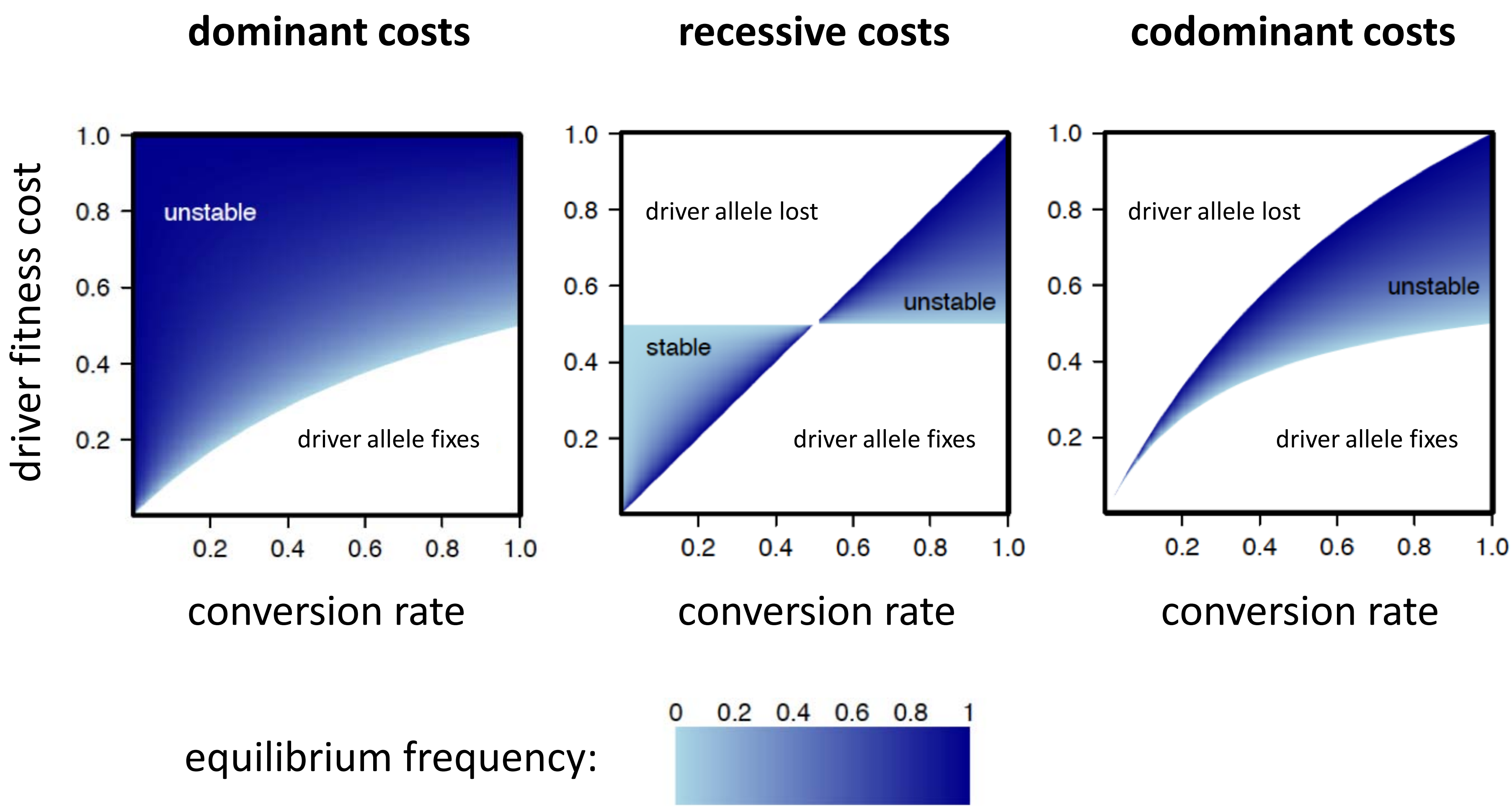
$$E[x'_{\mathrm{r}}] = \frac{x_{\mathrm{r}}x_0\omega_{\mathrm{r}0} + x_{\mathrm{r}}x_{\mathrm{d}}\omega_{\mathrm{dr}} + x_{\mathrm{r}}^2\omega_{\mathrm{rr}}}{\overline{\omega}(t)}, \quad (2)$$

$$E[x'_0] = 1 - E[x'_{\mathrm{d}}] - E[x'_{\mathrm{r}}]. \quad (3)$$

Model parameters:

- Conversion rate (*c*)
- NHEJ rate (δ)
- *De novo* mutation rate towards resistance alleles (μ)
- Fitness costs of various genotypes
- Effective population size (N_e)

Driver dynamics in absence of resistance



Unckless, Messer et al., *Genetics* 2015

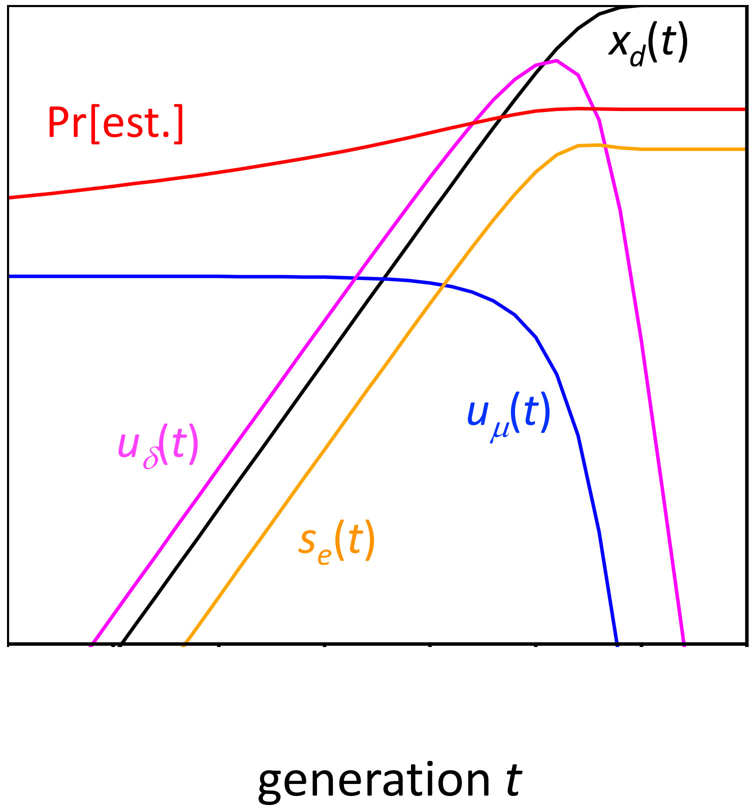
Evolution of resistance

Pr[resistance evolves] ~ resistance allele creation rate * Pr[establishment]

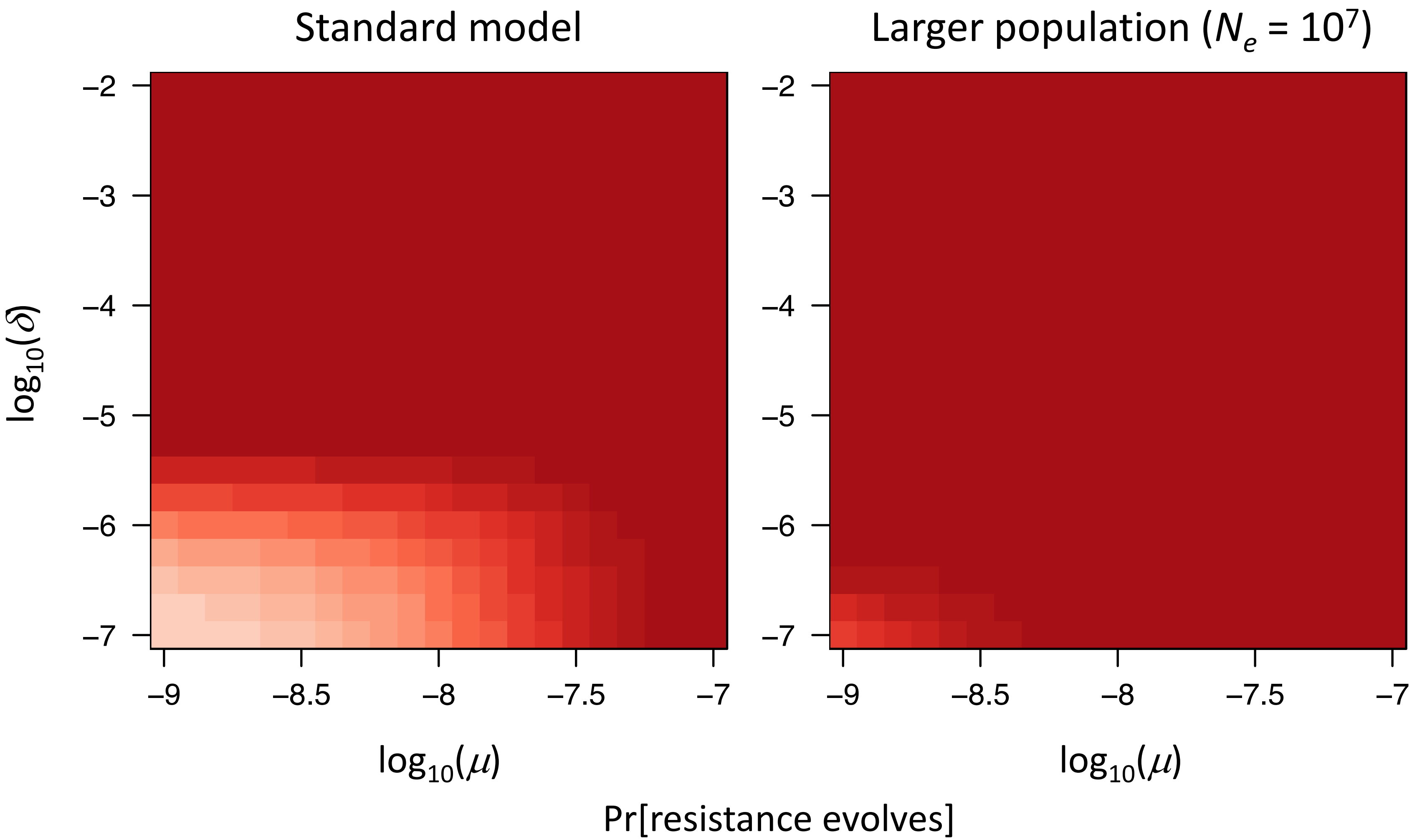
$$E[x_r'] \approx x_r[1 + s_e(t)] \quad \text{with} \quad s_e(t) = \frac{(1 - x_d)\omega_{r0} + x_d \omega_{dr}}{\overline{\omega}(t)} - 1.$$

“Standard” model:

- Conversion efficiency: 90%
- Driver fitness cost: *s* = 0.1 (codominant)
- Cost-free resistance
- $N_e = 10^6$
- Introduction freq. = 10^{-5}

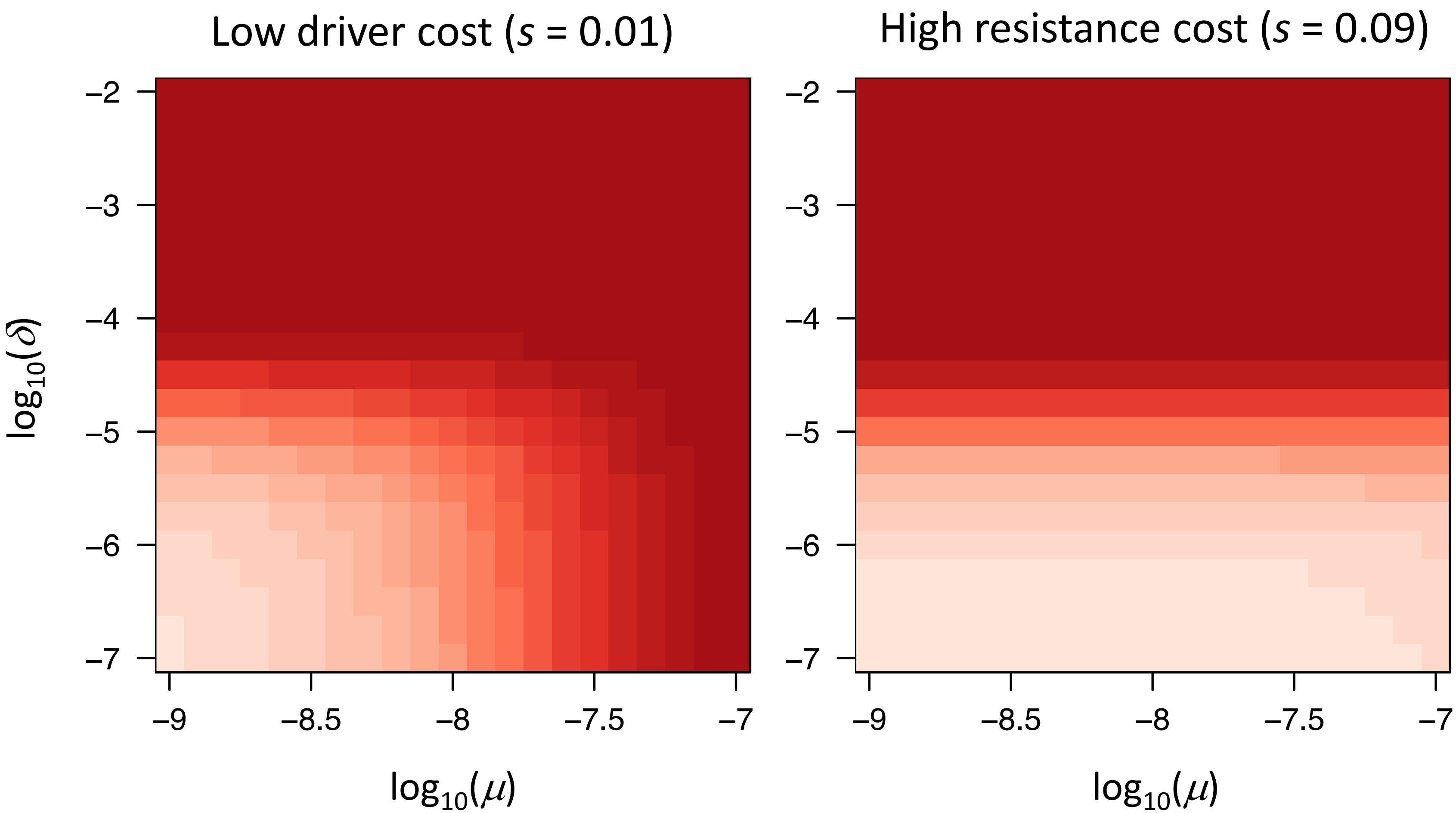


Resistance probability



Unckless, Clark, Messer, *Genetics* 2017

Resistance probability

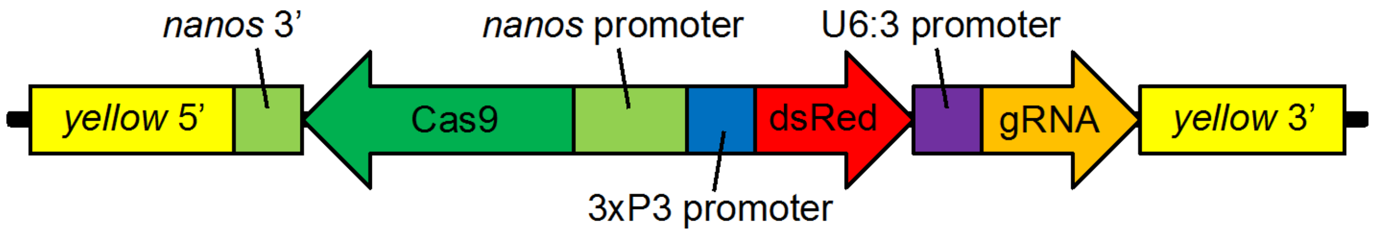


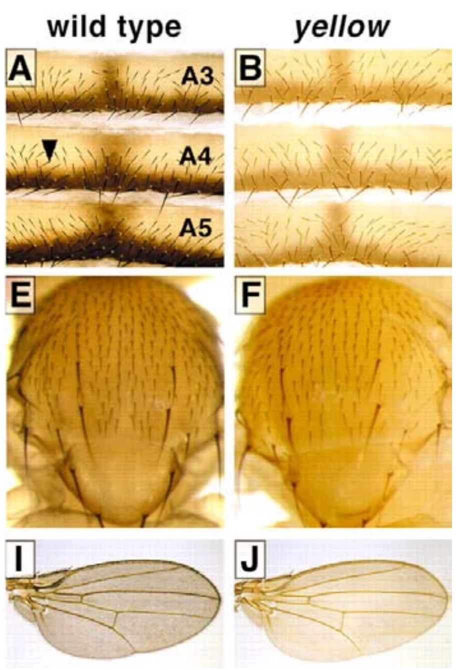
Pr[resistance evolves]

Unckless, Clark, Messer, *Genetics* 2017

Quantifying resistance allele formation

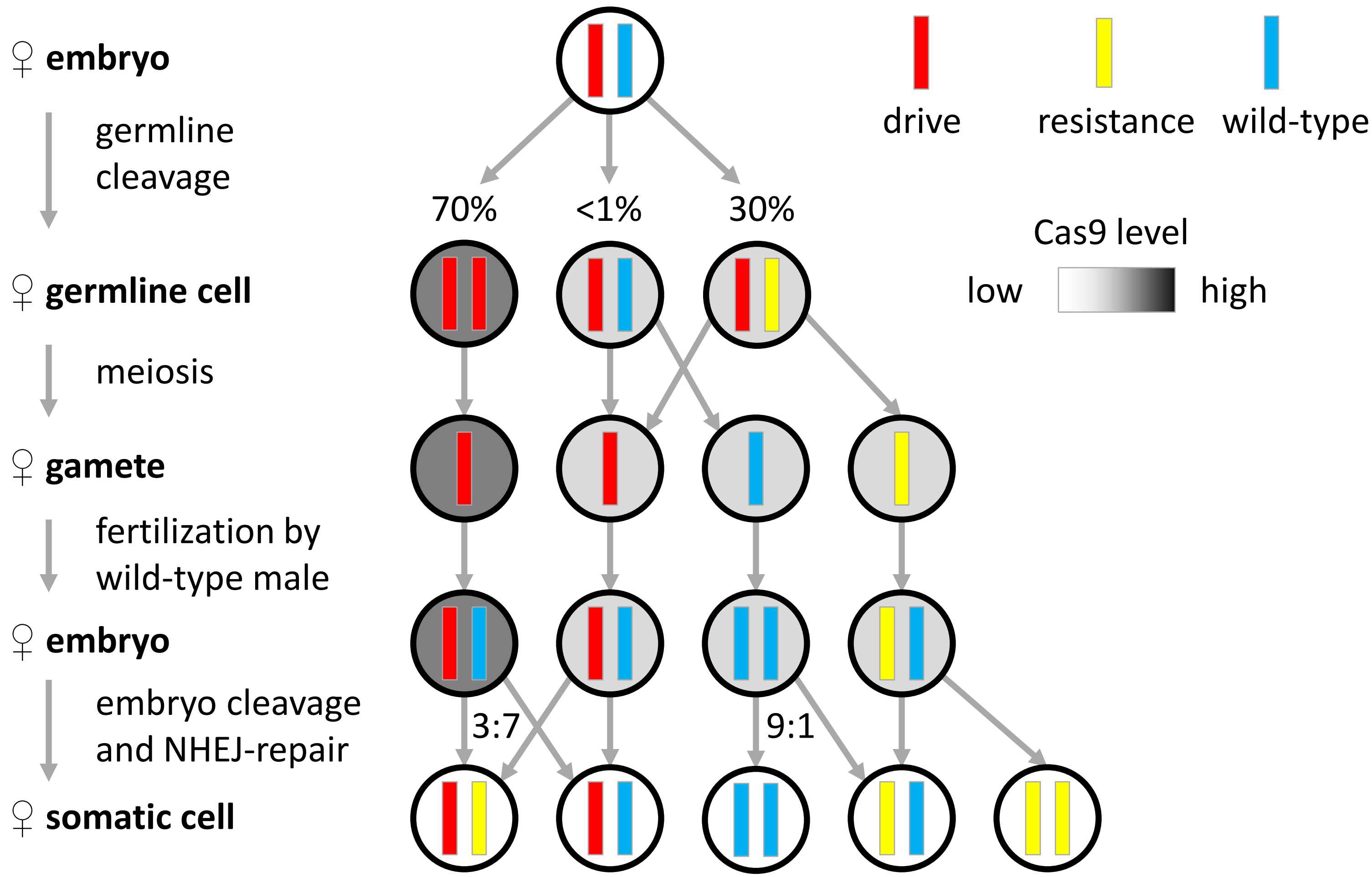
Nanos drive targeting *yellow* gene in *D. melanogaster*





Champer et al., *PLoS Genetics* 2017

Resistance mechanisms



Champer et al., *PLoS Genetics* 2017

Variation among diverse fly lines

Nanos-drive targeting *yellow* gene:

Fly line	Conversion rate	Germline resistance rate	Embryo resistance rate
w^{1118}	62%	29%	20%
Canton-S/w^{1118}	55%	35%	19%
GDL-Z/w^{1118}	54%	39%	28%
GDL-T/w^{1118}	51%	41%	56%
GDL-B/w^{1118}	50%	36%	26%
GDL-N/w^{1118}	46%	52%	22%
GDL-I/w^{1118}	40%	47%	3%
DGRP387/w^{1118}	63%	23%	49%
DGRP336/w^{1118}	58%	38%	85%
DGRP237/w^{1118}	56%	29%	78%
DGRP161/w^{1118}	55%	39%	40%
DGRP352/w^{1118}	52%	46%	81%
DGRP338/w^{1118}	50%	55%	32%
DGRP398/w^{1118}	48%	48%	38%
DGRP392/w^{1118}	40%	65%	84%

POLICY FORUM | BIOSAFETY

Safeguarding gene drive experiments in the laboratory

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]

+ Author Affiliations

↵*Corresponding author. E-mail: kevin.esvelt@wyss.harvard.edu (K.E.); ebier@ucsd.edu (E.B.)

Science 28 Aug 2015:
Vol. 349, Issue 6251, pp. 927-929
DOI: 10.1126/science.aac7932

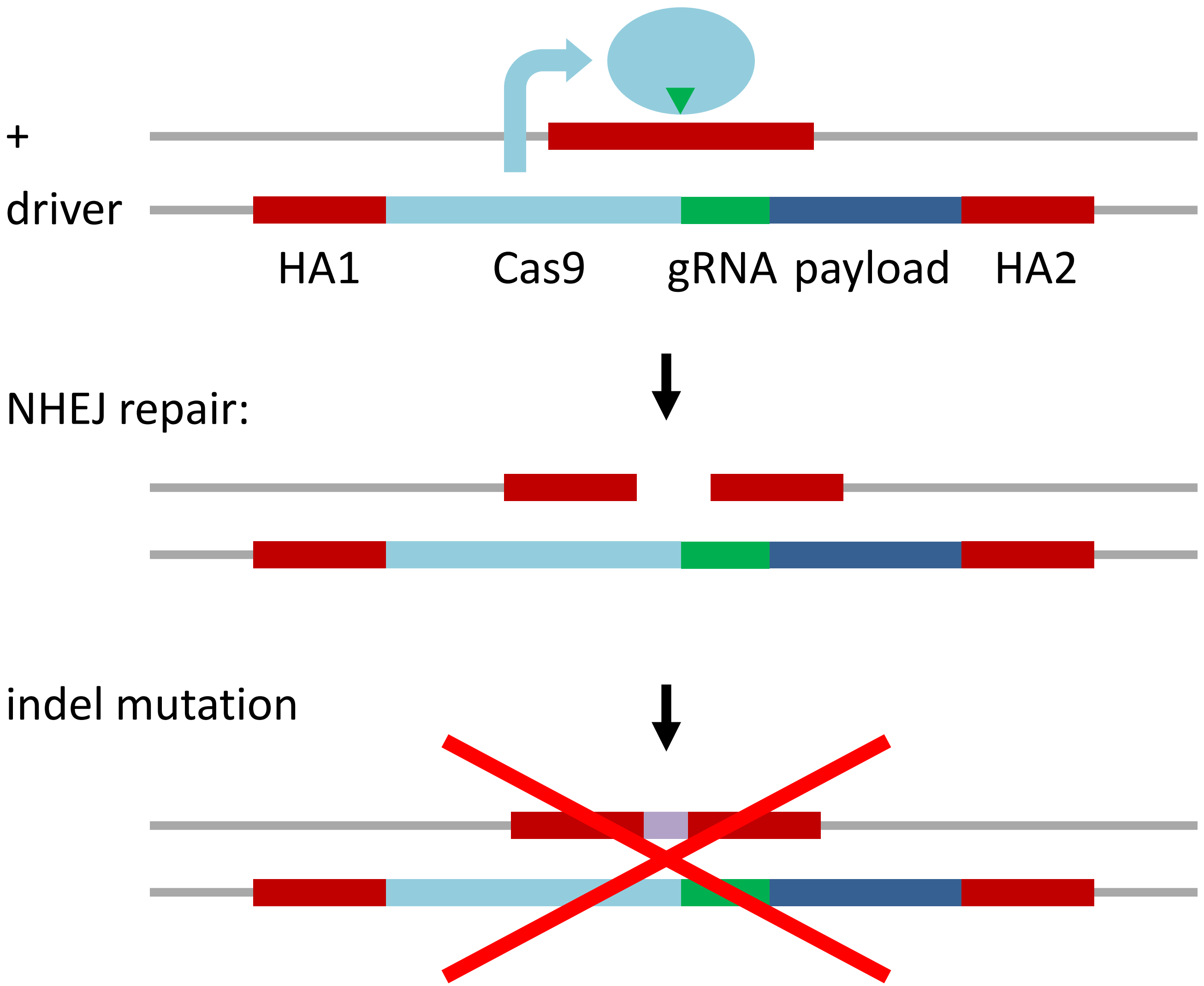
Proposed safety mechanisms / examples:

- Barrier: containment facility
- Ecological: experiments outside natural range
- Reproductive: strain with mating incompatibility
- Molecular: synthetic target sequence

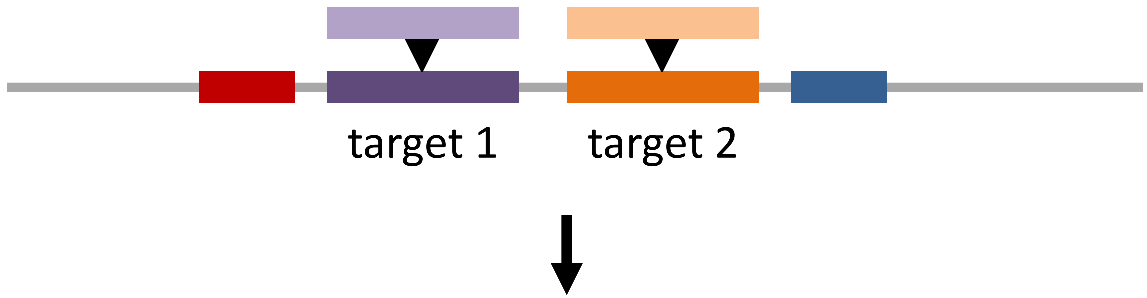
Sarkaria Arthropod Research Lab (SARL)

Cornell University
Agricultural Experiment Station

Targeting essential/haplolethal genes

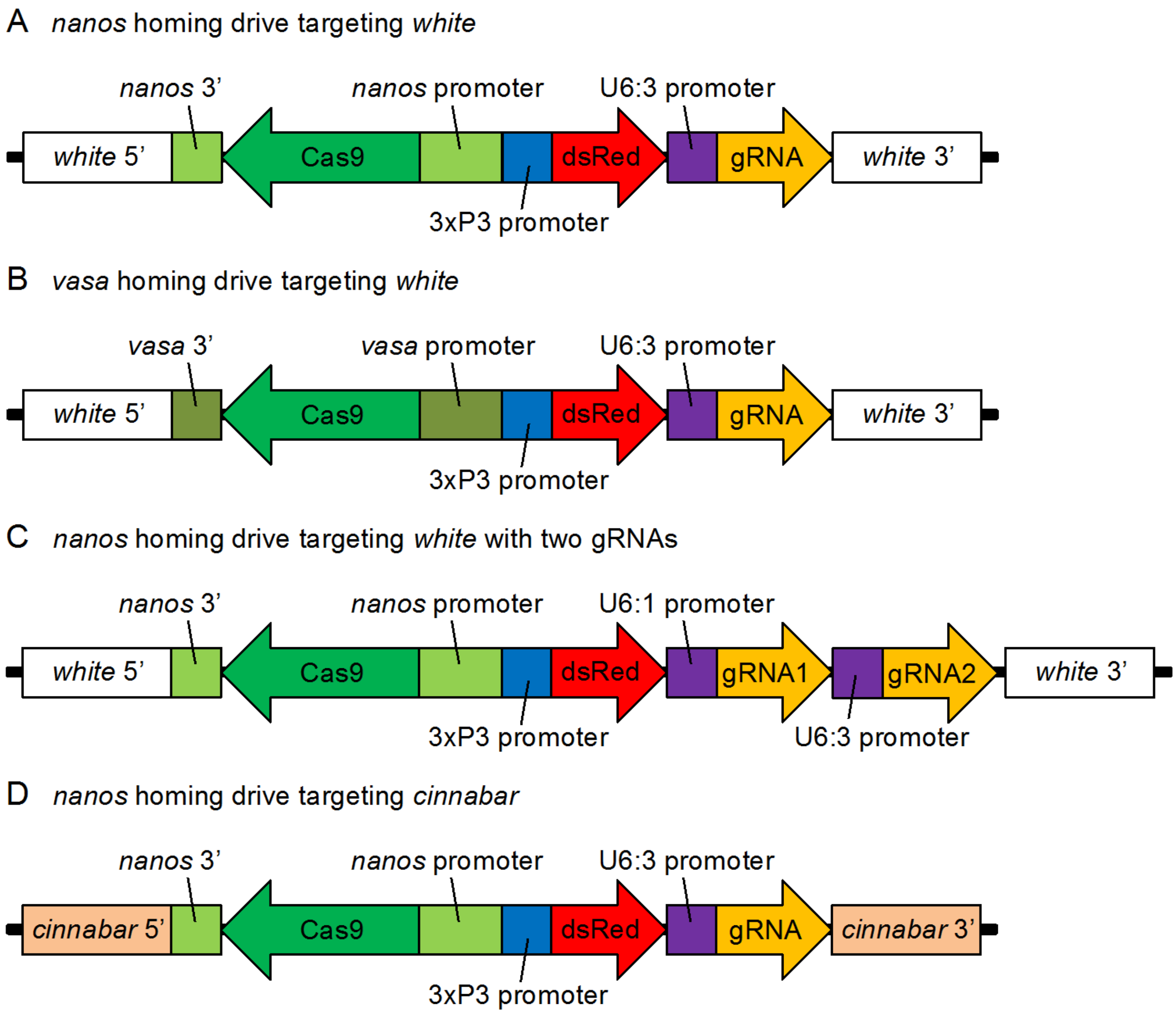


Using multiple gRNA targets



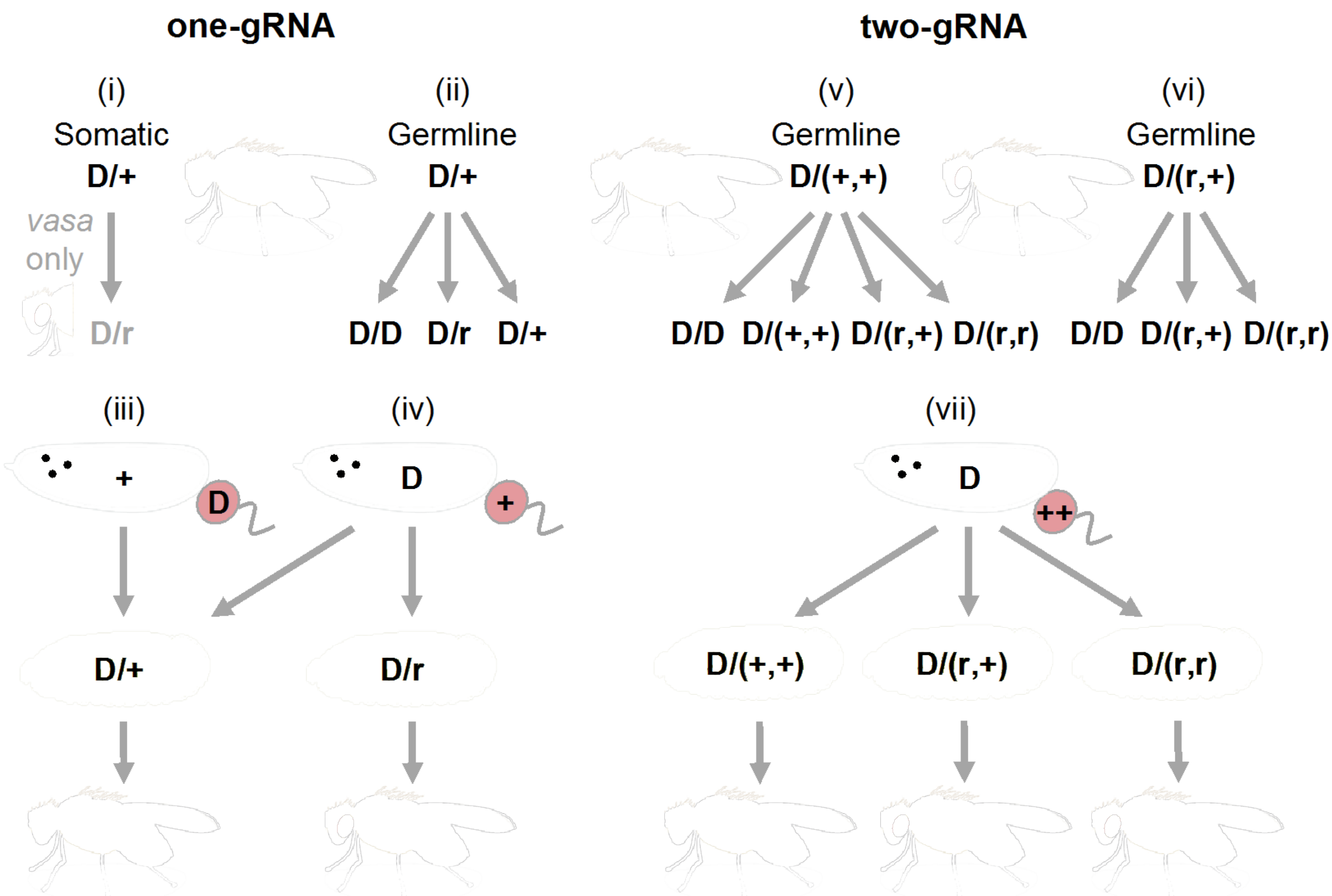
Homology-directed repair of cleavage at target site 1:

Homology-directed repair of cleavage at target site 2:



Champer et al., *bioRxiv*

Resistance mechanisms



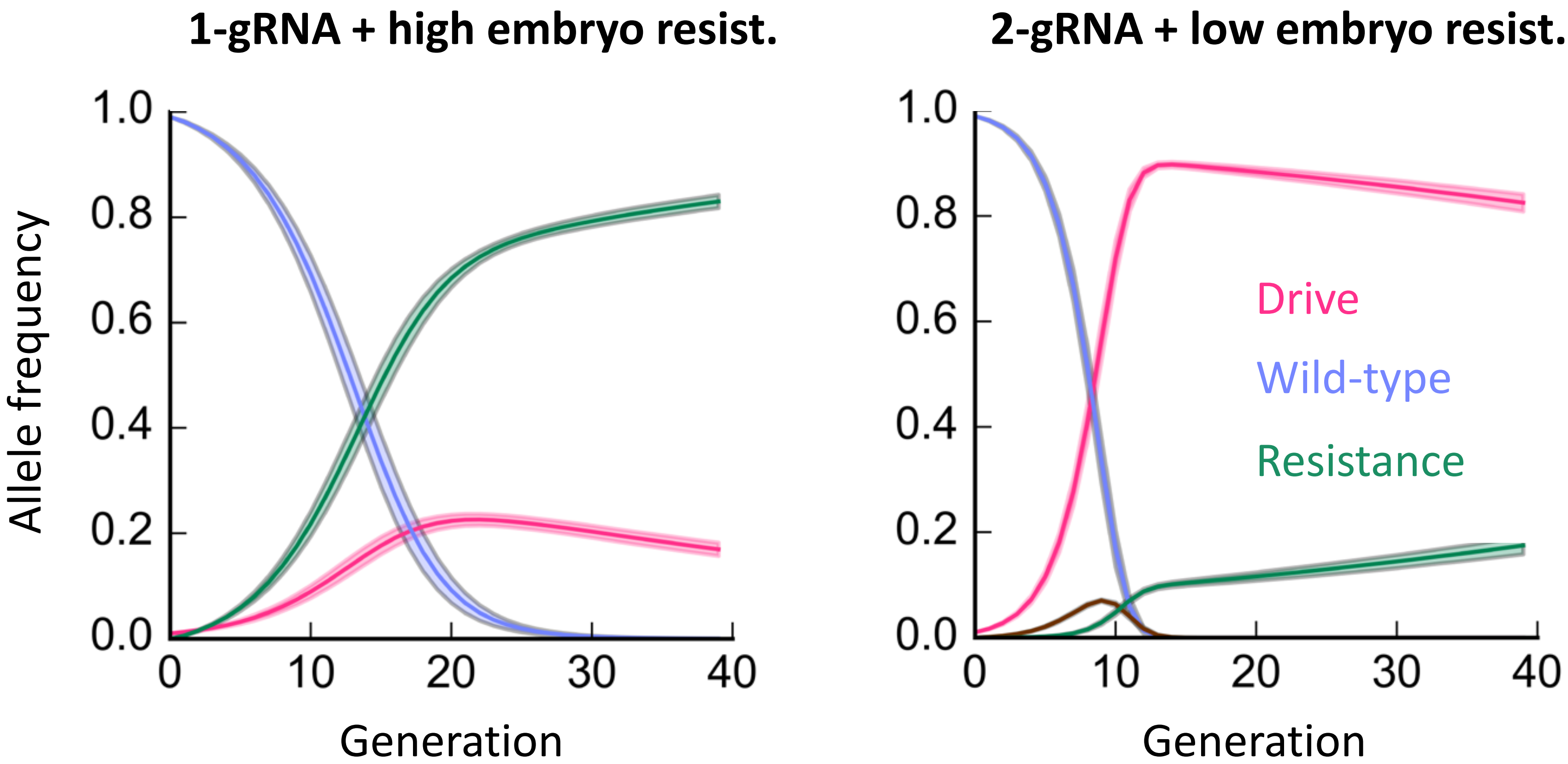
Champer et al., *bioRxiv*

Drive performances

Fly line	Cas9 promoter	Target gene	Conversion rate	Germline res. rate	Embryo res. rate
Canton-S	nanos	white	59%	36%	77%
Canton-S	vasa	white	56%	39%	75%
Canton-S	nanos	white-2gRNA	76%	16%	76%
Canton-S	nanos	cinnabar (female)	54%	46%	100%
Canton-S	nanos	cinnabar (male)	38%	62%	0%
w^{1118}	nanos	yellow coding	62%	29%	20%
w^{1118}	vasa	yellow promoter	53%	47%	20%

Champer et al., *bioRxiv*

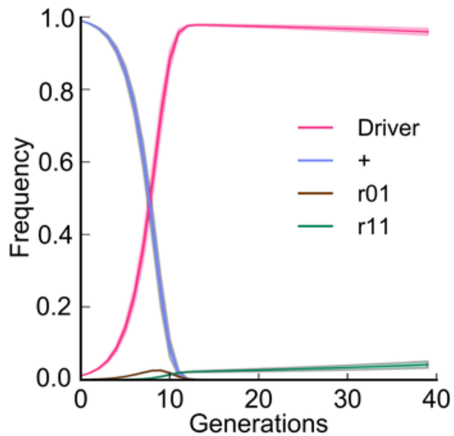
Population dynamics

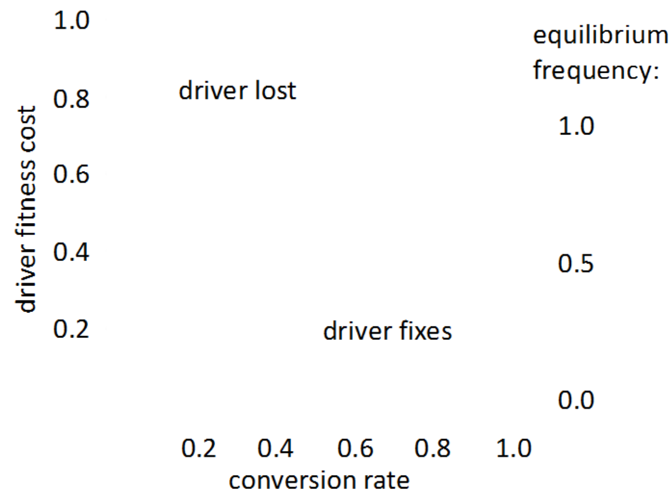


Champer et al., *bioRxiv*

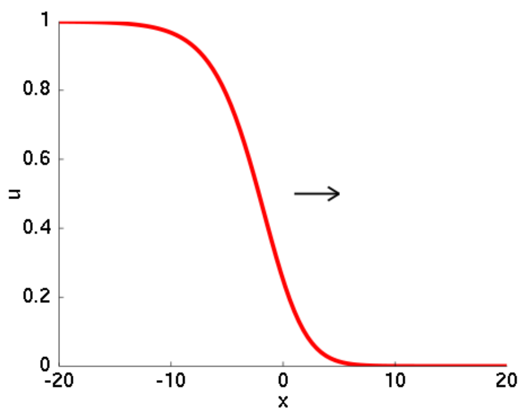
Modeling frameworks

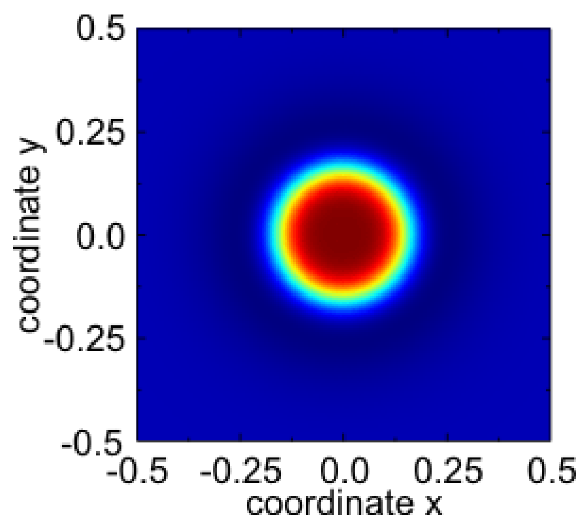
1. Panmictic population models:





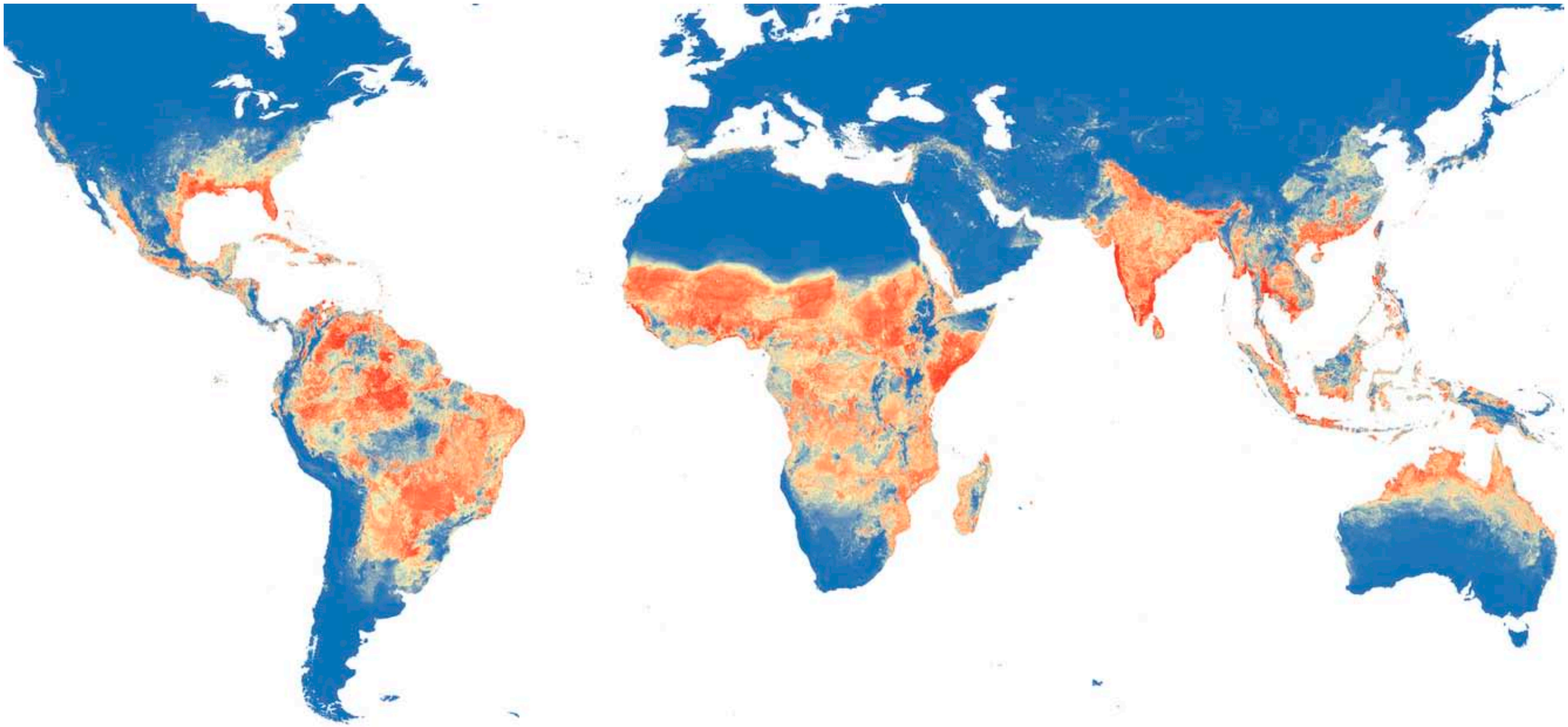
2. "Travelling wave" models:





Spatial population dynamics

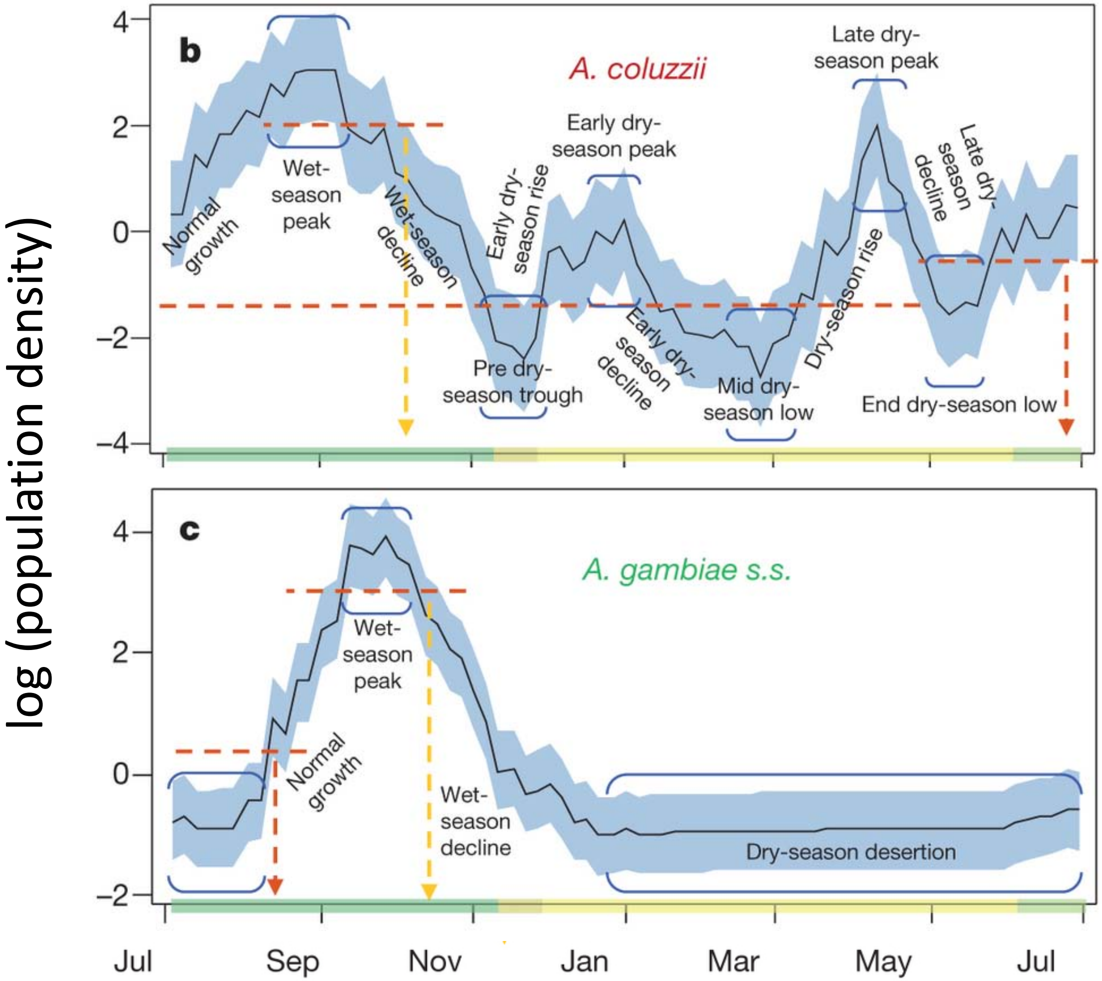
Global distribution of *Aedes aegypti* mosquitoes:



Kraemer et al., *eLife* 2014

Demography

Seasonal dynamics of *Anopheles* mosquitoes in Sahel:



Dao et al., *Nature* 2014

t = 10

t = 20

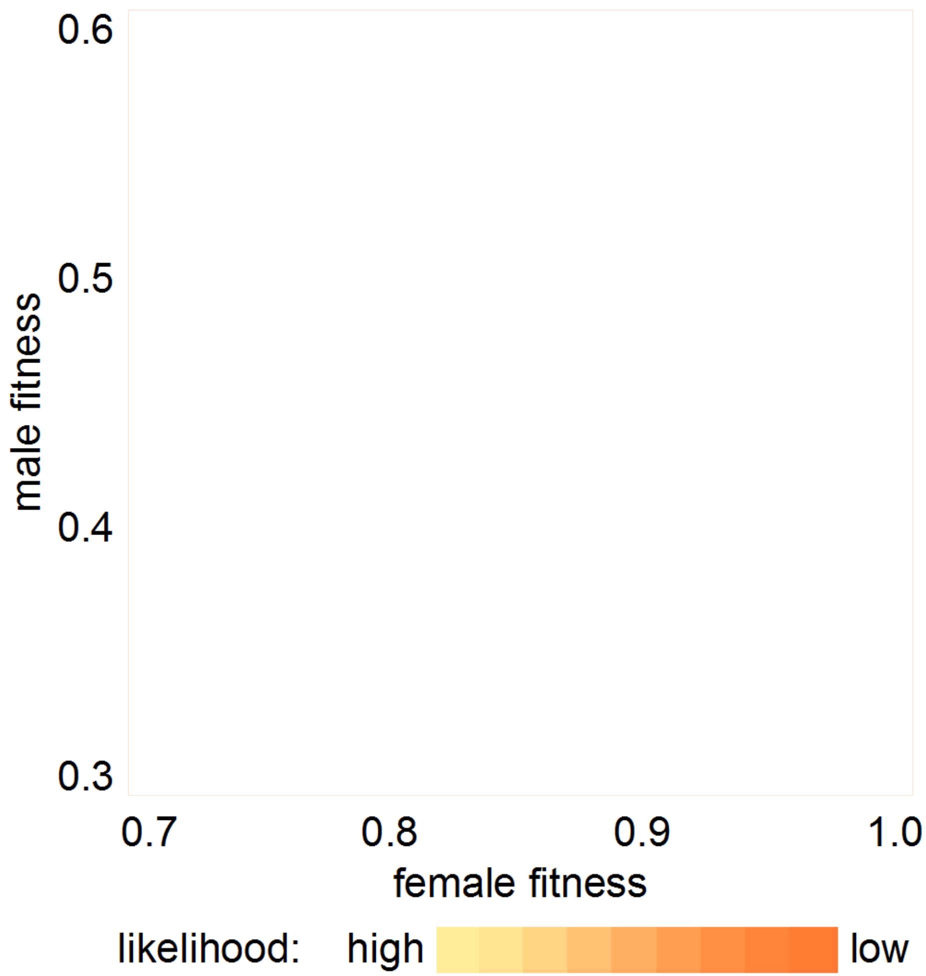
t = 40

t = 50

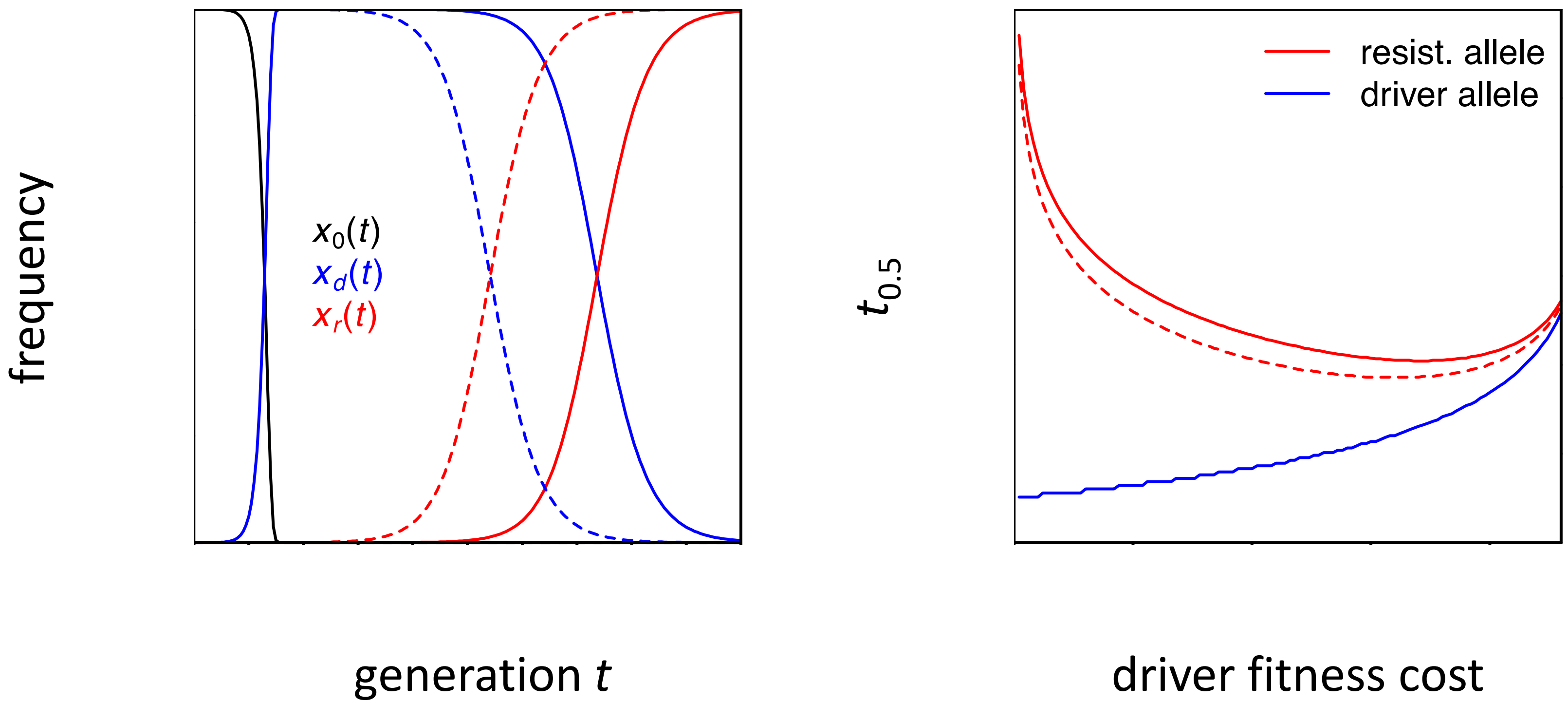
Fitness costs of a drive

Fitness costs of a drive

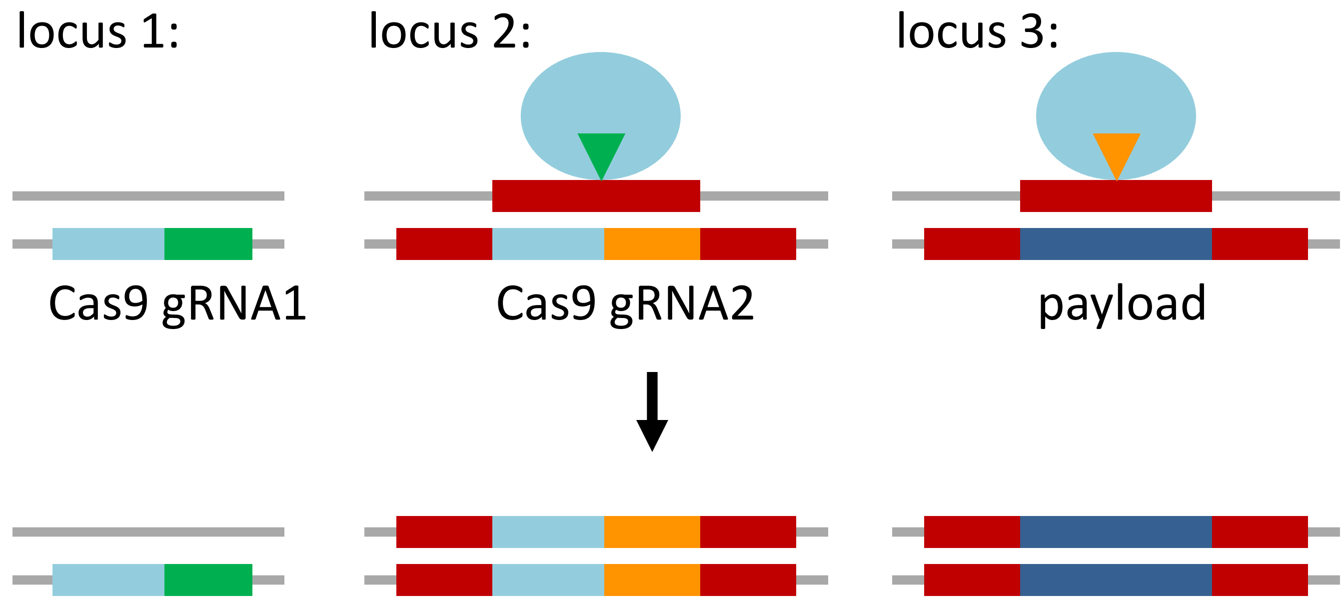
Fitness costs of disrupted *yellow* gene in *D. melanogaster*:

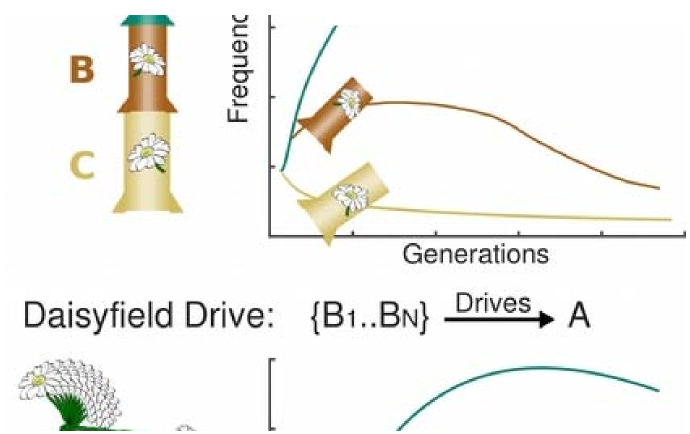


Resistance as a control mechanism?



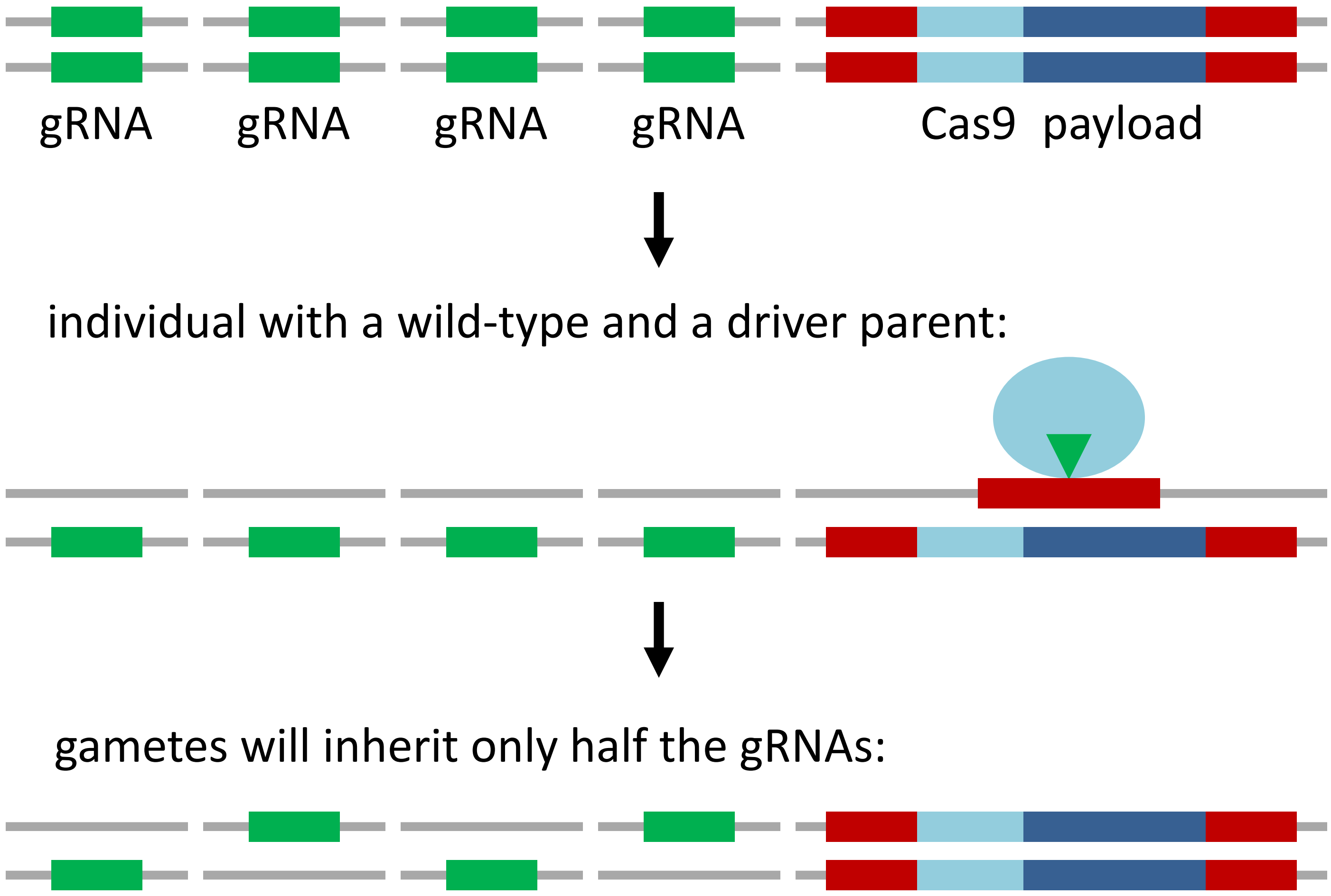
Daisy-chain drives





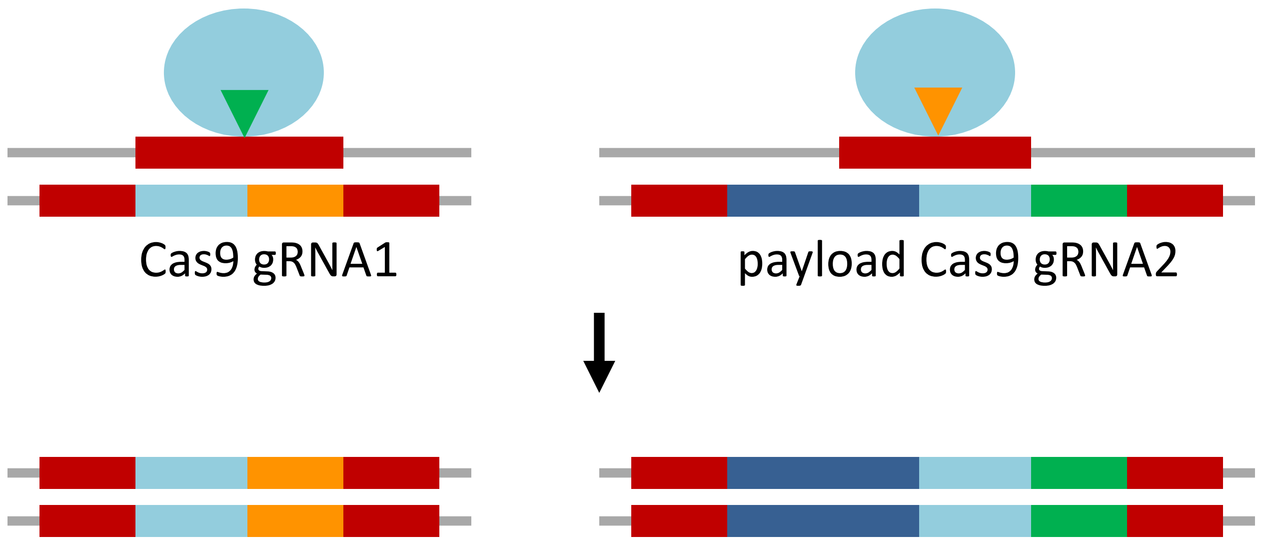
Noble et al., *bioRxiv* 2016

Daisy-field drives

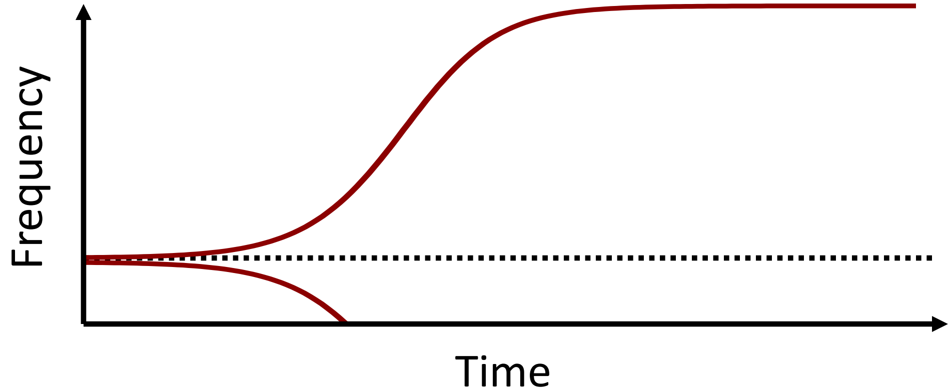


Min et al., *bioRxiv* 2017

Reciprocal drives



Frequency-dependent system → Quorum effect



Gene drive is an evolutionary process

Resistance will pose major obstacle (especially for population suppression approaches)

There may be strategies for confinement of CGDs, but many "moving" parts and robustness unclear

To understand and predict population dynamics, we need better population genetic models

Acknowledgments

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Publications:

Unckless, Messer, Connallon, Clark. *Genetics* 2015
Unckless, Clark, Messer. *Genetics* 2017
Champer, Reeves, Oh, Liu, Liu, Clark, Messer. *PLoS Genetics* 2017
Champer, Liu, Oh, Reeves, Luthra, Oakes, Clark, Messer. *bioRxiv*