Modeling/simulation of CRISPR-Cas9 based Controllable Gene Drive (CGD) system

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Outline

Introduction

Experimental methods

Modeling/Simulation results

Discussion

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Introduction

Engineering gene inheritance

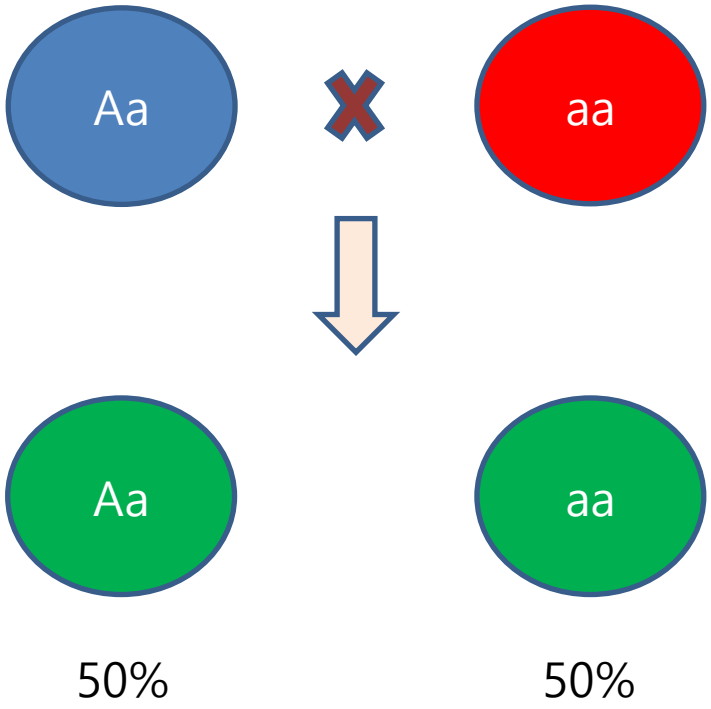
Many diseases can be tackled by developing strategies to alter gene inheritance.

e.g) Anti-malarial drug resistance is of enormous public health importance.

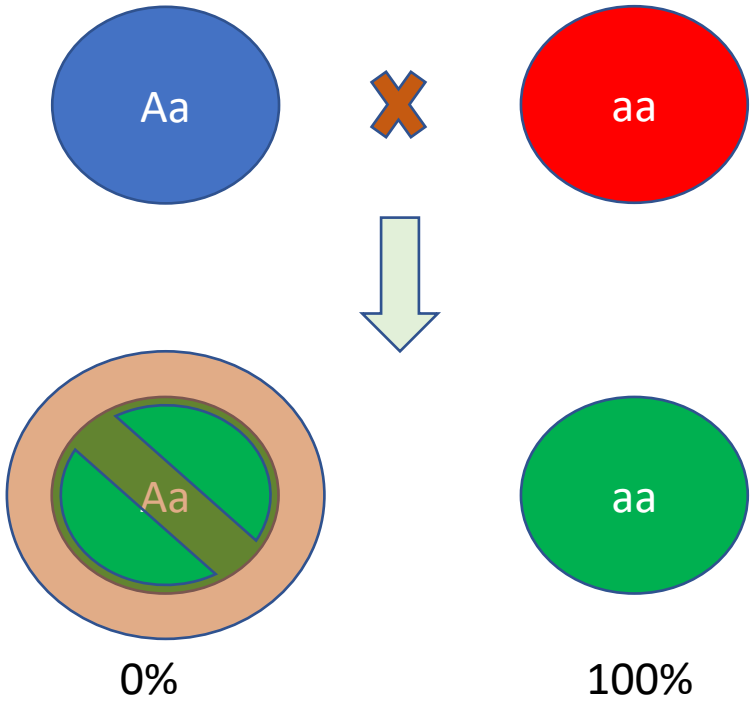
Strategy #1. Develop new drugs against the evolving resistance strains

Strategy #2. Alter the resistance genes and block their inheritance.

Mendelian inheritance

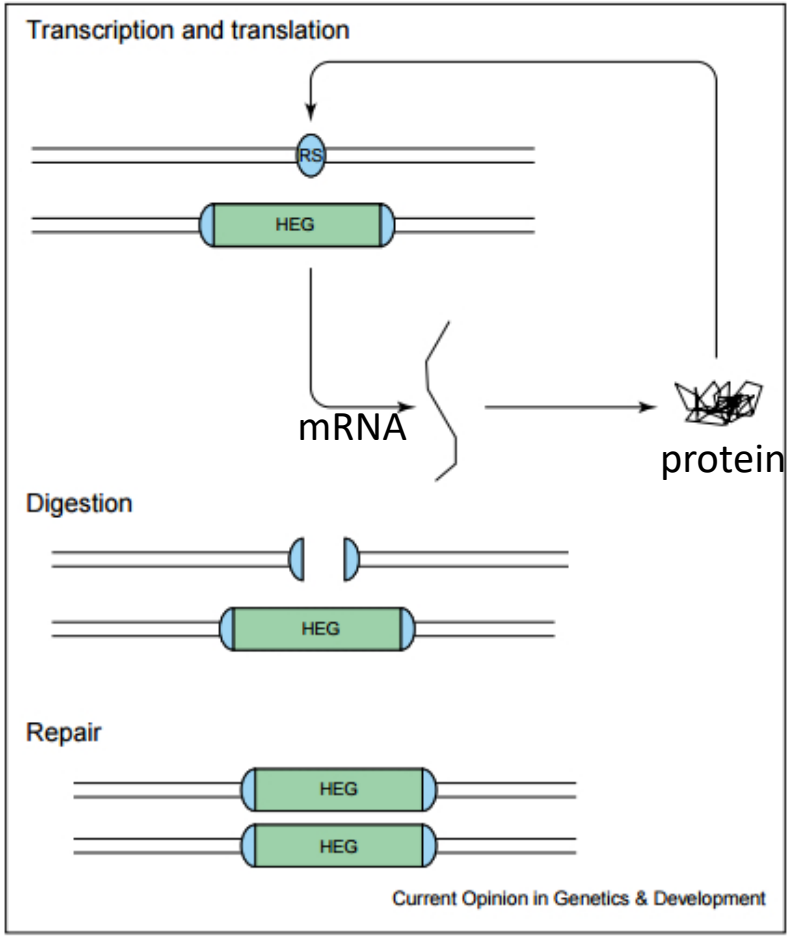


Super-Mendelian inheritance



Gene drives are genetic systems that greatly increase the odds that a particular allele will be passed on to offspring

How gene drive works



Austin Burt and Vassiliki Koufopanou (2004)

CRISPR/Cas9

A powerful tool that made the idea of engineered gene drives feasible.

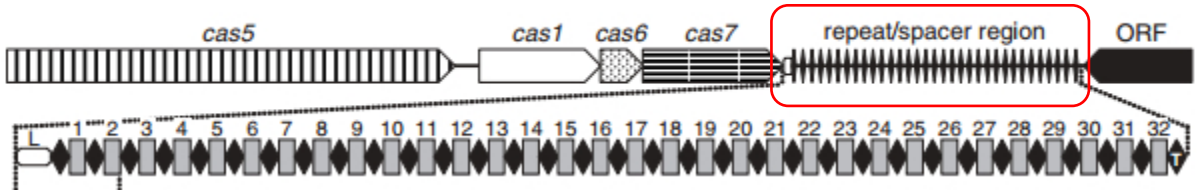
CRISPR is a genetic element that stores DNA from invading viruses.

Cas9 are enzymes that cut the foreign DNA at specific locations.

CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes

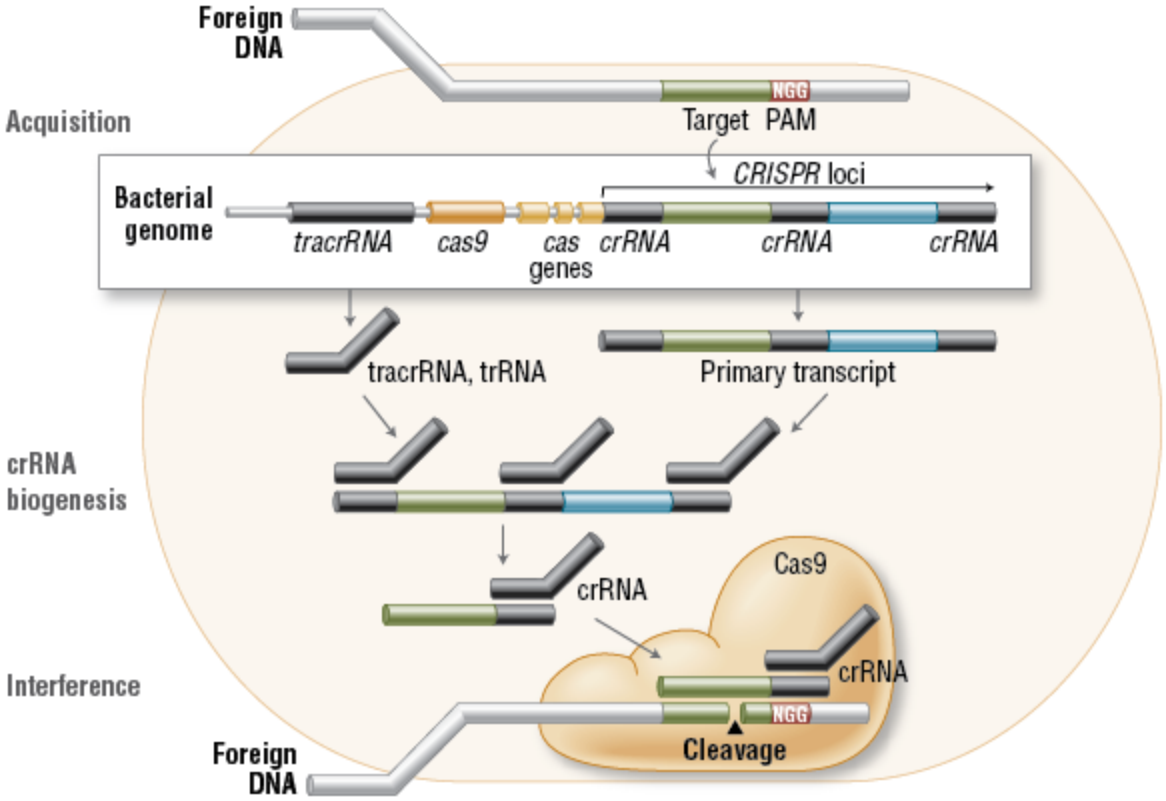
Rodolphe Barrangou,[1] Christophe Fremaux,[2] Hélène Deveau,[3] Melissa Richards,[1] Patrick Boyaval,[2] Sylvain Moineau,[3] Dennis A. Romero,[1] Philippe Horvath[2]*

Clustered regularly interspaced short palindromic repeats (CRISPR) are a distinctive feature of the genomes of most Bacteria and Archaea and are thought to be involved in resistance to bacteriophages. We found that, after viral challenge, bacteria integrated new spacers derived from phage genomic sequences. Removal or addition of particular spacers modified the phage-resistance phenotype of the cell. Thus, CRISPR, together with associated *cas* genes, provided resistance against phages, and resistance specificity is determined by spacer-phage sequence similarity.



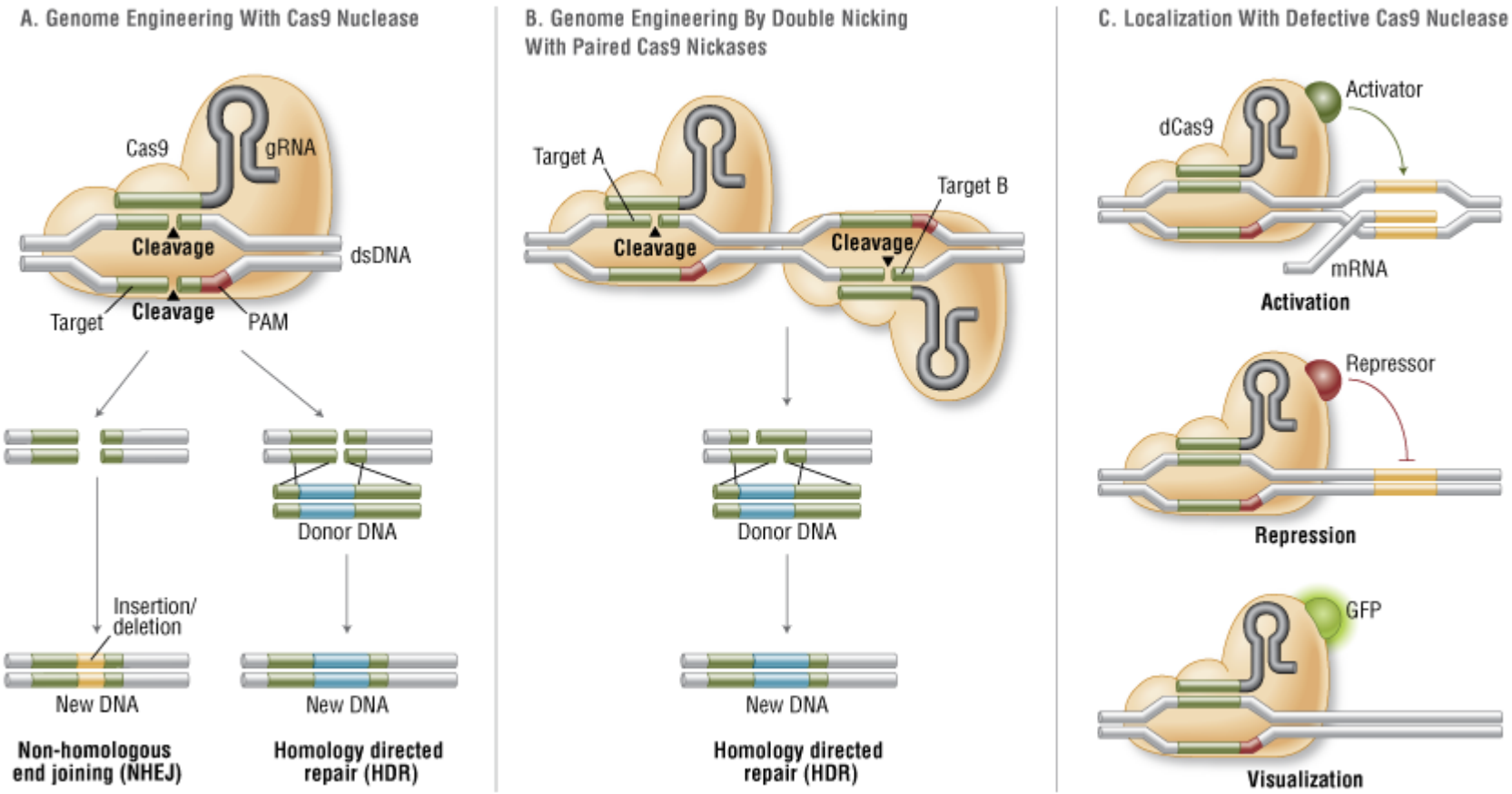
SCIENCE VOL 315 23 MARCH 2007

CRISPR/Cas9 mechanism



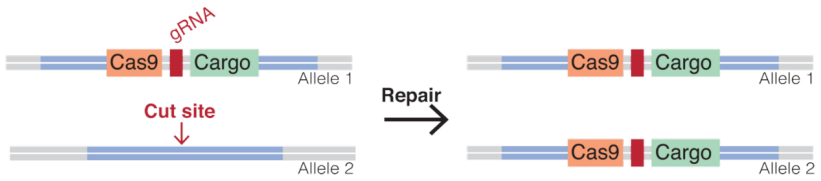
https://international.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology

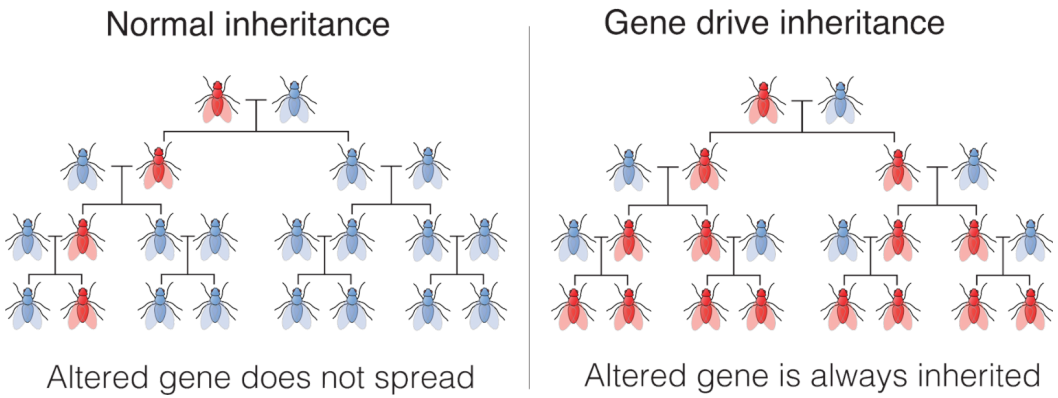
Applications of CRISPR/Cas9



https://international.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology

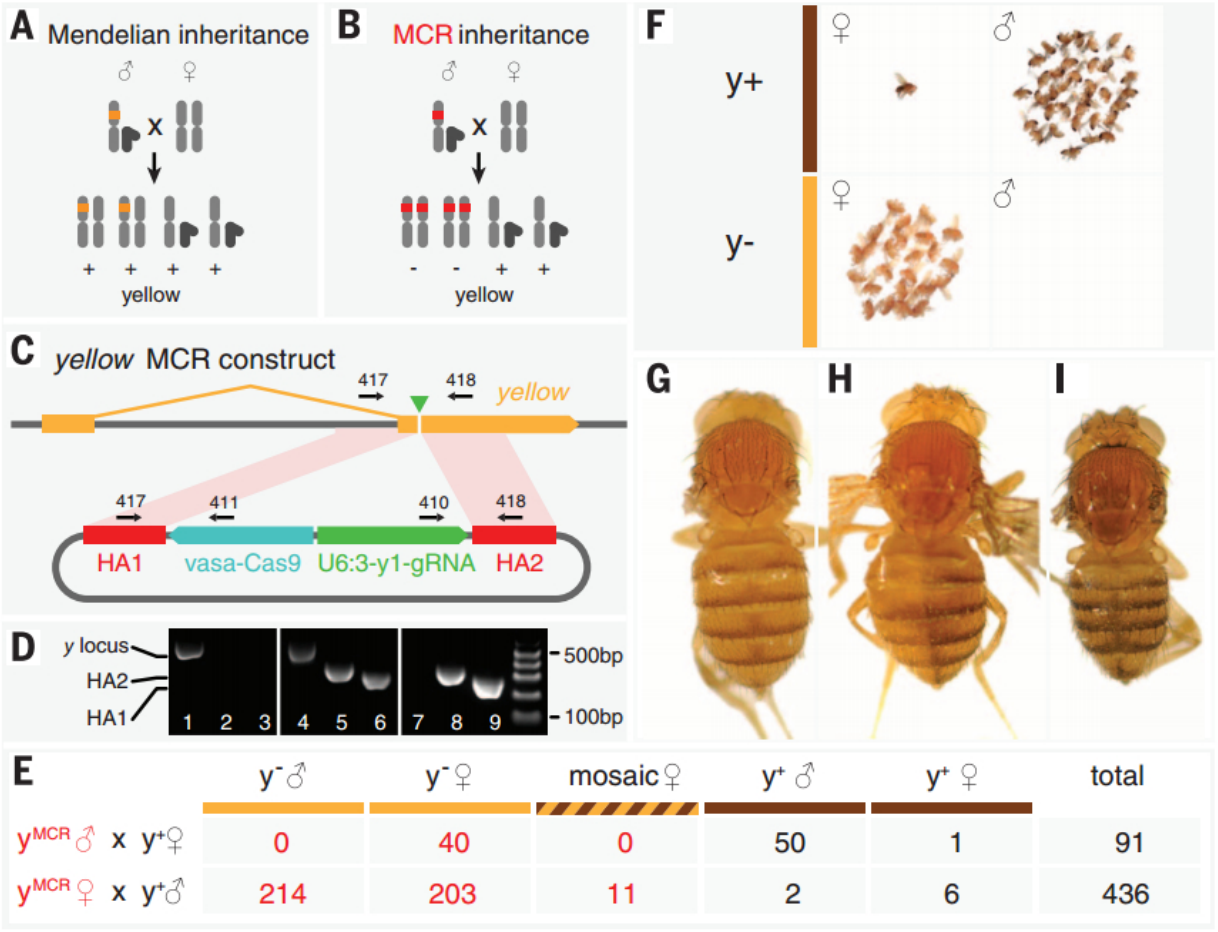
Cas-9 based gene drive





https://en.wikipedia.org/wiki/Gene_drive

Experimental demonstration



E

	y⁻♂	y⁻♀	mosaic♀	y⁺♂	y⁺♀	total
y^{MCR}♂ x y⁺♀	0	40	0	50	1	91
y^{MCR}♀ x y⁺♂	214	203	11	2	6	436

Gantz et al. Science, vol 348 issue 6233 (2015)

Proof-of-principle studies

Successful drive conversion were conducted in yeast, flies, and mosquitoes.

Highly variable conversion efficiencies – yeast: ~100%, flies: 19-62%, mosquitoes: 87-99%

Recently, successful drive conversion achieved in mice.

Rapid fixation of mutant allele

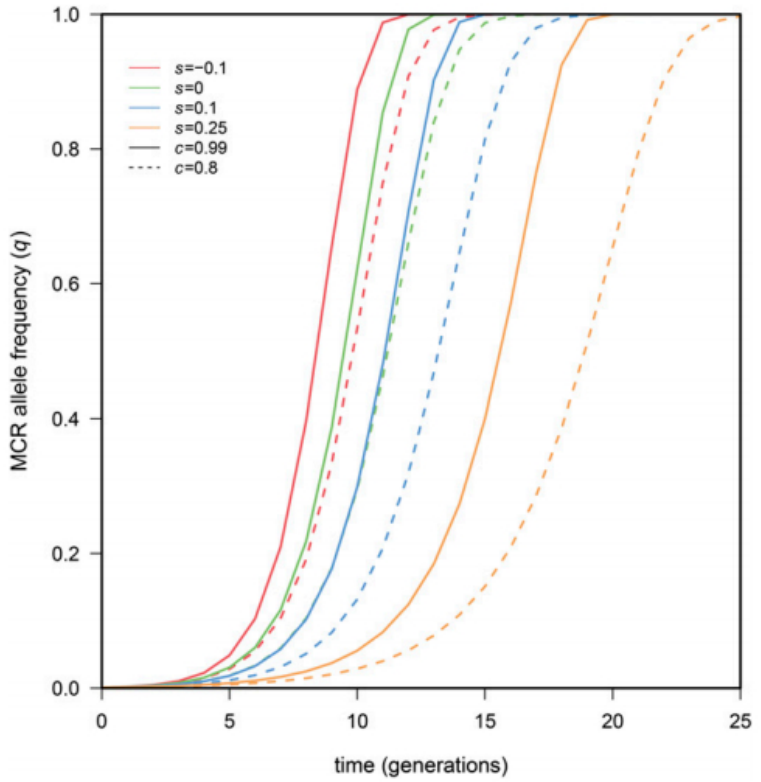


Figure 1 Trajectories of introduced MCR alleles reveal that even deleterious alleles sweep to fixation very quickly. Only parameter sets leading to fixation are presented, and all cases shown assume that fitness costs are recessive ($h = 0$).

Unckless et al. Genetics, Vol. 201, 425-431 (2015)

Introducing a novel gene into a population and having it spread to high frequency holds great promise for biological control (e.g. malaria).

Potential use of gene drive

Population suppression: the drive induces a major genetic load

Population replacement: The expressed gene induces an intended phenotypic alteration, such as blocked transmission of a pathogen.

e.g) Elimination of malaria, dengue, yellow fever, West Nile, sleeping sickness, Lyme, and others

ARTICLES

nature biotechnology

OPEN

A CRISPR–Cas9 gene drive targeting *doublesex* causes complete population suppression in caged *Anopheles gambiae* mosquitoes

Kyros Kyrou[1,2], Andrew M Hammond[1,2], Roberto Galizi[1], Nace Kranjc[1], Austin Burt[1], Andrea K Beaghton[1], Tony Nolan[1] & Andrea Crisanti[1]

In the human malaria vector *Anopheles gambiae*, the gene *doublesex* (*Agdsx*) encodes two alternatively spliced transcripts, *dsx-female* (*AgdsxF*) and *dsx-male* (*AgdsxM*), that control differentiation of the two sexes. The female transcript, unlike the male, contains an exon (exon 5) whose sequence is highly conserved in all *Anopheles* mosquitoes so far analyzed. We found that CRISPR–Cas9-targeted disruption of the intron 4–exon 5 boundary aimed at blocking the formation of functional AgdsxF did not affect male development or fertility, whereas females homozygous for the disrupted allele showed an intersex phenotype and complete sterility. A CRISPR–Cas9 gene drive construct targeting this same sequence spread rapidly in caged mosquitoes, reaching 100% prevalence within 7–11 generations while progressively reducing egg production to the point of total population collapse. Owing to functional constraint of the target sequence, no selection of alleles resistant to the gene drive occurred in these laboratory experiments. Cas9-resistant variants arose in each generation at the target site but did not block the spread of the drive.

Nature biotechnology, November 2018

Risk of gene drive based on MCR

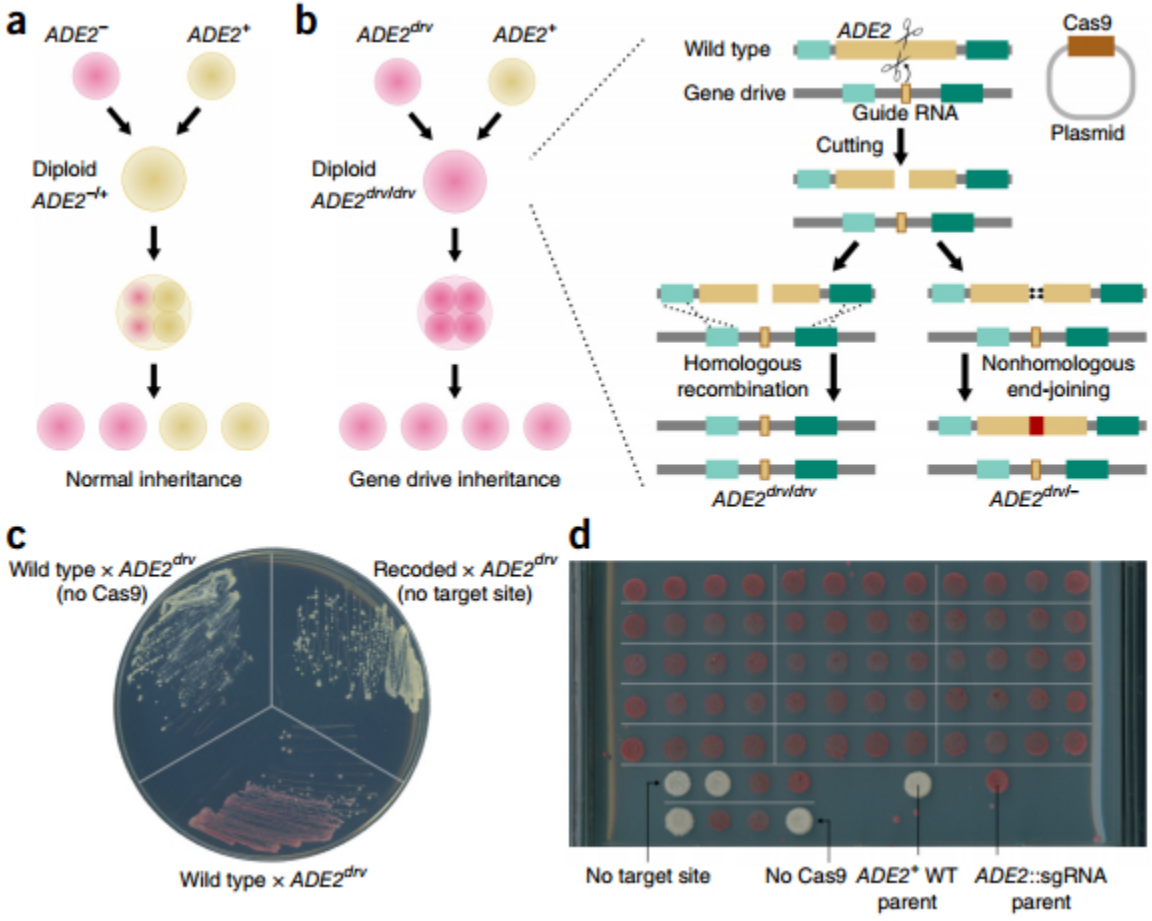
Despite their promise, gene drive can lead to unintended geographical spread.

Efforts to address such risk have been made.

- Church et al. (Nature biotechnology, 2015) proposed a molecular confinement strategy.

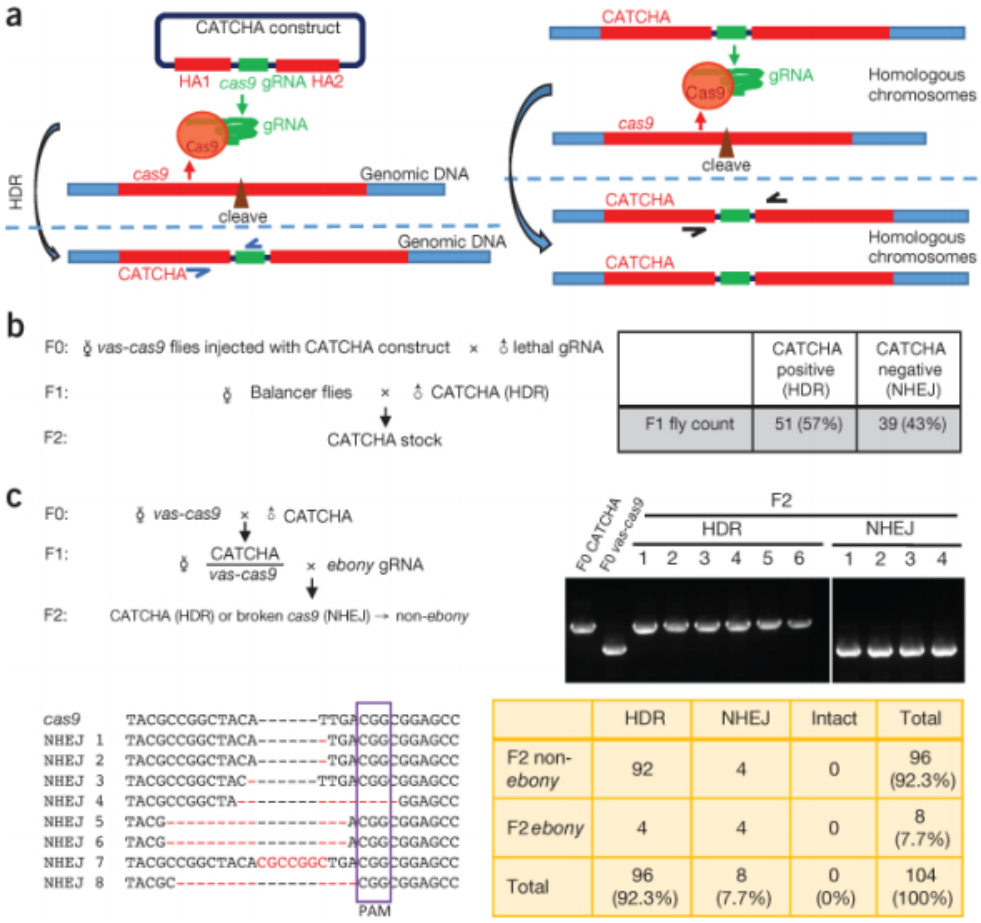
- Wu et al. (Nature biotechnology, 2017) proposed an overwriting strategy.

Molecular confinement



Church et al. Nature biotechnology, vol 33 no 12 (2015)

Overwriting (Reversal) "CATCHA"



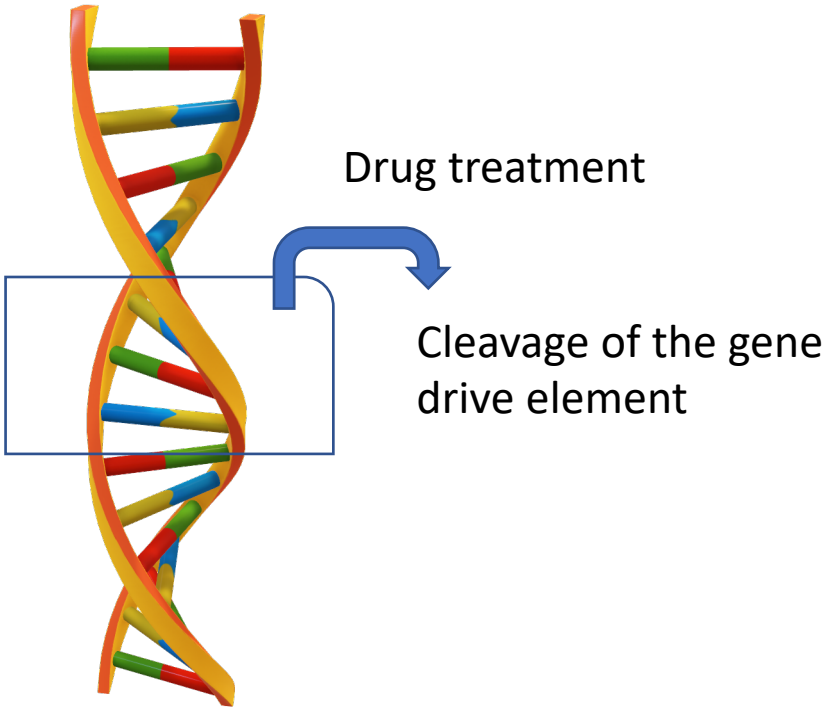
	CATCHA positive (HDR)	CATCHA negative (NHEJ)
F1 fly count	51 (57%)	39 (43%)

	HDR	NHEJ	Intact	Total
F2 non-*ebony*	92	4	0	96 (92.3%)
F2 *ebony*	4	4	0	8 (7.7%)
Total	96 (92.3%)	8 (7.7%)	0 (0%)	104 (100%)

Wu et al. Nature biotechnology 34(2): 137-138 (2016)

Objectives

We propose a new strategy of chemical control whereby drug treatment induces a cleavage of the gene drive element.



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Experimental methods

yellow gene

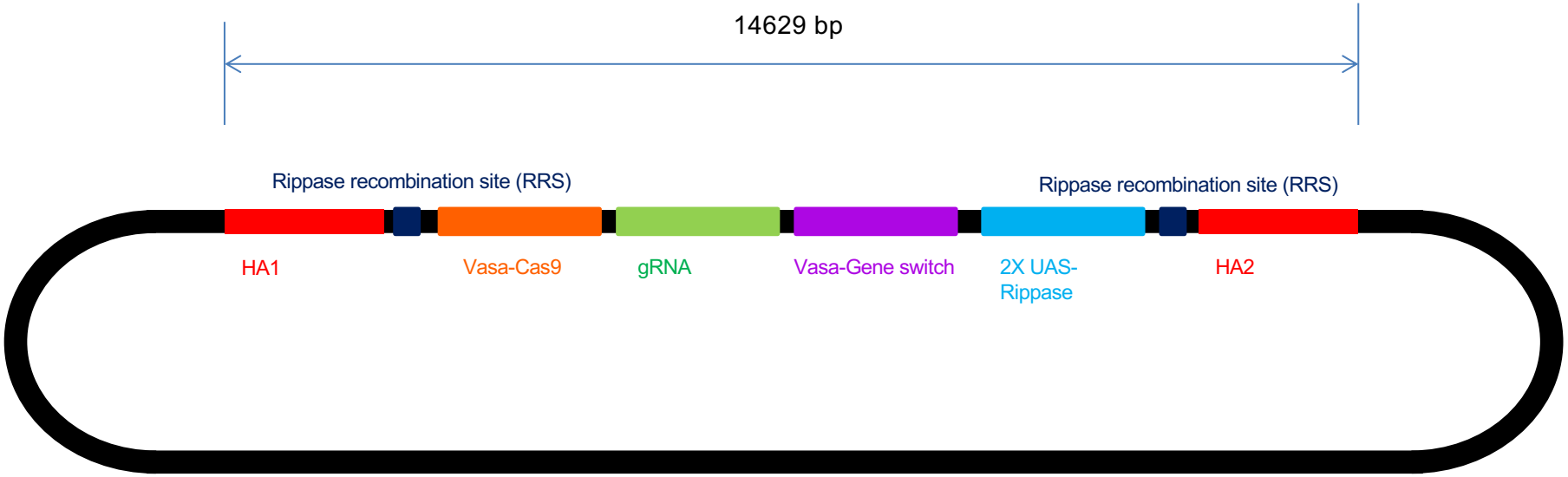
D. melanogaster.

The target gene = *yellow*.

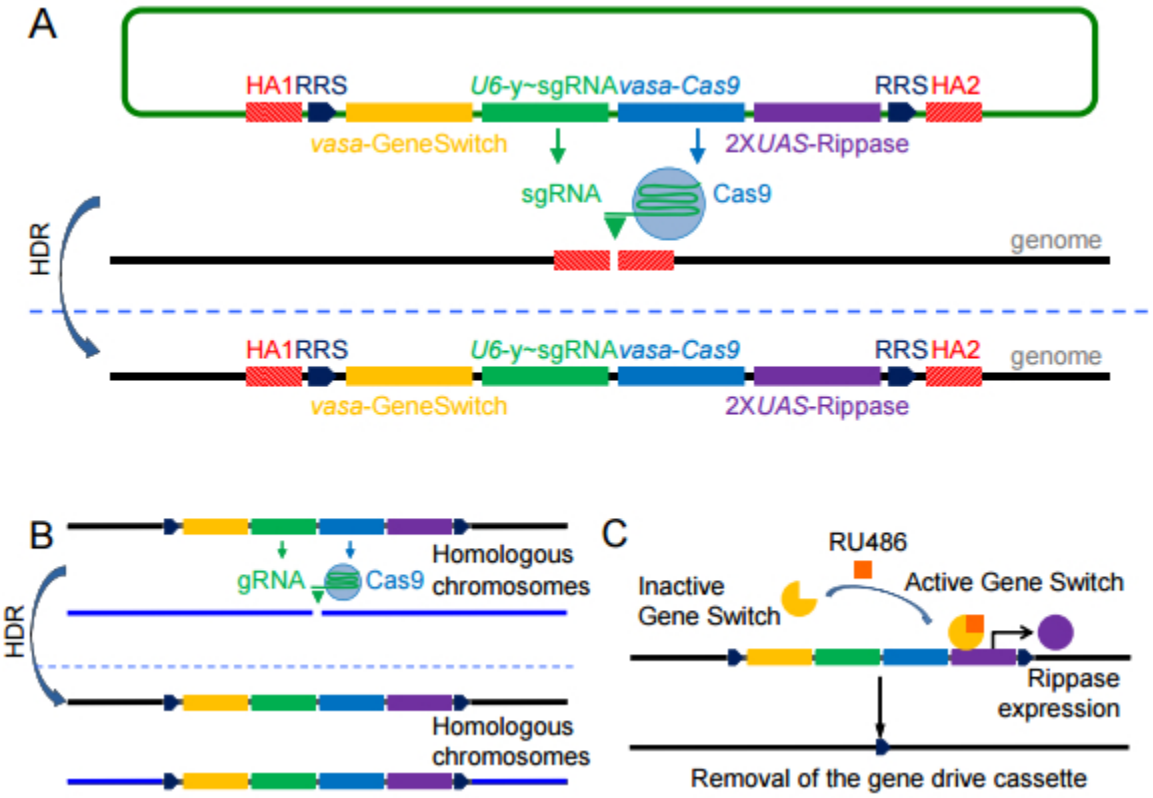
Located on the X-chromosome and produces a yellow cuticle when knocked out. (X-linked recessive)

A. mutant, B. mosaic, C. wild type

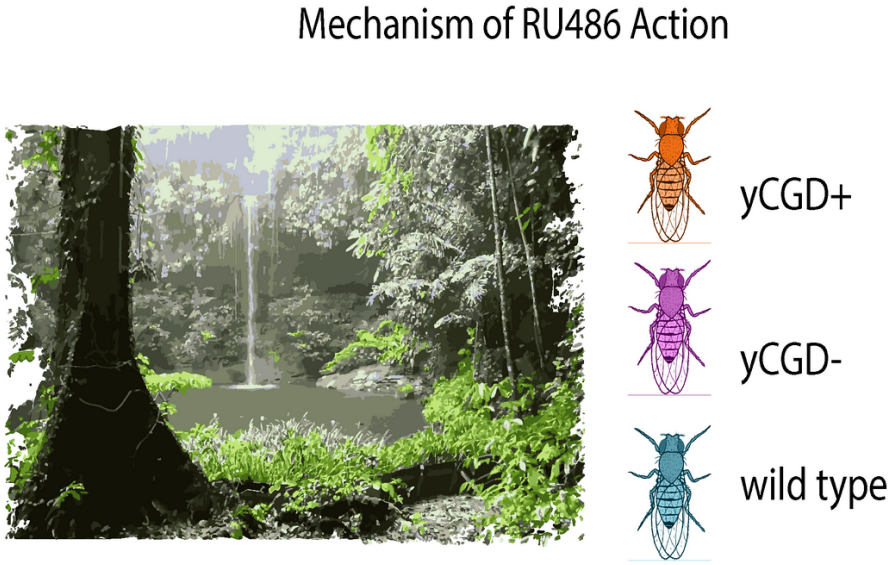
Controllable gene drive (CGD) construct



Mechanism of drug action



RU486 halts gene drive spread



Breeding results

Gender of y^{CGD} individual		RU486 dose (μM)	F_1 crosses [95% CI]	F_2 crosses [95% CI]
F1	F2			
Male	Female	0	Male *y*- progeny: 0% Female *y*- progeny: 100%	Male *y*- progeny: 83.46% Female *y*- progeny: 59.44%
		200	Male *y*- progeny: 0% Female *y*- progeny: 100%	Male *y*- progeny: 82.92% Female *y*- progeny: 52.94%
Female	Female	0	Male *y*- progeny: 85.79% Female *y*- progeny: 57.89%	Male *y*- progeny: 89.84% Female *y*- progeny: 56.89%
		200	Male *y*- progeny: 87.43% Female *y*- progeny: 54.42%	Male *y*- progeny: 90.09% Female *y*- progeny: 44.87%

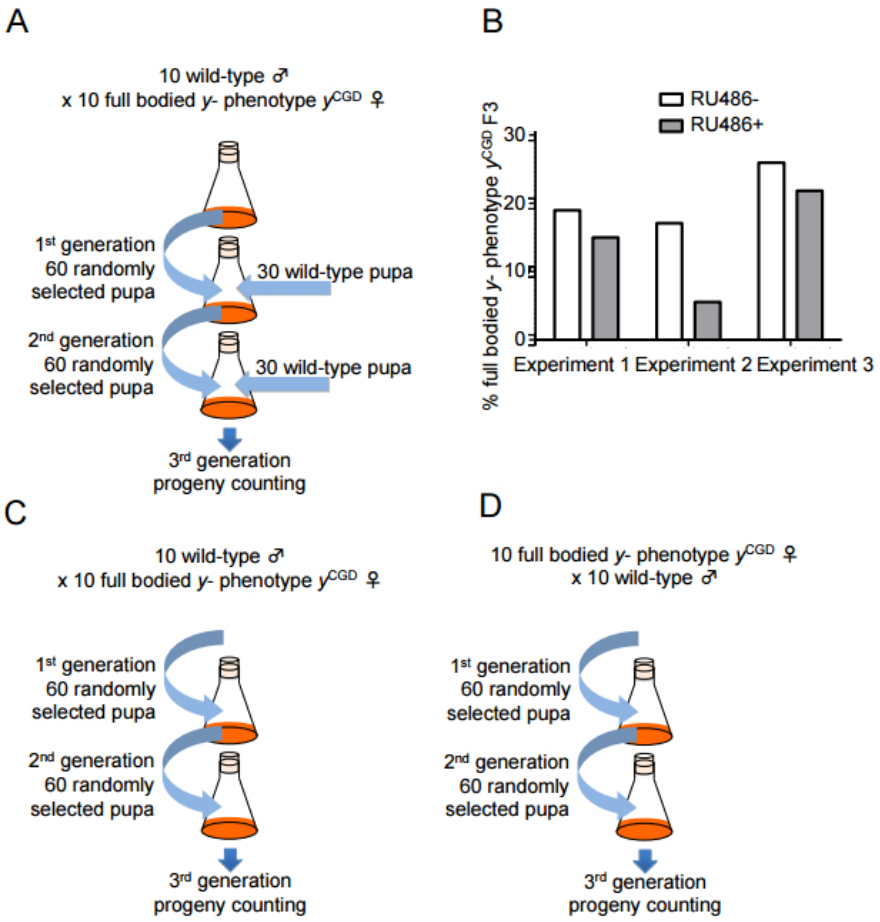
Interpretation

RU486 acts on the embryo to convert to yCGD+ to yCGD-.

Both yCGD+ and yCGD- are y- in phenotype, so % y- frequency in F2 is altered.

Since yCGD- cannot spread, % y- frequency in F3 is reduced in the RU486 treated group.

Cage population experiments



A. Open experiment

B. % full bodied y- phenotype in F3 in open experiment repeated three times

C. Closed experiment with F1: wild type male x mutant female

D. Closed experiment with F1: mutant male x wild type female

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Modeling results

Population genetics

p=wild type

q=mutant allele frequency

$(p + q = 1)$

$q(t+1) = (\text{homozygote q}) + 0.5(\text{heterozygote q}) + (\text{converted homozygote q})$

$$q(t+1) = q(t)^2 + p(t)\cdot q(t) + e\cdot p(t)\cdot q(t)$$
$$= q(t)(q(t) + p(t)(1 + e))$$
$$= q(t)(1 + e\cdot p(t))$$

(e: MCR efficiency)

Population genetics (2)

Sex-specific equations :

(q_m: mutant male, q_f: mutant female)

In females,

$$q_f(t+1) = q_m(t)\, q_f(t) + 0.5(p_m(t)\, q_f(t) + q_m(t)\, p_f(t))(1 + e)$$

In males,

$$q_m(t+1) = q_f(t)$$

Since y gene is on X-chromosome,

Population genetics (3)

Drug (RU486) converts yCGD+ to yCGD-

$$q(t+1) = q(t)(q(t)(1 - d_1) + p(t)(1 + e))(1 - d_2)$$
$$= q(t)(1 + p(t)(1 + e) - d_1 q(t))(1 - d_2)$$

d_1: Drug effect before fertilization (in the germ cells)

d_2: Drug effect after fertilization (in the embryo)

Modeling fitting

Model fitting to population phenotype frequency data using NONMEM.

Since phenotype frequency ranges between 0 and 1, beta regression was used.

```
$ERROR
;PHENO: y- phenotype frequency prediction
 X1 = TAU
 X2 = PHENO*TAU
 X3 = (1 – PHENO)*TAU

 COEFF = EXP(GAMLN(X1))/(EXP(GAMLN(X2))*EXP(GAMLN(X3)))
 LOGY = (X2-1)*LOG(DV+1E-06) + (X3-1)*LOG(1-DV) + LOG(COEFF)
 Y = -2*LOGY

$ESTIM COND -2LL LAPLACIAN
```

Beta regression method in NONMEM

J Pharmacokinet Pharmacodyn (2013) 40:537–544
DOI 10.1007/s10928-013-9318-0

SHORT REPORT

Mixed-effects beta regression for modeling continuous bounded outcome scores using NONMEM when data are not on the boundaries

Xu Steven Xu · Mahesh N. Samtani · Adrian Dunne · Partha Nandy · An Vermeulen · Filip De Ridder · The Alzheimer's Disease Neuroimaging Initiative

$$y_{ij}|\eta_i, \theta, \tau \sim beta\,(\mu_{ij}\tau, (1-\mu_{ij})\tau) \quad (1)$$

$$f(y_{ij};\theta,\eta_i,\tau) = \frac{\Gamma(\tau)}{\Gamma(\mu_{ij}\tau)\Gamma((1-\mu_{ij})\tau)} y_{ij}^{(\mu_{ij}\tau-1)}(1-y_{ij})^{(1-\mu_{ij})\tau-1} \quad (2)$$

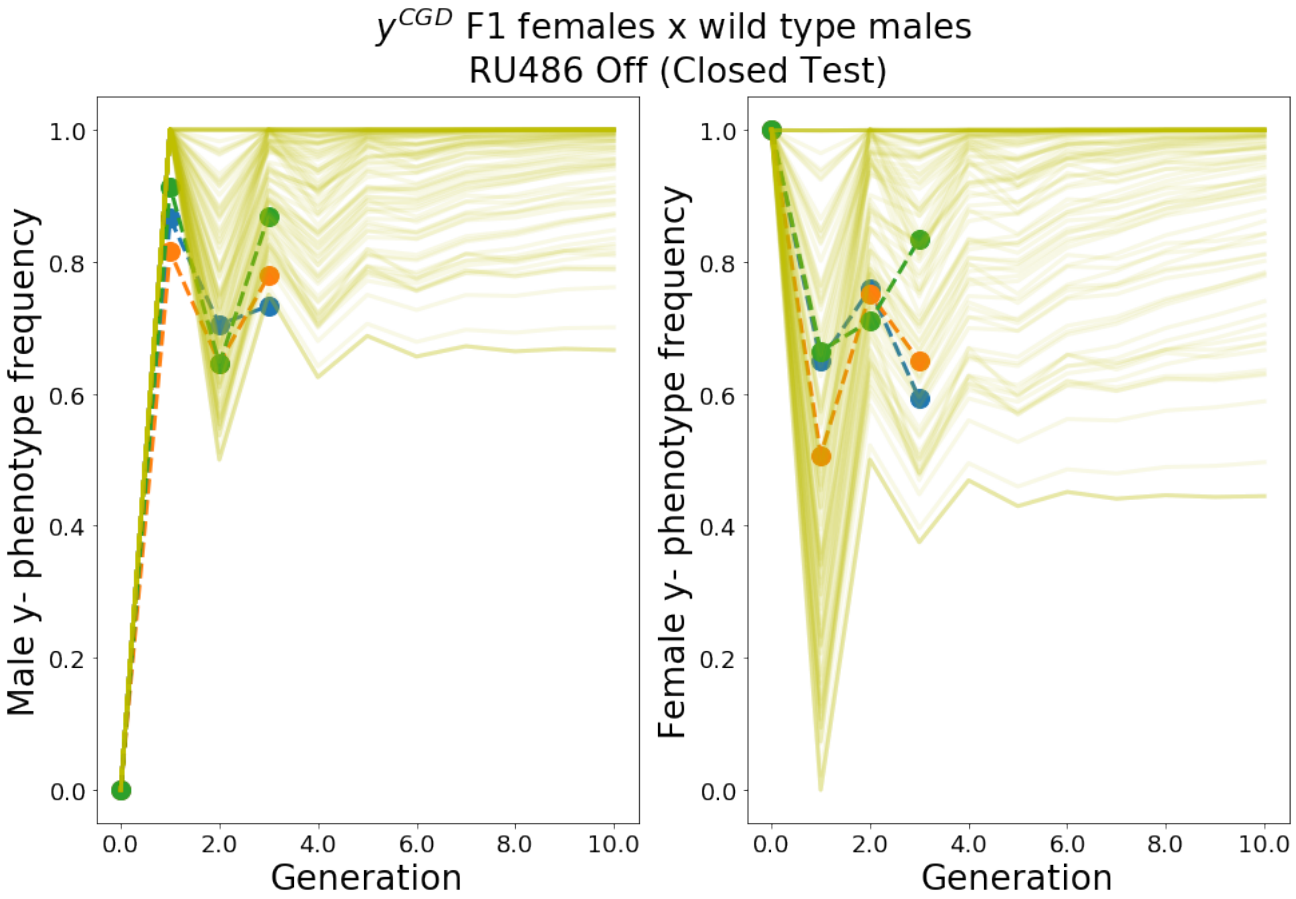
$$\log\left(\frac{\mu_{ij}}{1-\mu_{ij}}\right) = g(\theta,\eta_i,x_{ij}) \quad (3)$$

Estimation result

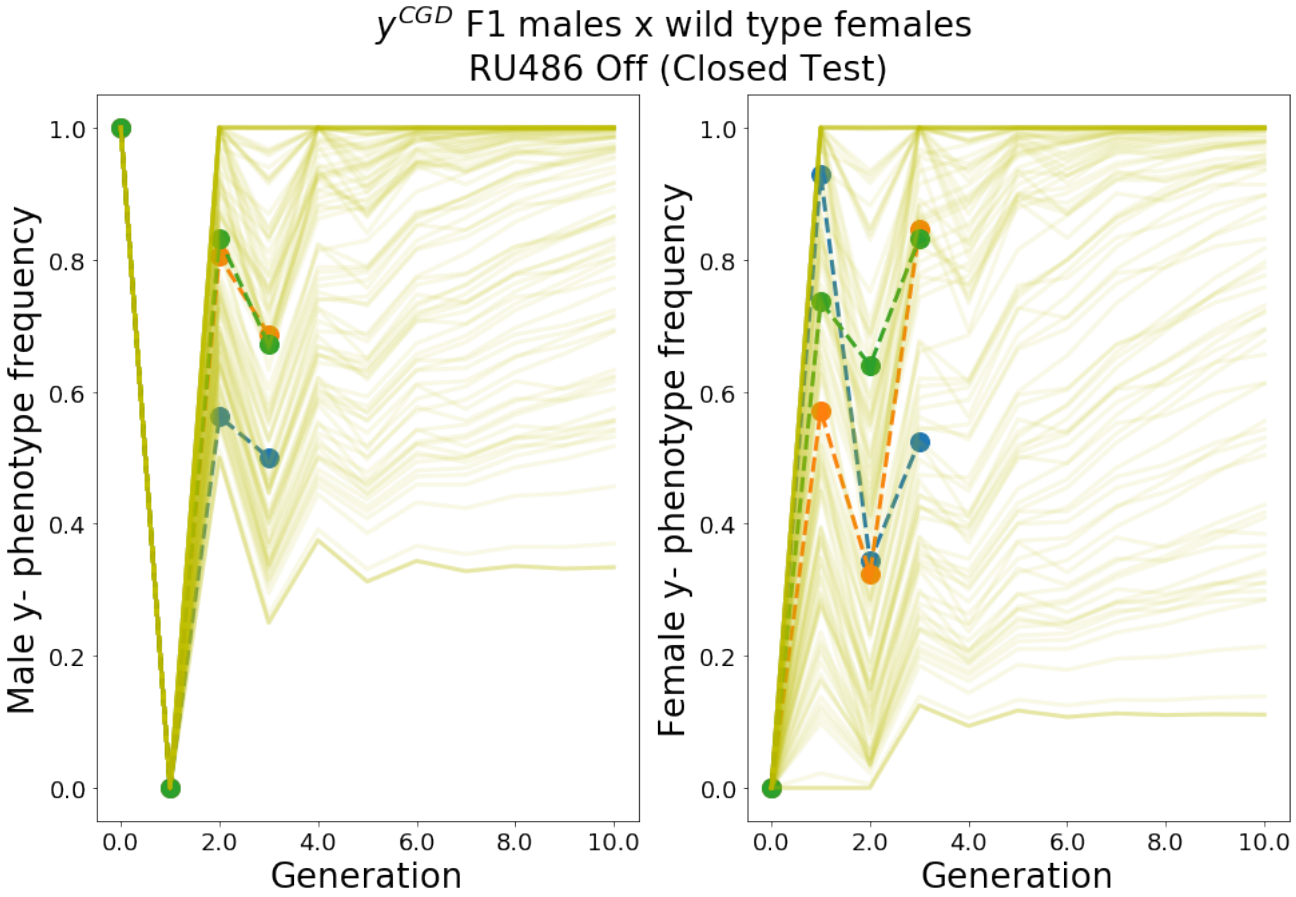
Model parameters	Estimate (Standard error, *CV%)
Fixed effect parameters	
Drug effect (δ) after fertilization	0.142 (15.22%)
MCR efficiency (e)	0.6713 (9.11%)
% Maternal germline y^{CGD}	0.8954 (1.79%)
Random effect parameters	
Variance of MCR efficiency (e) in random crossing	0.1896 (20.83%)
Variance of % maternal germline y^{CGD}	0.1032 (35.8%)

*CV%: coefficient of variation

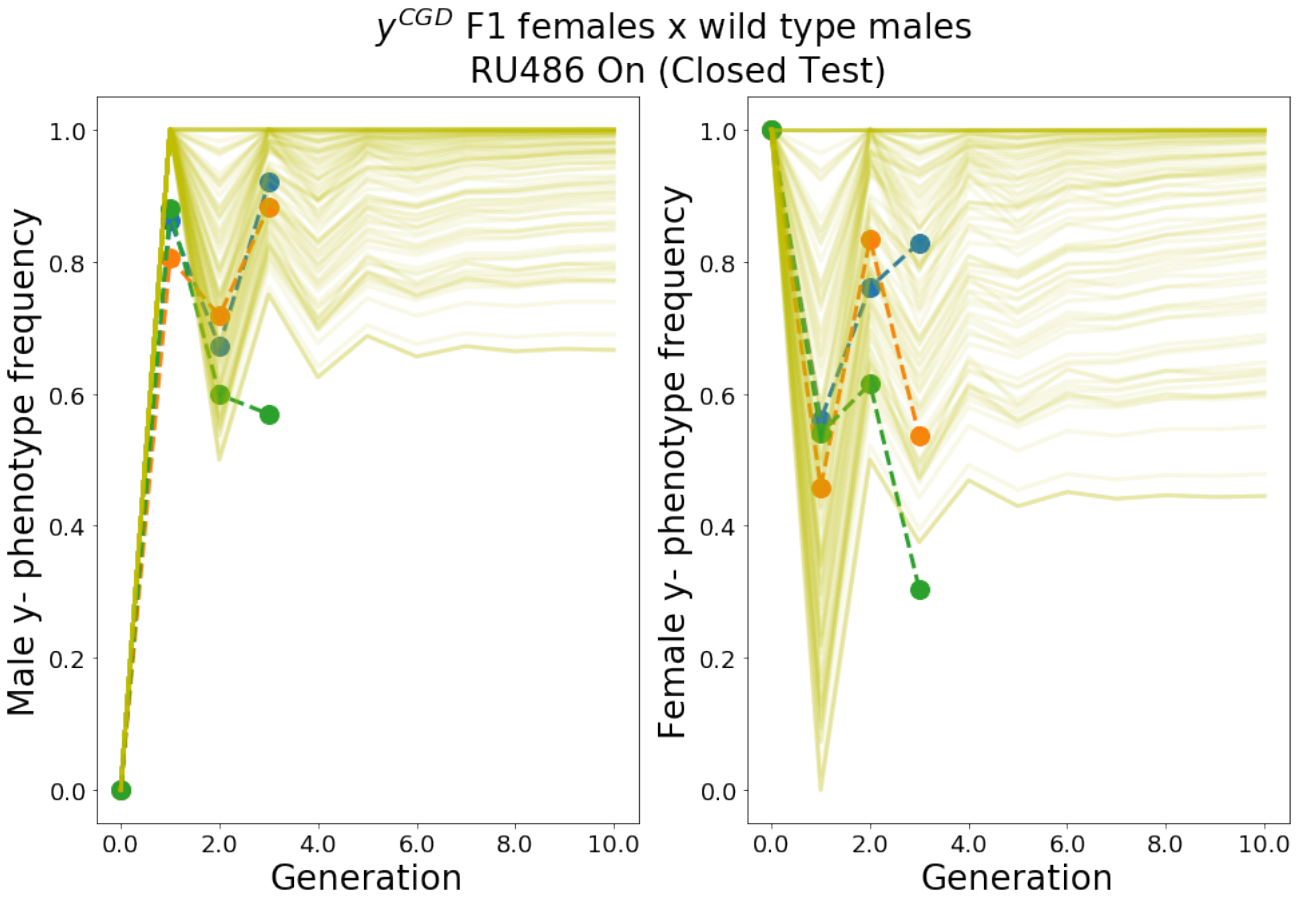
Visual predictive check (1)



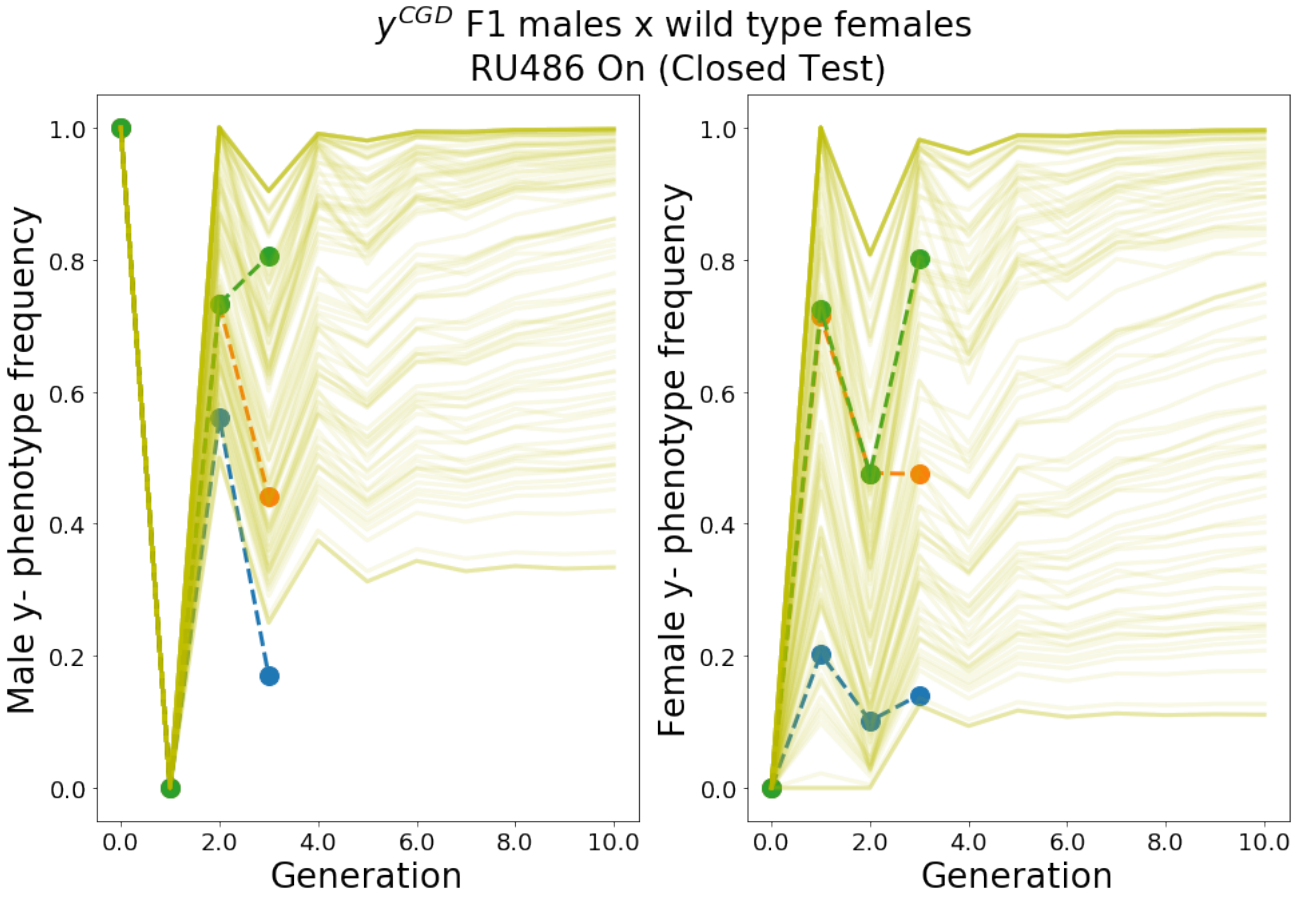
Visual predictive check (2)



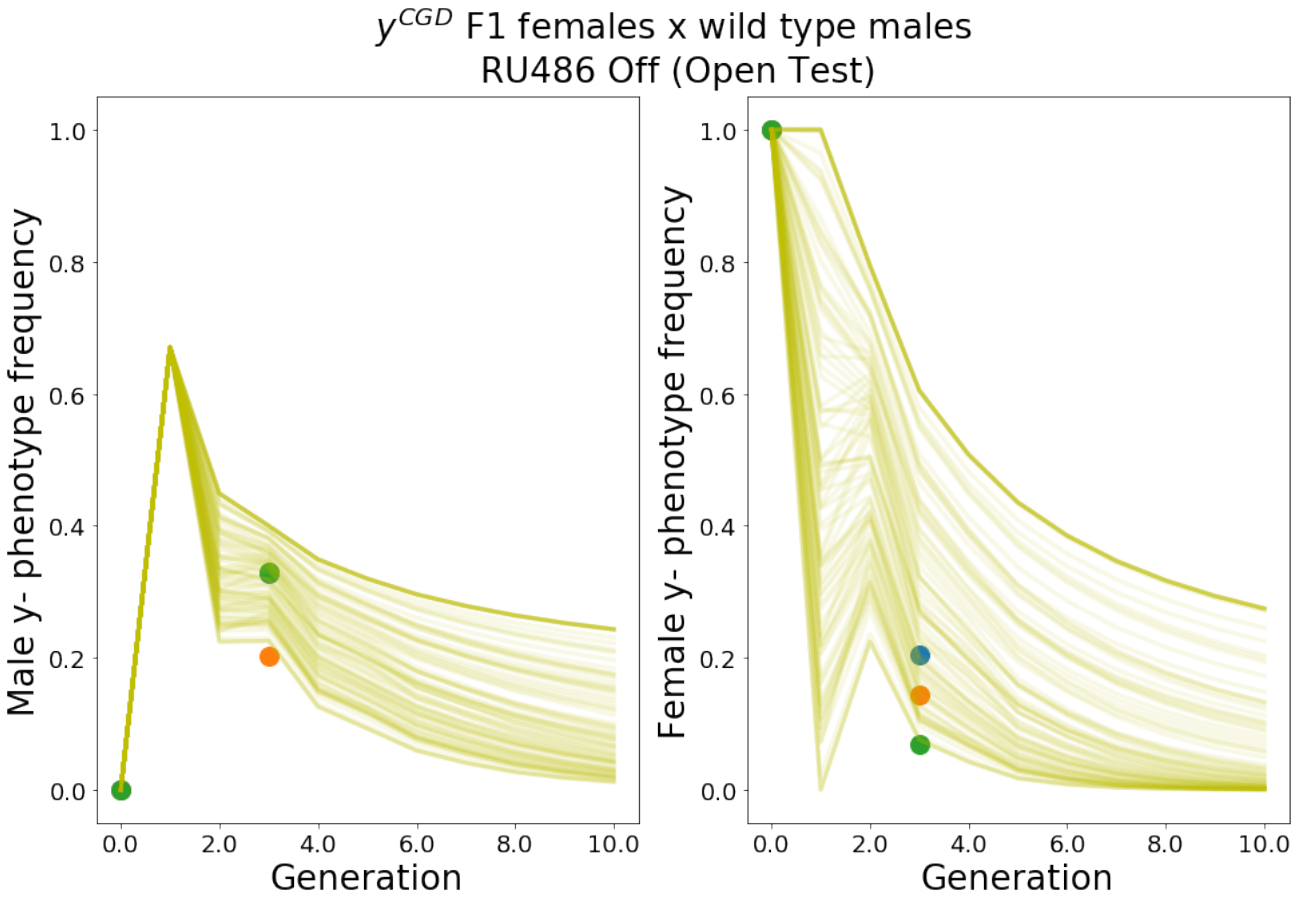
Visual predictive check (3)



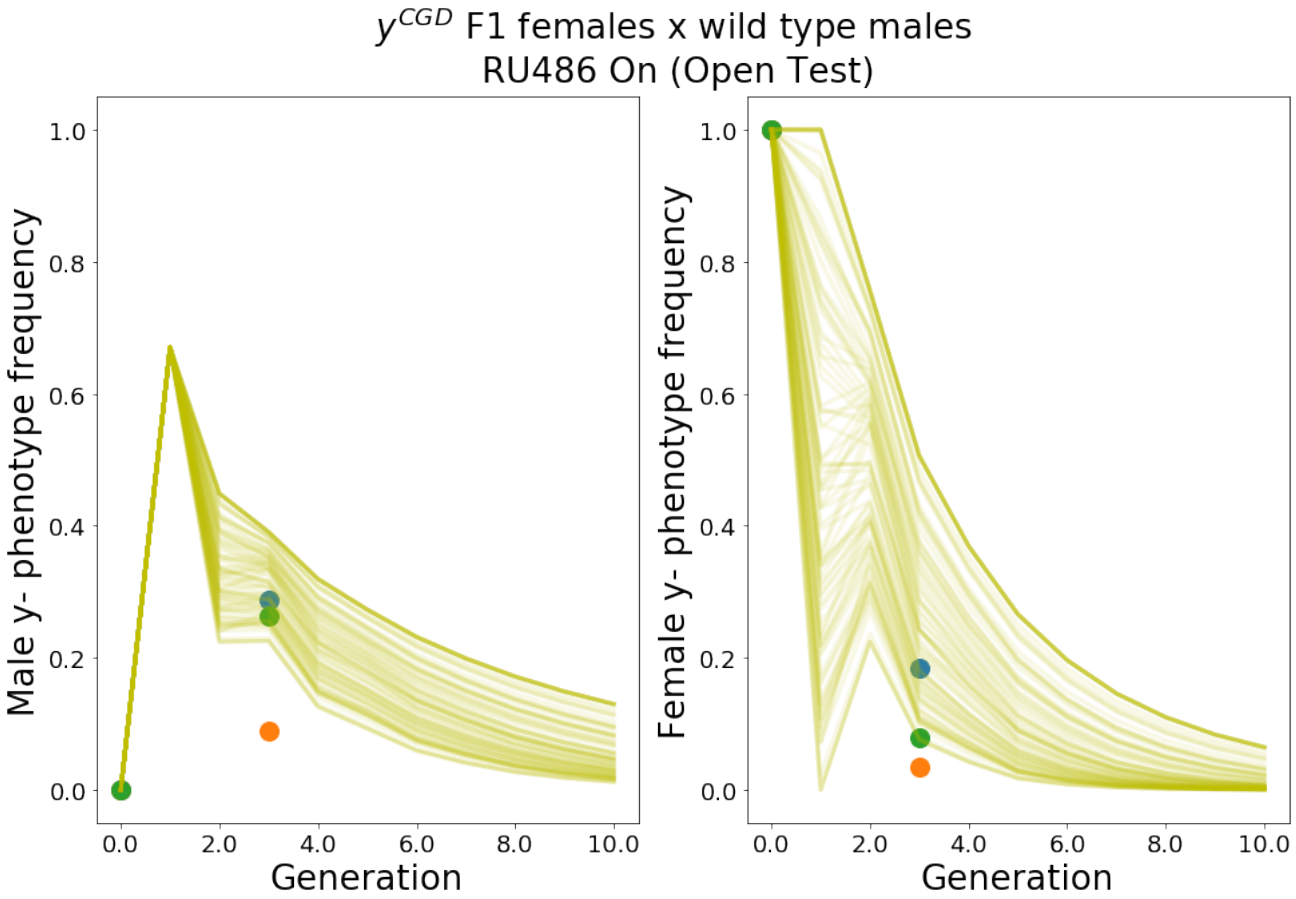
Visual predictive check (4)



Visual predictive check (5)



Visual predictive check (6)



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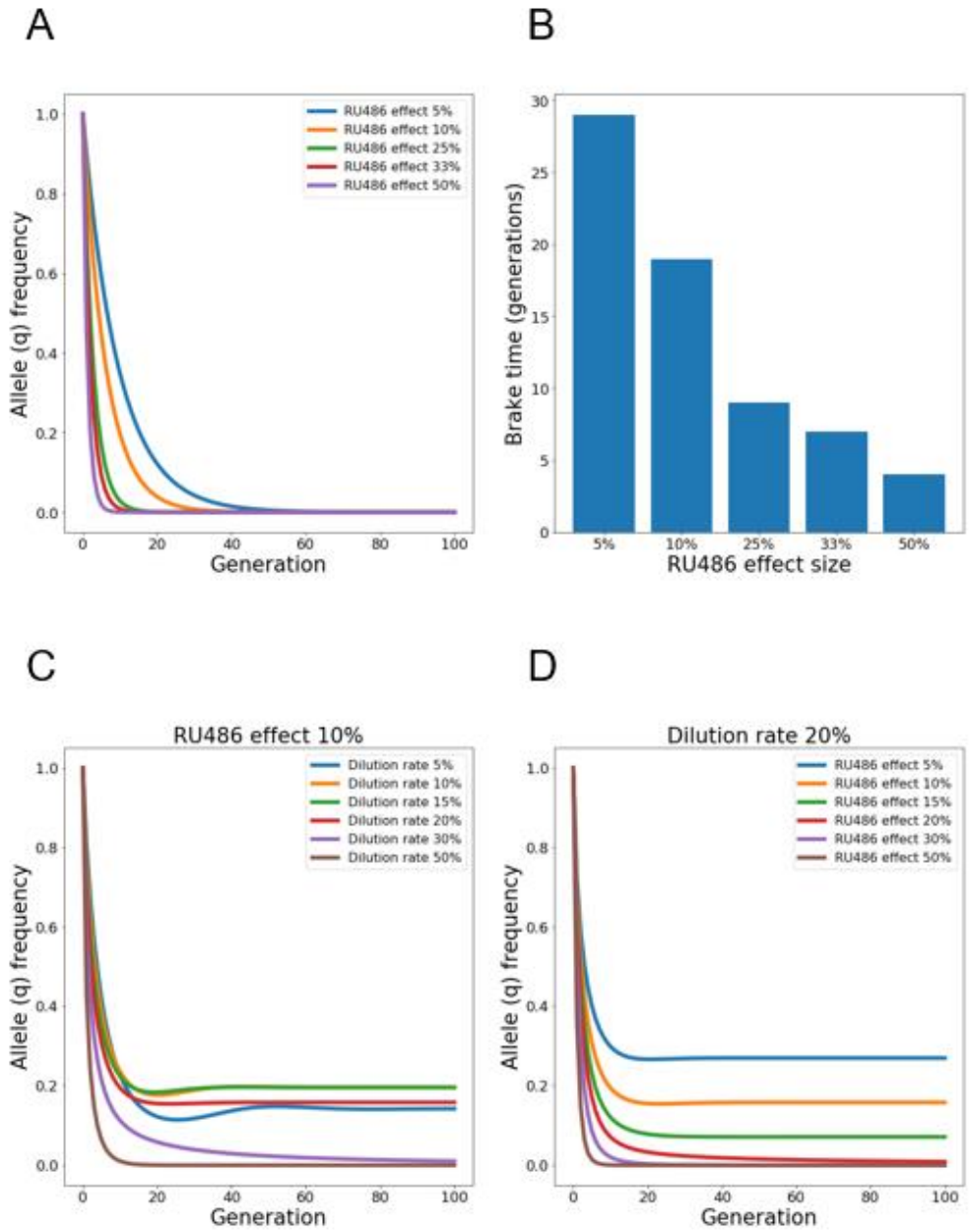
Simulation results

Definition of "brake time"

The time (generations) required for yCGD+ allele frequency to drop below 5%.

Magnitude of drug effect: The percentage of yCGD+ allele that is converted to yCGD-



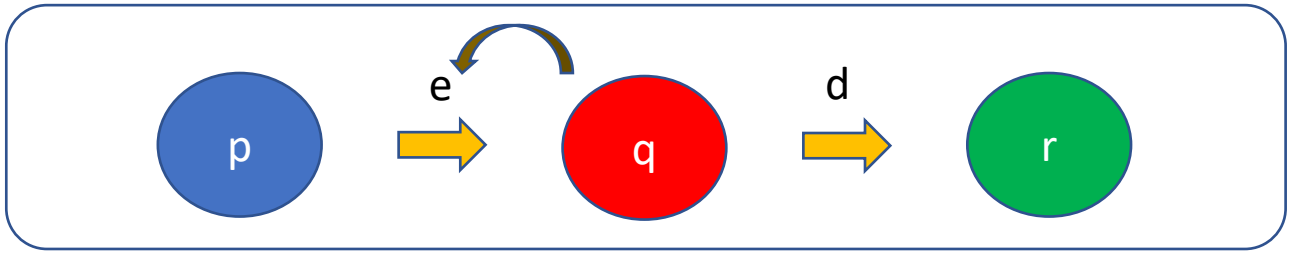
Closed system.

Increasing RU486 effect leads to shorter brake time.

Open system with wild type immigration and mutant dilution.

Dilution rate between 5-15% leads to failure of mutant extinction.

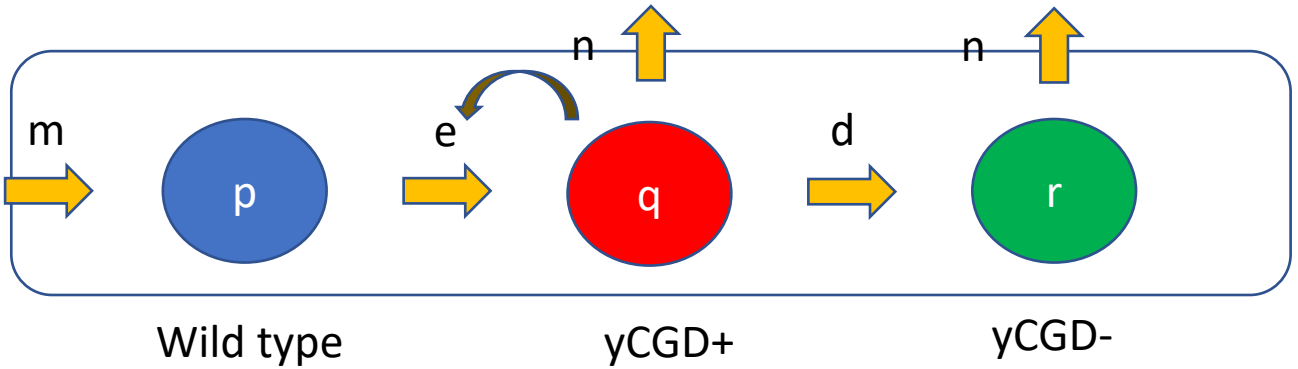
Why?



When there is no external input, the flow of p → q → r stops once wild type is depleted.

At steady state, p = 0 and r = 1.

Why? (2)

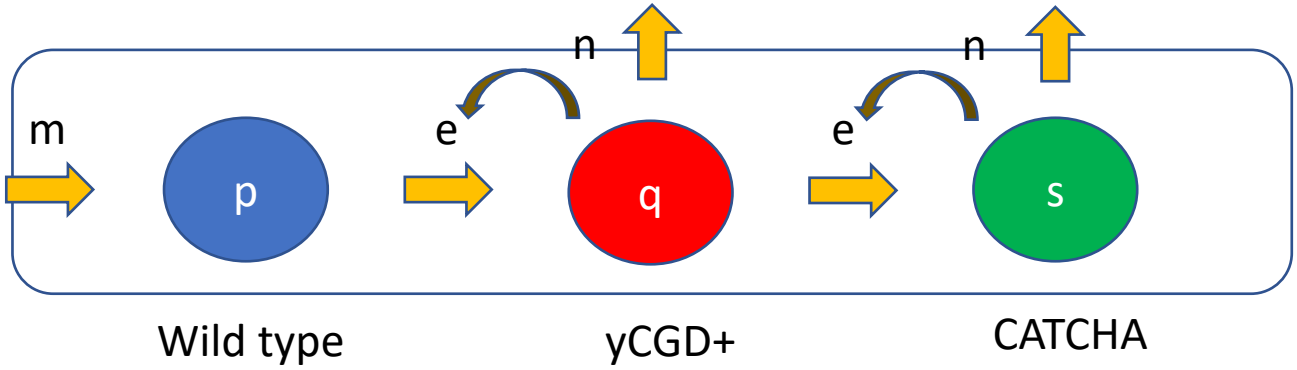


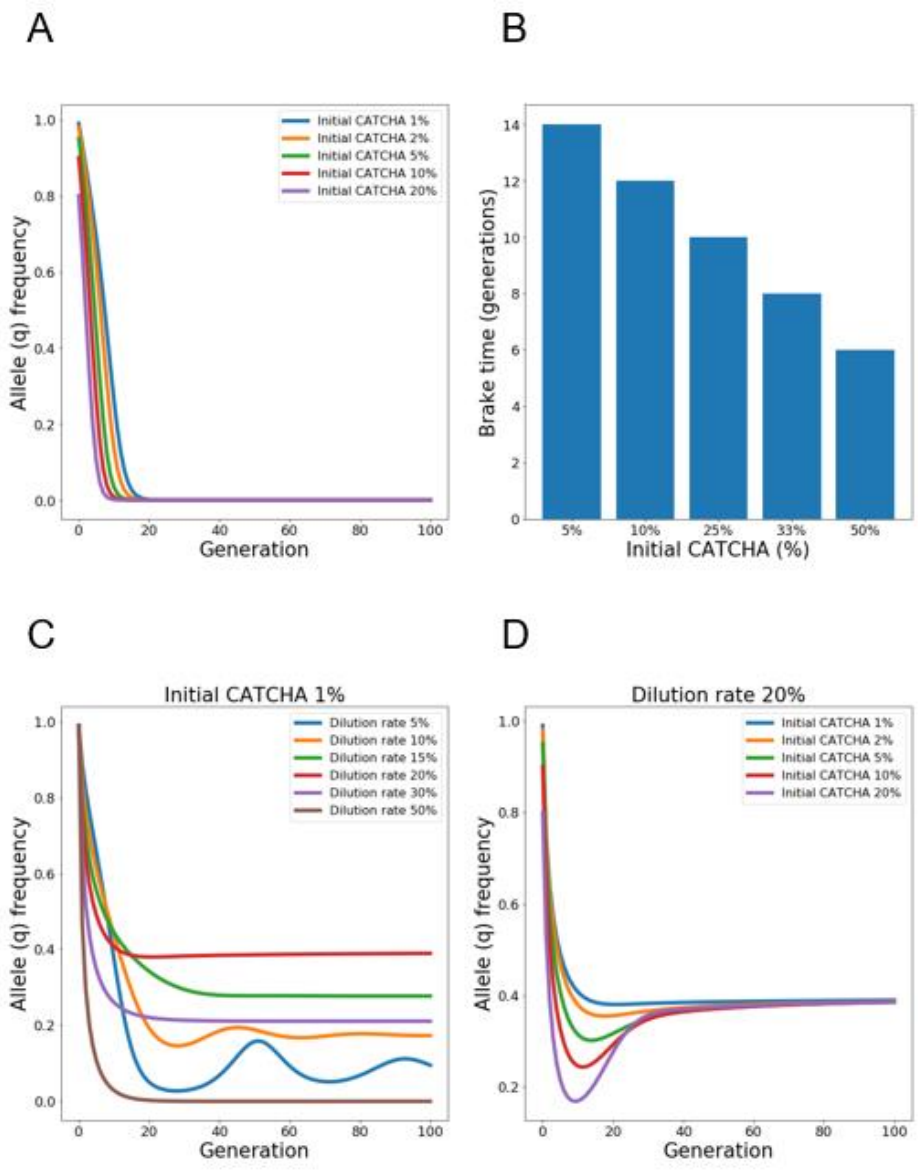
When wild type is infused at a rate m, q and r are diluted at a rate n such that a dynamic equilibrium results that leads to coexistence of p, q, and r.

Comparison with CATCHA

CATCHA “overwrites” the gene drive element.

Just as yCGD+ converts wild type to yCGD+, CATCHA converts yCGD+ to CATCHA.





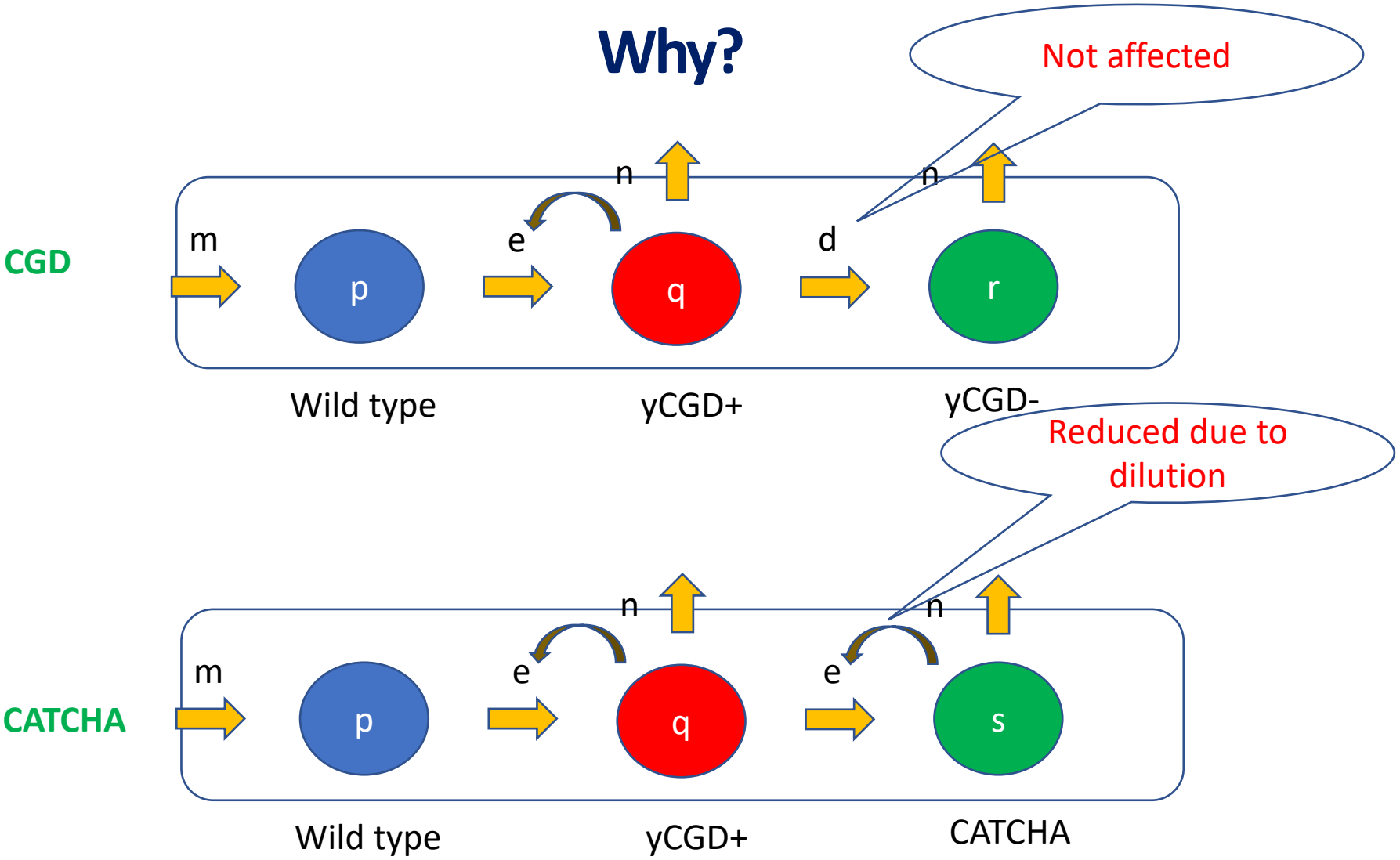
<u>Closed system.</u>

Significantly faster brake time.

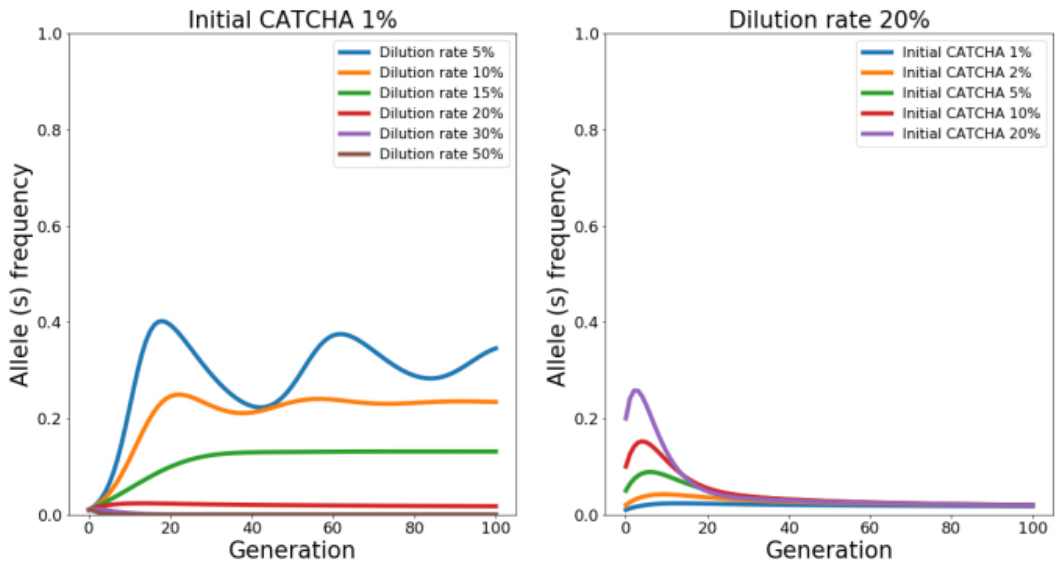
<u>Open system with wild type immigration and mutant dilution.</u>

Often leads to failure of gene drive reversal.

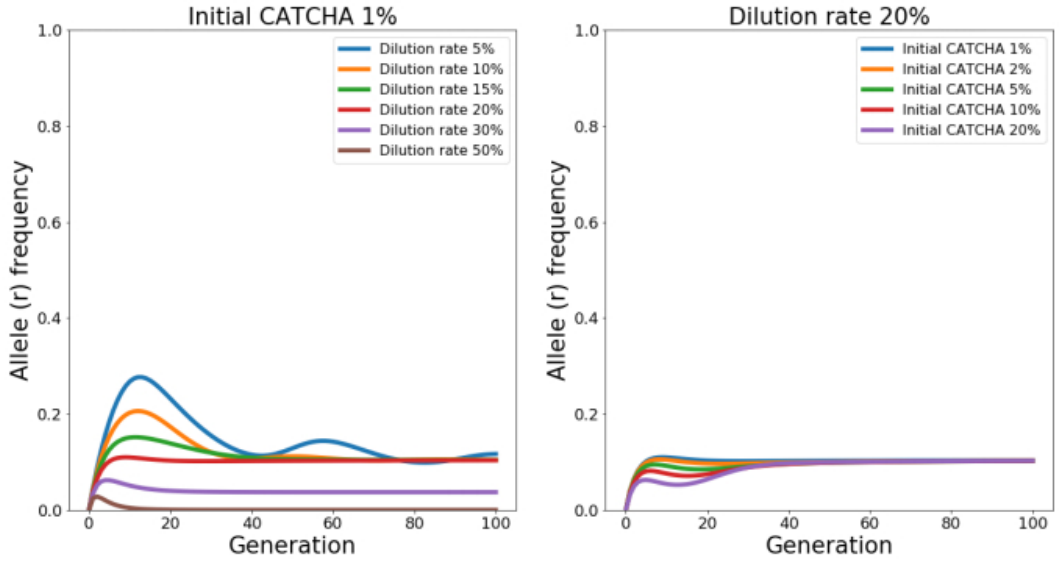
Why?


Not affected
CGD
m
p
n
e
q
d
n
r
Wild type
yCGD+
yCGD-
Reduced due to dilution
CATCHA
m
p
n
e
q
n
e
s
Wild type
yCGD+
CATCHA

(A) CATCHA allele frequency (s)



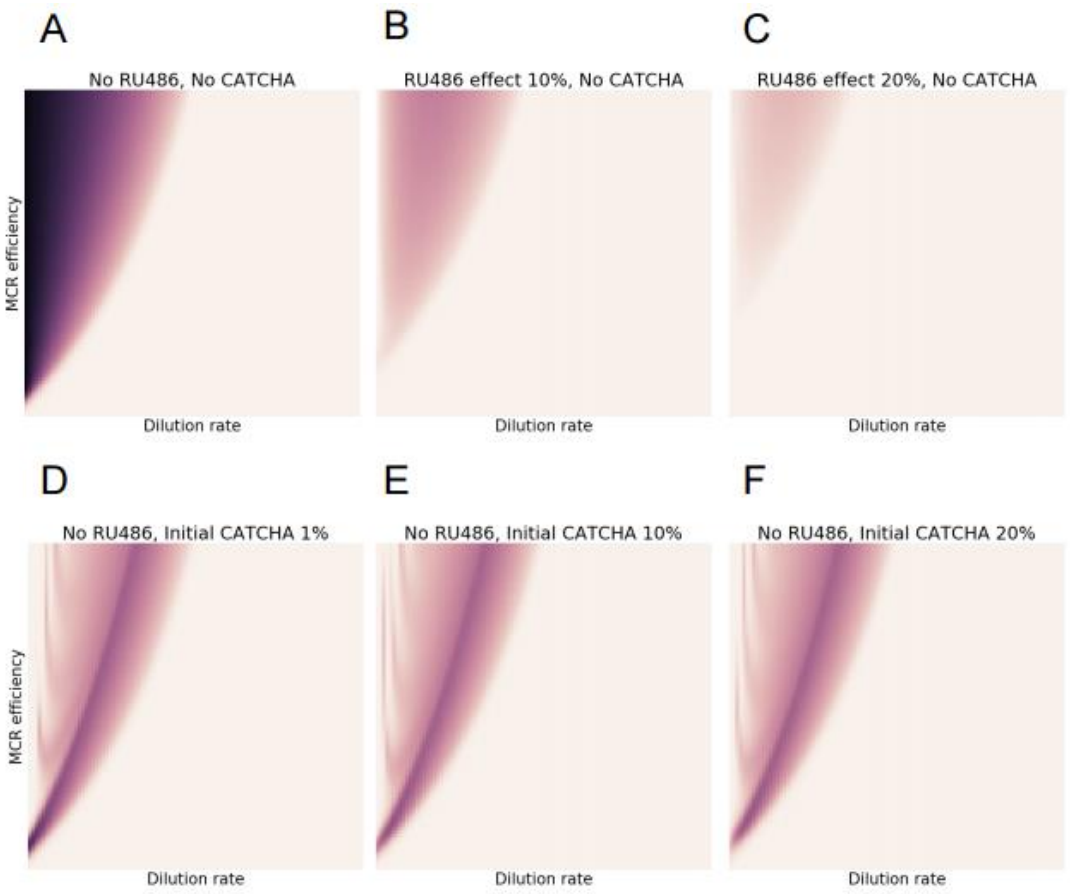
(B) y^{CGD-} allele frequency (r)

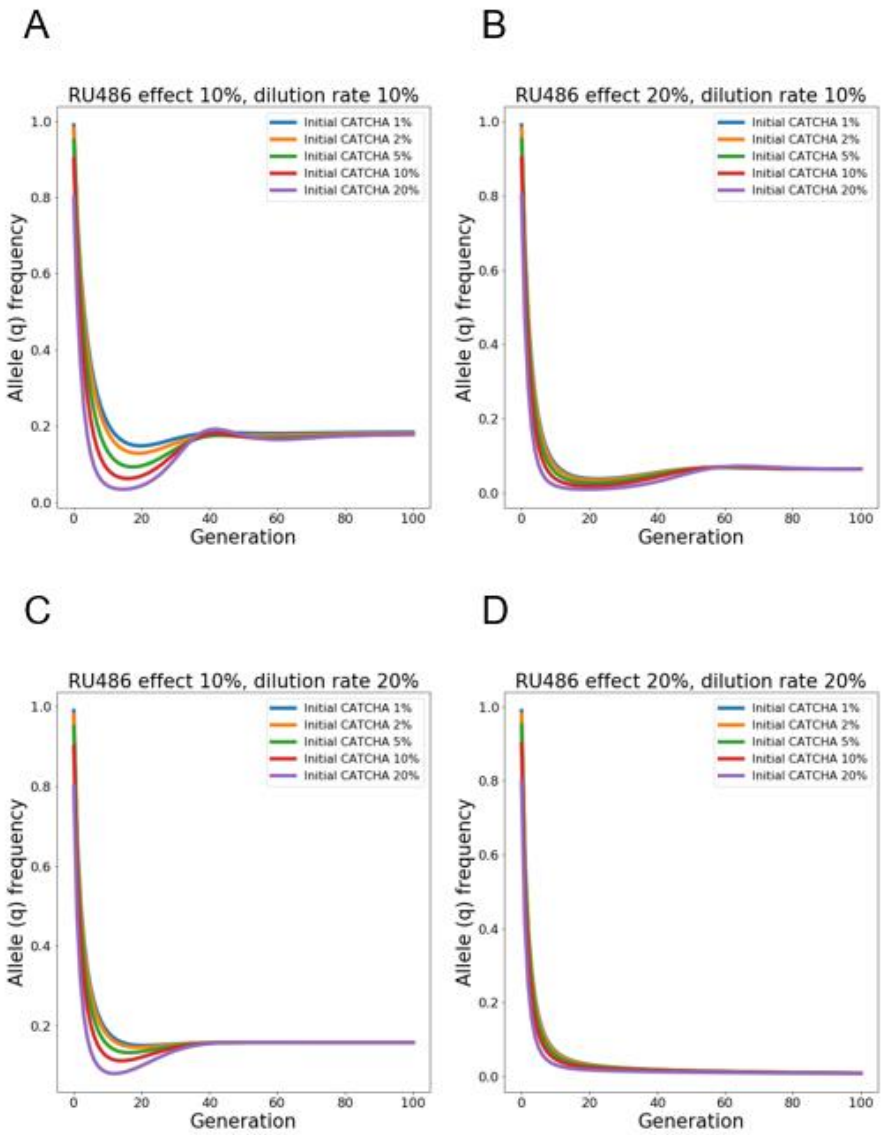


Higher dilution leads to extinction of CATCHA

RU486 effect is unaffected by dilution

Equilibrium frequency of yCGD+





Combining CGD with CATCHA leads to a synergistic effect

Fitness cost

Wild type fitness = 1

Mutant fitness = 1 – s (s: fitness cost)

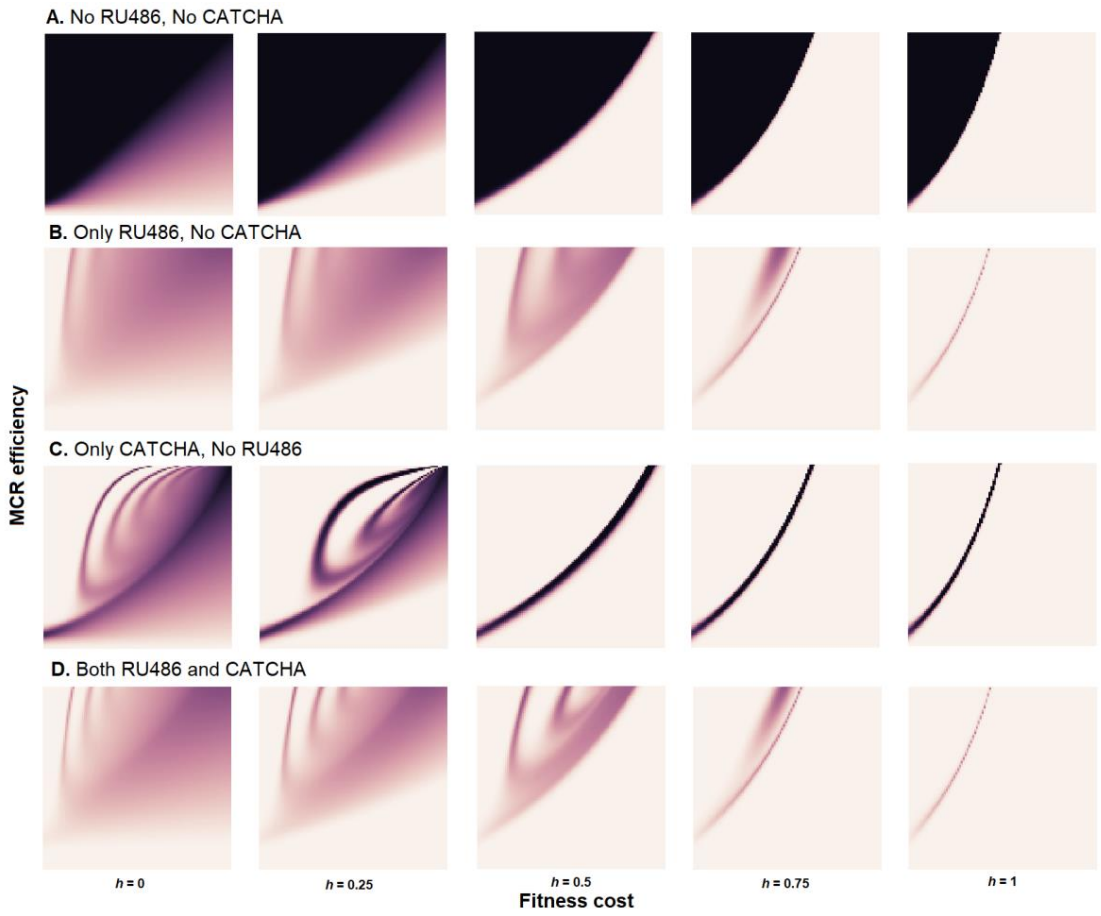
Heterozygote dominance (h):

A value ranging from 0 to 1 that dictates how close the heterozygotes are to the homozygote mutants.

i) h = 0 : Heterozygotes are phenotypically wild type

ii) h = 1: Heterozygotes are phenotypically mutant

Equilibrium frequency of yCGD+



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Discussion

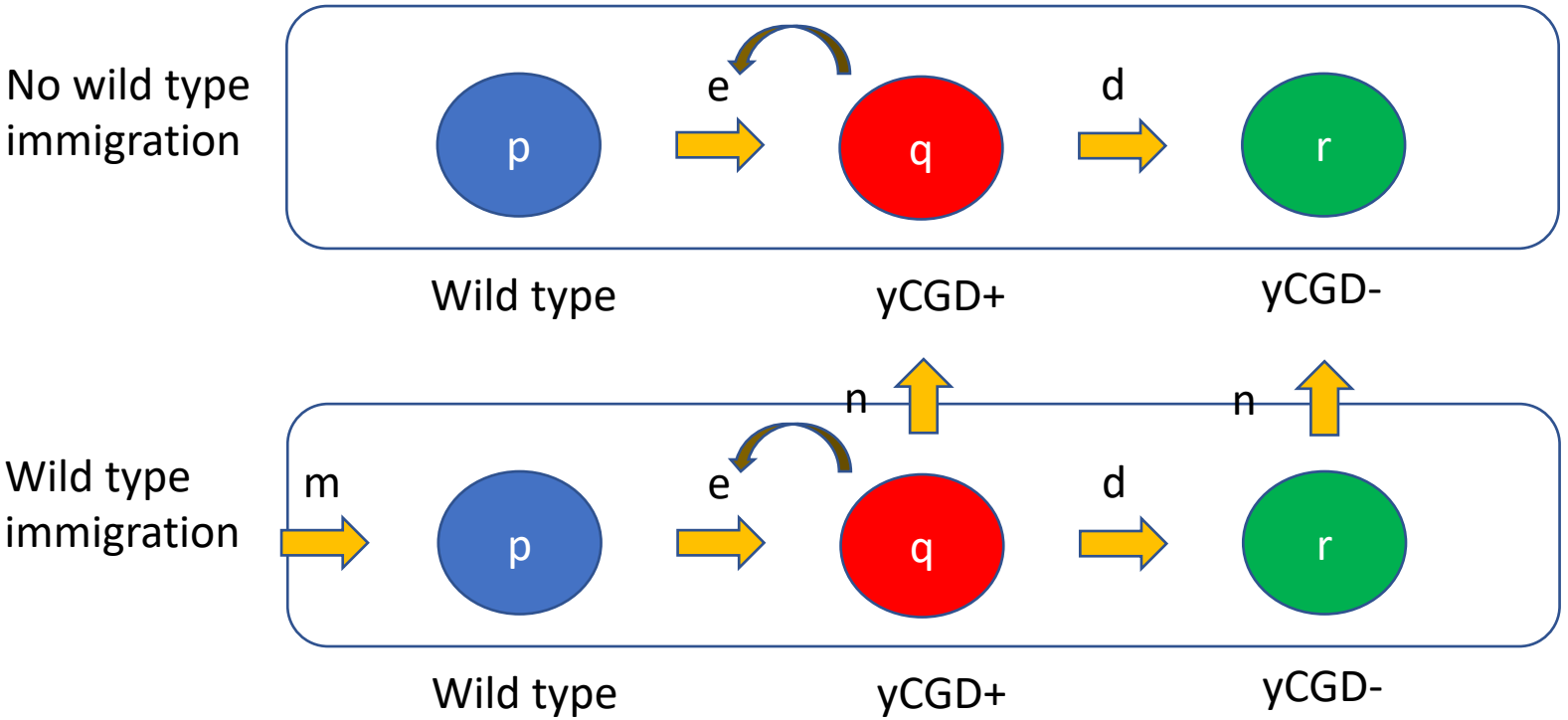
Mechanism of CGD and its effect

RU486 converts a fixed fraction of yCGD+ to yCGD-.

Analysis of individual crosses and model fitting showed that 9-15% of yCGD+ is converted to yCGD- given 200 μM of RU486 every generation.

Wild type immigration and fitness cost

Wild type immigration, or fitness cost can result in a dynamic equilibrium whereby yCGD+ is not eradicated.



CGD vs. CATCHA

CATCHA is a previously reported gene brake system that converts yCGD+ to itself.

Compared to CGD, it leads to a faster gene drive brake. This important property, however, has a downside.

CGD vs. CATCHA (2)

The rate of yCGD+ elimination by RU486 is constant per allele.

The rate of yCGD+ to CATCHA conversion, however, is proportional to CATCHA allele frequency.

Hence, wild type immigration or fitness cost that reduces CATCHA also reduces yCGD+ to CATCHA conversion.

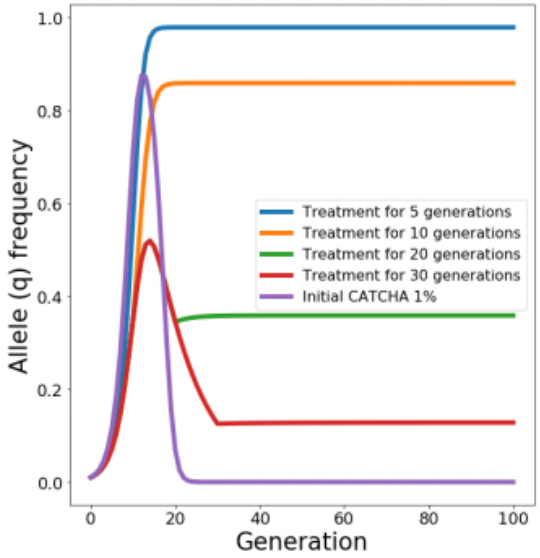
CGD vs. CATCHA (3)

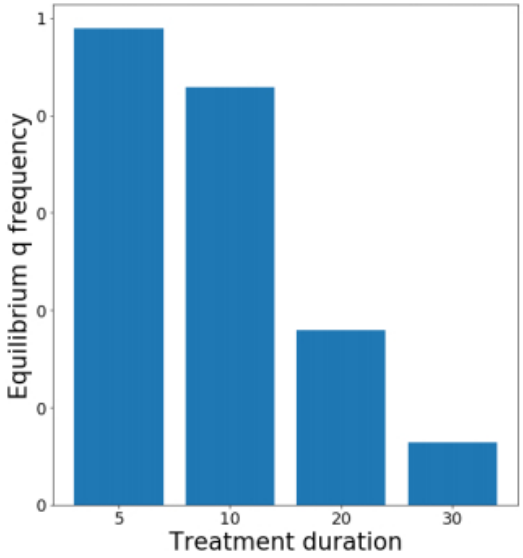
CATCHA is also an irreversible gene brake system.

Once CATCHA is released into the population, it preys on yCGD+ to self-propagate.

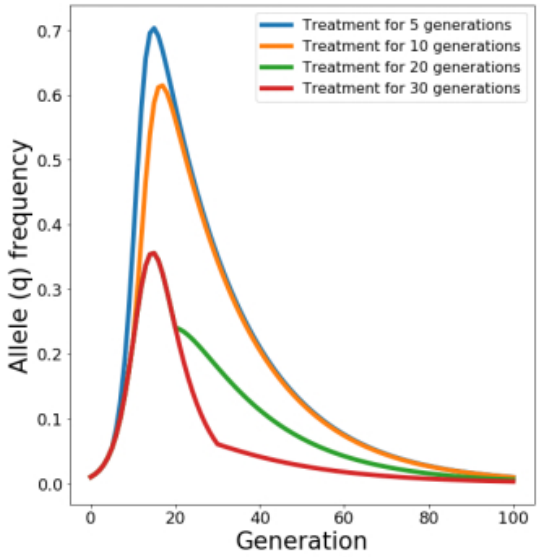
CGD, on the other hand, is a reversible system and enables the experimenter to fine-control the equilibrium yCGD+ allele frequency.

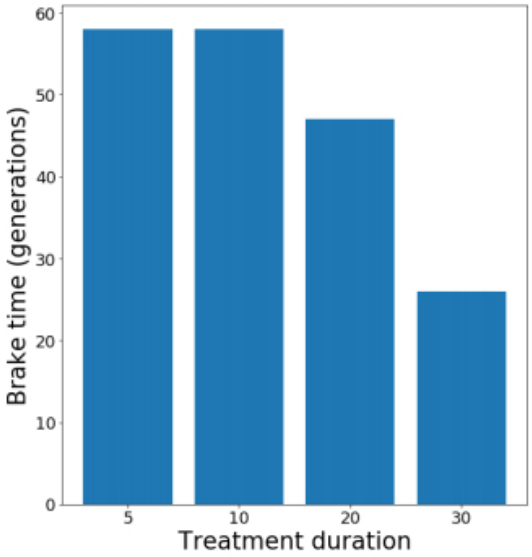
(A) NHEJ = 0%





(B) NHEJ = 5%





Withdrawing RU486 treatment stops yCGD+ decline.

CATCHA, on the other hand, continues to act until all yCGD+ is eradicated.

Limitations

Only a single dose level (200 μM) used → dose-response relationship remains elusive.

Practical aspects

1) Cost of RU486 associated with supplying the population with RU486
2) Treatment duration spanning several generations

Conclusion

We developed a reversible method to control gene drive in a chemically responsive manner.

Mathematical modeling showed that both CGD and CATCHA are capable of controlling gene drive.

However, CGD can reversibly control gene drive and is more robust to wild type immigration or fitness costs.

Acknowledgments

Kyungsoo Park

Junwon Lee and Nayoung Lee: Co-first authors who carried out all the experiments

Hyoungbum Kim and Seok Jun Moon : Co-corresponding authors who orchestrated the project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