Gene Drive Technologies: Beyond Mendelian Genetics

Disinsection Brigade

2022-9-29

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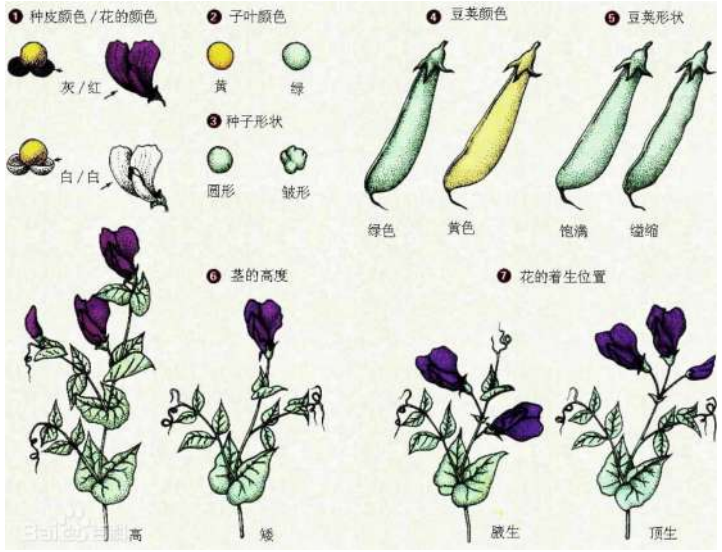
Introduction to gene drives: history and principle

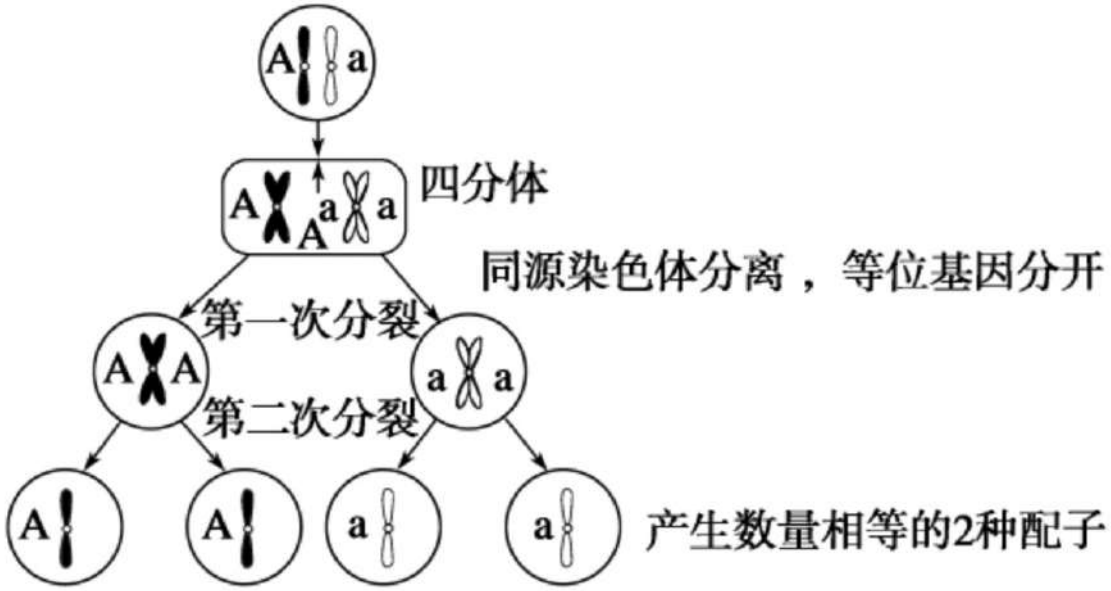
蒋昕钰

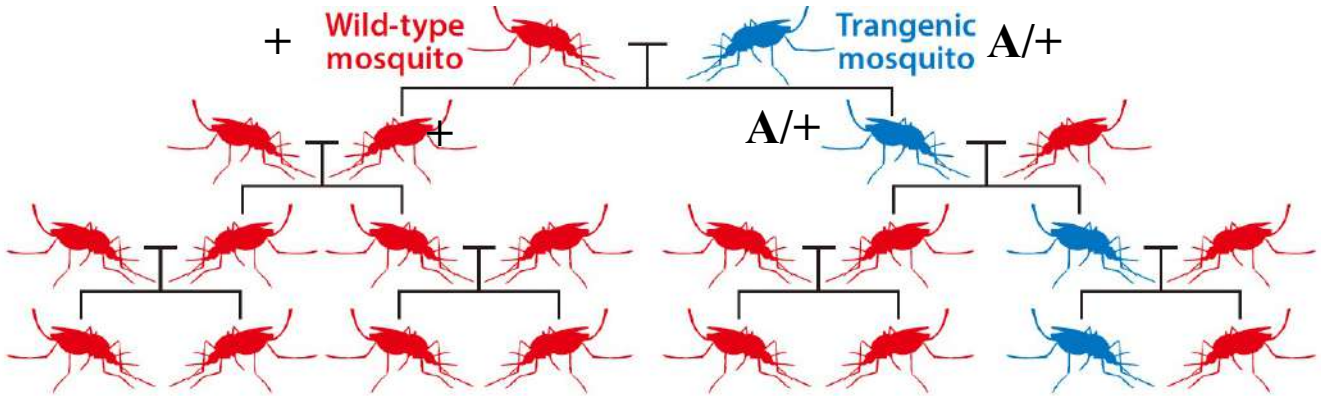
What are gene drives? How do gene drives progress? How do gene drives work?

Genes have a 50% chance of being passed from parent to offspring according to Mendelian Genetics

Gregor Johann Mendel，1822-1884





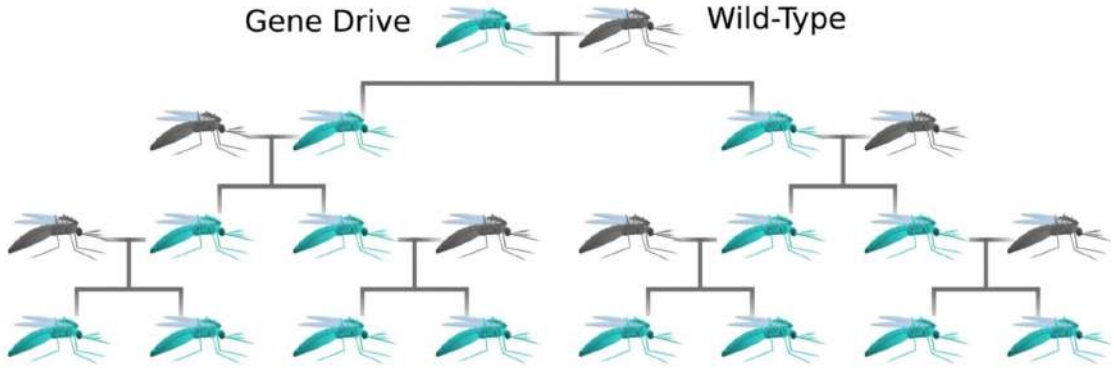


What are gene drives?

Selfish genetic elements are genetic segments that can enhance their own transmission at the expense of other genes in the genome, even if this has no or a negative effect on organismal fitness.

> 50% chance of inheritance

Gene drives are systems of biased inheritance that enhance the likelihood a sequence of DNA passes between generations through sexual reproduction and become a dominant one in a population.



Population suppression or Population replacement

"suppression drive" types

"modification drive" types

self-sustaining technology

edit genomes at the population level

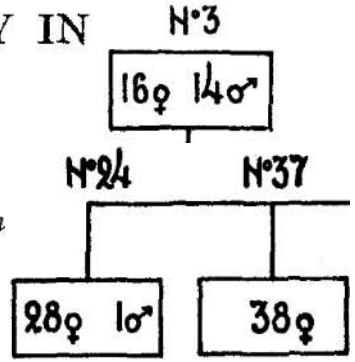
The study of selfish genetic elements has continued for a century

A NEW SEX-RATIO ABNORMALITY IN *DROSOPHILA OBSCURA**

S. GERSHENSON

Institute of Experimental Biology, Moscow,[1] Russia

Received February 27, 1928



"In many cases these chromosomes have no useful function at all to the species carrying them… [B chromosomes] need not be useful for the plants. They need only be useful to themselves."
—Gunnar Östergren in 1945

Coined the term "selfish genetic element"

TREE vol. 3, no. 11, November 1988

Selfish Genetic Elements

John H. Werren, Uzi Nur and Chung-I Wu

Introduced the concept of selfish genes to the wider scientific community

Nature Vol. 284 17 April 1980 601

Selfish genes, the phenotype paradigm and genome evolution

W. Ford Doolittle & Carmen Sapienza

Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7

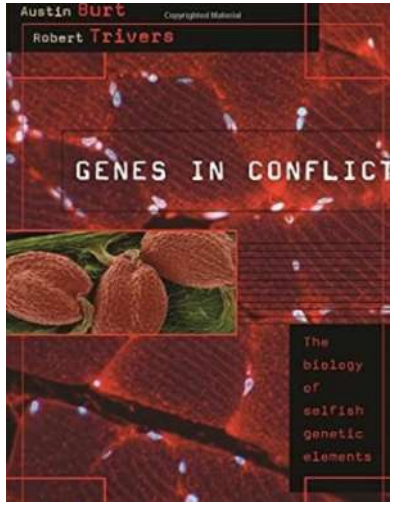
604 Nature Vol. 284 17 April 1980

Selfish DNA: the ultimate parasite

L. E. Orgel & F. H. C. Crick

The Salk Institute, 10010 N. Torrey Pines Road, La Jolla, California 92037

Published the first book in 2006



Rice WR.
Nothing in genetics makes sense except in light of genomic conflict.
Annu Rev Ecol Evol Syst. 2013.

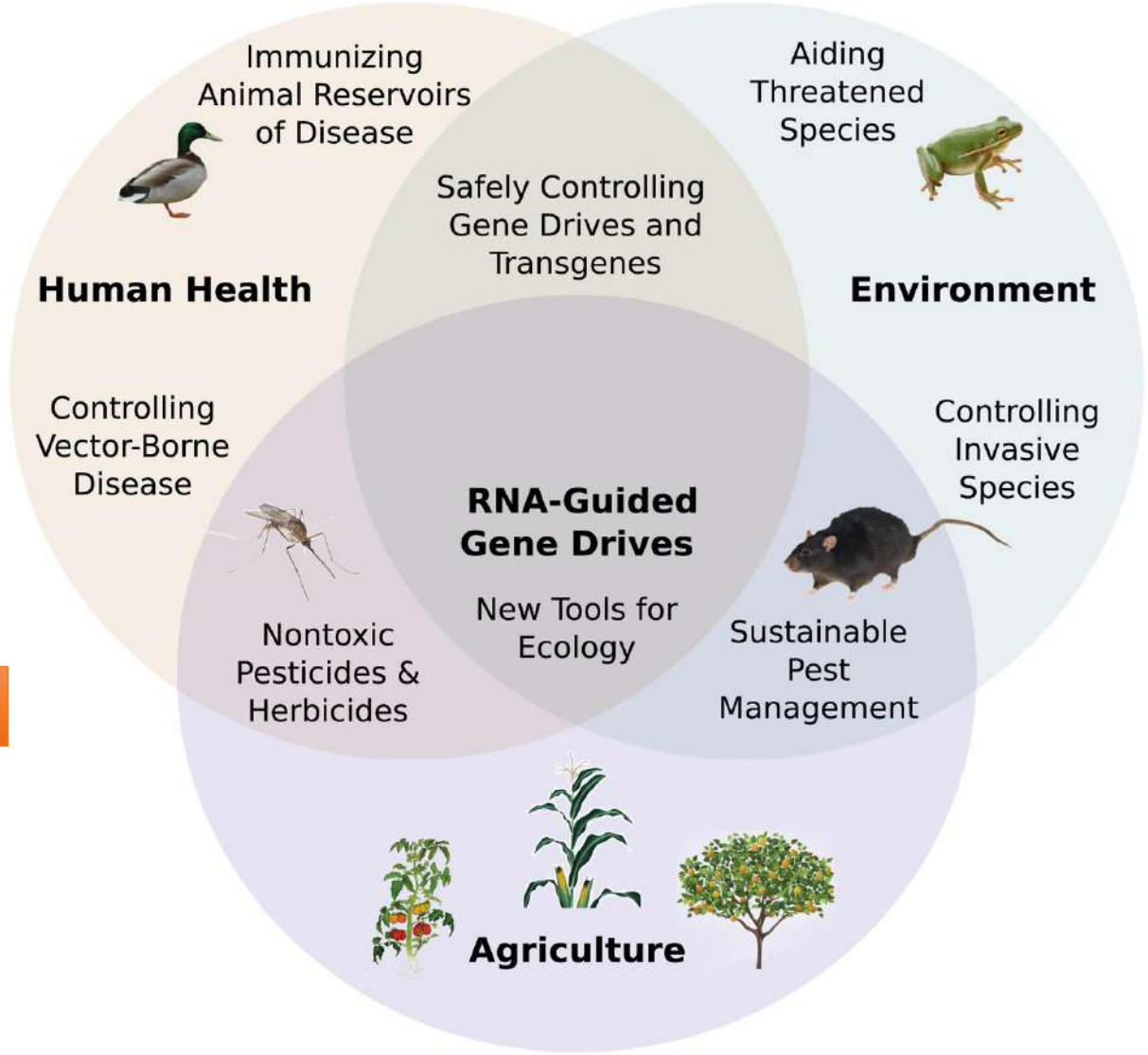
Application of gene drives ——solving environmental and public health challenges

- lead to the spread of fitness-reducing traits (including lethality and sterility)

- lead to the spread of fitness-increasing traits

overcome the evolutionary disadvantages

more quickly and thoroughly than natural selection



The idea of solving biological problems by gene drives evolved over decades

NATURE, VOL. 218, APRIL 27, 1968

Possible Use of Translocations to fix Desirable Genes in Insect Pest Populations

CHROMOSOME translocation heterozygotes $(T/+)$ are usually semisterile, but translocation homozygotes (T/T) if viable are usually fully fertile. If such a viable translocation were produced in an insect pest, T/T insects could be reared in captivity and released into the wild, where matings with wild types $(+/+)$ would produce $T/+$ progeny.

> Philos Trans R Soc Lond B Biol Sci. 1994 May 28;344(1309):313-24.
doi: 10.1098/rstb.1994.0069.

Selfish DNA as a method of pest control

I M Hastings [1]

THE ROYAL SOCIETY

Received 14 October 2002
Accepted 12 December 2002
Published online 19 March 2003

Site-specific selfish genes as tools for the control and genetic engineering of natural populations

Austin Burt

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Imperial College London

Home Publications Teaching

Professor Austin Burt

/// Faculty of Natural Sciences, Department of Life Sciences (Silwood Park)

Professor of Evolutionary Genetics

Summary

My primary research interests are in developing novel genetic approaches to control disease vectors and other pest species, with a specific focus on the mosquitoes that transmit malaria in sub-Saharan Africa.

Hundreds of thousands of people still die every year due to malaria, and new interventions are needed. Recent advances in molecular biology have opened up the possibility of completely new approaches based on genetic modification of the mosquitoes that transmit malaria. The most efficient such approaches use gene drive — a natural process by which some genes are preferential transmitted from one generation to the next — but I am also interested in more localisable interventions. In addition to their potential role in malaria control and elimination, these novel genetic strategies may be useful for controlling other vector-borne diseases, harmful invasive species, and other pests.

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> Silwood Park Campus
> Synthetic biology

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> Expert Directory

Several types of gene drives with different characteristics have been engineered

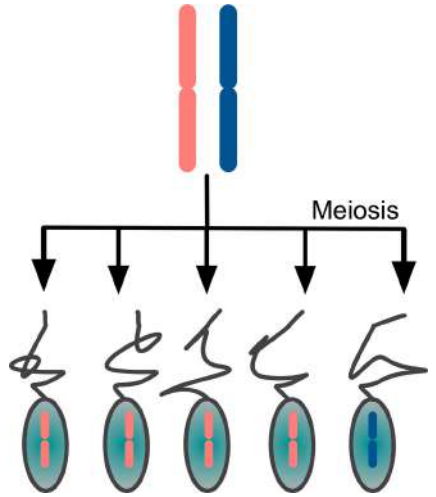
Most proposed engineered gene drives are based on naturally existing selfish genetic elements

- Sex-linked meiotic drives (MD)
- The maternal effect dominant embryonic arrest system (Medea)
- Underdominance drives (UD)
- Homing endonuclease-based gene drives (HEGD)

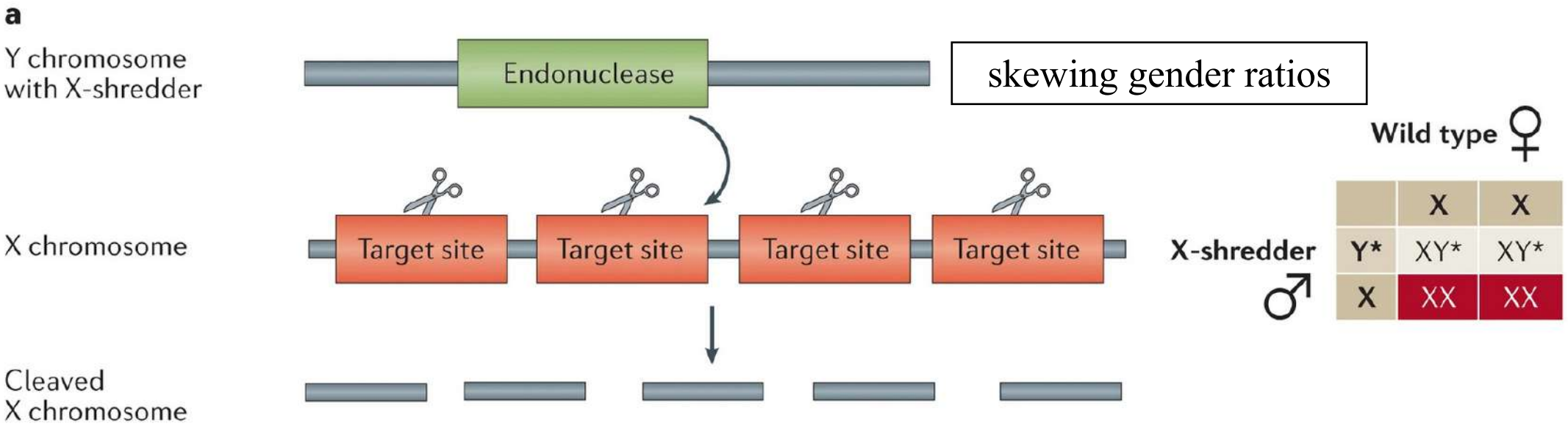
Attributes
Rate of spread
Species specificity
Fitness cost
Susceptibility to resistance
Removability or reversibility
Ease of manipulation in the laboratory

Mechanisms of sex-linked meiotic drives

Segregation distorters
transmission of certain alleles is biased during meiosis, resulting in increased frequencies of those alleles in the gametes



Attributes
Type: **Suppression**
Rate of spread: Moderate
Fitness cost: Low
Resistance generation rate: Low
Reversibility: second-generation drive



Mechanisms of *Medea*

Maternal-effect lethality to all hatchlings that do not inherit a copy of the factor itself.

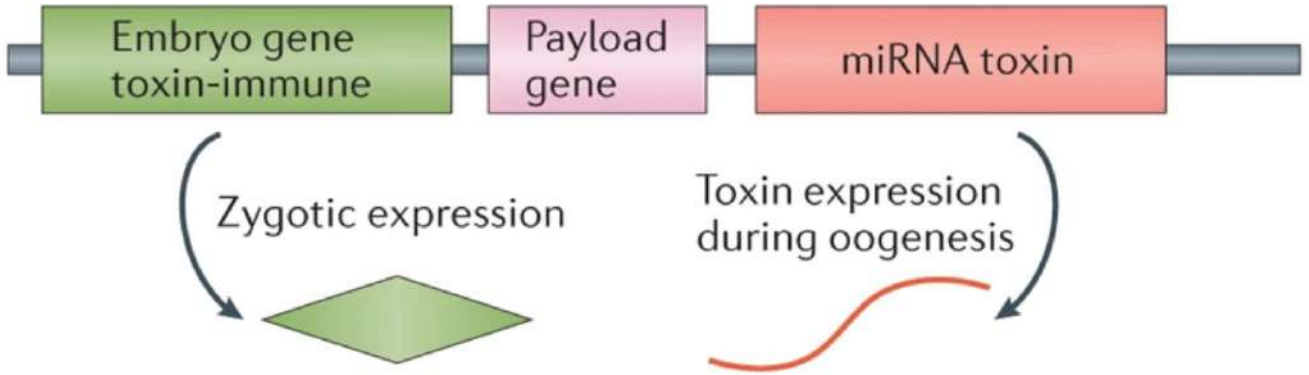
A survival of 50% of the embryos

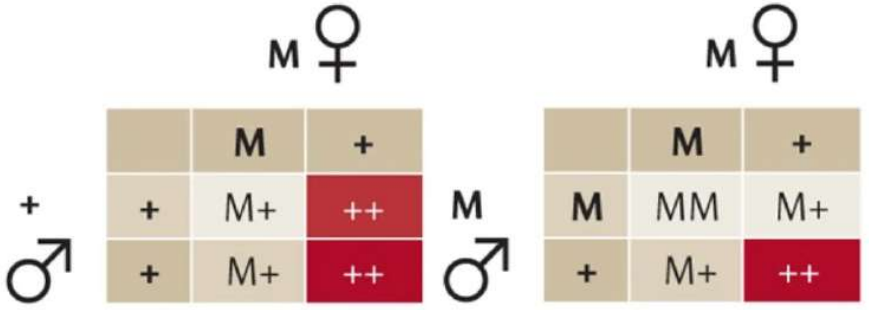
fitness advantage

↓

linked payload gene drive rapidly

Attributes
Type: **Replacement**
Rate of spread: Moderate
Fitness cost: Uncertain
Resistance generation rate: Low
Reversibility: second-generation drive





Toxin: a microRNA is expressed during oogenesis in *Medea*-bearing females, disrupting an embryonic essential gene in all embryos
Antidote: a toxin immune protein is expressed at the zygotic stage early in embryogenesis only in embryos that inherit the *Medea* element

Mechanisms of underdominance gene drives

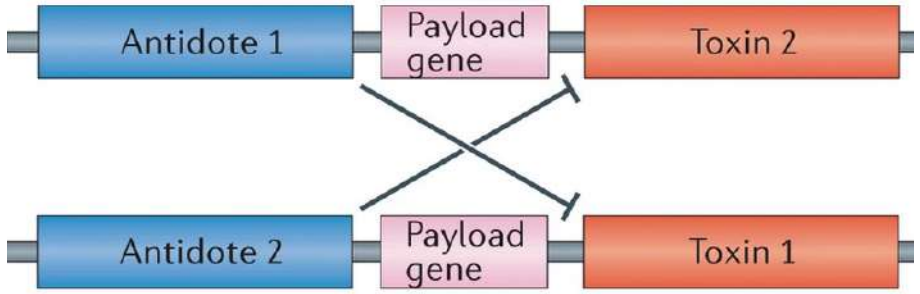
Underdominance: It is the selection against the mean of a population distribution causing disruptive selection and divergent genotypes

Heterozygote inferiority: heterozygotes have a lower fitness than parental homozygotes

Attributes
Type: **Replacement**
Rate of spread: **Slow**
Fitness cost: High, **locally confined**
Resistance generation rate: Moderate
Reversibility: **Removable with releasing large numbers of wild-type organisms**

1. **Chromosomal translocations**

2. **Combinations of toxins and antidotes**



Wild type
1+1+2+2+

Heterozygote
1*1+2*2+

	1+2+	1+2+	1+2+	1+2+
1*2*	1+1* 2+2*	1+1* 2+2*	1+1* 2+2*	1+1* 2+2*
1*2+	1+1* 2+2+	1+1* 2+2+	1+1* 2+2+	1+1* 2+2+
1+2*	1+1+ 2+2*	1+1+ 2+2*	1+1+ 2+2*	1+1+ 2+2*
1+2+	1+1+ 2+2+	1+1+ 2+2+	1+1+ 2+2+	1+1+ 2+2+

Heterozygote
1*1+2*2+

Heterozygote
1*1+2*2+

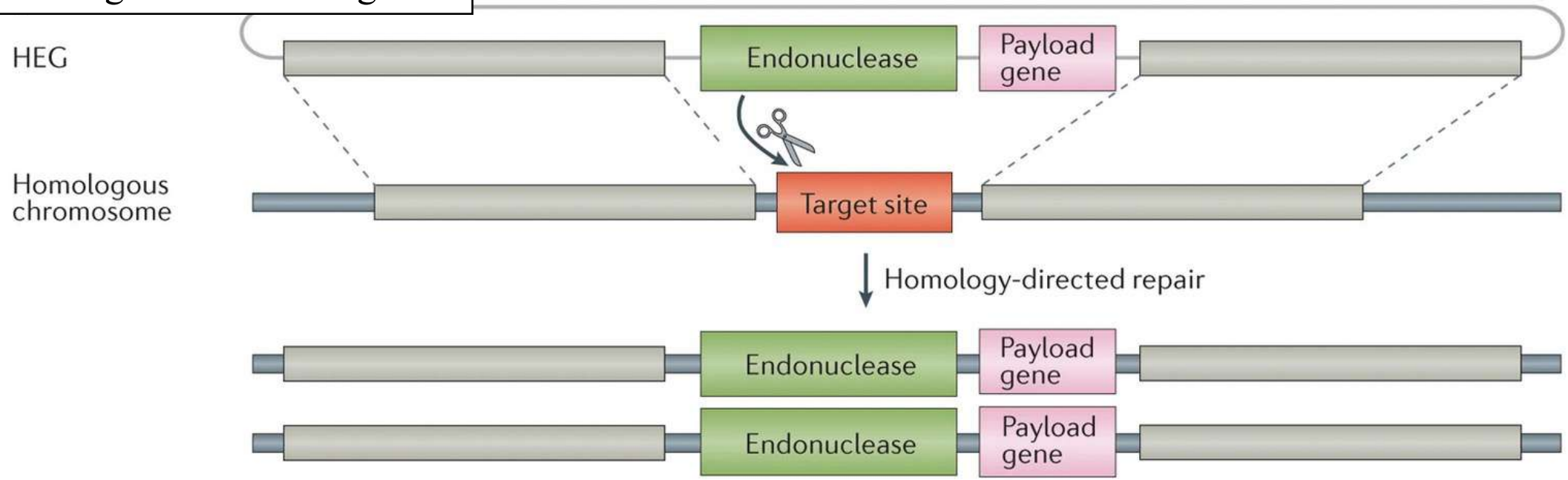
	1*2*	1*2+	1+2*	1+2+
1*2*	1*1* 2*2*	1*1* 2+2*	1+1* 2*2*	1+1* 2+2*
1*2+	1*1* 2*2+	1*1* 2+2+	1+1* 2*2+	1+1* 2+2+
1+2*	1*1+ 2*2*	1*1+ 2+2*	1+1+ 2*2*	1+1+ 2+2*
1+2+	1*1+ 2*2+	1*1+ 2+2+	1+1+ 2*2+	1+1+ 2+2+

Mechanisms of homing drives

Homing: copying themselves onto the opposite chromosome

Attributes
Type: **Suppression + Replacement**
Rate of spread: **Fast**
Fitness cost: Low
Resistance generation rate: **High**
Reversibility: second-generation drive

homing endonuclease genes



1. Encodes an endonuclease
2. Cleaves at a target site on the homologous chromosome opposite the HEG
3. Homology-directed repair (HDR) results in the HEG being copied to the homologous chromosome

The past ten years have seen only modest progress in gene drives development

> Science. 2007 Apr 27;316(5824):597-600. Epub 2007 Mar 29.

A synthetic maternal-effect selfish genetic element drives population replacement in Drosophila

The first engineered *Medea* gene drive system

> Nature. 2011 May 12;473(7346):212-5. doi: 10.1038/nature09937. Epub 2011 Apr 20.

A synthetic homing endonuclease-based gene drive system in the human malaria mosquito

The creation of a engineered homing drive system in mosquitoes

> Curr Biol. 2013 Apr 22;23(8):671-7. doi: 10.1016/j.cub.2013.02.059. Epub 2013 Mar 28.

A synthetic gene drive system for local, reversible modification and suppression of insect populations

The first engineered underdominance system

> Nat Commun. 2014 Jun 10;5:3977. doi: 10.1038/ncomms4977.

A synthetic sex ratio distortion system for the control of the human malaria mosquito

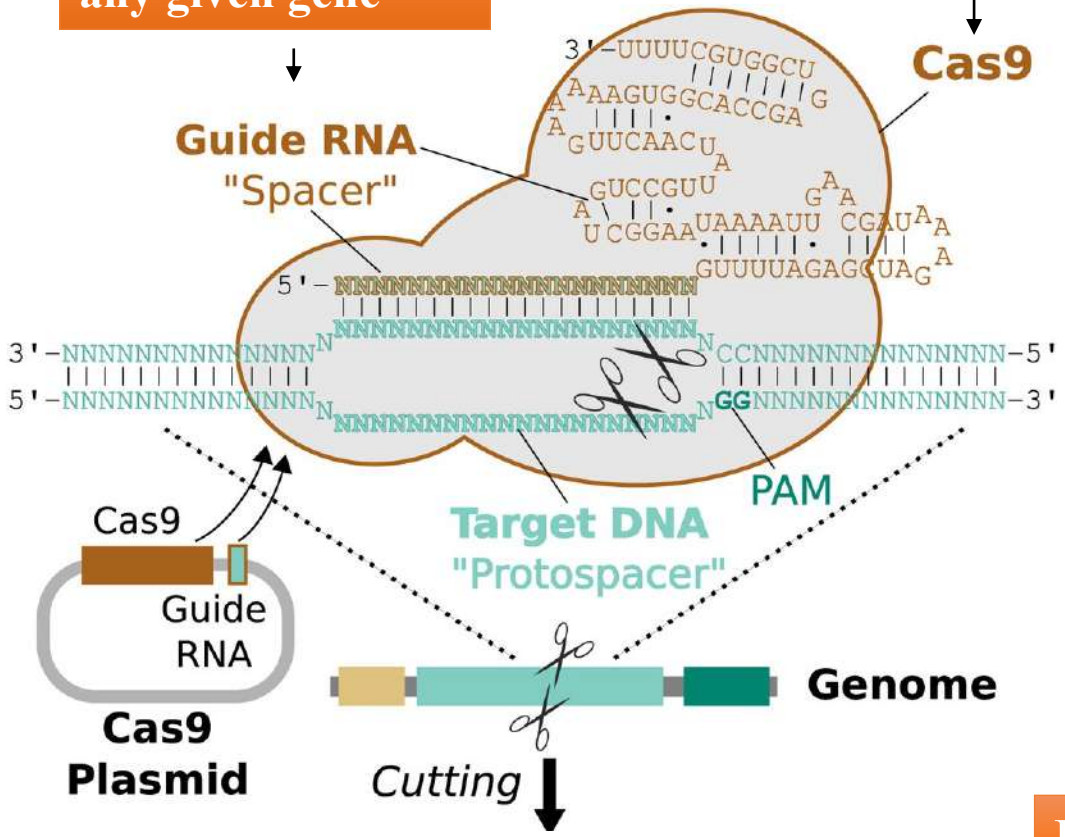
The creation of a fully functional X-shredder in mosquitoes

The use has remained largely theoretical due to technical constraints

The advent of the CRISPR/Cas9 technology gives a renewed impetus to developing gene drives in the laboratory for eventual application

Efficiently target any given gene

Active in a wide variety of organisms

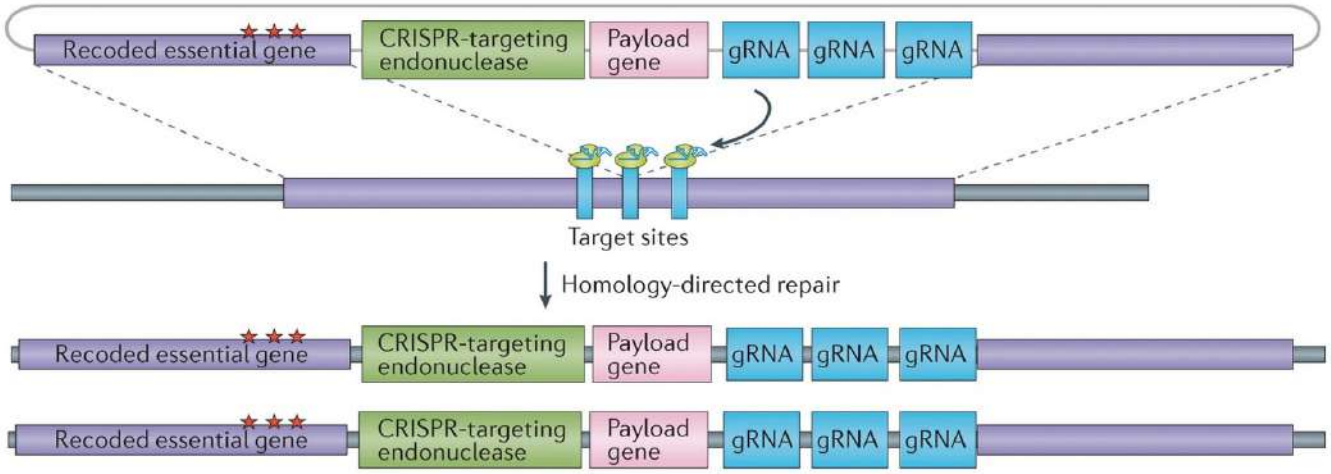


Review > Elife. 2014 Jul 17;3:e03401. doi: 10.7554/eLife.03401.

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

Kevin M Esvelt [1], Andrea L Smidler [1], Flaminia Catteruccia [2], George M Church [1]

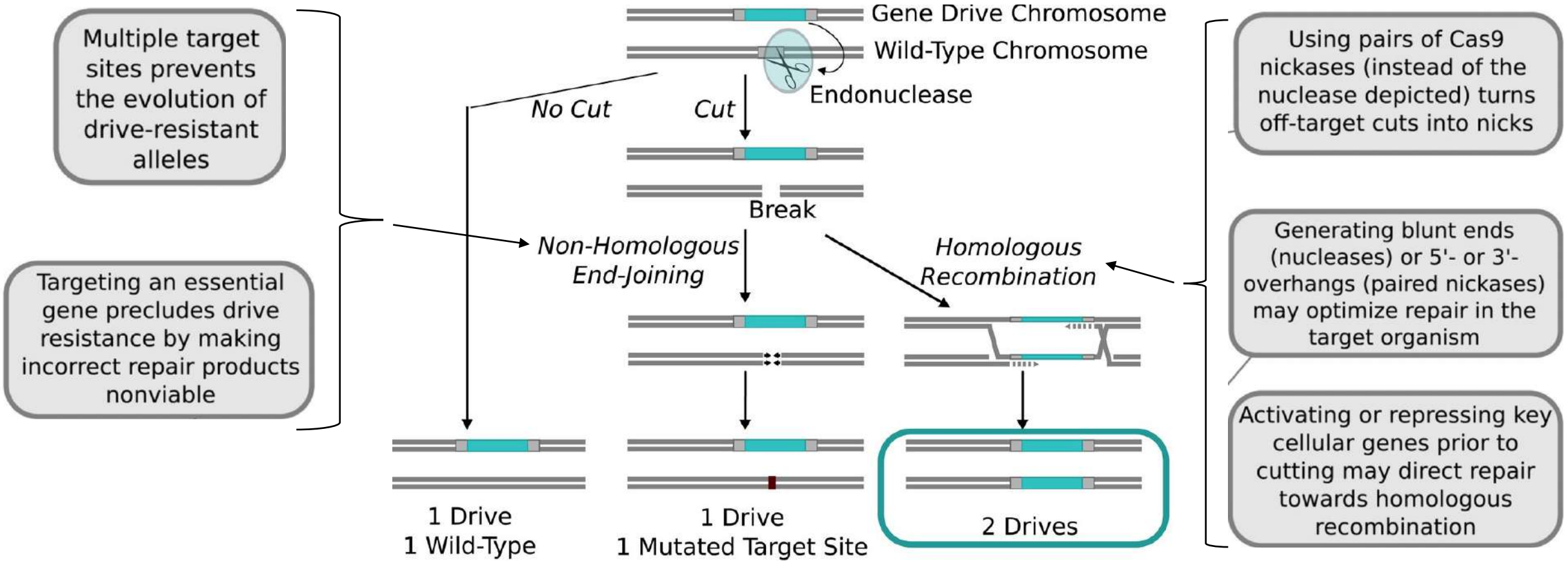
Substituting Cas9 for the homing endonucleases



Evolutionary stability

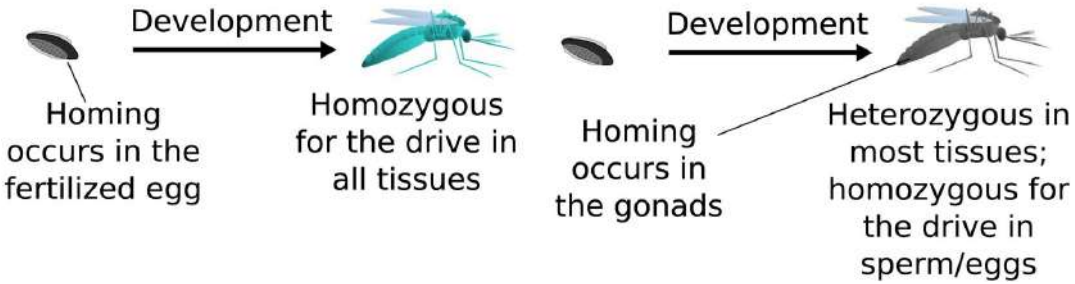
Multiple target sites increases the cutting rate

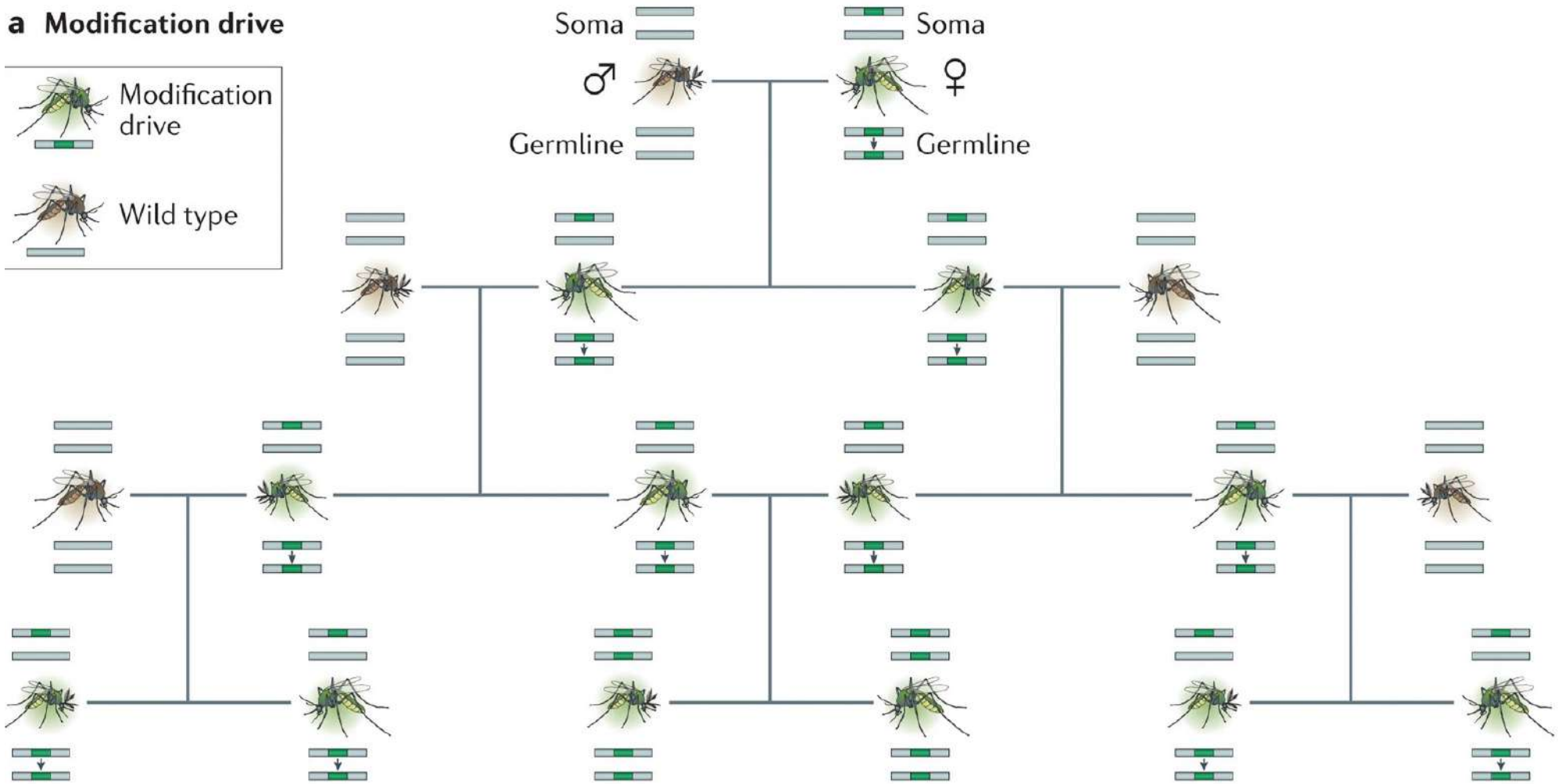
The advent of the CRISPR/Cas9 technology gives a renewed impetus to developing gene drives in the laboratory for eventual application



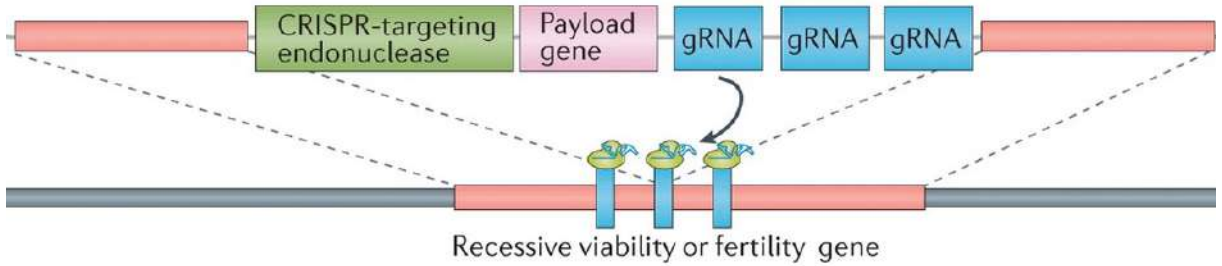
easier to use, faster to develop, more precise

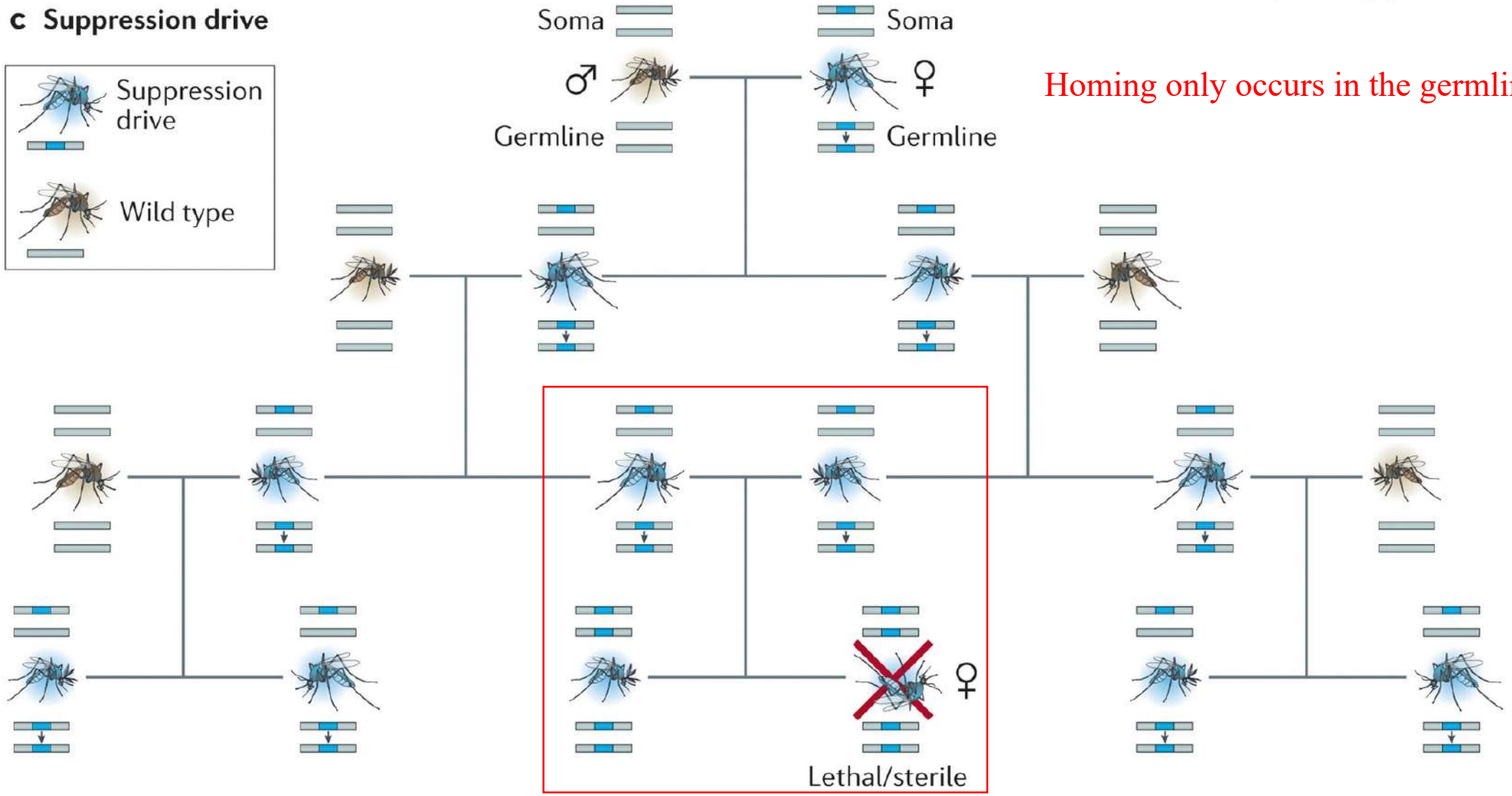
Homing drives result in most or all progeny of heterozygotes receiving the desirable genes and spread rapidly throughout the population





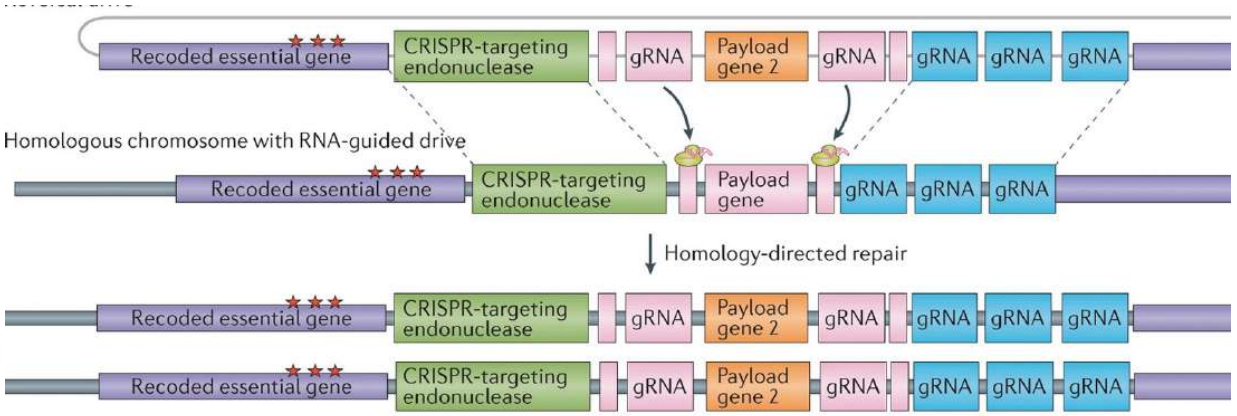
Suppression drives target the recessive genes required for viability or fertility

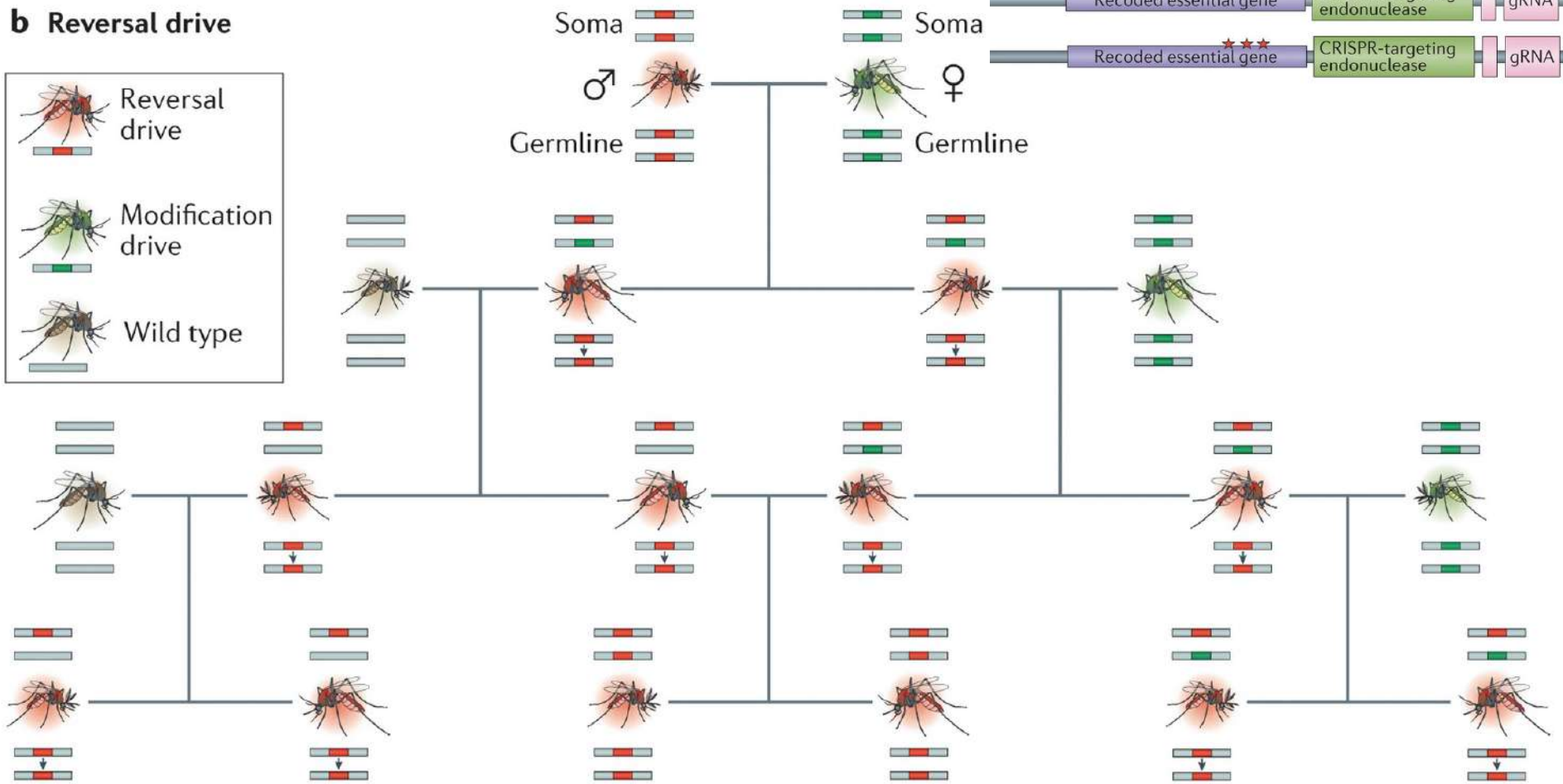




Homing only occurs in the germline cells

A second-generation reversal drive can overwrite a first-generation homing drive, replacing its payload gene





CRISPR/Cas9-based gene drives show great potential in diverse organisms

› Science. 2015 Apr 24;348(6233):442-4. doi: 10.1126/science.aaa5945. Epub 2015 Mar 19.

Genome editing. The mutagenic chain reaction: a method for converting heterozygous to homozygous mutations

Valentino M Gantz [1], Ethan Bier [1]

› Nat Biotechnol. 2015 Dec;33(12):1250-1255. doi: 10.1038/nbt.3412. Epub 2015 Nov 16.

Safeguarding CRISPR-Cas9 gene drives in yeast

James E DiCarlo [1 2 3], Alejandro Chavez [1 2 4 5], Sven L Dietz [1 2 4 6], Kevin M Esvelt [2 4], George M Church [1 2 4]

› Proc Natl Acad Sci U S A. 2015 Dec 8;112(49):E6736-43. doi: 10.1073/pnas.1521077112 Epub 2015 Nov 23.

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

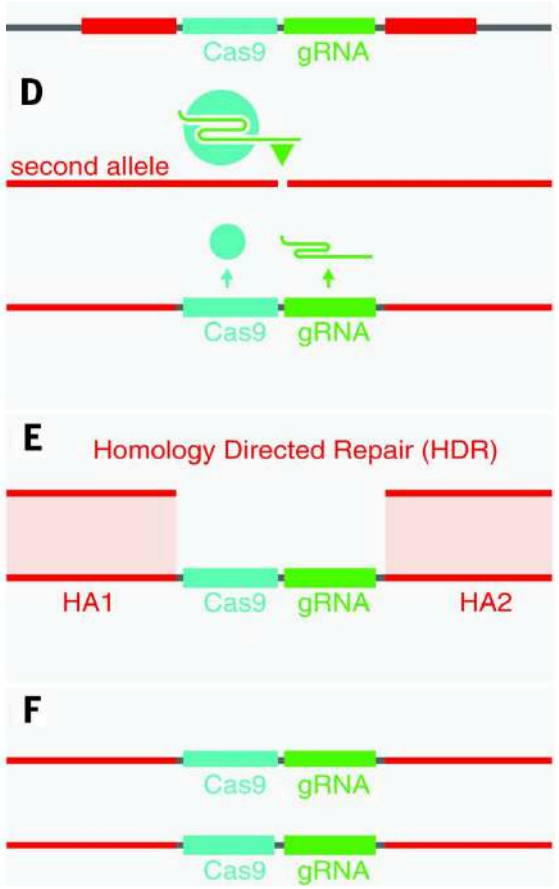
Valentino M Gantz [1], Nijole Jasinskiene [2], Olga Tatarenkova [2], Aniko Fazekas [2], Vanessa M Macias [2], Ethan Bier [3], Anthony A James [4]

› Nat Biotechnol. 2016 Jan;34(1):78-83. doi: 10.1038/nbt.3439. Epub 2015 Dec 7.

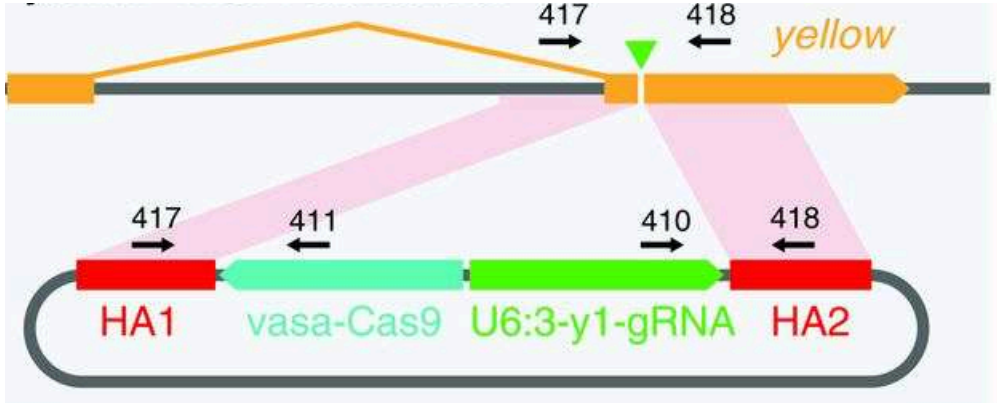
A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae

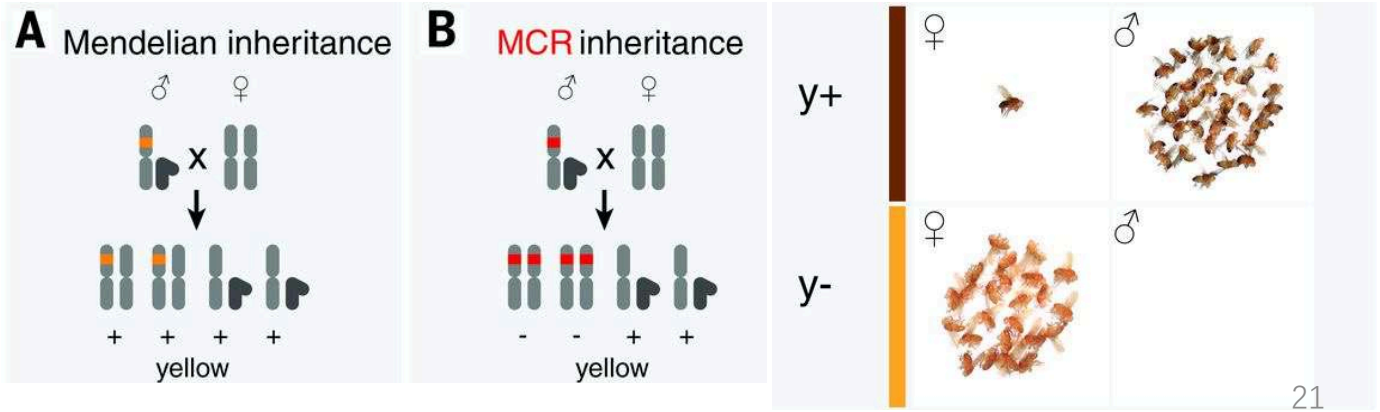
Andrew Hammond [1], Roberto Galizi [1], Kyros Kyrou [1], Alekos Simoni [1], Carla Siniscalchi [2], Dimitris Katsanos [1], Matthew Gribble [1], Dean Baker [3], Eric Marois [4], Steven Russell [3], Austin Burt [1], Nikolai Windbichler [1], Andrea Crisanti [1], Tony Nolan [1]

The first creation of homing drive by CRISPR/Cas9 efficiently drives allelic conversion in *Drosophila*



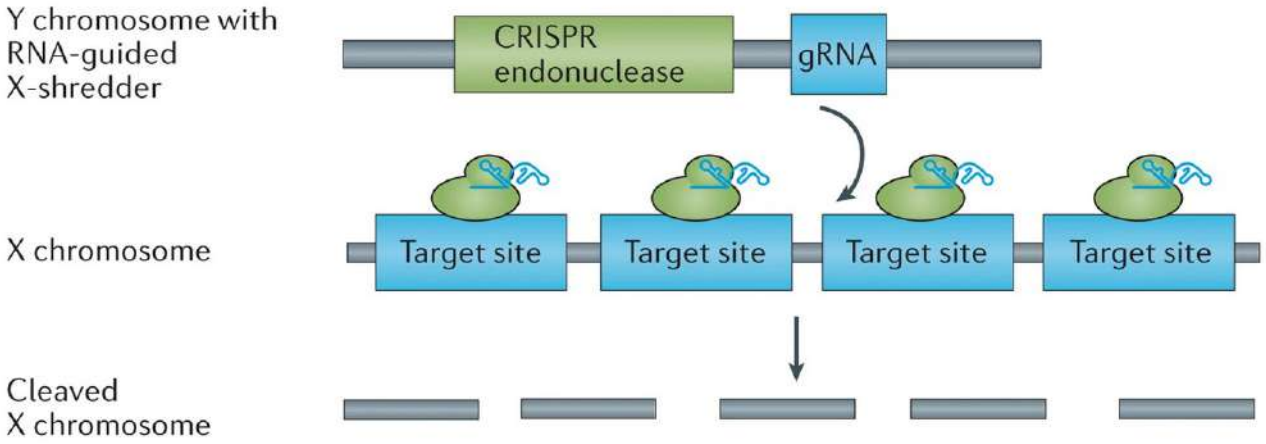
1. A *vasa-Cas9* gene expressed in both somatic and germline cells
2. A gRNA targeted to *y1* in the X-linked *yellow* locus
3. Homology arms

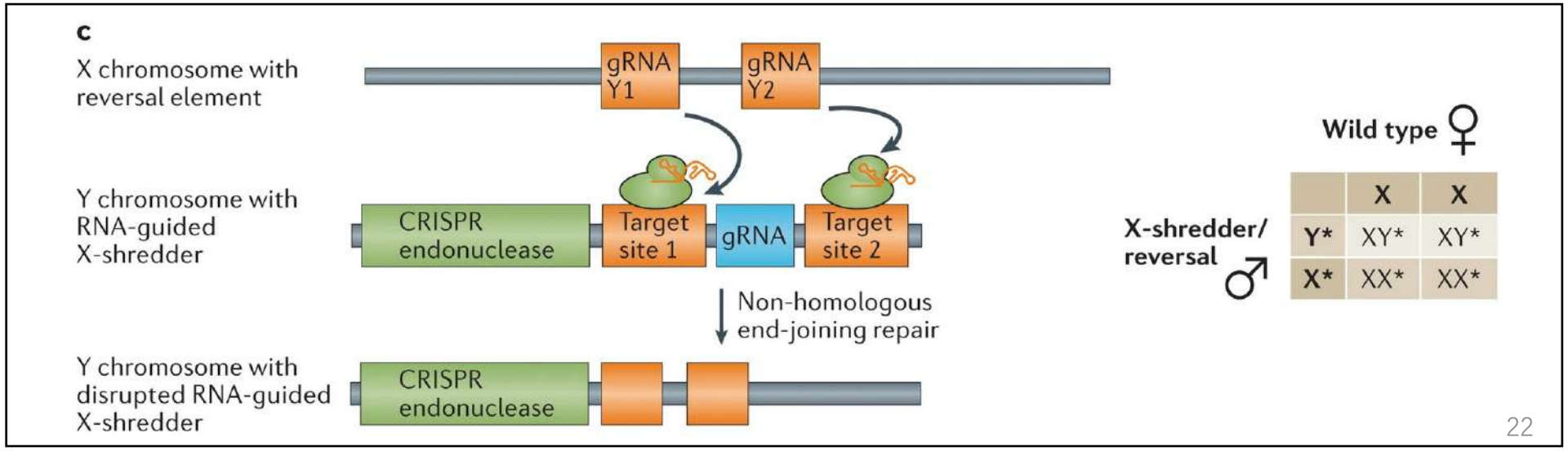




CRISPR/Cas9 technology provides a new tool for the effective development of a variety of engineered gene drives

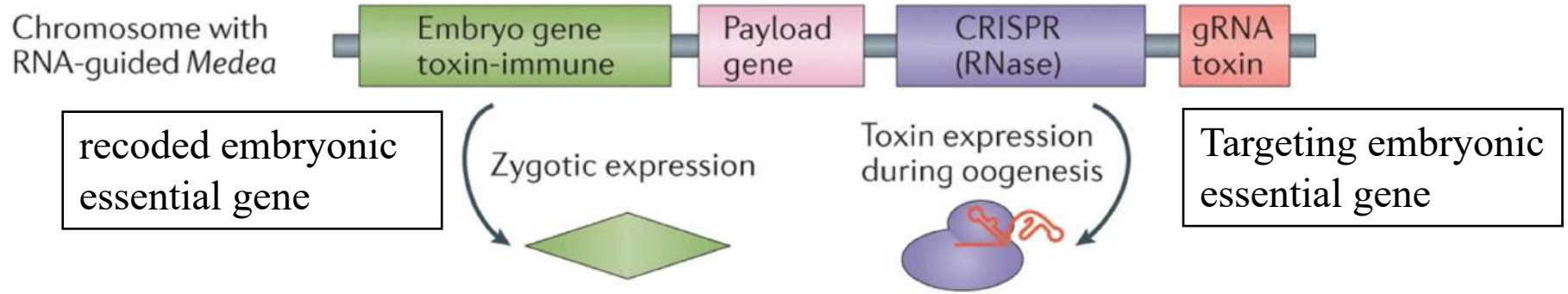
Sex-linked meiotic drives

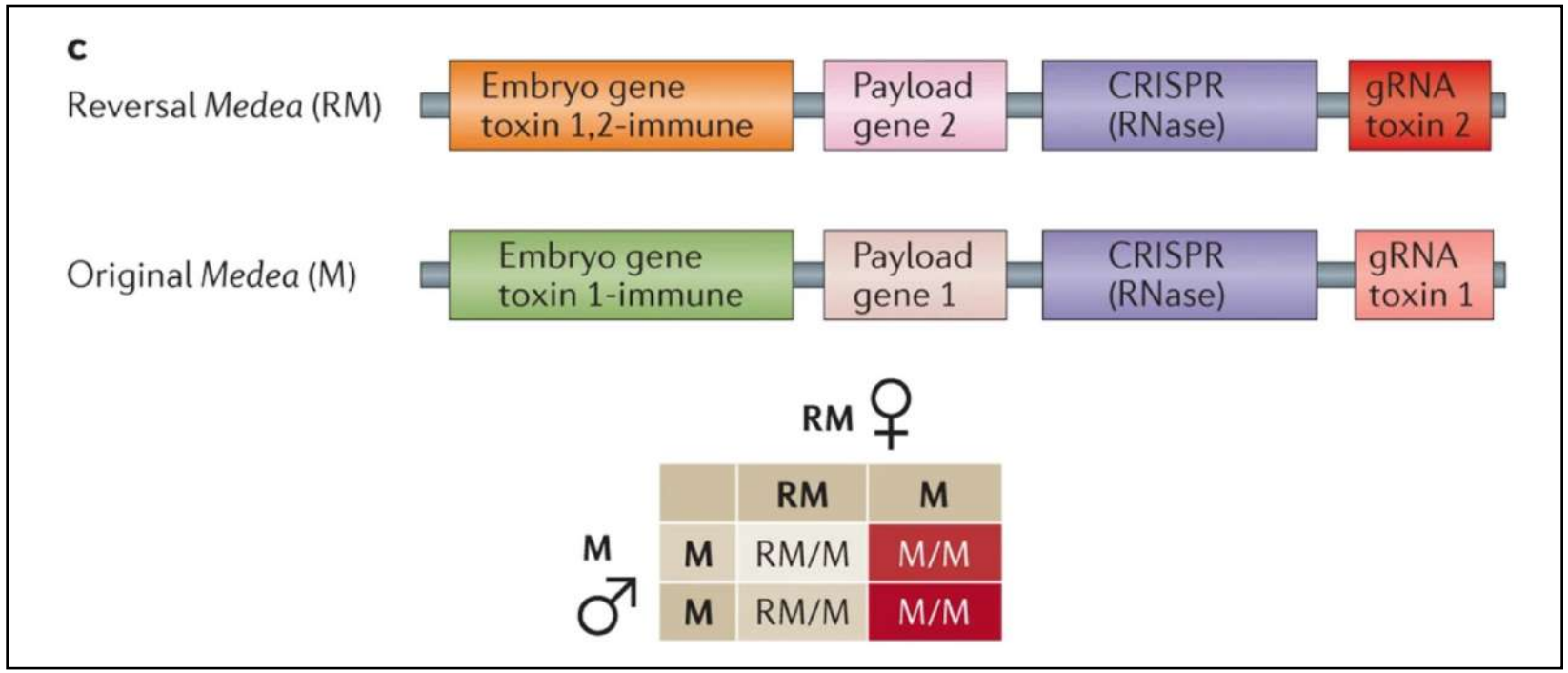




CRISPR/Cas9 technology provides a new tool for the effective development of a variety of engineered gene drives

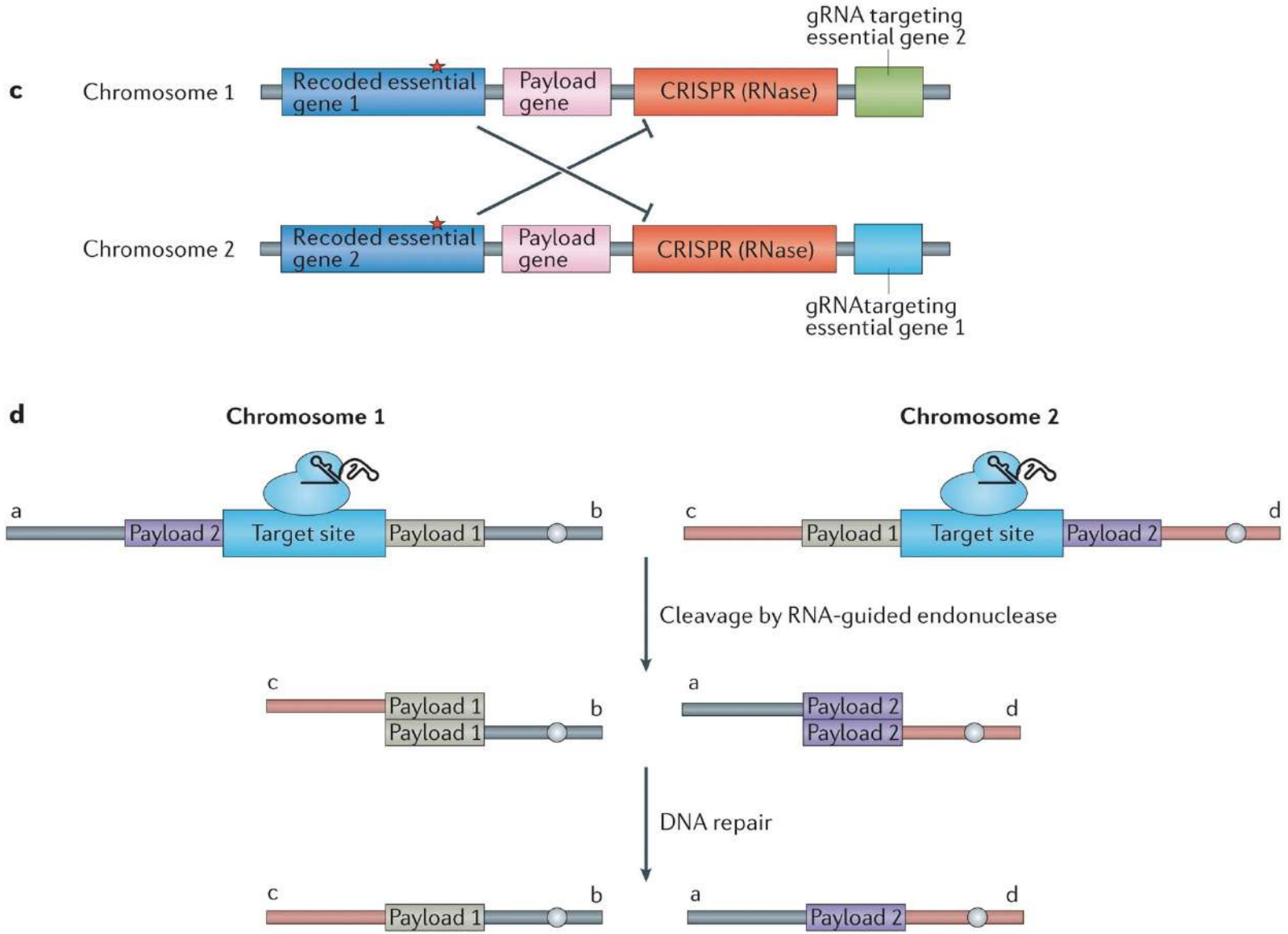
Medea

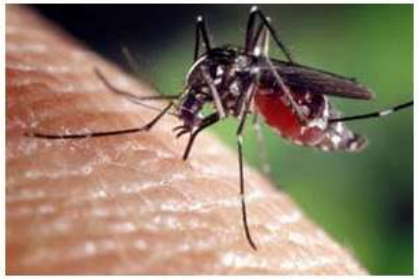




CRISPR/Cas9 technology provides a new tool for the effective development of a variety of engineered gene drives

Underdominance gene drives





联合国会议同意限制测试基因驱动

cnBeta.COM　　2018-12-03

在埃及沙姆沙伊赫举行的联合国生物多样性公约会议上，各国否决了一项暂时禁止释放携带基因驱动生物体的提议。基因驱动是一种基因工程技术，旨在于目标群体内迅速传播突变。比如我们想要消灭传播疟疾的蚊子，利用基因驱动可以使得蚊子的后代全部是雄性，从而导致它们的灭绝。

代表们同意对条约进行修改，但这些修改非常模糊，以至于基因驱动技术的支持者和怀疑者都在鼓吹胜利。

签署国同意有必要在个案基础上评估基因驱动释放的风险，并应咨询可能受这种释放影响的当地社区和土著群体。

Gene drives: not just about science, but at the interface of science and society

rapid spread, persistence and irreversibility

Developing such a technology cannot be separated cleanly from non-science and engineering-related issues

ethical, legal, and social dimensions

- Phase 0: Research preparation
- Phase 1: Laboratory-based research
- Phase 2: Field-based research
- Phase 3: Staged environmental release
- Phase 4: Post-release surveillance

Safety!

Multiple stringent confinement strategies should be used in laboratory for preventing the unintentional release

› Science. 2015 Aug 28;349(6251):927-9. doi: 10.1126/science.aac7932. Epub 2015 Jul 30.

BIOSAFETY. Safeguarding gene drive experiments in the laboratory

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

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*)

Summary

- **Gene drives are systems of biased inheritance** that enhance the likelihood a sequence of DNA passes between generations through sexual reproduction and become a dominant one causing population modification or suppression.

- Gene drives can be characterized by a number of **different attributes** which should be considered when evaluating the type of gene drive that is best suited for a particular application and assessing context-dependent risks.

- The **advent of the CRISPR/Cas9 technology** gives a renewed impetus to developing gene drives in the laboratory for eventual application in diverse organisms.

- **Numerous practical difficulties** must be overcome before gene drives will be in a position to address any of the suggested applications.

Applications of gene drive systems in mosquitoes

姜思梅

Malaria “打摆子”

Anopheles gambiae

How to eliminate malaria ?

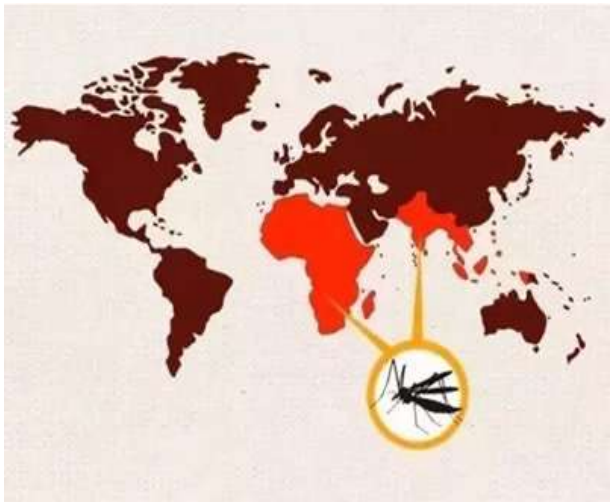
①Control the source of infection

②Cut off the transmission route

③Protect susceptible populations

Symptom ：

Disease area ：



Foreword

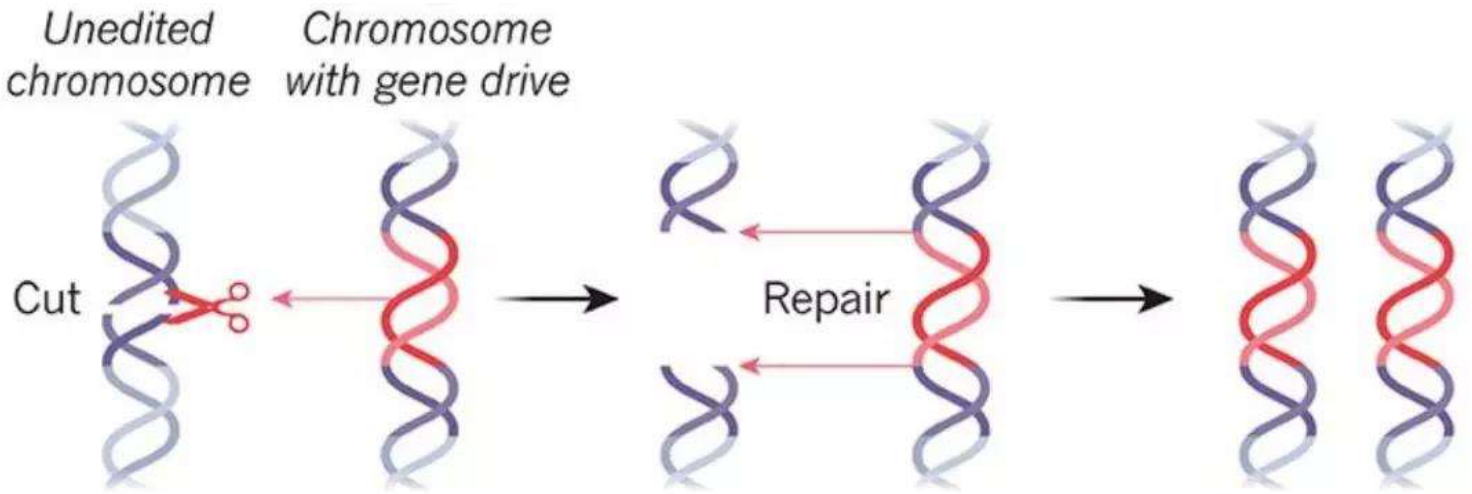
- **Purpose**：Using gene drives to reduce or even eliminate populations of *Anopheles gambiae*
- **Train of thought**：Find and validate suitable target genes→introduce gene drive into target loci and assess effects on fertility→kinetic models predict the spread of gene drive constructs in populations
- **Difference**：Gene drives constructed differently；targeted genes differ；different effects；different advantages and disadvantages

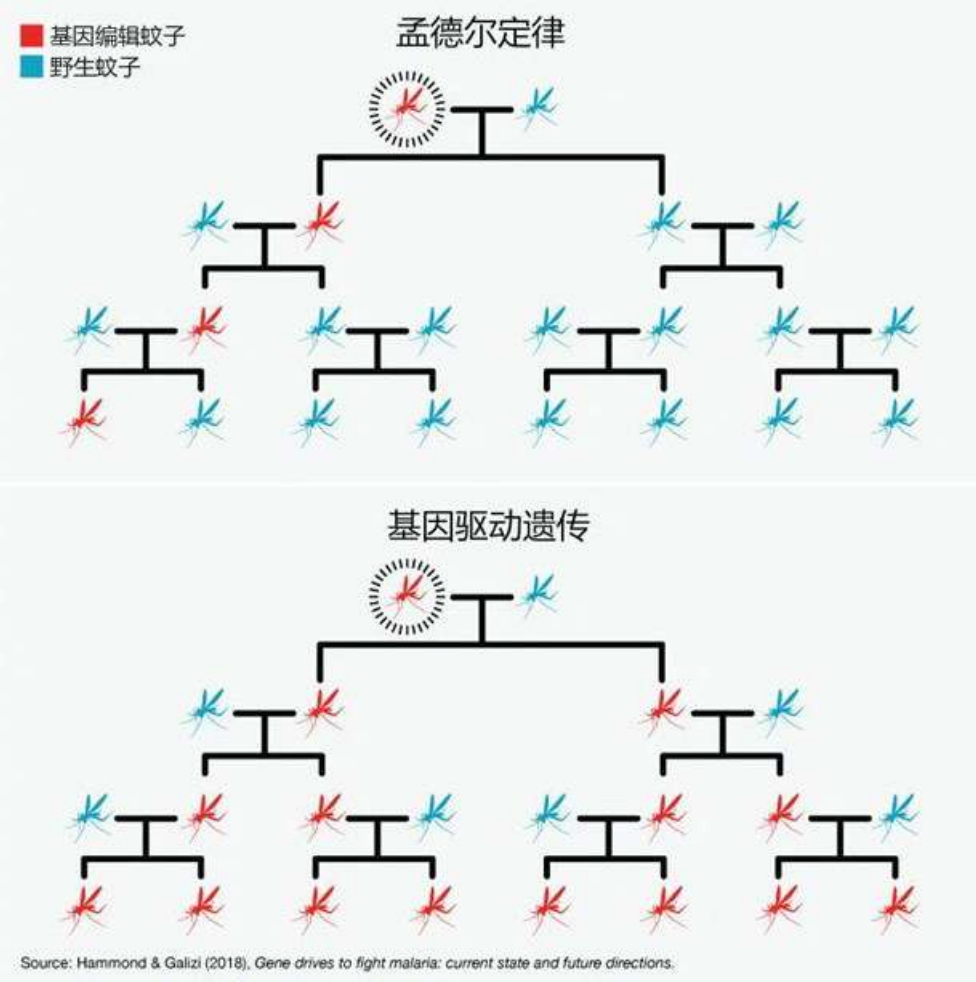
LETTERS

nature biotechnology

A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*

Andrew Hammond[1], Roberto Galizi[1], Kyros Kyrou[1], Alekos Simoni[1], Carla Siniscalchi[2], Dimitris Katsanos[1], Matthew Gribble[1], Dean Baker[3], Eric Marois[4], Steven Russell[3], Austin Burt[1], Nikolai Windbichler[1], Andrea Crisanti[1] & Tony Nolan[1]





归巢内切酶基因HEG　　归巢homing

Determination that the three female fertility genes in *A. gambiae* are haplosufficient

$$G_1 \xrightarrow{\text{cross}} G_2$$

$$G_2(\♀) \times WT(\♂)$$

detecting fertility (egg laying and hatching)

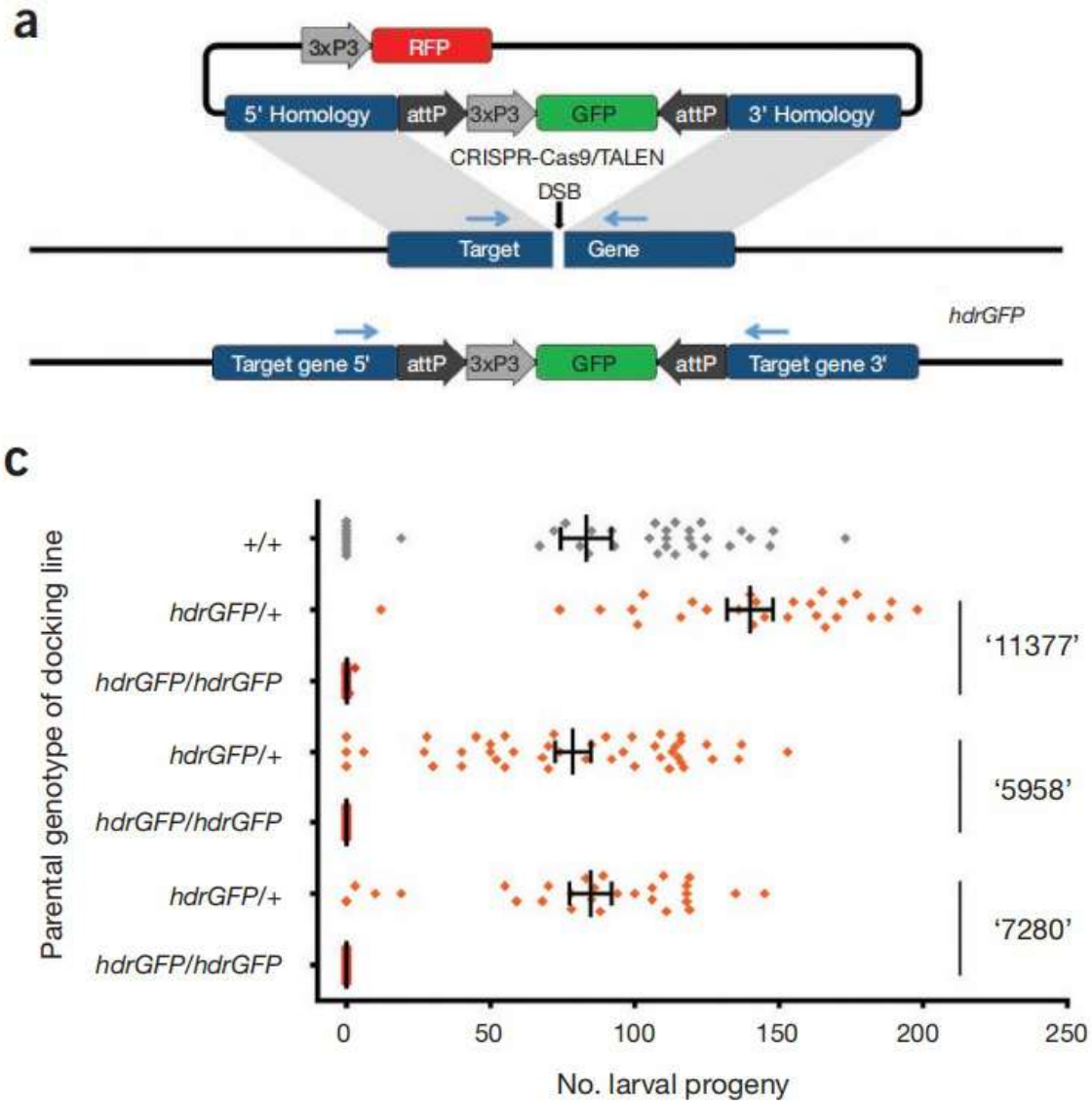
All homozygous female mosquitoes were sterile, whereas heterozygous females showed normal rates of egg laying and hatching

Target genes：

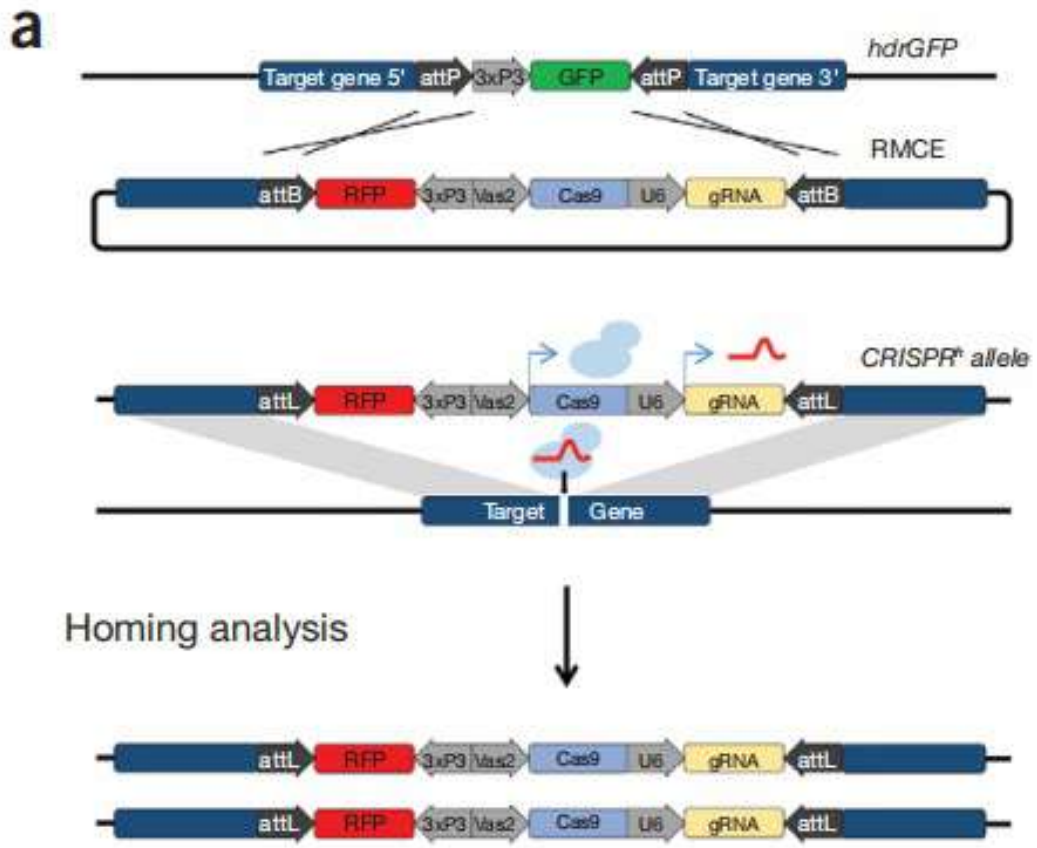
AGAP005958

AGAP007280 ⟹ haplosufficiency

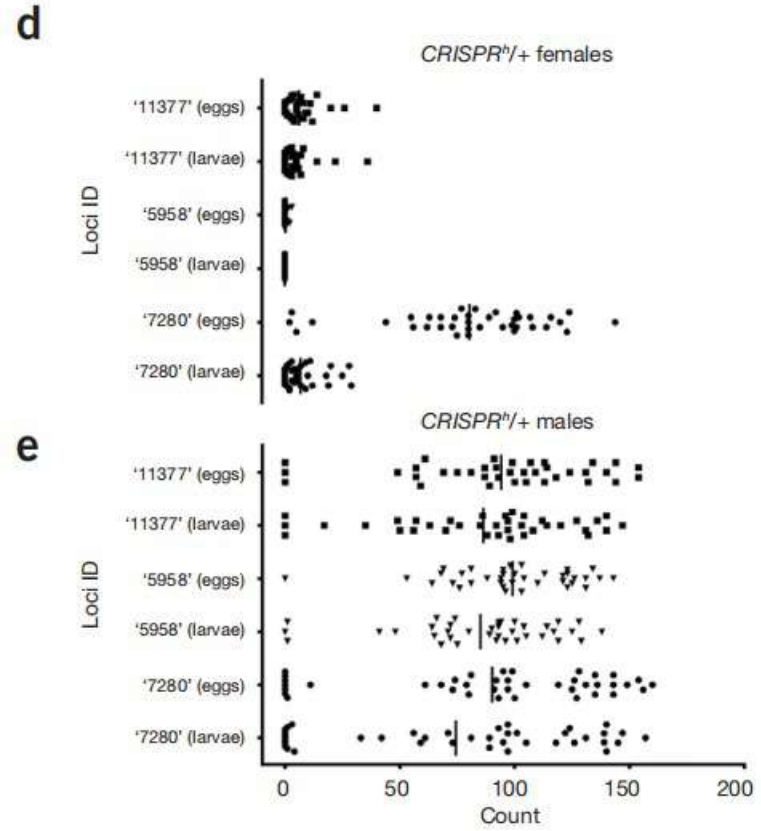
AGAP011377



The fertility of females heterozygous for *CRISPR^h* was markedly reduced,males heterozygous showed normal fertility



重组酶介导的盒式交换(RMCE)



the number of larvae produced：

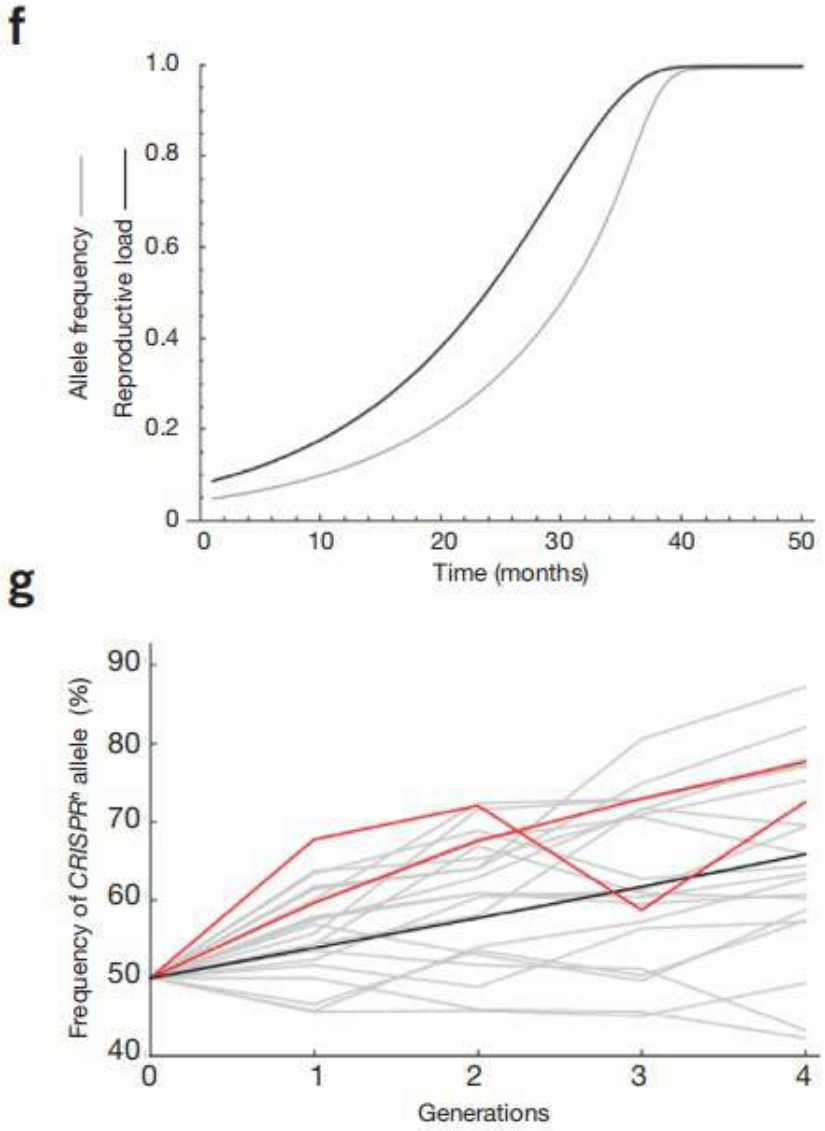
（♀）*AGAP011377* 4.6% of WT

AGAP007280 9.3% of WT

AGAP005958 no larvae

（♂）No significant difference

Dynamics model prediction shows that the construct can spread through the population



- the fitness cost in terms of reduced reproductive capability imposed by the *CRISPR*^{h} constructs at *AGAP011377* and *AGAP005958* outweigh the homing rate, and the constructs would be expected to disappear from a population over time.

*AGAP011377*和*AGAP005958*的*CRISPR*^{h}结构所带来的生殖能力下降导致的生殖负荷超过了归巢率，预计这些结构将随着时间的推移从种群中消失。

- the higher homing rates observed for *CRISPR*^{h} at *AGAP007280*, combined with the milder fertility reduction observed in heterozygous females indicate that this construct could spread through a population.

在*AGAP007280*观察到的*CRISPR*^{h}的较高归巢率，加上在杂合子雌性中观察到的较温和的生育力下降，表明该结构可以在种群中传播。

doublesex and sex differentiation in *A. gambiae*

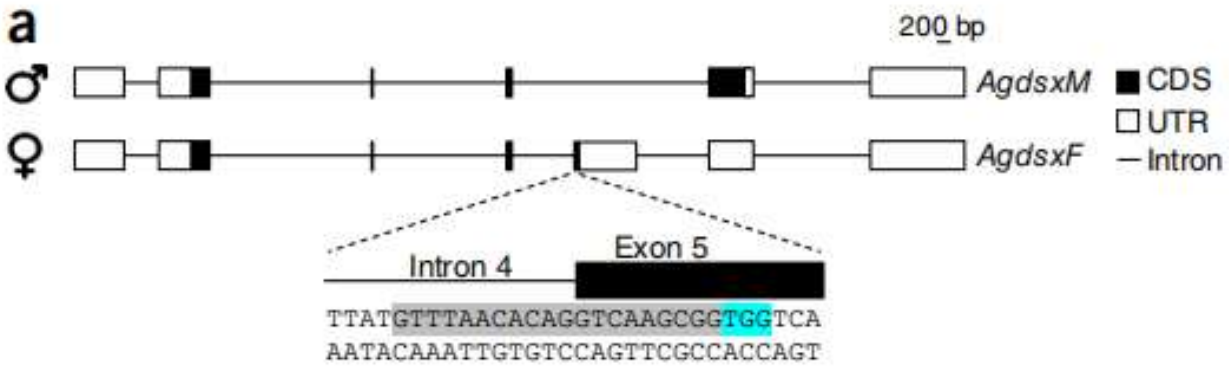
ARTICLES

nature biotechnology

OPEN

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

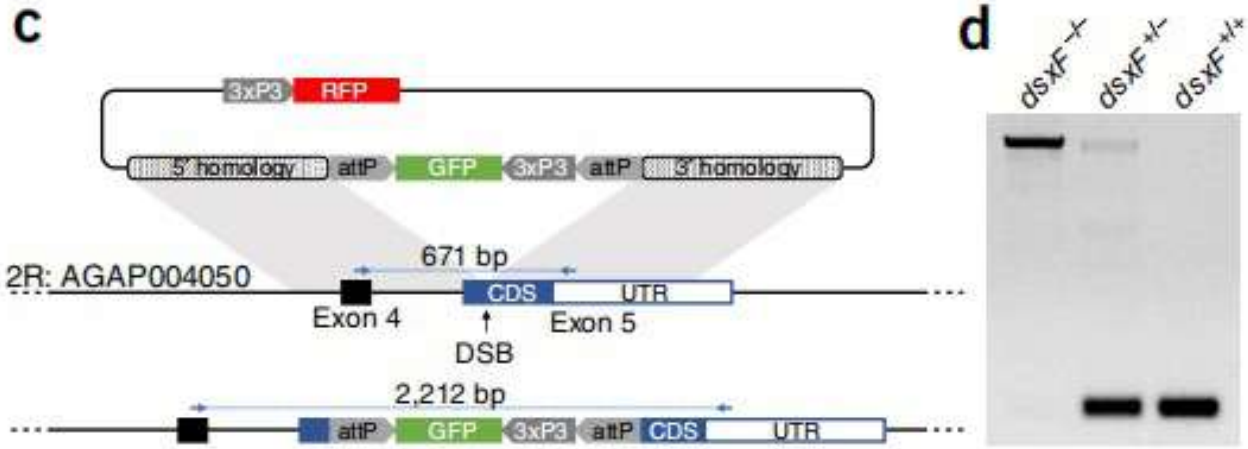
Kyros Kyrou[1,2], Andrew M Hammond[1,2], Roberto Galizi[1], Nace Kranjc[1], Austin Burt[1], Andrea K Beaghton[1], Tony Nolan[1] & Andrea Crisanti[1]



doublesex→control differentiation of the two sexes

In *A. gambiae*, *dsx* (*Agdsx*) consists of seven exons, distributed over an 85-kb region on chromosome 2R

Disruption of the intron 4–exon 5 boundary of *dsx* lead to intersex phenotype suggesting that the female-specific isoform of *dsx* is haplosufficient

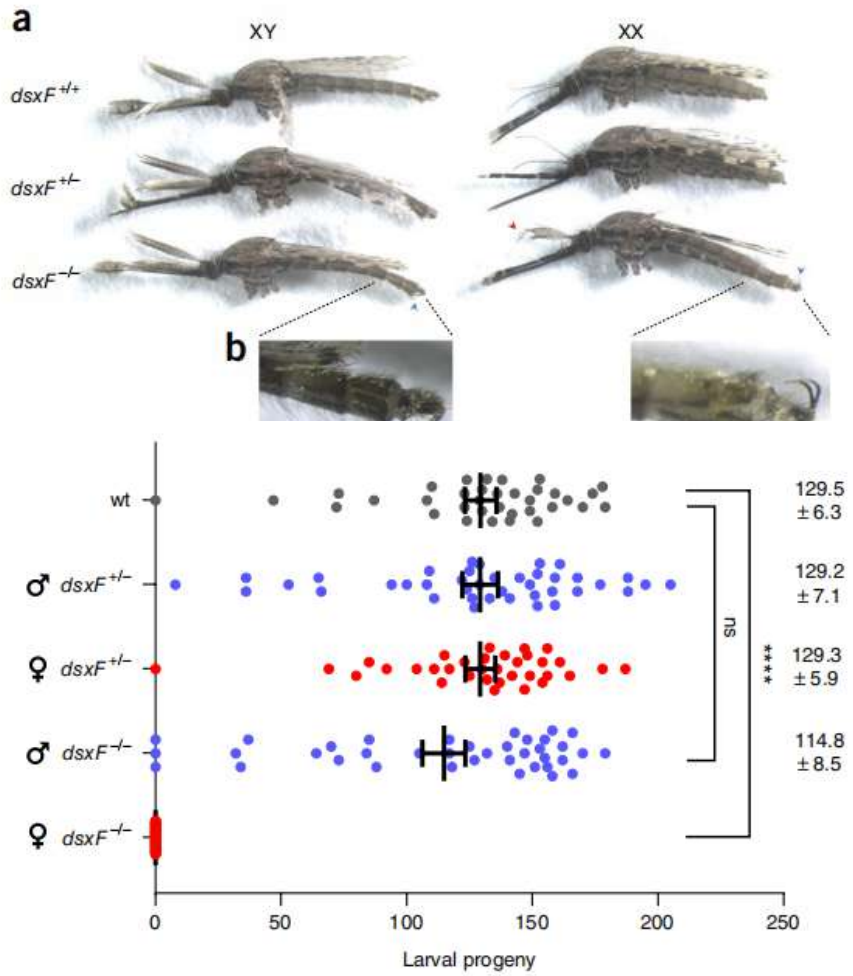


Supp Table S2 | Ratio of larvae recovered by intercrossing heterozygous *dsx* ϕC31-knock-in mosquitoes			
GFP strong (*dsxF*$^{-/-}$)	GFP weak (*dsxF*$^{-/+}$)	no GFP (*+/+*)	Total
262 (24.9%)	523 (49.7%)	268 (25.5%)	1053

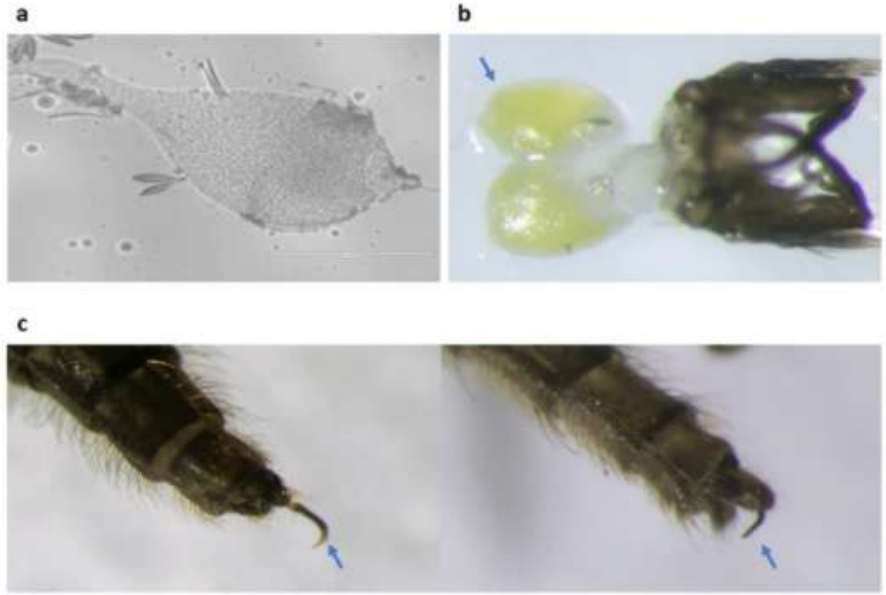
Crosses of heterozygous individuals produced wild-type, heterozygous and homozygous individuals at the expected Mendelian ratio 1:2:1, indicating that there was no obvious lethality associated with the mutation during development.

dsx^{F} → haplosufficiency

External and internal anomalous features of the *dsxF*$^{-/-}$ genotypic females



dsxF$^{-/-}$ and *dsxF*$^{+/-}$ males and females crossed with WT separately，recording the number of larvae progeny

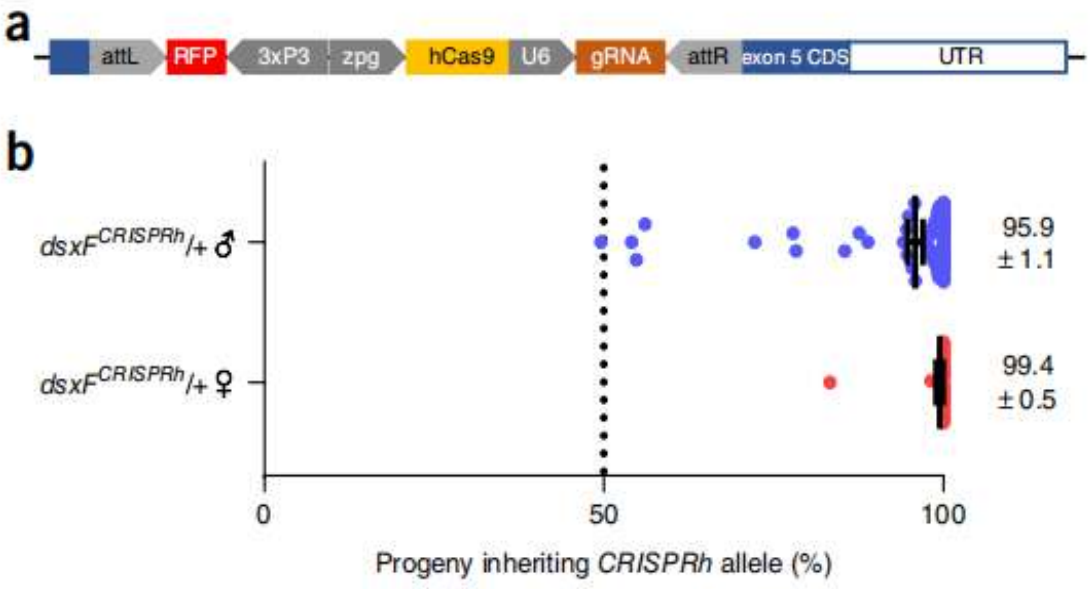


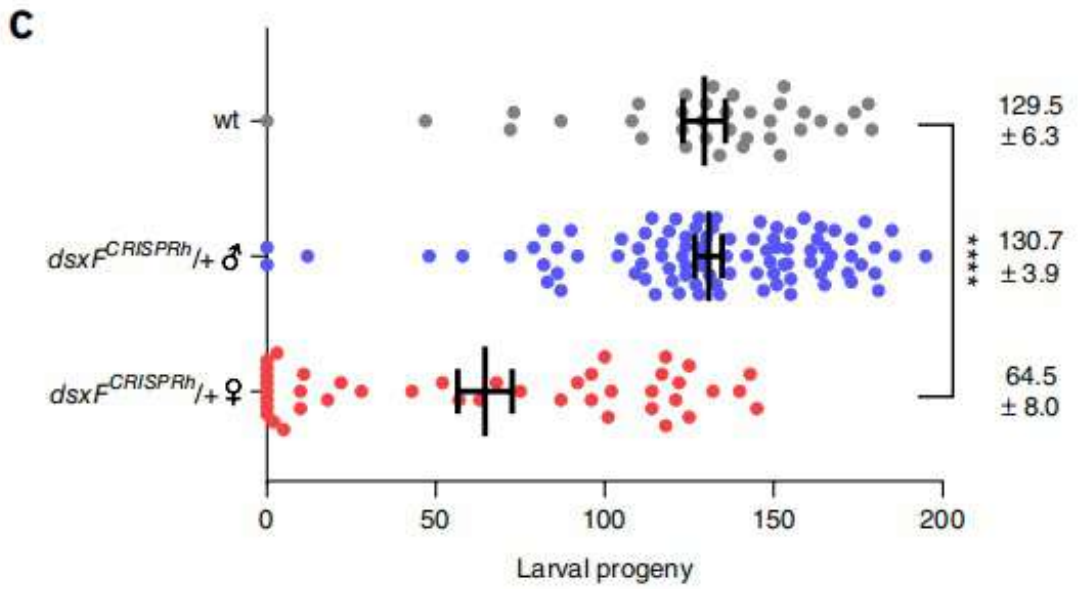
“intersex phenotype”

Intersex XX *dsxF*$^{-/-}$ female mosquitoes,although attracted to anesthetized mice, were unable to take a blood meal and failed to produce any eggs

The fertility of heterozygous （$dsxF^{CRISPRh}$/+） females was reduced，heterozygous males showed normal fertility

RMCE

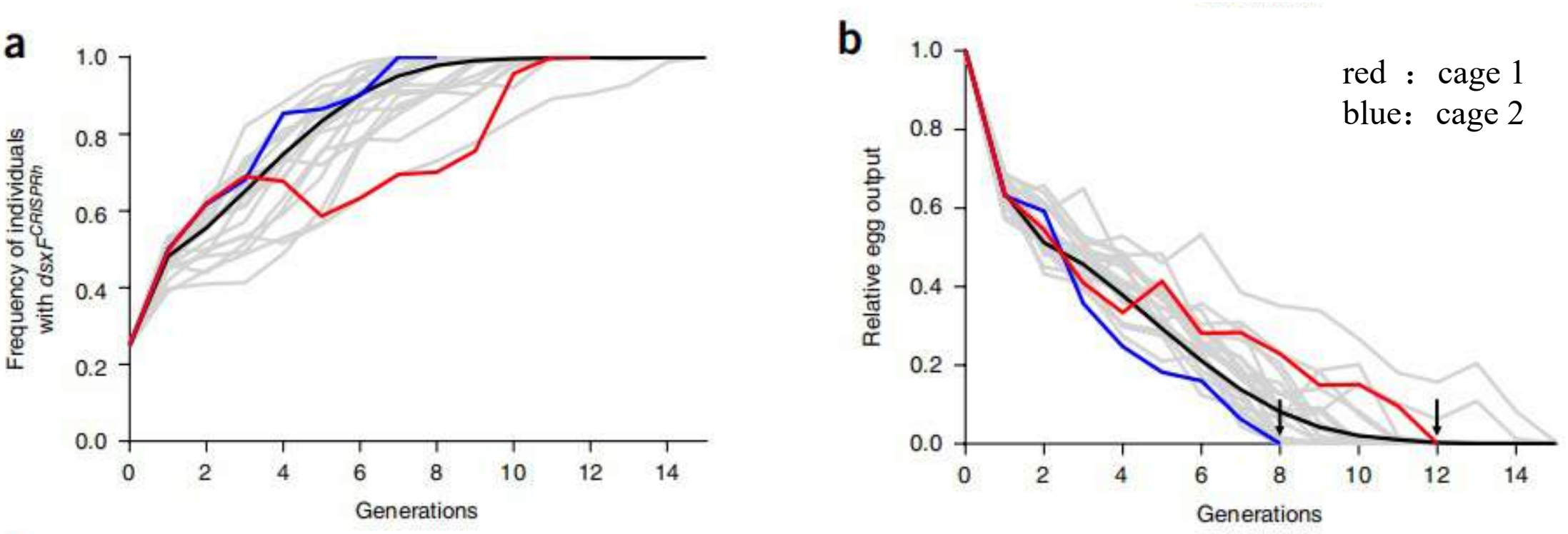




Heterozygous parents （$dsxF^{CRISPRh}$/+） crossed with WT，detecting RFP markers on progeny

Heterozygous parents （$dsxF^{CRISPRh}$/+） crossed with WT，recording the number of larvae produced by single females

Model prediction that the *dsxF*^{CRISPRh} had the potential to reach 100% frequency in caged population in 9–13 generations



Two cages were set up with a starting population of 300 wild-type females, 150 wild-type males and 150 *dsxF*^{CRISPRh}/+ males, seeding each cage with a *dsxF*^{CRISPRh} allele frequency of 12.5%.

The drive allele reached 100% prevalence in both cage 2 and cage 1 at generation 7 and 11, respectively.The population completely collapses at generation 8 (cage 2) or generation 12 (cage 1).

Designing an SDGD（sex-distorter gene drive）

ARTICLES
https://doi.org/10.1038/s41587-020-0508-1
nature biotechnology
Check for updates

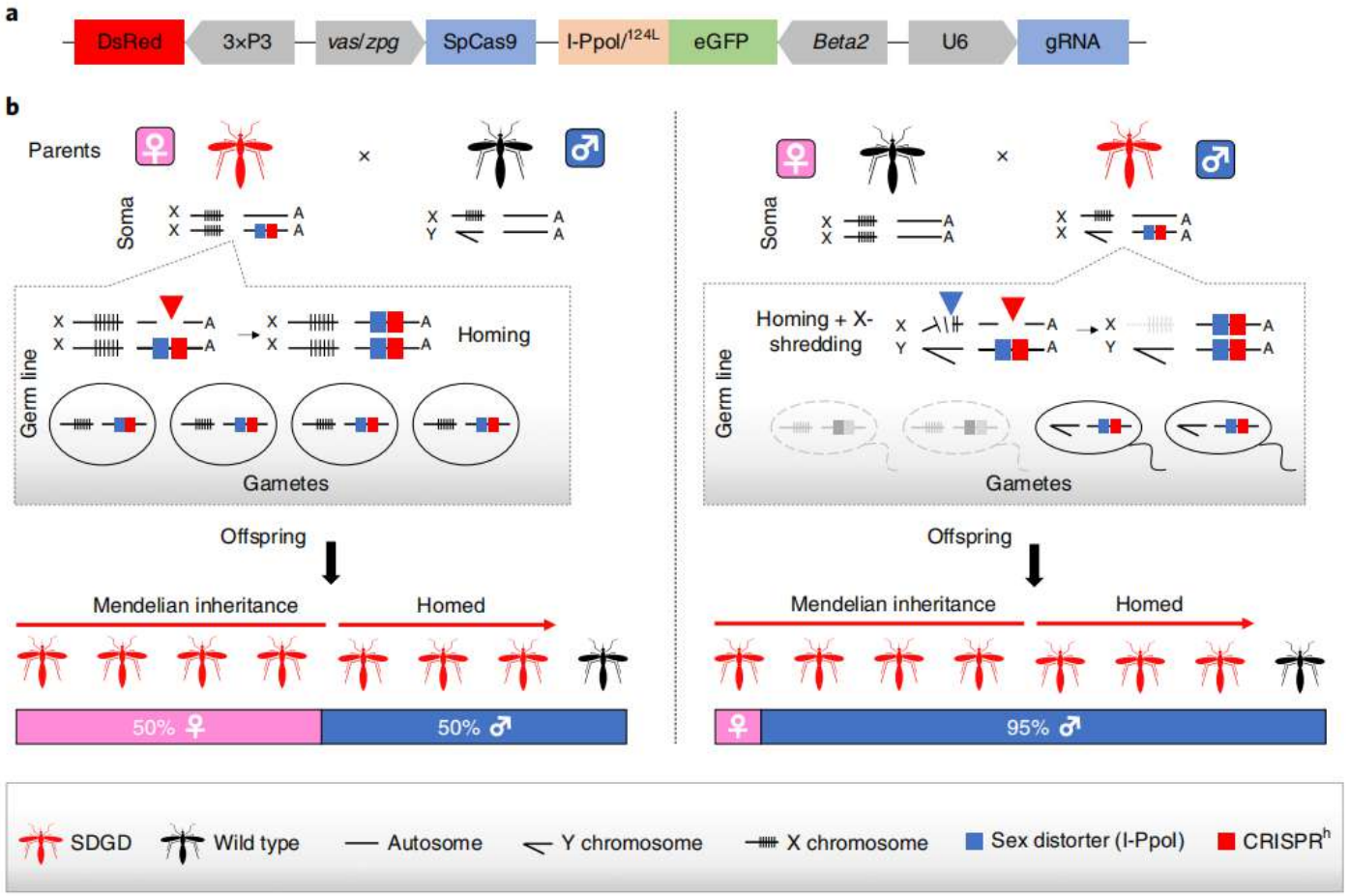
OPEN

A male-biased sex-distorter gene drive for the human malaria vector *Anopheles gambiae*

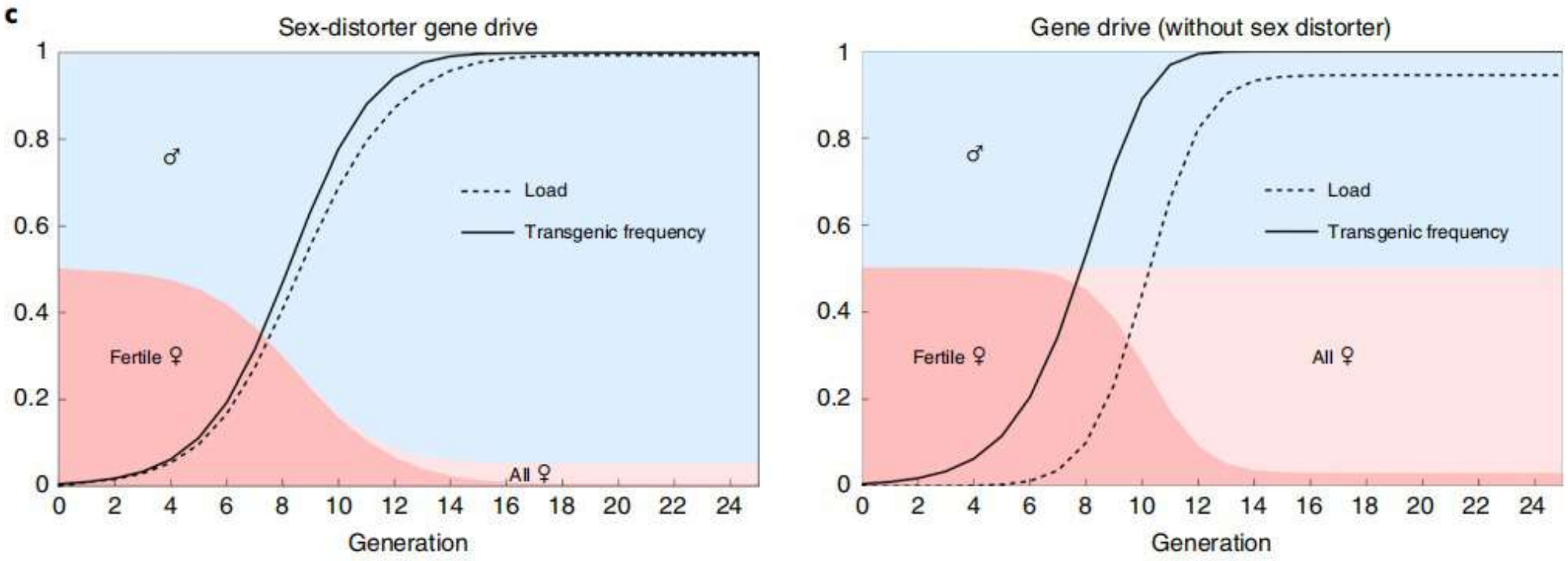
Alekos Simoni[1,2,8], Andrew M. Hammond[1,3,8], Andrea K. Beaghton[1], Roberto Galizi[1,4], Chrysanthi Taxiarchi[1], Kyros Kyrou[1], Dario Meacci[1], Matthew Gribble[1], Giulia Morselli[1], Austin Burt[5], Tony Nolan[1,6] and Andrea Crisanti[1,7]✉

Female： the CRISPR^{h} component is active→homing of the construct

Male： both the gene-drive and sex-distorter transcription units are active→homing of the construct ＋ shredding of the X chromosome



Model prediction that SDGD could spread rapidly from a low starting frequency to produce a largely unisex male population and impairing female fertility



SDGD^{011377} and SDGD^{005958} show a high rate of transgene transmission and male bias

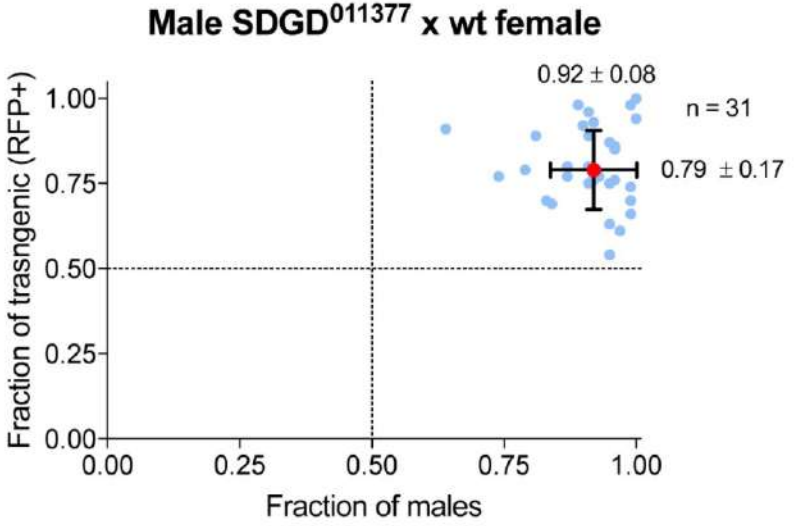
Target genes(haplosufficiency)：

AGAP005958

AGAP007280

AGAP011377

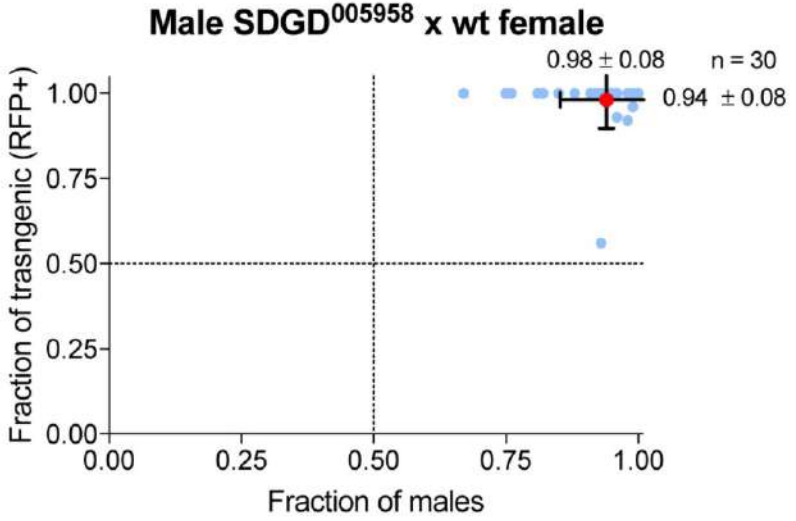
→ SDGD^{005958}, SDGD^{007280}, SDGD^{011377}



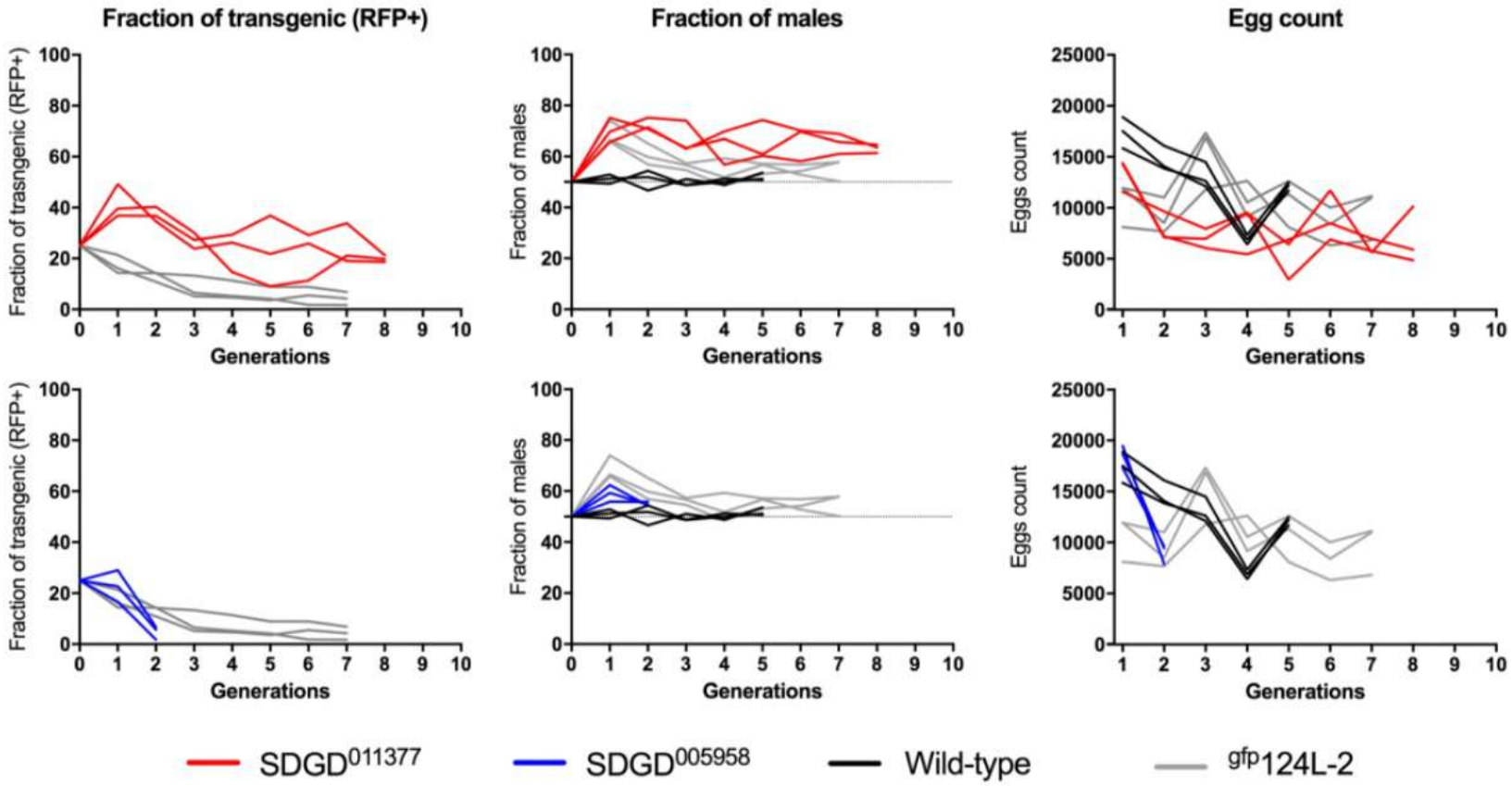
SDGD-heterozygous(♂) × WT(♀)

detecting RFP markers and fraction of males on progeny

SDGD^{007280} had severely reduced fertility, and we did not recover enough progeny to assess drive activity



Kinetics of SDGD^{011377} and SDGD^{005958} spread in target mosquito populations



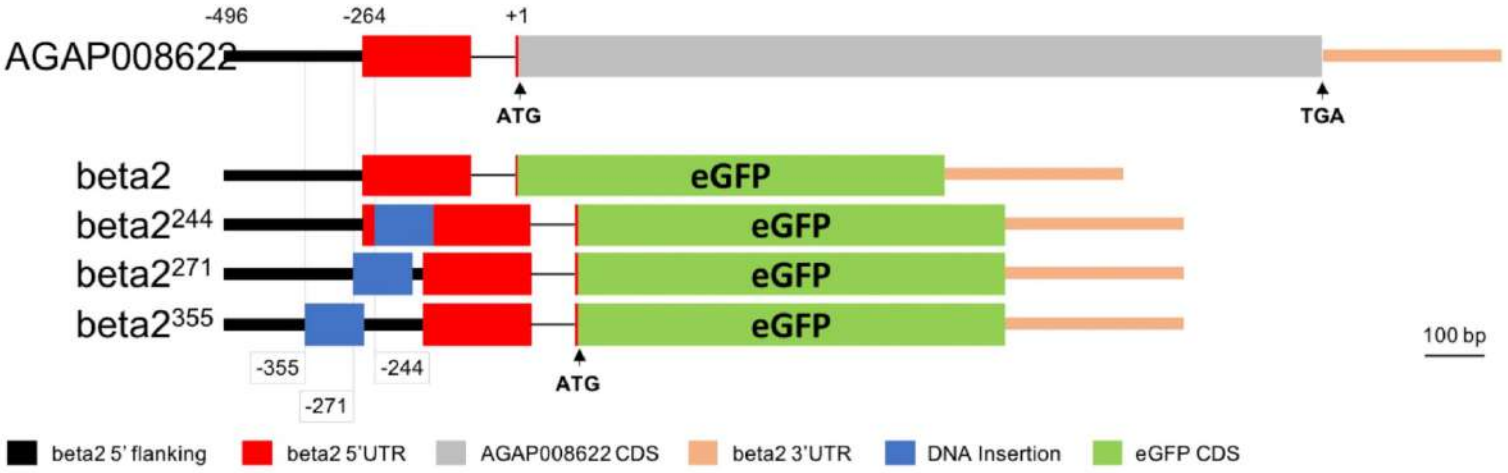
100 heterozygous transgenic males were introduced into a population of 100 wild-type males and 200 wild-type females (transgenic allele frequency of 12.5%).
The frequency of the transgene was monitored every generation together with the fraction of males in the population and the total number of eggs laid. A random selection of 450 eggs was seeded for the next generation.Three repeats.

Optimization of temporal and spatial characteristics and level of expression of Cas9 and I-PpoI

Fitness costs, most likely associated with non-optimal spatial and temporal activity of both the Cas9 and I-PpoI genes, impaired SDGD spread into mosquito populations.

Method:①To minimize the ectopic activity of Cas9→replac the *vasa* promoter with the *zpg* promoter.

②To reduce the transcriptional activity of the beta2-tubulin promoter→generat three variants by inserting a G+C-rich sequence of 100bp in proximity to conserved sequences at position −244, −271 or −355 with respect to the ATG start codon.

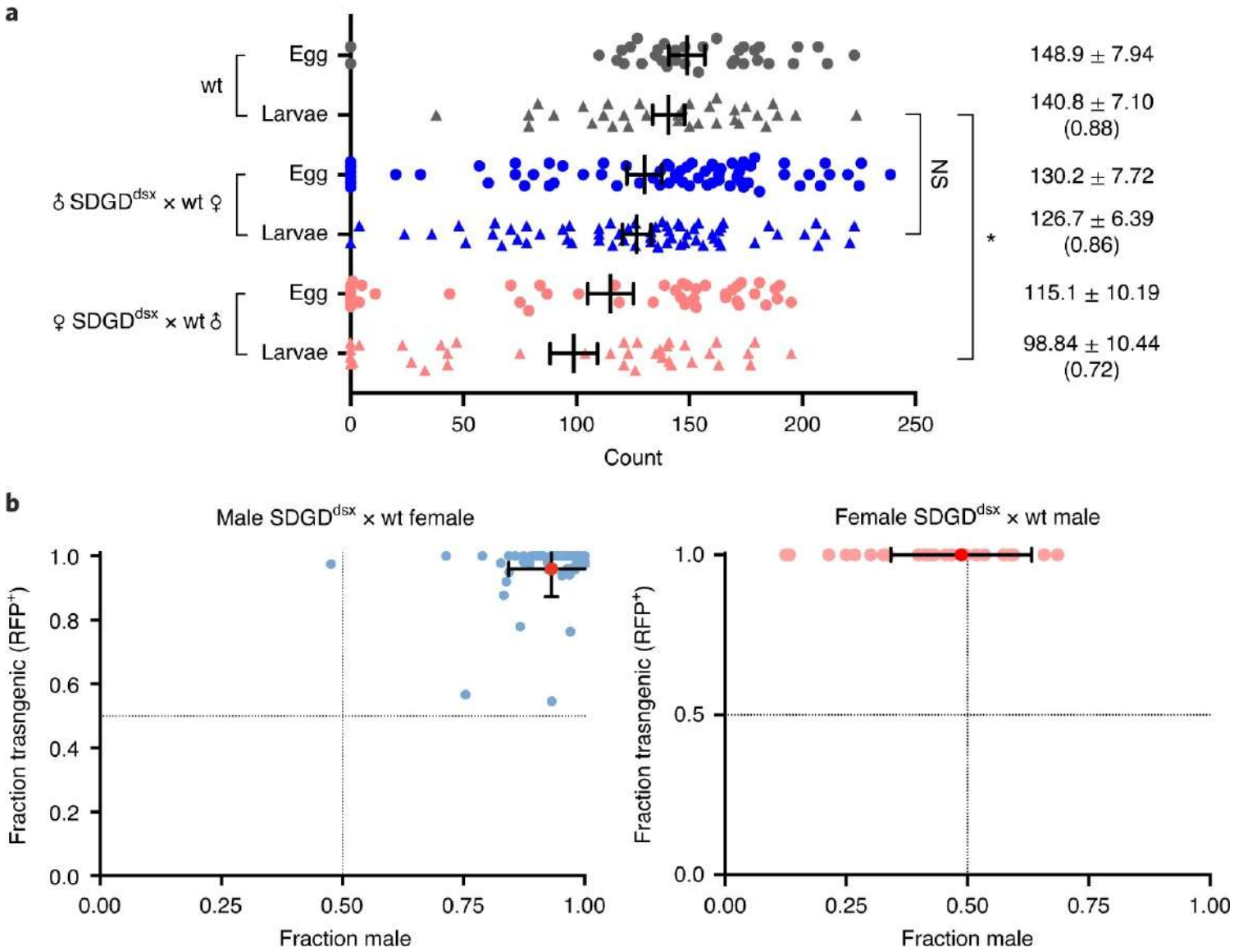


DNA spacer

GACTCGGACCCGAGTTGGCCAACGACCCACGGGCGGAG
TTAGGGCGGATGGTGAGAAGTGCGCGTCTCGTTCCCGC
AGCTCGCCAGCACTCTCAGACTCA

100 bp

SDGD^{dsx} had no significant impact on the fertility of heterozygotes and SDGD^{dsx} heterozygous males had a marked male bias in the offspring



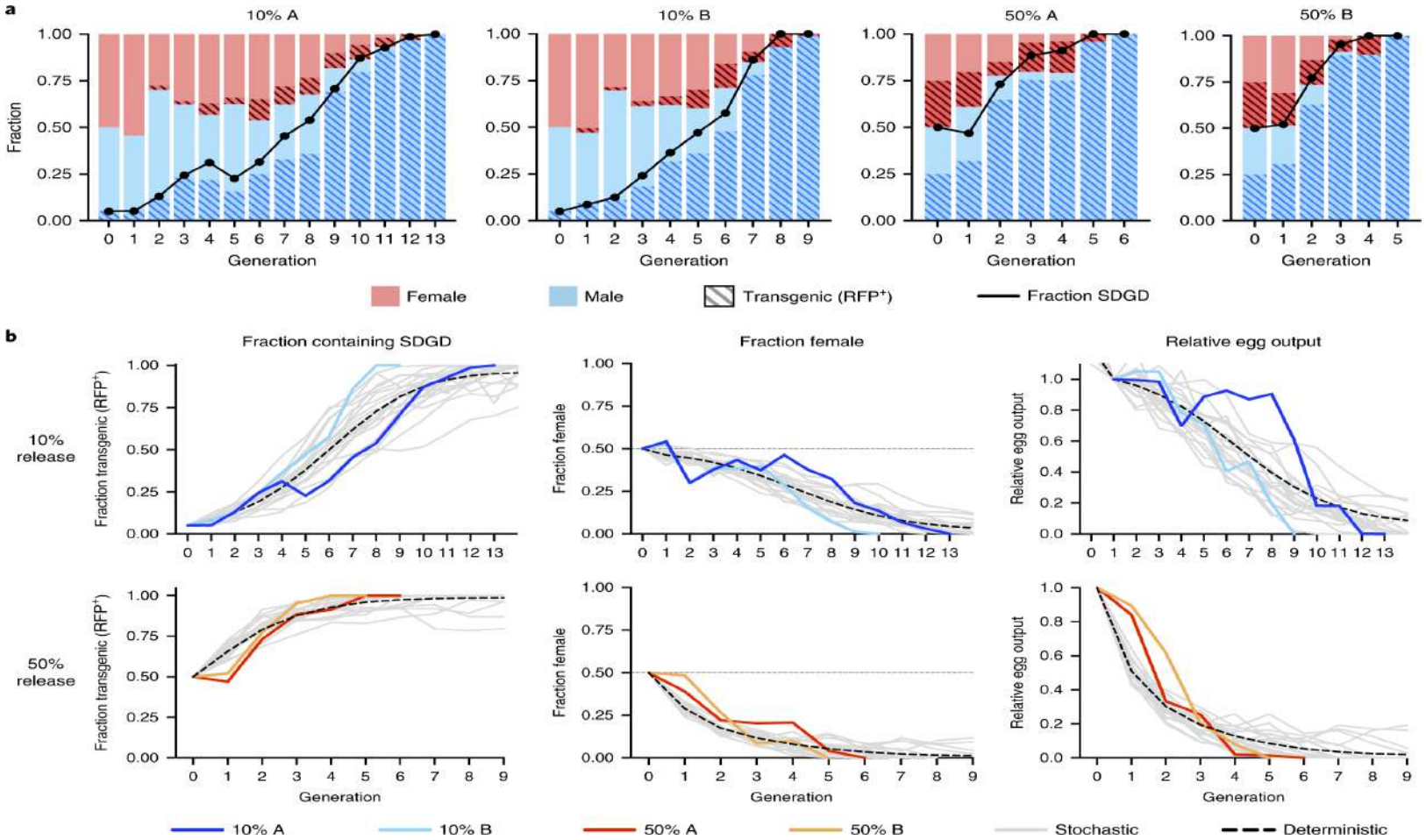
The larval output of SDGD^{dsx} males was comparable to that of controls.

The fertility of SDGD^{dsx} heterozygous females, measured as viable offspring, was reduced as compared to controls,although it was still sufficient to produce a large number of fertile individuals.

Fraction RFP$^+$： ♂ 96.0%

♀ 99.9%

Model prediction that SDGD^{dsx} would quickly invade the population, reaching 100% allelic frequency and leading to collapse of the population



two cages：A-300 wild-type females, 270 wild-type males and 30 SDGD^{dsx}-heterozygous males(allelic frequency of 2.5%，10% male release)

B-150 wild-type females, 150 wild-type males, 150 SDGD^{dsx}-heterozygous males and 150 SDGD^{dsx}-heterozygous females (allelic frequency of 25%，50% male and female release)

Summary

- A CRISPR-Cas9 gene drive system targeting female-fertility genes
  - Efficient spread of gene drive，not too conservative，develop resistance

- A CRISPR-Cas9 gene drive system targeting *doublesex* gene
  - Efficient spread of gene drive，very conservative，extremely difficult to develop resistance

- A sex-distorter gene drive targeting female-fertility genes and *doublesex* gene
  - Very conservative，introduction of sex-distorter，male-biased，reduce malaria transmission

Applications of gene drive systems in rodents

彭琼琳

Animal models play a critical role in translational research and advancement of human and animal health

Recent Milestones in Animal Modeling

Years	Researcher(s)	Milestone
1902	William Castle	Begins breeding mice for genetic studies
1909	Clarence Little	Begins inbreeding mice to eliminate variation
1920s	Frederick Banting	Isolated canine insulin and effectively treated diabetic dogs
ca. 1930	Little and MacDowell	First fully inbred mouse (20 brother × sister matings) achieved
1940s	John Cade	Studied the use of lithium salts as an anticonvulsant in guinea pigs and translated his findings to treatments of depression
1976	Rudolf Jaenisch et al.	Developed first transgenic mouse
1980s	Several	Extensive testing of drug safety and dosing regimens for HIV performed in rhesus macaques
1987	Capecchi, Evans, and Smithies	Developed first knockout mouse
1997	Wilmut and Campbell	First animal cloned from an adult somatic cell, Dolly the sheep
2002	Several	Mouse genome sequenced
2004	Several	Rat genome sequenced
2009	Aron Geurts et al.	Developed first knockout rat

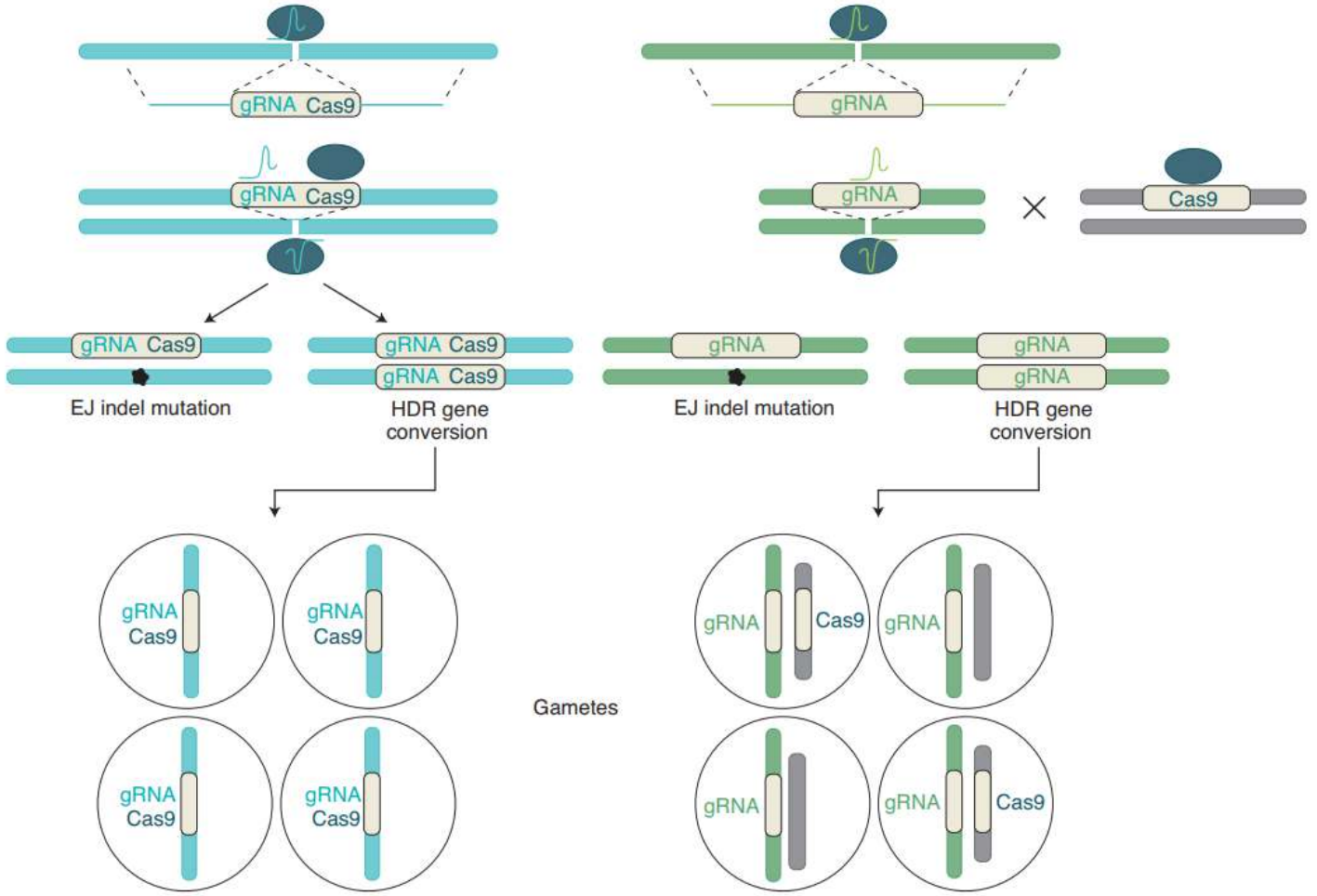
Ericsson, A. C., et al. (2013)

Active genetics:

self-propagating system **split system**

Genetic manipulations in which a genetic element is copied from one chromosome to the identical insertion site on the sister chromosome using *cas9* and gRNA elements.

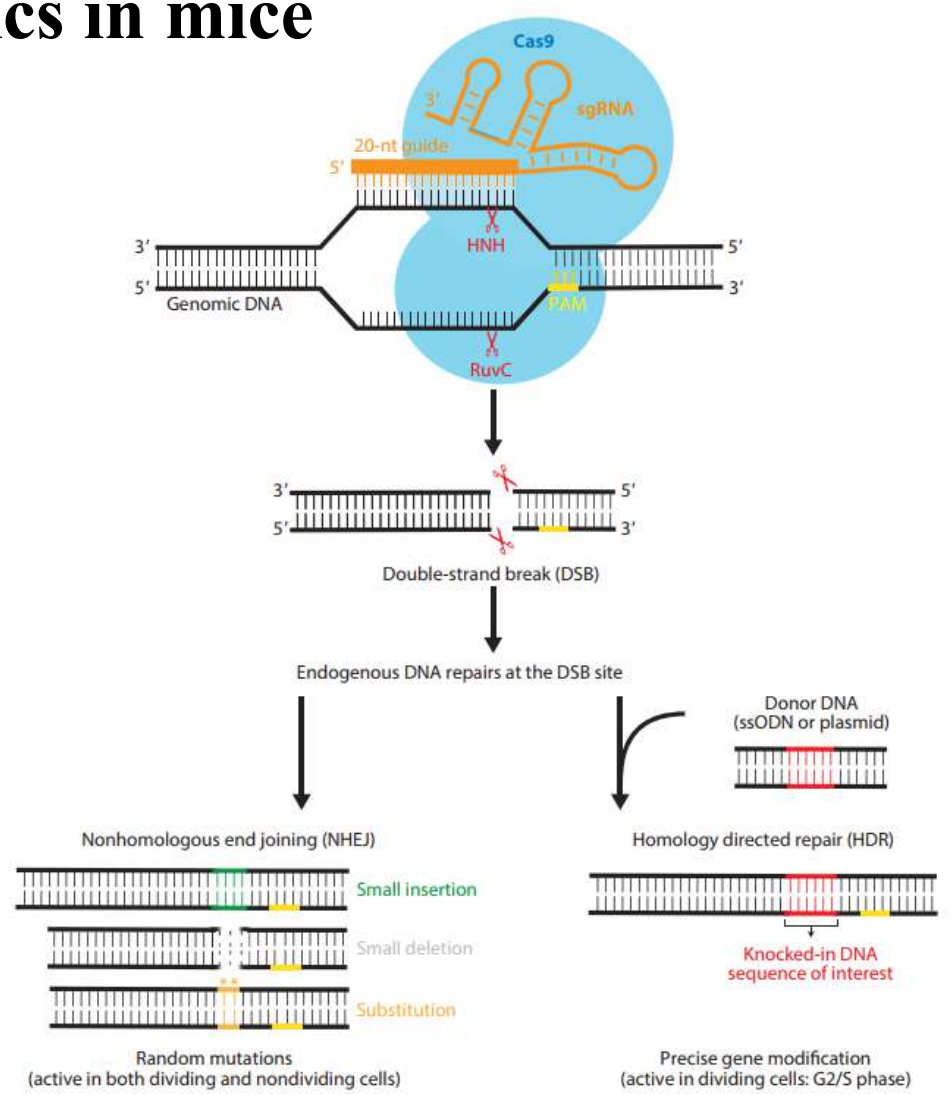
"super-Mendelian"



Grunwald, H. A., et al. (2022)

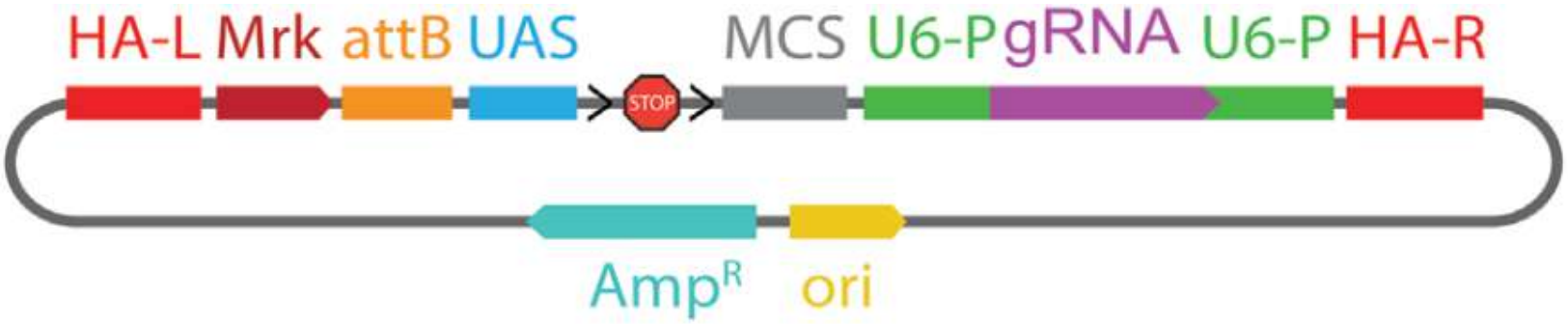
Two potential obstacles to the implementation of active genetics in mice

- The frequency of DSB formation using a genetically encoded Cas9 and gRNA.
- The frequency of HDR may prevent efficient gene conversion.



Jiang, F. and J. A. Doudna (2017)

CopyCat cloning vectors



Plasmid cloning vectors that in addition to having **standard features** (e.g., origin of replication, antibiotic resistance genes, multiple cloning sites) also carry a **gRNA** flanked by **homology arms** that direct insertion of the element into defined locations.

Transgenes inserted into cc vectors can be readily rendered homozygous by providing a source of Cas9 *in trans*.

Gantz, V. M. and E. Bier (2016)

LETTER

https://doi.org/10.1038/s41586-019-0875-2

Super-Mendelian inheritance mediated by CRISPR-Cas9 in the female mouse germline

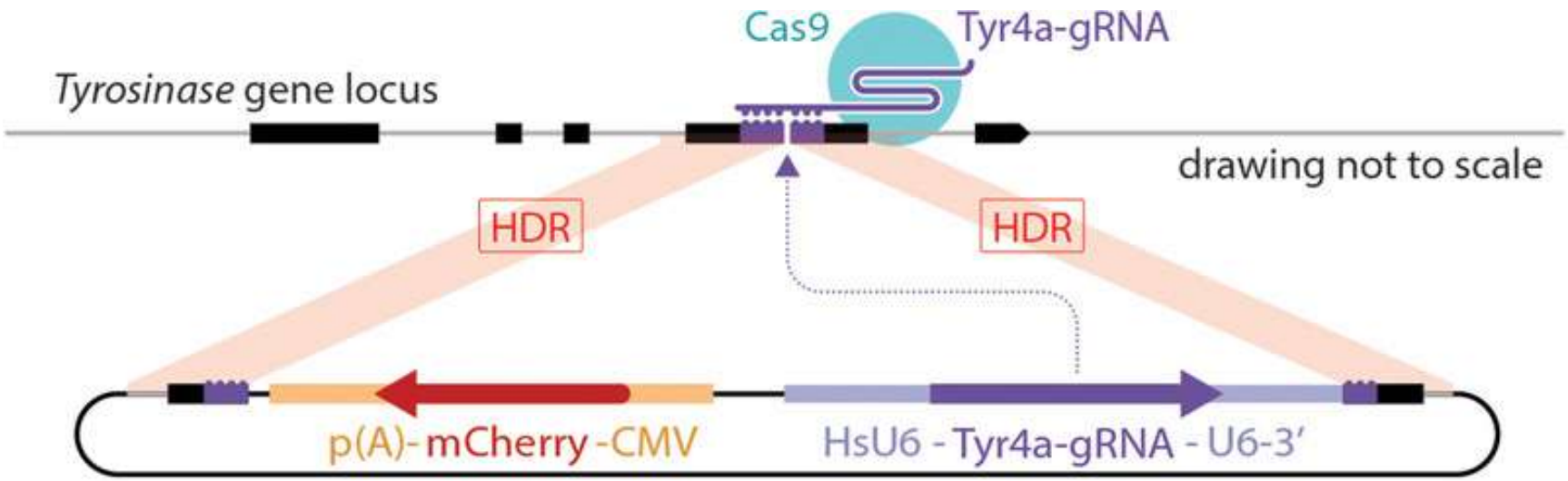
Hannah A. Grunwald[1,5], Valentino M. Gantz[1,5], Gunnar Poplawski[2,4,5], Xiang-Ru S. Xu[1], Ethan Bier[1,3] & Kimberly L. Cooper[1,3]*

This study provided the first proof-of-principle gene drive system in mammals, which selectively sustained drive via the female germline.

Grunwald, H. A., et al. (2019)
Nature 566(7742): 105-109.

How to prove the active genetic system is feasible in mice?

Tyr^{CopyCat} : a split-drive element inserted into the mouse *Tyrosinase* locus

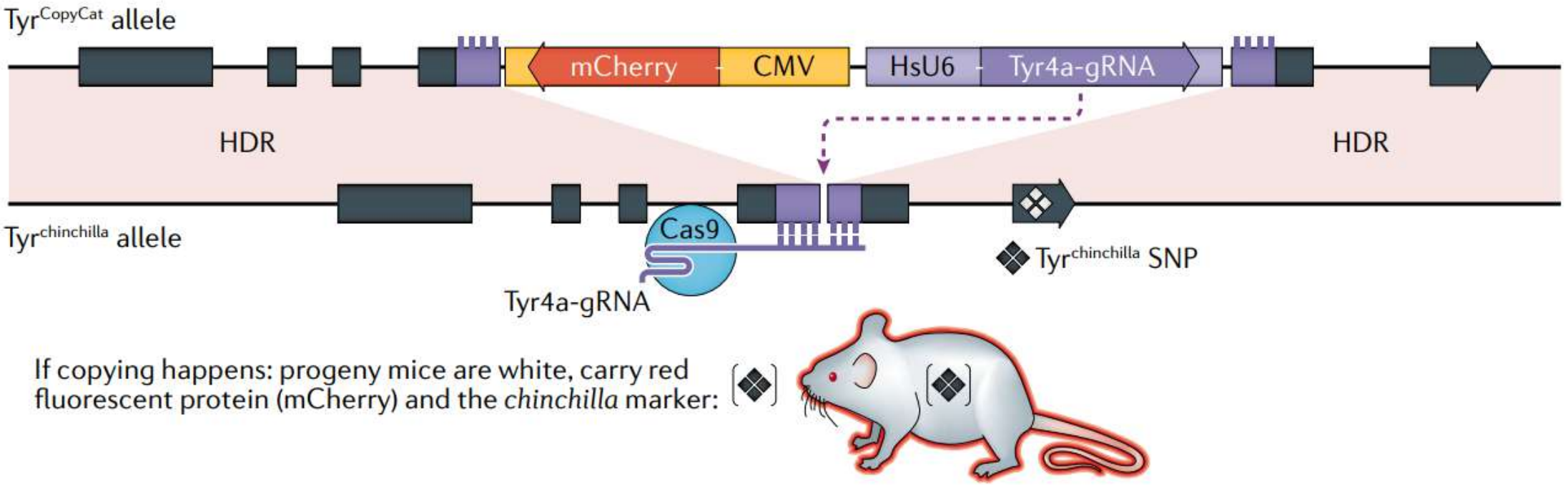


Why *Tyr* ?

-Because of the obvious albino phenotype of homozygous *Tyr* loss-of-function mice.

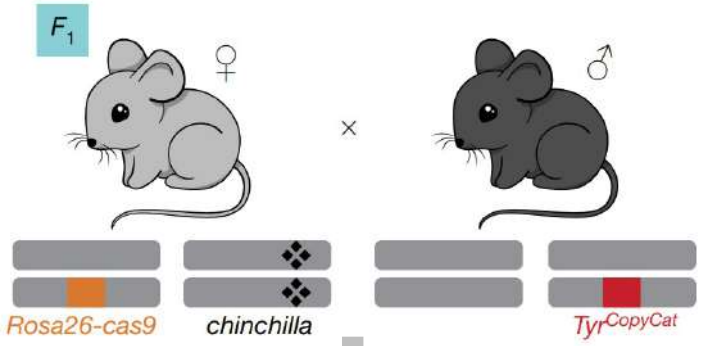
Grunwald, H. A., et al. (2019)

Tyr^{CopyCat} : a split-drive element inserted into the mouse *Tyrosinase* locus



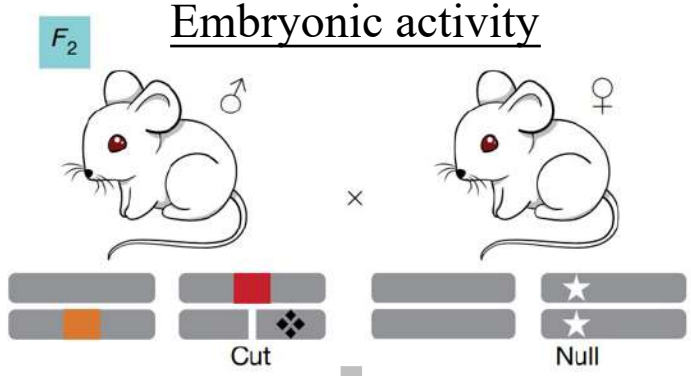
Bier, E. (2022)

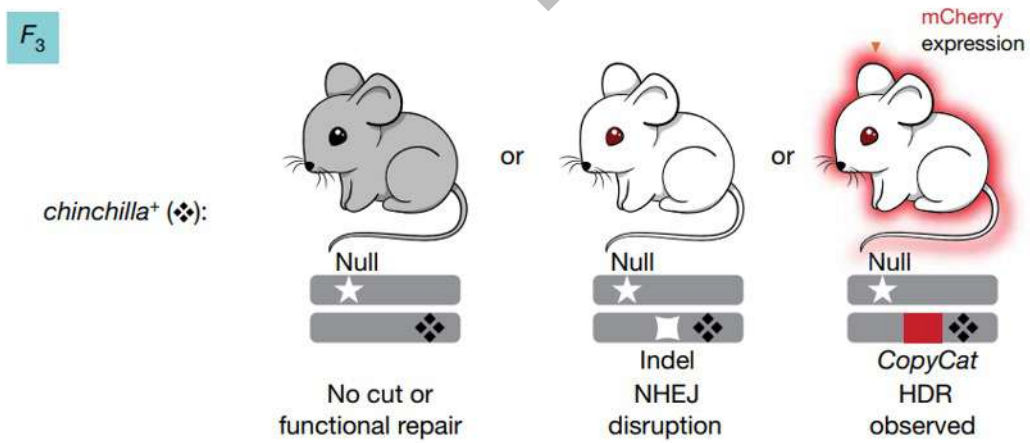
Embryonic Cas9 activity does not copy the *Tyr*^{CopyCat} allele from the donor to the receiver chromosome (*Rosa26-cas9*)



(white mice) *Rosa26-cas9/+; Tyr*^{chinchilla}*/Tyr*^{CopyCat}
(grey mice) *+/+; Tyr*^{CopyCat}*/Tyr*^{chinchilla}
(black mice) *Rosa26-cas9/+; Tyr*^{chinchilla}*/+*
+/+; Tyr^{chinchilla}*/+*

Embryonic activity





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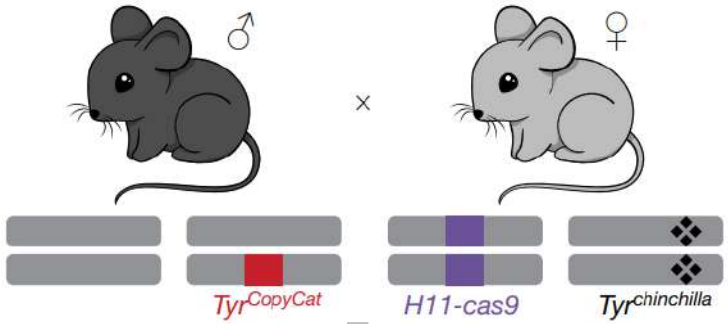
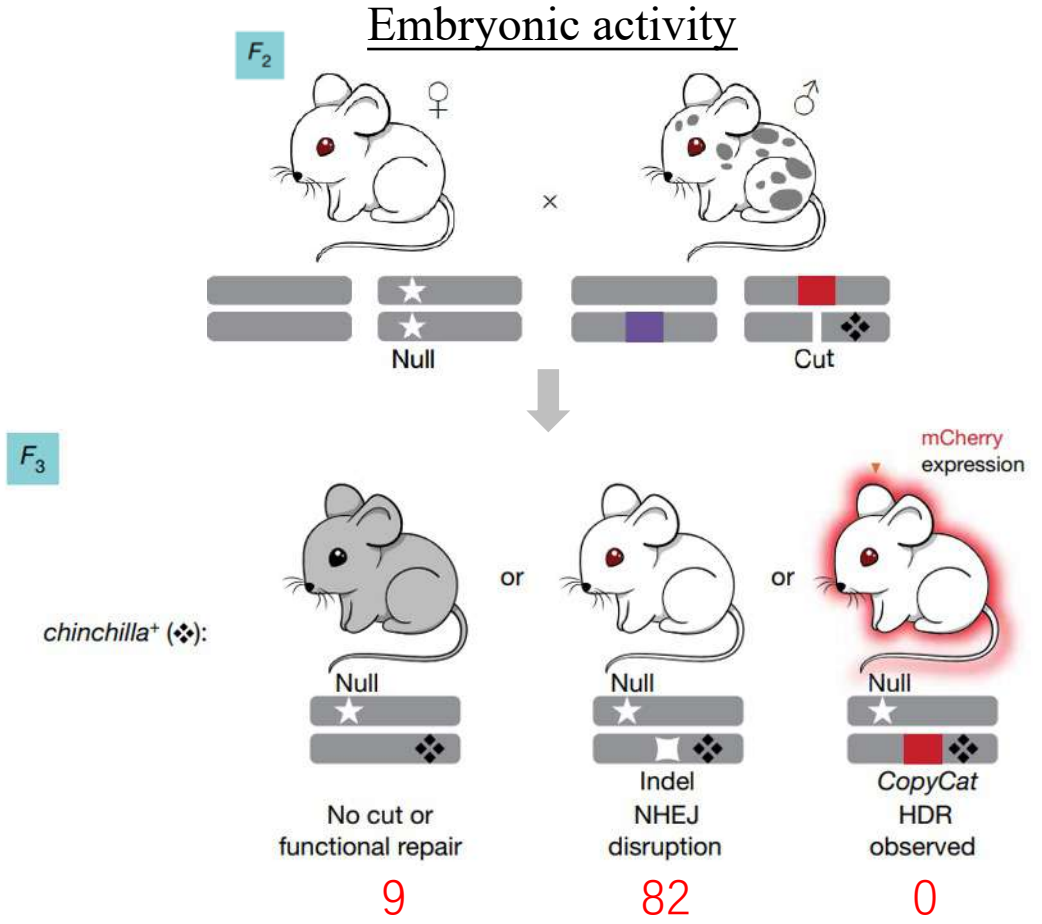
Grunwald, H. A., et al. (2019)

Embryonic Cas9 activity does not copy the *Tyr*^{CopyCat} allele from the donor to the receiver chromosome (*H11-cas9*)



(mosaic mice) *H11-cas9/+; Tyr*^{chinchilla}*/Tyr*^{CopyCat}



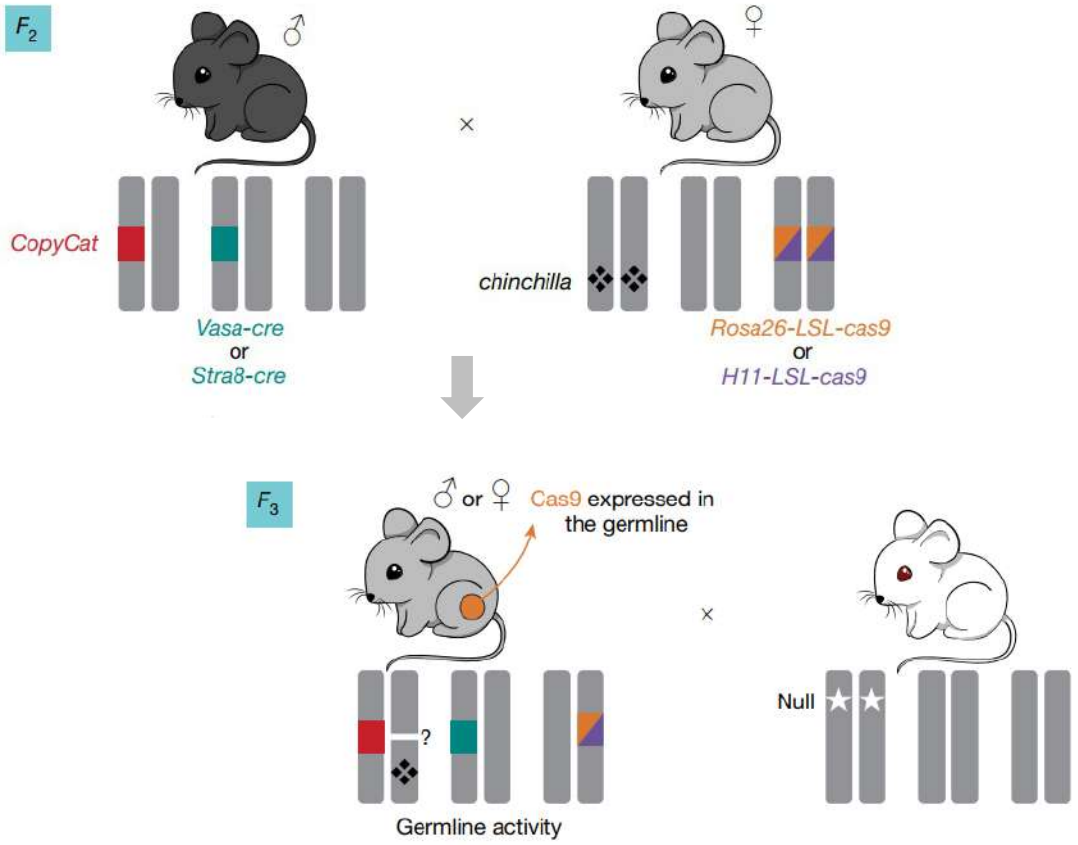
Grunwald, H. A., et al. (2019)

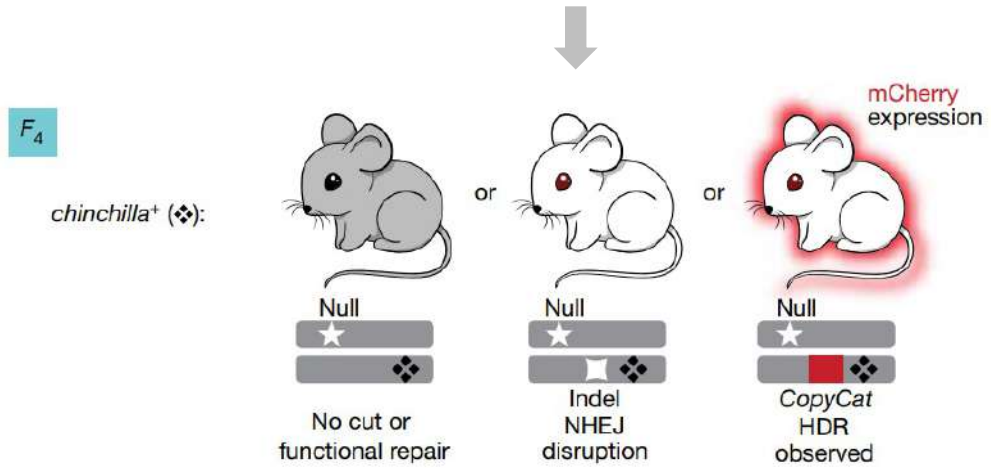
- The active genetic system is feasible in mice.

- Why *Tyr*^{CopyCat} was not copied to the receiver chromosome in the early embryo?

➢ Homologous chromosomes are not aligned for inter-homologue HDR to repair DSBs.
➢ The DNA repair machinery in somatic cells typically favours NHEJ over HDR.

Another crossing scheme to initiate Cas9 expression during germline development in *Tyr*$^{CopyCat/ch}$ mice





eg. *Tyr*^{CopyCat} */Tyr*^{ch}*; Vasa-Cre/+; Rosa26-LSL-cas9/+*

Grunwald, H. A., et al. (2019)

Gene conversion upon Cas9 expression in the female germline

*F*4 outcomes of germline Cas9 strategies

cre	*cas9*	F_3 parent		No cut or functional repair	NHEJ disruption	HDR conversion	Observed HDR conversion (%)
Vasa	*Rosa26*	F	1	–	15	–	–
			2	1	2	–	–
			3	–	3	1	25
			4	4	5	3	25
			5	6	6	1	8
Vasa	*Rosa26*	M	1	–	25	–	–
			2	–	17	–	–
Vasa	*H11*	F	1	4	1	13	72
			2	10	7	4	19
			3	4	–	5	56
			4	8	4	4	25
			5	8	3	10	48
Vasa	*H11*	M	1	–	15	–	–
			2	–	5	–	–
			3	–	2	–	–
			4	–	3	–	–
Stra8	*Rosa26*	M	1	–	19	–	–
			2	–	3	–	–
Stra8	*H11*	M	1	3	21	–	–

F, female. M, male.

F3: Female

F3: Female

Cas9 expression limited to the female germline induces DSB that are corrected by HDR.

Why in the female germline ?

Grunwald, H. A., et al. (2019)

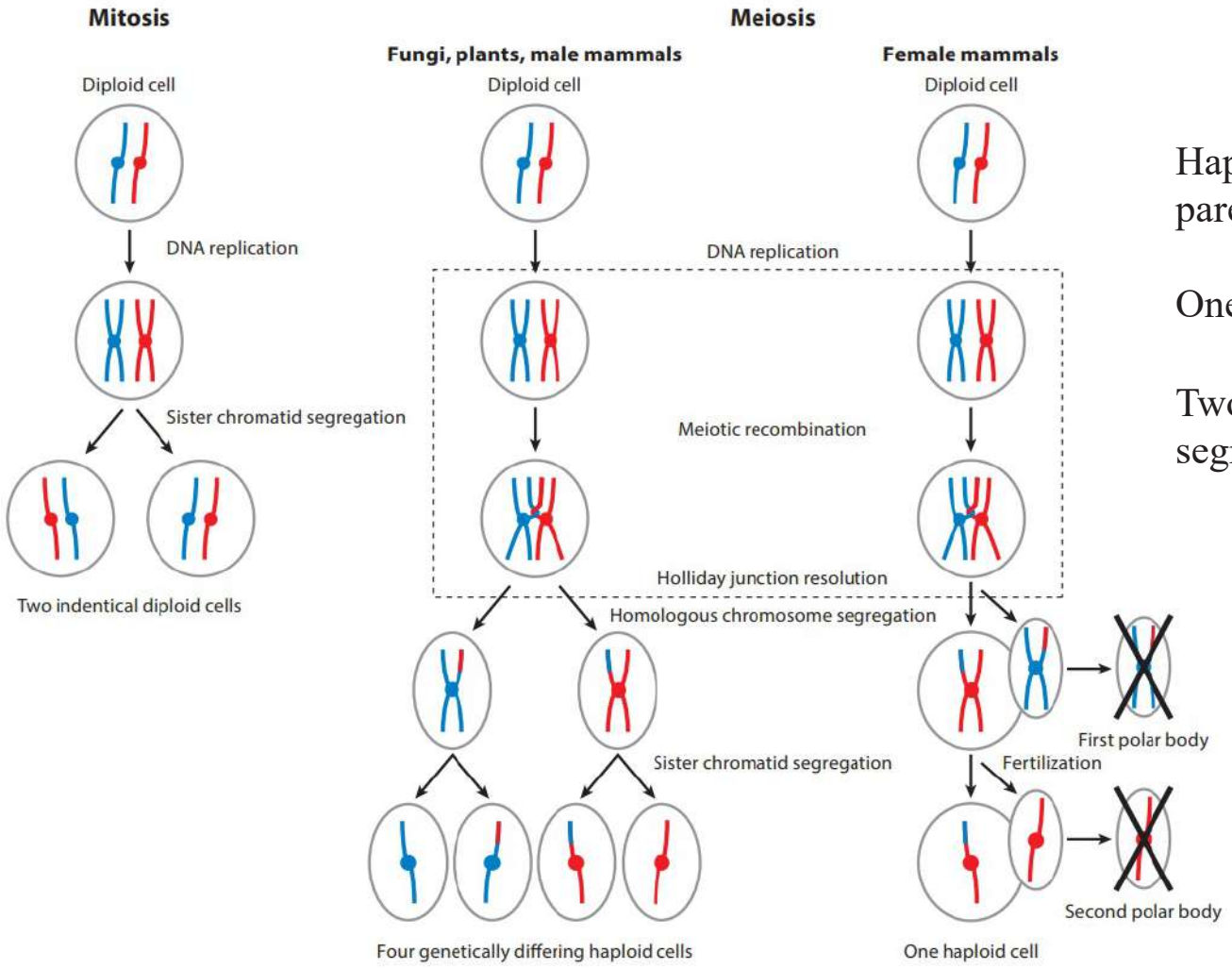
Mitosis vs. Meiosis

Mitosis

Genetically identical diploid daughter cells from a diploid progenitor cell

One round of DNA replication

One chromosome segregation



Meiosis

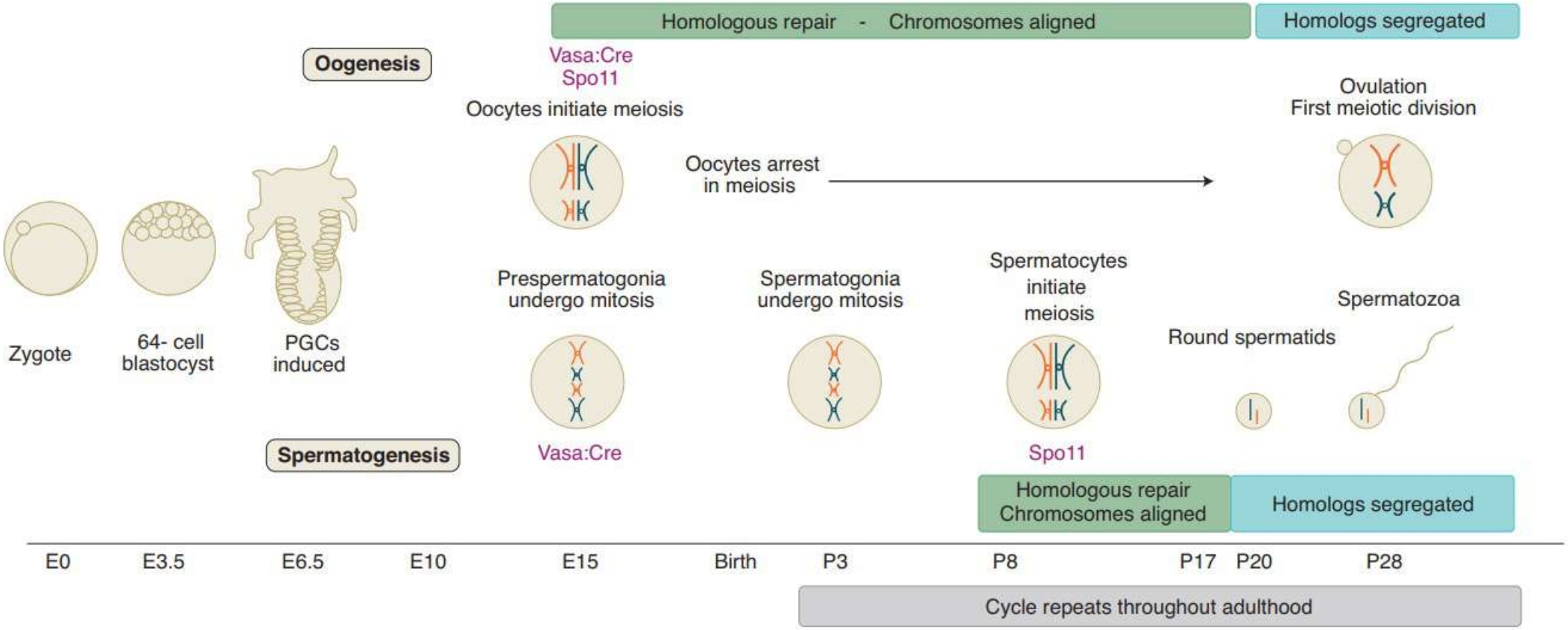
Haploid gametes from a diploid parental cell

One round of DNA replication

Two rounds of chromosome segregation

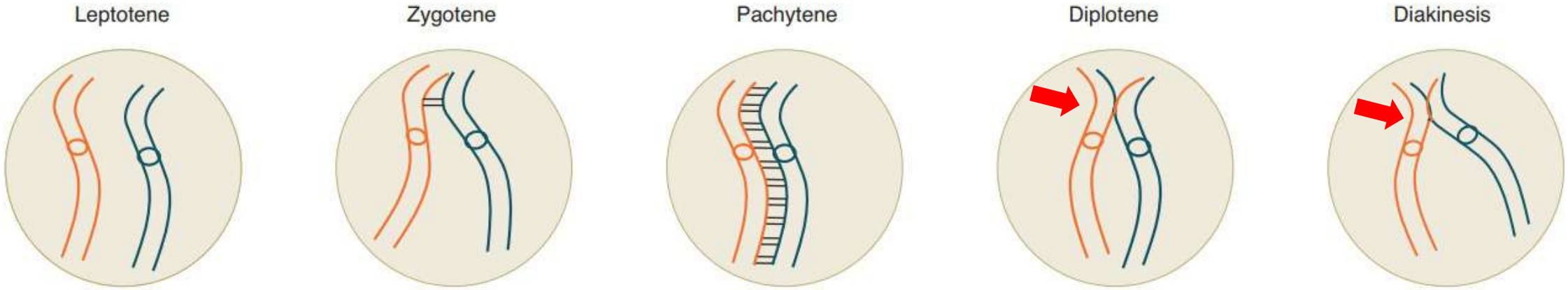
Gray, S. and P. E. Cohen (2016)

Meiotic timelines differ between male and female germlines



Grunwald, H. A., et al. (2022)

Differential homolog alignment during the five stages of prophase I



The crossovers maintain a strong connection between homologs.

Leptotene: replicated chromosomes condense.

Zygotene: synaptonemal complexes (black bars) form at distinct loci along the chromosome, joining homologs.

Pachytene: the synaptonemal complex zips homologous chromosomes together to form a bivalent chromosome.

Diplotene: the synaptonemal complex is degraded. Chromosomes are tied together by chiasmata at points of recombination.

Diakinesis: chromosomes are still joined by chiasmata but repel one another.

Grunwald, H. A., et al. (2022)

Summary

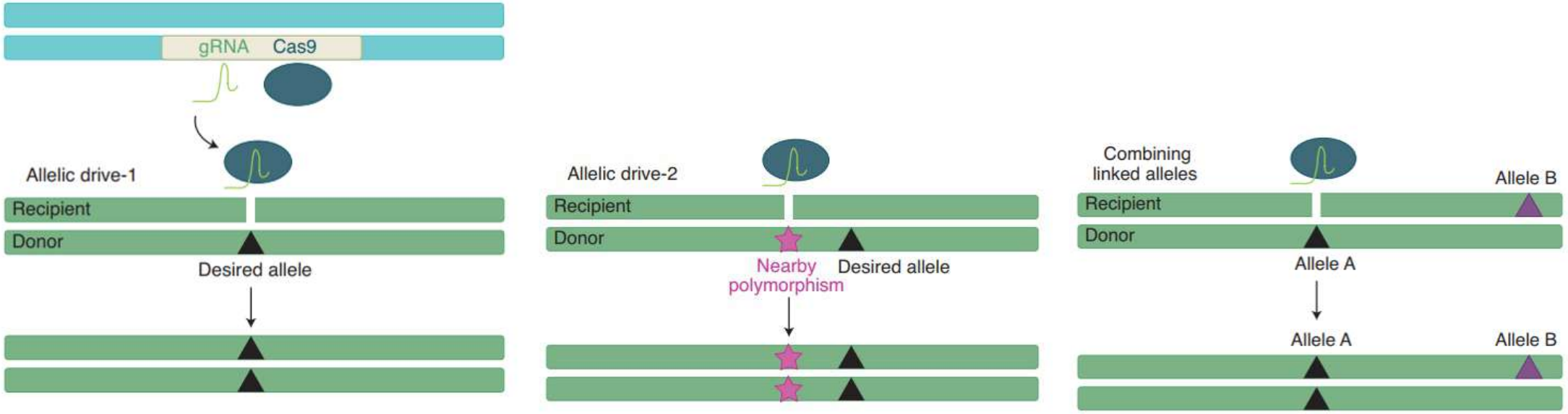
- The fundamental mechanism of a CRISPR–Cas9-mediated gene drive is feasible in mice.

- The precise timing of Cas9 expression present a greater challenge in rodents than in insects to restrict DSB formation to a window.

How to use gene drive for the production of a variety of mouse models in laboratories?

Active genetic strategies for the production of a variety of mouse models

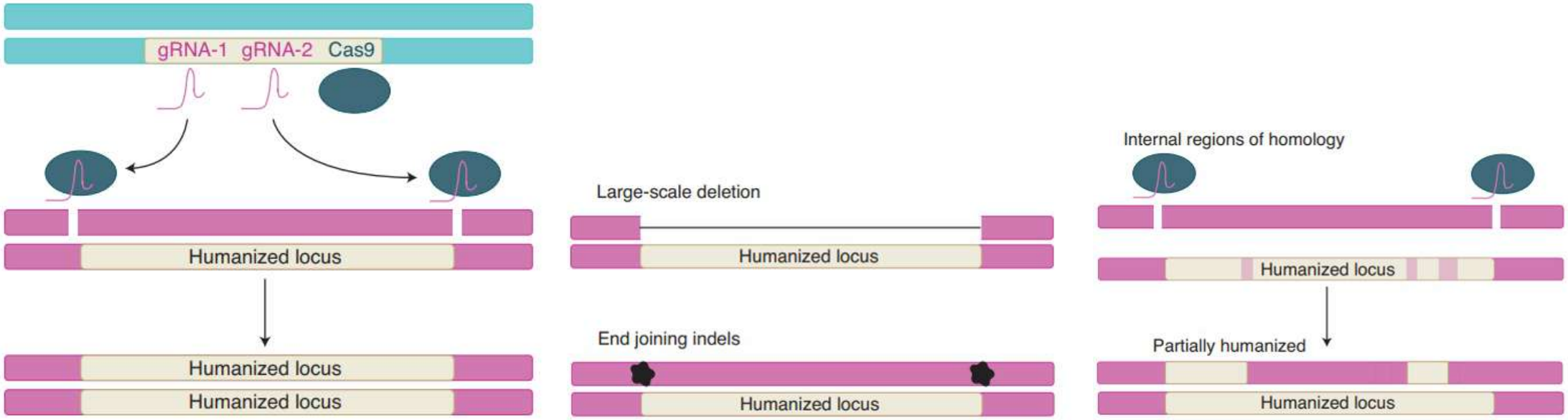
Strategies that make use of existing alleles:



Grunwald, H. A., et al. (2022)

Active genetic strategies for the production of a variety of mouse models

Humanization of a mouse locus:



Grunwald, H. A., et al. (2022)

Take-home message

- Gene drive is feasible in mice; however, the active genetic elements are inherited in the female germline.

- Complex genotypes could be produced by using CRISPR-Cas9-mediated gene conversion systems.

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