# CRISPR/Cas9-based Gene Drive to Suppress Agricultural Pests

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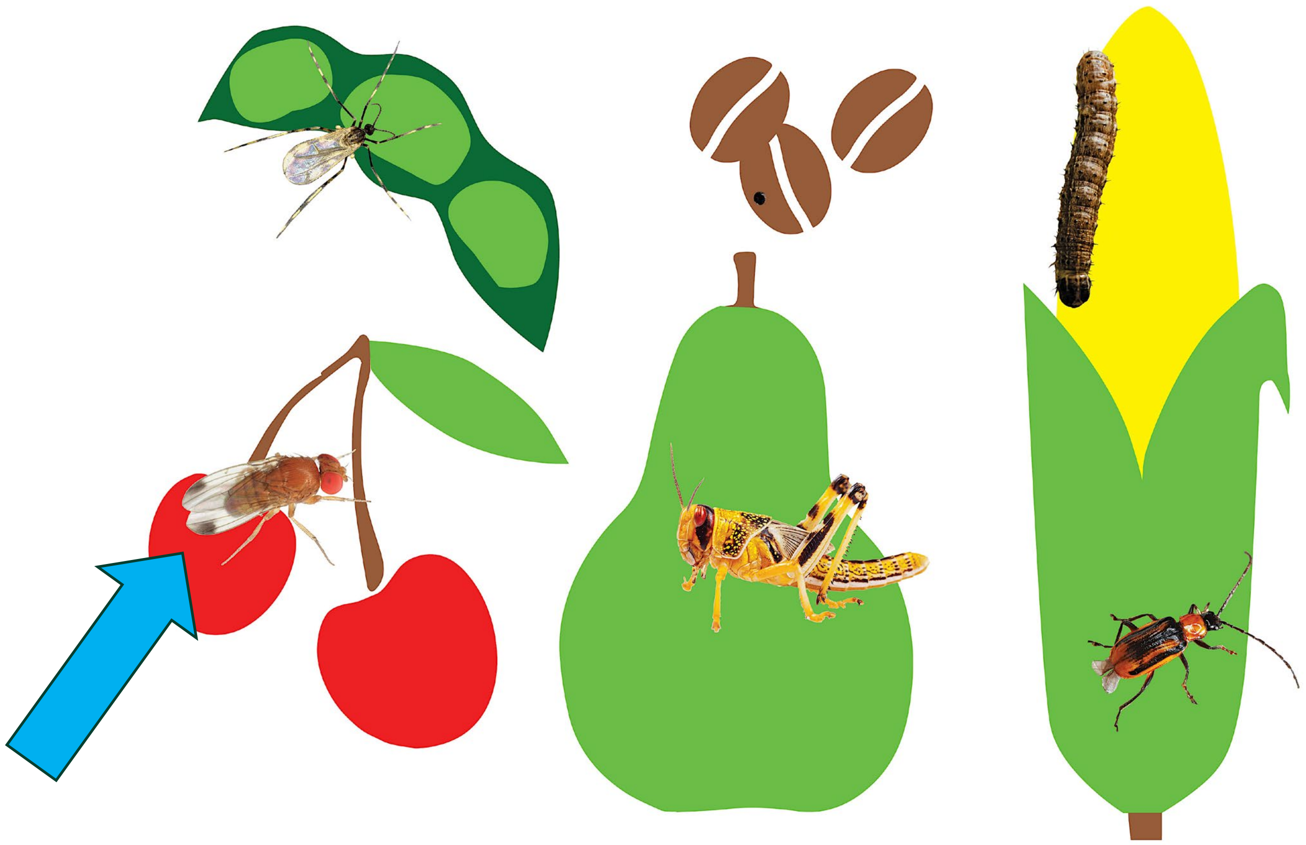
# Talk Overview

- Agricultural pests **genetic biocontrol**
- CRISPR/Cas9-based **gene drives development (in SWD)**
- **Challenges** in gene drives development and its application

# Agricultural Pests impact

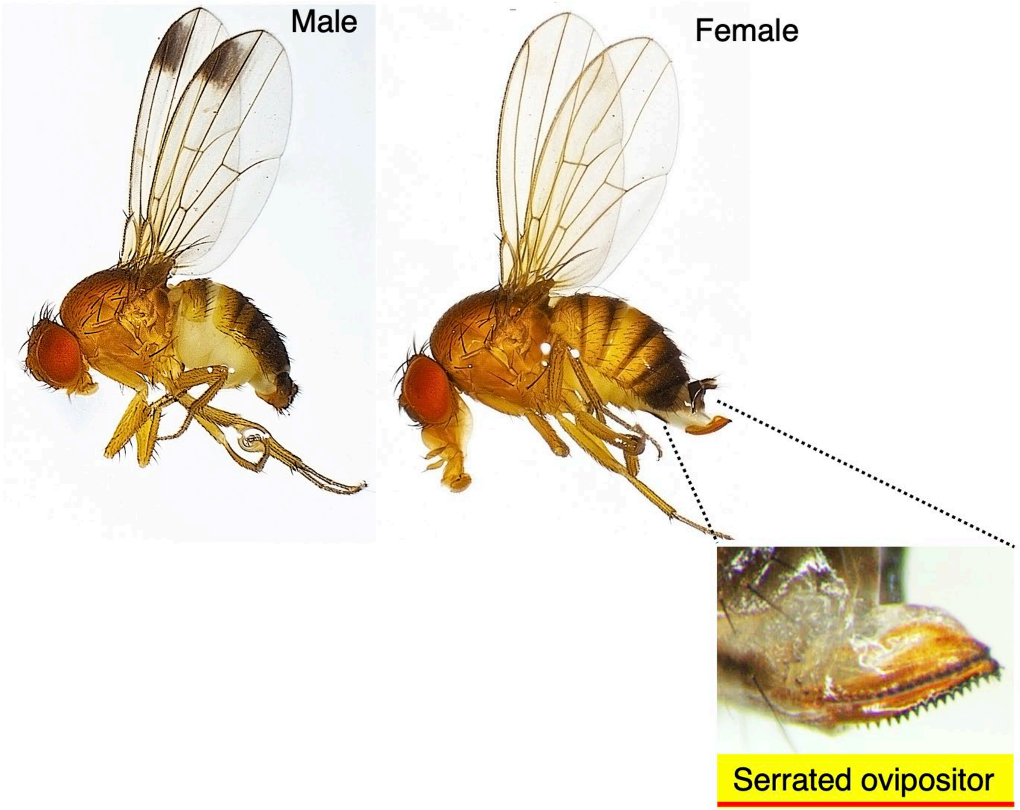
- Threat to agricultural productivity and food-security
- Each year, **20% to 40%** of the global crop production lost to pests
- Annual economic loss ∽$540 billion

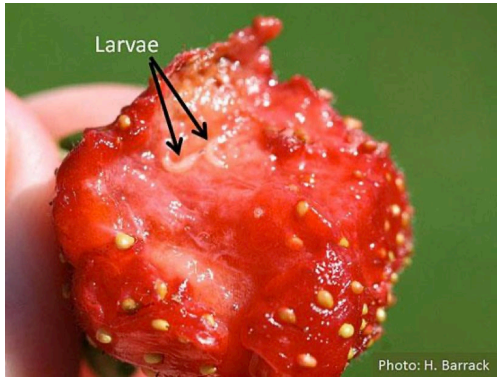


*Picture credit and source: Waldo Swiegers/Bloomberg. Nigel Cattlin/Alamy. Justin McMechan. Tomasz Klejdysz/Alamy. Getty Images. Blickwinkel/Alamy .* Bloomberg.com

# Spotted wing drosophila (SWD)

- Native to Southeast Asia
- Single Female can lay up to 300 eggs
- 3-16 overlapping generations in a year



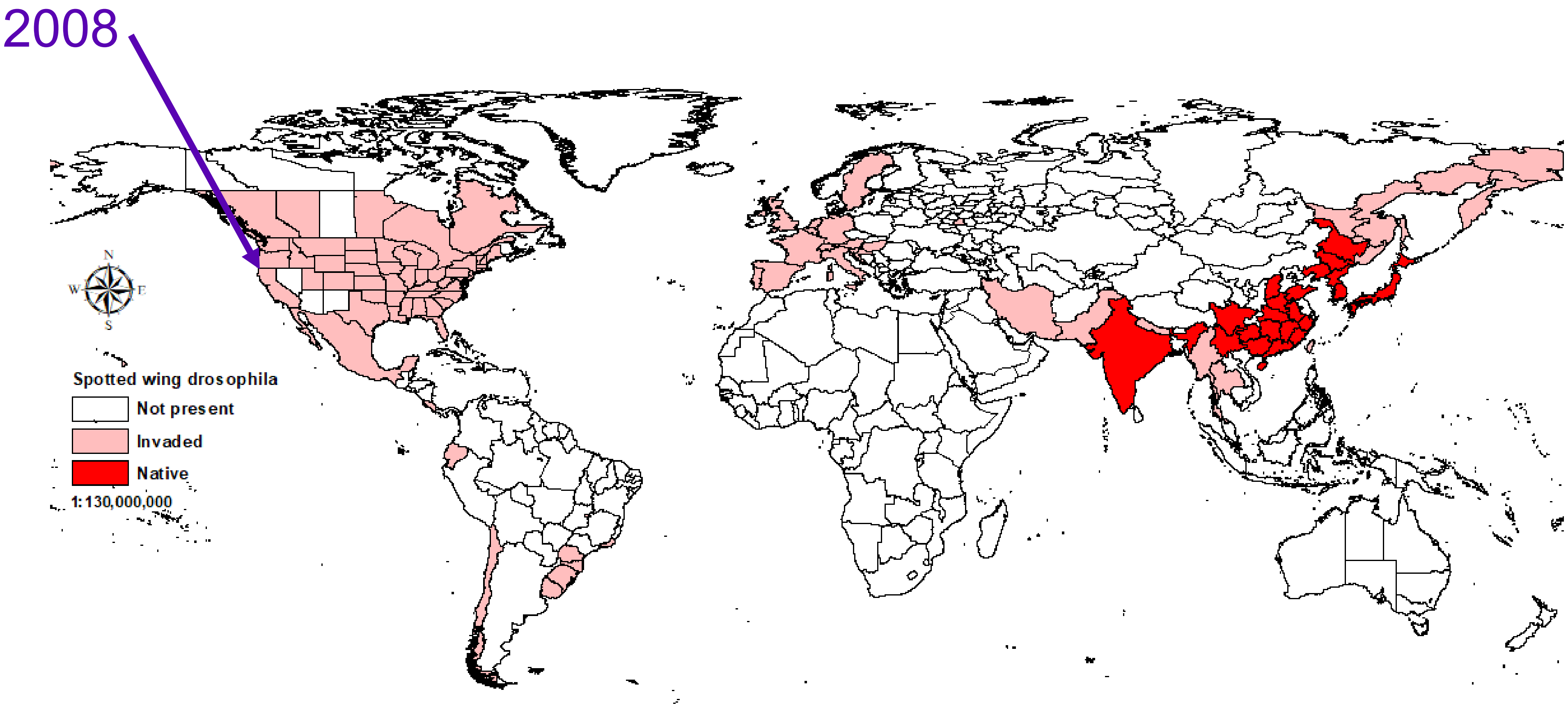




Damaged fruits, and growing larvae and Pupae inside fruits

Picture source: *SWD male and female flies, webpage CALS, Oregon State University*

# Spotted wing drosophila: **Global distribution**

- Becoming a major agricultural **invasive pest** species in the USA and Europe

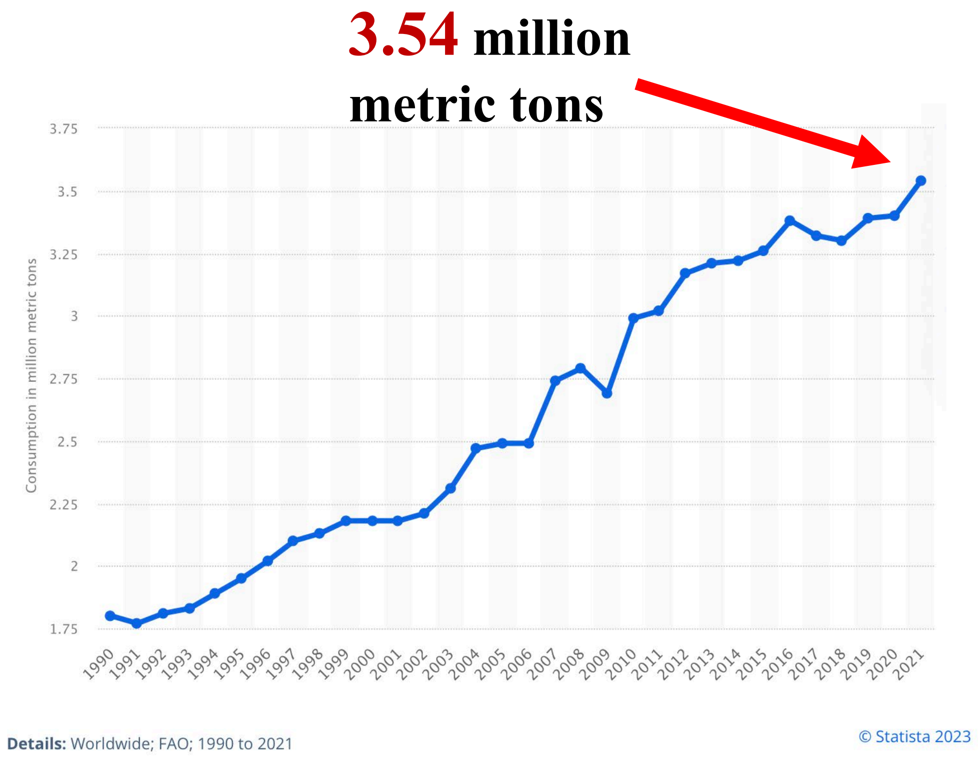


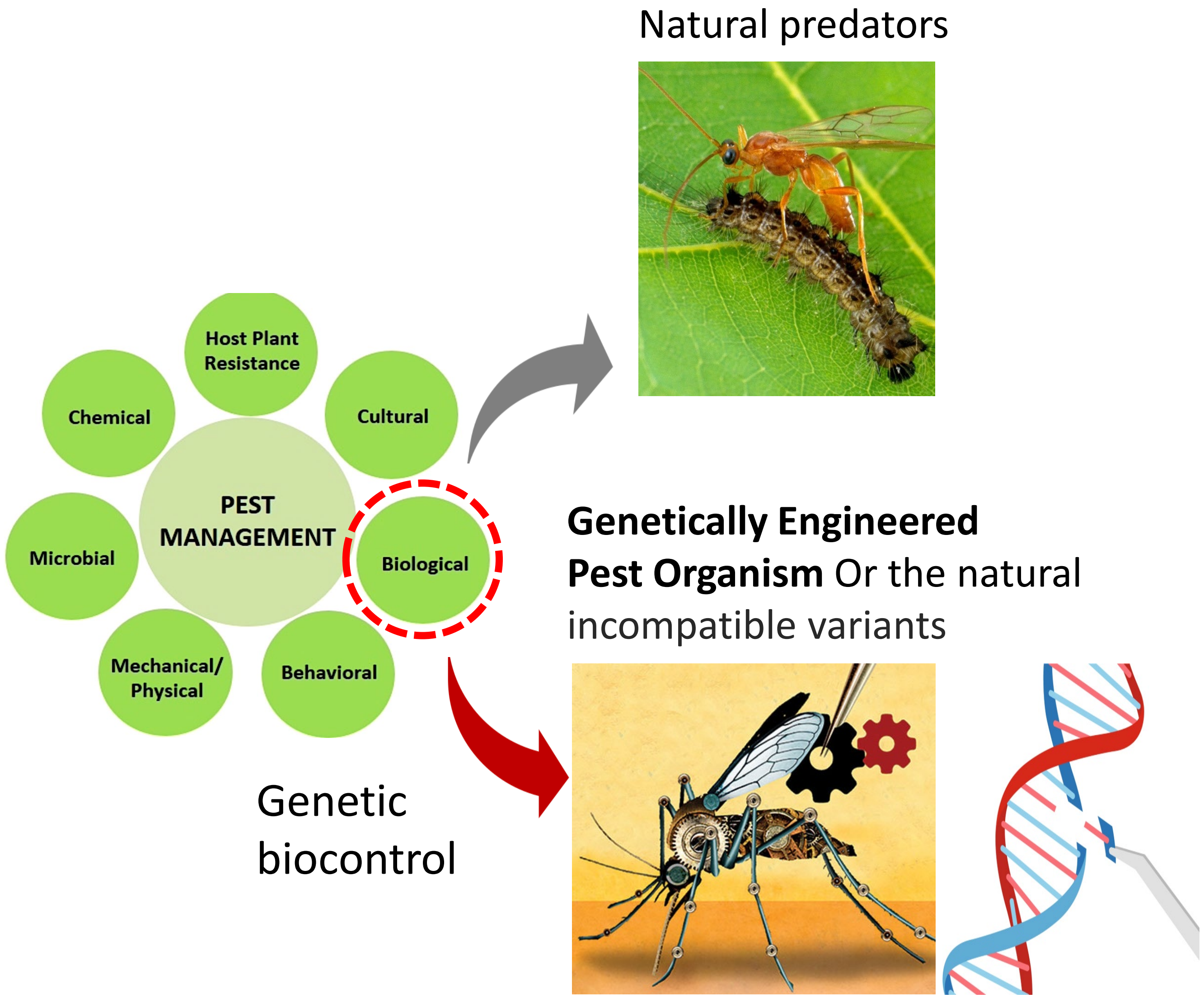

Biosecurity.co.nz

# Genetic Biocontrol an alternative to chemicals…

- Genetic control Reduces the reliance on pesticides

**Consumption of pesticides worldwide from 1990 to 2021**



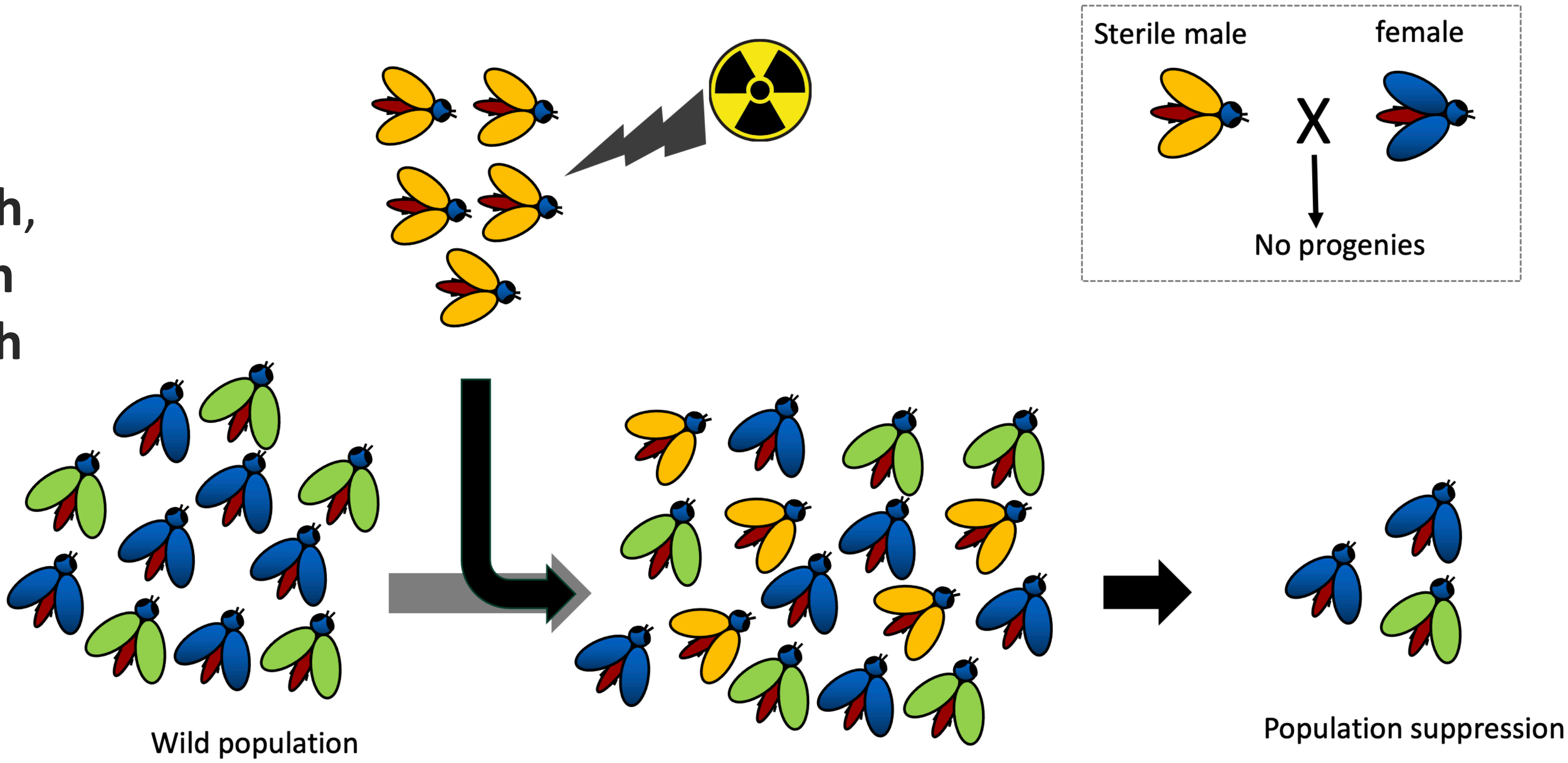



# The Sterile Insect Technique (SIT)

- Environmentally-friendly

- Successful eradication of **screw worms** from the southeastern United States
- Control of **codling moth**, **med fly, pink bollworm** and **painted apple moth** etc..

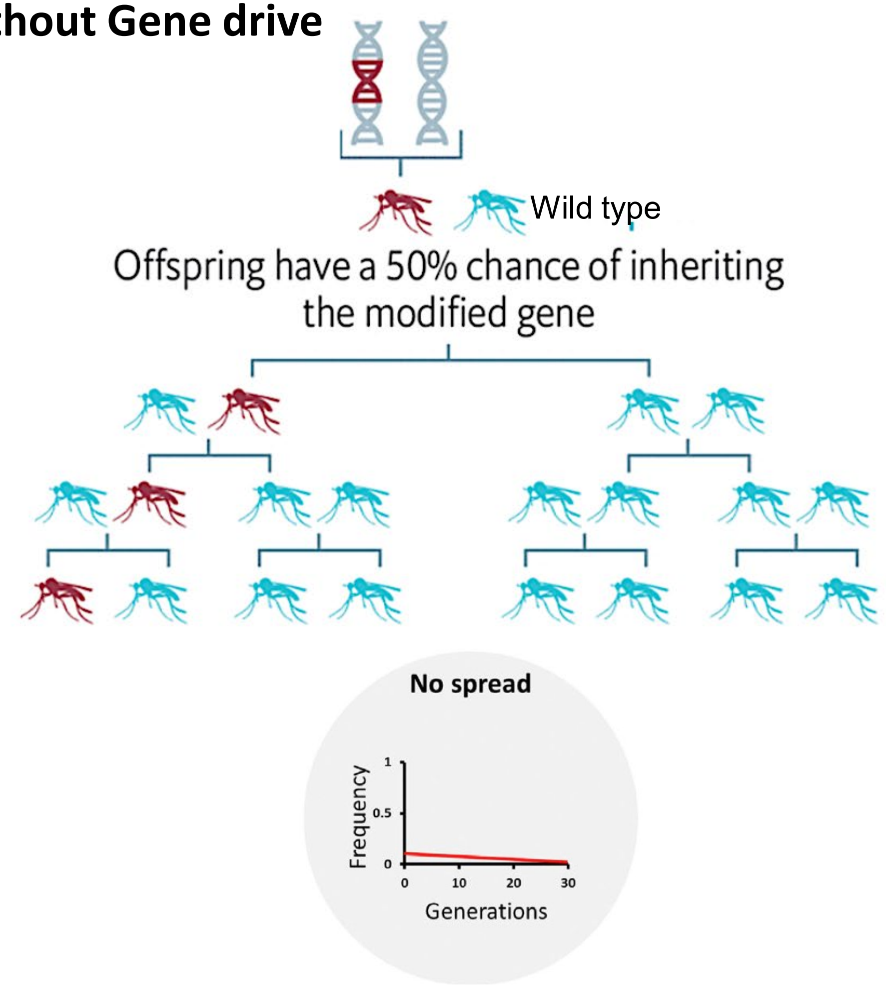
- Other methods: pgSIT, fsRIDL



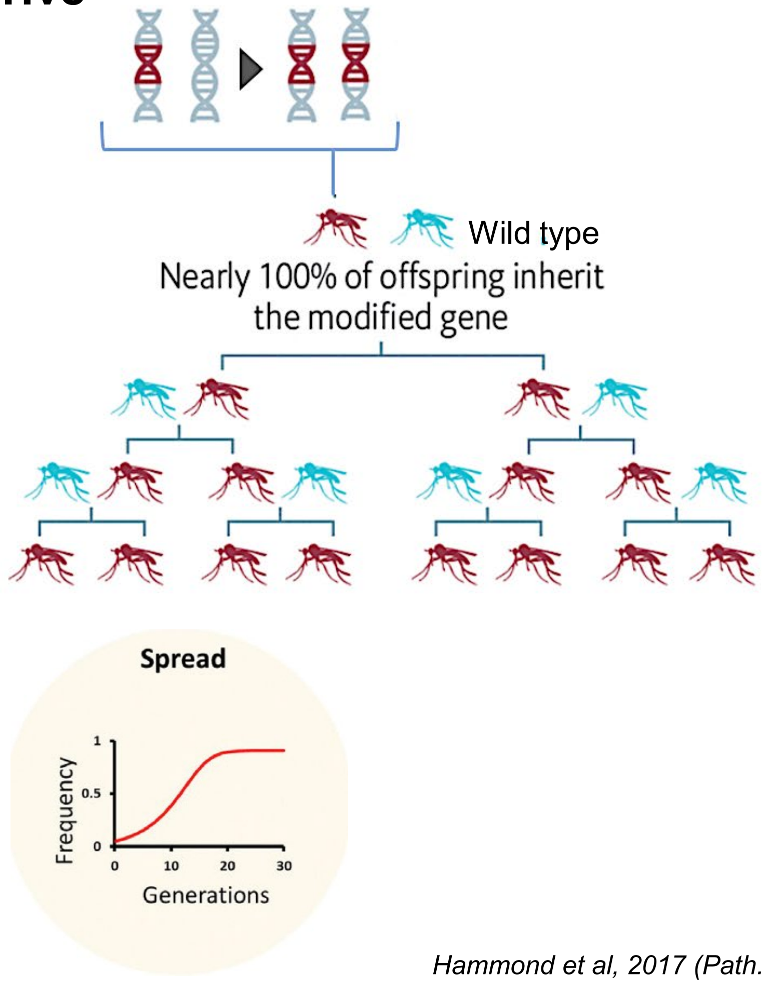
# Gene drive

- A gene drive is a **phenomenon of biased inheritance** to introduce a genetic change into a population **at a rate higher** than normal inheritance.

**Without Gene drive**



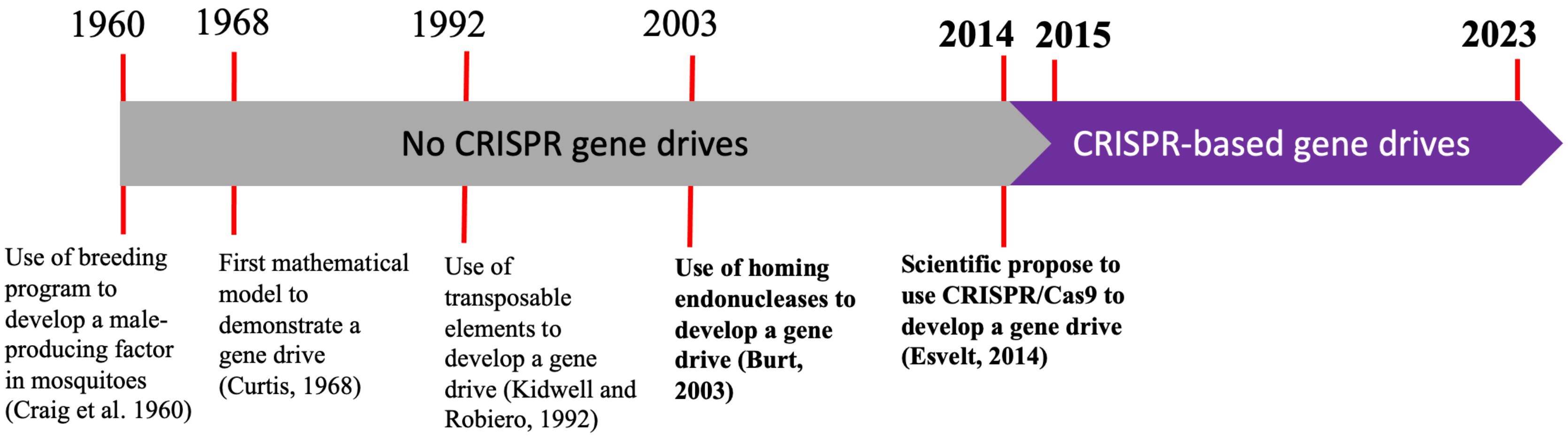

**Gene Drive**



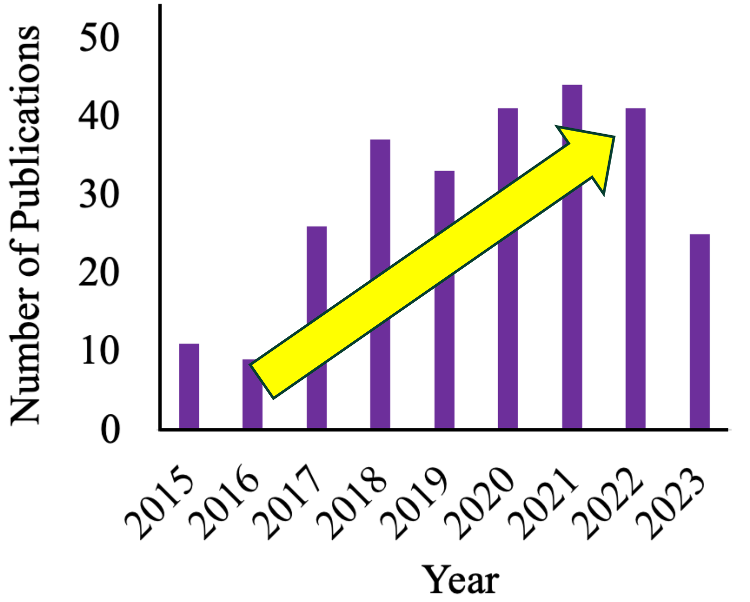

*Hammond et al, 2017 (Path. Glob. Health)*

# The timeline of Gene-drives

- Natural gene drives
- Synthetic gene-drives



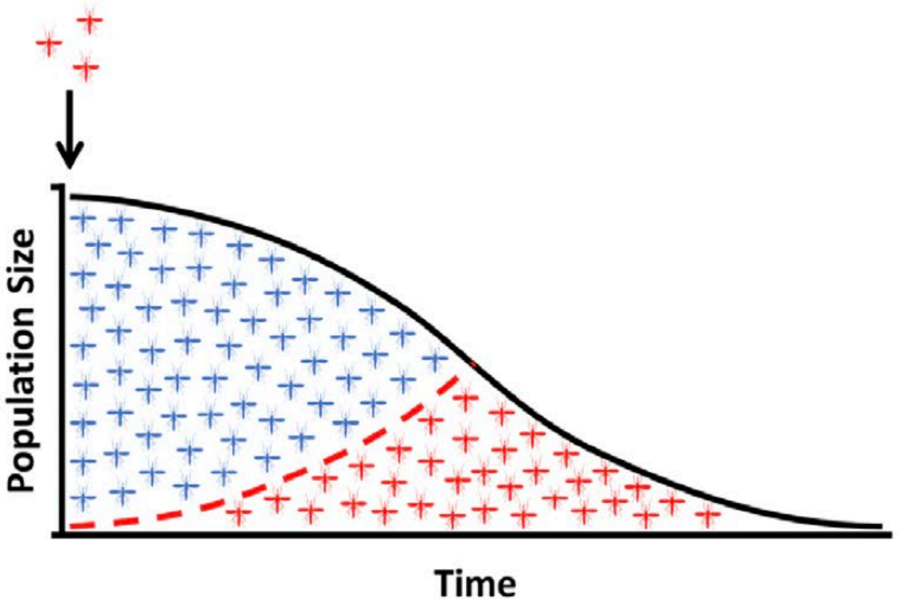

Research/Review articles on CRISPR-based gene drives <u>since 2015</u>




*Source: Modified from Gene Drives on the Horizon ..(doi: 10.17226/23405)*

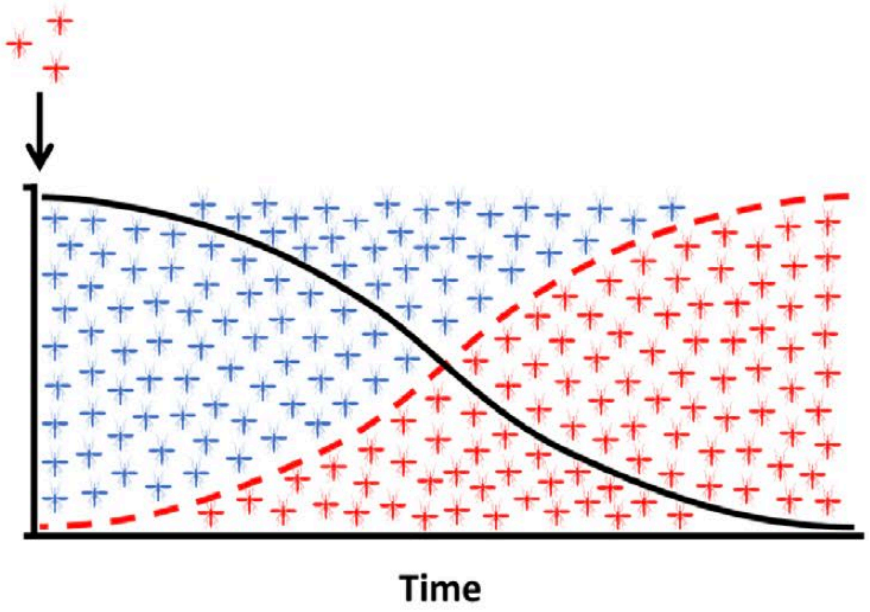
# Gene drives potential Applications

Population Suppression




- Targets gene essential for development/female fertility
- Bias sex ratio

Population Replacement

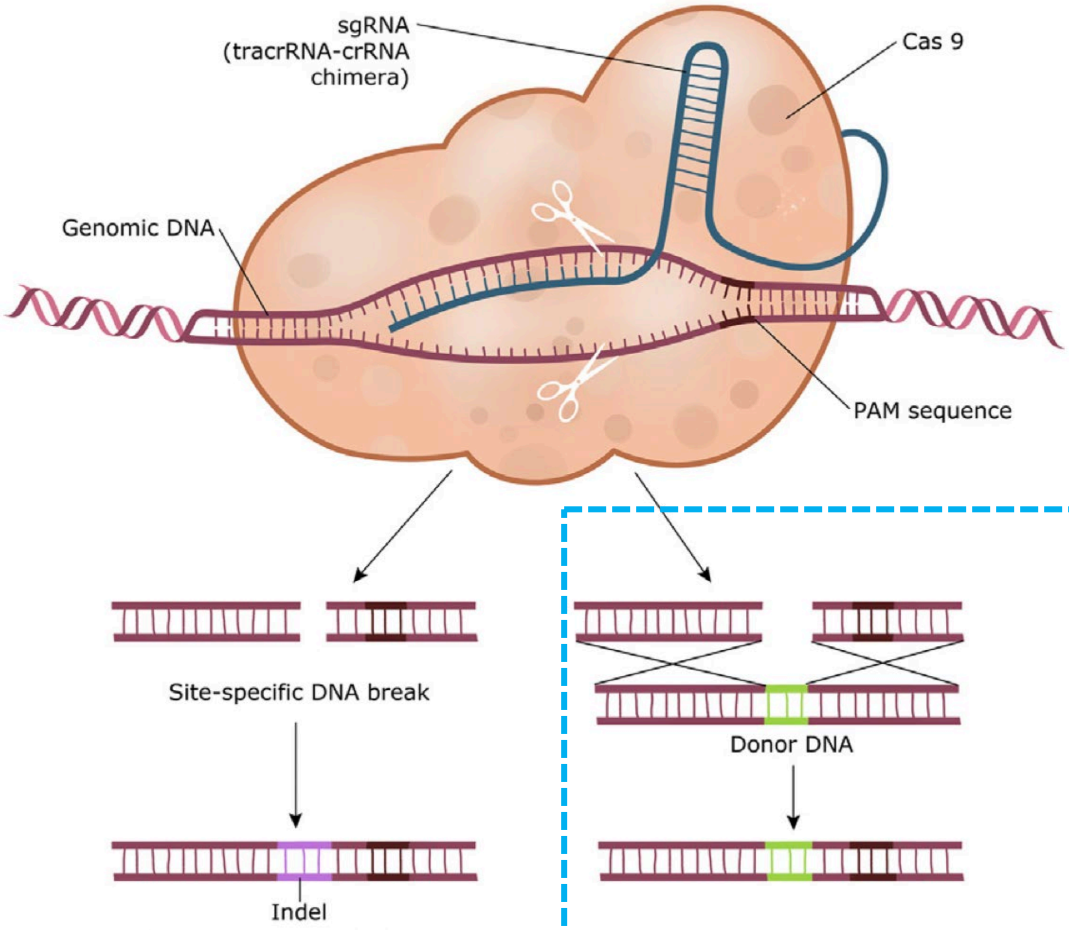



- Alter genes or add new gene to stop the pathogen transmission in a disease vector

*Hammond et al, 2017*
*(Pathogens and Global Health)*

# ‘CRISPR/Cas9’ system

- Derived from the immune system of  bacteria and archaea
- Uses RNA guided DNA endonuclease

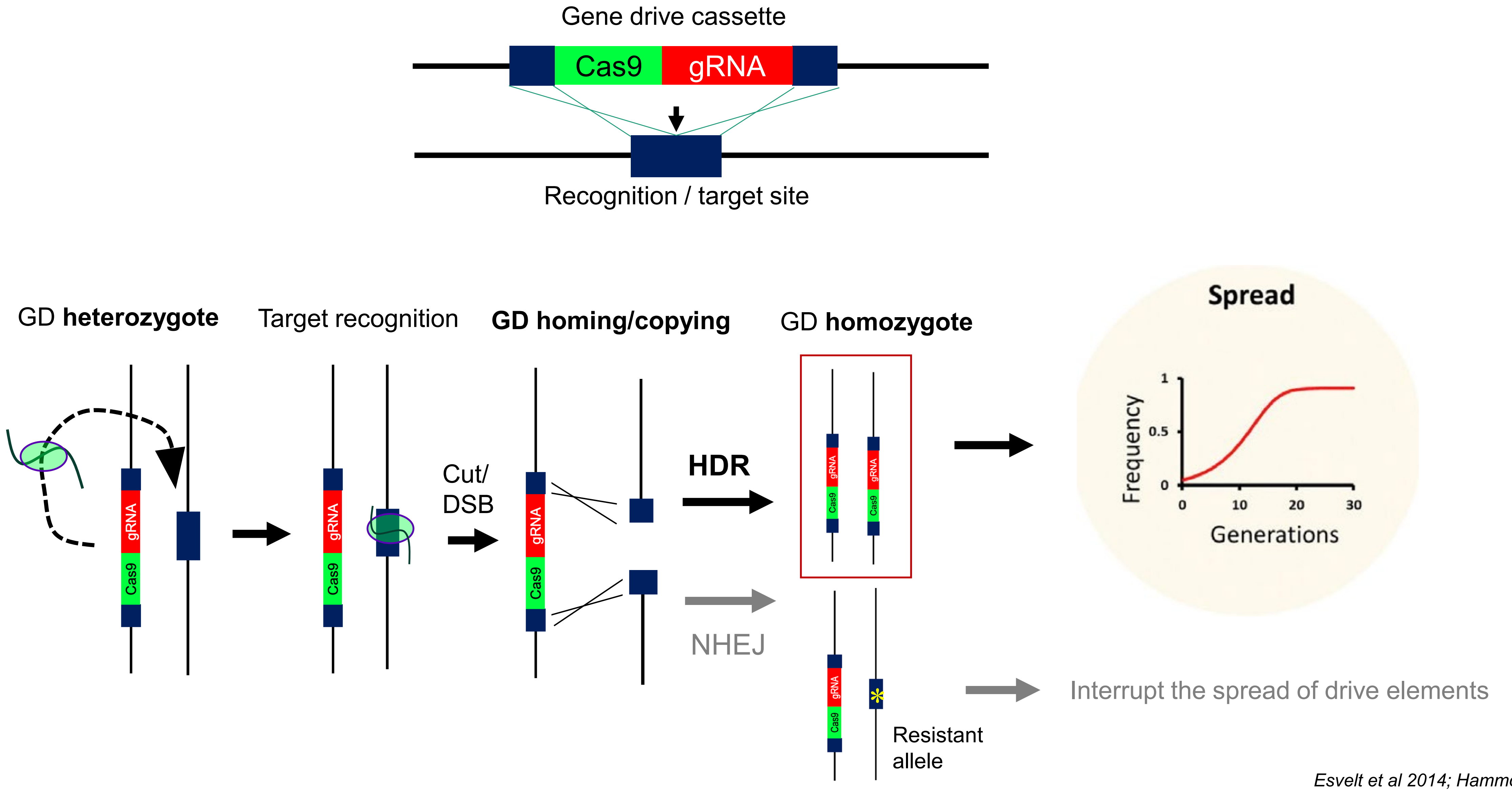



Non-homologous end joining (NHEJ)

Homology directed Repair (HDR)

# CRISPR/Cas9-based Gene drives design

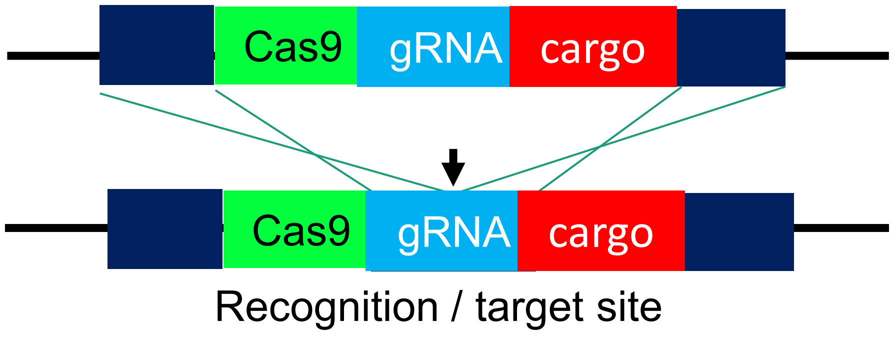
- CRISPR/Cas9-based gene drive relies on **Homology Directed Repair** in Germ cells

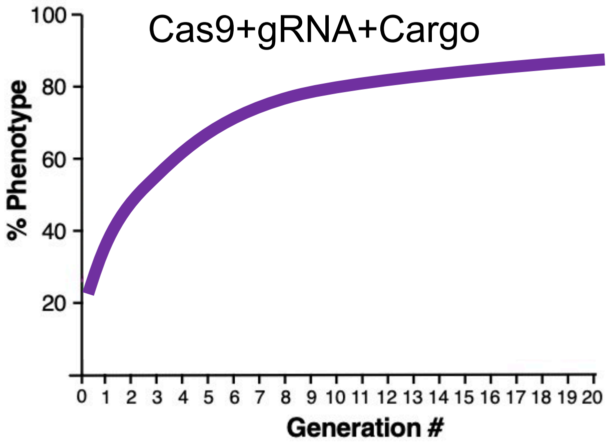



*Esvelt et al 2014; Hammond et al 2017*

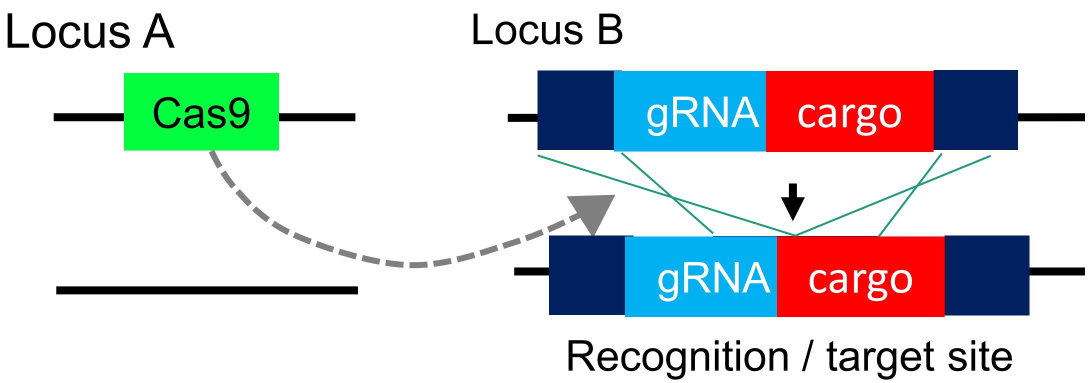
# homing Gene drive: types

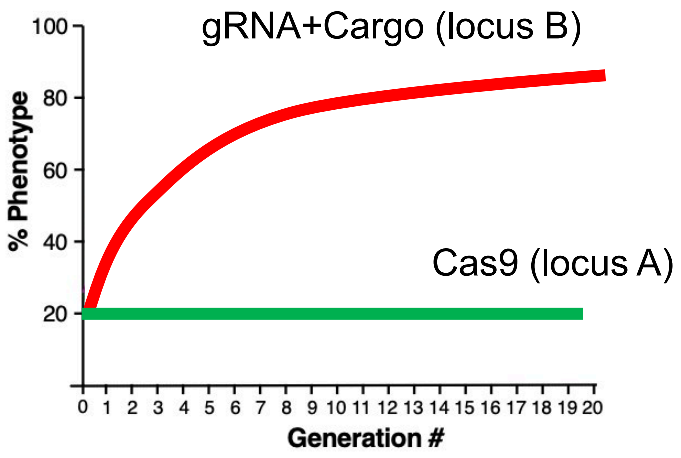
## **A.** Gene drive (All in one)



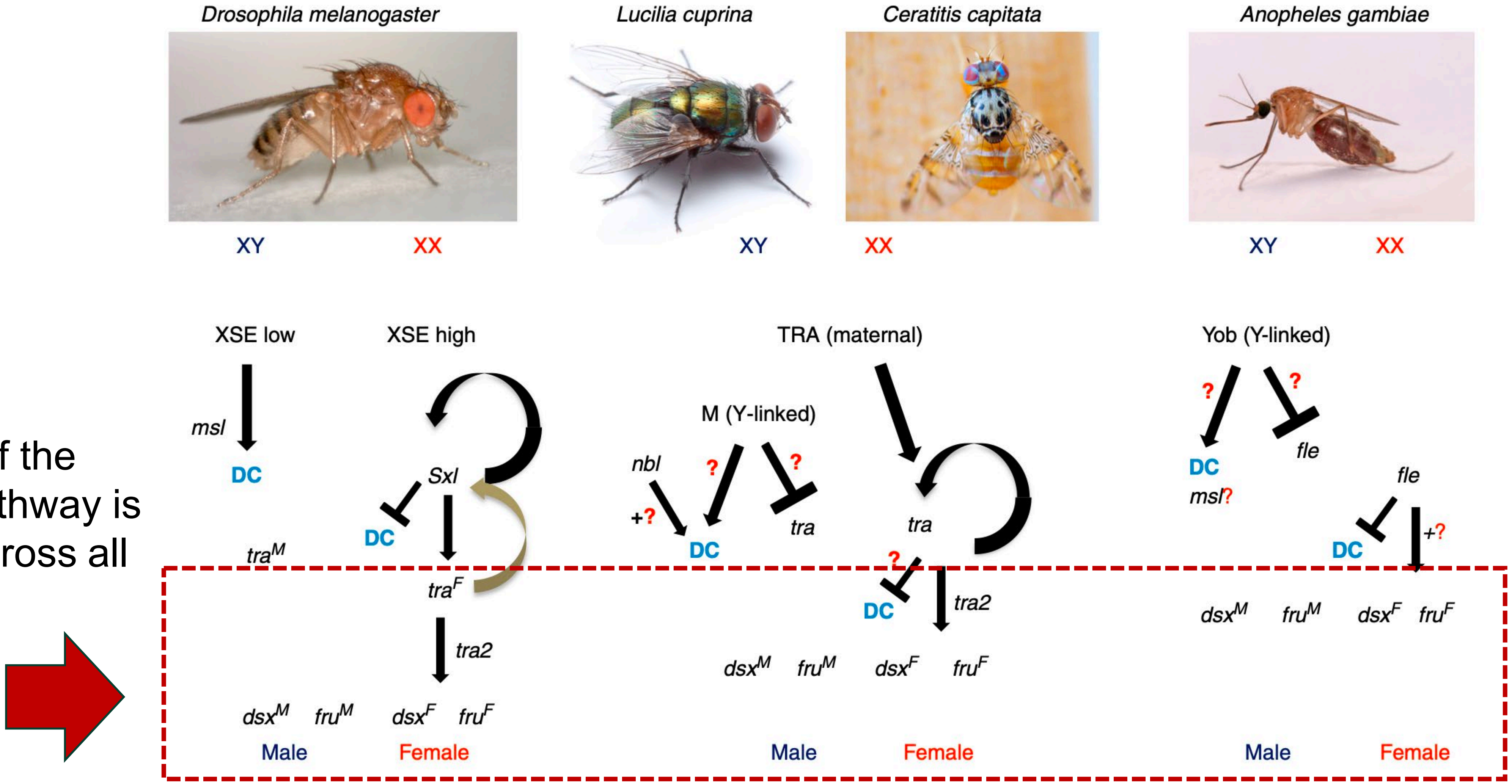

## **B.** Gene drive **(Split drive)**




*Modified from Terradas et al, 2021*

# Sex determination genes as target for the suppression drives




The bottom of the regulatory pathway is conserved across all species.

Scott M, 2021 (Current Biology)

# Homing Gene drives demonstrated

### *D. melanogaster*

| Target | Drive |
|---|---|
| ***yellow*** | *97%* |
| ***yellow-g*** | 86.4♀-90.4%♂ |
| ***RpL35A*** | 91% |
| ***rab5*** | 76.1♂-97.1%♀ |
| ***prosalpha2*** | 99.3♀-99.9%♂ |
| ***tra*** | 78% |
| ***dsx*** | 70%♂-85%♂ |

….many more!

### Diamondback Moth

| Target | Drive |
|---|---|
| ***yellow*** | *~50%* |
| ***kmo*** | ~50% |

### Medfly

| Target | Drive |
|---|---|
| ***white*** | **85.6%* |
| ***tra*** | *83.1%* |

### Spotted wing drosophila

| Target | Drive |
|---|---|
| ***dsx*** | *95-99%* |

### *Anopheles gambiae*

| Target | Drive |
|---|---|
| ***dsx*** | *95.9-99.4%* |

### *Anopheles stephensi*

| Target | Drive |
|---|---|
| ***kh*** | *99.7%* |

### *Culex quinquefasciatus*

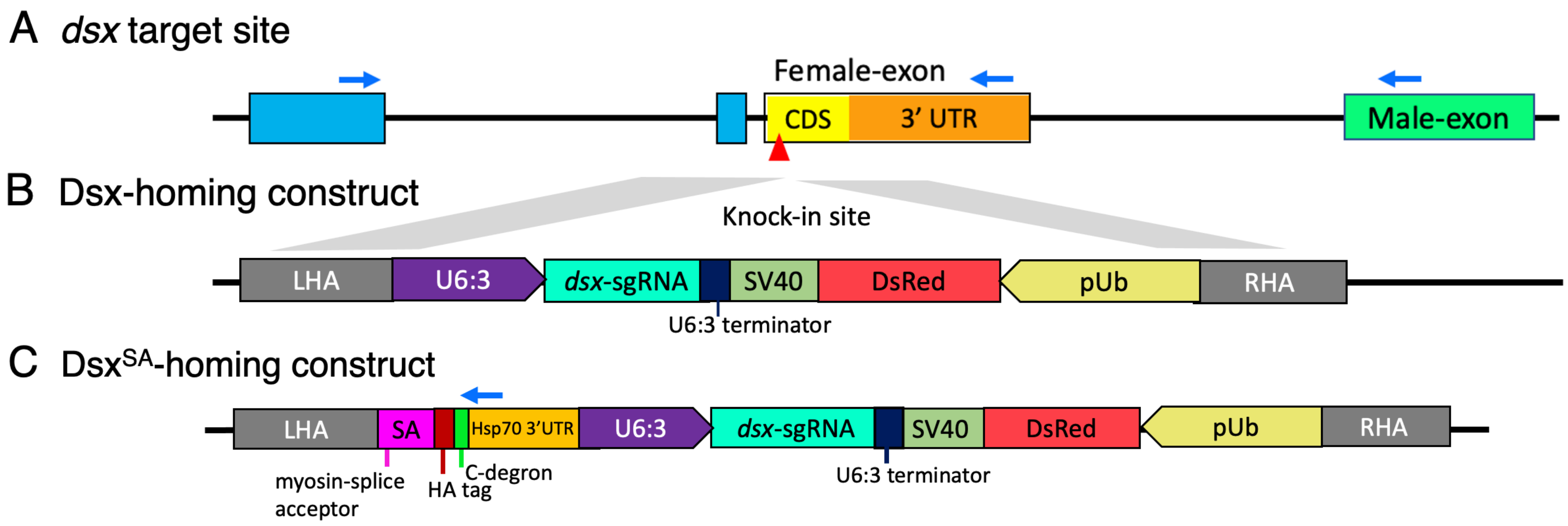
| Target | Drive |
|---|---|
| ***white*** | *56%* |
| ***kmo*** | *57.5%* |

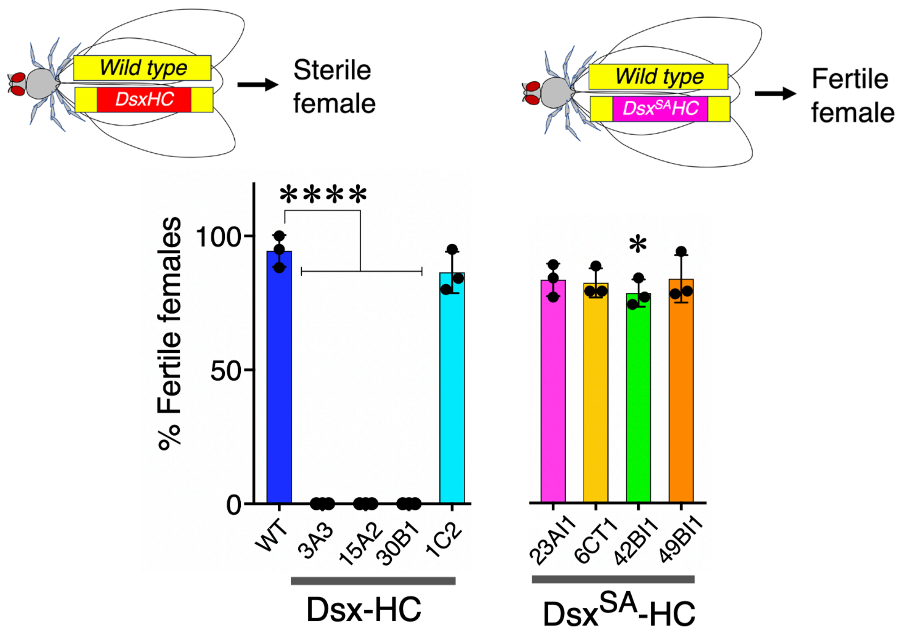
### *Aedes aegypti*

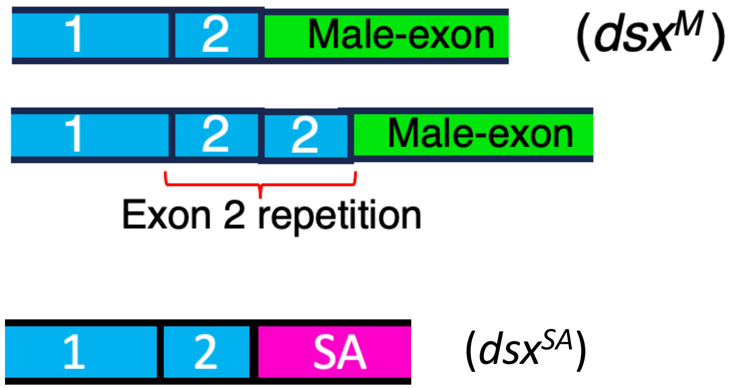
| Target | Drive |
|---|---|
| ***white*** | **94%* |

Gantz VM et al, 2015 (Science); Kyrou K, 2018 (Nat. Biotech); Champer J et al, 2020 (PNAS); Yang E et al, 2022 (G3); Kandul NP 2020 (G3); Terradas G et al; 2021 (Nat. Comm.); *Meccariello A, 2023 (BioRxiv); Xu X, 2022 (CRISPR J),* Carrami EM et al, 2018 (PNAS); Li M et al, 2020 (eLife); Adolfi A et al, 2020 (Nat. Comm); Yadav et al 2023 (PNAS); *Harvey-Samuel T et al, 2023 (BioRxiv);* Chen W, 2023 (BioRxiv)

# SWD homing strains **development**

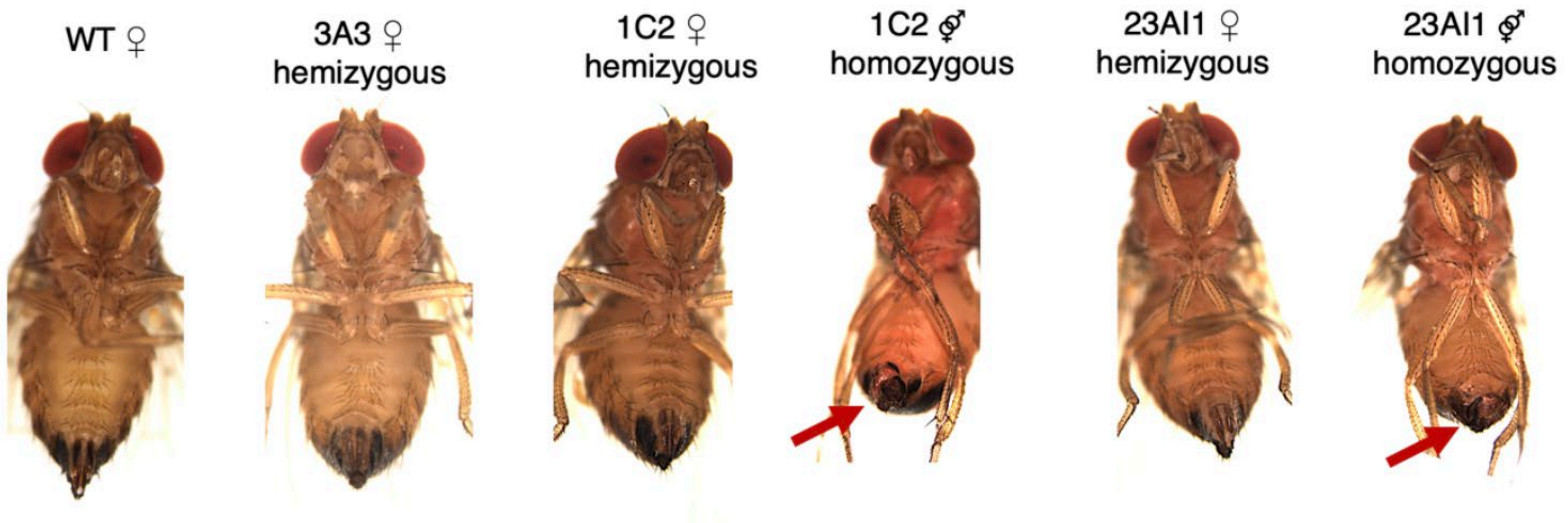


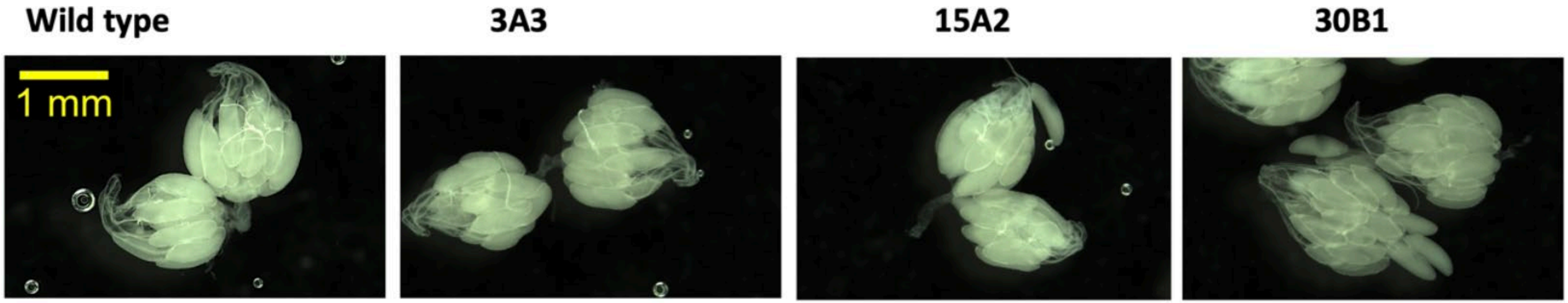




*Yadav et al, 2023; PNAS*

# SWD Homing strains morphology




Ovaries

Dominant sterile hemizygous females

# SWD Cas9 strains established

*Dmnos-Cas9-NLS*

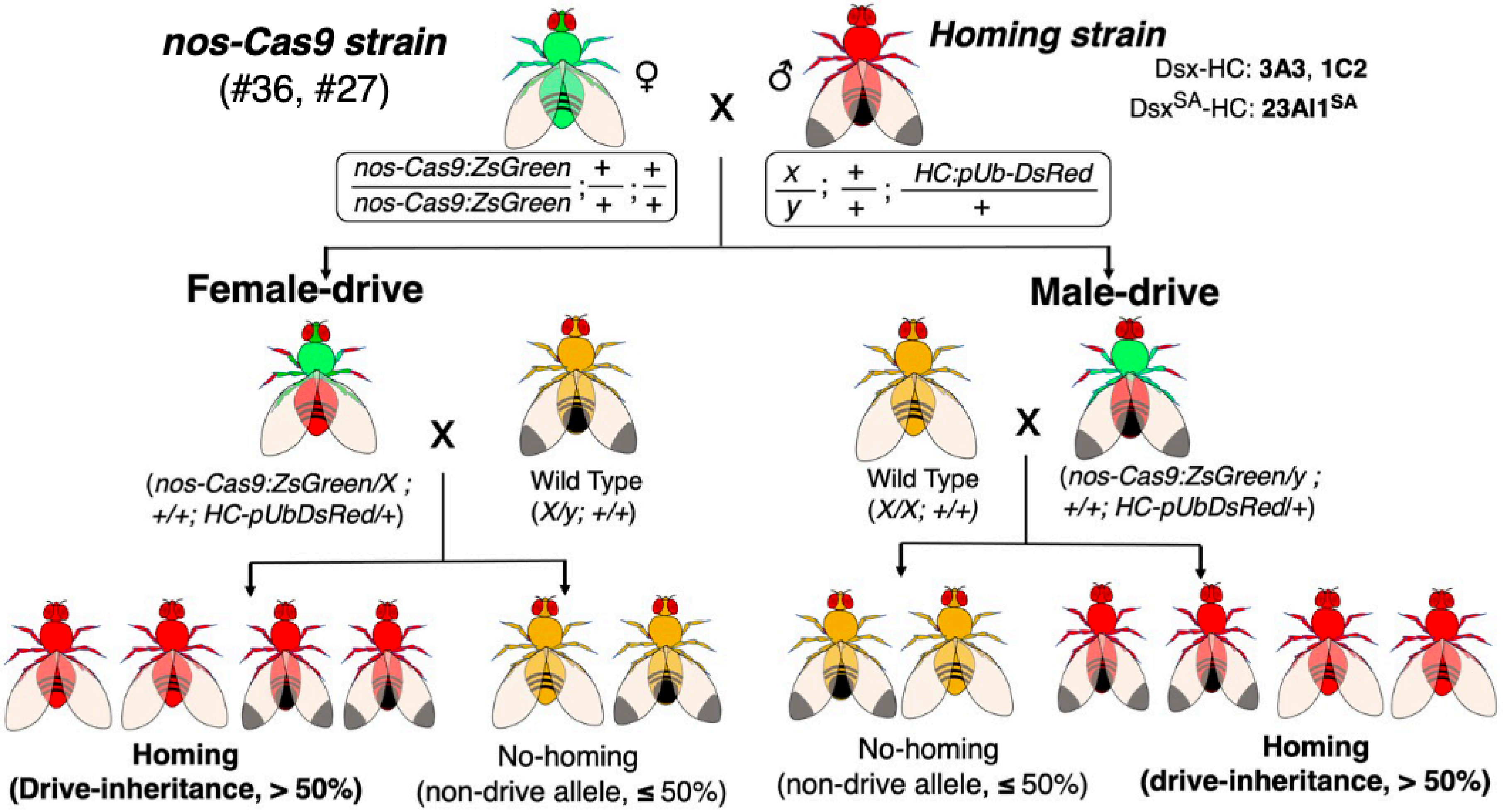

- X chromosome linked
- Cas9 expression under D. melanogaster *nanos* promoter and 3'UTR
- Cas9 has single NLS
- Low editing efficiency relatively

*Dsnos-NLS-Cas9-NLS*


- X chromosome linked
- Cas9 expression under D. suzukii *nanos* promoter and 3' UTR
- Cas9 has two NLS
- High editing efficiency relatively

*Yadav et al, 2023; PNAS*

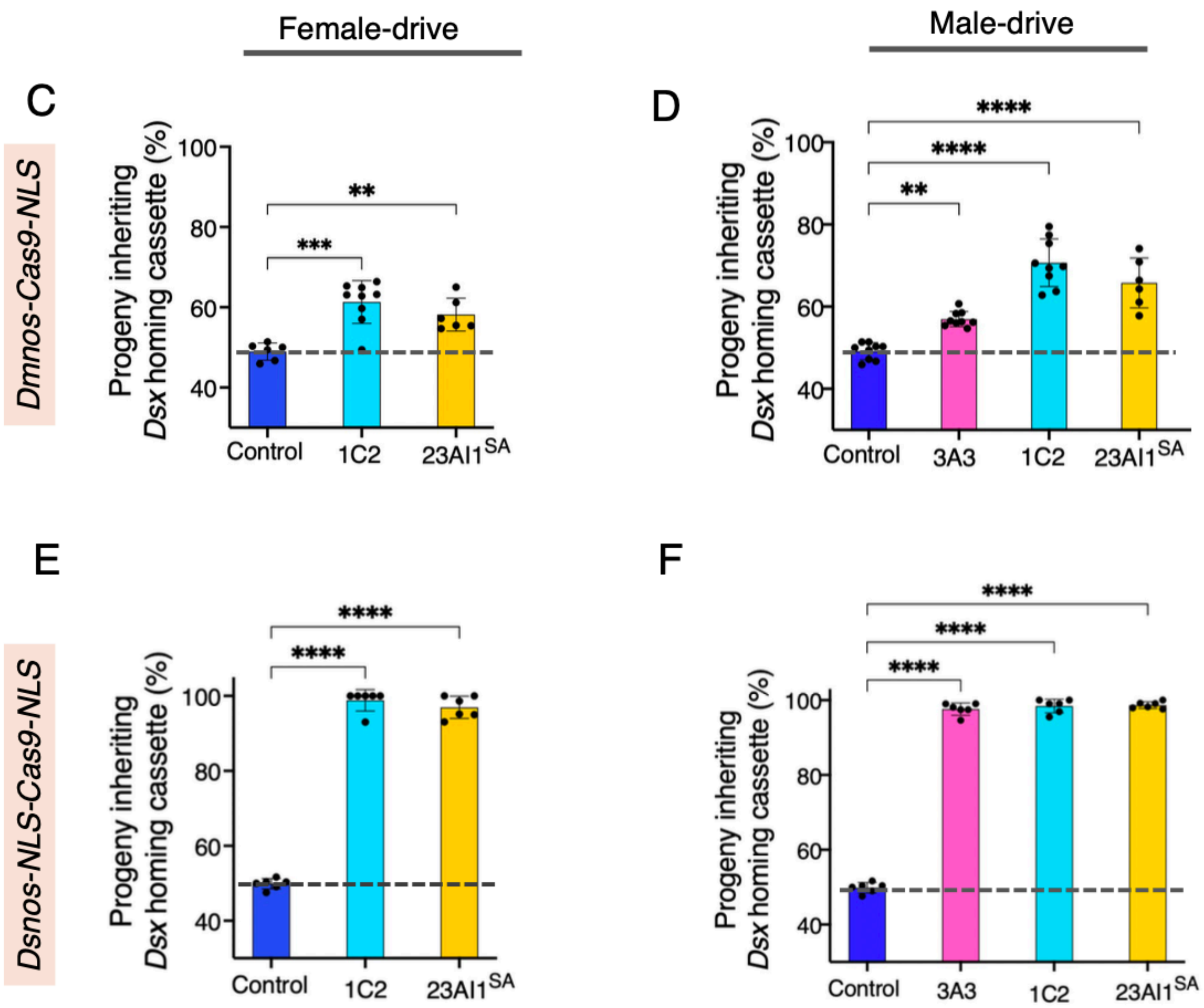
# Genetic crosses to score the homing




*Yadav et al, 2023; PNAS*

# *dsx* homing gene drive inheritance

- **Improved Drive**: 94-99% inherit *dsx* with DsRed and sgRNA gene!




Yadav et al, 2023; PNAS

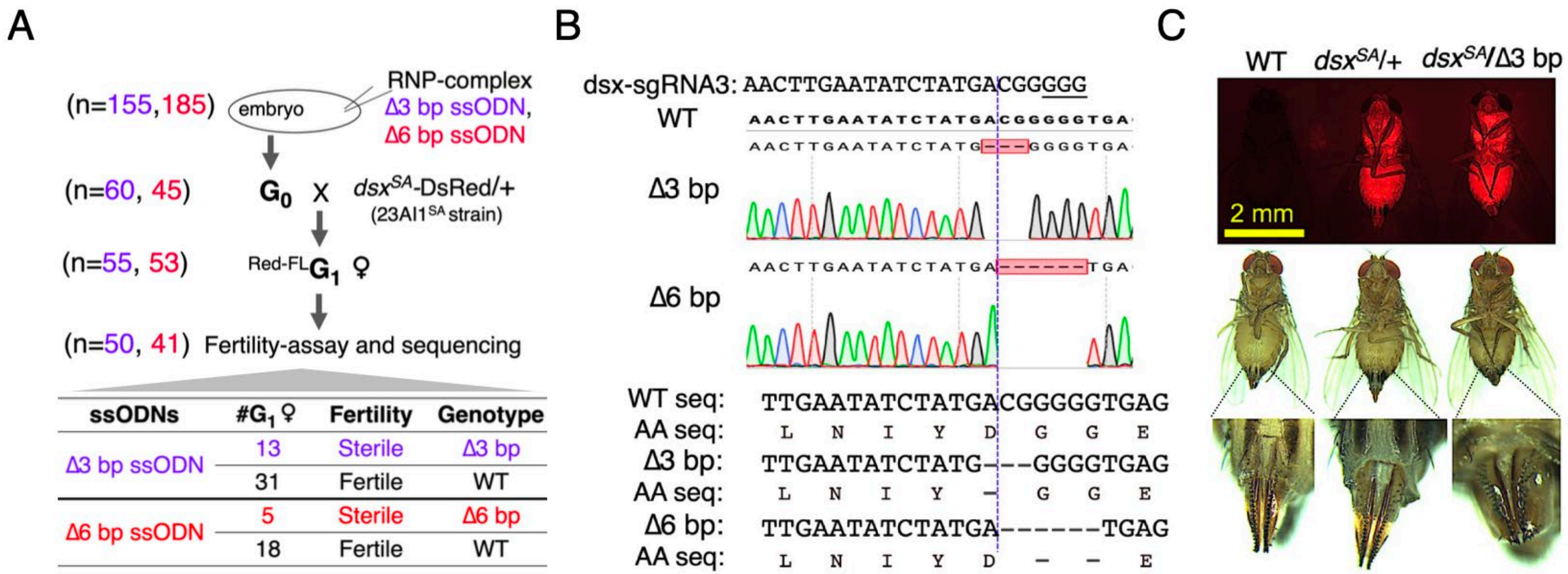
# Potential **Resistance Alleles** Development

- NHEJ repair pathways can generate a resistance allele
- Possible, in-frame deletions or insertion would resistant to Cas9/sgRNA3 cleavage but produce functional protein!

| | | Indel | n/total |
|---|---|---|---|
| sgRNA3 | AACTTGAATATCTATGACGG**GGG** | | |
| WT | AACTTGAATATCTATGACGG**GGG** | | |
| ***3A3 male drive*** | AACTTGAATATCTATGACGG**GGG** | **WT** | 7/13 |
| | AACTTGAATATCTATG----**GGG** | -4 bp | 2/13 |
| | AACTTGA-------------------GCTTCGCAA | -19 bp | 1/13 |
| | AACTTGAATA-------------TGAGCTTCGCAA | -13 bp | 1/13 |
| | AACTTGAATATCTATGANNNCGG**GGG** | +3 bp | 1/13 |
| | AACTTGAATATCTA---CGG**GGG** | -3 bp | 1/13 |
| ***1C2 male drive*** | AACTTGAATATCTATGACGG**GGG** | **WT** | 1/9 |
| | AACTTGAATATCTATG----**GGG** | -4 bp | 1/9 |
| | AACTTGA-------------------GCTTCGCAA | -19 bp | 4/9 |
| | AACTTGAATATCTATG-CGG**GGG** | -1 bp | 3/9 |
| ***1C2 female drive*** | AACTTGAATATCTATGA-GG**GGG** | -1 bp | 2/3 |
| | AACTTGAATATCTATG--GG**GGG** | -1 bp | 1/3 |
| ***23AI1 SA male drive*** | AACTTGAATATCTATGACGG**GGG** | **WT** | 7/8 |
| | AACTTGAATATCTAT---GG**GGG** | -3 bp | 1/8 |
| ***23AI1 SA female drive*** | AACTTGAATATCTA---CGG**GGG** | -3 bp | 3/12 |
| | AACTTGAATATC-----CGG**GGG** | -5 bp | 3/12 |
| | AACTTGAATATCTATGA---**GGG** | -3 bp | 1/12 |
| | AACTTGAATATCTATGA------TGAGCTTCGCAA | -6 bp | 1/12 |
| | AACTTGAATATCTATGA---------GCTTCGCAA | -9 bp | 1/12 |
| | AACTTGAATATCTATGANNNNNNNCGG**GGG** | +7 bp | 1/12 |
| | CGTCAGCA-------------------GG**GGG** | -19 bp | 1/12 |
| | AACTTGAATAT-------GG**GGG** | -7 bp | 1/12 |
| | GCAT----------------------**GG**TGAGCTTCG | -21 bp | 1/12 |

*Yadav et al, 2023; PNAS*

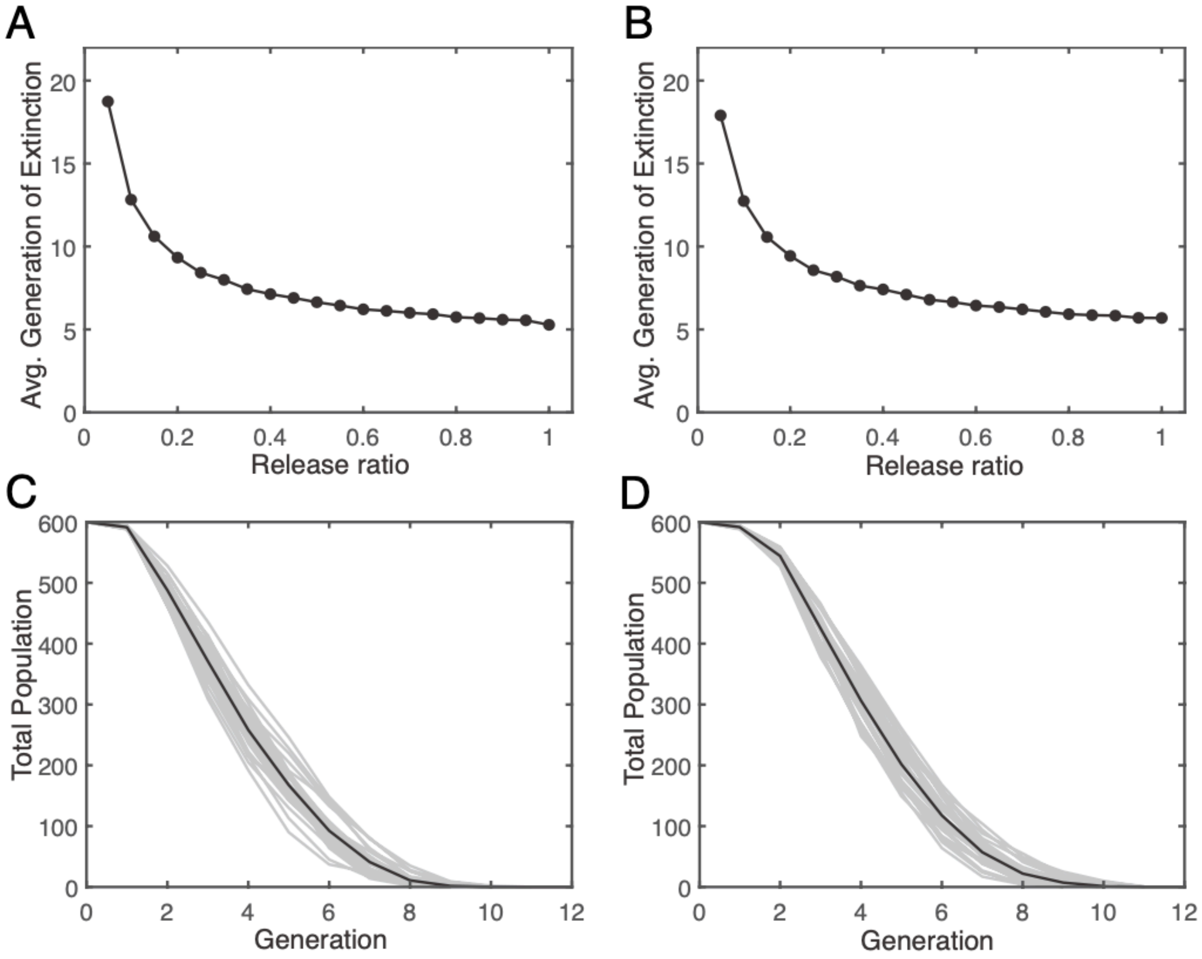
# In-frame deletion mutations are resistant to cleavage but are not functional




*Yadav et al, 2023, PNAS*

# Mathematical Modelling of split drives

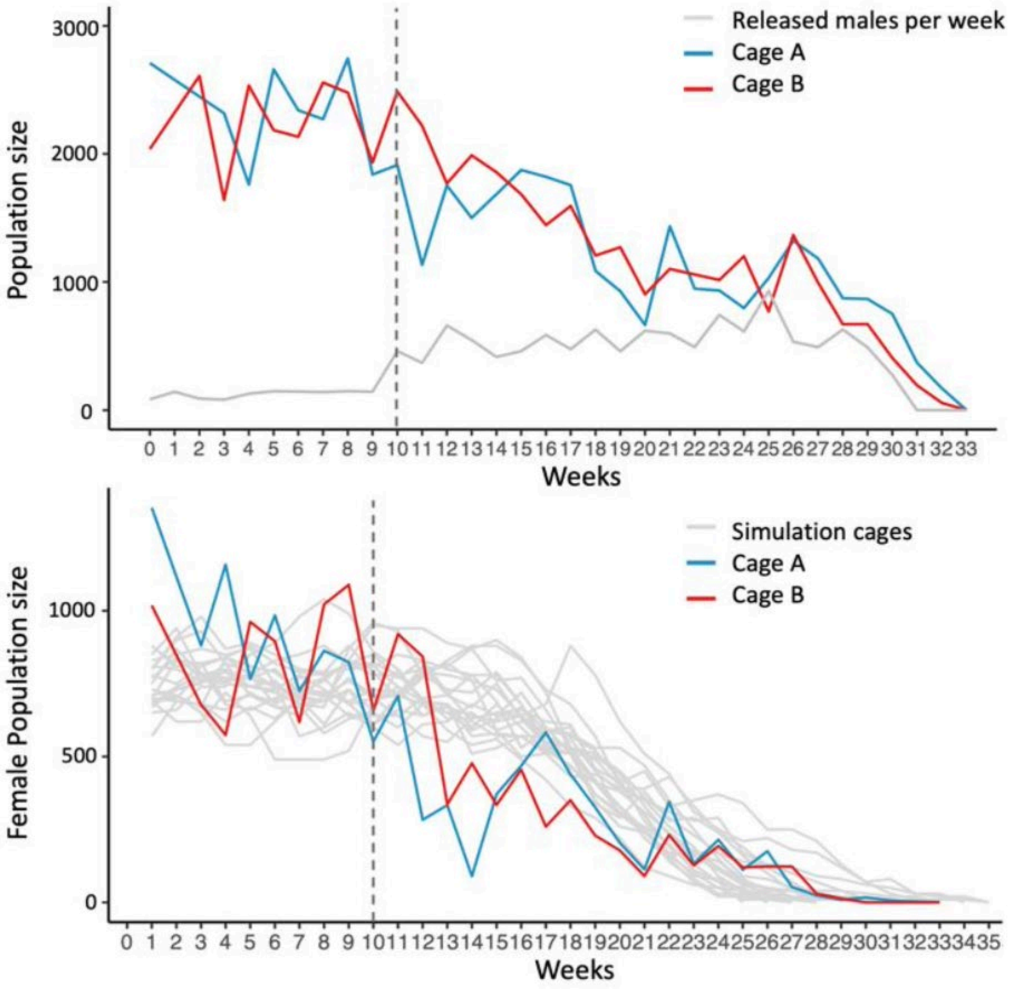
- Discrete generation lab cage population
- Repeated Releases, 1: 4 ratio (transgene: wild type)
- Suppression within 10 generations




*Yadav et al, 2023, PNAS*

# Population dynamics of Dominant sterile Drive in caged populations of ***D. melanogaster***

- Cage Population size: 2500
- Weekly releases of drive
- Population collapsed in 29 weeks




Chen W, 2023 (BioRxiv)

# Challenges

- Precision at genetic and molecular level
  -**Efficiency and fitness cost**

- Population dynamics of the target pest
  **-Pest population size and its distribution**

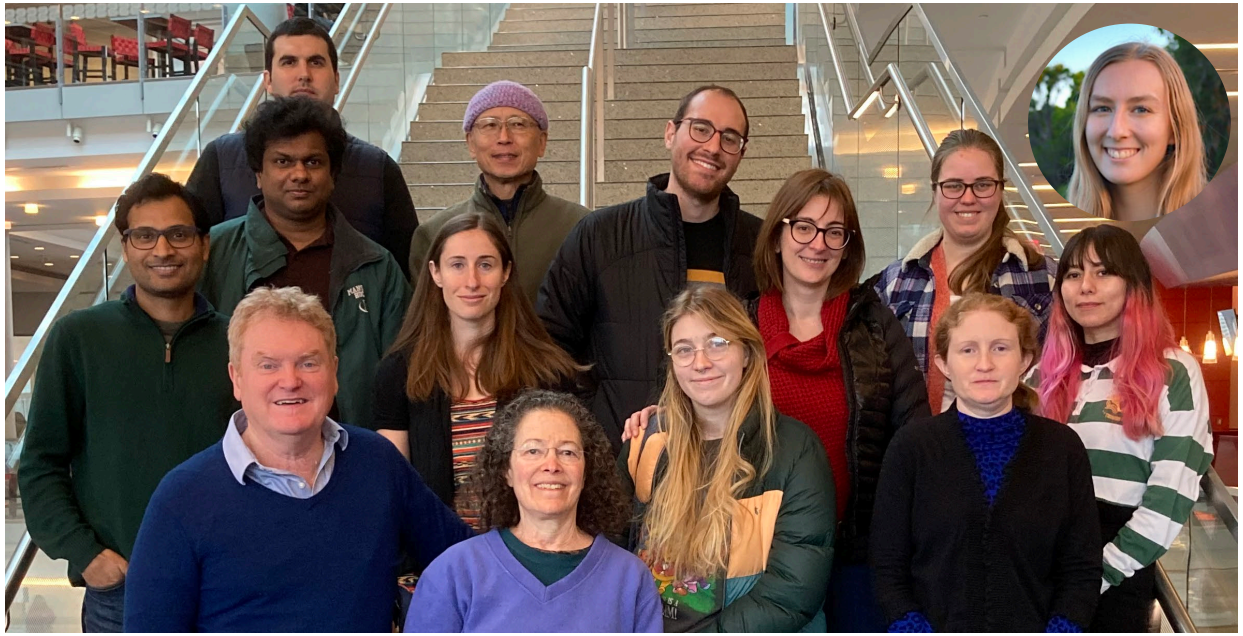
- Mass rearing and field releases

# Acknowledgements

**Scott lab members**
Prof. Max Scott (PI)
Esther Belikoff
Dr. Aki Yamamoto
Dr. Tatiana Basika
Dr. Rossina Pelaez
Dr. Kalindu Ramyasoma
Dr. Juan wulff
Alexis Kriete
Sarah Hudadoff
Rachel Laminack
Ariel Terrand
Laura Welsh

**Recent past lab members**
Dr. Mellina Florez-Cuadros
Dr. Anandrao Patil
Dr. Megan Williamson
Dr. Sophia Webster
Pedro Mariano
Karla Garavito

**Collaborations**
Prof. Alun Llyod (NCSU)
Cole butler (NCSU)



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**Funding**
USDA-APHIS
USDA-ARS
USDA-NIFA, BRAG program