

# Engineering biosafe gene drives in *Drosophila suzukii* for population suppression.

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## Overview

- Drosophila suzukii*, also known as spotted wing drosophila (SWD), is an economically important agricultural pest of soft skinned fruits like peach, cherries, blueberries, and strawberries etc.
- We have been developing species-specific genetic methods for control of this pest include Cas9-based homing gene drive.
- Our research will provide valuable data to assist regulatory agencies in making decisions about gene drives.

## Background

### Spotted Wing *Drosophila* (*D. suzukii*)

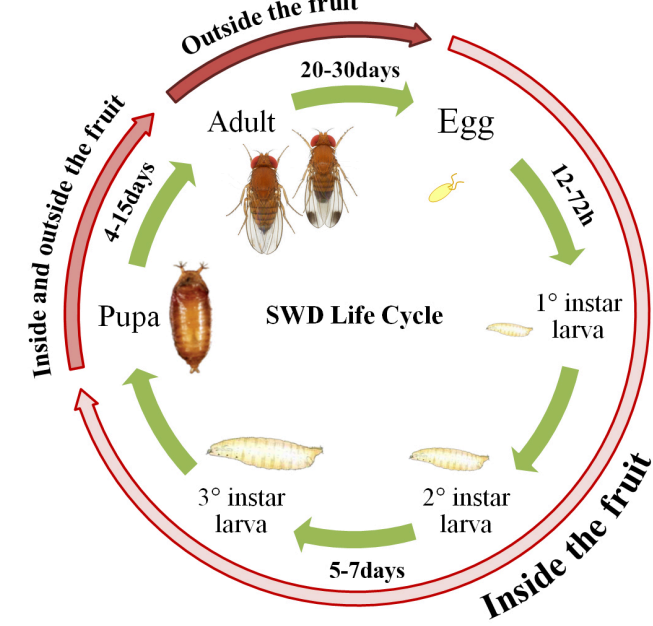
- Invasive species first found in the U.S. in 2008.
- Rapid generation time (2 weeks); growers must apply pesticides multiple times throughout the growing season.



A male SWD with wing spots and the saw-like ovipositor of females (inset).



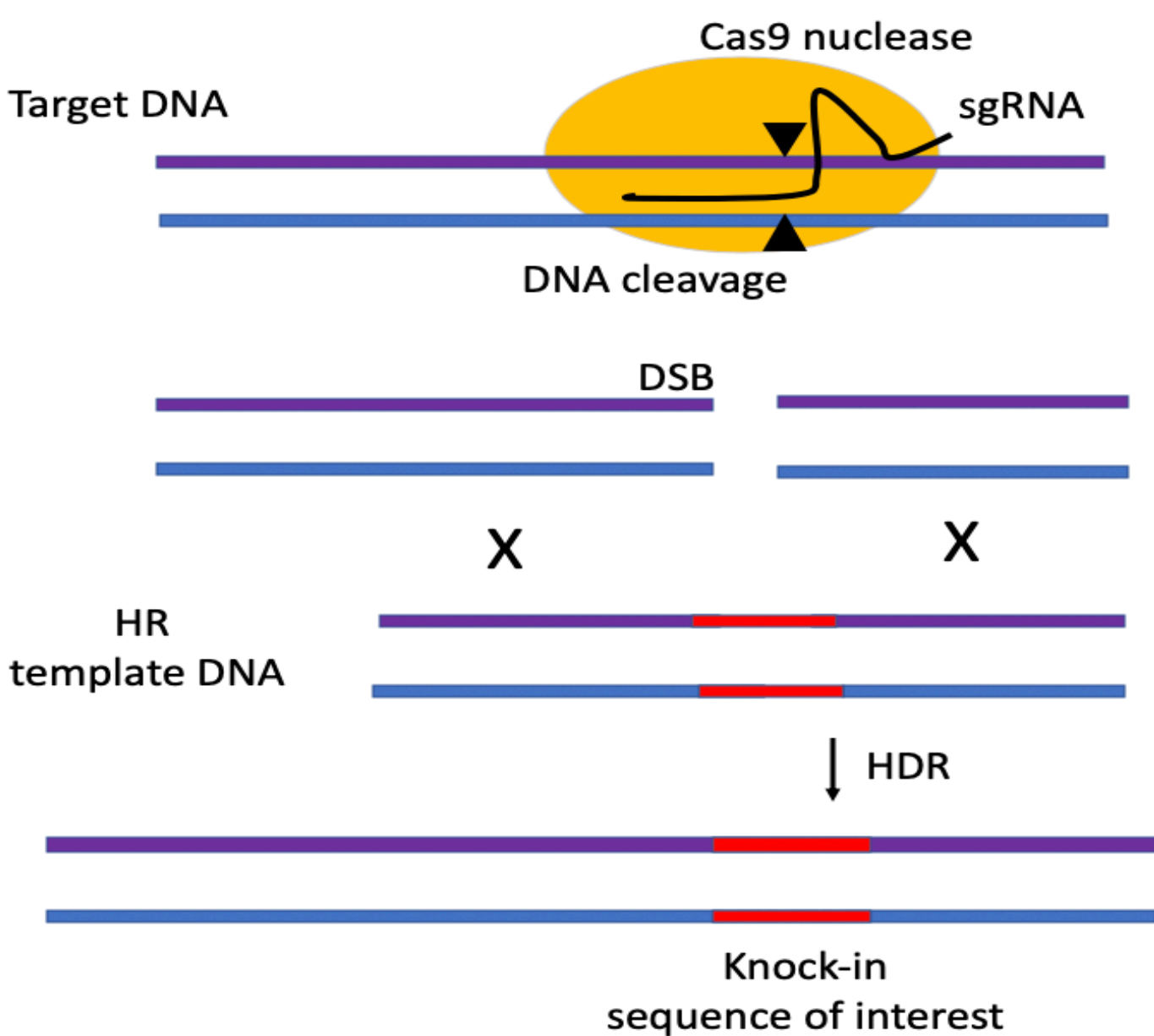
Females lay eggs in healthy, soft-skinned fruits.



SWD life cycle depends upon temperature and humidity. (Pictures from Oregon State Uni Extension Service).

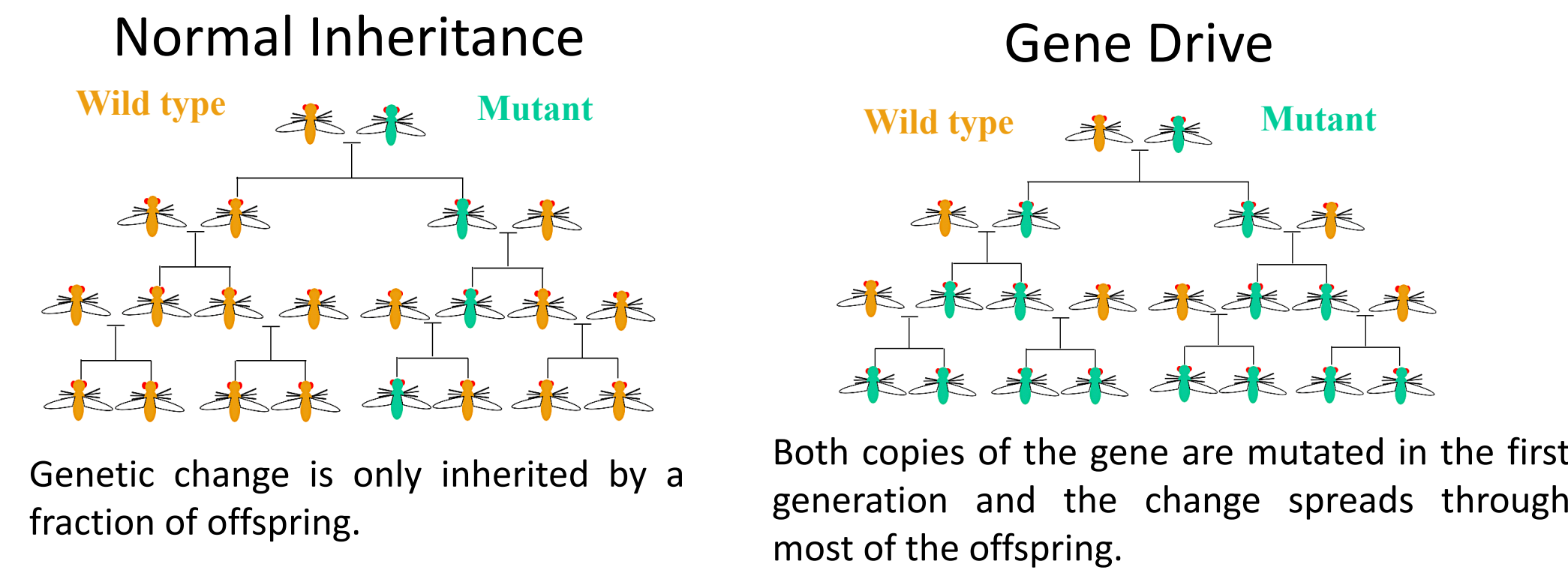
### CRISPR/Cas9 and gene editing

- CRISPR/Cas9 gene editing system is adopted from bacterial immune system.
- Cas9 is an RNA guided endonuclease induces DNA double strand breaks.
- Homology directed repair of DSBs enables targeted knock-in sequence of interest.



## Gene Drives

A gene drive is a heritable mechanism to introduce a genetic change into a population at a rate higher than normal inheritance.



### Benefits of Gene Drives

- Rapid.
- Fewer flies needed compared to sterile insect method.
- Complement and enhance traditional pest management.
- Species-specific.

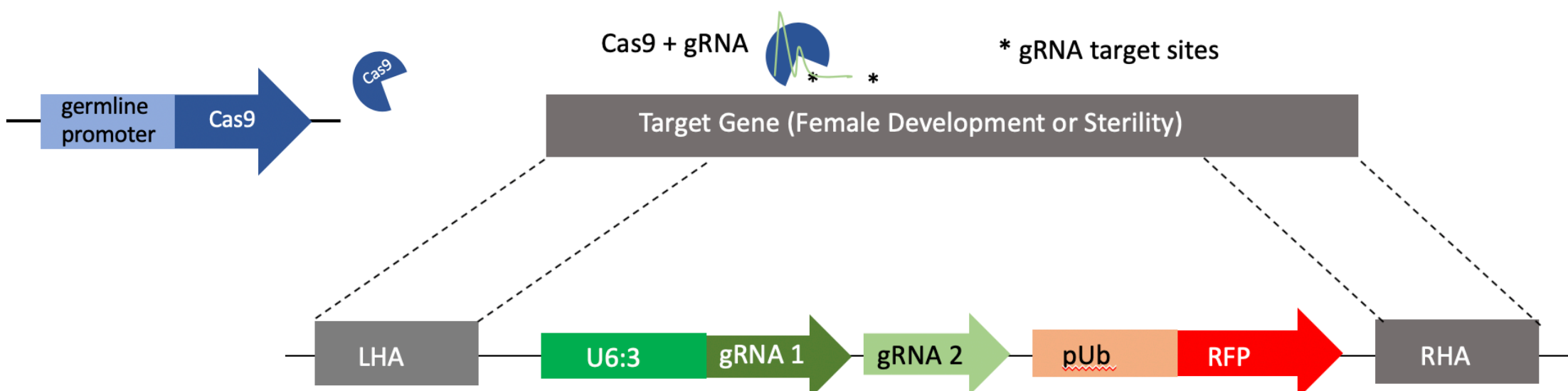
### Potential Problems

- Uncontrolled drive could wipe out entire pest populations.
- Non-specific cutting by Cas9 could make undesired mutations.
- Resistance development.

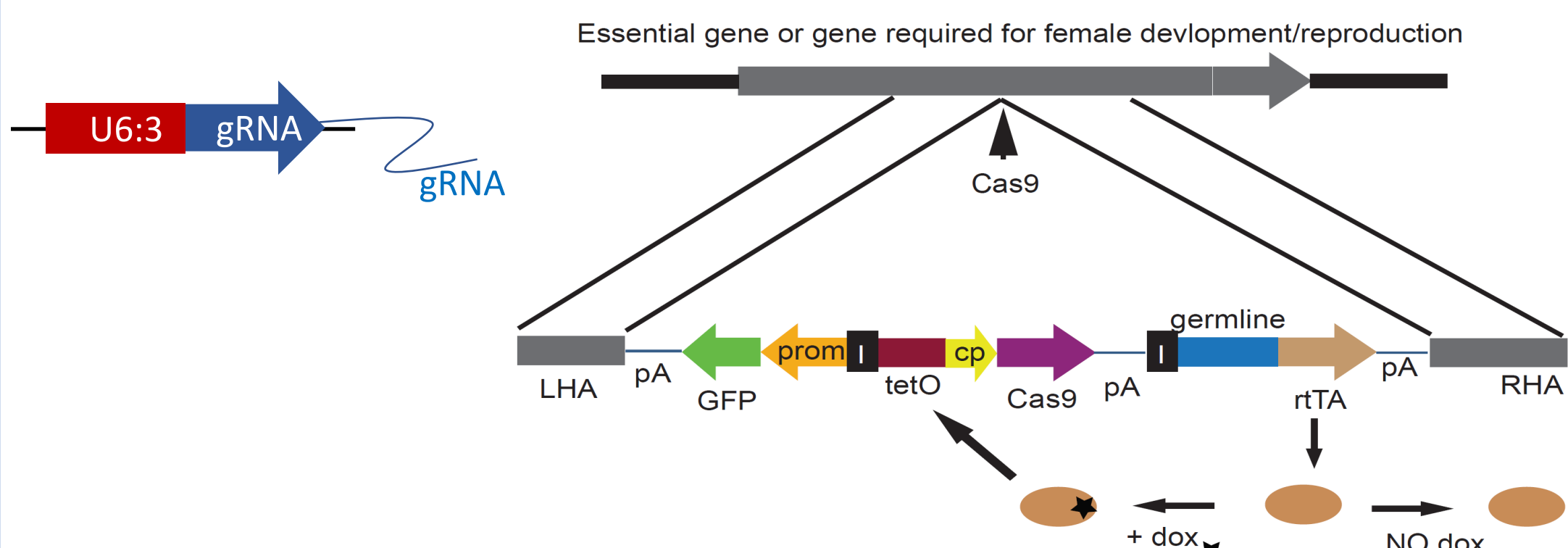
## Methods and Results

### Biosafe Gene Drive design: Approaches

- Split gene drive system



- Controlling Cas9 expression using conditional system eg. Tet-On/Off system



## CRISPR/Cas9 mediated Sxl gene disruption in germ cells of SWD

- Sxl* is essential for female development
- Sxl* gRNA lines were crossed with Cas9 lines
- vasa-Cas9*: 100 % female offspring have ovipositor structural deformation (Fig 1A)
- nos-Cas9*: female offspring show no morphological deformation (Fig1B), however they were sterile (94.4% flies), suggesting loss of *Sxl* function in the female germline.
- The confocal microscopy revealed the development of small ovaries filled with a large number of cells, similar to the “bag of marbles” phenotype described in *D. melanogaster* (Fig. 2).

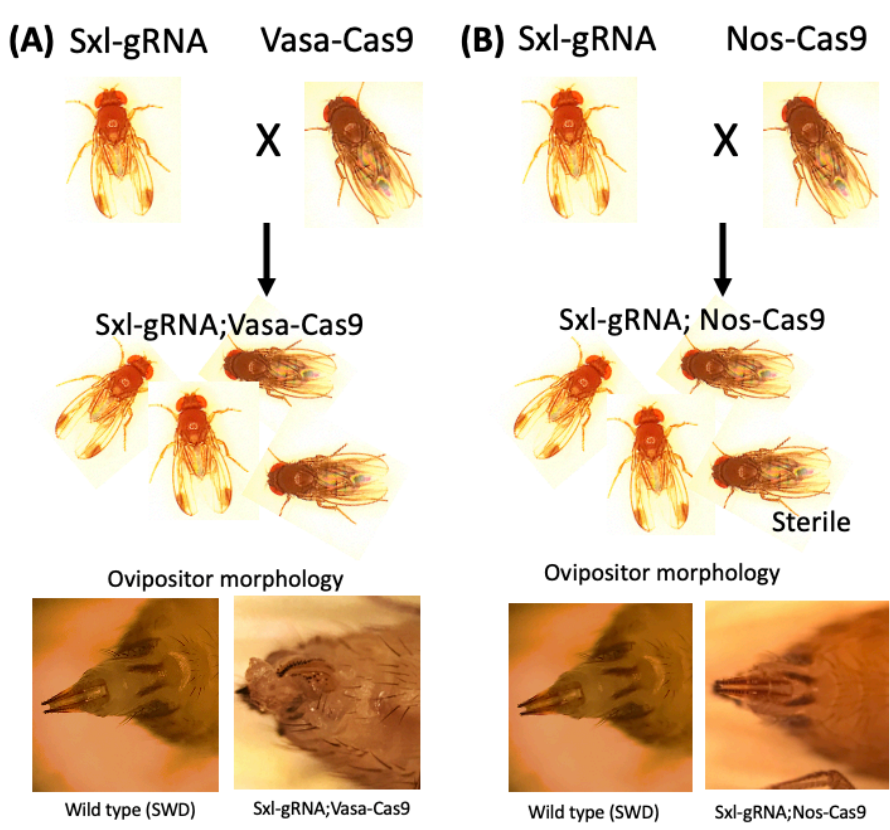
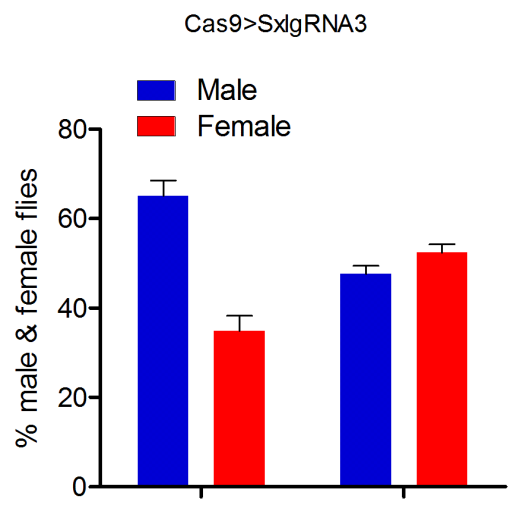


Fig.1. Cas9 lines were crossed with Sxl gRNA line.



Histogram1: Representing percentage of offspring enclosed.

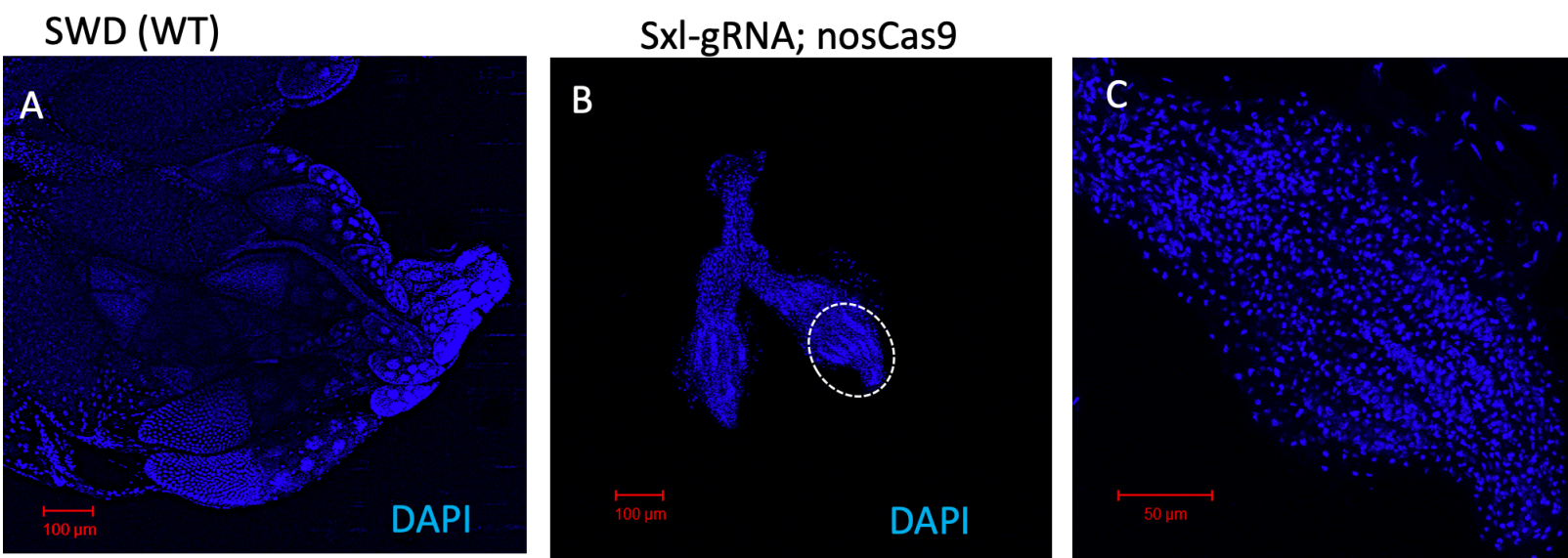


Fig.2. Confocal projections showing SWD (A) and Sxl gRNA; nosCas9 (B) ovaries structure. Magnified (white circle) shown in panel C. Representing 'bag of marbles' phenotype.

### Dsx gRNAs efficiency tested: *In-vitro*

- In vitro* Cas9 assays were conducted to determine the most efficient sgRNAs.
- Reactions contained Cas9 protein, sgRNA, and a PCR product containing the PAM site.
- gRNA3 and gRNA5 appear relatively more efficient than gRNA 1, 2 & 4.
- Generated Dsx split gene drive construct for SWD microinjections

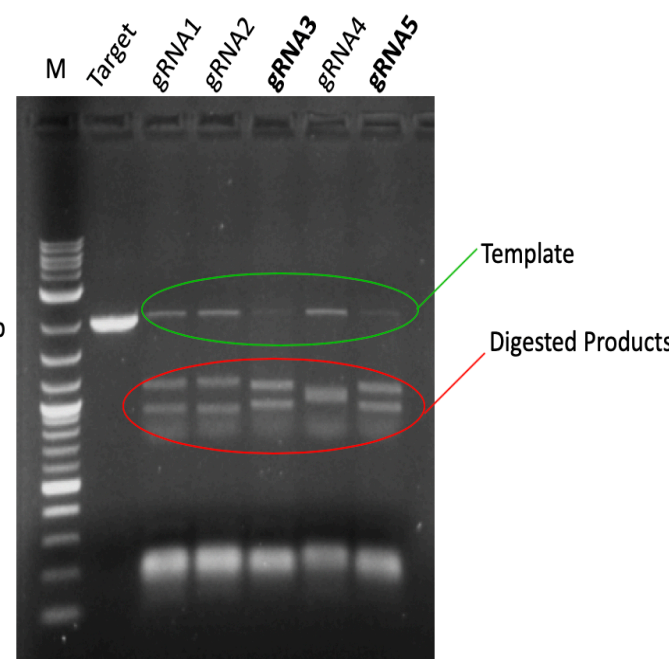
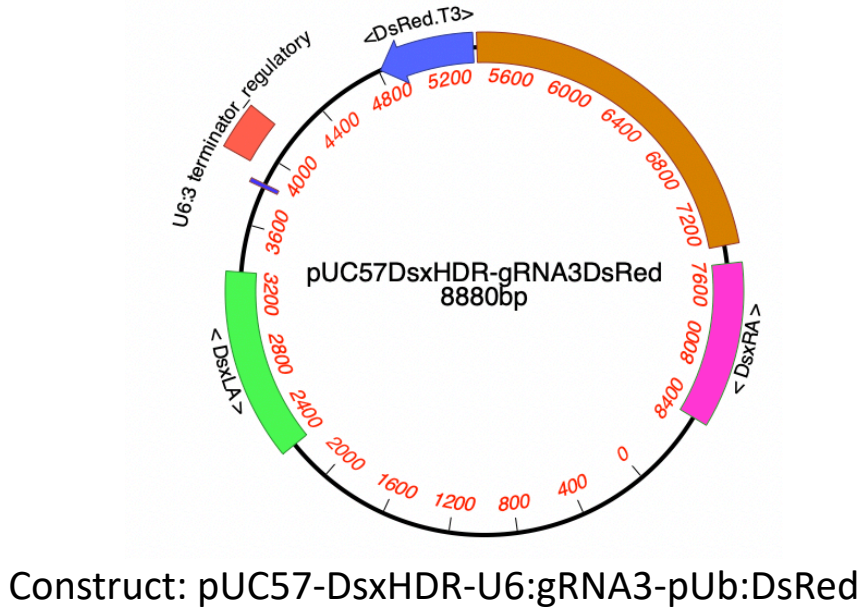


Fig.3. In-vitro Cas9 activity

## Conclusions and Future Directions

- We have determined the activity levels of Cas9 in transgenic SWD.
- The most efficient gRNAs for Dsx in SWD have been identified.
- We have generated the construct for the gene drive for Dsx.
- “Split” drives will be tested in cage studies to follow the spread of the drive in a contained population.

## Acknowledgement

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