The CRISPR toolkit for genetic biocontrol of invasive species

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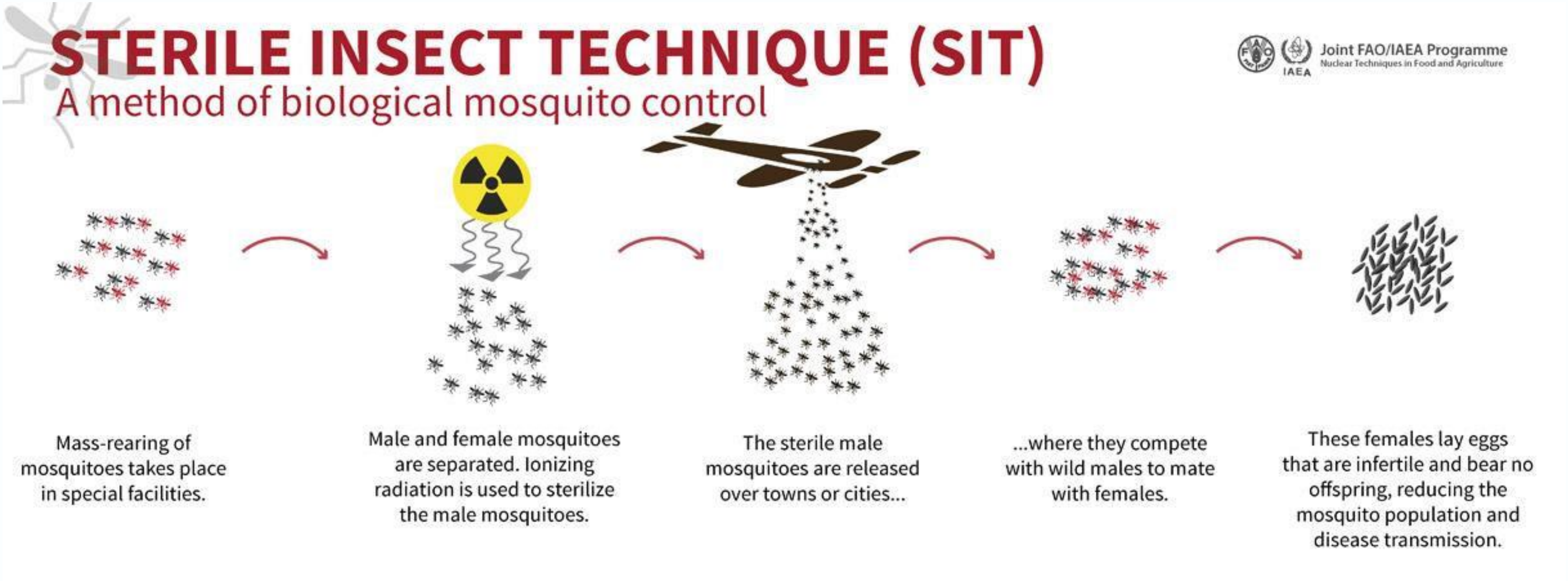
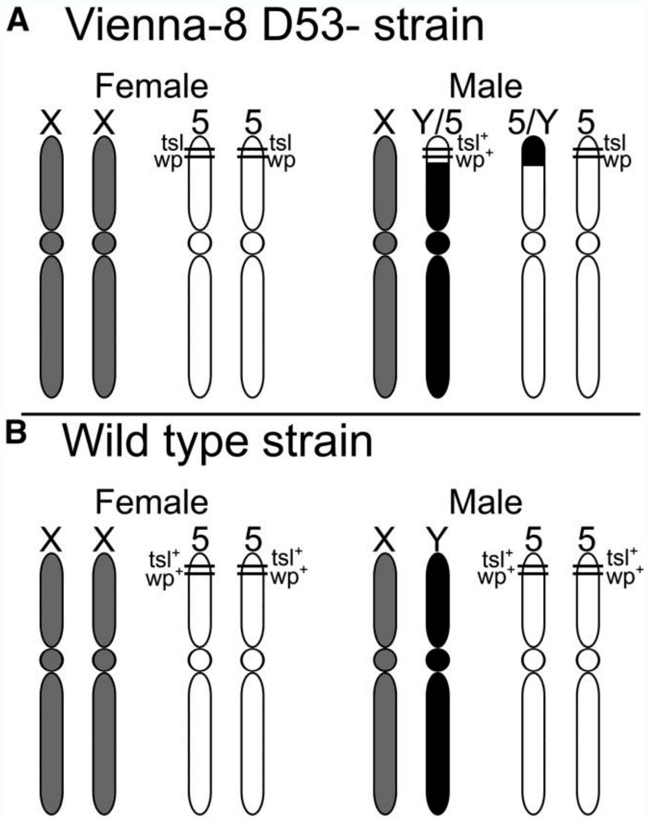


<u>Limitations</u>: Sex separation, Large release numbers, single matings, radiation effects

Genetic Sex Selecting (GSS) Strains

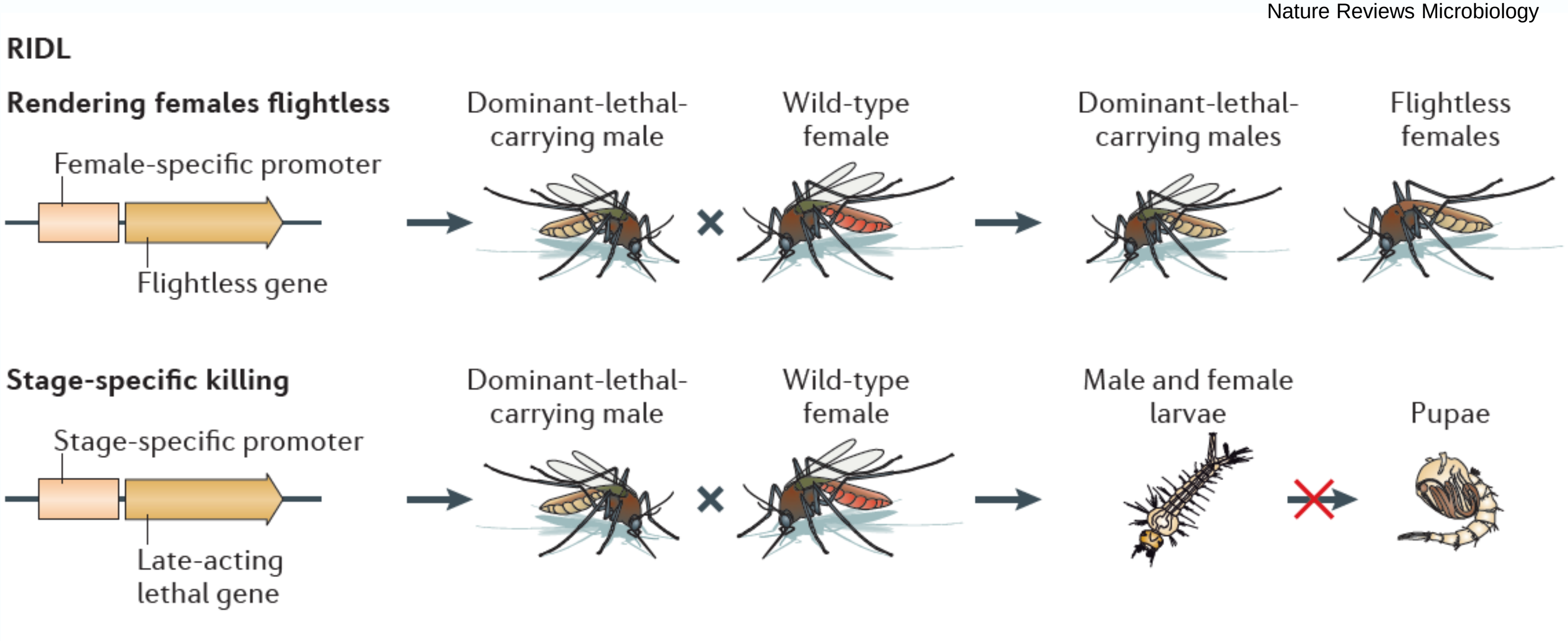


Mediterranean fruit fly, *Ceratitis capitata*

<u>Limitations</u>: Massive investment, Large release numbers, single matings, radiation effects, unstable

Release of Insects with Dominant Lethal (RIDL)

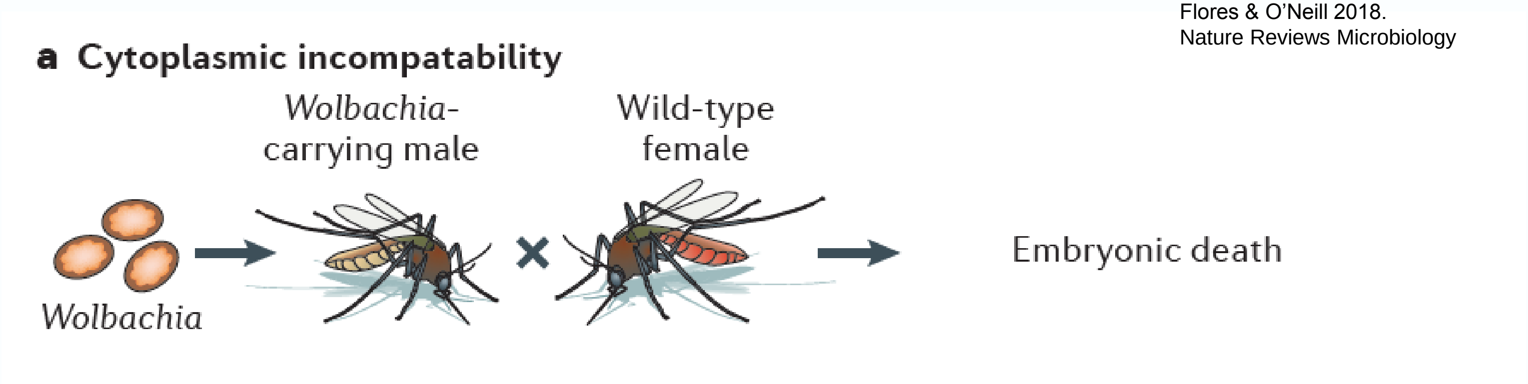
Flores & O'Neill 2018.
Nature Reviews Microbiology



<u>Limitations</u>: Massive investment, Large release numbers

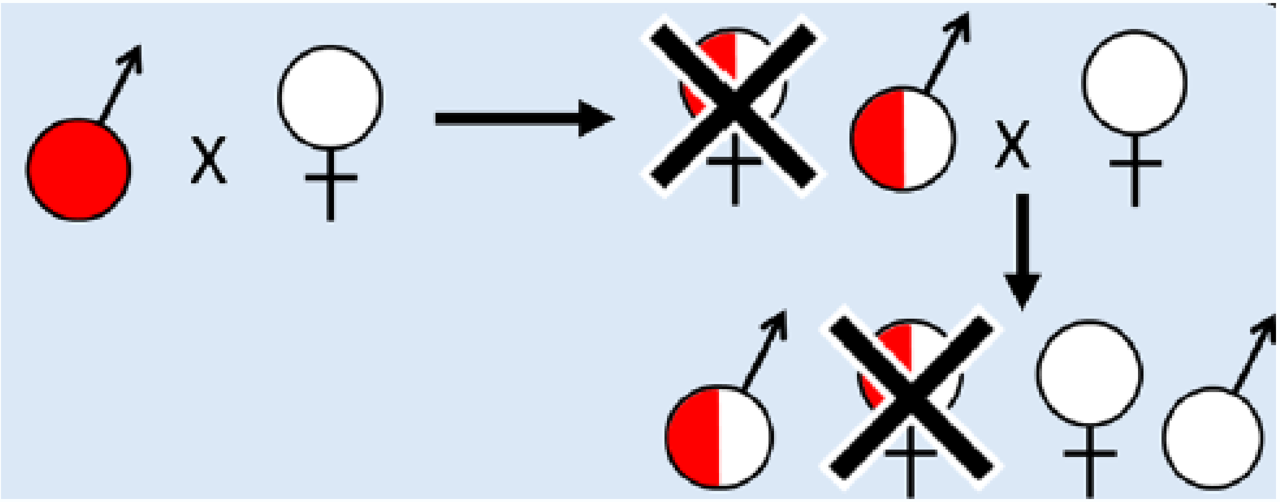
Wolbachia symbiotic bacteria

Flores & O'Neill 2018.
Nature Reviews Microbiology



<u>Limitations</u>: Large investment, Amenable to Wolbachia, sex separation

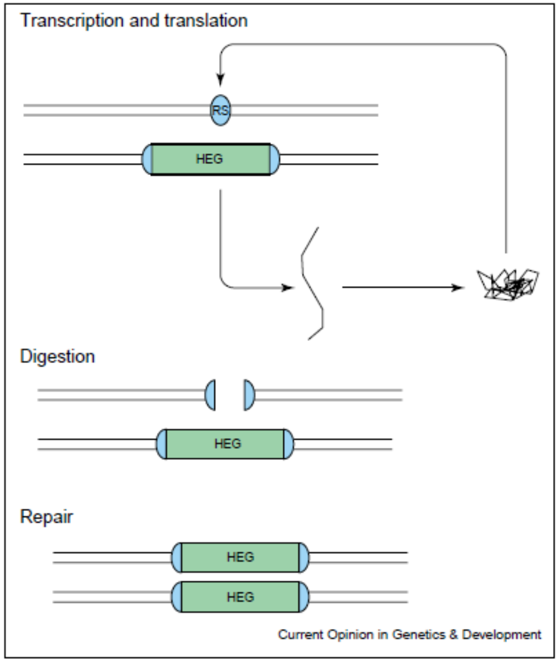
“Daughterless” technology



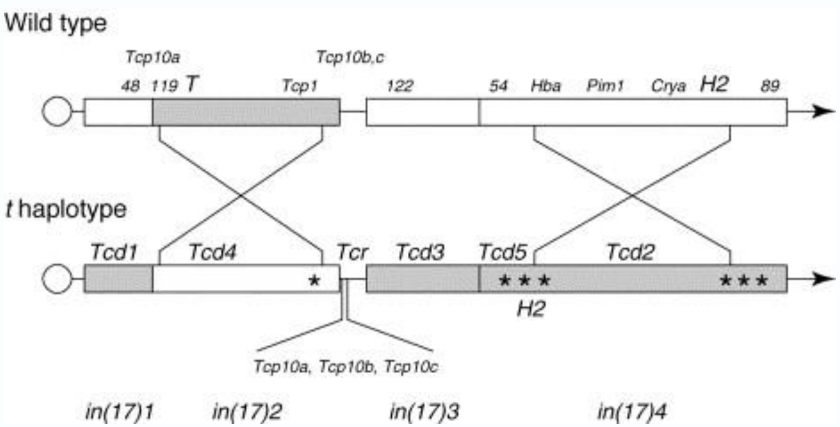
<u>Limitations</u>: Large investment, Repeated releases, unstable

Natural gene drives

Homing endonucleases



Mouse *t* haplotype

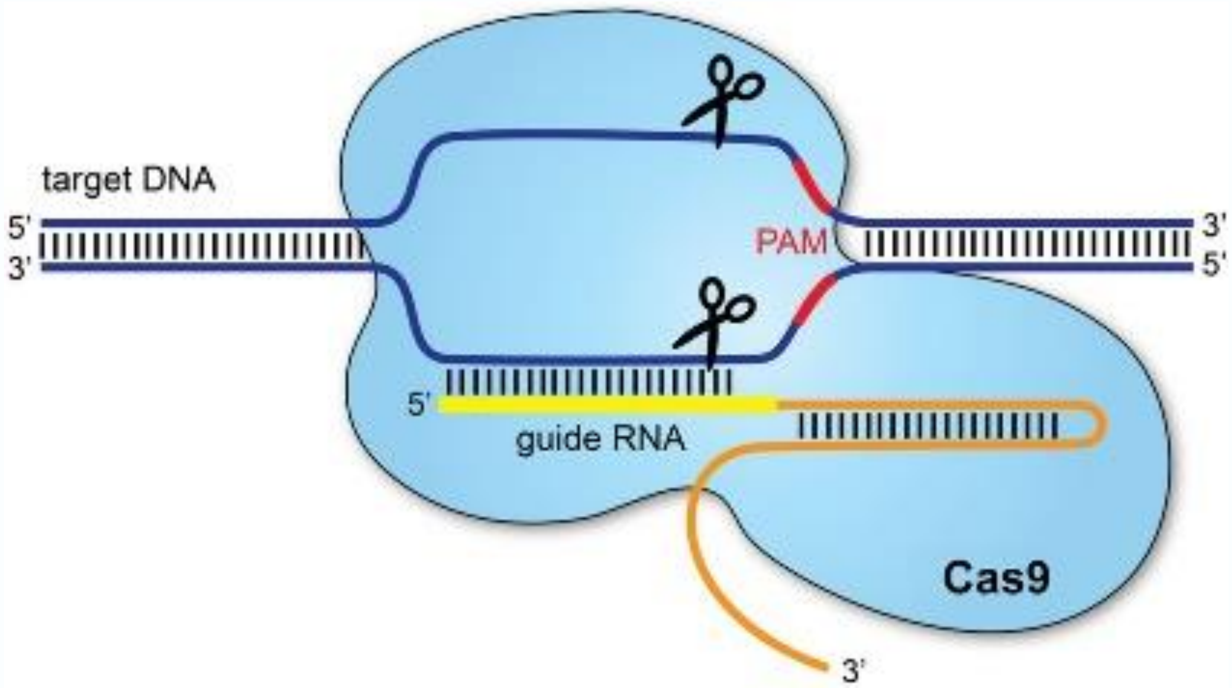


Limitations: Large investment, limited target species, self-propagating

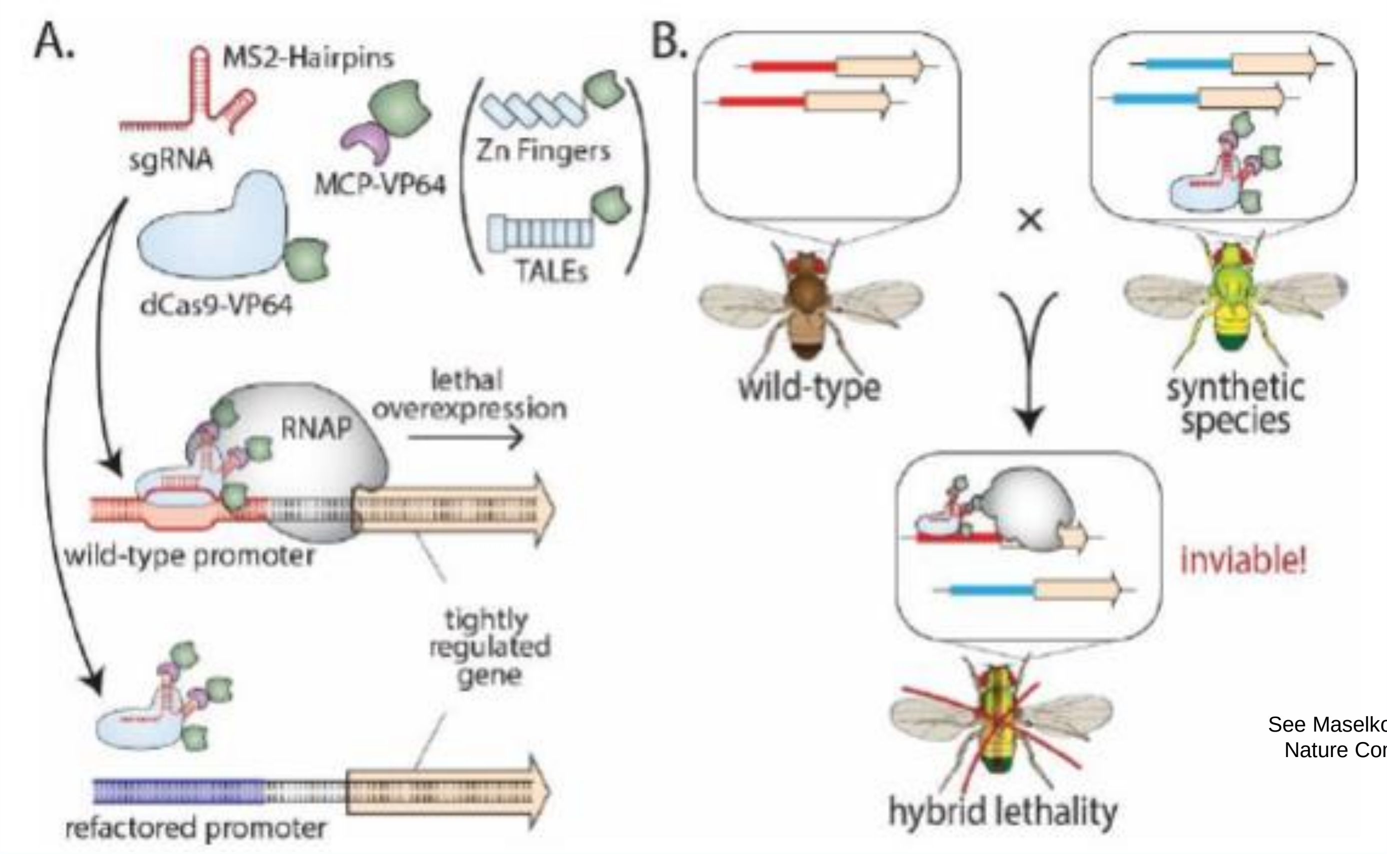
Summary: Early genetic control methods

1. CSIRO/Australia have been world leaders in developing and implementing genetic technologies
2. The utility of many methods is limited to targets with particular biology
3. Most methods require the rearing and release of large numbers
4. Early methods required efficient sex sorting to be effective
5. GM methods had huge development costs, and the technology was not easily adapted to other species
6. Male sterility/mating incompatibility and female-killing (sex ratio bias) are the most popular mechanisms used to achieve genetic control

CRISPR-Cas9



Engineered incompatibility

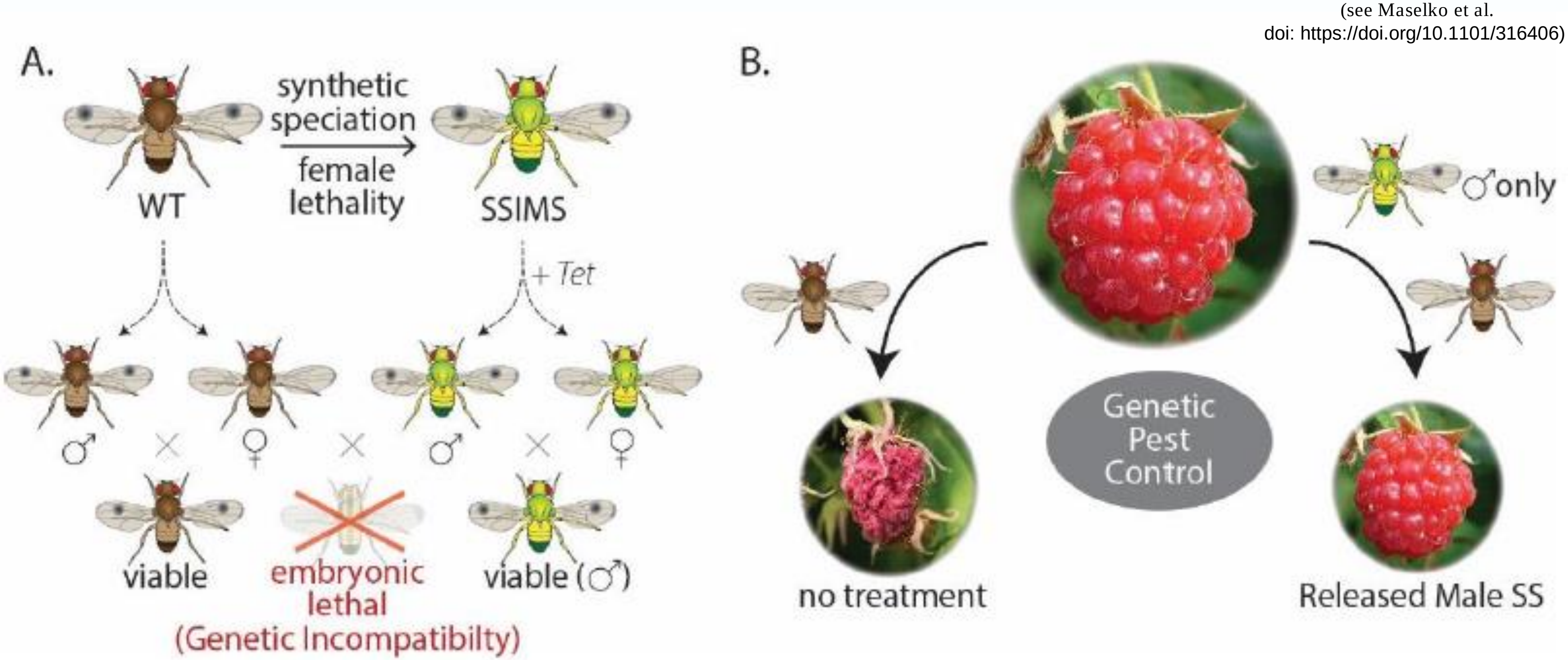


See Maselko et. al, 2017. Nature Comms 8: 883

- Can be used like Wolbachia infections, but easily transferrable

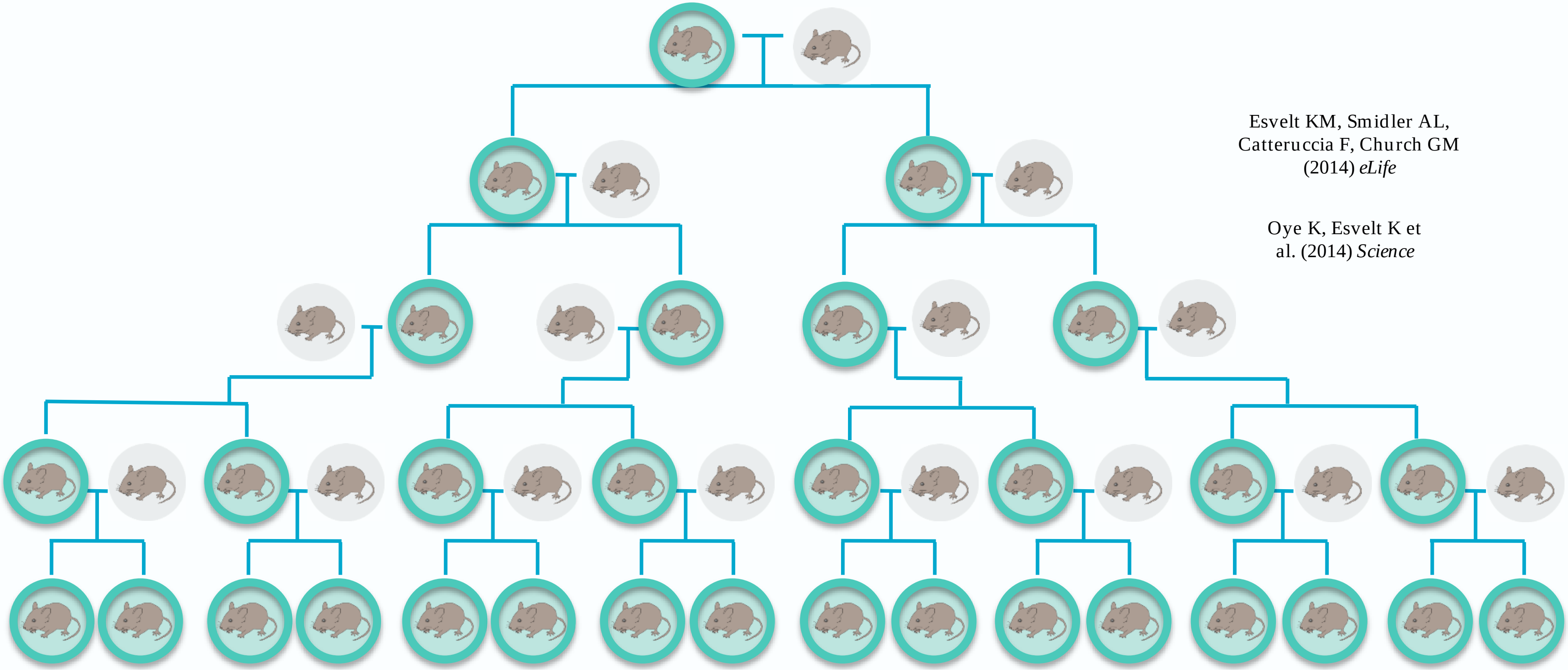
Self-sorting incompatible male system (SSIMS)

Courtesy of M. Maselko
(see Maselko et al.
doi: https://doi.org/10.1101/316406)



- Works like RIDL, but much more flexible and adaptable

Synthetic gene drives



Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*

Oye K, Esvelt K et al. (2014) *Science*

- A much more flexible and adaptable form of HEG

CRISPR-Cas9 has transformed genetic control

1. All previous genetic control methods can be replicated using CRISPR-Cas9 with far reduced development costs and time

2. CRISPR-engineered methods can usually be adapted to the biology of any target

3. CRISPR-Cas9 approaches are more easily transferred between species

4. Male sterility/mating incompatibility and female-killing (sex ratio bias) remain the most popular mechanisms used to achieve genetic control using CRISPR-Cas9

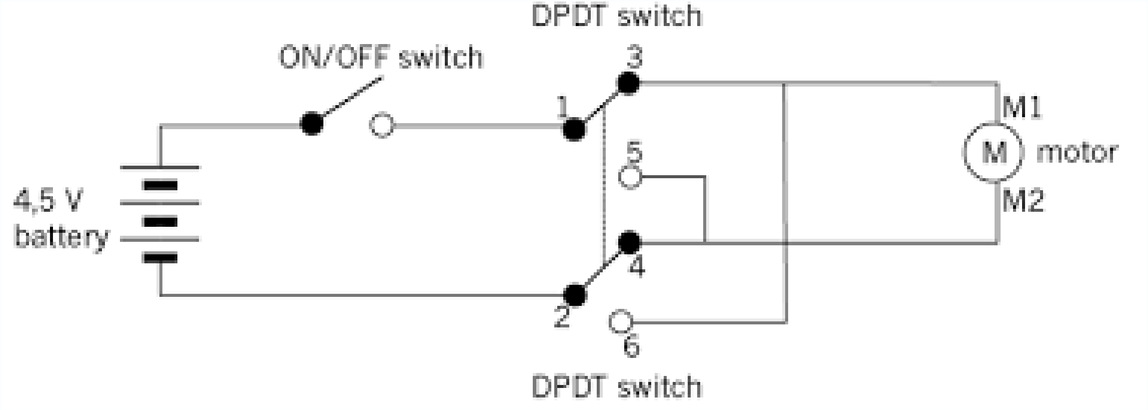
Synthetic Biology: Applying engineering principles to biology

1. Functional biological components (“biobricks”) connected by switches, logic gates, and other regulatory components

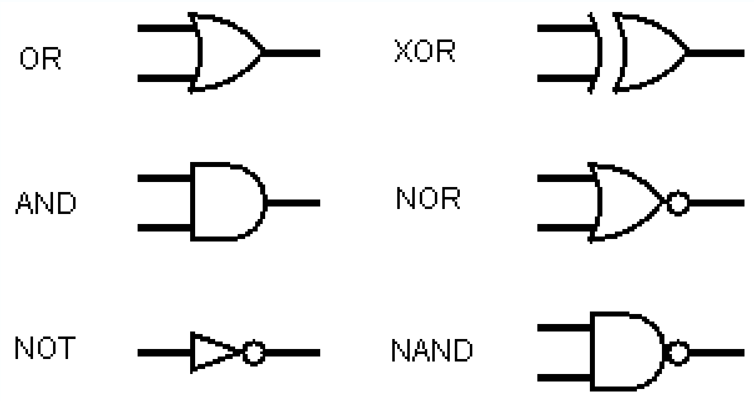
2. Synthetic biology approaches are generally more tuneable to the characteristics of the organism and environment

3. “Synthetic speciation”, “female-killing”, “gene drive” biobricks can be engineered to work together in the same genetic control construct

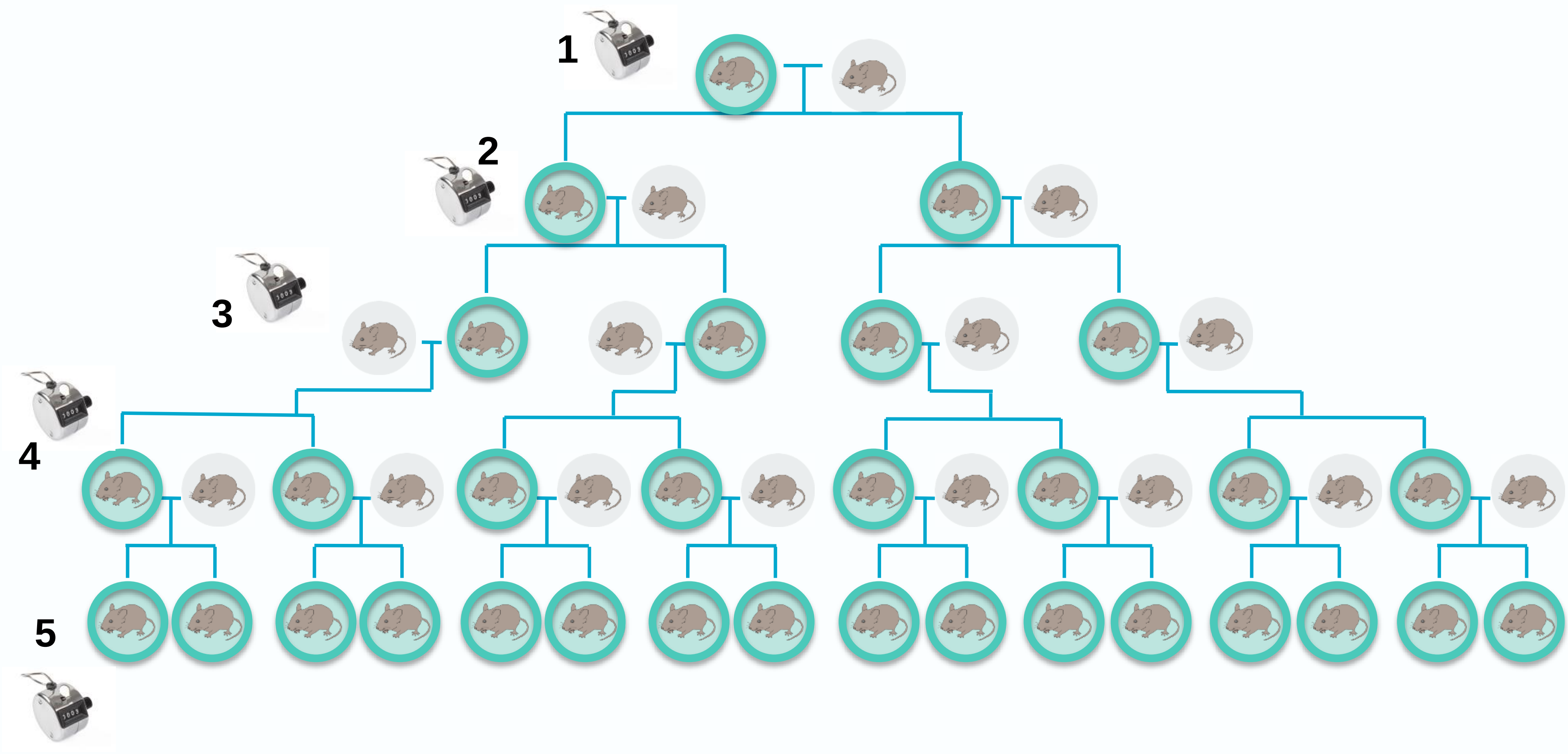
Synthetic Biology: Applying engineering principles to biology



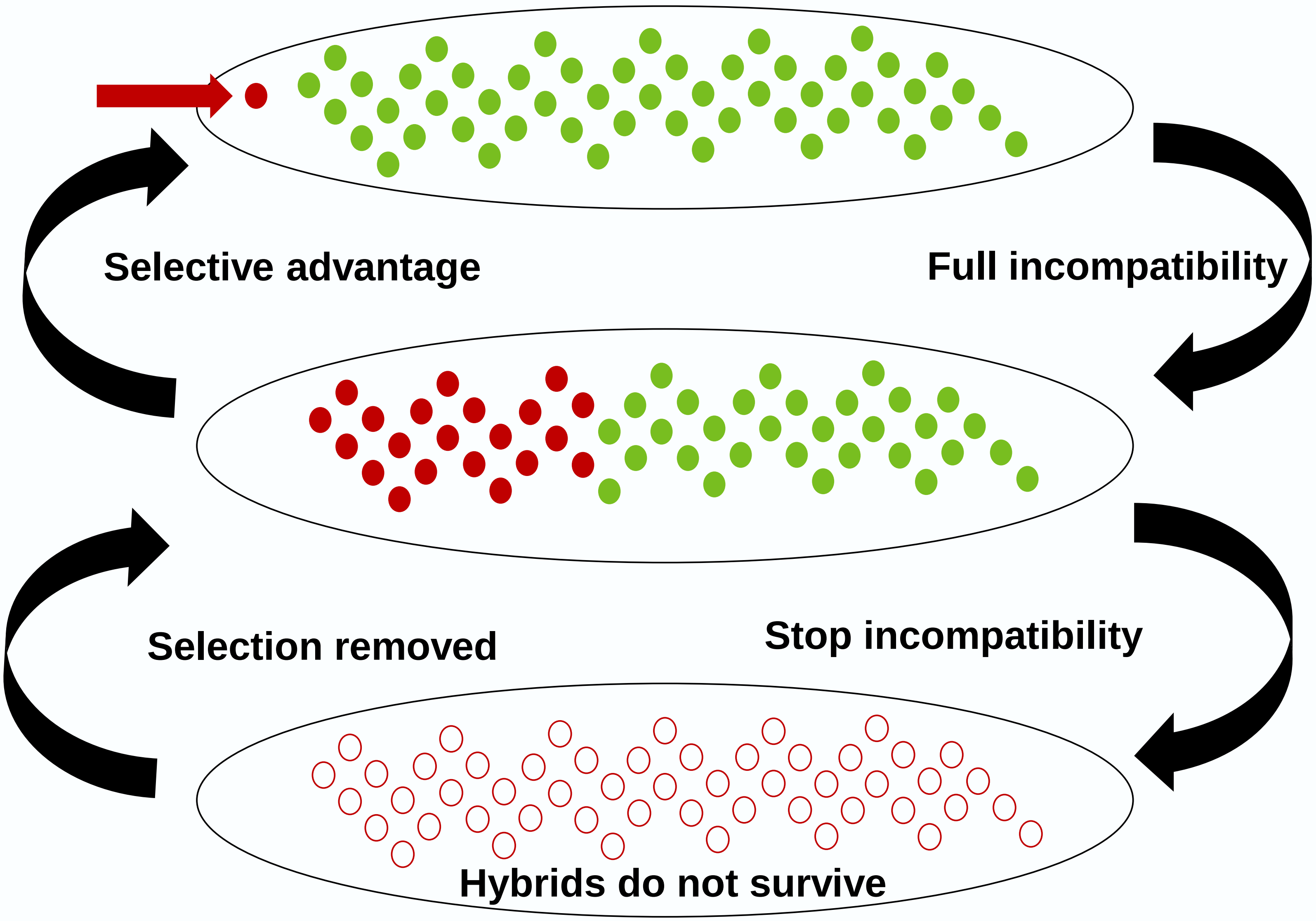
<u>Control elements:</u>
Switches and logic gates



Generation counter



Field Amplification to Achieve Frequency Threshold for Control



Take home messages

1. CRISPR-Cas9 technology has made genetic control technologies more readily available for more target species

2. Synbio-engineered genetic control technologies will use switches and logic gates to regulate the functional components to achieve population control (mating incompatibility, female-killing remaining the best options)

3. Synthetic gene drives by themselves will not be deployed as a genetic control technology, but rather as a component of a synbio-engineered genetic control construct