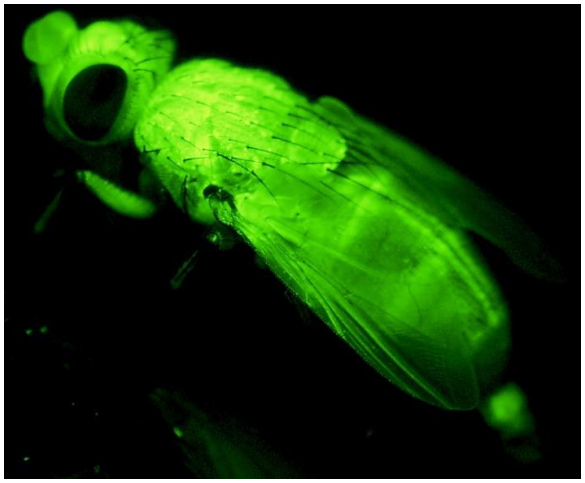
Insect Biotechnology: Current Uses and Future Developments

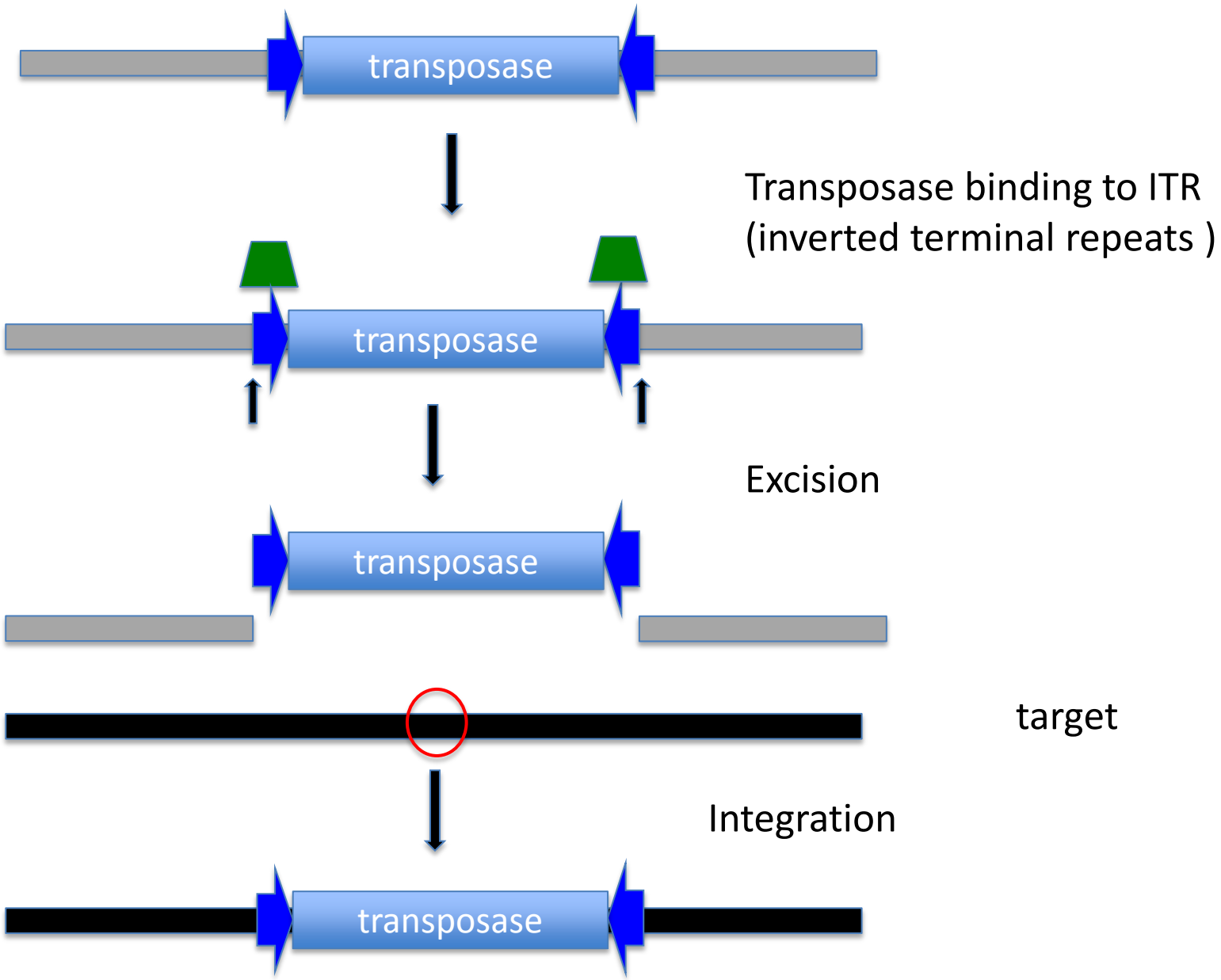


This presentation is mostly based on that of Max Scott
Thanks for sharing the slides

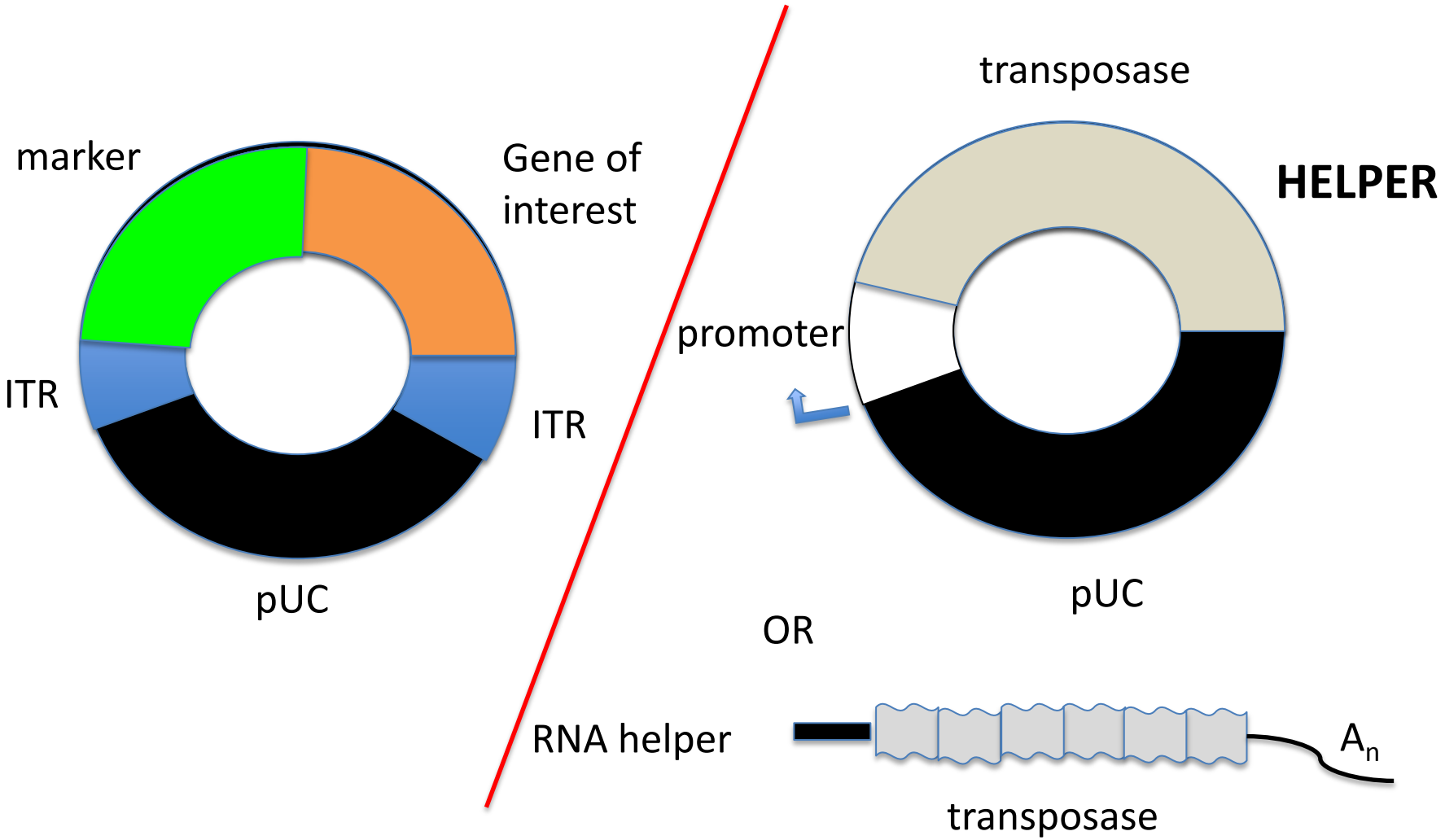
DNA transposons for making GM insects

- ***piggyBac*** from the cabbage looper moth *Trichoplusia ni*
- ***Mos1*** a *mariner* family element from *Drosophila mauritiana*
- ***Minos*** a *mariner family* element from *Drosophila hydei*
- ***P element*** from *Drosophila melanogaster*
- ***Hermes*** *a hAT* family element from the housefly *Musca domestica*

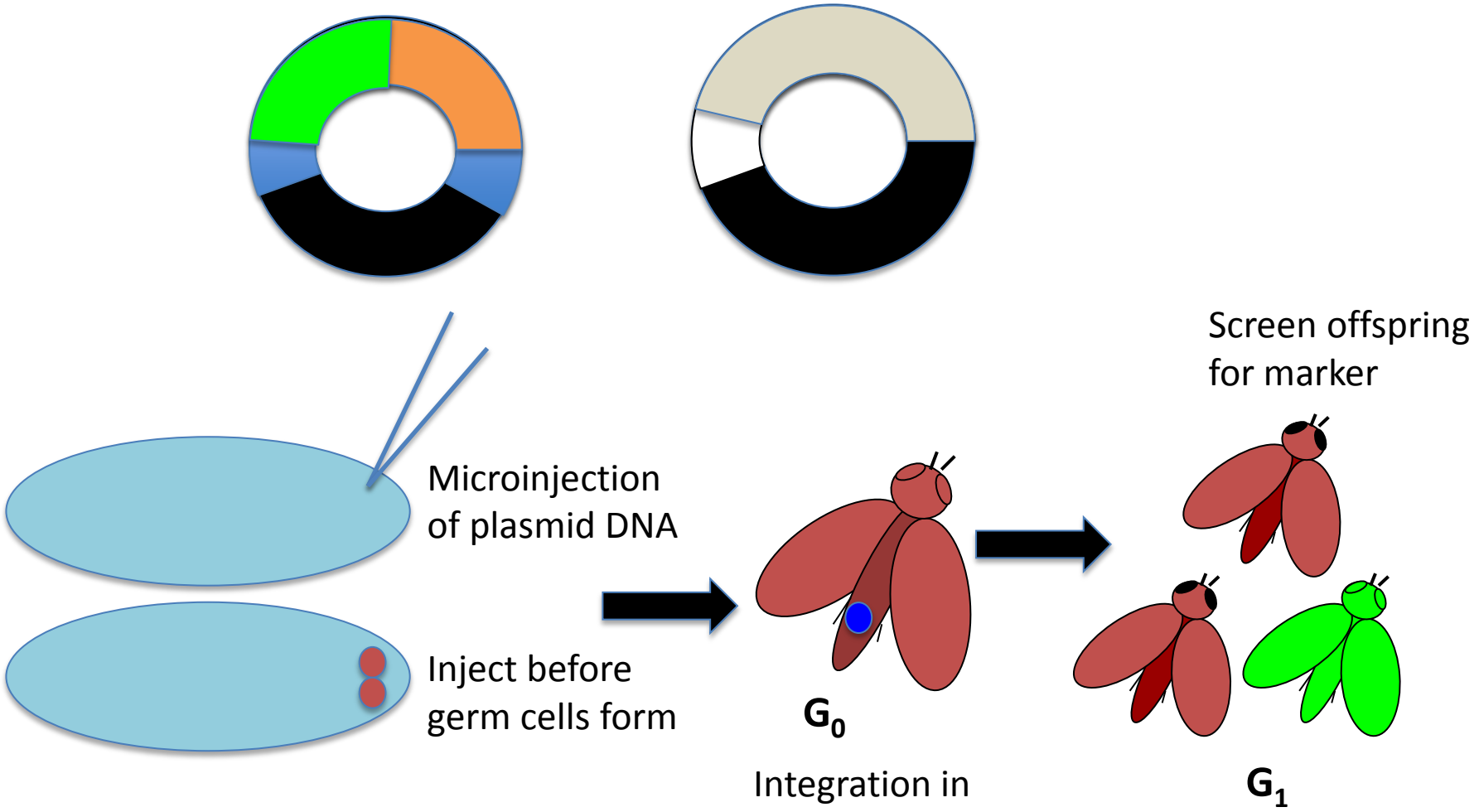
Cut-out, Paste-In transposition mechanism



For germ-line transformation: ITRs and transposase are separated



Generation of GM insect



Other systems for making transgenic insects

- site-specific recombination. e.g. phiC31 recombinase catalyses recombination between attB and attP sequences. Requires prior integration of a attP or attB site
- CRISPR/Cas9 (gene editing). Transgene can be inserted by flanking with 1kb “arms” homologous to a specific region of the genome

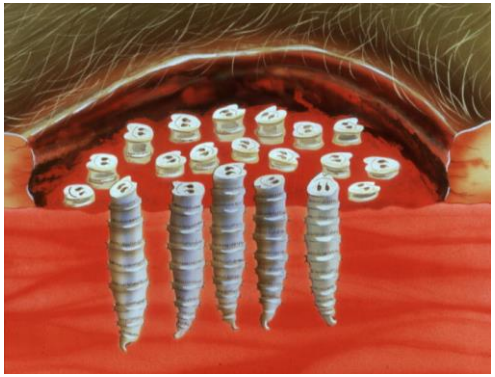
New World Screwworm (NWS) fly: *Cochliomyia hominivorax* (Coquerel)

- A major pest of warm-blooded animals
- Eradicated from North and Central America using the Sterile Insect Technique (SIT)
- There is not anywhere else in the world

Photos from Steve Skoda, USDA

THE SCREWWORM LIFE CYCLE

EGGS



LARVAE

PUPAE

ADULT

21 DAYS

HISTORY OF SCREWWORM

- ***Cochliomyia hominivorax* (Coquerel)**
  - *"hominivorax"* – "man eater"
  - Primary or New World Screwworm

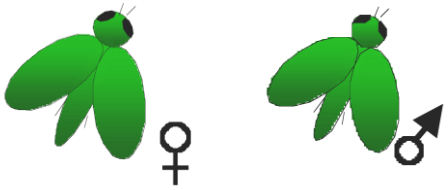
- First identified by Charles Coquerel (1858)

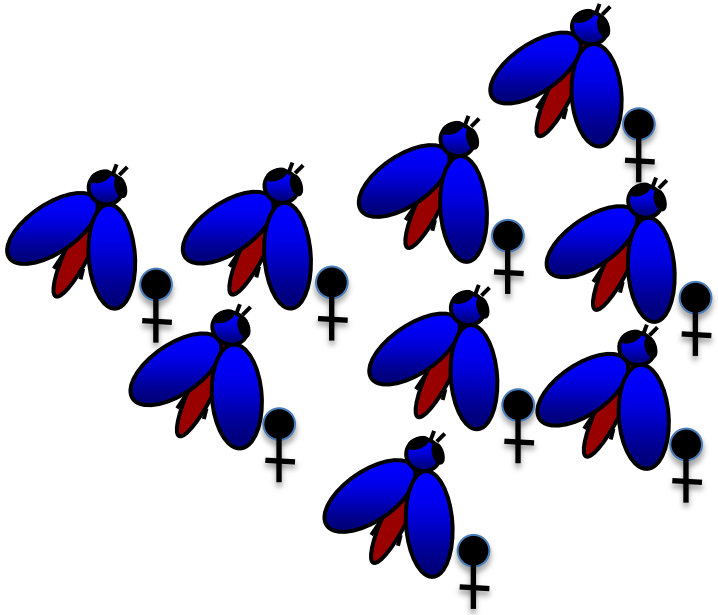
- Cushing & Patton (1933) – *C. hominivorax* recognized as an obligate parasite, separate from *C. macellaria*, the secondary screwworm.

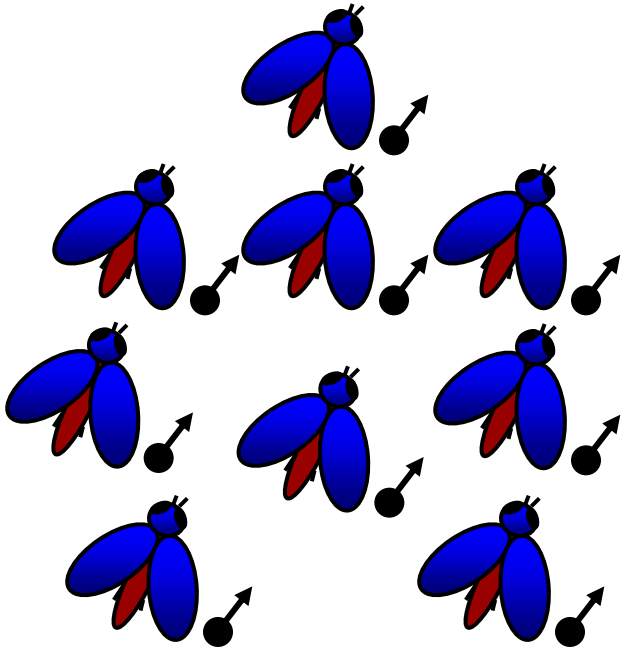
C. macellaria is present at much higher densities in the field than *C. hominivorax*

Charles Coquerel

NWS SIT Program: Repeated releases of sterile males and females





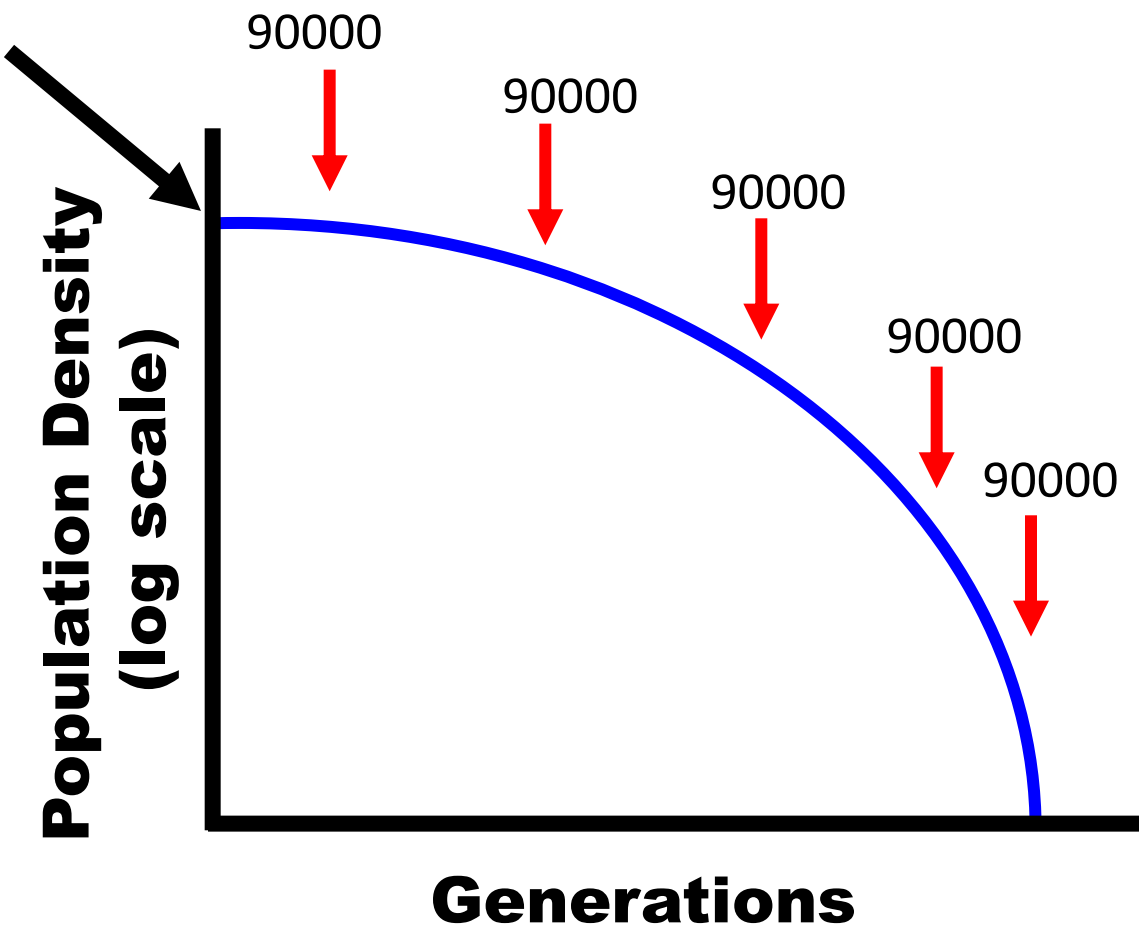


During winter months the adult population in nature would not average more than 20 flies per square mile

A 10X excess of irradiated males, in multiple releases, necessary to control the species.

Release of females too risky. Therefore, separation before release

Local population
10,000 males



HISTORY OF SCREWWORM PROGRAM

Área desde México hasta Panamá: 2.5 millón km²

Mass Rearing Plant, Pacora Panama

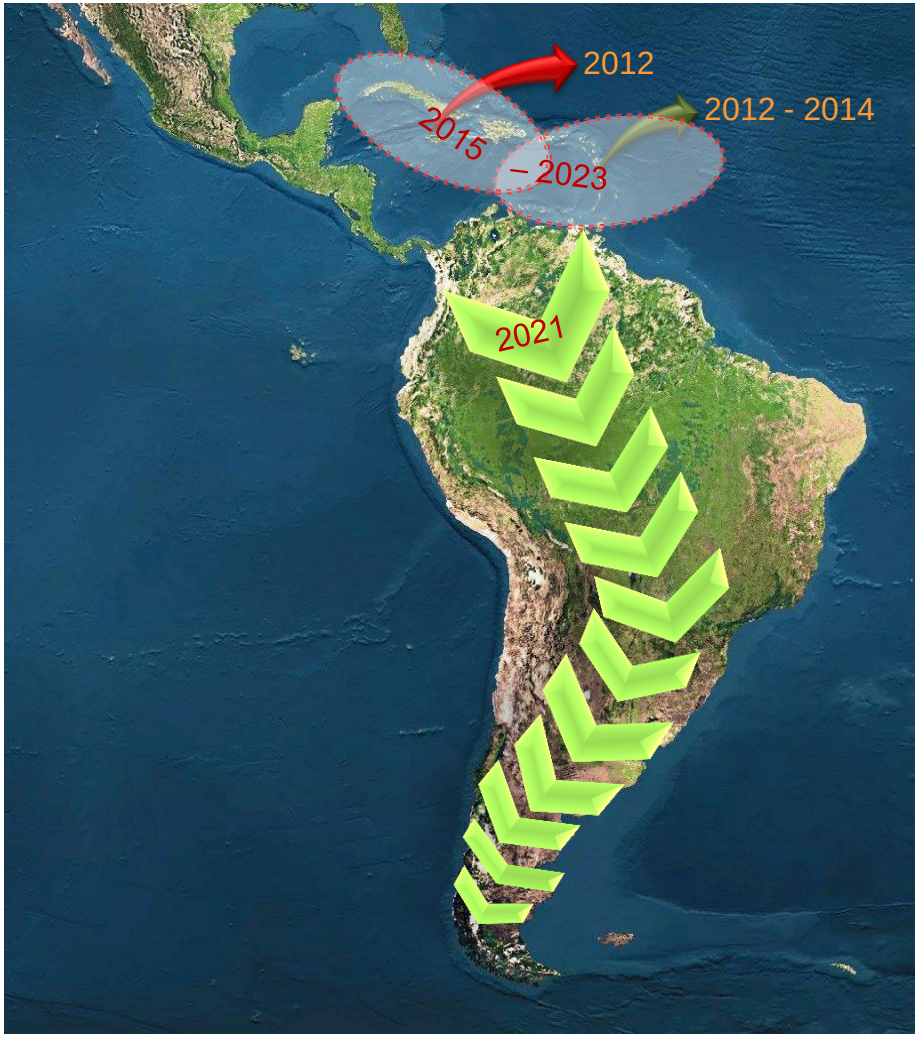
Before release the pupae are irradiated
High dose necessary to ensure 100% sterility of females (and males).
Some loss of male fitness

Keys to SIT success

- Area wide control program. Aerial or ground distribution. GPS-guided.
- Mass rearing economical.
- Sterile flies (males) competitive in the field.
- Green technology as species-specific.
- Multiple, regular releases of sterile flies in 10-fold excess required for effective suppression
- Relatively expensive costing about $1 billion over 60 years. However… annual savings $1.3-$1.5 billion in lost production *(and animal suffering, both livestock and wildlife)*

Strategic Goals

- **2012** Eradication from Jamaica
- **2012** – **2014** Diagnose situation in the Caribbean and initiate funding projects
- **2015** – **2023** Eradication from the Caribbean
  - 2012 – 2021 Collect information on incidence & geographic distribution in South America
- **2021** Initiate operations in South America



HOJA DE RUTA

“Of course it would be a tremendous amount of work to separate males from females…. The efficiency of the treated males as competitors of the males would of course be reduced since they would only engage in a fraction of as many matings with the wild females if they had the artificially bred ones with them“ (HJ Muller, back in 1950)

- Sterile females do not contribute to genetic suppression and compete with fertile females for matings with sterile males
- Releasing only males increases efficiency of genetic suppression at least 3 fold.

The logical, modern approach, would be to develop a male-only strain: less rearing costs, increased safety

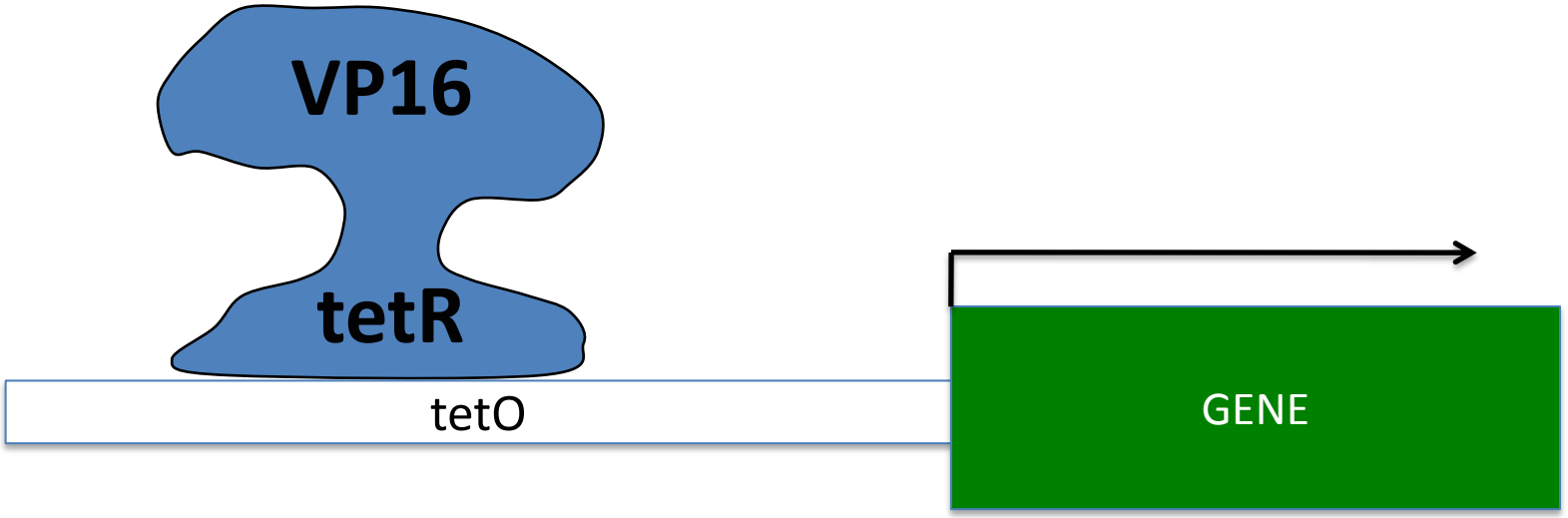
Male-only strain

Transgenic sexing strain: It has a female-specific conditional lethal gene that is switched off in the mass rearing factory by addition of tetracycline to the diet.

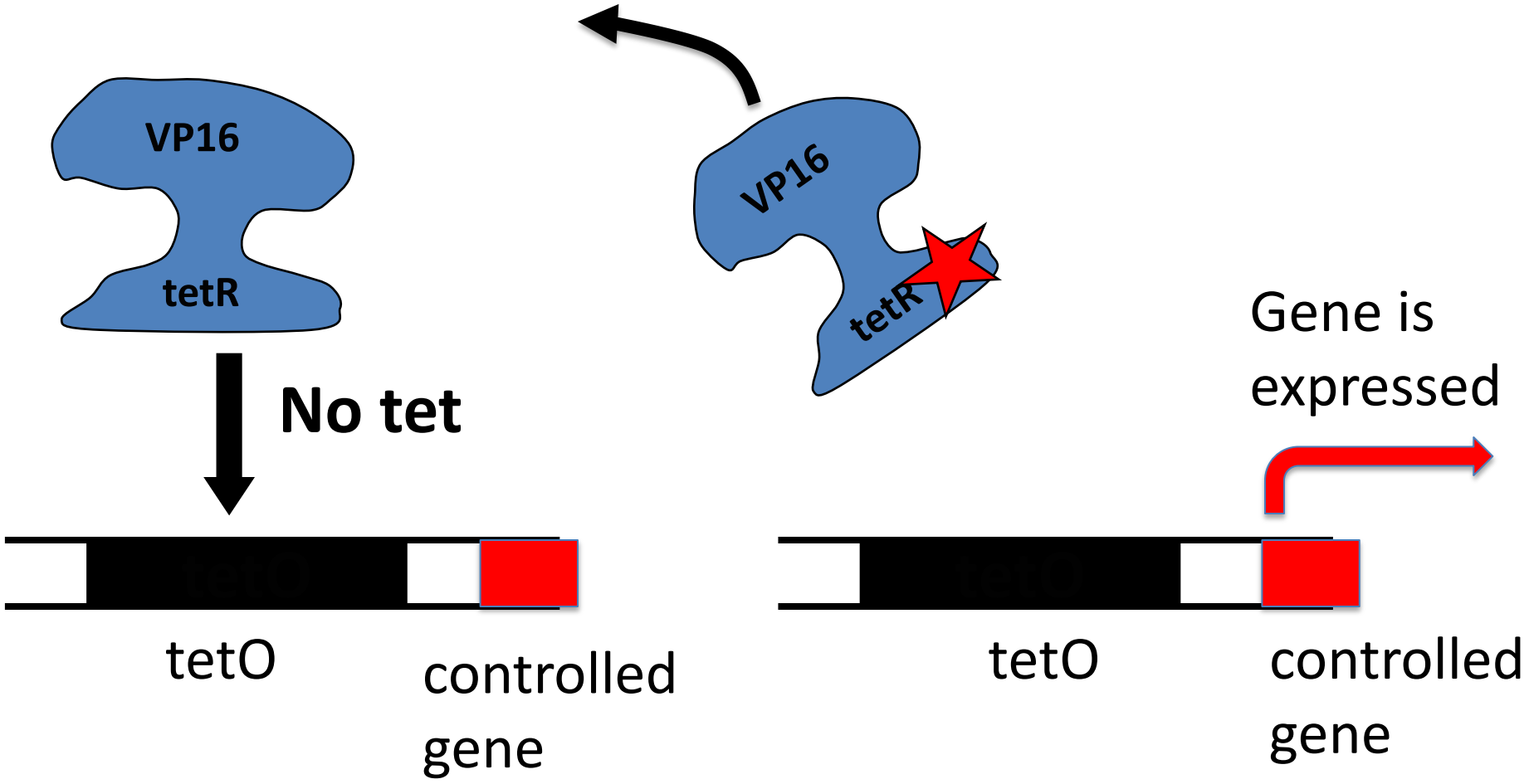
Condition Expression Systems: tet-OFF

A fusion of the DNA binding domain of the *E. coli* tet repressor (tetR) and the transcription activation domain from the HSV1 VP16 protein. DNA binding site is called tetO or TRE.

Whatever gene downstream from the tetO promoter **will be under control (repression)** of tetracycline

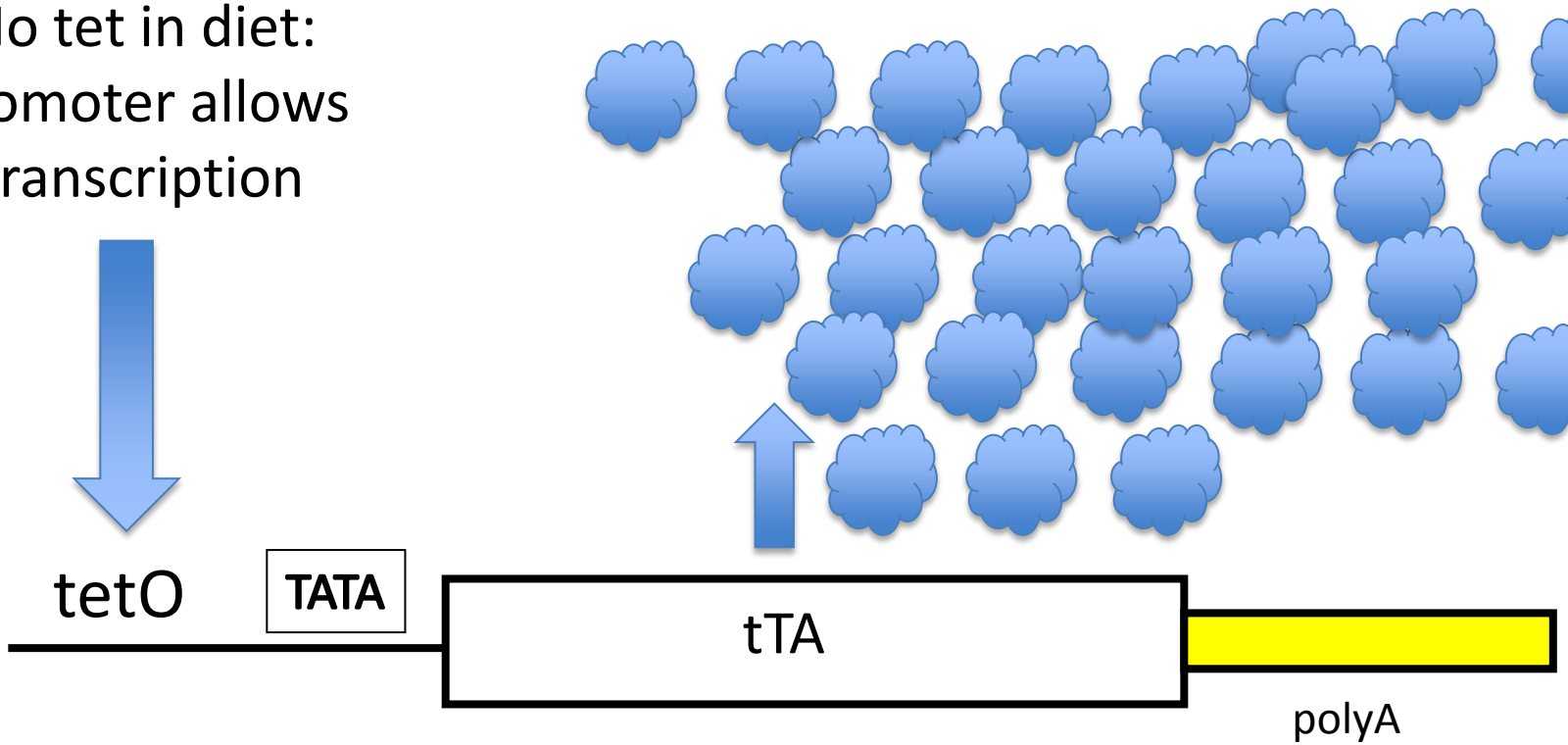


Binding of the tTA Fusion Protein to DNA is Inhibited by Tetracycline: tet OFF



Transcription (and translation) of the tTA gene leads to high intracellular concentration of the transcription activator (TA)

No tet in diet: promoter allows transcription



Insect die late in development (pupae) due to "transcription squelching" because yoo much of one transcription activation factor causes a general interference in gene transcription

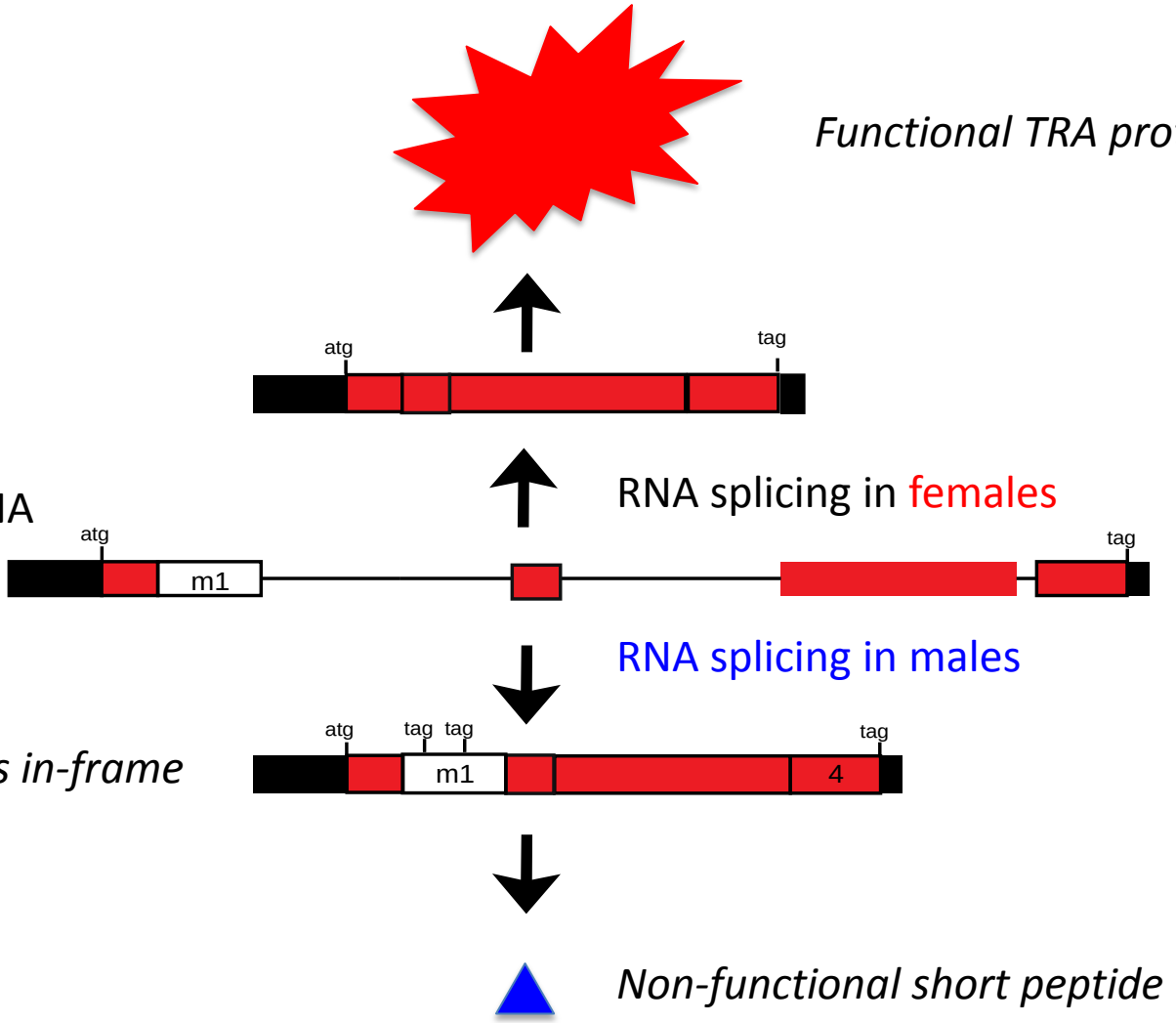
Gong et al [2005] Nature Biotech, 23, 453. medfly
Phuc et al [2007] BMC Biol, 5, 11. Aedes aegypti

- Brazil has approved the commercial release of transgenic Aedes aegypti with tTA overexpression system(since 2014)
- FDA has recently approved its controlled release

Florida Keys Approves Trial Of Genetically Modified Mosquitoes To Fight Zika

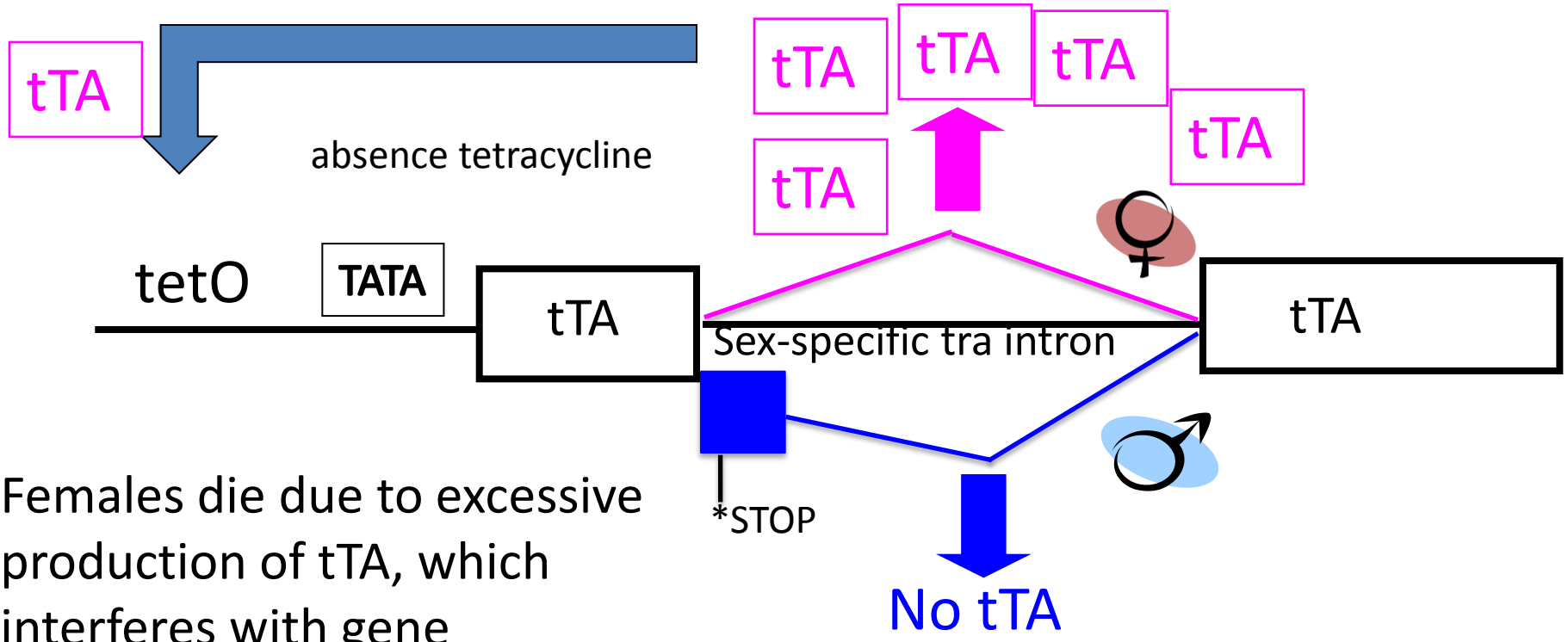
Company site: http://www.oxitec.com/programmes/united-states/
Opposition: http://www.genewatch.org/sub-566989
FDA: https://www.fda.gov/animalveterinary/developmentapprovalprocess/geneticengineering/geneticallyengineeredanimals/ucm446529.htm

Sex-specific RNA splicing of *transformer* (*tra*) in flies



Regulatory elements required for sex-specific splicing are <u>all</u> in the first intron

Sexing strain: Only females make tTA and they die due to feedback loop producing high levels of tTA.

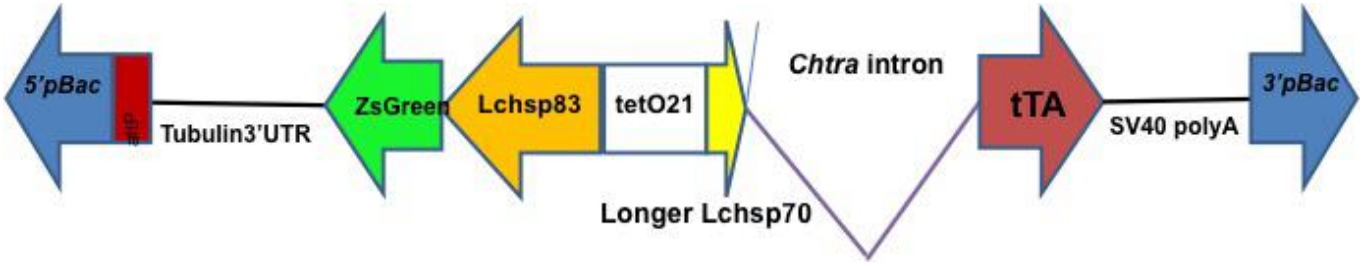


Females die due to excessive production of tTA, which interferes with gene transcription

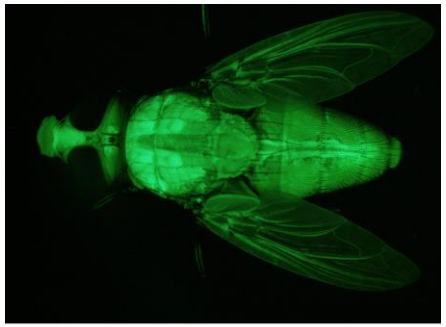
100% female lethal in screwworm, sheep blow fly and Drosophila

Li et al [2014] Insect Biochem Mol Biol; Concha et al [2016] BMC Biology

GM *Cochliomyia hominivorax*



FL12



Transgenic sexing strains have been developed for several insect species but not field tested*

Sheep blowfly and screwworm*

Moths: diamondback*, silk and pink bollworm

Ae. aegypti and *Ae. albopictus*

Fruit flies: medfly, mexfly and olive fly

Other systems

- **Female embryo sexing systems**. Two component. tTA expressed mostly in early embryo stage only. Female death due to expression of cell death gene. Early female death saves diet. Developed for sheep blowfly, screwworm and medfly.
- **Chromosome shredding**. Chromosomes degraded by nuclease in sperm. All offspring die. Analogous to radiation. X-shredder in *An. gambiae* a special case version of this approach.
- **Gene drives.** Next talks…

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