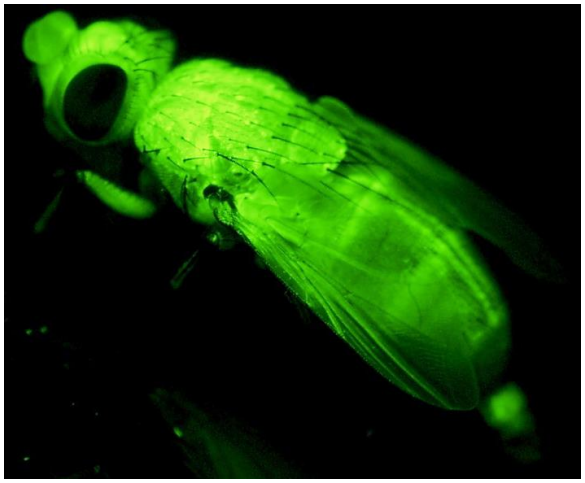
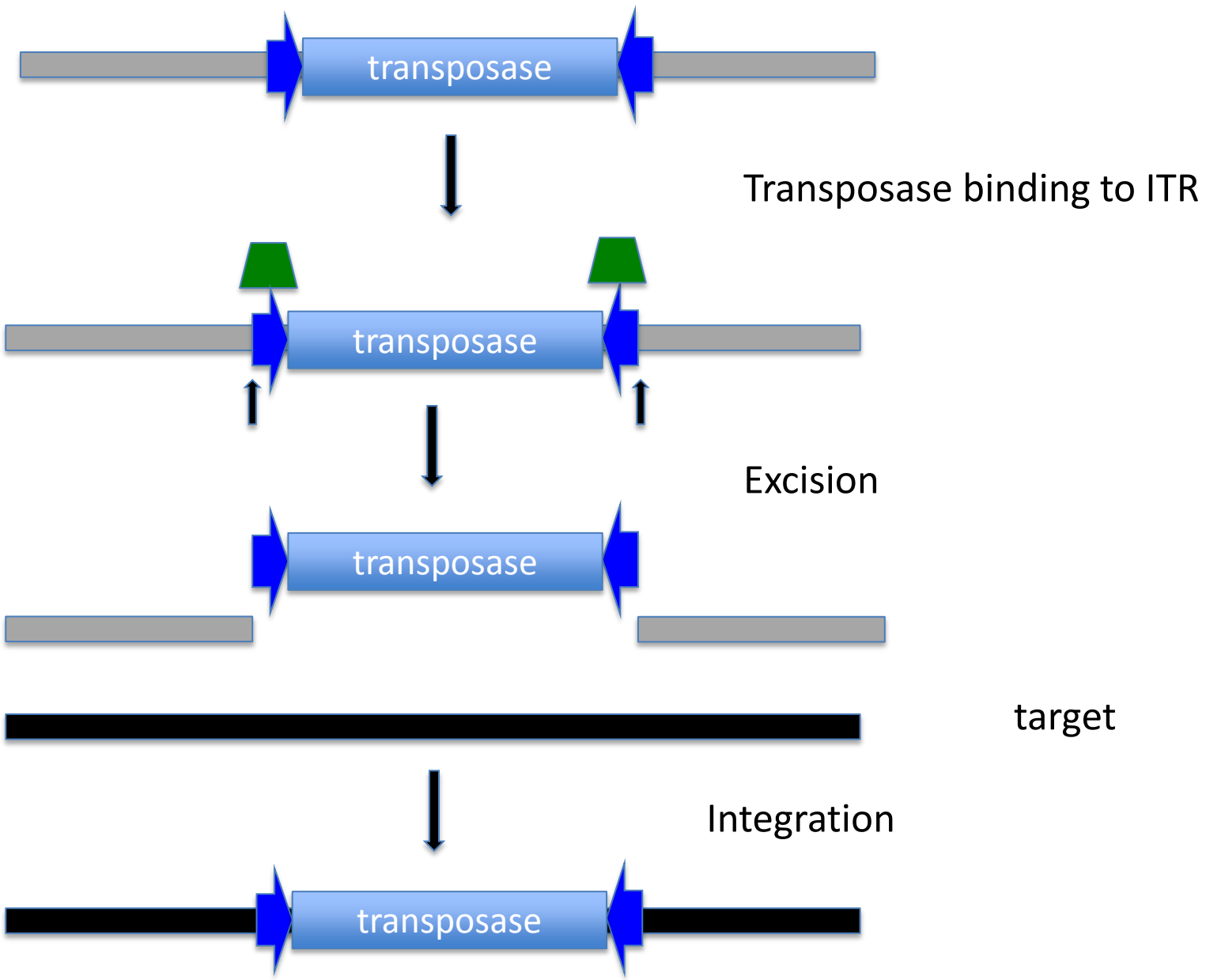
Insect Biotechnology: Current Uses and Future Developments



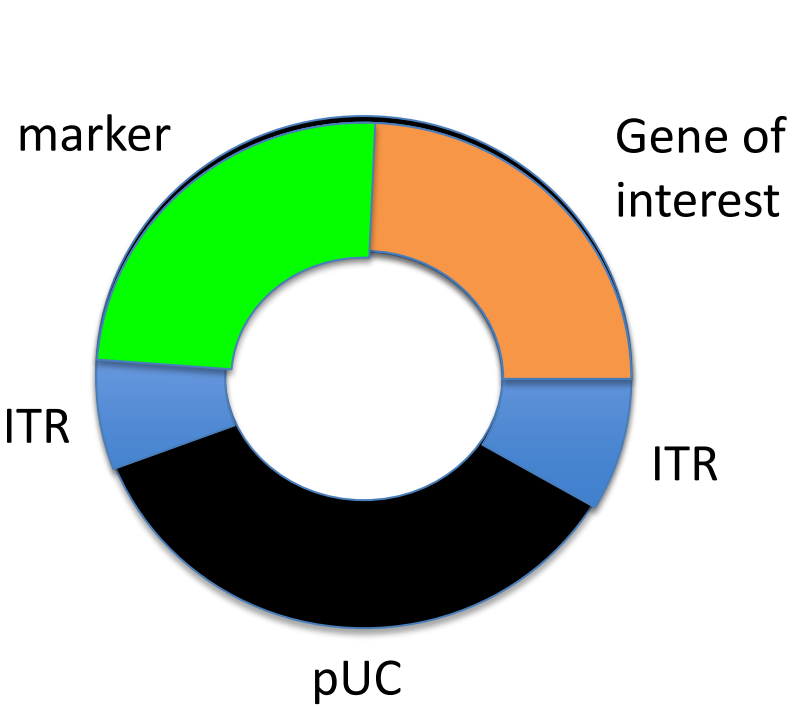
DNA transposons for making GM insects

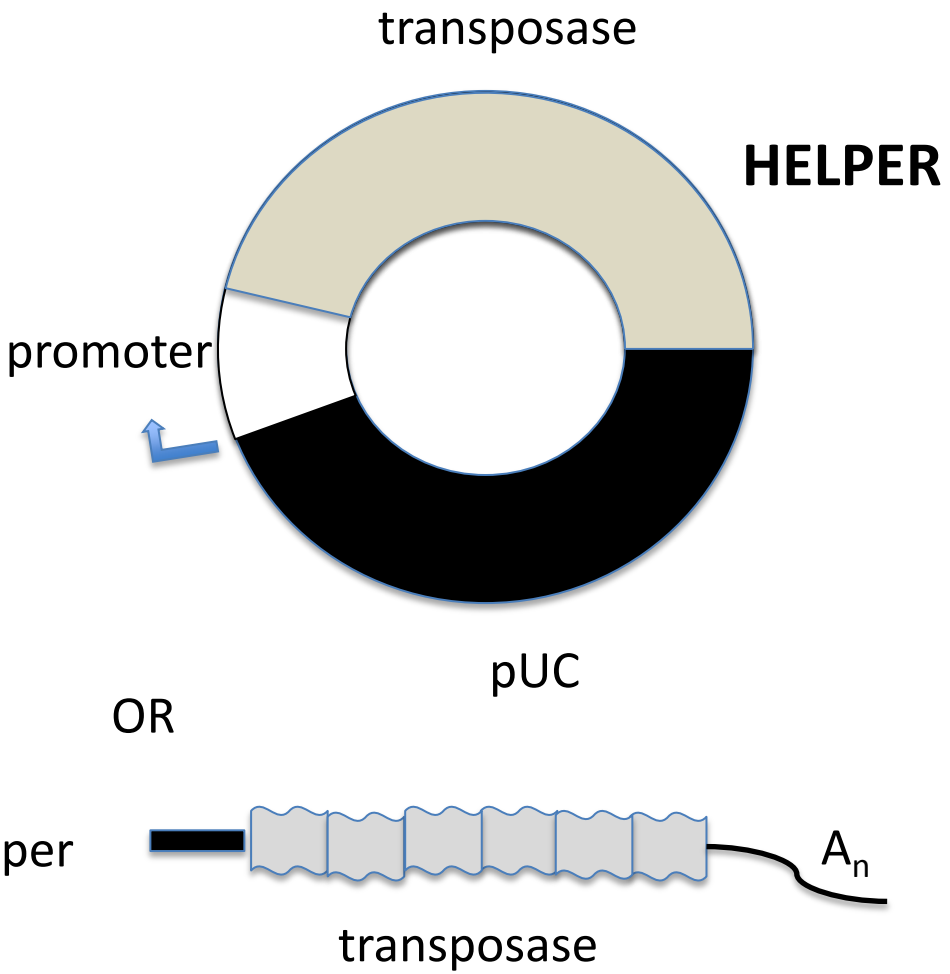
- ***piggyBac*** from the cabbage looper moth *Trichoplusia ni*
- ***Mos1*** a *mariner* family element from *Drosophila mauritiana*
- ***Minos*** a *mariner family* element from *Drosophila hydei*
- ***P element*** from *Drosophila melanogaster*
- ***Hermes*** *a hAT* family element from the housefly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

Cut-out, Paste-In transposition mechanism

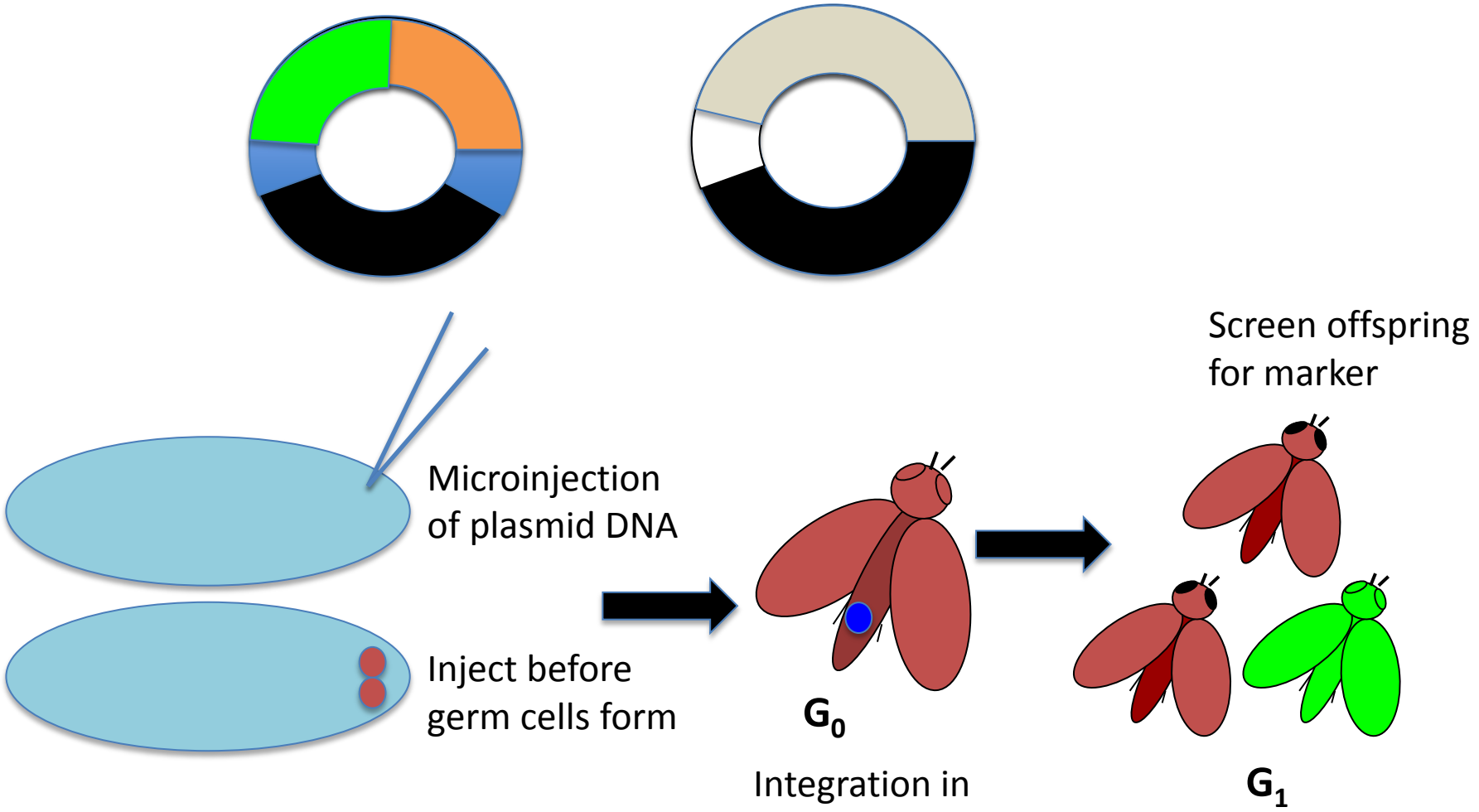


For germ-line transformation: ITRs and transposase are separated

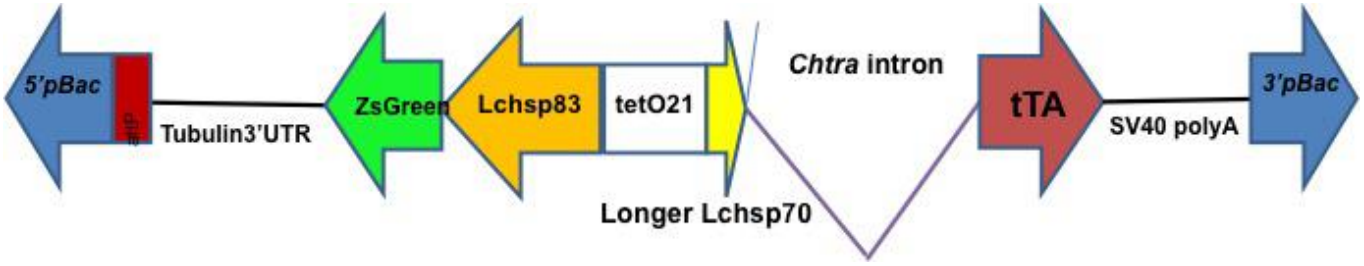




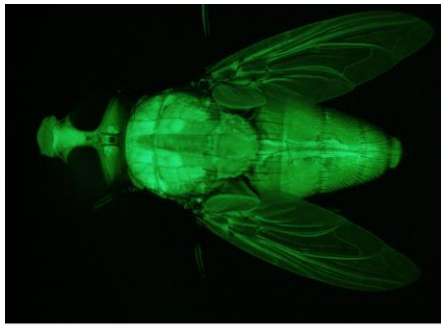
Generation of GM insect



GM *Cochliomyia hominivorax*



FL12



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. Transgene can be inserted by flanking with 1kb “arms” homologous to a specific region of the genome

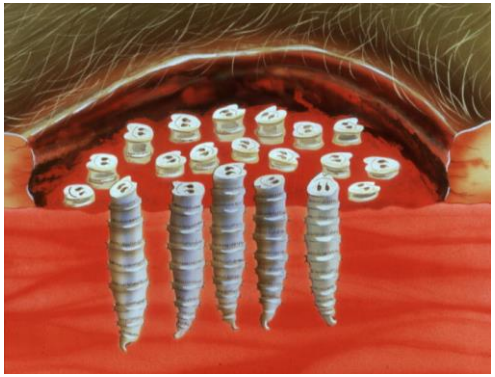
New World Screwworm (NWS) fly: *Cochliomyia hominivorax* (Coquerel)

- A major pest of warm-blooded animals
- Eradicated from North and Central America using the Sterile Insect Technique (SIT)

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

March 6, 1950

Dr. H. J. Muller
Indiana University
Bloomington, Indiana

Dear Dr. Muller:

I have read with interest your article entitled "Radiation Damage to the Genetic Material" published in the January 1950 issue of American Scientist.

Some of my associates and I have for some years given thought to the possibility of utilizing damaging effects to genetic material as an aid in the control of certain insects. I shall attempt to explain the possibilities that have been considered, and I would appreciate an opinion from you as to possible merits of such approach and whether or not research along this line to determine its feasibility would be warranted.

There are a number of insect species of economic importance which at times at least are present in nature in what might be considered small numbers. Yet under controlled laboratory conditions some of these insects can be reared in large numbers.

Copies sent to Rohwer, Lindquist, Parker 3-15-50

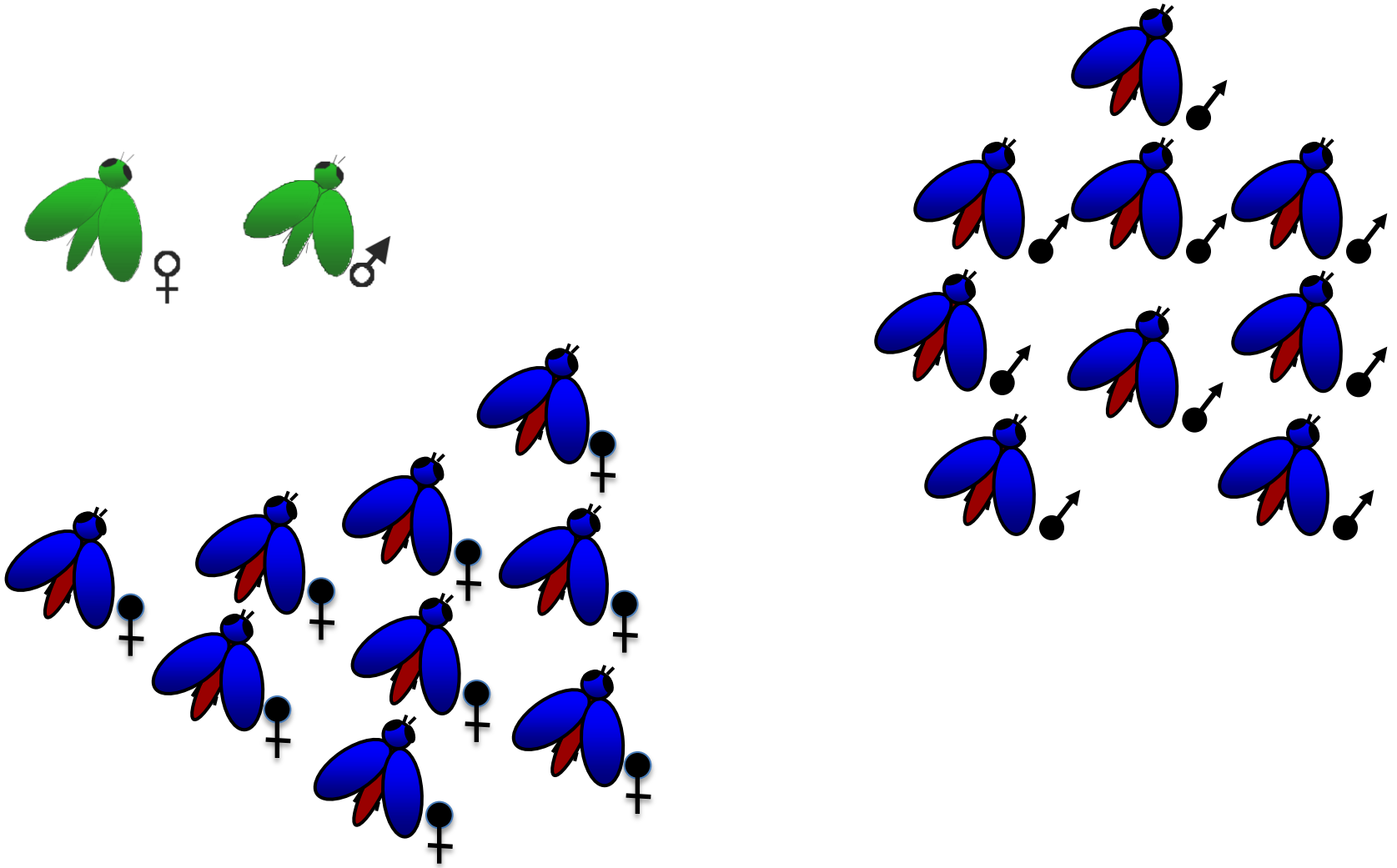
Copy to Pearson AEC 3-20-50

One insect in this category is the blow fly, Cochliomyia americana, which is an extremely important pest of livestock, particularly in the Southern states. In nature it develops only as a parasite of warm-blooded animals. The flies deposit eggs on wounds of living animals, and the larvae feed on the flesh. Multiple infestations due to repeated attack by flies may occur in the wound, and if animals are not treated, the parasite will eventually cause death of the host. The developmental periods of the different stages are as follows: eggs, 1 day; larvae in wounds, 5 days; pupae, 10 days. (During cold weather pupae might require 30-40 days.) Actual death losses or serious injury are exceedingly high among domestic and wild mammals in certain parts of the nation. The fly is about three times as large as the common house fly. Although precise information is not available, we estimate that during winter months the adult population in nature would not average more than 20 flies per square mile. The range of distribution during this unfavorable

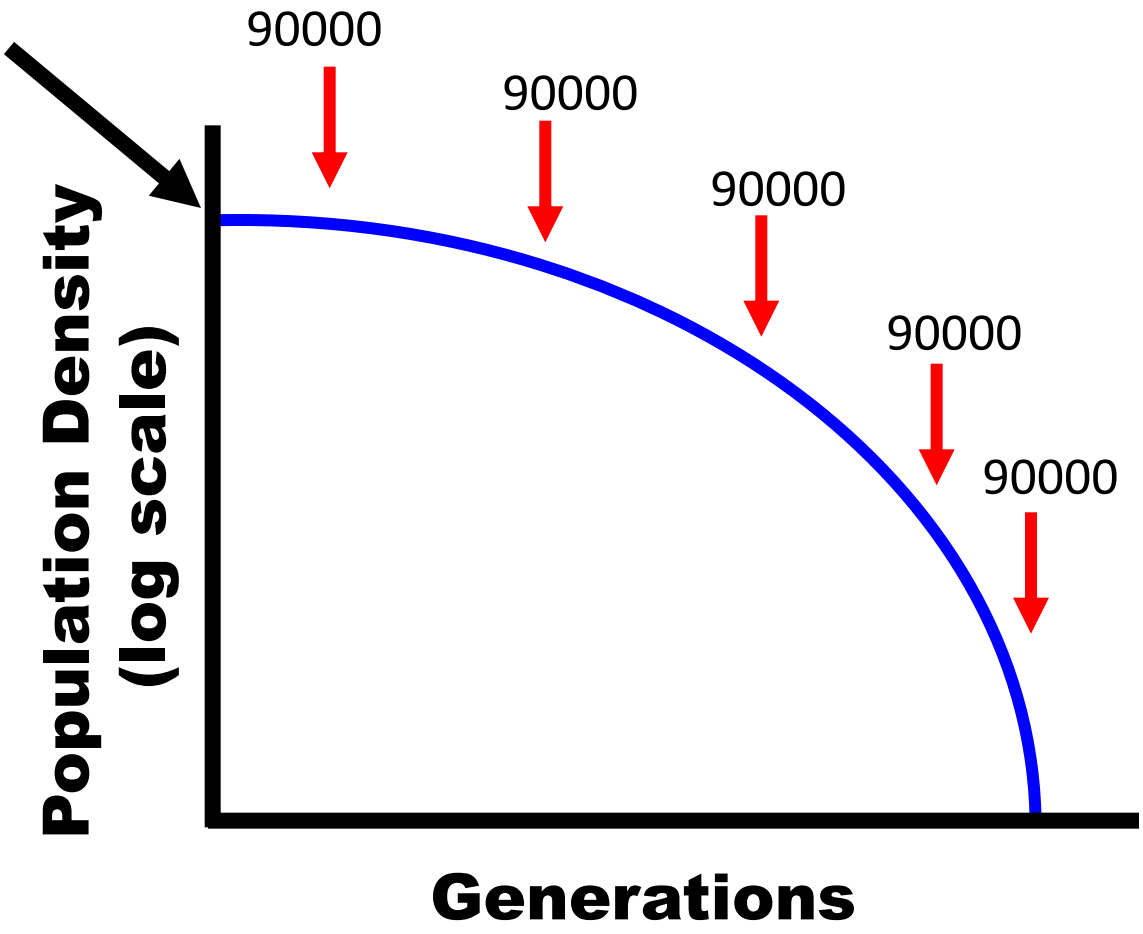
and to the lower third of Texas in the Southwest. In the spring and summer it increases in numbers and spreads to one-fourth to one-third the nation. If the species could be eradicated in Florida,

"we estimate that during winter months the adult population in nature would not average more than 20 flies per square mile"

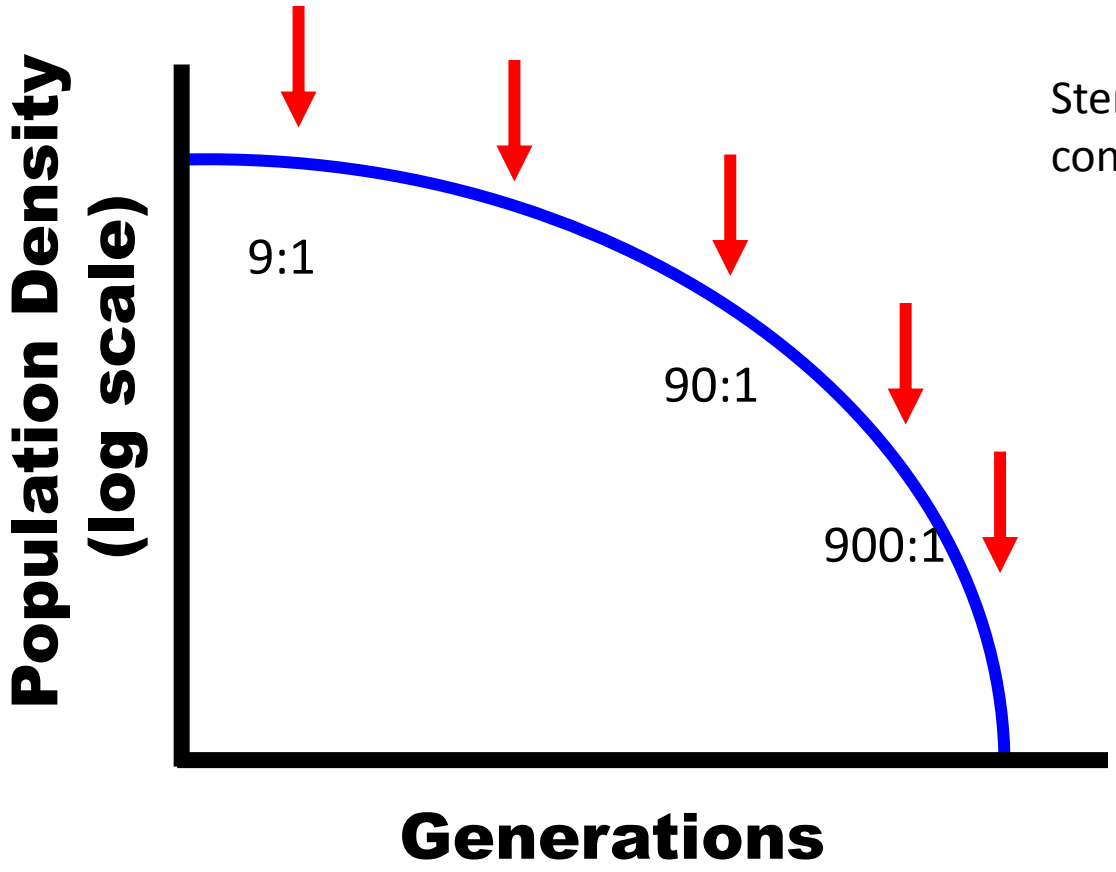
NWS SIT Program: Repeated releases of sterile males and females



Local population
10,000 males



Species-specific population decline:
A GREEN technology!

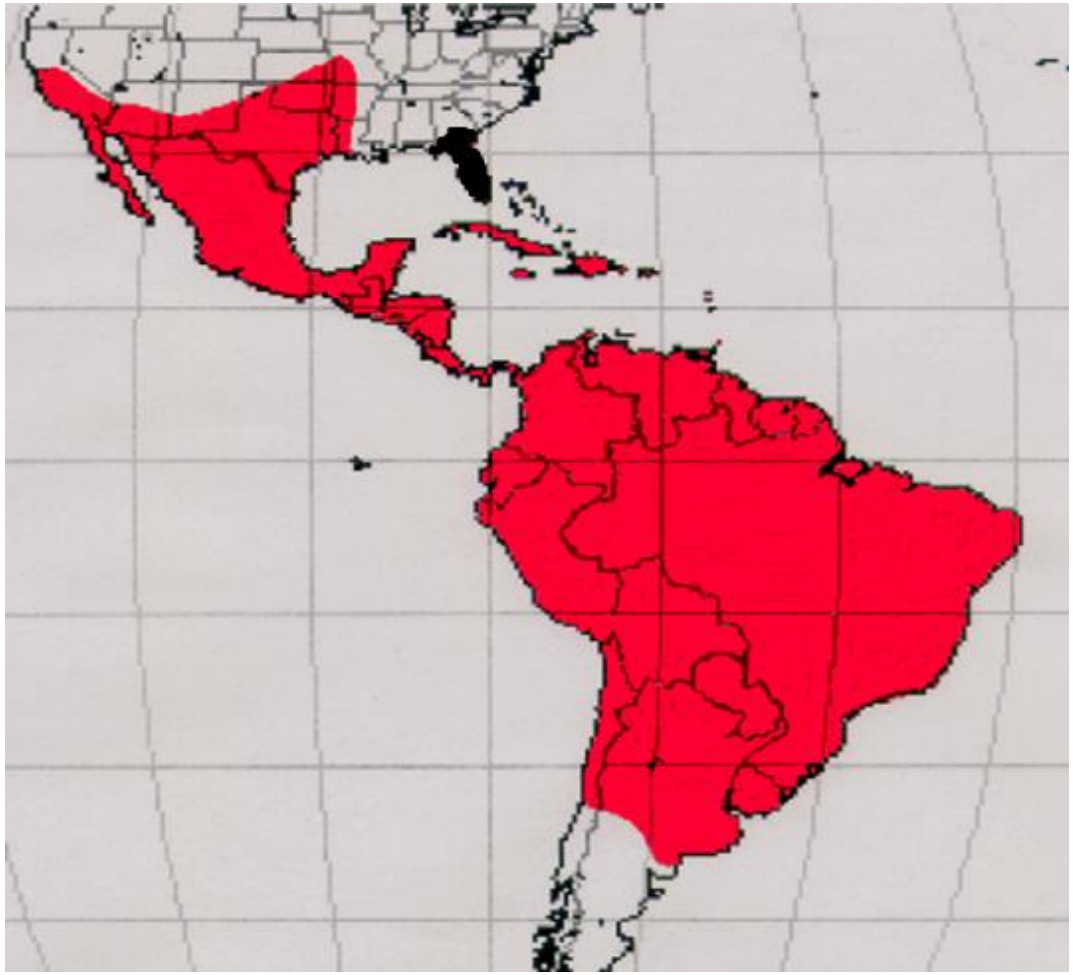


Sterile flies are the control agent

HISTORY OF SCREWWORM PROGRAM

NWS

- Original Distribution 1933
- Expanded into SE US, including most of Florida, by 1950's



HISTORY OF SCREWWORM PROGRAM

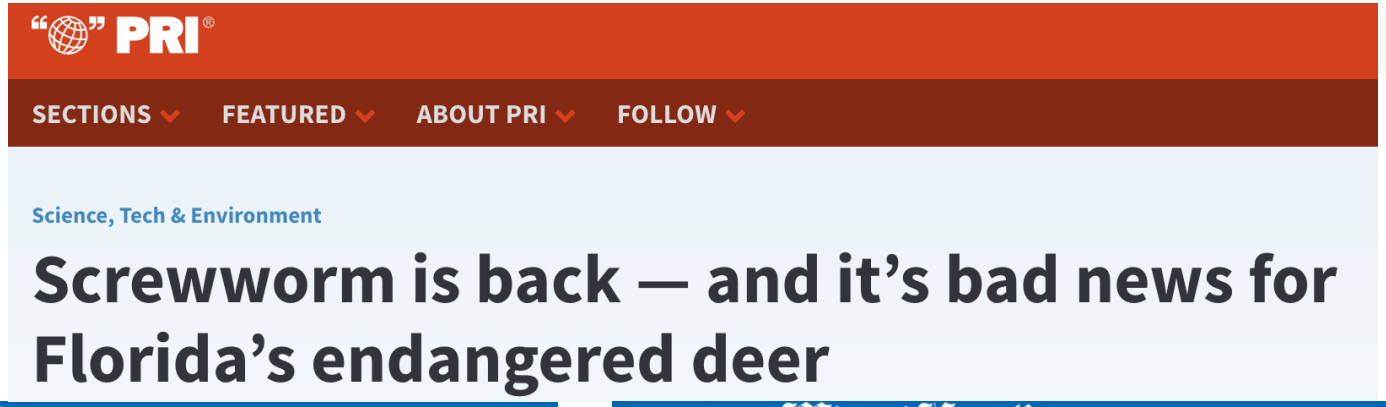
Área desde México hasta Panamá: 2.5 millón km²

LOCAL NOVEMBER 2, 2016 9:13 AM

Sterile screwworm flies now released in Marathon

FLORIDA KEYS NEWS

Screwworm Questions & Answers



ENVIRONMENT JANUARY 10, 2017 5:31 AM

Flesh-eating screwworm appears outside Florida Keys in stray dog

FLORIDA KEYS FEBRUARY 4, 2017 1:06 PM

More than 101 million sterile screwworm flies have been released in the Keys

The Special Brand of Horror that is the New World Screwworm

Sen. Nelson calls for increased funding to fight screwworm

Mass Rearing Plant, Pacora Panama

Mass Rearing

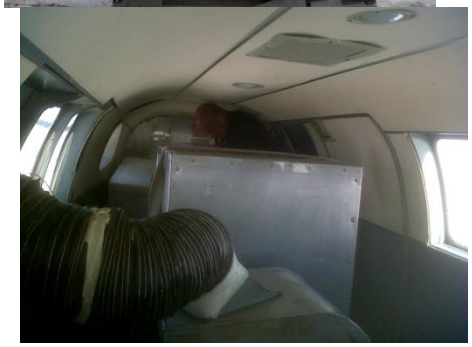
60,000 flies per cage

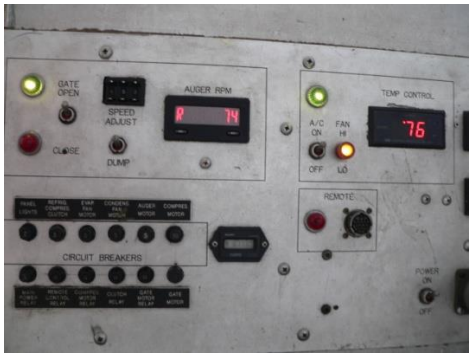
20-40 million pupae per week

Before release the pupae are irradiated.
High dose ensures 100% sterility of females and males.
Some loss of male fitness

Pupae taken to the Dispersal Center in Coolers, Each cooler holds 22 liters

Dispersal Equipment

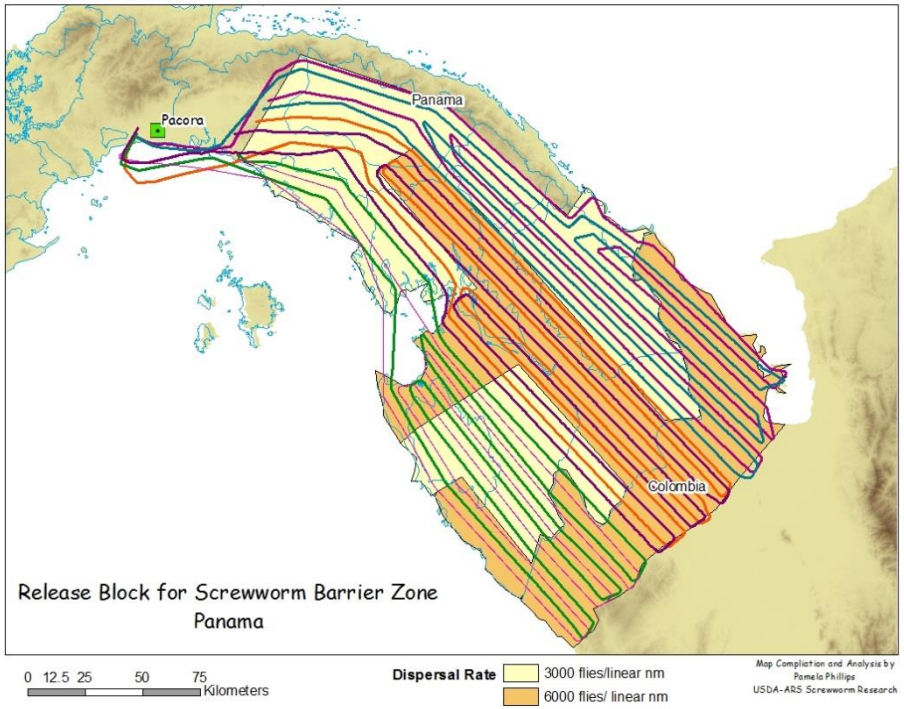




- 4 Beechcraft King Air aircraft
  - Equipped with APHIS PPQ designed dispersal machines (1996)
  - Motor-driven screw auger mounted with 2 air conditioned release boxes –hold approximately 230 liters of **chilled flies**
  - Release rate controlled by varying the speed of auger or aircraft

Aerial Release of Sterile Screwworms in Panama Barrier

SIT Release Protocol for Screwworm:



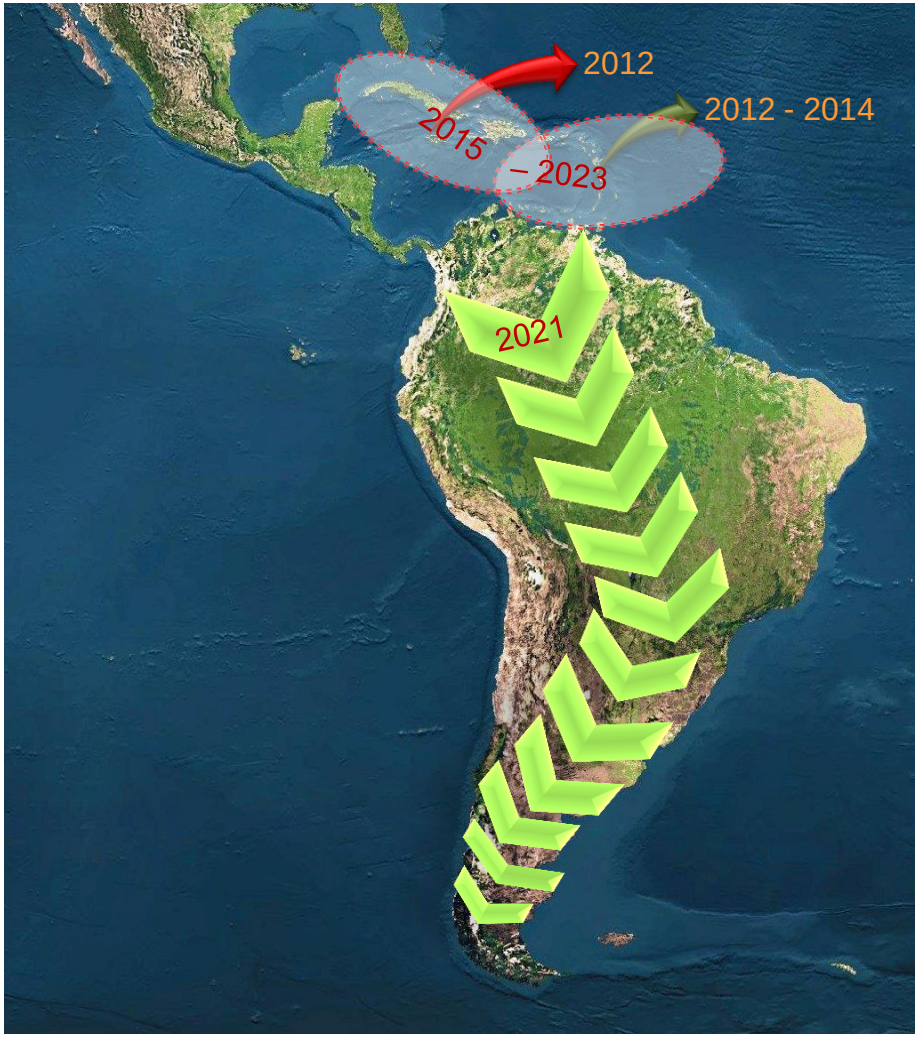
- Flight lines based on compass directions to cover entire barrier zone
- Dosage of 6,000 per linear nautical mile in Colombia And areas of high cattle density. Dosage of 3,000/lnm for remaining areas.
- During normal dispersal schedule (no outbreaks) 8 dispersal flight per week

Keys to SIT success

- Area wide control program. Aerial or ground distribution. GPS-guided.
- Mass rearing economical.
- Sterile flies 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. Annual savings $1.3-$1.5 billion in lost production.

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

H. J. Muller

INDIANA UNIVERSITY
BLOOMINGTON, INDIANA

Science Hall 101
DEPARTMENT OF ZOOLOGY

Atomes Radiation

Dr. Knipling EFK.
Mr. Stage
Dr. Henderson EFK
Miss Lynch

March 10, 1950

xc attached

Dr. E. F. Knipling
Division of Insects Affecting Man and Animals
Bureau of Entomology and Plant Quarantine
Agricultural Research Administration
U. S. Department of Agriculture
Washington 25, D. C.

screw worm EFK.

Dear Dr. Knipling:

I think that your very ingenious idea for reducing and perhaps eventually eliminating the blow fly in the southern states might work, provided the released sterilized males could really be gotten into such a situation as to compete effectively with those that had been breeding in "nature". That would, in my opinion, be the chief obstacle to be overcome since the phenomenon of reinfestation that you mention indicates that the chief multiplying is done "on the spot", so to speak.

Copies sent to Lauder Knipling Reiver 3-15-50

Copy to Pearson AEC 3-20-50

There is no question, I think, but that the adult males could be given a dose of radiation sufficient to practically or completely sterilize them without being materially damaged so far as their ability to live and their mating behavior are concerned. Judging by Drosophila, this would be accomplished with a dose of 10,000 roentgen units, whereas it takes about 17,000 to kill them. On the other hand, it might be more feasible to give them only about 5,000 units, which in Drosophila reduces their fertility to something like a sixth of normal, if tests showed that a dose of 10,000 was too injurious to them

Of course it would be a tremendous amount of work to separate the males from the females, for I figure that the number of flies raised would have to run into the hundreds of thousands or nearly to the millions for Florida alone if you wanted to outnumber the natural male population by some ten times. On the other hand, it would probably not be practicable to irradiate and release the females along with the males to save labor, unless it should by good fortune be found that the females were completely sterilized by the dose used. The efficiency of the treated males as competitors of the other males would of course be reduced in this way 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, whereas the competition of the sterilized females with the wild ones for the wild males would not be as great. Might there perhaps be a way of getting partial separation by some

Of course it would be a tremendous amount of work to separate males from females…. But.. 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

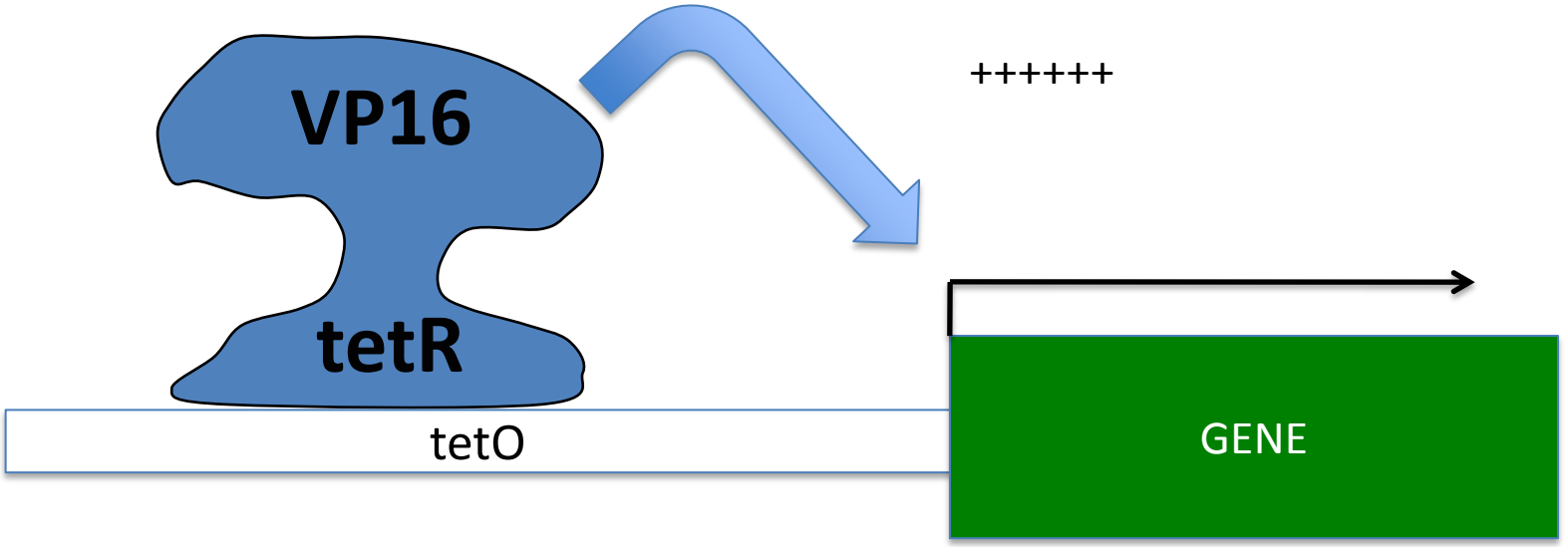
Male-only strain

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

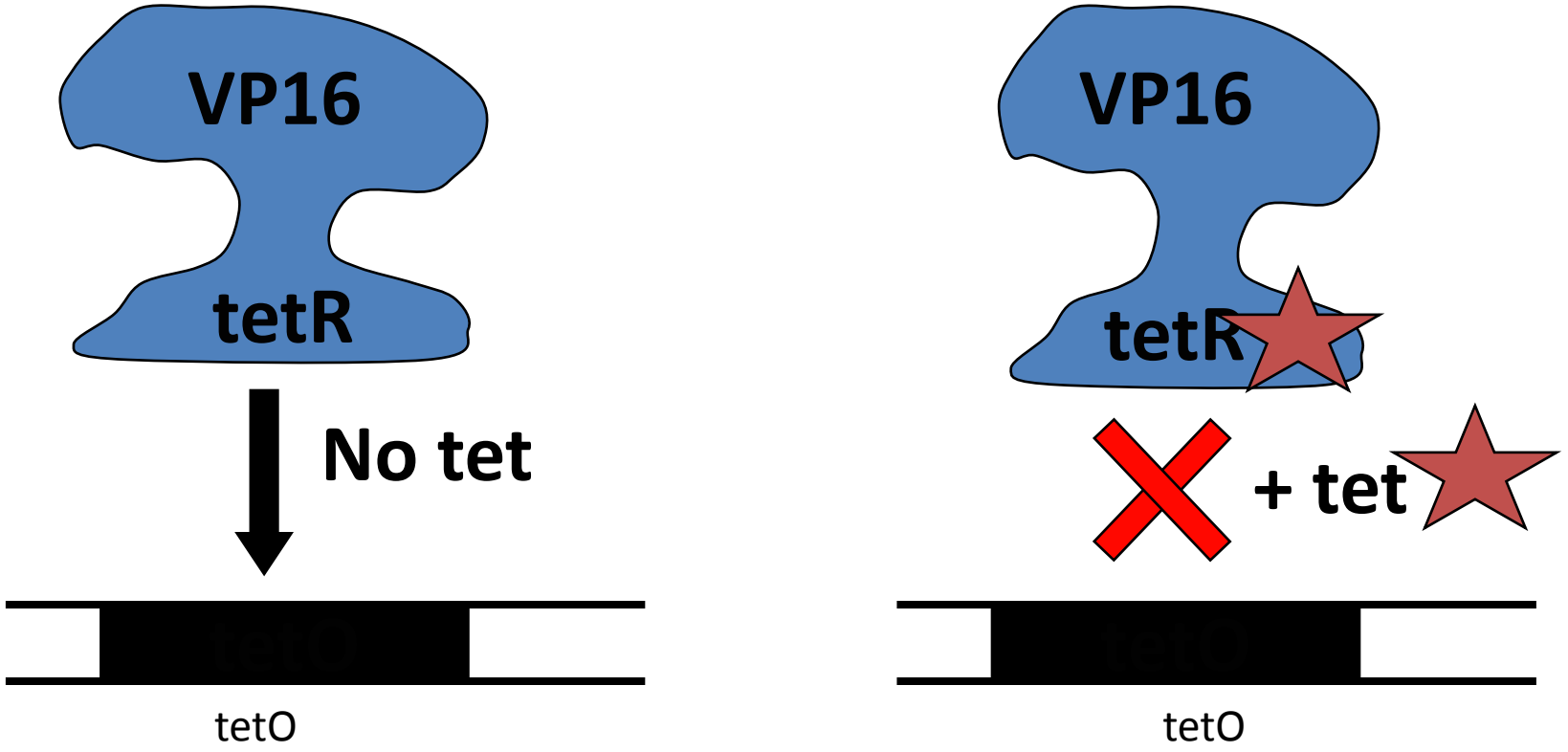
Condition Expression Systems: tet-OFF

The tetracycline dependent transactivator (tTA):

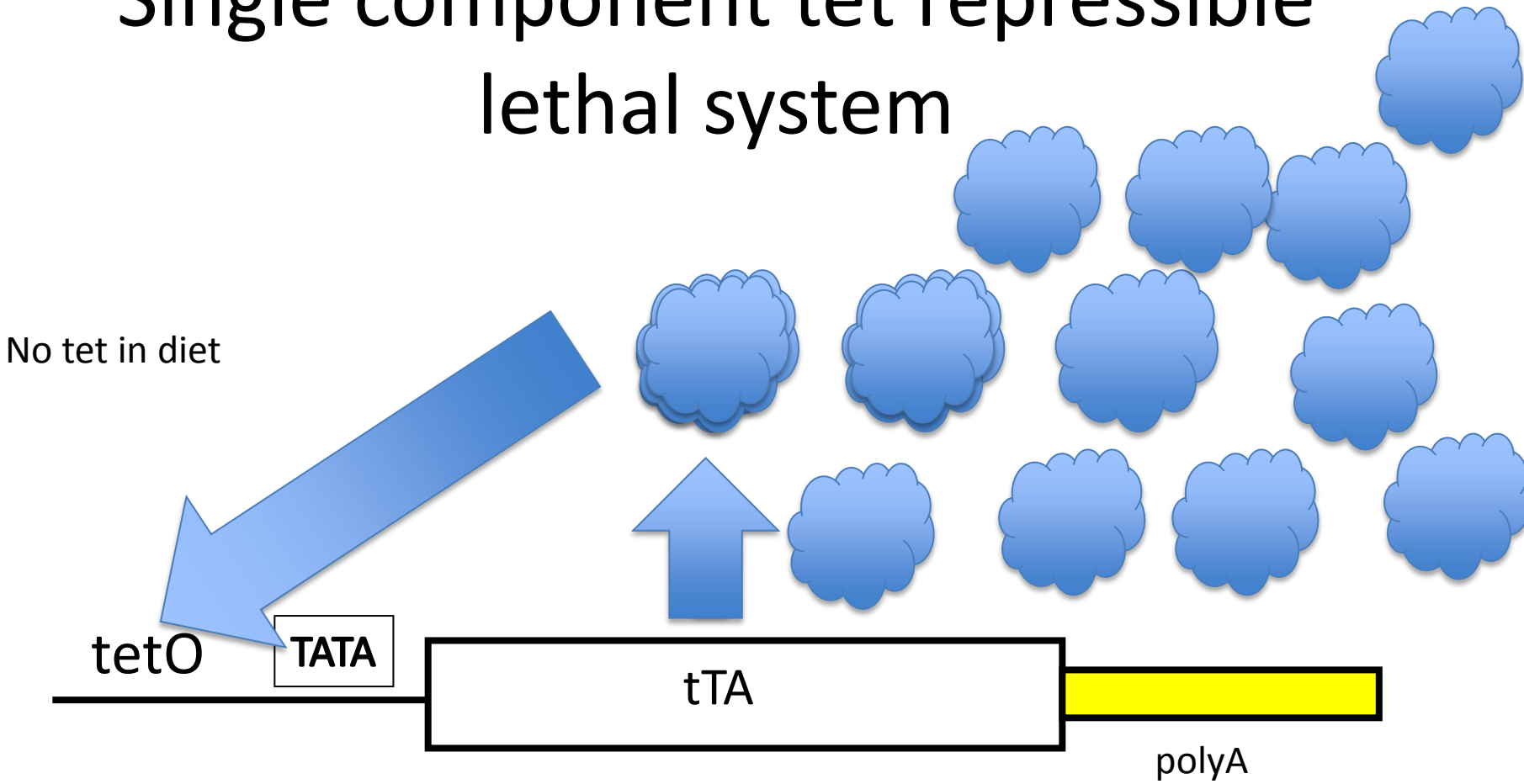
A fusion of the DNA binding domain of the *E. coli* tet repressor (tetR) and the transcription activation domain from the HSV1 VP16 protein. DNA binding site is called tetO or TRE.



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



Single component tet repressible lethal system



Insect die late in development (pupae) due to "transcription squelching"
Too much of one transcription 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

FDA has approved release of transgenic *Aedes aegypti* with tTA overexpression system

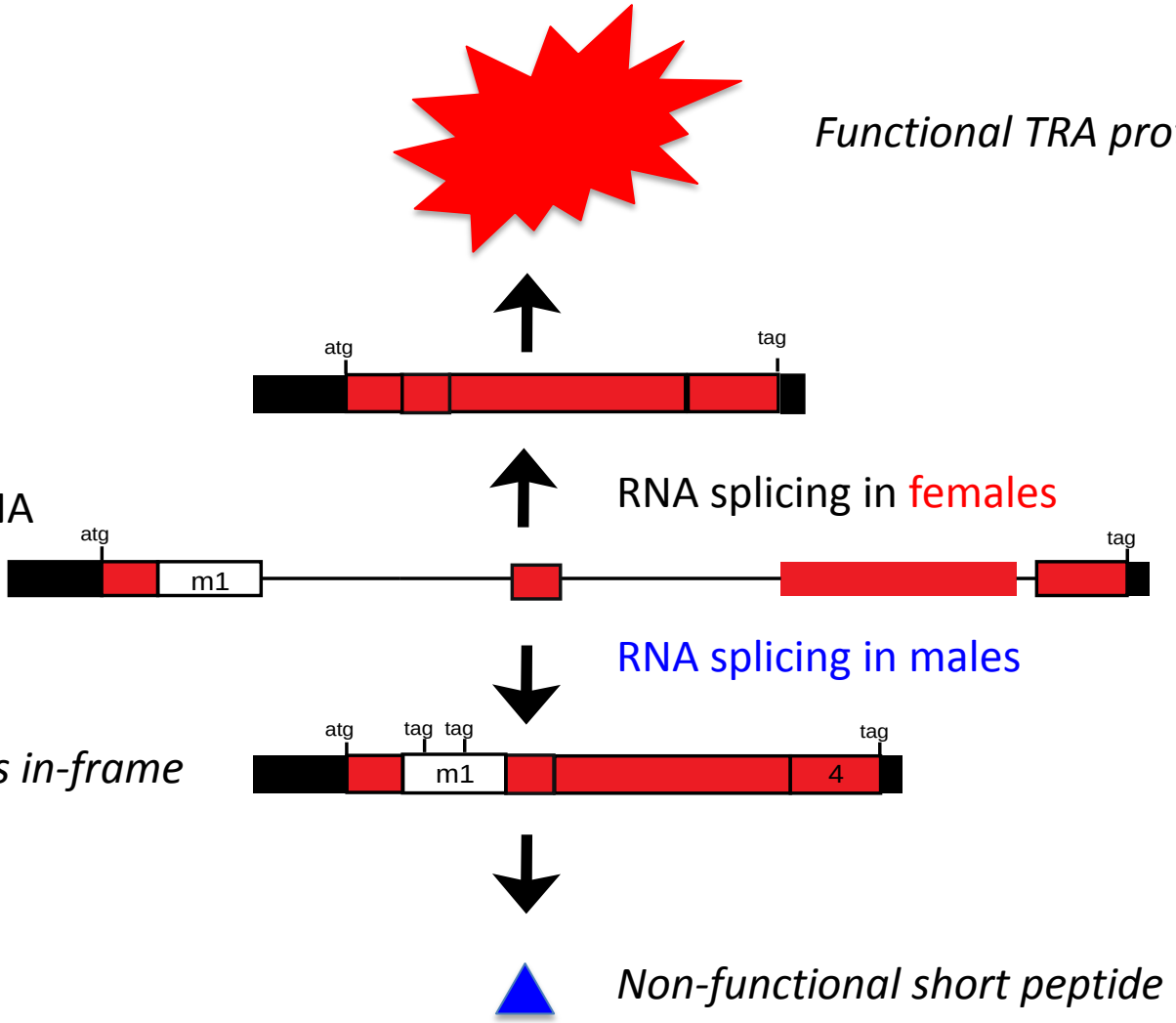
Florida Keys Approves Trial Of Genetically Modified Mosquitoes To Fight Zika

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

Transgenic sexing systems

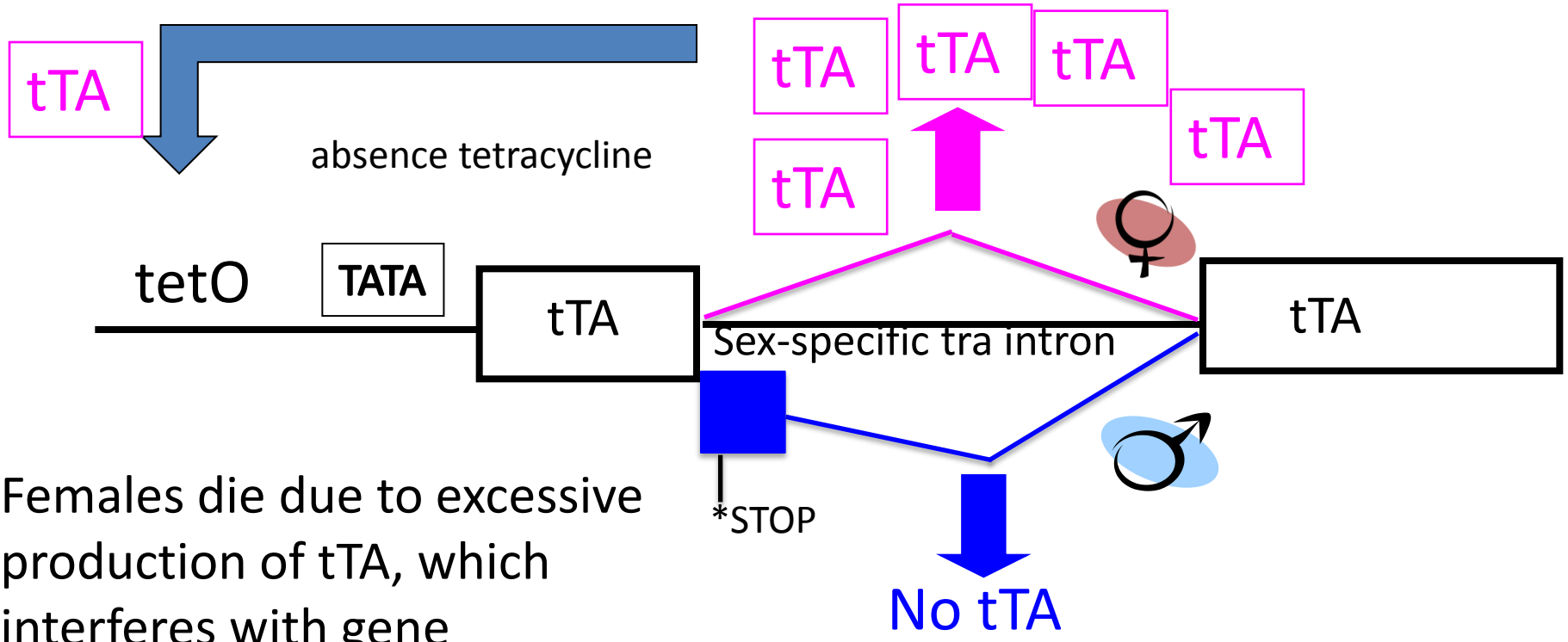
- For *Aedes aegypti*, males and females are separated mechanically based on differences in pupal size
- For other insects this may not be possible. Develop transgenic systems that have sex-specific gene expression.

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

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

Sheep blowfly and screwworm*

Moths: diamondback*, silk and pink bollworm

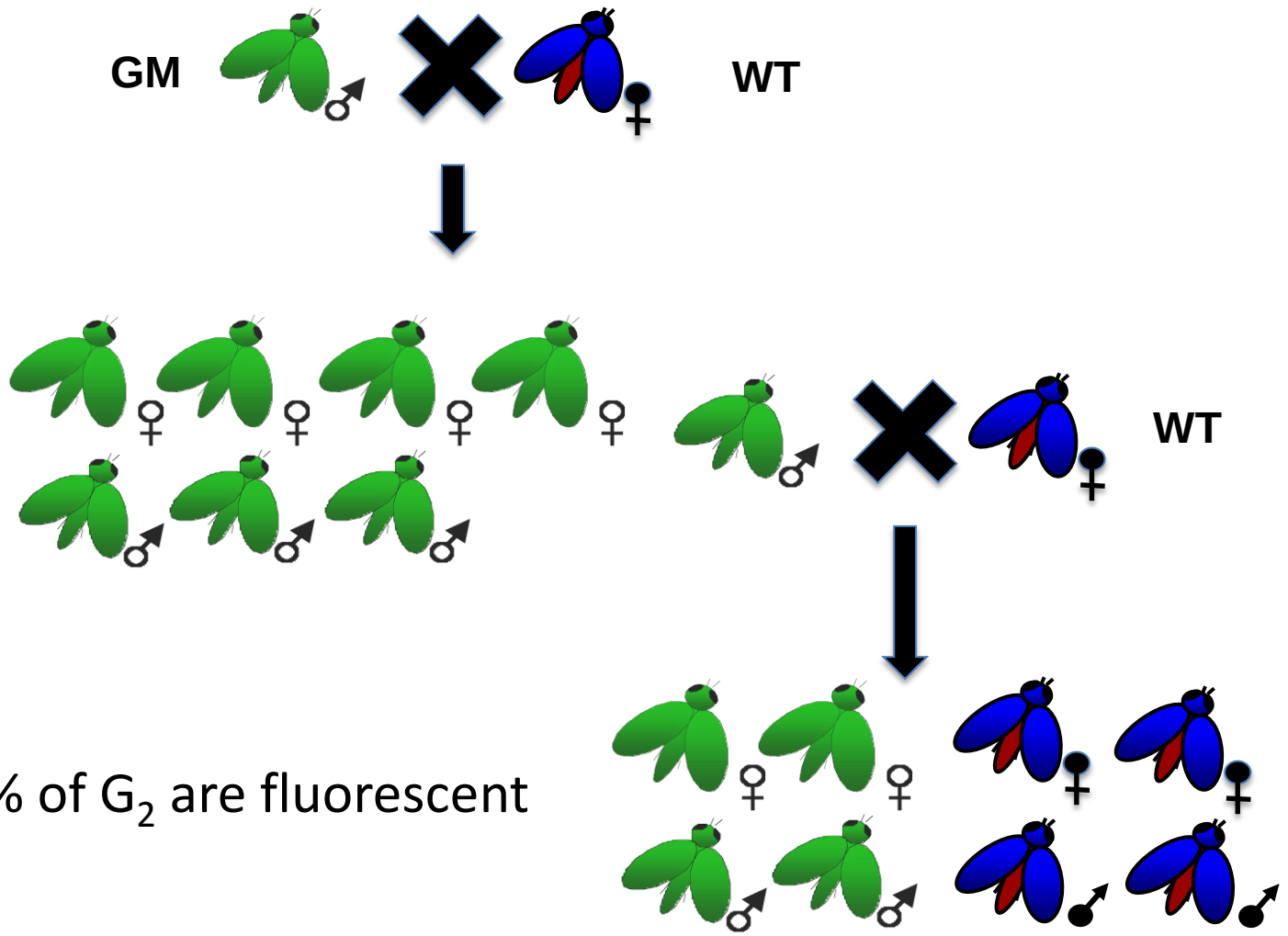
Ae. aegypti and *Ae. albopictus*

Fruit flies: medfly, mexfly and olive fly

Other systems

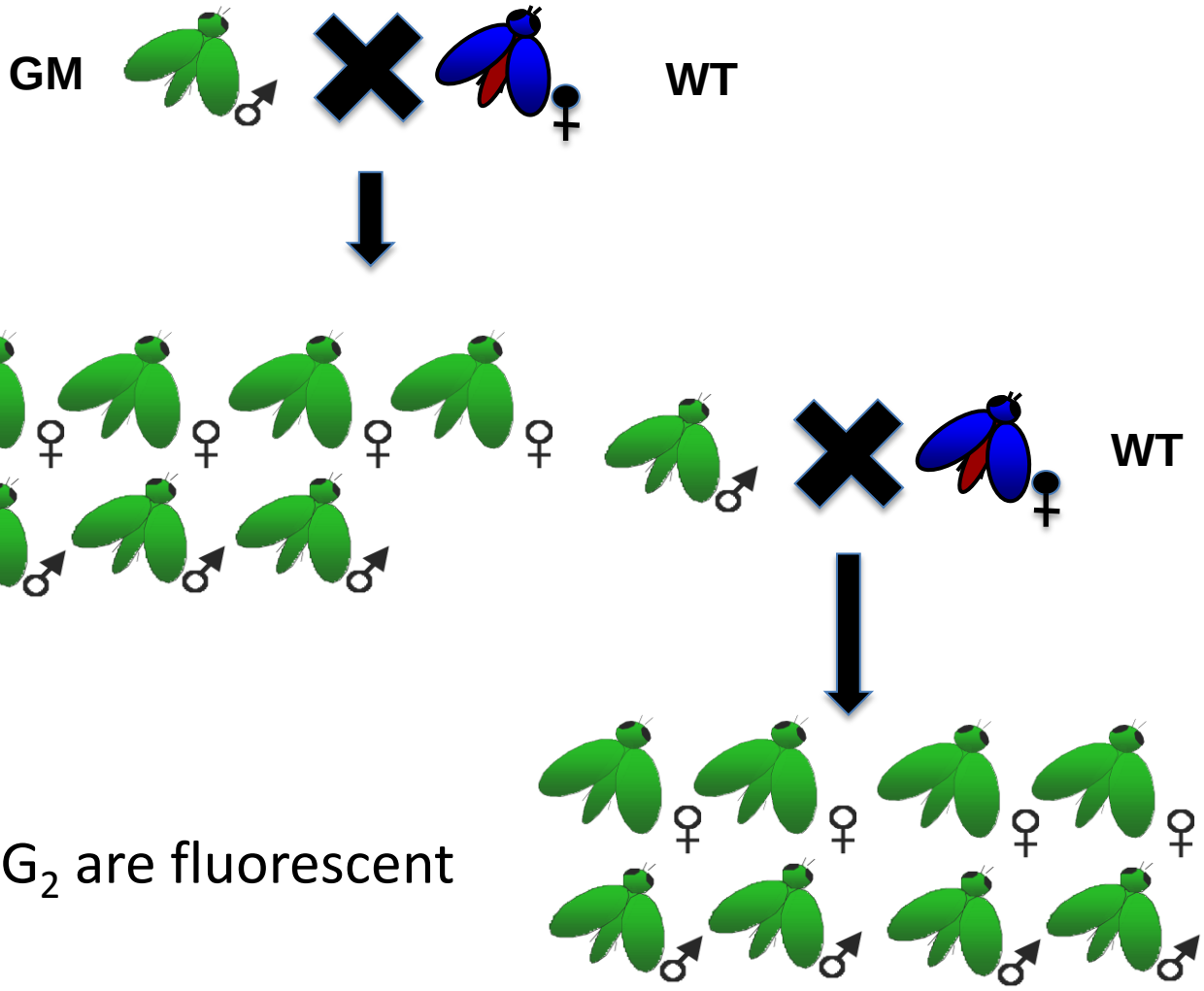
- **Female embryo sexing systems**. Two component. tTA expressed mostly in early embryo stage only. Female death due to expression of cell death gene. Early female death saves diet. Developed for sheep blowfly, screwworm and medfly.
- **Chromosome shredding**. Chromosomes degraded by nuclease in sperm.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

Intro to Gene Drive:
Release of GM fly homozygous for GFP with no drive



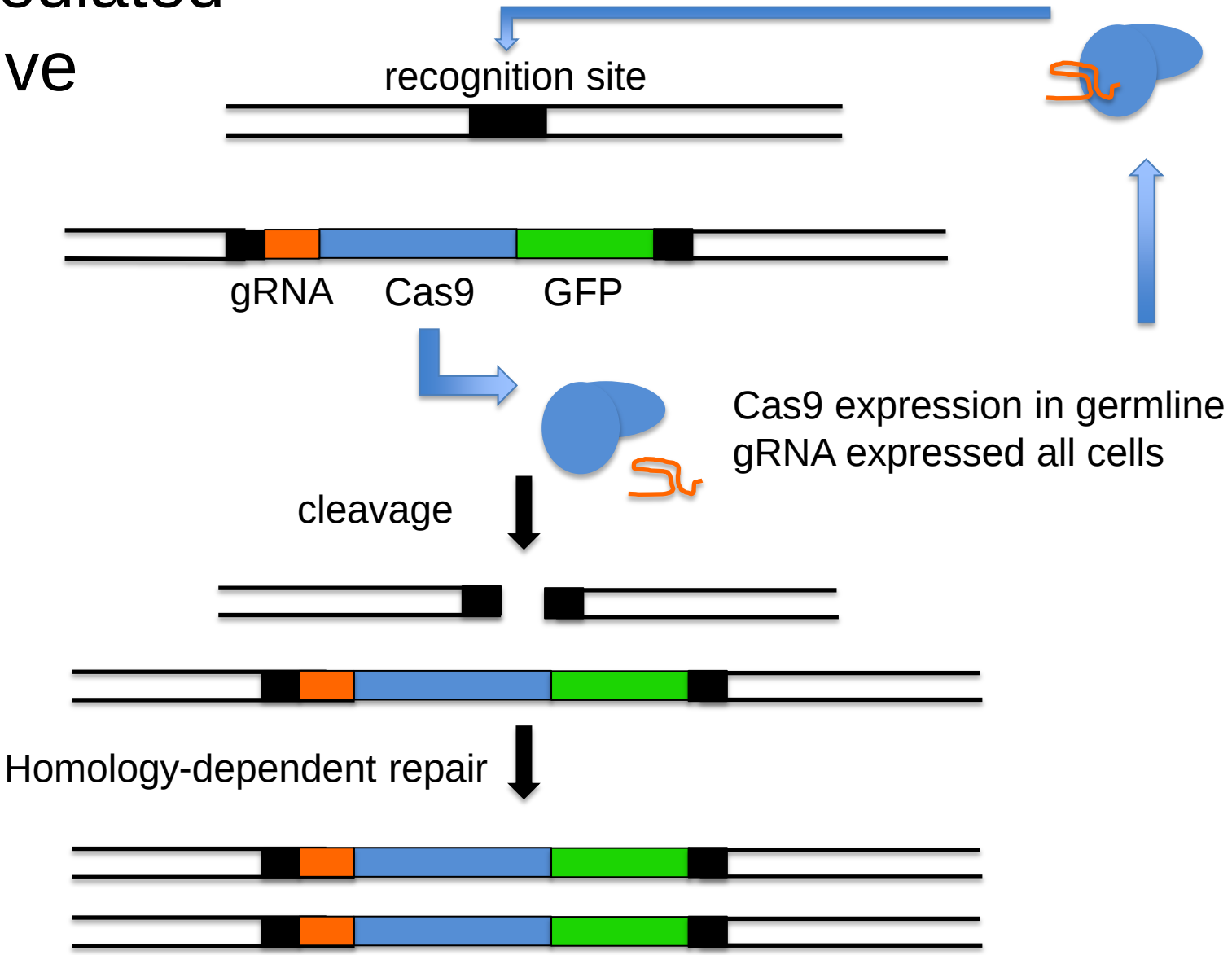
50% of G_2 are fluorescent

Intro to Gene Drive:
Release of GM fly homozygous for GFP coupled to drive



100% of G_2 are fluorescent

Cas9-mediated gene drive



Cas9 gene drive in insects

- **Population suppression.** Targets would be genes required for female development or female fertility or an essential gene in both sexes. Under development for *Anopheles gambiae* and other insects.
- **Population replacement**. Target a neutral region of genome. Link anti-parasite (malaria) or anti-viral (Dengue) gene to drive. Under development for mosquito diseases vectors.

HIGHLIGHTED ARTICLE
GENETICS | INVESTIGATION

Evolution of Resistance Against CRISPR/Cas9 Gene Drive

SCIENCE ADVANCES | RESEARCH ARTICLE

EVOLUTIONARY GENETICS

Evolutionary dynamics of CRISPR gene drives

Resistance arises either due to natural nucleotide polymorphism in target sequence or indels induced by NHEJ repair.

Could be minimized through construct design (eg multiple gRNAs).

Nucleotide polymorphism in insect populations could act as a natural barrier for a drive.

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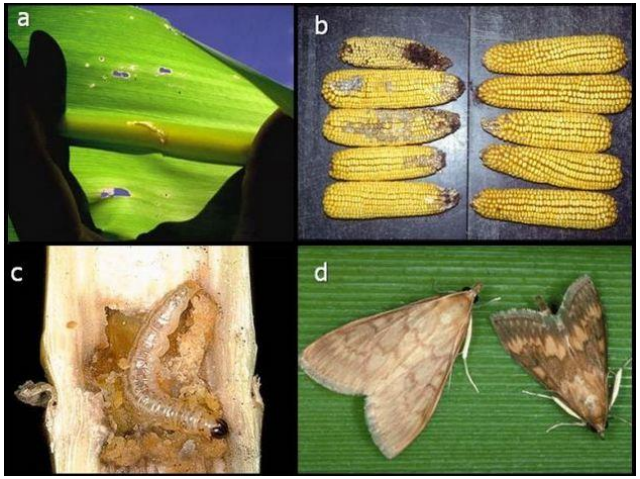
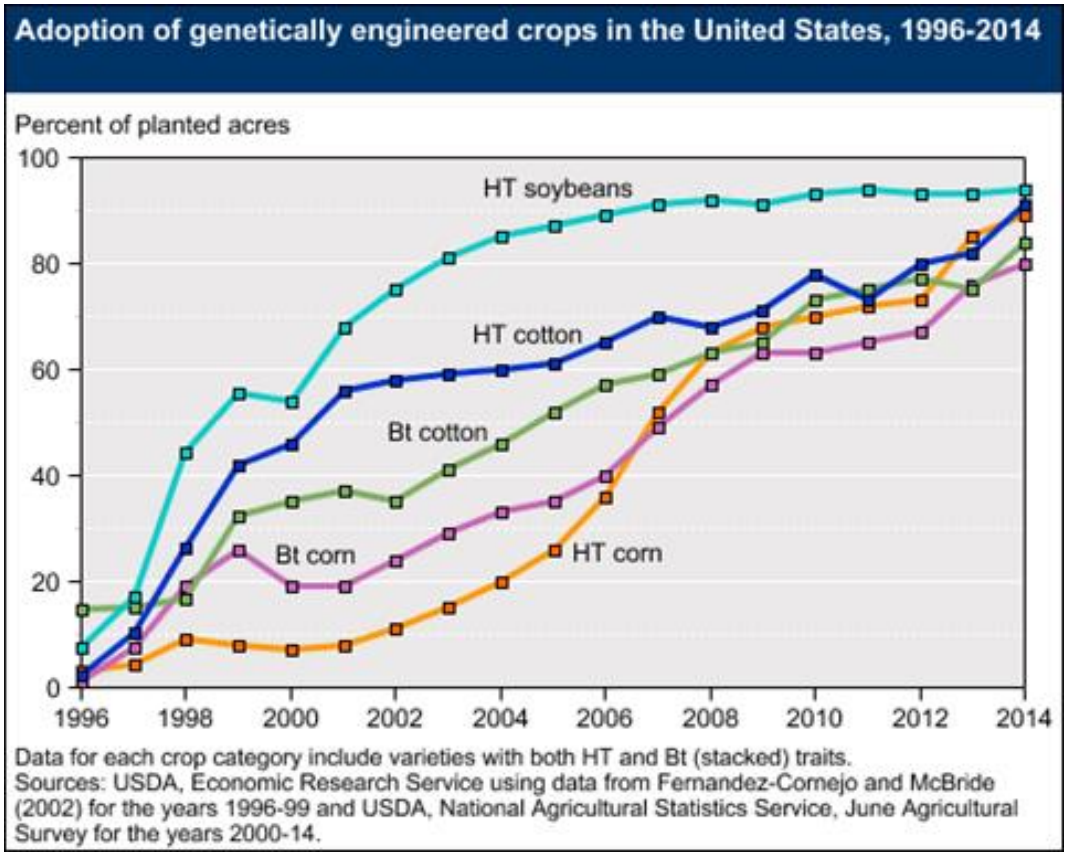
Trinidad Pardo
Gladys Quintero
Agustin Sagel
Mario Vasquez
Pamela Phillips

Funding

NIFA-BRAG
COPEG
USDA-ARS
NC Biotech Center
NCSU

GM crops widely adopted in USA-but not specialty crops (eg berries, broccoli etc)



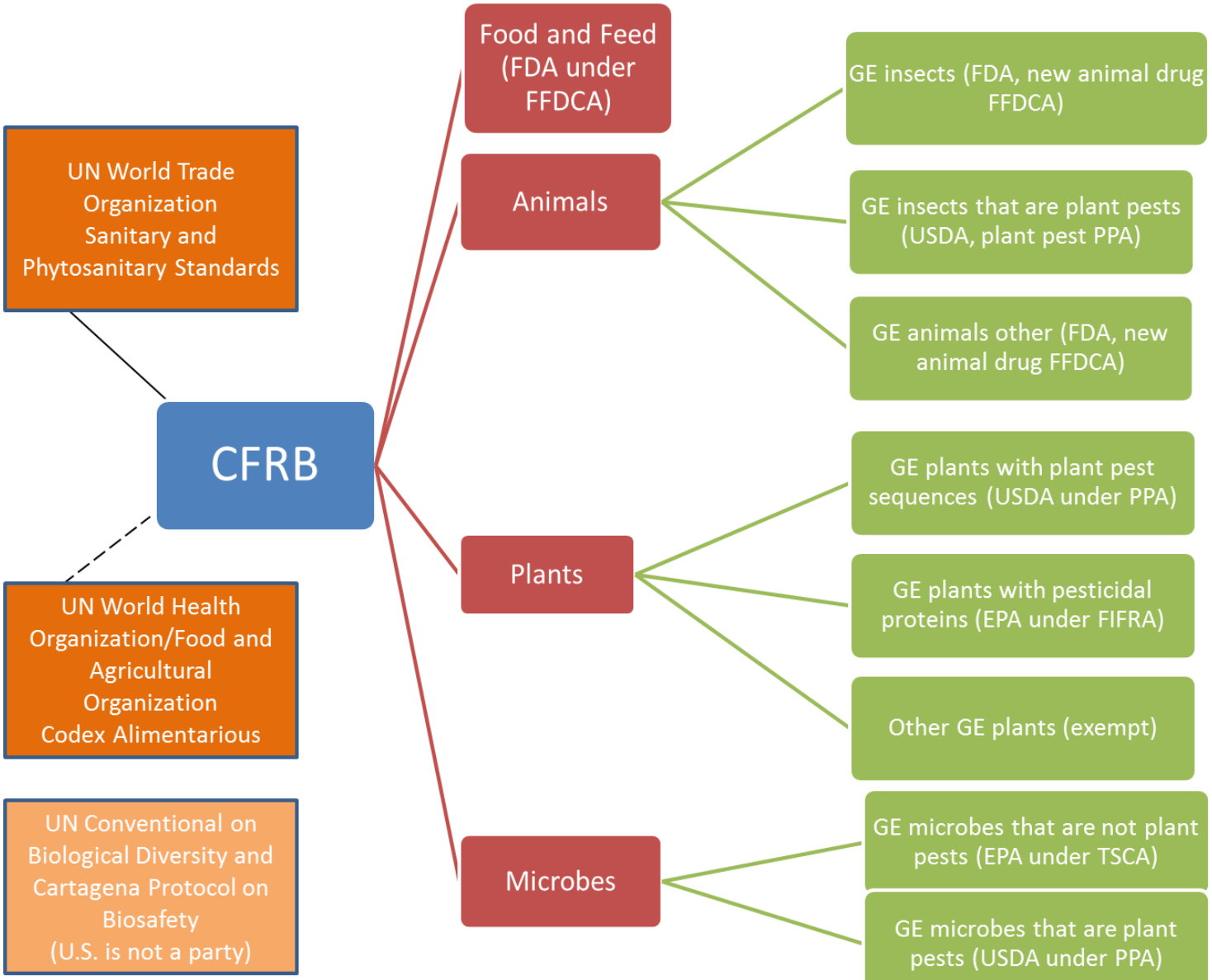


Coordinated Framework for the Regulation of Biotechnology Products (1986-present)

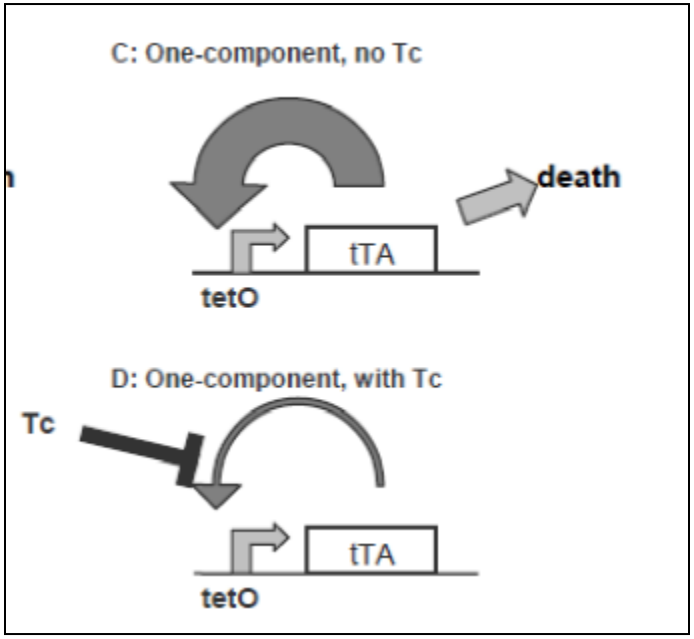
Agency	Jurisdiction	Laws
US Dept. of Agriculture (USDA)	Plant pests, plants, veterinary biologics	Federal Plant Pest Act (FPPA)—(Revised to Plant Pest Act)
Food and Drug Administration (FDA)	Food, feed, food additives, vet. Drugs, human drugs, medical devices	Federal Food, Drug and Cosmetic Act (FFDCA)
Environmental Protection Agency (EPA)	Microbial and plant pesticides; novel microbes	Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)--1947; Toxic Substances Control Act (TSCA)

No new categories of risks, no new laws needed, "product not process"

Kuzma, CAST report, in press



GM mosquito release in Florida



<u>On diet without tetracycline:</u>
96% dominant lethal
Both sexes die

Male and female pupae can be mechanically separated by size with 99.8-100% accuracy

<u>Proposal:</u> Successive releases of fertile males (incl few females) over one location for a 2 year period. Prediction is collapse of *Aedes aegypti* population

Island suburb of Key Haven is the proposed site

FDA FONSI for Oxitec mosquito

- Release of GM mosquito not expected to have any adverse impacts on the environment or human and non-target animal health beyond that caused by wild type mosquitoes
- Issue finding of no significant impact (FONSI) and will not prepare an environmental impact statement (EIS)
- Is FDA the best agency to consider environmental impact?

Factors that influenced finding of no significant impact

- 99.8-100% of mosquitoes released would be male. Very low risk anyone would be bitten by GM mosquito
- Transgene proteins, tTA and DsRed not detected in mosquito saliva. No risk that GM mosquito would be more allergic than wild type mosquito
- Transgene stably integrated. Negligible risk transgene could be horizontally transferred to humans.
- Negligible risk of increased disease transmission. GM mosquito likely less competent vector as females longevity less than wild type and too short to transmit virus

Factors that influenced finding of no significant impact

- Negligible risk that release of GM mosquito would impact the one endangered species in the trial site (a snail) as no overlap in habitats.
- Negligible risk of introducing insecticide resistance genes as GM mosquito susceptible to insecticides
- Negligible risk of GM mosquito persisting in the environment as carries a self-limiting dominant lethal gene