Modified Insect Product Identity Evaluation

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Summary of Product Identity Evaluations

- Define the active ingredient
  - What is the insect?
  - What are the characteristics that make it a pesticide?
    - What is the mechanism being used to prevent, destroy, repel, or mitigate?

- Define the mechanism of modifications
  - What are the processes used to change the insect?
  - How are the modifications stabilized in the insect to prevent loss of the modification?
  - How are the modifications to the insect monitored and controlled?

- Define the persistence of modifications
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  - Are the modifications in the insects restricted to an individual or does it spread into the insect population?

What is the Insect?

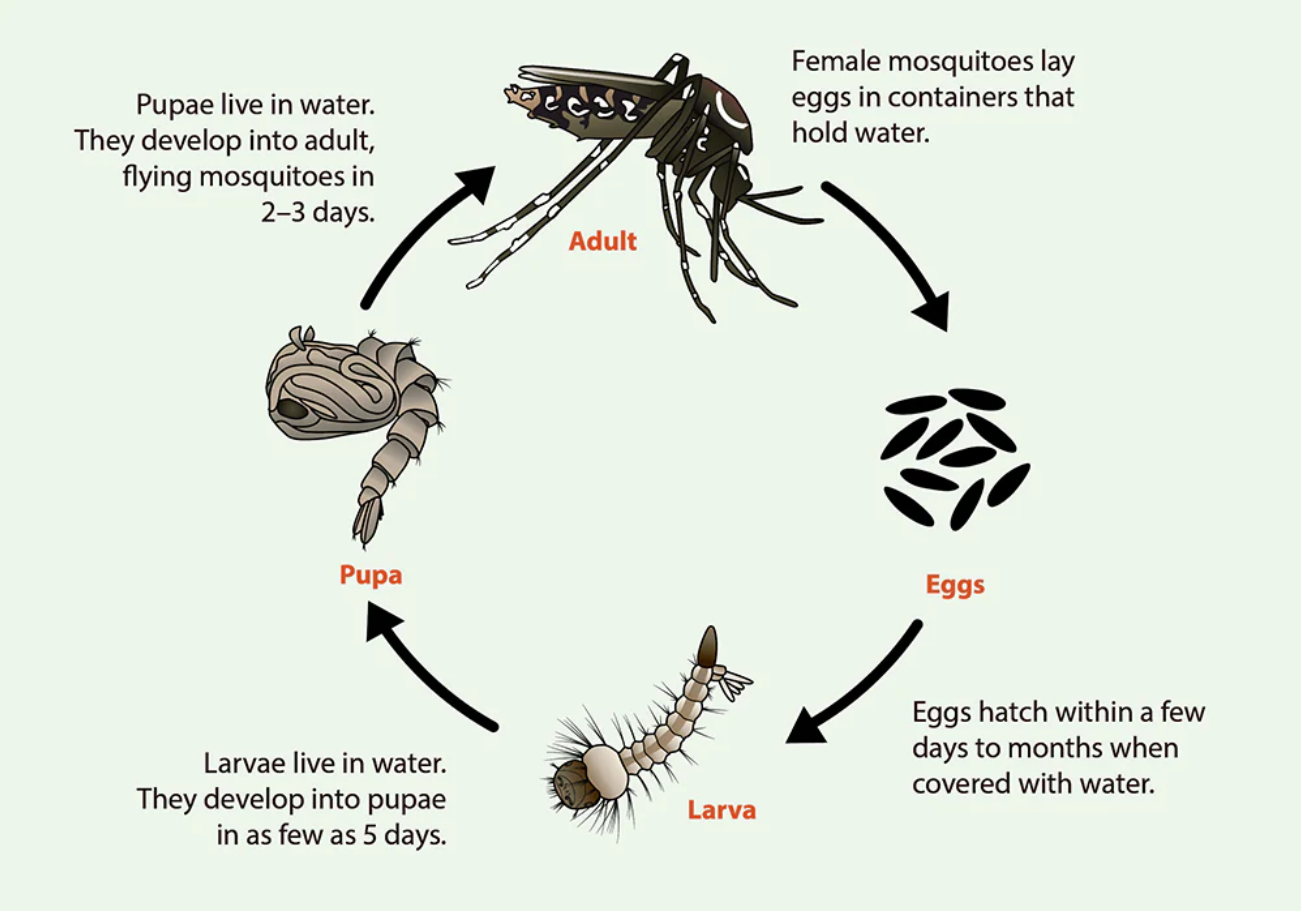
Description of the Insect, Insect Biology and Insect Lifecycle

- Invasive *Aedes aegypti* mosquitoes
  - Multiple introductions to California
- Potential diseases associated with *Aedes aegypti* mosquitoes include
  - Yellow Fever Virus
  - Dengue Fever Virus
  - Chikungunya Virus
  - Zika Fever Virus
- Prefer to live near and bite people

What is the Insect?

Description of the Insect, Insect Biology and Insect Lifecycle

- *Aedes aegypti* mosquitoes eggs
  - 7 – 10 days to develop into adult
- Eggs are laid above the waterline
  - Eggs can survive drying
  - Eggs hatch when covered with water
- Larvae active in water
  - “Wigglers”
- Live indoors and outdoors
  - Only females bite
  - Very aggressive day time biters



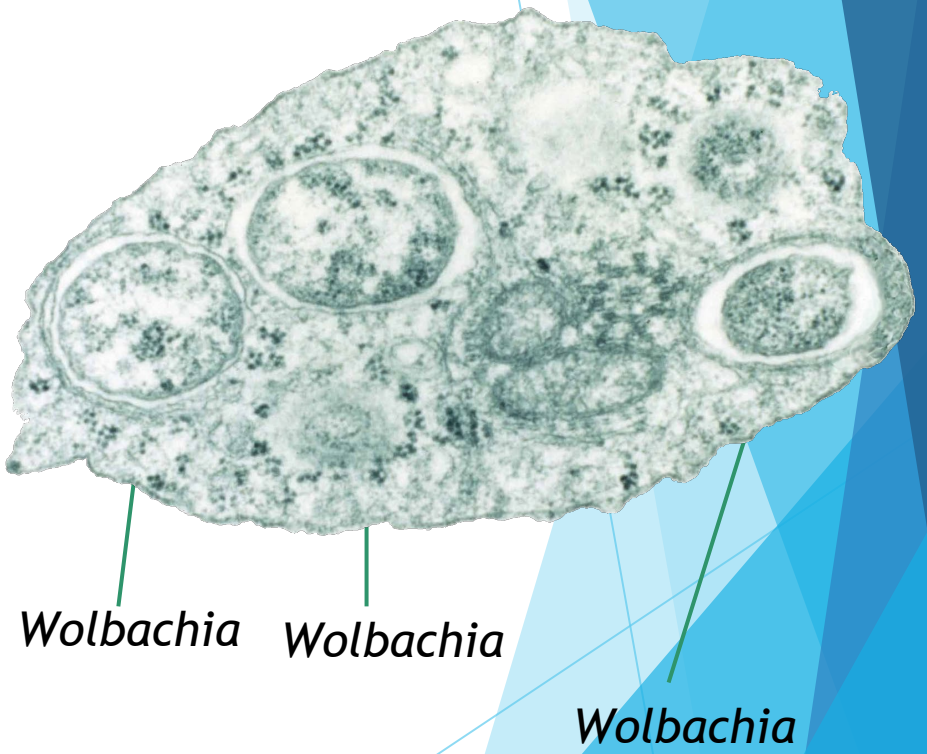
What are the Characteristics that Make the Active Ingredient a Pesticide?

What is the mechanism being used to prevent, destroy, repel or mitigate?

- Incompatible Insect Technique (IIT)
  - *Wolbachia* species microinjected into males
  - Mating with wild incompatible females results in nonviable offspring due to incompatibility
  - Insect population declines overtime

- Dominant Lethal gene systems
  - Released insects carry lethal mutation(s)
  - Progeny inherit dominant lethal mutation(s)
  - Insect population declines overtime

Wolbachia within an insect cell

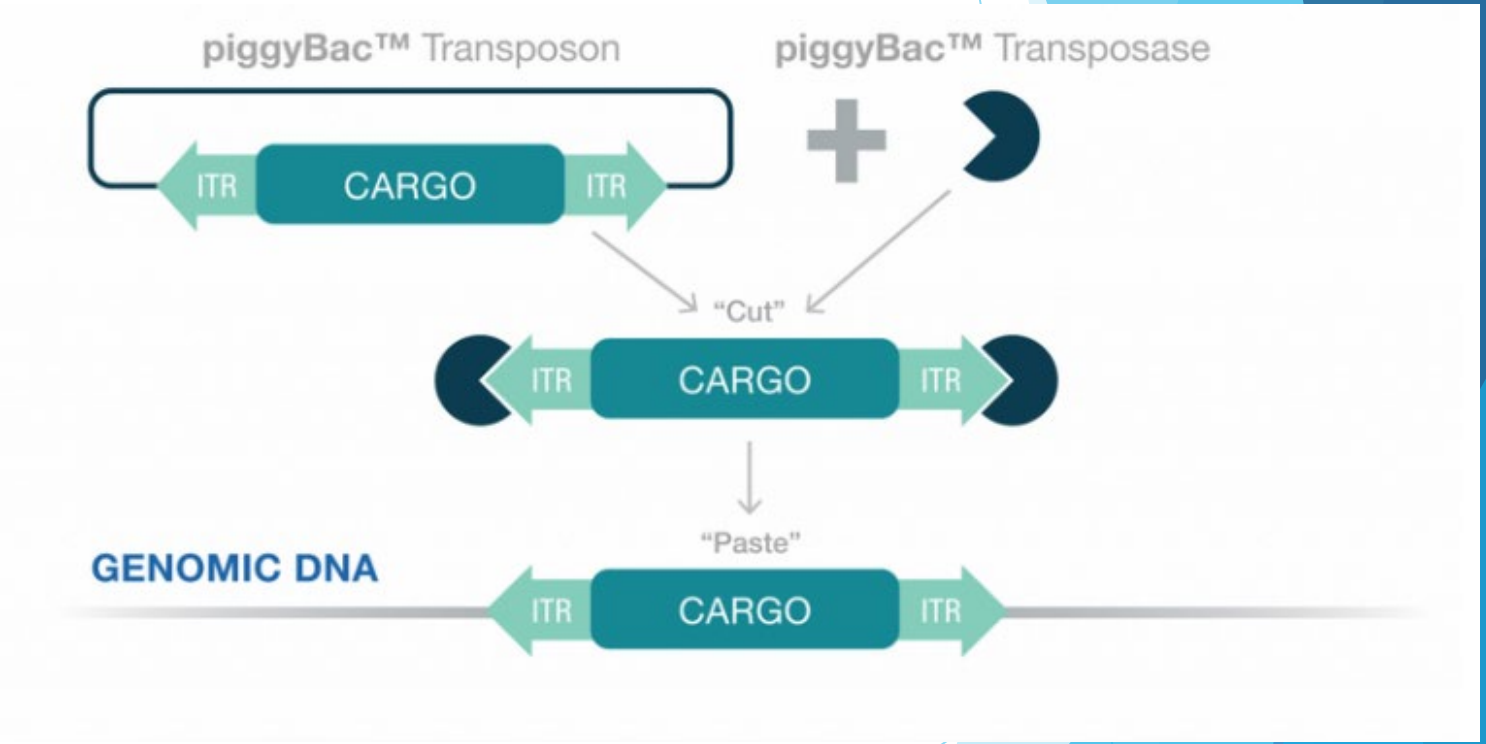


Mechanism of Modifications

What are the processes used to change the insect?

Transposon Based Genetic Modification Systems

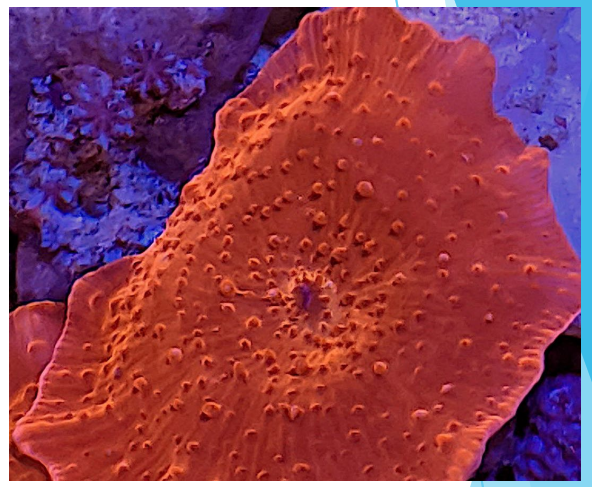
- Transposon
  - Modified Gene/Reporter Gene Sequence
  - Sequence analysis needed for review
- Transposase
  - Enzyme catalyzing DNA transfer
  - Characterization of Transposase needed
- “Cut & Paste”
  - Inverted Terminal Repeats (ITR) in ‘TTAA’ sites on genomic DNA
  - How characterized and selected for the desired trait?
- Transposase enzyme needed to move genetic sequence
  - How stable is transposon sequence in genomic DNA?



How are the Modifications in the Insect Monitored?

- Detection method(s) used to differentiate modified insects from non-modified insects
  - Comparisons between modified insects and unmodified insects needed to support the claim of no-effect
  - How stable is the monitoring system in the modified insects
  - Can monitoring system be passed on to subsequent generations?
- Reporter systems
  - "DsRED2"
    - Red fluorescent protein from sea anemone *Discosoma*
    - Red Fluorescent under yellow light
  - "GFP"
    - Green fluorescent protein from jellyfish *Aequorea* Victoria
    - Green fluorescent under blue to ultraviolet (UV) light

DsRED2



GFP

Genotype versus Phenotype

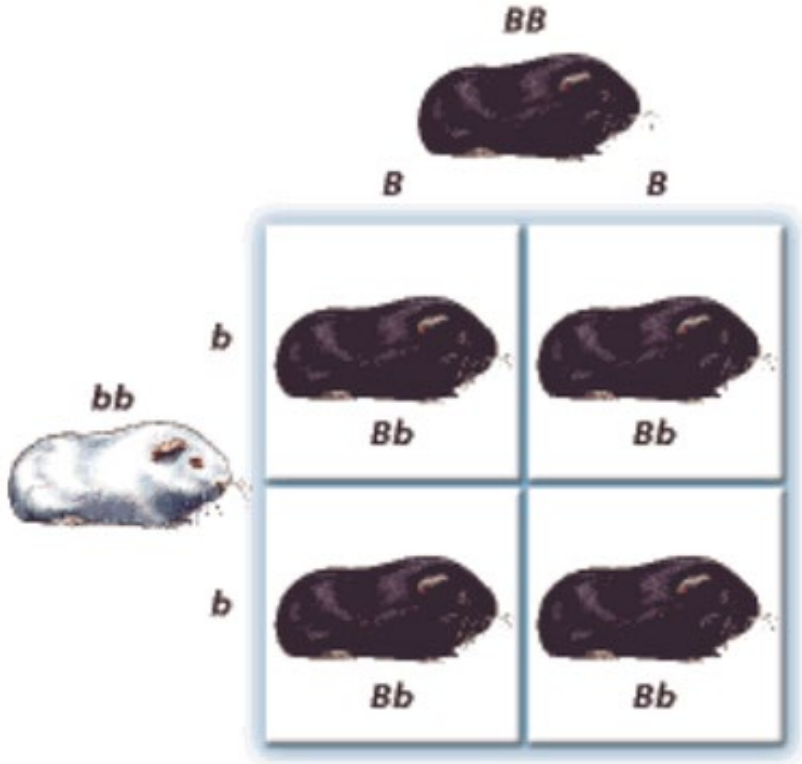
Genotype is the genetics of a trait

	A	B	O
A	AA	AB	AO
B	AB	BB	BO
O	AO	BO	OO

Blood can be A, B, O, and AB based on genetic trait.

Blood's physical appearance does not indicate blood type

Phenotype is the expression of a trait



Dominant hair color (B – Black)
Recessive hair color (b – White)
Dominant phenotype expression is black hair

Types of Gene Expression
One Gene versus Multiple Genes

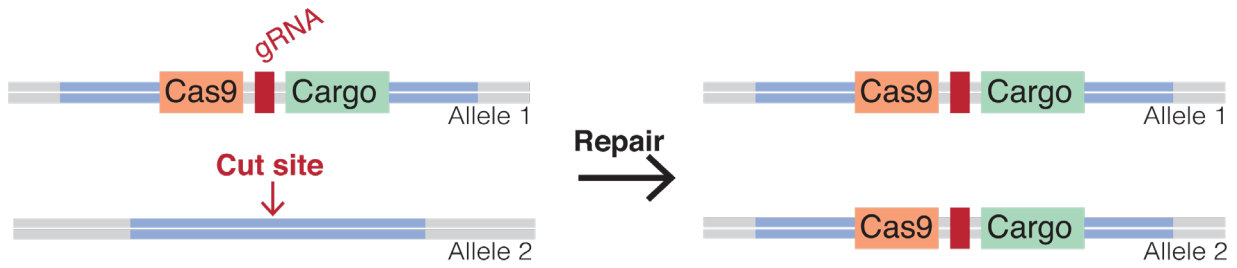
Mendelian-Based Single Gene Trait

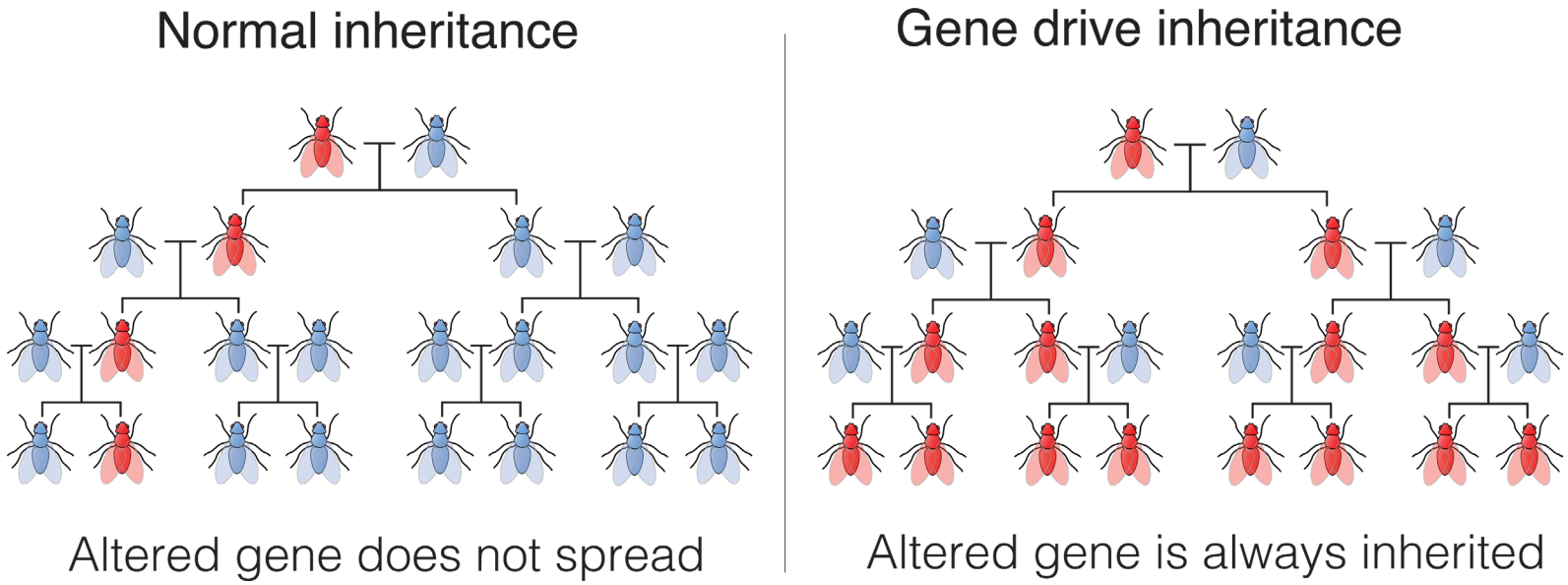
- One gene controls phenotypic expression
- Dominant genotype results in dominant phenotypic expression
- Recessive phenotypic traits not expected to be expressed
  - Expression of recessive traits under <u>one</u> system of control

Non-Mendelian-Based Gene Trait

- Multiple genes control phenotypic expression
- Dominant genotype not directly controls phenotypic expression
- Recessive phenotypic traits possibly expressed
  - Expression of recessive traits under <u>multiple</u> systems of control

Standard Inheritance versus Gene Drive





Genetic modification spreads limited in insect population

CRISP-Cas9 Transposon System using guide RNA (gRNA)
Genetic modification spreads throughout insect population

Persistence in the Environment
Chemical or Biologic Regulators

- Genetic modifications can be regulated by chemical or biologic “switches”

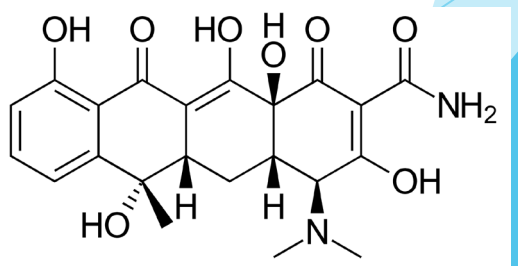
- Regulator chemicals can include:
  - Antibiotics and antibiotic analogs
  - Steroids and steroid analogs compounds
  - Metals and metal complexes
  - Other biologically active chemicals and complexes

- Distribution of the regulator chemicals and their analogs can alter genetically modified insects biology to either decrease survival or increase survival
  - Distribution of the regulator chemical and their analogs information is needed prior to release of modified insects into the environment.
  - Minimum concentration of regulator chemical and their analogs that is needed to “rescue” insect survival needs to be defined prior to release of insects into the environment.

Chemical or Biologic Regulator

Example

- Mosquitoes genetically modified with female lethal mutation controlled by the antibiotic tetracycline
  - **Presence of antibiotic:** Both male and female mosquitoes survive and reproduce
  - **Absence of antibiotic:** Only male mosquitoes survive
- Distribution of the antibiotic in the environment effects expression of the gene system
  - Tetracycline is in the “cycline” family of antibiotics from soil bacteria *Streptomyces aureofaciens*
    - Distribution and concentration of environmental sources of “cycline” antibiotic need to be known
      - To prevent “rescue” events by understanding environmental sources of “cycline” antibiotics
  - Detectable levels of “cycline” antibiotics:
    - Healthcare Facilities
    - Human Wastewater/Sludge
    - Agricultural Orchards
    - Livestock Liquid/Solid Waste
    - Aquaculture (some)



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Questions?