

# Female Conditional-Lethal Strains of Diptera: Microinjection of horn fly, secondary and primary screwworm

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presented by Anna Rachinsky



# Overall project objective

Transgenic strain of flies in which female lethality can be selectively turned on early in the life cycle

→ "male-only strain"

advantages: no / less irradiation  
reduced rearing cost

→ Secondary screwworm *C. macellaria* as 'transitional' species (Kerrville, TX)

→ in progress:

transformation of secondary screwworm

→ next step:

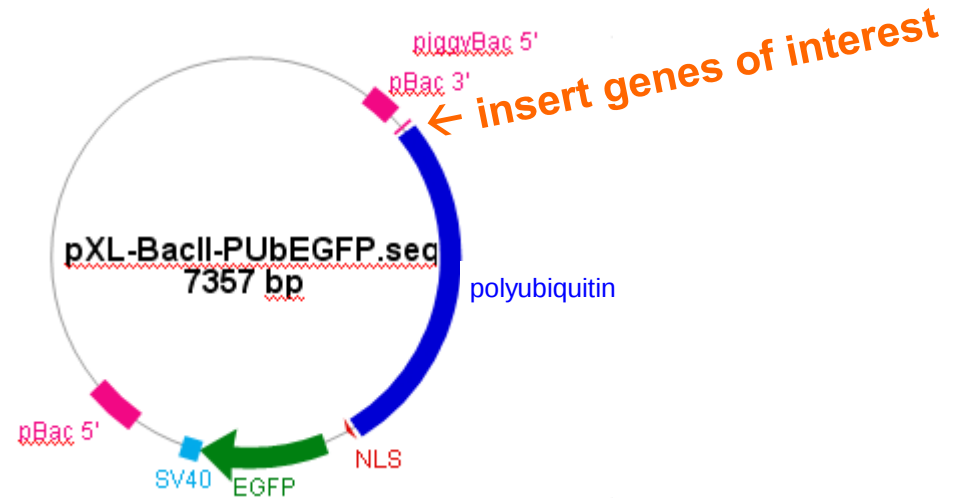
develop & inject components of female-lethal system

→ Transgenesis of *New World Screwworm* in Panama

## Plasmids used:

from Dr. Al Handler's lab  
(USDA-ARS, Gainesville, FL)

- circular DNA
  - transposable element
  - marker
  - control gene
  - genes of interest



Secondary screwworm,  
*Cochliomyia macellaria*



# Secondary screwworm, *Cochliomyia macellaria*

- occur naturally in Texas
- larvae consume necrotic tissue of carrion or animal host, do NOT feed on live tissue
- easy to rear in the lab
  - larvae: artificial diet & liver
  - adults: sugar & water  
larval diet = protein for egg development  
egg laying on larval diet



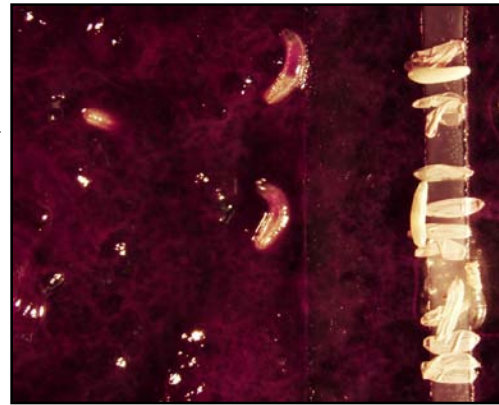
- Transformation permit updated 2/13/2009
- 'Texas' strain used for microinjections
- 'Mixed age colonies' allow daily egg collection



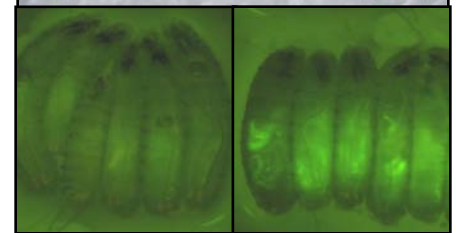
# Microinjection and survival of *C. macellaria* embryos 1



injected eggs on larval diet



larval hatch → empty chorion stays behind



- some larvae abnormal  
- auto-fluorescence



mature larvae leave diet



pupation in sand



adults mated  
with colony flies

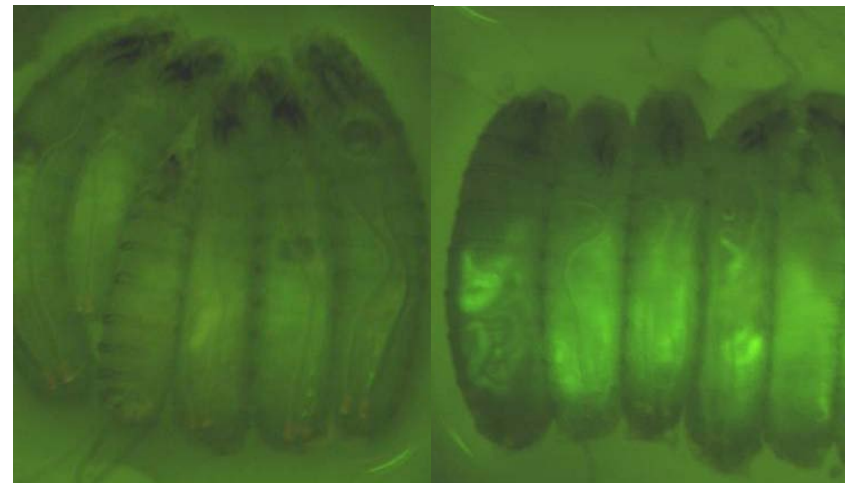


## Screening of injected GO larvae for fluorescent marker:

→ **transient expression**

→ indicates successful DNA injection  
... does NOT indicate insertion of  
genetic material into fly genome

Challenge:  
varying levels of  
auto-fluorescence  
in control L1





# Accomplishments fly transgenesis project

## (February - June 2009)

- ❑ Started microinjection of secondary screwworm embryos (permit update 2/13/09)
  - ➔ demonstrated successful injection of plasmid mix & acceptable survival rates
  - ➔ produced 2 groups of potentially transformed G1 flies
  - ➔ produced 1 group of potentially transformed G2 offspring

### Next:

- confirmation of successful transformation by PCR
- development of transgenic lines from so far transformed flies
- microinjection training for Panama staff
- injection of *C. macellaria* embryos with plasmids containing components of female-lethal system
- development of transgenic lines from flies injected with female-lethal components

# Microinjection training: Preparing fly embryos

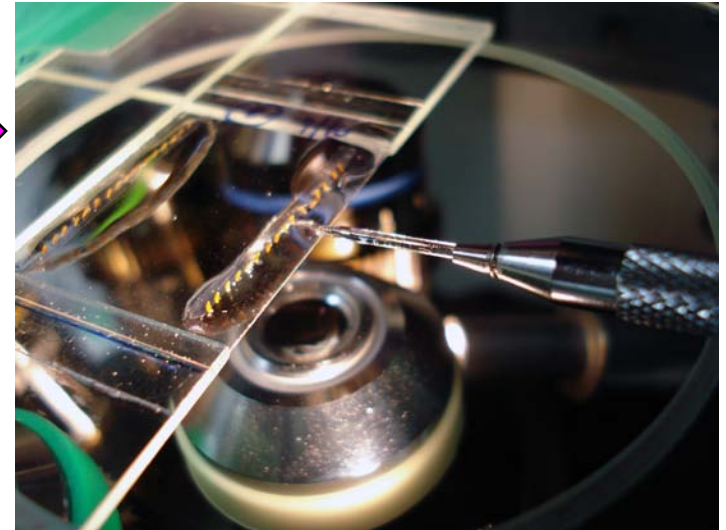


collect and  
dechorionate eggs

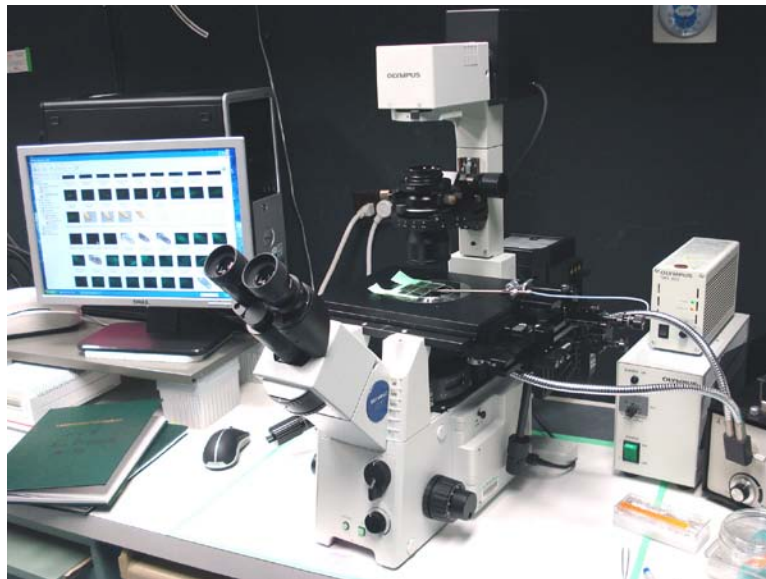


line up for injection

← *injection site*  
= *posterior pole*



micromanipulator-controlled injection

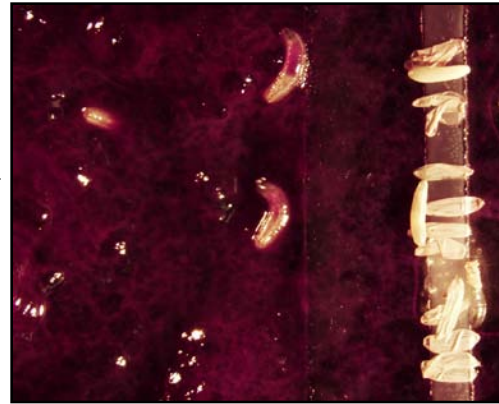


microscope with micromanipulators for  
controlling injection needle

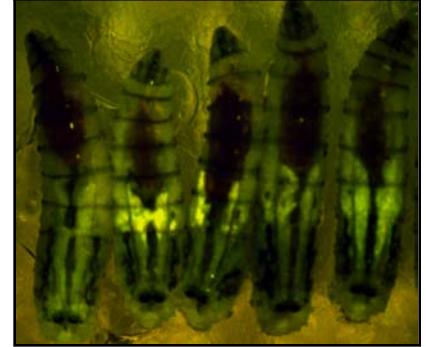
# Rearing and screening of injected individuals



injected eggs on larval diet



larval hatch



screening & selection  
of positive larvae



rearing of small groups



facilitate pupation



collect offspring from  
positive adults

# Microinjection training – Outline

*Based on training by Insect Transformation Facility, U of MD, Rockville, MD*

- **Background information:**
  - process of genetic transformation; egg timing (development)
  - microinjection equipment; injection strategies (1 vs. 2 person)
- **Microinjection preparation**
  - DNA prep
  - needle pulling & beveling
  - embryo slide / coverslip prep
- **Injection station**
  - egg collection and dechoriation; egg set up
  - micromanipulator & microinjector operation
  - needle filling & needle filler creation
  - injection demonstration
  - injection practice
- **Post-injection**
  - transient expression
  - hatch & adult survival
  - G0 crosses and rearing of G1 offspring
  - screening of G1
- **Discussions**
  - equipment pros & cons
  - precautions for working with the 'real screwworm', *C. hominivorax*