RNAi strategies in support of mosquito SIT applications

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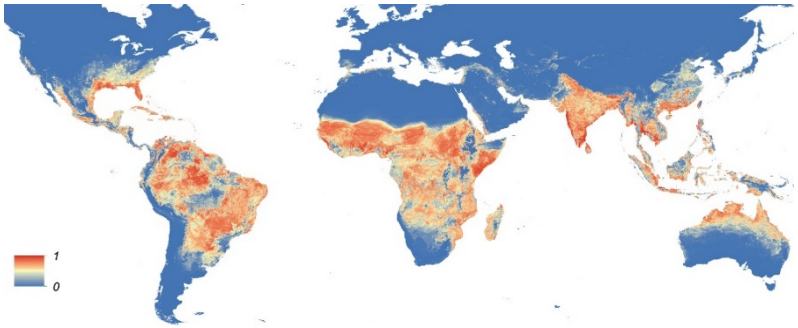
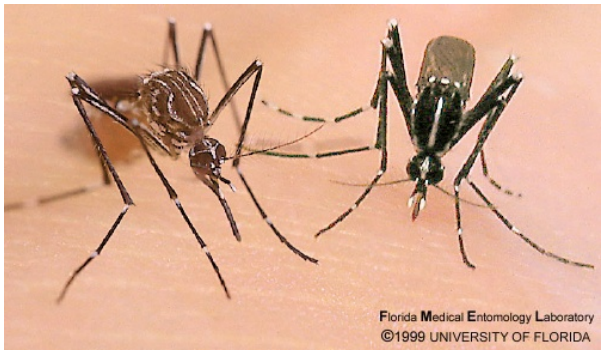
Two serious disease vectors: *Aedes aegypti* and *Ae. albopictus*

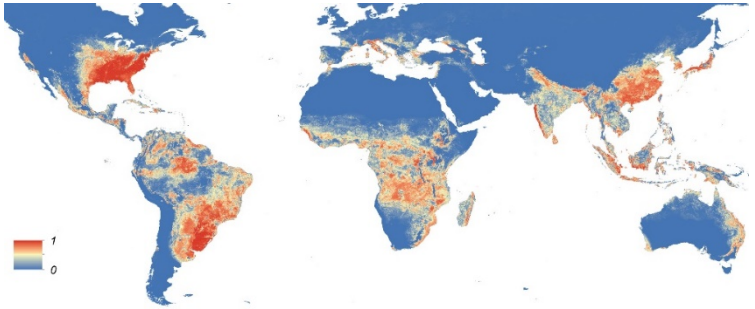
- *Aedes aegypti*
  - Urban, endophilic vector
  - Preferentially bites humans
  - Tropical & semitropical



- *Aedes albopictus*
  - Peri-urban and rural vector
  - Feeds readily on mammals and birds
  - Invasive species to Americas
  - Tropical to temperate



- Both transmit dengue, yellow fever, Chikungunya, and Zika viruses



Mosquito control

- Biocontrol
  - Mosquitofish (*Gambusia affinis*)

- Trapping/Baiting
  - Chemical attractants to lure mosquitoes

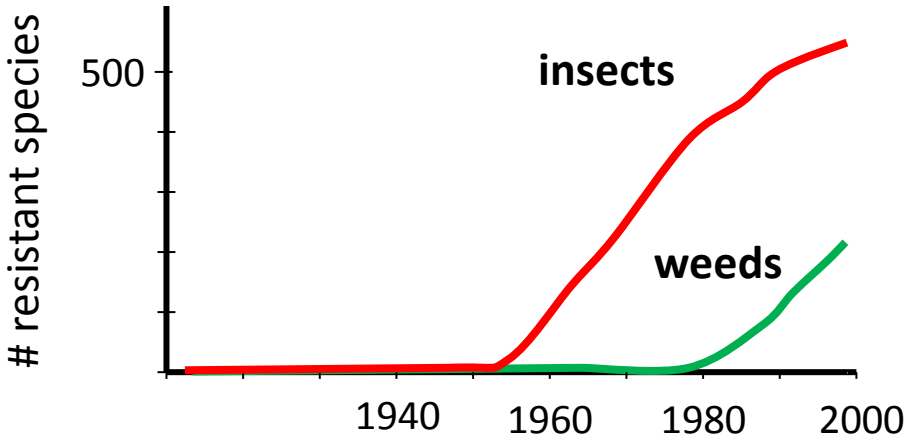
- Chemical control
  - Larvacides
  - Adulticides

Current chemical pesticide issues:

1. Increasing resistance to pesticides

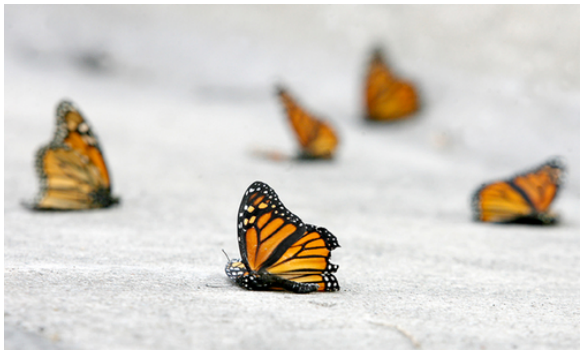
More resistant species

Higher levels of resistance



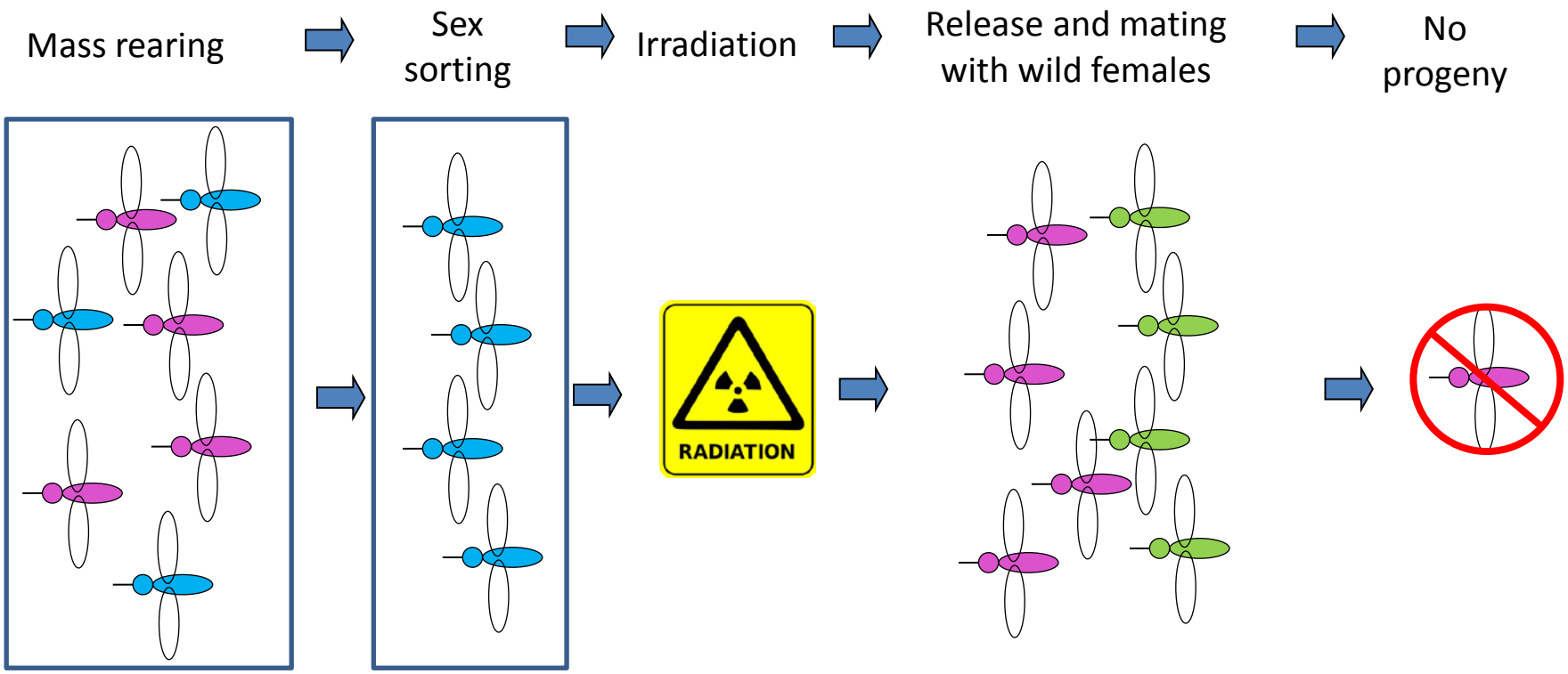
2. Off-target effects of pesticides

Broad-spectrum kill many non-target species



Sterile Insect Technique - a biological, species-specific control method

Conventional SIT



Problems associated with previous mosquito SIT programs:

- Radiation-induced sterilization might weaken males
- Sex sorting was time-consuming and not fail-safe

Oxitec (and others) - producing genetically-modified sterile mosquitoes

- GM technology will require regulatory and public approval

Can we enhance the Sterile Insect Technique, without using Genetic Modification?

- Non-radiation approach

- Non-GM approach

- Male-only production

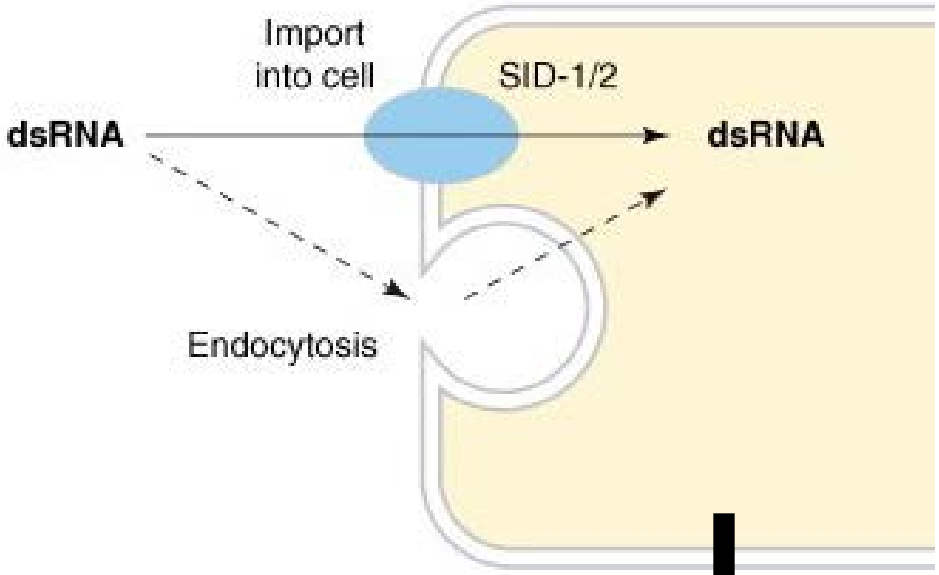
- Adaptable to other species?

+

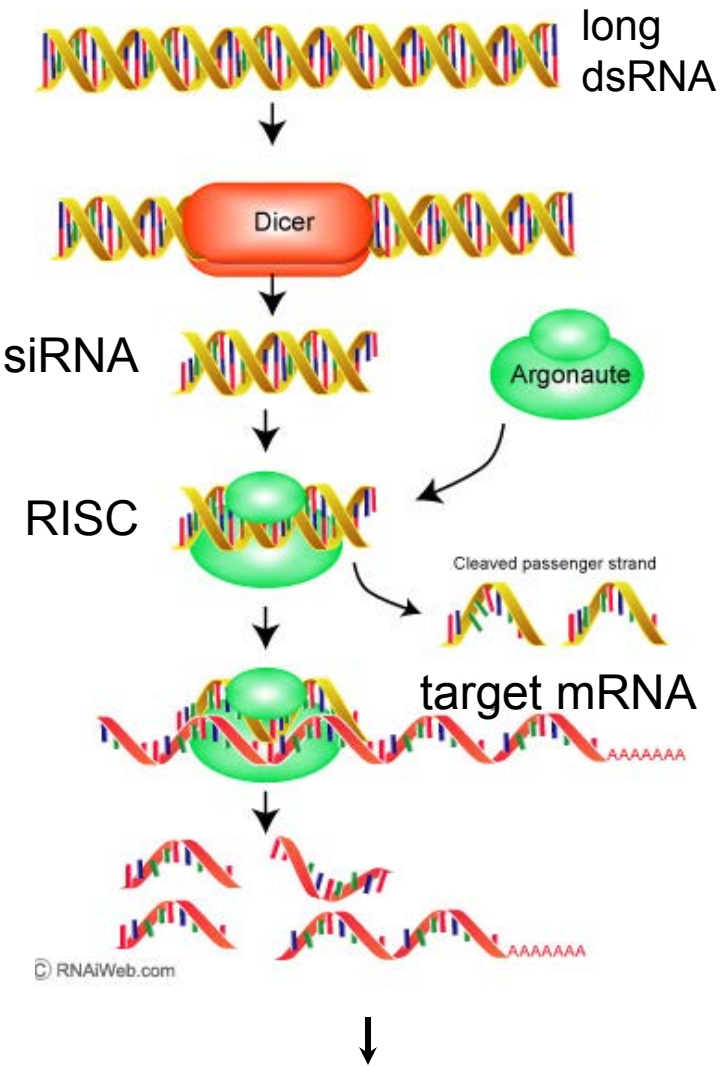
One approach – RNA interference-mediated sterilization and sex-sorting

RNA interference in insects: what we know and don't know…

- RNAi - targeted destruction of mRNA = gene silencing



dsRNA or siRNA export?

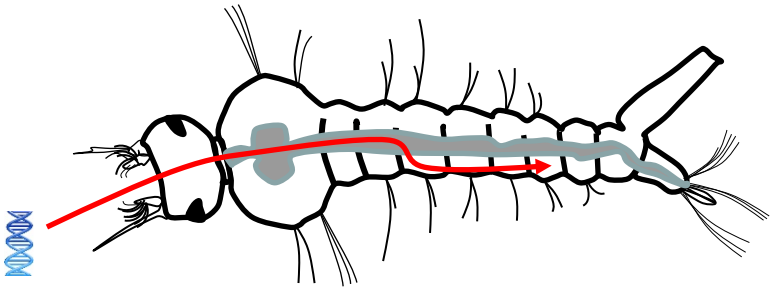


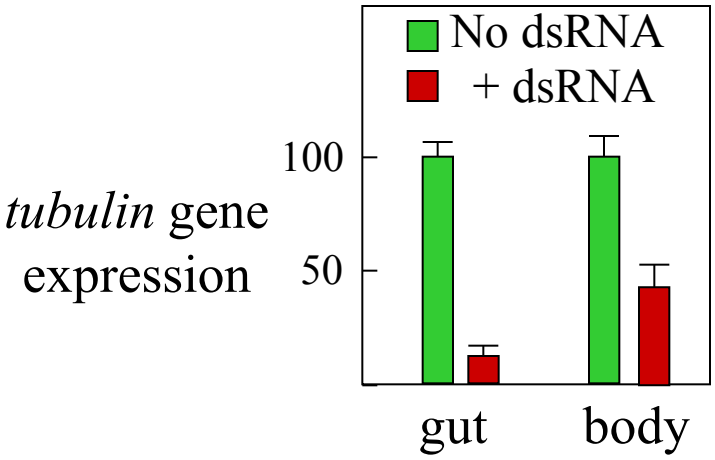
No protein translation

Feeding dsRNA to mosquitoes

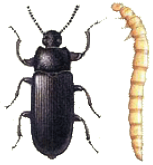
- Ingested dsRNA can silence genes in the mosquito gut
- The dsRNA can escape the gut and silence genes in other tissues (systemic RNAi)

mosquito larva





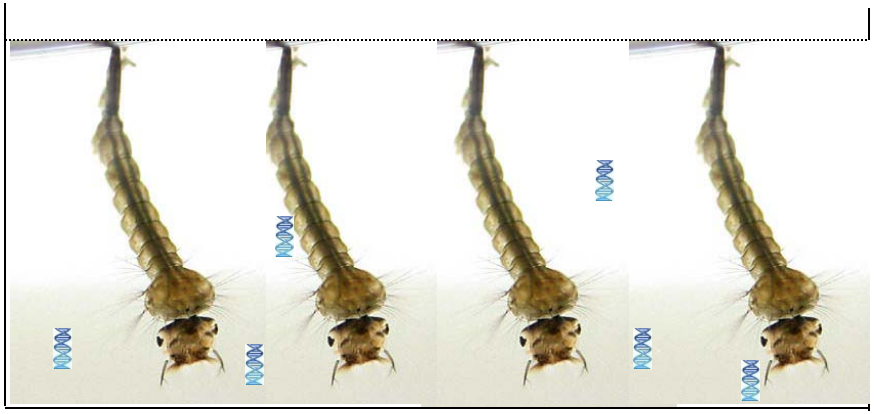
Delivery of dsRNA to insects: Feeding – transient RNAi /pest control

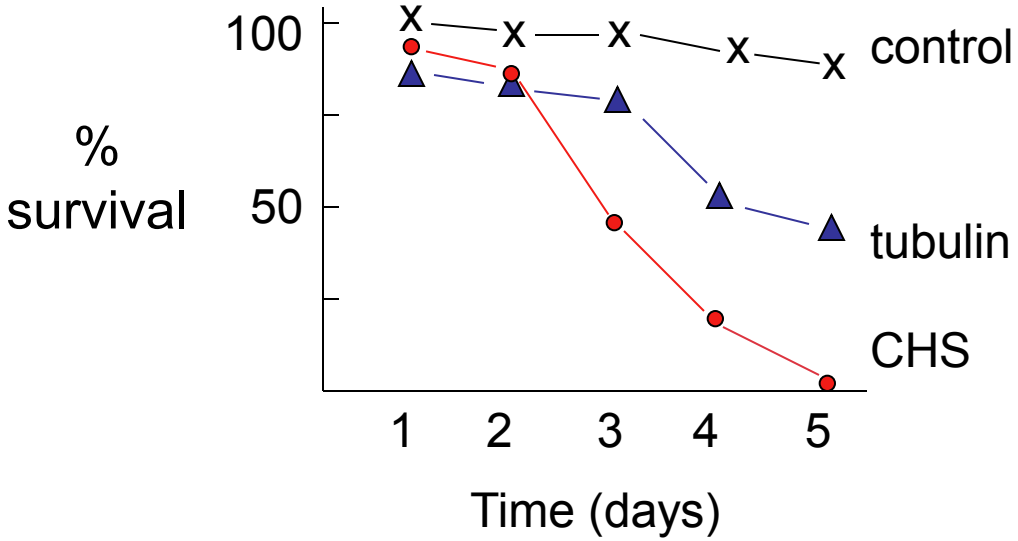


		Extent of RNAi after feeding		
Order	Genus	Gut genes	Non-gut genes	# genes
Diptera	Drosophila	+++	+	19
	Aedes	+++++	++	>100
	Culex	++	+	8
Coleoptera	Coccinella	+++	+	3
	Tribolium	+++	++	7
	Tenebrio	+++	?	2
Lepidoptera	Manduca	+	?	2
	Spodoptera	+	?	2
	Plutella	+	?	2
Hemiptera	Aphis	+	+	4
	Acyrthosiphon	+	+	8
	Myzus	++	+	8
	Lygus	+	+	1

Ingested dsRNA can kill mosquito larvae - not all genes are equally affected

dsRNA

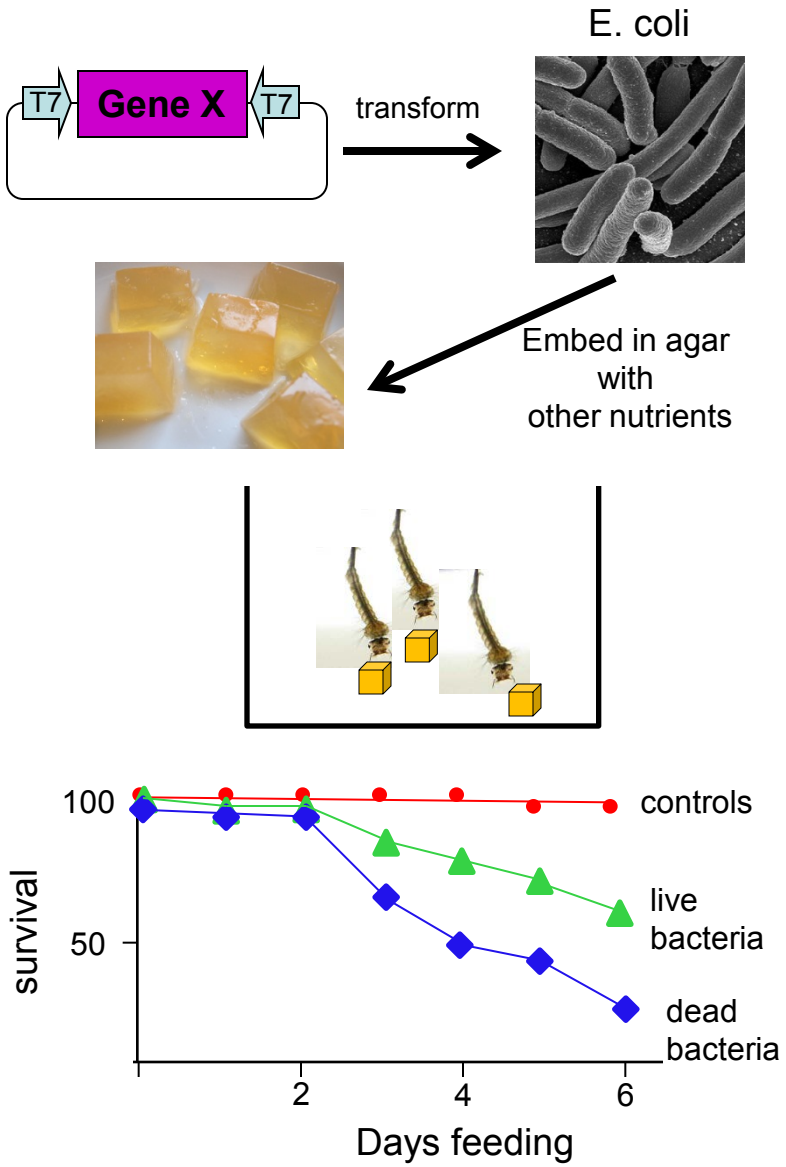




Singh et al. 2013 J Insect Sci

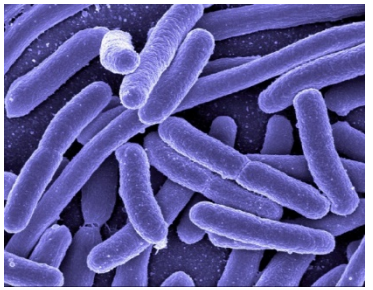
Producing dsRNA in microorganisms

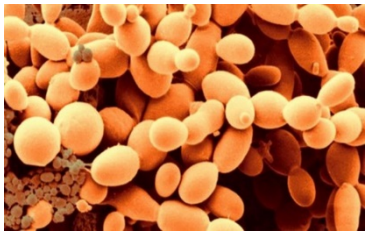
- Microorganisms could serve as biofactories to produce dsRNA
- Some insects readily consume bacteria, yeast
- Release of transgenic organisms may not be acceptable
- Heat-killed bacteria still provided sufficient dsRNA to kill mosquito larvae



Mass production of dsRNA

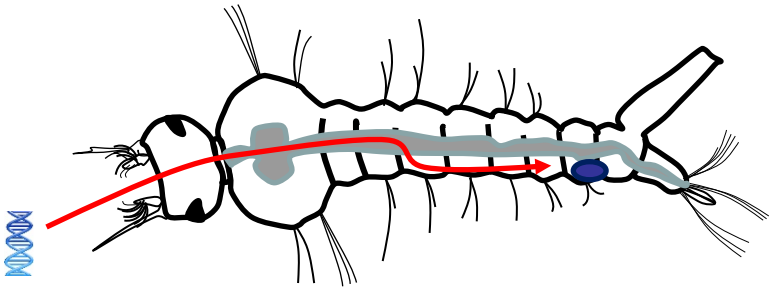
- Intact bacteria expressing dsRNA
  - 1 L culture – enough to treat 10,000 mosquitoes
- RNA extracted from bacteria
  - 1 L culture - enough to treat 5,000 mosquitoes
- Intact yeast expressing dsRNA
  - Currently testing whether mosquitoes prefer yeast or bacteria and which vector can provide more dsRNA
- Buy dsRNA – companies now making dsRNA cheaply - $100/g

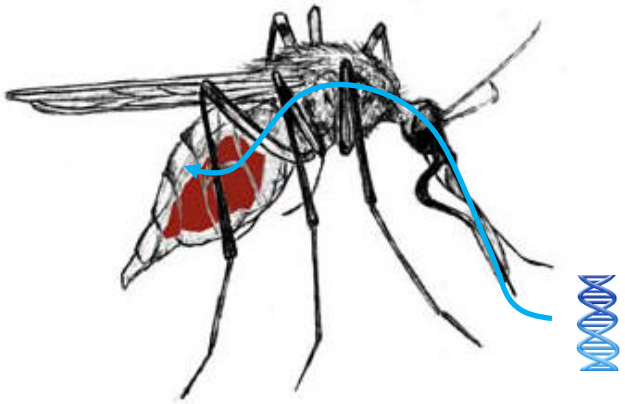




Ingested dsRNA to produce sterile male mosquitoes

- Target mRNAs for spermatogenesis
- Target female-specific mRNAs





- larva ingest dsRNA
- dsRNA exits gut and enters target tissues
- **Goals:**
  - female larvae die
  - males are sterile

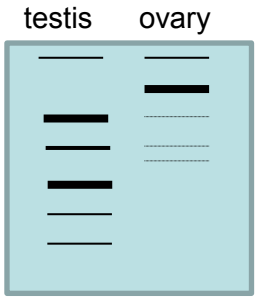
- adult females ingest dsRNA
- dsRNA exits gut and enters ovaries
- **Transgenerational RNAi:**
  - female progeny die
  - male progeny sterile

Knockdown of male fertility genes in mosquitoes

ovaries

Extract RNA

Subtractive suppression hybridization



35 male-limited reproductive genes

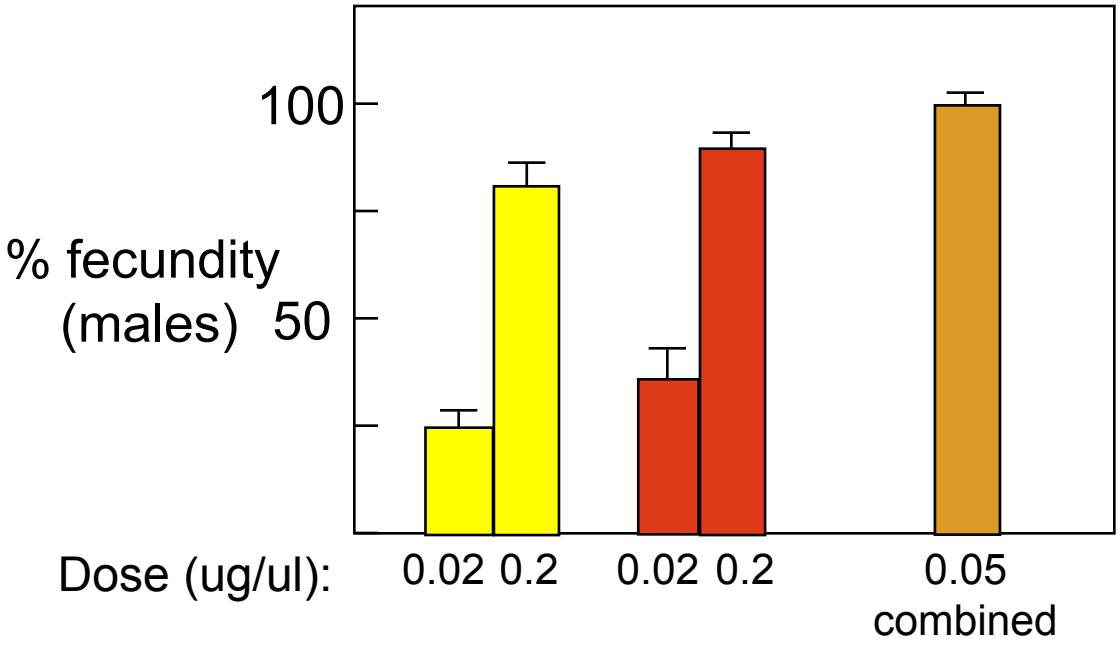
dsRNA injection or feeding

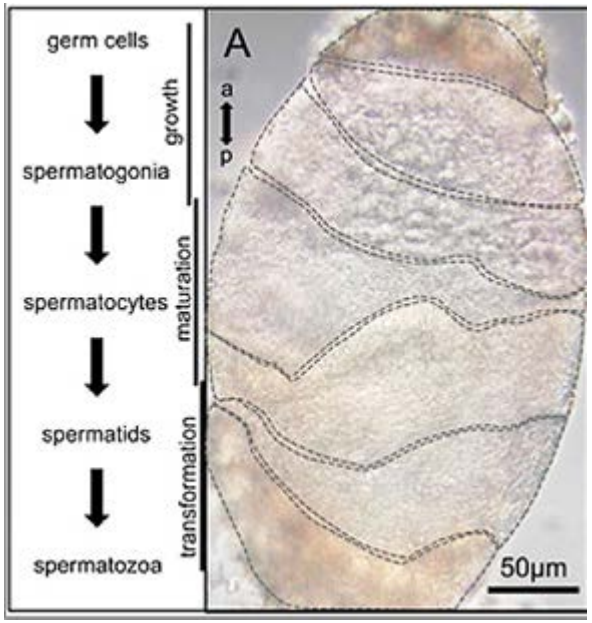
gene	Sterile / competitor?
bol	Yes / Yes
tud	Yes / No
zpg	Yes / Yes
AAEL004231	Yes / Yes
AAEL006975	No
AAEL007434	Yes / No

Ideal target genes:

- Late stage spermatogenesis e.g. sperm motility
- Genes not expressed in other tissues in males

1. RNAi is dose-dependent
2. Combining different dsRNAs improves impact



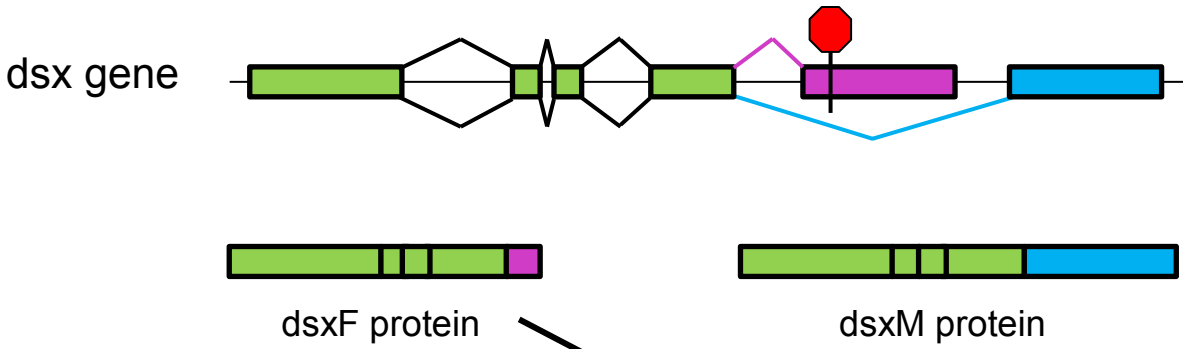


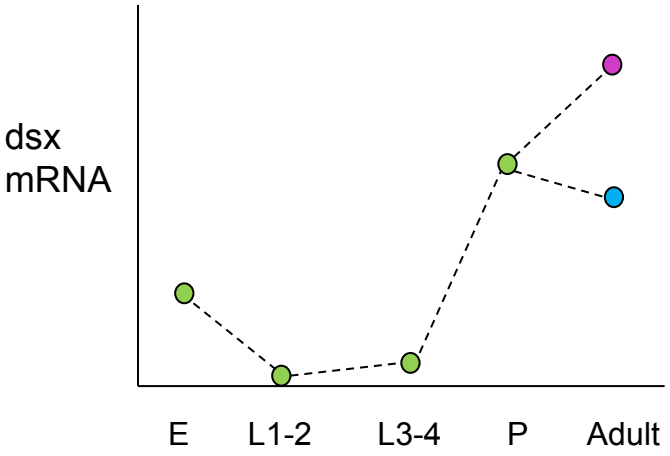
Still need to identify the precise functions of the various spermatogenesis genes

Feeding dsRNA to larvae to prevent female development

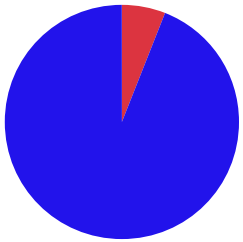
Alternative approach to transgenics

- feeding female-specific dsx dsRNA to insects:





Fed dsxF
dsRNA



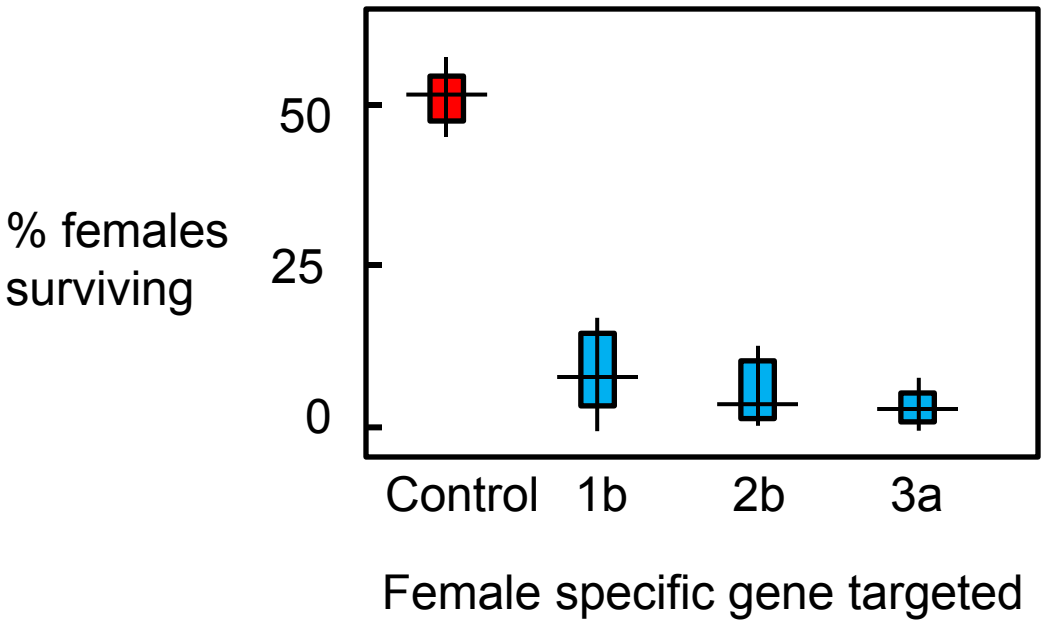
96% males

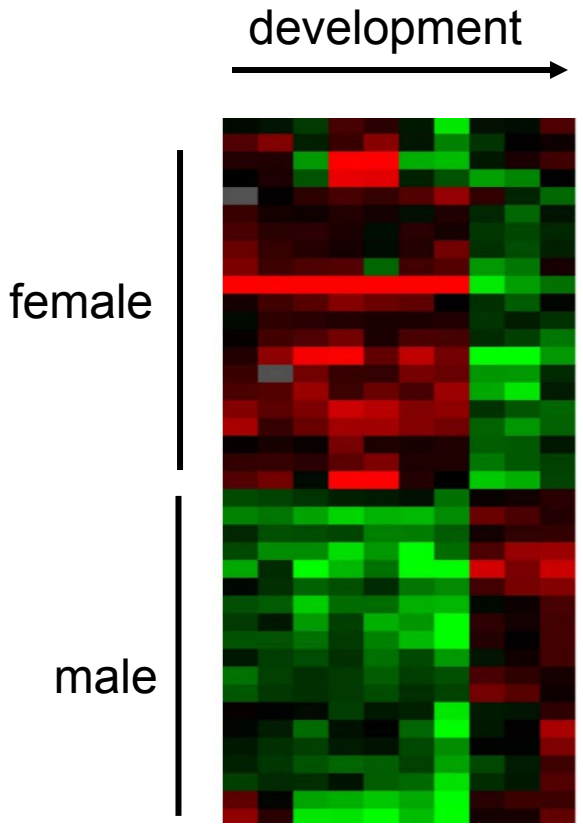
4% females
- all sterile
- None blood-fed

Why is dsx-F knockdown lethal?

Other female-specific target genes

- Transcriptomic analyses to identify female-specific transcripts in larvae or pupae
- Currently testing several new candidate genes

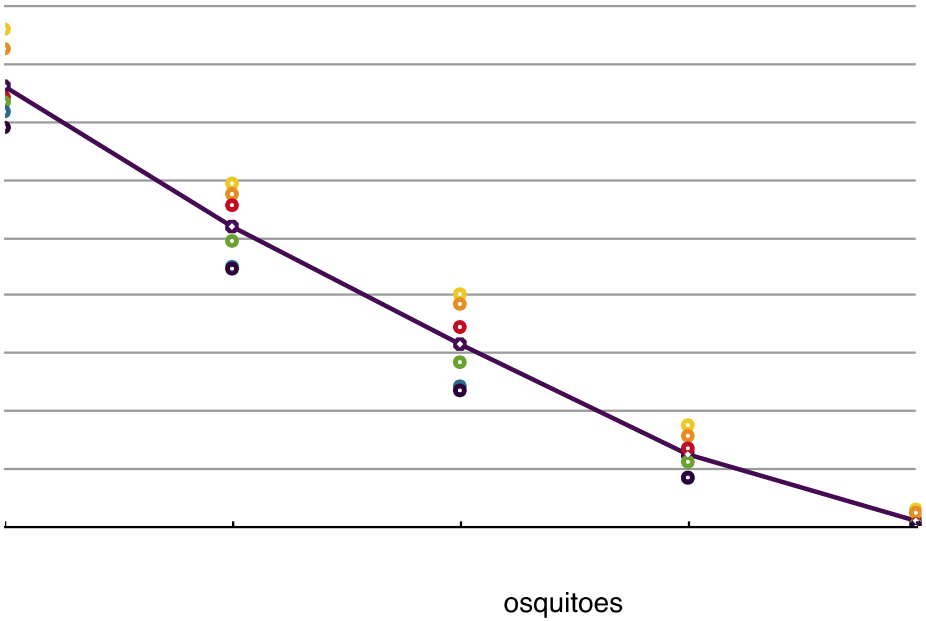




Testing the sterile males in population cages

1. Fed larvae with dsRNAs
- Targeting sperm motility
- Female isoform of dsx

2. Set up mating competition cages with different densities of sterile males

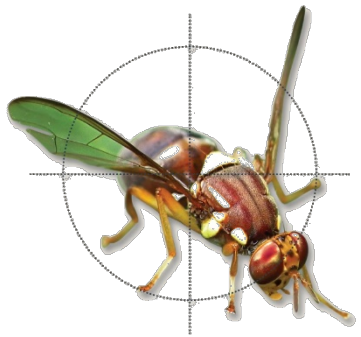


Aedes aegypti vs *Aedes albopictus*

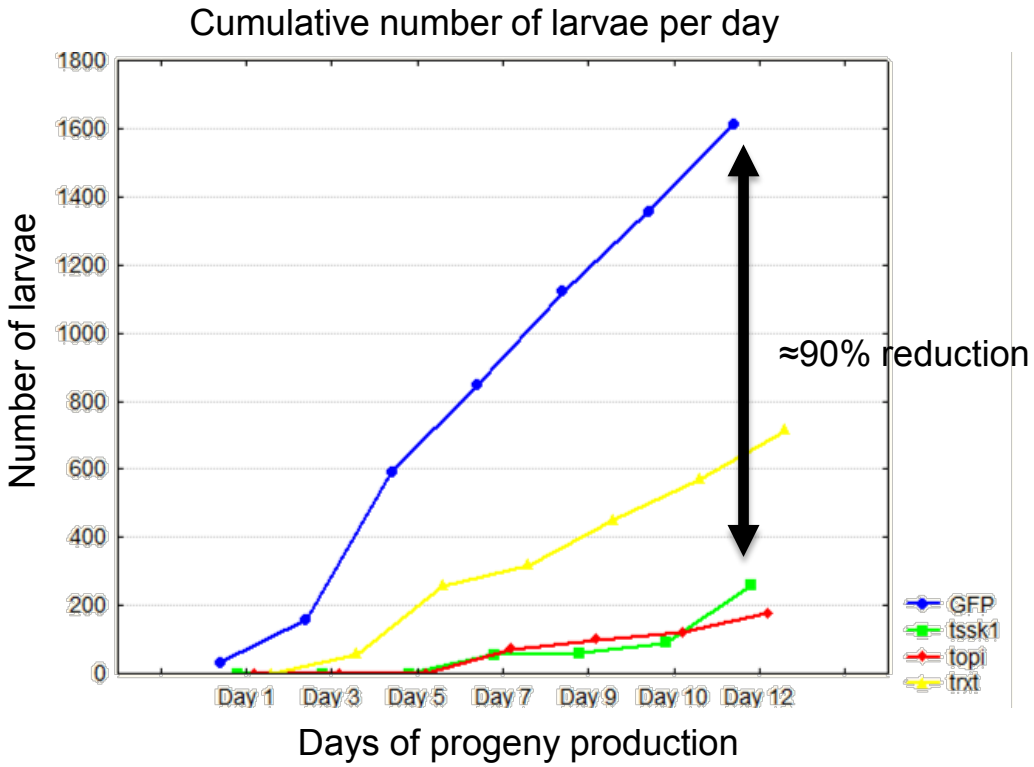
Developing SIT for other mosquito species

	Current progress:
• Find orthologues of target genes – Using bioinformatics to search available databases, or: – Designing primers for degenerate, low stringency PCR	14
• Prepare dsRNA	8
• Injecting larvae/pupae	3
• qRT-PCR to validate RNAi	3
• Mating bioassays	-

Applying RNAi sterility to other insects: Queensland fruit fly

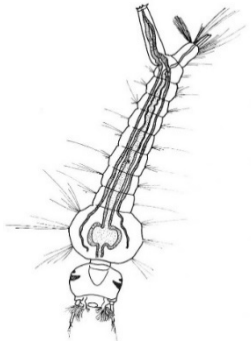


- Feeding dsRNA to young adult males reduced their fecundity by as much as 90%
- Feeding larvae also can sterilize males

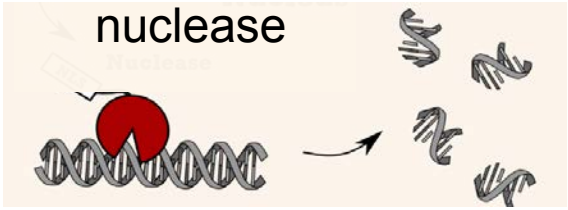


Technical challenges for oral RNAi (1)

Some insects can degrade dsRNA using dsRNA-specific nucleases

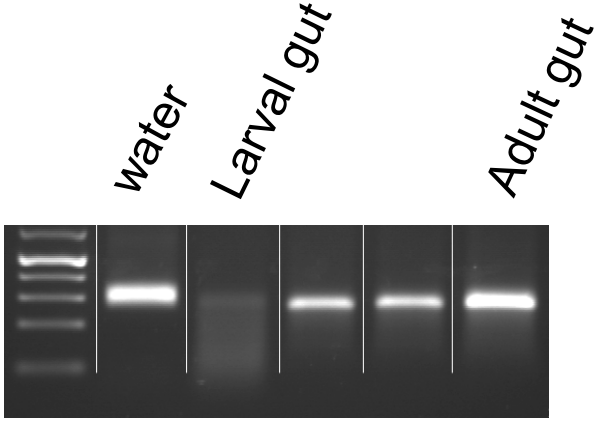


Gut-specific, larval nuclease



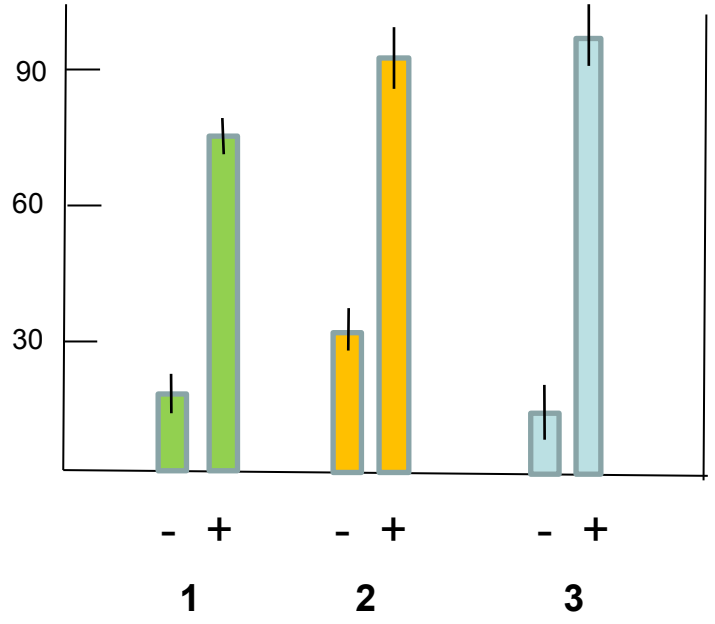
Counter-measures

- Target different developmental stage
- Dual knockdown of nuclease and target RNA
- Microcarriers that protect dsRNA



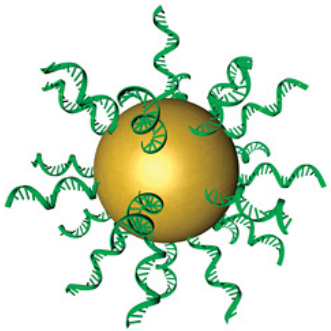
Chitosan nanoparticles improves RNAi efficacy

Extent of RNAi in gut



Up to 10 x more RNAi with chitosan nanoparticles

Ingested dsRNA in mosquito larvae

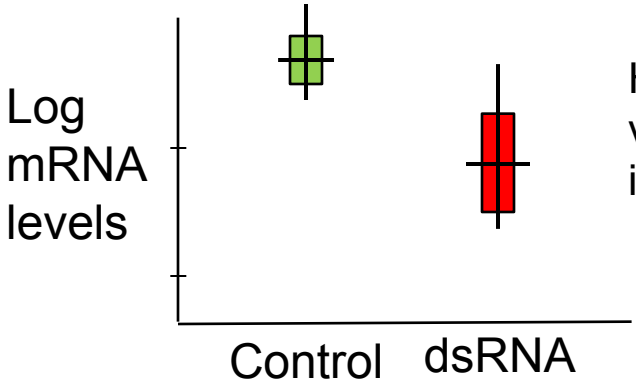


Now testing other organic microparticles

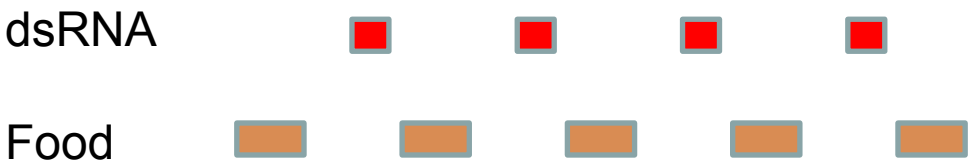
Technical challenges for oral RNAi (2)

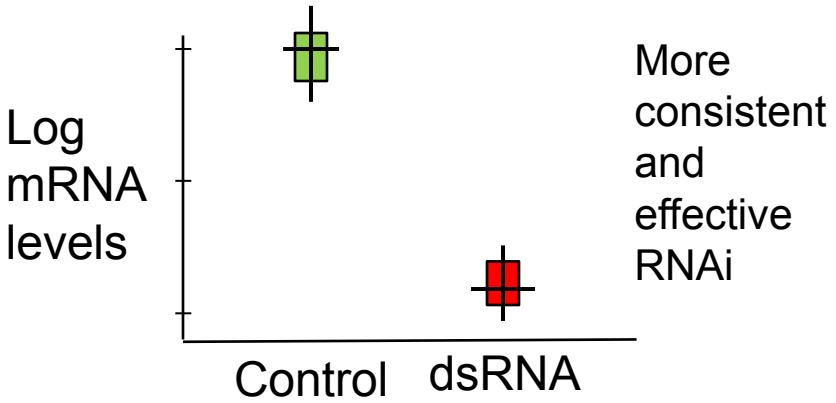
Balancing time of feeding with timing of dsRNA exposure

Continuous dsRNA supply:

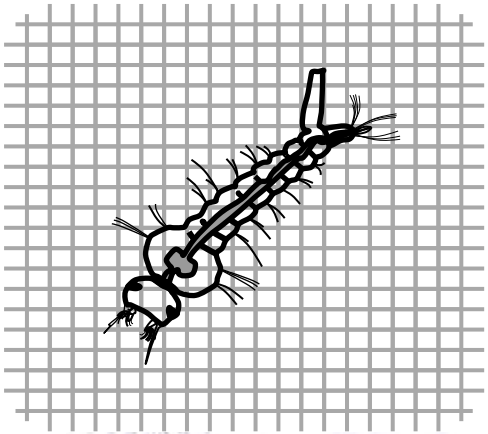


dsRNA after starving:





How might RNAi-mediated SIT mosquitoes be used?



- Insect factories – fully automated!
  - Need to develop automated dsRNA treatments

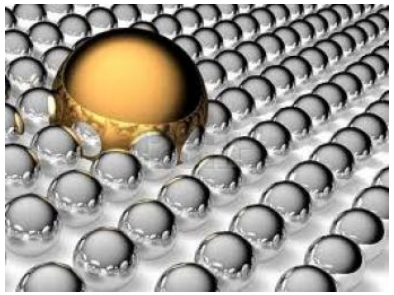
- Could be adapted for any population (not just lab colonies)
  - might improve efficacy if females show preference for local males

- Baited oviposition traps
  - Lure females to oviposition traps
  - Larvae reared in traps fed sterilizing dsRNAs

What's next?

- Identifying more target genes
  - Looking for more genes (essential female genes, male fertility genes)
  - More combinations of dsRNAs

- Developing feeding formulations & microcarriers that maximize RNAi
  - Understanding how dsRNA moves from cell to cell



- Developing higher throughput production methods

RNAi-mediated SIT

- A non-radiation method of producing sterile males
- A simpler method of sex-sorting than mechanical methods
- A non-GM approach to the new SIT methods
- Adaptable to many species
- Can be used with field-caught strains – minimizes assortative mating issues

Thanks to:

- My students and staff:

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Horticulture Innovation Australia

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