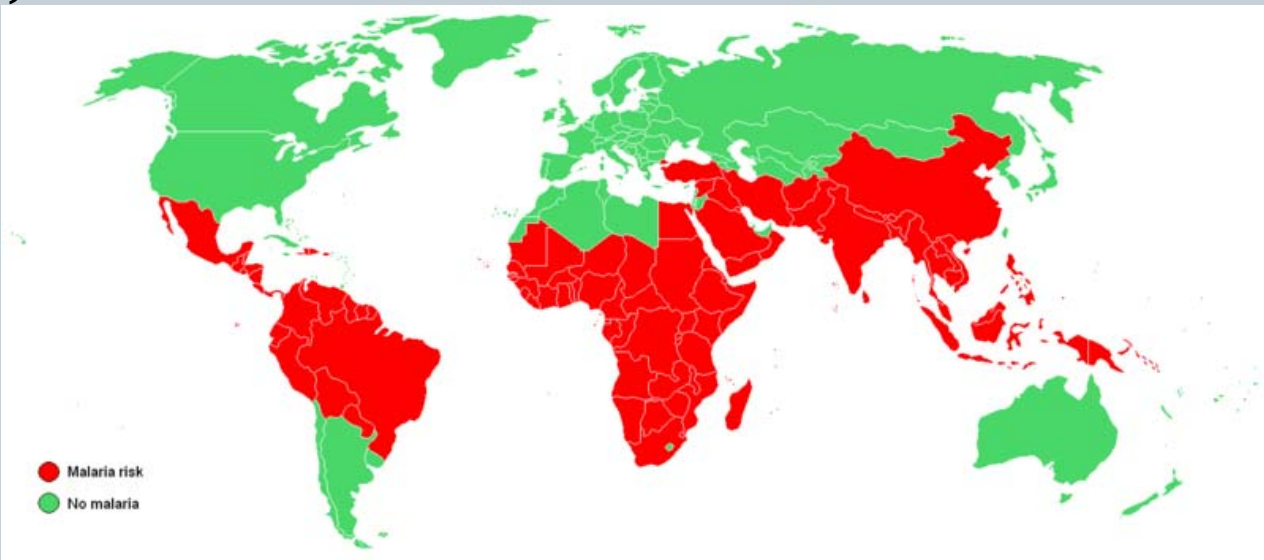
Expression of dsRNA in *Spirogyna* algae to inhibit development of Malaria carrying mosquitoes

KAT PAK AND MATTHEW LOPER

20.109 FINAL PRESENTATION
DECEMBER 7, 2007

Malaria

- Caused by *Plasmodium* protozoan parasites
  - Once a host is infected, the parasite remains indefinitely
- Infects 515 mil people a year and kills between 1 and 3 million including South America, **Africa,** India, Asia, and the Middle East



Map from Wikiped a, using 2003 data from U.S. CDC.

Malaria

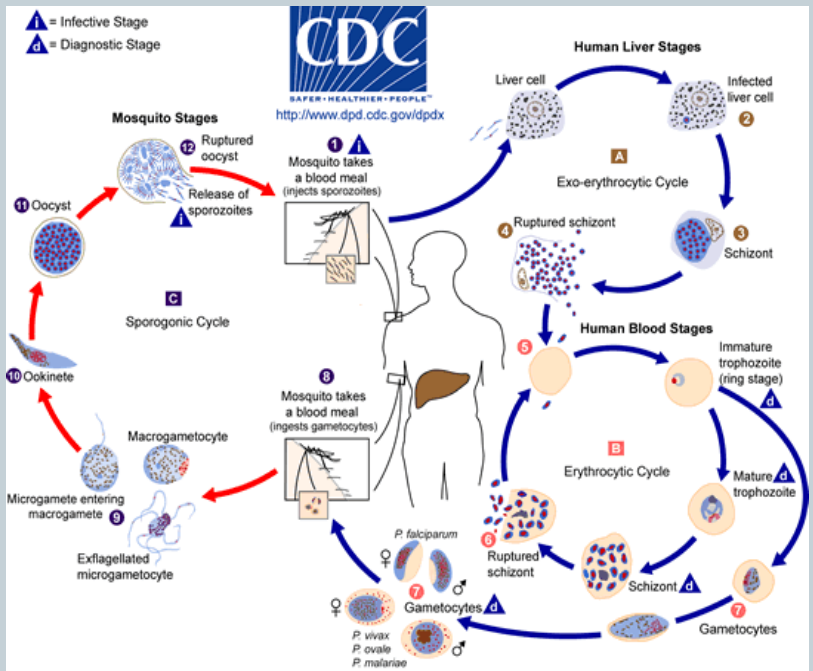
- Associated with poverty and possible huge hindrance to economies
- Estimated $12B malaria economic costs in Africa per year

1. Sachs J, Malaney P (2002). "The economic and social burden of malaria". *Nature* **415**: 680-5.

Image courtesy of "dosh dosh."

Anopheles mosquitoes: A deadly vector

- Female *Anopheles* mosquitoes primary *Plasmodium* carrier
- Larvae hatch and feed on *Spirogyna* algae[2]
- Grow and develop to into pupa and become adults



Courtesy of US CDC.

2. Rejmankova, E. H. et al. 1993 Environmental and regional determinants of Anopheles (Diptera: Culicidae) larval distribution in Belize, Central America. Environ. Entomol. 22, 978–992.

Malaria: Current Treatments

- No effective, approved vaccine
  - Huge amount of research being done to discover effective vaccines
  - Current drugs not affordable or practical for chronic use in malaria regions
  - Recently, a group at Cal used Synthetic Biology to developed yeast to produce artemisinin[2] much cheaper than current synthesis methods

2. Keasling, Jay D et al. **Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature [Nature]. Vol. 440, no. 7086, pp. 940-943. 14 Apr 2006.**

Malaria: Prevention and Vector Control

- Widespread use of insecticides
- Domestic spraying of fungus *Beauveria bassiana*[3]
- Drainage and filling of ponds/rivers
  - Huge economic and environmental costs[4]
- Clearing of algae from ponds[5]
- Biological (predator and microbe) larvicidal controls

Photo removed due to copyright restrictions.
People working in a river.

3. Simon Blanford, et al. Fungal Pathogen Reduces Potential for Malaria Transmission. (10 June 2005) *Science* **308** (5728), 1638.

4. Collins, F. H. & Paskewitz, S. M. 1995. Malaria: current and future prospects for control. A. Rev. Entomol. 40, 195–219.

5. WALKER, K. (2007) Contributions of Anopheles larval control to malaria suppression in tropical Africa: review of achievements and potential. *Medical and Veterinary Entomology* 21(1)

Research Problem & Goals

Goal:

Reduce the *Anopheles* mosquito population by targeting key larvae genes with dsRNA expressed in transgenic algae

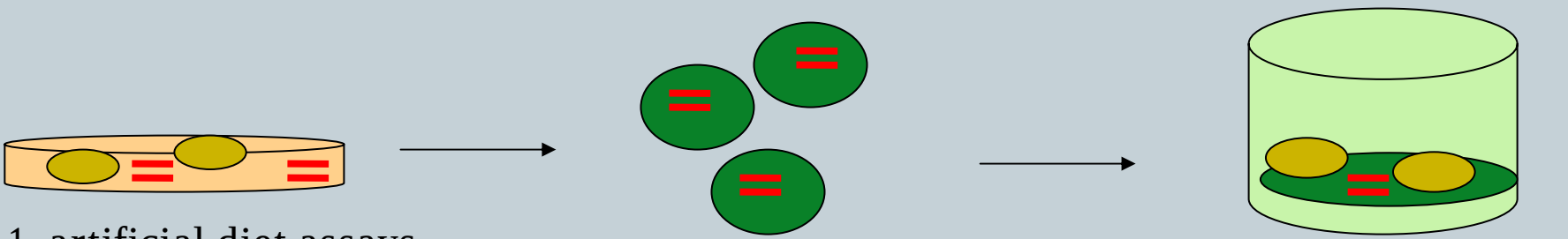
Benefits:

- Minimize harm to ecosystem
- Directly-targeted approach
- Reduce maintenance

Photo removed due to copyright restrictions. Person spraying for mosquitos.

Research Outline

1) Determine larvae key developmental genes
2) Create transgenic algae expressing dsRNA
3) Test if larvae fed on transgenic algae have increased mortality

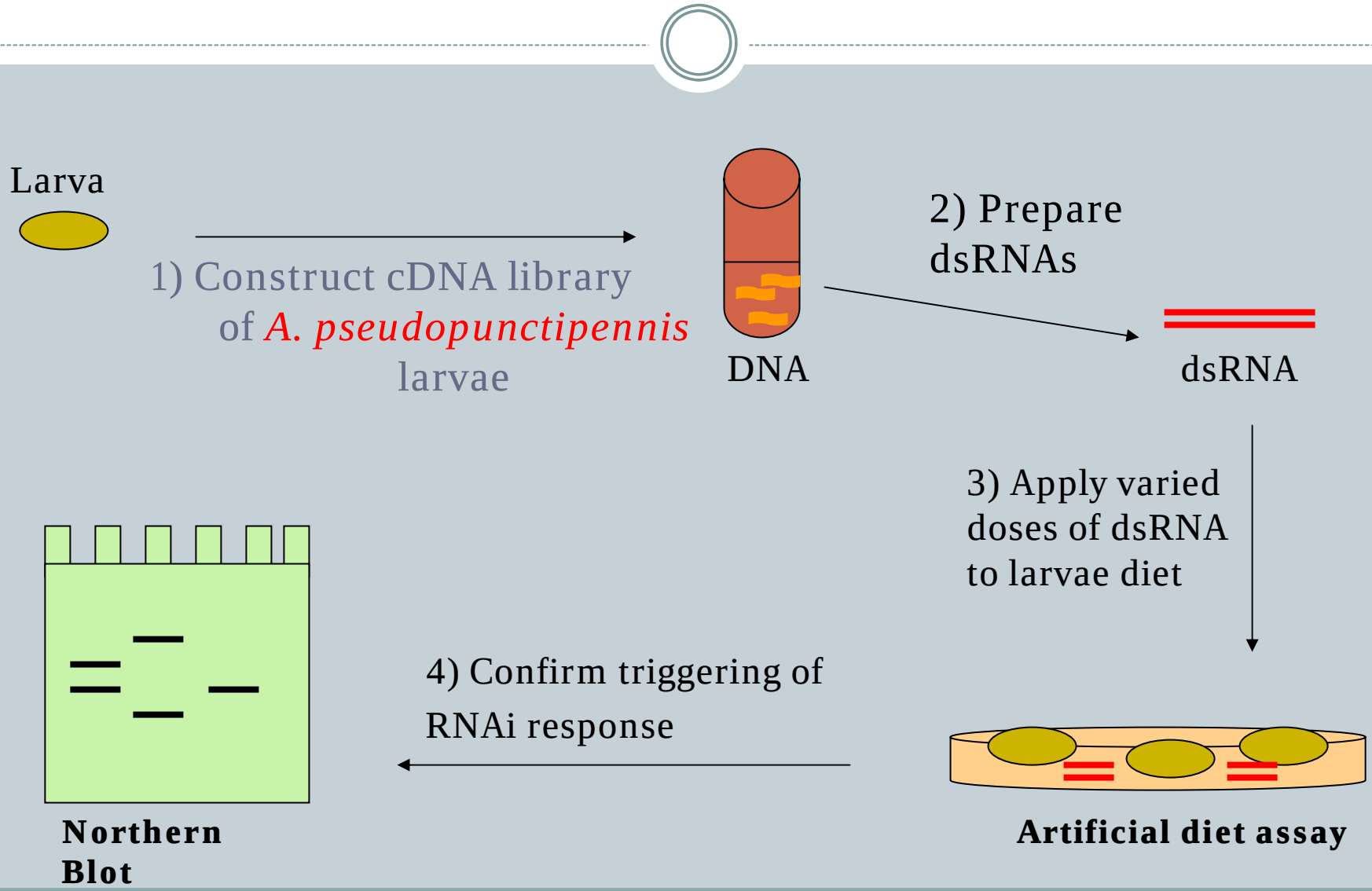


1. artificial diet assays

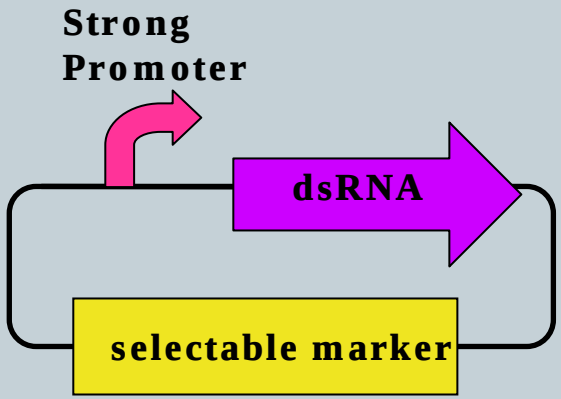
2. transgenic algae

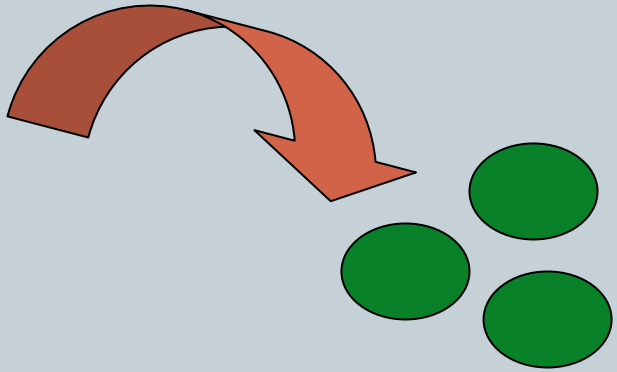
3. growth assays

Determine Key Developmental Genes of Larvae



Test Mortality of Larvae Fed on Transgenic Algae

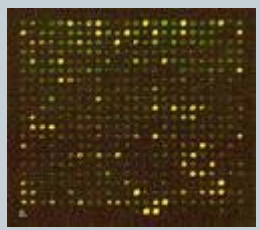




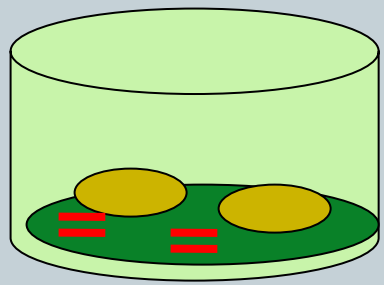
1. Assemble transformation expression cassette

2. Ensure no off-target effect on algae

3. Perform growth chamber assays



microarray



growth chamber

Outcomes

<u>If all goes to plan:</u>

- Test compatibility of transgenic algae with environment
- Introduce transgenic algae to natural habitat
- Test prevalence of transgenic algae over time after introduction
- Determine if technique can be applied to different food sources, mosquito species, and locations

<u>If nothing goes to plan:</u>

- Test other dsRNA sequences
- Target a food source of the mosquito

Needed Resources

- 400 *A. pseudopunctipennis* mosquitoes of the *G3* strain
- Collected algae
- CD1 mice
- Growth Chambers
- dsRNA – order
- Plasmid constructs for Transformation
- Microarrays with larvae DNA
- General Lab materials & Machines (e.g. for microarrays, RT-PCR)

Study Implications

If successful:

- Create a form of Malaria vector control that is **less environmentally harmful** than current practices
- A **cheaper alternative** to current practices
- When combined with vaccine/cheaper drug development, **significantly improve the quality of life** in an impoverished area
- Creates a **profitable** product

Questions?

References

Sachs J, Malaney P "The economic and social burden of malaria". *Nature* **415**: 680-5.

Keasling, Jay D et al. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature Vol. 440, no. 7086, pp. 940-943. 14 Apr 2006.

Simon Blanford, et al. Fungal Pathogen Reduces Potential for Malaria Transmission. *Science* 2005; **308** (5728), 1638.

Collins, F. H. & Paskewitz, S. M. Malaria: current and future prospects for control. A. Rev. Entomol. 1995; 40, 195–219.

Walker, K. Contributions of Anopheles larval control to malaria suppression in tropical Africa: review of achievements and potential. *Medical and Veterinary Entomology* 2007; 21(1)

Brown, A., Crisanti, A., Catteruccia, F. Comparative analysis of DNA vectors at mediating RNAi in ***Anopheles*** mosquito cells and larvae. The Journal of Experimental Biology 2003; 206: 1817-1823

Holt et al. The Genome Sequence of the Malaria Mosquito *Anopheles gambiae. *Science 2002; 298: 129- 149

Baum, J., Bogaert, T., Clinton, W., Heck, G., Feldmann, P., Ilagan, O., Johnson, S., Plaetinck, G., Munvikwa, T., Pleu, M., Vaughn, T., Roberts, J. Control of coleopteran insect pests through RNA interference. Nature Biotechnology 2007; 11: 1322-1326