Modeling Genetic Strategies for Controlling Mosquito-Borne Diseases

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Mosquito-Borne Diseases

Mosquitoes are vectors for many of the most important human infections

- Malaria
  
  Protozoan parasite: *Plasmodium spp.*
  
  Vector: *Anopheles spp.*

  300 to 500 million clinical cases per year…
  
  … leading to between one and two and a half million deaths

Photo credits: C. Curtis, WHO, USAID, AP Photo/Charles Dharapak
Mosquito-Borne Diseases

Mosquito-Borne Diseases

- Dengue
  - Virus (flavivirus: an arbovirus)
  - Four serotypes
  - Vector: *Aedes aegypti* (and others)

- 50 million cases per year
- Severe flu-like illness with severe joint pain
  - “classic dengue”: “break-bone fever”

- About 1% of cases lead to dengue haemorrhagic fever (DHF)
  - Untreated, DHF death rate can be 20%+, but treatment reduces this to 1%.
Mosquito-Borne Diseases

(A. aegypti is also the vector for yellow fever)

- Yellow fever, West Nile virus, Eastern & Western equine encephalitis, LaCrosse encephalitis, …
Control of Mosquito-Borne Infections

• Prevent mosquitoes biting people
  Insecticide-laced bed nets

  *Ineffective against mosquitoes that mainly bite during the day (e.g. A. aegypti)*

• Drug treatment
  Not always available
  (e.g. dengue, although ribavirin?)
  Major problems with drug resistance
  (e.g. malaria)
  Issues with some drugs…

• Vaccines
  Antigenically diverse pathogens
  (malaria especially; dengue has four serotypes)
Vector Population Suppression

Reduce vector population: Population Suppression or Elimination

- Make environment less mosquito-friendly by draining standing water
  Malaria (“marsh fever”) was a major problem in South-East England before marshlands were drained

- Use insecticides
  
  *Not without problems: e.g. DDT, insecticide resistance, impact on non-target insect species*

Image credits: unknown, C. Curtis
Vector Population Suppression

- **Sterile release**
  Introduce a large number of sterile mosquitoes (e.g. radiation sterilized):
  Sterile females will have no offspring
  Some wild-type females will mate with released sterile males, but have no offspring

Problems:
- Genes for sterility do not get passed on (!)
- Density-dependence
  Mosquito population is regulated by density-dependence. Having some females produce no offspring increases the reproductive success of those females that manage to mate with wild-type males

In time, effect of a sterile release would be countered

The success of this approach requires the production and release of a **large** number of sterile insects on an ongoing basis

It can work: the screwworm fly has been eradicated from the US, and as far south as the Panama Canal

Image credit: John Kucharski ARS-USDA
Screwworm fly Eradication

Screw-worm fly, *Cochliomyia hominivorax*, distribution and eradication

- 1953 Infestation in USA
- 1985 Re-introductions
- 1992

**Map Details:**
- MEXICO 1981
- Isthmus of Tehuantepec 1984 - 86
- Sanibel 1953
- Yucatan Peninsula 1991
- PUERTO RICO 1974 - 75
- BELIZE 1989 - 92
- HONDURAS 1991 - 93
- NICARAGUA 1992 - 94
- PANAMA 1994 - 96
- EL SALVADOR 1991 - 93
- COSTA RICA 1993 - 95

**Image:**
- A close-up image of a screwworm fly.
Vector Population Replacement

Produce a mosquito that is unable to transmit infection, and cause it to become more widespread than (i.e. replace) the wild-type mosquito

Genetic engineering (transgenics)

What has to be done?

1. Create mechanism to get genes into the mosquito genome (transformation system)
2. Create an anti-pathogen gene
3. Identify a promoter that can express the desired gene in the correct location within the mosquito and at the correct time
4. Get the transgenic mosquito to replace wild-type population
Progress in Creating Transgenics

1. Create mechanism to get genes into the mosquito genome (transformation system)
   *Done:* Mariner transposable element (Tony James, U.C. Irvine)
   Coates et al. (1998) PNAS 95: 3748

2. Create an anti-pathogen gene
   *Done,* at least for DENV-2: RNA Interference (Ken Olson, Colorado State)
   Franz et al. (2006) PNAS 103: 4198

   co-opts the RNAi pathway, which is an immune mechanism against dsRNA (e.g. of viral origin)
   - Dicer cuts dsRNA into siRNA (small interfering RNA)
   - siRNA incorporated into RNA silencing complex (RISC)
   - RISC targets mRNA, preventing translation

   Idea: anti-pathogen construct expresses DENV2-like dsRNA
Progress in Creating Transgenics

3. Identify a promoter that can express the desired gene in the correct location within the mosquito and at the correct time

**Done:** *Aedes aegypti* carboxypeptidase A promoter (AeCPA) (Marcelo Jacobs-Lorena, Case Western, now Johns Hopkins)


GFP under the control of AeCPA is expressed in the mid-gut of the mosquito 24 hours after a blood meal, but not in control (expression in eyes is due to GFP that is inserted as part of the Mariner construct)

1. Ensure that transgenic mosquito can replace the wild-type population
Vector Population Replacement

Problem: resulting mosquito is typically less fit (has fewer offspring), so trait will not spread through the population (outcompeted by wild-type)
(sterile male is an extreme example of this)

Need a genetic drive mechanism to aid the spread of the desired gene
Such drive mechanisms exist in nature:

- **Transposon**: “jumping gene”: selfish DNA that replicates within genome, increasing its chances of being transmitted to offspring
  
  P-element spread rapidly through wild *Drosophila melanogaster* population

- **Wolbachia**: maternally inherited intracellular bacterium
  
  Spread through *Drosophila simulans* population in California (Turelli & Hoffmann)

Will such approaches work? We use population genetic models to address whether genes can spread through a population
Underdominance (1940s: Serebrovskii, Vanderplank) when the offspring of strain A and strain B is less fit than either

- Can lead to the invasion of a less fit strain, provided that the less fit strain is present in high enough numbers (invasion threshold)

Example (Gould et al., 2006)
Imagine that the offspring of AxA produce 100 eggs, offspring of BxB 50, offspring of AxB 20

If 20% of the population is strain A, 80% strain B, and mating is random, then

- offspring of A produce an average of 0.20(100) + 0.80(20) = 36 eggs
- offspring of B produce an average of 0.20(20) + 0.80(50) = 44 eggs

Strain B is more fit

Vanderplank showed that this worked experimentally, using tsetse flies (vector of sleeping sickness), causing the replacement of the more fit fly by a less fit one
Engineered Underdominance

Davis et al., 2001, Magori and Gould, 2006

Two genetic constructs inserted at unlinked loci
Each construct contains:
- a lethal gene that is suppressed by the other construct
- and the desired gene, with its promoter

Label alleles:
- locus 1: A (wild-type), α (engineered construct 1)
- locus 2: B (wild-type), β (engineered construct 2)

Individuals with only one type of construct are non-viable
- (AABβ, AAββ, AαBB, ααBB)

Individuals are viable if they have both (AαBβ, Aαββ, ααBβ, ααββ) or neither (AABB)

Fitness cost c for carrying each copy of the construct
- (1 - c) for one copy, (1 - c)^2 for two, …
Engineered Underdominance

Parents produce gametes:
  e.g. genotype $A\alpha B\beta$ produces $AB$, $A\beta$, $\alpha B$, $\alpha\beta$
  genotype $AABB$ produces just $AB$

  1/4 of its gametes will be of each type
  all its gametes will be of this type

Gametes combine to produce offspring:
  e.g. for the two individuals above, offspring would be
  $AABB$, $AAB\beta$ ($\overline{\tau}$), $A\alpha BB$ ($\overline{\tau}$), $A\alpha B\beta$
  not all offspring are viable

Cycle repeats

Write genotype frequencies in generation $k$ as $AABB_k$, $A\alpha B\beta_k$, $\alpha\alpha\beta\beta_k$, $\alpha\alpha B\beta_k$, $A\alpha\beta\beta_k$

Gamete frequencies as $AB_k$, $A\beta_k$, $\alpha B_k$, $\alpha\beta_k$

It’s slightly easier to work with equations that describe gamete frequencies because there are fewer types of gametes
Difference Equations for Gamete Frequencies

(Ignore fitness costs here)

\[ \begin{align*}
AABB_{k+1} &= AB_k^2 \\
A\alpha B\beta_{k+1} &= 2AB_k\alpha\beta_k + 2A\beta_k\alpha B_k \\
A\alpha\beta\beta_{k+1} &= 2A\beta_k\alpha\beta_k \\
\alpha\alpha B\beta_{k+1} &= 2\alpha B_k\alpha\beta_k \\
\alpha\alpha\beta\beta_{k+1} &= \alpha\beta_k^2
\end{align*} \]

Gamete frequencies → genotype frequencies

\[ \begin{align*}
AB_{k+1} &= \frac{1}{V} \left( AABB_{k+1} + \frac{1}{4} A\alpha B\beta_{k+1} \right) \\
A\beta_{k+1} &= \frac{1}{V} \left( \frac{1}{2} A\alpha\beta\beta_{k+1} + \frac{1}{4} A\alpha B\beta_{k+1} \right) \\
\alpha B_{k+1} &= \frac{1}{V} \left( \frac{1}{2} \alpha\alpha B\beta_{k+1} + \frac{1}{4} A\alpha B\beta_{k+1} \right) \\
\alpha\beta_{k+1} &= \frac{1}{V} \left( \alpha\alpha\beta\beta_{k+1} + \frac{1}{2} A\alpha\beta\beta_{k+1} + \frac{1}{2} A\alpha B\beta_{k+1} + \frac{1}{4} A\alpha B\beta_{k+1} \right)
\end{align*} \]

Genotype frequencies → gamete frequencies

where \( V = AABB_{k+1} + A\alpha B\beta_{k+1} + A\alpha\beta\beta_{k+1} + \alpha\alpha B\beta_{k+1} + \alpha\alpha\beta\beta_{k+1} \)

(Normalization accounts for only five genotypes being viable, and ensures gamete frequencies sum to one)

Four difference equations in the four gamete frequencies:

• Only need three of these since gamete frequencies sum to one
• If \( A\beta_0 = \alpha B_0 \), symmetry reduces this to two since \( A\beta_0 = \alpha B_0 \) implies \( A\beta_k = \alpha B_k \) for all \( k \)
Dynamics: Invasion threshold

DeFinetti diagram

Three equilibria

- **Co-existence** (saddle)
- Wild-type only \( AB=1 \)
- Engineered only \( \alpha\beta=1 \)

Invasion threshold

Introduction with engineered type \( \alpha\alpha\beta\beta \) into wild-type population (AABB) corresponds to starting on the bottom edge of the DeFinetti diagram.

Invasion threshold determined by location of stable manifold of the co-existence equilibrium.

This corresponds to a 27% release fraction! If this is achieved, engineered type will replace wild-type, otherwise engineered type will go extinct (Davis et al., 2001).

This already high release threshold is higher still if the constructs incur fitness costs.
Medea

Maternal-effect dominant embryonic arrest

Offspring of a Medea female that does not inherit the Medea gene (either from mother or father) dies

Leads to non-Mendelian inheritance

Named for the Greek mythological figure who killed her children when her husband (Jason of Argonaut fame) deserted her

Phenomenon first discovered in *Tribolium* flour beetle

Richard Beeman, 1992

Molecular mechanism still unclear, but close to being found…

Believed to involve two tightly linked loci: one causes a toxin to be laid down in eggs, second encodes for an antidote that rescues the embryo
Synthetic Medea

Synthetic Medea element created in Drosophila by Bruce Hay (Caltech)

Maternally inherited Myd88 is required for normal embryo development

1. Female germline-specific bicoid promoter drives the expression of two microRNAs (miRNA) that silence expression of myd88 (maternally inherited toxin)

2. Bottleneck (Bnk) promoter, active during early embryo development, drives expression of a Myd88 transgene that is insensitive to the miRNA (zygotic antidote) (timing is very important here!)

Science (2007) 316: 597
From the table, we see that $\frac{2}{3}$ of the offspring of matings between female heterozygotes and male heterozygotes inherit Medea (c.f. $\frac{1}{2}$ predicted by Mendelian inheritance)

All offspring of homozygous females (Medea+ or Medea-) survive

Non-Mendelian inheritance: “selfish genetic element”

If there is no cost to having Medea, it will spread through a population

Wade & Beeman (1994)

[went further: showed that Medea will go to fixation even if killing is incomplete]
If Medea incurs a cost, things are a little more complex: invasion threshold

Population genetic model:
Introduce homozygous Medea+ males into WT population, so that half of males are Medea+

Medea can invade, even in the face of a high fitness cost

Experimental results are in agreement:

Medea is an efficient gene-drive system

Other Drive Mechanisms

Sex-linked meiotic drive:
Product of a drive gene (which sits on a sex chromosome) blocks the maturation of gametes that carry a sensitive allele of the response gene. 
*Distorts sex ratio of the offspring* and increases frequency of drive gene.

Male-biased Y chromosome meiotic drive exists in *Aedes aegypti*.

**Wolbachia**: Maternally inherited intracellular bacterium.
Many different types in different insect species.

Cytoplasmic Incompatibility (CI): a cross between an infected male and an uninfected female leads to fewer offspring. Infected females’ offspring more likely to survive.

*Wolbachia* spreads through a susceptible population.

Combined strategies?

We have used simple population genetic models to examine the effectiveness of these drive mechanisms, and strategies that combine two of them.
More Detailed Models…

Simple population genetic models assume large population, random mixing, no spatial structure, no density-dependence

The real world most definitely is not like this

Incorporate genetics into “realistic”, density-dependent model for mosquito population dynamics ( “Skeeter Buster”)

(We also look at models of intermediate complexity, e.g. spatial or age structure, but simple population dynamics)
Important point about *Aedes aegypti*: eggs are laid in clean water, so main habitat for immature stages is water containers.

Field studies of immatures focus on containers.

Pictures courtesy of Amy Morrison, Fred Gould.
General characteristics:

- Species-specific
- Cohort and stage based (eggs, larvae, pupae, adults)
- Detailed biology (larval and pupal development, track weights of cohorts)
- Weather-dependent (temperature and rainfall)
- Spatially explicit (containers and houses)
- Stochastic

Based on an earlier model (CIMSiM: Focks, 1983)
More details:

- Daily timestep
- Each container’s water level and food content are tracked:
  - Water gain (rain, human filling), loss (evaporation, human emptying)
  - Nutrient input (falling from vegetation, dead pupae), output (consumption)
- Water level important for egg laying and egg development (dessication)
- Larvae compete for food
- Enzyme kinetics-based equations model growth and development of immatures
- Female adult weights and gonotrophic cycle are tracked:
  - Female mosquitoes bite when they need blood (allows egg production)
- Mating between males and females, depends on sizes:
  - Larval and pupal development
  - Track weights of cohorts
- Movement: currently random, but could depend on resident population (e.g. females might migrate in search of a mate if no males are present)
- Fertilization of females? One-time deal, or multiple matings? Sperm choice?
Skeeter Buster

Highly complex model (an understatement)
  Gives us a way to ask whether the details matter and to guide data collection

Requires lots of data to parameterize, but fortunately…

Long-term studies of mosquito population dynamics in Iquitos, Peru (since 1999) (Amy Morrison, UC Davis)

Isolated town in the Amazon basin

Population ~ 400,000
Skeeter Buster

Available data:

GIS mapping of all houses in the city

Entomological circuits:
census of immature and adult mosquitoes within individual houses
Effects of container heterogeneity and spatial structure are crucial.

Mosquito dispersal pattern is very important for the spread of a gene. Typically, *A. aegypti* disperse over short distances (nearby houses), but any long-range dispersal has a major impact.

Age structure is important. Releases of different-aged individuals (e.g. eggs, pupae, adults) can have dramatically different outcomes: reproductive value.

Stochastic effects play an important role.

Multiple releases may be beneficial for many strategies.
Impact on Dengue?

Work in progress: linking ecological model to epidemiological model

How effective does the anti-pathogen gene need to be?

What fraction of the wild-type population needs to be replaced?

Even if we cannot achieve fixation of the transgene, can we significantly impact transmission, e.g. reduce $R_0$ below one?

Investigate negative consequences (e.g. recent paper by Koella that suggested vector control could, under certain circumstances, lead to an increase in dengue hemorrhagic fever)
So Everything is Looking Good?

Use of this technology raises important ethical and societal questions

- Government regulation (look to transgenic crops as a model)
- Public acceptance (again, transgenic crops…)

Project involves working with regulatory bodies, health officials, engaging the public, education and honestly evaluating the risks (e.g. virus evolving resistance to anti-pathogen gene, loss of linkage between drive and effector genes)

Very cautious approach
Field Trials and Ecological Studies

Very cautious approach:
Initial work involves releases in controlled, enclosed, environments (large cages)

Field site: Tapachula, Mexico (Hurricane risk…)
cultural sensitivity

Modelers need to know detailed information about the ecology of the mosquito in Tapachula

Field work will be carried out by a large team of entomologists and ecologists (US and local)
If we are going to be cautious, maybe the use of a not so efficient gene drive mechanism would be prudent.

Medea appears to be so efficient that it would spread rapidly and far. Even if Mexico gives approval, what do its neighbors think?

Killer-Rescue: two unlinked genes (K/k, R/r)
- K allele kills offspring
- R allele rescues offspring that carry K

Link anti-pathogen gene to R

Costs to K or R typically lead to both being lost from the population in the long-run, however R may reach high levels for a transient period.

Self-limiting in time and space
- but repeated release of K individuals could keep R at a high level, if wanted
Killer-Rescue

Difference equations

\[ Wx_1 = (1 - c_{R2} - c_{K2})x_1^2 + (1 - c_{R1} - c_{K2})x_1x_2 + (1 - c_{R2} - c_{K1})x_1x_3 + \frac{1}{2}(1 - c_{R1} - c_{K1})x_1x_4 + \frac{1}{2}(1 - c_{R1} - c_{K1})x_2x_3 \]

\[ Wx_2 = (1 - c_{R1} - c_{K2})x_1x_2 + \frac{1}{2}(1 - c_{R1} - c_{K1})x_1x_4 + \frac{1}{2}(1 - c_{R1} - c_{K1})x_2x_3 \]

\[ Wx_3 = (1 - c_{R2} - c_{K1})x_1x_3 + \frac{1}{2}(1 - c_{R1} - c_{K1})x_1x_4 + \frac{1}{2}(1 - c_{R1} - c_{K1})x_2x_3 + (1 - c_{R2})x_3^2 + (1 - c_{R1})x_3x_4 \]

\[ Wx_4 = \frac{1}{2}(1 - c_{R1} - c_{K1})x_1x_4 + \frac{1}{2}(1 - c_{R1} - c_{K1})x_2x_3 + (1 - c_{R1})x_3x_4 + x_4^2. \]

\( W \) is the sum all of the terms on the right hand sides of the four equations

mean fitness of the population
Killer-Rescue

Killer-Rescue Dynamics

Cost to both K and R:

- Introduction corresponds to points on black line
- Three equilibria, two unstable, one stable
- Heteroclinic connections
- Almost all trajectories end up at all WT (both K and R lost)

Both K and R lost: WT
Broader Use of Genetic Pest Management

Approach could be used for many other pest species and/or diseases: malaria, mice, flour beetle, Lyme disease, …

New Genetic Pest Management Program at NC State
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