IDEAS Fundamentals: Mosquito – virus interactions and within host mechanisms

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The World's Deadliest Animals
Number of people killed by animals per year

725,000
Mosquito

475,000
Human

50,000
Snake

25,000
Dog

10,000
Tsetse fly (sleeping sickness)

10,000
Assassin bug (Chagas disease)

10,000
Freshwater snail (schistosomiasis)

2,500
Ascaris roundworm

2,000
Tapeworm

1,000
Crocodile

500
Hippopotamus

100
Elephant

100
Lion

10
Wolf

10
Shark

Source: Gatesnotes
Hundreds of millions of humans are infected with mosquito-borne viruses, **arboviruses**

- **5 genera of arboviruses**
  - **(Flaviviridae)**  
    - *Flavivirus*  
  - **(Togaviridae)**  
    - *Alphavirus*  
  - **(Bunyaviridae)**  
    - *Phlebovirus*  
  - **(Bunyaviridae)**  
    - *Orthobunyavirus*  
  - **(Reoviridae)**  
    - *Seadornavirus*
Hundreds of millions of humans are infected with mosquito-borne viruses, **arboviruses**

5 genera of arboviruses

- **(Flaviviridae)**
  - *Flavivirus*
    - Dengue
    - Zika
    - West Nile virus
    - Japanese Encephalitis virus

- **(Togaviridae)**
  - *Alphavirus*
    - chikungunya

- **(Bunyaviridae)**
  - Orthobunyavirus

- **(Reoviridae)**
  - Seadornavirus

- **(Bunyaviridae)**
  - Phlebovirus

**West Nile virus**
Importance of the Problem (3.9 billion at risk)
Many of the most significant, recent invasions of emerging human diseases are mosquito-transmitted viruses, “arboviruses”

Dengue fever: “break bone fever” began emerging globally 30 years ago and now affects 400 million annually (significant mortality and morbidity)
Importance of the Problem (3.9 billion at risk)
Many of the most significant, recent invasions of emerging human diseases are mosquito-transmitted viruses, “arboviruses”

chikungunya: “to contort or bend up” introduced to the Americas in 2013 and has caused 1.8 million cases in 44 countries / territories (significant morbidity)
Importance of the Problem (3.9 billion at risk)
Many of the most significant, recent invasions of emerging human diseases are mosquito-transmitted viruses, “arboviruses”

Zika fever
- Fever
- Rash
- Joint pain
- Red eyes
- Spread through mosquito bites

Zika: introduced to the Americas in 2014 and is rapidly expanding with 360,000 suspected cases in 2015-2016 with likely many more going unreported (significant morbidity in pregnant women)
Many emerging arboviruses (DENV, CHIKV, and ZIKV) have non-human primate reservoirs in native zones.

Weaver et al. 2017
Aedes aegypti; Yellow Fever mosquito
African, introduced with European colonists

Aedes albopictus; Asian Tiger mosquito
Asian, introduced via tires / lucky bamboo
Environmental, genetic, and human factors are all likely relevant for arbovirus escape from sylvatic cycles, global emergence, and transmission.
A better understanding of mosquito – virus interactions may provide novel strategies to target virus transmission in nature.
Antiviral immunity modulates viral replication, dynamics, and the extrinsic incubation period
Schematic Overview of Mosquito Antiviral Mechanisms

(A) Systemic antiviral strategies

- Unknown PRR
- Toll
- Myd88
- IMD
- Unknown receptor
- FADD
- Rel1
- Rel2
- R2D2
- Dcr-2
- Viral dsRNA
- Viral genome
- viRNAs
- RISC
- Target RNA cleavage

(B) Midgut
- RNAI
- JAK-STAT
- Toll
- Microbiota

(C) Hemolymph hemocytes
- PO
- AMPs
- AaMCR
- AaSR-C
- AMPs

- Vago
- Hop
- Upd
- Dome
Schematic Overview of Mosquito Antiviral Mechanisms

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(D) Salivary glands
- CEC-like peptide
- Extracellular
- Cyst
- AnkP
Schematic Overview of Mosquito Antiviral Mechanisms

(A) Systemic antiviral strategies

(B) Midgut
- RNAi
- JAK-STAT
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- Microbiota

(C) Hemolymph hemocytes
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- AMPs

(D) Salivary glands
- CEC-like peptide
- Extracellular
- Cyst
- AnkP

(E) Neural system
- AaHig
- Blocking endocytosis

Trends in Parasitology
There are significant decreases in the proportion of mosquitoes that become infectious with Zika from those that initially were infected.
Identifying Genes that are Relevant

1. Use sequencing technology to identify genes that are differentially expressed (up- or down-regulated) throughout infection

2. Use reverse transcription PCR across a wider range of conditions to assess gene expression dynamics of those identified genes

3. Design double stranded RNA silencing techniques to assess gene function
RNA interference
Gene silencing by nuclear-encoded miRNA

pre-miRNA

DICER

cleavage by DICER

RNA-RISC complex

target mRNA

enzymatic cleavage of target mRNA

cleaved, nonfunctional mRNA

Gene silencing by synthetic dsRNA

shRNA

siRNA

© 2010 Encyclopædia Britannica, Inc.
dsRNA silencing (B2) of the RNAi response resulted in increased burdens of dengue virus.

Cirimtoch et al. 2009 BMC Microbiology
The mosquito microbiota can also play an important role in modulating mosquito immunity and viral infection.
Members of the microbiome can directly impede viruses.
Or, can stimulate basal immunity leading to virus suppression
Conversely, some microbiota can enhance arboviruses.
Intracellular bacteria, like *Wolbachia*, and the host can stimulate immunity via the generation of reactive oxygen species (ROS).
Intracellular bacteria can also manipulate host miRNA expression
Arboviruses can suppress and enhance members of the microbiome.
Connecting within mosquito processes that limit arbovirus infection to transmission dynamics is difficult

- Lack information on natural variation in immunity across field populations of mosquitoes
- Have little sense of how environmental factors (abiotic and biotic) might mediate vector immunity
- Treat mosquito immune systems as an isolated response instead of part of a network of interacting systems (e.g. digestion, reproduction, nervous, etc.)
Transmission

- Environment:
  - abiotic, biotic factors

- Host:
  - genotype: host defense, resistance

- Parasite:
  - genotype: invasion, virulence factors
Ecoimmunology
Seeks to understand the physiological, ecological, and evolutionary causes and consequences of variation in host immune systems

Vector Biology / Ecology
Seeks to understand the physiological, ecological, and evolutionary drivers of variation in vector-borne disease transmission

The Intersection
Seeks to understand how variation in vector immune systems impact disease transmission
**Vectorial Capacity**

is a measure of transmission potential, and is comprised of both vector and parasite / pathogen traits.

\[
\text{Vectorial Capacity} = \frac{ma^2 bce^{-\mu n}}{\mu}
\]

- **Vector**
  - genotype: host defense, resistance

- **Parasite**
  - genotype: invasion, virulence factors

- **Environment**
  - abiotic, biotic factors

**m** = vector density (determined by multiple factors, e.g. no. eggs laid / female, larval development, larval survival)

**a** = biting rate (1 / gonotrophic cycle)

**bc** = vector competence (proportion of infectious vectors)

**µ** = daily vector mortality rate

**n** = extrinsic incubation period (1 / parasite development rate)
Genotype x genotype interactions impact vector competence ($bc$, the probability of becoming infectious) in the mosquito: CHIKV as an example.
Genotype x genotype x environment interactions

6 populations of *Aedes albopictus* (temperate vs. tropic)

2 strains of CHIKV
1909 = temperate
06-021 = tropic

2 mean temperatures
20 C = blue
28 C = red

Zouache et al. 2014
Environmental variation can **directly** affect vector competence ($bc$) and EIP ($n$) via changes in the rates of vector / pathogen responses.
We know that variation in temperature impacts vector competence \((bc)\) and \(EIP\), and that the relationship with temperature is non-linear.

\[
\text{Vectorial Capacity} = \left( \frac{Ma^2bc - \mu}{\mu/EIR} \right)^{1/2}
\]

Mordecai et al. (under review) PLoS NTDs
We know that variation in temperature is important for transmission, and that the relationship with temperature is non-linear.

\[ \text{Vectorial Capacity} = \left( \frac{Ma^2bc - \mu/EIR}{\mu} \right)^{1/2} \]

Mordecai et al. (under review) PLoS NTDs
Yet, we do not know how temperature is affecting vector competence and EIP: via direct effects on pathogen infection, indirect effects through changes in mosquito immune mechanisms, or both.
Immune profiles of *Anopheles* mosquitoes change dynamically with temperature

Murdock et al. 2012 *Proc Roy Soc B*
Murdock et al. 2012 *Nat Rev Microbiol*
Ae. aegypti infection with CHIKV is enhanced at cooler temperatures due to the destabilization of the RNAi defense pathway

Adelman et al. 2013 PLoS NTDs
CHIKV replicates fastest in *Ae. albopictus* cells (C6/36) at intermediate temperatures, 24 and 28°C (A)...

![Graph showing replication of CHIKV at different temperatures](image)
CHIKV replicates fastest in *Ae. albopictus* cells (C6/36) at intermediate temperatures, 24 and 28°C (A)... due to the effects of low and high temperatures on host cell physiology (B)
Implications

• Vector immunity characterized in the lab may not reflect immune phenotypes in the field

• Pathogens with critical windows of time for establishment may be particularly sensitive to rapid changes in environmental conditions

• Transgenic technologies that manipulate mosquito resistance mechanisms may also be vary with environmental conditions
Transmission

\[ m a^2 b e^{-\mu n} \]

\[ \mu \]

Vector
- genotype: host defense, resistance

Parasite
- genotype: invasion, virulence factors

Environment
- abiotic, biotic factors

Variation in vector immune investment can indirectly impact other transmission traits via physiological and functional trade-offs...

...and these changes could result in much larger effects than changes in vector competence.
ABIOTIC FACTORS
- Temperature
- Humidity
- Salinity

BIOTIC FACTORS
- Food Resources
- Competitors
- Predators / Parasites

BODY CONDITION
- Size, Mass
- Nutritive Stores

probes = energetic / molecular resources

VECTORIAL CAPACITY

Mosquito Fitness
- Development Time
- Gonotrophic Cycle
- Fecundity
- Survival

Pathogen Fitness
- Pathogen development
- Immunity / Physiology
- Microflora
Ecological immunology assumes that what energy is invested in immune function cannot be invested elsewhere.

Differential investment in immunity could result in substantial variation in vectorial capacity.
How trade-offs could be important: using invasive mosquitoes as an example
The enemy release hypothesis: invasive species are successful in their invasive range due to a loss of biological enemies

77% fewer pathogens in invasive range

Mitchell & Power 2003 Nature
53% fewer helminths in invasive range for mollusks, crustaceans, fish, amphibians, and mammals

Parasite species richness

Parasite prevalence

Torchin et al. 2003 Nature
• Organisms on the edge of the invasion also lack parasites relative to those at the core

• Result in differential allocation of energy – from immunity to increased growth and reproduction

• Could result in variation in susceptibility across a zone of invasion, with actively invading populations being more susceptible

• Might help explain variation in susceptibility observed in invasive mosquitoes
**Aedes aegypti;**
Yellow Fever mosquito
African, introduced with European colonists

**Aedes albopictus;**
Asian Tiger mosquito
Asian, introduced via tires / lucky bamboo
Implications

• Selection acts on the whole organism –
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• Selection acts on the whole organism – immunity is one component of an interacting network of physiological systems

• Environmental variation will shape the strength and potentially the direction of these trade-offs by changing the balance of investment
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• Environmental variation will shape the strength and potentially the direction of these trade-offs by changing the balance of investment

• Transgenic manipulation of mosquito resistance mechanisms could result in changes in the entire physiological system – need to develop better fitness assays