Mosquito resistance to the malaria parasite *Plasmodium*

- Plasmodium’s development in the mosquito
- Mosquito immunity to *Plasmodium*
- The mosquito’s natural intestinal bacteria can influence transmission of *Plasmodium*

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Plasmodium’s development in the mosquito

*Plasmodium* gametogenesis

Initiation is triggered in the mosquito gut by:

- Temperature drop of $5^\circ C$
- Xanthurenic acid concentration increase
- pH: 7.4 – 8.3
- $>20$ mM bicarbonate
Plasmodium’s development in the mosquito

The ookinete -stage *Plasmodium*

Blood meal → Peritrophic matrix → Ectoperitrophic space → Midgut epithelium → Basal laminae → Hemocoel

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Plasmodium’s development in the mosquito
The oocyst -stage *Plasmodium*

Day 4  
Day 6  
Day 8

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Plasmodium’s development in the mosquito
The sporozoite-stage *Plasmodium*
Bottlenecks of *Plasmodium* development in *Anopheles*

Contractions and expansions of parasite populations

Sinden *et al.*, Insect Biochem. & Mol. Biol. 2004
CONCLUSIONS:

• *Plasmodium* has to complete complex developmental processes in the mosquito in order to complete its life cycle.

• Critical bottlenecks exist and result in contractions of parasite populations in the mosquito vector.
Mosquito immunity to *Plasmodium*

Mosquito innate immune system

**Pattern Recognition Receptors**

**Signal Amplification Pathways**
- (serine proteases)

**Signal Transduction Pathways**
- (Toll, IMD)

**Effectors Genes (AMP)**

**Phagocytosis**

**Melanization**

**Defense**
Mosquito immunity to *Plasmodium*
Assaying *Anopheles* defenses to *Plasmodium* in the midgut

Dong et al., *PLoS Pathogens* 2006
Many of the discovered genes had anti-*Plasmodium* function. Inactivation of these genes in the mosquito resulted in a lesser resistance to infection.

Dong et al., *PLoS Pathogens* 2006
CONCLUSION:

The *Anopheles* mosquito uses multiple factors of its immune system to fight against malaria parasite infection.
Implication of immune pathways in anti-*Plasmodium* defense

Positive regulators

Negative regulators
Implication of immune pathways in anti-*Plasmodium* defense
Anopheles defenses to *Plasmodium* in the midgut
Implication of Imd and Toll pathways in anti-Plasmodium defense

The Imd pathway controls Anopheles gambiae resistance to Plasmodium falciparum

Garver et al., PLoS Pathogens 2009
Implication of the Imd pathway in anti-*Plasmodium* defense

**EFFECTORS?**

- **Known anti-*Plasmodium* factors**
  - FBN9
  - LRRD7
  - TEP1

Dong & Dimopoulos *JBC* 2009
Garver *et al.*, *PLoS Pathogens* 2009
Transient Imd pathway activation has insignificant impact on longevity

Garver et al., PLoS Pathogens 2009
CONCLUSIONS:

• The Imd pathway is a more potent regulator of anti-\textit{P. falciparum} defense in multiple mosquito vector species.

• The transient activation of the Imd pathway does not cause a significant fitness cost at laboratory conditions.

Garver et al., \textit{PLoS Pathogens} 2009
Can the Imd pathway be used for malaria control?

- **Active against *Plasmodium* in multiple vector species**
  - *A. gambiae/P. falciparum*
  - *A. albimanus/P. falciparum*
  - *A. stephensi/P. falciparum*

- **Can be used at multiple locations and vectors.**

- **Activation has insignificant impact on vector fitness**
  - **LONGEVITY**
    - Sucrose fed
    - Blood fed
    - *P. f. Infected*

- **Spread of transgene Rel2 in a natural population.**

- **Control multiple anti-Plasmodium factors**

- **Plasmodium cannot develop resistance.**
Plasmodium resistant GM mosquitoes based on the IMD/Rel2 system

Midgut & fatbody specific promoter driven Rel2

- REL2
- Imd
- Caspar
- CYTOPLASM
- NUCLEUS

Gene activity over time:
- Blood ingestion
- 15 hr
- 30 hr

Promoters:
- Fat body promoter
- Gut promoter
Plasmodium resistant GM mosquitoes based on the Rel2 system

Genetically modified mosquito lines

Gut promoter -driven Rel2

Fatbody promoter -driven Rel2

A. stephensi transgenic lines (generation 3)

*\(p < 0.0001\)

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The mosquito’s natural intestinal bacteria can influence transmission of *Plasmodium*

~2,000-fold expansion of mosquito gut bacteria after a blood meal

*Plasmodium*

*Bacteria*

Richman et al., 1997
The microbial flora stimulates anti-Plasmodium activity

Reduction of the bacteria flora render mosquitoes more susceptible to Plasmodium infection

Dong et al., PLoS Pathogens 2009
The microbial flora stimulates immune gene expression

antibiotic treatment

bacteria feeding

The mosquito’s natural bacteria flora activate the immune system

Dong et al., *PLoS Pathogens* 2009
Anti-Plasmodium genes control midgut bacteria flora

The presence of the microbial flora activates immune genes that control the proliferation of the flora and Plasmodium infection.

Dong et al., PLoS Pathogens 2006
Dong et al., PLoS Pathogens 2009
CONCLUSION:

- The microbial flora is a regulator of mosquito susceptibility to *Plasmodium*
What happens in the field?

JHMRI field laboratory, Macha - southern Zambia
An *E. cloaca*–like bacteria is able to completely inhibit *Plasmodium falciparum* development in the mosquito gut.

**Graphical Data:**
- PBS
- LIVE
- HIA
- HIA10
- HIA100
- Number of parasites per mosquito

- **=p<0.01**
- **=p<0.001**

- Compared to PBS

- Number of parasites per mosquito: ~10^9, ~10^8, ~10^7
www.dimopoulosgroup.org

• Ruth Aguilar
• Chris Cirimotich
• April Clayton
• Shuchismita Das
• Yuemei Dong
• Lindsey Garver
• Fabio Manfredini
• Musapa Mulenga
• Jose Ramirez
• Shuzhen Sim
• Jayme Souza-Neto
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