Understanding the tripartite parasite-killing relationship between midgut bacteria, mosquito and *Plasmodium* using transcriptomics.

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Mosquito-borne disease elimination targets

The mosquito midgut as bottleneck

Isaacs AT et al. 2012. PNAS
Mosquito midgut bacteria affect parasite development

Natural Microbe-Mediated Refractoriness to *Plasmodium* Infection

*Chromobacterium Csp_P* Reduces Malaria and Dengue Infection in Vector Mosquitoes and Has Entomopathogenic and *In Vitro* Anti-pathogen Activities

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How is *Plasmodium* affected by mosquito microbiota?

Ingested *Plasmodium* → Reduced parasite intensity

**Bacteria**
- Direct (reactive oxygen species. eg. *Enterobacter*)
- Indirect (priming mosquito immunity eg. *Serratia*)

Gene targets

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Transcriptomics and RNA-seq

RNA-seq: High-throughput sequencing technology to assess the transcriptome.

Transcriptomics: Study of the RNA (transcripts) content of a sample.
Previous studies

Fig 1: Bacterial family proportions in mosquito midguts sampled from breeding sites during the dry (A) and rainy (B) seasons. Families representing ≥0.01 proportions in each site are shown; all families <0.01 are grouped as ‘Others’.

Fig 2: Effect of sugar and blood on bacteria. **** ( <0.0001); ***(0.0001-0.001)

Simultaneously profile the transcriptome of mosquito midgut and *Plasmodium* parasites to explore differential global gene expression in the presence of *Enterobacter* and *Serratia*. 
Experimental design

Treat day old mosquitoes with cocktail of antibiotics to clear midgut bacteria

350-400 females  350-400 females  350-400 females

Separate ‘treated’ mosquitoes into 9 cages for reintroduction of bacteria

Enterobacter

100-130  100-130  100-130

Serratia

100-130  100-130  100-130

No reintroduction (control)

100-130  100-130  100-130

Dissect 10% each to check Plasmodium ingestion

Dissect ~10-18 mosquitoes from each cage after 24 hours for ookinete development

Dissect midgut of remaining mosquitoes (~25-35) from each cage for RNAseq
Progress

• Successful infection of ‘field’ mosquitoes with *P. berghei*

• Bacteria clearing and re-introduction

Stained midgut showing *Plasmodium* oocysts

Antibiotic-treated

*Enterobacter* re-introduced
Challenges

• Mosquito numbers
  – Lost mosquito populations in the dry season
    • More sampling during the rainy seasons (ongoing)
  – Inability to obtain numbers to set-up one big experiment
    • Blocks of smaller set-ups, and pooling of similarly-treated samples (ongoing)
Expected outcome

• Examine how the gut microbiota alters both constitutive gut immunity and the induced response to malaria parasites.

• Characterization of how the gut microbiota modulates mosquito-Plasmodium interactions at the transcriptomic level.

• Gene targets to explore further for potential disease transmission-blocking
Summary

Immune functions

Transcriptional effect

Transcriptional effect
Acknowledgement

Collaborator
Prof. Francis Jiggins
Dept. of Genetics, Cambridge

Mentor
Prof. Michael Wilson
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Thank you