Epidemiological and parasite-genomic evidence for selection on *Schistosoma mansoni* in the context of mass drug administration

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Postdoctoral Researcher

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Academic Background

UNIVERSITY OF CAMBRIDGE

2008-11 Bachelor's Degree:
Biological Anthropology

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

2012-13 Master's Degree:
Medical Parasitology
Project on diagnostics for soil-transmitted helminths

Imperial College London

wellcome sanger institute

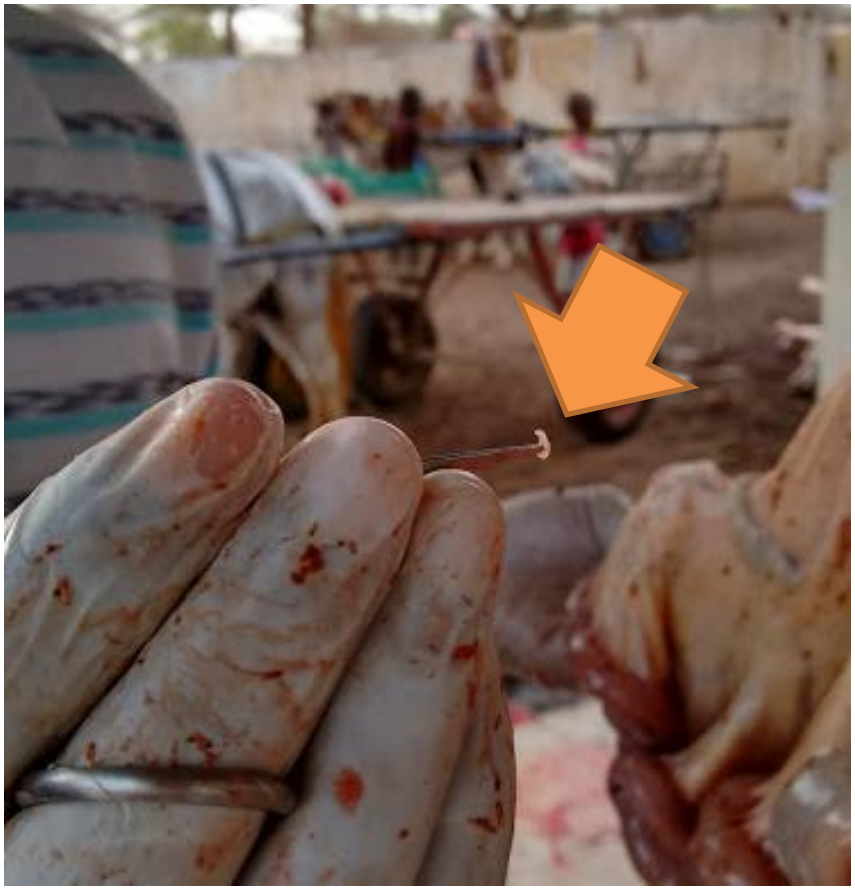
2013-17 PhD Imperial College:
Dept. Infectious Disease Epidemiology
Thesis Title: Epidemiology and population genomics of *Schistosoma mansoni*

MORU Tropical Health Network

2017- Present Postdoctoral Researcher
Dept. Mathematical and Economic Modelling (Bangkok)

Schistosoma mansoni adult worm

Photo credit:
Gabriel Rinaldi



Schistosome adult worm (zoonotic species)

Photo credit:
Thomas Crellen

Outline of Presentation

Part 1) Introduction to *Schistosoma mansoni* and schistosomiasis

Part 2) Epidemiological evidence for selection caused by mass drug administration (MDA)

Part 3) Parasite genomic evidence for selection caused by MDA

Parasite Lifecycle

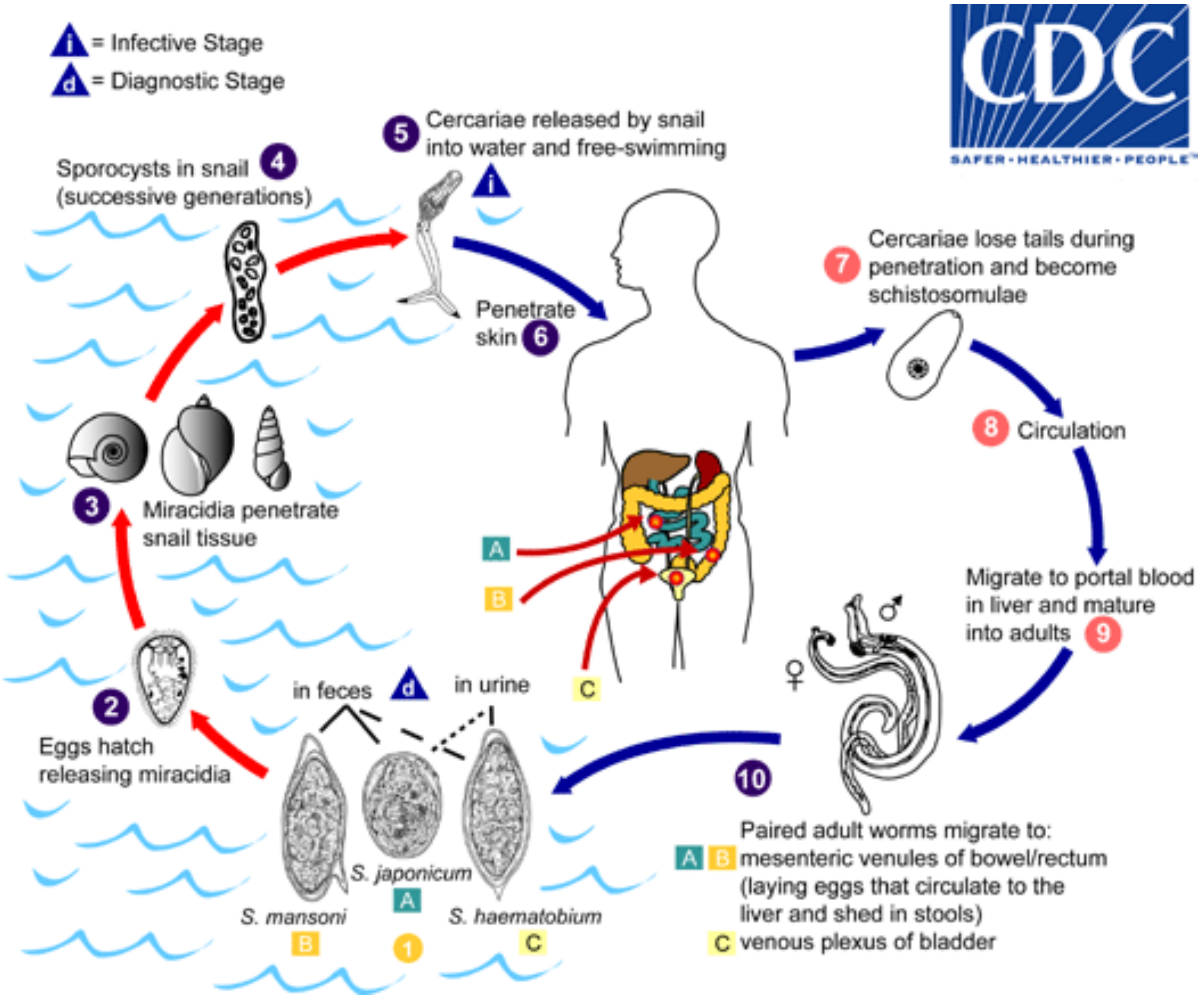
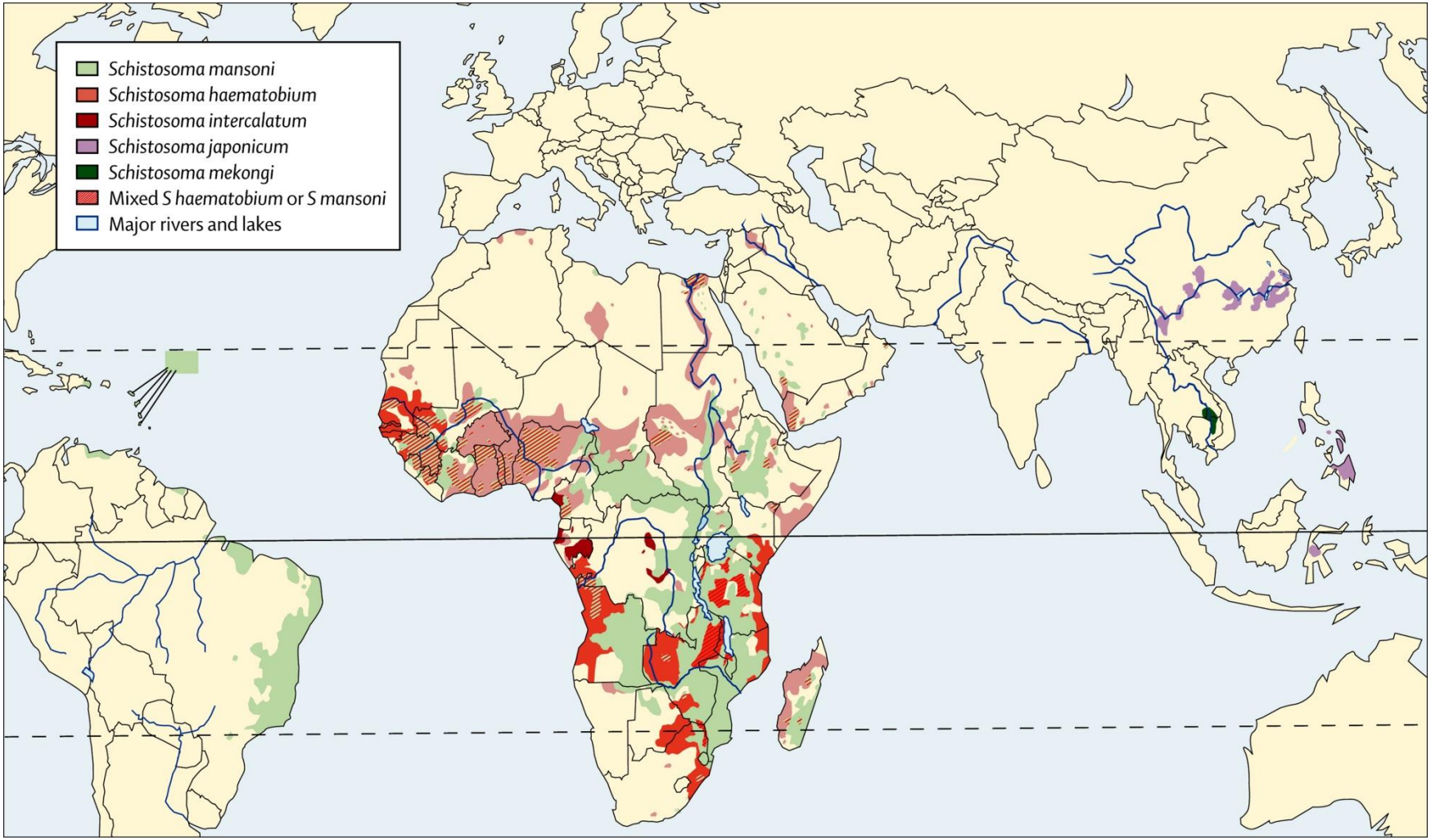


Image credit: CDC

Spatial Distribution of Schistosomiasis



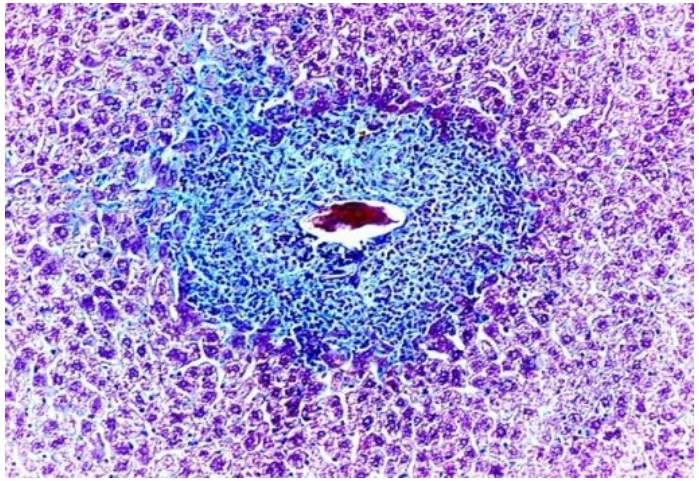
Colley, Daniel G., et al. "Human schistosomiasis." *The Lancet* 383.9936 (2014): 2253-2264.

Morbidity from schistosomiasis

1) Eggs from adult worms become trapped in tissue

2) Leads to inflammatory immune response, and causes granuloma around the egg

3) Fibrous tissue in liver or spleen can cause chronic damage and organ failure



Colley, Daniel G., et al. "Human schistosomiasis." *The Lancet* 383.9936 (2014): 2253-2264.

Historical Parasite Control

Japan: Last human case of *Schistosoma japonicum* in 1977. Control achieved through destroying snail environments

Image credit: WHO

Sudan and Egypt (1979-1990): tried to eliminate *Schistosoma haematobium* through sanitation, health education and snail control. Failed due to conflict.

Sub-Saharan Africa (2003- present): Reliance on mass drug administration with praziquantel. Low cost as drug is donated. National programs are often supported by international NGOs (charities).

Mass Drug Administration (MDA)

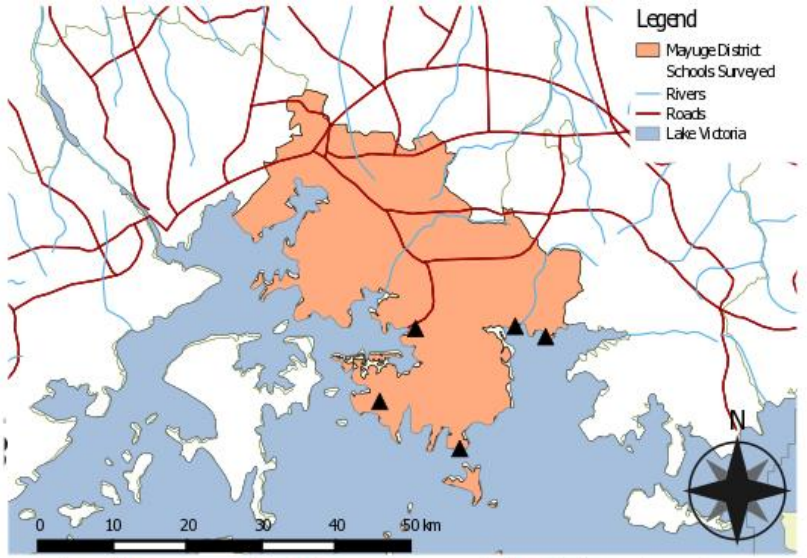
- MDA is being used to eliminate schistosomiasis by 2025
  - 61 million treated in 2014 (out of ~260 million infected)
  - Evidence that MDA can reduce prevalence in low transmission settings (Burkina Faso)
  - Praziquantel generally safe / well tolerated

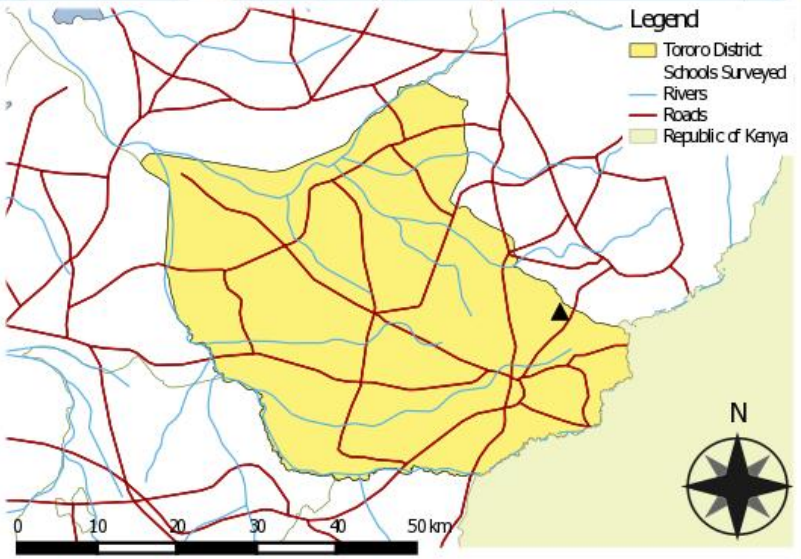
Image credits: The END Fund, Schistosomiasis Control Initiative

- **My research question – are repeated rounds of treatment leading to drug resistance or selection on the parasite genome?**

Part 2) Epidemiological evidence for selection caused by MDA

Sampling schools in Uganda





3 schools with 9 previous rounds of MDA (“High”)

2 schools with 5 previous rounds of MDA (“Medium”)

1 school with 1 previous rounds of MDA (“Low”)

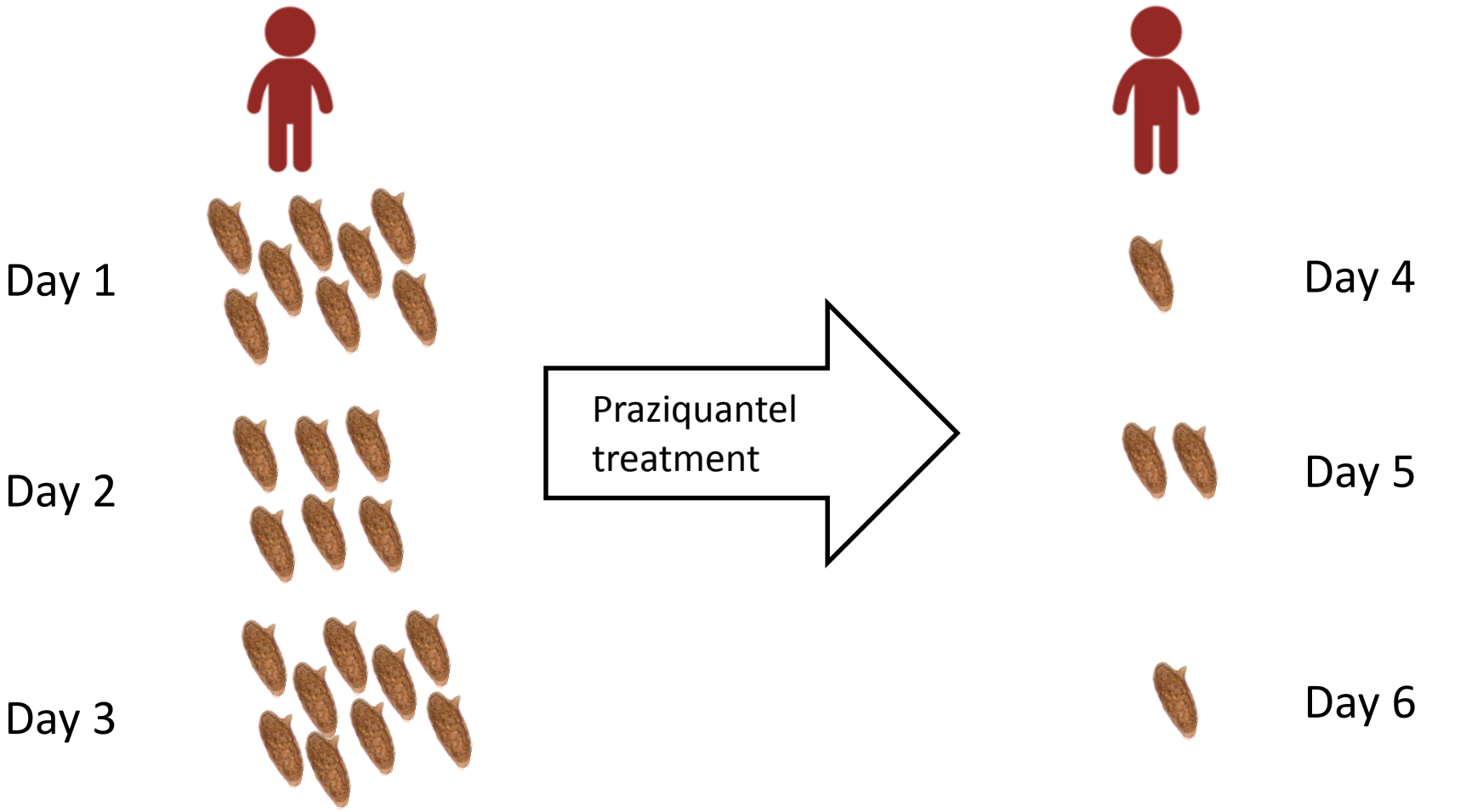
Sampling schools in Uganda

Image credits: Thomas Crellen

Baseline prevalence and infection intensity

School	Recruited	Tested at Baseline	Positive for *S. mansoni* (%)	Mean EPG (95 % CI)
Bwondha	98	96	87 (90.63)	742 (518-973)
Bugoto	183	170	144 (84.71)	436 (339-534)
Musubi	128	127	120 (94.49)	465 (362-600)
Bukoba	120	118	53 (44.92)	92 (67-128)
Bukagabo	120	118	57 (48.31)	306 (197-442)
Kocoge	120	120	81 (67.50)	347 (245-456)
Total	769	749	542 (72.36)	382 (338-446)

Statistical model for egg count reduction (1)



Statistical model for egg count reduction (2)

Hierarchical regression models

Statistical models that account for **repeated egg counts** and permit the estimate of egg count reduction to vary by **individuals**, **schools** or **MDA exposure** (random effects).

Review

CellPress

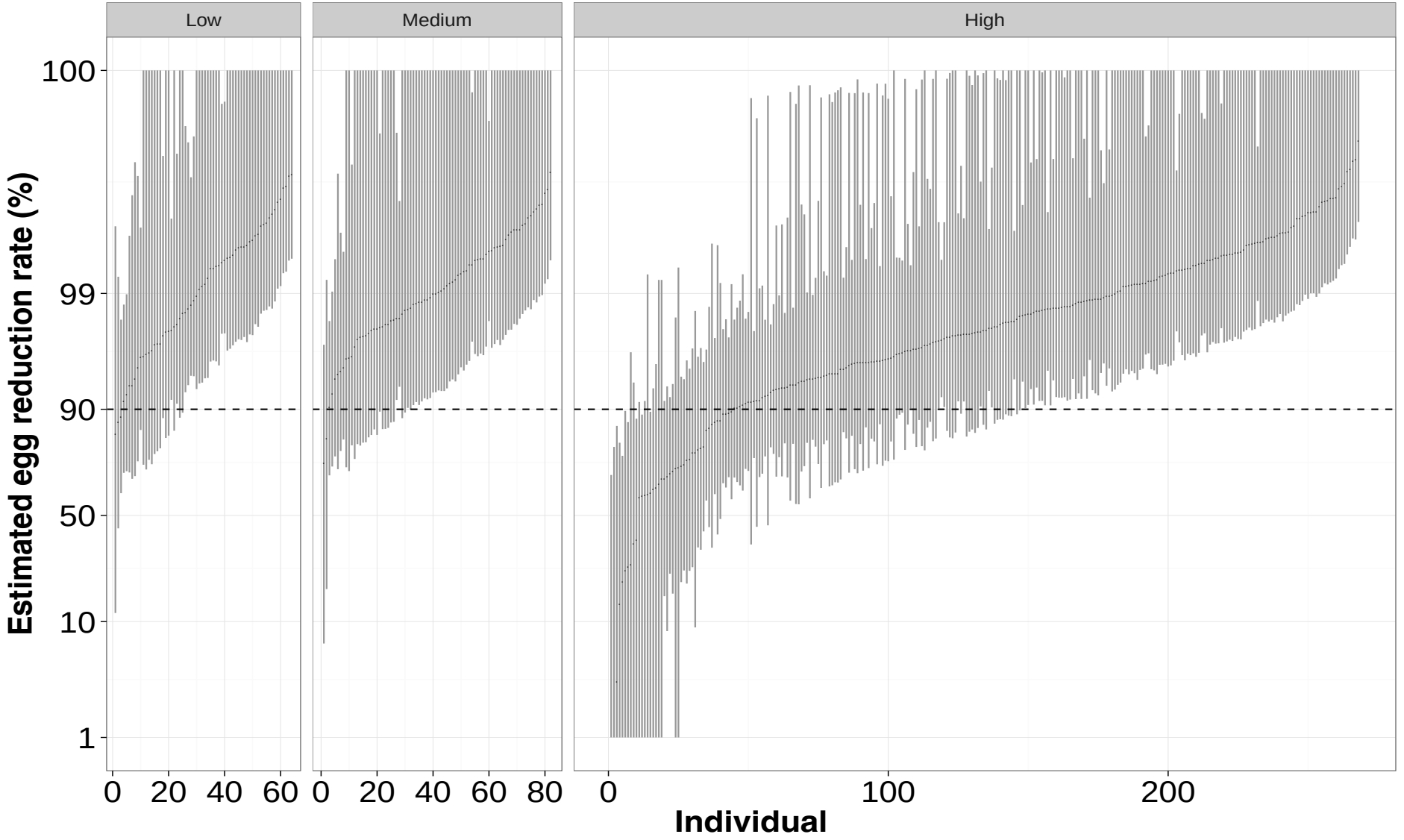
Models for measuring anthelmintic drug efficacy for parasitologists

Martin Walker, Thomas S. Churcher, and María-Gloria Basáñez

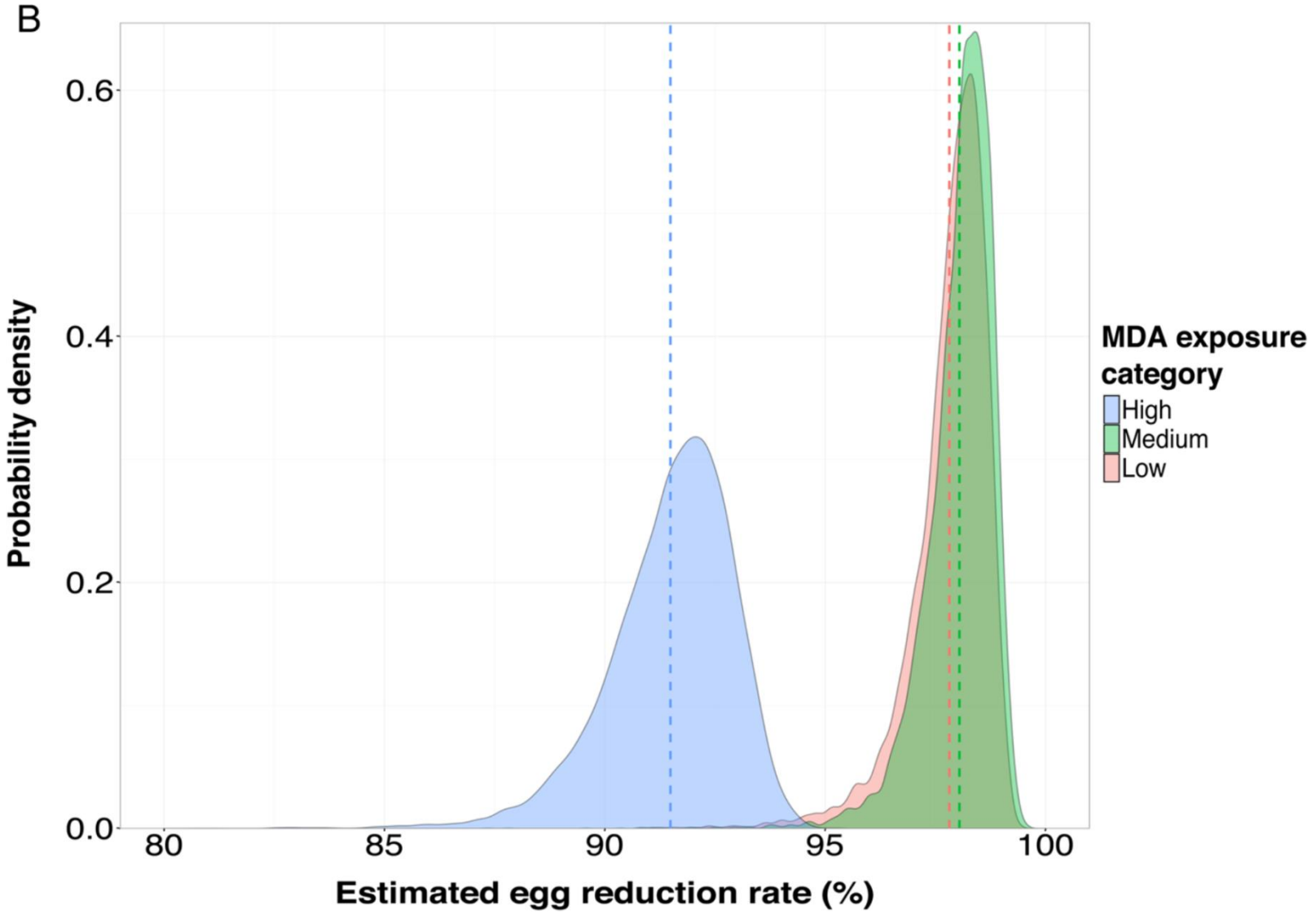
Department of Infectious Disease Epidemiology, London Centre for Neglected Tropical Disease Research, School of Public Health, Faculty of Medicine (St Mary's Campus), Imperial College London, Norfolk Place, London, W2 1PG, UK

Trends in Parasitology 2014 30:11

Individual-level Egg Reduction by MDA category (414 patients)



Posterior Distribution of Egg Reduction by MDA category



Paper published 2016

Clinical Infectious Diseases

MAJOR ARTICLE

Reduced Efficacy of Praziquantel Against *Schistosoma mansoni* Is Associated With Multiple Rounds of Mass Drug Administration

Thomas Crellen,[1,2,3,a] Martin Walker,[1,a] Poppy H. L. Lamberton,[1,4] Narcis B. Kabatereine,[5] Edridah M. Tukahebwa,[5] James A. Cotton,[2] and Joanne P. Webster[1,3]

[1]Department of Infectious Disease Epidemiology and the London Centre for Neglected Tropical Disease Research, Imperial College London, St Mary's Campus, [2]Wellcome Trust Sanger Institute, Hinxton, [3]Department of Pathology and Pathogen Biology, Royal Veterinary College, University of London, Hertfordshire, and [4]Institute of Biodiversity, Animal Health & Comparative Medicine and Wellcome Trust Centre for Molecular Parasitology, University of Glasgow, United Kingdom; and [5]Division of Vector Bourne Diseases, Ministry of Health, Kampala, Uganda

Background. Mass drug administration (MDA) with praziquantel is the cornerstone of schistosomiasis control in sub-Saharan Africa. The effectiveness of this strategy is dependent on the continued high efficacy of praziquantel; however, drug efficacy is rarely monitored using appropriate statistical approaches that can detect early signs of wane.

Methods. We conducted a repeated cross-sectional study, examining children infected with *Schistosoma mansoni* from 6 schools in Uganda that had previously received between 1 and 9 rounds of MDA with praziquantel. We collected up to 12 *S. mansoni* egg counts from 414 children aged 6–12 years before and 25–27 days after treatment with praziquantel. We estimated individual patient egg reduction rates (ERRs) using a statistical model to explore the influence of covariates, including the number of prior MDA rounds.

Part 3) Parasite genomic evidence for selection caused by MDA

Image credit: Thomas Crellen

- Collected >11,000 miracidia in Uganda
- Miracidia hatched from eggs in stool (process of washing and filtering stool)
- Miracidia picked by pipette and washed in “nuclease free water” before stored on FTA cards

Generating whole-genome sequences

Miracidia collected on filter paper (binds DNA)

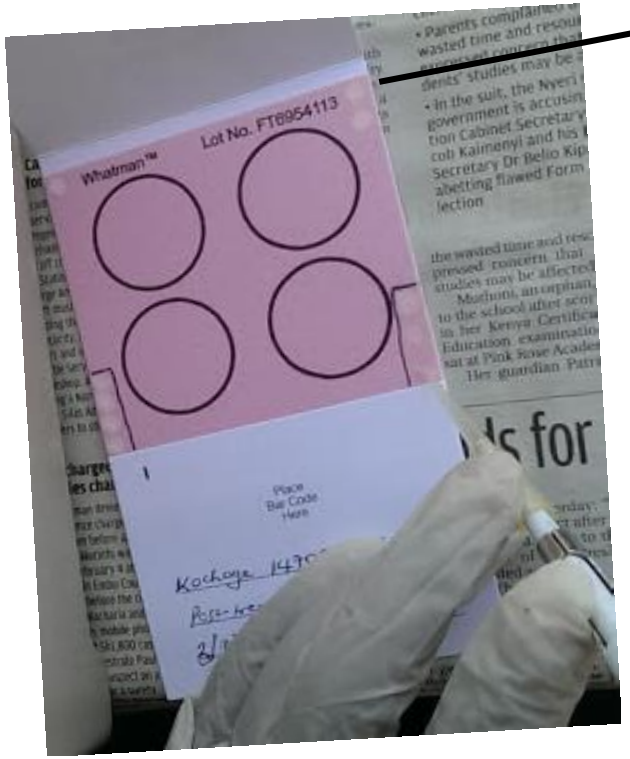


Image credit: Thomas Crellen

DNA extraction, purification and amplification

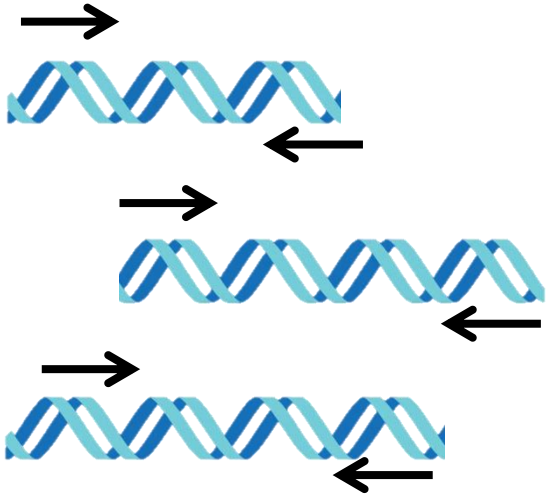
Preparation of DNA for “next gen” (Illumina) sequencing

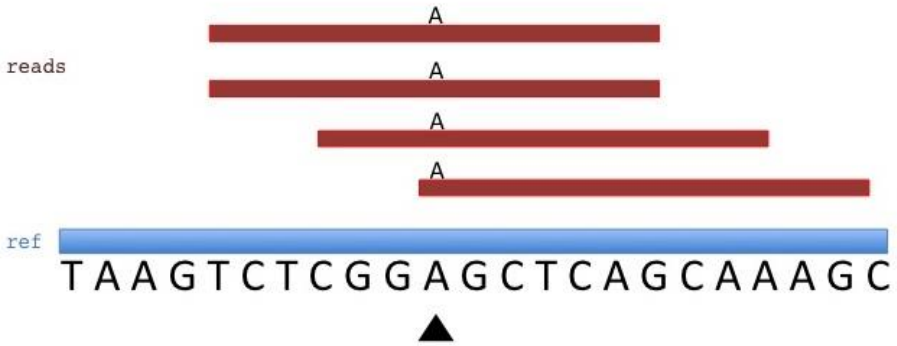
Image credit: Illumina

Short read sequencing and bioinformatics

Genomic DNA is fragmented

Sequence 150bp in forward and reverse direction



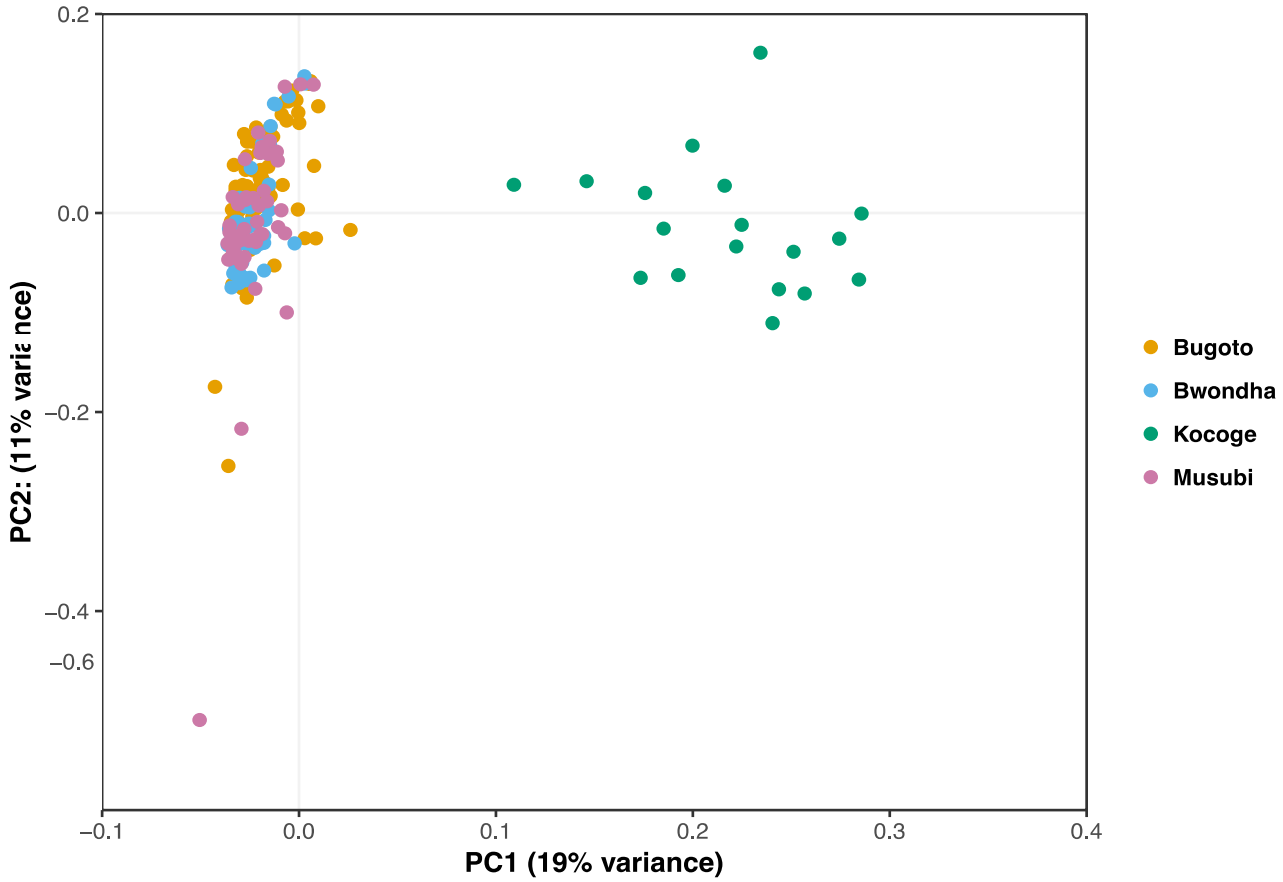


Map reads to reference genome, determine SNPs

Image credit: Wikipedia, Pixabay

Parasite population genomics

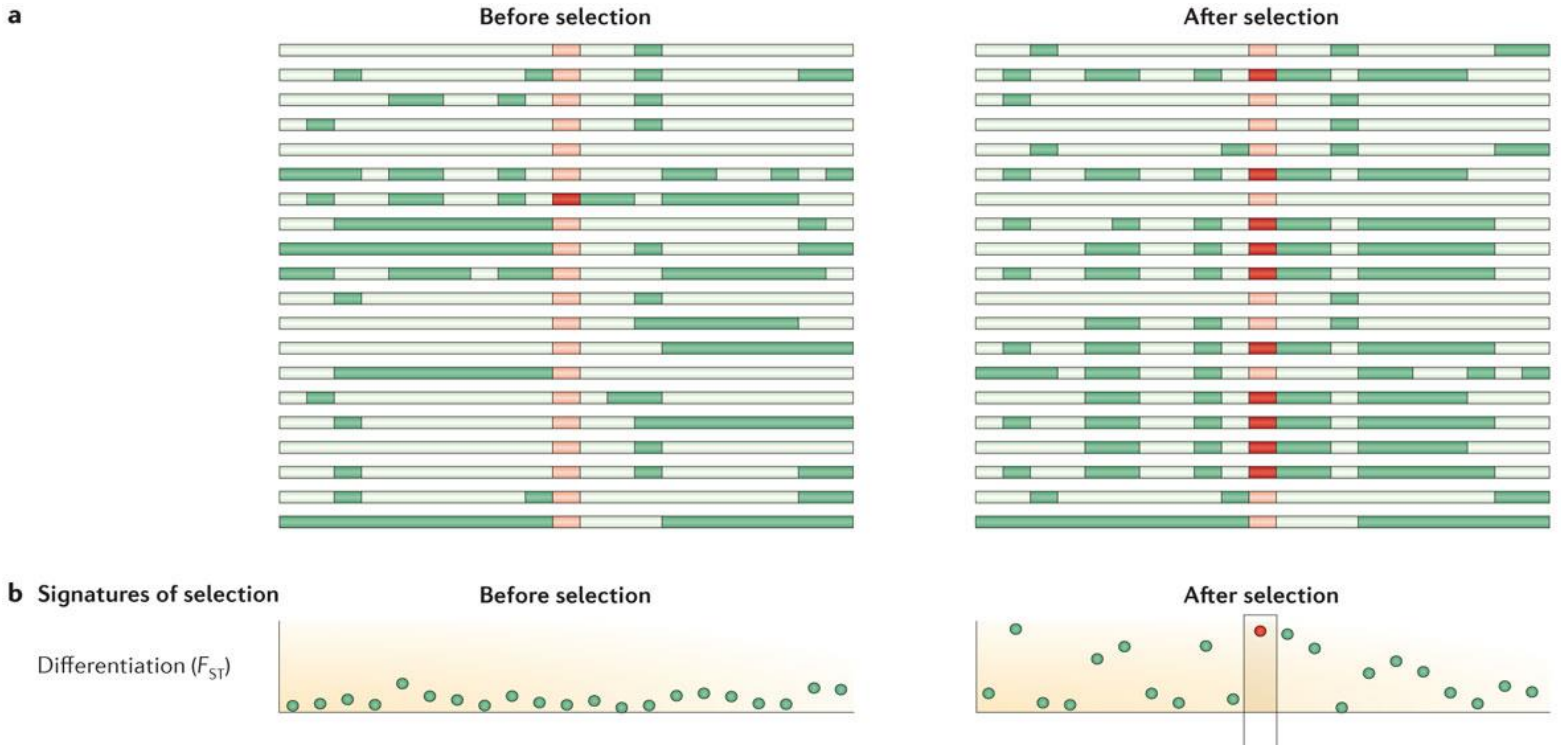
After filtering, we have ~4 million SNPs from 200 miracidia (taken from 38 individuals in 4 of the schools)



Unpublished data, Crellen, Berger et al.

Testing for selection: Linkage Disequilibrium

In eukaryotic organisms (humans, *Plasmodium* etc) selection causes a reduction in diversity around the causal variant, which is gradually broken up by recombination



Karlsson, E.K., et al. 2014. Natural selection and infectious disease in human populations. *Nature Reviews Genetics*, *15*(6), p.379.

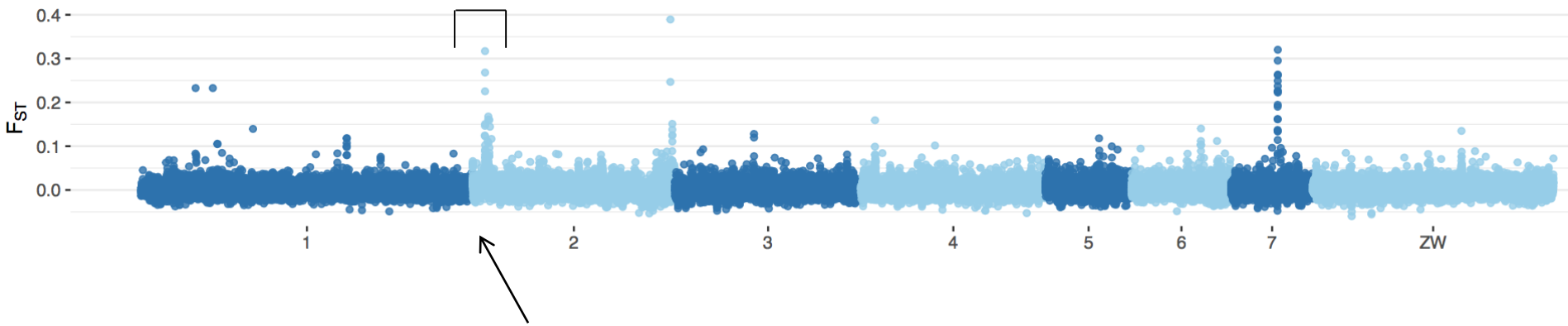
Testing for selection: F_{ST}

F_{ST} measures the differences between groups of samples along a genome

When F_{ST} is higher, this indicates an unusual region where selection may be occuring

We expect selection in miracidia from areas with high MDA pressure, but not in areas of low pressure

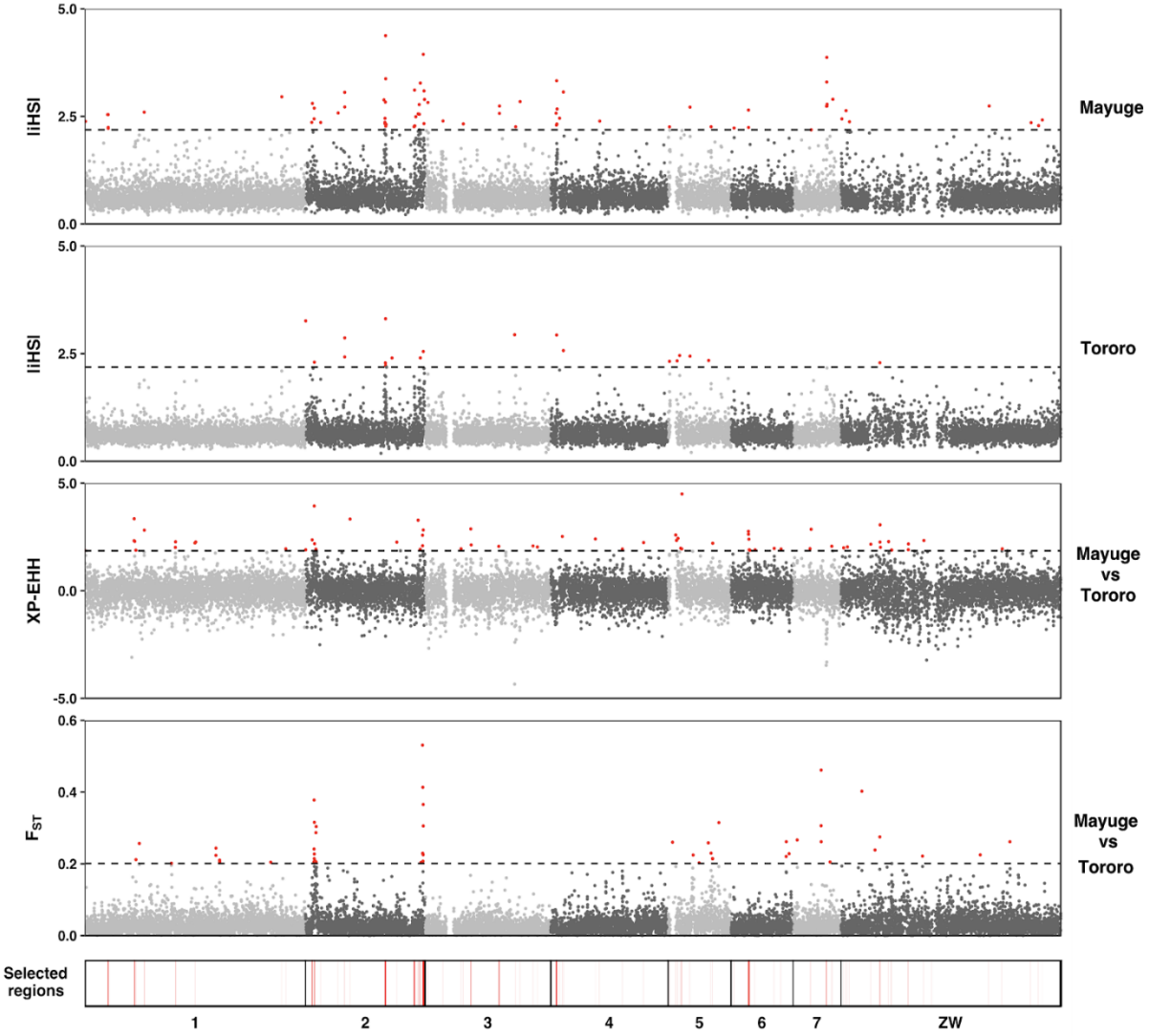
Each point represents an F_{ST} average from 100 SNPs



Unpublished data, Crellen, Berger et al.

This region contains a calcium transporter.
Undergoing RNAi knockdown to determine function

Testing for selection: Combining tests



Unpublished data, Crellen, Berger et al.

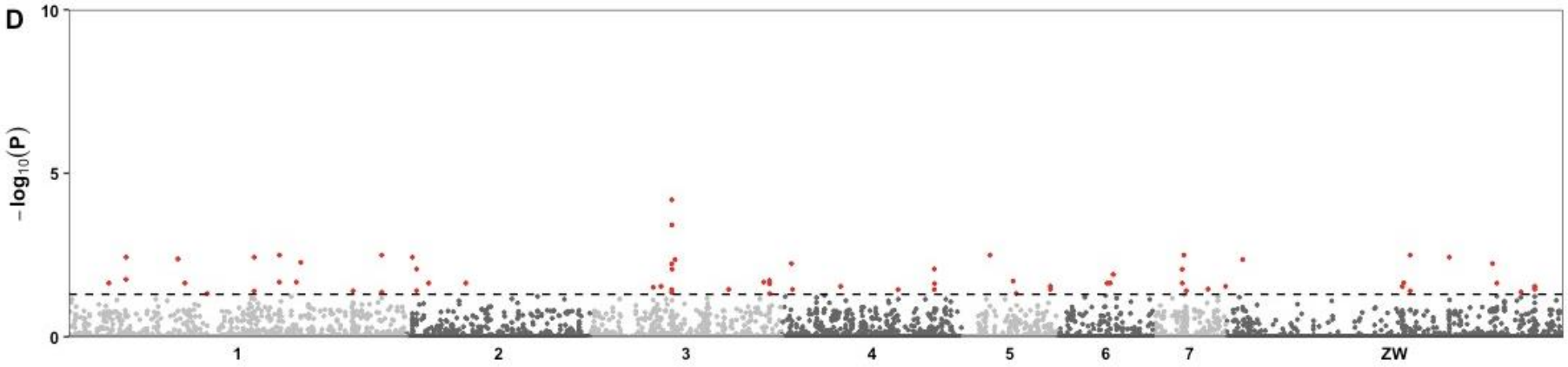
Testing for association with egg reduction rate

An alternative is to measure the association between SNPs and a phenotype

Here we are associating variants with the egg reduction rate from the model

Also able to control for population structure. The y axis is -$\log_{10}$ corrected *p* values (higher points = greater association with ERR). Each point is a SNP

A major challenge in interpretation is a lack of biological knowledge about gene function



Unpublished data, Crellen, Berger et al.

Conclusions

- We observed prevalence of >90% and high infection intensity in schools that have been treated 9 times with MDA
  - Suggests additional strategies (sanitation, snail control) will be necessary to halt transmission

- A statistical model of egg count reduction showed that schools with higher previous MDA have significantly lower reduction rates
  - Provides evidence for emerging resistance to praziquantel

- Genomic evidence has shown signals of selection along the genome and SNPs correlated with egg reduction rate
  - These are potential candidates for praziquantel resistance and are being followed up by functional analysis (RNAi)

Acknowledgements

Imperial College London

David Aanenson
Poppy Lamberton

Royal Veterinary College University of London

Joanne Webster
Martin Walker

wellcome trust sanger institute

James Cotton
Matthew Berriman
Nancy Holroyd
Hayley Bennett
Duncan Berger
Alan Tracey
Gabriel Rinaldi

NATURAL HISTORY MUSEUM

Fiona Allan

THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

Edridah Tukahebwa
Narcis Kabatereine
Fieldwork team

MRC Medical Research Council