Population genomics of anthelmintic response in *Onchocerca volvulus*
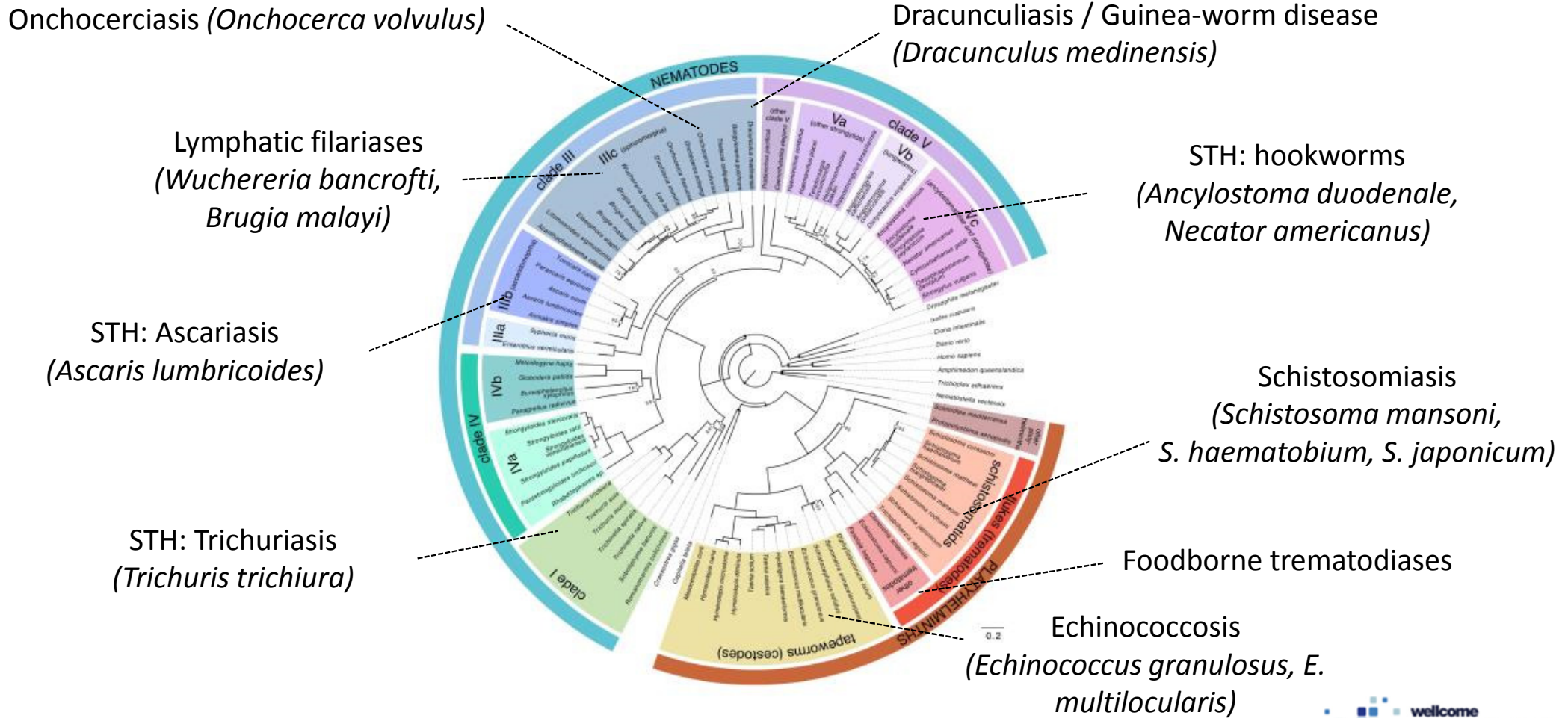
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Genetic diversity of NTD pathogens, LCNTDR

23 May 2018, Natural History Museum, London

Genomic resources are increasingly important for NTD research

Onchocerciasis *(Onchocerca volvulus)*

Dracunculiasis / Guinea-worm disease *(Dracunculus medinensis)*

Lymphatic filariases *(Wuchereria bancrofti, Brugia malayi)*

STH: hookworms *(Ancylostoma duodenale, Necator americanus)*

STH: Ascariasis *(Ascaris lumbricoides)*

Schistosomiasis *(Schistosoma mansoni, S. haematobium, S. japonicum)*

STH: Trichuriasis *(Trichuris trichiura)*

Foodborne trematodiases

Echinococcosis *(Echinococcus granulosus, E. multilocularis)*

International Helminth Genomes Consortium. BioRxiv. https://doi.org/10.1101/236539

Genomic resources are increasingly important of NTD research

Onchocerca volvulus, agent of river blindness

Onchocerciasis (Onchocerca volvulus)
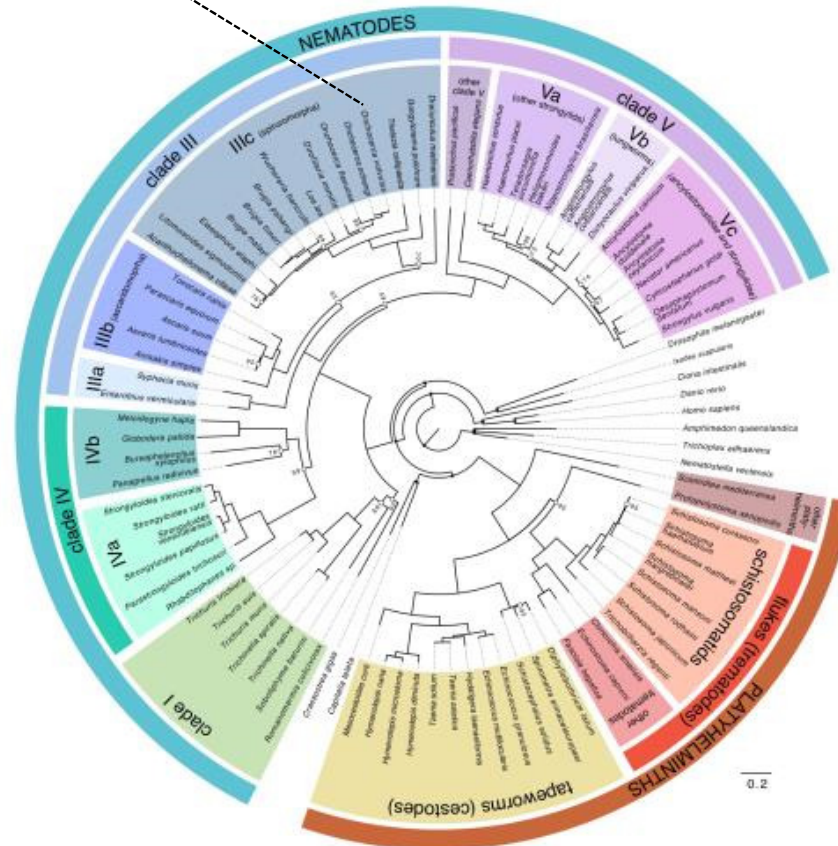
Complete genome of *Onchocerca volvulus*

- Adults are long living: < 15 yrs
- Intermediate host: Blackfly
- Prepatent period: 12-18 mths
- Causes severe **morbidity**

- Target: Glutamate-gated chloride channels

- Effect: Does **not** kill adult worms….

  - >99% of microfilaria disappear

  - Inhibits reproduction

  - Interrupts transmission

HOWEVER

1977

2002

| | <=5% | | 15% | | 30% | | 45% | | >=60% |
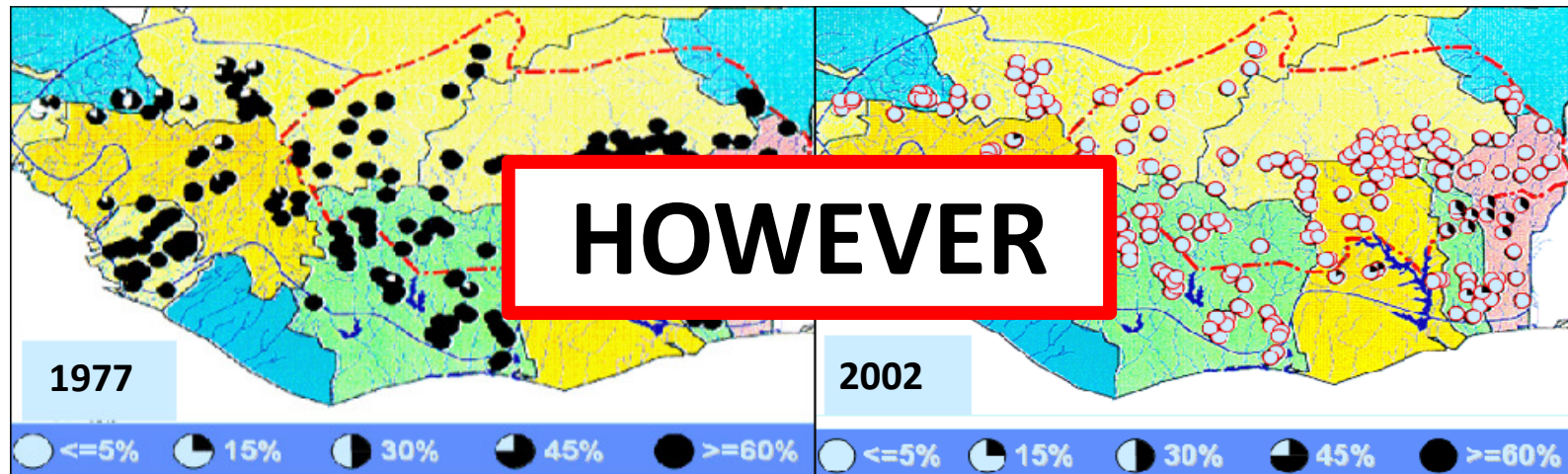
Phenotypic variation in ivermectin response by *O. volvulus*

Sub-optimal or atypical response
- HOST: High skin microfilaria density
- HOST: Faster repopulation rates
- PARASITE: embryograms show live / developing microfilaria
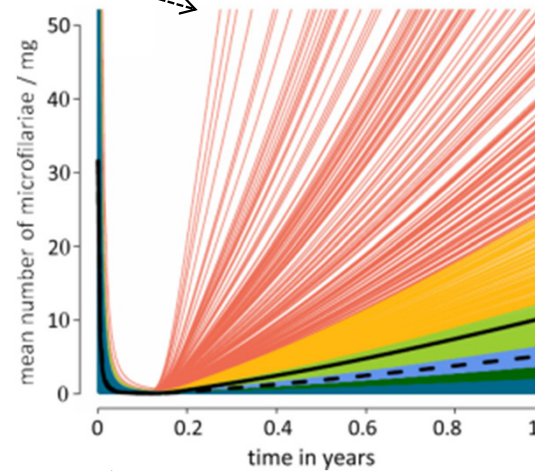
Ivermectin

Susceptible response
- HOST: Low skin microfilaria density
- HOST: Slower repopulation rates
- PARASITE: Embryograms are clear or show only degenerate microfilaria

Churcher *et al. (*2009) PNAS

Sampling and experimental design

(Gardon et al., 2002; Nana-Djeunga et al., 2014; Pion et al., 2013)

(Osei-Atweneboana et al., 2011; Osei-Atweneboana et al., 2007)

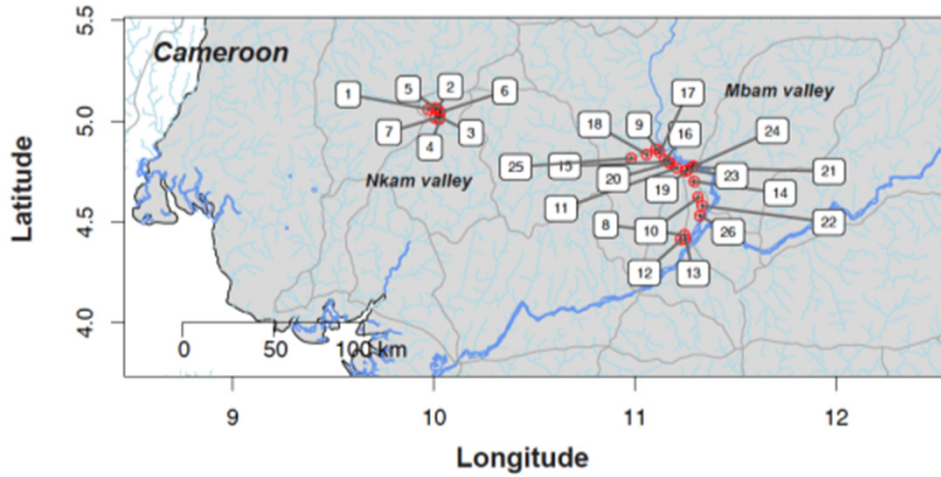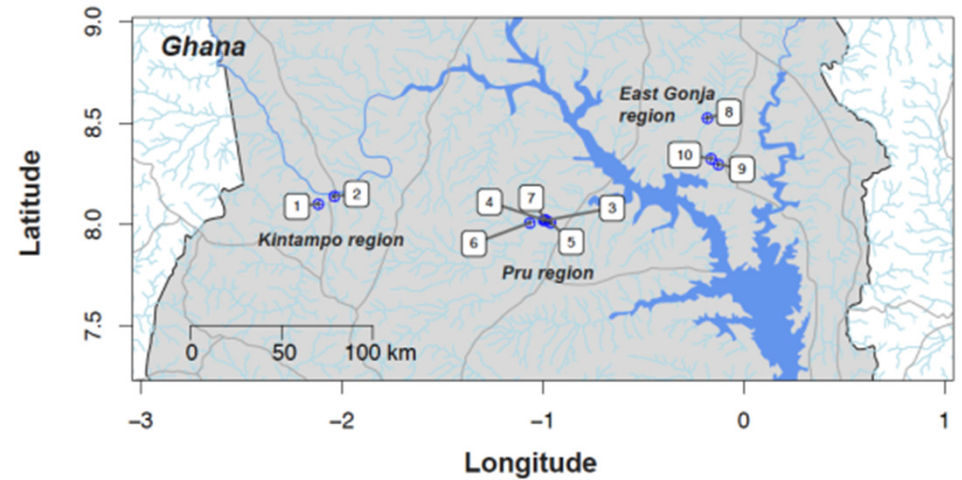| Phenotype	Defined at the host level (microfilarial (mf) density in skin determined by skin snip)	Defined at parasite level (intra-uterine microfilariae determined by embryogram of adult female parasites)
Good response (GR)	Day 0 [a]: < 25 mf/mg skin Day 80/90 [b]: 0 mf/mg skin Day 180: < 6% of pre-Tx value	Day 80/90 [b]: Viable stretched microfilariae absent in utero
Sub-optimal response (SOR)	Day 80/90 [b]: > 7% of pre-Tx value	Day 80/90 [b]: Viable, stretched microfilariae present in utero
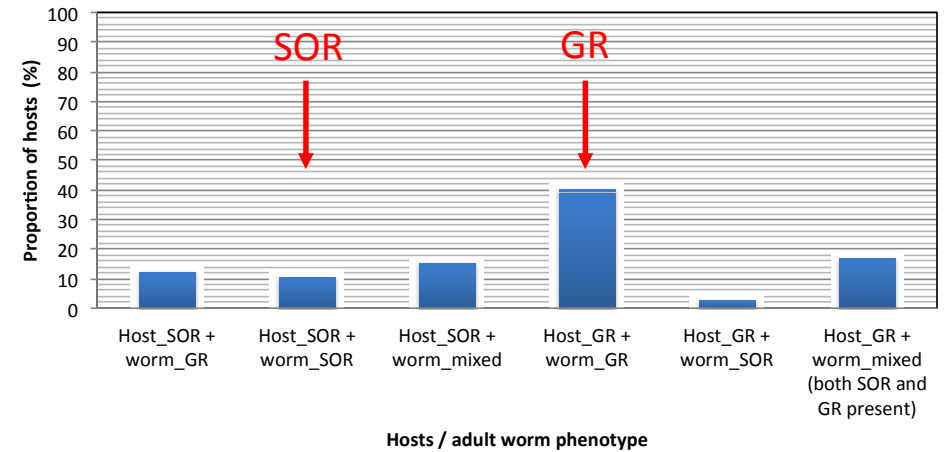
Doyle et al. (2017) PLoS NTD 11(7)

Sampling and experimental design

(Gardon et al., 2002; Nana-Djeunga et al., 2014; Pion et al., 2013)

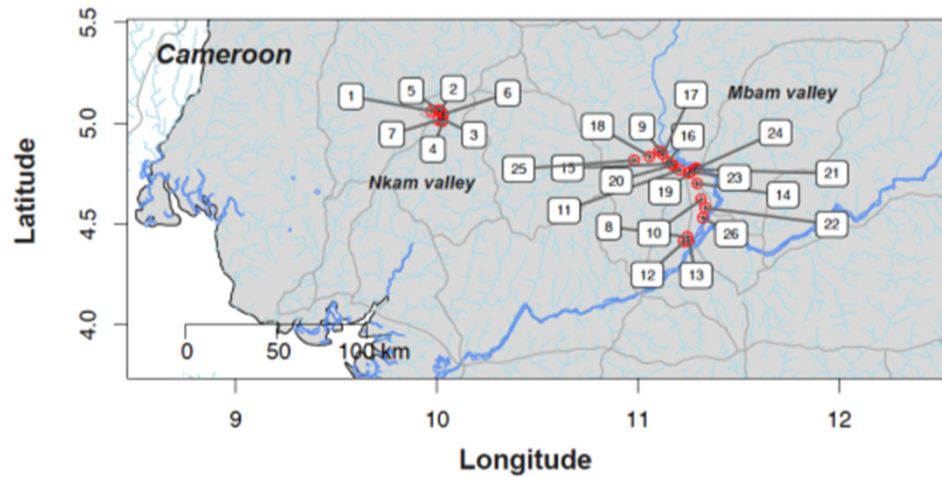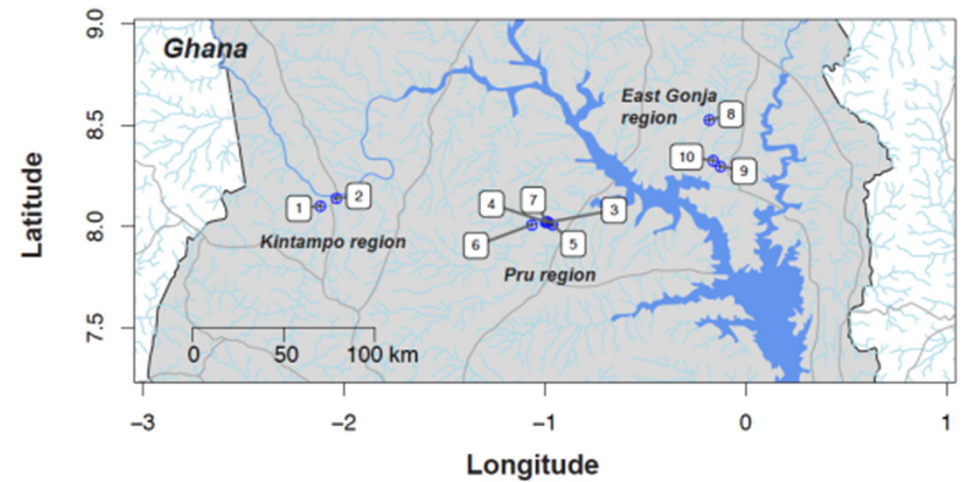(Osei-Atweneboana et al., 2011; Osei-Atweneboana et al., 2007)

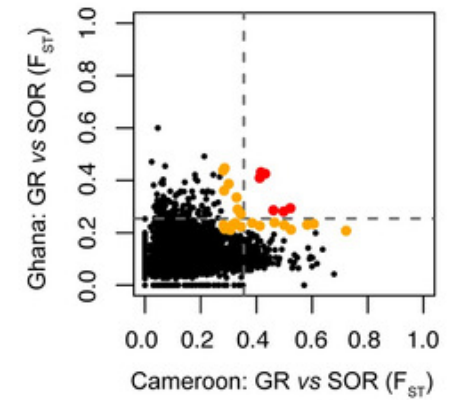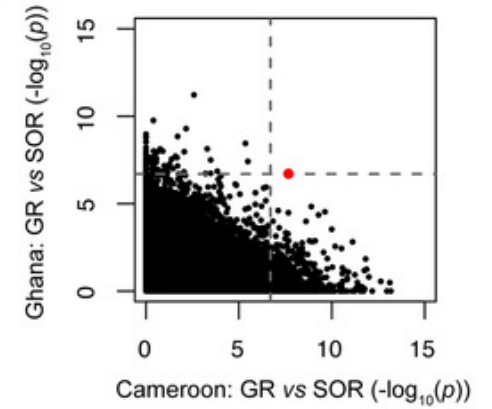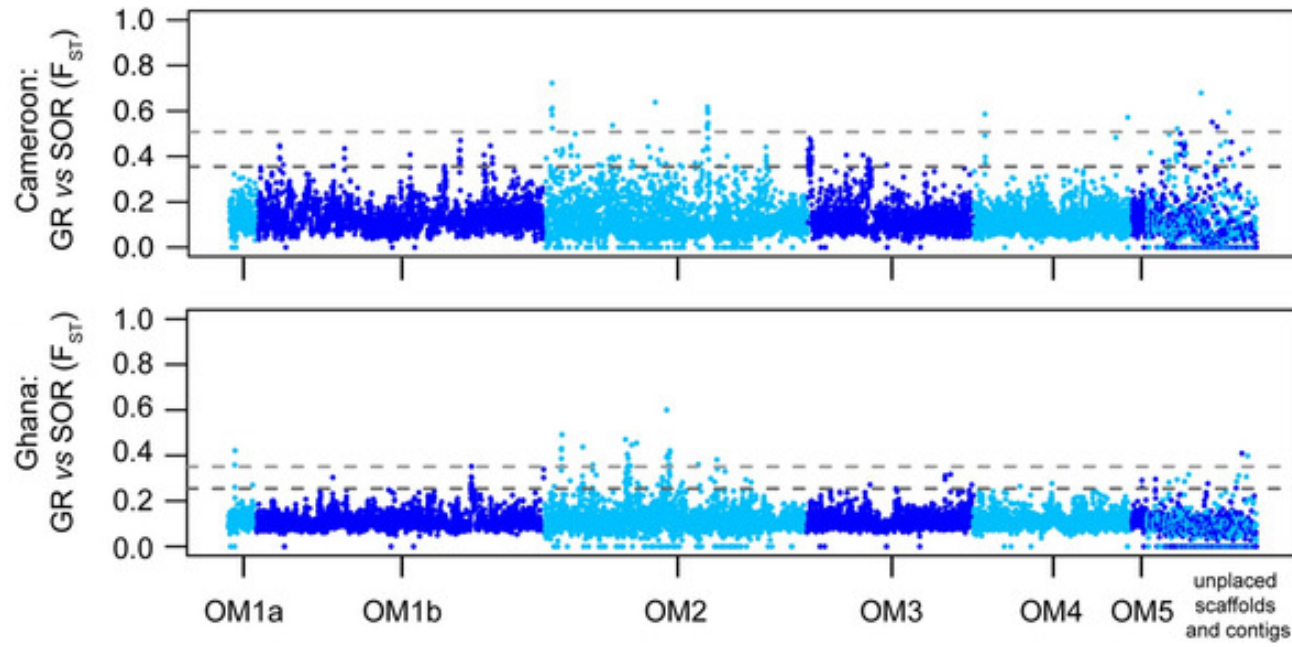**GR** **SOR** **Naive**

GR **SOR** **Naive**

Sequencing: 15-20 worms per pool, 20-30X coverage for each group (8 lanes GAII, ~280 x 10^6 reads)

Analysis: variant read frequency ≈ allele frequency, scan genome for regions where GR and SOR differ

Doyle et al. (2017) PLoS NTD 11(7)

Doyle et al. (2017) PLoS NTD 11(7)

Enrichment of genes in a limited number of functional pathways

Cameroon
Ghana

- Known IVM sensitivity alleles
  - *unc-44, klp-11, inx-5*

- Neurotransmission, particularly ACh
  - ion channels (*acc-1, lgc-46, lgc-47*)
  - acetylcholine synthesis (*cha-1*),
  - transport (*unc-17, aex-3*),
  - regulation (*stg-1, nrfl-1, emc-6, snb-1, nud-1, kin-2, unc-26*)
  - structural (*inx-5*)

- Lipid synthesis (*acs-16, fat-4*) and regulation and storage (*acs-16, math-46, obr-2, cuc-1, sms-1, tat-2*)

- Suppression (*sel-7, bre-5*) or cleavage (*pen-2, crb-1*) of the LIN-12 receptor

Geography rather than drug response defines population genetic structure

Doyle et al. (2017) PLoS NTD 11(7)

Geography rather than drug response defines population genetic structure

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Geography rather than drug response defines population genetic structure

Doyle et al. (2017) PLoS NTD 11(7)
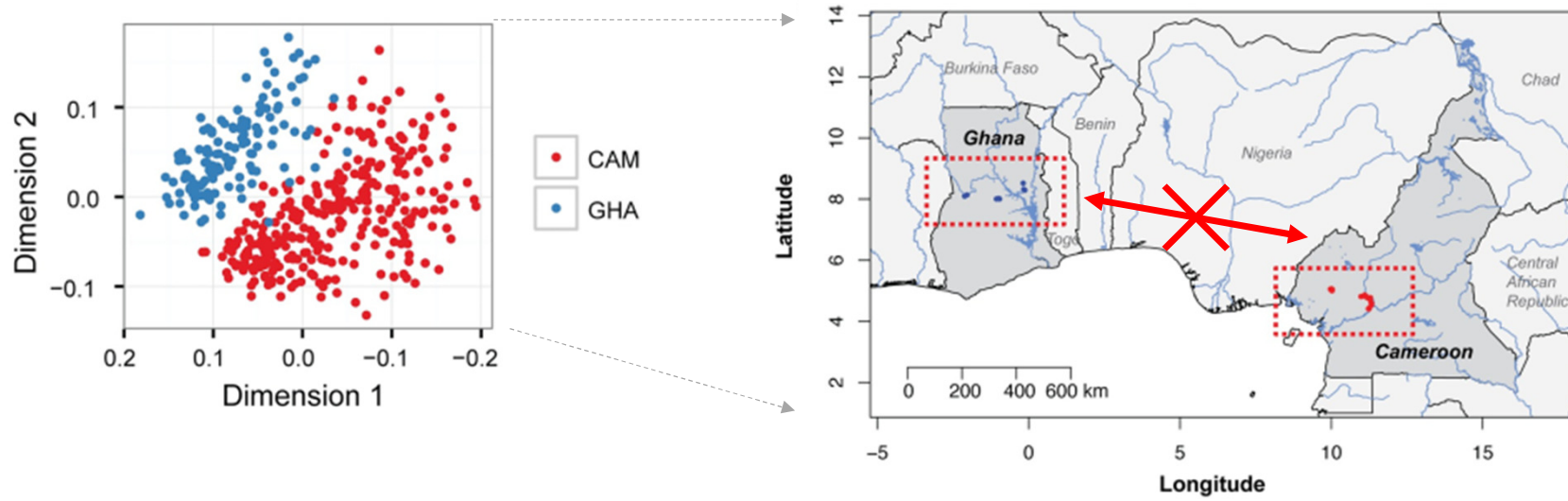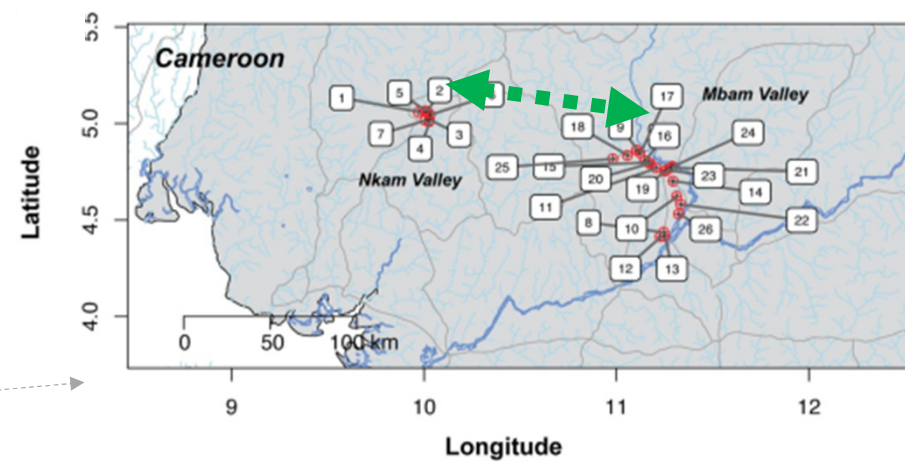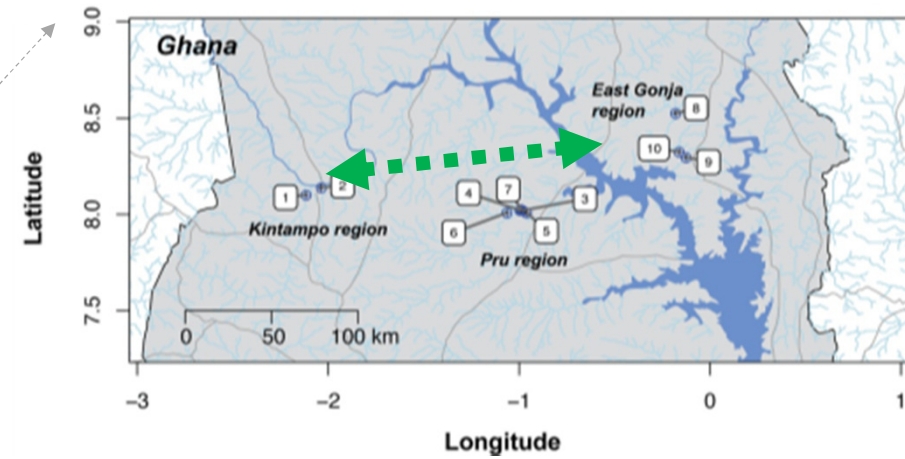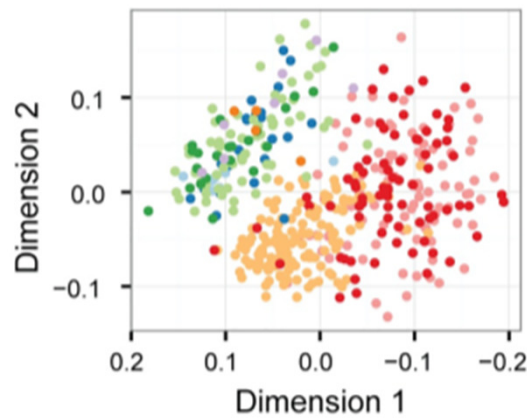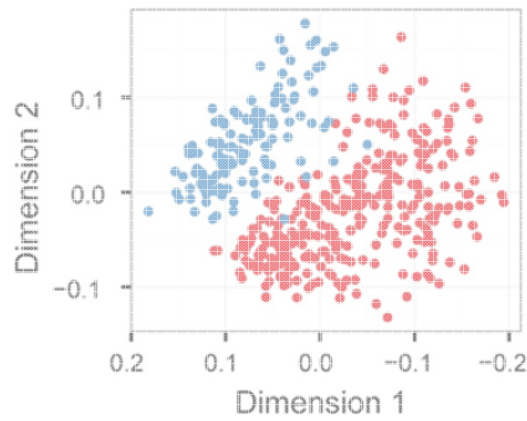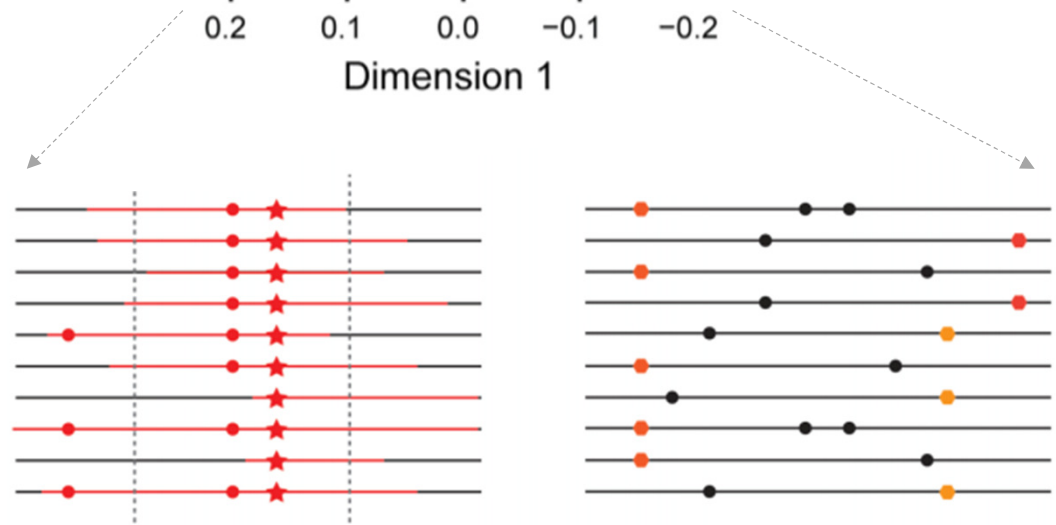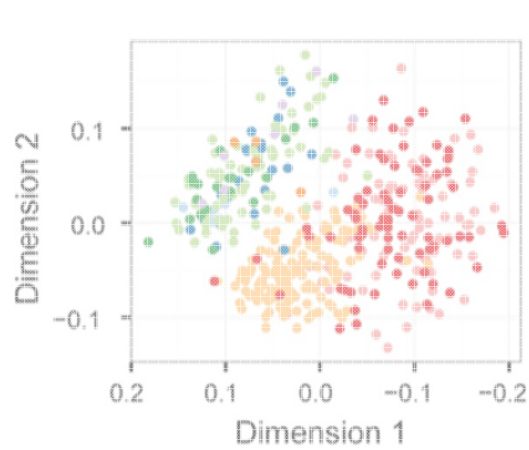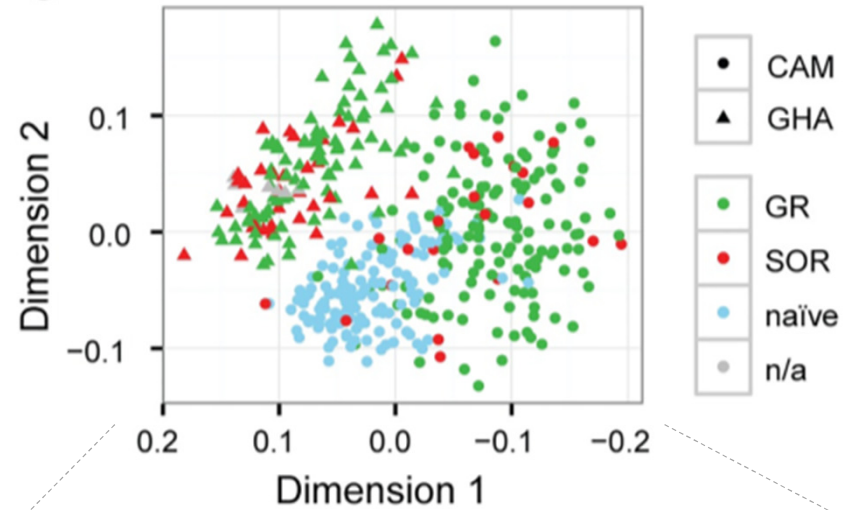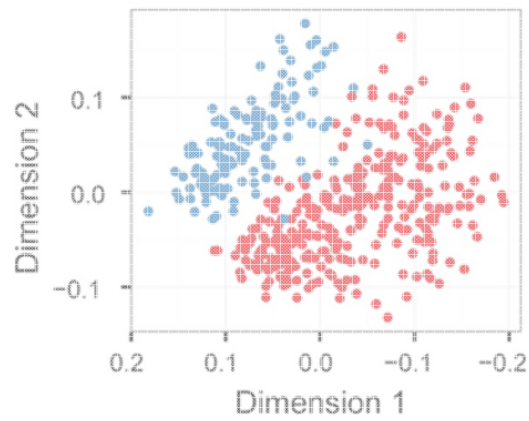
"hard" selection

"soft" selection

Genetic determinants of SOR are likely derived from pre-treatment standing genetic variation
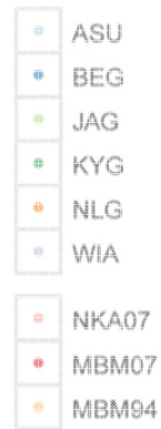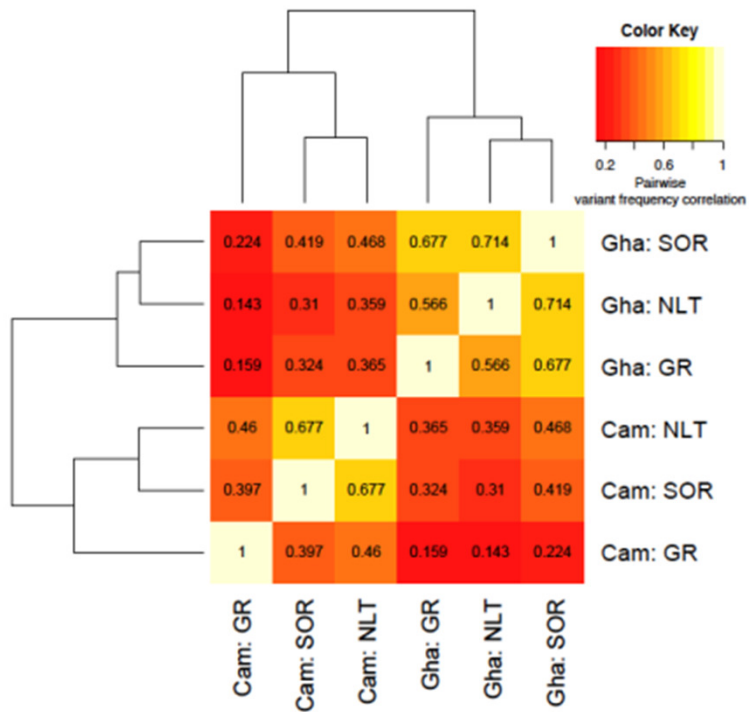

1. Allele frequency correlation

2. Genetic similarity between groups
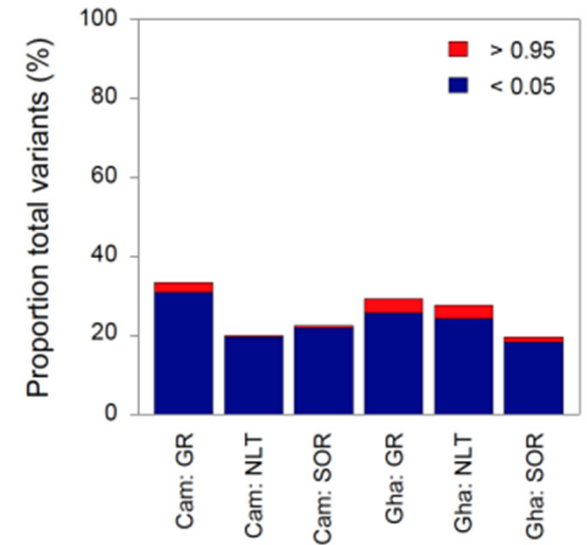
3. Proportion of invariant sites

Doyle et al. (2017) PLoS NTD 11(7)

- Multiple regions of the genome differentiate GR and SOR parasites, and these regions differ between Ghana and Cameroon
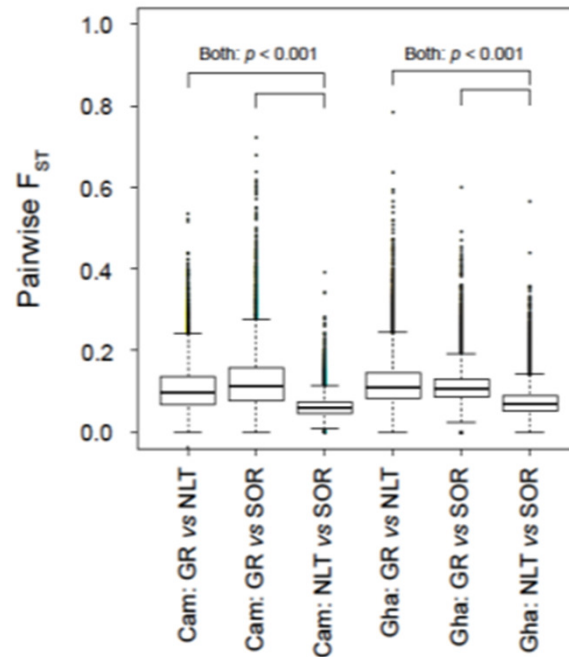 - Consistent with soft- rather than hard-selective sweeps on multiple loci

- Different genes differentiate GR form SOR in each country, but pathways involving neurotransmission & stress response are shared

- Understanding underlying genetic variation and its distribution is critically important in drug response analyses

Acknowledgements

Matt Berriman
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Laurent Toe

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Jean Bopda

Mike Osei-Atweneboana
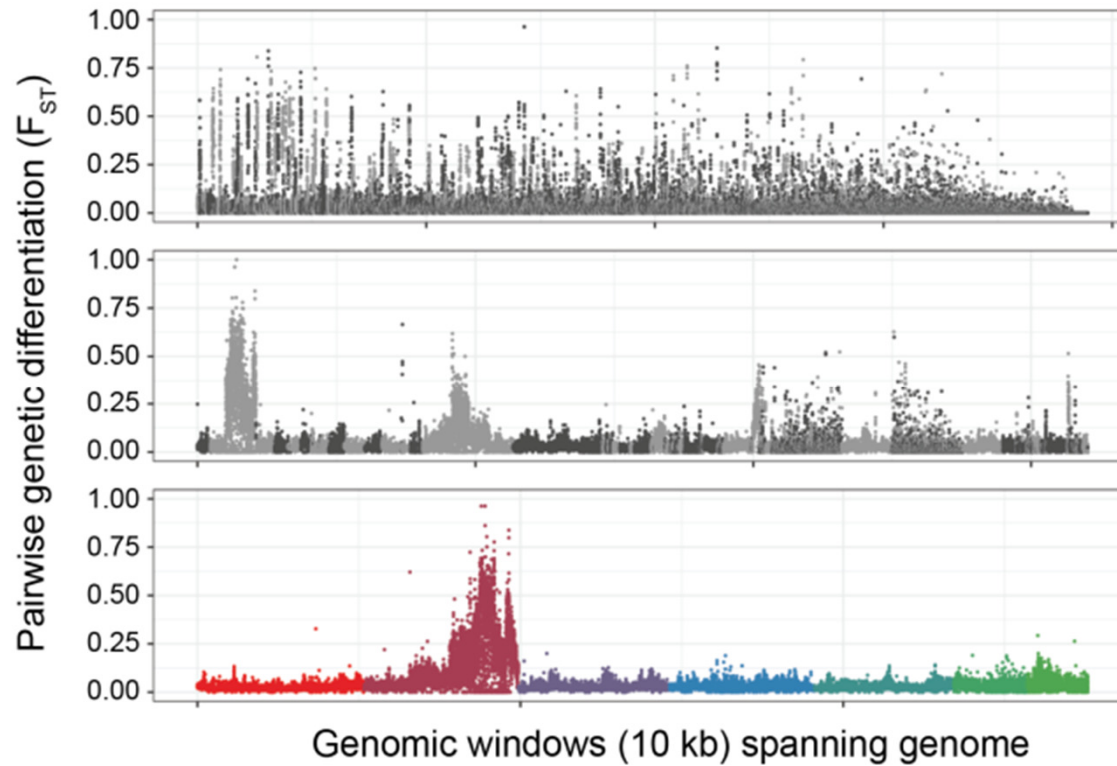Daniel Boakye

REFOTDE (Cameroon)
Samuel Wanji
Jonas Kengne-Ouafo

Annette Kuesel

Maria-Gloria Basáñez
Martin Walker

Interpretation of genetic diversity critically relies on a contiguous genome sequence

Published V1 (Laing 2013)
- N50 = 0.083 Mb
- N50(n) = 1,151
- n = 23,860

Intermediate assembly
- N50 = 5.2 Mb
- N50(n) = 16
- n = 6668

Complete chromosomes
- N50 = 47.4 Mb
- N50(n) = 3
- n = 8