

Long-Lasting Insecticidal Nets in Africa: Combining Experimental Huts and Genetics to Inform Vector Control Tool Choice

On Behalf of RAFT Workstream 1 – Current Threats



globalvectorhub.lshtm.ac.uk/raft

Combining Genetics with Experimental Hut Trials to Predict Relative Performance of LLIN Products Across Distinct Resistance Settings

Dr. Jackline Martin

NIMR MWANZA, TANZANIA

On Behalf of RAFT Workstream 1 – Current Threats

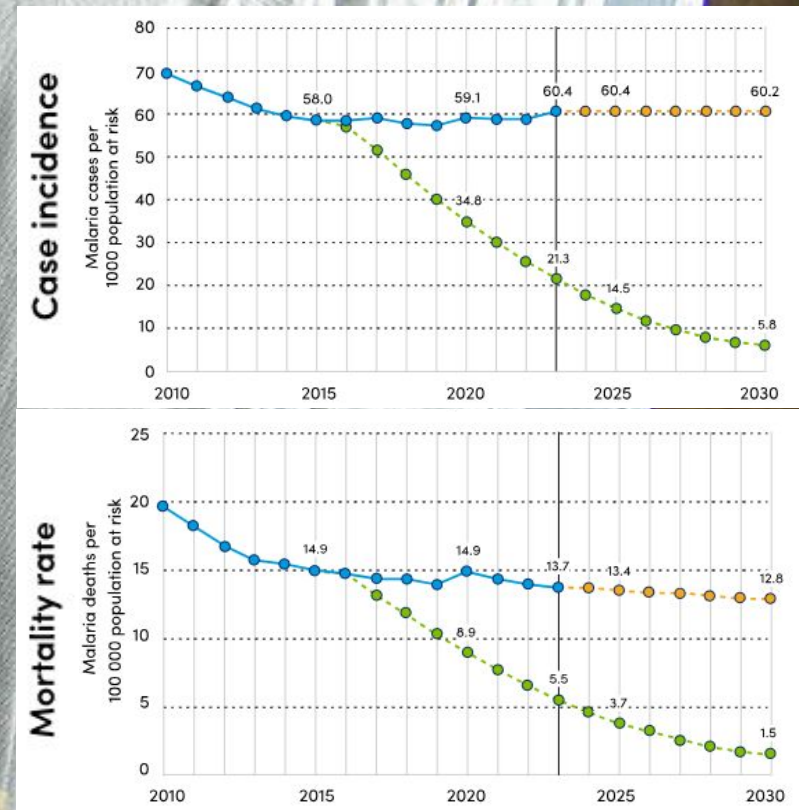
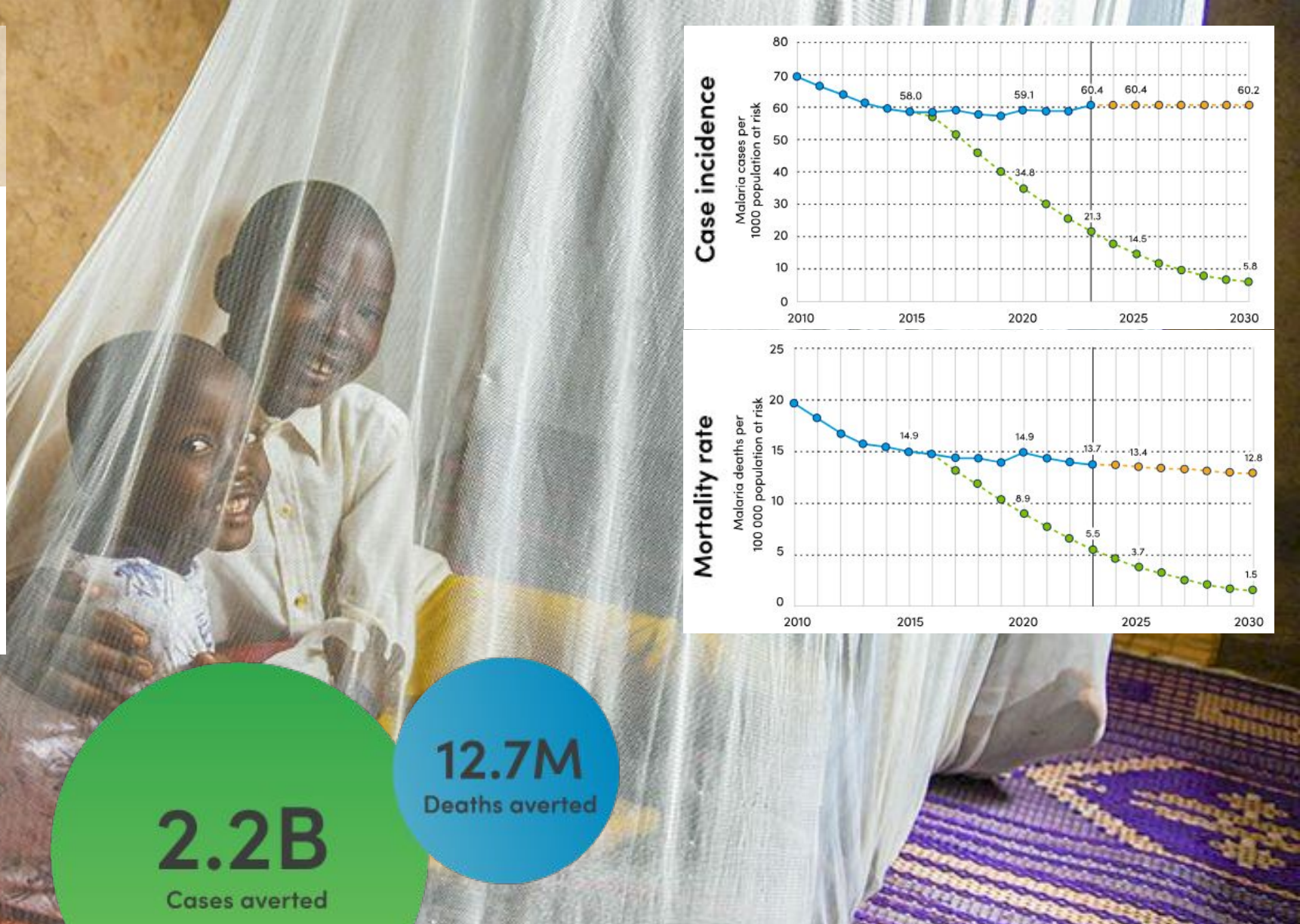
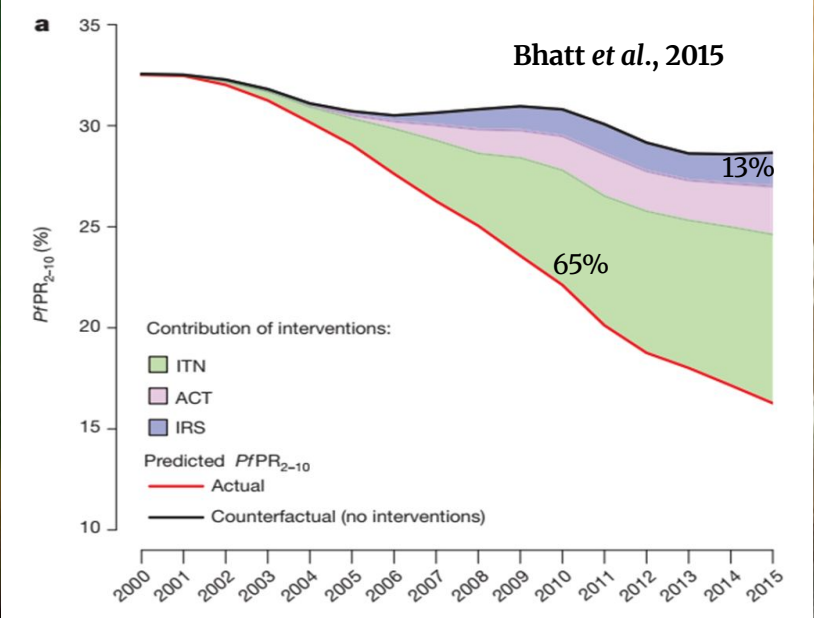
RAFT: Resilience Against Future Threats

- RAFT is a multi-partner programme uniting leading researchers and implementers from eight institutions across Africa, Asia, and UK
- Research Themes:
 - Current Threats – Managing Insecticide Resistance
 - Inform LLIN selection
 - Future Threats - *Aedes*-borne viruses
 - Urbanization
 - Development & VBD
 - Rice & malaria



RAFT is funded with UK International Development from the UK Government

LLINs have been responsible for 2/3s reduction of malaria burden since 2000



Relative LLIN Efficacy

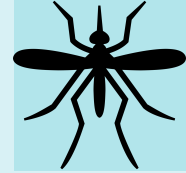


LLIN Specifications

- Location and loading dose of PBO / partner AI
- Pyrethroid type and content
- Wash-fastness
- AI bioavailability

Vector Species Composition

- Predominant *Anopheles* species
- Sympatric major vector species
- Seasonality



Vector Insecticide Resistance

- Levels of phenotypic resistance intensity
- Molecular and metabolic resistance mechanisms
- Cross-resistance between chemical classes

Vector Behaviour and Adaptation

- Anthropophilic / zoophilic
- Endophagic / exophagic
- Endophilic / exophilic
- Shifts in biting times



Components of the Task I

NMCP

- ❑ Must choose most cost-effective LLIN



- ❑ LLINs kill female vectors and prevent feeding
- ❑ Experimental huts directly measure these
- ❑ BUT only locally



- ❑ Sub-national tailoring
- ❑ Different endemic areas have very different vectors
- ❑ 2 or 3 species each with various resistance genes

Components of the Task II

Q

- How to generalize from results in a few experimental hut locations...
- ...to many diverse vector populations?

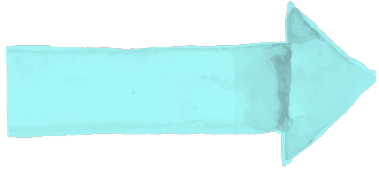
A

- **GENETICS!** Species by species and gene by gene
- Measure comparative performance of products against each common resistance gene

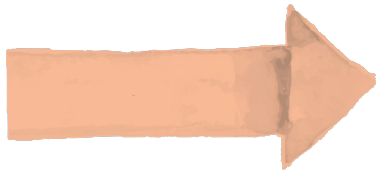
General Methods



Three major experimental hut sites



Conventional experimental hut methods



Genetic analysis using PCR genotyping,
RNA-expression and DNA pool-sequencing



Multivariate analysis and mathematical
modelling

LLIN Brand	Dose AI/m ² of Netting Fabric	Fibre and Denier	Mode of Action
MaGNet	1. Alpha-cypermethrin 216 mg (Pyrethroid-only ITN - Control)	150 polyethylene	Hyper-excitation of nerve system
Veeralin [®]	1. Alpha-cypermethrin 216 mg 2. PBO 79 mg	130 Polyethylene	Enhance the potency of the PY insecticide
PermaNet 3.0	1. Deltamethrin 120 mg 2. PBO 800 mg	75-100 polyester side part and 180 polyethylene roof part	Enhance the potency of the PY insecticide
Olyset [™] Plus	1. Permethrin 800 mg 2. PBO 400 mg	150 Polyethylene	Enhance the potency of the PY insecticide
Interceptor [®] G2	1. Alpha-cypermethrin 100 mg 2. Chlorfenapyr 200 mg	100 Polyester	Disrupts the insect's ability to produce energy

Major Mechanisms Driving Contemporary Pyrethroid Resistance in Malaria Vector Populations

Dr. Louisa Messenger

UNLV, USA

On Behalf of RAFT Workstream 1 – Current Threats

Persistence of Malaria and Challenges



Vector insecticide resistance

Vector resistance to the insecticides used for vector control threatens malaria control efforts.



Invasive vector species

The spread of certain anopheline vector species and their establishment in new ecosystems poses a threat to malaria control.



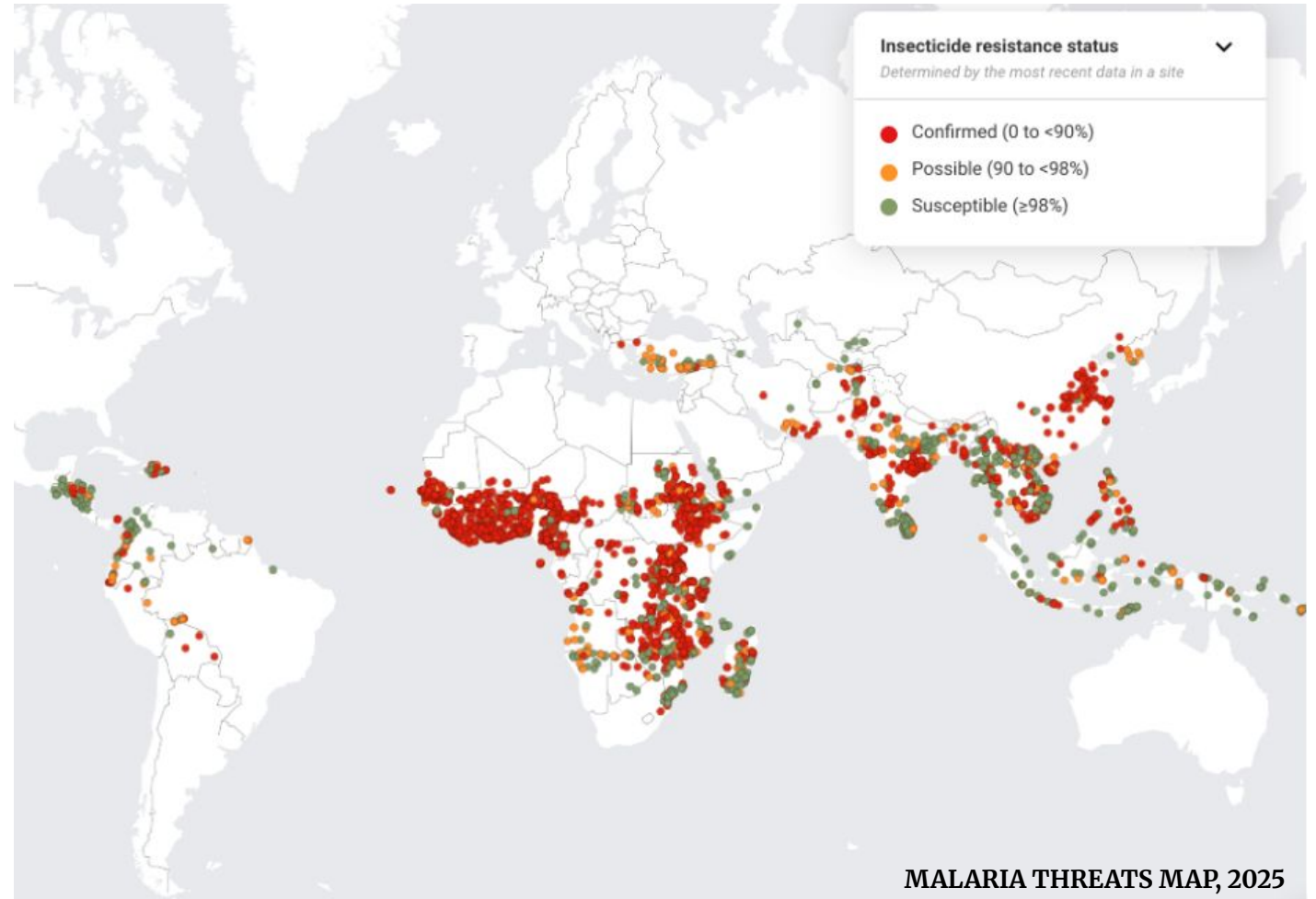
Antimalarial drug efficacy and resistance

Antimalarial drug resistance remains one of the key threats to global malaria control efforts, particularly in the Greater Mekong Subregion.

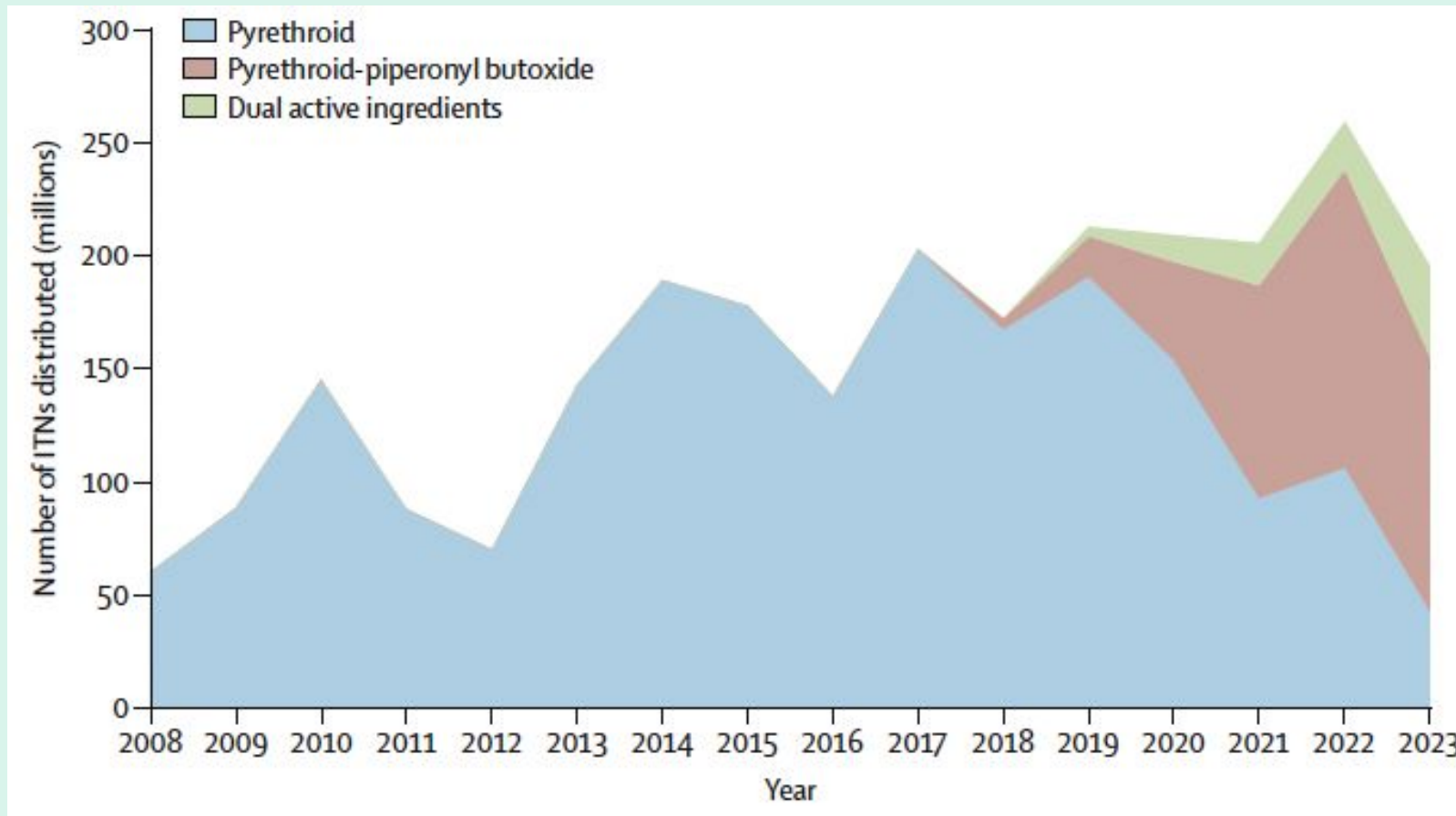


Parasite pfhrp2/3 gene deletions

Gene deletions among some malaria parasites cause false negative diagnostic test results, complicating case management and control.



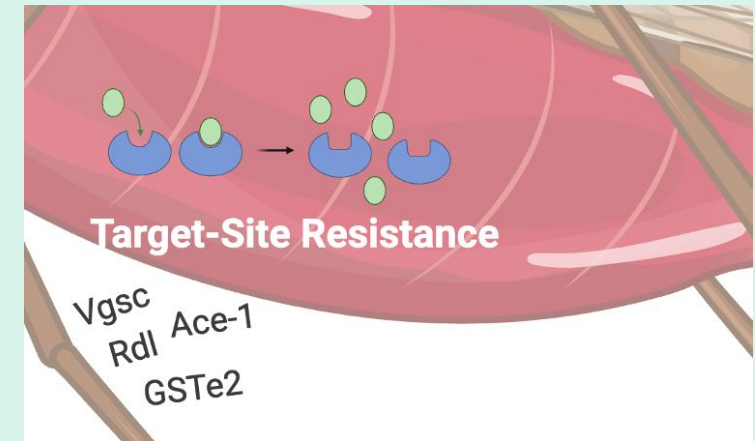
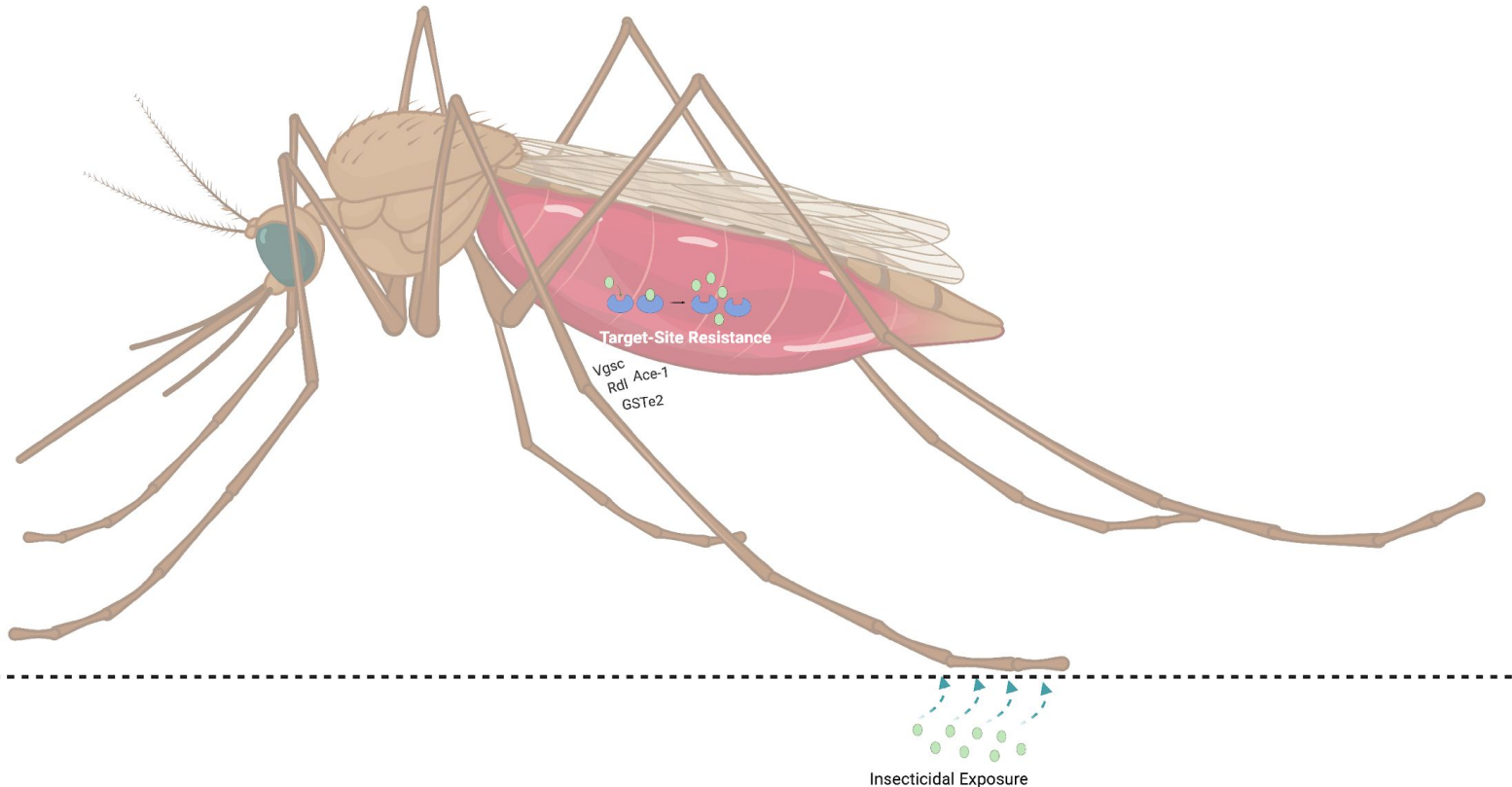
Pyrethroid Resistance Selection



- 2.13 billion pyrethroid-only LLINs distributed in sub-Saharan Africa between 2004 – 2022
- Imposed significant, long-term selection for the evolution and spread of pyrethroid resistance
- Pyrethroid resistance is now ubiquitous and entrenched across sub-Saharan Africa
- Continued distribution of pyrethroid-only LLINs and PBO-pyrethroid LLINs continues to intensify pyrethroid resistance

Source: Oxborough, Chilito, Tokponnon and Messenger. Lancet Planet Health 2024;8:e804-12.

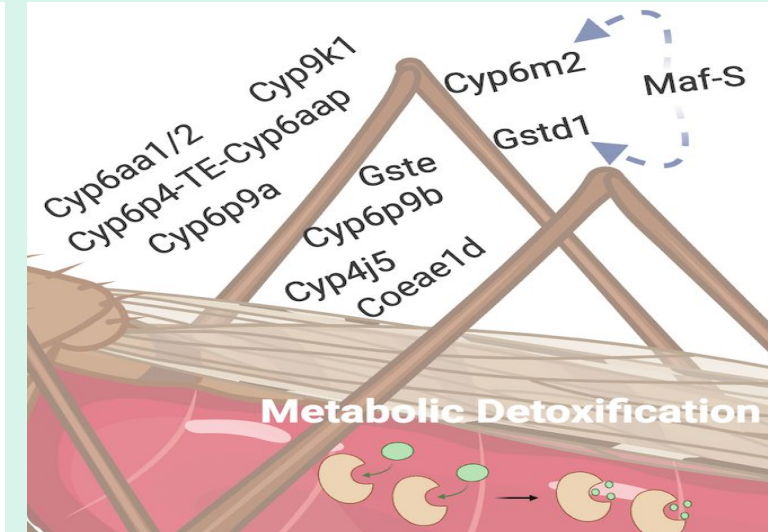
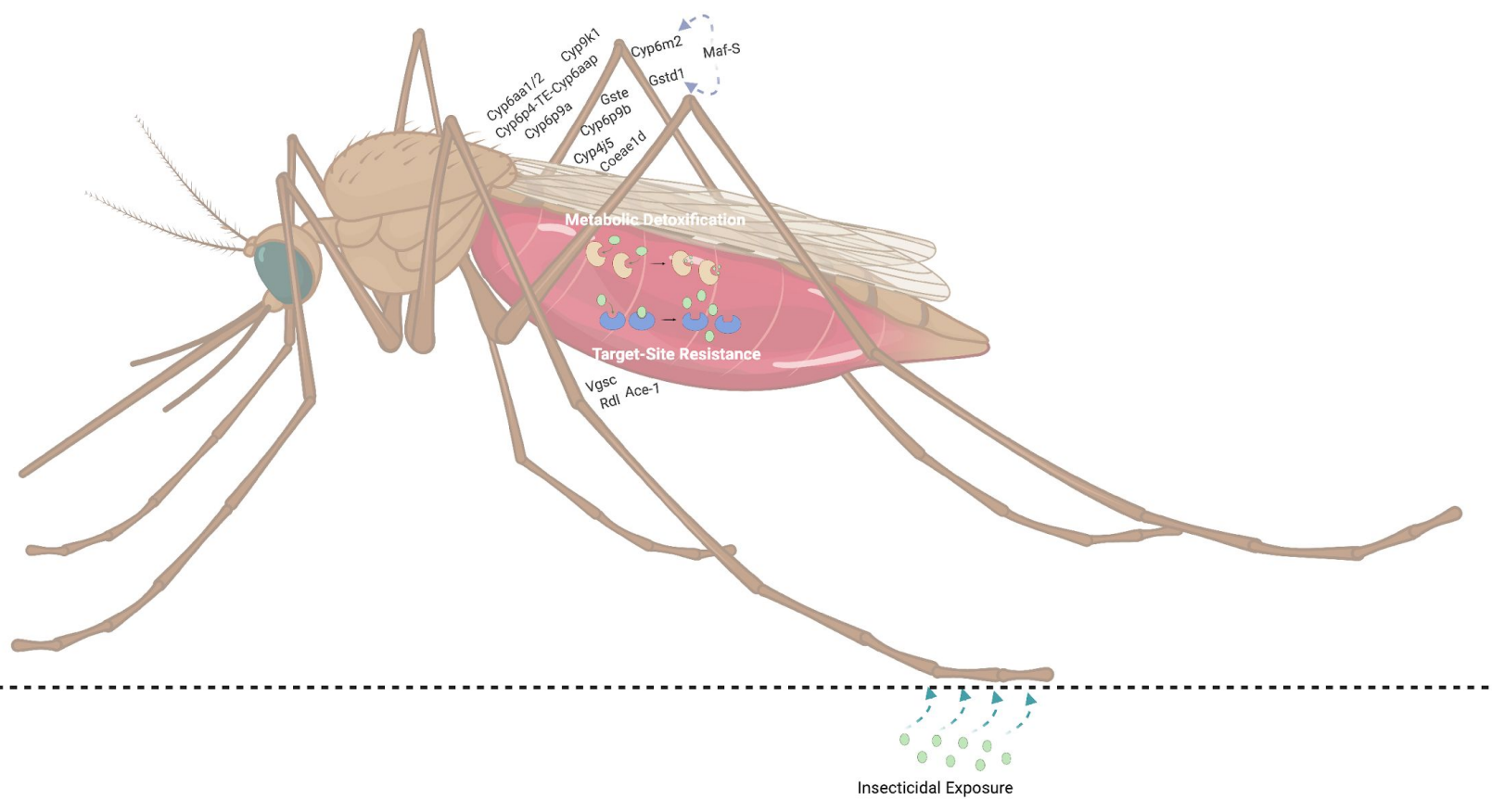
Target Site Mutations



- Non-synonymous point mutations result in amino acid change, leading to alteration of 3D protein structure and insecticide binding
- Copy number variants (duplications or deletions) result in gene overexpression
- In this study: *vgsc*-L1014F/S, *vgsc*-V402L, *vgsc*-I1527T, *vgsc*-N1575Y

Source: Hancock, Ochomo and Messenger. Trends Parasitol 2024; 40(7):604-618.

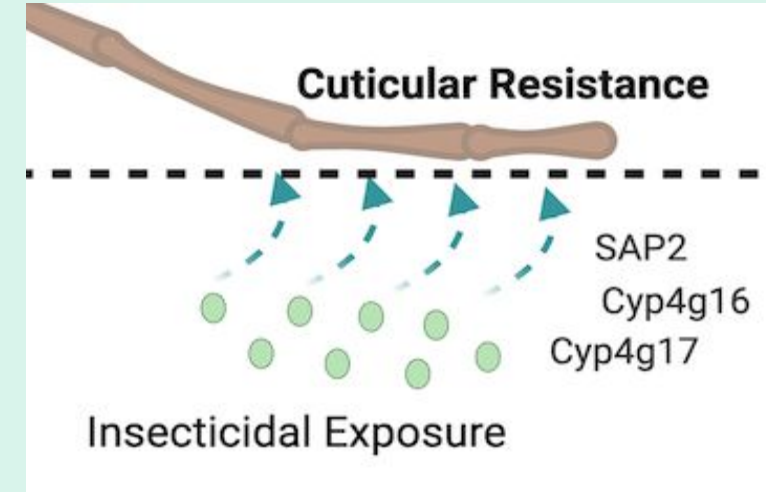
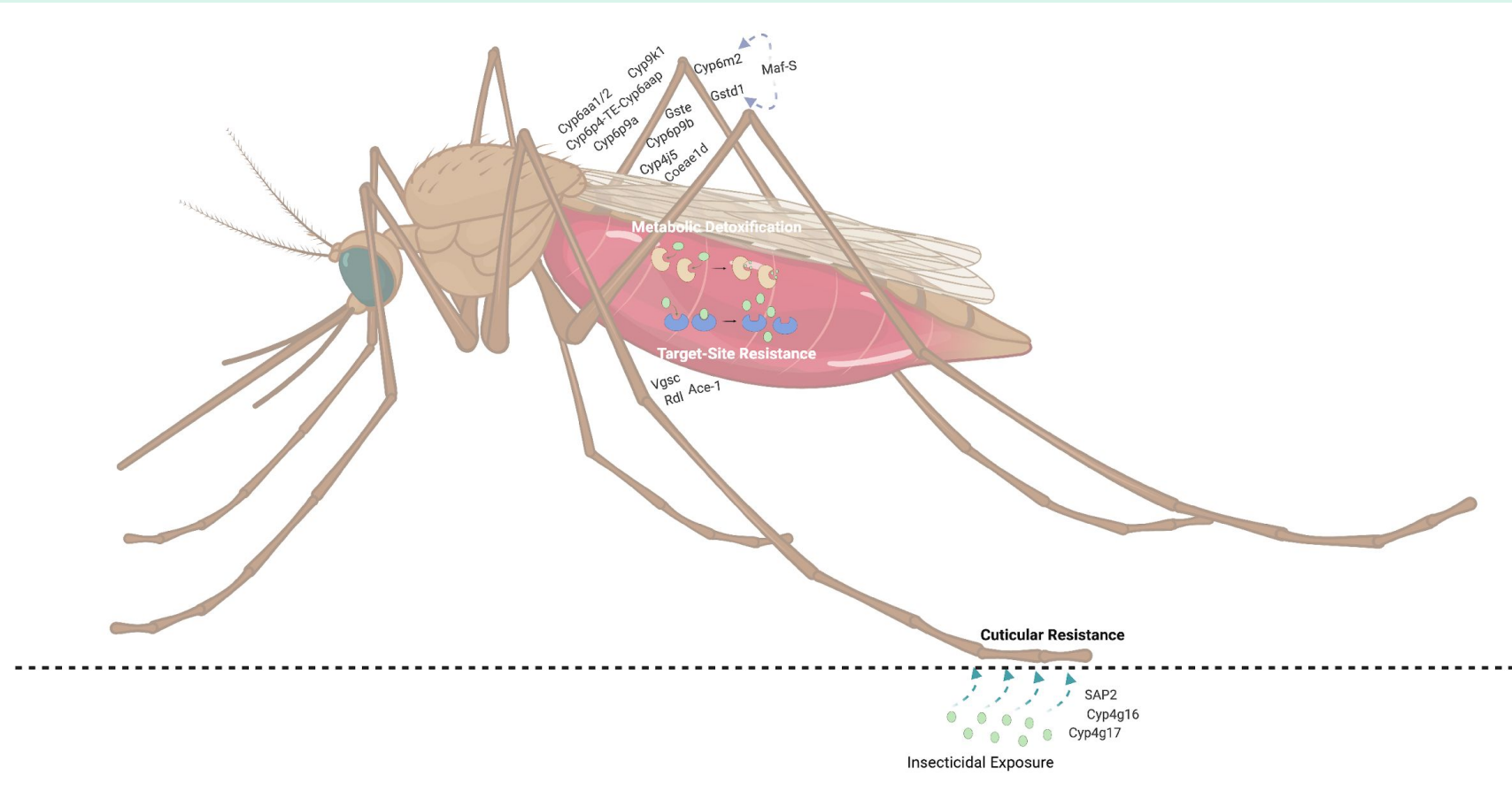
Metabolic Mechanisms



- Increased RNA transcript levels leads to overexpression of detoxification enzymes, cuticular proteins and transcription factors
- Shared metabolic mechanisms between vector populations and population-specific mechanisms
- In this study: *gste2*-I114T, *cyp6p3*-E205D

Source: Hancock, Ochomo and Messenger. Trends Parasitol 2024; 40(7):604-618.

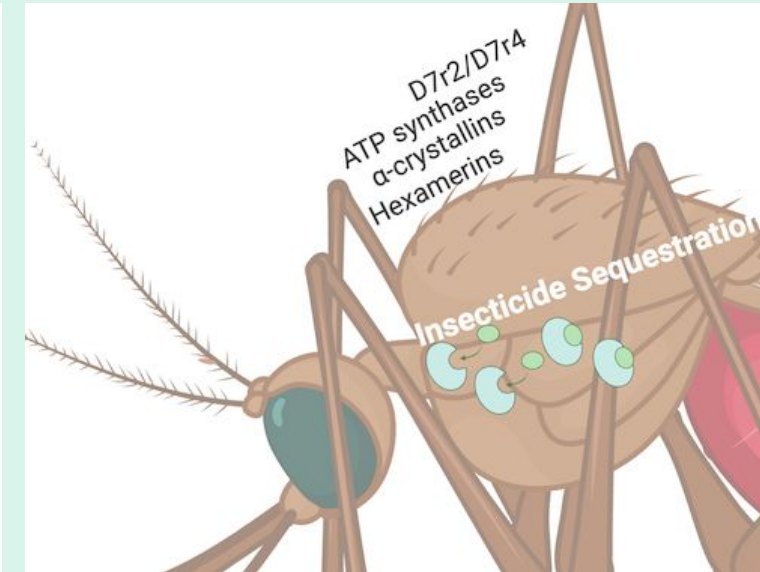
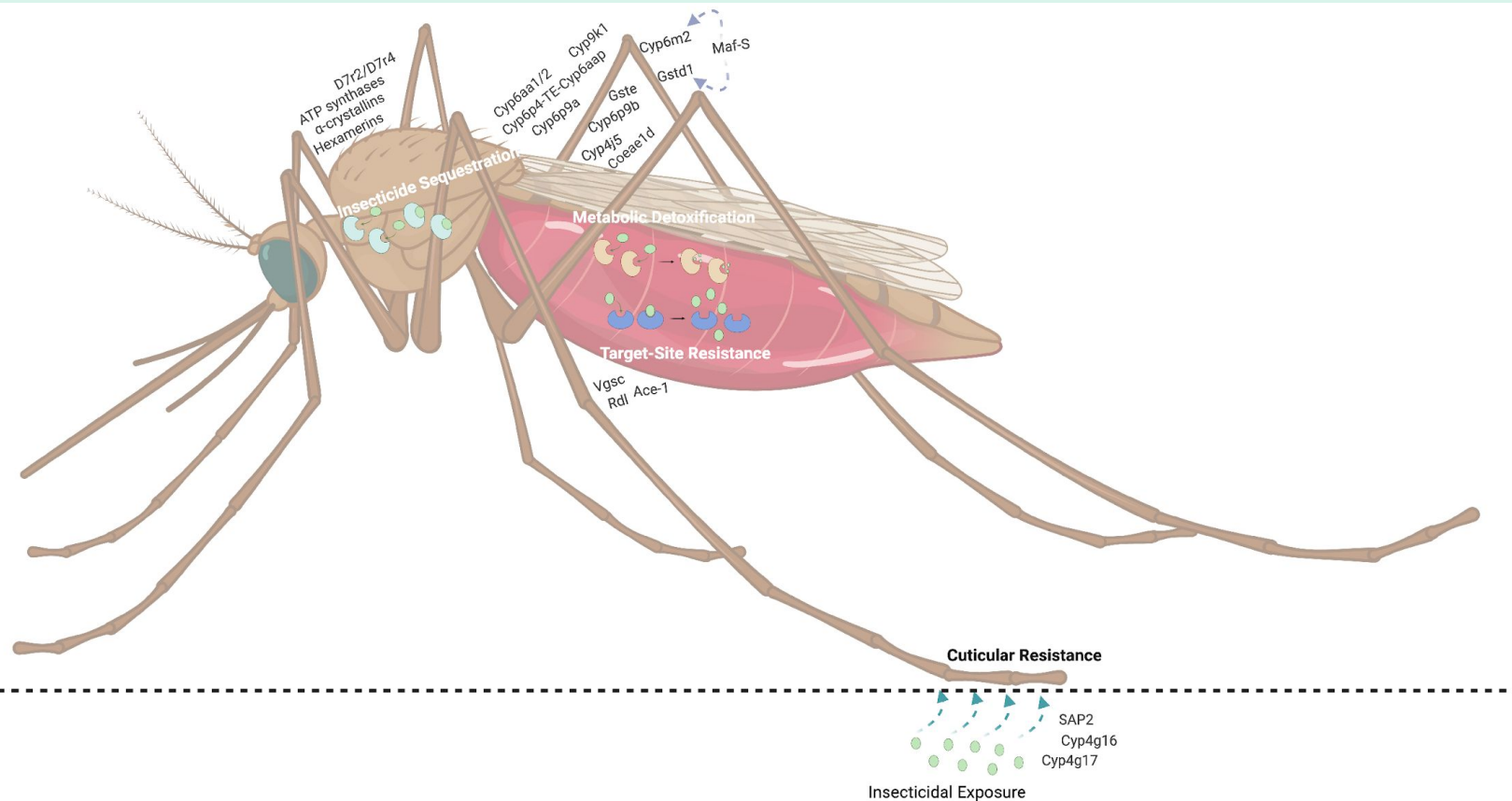
Cuticular Resistance



- Changes to mosquito cuticle reduces insecticide rate penetration
- Increased cuticle thickness
- Changes to cuticle hydrocarbon composition
- Upregulation of detoxification enzymes in mosquito tarsi

Source: Hancock, Ochomo and Messenger. Trends Parasitol 2024; 40(7):604-618.

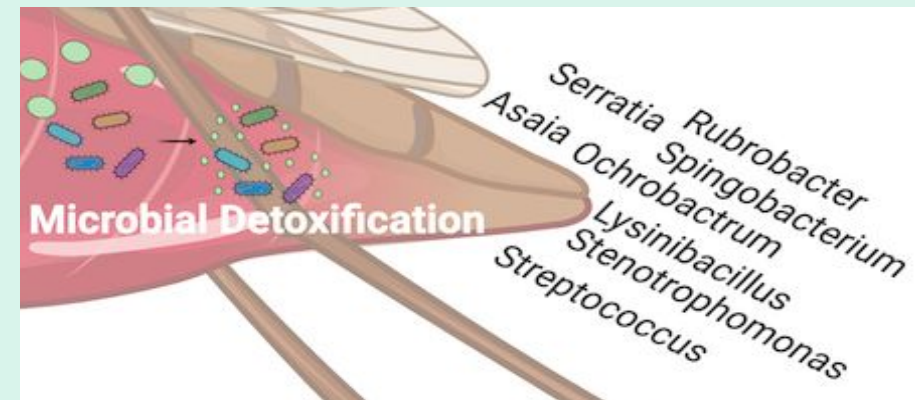
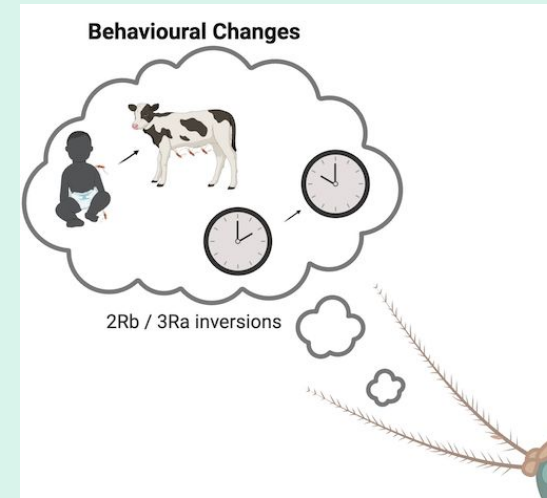
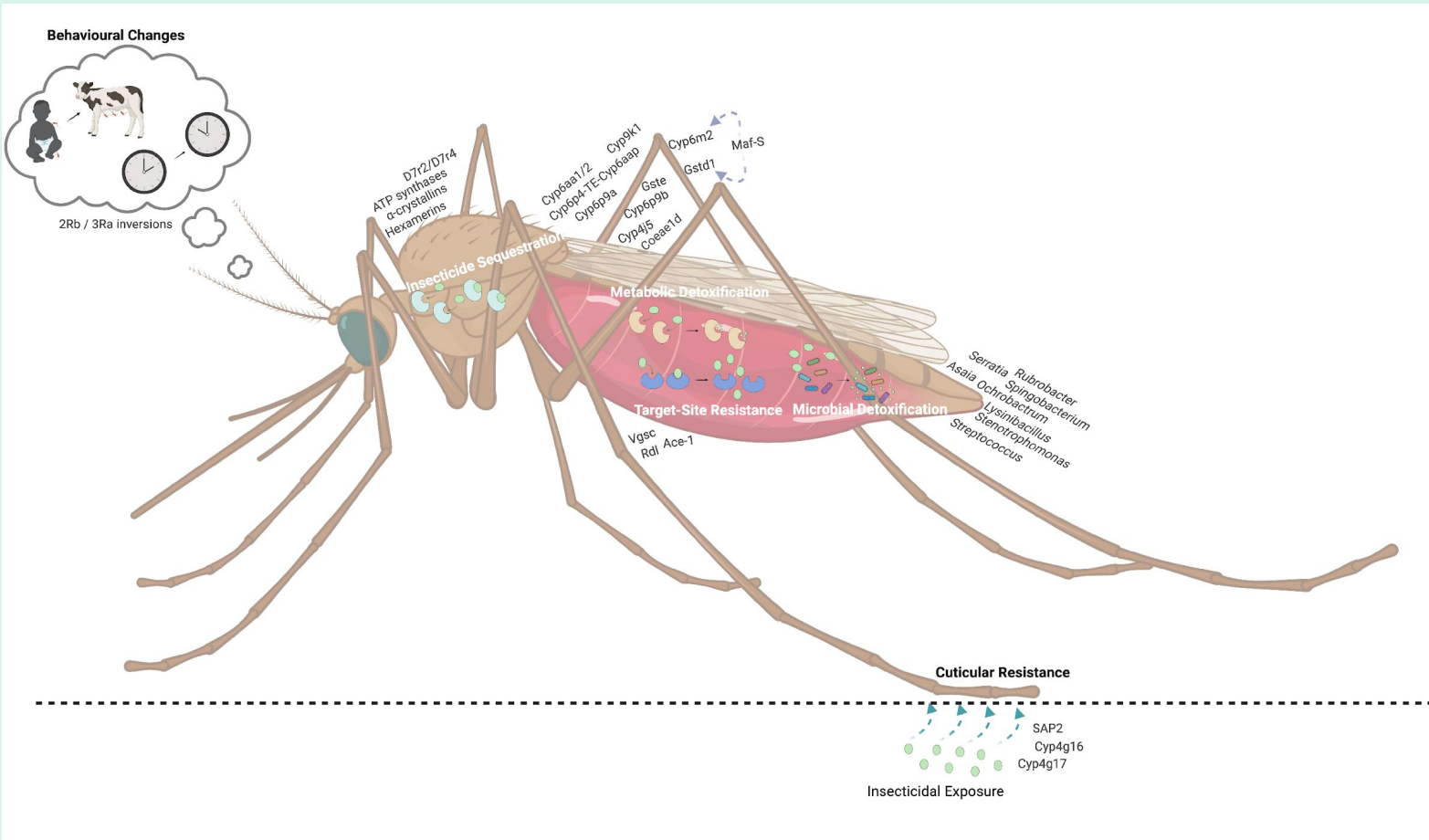
Insecticide Sequestration



- Genes involved in transportation or efflux of foreign components are overexpressed and may sequester insecticides

Source: Hancock, Ochomo and Messenger. Trends Parasitol 2024; 40(7):604-618.

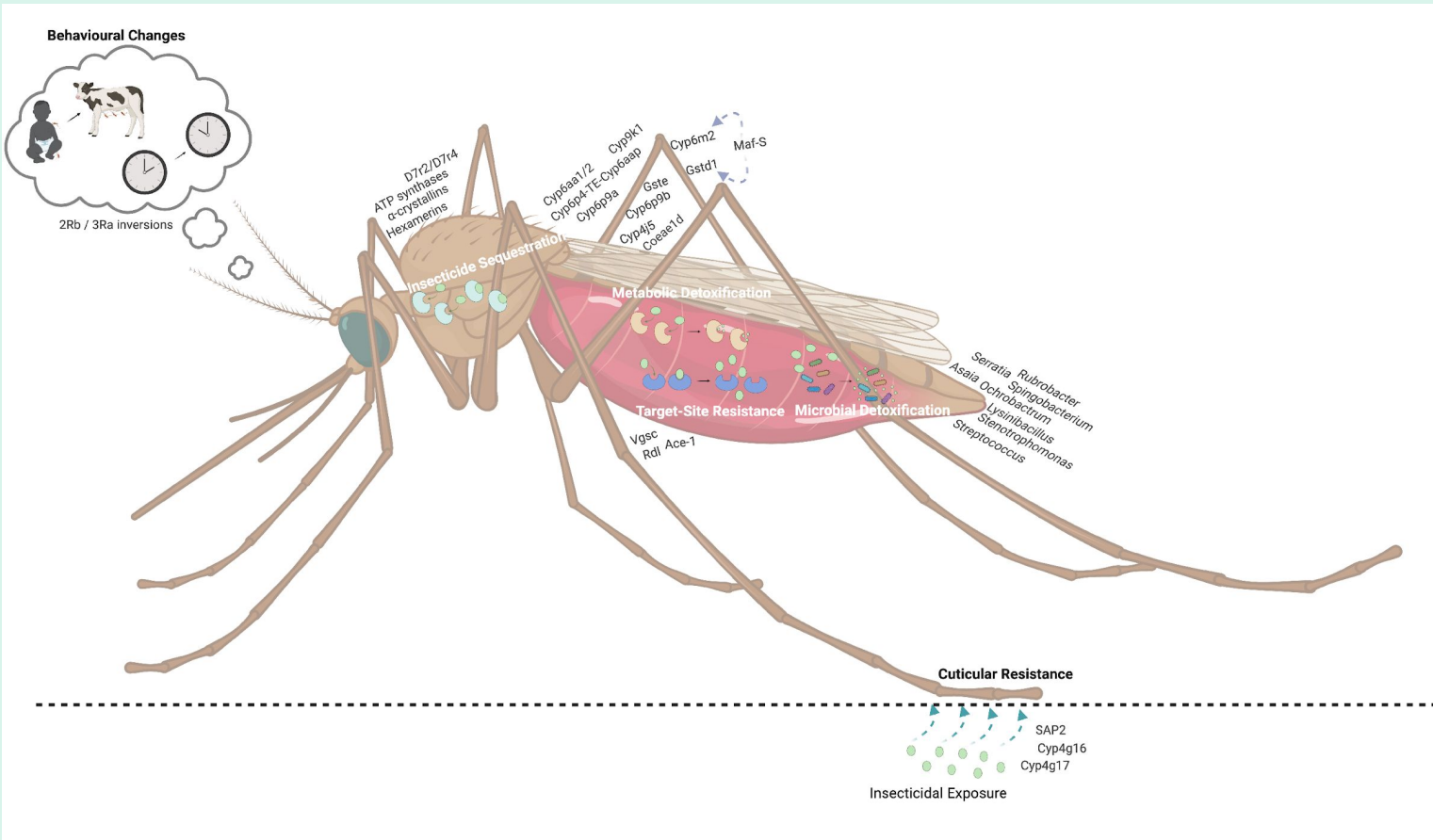
Other Resistance Mechanisms



Source: Hancock, Ochomo and Messenger. Trends Parasitol 2024; 40(7):604-618.

Insecticide Resistance Mechanisms

- Insecticide resistance driven by multiple, overlapping, distinct mechanisms, reflecting vector population evolution and life history
- Resistance mechanisms commonly confer cross-resistance within and between chemical classes
- Disentangling relative contribution of each resistance mechanism per phenotype is complex
- Depreciating costs of next-generation sequencing now facilitates more comprehensive characterization of resistant vector populations
- Some mechanisms linked functionally to phenotype but no clear thresholds relating resistance to vector tool operational efficacy



Source: Hancock, Ochomo and Messenger. Trends Parasitol 2024; 40(7):604-618.

Molecular Characterization of Experimental Hut Trial Samples

PCR Genotyping

- LNA qPCR targeting known mutations
- *vgsc*-L1014F/S, *vgsc*-V402L, *vgsc*-N1575Y, *gste2*-I114T

RNA-Sequencing

- Whole transcriptome sequencing to measure expression levels of mRNA transcripts

DNA Pool-Sequencing

- Whole genome sequencing of pooled individuals to estimate allele frequencies per locus

Elucidating Insecticide Resistance Mechanisms



Entomological Performance of New-Generation LLINs Across Three African Resistance Settings

Dr. Rosine Z. Wolie

Institut Pierre Richet (IPR), Côte d'Ivoire
Nangui Abrogoua University (UNA), Côte d'Ivoire

on behalf of RAFT Workstream 1 – Current Threats

Objectives

Obj. 1

□ **Assess differences in efficacy between different type of ITNs in experimental hut using free flying mosquitoes**

Obj. 2

□ **Assess the intensity of resistance and investigate the mechanism of resistance in wild *Anopheles* mosquitoes**

Methodology Overview

- **Experimental hut trials** were conducted in **Côte d'Ivoire (M'bé)**, **Cameroon (Mangoum)**, and **Tanzania (Magu & Mwagagala)**, all showing high pyrethroid resistance
- **Trials used six WHO-standard huts** with rotating treatments in a **latin square design**
- Adult volunteers slept under LLINs; mosquitoes were collected each morning to assess mortality, blood feeding, and exophily

- **LLINs tested:** Control (untreated), MaGNet, PermaNet® 2.0 (both pyrethroid-only) Veeralin®, PermaNet® 3.0, Olyset™ Plus (pyrethroid + PBO), and Interceptor® G2 (α -cypermethrin + chlorfenapyr)

- **Bioassays:** WHO and CDC tests with pyrethroids ($\pm$ PBO) and chlorfenapyr (100–200 μ g/ml)
- **Molecular assays:** *Anopheles* species identified by SINE-PCR
- Resistance markers genotyped by TaqMan/LNA-qPCR, including *kdr*-West (L1014F), *kdr*-East (L1014S), V402L, N1575Y, G119S (Ace-1), I114T (GSTe2), and E205D (CYP6P3)

Malaria Vector Species Collected in Experimental Hut Trials



In Cameroon (Mangoum)

A total of 2,119 mosquitoes were collected

- *An. gambiae* s.s.: 36%



Côte d'Ivoire (M'bé)

A total of 6,586 mosquitoes were collected

- *An. gambiae* s.l. : 49%
 - ✓ *An. coluzzii* : 91%
 - ✓ *An. gambiae* s.s. : 9%
- *An. funestus* : <1%

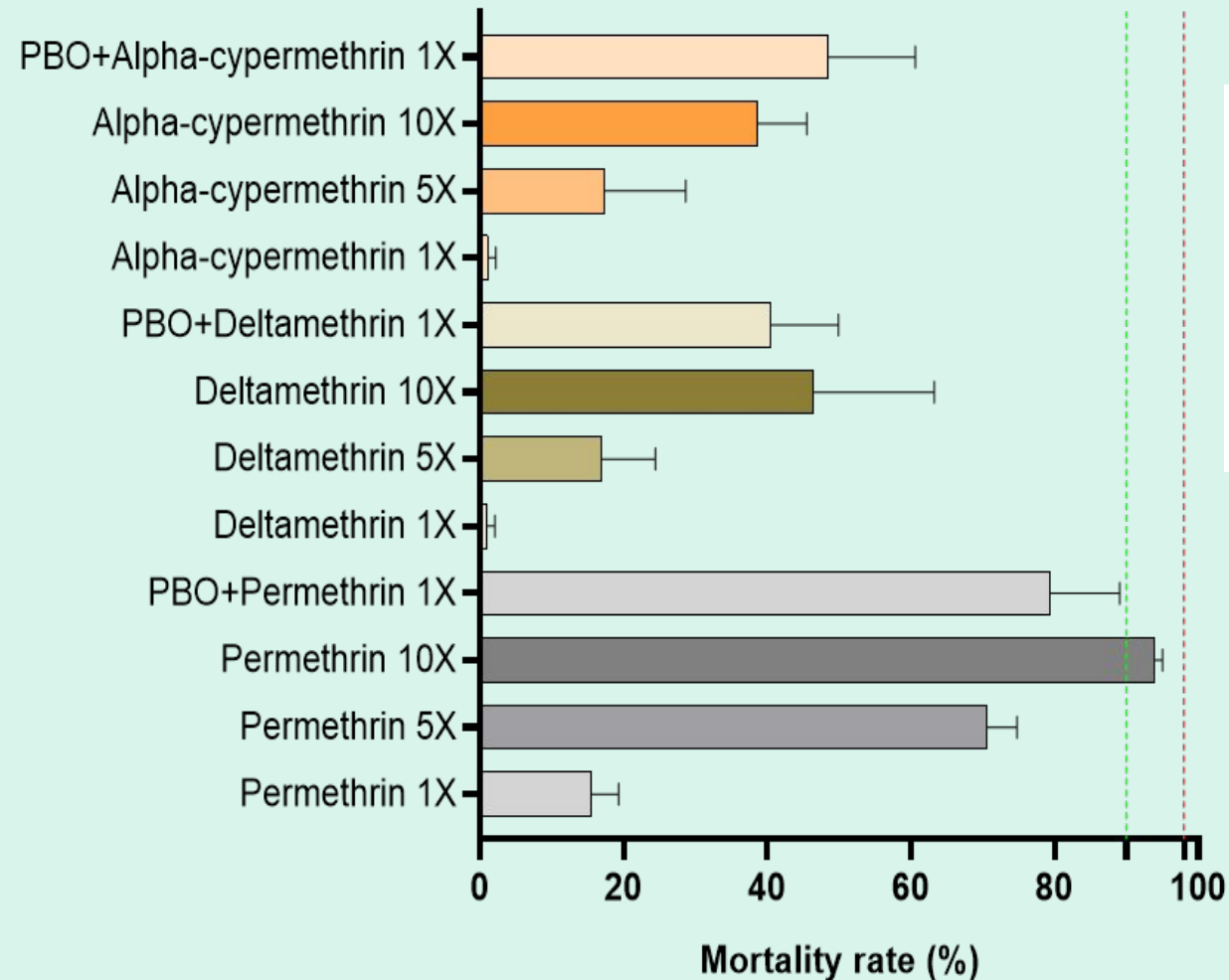


Tanzania (Magu)

A total of 3,548 mosquitoes were collected

- *An. gambiae* s.l. : 82 %
 - ✓ *An. arabiensis* : 77%
 - ✓ *An. gambiae* s.s. : 23%
- *An. funestus* s.s. : 18%

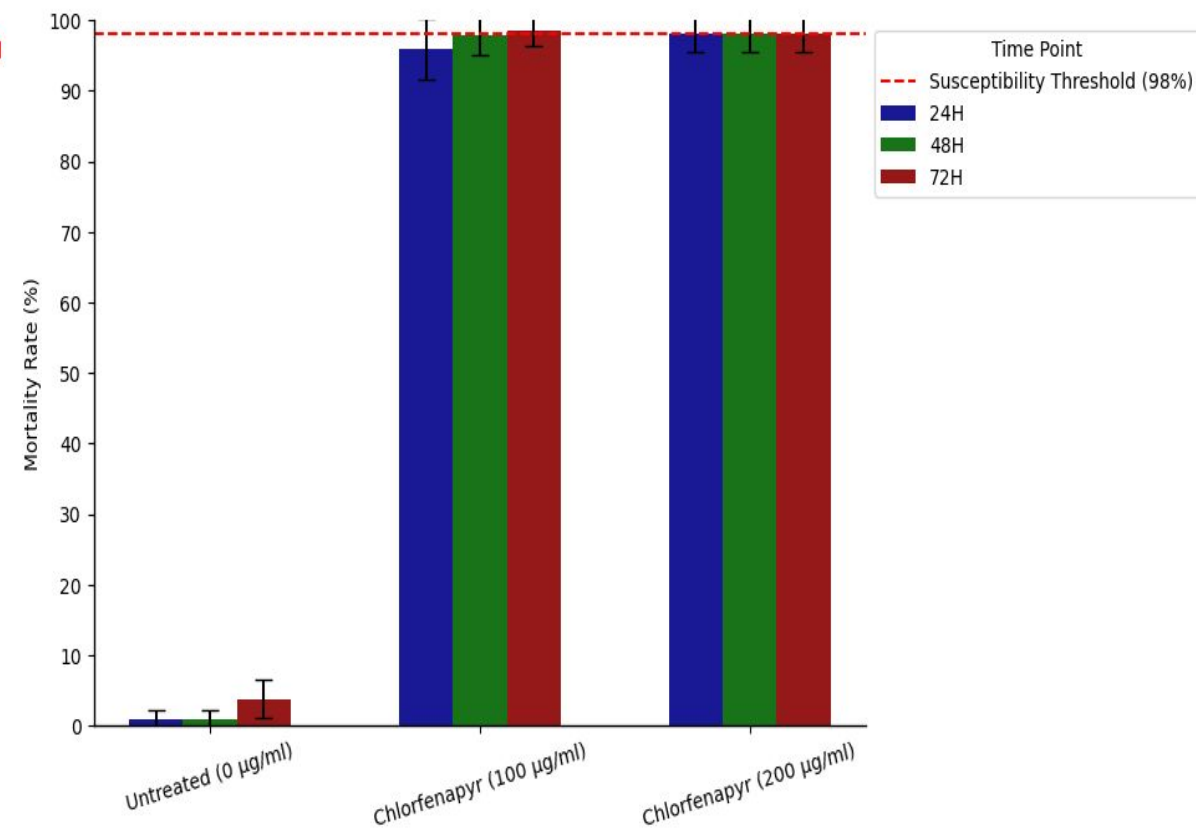
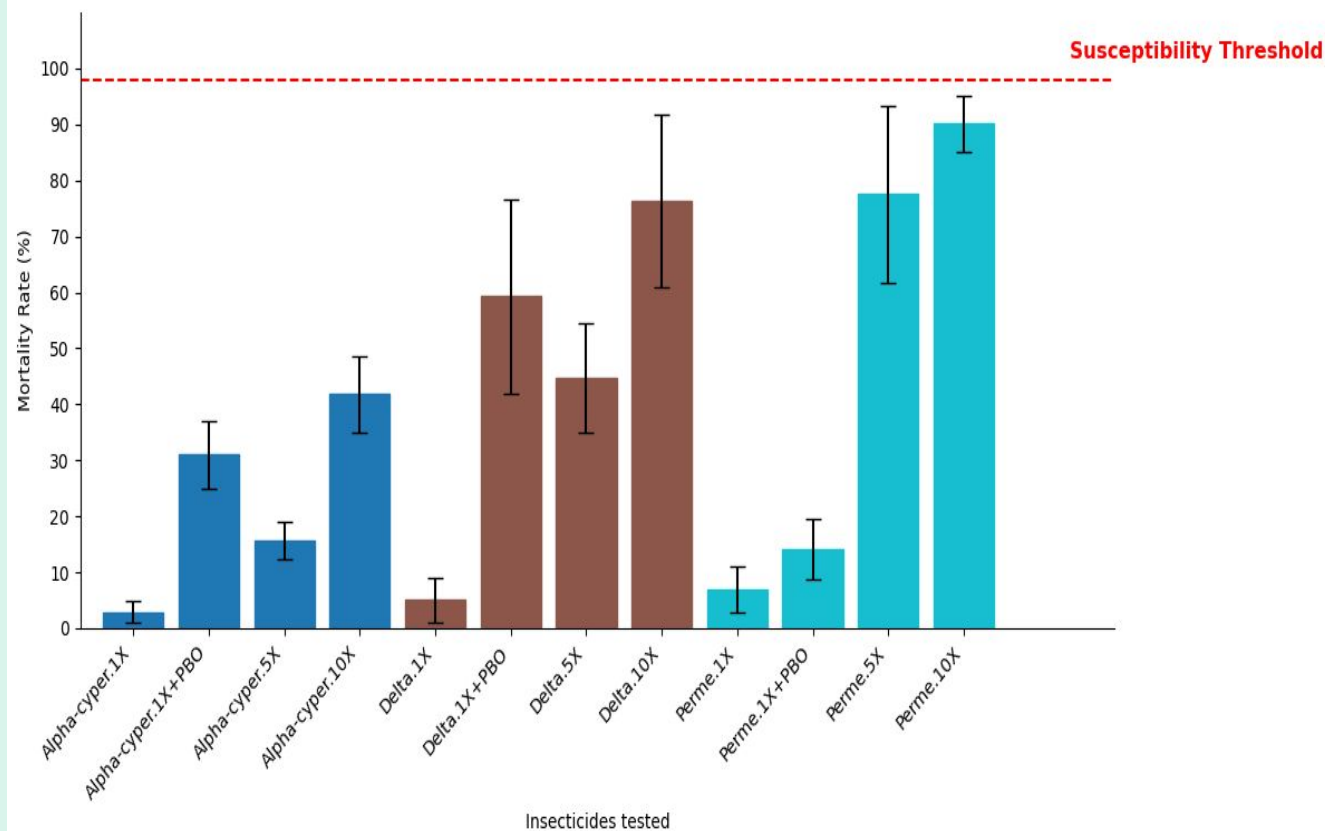
Insecticide Susceptibility & Characterization in Cameroon



	Genotypes			Total	Phenotypes	
	RR	RS	SS		f(R)	f(S)
L995F-kdr	38	0	0	38	100.00	0.00
L995S-kdr	0	1	37	38	1.32	98.68
E205D-Cyp6p3	20	11	7	38	67.11	32.89

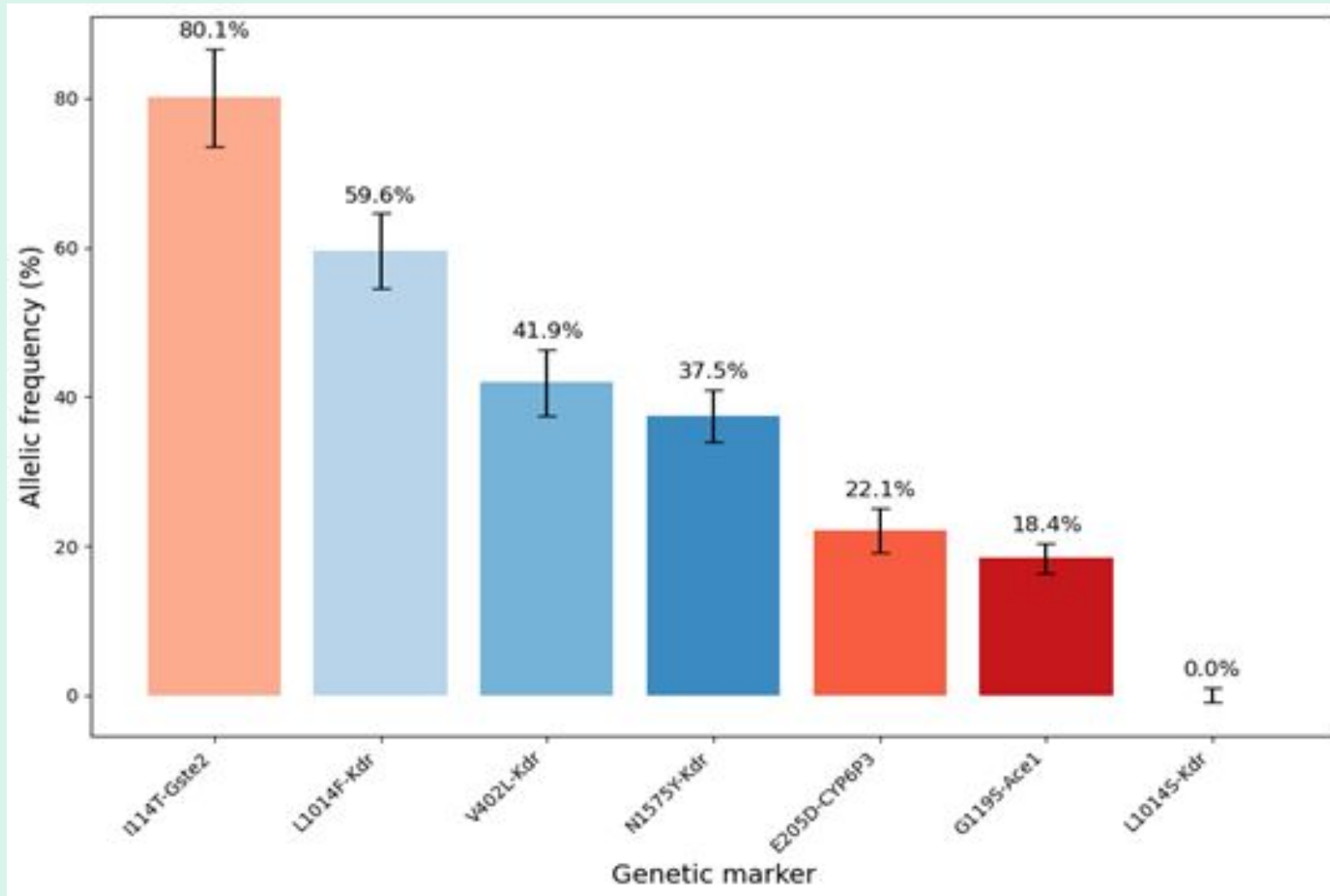
- Strong pyrethroid resistance (<40% mortality at DC)
- Partial restoration with PBO
- L995F fixed, E205D-CYP6P3 (67%)
- → target-site + metabolic resistance

Insecticide Resistance Profile & Molecular Characterization of *An. gambiae* s.l. in Côte d'Ivoire



- High resistance intensity (<10% mortality at DC)
- PBO ≤60 % → oxidase-based metabolic resistance
- Full susceptibility to chlorfenapyr (100 and 200 µg/ml) >98% mortality within 24-72h

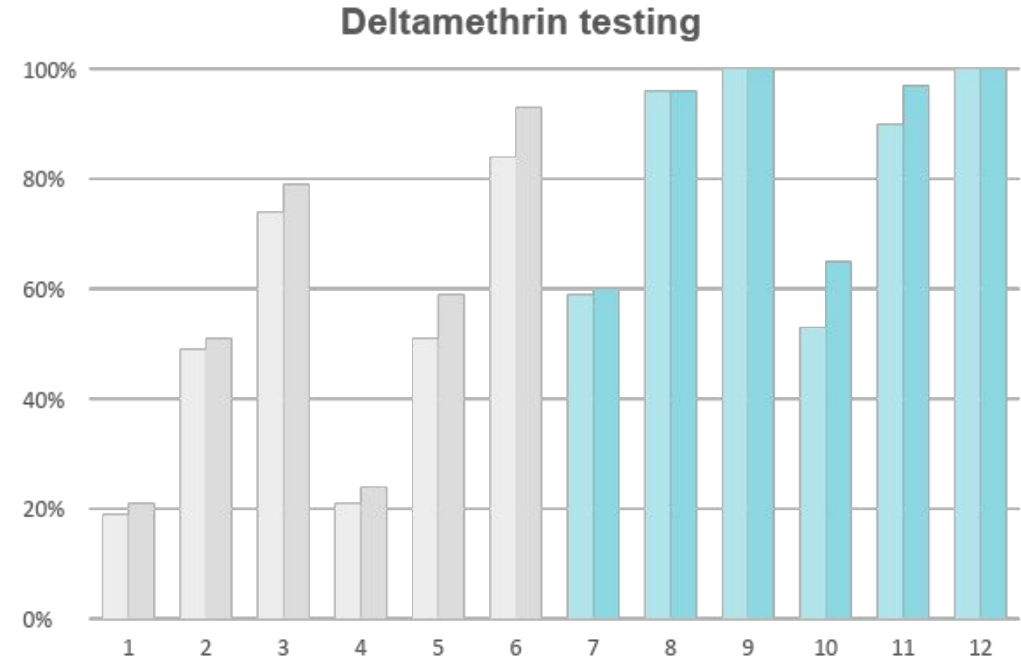
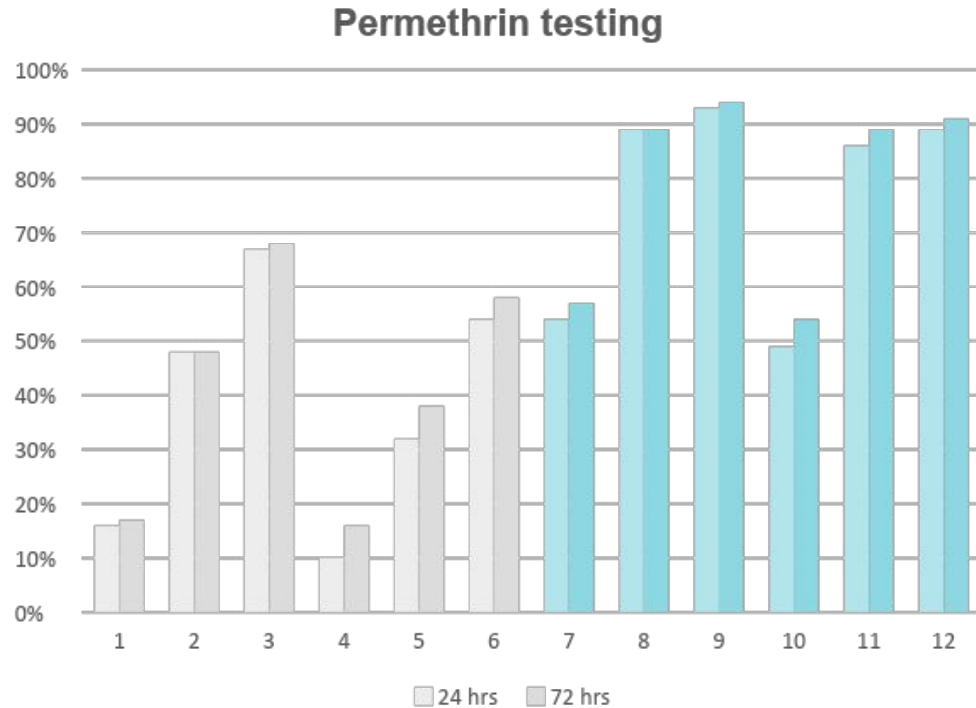
Insecticide Resistance Profile & Molecular Characterization of *An. gambiae* s.l. in Côte d'Ivoire



- **Key alleles: L1014F (53-100%), V402L (48%), E205D (21-42%), I114T (75-81%), N1575Y (33-39%)**

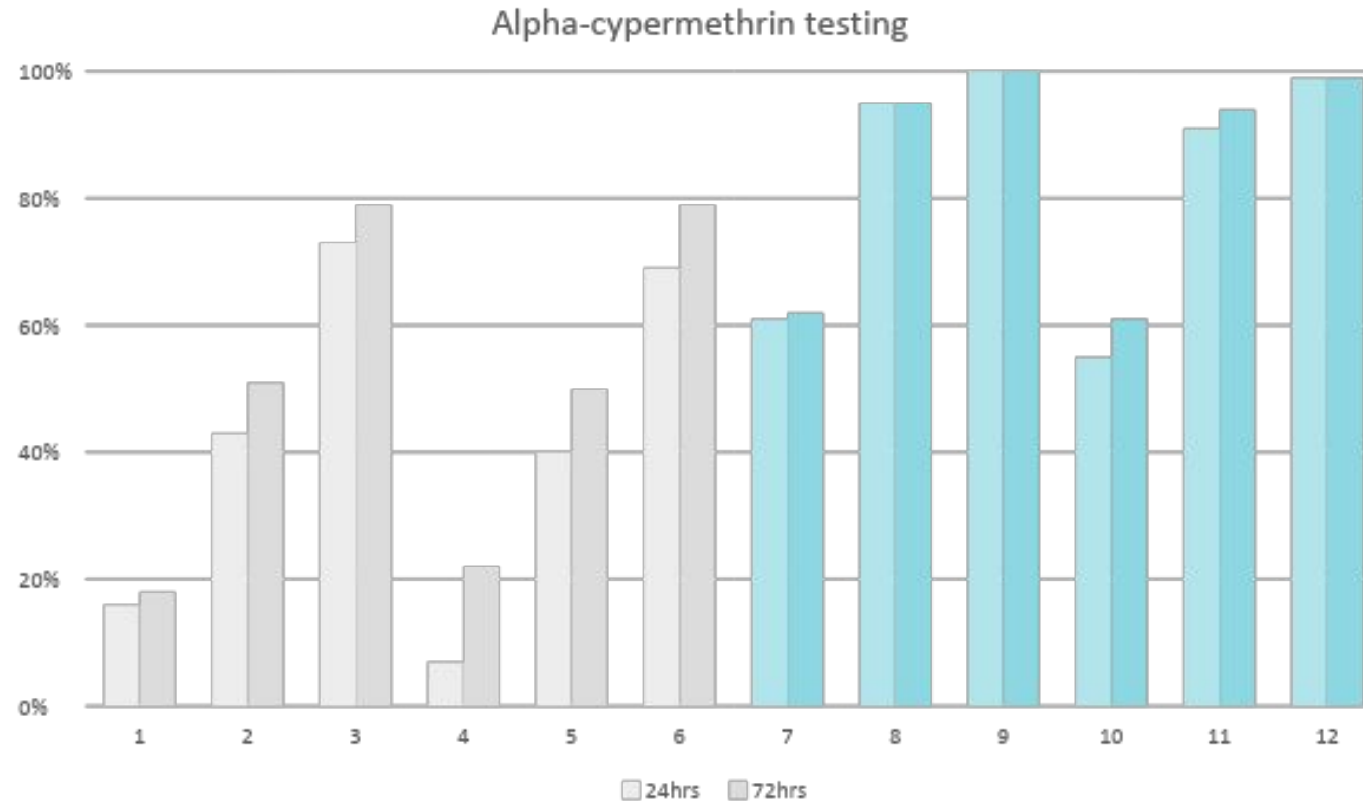
→ **Combined target-site + metabolic mechanisms**

Insecticide Resistance Profile of *An. gambiae* s.l. and *An. funestus* in Tanzania



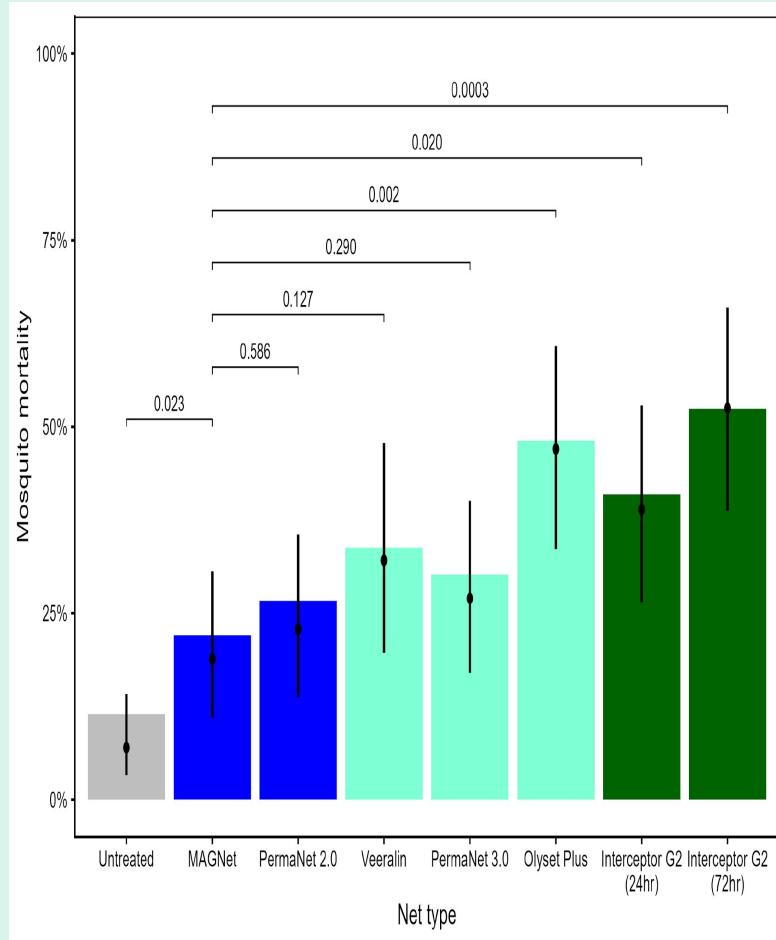
- High resistance intensity using permethrin against *An. gambiae* and *An. funestus*
- Pre-exposure to PBO could not restore full susceptibility
- High resistance intensity using deltamethrin against *An. gambiae* and *An. funestus*
- Pre-exposure to PBO restore full susceptibility using 10X deltamethrin

Insecticide Resistance Profile of *An. gambiae* s.l. and *An. funestus* in Tanzania

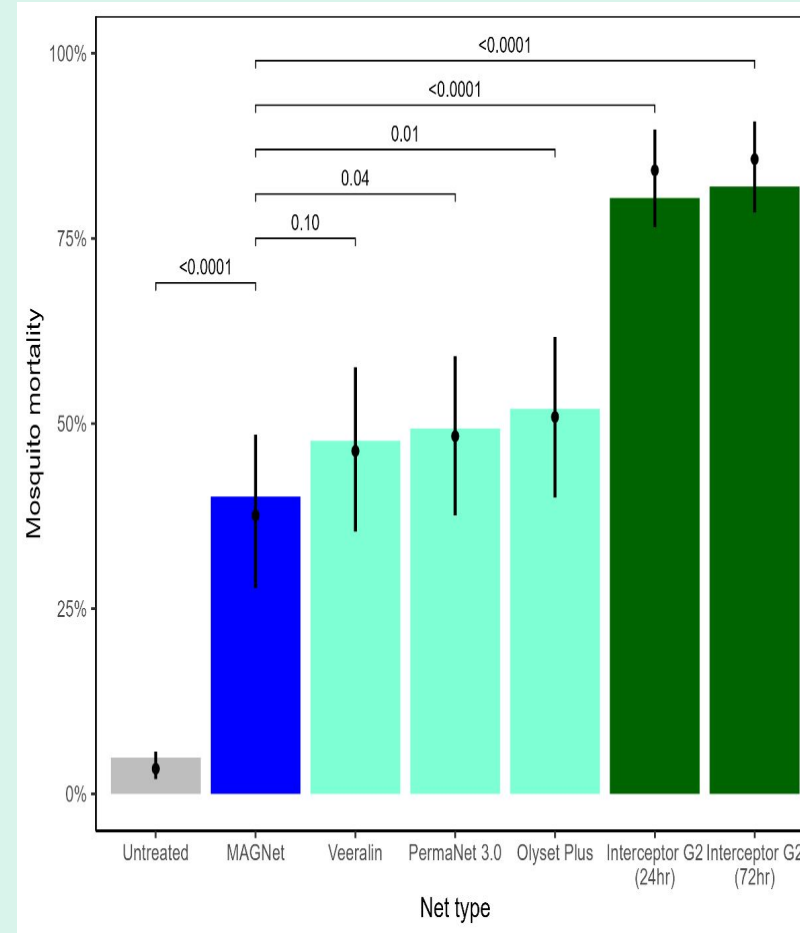


- High resistance intensity using alpha-cypermethrin against *An. gambiae* and *An. funestus*
- Pre-exposure to PBO restore full susceptibility at 10X dose

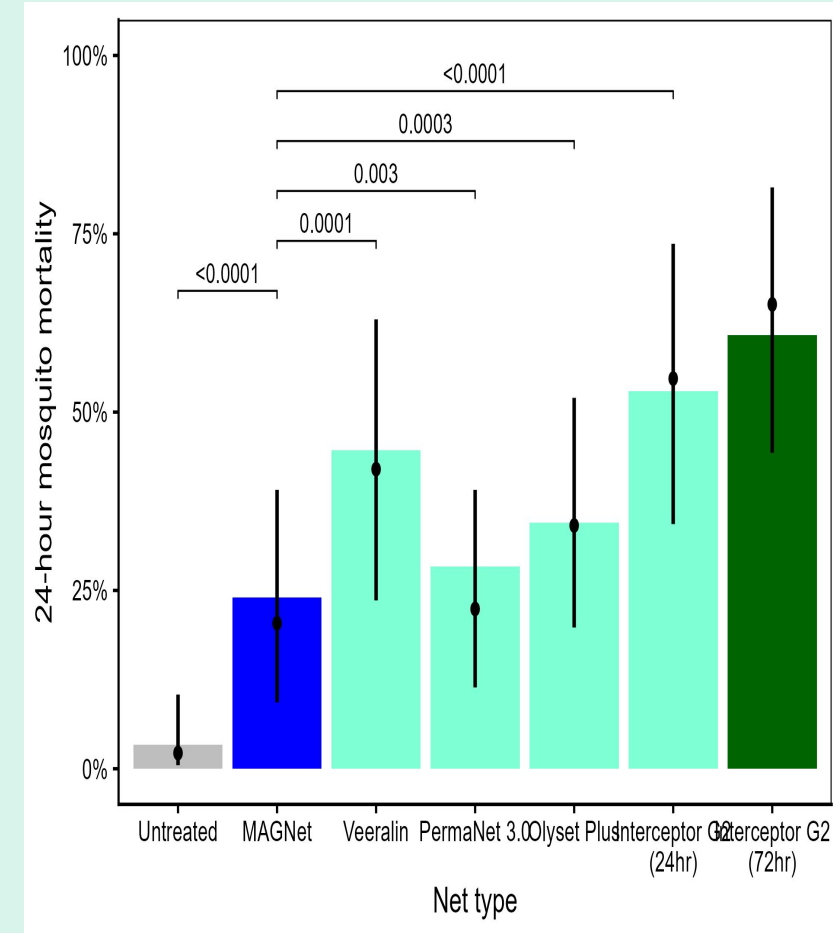
Mortality Outcomes Across Three Experimental Hut Sites



- **Cameroon (Mangoum)**
Mortality highest with Olyset Plus & Interceptor G2 (>50%)
Pyrethroid-only nets induced <40% mortality

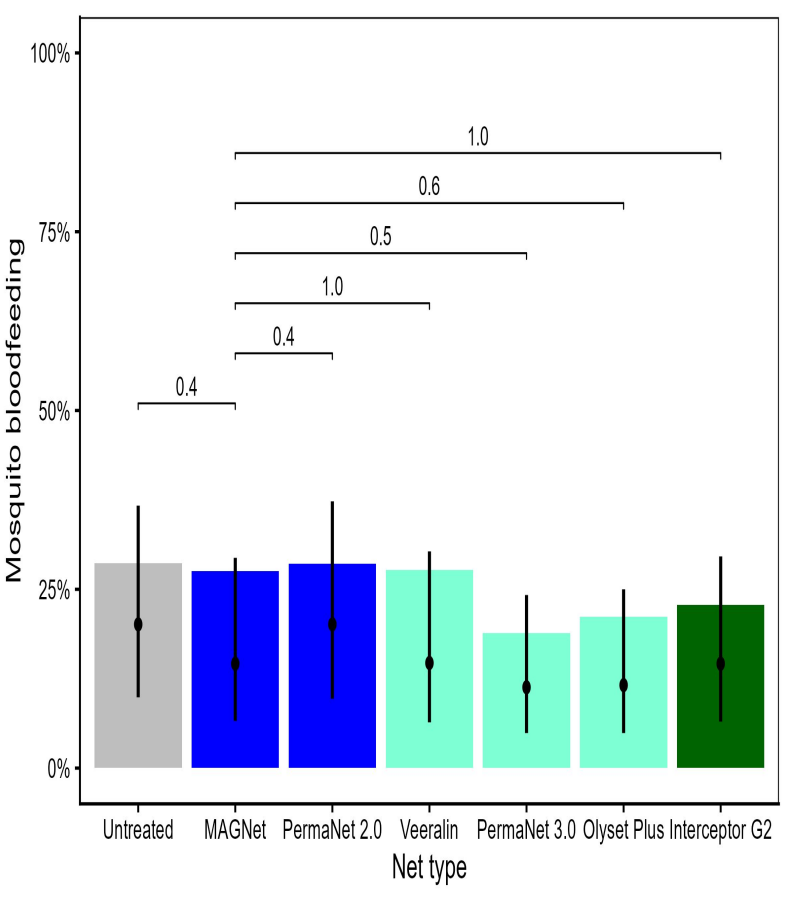


- **Côte d'Ivoire (M'bé)**
Interceptor G2 achieved ≥80% mortality at 72h
PBO nets (Olyset Plus, PermaNet 3.0) ≈ 50%
Pyrethroid-only nets <45%

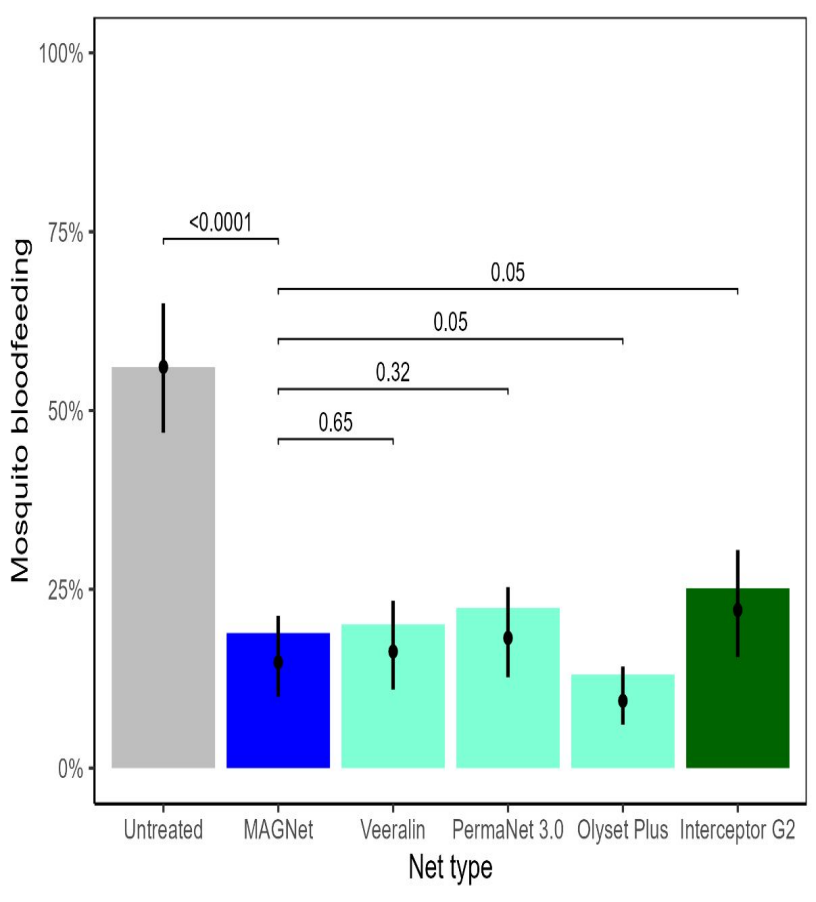


- **Tanzania (Magu)**
Mortality <60% for all nets
Interceptor G2 & Olyset Plus slightly higher but not significant vs PBO nets

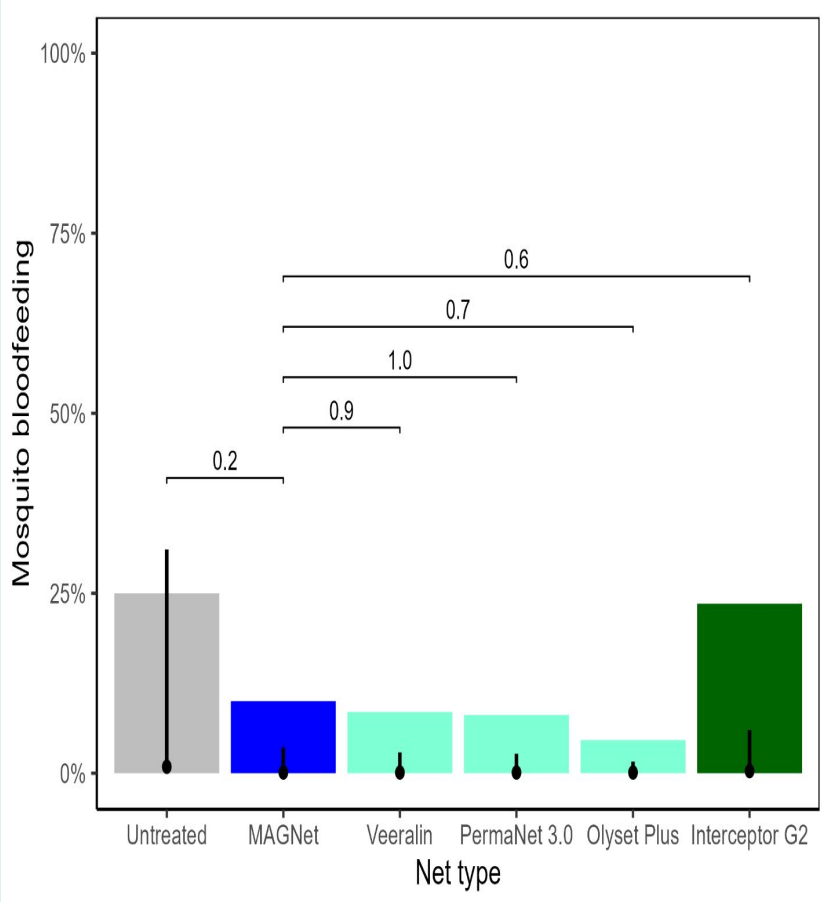
Blood-feeding Outcomes Across Three Experimental Hut Sites



- **Cameroon (Mangoum)**
Blood-feeding <30% for all nets
Slightly lower with PermaNet 3.0 and Olyset Plus

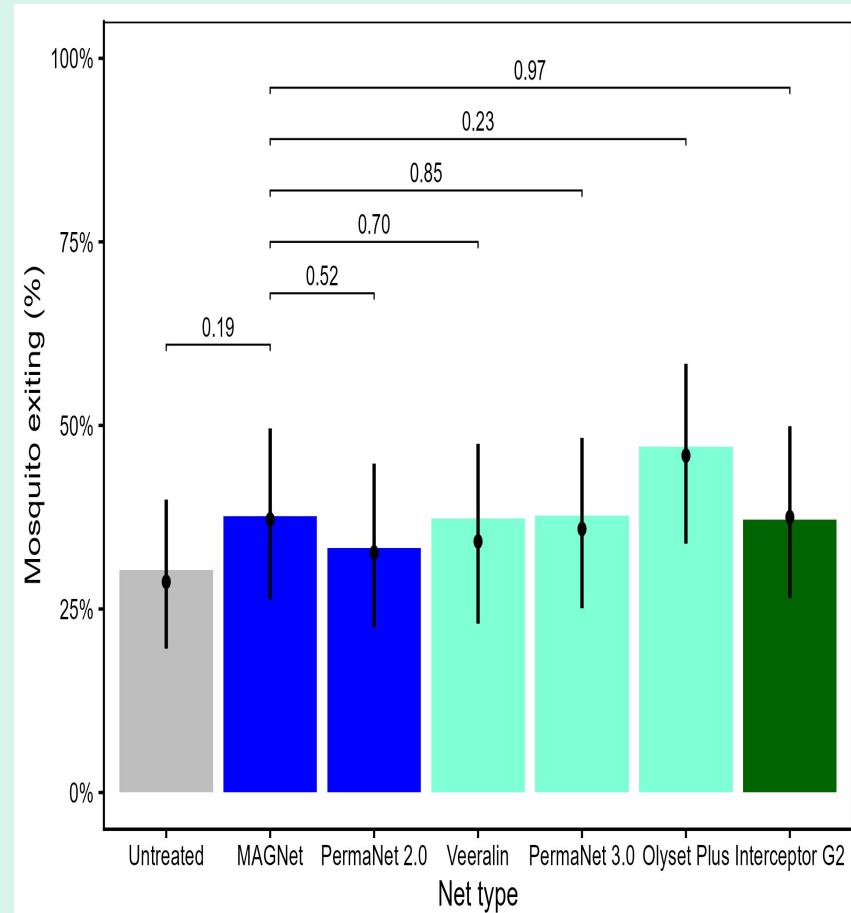


- **Côte d'Ivoire (M'bé)**
Untreated: 57% blood-fed
All nets reduced feeding by <50%, strongest with Olyset Plus

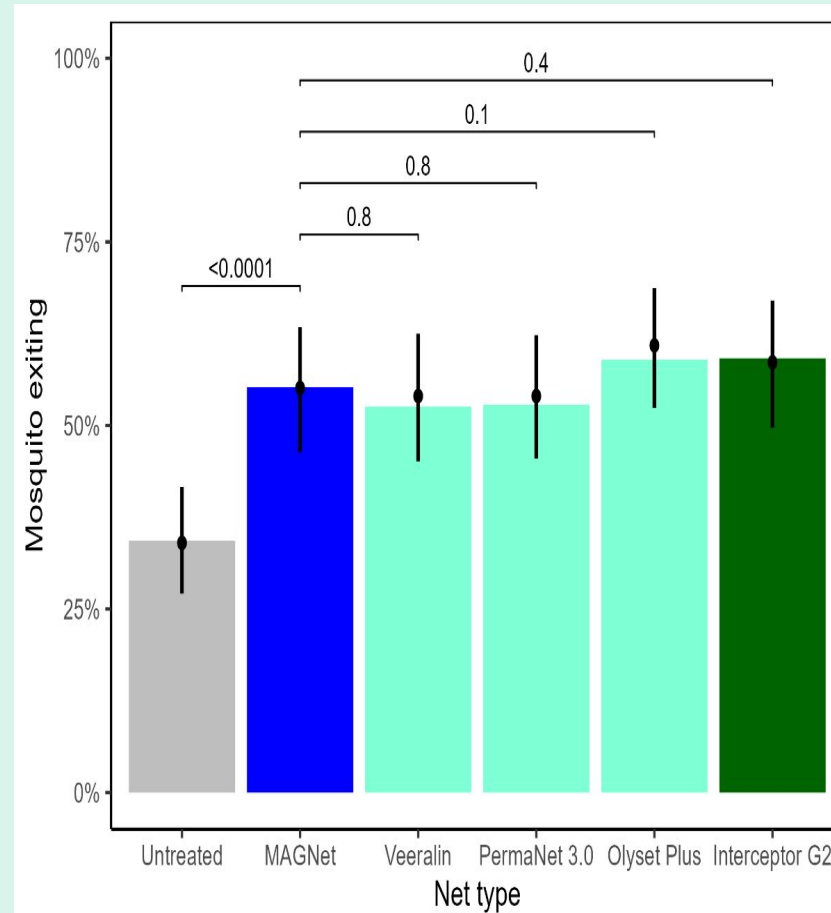


- **Tanzania (Magu)**
Very low feeding (<10%) across nets
Olyset Plus performed best

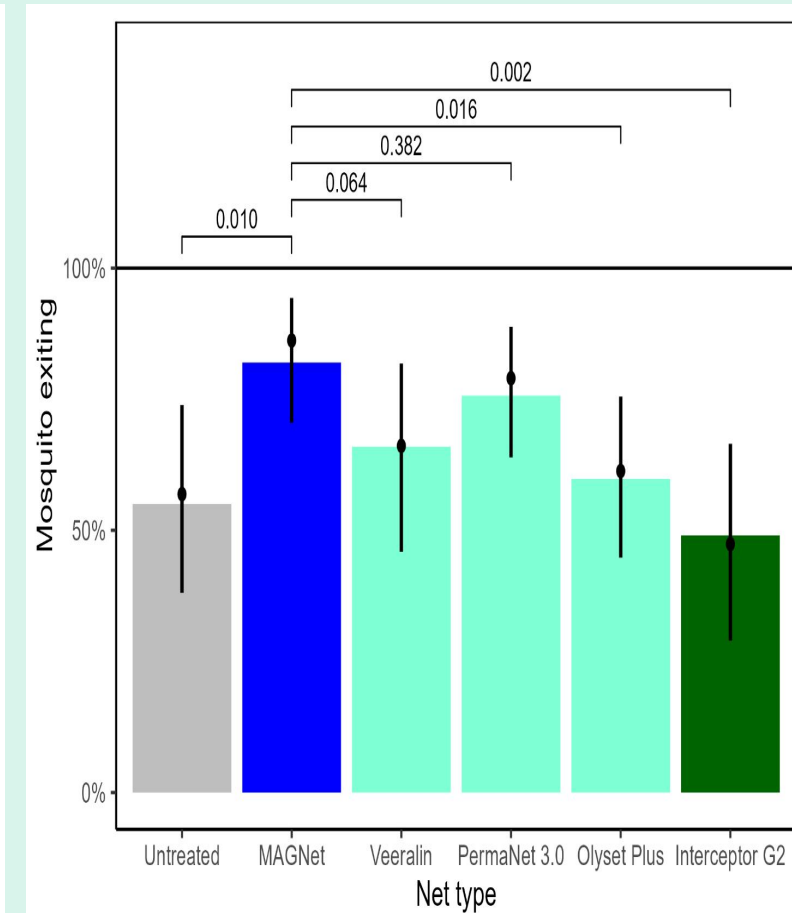
Exiting Outcomes Across Three Experimental Hut Sites



- **Cameroon (Mangoum)**
No significant differences in exiting for any LLIN



- **Côte d'Ivoire (M'bé)**
Exit rates 50-60% for all treated huts vs 30% Untreated
No major difference among LLINs



- **Tanzania (Magu)**
Exit rates >50% for all nets
MAGNet and PermaNet 3.0 showed slightly higher exophily

Summary / Take-Home Messages



Widespread pyrethroid resistance confirmed in all sites, with mixed target-site and metabolic mechanisms



PBO and dual-AI LLINs (especially Interceptor® G2) provided higher mortality and protection than pyrethroid-only nets



Chlorfenapyr efficacy across all settings highlights its crucial role in sustaining LLIN performance against resistant vectors

Transcriptomic Signatures of Vector Responses to LLINs During the Experimental Hut Trials

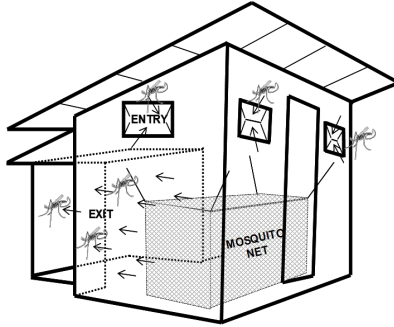
Dr. Yvan Fotso-Toguem

CRID, Cameroon

on behalf of RAFT Workstream 1 – Current Threats

Study Design

1



Nets used

- ❖ Untreated
- ❖ MAGNet
- ❖ PermaNet 3.0
- ❖ Olyset Plus
- ❖ VeeraLIN
- ❖ Interceptor G2

Experiments Hut Trials

Using free flying mosquito from 3 countries:

2

Sorted:

- ❖ Alive from treated hut
- ❖ Alive from untreated

Susceptible ref. strain:

- ❖ CIV: *An. coluzzii* Ngousso
- ❖ CMR: *An. gambiae* Kisumu
- ❖ TZA: *An. arabiensis* Dongola

Sorting

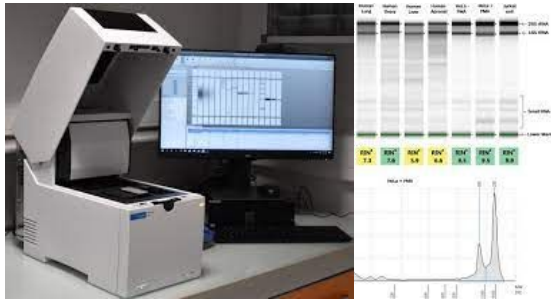
3



RNA extraction

Picopure kit

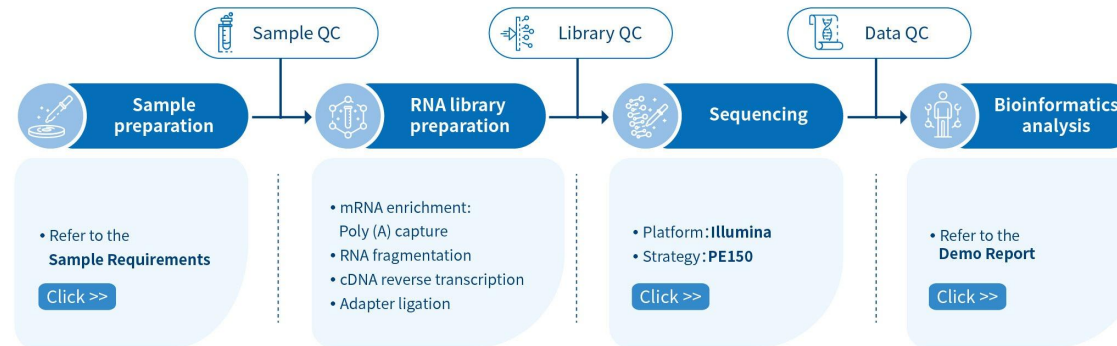
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Quality control

Using the bioanalyser

5



RNA Sequencing

6

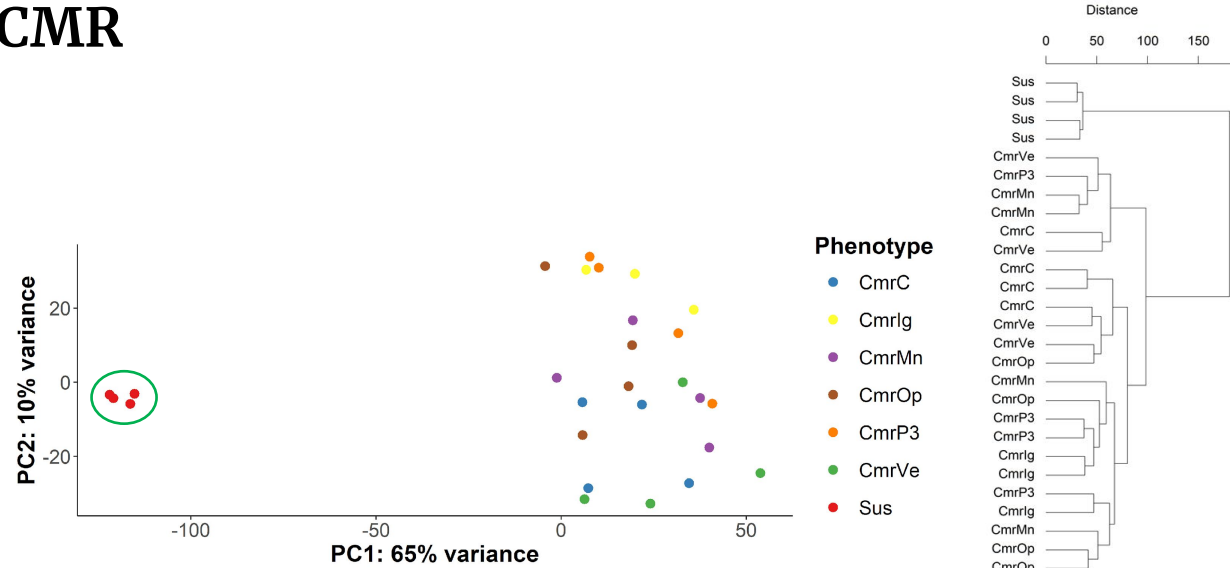


Analysis

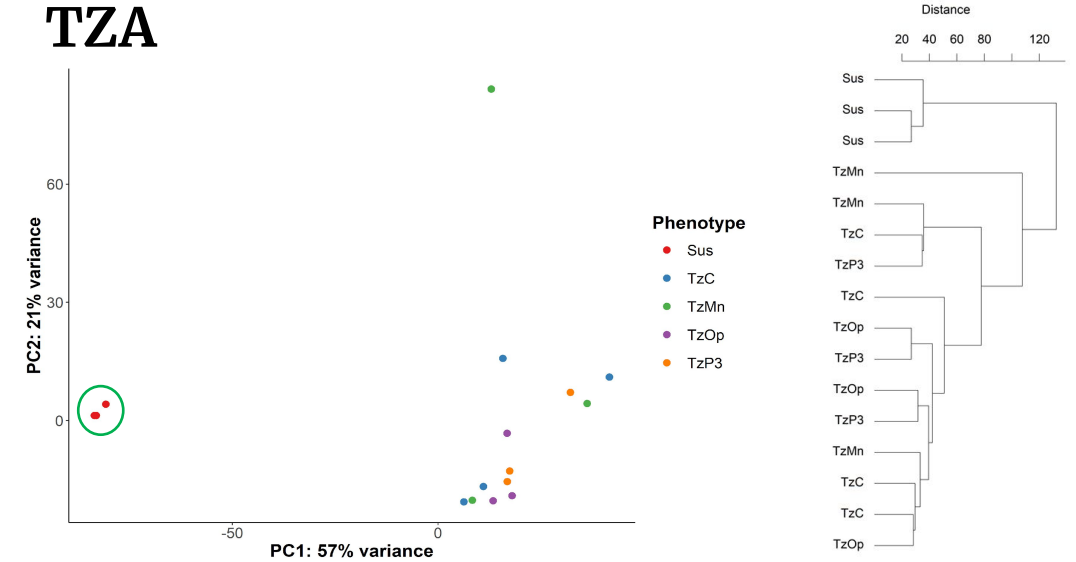
bioinformatic tools

Results: Population Structure with PCA

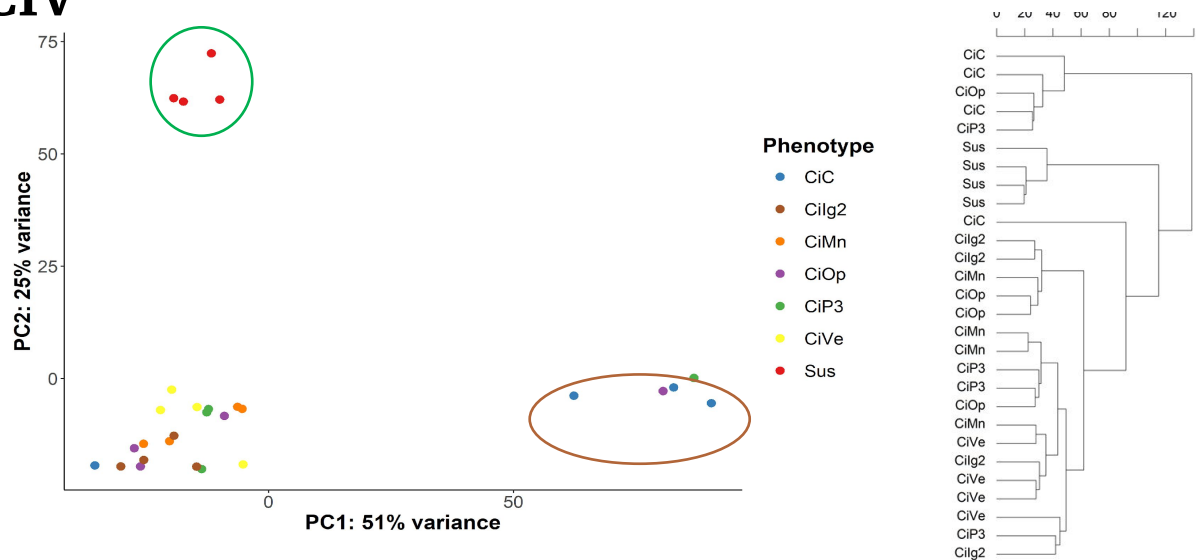
CMR



TZA

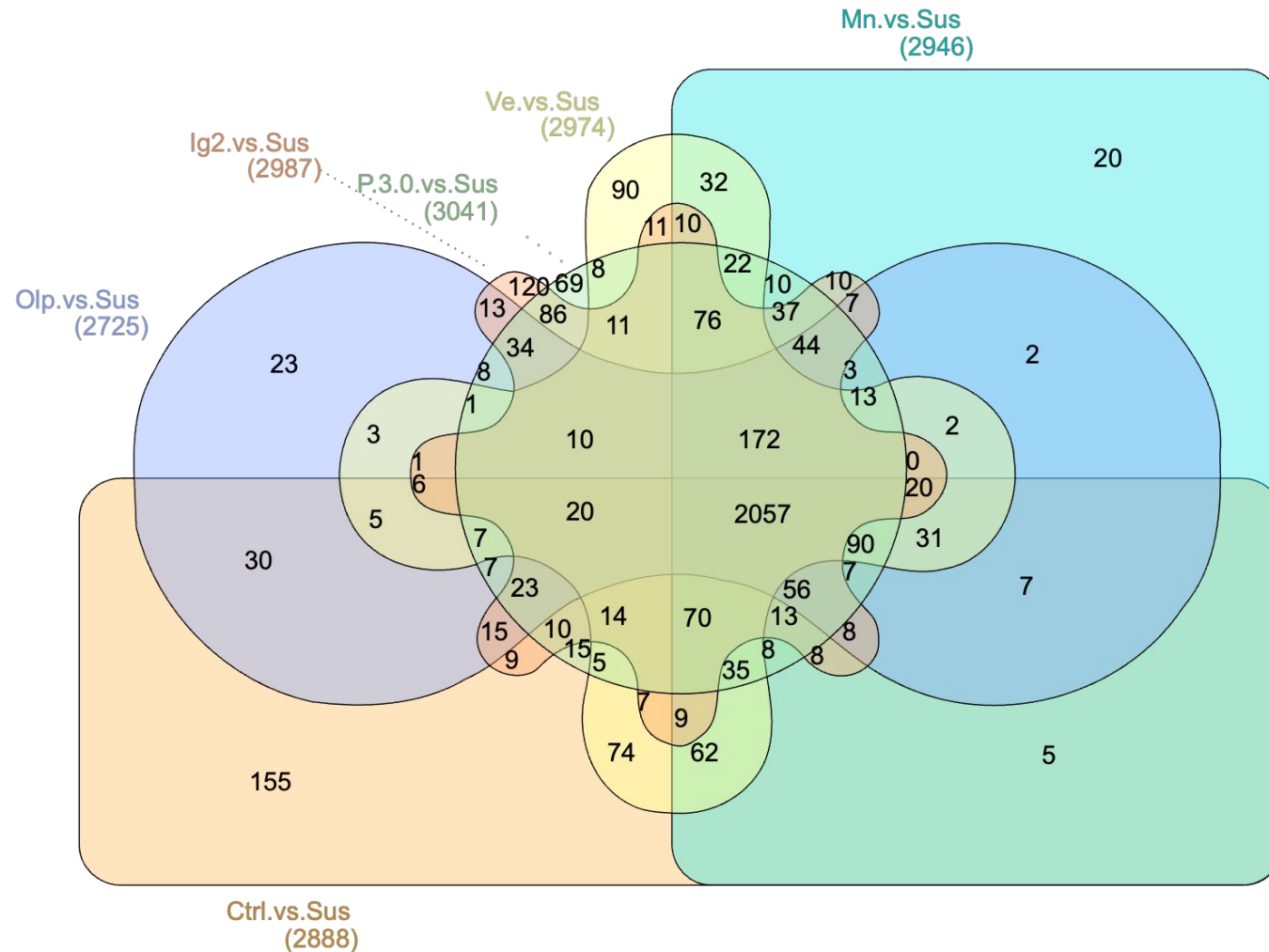


CIV



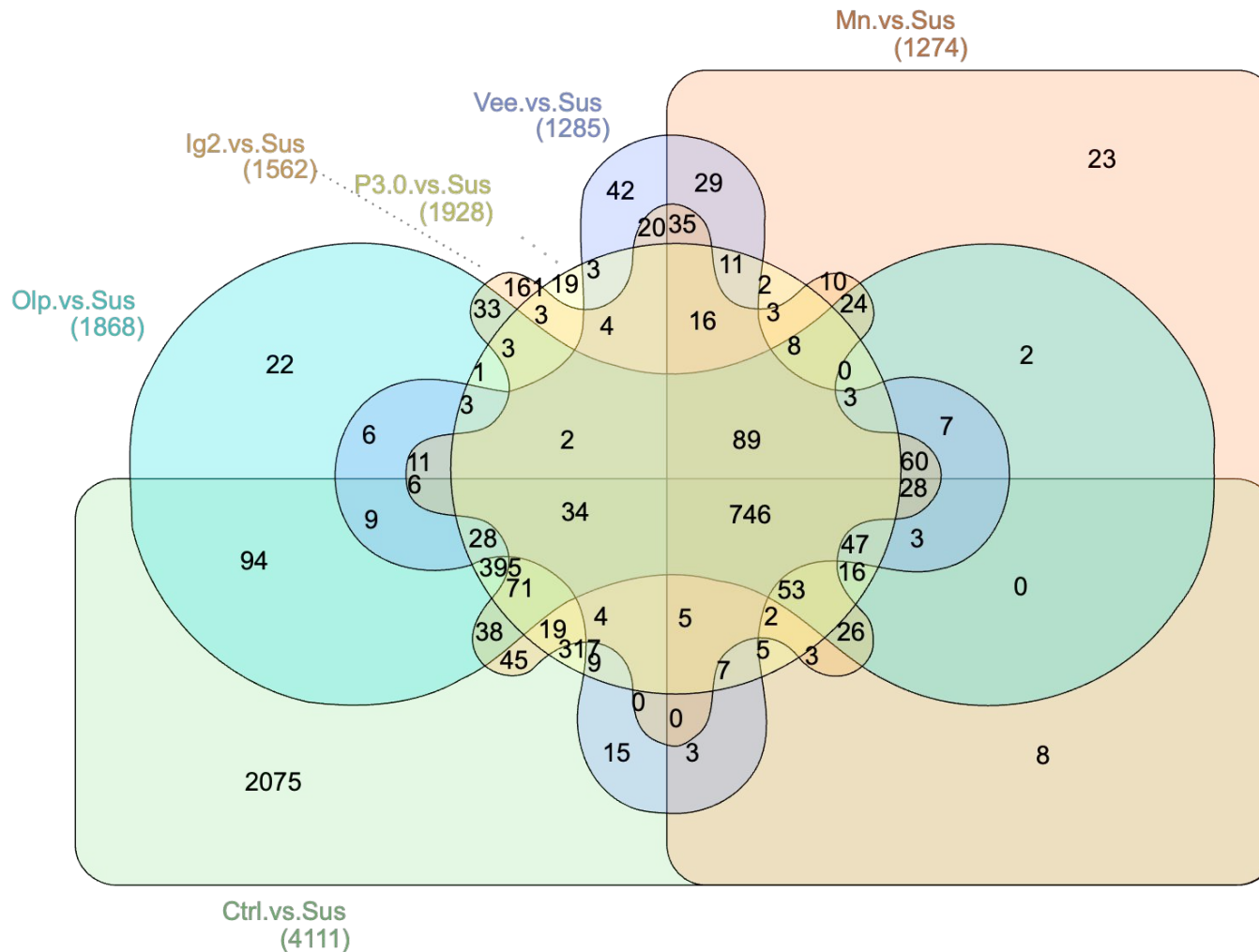
- ❑ Clear differentiation between the susceptible (in red) and the exposed populations
- ❑ No cluster between untreated and exposed samples population across the site
- ❑ Confirms by the dendrogram

Results: Differential Upregulated Genes in CMR Samples



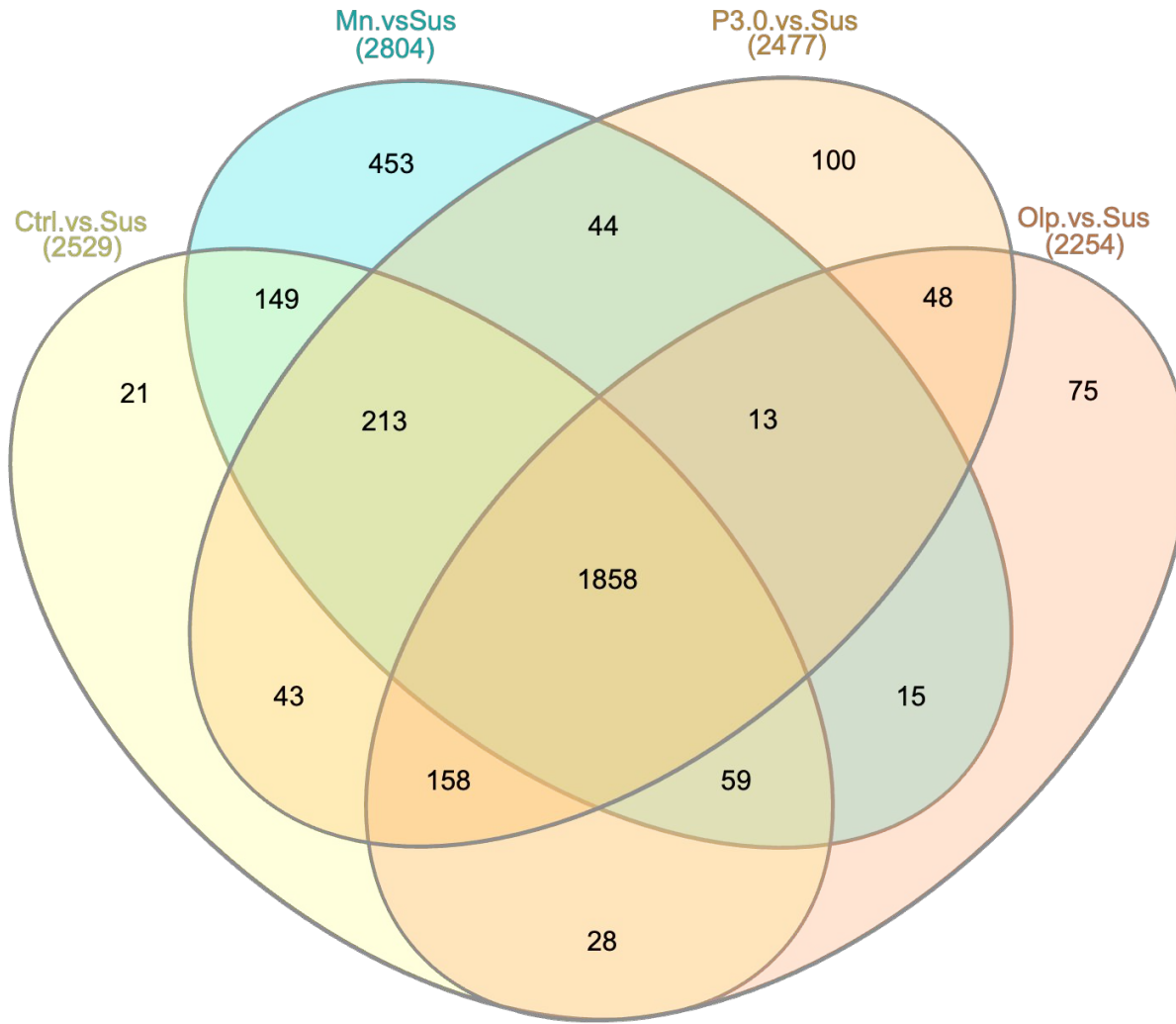
- ❑ 2057 was commonly upregulated genes across all groups
- ❑ No unique was shared between Mn.vs.Sus and Ctrl.vs.Sus.
- ❑ No unique upregulated genes were identified between P3.0.vs.Sus, Olp.vs.Sus and Vee.vs.Sus.
- ❑ 23 upregulated genes was unique to Ig2.vs.Sus.

Results: Differential Upregulated Genes in CIV Samples



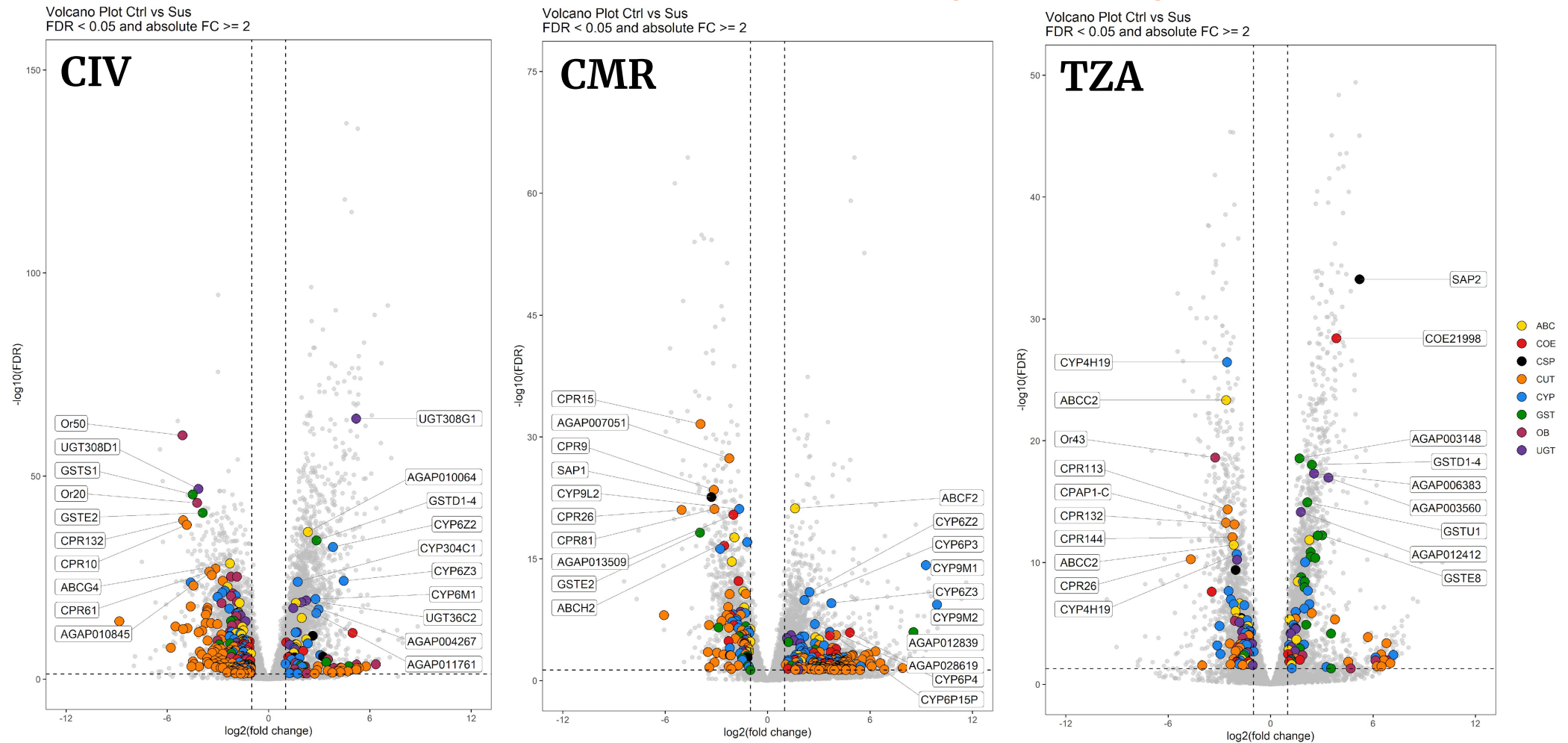
- ❑ 746 was commonly upregulated genes across all groups
- ❑ 34 upregulated genes was shared between P3.0.vs.Sus, OIp.vs.Sus and Vee.vs.Sus.
- ❑ 22 upregulated genes was unique to Ig2.vs.Sus.

Results: Differential Upregulated Genes in TZA Samples



- ❑ 1858 was commonly upregulated genes across all groups
- ❑ 149 was shared between Mn.vs.Sus and Ctrl.vs.Sus.
- ❑ 158 between P3.0.vs.Sus and Olp.vs.Sus.

Results: Differential Expressed Genes by Family in Untreated Nets



- ❑ In general, the most abundant genes were the cuticular proteins (orange), followed by the P450s proteins (blue), the odorant binding (maroon) and carboxylesterases proteins (red).

Results: Candidates Detox Genes in CMR Samples

Gene_Name	Ctrl.vs.Sus	Mn.vs.Sus	P3.0.vs.Sus	Op.vs.Sus	Ve.vs.Sus	Ig2.vs.Sus	Chr.	Description
* UGT308G1	36.50	37.33	48.20	53.57	34.31	50.25	3R	UDP-glucosyltransferase 2
AGAP028619	31.50	25.60	15.10	25.00	42.20	13.73	3L	Carboxylesterase 3
* CYP6Z3	21.79	24.08	23.70	30.07	15.61	32.20	3R	Cytochrome P450 6d3
* CYP6Z2	14.02	10.80	10.58	12.34	7.23	14.41	3R	Cytochrome P450 6d3
* SAP2	9.35	6.64	10.44	11.65	8.21	11.08	3R	Ejaculatory bulb-specific protein 3
CYP4C26	8.32	8.73	8.08	14.10	10.21	8.00	X	Cytochrome P450 4V2
AGAP001789	7.78	11.49	10.86	7.36	8.82	10.75	2R	NADPH--cytochrome P450 reductase
* GSTD1-4	7.16	8.89	8.53	7.84	8.96	8.14	2R	Glutathione S-transferase 1-1
CYP4H17	7.11	6.77	6.73	8.19	4.70	8.95	3R	Cytochrome P450 4V2
CYP6M1	6.87	4.40	5.00	4.71	2.94	4.52	3R	Probable cytochrome P450 6a14
AGAP008096	6.54	7.25	7.80	6.45	7.32	6.98	3R	Cyclic GMP-AMP phosphodiesterase SMPDL3A
AGAP010064	5.04	5.30	5.30	4.31	5.30	5.41	3R	DNA-directed RNA polymerases I, II, and III subunit RPABC1
CYP307A1	5.00	6.76	4.62	6.20	6.87	3.69	X	Cytochrome P450 1A1
ABCF3	4.69	3.42	3.01	3.43	4.96	3.02	3L	ATP-binding cassette sub-family F member 3
UGT36C2	4.68	3.93	6.19	5.67	3.37	6.08	3R	UDP-glycosyltransferase UGT5
Ace	4.13	2.51	3.53	2.55	2.62	3.94	2R	Acetylcholinesterase
COEAE8O	4.13	4.14	4.09	5.13	5.47	3.55	2L	Acetylcholinesterase
AGAP006160	2.20						2L	Ubiquitin thioesterase OTU1
COE09916	2.06						3L	Carboxylesterase 3
AGAP004665		2.06					2R	Cytochrome P450 2U1
CYP6S1		2.06					3R	Probable cytochrome P450 6a20
AGAP009893			2.37				3R	2',5'-phosphodiesterase 12
AGAP006227			2.17				2L	Acetylcholinesterase
* GSTD6			2.14				2R	Glutathione S-transferase 1-1
AGAP000434				2.10			X	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase
AGAP009304				2.03			3R	Metallophosphoesterase 1
CPR131						3.846227385	3R	Cuticle protein
* CYP9J5						2.513382294	3L	Probable cytochrome P450 9f2
* CYP6N2						2.396427051	3R	Cytochrome P450 6A1
AGAP003148						2.110919589	2R	Glutathione S-transferase LANCL1
AGAP013509						2.069544641	2R	Acetylcholinesterase

- ❑ Increase fold-change compared to untreated comparison
- ❑ UGT308G1, CYP6Z3, CYP6Z2, SAP2 and GSTD1-4 was common to all the 6 comparisons
- ❑ GSTD6 for PBO base net comparison: P3.0.vs.Sus.
- ❑ CYP9J5 and CYP6N2 for dual AI nets comparison: Ig2.vs.Sus.

Tene-fossog *et al.*, 2013; Fotso-Toguem *et al.*, 2023; Tepa *et al.*, 2025; Oruni *et al.*, 2025

Results: Candidate Detox Genes in CIV Samples

Gene Name	Ctrl.vs.Sus	Mn.vs.Sus	P3.0.vs.Sus	Op.vs.Sus	Ve.vs.Sus	Ig2.vs.Sus	Chr.	Description
* CYP6Z3	13.29	25.11	39.21	27.57	35.71	32.27	3R	Cytochrome P450 6d3
* CYP6P4	6.79	8.82	8.85	8.36	6.00	7.87	2R	Probable cytochrome P450 6a14
AGAP010577	5.51	2.59	4.08	4.47	3.15	2.89	3L	Tyrosyl-DNA phosphodiesterase 1
* CYP6Z2	5.47	10.07	13.62	9.87	13.43	12.79	3R	Cytochrome P450 6d3
CYP307A1	4.54	3.09	3.72	4.25	2.89	2.57	X	Cytochrome P450 1A1
* CYP6P3	4.47	8.86	6.04	6.00	5.89	7.84	2R	Probable cytochrome P450 6a14
CYP4H17	4.44	4.03	4.61	3.16	2.43	2.25	3R	Cytochrome P450 4V2
AGAP002416	3.97	2.75	2.99	2.89	2.21	2.26	2R	Cytochrome P450 4V2
* CYP6M2	3.97	11.85	11.43	6.08	7.16	7.40	3R	Probable cytochrome P450 6a14
UGT302A2	3.80	5.87	4.21	3.32	3.34	2.74	2L	UDP-glucuronosyltransferase 2A3
CYP4H18	3.19	2.96	3.21	3.47	3.36	2.84	3R	Cytochrome P450 4V2
ABCF2	3.04	2.70	3.12	3.01	3.15	3.07	3L	ATP-binding cassette sub-family F member 3
* UGT308G1	2.93	8.39	9.50	4.62	6.95	6.87	3R	UDP-glucosyltransferase 2
UGT302A1	2.88	7.51	5.13	3.97	4.62	5.33	2L	UDP-glucuronosyltransferase 2A3
AGAP012811	2.72	2.11	3.05	2.53	2.85	2.56	UNKN	cGMP-specific 3',5'-cyclic phosphodiesterase
AGAP004119	2.59	2.36	2.59	2.41	2.56	2.54	2R	cGMP-specific 3',5'-cyclic phosphodiesterase
AGAP000434	2.58	2.98	2.96	2.84	3.30	2.76	X	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase
* CYP9K1	2.28	3.73	3.85	3.65	3.81	4.85	X	Cytochrome P450 9e2
* CPR30	2.09	2.66	2.35	2.84	3.56	3.47	2L	Endocuticle structural glycoprotein ABD-5
AGAP009435	2.07	3.00	2.71	2.35	2.58	2.81	3R	Pimeloyl-[acyl-carrier protein] methyl ester esterase
ABCC9		2.14					3R	Multidrug resistance-associated protein 1
* CYP6AA2		2.06					2R	Probable cytochrome P450 6a14
* CYP6AA1		2.04					2R	Cytochrome P450 6a2
CYP315A1			2.06				X	Cholesterol side-chain cleavage enzyme, mitochondrial
AGAP000467					2.06		X	Neuropathy target esterase sws
ABCC7					2.20		2L	ATP-binding cassette sub-family C member 12
* UGT308A2						2.16	2L	2-hydroxyacylsphingosine 1-beta-galactosyltransferase
* CYP6Z1						2.10	3R	Cytochrome P450 6d3
AGAP009079						2.10	3R	CCR4-Not complex 3'-5'-exoribonuclease subunit Ccr4
AGAP008096						2.07	3R	Cyclic GMP-AMP phosphodiesterase SMPDL3A
AGAP002220						2.03	2R	U6 snRNA phosphodiesterase 1
AGAP009695						2.02	3R	Palmitoyl-protein thioesterase 1
AGAP008336						2.02	3R	Protein phosphatase methylesterase 1

- ❑ Similar pattern:
- ❑ These include *CYP6Z3*, *CYP6P4*, *CYP6Z2*, *CYP6P3*, *CYP6M2*, *CYP9K1*, *CPR30*, *UGT308G1* common to all the 6 comparison;
- ❑ *CYP6AA2* and *CYP6AA1* for Pyr net comparison: Mn.vs.Sus
- ❑ *CYP6Z1* and *UGT308A2* for dual AI nets comparison: Ig2.vs.Sus.

Edi constant *et al.*, 2014;
Vontas *et al.*, 2020

Results: Candidate Detox Genes in TZA Samples

	GeneID	Gene_Name	Ctrl.vs.Sus	Mn.vs.Sus	P3.0.vs.Sus	Op.vs.Sus	Chro.	Description
*	AARA21_001434	SAP2	36.97	39.79	41.94	26.54	3R	Ejaculatory bulb-specific protein 3
	AARA21_006767	COE21998	14.46	16.37	13.33	12.78	3L	Carboxylesterase 4A
*	AARA21_004473	ACE2	13.99	47.19	12.02	13.89	X	Acetylcholinesterase
	AARA21_006936	CPR131	13.62	6.53	11.72	9.28	3R	Cuticle protein
	AARA21_012041	GSTT1a	11.58	46.43	9.13	8.61	X	Glutathione S-transferase theta-1
	AARA21_012426	AGAP003560	10.49	7.39	10.27	10.66	2R	UDP-glucose:glycoprotein glucosyltransferase
	AARA21_004010	GSTe8	8.08	9.92	7.01	8.45	3R	Glutathione S-transferase 1-1
	AARA21_004780	GSTT1b	6.76	6.35	6.38	5.10	X	Glutathione S-transferase theta-1
	AARA21_003303	GSTT1c	6.13	6.26	6.42	5.17	X	Glutathione S-transferase theta-1
	AARA21_002218	AGAP006383	5.79	4.98	6.42	5.45	2L	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase
	AARA21_012290	AGAP008096	5.51	4.70	4.16	4.75	3R	Cyclic GMP-AMP phosphodiesterase SMPDL3A
*	AARA21_013126	GSTD1-4	5.35	4.73	5.41	4.72	2R	Glutathione S-transferase 1-1
	AARA21_003890	CPLCX1	5.30	4.53	5.38	4.25	2R	unspecified product
	AARA21_014464	GSTMS2	5.09	6.45	4.30	4.13	X	Microsomal glutathione S-transferase 1
	AARA21_011577	GSTT1d	5.08	6.06	5.02	3.46	X	Glutathione S-transferase theta-1
	AARA21_002783	ABCG2	4.83	4.77	5.62	6.50	X	Protein white
	AARA21_007769	CYP314A1	4.81	4.79	5.15	5.89	2R	1,25-dihydroxyvitamin D(3) 24-hydroxylase, mitochondrial
	AARA21_004905	AGAP006227	4.75	4.03	4.33	2.46	2L	Acetylcholinesterase
*	AARA21_004620	CYP9K1	4.52	5.21	4.37	3.17	X	Cytochrome P450 9e2
	AARA21_009457	AGAP001523		4.51			2R	ATP-binding cassette sub-family A member 2
	AARA21_004074	CPR128		3.96			X	Pro-resilin
	AARA21_011288	CPR147		3.26			UNKN	Pro-resilin
	AARA21_012502	CYP4H19a		3.09			X	Cytochrome P450 4V2
	AARA21_008301	COEBE4C		3.01			2L	Esterase-5B
	AARA21_013477	CYP4H19		2.89			X	Cytochrome P450 4V2
	AARA21_000825	COEunkn		2.81			3L	Acetylcholinesterase
	AARA21_014966	ABCG2		2.41			X	Protein white
	AARA21_013998	CYP4H19		2.40			X	Cytochrome P450 4V2
	AARA21_003258	UGT50B2		2.38			2R	2-hydroxyacylsphingosine 1-beta-galactosyltransferase
	AARA21_004292	COEAE6G		2.24			2L	Cholinesterase
	AARA21_009302	CPR9			2.56		2R	Larval cuticle protein 1
	AARA21_015164	CYP4H19b			2.45		X	Cytochrome P450 4V2
	AARA21_007372	ACE2			2.42		X	Acetylcholinesterase
	AARA21_013129	CPLCG1			2.42		3R	unspecified product
	AARA21_003031	AGAP000434			2.03		X	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase
	AARA21_001842	COEJHE2E				2.55	2L	Esterase-5A

- ❑ Increased fold-change marks for **Mn.vs.Sus.** comparison
- ❑ **SAP2, Ace2, GSTT1, GSTe8** and **CYP9K1** common to all the 6 comparison
- ❑ **CYP4H19** common to all the PBO-based comparison;
- ❑ Many esterase gene for **other** comparison

CPR involvement:
 Messenger *et al.*, 2021,
 Omoke *et al.*, 2024

Take Home Messages

- ❑ **Overexpression of detoxification genes** such as cytochrome P450s, glycosyltransferases, and ABC-type xenobiotic transporters, **which are previously linked to insecticide resistance**
- ❑ **Increased fold change when comparing treated nets to untreated comparison:** *UGT308G1*, *CYP6Z3* for both Cameroon and Cote d'Ivoire, *CYP6Z2*, *CYP6P3* and *CYP6M2* for Cote d'Ivoire, *SAP2* and *GSTD1-4* for Cameroon and Tanzania
- ❑ This study provides insights into the genetic mechanisms underlying vector survivorship to LLINs, with potential implications for developing targeted vector control strategies

Genomic Signatures of Vector Responses to LLINs During Experimental Hut Trials

Prof. Charles Wondji

CRID, Cameroon & LSTM, UK

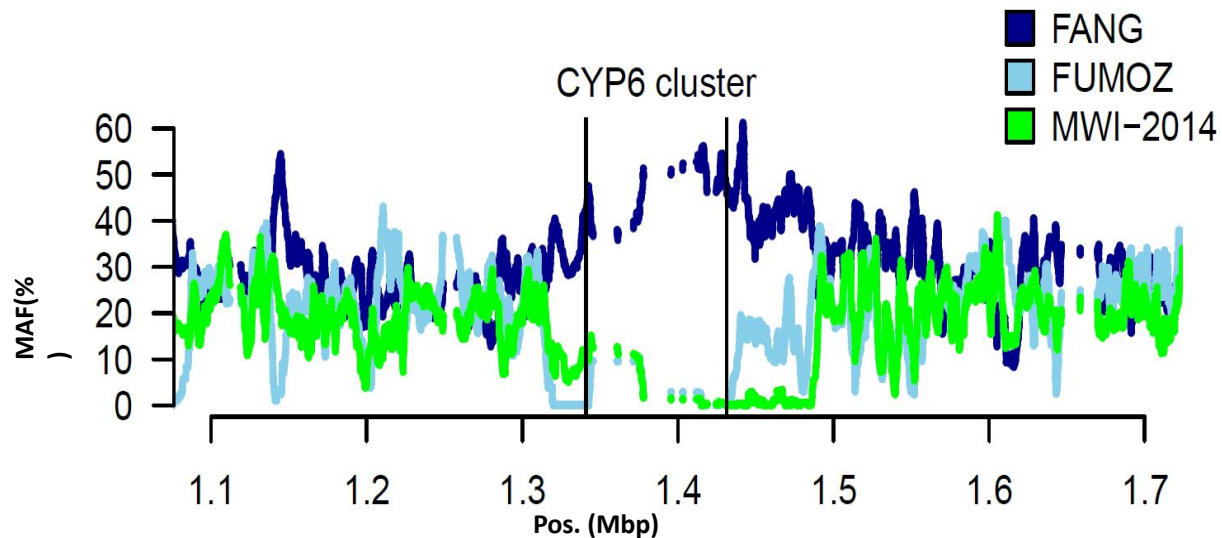
on behalf of RAFT Workstream 1 – Current Threats



globalvectorhub.lshtm.ac.uk/raft

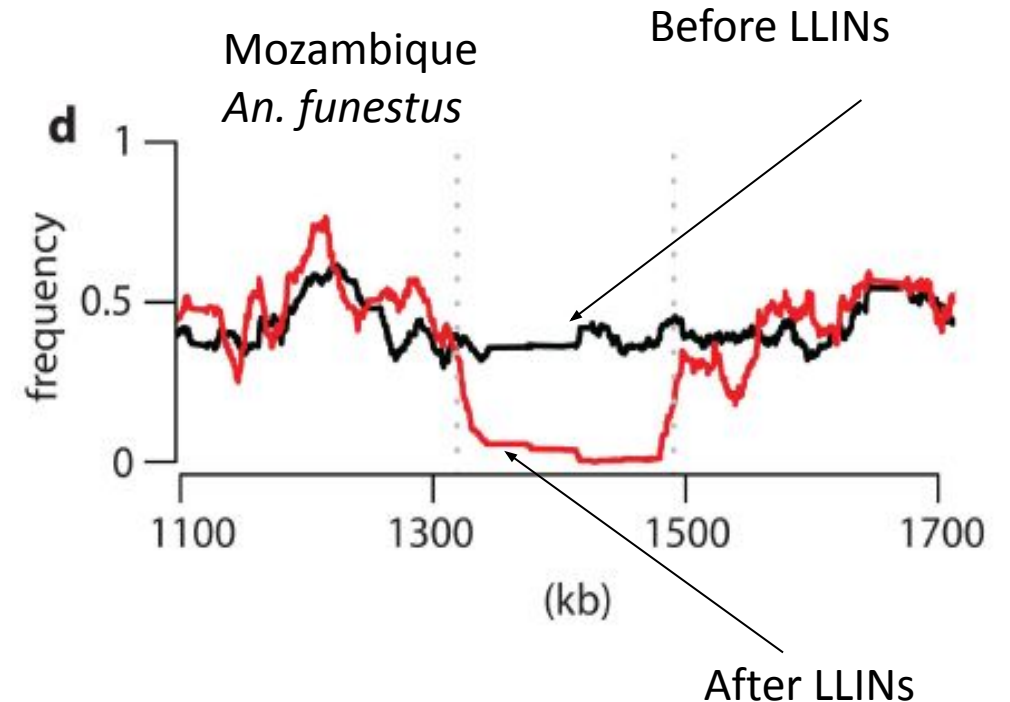
Can a whole genome analysis reveal footprints of mosquito's adaptation to bed nets?

Pooled-template Whole genome sequencing (WGS-PoolSeq)
sequencing



Strong reduced genetic diversity in resistant around rp1 QTL

Strong evidence that a WGS-PoolSeq could help detect genetic changes associated with ITNs deployment



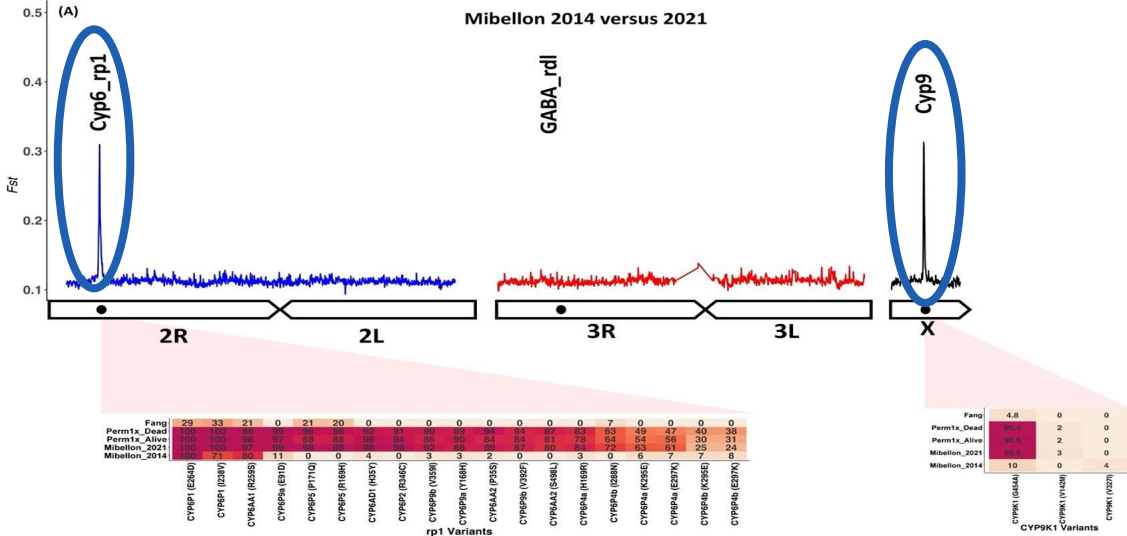
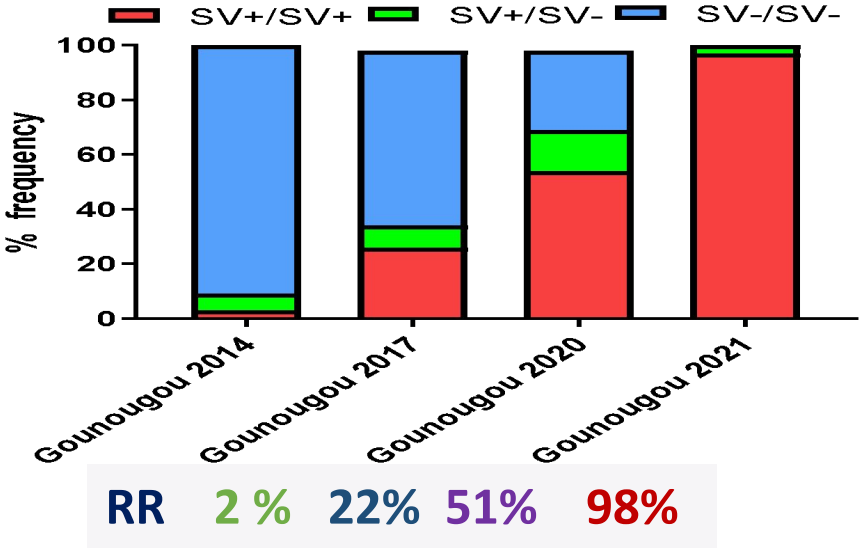
Genomic signatures of mosquitoes' adaptation to bed nets scale up detected in *An. funestus* in Central Africa using WGS-PoolSeq

Molecular Biology and Evolution, 2025, 42, 1–20
<https://doi.org/10.1093/molbev/msaf251>
Advance access publication 24 October 2025
Discoveries

MBE OXFORD

Genomic Drivers of Pyrethroid Resistance Escalation in the Malaria Vector *Anopheles funestus* Across Africa

Mahamat Gadji ^{1,2,†*} Hervé Raoul Tazokong ^{1,2,†} Mersimine F.M. Kouamo ¹
Magellan Tchouakui ¹ Murielle Wondji ^{1,3} Leon M.J. Mugenzi ⁴ Helen Irving ³
Jack Hearn ⁵ Sulaiman S. Ibrahim ^{1,6} Charles S. Wondji ^{1,3,*}

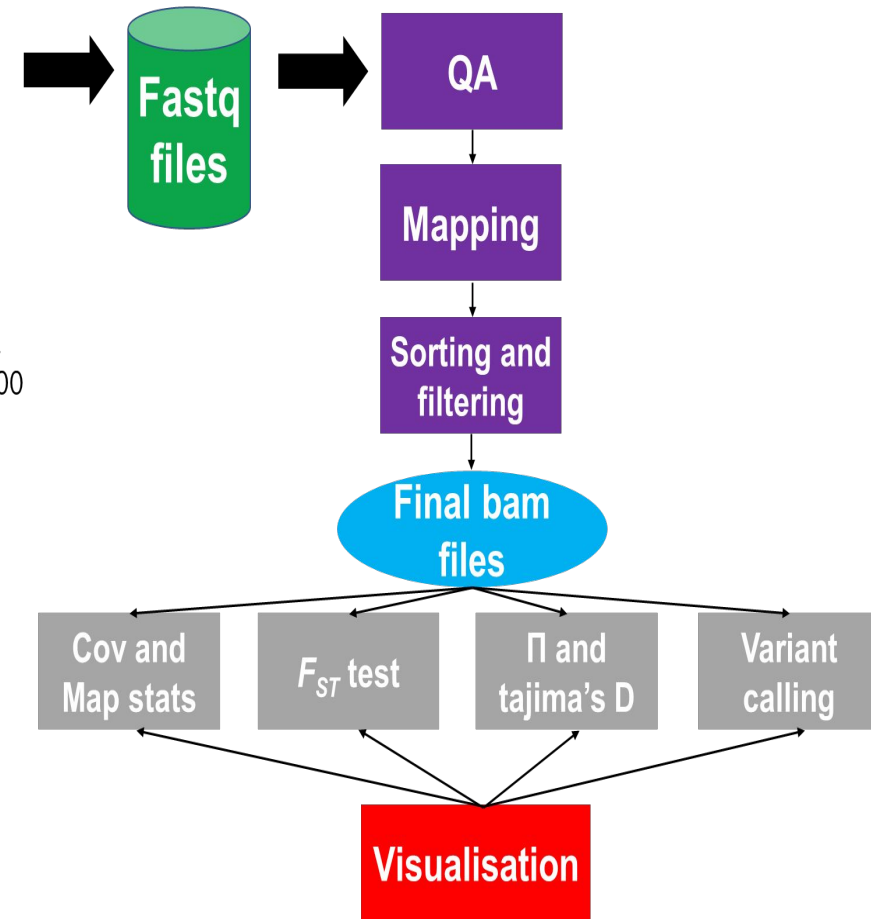


Use of WGS-PoolSeq to detect genetic variants linked with mosquitoes survival to different bed nets exposure



Nets used

- ❖ Untreated
- ❖ MAGNet
- ❖ PermaNet 3.0
- ❖ Olyset Plus
- ❖ VeeraLIN
- ❖ Interceptor G2



Experiments Hut Trials

free flying mosquito from 3 countries

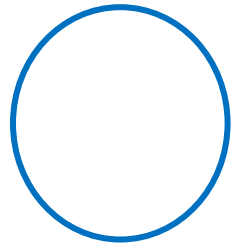
Sorting

- ❖ Alive from treated hut
- ❖ Alive from untreated
- ❖ Dead from treated huts
- ❖ **Susceptible ref. strain**
 - CIV: *An. coluzzii* Ngousso
 - CMR: *An. gambiae* Kisumu
 - TZA: *An. arabiensis* Dongola

DNA extraction

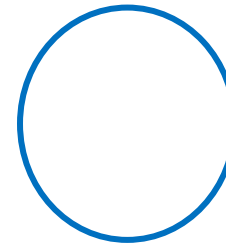
using DNAeasy (QIAGEN) and quality control (Bioanalyser)

Principal Component Analyses of the 3 experiments

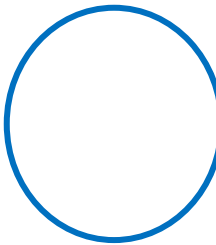


Cameroon

Ivory Coast



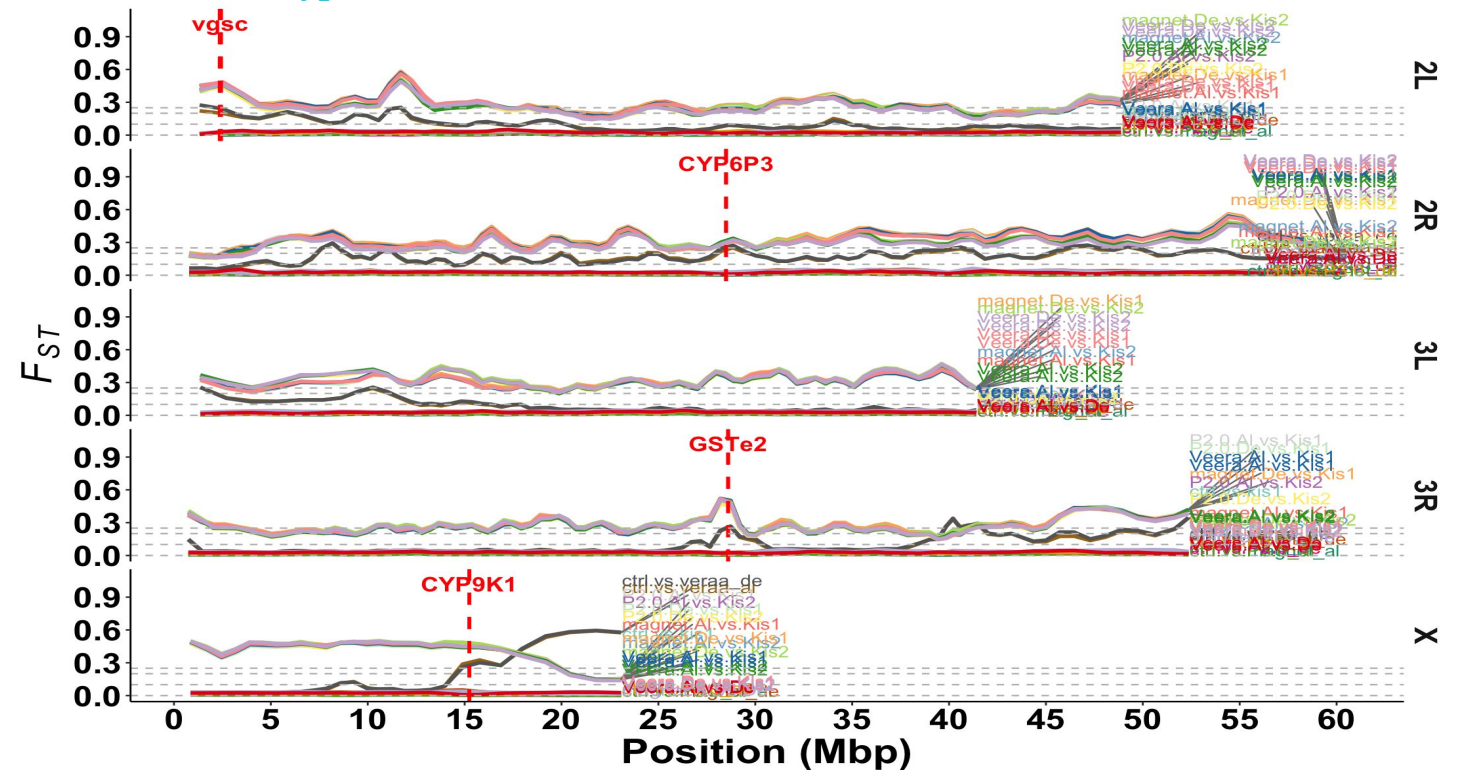
Tanzania



Mosquitoes clustered more by origin than by ability to survive to the ITN exposure

Genome-wide F_{ST} across *An. gambiae* from Cameroon

- Mosquitoes that survived bed net exposure have a similar genetic makeup to those that died post-exposure and to control
- There is strong genomic differentiation vs the susceptible Kisumu strain, with F_{ST} values above 0.3
- The lack of differentiation likely due to extreme field resistance, with key resistance alleles already fixed



Tazokong et al. *Malaria Journal* (2025) 24:308
<https://doi.org/10.1186/s12936-025-05542-z>

Malaria Journal

RESEARCH

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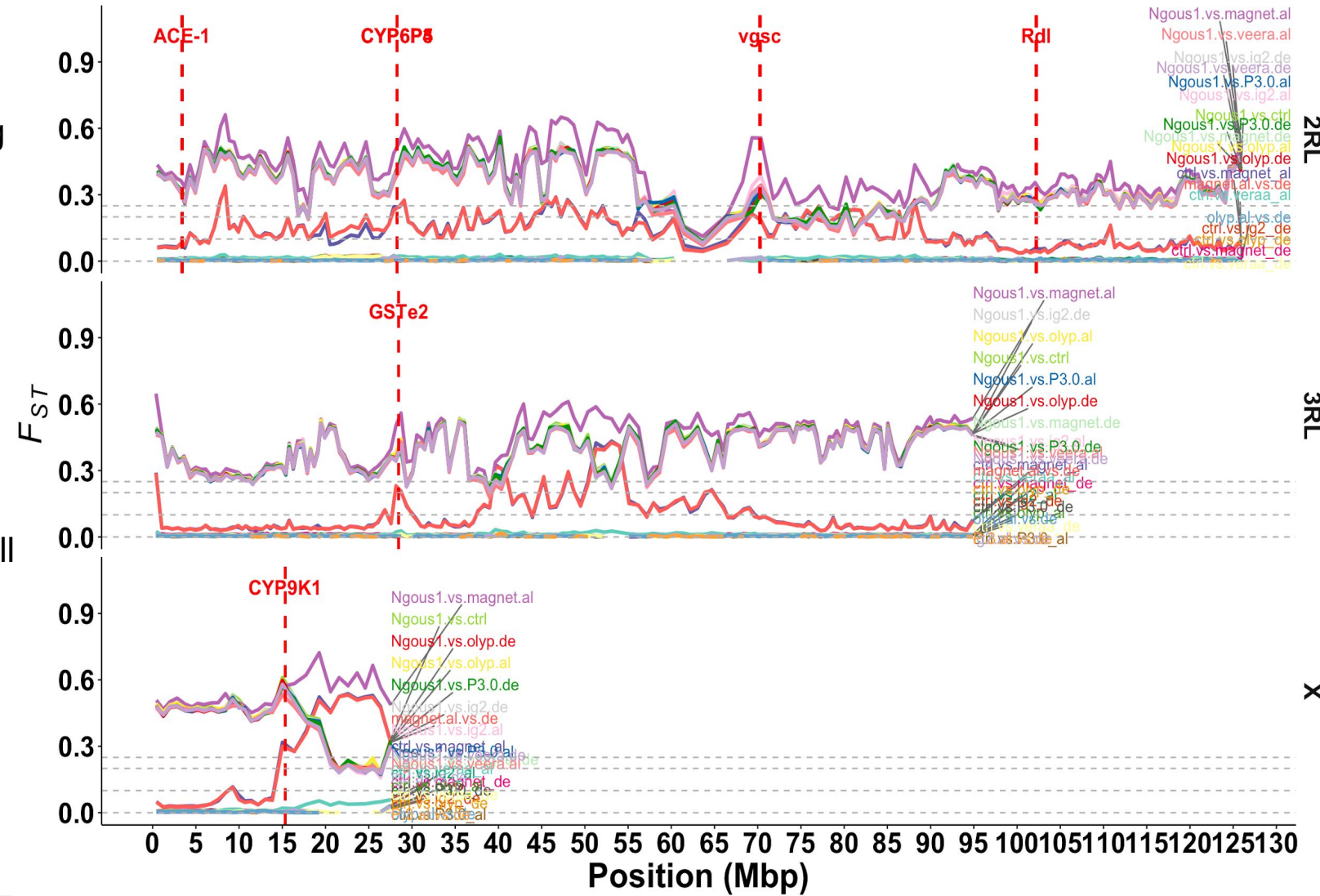


Characterizing the escalation of pyrethroid resistance and its impact on bed nets efficacy alongside molecular basis in *Anopheles funestus* from Cameroon

Hervé Raoul Tazokong^{1,2*}, Stevia Ntadoun Tchamga^{1,3}, Magellan Tchouakui¹, Tatiane Assatse^{1,4}, Steve Valdi Djova¹, Leon M. J. Mugenzi¹, Gadji Mahamat^{1,2}, Onana Boyomo² and Charles Sinclair Wondji^{1,5*}

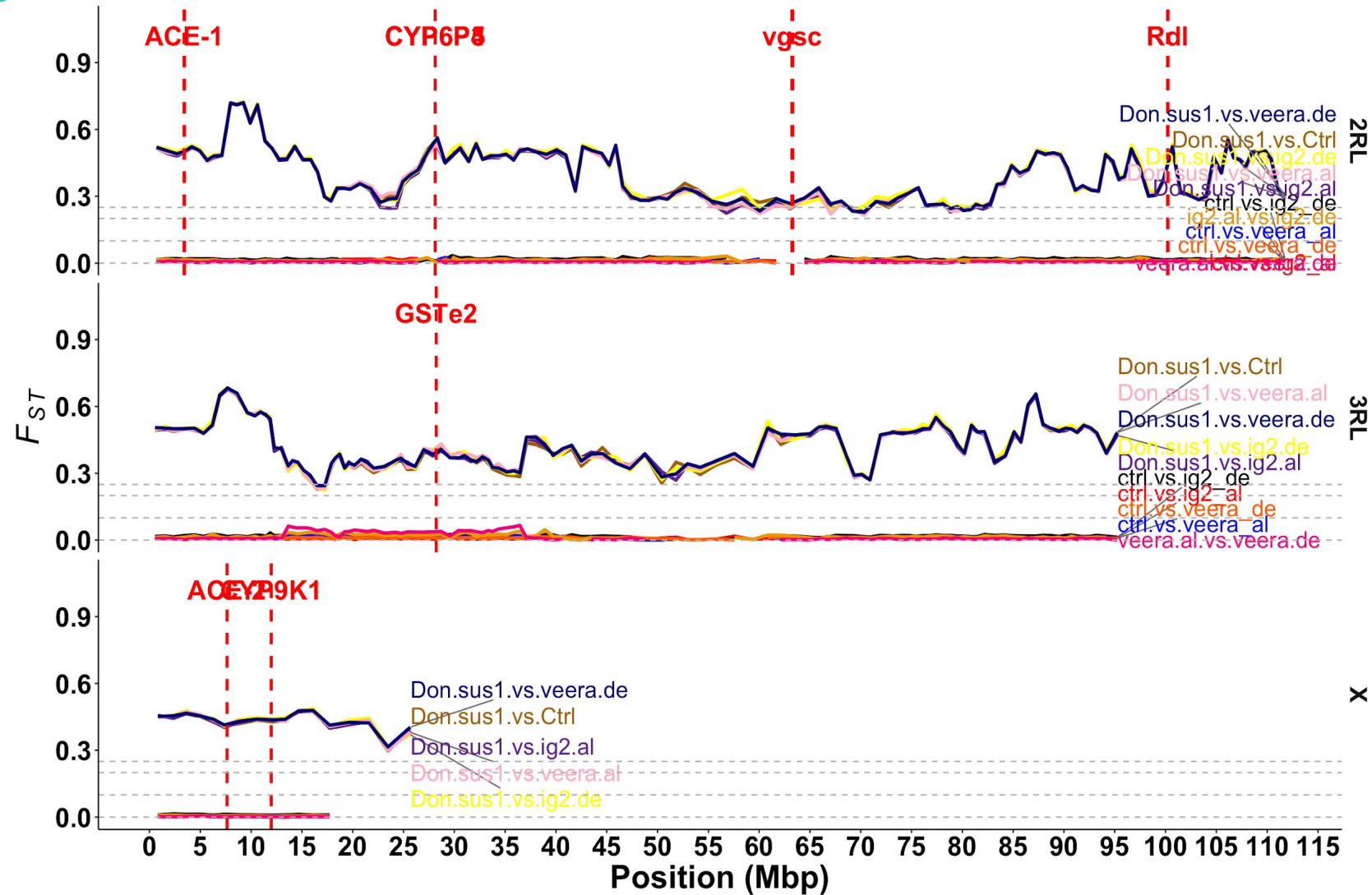
Genome-wide F_{ST} across *An. coluzzii* from Côte d'Ivoire

- No significant difference between mosquitoes that survived bed net exposure and those that died except for Veeralin- and Magnet-surviving mosquitoes
- Moderate genomic differentiation across the genome between Magnet-surviving and Veeralin-surviving mosquitoes versus other populations, with F_{ST} values ranging from 0.1 to 0.3
- Strong genomic differentiation is observed between the Ngousso susceptible strain and all other populations, with F_{ST} values above 0.3
- These findings are fully consistent with the PCA plot

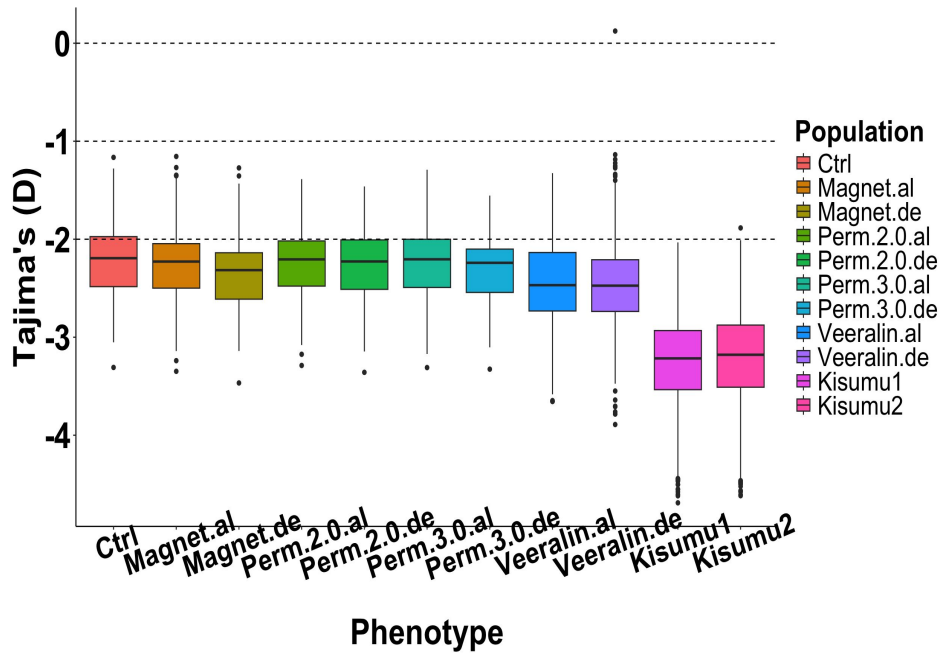


Genome-wide F_{ST} across *An. arabiensis* from Tanzania

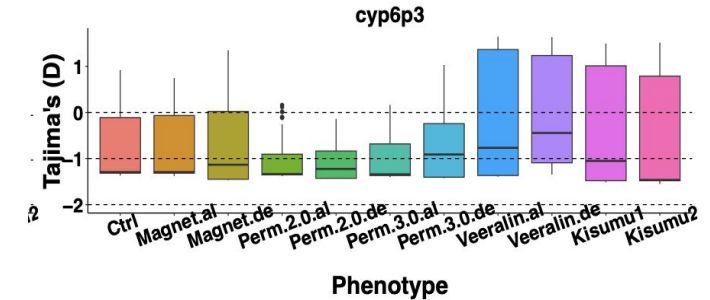
- All bed net–exposed mosquitoes share a similar genetic makeup, including the control group
- There is strong genomic differentiation across the genome between the Dongola susceptible strain and all exposed mosquitoes, with F_{ST} values ranging from 0.3 to 0.8
- These findings are fully consistent with the PCA plot



Genome-wide Tajima's D in *An. gambiae* from Cameroon



- Similar low Tajima's D values suggesting recent directional selection, potentially driven by insecticide resistance, but that this selection is consistent across phenotypes

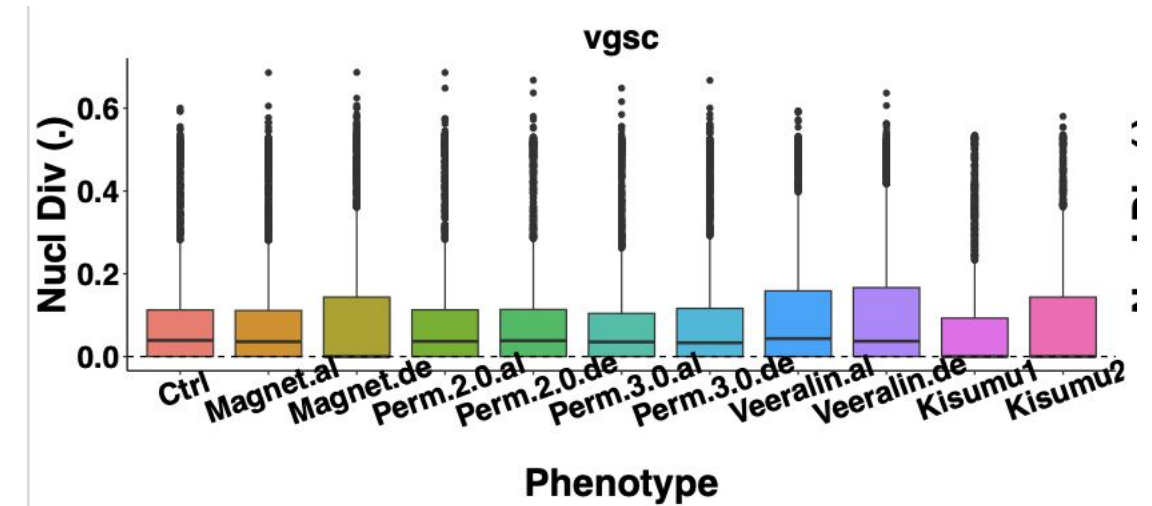


Tajima's D was computed per IR gene to detect evidence of selection acting on the gene

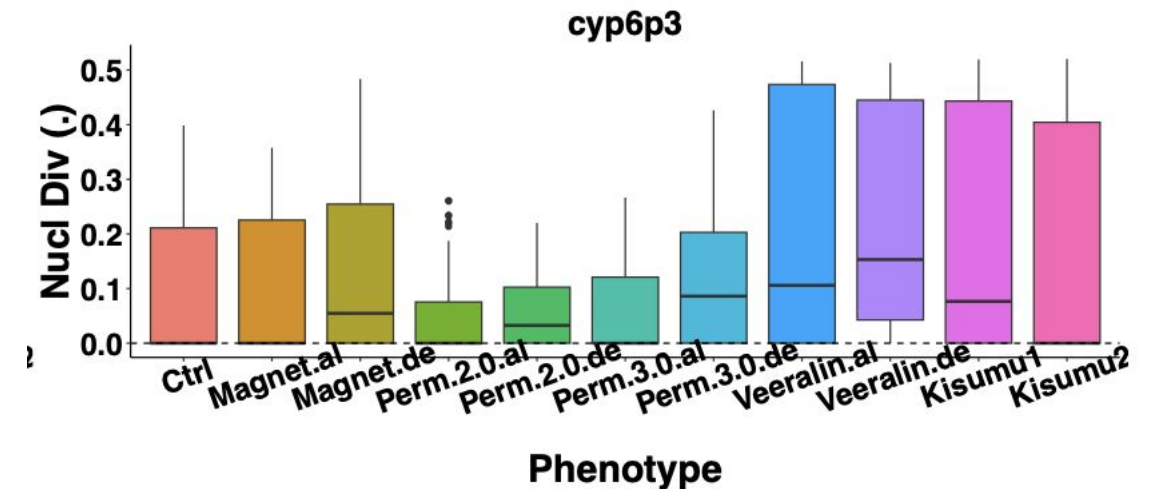
- The median negative Tajima's D values observed particularly for *vgsc*, *CYP9K1*, and *CYP6P3*, for some phenotypes suggest recent positive selection, reflecting selective sweeps associated with insecticide resistance evolution

Genomewide nucleotide diversity in *An. gambiae* from Cameroon

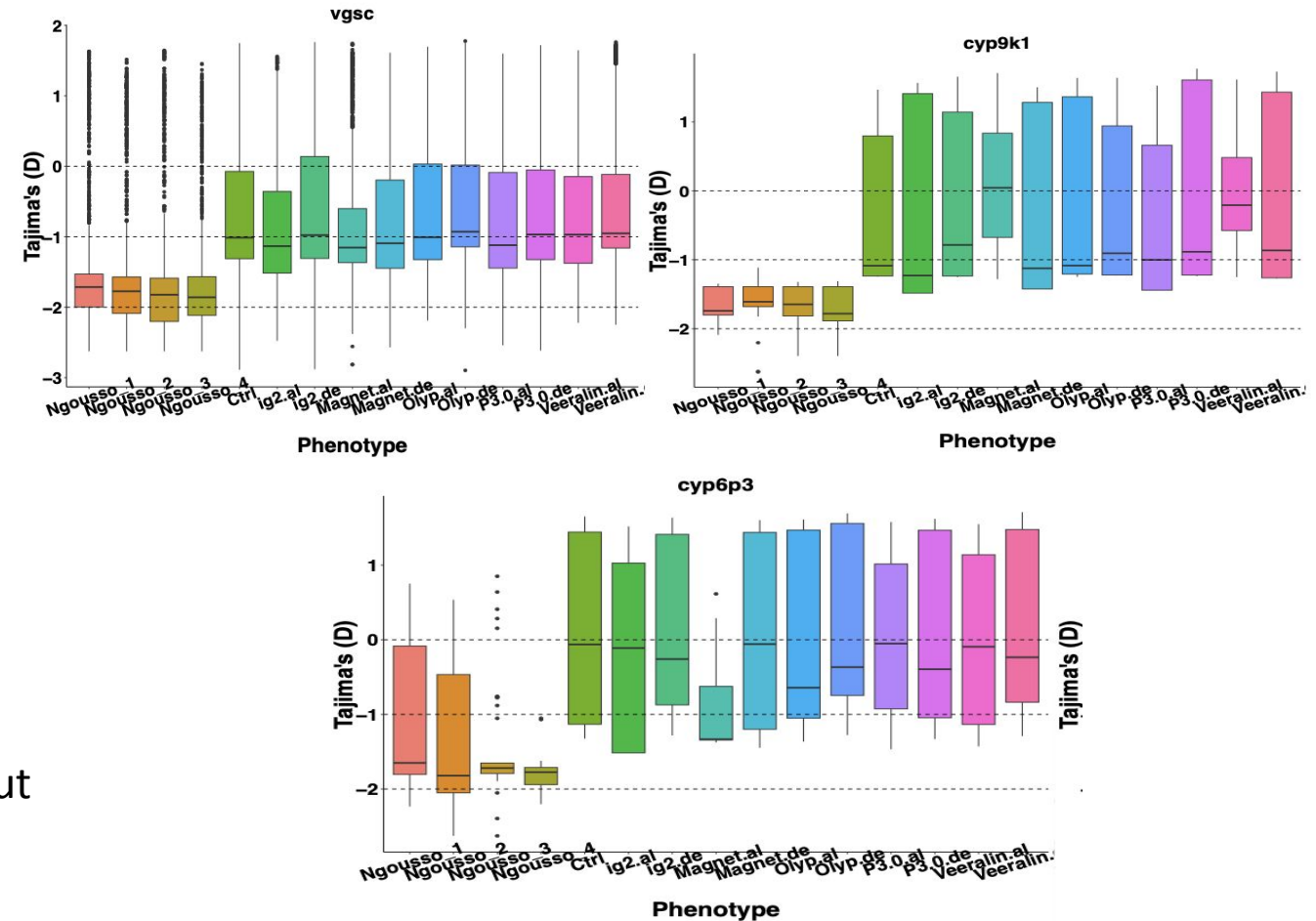
- Nucleotide diversity was similar across all exposed mosquitoes, but it was much lower in the Kisumu susceptible strain.



- VGSC: Low diversity in most insecticide-exposed mosquitoes, suggesting strong selection pressure from pyrethroids targeting VGSC
- Lower genetic diversity at key P450 resistance genes e.g CYP6P3



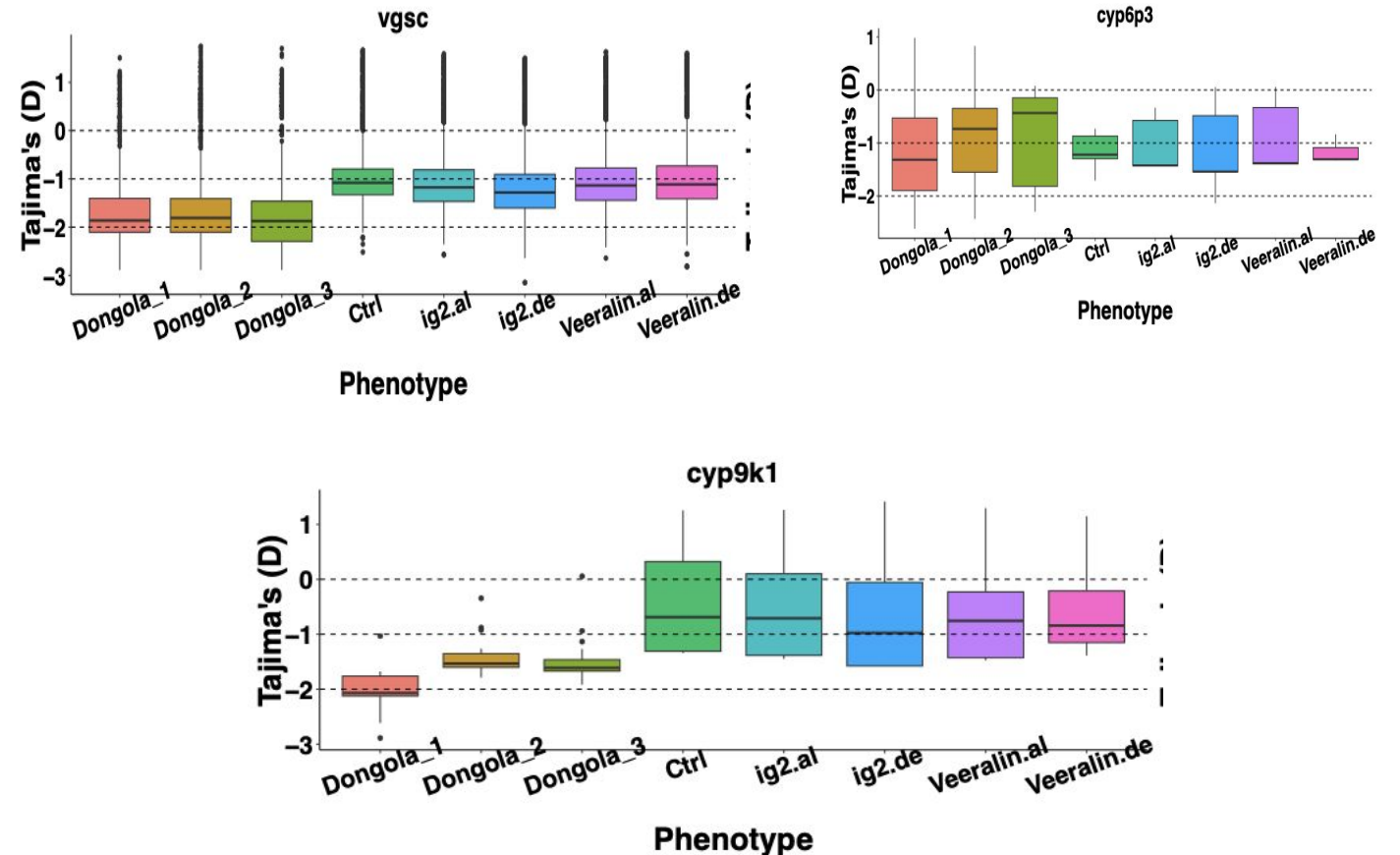
Genome-wide Tajima's D in *An. coluzzii* from Côte d'Ivoire



A similar pattern was observed as in *Anopheles gambiae*, but with slightly higher Tajima's D values.

- VGSC : Negative Tajima's D values observed across all treatments, suggesting selection pressure
- CYP9K1: Lower Tajima's D values were observed in IG2, Magnet, Olyset, and Perm 3.0 survivors compared to dead mosquitoes
- CYP6P3/P4/P5: Magnet survivors showed lower values compared to dead mosquitoes

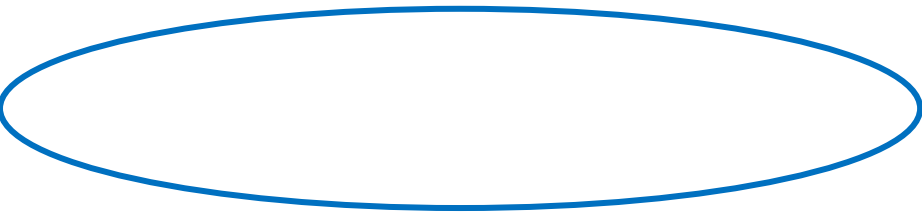
Genome-wide Tajima's D in *An. arabiensis* from Tanzania



Similar negative Tajima's D values were observed across all treatments, with lower values recorded in IG2 dead and Veeralin-surviving mosquitoes

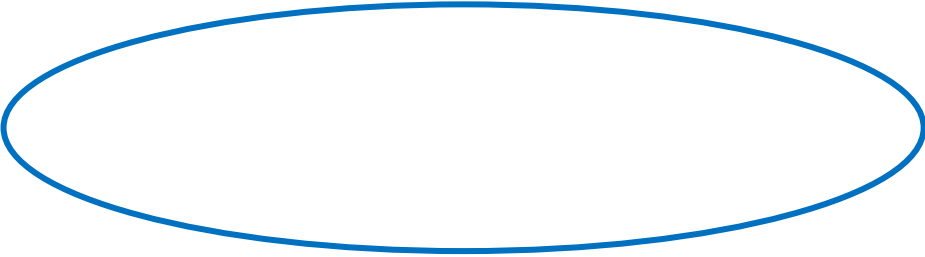
- VGSC: Evidence of selection across all phenotypes
- CYP6P3: under strong selection in all phenotypes, with IG2 dead again showing the lowest Tajima's D value
- CYP9K1: Populations appear near equilibrium, with the IG2 dead group showing the lowest Tajima's D value

Key insecticide resistance SNPs in *An. gambiae* from Cameroon



- The Vgsc gene, carrying the L995F mutation, was fixed in all phenotypes except mosquitoes exposed to Veeralin
- Three key GSTe2 mutations (N3K, T145S, and L119V) also occurred at high frequencies under most conditions, except in Veeralin-exposed mosquitoes, where frequencies were markedly reduced
- Similarly, the CYP6P3 E205D mutation-previously associated with pyrethroid resistance-was present at high frequencies in all phenotypes except Veeralin-exposed mosquitoes
- As expected, the susceptible Kisumu strain showed very low frequencies for all resistance-associated SNPs

Insecticide resistance SNPs in *An. coluzzii* from Côte d'Ivoire

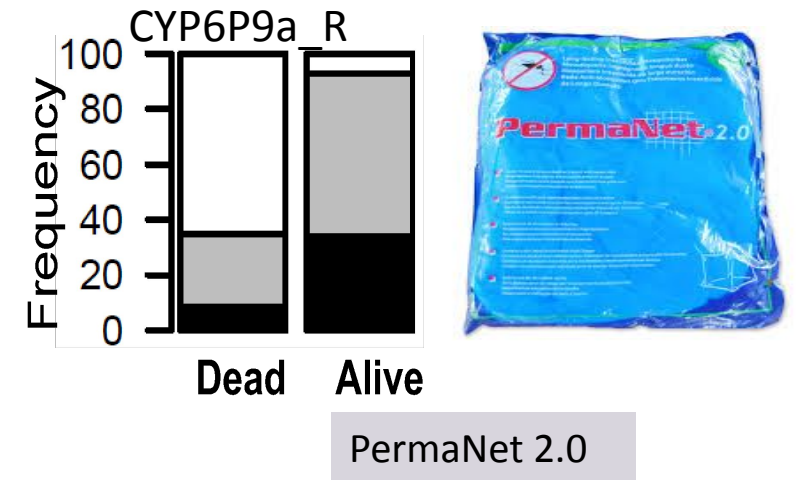
- 
- Five key SNPs were near fixation in all treatments except the Ngousso susceptible strain—specifically Rdl (S296A, S345T), CYP9K1 (I409M), and CYP6P5 (V409M, I4V)—indicating strong selection pressure and high resistance likely driven by multiple insecticide resistance mechanisms
 - Difference between Magnet-alive and Magnet-dead, with several resistance-associated SNPs at higher frequencies in alive mosquitoes compared to dead
 - These included GSTe2 (T114I, L119V), CYP6P4 (I168V), Vgsc (L1012F), CYP6P5 (N27K), and CYP6P3 (E205D)—further supporting the involvement of multiple loci in conferring pyrethroid resistance
 - Such markers could be used to design simple diagnostic tools to track resistance and its impact in *An. coluzzii*

Key insecticide resistance SNPs in *An. arabiensis* from Tanzania

- Major resistance genes are present at moderate to high frequencies, with some nearly fixed in *An. arabiensis* mosquitoes that survive exposure to various bed nets
- Four key SNPs-CYP6P5 (T263A), CYP6P4 (N55S), CYP6P3 (K114Q), and GSTe2 (V9I)-are at high frequencies or near fixation in all treatments
- This suggests that high resistance is driven by metabolic resistance in *An. arabiensis* through multiple genes
- Such markers could be used to design simple diagnostic tools to track resistance and its impact in *An. arabiensis*

Take Home Messages

- For all species, there is little genomic differentiation across pairwise comparisons within each treatment likely due to resistance being nearly fixed in the field
- But strong genomic differences are observed when comparing field populations to susceptible laboratory strains allowing to detect key genetic drivers of resistance
- Resistance is multigenic and key resistance loci appear to impact most pyrethroid-based nets
- Individual whole genome sequencing should be explored to provide a more refined segregation between mosquitoes exposed to various nets
- Existing DNA-based markers could also help assess impact of resistance on ITNs efficacy as done for CYP6P9a in *An. funestus*



SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

MALARIA VECTOR CONTROL

A cytochrome P450 allele confers pyrethroid resistance on a major African malaria vector, reducing insecticide-treated bednet efficacy

Gareth D. Weedall^{1,2}, Leon M. J. Mugenzi^{3,4}, Benjamin D. Menze^{1,3,4}, Magellan Tchouakui^{3,4}, Sulaiman S. Ibrahim^{1,5}, Nathalie Amvongo-Adjia^{4,6}, Helen Irving¹, Murielle J. Wondji^{1,3,4}, Micareme Tchoupo^{3,4}, Rousseau Djouaka⁷, Jacob M. Riveron^{1,3,4}, Charles S. Wondji^{1,3,4*}

Mathematical models integrating genetic data to inform LLIN procurement by NMCPs and other key stakeholders

Ciara Hamilton

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on behalf of RAFT Workstream 1 – Current Threats



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Selecting Genetic Markers for Modelling

- A number of markers tested at baseline were fixed

	Cote d'Ivoire			Cameroon			Tanzania		
Gene	Proportion RR (number)	Proportion RS (number)	Proportion SS (number)	Proportion RR (number)	Proportion RS (number)	Proportion SS (number)	Proportion RR (number)	Proportion RS (number)	Proportion SS (number)
KDR West	0.38 (26)	0.43 (29)	0.19 (13)	1 (38)	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)
KDR East	0 (0)	0 (0)	1 (42)	0 (0)	0 (0)	1 (38)	0 (0)	0 (0)	1 (4)
6P3	0.06 (4)	0.32 (22)	0.62 (42)	0.53 (20)	0.29 (11)	0.16 (6)	NA	NA	NA
402L	0.22 (15)	0.40 (27)	0.38 (26)	0 (0)	0 (0)	1 (37)	NA	NA	NA
ACE_1	0 (0)	0.37 (25)	0.63 (43)	NA	NA	NA	NA	NA	NA
GSTe_2	0.69 (47)	0.22 (15)	0.07 (5)	NA	NA	NA	NA	NA	NA
I1527T	0 (0)	0.75 (51)	0.25 (17)	0 (0)	0 (0)	1 (37)	NA	NA	NA

Suspected linkage

Literature suggests that there is tight linkage between 402L and I1527T (Williams, et al. 2021)

Combination	No. times alleles have same genotype	Proportion of observations from this combination that have the same genotype
402L & I1527T	246	0.76
6P3 & I1527T	170	0.53
6p3 & 402L	144	0.45
6P3 & KDR W	98	0.30
402L & KDR W	88	0.27

Benefits of adding genetics to the model

- We began the analysis with a simple glm that did not include genetic information

$$mortality \sim \beta_0 + PBO * \beta_1 + geneticsdone * \beta_2$$

- We then added the available genetic information from Cote d'Ivoire
- **Adding 6P3, KDR West, and 402L to the model was shown to improve the ability of the model to predict mosquito mortality (p-value < 0.0001)**

Refining the model

Interaction between 6P3 and PBO nets

- As LNA 6P3 was suggested to impact metabolic resistance, an interaction between this gene and PBO nets was tested.
- There was no evidence of an interaction between LNA 6P3 and PBO nets (p-value = 0.38).

Dominant or recessive traits

- Need to reduce the number of genotypic combinations so tested whether evidence of resistance genes being recessive, partially dominant or dominant.
- Unfortunately, insufficient samples to allow independent assessment of each loci, so comparisons made assuming dominance is the same for all genes.
- Marginal significance for the benefit of the semi-dominant model (p value = 0.0596) so results presented in this instance for fully recessive model.
- There was no evidence that the model with the heterozygotes was significantly better at predicting mosquito mortality than the R recessive model.

PRELIMINARY DATA: Mortality Estimates

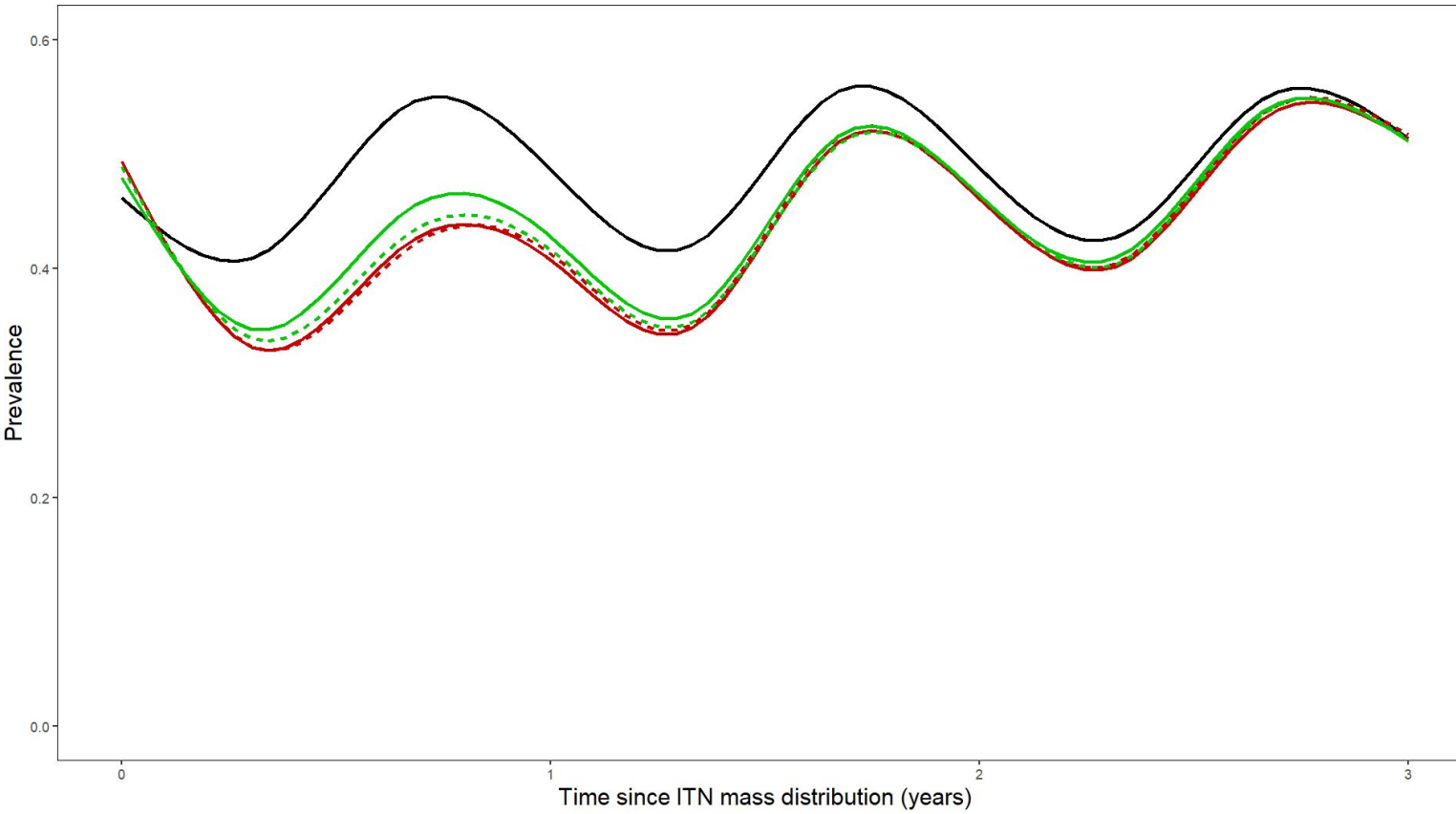
Based on the GLM with no IG2 nets and assuming **R is recessive**

Genotype			All Nets	Pyrethroid-only nets		Pyrethroid-PBO nets	
KDR West	402L	6P3	total sampled	Total sampled	Mortality estimates	Total sampled	Mortality estimates
SS/RS	SS/RS	SS/RS	135	36	0.544	99	0.641
RR	SS/RS	SS/RS	128	20	0.430	108	0.529
SS/RS	SS/RS	RR	1	0	NA	1	0.722
SS/RS	RR	SS/RS	105	35	0.414	70	0.513
SS/RS	RR	RR	1	0	NA	1	0.605
RR	RR	SS/RS	8	4	0.308	4	0.399
RR	SS/RS	RR	7	2	0.523	5	0.620

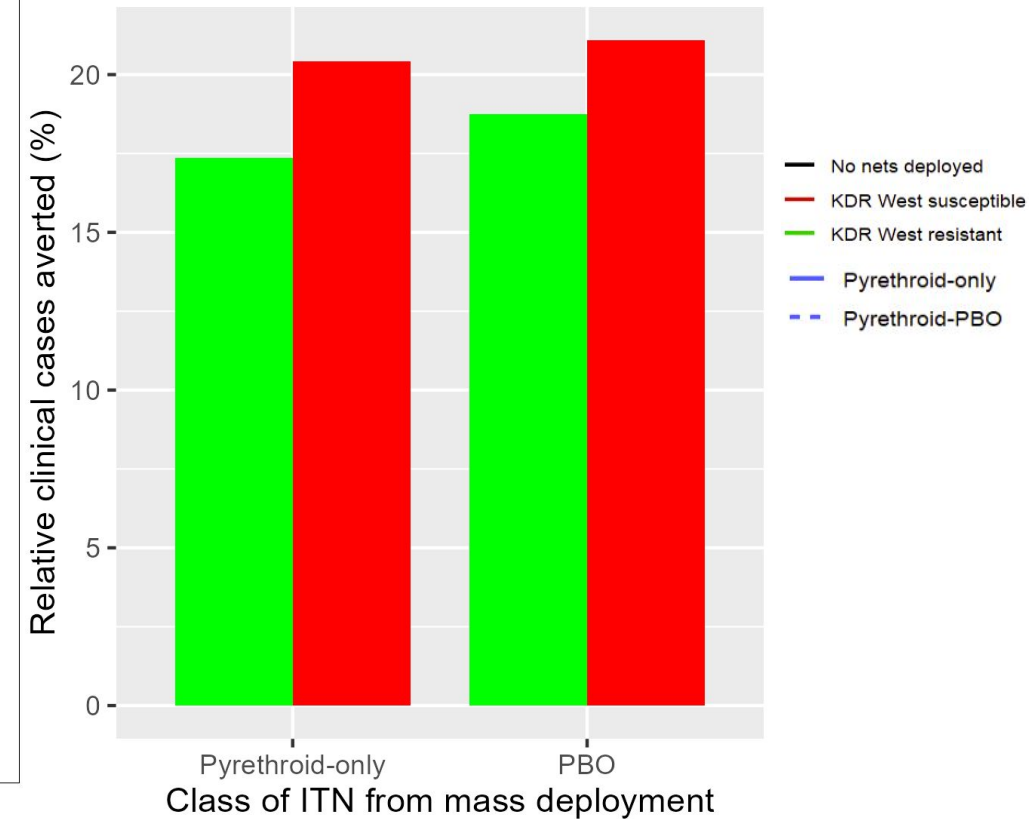
Epidemiological Impact

Model parameterise with experimental hut trial data assuming 0 or 100% of mosquitoes have KDR W resistance allele

Predicting epidemiological impact of different ITNs assuming the mosquito population is either susceptible or resistant at the KDR W loci



Relative clinical cases averted over 3 years compared to no intervention scenario



This is an illustrative example from Bouake, in Cote d'Ivoire, impact will vary between settings

Conclusions

- First evidence that including genetic information can improve the predictions of mosquito mortality in an experimental hut trial.
 - Models predict substantially lower mortality in mosquitoes with resistant genes.
- This difference is likely to have large epidemiological impact.
- Diversity of genetic mechanisms means disentangling contribution of different genes requires many more mosquitoes to be analysed.
- Need to select small number of highly informative markers, though currently unclear how this can be used to inform decision-making in areas with high pyrethroid resistance.
- The techniques will have much greater impact if extended to resistance markers for newer insecticides such as chlorfenapyr.

What Does It All Mean?

Prof. Jo Lines

LSHTM, UK

on behalf of RAFT Workstream 1 – Current Threats



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Question: How to Generalize from Experimental Huts to Other Places

Problem:



Experimental huts compare products against **local** vectors



Many other districts each with different mix of species and genes

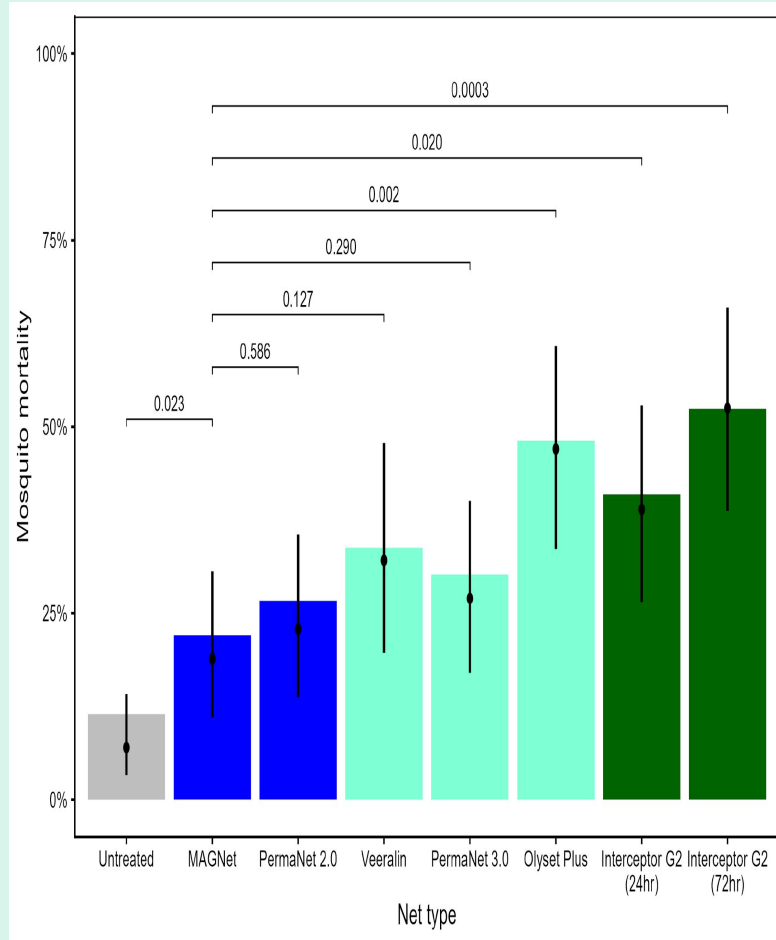


Overlapping species and R genes but in different proportions

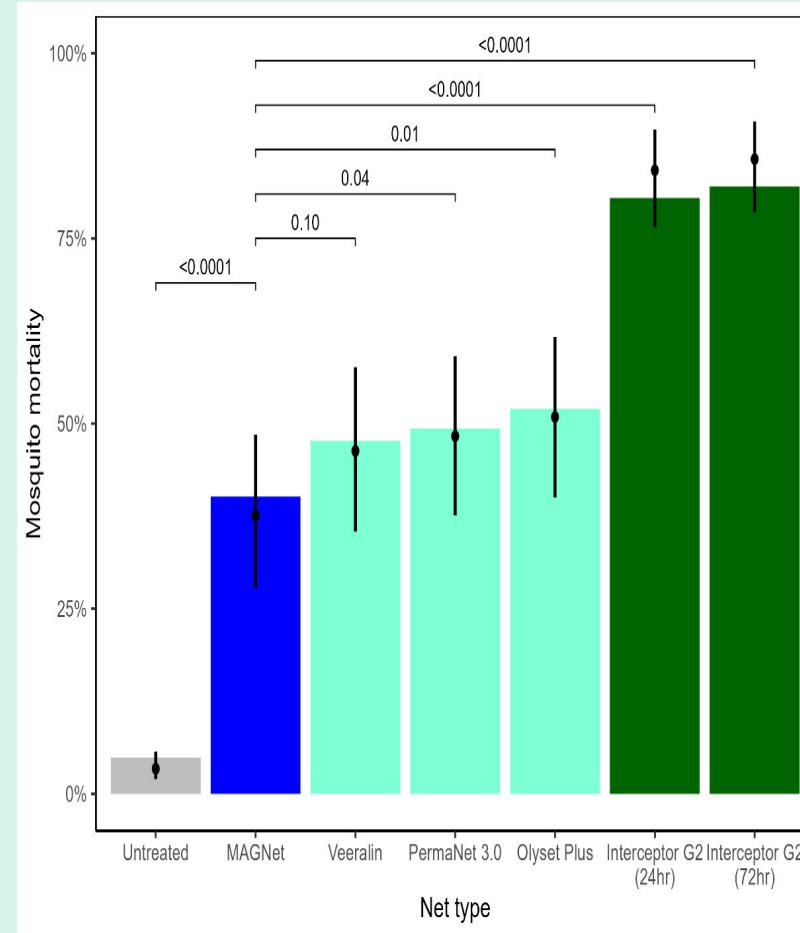


How we did it on the 1980s with DDT, OPs

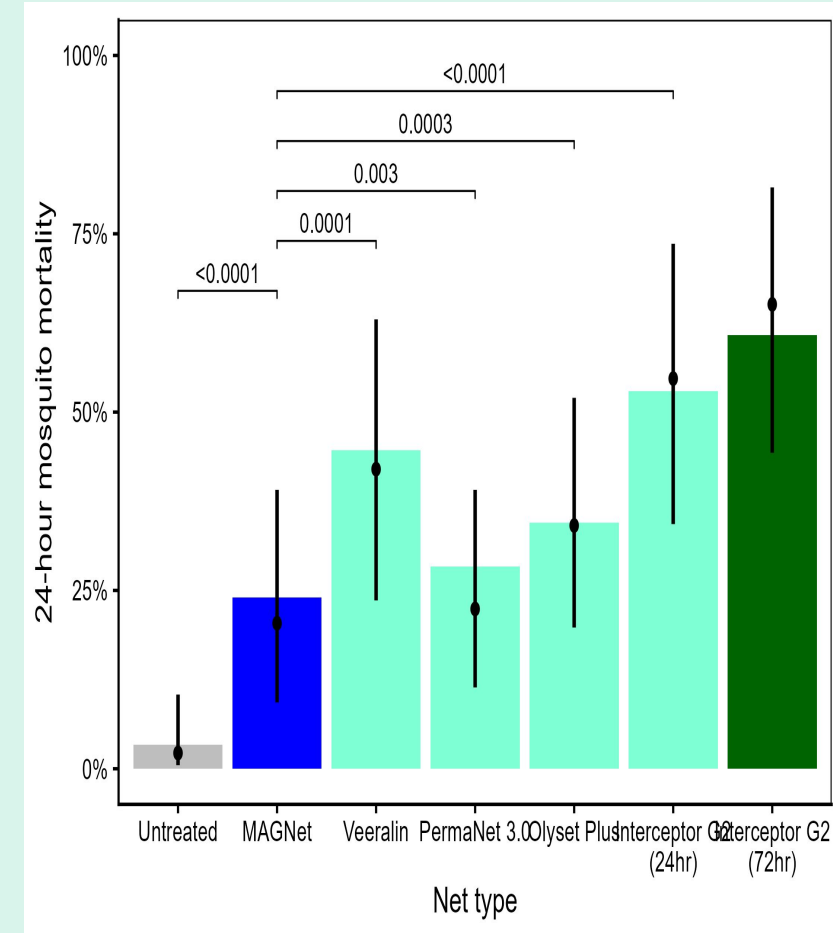
Mortality Outcomes Across Three Experimental Hut Sites



- **Cameroon (Mangoum)**
Mortality highest with Olyset Plus & Interceptor G2 (>50%)
Pyrethroid-only nets induced <40% mortality



- **Côte d'Ivoire (M'bé)**
Interceptor G2 achieved ≥80% mortality at 72h
PBO nets (Olyset Plus, PermaNet 3.0) ≈ 50%
Pyrethroid-only nets <45%



- **Tanzania (Magu)**
Mortality <60% for all nets
Interceptor G2 & Olyset Plus slightly higher but not significant vs PBO nets

PRELIMINARY DATA: Mortality Estimates

Based on the GLM with no IG2 nets and assuming **R is recessive**

Genotype			All Nets	Pyrethroid-only nets		Pyrethroid-PBO nets	
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RR	SS/RS	RR	7	2	0.523	5	0.620

Did it work?

YES,
sort of,
but...

- Fewer products
 - We expected other good new-gen LLINs
 - At the moment, IG2 is best everywhere
- Complex genetics – crazy complexity!
 - Linkage disequilibrium
 - Major sample size issues – assume dominance
- Many fixed loci – but can still compare products
- Many important genes still lack DNA-level markers
- Will we soon have CFP resistance?



Thank you
for more information, visit

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