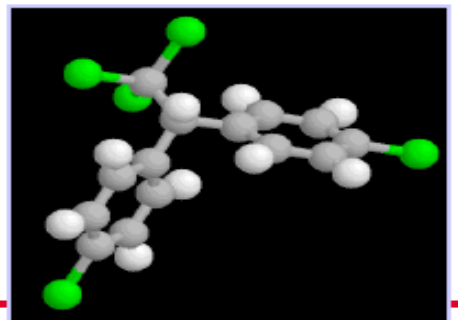
Molecular drivers of insecticide resistance in malaria vectors

LSTM
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Prof Charles Wondji, LSTM/CRID; charles.wondji@lstmed.ac.uk

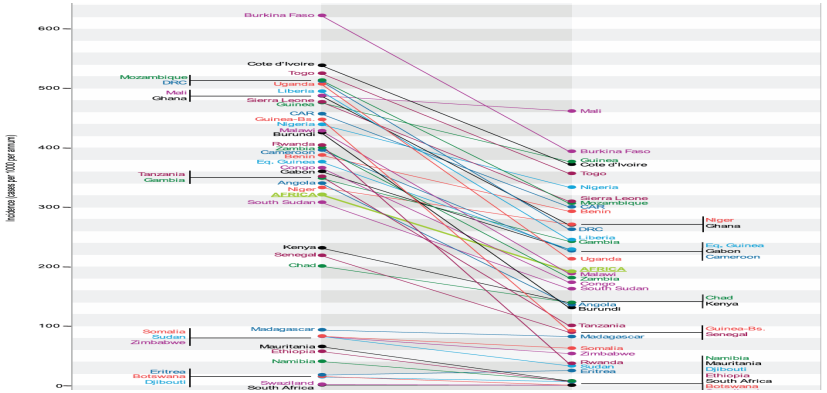


Vector control a cornerstone of prevention of vector-borne diseases

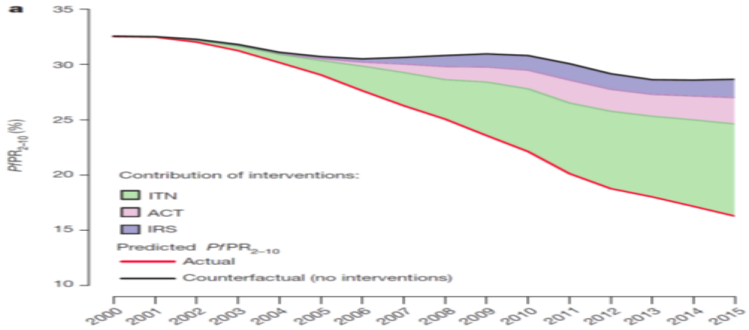
Long Lasting Insecticidal Nets

Indoor Residual Spraying

Larviciding



2000 2015

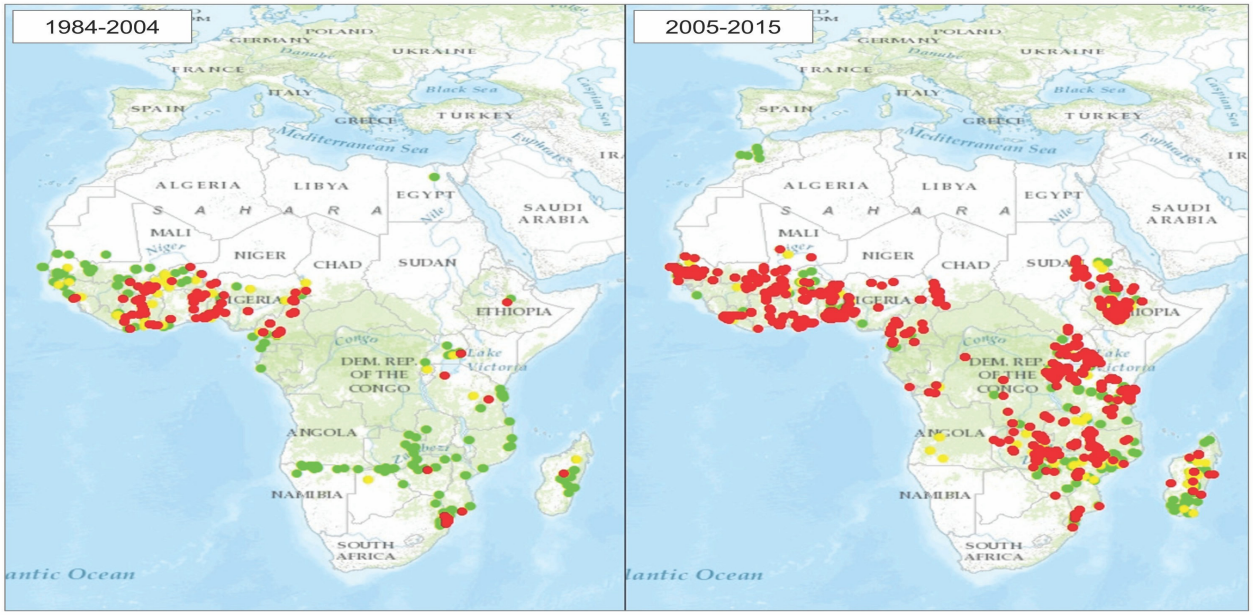


Decline in Burden of Malaria across Africa been largely attributed to scale up of ITNs (68%)

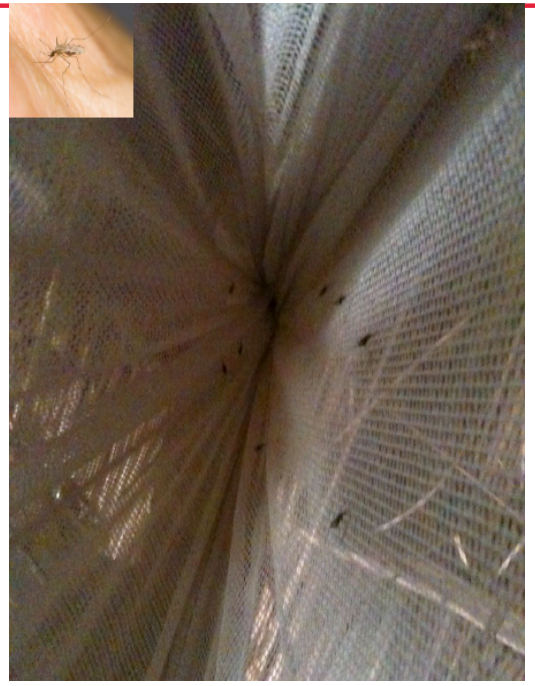
Bhatt et al 2015 Nature

Increasing report of insecticide resistance

Resistance in African malaria vectors



Pyrethroid resistance in malaria vectors in Africa from 1984–2004 (left map) and 2005–2015 (right map). Red dots show resistant populations according to WHOs definition following exposure to a discriminating dose; yellow dots show possible resistance; green dots show susceptible populations. Source: R Mapper

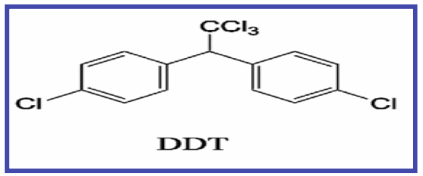


Resistant mosquitoes resting in bed nets

An insecticide is a chemical use to control insects

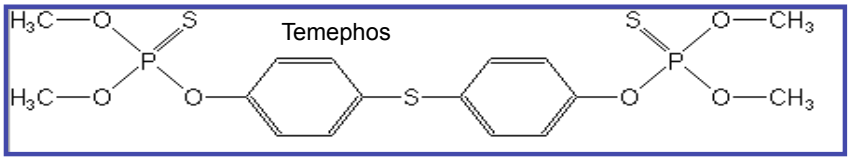
Very limited choice of insecticides for control of public health pests

Organochlorines

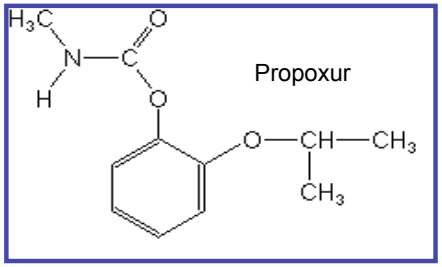


dichlorodiphenyltrichloroethane

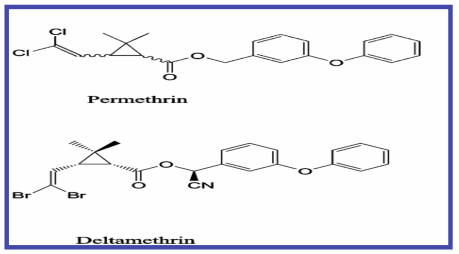
Organophosphates



Carbamates



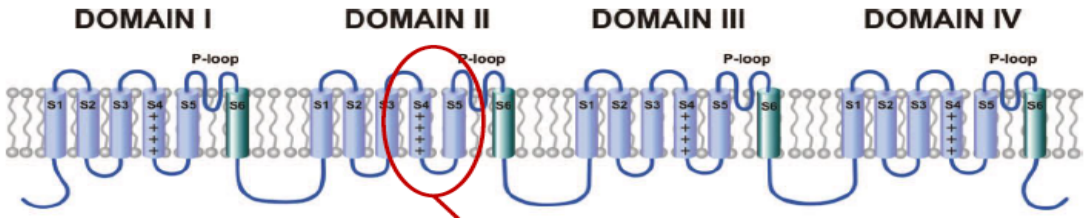
Pyrethroids



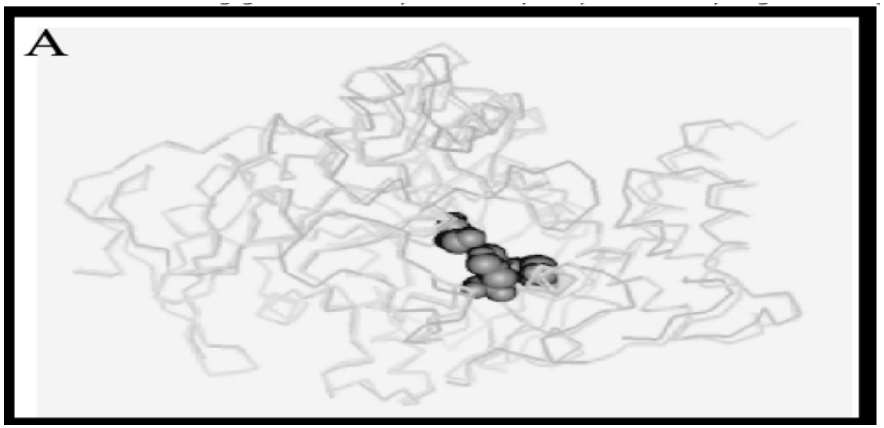
Pyrethroids are the most used for control program because of their low toxicity to humans.

All of these insecticides target the insect's nervous system

- DDT and pyrethroids bind to the sodium channel
- Keep the channel in open state causing repetitive firing of neurones, paralysis and death
- Organophosphates and Carbamates bind to acetylcholinesterase (*AChE*)

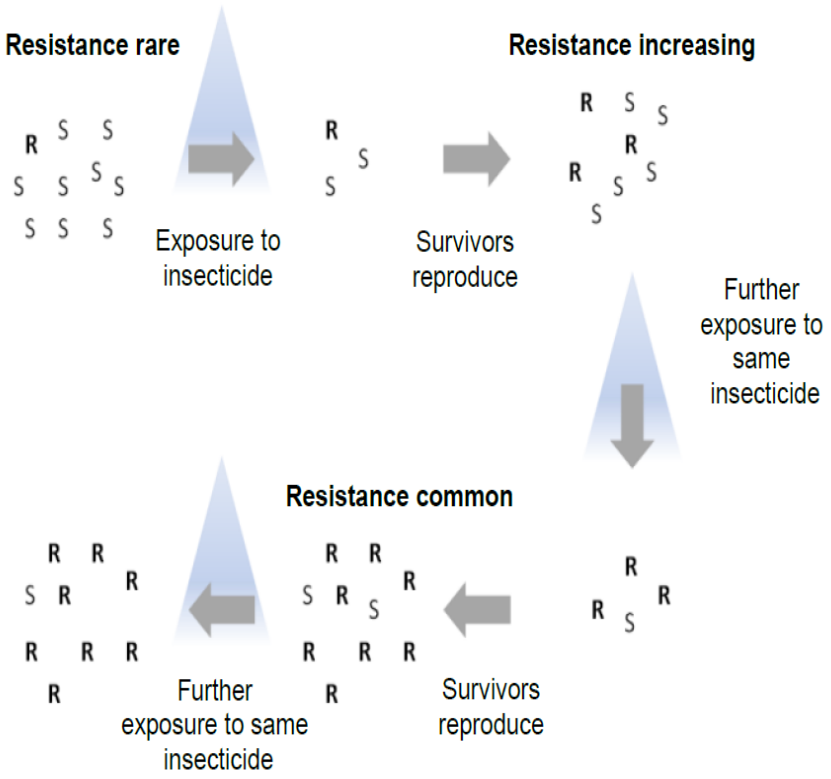


Sodium channel gene



Acetylcholinesterase modelling

Genetic basis of resistance



- Naturally occurring genetic mutations allow a small proportion of the population to survive insecticide exposure.
- The resistant insects will reproduce and the genetic changes that confer resistance are transferred from parents to offspring and resistant insects become predominant.
- The process will take longer if the gene conferring resistance is rare or present at a low frequency.
- Resistance genes can range from dominant, semi-dominant and recessive.
- If the resistance is genetically dominant, it can rapidly become established within the population and will be difficult to manage

Sources of resistance: Impact of agriculture

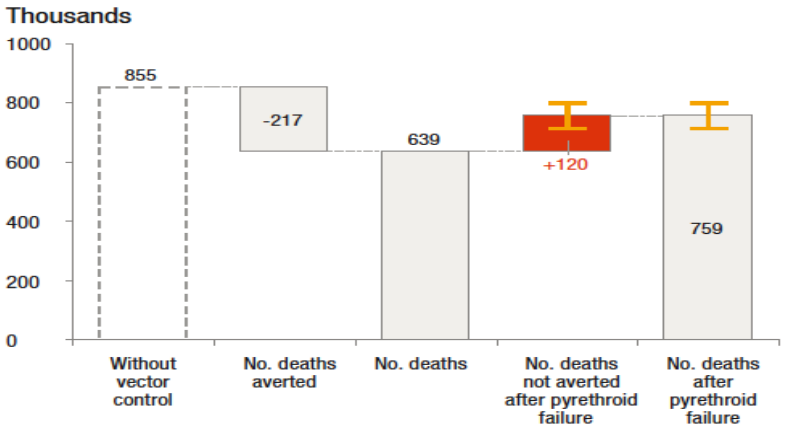
- **Agriculture**
  - Same insecticides used in agriculture as in public health
  - Insecticides used in agriculture can contaminate vector breeding sites and induce resistance to insecticides even before they are used in public health. Eg. High insecticide resistance in malaria vectors due to high usage of insecticide in cotton field.

- **Need of collaboration between agriculture and public health sector for better vector control**

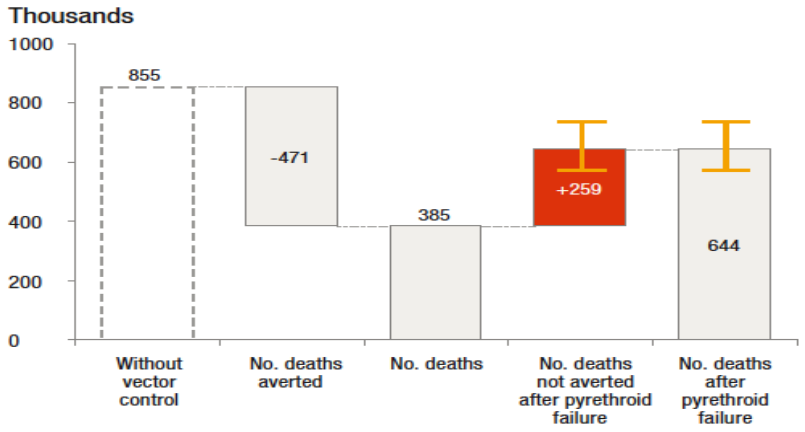
Why insecticide resistance matters?

(GPIRM WHO, 2012) Global Plan for Insecticide Resistance Management

At current coverage[a]: ~56% of vector control benefits would be lost (~120 000 yearly deaths)



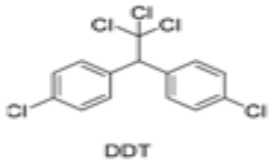
At universal coverage[b]: ~260 000 yearly deaths of children under 5 would not be averted

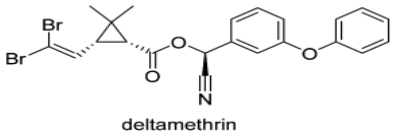


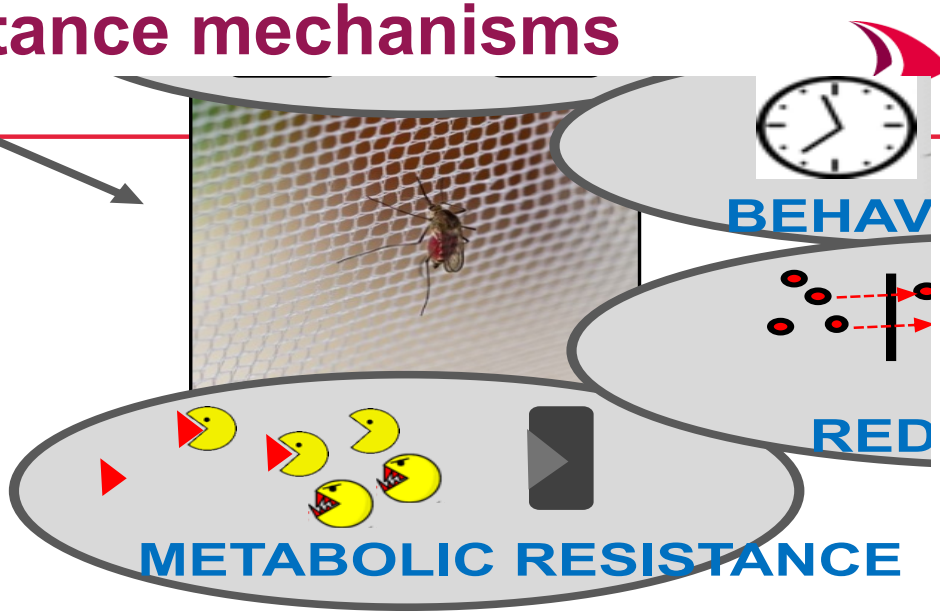
a Current coverage with LLINs and IRS
b Assuming 100% coverage with LLINs and IRS, with current distribution between the two interventions maintained

Pyrethroid resistance can lead to 260000 deaths/year if nothing is done.
Insecticide resistance a major public health issue not just academic topic

Insecticide resistance mechanisms



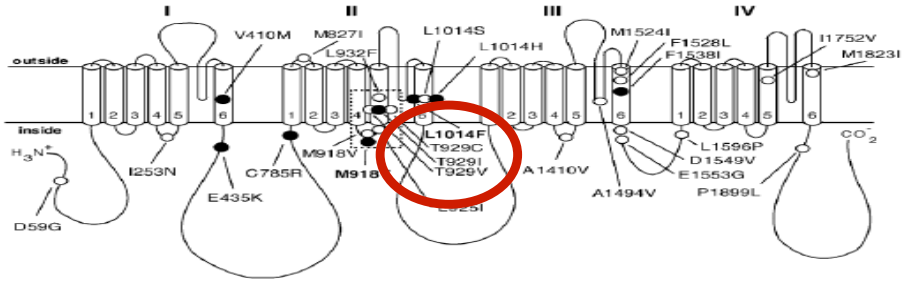


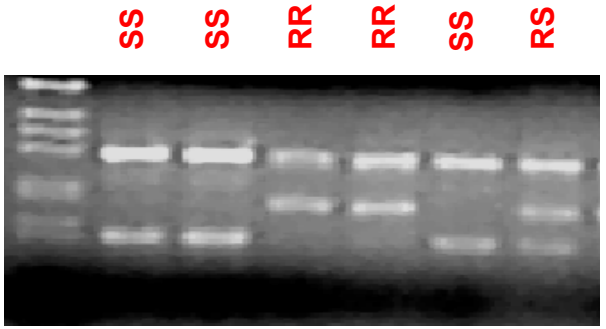


Biochemical mechanism of resistance					
	Metabolic			Target-site	
	Esterases	Monooxygenases	GSH S-Transferases	kdr	MACE
Pyrethroids	●	●		●	
DDT		●	●	●	
Carbamates	●				●
Organophosphates	●	●			●

Target-site resistance: knockdown resistance (*kdr*) mutations

- Single amino acid substitution in the sodium channel transmembrane protein can result in resistance known as *kdr (knockdown resistance)*
- These mutations confer resistance to Pyrethroids and DDT
- **Leu1014Phe substitution** been found in many mosquito species e.g. widespread in *An. gambiae* in Africa, L1014S
- But no *kdr* mutations in other species such as *An. funestus* (Irving and Wondji 2017).

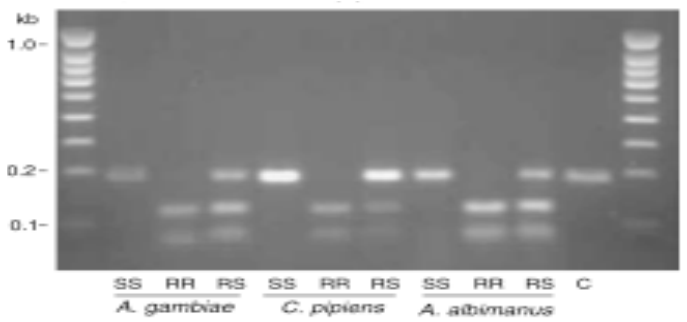




First DNA-based diagnostic for *target-site resistance* established 2 decades ago (Martinez-Torres et al. 1998; Ranson et al 2000).

Altered Acetylcholinesterase

- A glycine to serine G119S mutation in *ace-1* responsible for resistance to organophosphates and carbamates in *Anopheles* and *Culex*
- G119S absent in other species such as *An. funestus* where new mutation present (Asparagine to Isoleucine at codon N485I) Ibrahim et al 2016.

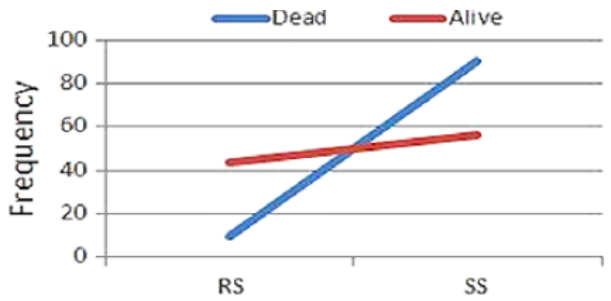


Weill et al, Insect Mol Biol, 13 2004

MOLECULAR ECOLOGY
Molecular Ecology (2016) doi: 10.1111/mec.13673

The P450 *CYP6Z1* confers carbamate/pyrethroid cross-resistance in a major African malaria vector beside a novel carbamate-insensitive N485I *acetylcholinesterase-1* mutation

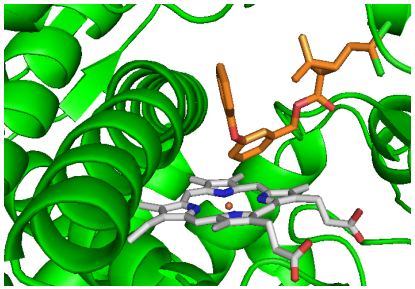
SULAIMAN S. IBRAHIM, MIRANDA NDULA, JACOB M. RIVERON, HELEN IRVING and CHARLES S. WONDJI
Vector Biology Department, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK



N485I correlates with bendiocarb resistance

Genetic basis of metabolic resistance

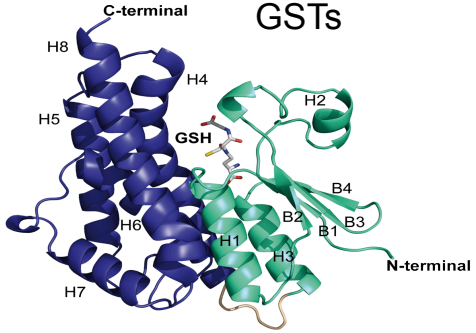
P450s



Increased detoxification of insecticides

(Metabolic resistance)-most operationally important

GSTs



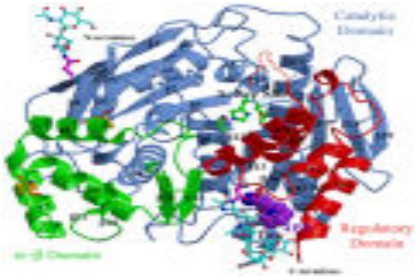
Over-expression of 3 enzyme families: Cytochrome P450s, Glutathione S-Transferases, esterases

Molecular complexity of metabolic resistance

-Multiple mechanisms (e.g. point mutations in coding regions, mutations in cis/trans regulatory loci or in transcription factors); gene amplification

> 200 enzymes - which ones are involved in resistance and how?

Esterases



Role of over-expression of detoxification genes in Metabolic resistance to pyrethroids in *An. gambiae*

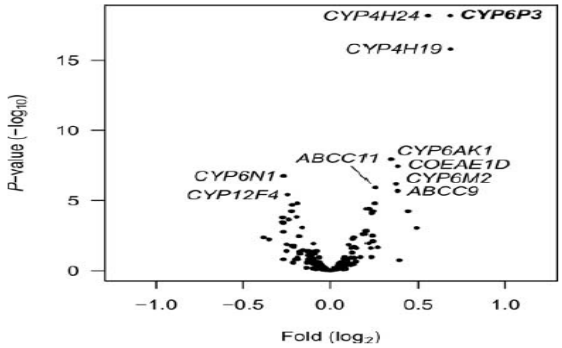
Over-expression of Cytochrome P450s of subfamily CYP6 mainly involved in *An. gambiae* CYP6P3 and CYP6M2 playing main role

OPEN ACCESS Freely available online PLOS GENETICS

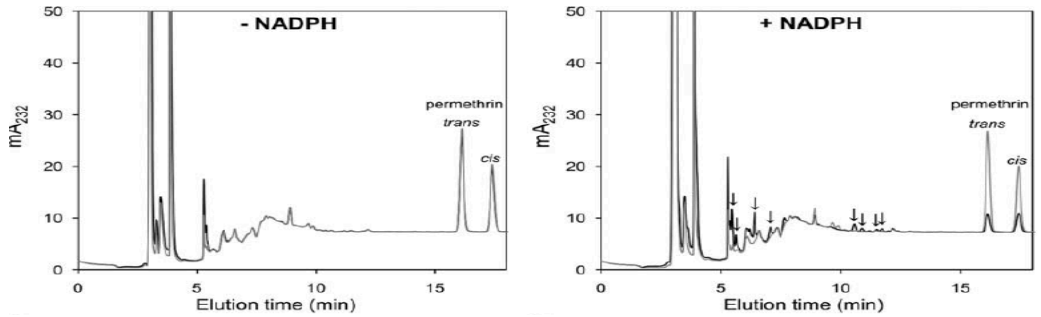
Field-Caught Permethrin-Resistant *Anopheles gambiae* Overexpress CYP6P3, a P450 That Metabolises Pyrethroids

Pie Müller[1☯*], Emma Warr[1☯¤], Bradley J. Stevenson[1☯], Patricia M. Pignatelli[1], John C. Morgan[1], Andrew Steven[1], Alexander E. Yawson[2], Sara N. Mitchell[1], Hilary Ranson[1], Janet Hemingway[1], Mark J. I. Paine[1], Martin J. Donnelly[1]

Muller et al 2008
Djouaka et al 2008
Mitchell et al 2012

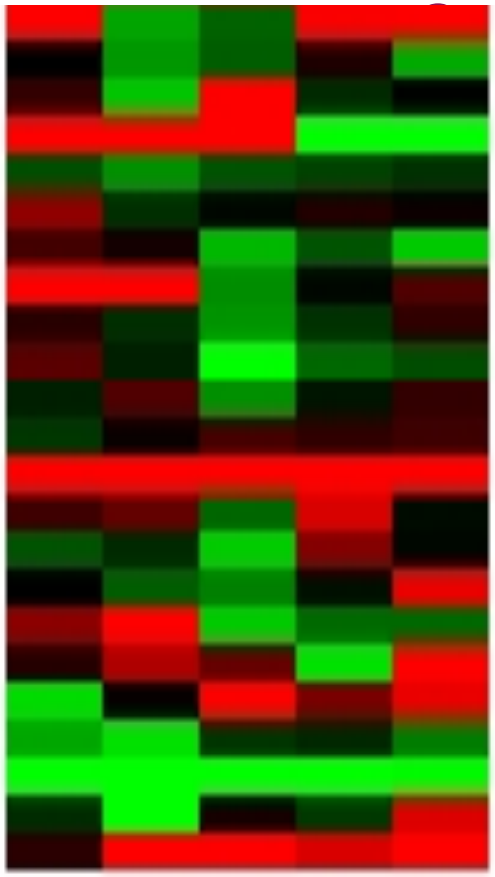


Microarray experiments showing over-expression of CYP6P3



In vitro recombinant assay showing that CYP6P3 can metabolise permethrin

r-expression of key detoxification es varies regionally



RNAseq Profile

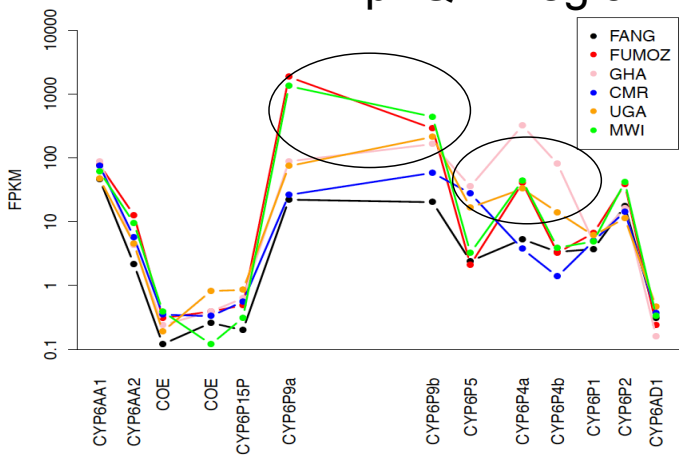
CYP6P4
CYP9J11
CYP9K1
CYP6P5
GSTD1
GSTD3
GSTE1
GSTE2
GSTE3
GSTE4
GSTE5
GSTE6
ATPase
UGT
SULT1
SULT2
COE1
COE2
COE3
CPFL
CPLCA
CPR51
CPR71

Glutat
S-tran
UDP gl
Transfe
Sulfotr
Carbox
Cuticul

Over-expression of key detoxification genes varies regionally

East Africa

rp1QTL region



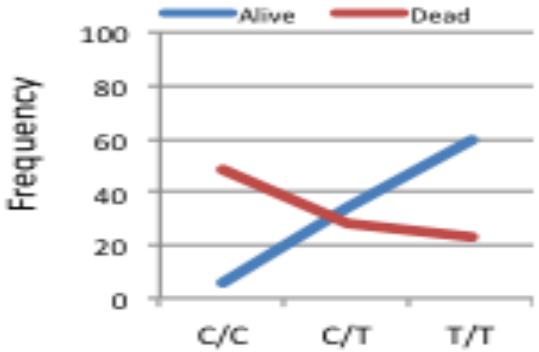
Underlying molecular basis of resistance not the same across Africa

Weedall et al, 2019 Science TM

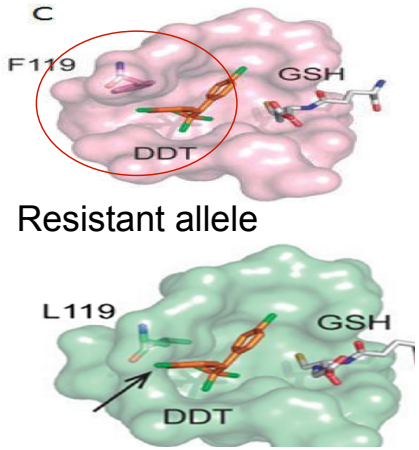
First DNA-based metabolic resistance markers: GST-mediated Metabolic resistance to DDT/Pyrethroid

-**A single amino acid change (L119F) in GSTe2 (Glutathione-S transferase)** confers high level of DDT resistance (and some pyrethroid resistance) in *An. funestus*
-First DNA-based diagnostic designed for metabolic resistance to detect and map this resistance

119F enlarges substrate binding pocket inducing increased DDT metabolism



OR=22 P<0.001



Resistant allele

Susceptible allele

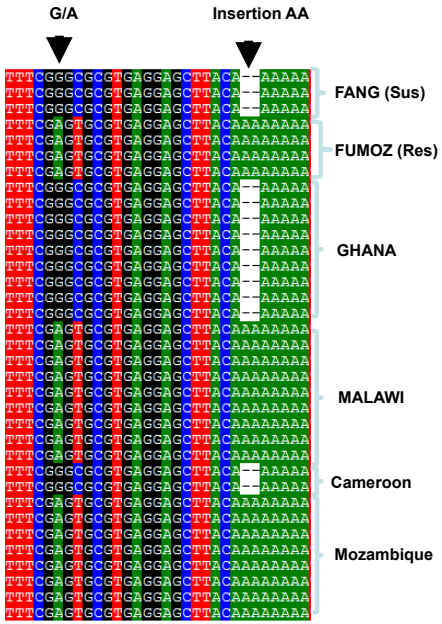
(Riveron et al 2014a, Genome Biology)

A similar mutation also found in *An. gambiae* I114T (Mitchell et al 2014 PLoSONE)

Distribution not uniform across Africa; barriers to gene flow???

Design of DNA-based diagnostic tool for P450 resistance

TaqI restriction site



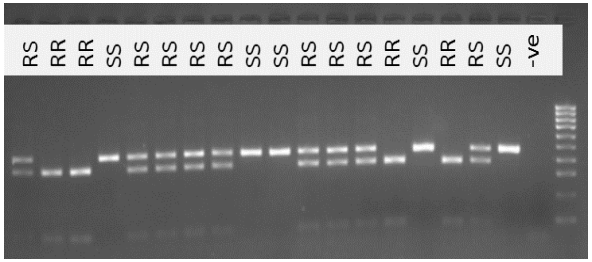
CYP6P9a cis-promoter

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

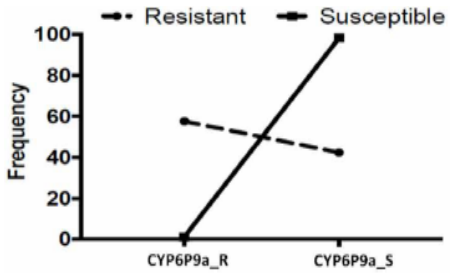
Gareth D. Weedall[1,2], Leon M. J. Mugenzi[3,4], Benjamin D. Menze[1,3,4], Magellan Tchouakui[3,4], Sulaiman S. Ibrahim[1,5], Nathalie Amvongo-Adjia[4,6], Helen Irving[1], Murielle J. Wondji[1,3,4], Micareme Tchoupo[3,4], Rousseau Djouaka[7], Jacob M. Riveron[1,3,4], Charles S. Wondji[1,3,4]*



Weedall et al 2019

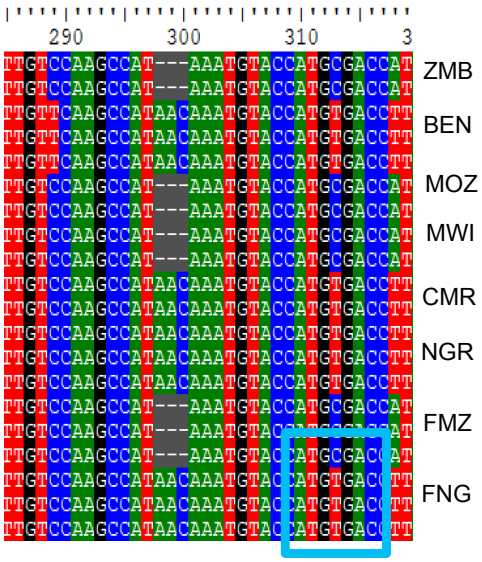
Marker shows strong correlation with pyrethroid resistance

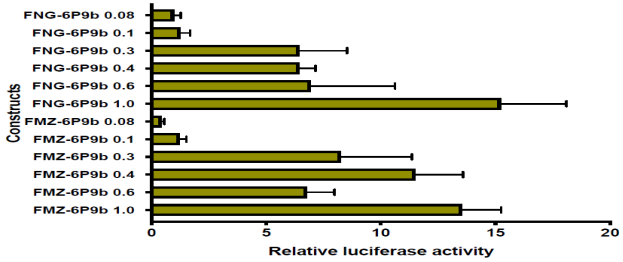
2019



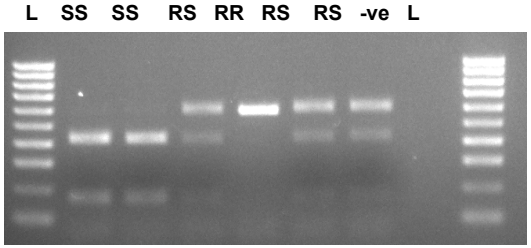
1st P450 mediated DNA-based diagnostic tool for metabolic resistance 20 years after 1st kdr assay

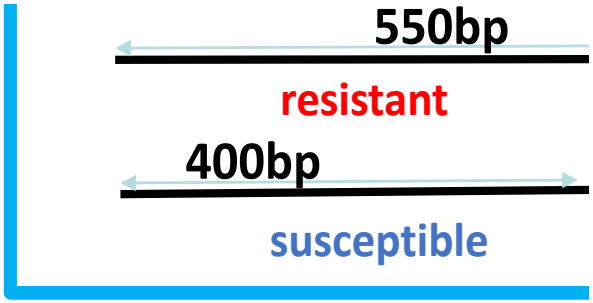
Detection of a second P450-based DNA marker from CYP6P9b

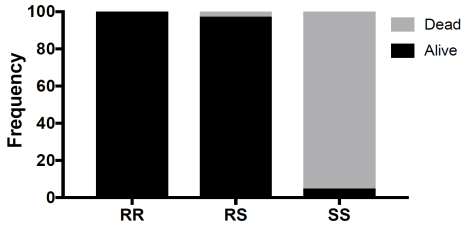




Promoter analysis





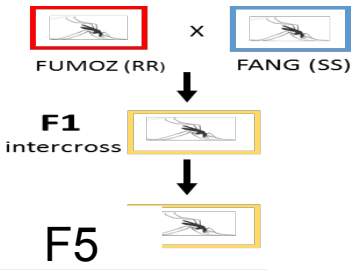


Strong correlation with resistance phenotype

A simple PCR-RFLP to detect *CYP6P9b* P450 resistance

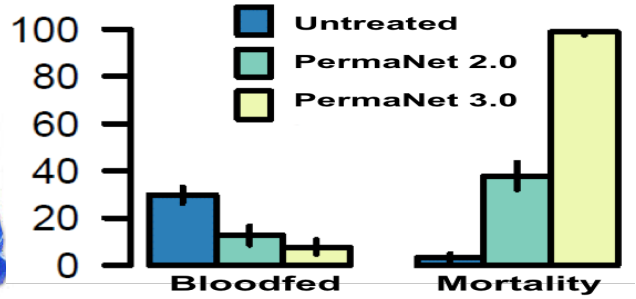
Mugenzi et al 2019 Nature Comm

Impact of the CYP6P9a-based metabolic resistance on the efficacy of bed nets using semi-field experimental hut trials



LLINs:
PermaNet 2.0 vs
PermaNet 3.0
(PBO)

Experimental hut



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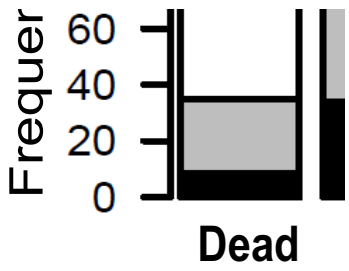
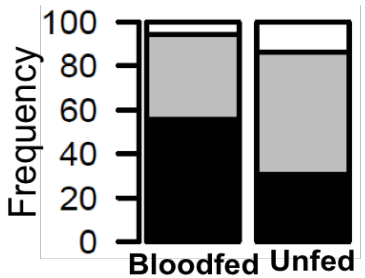


PermaNet 2.0



PermaNet 3.0

Homozygote Resistant CYP6P9a-R mosquitoes significantly more able to survive exposure to the PermaNet 2.0 than the homozygote susceptible mosquitoes
But RR also significantly more able to blood feed when exposed to PBO-based Net (PermaNet 3.0)

Weedall et al 2019 Science TM

Impact of multiple P450-based resistance on effectiveness of LLINs; Case of CYP6P9a and CYP6P9b

ARTICLE

https://doi.org/10.1038/s41467-019-12686-5 OPEN

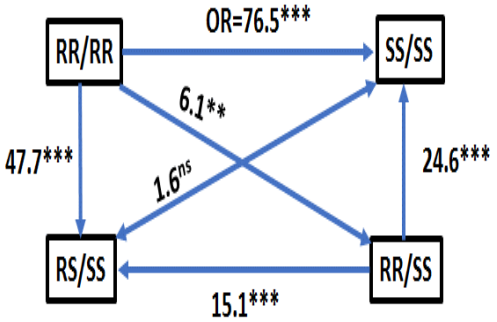
nature COMMUNICATIONS

Cis-regulatory *CYP6P9b* P450 variants associated with loss of insecticide-treated bed net efficacy against *Anopheles funestus*

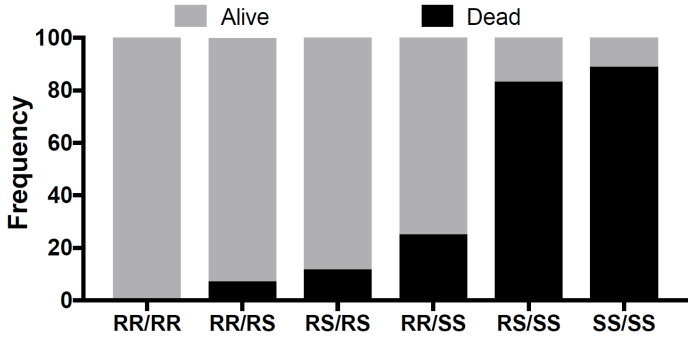
Leon M.J. Mugenzi[1,2,3], Benjamin D. Menze[1,2], Magellan Tchouakui[2], Murielle J. Wondji[1,2], Helen Irving[1], Micareme Tchoupo[2], Jack Hearn[1], Gareth D. Weedall[1,4], Jacob M. Riveron[1,2] & Charles S. Wondji[1,2*]

PermaNet 2.0

Survival odds ratio

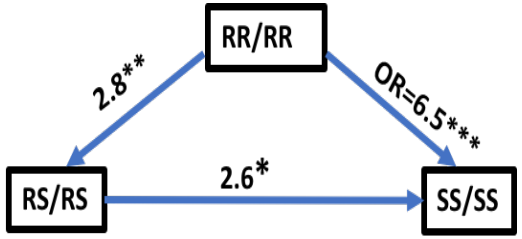


CYP6P9b combines with CYP6P9a to Exacerbate loss of efficacy of LLINs



Double homozygotes resistant survive more

Blood feeding odds ratio



Double homozygotes resistant blood feed more

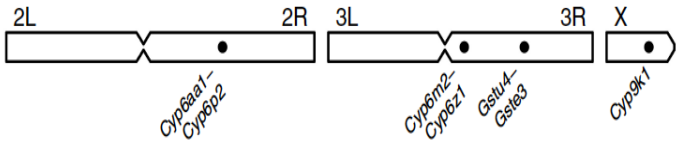
Role of Copy Number variation in insecticide resistance in *An. gambiae*

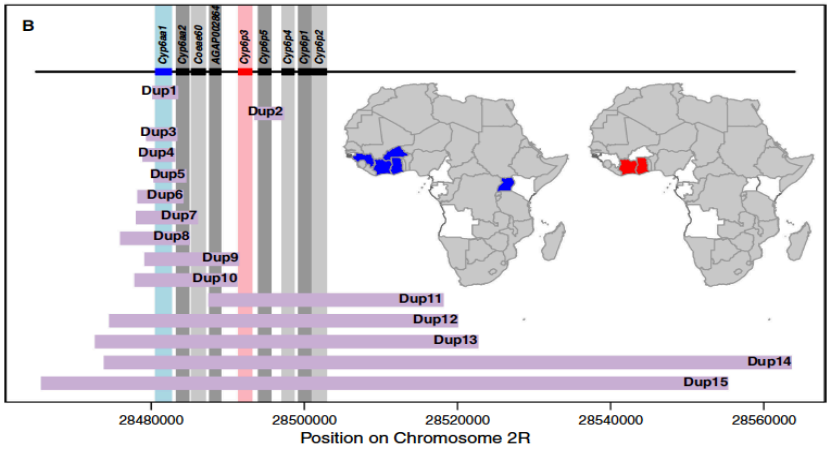
LSTM

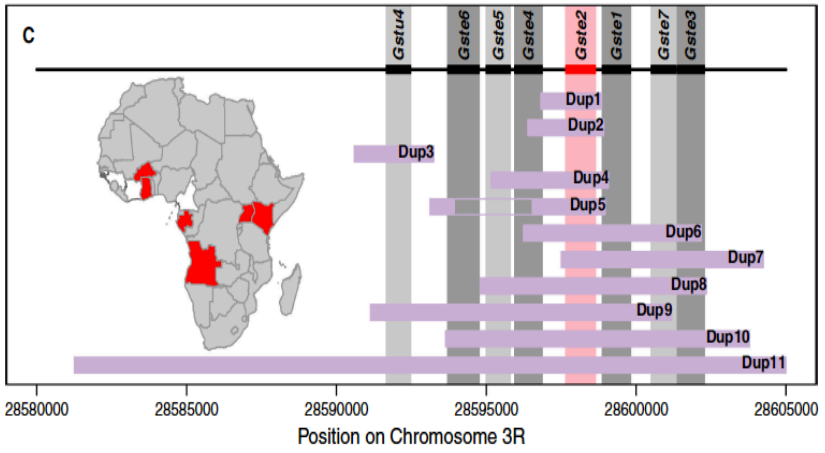
Research

Whole-genome sequencing reveals high complexity of copy number variation at insecticide resistance loci in malaria mosquitoes

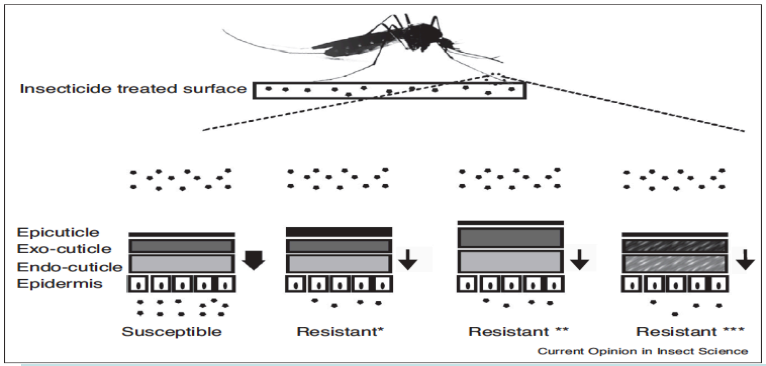
Eric R. Lucas,[1] Alistair Miles,[2,3] Nicholas J. Harding,[3] Chris S. Clarkson,[2] Mara K.N. Lawniczak,[2] Dominic P. Kwiatkowski,[2,3] David Weetman,[1] Martin J. Donnelly,[1,2] and The *Anopheles gambiae* 1000 Genomes Consortium[4]







Cuticular resistance



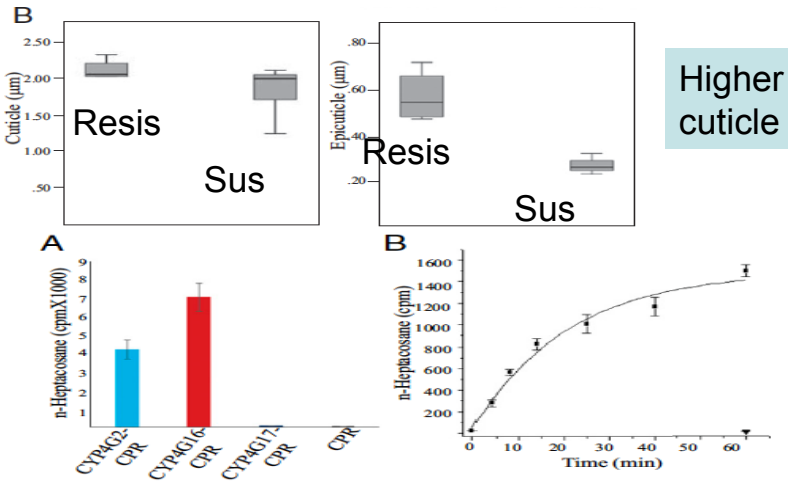
Cuticle thickening → slower rates of insecticide absorption
(reduced penetration) → efficiency of metabolic resistance

In vitro assays showed that CYP4G16 can contribute to production of cuticular hydrocarbon

Cytochrome P450 associated with insecticide resistance catalyzes cuticular hydrocarbon production in *Anopheles gambiae*

Vasileia Balabanidou[a,b], Anastasia Kampouraki[b], Marina MacLean[c], Gary J. Blomquist[c], Claus Tittiger[c], M. Patricia Juárez[d], Sergio J. Mijailovsky[d], George Chalepakis[b], Amalia Anthousi[b], Amy Lynd[e], Sanou Antoine[e], Janet Hemingway[e,1], Hilary Ranson[e], Gareth J. Lycett[e], and John Vontas[a,f,1]

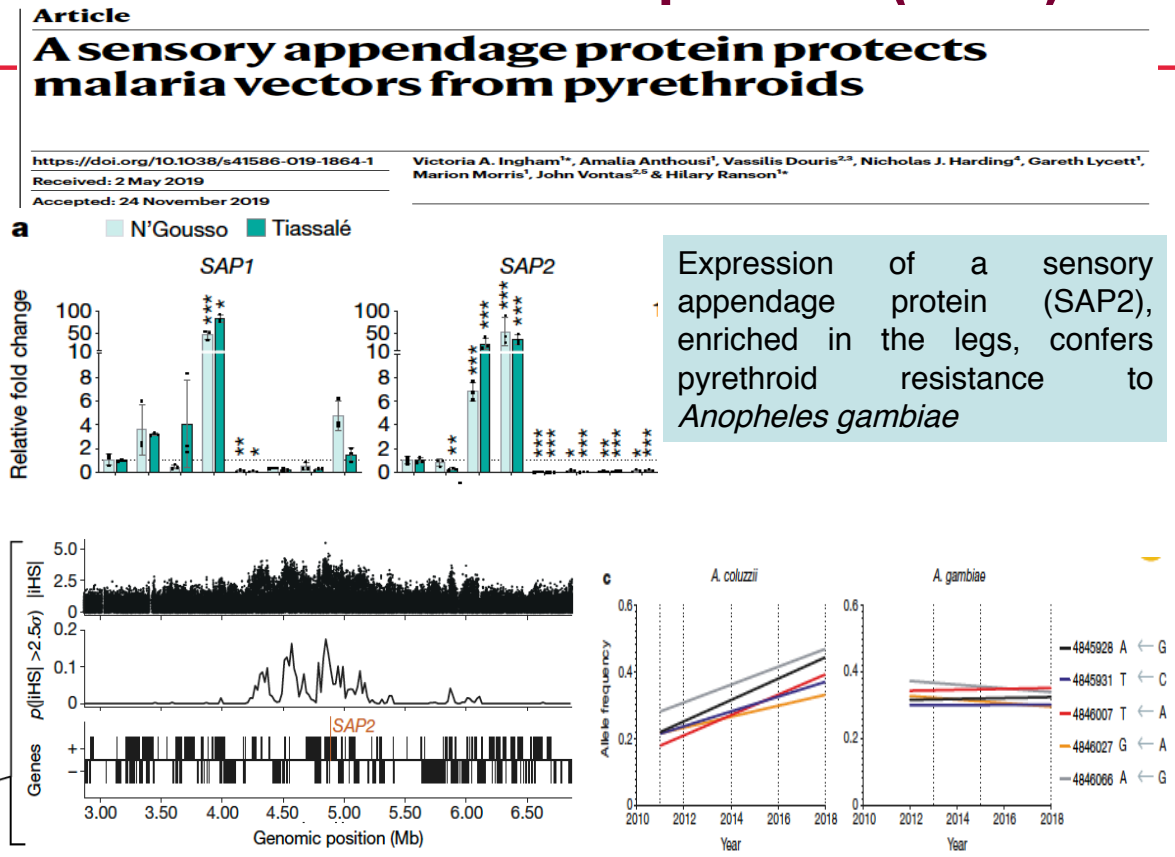
2016



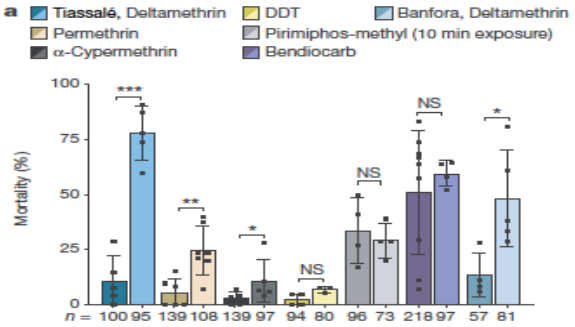
Higher amount of cuticle in resistant

No molecular marker yet

Novel resistance mechanism: Sensory appendage protein (SAP)



Expression of a sensory appendage protein (SAP2), enriched in the legs, confers pyrethroid resistance to *Anopheles gambiae*



RNA interference increases susceptibility to permethrin

Detection of selective sweep in West/Central Africa showing gradual selection of a resistance SAP2 allele

Is resistance increasing malaria transmission?

Genotyping of field collected mosquitoes and detection of *Plasmodium* infection

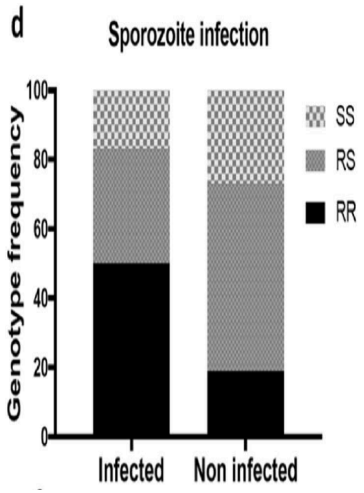
SCIENTIFIC REPORTS

OPEN

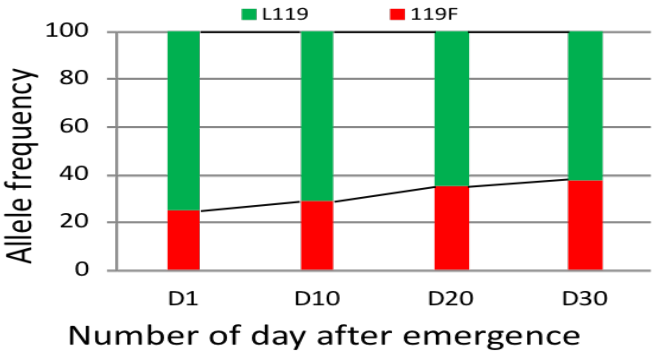
d: 18 June 2018
d: 13 March 2019
:d online: 08 April 2019

A marker of glutathione S-transferase-mediated resistance to insecticides is associated with higher *Plasmodium* infection in the African malaria vector *Anopheles funestus*

Magellan Tchouakui[1,2,3], Mu-Chun Chiang[4], Cyrille Ndo[1,2,5], Carine K. Kuicheu[1,2,3], Nathalie Amvongo-Adjia[1,3,6], Murielle J. Wondji[1,2,4], Micareme Tchoupo[1,2], Michael O. Kusimo[2], Jacob M. Riveron[1,2,4] & Charles S. Wondji[1,2,4]



Tchouakui et al 2018, Gene

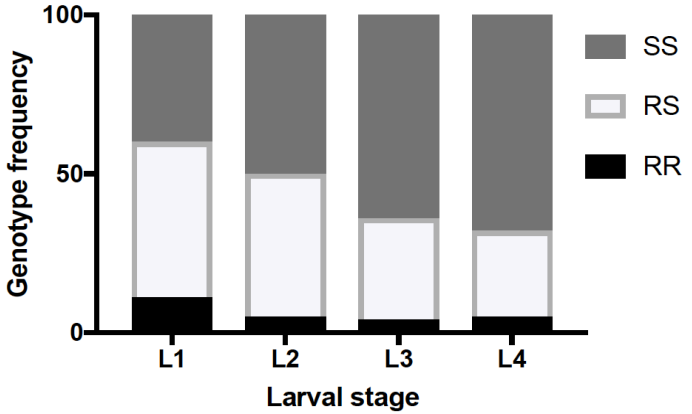


GSTe2 increases longevity of resistant mosquitoes

GSTe2 Resistant mosquitoes are more infected than susceptible- Increase risk of malaria transmission due to this resistance

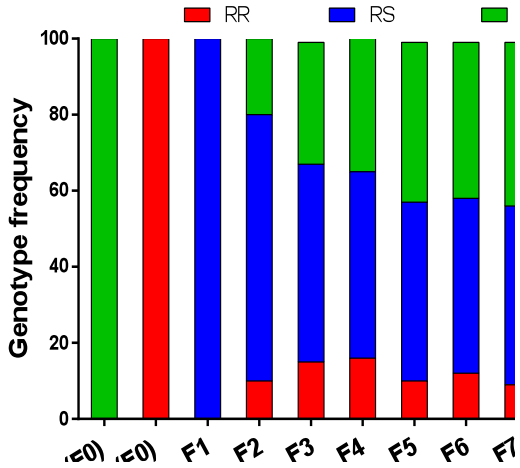
Is there a fitness cost of resistance due to P450 resistance?

CYP6P9a metabolic resistance induces a significant fitness cost on pyrethroid resistant mosquitoes

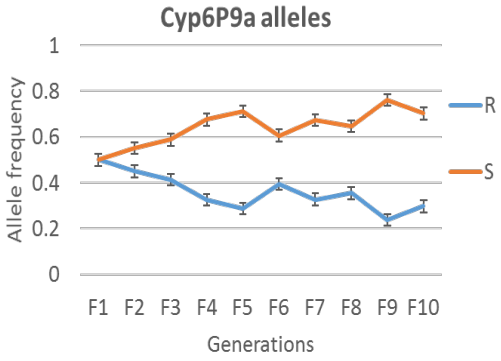


Slower development and higher mortality of resistant mosquitoes at larval stage

Restauration of resistance



Increase frequency of SS



Decrease freq of R allele

Loss of resistance if no selection after 10 generations in lab

Fitness cost of CYP6P9a suggests implementation of insecticide resistance management (rotation, etc) could help manage resistance

Tchouakui et al, 2020 Heredity

Scale-up of insecticide-based interventions is driving resistance to fixation in mosquito populations

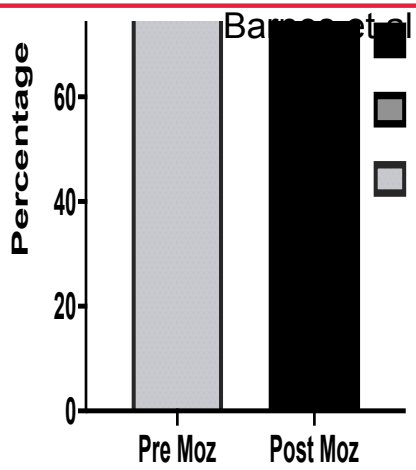
	CYP6P9a	CYP6P9b	6.5kb INS
1	SS	SS	SS
2	SS	SS	SS
3	SS	SS	SS
4	SS	SS	SS
5	SS	SS	SS
6	SS	SS	SS
7	SS	SS	SS
8	SS	SS	SS
9	SS	SS	SS
10	SS	SS	SS
11	SS	SS	SS
12	SS	SS	SS
13	SS	SS	SS
14	RS	RS	SS
15	SS	SS	SS
16	RS	RS	SS
17	SS	SS	SS
18	SS	SS	SS
19	SS	SS	SS

Mozambique 2002

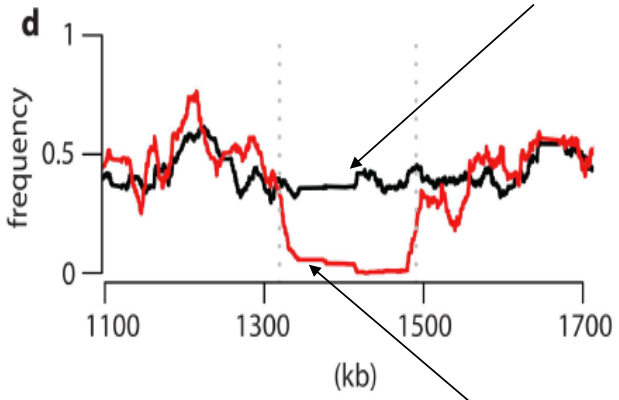
	CYP6P9a	CYP6P9b	6.5kb INS
1	RR	RR	RR
2	RR	RR	RR
3	RR	RR	RR
4	RR	RR	RR
5	RR	RR	RR
6	RR	RR	RR
7	RR	RR	RR
8	RR	RR	RR
9	RR	RR	RR
10	RR	RR	RR
11	RR	RR	RR
12	RR	RR	RR
13	RR	RR	RR
14	RR	RR	RR
15	RR	RR	RR
16	RR	RR	RR
17	RR	RR	RR
18	RR	RR	RR
19	RR	RR	RR

Mozambique 2017

Mugenzi et al 2020 Mol Ecol



Barnes et al 2017; PLoS Genetics



Whole genome sequencing

Before massive scale up of LLINs most mosquitoes were predominantly homozygote susceptible

But in 2017 All Mosquitoes are homozygotes resistant to all resistance alleles

New insecticides and new generation LLINs

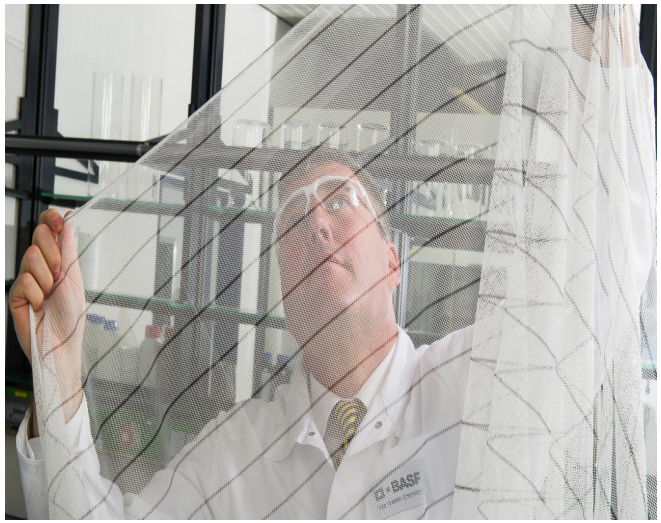
Indoor Residual Spraying

SumiShield 50WG is based on the neonicotinoid insecticide clothianidin.

BAYER Fludora® FUSION

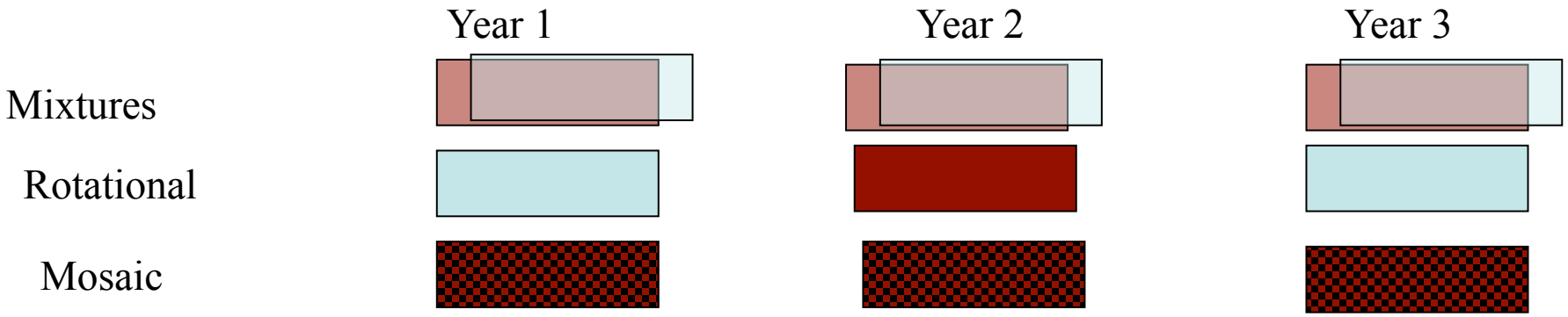
First two-way indoor residual spray solution combining the neonicotinoid clothianidin with a second insecticide with an unrelated mode of action – the pyrethroid deltamethrin.

New LLINs



Interceptor G2: A new net combining chlorfenapyr, a pyrrole class insecticide with alpha-cypermethrin
Royal Guard: alpha-cypermethrin and Pyriproxyfen

Need to deploy resistance management strategies to prevent loss of new insecticides



- Mixtures: application of two mixed compounds so individuals are exposed simultaneously to each compound
- Rotations: temporal alternation of unrelated pesticides
- Mosaics: adjacent areas are treated simultaneously with different pesticides

Summary

- Insecticide resistance is compromising the control of vector borne diseases
- Metabolic resistance, the major route of resistance, is molecularly complex but gradually been elucidated with key features detected although more remain to do
- Recent progress has detected first DNA-based markers of metabolic resistance allowing to track spread of resistance
- **Such diagnostic tools** are crucial for the implementation of suitable resistance management strategies by providing key information on:
  - Frequency of resistance allele allowing early detection of resistance
  - Evolution of resistance, cross-resistance to current and new insecticides, fitness cost of resistance
  - Impact of resistance on outcome of control interventions etc
- New mode of action insecticides are been made, knowledge generated by these research activities will help anticipate resistance and prolong their efficacy

Acknowledgments

LSTM, Liverpool

Team members:

Dr Sulaiman Ibrahim
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