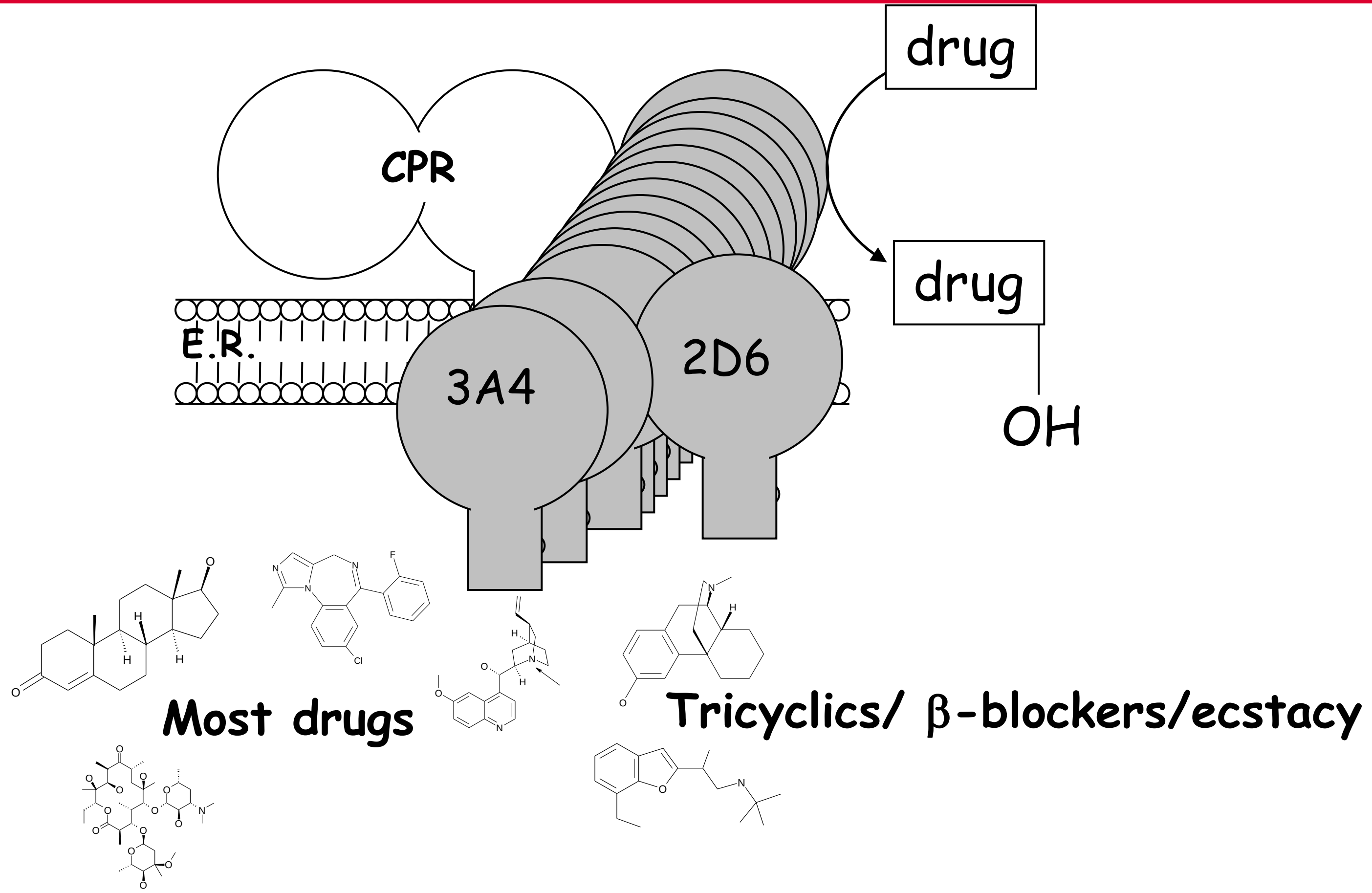
LSTM
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Metabolic Resistance Mechanisms: of man and mosquitoes

Mark Paine - IRAC 19th March 2013 14.30 – 15.00

Drug Discovery: The first Commandment

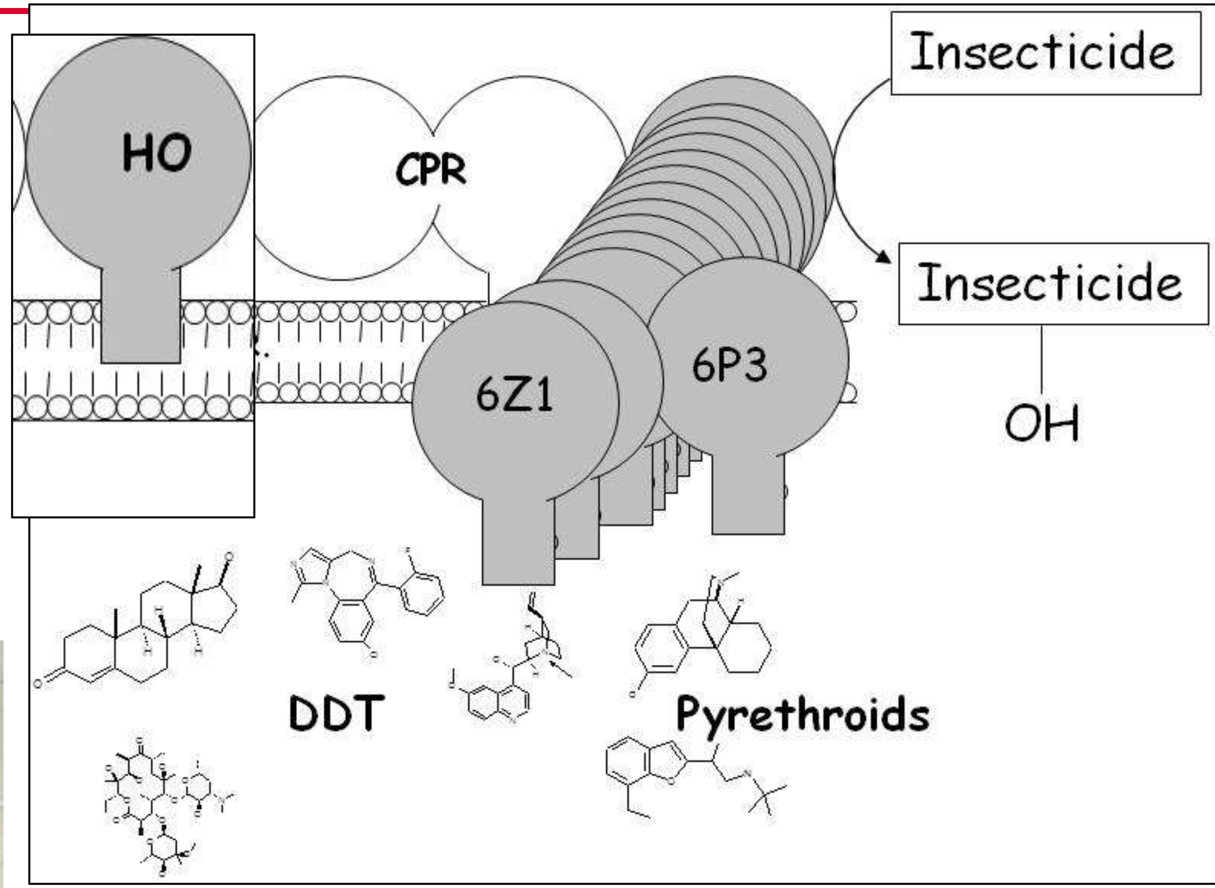
Thou shalt determine if your drug is a P450 ligand

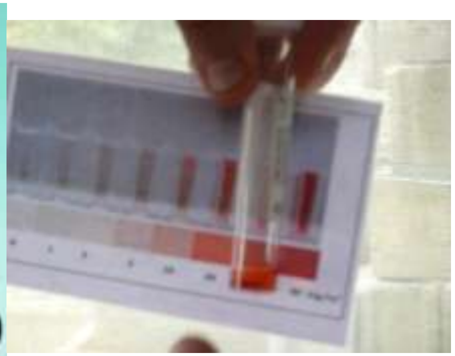


Insecticide Resistance:
Mechanisms, Monitoring, New Tools

Enzyme Characterisation Group

- Resistance
  - Validation
  - Mechanism
  - Translation
    - P450
    - Heme oxygenase
    - Diagnostic kits





Mosquitoes, Sand flies, Bees, Spidermites, Whitefly, Diamondback moth

How metabolic resistance mechanisms vary amongst mosquitoes (Anophelines)

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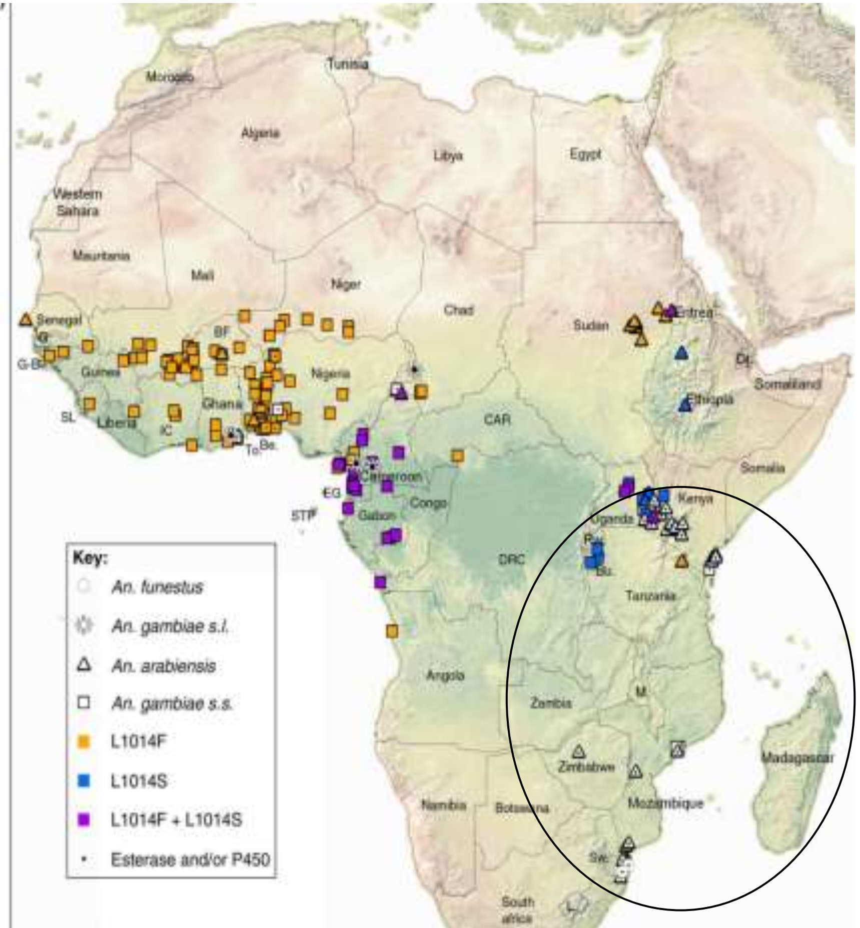
Pyrethroid Resistance mechanisms



Source: Ranson et al. 2010

Pipeline for identification of resistance candidates

Phenotype

Candidate

Validate

Technology

Field test

Source: Coleman et al. 2006

Pyrethroid characterisation pipeline 2006 - 2008

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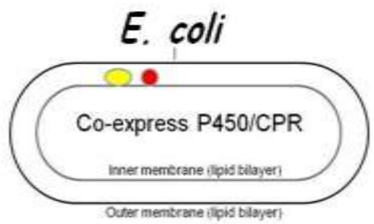
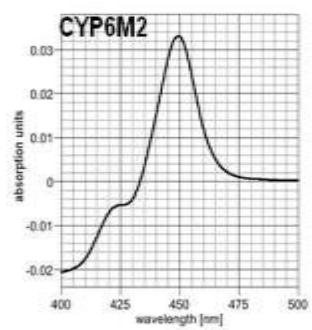
Candidates → Cloning → Expression → Metabolism

Organism	Gene
A. gambiae	cyp325a3
	cyp4g16
	cyp4h19
	cyp4h24
	cyp6m2
	cyp6p3
	cyp6z1
	cyp6z2
	gsts1-1
	gsts1-2
A. funestus	cyp6p4
	cyp6p9
A. aegypti	cyp6cb1
	cyp9j10
	cyp9j19
	cyp9j24
	cyp9j26
	cyp9j28
	cyp9j32
	cyp9j9
	gste4

pCW ompA



Isolate membranes for metabolism assay



CYP6Z2, CYP6M2 and CYP6P3
300 – 400 assays/litre culture

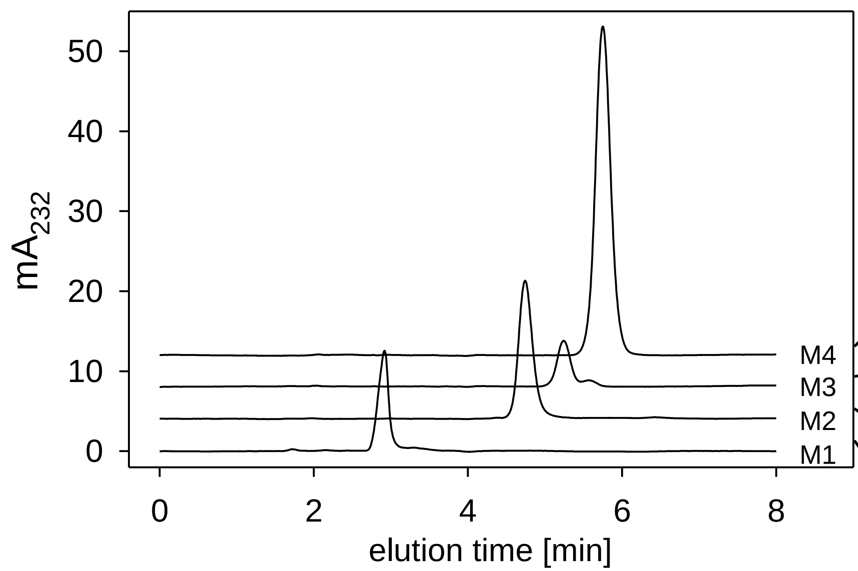
P450	Permeth (min^{-1})	Deltameth (min^{-1})
CYP6P3	3.1	2.6
CYP6M2	6.0	1.2
CYP6Z2	0	0
CYP6P9a	Yes	Yes
CYP9J32	0.8	3.0

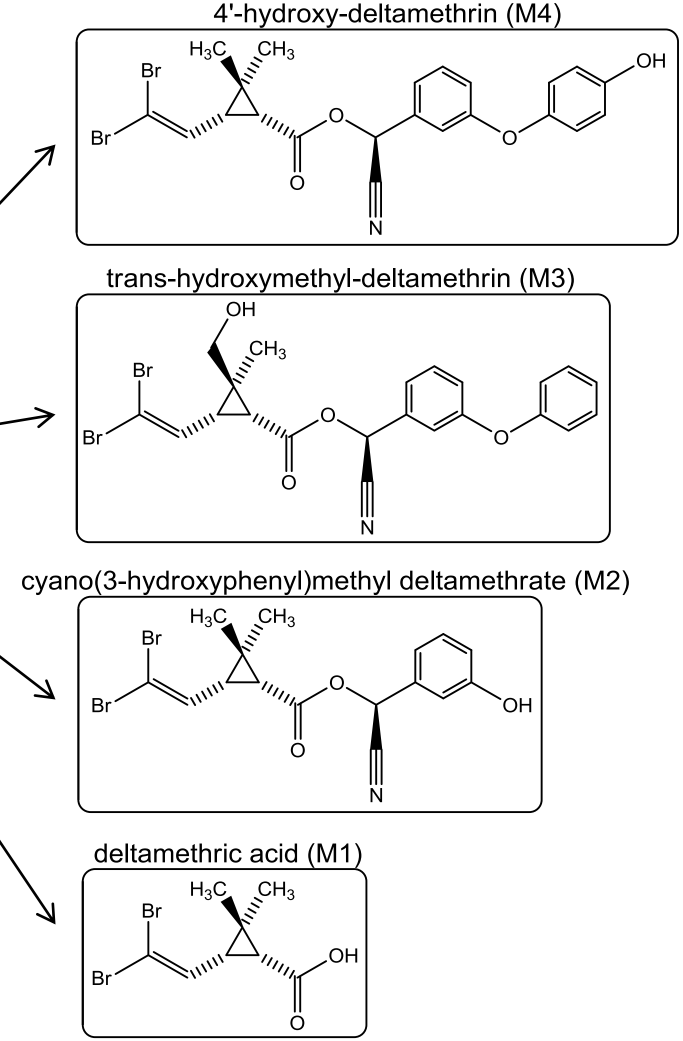
T

Muller et al, 2008 PLoS Gen
Stevenson et al 2011, IBMB
Stevenson et al 2012, PLoS NTD
McLaughlin et al, 2006, IMB
Riveron et al, 2013, PNAS

CYP6M2: How is deltamethrin metabolised?

Reaction volumes and times were increased and metabolites were isolated by HPLC with fraction collection. These were then studied by mass spectrometry and NMR.

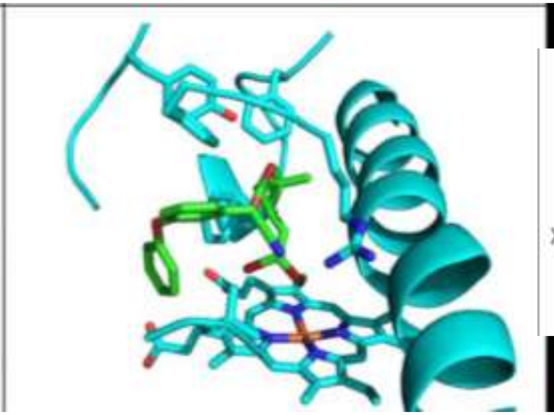




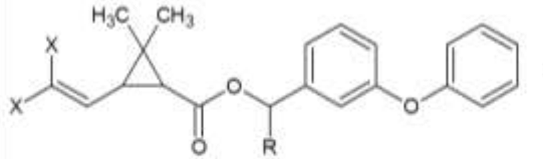
Secondary metabolism needs to be considered

Stevenson et al 2011 IBMB

Why is CYP6Z2 overexpressed?

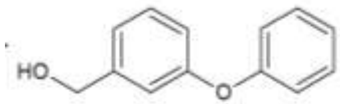


Pyrethroids



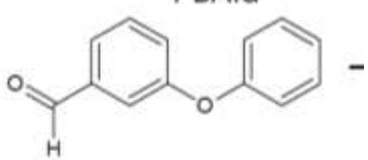
Clashes prevent metabolism of pyrethroids

PBAlc



But not metabolic products

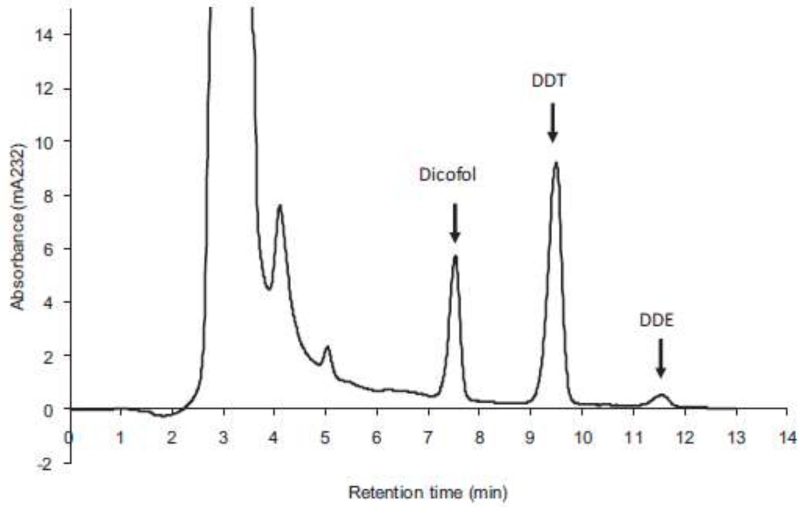
PBAld



Does secondary metabolism contribute to resistance?

J-P David *et al submitted*

Evidence that cooperativity may be important



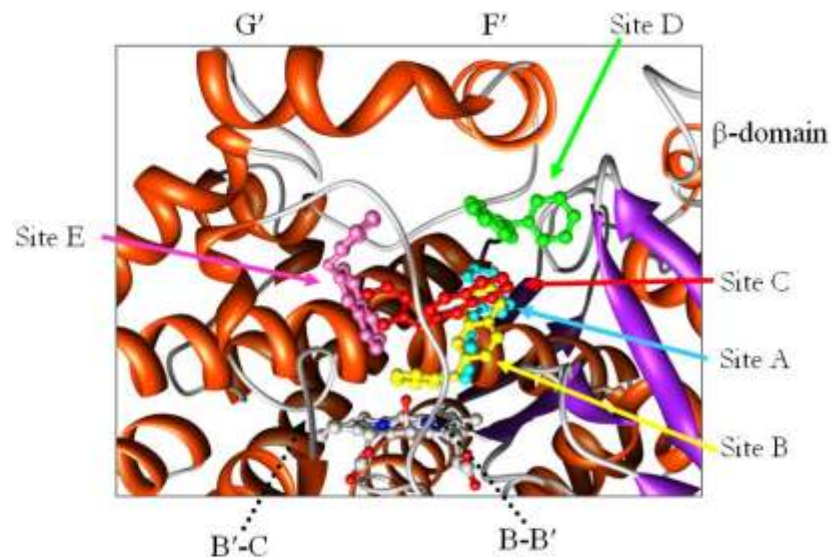
CYP6M2 metabolises DDT to dicofol and DDE in the presence of sodium cholate

0090-9556/08/3610-2136–2144$20.00
DRUG METABOLISM AND DISPOSITION
DMD 36:2136–2144, 2008

Vol. 36, No. 10
21733/3388107
Printed in U.S.A.

Multiple Substrate Binding by Cytochrome P450 3A4: Estimation of the Number of Bound Substrate Molecules

Yury Kapelyukh,[1] Mark J. I. Paine,[2] Jean-Didier Maréchal,[3] Michael J. Sutcliffe,[4] C. Roland Wolf, and Gordon C. K. Roberts

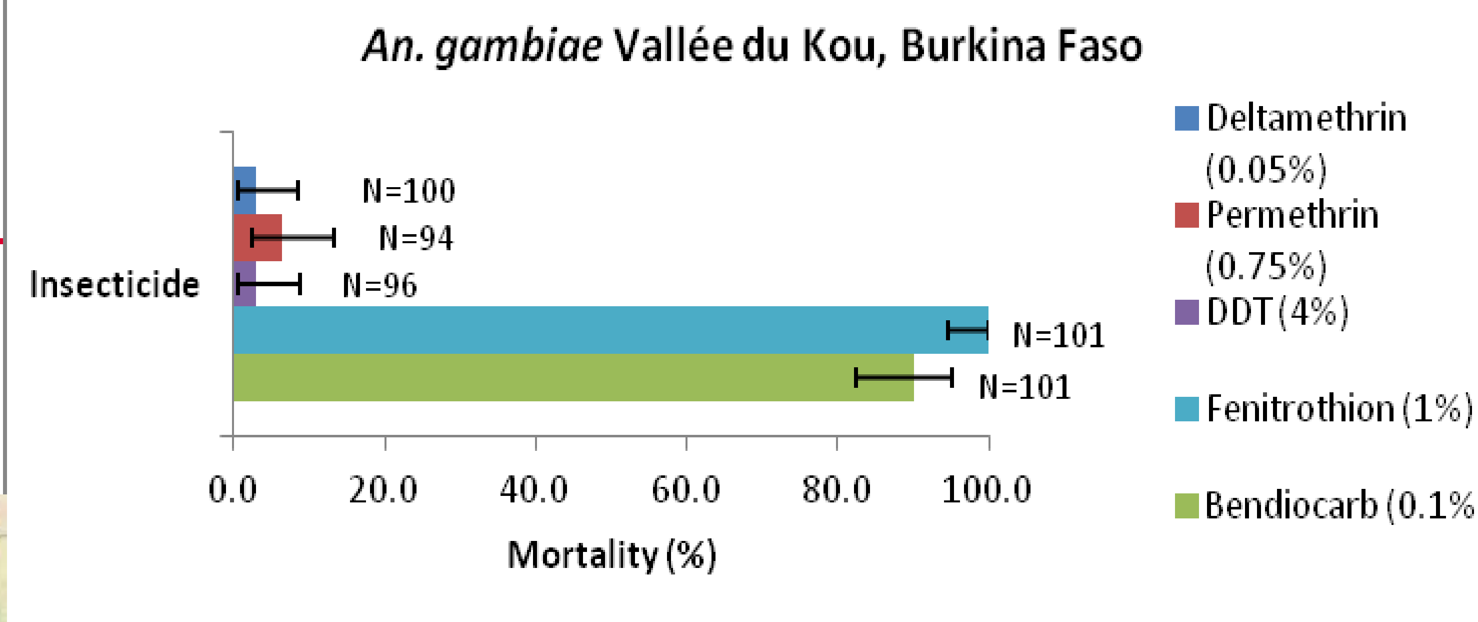


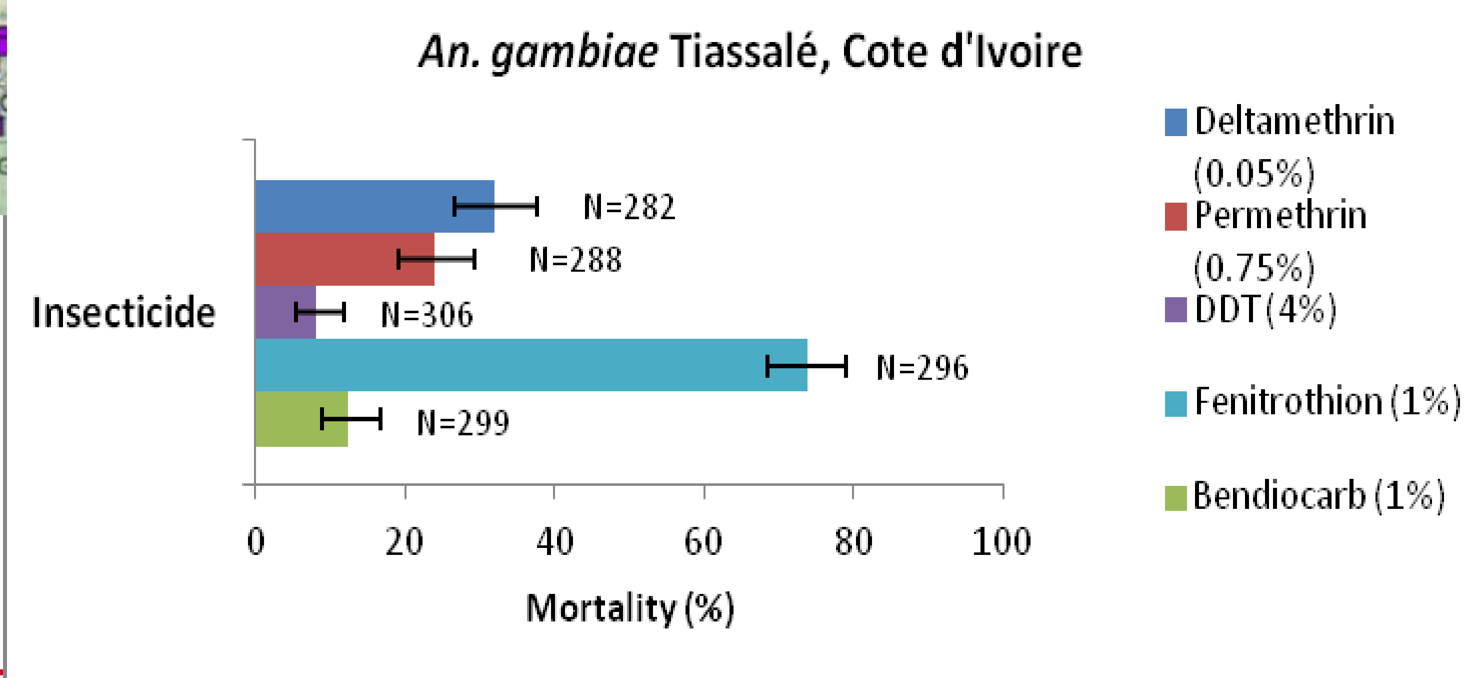
CYP3A4 binds at least 4 7BQ's

Mitchell *et al* 2012 PNAS

2012-13

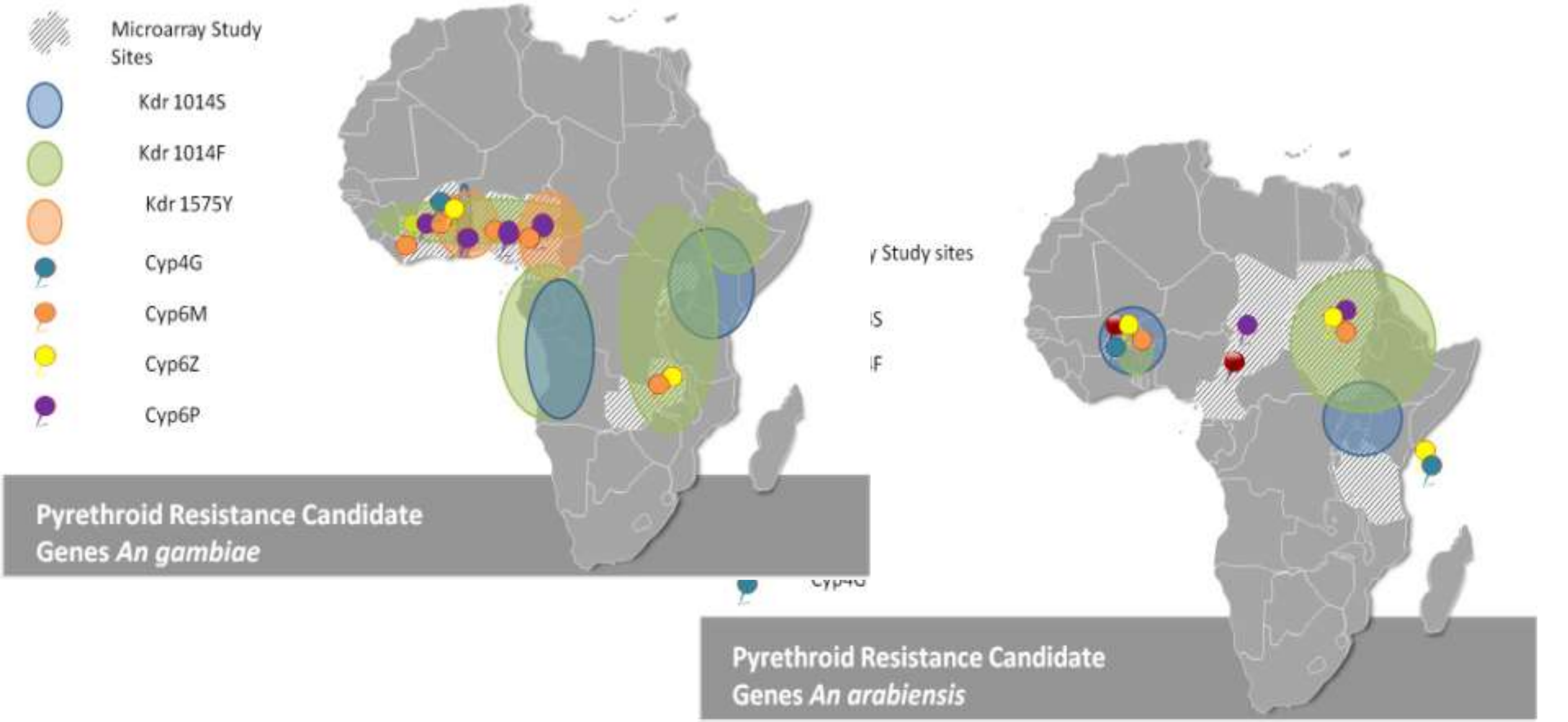
WHO insecticide resistance bioassays from Burkino Fas and Ivory Coast





Chris Jones, Hilary Ranson
Edi Constant
Toe Hyacinthe
Dave Weetman

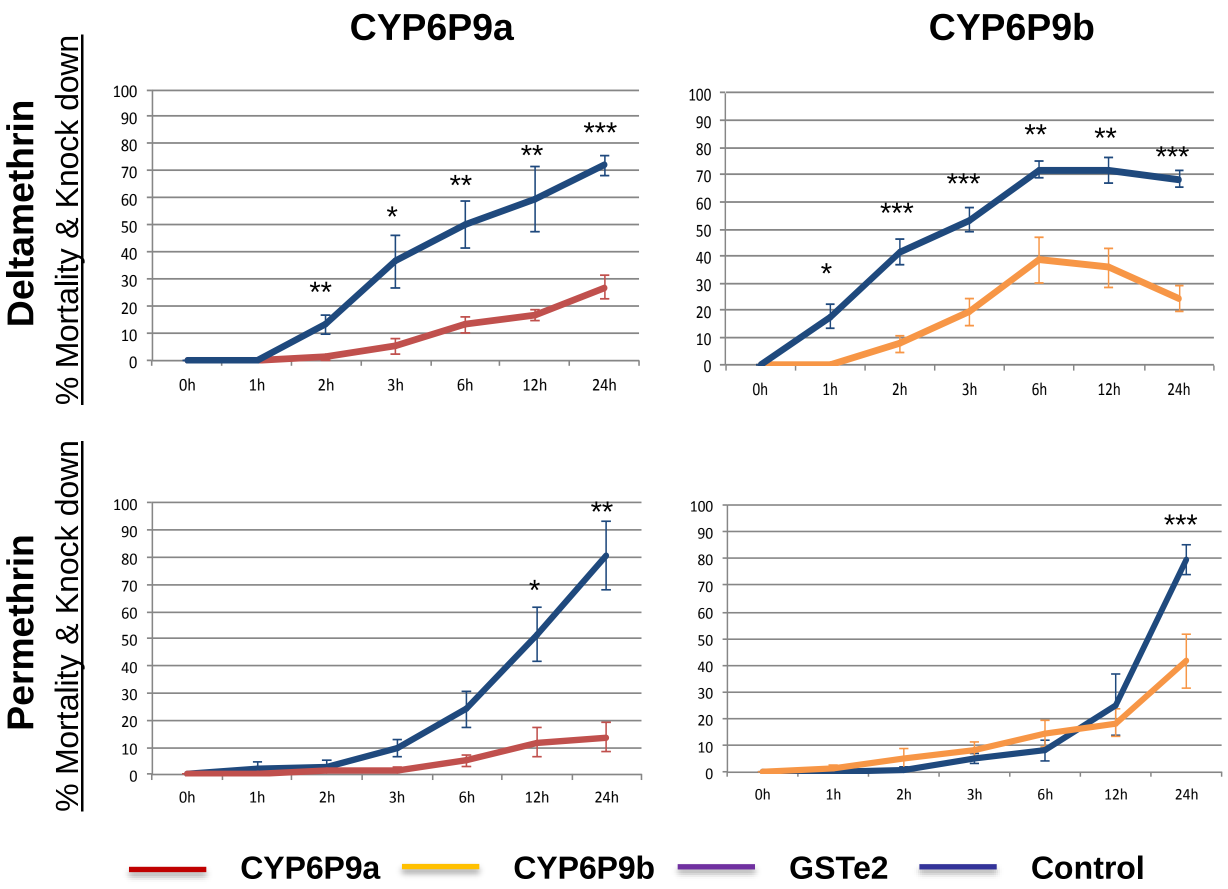
Current status - distribution of Pyrethroid Resistance Genes in *An gambiae* and *arabiensis*



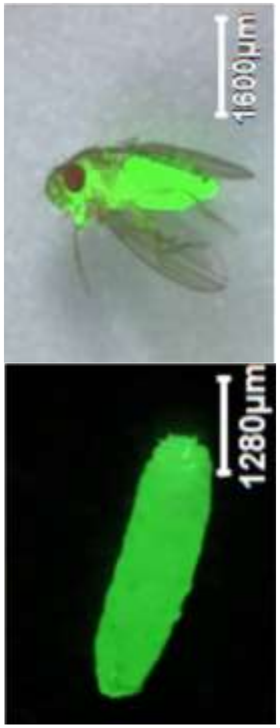
Kdr and metabolic resistance, mostly overlapping
Caution – limited set of data

Contributed by Chris Jones

Validation of *An funestus* resistance genes using transgenic *Drosophila melanogaster*



GAL4-Actin 5C
broad tissue expression

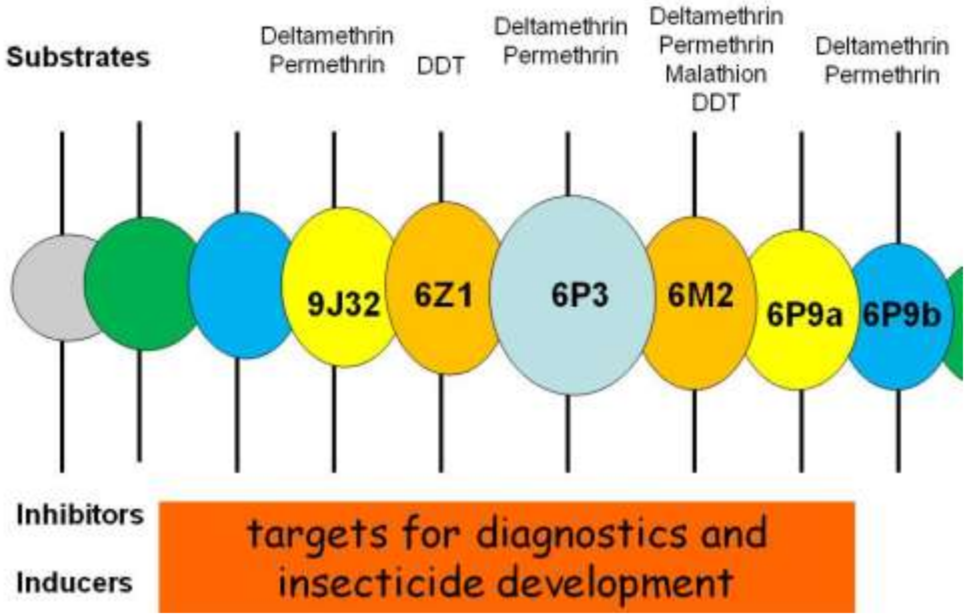


Shen et al; 2009

Riveron et al, 2013 PNAS.

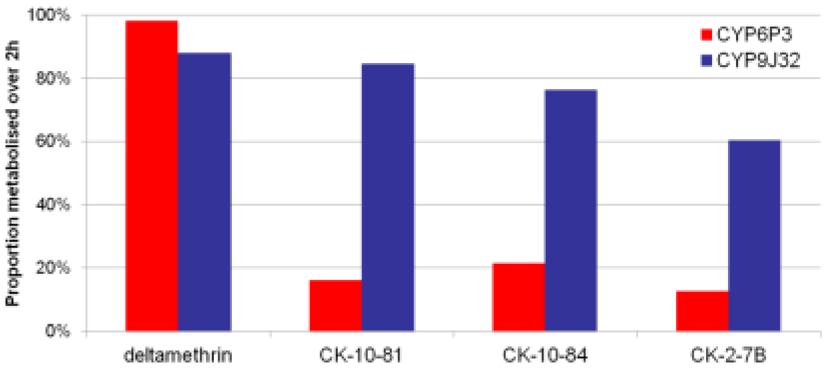
In progress:
CYP6M4 and 7
CYP9J11

✓Core metabolic resistance genes for pyrethroids have been identified



Commercially Available

Mosquito CYP6P3, 6P9a, 9J32 from LSTM/LITE
Mammalian from Cypex Ltd, Dundee



using CYPs to validate pyrethroid SAR (Ward, O'Neill, Hong)

Variation in pyrethroid resistance across Africa

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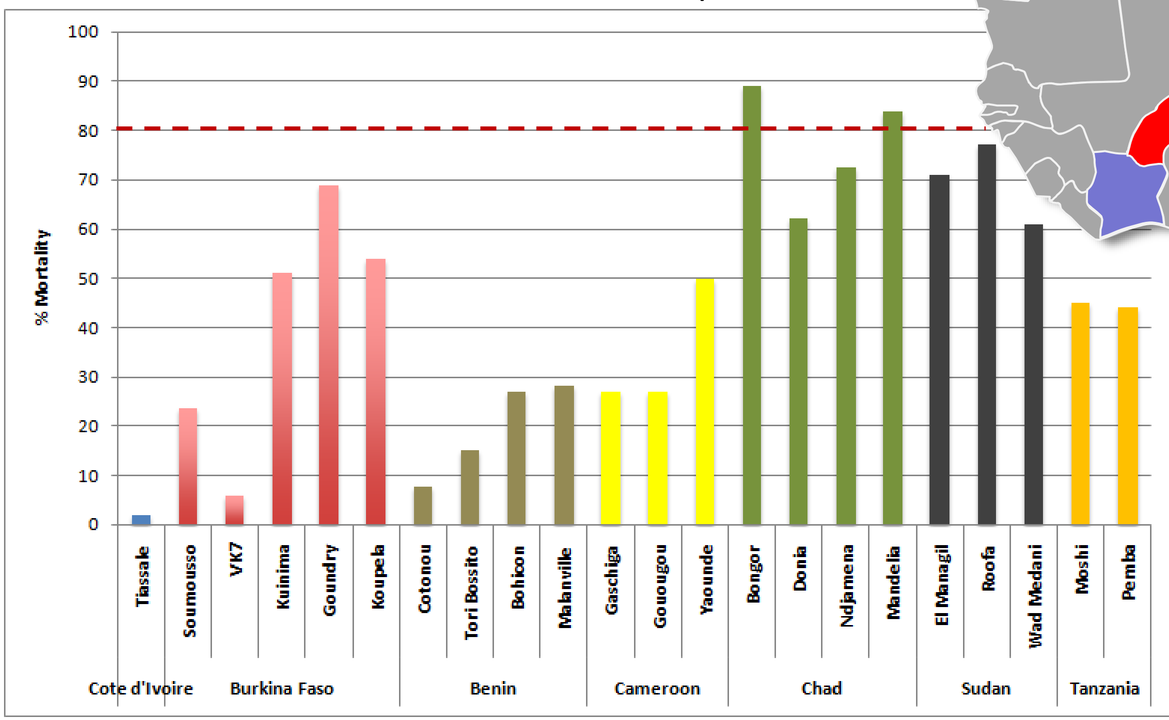
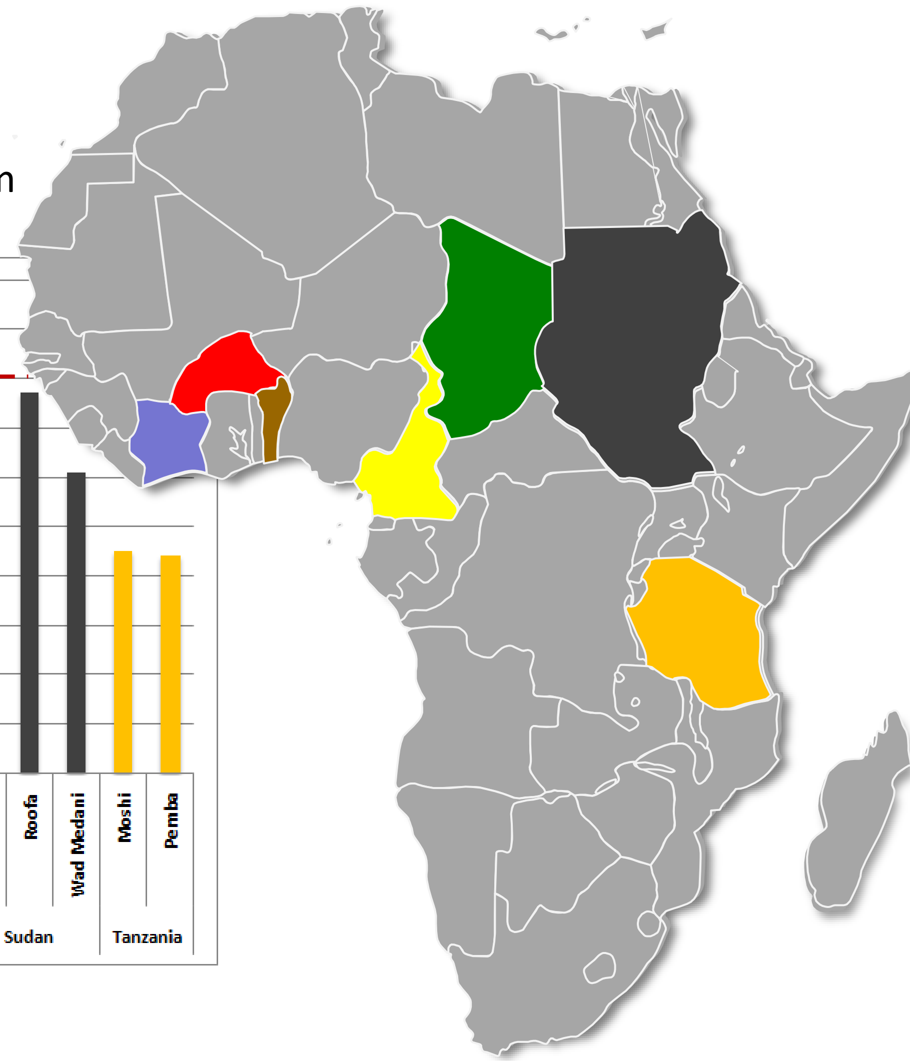
Variation clearly exists

Permethrin Mortality in *An gambiae s.l.* from Field Studies in 2010/2011*





WHO criteria for ' insecticide resistance'

*Data from ZMCP, TDR Network, AvecNet, Vestegaard, MCPC

How does variation in metabolic resistance mechanisms lead to variations in resistance levels amongst insecticides from the same and different chemistries ?????

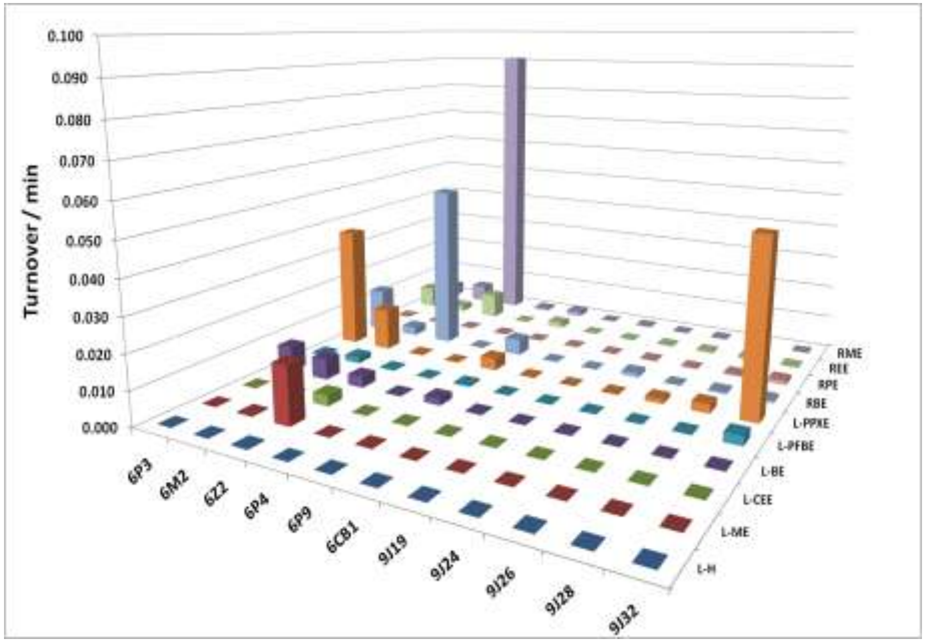
Work in progress

Bioassays

Strain	Insecticide	LD50	RR
Ngousso	Permethrin		
(An. gambiae)	Bifenthrin	0.04	
	Deltamethrin	0.0196	
	Lambda-cyhalothrin	0.017	
VK7	Permethrin	6.3246	
(An. gambiae)	Bifenthrin		
	Deltamethrin	0.2601	13.266
	Lambda-cyhalothrin		

Determine relative resistance levels

P450 profiling



Identification of diagnostic probes

Poupardin unpublished

Stevenson et al 2012 PlosNTD

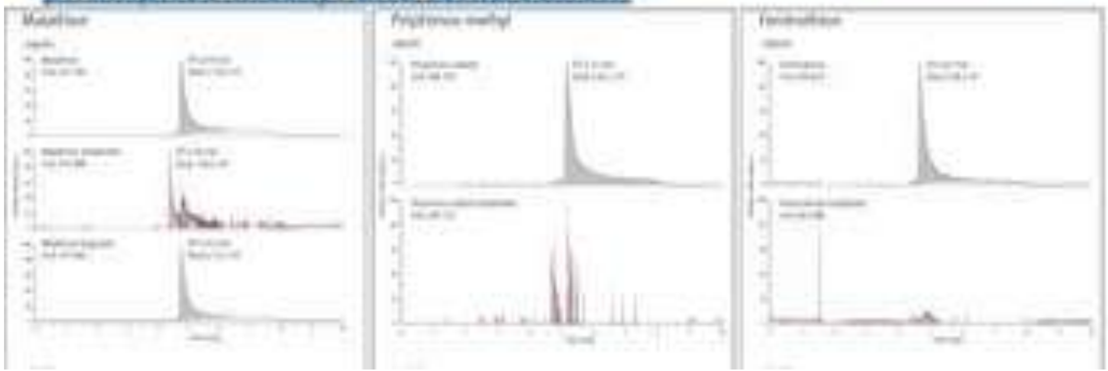
Profile of CYP6M2 interactions with WHO insecticides

Table 2. IC50 for CYP6M2 inhibition by 15 insecticides.

Compound	IC_{50} (μM)	Class
DDT	2.31 ± 0.56	OC
Chlorpyriphos	0.94 ± 0.19	OP
Diclorvos	> 10	OP
Fenitrothion	2.30 ± 0.72	OP
Malathion	0.14 ± 0.05	OP
Pirphimos methyl	1.22 ± 0.48	OP
Bendiocarb	> 100	C
Propoxur	> 100	C
Alpha-cypermethrin	0.32 ± 0.12	P
Bifenthrin	1.98 ± 0.37	P
Cyfluthrin	0.31 ± 0.08	P
Deltamethrin	0.51 ± 0.03	P
Etofenprox	1.84 ± 0.67	P
Lambda-cyhalothrin	1.04 ± 0.26	P
Permethrin	1.19 ± 0.25	P

n = 3 different CYP6M2 preps. OC Organochlorine, OP, organophosphate, C, carbamate, P, pyrethroid

Fig 2. LC-MS analysis of CYP6M2 metabolism of malathion, pirimiphos methyl and fenitrothion.



Rapid inhibition screens provide an indication of susceptibility to metabolism

Voice *et al* Poster ISSX USA 2012

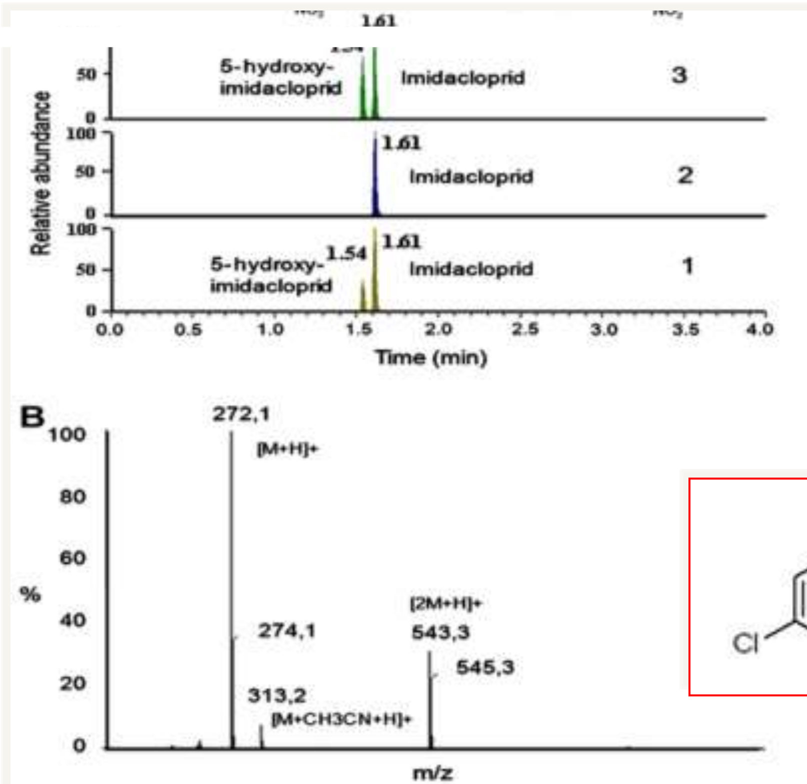
Cypex Ltd Dundee

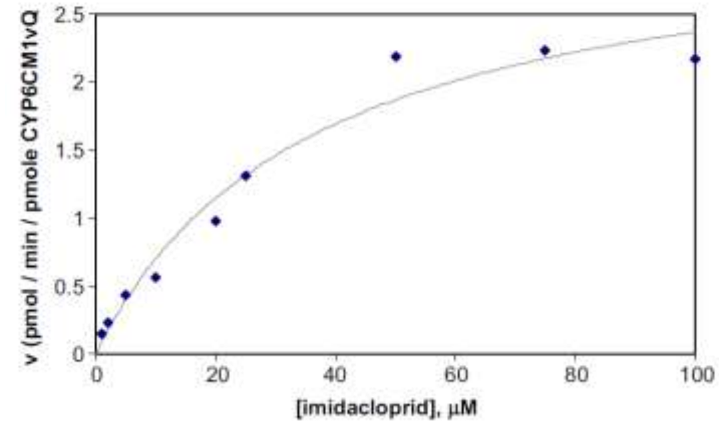
Core P450s: metabolism profile against WHO insecticides

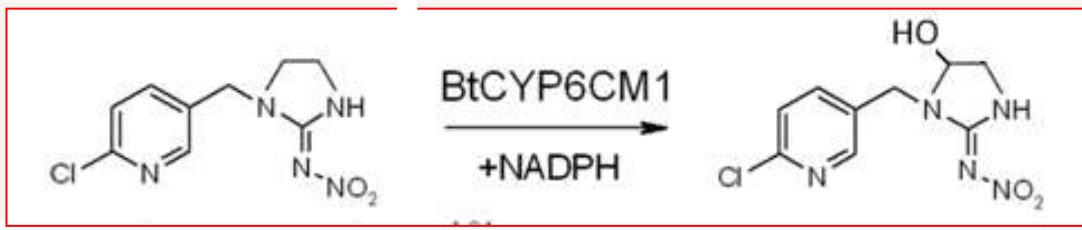
% Turnover

	An. gambiae			*An. funestus*		*Ae. aegypti*	
Insecticide	*CYP6M2*	*CYP6P3*	*CYP6Z2*	*CYP6P9a*	*CYP6P9b*	*CYP9J32*	*CYP9J24*
Deltamethrin	**60**	**92**	**15**	**74**		**90**	**30**
Permethrin	**49**	**98**	**20**	**85**		**61**	**76**
Lambda-cyhalothrin				**94**			
Bifenthrin		**98**	**0**	**78**		**25**	**0**
Alpha-cypermethrin	**88**	**99**	**0**	**74**			**3**
Etofenprox		**98**	**13**	**90**			**22**
DDT			**0**	**0**			
Malathion							
Bendiocarb							
Propoxur							

Identification of metabolite: the CYP6CM1vQ enzyme catalyses the hydroxylation of imidacloprid to its 5-hydroxy form with a high conversion rate







P450s play a key role in metabolic resistance in agricultural pests

Contents lists available at ScienceDirect

Insect Biochemistry and Molecular Biology 39 (2009) 697–706

journal homepage: www.elsevier.com/locate/ibmb

ELSEVIER

Structural model and functional characterization of the *Bemisia tabaci* CYP6CM1vQ, a cytochrome P450 associated with high levels of imidacloprid resistance

Iris Karunker[a,1], Evangelia Morou[b,c,1], Dimitra Nikou[b,c], Ralf Nauen[d], Rotem Sertchook[e], Bradley J. Stevenson[c], Mark J.I. Paine[c], Shai Morin[a,*], John Vontas[b,f,**]

Conclusions

- **Metabolic resistance clearly important factor in insecticide resistance**
- **As yet no easy diagnostic to determine its impact – reliance on microarrays**
- **However, starting to understand the core P450s involved in resistance**
- **Leading to new tools and Paradigms**