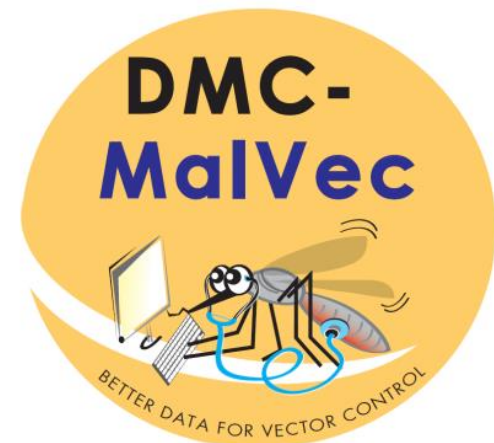


# Evidence based IRM: the value of molecular diagnostics and bioassays

John Vontas



12th Annual RBM VCWG, Geneva, Switzerland 9 February 2017

# Striking resistance phenotypes evolving ..

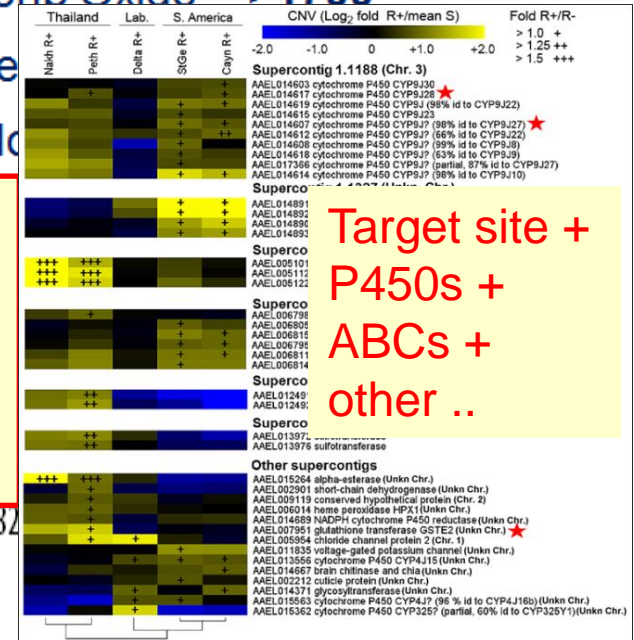


## Why ?

**Chemical** **RR**

Abamectin **1644**

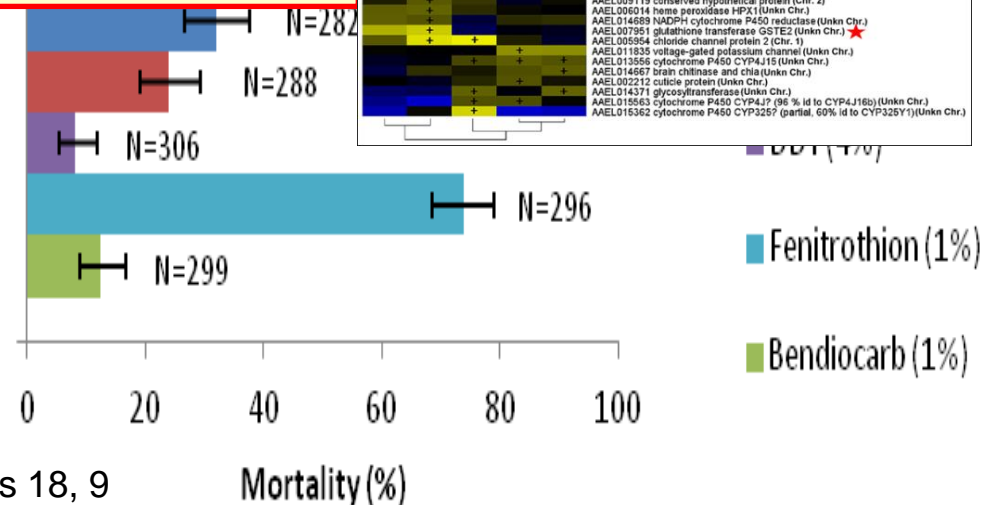
Fenb Oxide **>1700**



Target site +  
P450s +  
ABCs +  
other ..



Insecticide

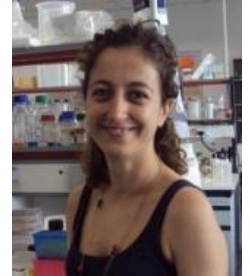
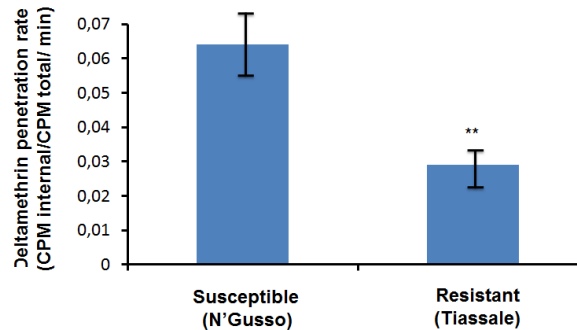


# Cytochrome P450 associated with resistance catalyzes cuticular hydrocarbon production: a mechanism that slows the uptake of pyrethroids, contributing to resistance and broadening the phenotype

Substantially reduced insecticide uptake in resistance insects



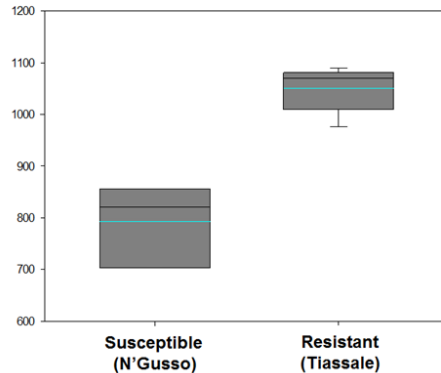
1



elevated epicuticular HCs

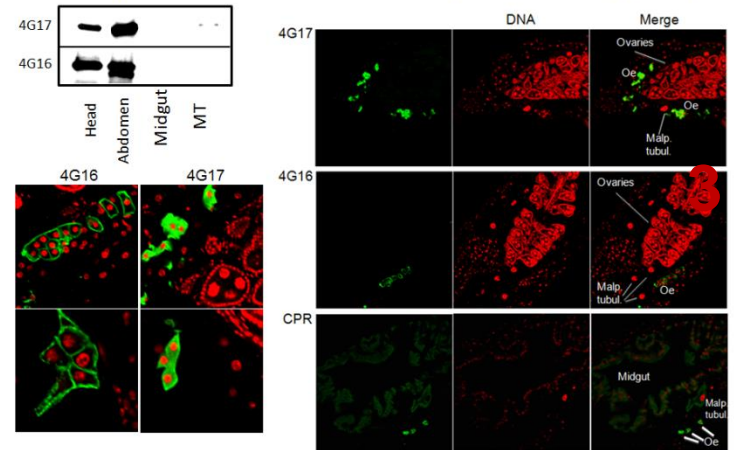
2

ng epicuticular HC/insect



CYP4G16/17 are over-expressed on the oenocytes of R mosquitoes and have decarbonylases activity (HC)

3



# Resistance mutation conserved between insects and mites unravels the benzoylurea insecticide mode of action on chitin biosynthesis

Vassilis Douris<sup>a,b,1</sup>, Denise Steinbach<sup>c,d,1</sup>, Rafaela Panteleri<sup>a,b</sup>, Ioannis Livadaras<sup>a</sup>, John Anthony Pickett<sup>e,2</sup>, Thomas Van Leeuwen<sup>f,g</sup>, Ralf Nauen<sup>c,2</sup>, and John Vontas<sup>a,h,2</sup>

Dec 2016, PNAS 113, 14692–14697



**Vassilis Douris**

A single mutation confers **striking** insecticide resistance against 3 different MoAs (Benzoylureas, buprofezin and etoxazole), which in fact share a common MoA (direct interaction with chitin synthase I)

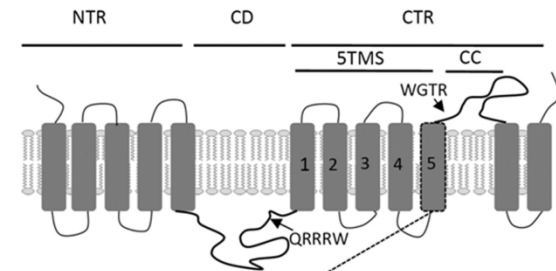
**Table 3:** Bioassay results (LC<sub>50</sub> values and associated resistance ratios) of genome modified flies (Et15, Px39) versus relevant unmodified controls (nos.Cas9) for five different insecticides

Insecticides	strains	LC <sub>50</sub> , ppm (CL95%)	Resistance Ratio
Etoxazole	Et15 (I1017F)	>10000	>1077
	Px39 (I1042M)	>10000	>1077
	nos.Cas9	9.28 (0.73 - 14.00)	1
Diflubenzuron	Et15 (I1017F)	>5000	>15625
	Px39 (I1042M)	>5000	>15625
	nos.Cas9	0.32 (0.24 - 0.42)	1
Lufenuron	Et15 (I1017F)	16.66 (8.70 - 66.47)	111.06
	Px39 (I1042M)	>20	>133
	nos.Cas9	0.15 (0.11 - 0.18)	1

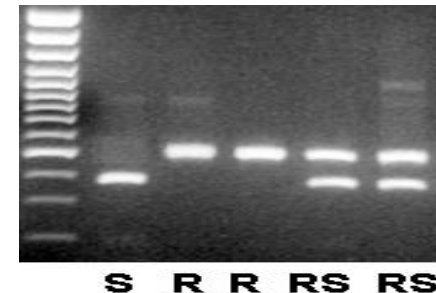
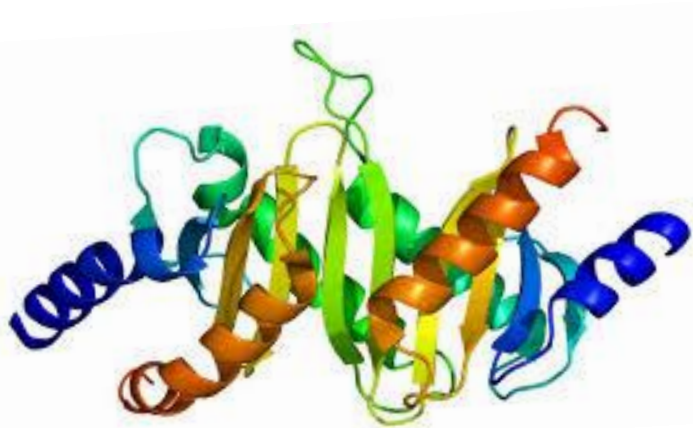
!



# Molecular analysis of resistance, provides markers for diagnostics

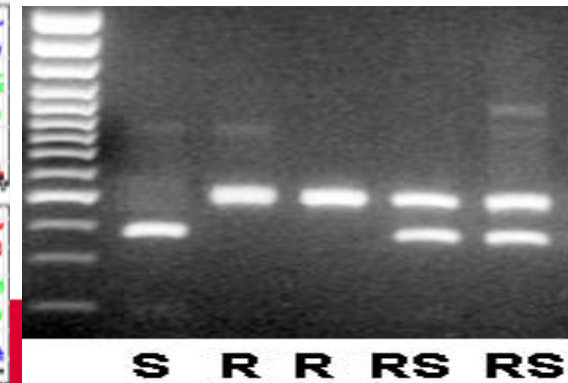
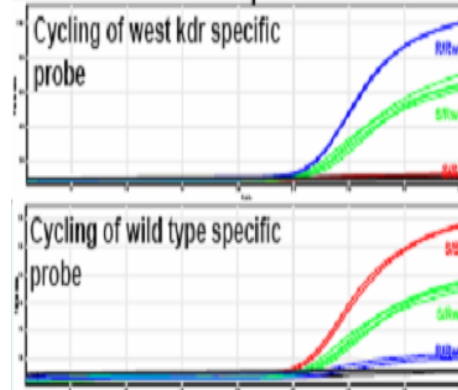
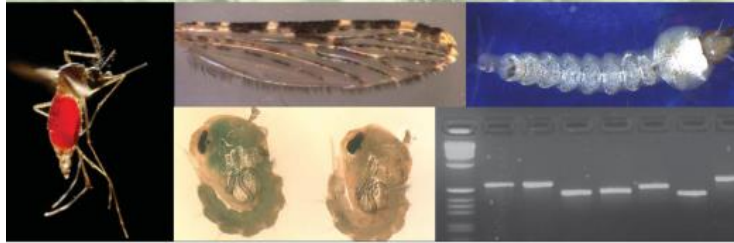


	TMS-5																															
Consensus	V	P	G	I	I	Y	L	L	S	X	P	S	M	Y	L	L	I	L	Y	S	I	I	N	L	N	V	S	W	G	T	R	
DmChs1 ( <i>kkv</i> )	T	C	.	L	.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-1079
MsChs1	.	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-1063
PxChs1-BCS-S	.	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-1065
PxChs1-Sudlon	.	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-1065
PxChs1-Sud-Tfm	.	.	.	.	.	.	.	.	.	.	M	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-1065
PxChs1-Japan	.	.	.	.	.	.	.	.	.	.	M	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-1065
PxChs1-India	.	.	.	.	.	.	.	.	.	.	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-1065
PxChs1-China	.	.	.	.	.	.	.	.	.	.	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-1065
TuChs1S	Y	.	O	L	L	.	F	.	.	I	.	C	.	.	.	M	I	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-1040
TuChs1R	Y	.	C	L	L	.	F	.	.	F	.	C	.	.	.	M	I	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-1040





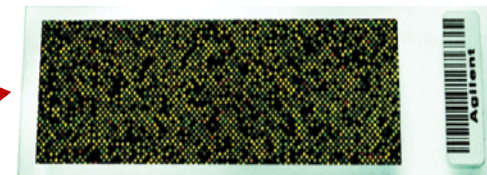
# Methods in *Anopheles* Research



Accurate & early detection,  
mechanism,  
dried specimens

But lack of clear links with decision making ....

- “Sophisticated” platform – multiple protocols
- Predictive value of target site mutations / markers?
- Markers for **metabolic resistance**? (RNA markers?) →
- high cost: 10 markers, the cost is approximately 15-20 USD per mosquito !! (1500-2000 / population)
- Data interpretation more challenging



Weetman and Donnelly (2015),  
Trans Roy Soc Trop Med Hyg 105,

# Stick with bioassays only for IRM ?



Maybe not ...  
Molecular diagnostics are highly complementary tools, to support, and substantially improve, evidence – based IRM

## Discriminatory

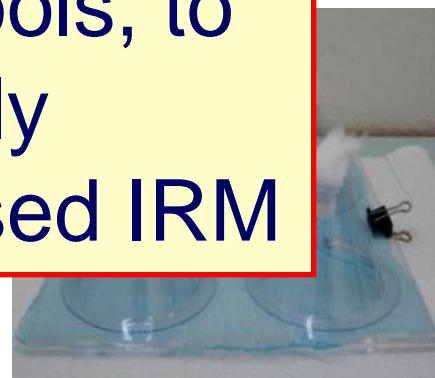
- ✓ Detect presence
- ✓ Estimate proportion in the population

## Quantitative CDC Bioassays

Intensity of resistance (strength)

Closer to operationally relevant data

- Number of mosquitoes, cross resistance,
- Late resistance detection, no mechanism



# Molecular diagnostic can detect underlying mechanism: an important evidence for IRM

Insecticide	Class	B. tabaci
		CYP6CM1
Imidacloprid	Neonicotinoids	+++
Thiacloprid		+++
Acetamiprid		No
Thiamethoxam		No
Flonicamid	Pyrid/carbox	++
Pymetrozine	Pyrid/azometh	++
Spirodiclofen	Ketoenols	
Spiromecifen		
Spirotetramat		
Abamectin	Macr. Lact.	
Bifenthrin	Pyrethroids	
Cypermethrin		no

✓**CYPs: Not always predictable**  
**cross spectrum catalytic efficiency**



The Cytochrome P450 from *B. tabaci* metabolises imidacloprid and thiacloprid, but not acetamiprid or thiamethoxam; CYP6CM1 also metabolises pymetrozine (unrelated)





# BIOKO Island: One of the most well organized vector control programs in Africa

## Country-level operational implementation of the Global Plan for Insecticide Resistance Management

Janet Hemingway<sup>a,1</sup>, John Vontas<sup>b</sup>, Rodolphe Poupardin<sup>a</sup>, Jaishree Raman<sup>c</sup>, Jo Lines<sup>d</sup>, Chris Schwabe<sup>e</sup>, Abrahan Matias<sup>e</sup>, and Immo Kleinschmidt<sup>d</sup>

<sup>a</sup>Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom; <sup>b</sup>Department of Biology, University of Crete, 71409 Heraklion, Greece; <sup>c</sup>Malaria Research Unit, South African Medical Research Council, Durban 4001, South Africa; <sup>d</sup>London School of Hygiene and Tropical Medicine, London WC1E 7HT, United Kingdom; and <sup>e</sup>Medical Care Development International, Bioko Island Malaria Control Project, Equatorial Guinea

Contributed by Janet Hemingway, April 26, 2013 (sent for review February 16, 2013)

Malaria control is reliant on the use of long-lasting pyrethroid-

Bioko, the main island of Equatorial Guinea, has a population

Use of Bendiocarb IRS for many years

Rotation with pyrethroids, but what if resistance appears, can we plan

Ace inhibitors IRS, even if bioassays don't show resistance ?

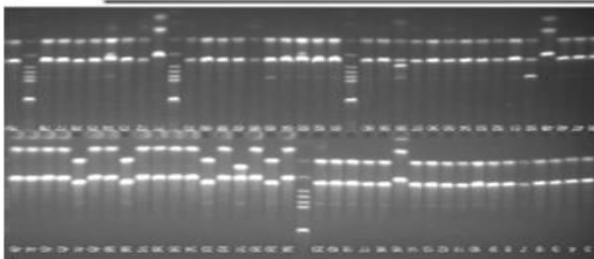
### Molecular data for evidence based IRM:

- ✓Is insensitive acetylcholinesterase (*ace-1<sup>R</sup>* mutation) present in the island, even at very low frequency?
- ✓Is resistance due to pyrethroid metabolisers, which also detoxify bendiocarb/OPs?

# Bioassays vs molecular diagnostics

Highly complementary not mutually exclusive tools, which should both be used to support evidence based IRM

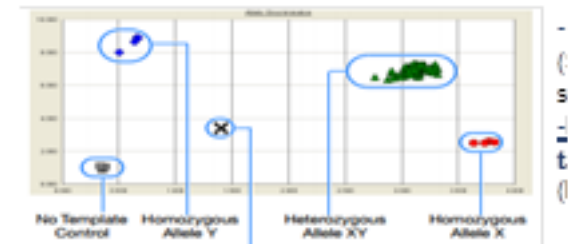
# Molecular assays: individual mosquitoes or pools? (for IRM)?



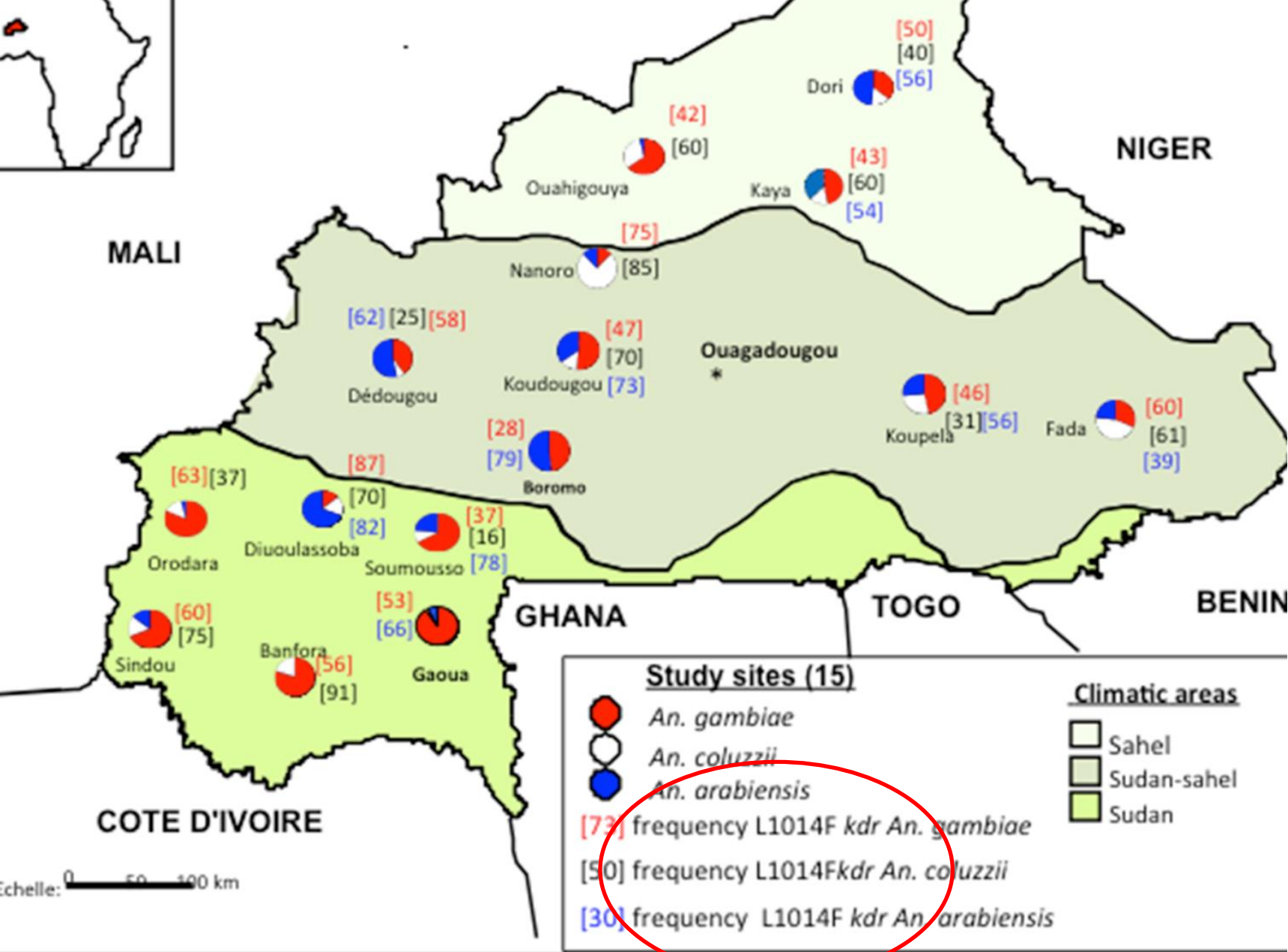
?



~20% KDR S  
~75% KDR W  
~ 5% KDR E



Genotype	BS	GG	GL	HC	HZ	LZ	NN	WZ	YL
1014 L/L	94.6	6.7	29.1	77.0	5.3	18.5	91.3	41.6	73.4
1014 L/F		6.7	15.7	2.1		25.9		4.2	5.7
1014 L/S	5.4			18.8		3.7	8.7	20.9	15.2
1014 L/C		20.0	26.3	2.1	31.6	22.2		25.0	5.7
1014 F/F						3.7			
1014 C/C		60.0	15.8		42.1	22.3		8.3	
1014 F/C		6.7	13.1		21.0	3.7			
Size (N)	55	15	38	48	19	27	35	24	52





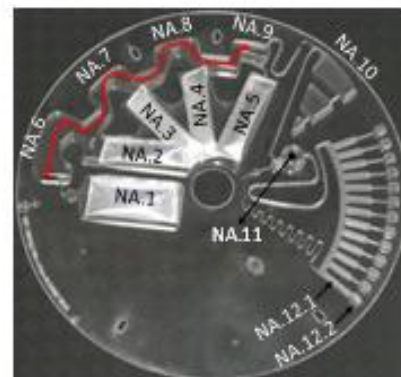
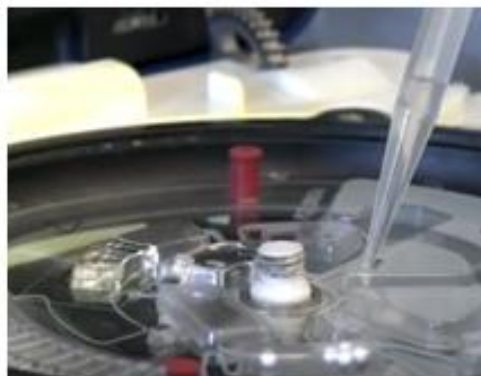


Mosquito  
pools

Species ID,  
Infection status  
**IR (target & metabolic)**



Step 2: Sample loading to the LabDisk



INFRAVEC2 — Horizon2020

**LabDisk:** sample-to-answer for monitoring the species ID, the infection status and **the insecticide resistance profile (incl. metabolic) of mosquito pools.**

**.. Please visit our poster ..**

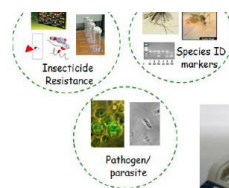
# Diagnosis Management Communication – Malaria Vector Control



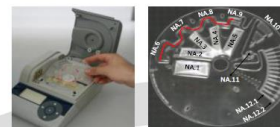
Horizon 2020  
2016-2019



«Automated diagnostic platform, data management and communication tool, for improving the impact of mosquito vector control interventions»



The LabDisk



A fully integrated automated diagnostic platform, to monitor mosquito population

The challenges



Malaria: 500,000 deaths/y;  
Insecticides: best control tool;  
Vector monitoring: prerequisite

**MALARIA CONTROL**

DDMS: Disease Data Management System



Bringing data together, to make informed decisions



Serious GAME



Effectively communicating vector disease data & guidelines to national malaria control programmes

Optimize impact of interventions



Swiss TPH

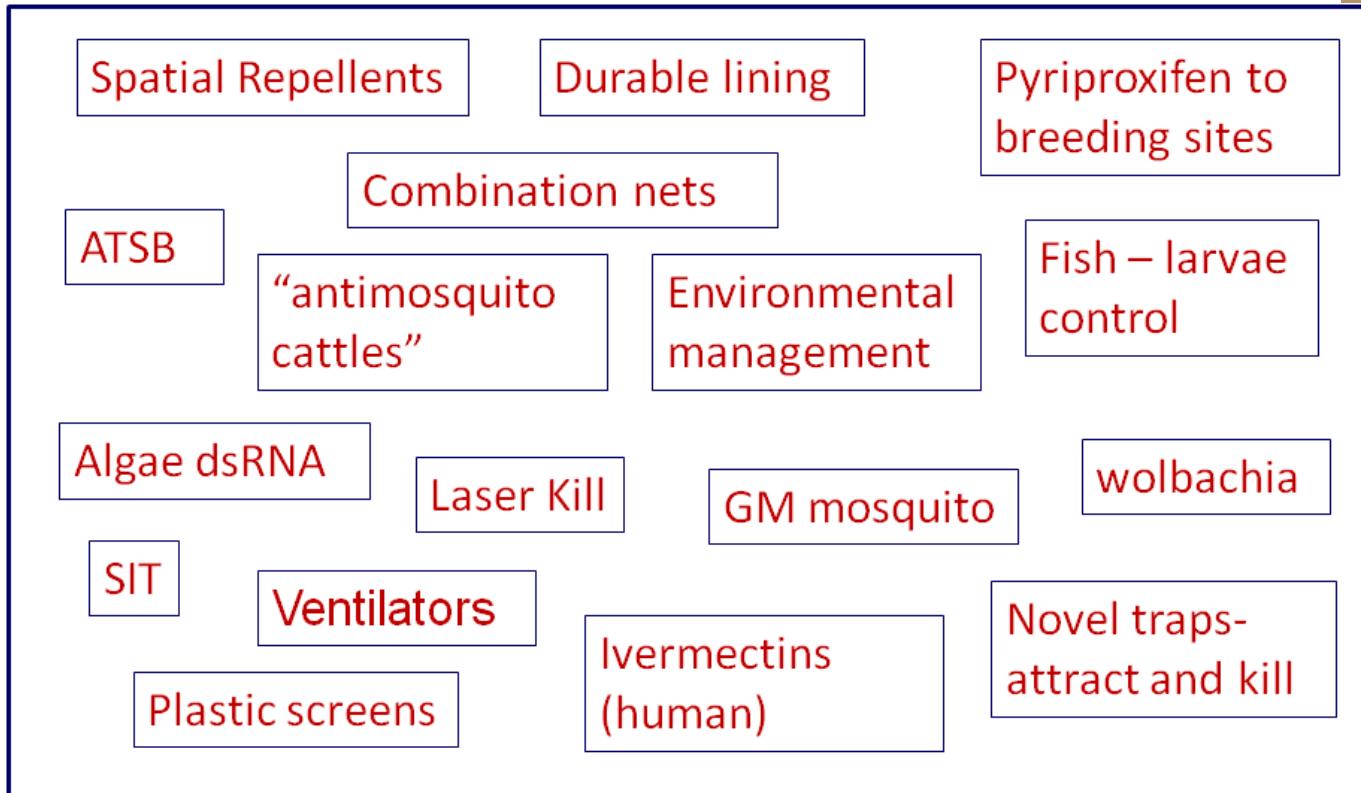
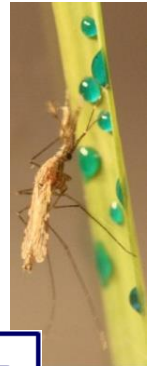
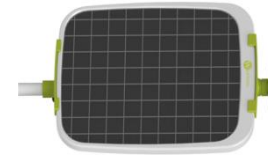
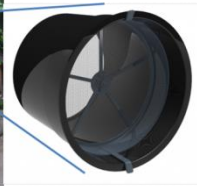
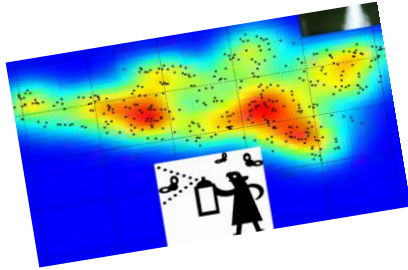


JIMMA UNIVERSITY  
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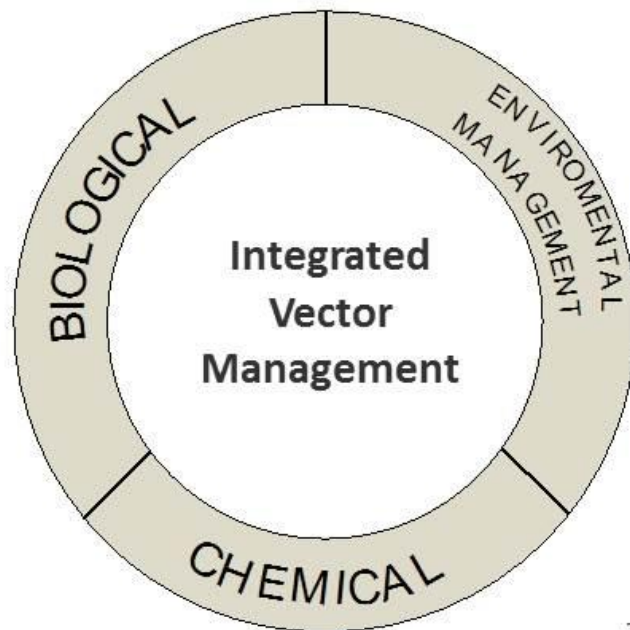
**CSRS**  
Centre Suisse de Recherches  
Scientifiques en Côte d'Ivoire



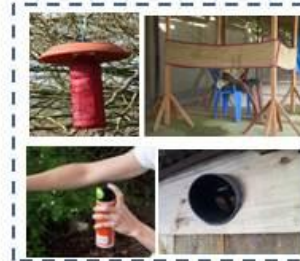
# Introduction of new vector control tools & IRM



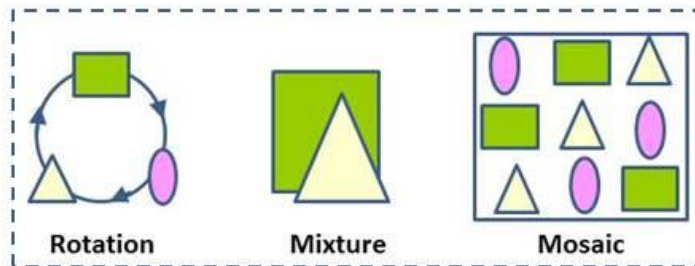




Right intervention for the right context



Evidence based  
Insecticide Resistance  
Management



Coordinate with  
tactics in agriculture



# Lab - People



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## Collaborators



Swiss TPH



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## Funding

EU Horizon2020

INFRAVEC2

