The Academy of Medical Sciences

International Health Lecture 2012

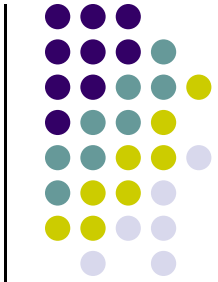
Health impacts of product development partnerships

Professor Janet Hemingway FRS FMedSci
Director of the Liverpool School of Tropical Medicine

The Academy of Medical Sciences

The Impact of PDPs on Health

Malaria Focused PDPs



Medicines for Malaria Venture

Malaria Vaccine Initiative

Innovative Vector Control Consortium

IVCC
COMBATING INSECT BORNE DISEASE

- **Why do we need Product Development Partnerships**
- **The role of PDPs in Vector Borne Disease**
- **Progress in new Vector Control Interventions**

Market Rupture Needed

	1956	2006
Music:	***Vinyl Disc***	  ***CD and MP3***
Telephone:	***Wire Phone*** 	 ***Mobile+Camera+***
Television:	***Cathode Ray Tube*** 	 ***Plasma/LCD HD***
IRS:	***Hudson Sprayer WP formulation 2000 mg DDT/m²***  	  ***Hudson Sprayer WP formulation 2000 mg DDT/m²***

BMGF supports IVCC as the leading Product Development Partnership in Vector Control

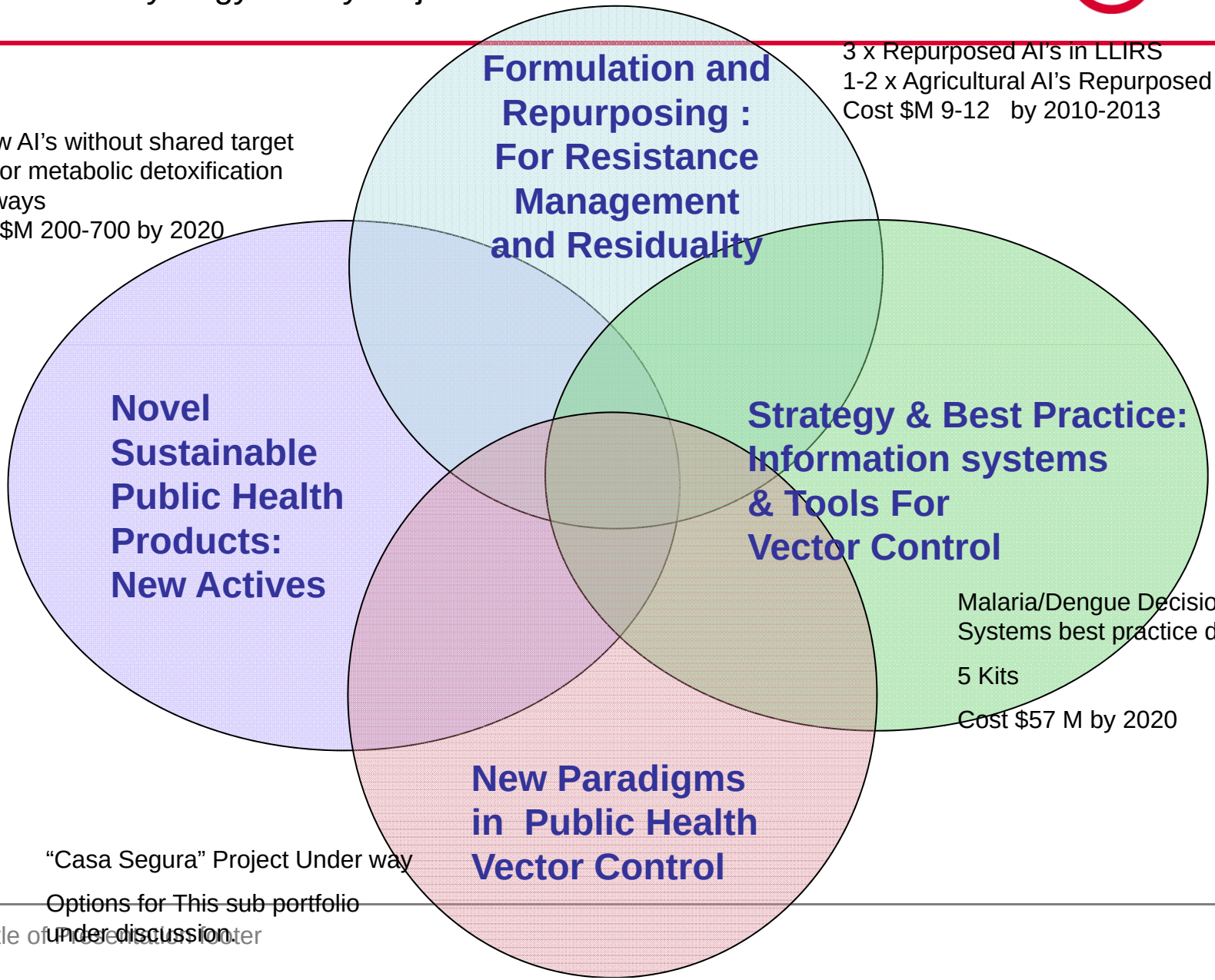
"Your promising research, and the rising concern of people around the world, represent an historic opportunity not just to treat malaria or to control it—but to chart a long-term course to eradicate it"

- BMGF / Boston Consulting Group Study
  - Eradication will cost $ 4-6 Bn per year until it is complete.
  - Vector Control is ~30 % of that.

The Ideal Portfolio;
Synergy of Key Objectives

3 x Repurposed AI's in LLIRS
1-2 x Agricultural AI's Repurposed in LLIN
Cost $M 9-12 by 2010-2013

3 New AI's without shared target sites or metabolic detoxification pathways
Cost $M 200-700 by 2020



Malaria/Dengue Decision Support Systems best practice disseminated.

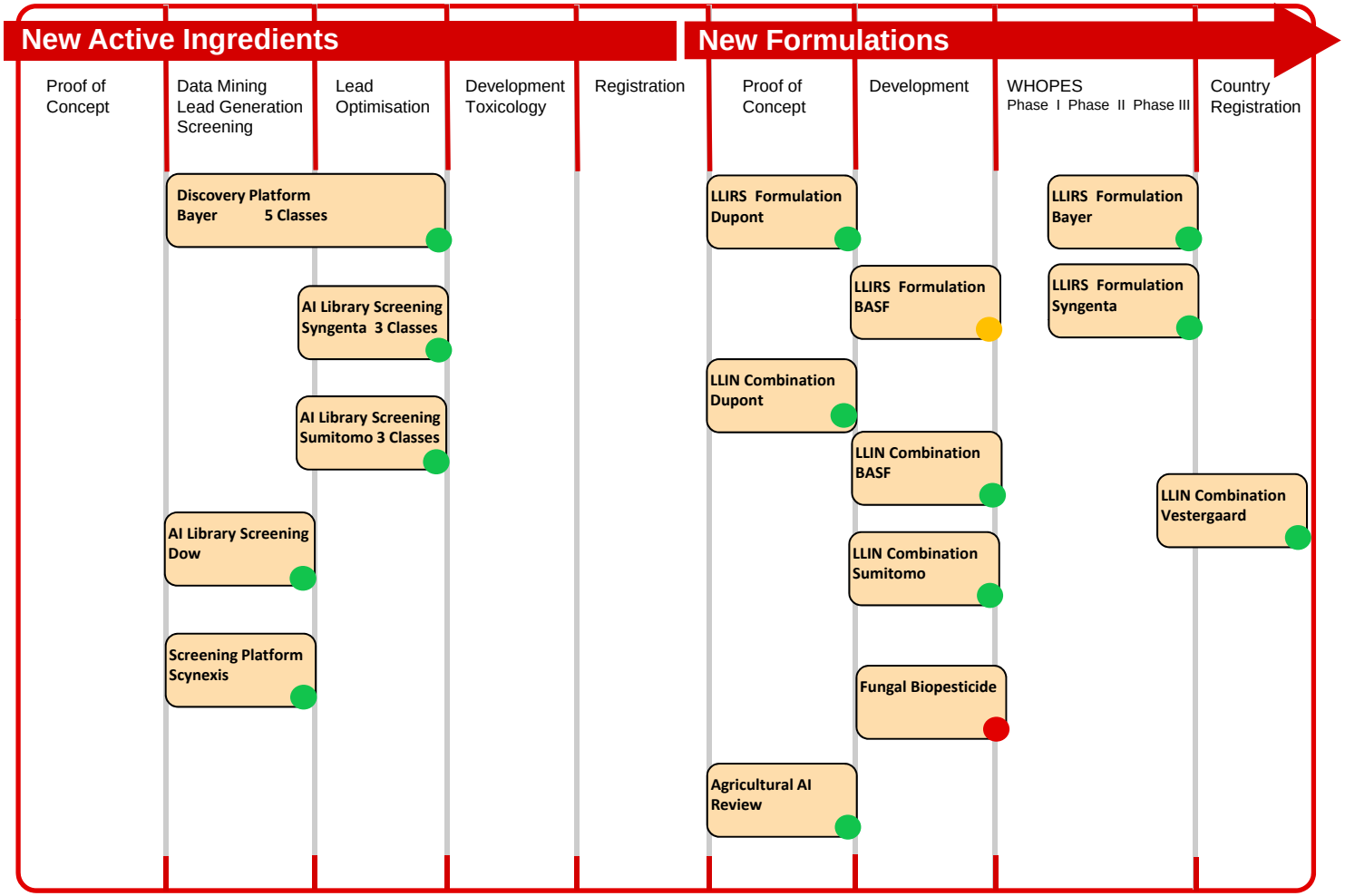
5 Kits

Cost $57 M by 2020

"Casa Segura" Project Under way

Options for This sub portfolio under discussion.

IVCC Public Health Insecticides Portfolio



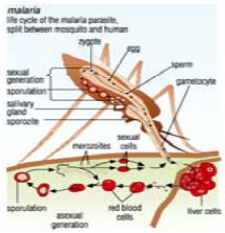
How do we assess value?

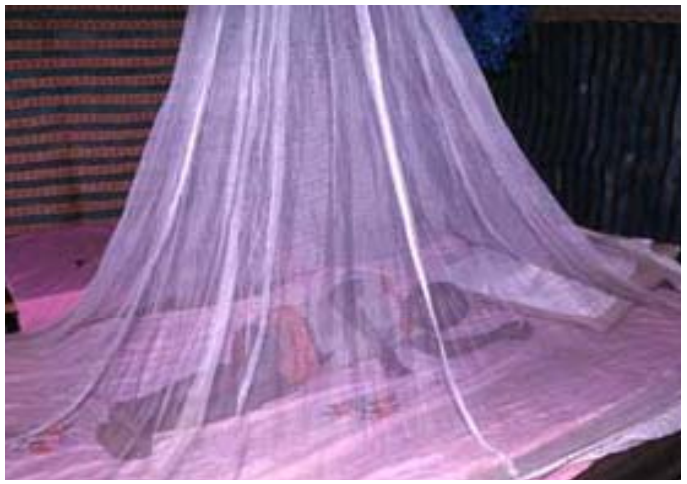
The long term role of competition

Cost of Goods Example

DDT → Malathion → Pyrethroids

Insecticide treated nets (ITNs) - indoor residual spraying (IRS):
major control measures in the fight against malaria.





Vector Data and insecticide Quality Assurance:
vital elements in planning successful - sustainable interventions

The IVCC is funding the development of diagnostic tools for these traits
which can dramatically improve insecticide based malaria control

The IVCC is sponsoring the development of Simple tests for measuring insecticides *in situ.*

IVCC
COMBATING INSECT BORNE DISEASE
The Innovative Vector Control Consortium

As - Is

Uganda 2009






Days
Variation
Complicate
Expensive

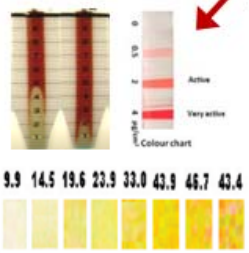
IRS - QC

No.	City	Area/Loca	Hom	Surface	DDT
					mg/m2
1	Kwazulu	Ophansi	14	Cement (unplastered)	48,0
2	Kwazulu	Ophansi	178	Cement (painted)	11,3
3	Kwazulu	Ophansi	178	Cement (unpainted)	79,3
4	Kwazulu	Ophansi	28	Mud (house)	17,8

To be







Quick
Accurate
Easy
Cheap

Detection technologies

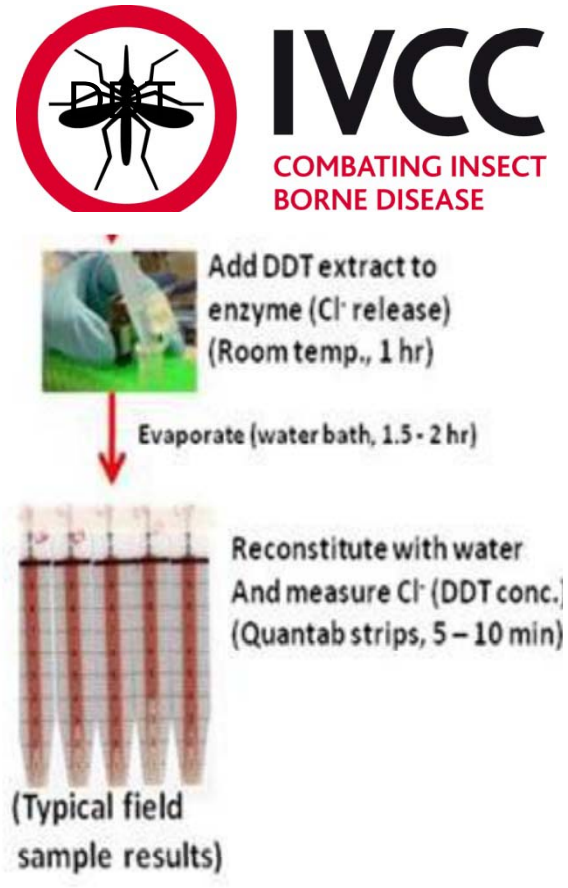
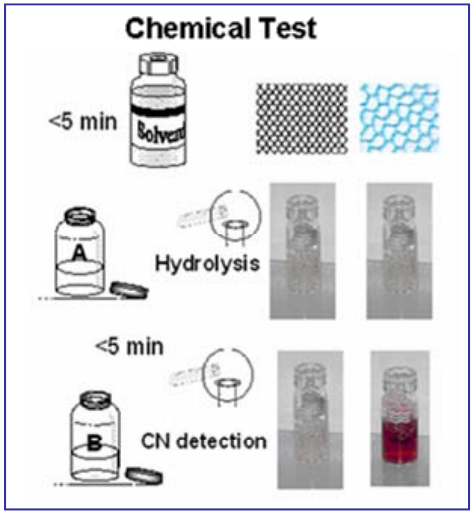
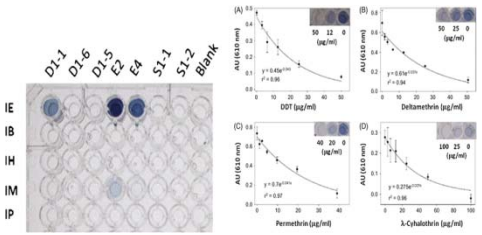


Fig 2. Basic components of the DQK

Morou et al (2008) Anal Biochem **378**, 60-64

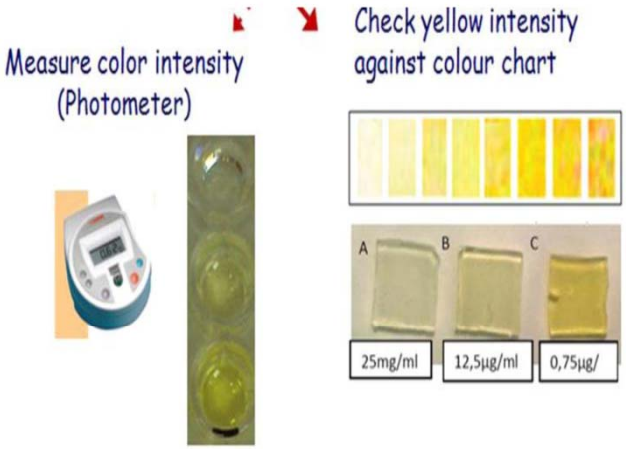
Pyrethroids (type II)





Kaur et al, MIM 2009
Dowd et al 2009

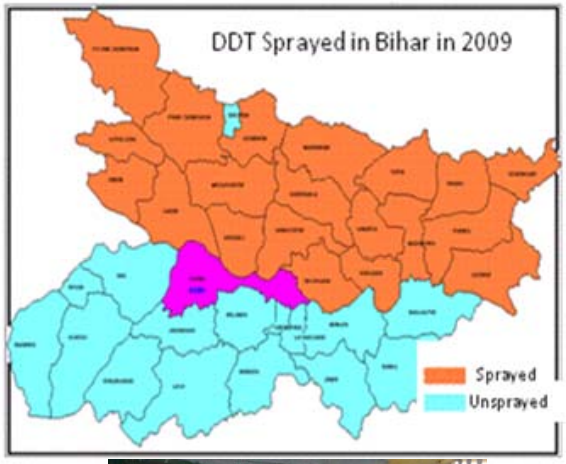
OPs / Carbamates

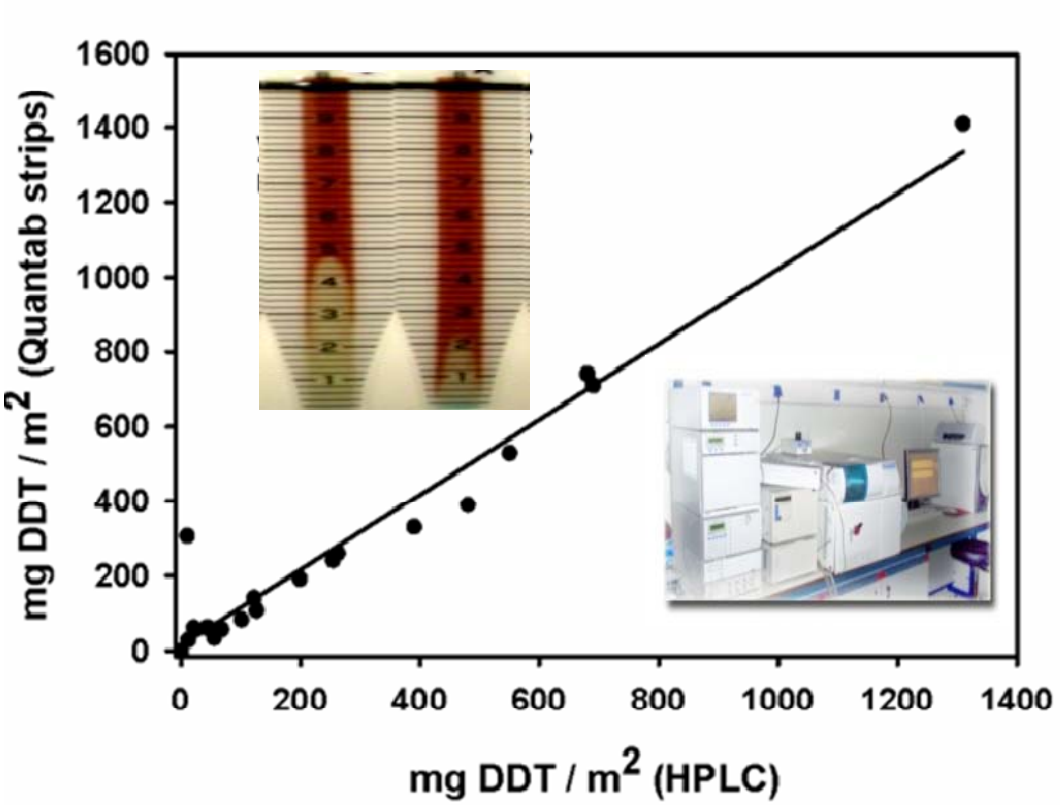


Morou, Vontas et al (in preparation)

Evaluation of the GST-based biosensor DDT “Kit” in the field: WHO – India & Africa

(Leishmaniasis – sandflies)



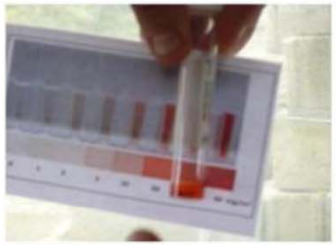


Pad Removal

House #1:

Bad spraying

Lambda Measurement



House #6

Good spraying

Heat Map of Insecticide Concentration

		Individual Pads and Wall Position								
Pooled	House	High			Middle			Low		
3	1	3	3	5	3	20	3	3	3	3
10	2	10	10	20	3	5	5	20	5	10
30	3	30	30	20	30	30	30	30	20	30
30	4	20	10	20	20	30	30	30	20	20
20	5	20	20	20	5	5	20	20	10	20
20	6	20	20	20	20	20	5	20	40	20
20	7	30	10	20	20	20	20	30	10	10
30	8	30	20	20	20	20	20	20	30	30
30	9	30	20	30	20	30	20	20	40	30
30	10	20	20	10	20	30	30	40	40	30
20	11	40	10	50	20	30	10	20	10	20
20	12	10	5	20	30	20	20	10	20	20
30	13	10	1	30	20	20	10	20	20	3
20	14	10	20	20	10	40	30	20	5	3
20	15	3	20	5	10	0	10	0	3	0
20	16	30	30	10	40	30	20	20	1	5
10	17	20	5	3	3	20	20	20	20	10
30	18	30	20	20	20	30	10	20	40	20
20	19	10	10	10	5	30	30	30	10	10
20	20	5	10	10	10	10	20	10	5	40
20	21	20	5	5	20	30	10	20	40	20
20	22	10	20	10	20	20	30	30	20	20
30	23	10	30	40	20	30	20	40	30	20
30	24	20	10	20	30	5	10	30	20	40
30	25	20	30	40	30	30	20	30	30	40
30	26	30	30	30	20	20	30	20	20	10
30	27	10	20	10	10	20	40	30	30	30
30	28	20	20	20	30	20	30	40	40	20
40	29	30	40	30	40	40	30	40	40	30
40	30	40	30	40	40	30	30	20	40	30

Insecticide (lambda cyhalothrin) content measured on small (R=1cm) pads (9/house) by IQK the day after spraying. Representative bad (House #1) and good (House #6) spraying is shown, as well as pads being removed and lambda cyhalothrin content measured. Heat map on right indicates some patchy spraying (i.e varied concentrations at individual positions), however, **85%** of averaged (pooled) concentrations of houses (N=30) were sprayed at or above required rate (20 mg/cm^2) indicating effective coverage. Mop-up operations to include re-spraying House #1, identify spray teams responsible for sub-optimal spraying and consider retraining if poor quality consistently linked with a particular team(s).

The IQK for bendiocarb was integrated into the routine surveillance of the Bioko Island vector control program

<u>WP 1: Quality control of IRS intervention using IQK, to inform the BIMCP-EGMCI about the effectiveness of the activity</u>

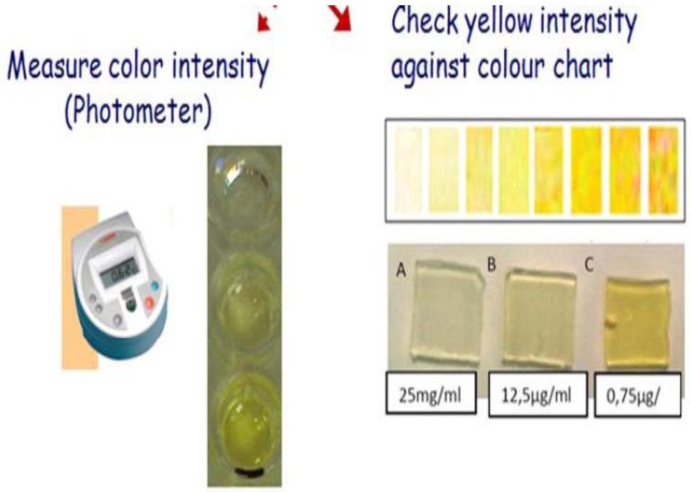
More than 1500 samples from both pre and post sprayed surfaces were taken and analyzed at Bioko

IQK vs HPLC for measuring insecticide from IRS

v. good correlation; IQK doesn't have the ability to calculate the exact amount of insecticide, but it can detect the walls that have insecticide above certain thresholds

House		**IQK test** ($\mu g/cm^2$)	**HPLC** ($\mu g/cm^2$)
House no 0097534 (b.s.)	Living room high	<0,1	0,1
House no 0097534 (b.s.)	Living room medium	<0,1	0,13
House no 0097534 (b.s.)	Living room low	<0,1	0,04
House no 0097534 (b.s.)	Bedroom high	<0,1	0,01
House no 0097534 (b.s.)	Bedroom medium	<0,1	0,01
House no 0097534 (b.s.)	Bedroom low	<0,1	0,01
House no 112898	Living room	1-5	3,3
House no 112898	Bedroom	1-5	2,4
House no 162544	living room	1-5	4,6
House no 162544	Bed-room	1-5	0,9
House no 0097534 (a.s.)	Living room high	>10	27,89
House no 0097534 (a.s.)	Living room medium	>10	23,48
House no 0097534 (a.s.)	Living room low	>10	28,64
House no 0097534 (a.s.)	Bedroom high	>10	28,13
House no 0097534 (a.s.)	Bedroom medium	>10	24,82
House no 0097534 (a.s.)	Bedroom low	>10	26,17

The IQK was found easy to use by spray supervisors



feedback for further simplification / optimisation obtained (easy of use; most directly informative scale)

Vector Population Monitoring

- Species
- Infection status
- Insecticide Resistance status

Infection and insecticide resistance detection in *Anopheles gambiae* and *Aedes aegypti*

Phenotype

Candidate

Validate

Technology

Field test

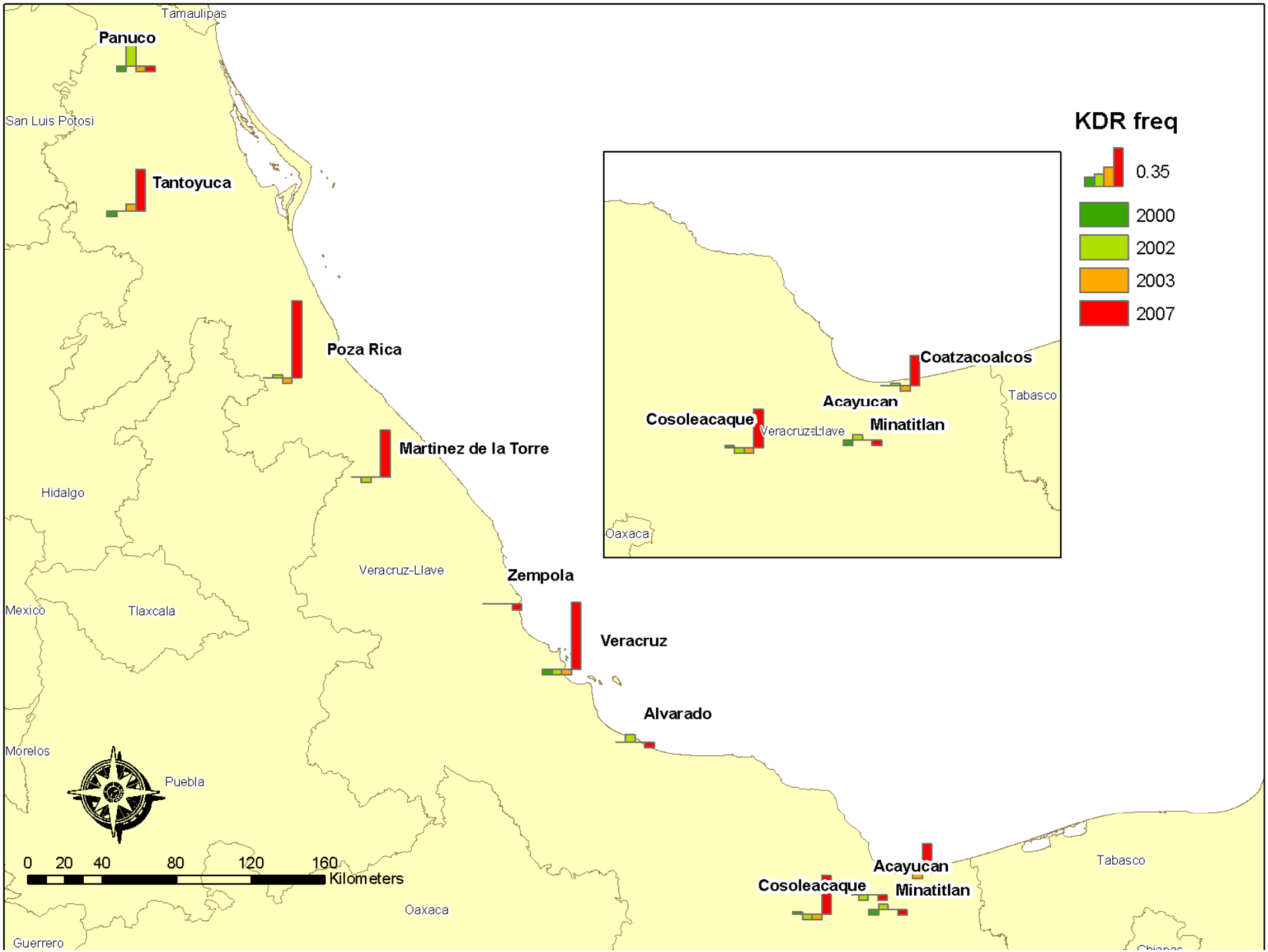
Phenotype populations for insecticide resistance

Identify candidate loci associated with insecticide resistance
Gene expression group- microarrays, qPCR
Genotyping group- association mapping

Validate that the candidates are the likely cause of resistance
Enzyme characterization group

Develop DEC appropriate technology for detecting the validated resistance locus

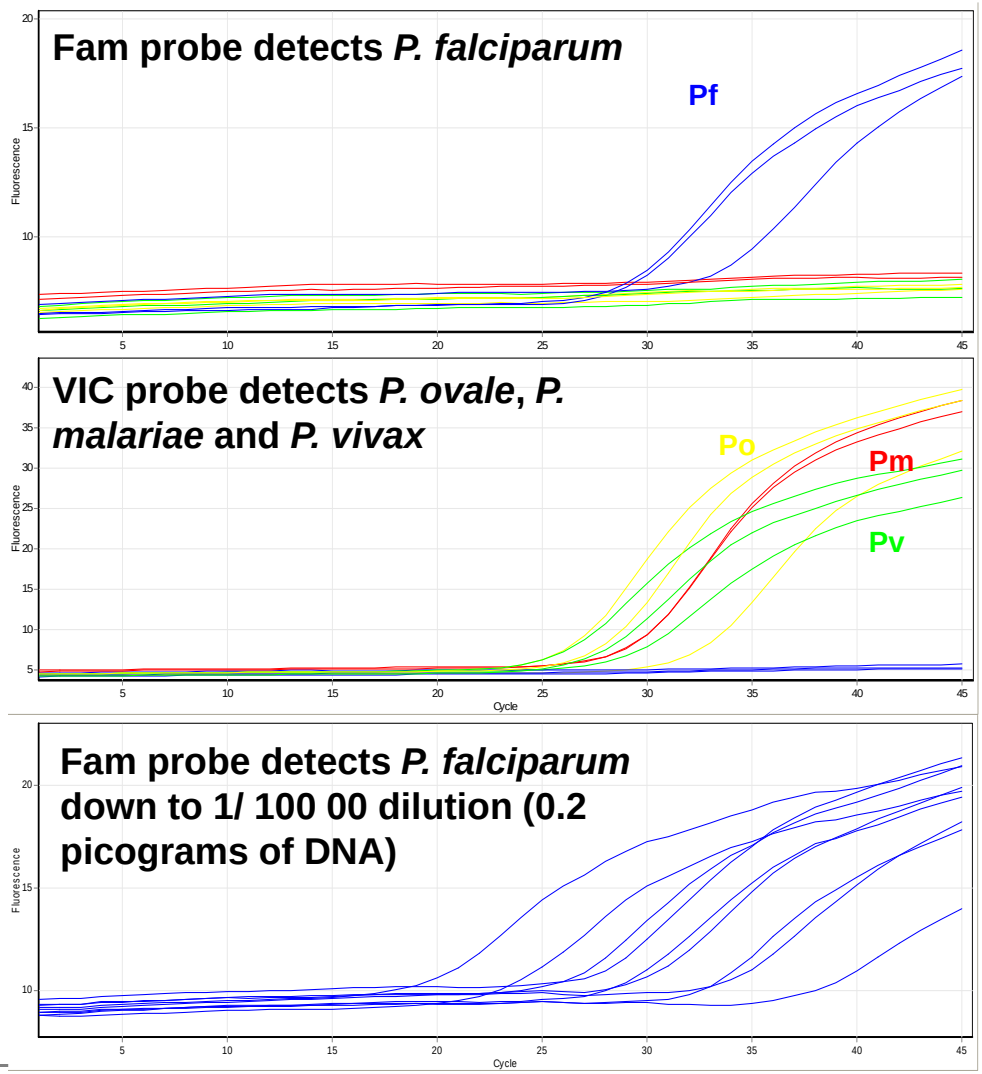
Field test the kits in DEC setting Africa, Latin America and South East Asia


Tamaulipas
Panuco
San Luis Potosi
Tantoyuca
Poza Rica
Martinez de la Torre
Hidalgo
Veracruz-Llave
Zempola
Veracruz
Alvarado
Mexico
Tlaxcala
Morelos
Puebla
Oaxaca
Guerrero
Tabasco
Chiapas
Acayucan
Cosoleacaque
Minatitlan
Coatzacoalcos
0 20 40 80 120 160
Kilometers
KDR freq
0.35
2000
2002
2003
2007

TaqMan Plasmodium assay

Assay tested with artificially infected mosquitoes (Bob Sinden) and field collected mosquitoes stored in Ethanol, Isopropanol, on Silica

- **More sensitive than nested gold standard**
- **Not inhibited by three storage conditions**
- **Cheaper than nested PCR (US$ ~0.5)**
- **Much higher throughput than PCR**
- **Now main diagnostic used at MRC, Durban**



Diagnostics for resistance management

Anopheles gambiae

Knockdown-resistance

Bass *et al Malaria Journal* 2007, **6**:e111 (13 August 2007)
Mzilahowa *et al* 2008 *Med Vet Entomol* 2008 **22**: Pages: 258-263 DEC trial
Ridl *et al Malaria Journal* 2008 **7**: e194 DEC trial (also *Plasmodium* detection)

iAcetylcholinesterase

Bass *et al* 2010 *Pesticide Biochemisty and Physiology* (in press)

GABA receptors (Dieldrin/cyclodienes)

Nikou *et al* (in prep)

Species identification

Bass *et al Malaria Journal* 2007, **6**: e155 (22nd November 2007)
Bass *et al Acta Tropica* 2008 **107**: Pages: 50-53

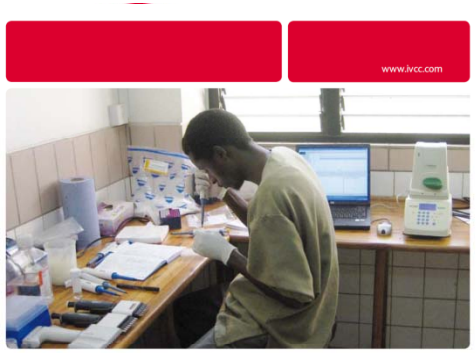
Plasmodium infection

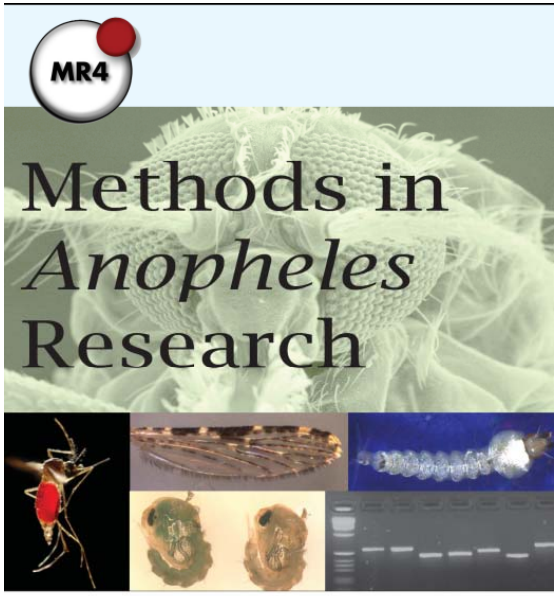
Bass *et al Malaria Journal* 2008 **7**: 177 (15th September 2008)

Anopheles funestus

Species identification

Vezenegho *et al Malaria Journal 2009* **8**:e 282

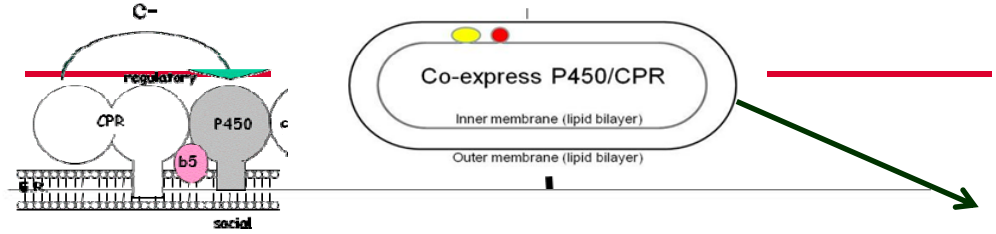


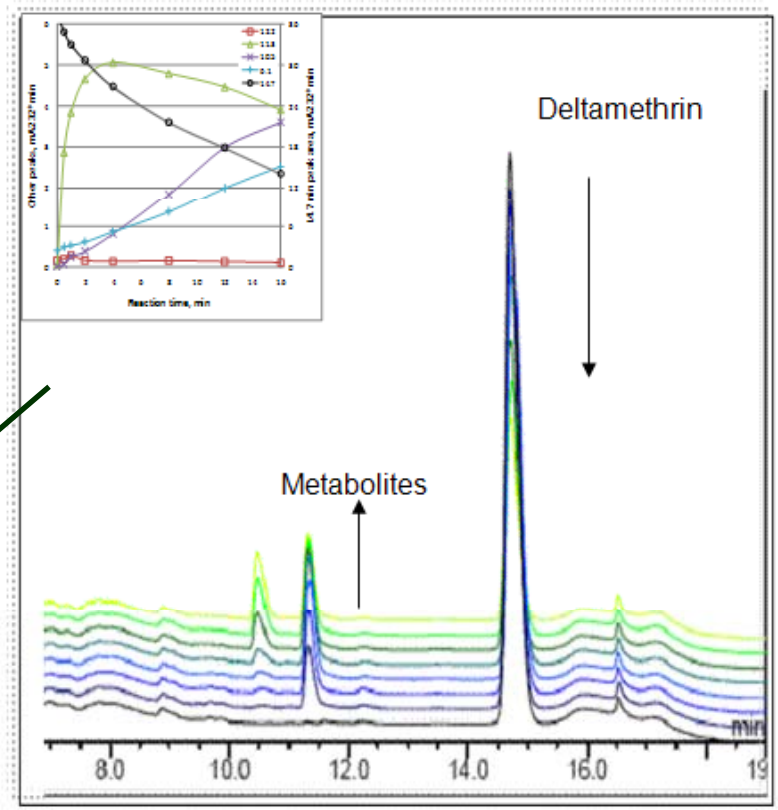


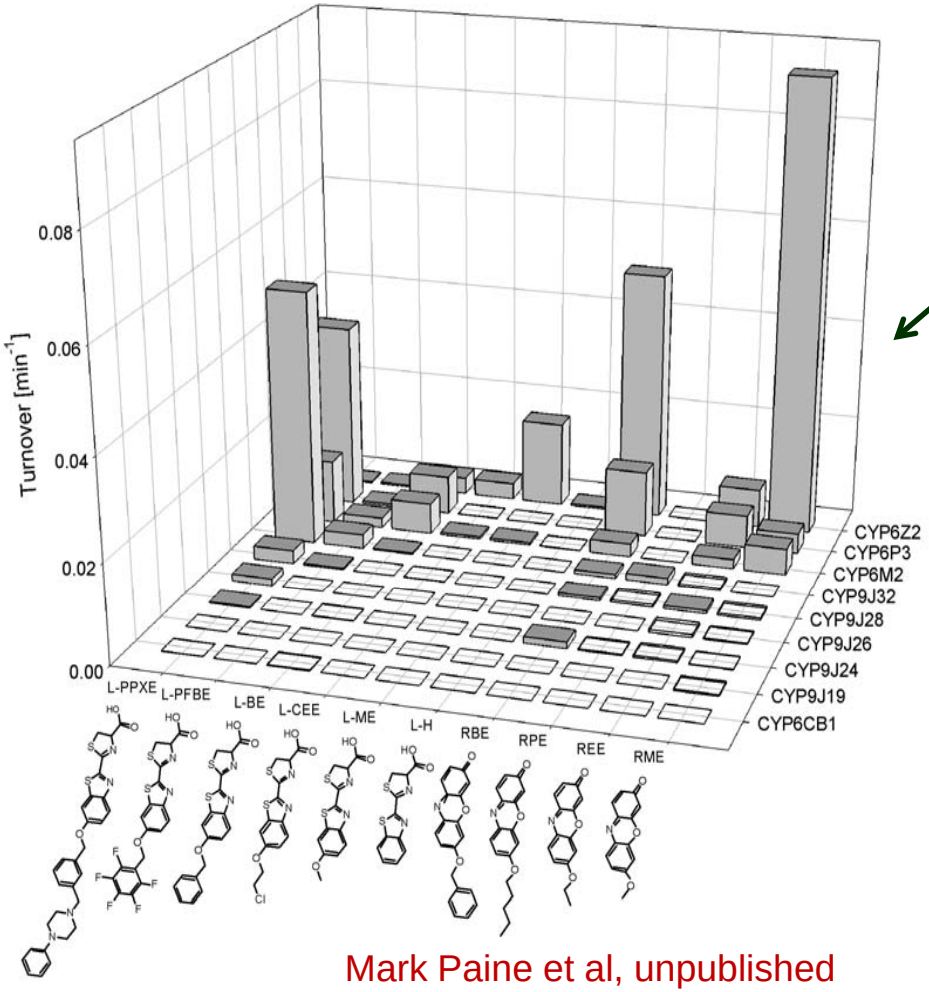
IVCC COMBATING INSECT BORNE DISEASE

National Institute of Allergy and Infectious Diseases · National Institutes of Health · CDC Centers for Disease Control and Prevention · ATCC American Type Culture Collection

CYPs that detoxify deltamethrin







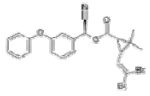
Mark Paine et al, unpublished

Experimental approach

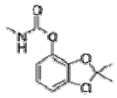
Larvae sampling in Malabo

Reared to adulthood

Tube bioassays on 3 day old non blood fed females



1 h exposure deltamethrin



10-15 min exposure bendiocarb

Control tube = non exposed mosquitoes

- Mortality recorded 24 hours after
- Survivors were stored in RNA later
- Deads were dried with silica gel.

Bioko

LSTM

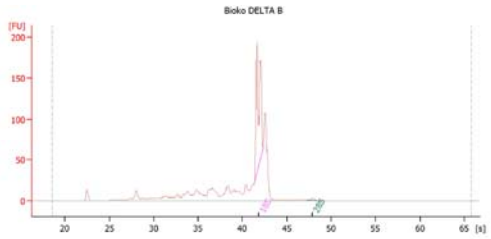
RNA extractions 3 pools of 16-17 females/treatment

RNA control quality

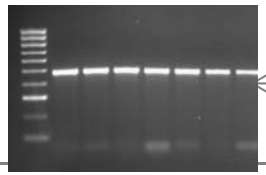
RNA labeling (cy3/cy5)

Microarrays

Bioanalyser



All M form



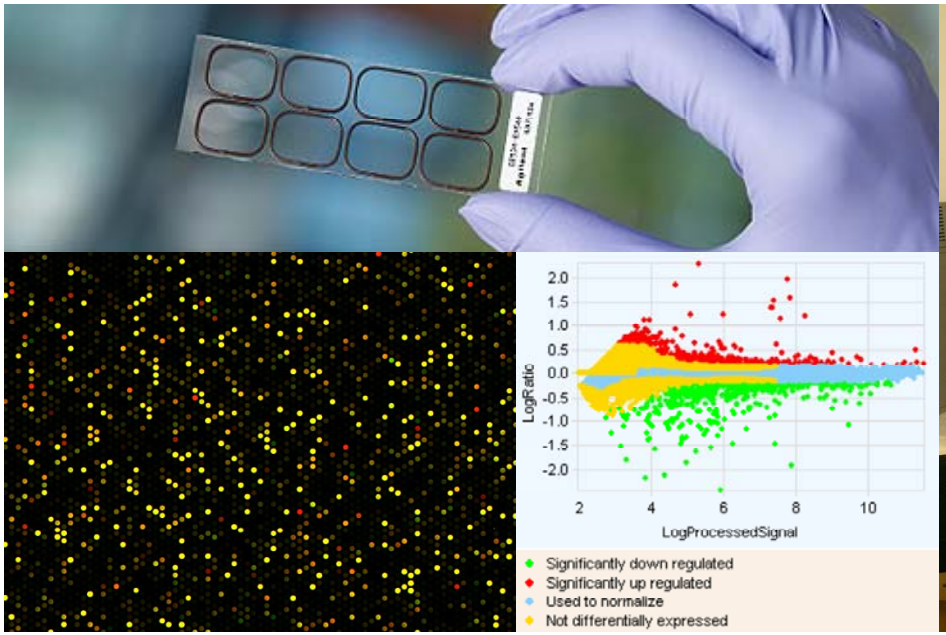
DNA extractions Species ID (SINE PCR)

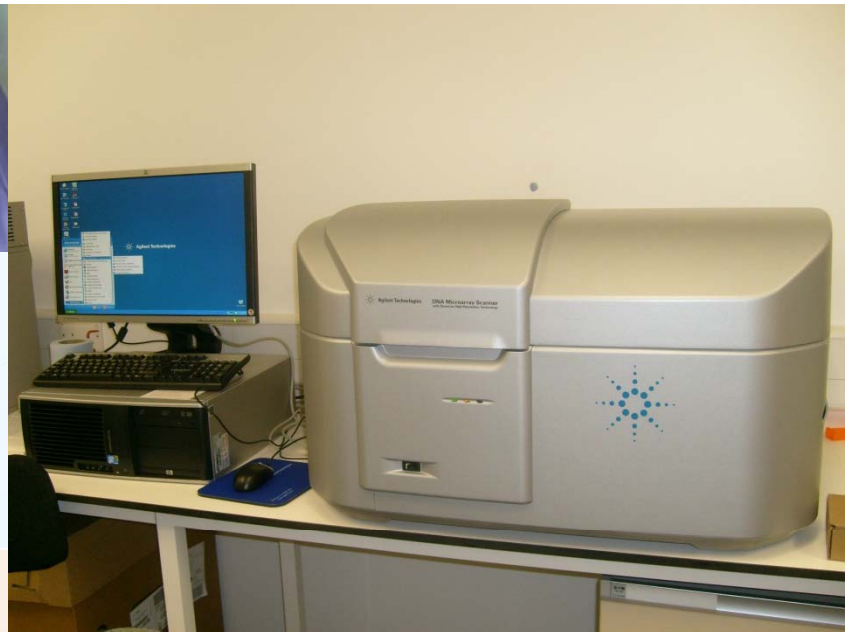
Bioassay results

Bioassays showed very low resistance to Bendiocarb, but resistance against deltamethrin (mortality after 1hour exposure: **40%)**

What is causing pyrethroid resistance?

Experimental approach : Microarrays



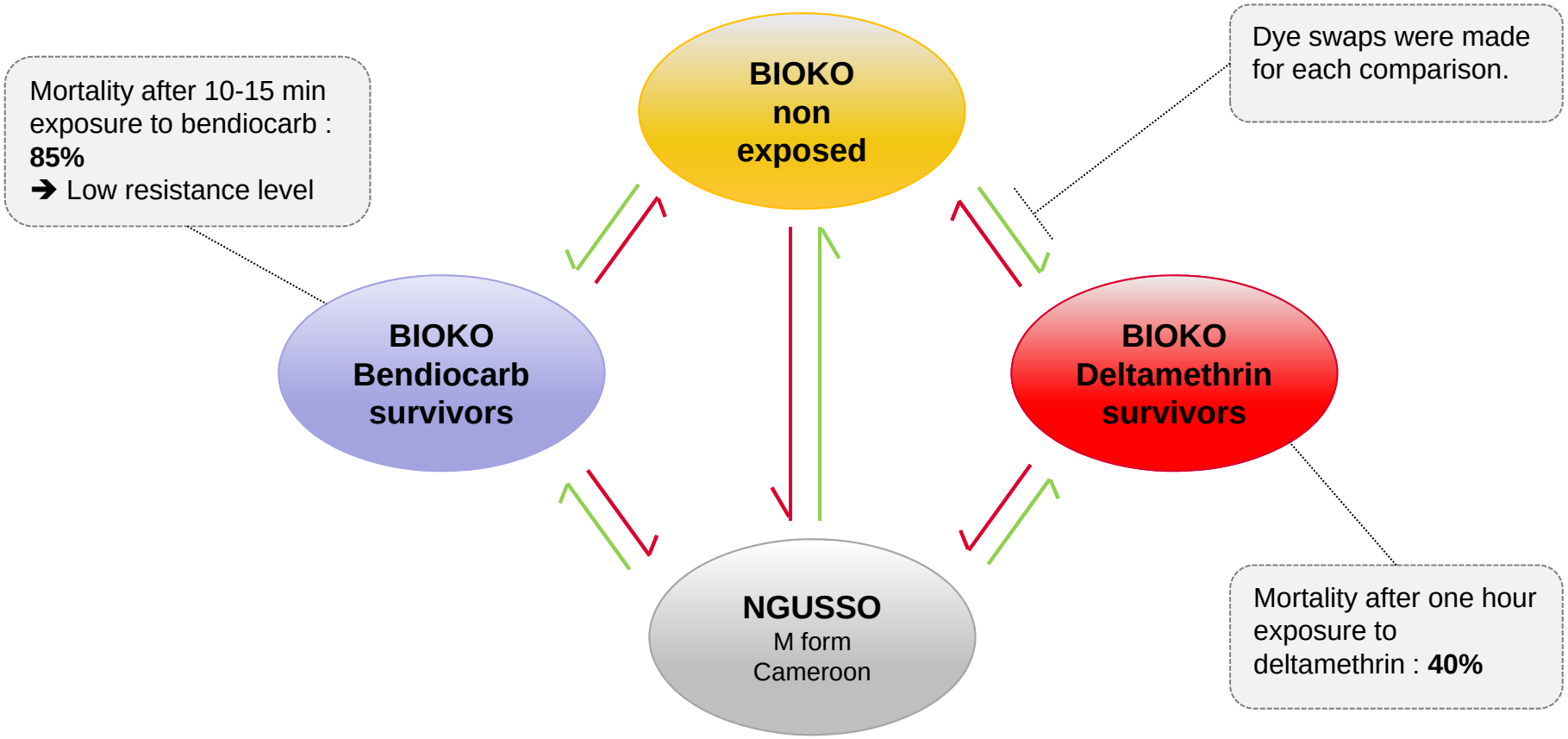


Use of the "*Agilent Anopheles chip*"

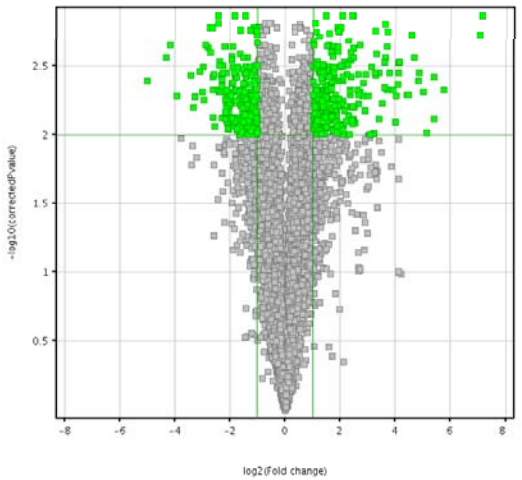
- Per slide: eight arrays containing 15 000 probes.

This approach allows to work on the whole transcriptome

Experimental approach : Microarrays



Microarray_results



Genes are considered significantly over/under-transcribed with a **absolute fold change > 2** and and a **corrected p-val < 0.01** (Benjamini Hochberg correction)

553 differentially transcribed genes

BIOKO non exposed

No significant differentially transcribed genes

BIOKO Bendiocarb survivors

BIOKO Deltamethrin survivors

NGUSSO M form Cameroon

608 differentially transcribed genes

512 differentially transcribed genes

Microarray results

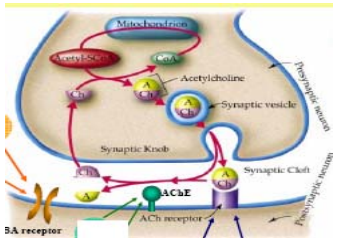
Transcript ID	Description (Blast2GO annotation)	Ngusso vs Non exposed Fold change	Ngusso vs Deltamethrin survivors Fold change	Ngusso vs Bendiocarb survivors Fold change
Detoxification genes				
AGAP012296-RA	CYP9J5 - Cytochrome P450 monooxygenase	17		7.61
AGAP002113-RB	cytochrome b5	4.00		
AGAP005992-RA	CYP302A1 - Cytochrome P450 monooxygenase	3.26	3.59	3.25
AGAP002863-RA	COEAE6O - Carboxylesterase	2.59		
AGAP000284-RA	cytochrome P450 (Novel?)	2.39	2.21	
AGAP002113-RC	cytochrome b5	2.33		
AGAP004380-RA	glutathione transferase GSTD12	2.30	2.52	2.35
AGAP002429-RA	CYP315A1 - Cytochrome P450 monooxygenase	2.27	2.22	
AGAP005371-RA	COEBE2C - Carboxylesterase	2.06		
AGAP002416-RA	CYP4K2 - Cytochrome P450 monooxygenase			2.01
AGAP002417-RA	CYP4AR1 - Cytochrome P450 monooxygenase			2.68
AGAP002419-RA	CYP4D22 - Cytochrome P450 monooxygenase		3.48	3.80
AGAP004164-RC	GSTD1_4 - Glutathion S-transferase			2.23
AGAP004383-RA	GSTD10 - Glutathion S-transferase			2.97
AGAP007480-RA	CYP6AH1 - Cytochrome P450 monooxygenase		4.59	5.85
AGAP008209-RA	CYP6M1 - Cytochrome P450 monooxygenase			2.54
AGAP012295-RA	CYP9L1 - Cytochrome P450 monooxygenase			10.82
Cuticular genes				
AGAP006497-RA	CPR134: cuticle protein			3.71
AGAP003385-RA	CPR123 : cuticle protein			3.20
AGAP003379-RA	CPR 117 : cuticle protein			3.08
AGAP012795-RA	cuticle protein putative			2.10
AGAP010906-RA	CPFL5 : cuticular protein 5 from CPFL family		4.28	
AGAP010908-RA	CPFL7 : cuticular protein 7 from CPFL family	2.80		
Oxidative stress				
AGAP006226-RA	aldehyde oxidase	46.13	41.74	35.40
AGAP011054-RA	TPX2 - Thioredoxin dependent peroxidase	21.99	21.63	24.37
ABC transporters				
AGAP011518-RA	atp-binding cassette sub-family a member = ABCA1	4.37		3.51
AGAP010416-RA	abc transporter	2.01		
AGAP007504-RA	atp-binding cassette sub-family a member			2.68

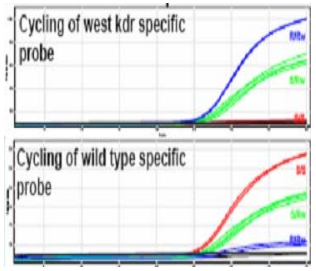
Microarray results overview (preliminary)

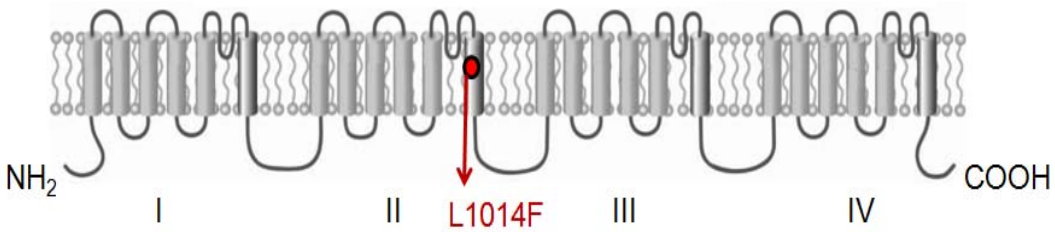
- ✓ The common known pyrethroid metabolizers in *An gambiae* CYP6P3 and CYP6M2 are NOT significantly overexpressed in Anopheles populations from Bioko.

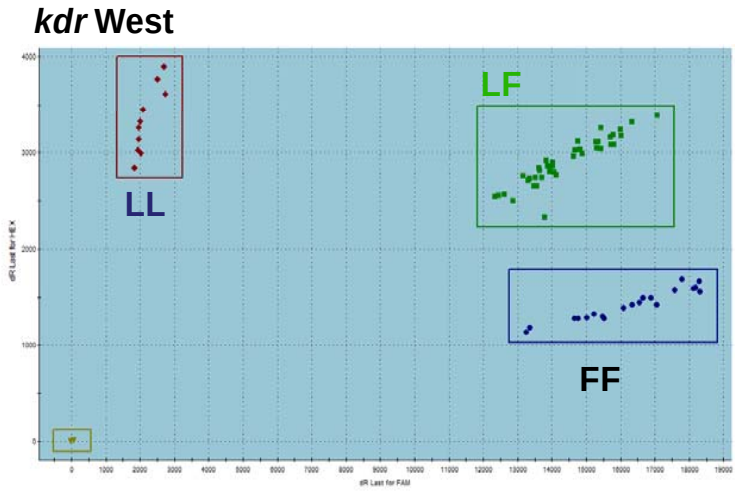
- ✓ Up-regulations of some other putative detoxification enzymes, such as monooxygenases CYP9J5

Target site resistance mutations



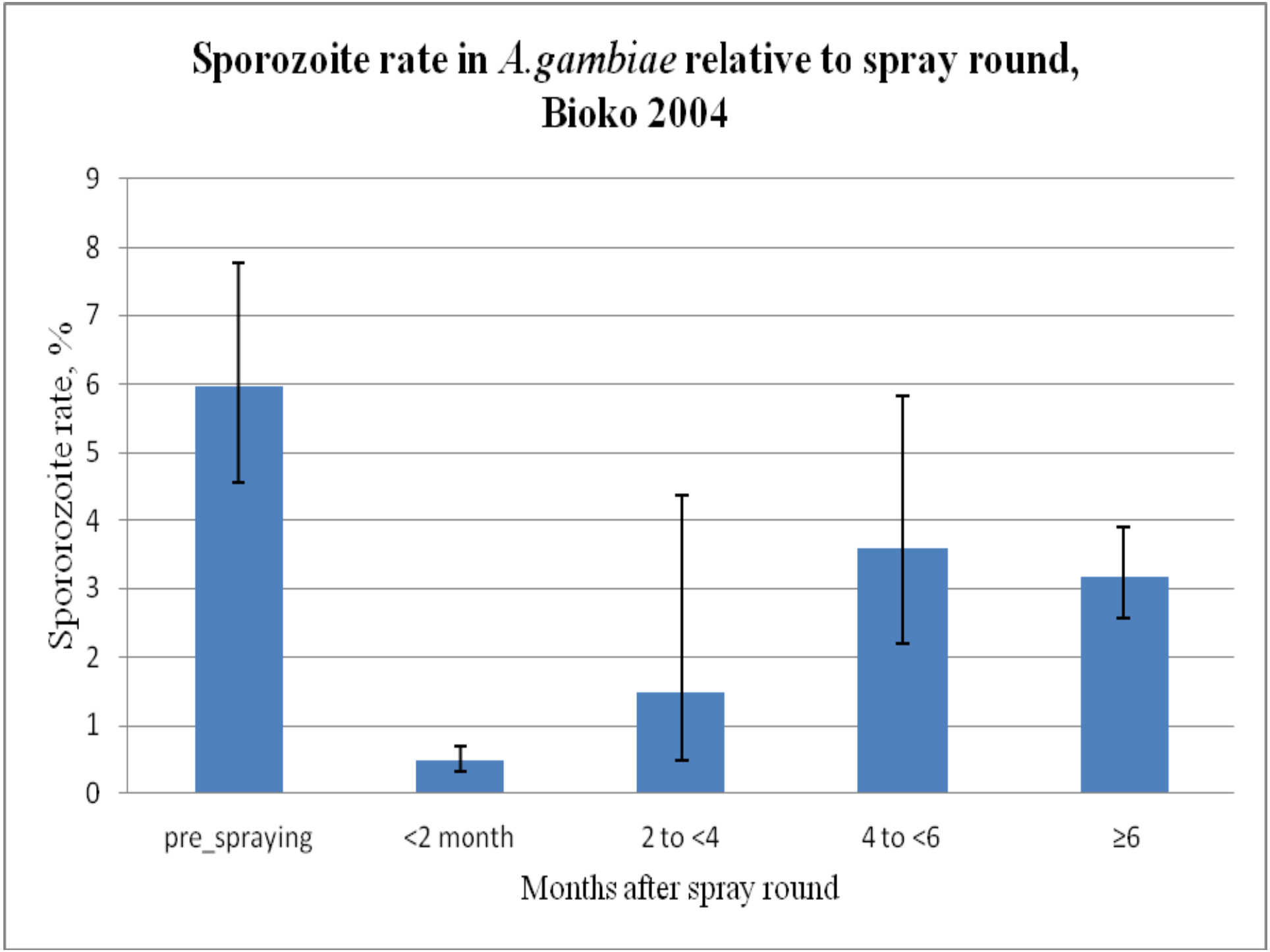






No AChE resistance mutations present (also no apparent metabolic resistance to Bendiocarb; it remains a good option)

Pyrethroid resistance mutations present at moderate frequency (F = 58% and L = 43%)


Sporozoite rate in A.gambiae relative to spray round, Bioko 2004
Sporozoite rate, %
0
1
2
3
4
5
6
7
8
9
pre_spraying
<2 month
2 to <4
4 to <6
≥6
Months after spray round

What Does this Mean for Insecticide Choice?

The Academy of Medical Sciences

International Health Lecture 2012

Health impacts of product development partnerships

Professor Janet Hemingway FRS FMedSci
Director of the Liverpool School of Tropical Medicine