

# Microarray analysis of genes mediating metabolic resistance in a pyrethroid resistant *Anopheles gambiae* s.s. strain from Ghana

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# Introduction



- Pyrethroid-treated bednets are the mainstay of malaria control programmes in sub-Saharan Africa
- Pyrethroids: fast, effective, low mammalian toxicity
- Currently no other insecticide class with similar efficacy and specificity is available
- The emergence of pyrethroid resistance threatens to compromise the successful use of insecticide-treated materials

# Mechanisms of insecticide resistance

## Site insensitivity

Acetylcholinesterase

- Organophosphates
- Carbamates

Knockdown resistance (*kdr*)

- DDT
- Pyrethroids

GABA receptor

- Cyclodienes
- Fipronils

## Metabolic resistance

Cytochrome P450 monooxygenases (*P450s*)

- Pyrethroids
- Carbamates
- Organophosphates

Carboxylesterases (*COEs*)

- Organophosphates
- Pyrethroids

Glutathione S-transferases (*GSTs*)

- DDT
- Organophosphates
- Pyrethroids

# Metabolic resistance to pyrethroids in *A. gambiae* s.s.

- Elevated transcript levels measured in the permethrin resistant *RSP* strain from Western Kenya:
  - *Glutathione S-transferase*: *GSTe2*
  - *Cytochrome P450s*: *CYP6z1* and *CYP325a3*

David *et al.*, 2005: PNAS 102, 4080-4

Nikou *et al.*, 2003: *Gene* 318, 91-102

- Do metabolic resistance mechanisms vary accross populations?
- Here, we measured constitutive gene expression patterns between a susceptible standard strain and a resistant strain from West Africa.

# Mosquito strains

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- *Anopheles gambiae* s.s., S-form

## ODUMASY

- Odumasy, Southern Ghana, West Africa
- Resistant to permethrin
  - Repeated selection by 1h exposure to 0.75% permethrin (1-day old adults, WHO test tubes)
  - Survival rate females: 74.1% ( $N=297$ )
  - Survival rate males: 62.1% ( $N=224$ )

## KISUMU

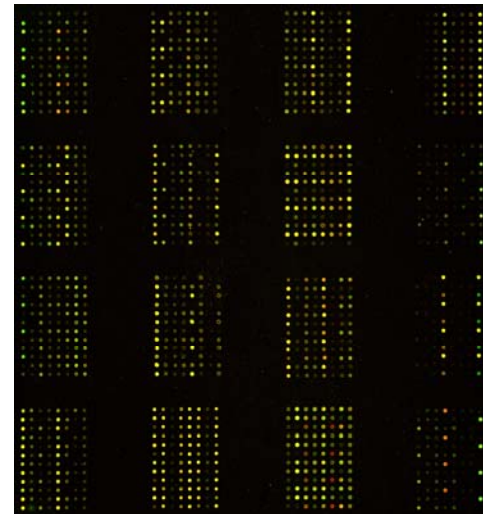
- Kisumu, Western Kenya, East Africa
- Susceptible to permethrin

# Detox Chip

- Custom made cDNA microarray for two-colour experiments

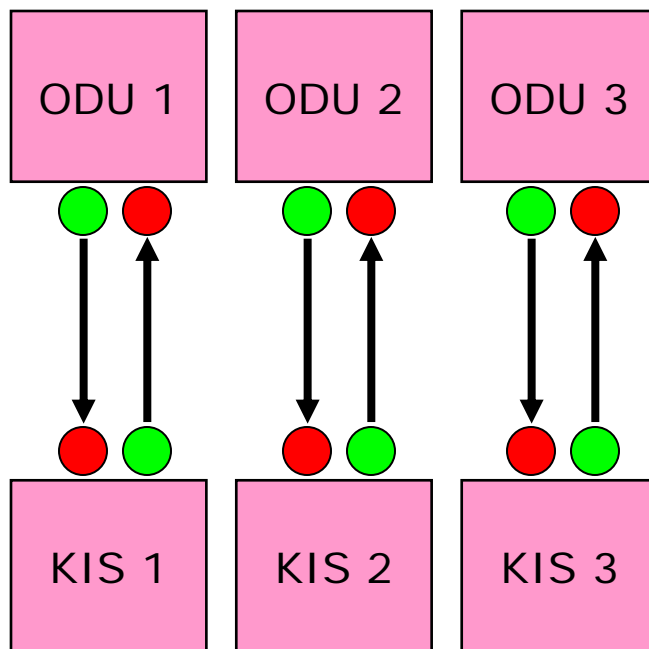
David *et al.* (2005): PNAS 102, 4080-4

- 230 gene fragments
  - 103 Cytochrome P450s
  - 31 Carboxylesterases
  - 35 Glutathione S-transferases
  - 41 Red/Ox genes
  - 5 ATP-binding-cassette transporters
  - Various control genes
- Four replicate spots

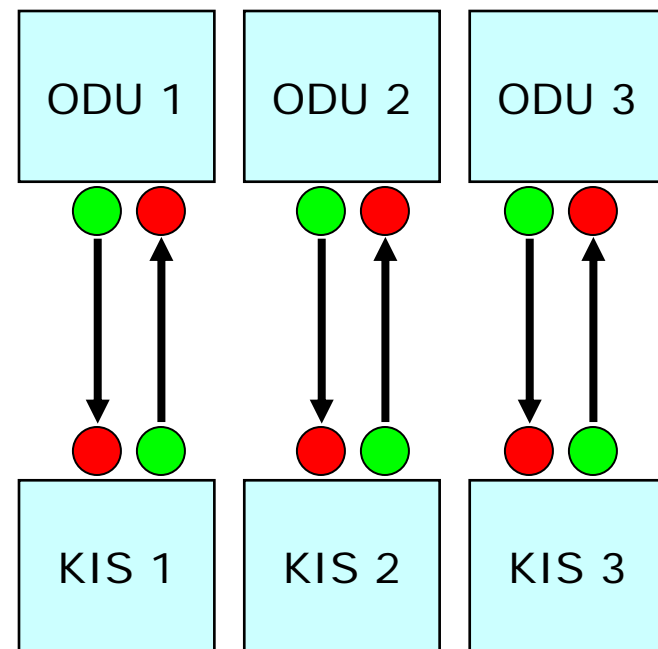


# Experimental design

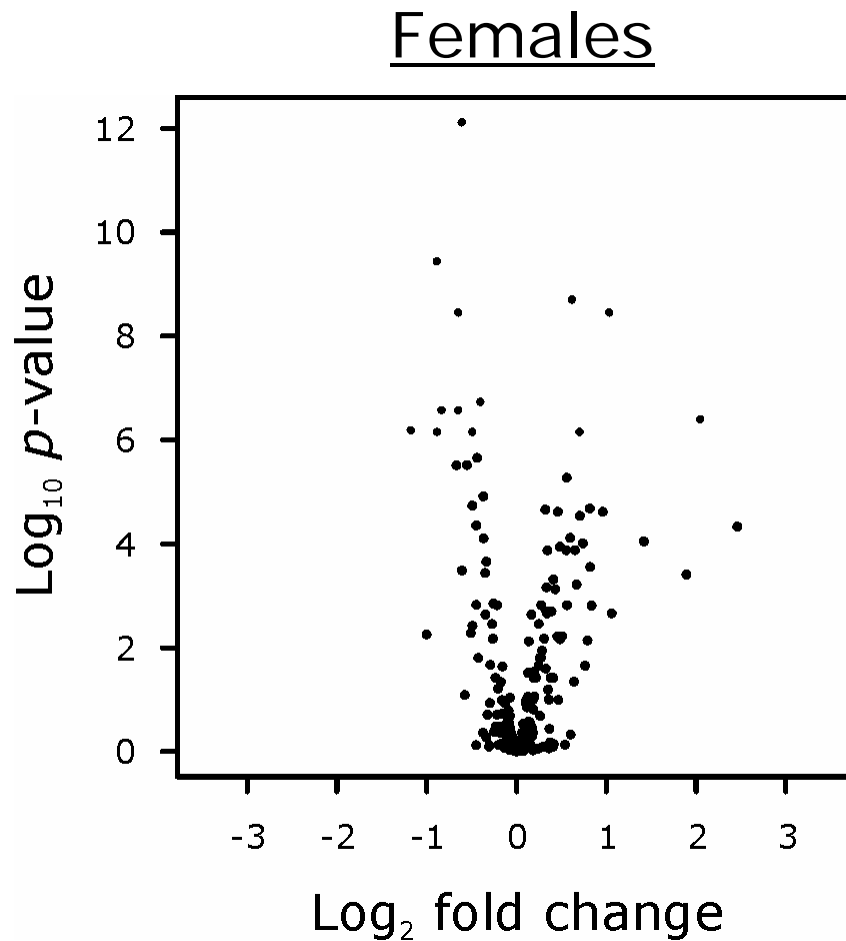
Adult females



Adult males

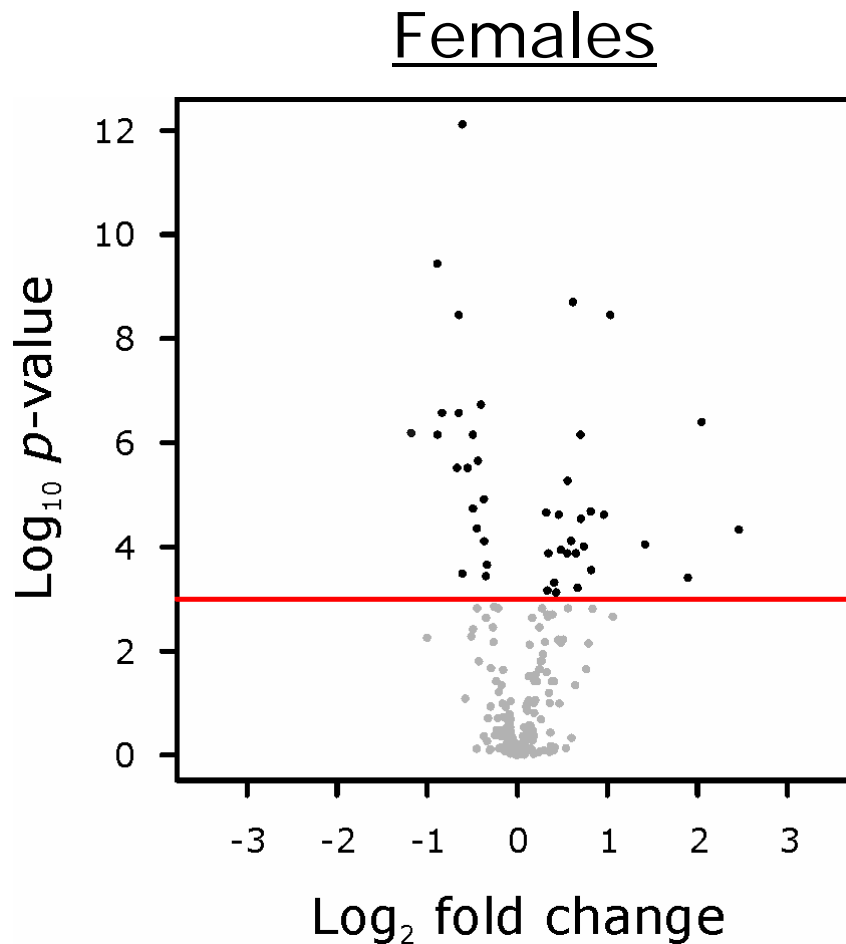


# Gene-wise comparison



- The association of gene expression with strain was assessed by fitting linear models to estimate the gene expression for each gene on the array

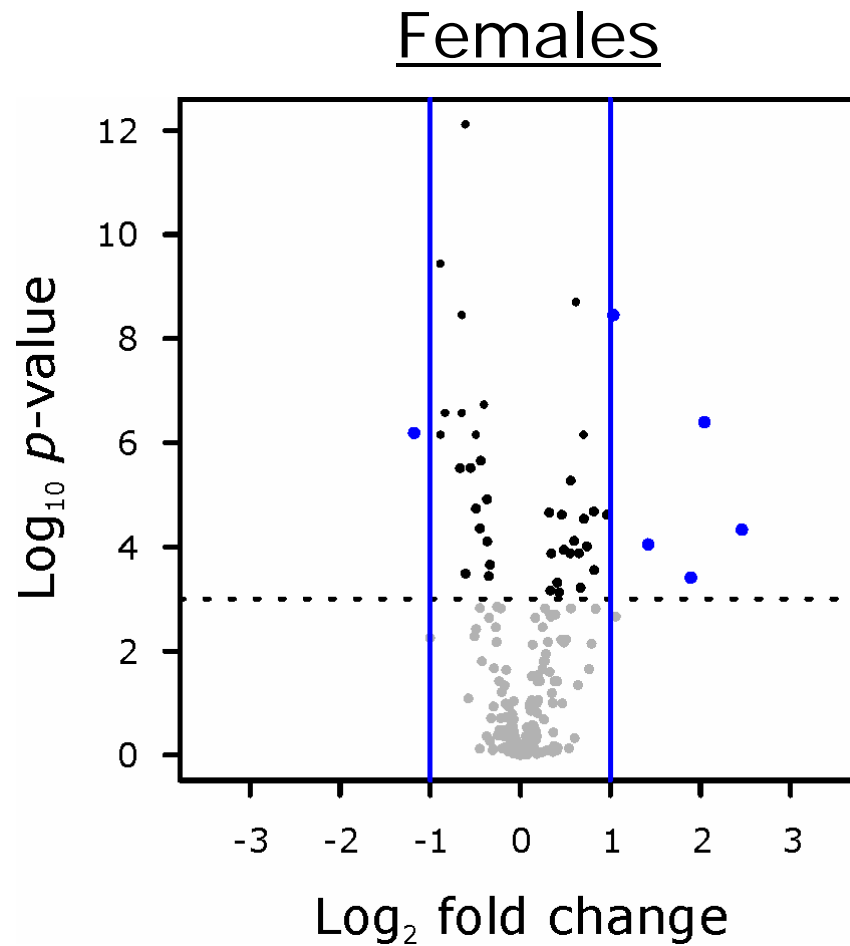
# Gene selection



Level of significance,  
 $\alpha = 0.001$

*P*-values were corrected  
for multiple testing  
(False discovery rate:  
Benjamini & Hochberg)

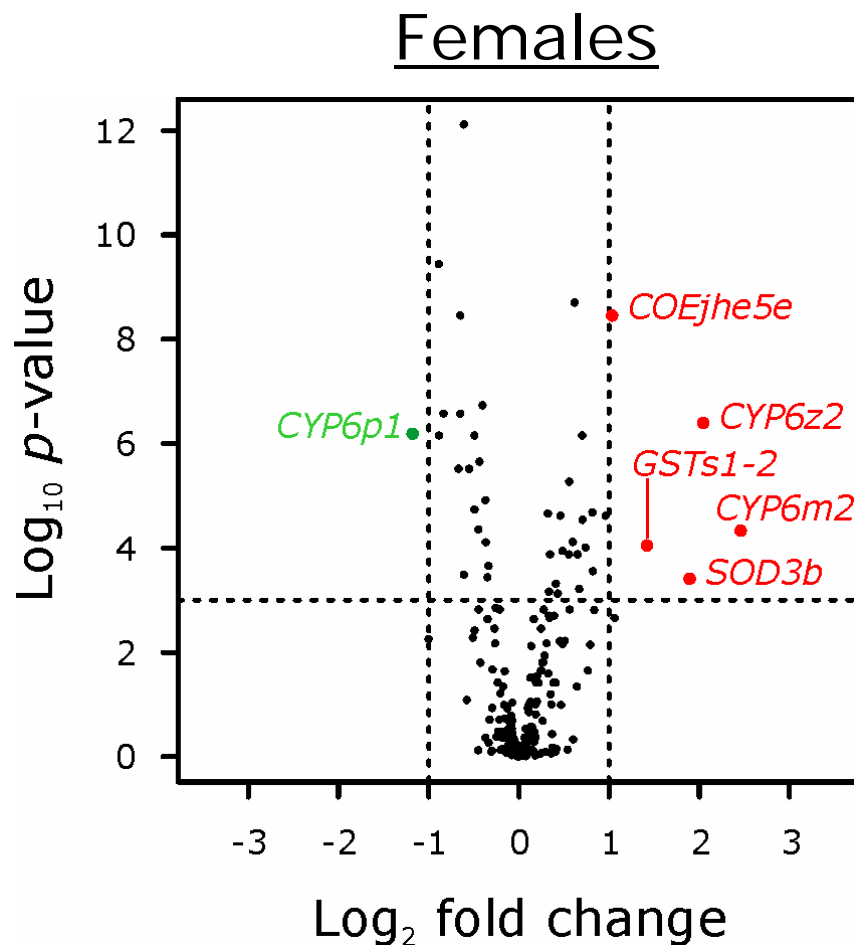
# Gene selection



Two-fold change

# Gene expression

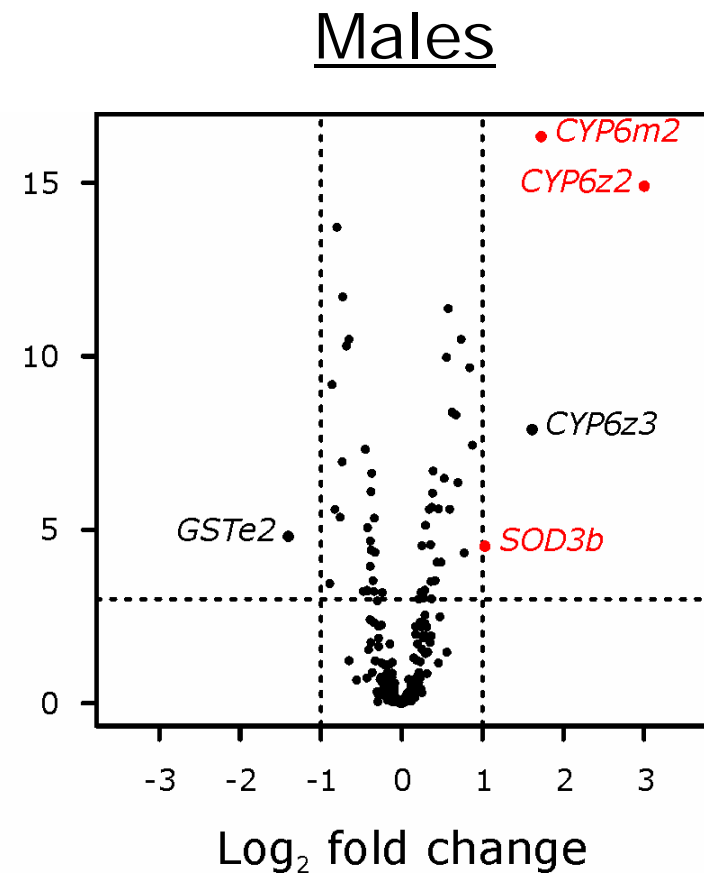
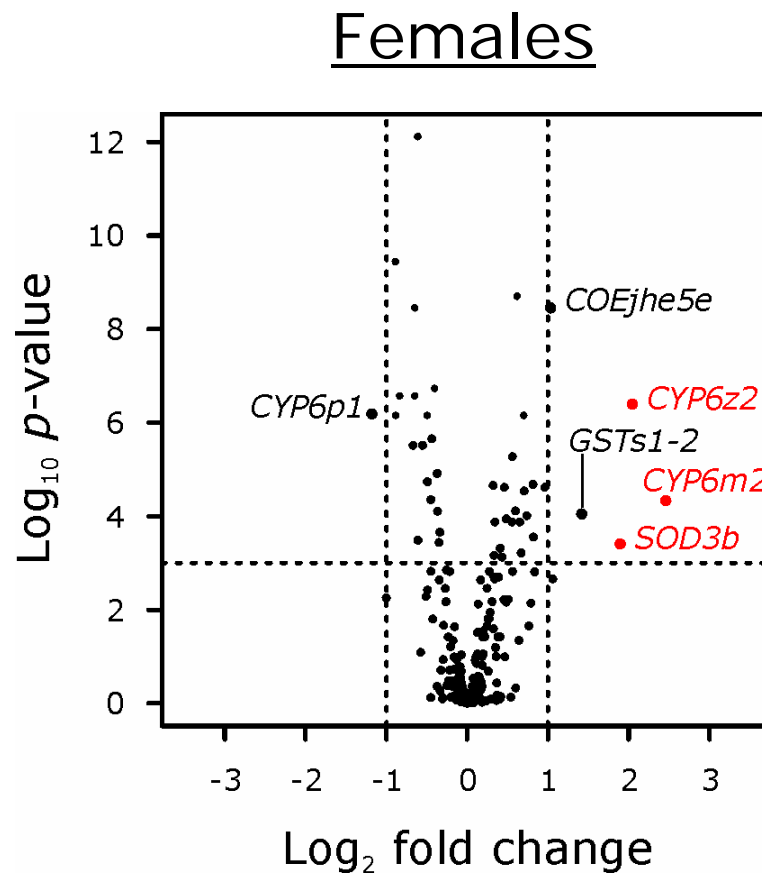
## *Odumasy vs. Kisumu*



Over-expression in  
*Odumasy* females

Over-expression in  
*Kisumu* females

# Gene expression *Odumasy vs. Kisumu*



# Conclusions

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- Two P450 monooxygenases *CYP6z2* and *CYP6m2* stand out as candidate genes conferring permethrin-resistance in the West African *Anopheles gambiae* s.s. strain from Odumasy
- *CYP6z2* and *CYP6m2* constitute a novel set of candidate genes implicated in insecticide detoxification
- Metabolic insecticide detoxification mechanisms may differ between population

# Ongoing studies

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- Extension from lab to field populations
- Development of simple assays such as allele-specific PCR to monitor resistance in field populations

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