Trends in the selection of insecticide resistance in *An. gambiae s.l.* in north-western Tanzania during a community randomised trial of long lasting insecticidal nets and indoor residual spraying

Matowo *et al.*, In press: *Med Vet Entomol*.

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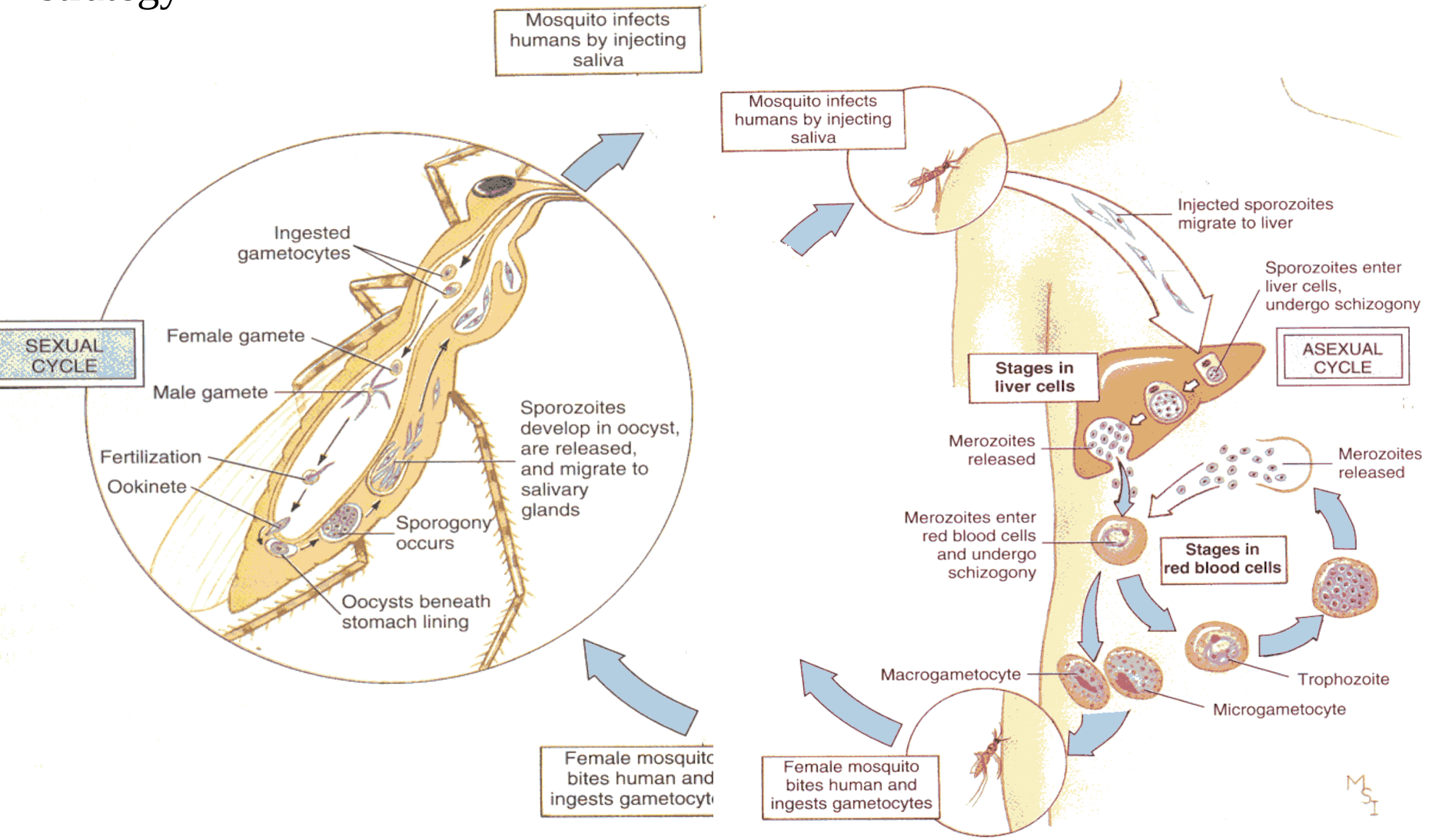
Introduction

Since the discovery of the relationship between *Anopheles* mosquitoes and malaria transmission, vector control has been widely used as a malaria control strategy



Background

- 1950s Global malaria-eradication program
- As a result, malaria was eradicated from many countries
- 1960s global eradication stopped
  - Insecticide resistance (DDT), Switch to newer insecticides (OPs, CBs, PYs)
  - Drug resistance
  - Poor infrastructure, particularly in Africa
- Eradication program changed to malaria control

- During 1970s and 1980s malaria received little attention

- In the 1990s malaria reemerged as a major international health issue, Global **malaria control** strategy adopted 1992

Background

- Global **malaria control** strategy:

1.Early diagnosis and prompt treatment

2. Insecticide treated nets

3. Indoor residual spraying

4. Intermittent preventive treatment during pregnancy

5. Early detection to contain or prevent epidemics

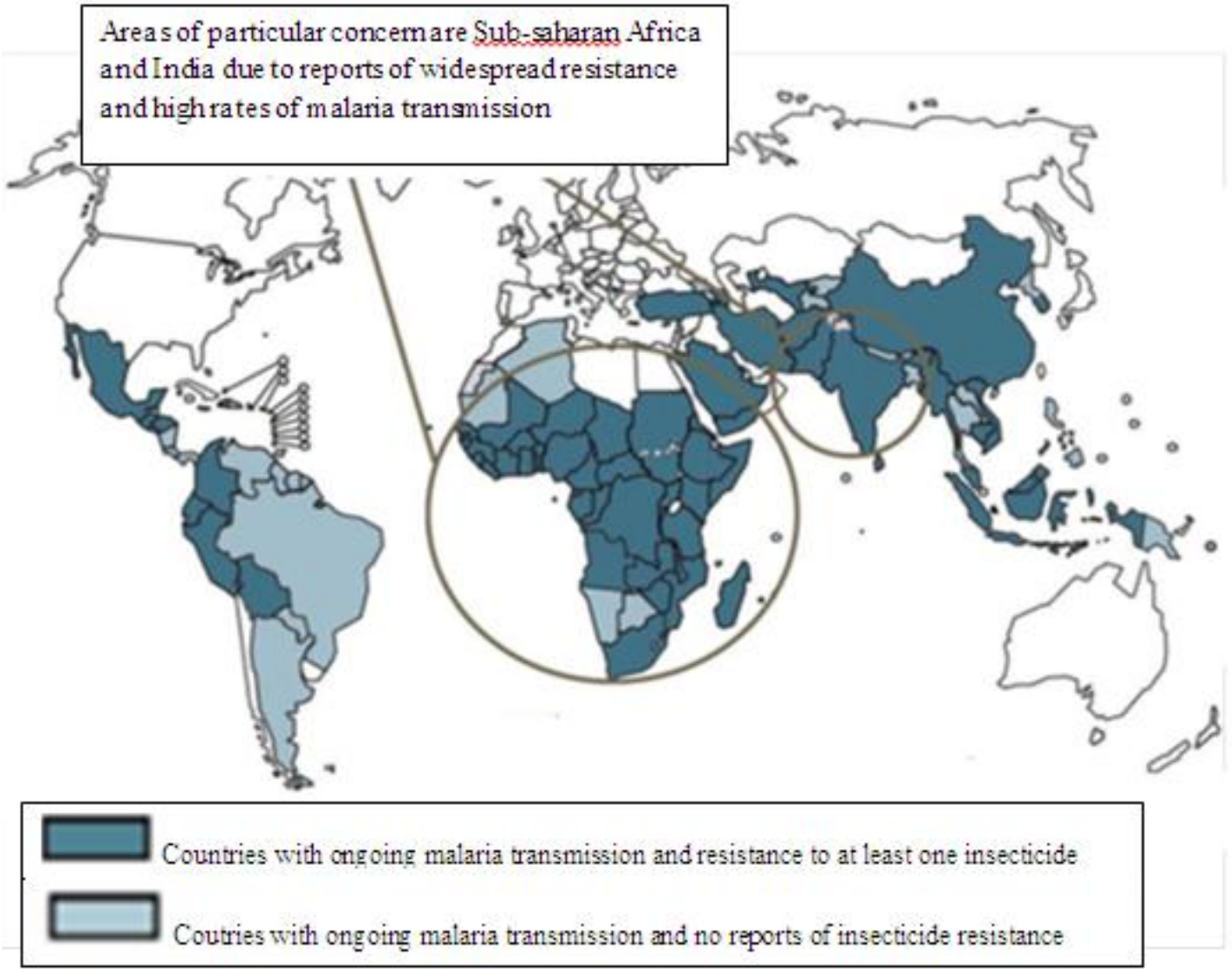
6. Strengthening of local capabilities in basic and applied research

➢ With the emergence and spread of insecticide resistance among malaria vectors, our global control efforts are at high risk

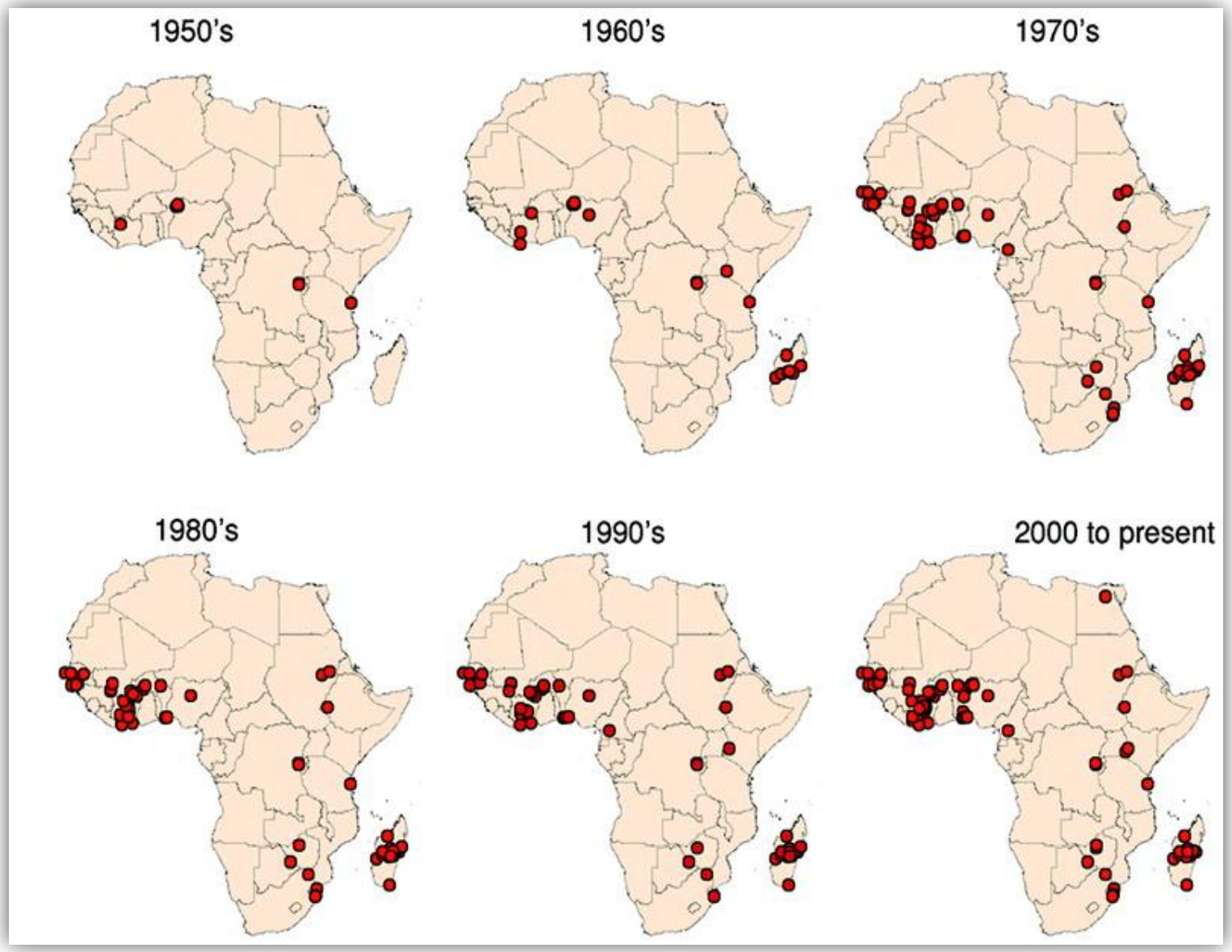
- LLINs and IRS combination interventions constitute a potential tool for vector-resistance management [1,2]

1,Djènontin *et al.*, 2010; 2,Ngufor, 2011, 2014

65 endemic Countries reporting insecticide resistance in at least one of their main malaria vectors, as indicated by bioassays

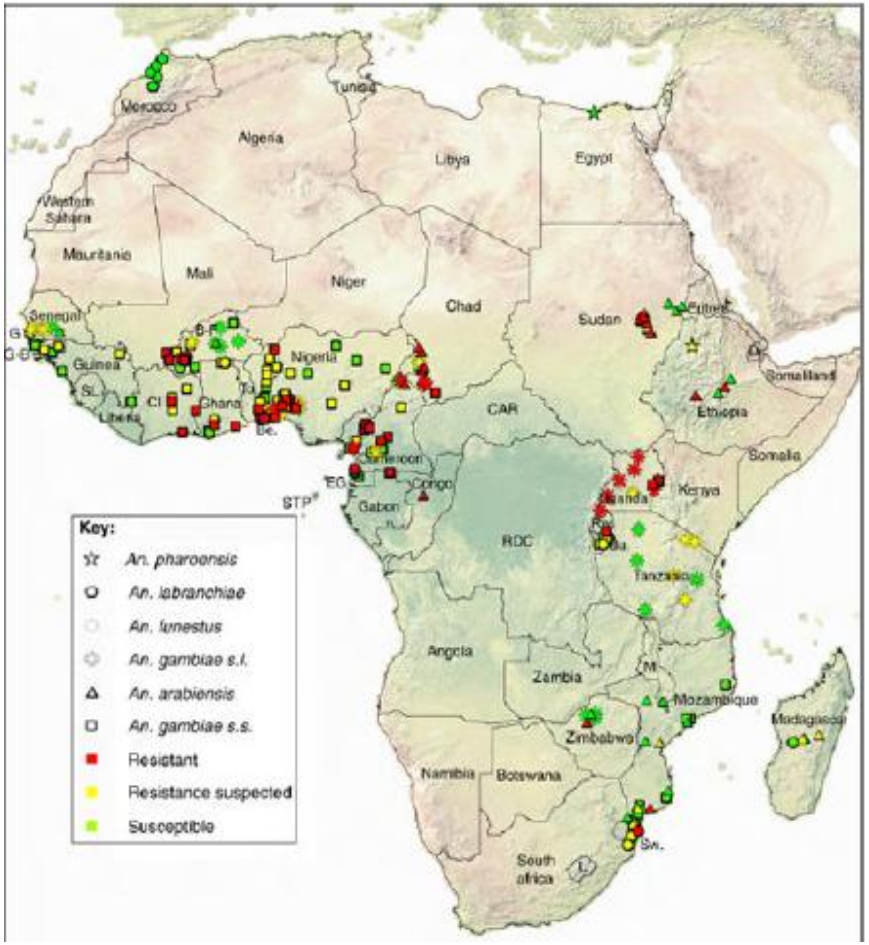


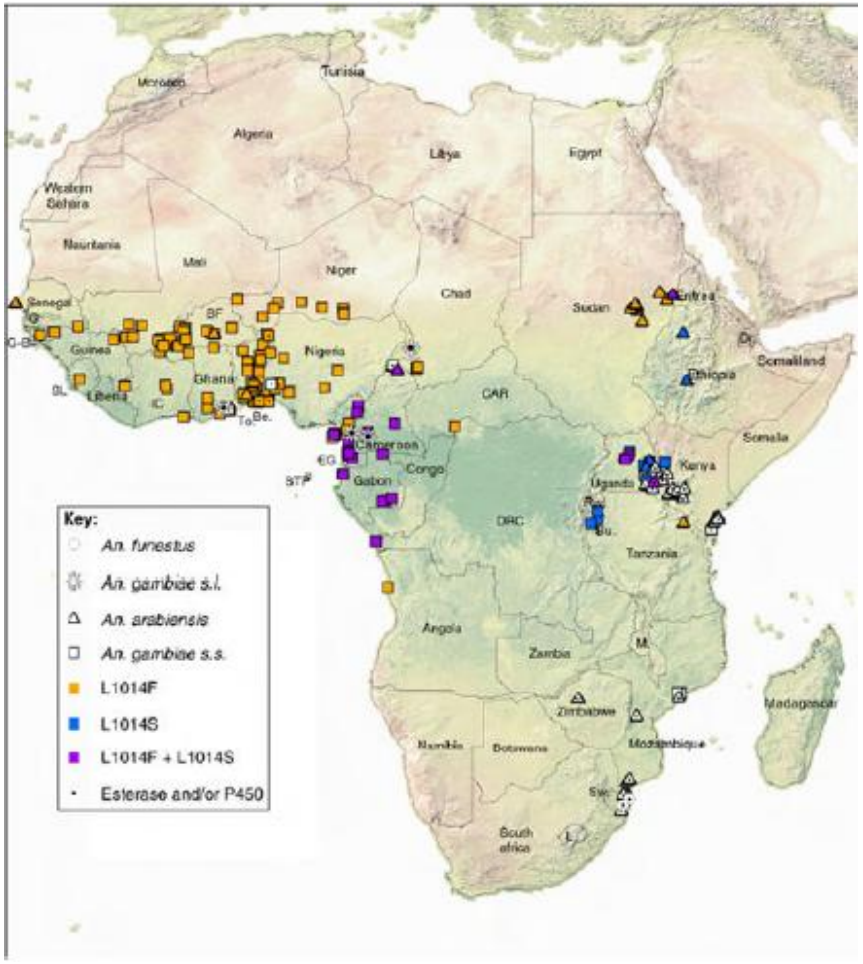
Background



Time series showing the sites that have reported insecticide resistance ;
Coleman *et al.,2006*

Maps showing the distribution of pyrethroid-resistance in African malaria vectors





A) status of pyrethroid resistance according to WHO criteria ; B) Target site (*kdr*) and metabolic resistance reported for a given mosquito species (Source; Ranson *et al.*, 2011).

Rationale

- *An. gambiae s.l.* in NW Tanzania has developed high levels of resistance to most insecticides currently advocated for malaria control [1]
  - The *kdr* mutation has almost reached fixation in *An. gambiae s.s*

- This change has potential to jeopardize malaria control interventions carried out in the region

- The present study was carried out in NW Tanzania to assess the effect of combining LLINs and IRS against pyrethroid resistant malaria vectors

- Additional mechanisms contributing to observed phenotypic resistance were also investigated

Protopopoff *et al.*, 2013

Methods

Study site: NW Tanzania (Muleba)

Methods …

Muleba District

- Malaria epidemics prone area
- PMI-funded spray operations with lambdacyhalothrin started in 2006, switched to bendiocarb in 2011 following high PY resistance
- ITNs ownership increasing from 63% in 2009 to 91% in 2011 due to UCC [1]
- Prevalence 8% in February and 23% in June 2011(High heterogeneity from 1% to 62% in some villages)

1, West *et al.*,2012

Methods...

LLINs /IRS combination

- Part of cluster randomized trial of two arms that was conducted in Muleba in 2012 where one arm (Kiteme village) received LLINs only following UCC in 2011.

- The second arm (Kyamyorwa village) received both LLINs and carbamate based IRS.
  - Bendiocarb IRS replaced pyrethroid based IRS in Dec.2011 following high pyrethroid resistance in the area.

- Monitoring of insecticide resistance which had started in 2011 was continued in the two intervention arms through WHO susceptibility testing

Methods …

- **Mosquito sampling**
  - Indoor adult mosquito collection using a mouth aspirator

- **Susceptibility testing**
  - Using the standard WHO susceptibility test kits[1]
  - The wild mosquitoes were exposed to diagnostic dosages of various insecticides

- **Synergy tests**
  - Piperonyl butoxide (PBO) and s,s,s-tributyl phosphorotrithioate (DEF) ,inhibitors for P450s and NSEs were used, followed CDC guidelines [2]
- ***kdr* and *Ace-1* genotyping**
  - Conducted using real-time PCR TaqMan assays

1, WHO, 1998; 2, CDC,2010

Methods …

Data analysis

- Interpretation of WHO susceptibility tests was based on WHO criteria [1]

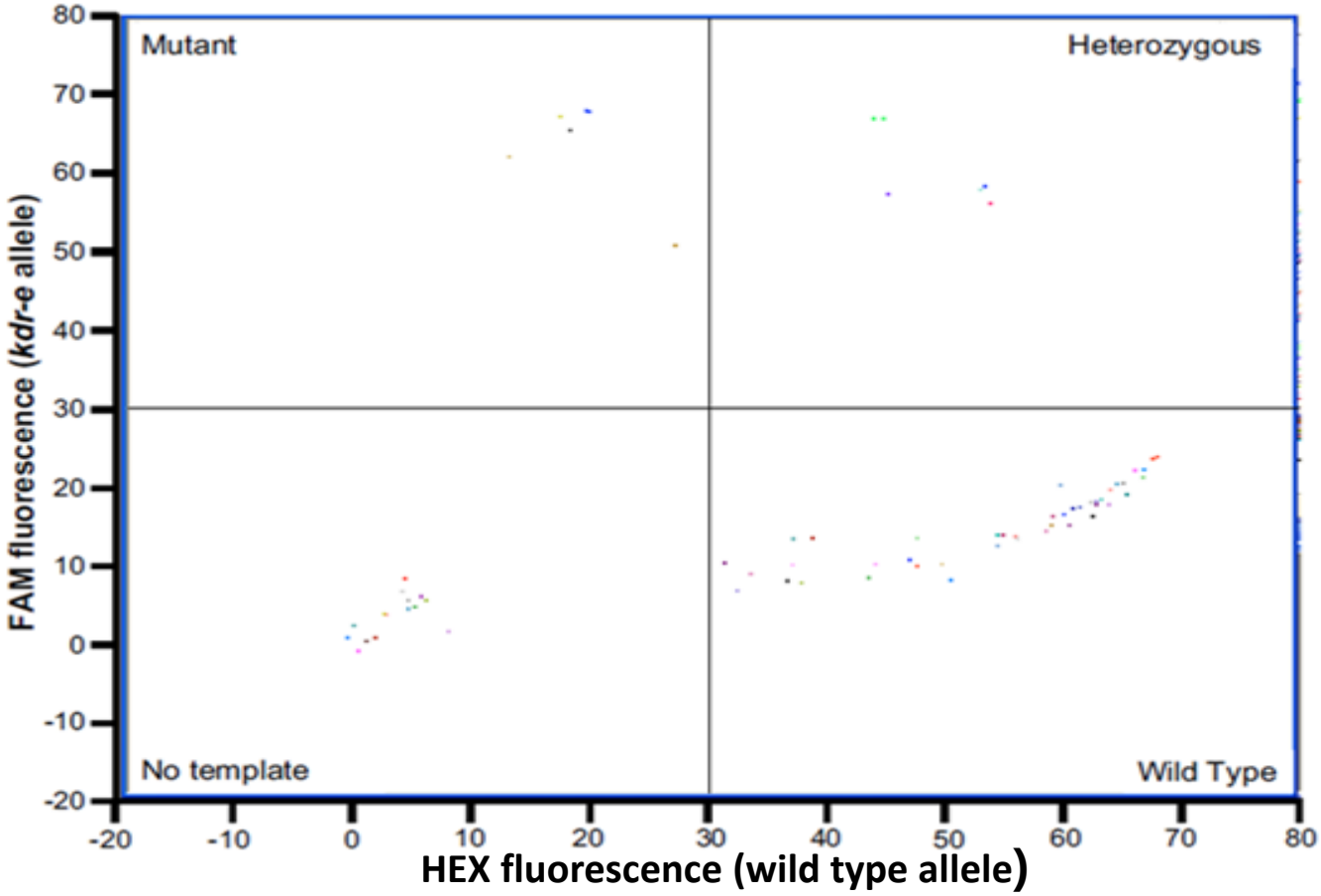
- The KdT_{50} and their 95% confidence interval values in synergy studies were calculated for each treatment using Probit analysis in SPSS 18.0 for windows

- The *kdr* and *Ace-1* genotyping data were analyzed using MXPro software

WHO, 1998

Methods …

Data analysis in MXPro



Scatter plot analysis of TaqMan fluorescence data

WHO, 1998

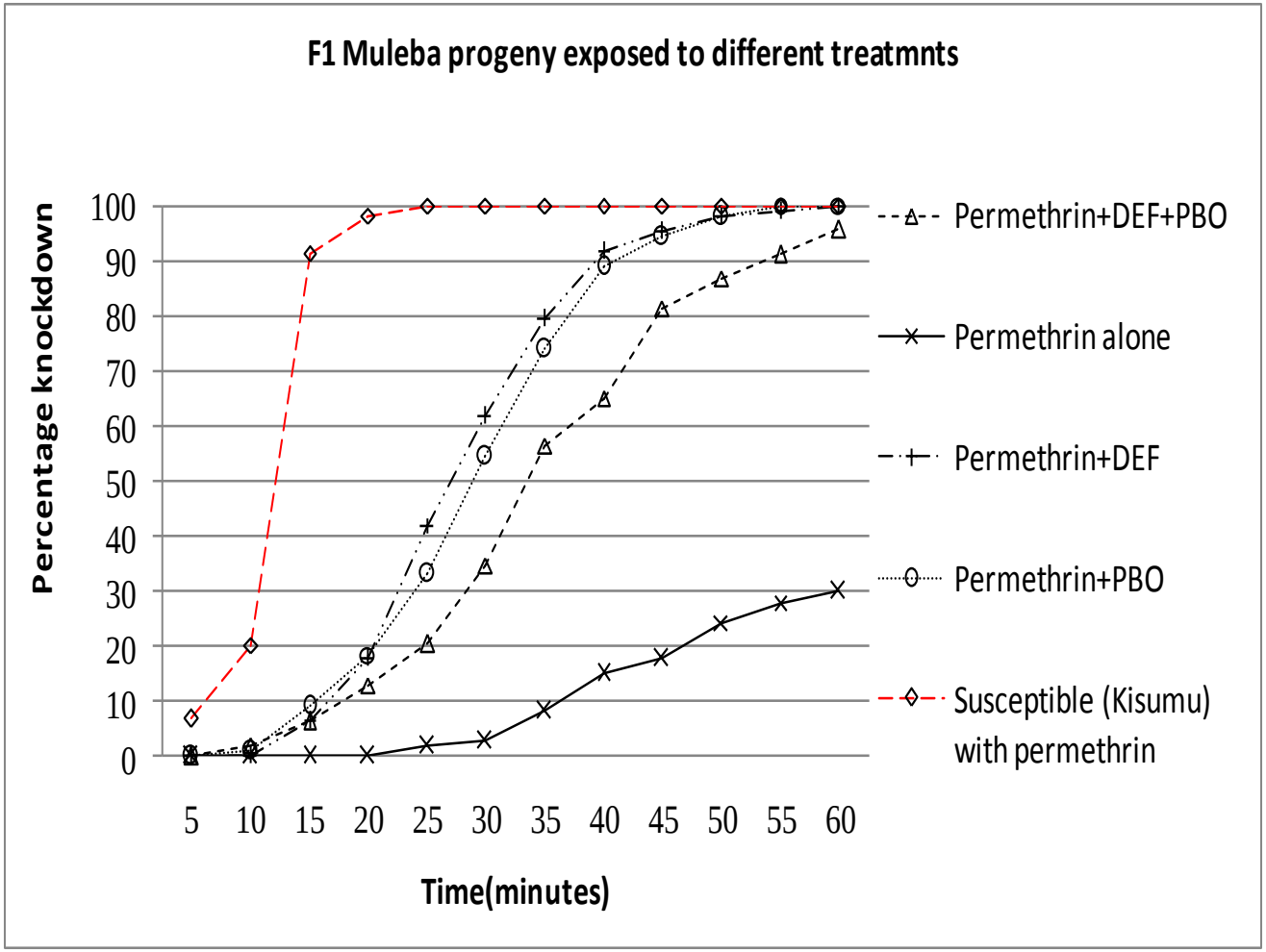
Results

- High phenotypic resistance was observed in both study villages to several pyrethroids and DDT, with 12-23% mortality
- *An. gambiae s.l.* remained susceptible to pirimiphos-methyl (organophoshate)
- There was no evidence for selection for *Ace-1* resistance alleles
- The frequency of *kdr* mutation was the same in the two villages at baseline (high allelic frequency= 0.98) (fisher exact test p= 0.596) and during the intervention year (Fisher exact test p= 0.635)
- Partial abolition of permethrin by PBO and DEF

Results …

CDC synergy bioassays

- Resistance to permethrin was partially abolished by PBO, DEF



Exposure of F1 F1 *An. gambiae s.l.* from Muleba to permethrin plus DEF or PBO or both

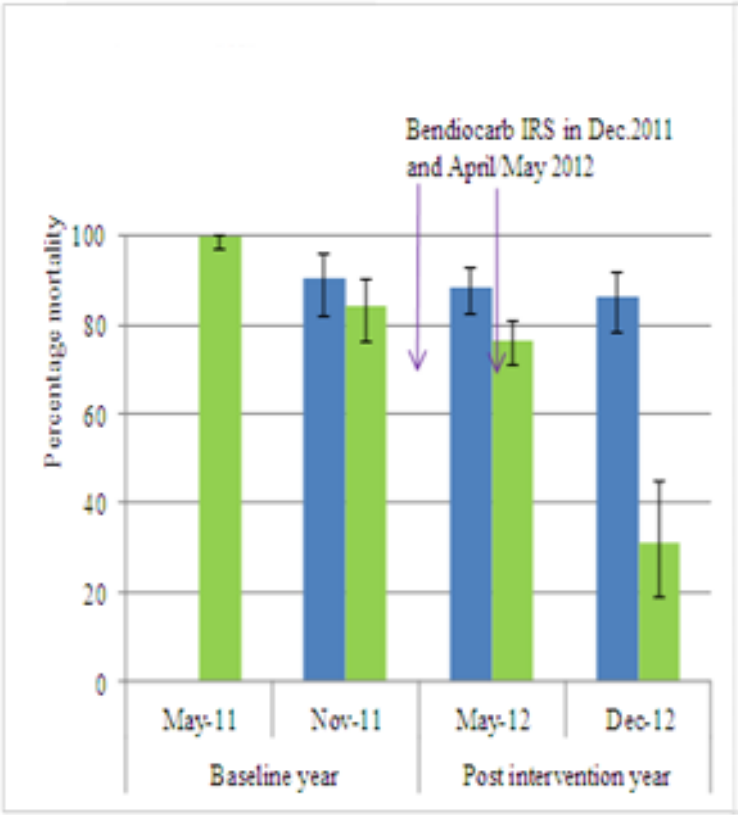
Results …

- There was a sharp decrease in mortality in *An.gambiae s.l.* exposed to bendiocarb (carbamate) from 84% in November 2011 to 31% in December 2012

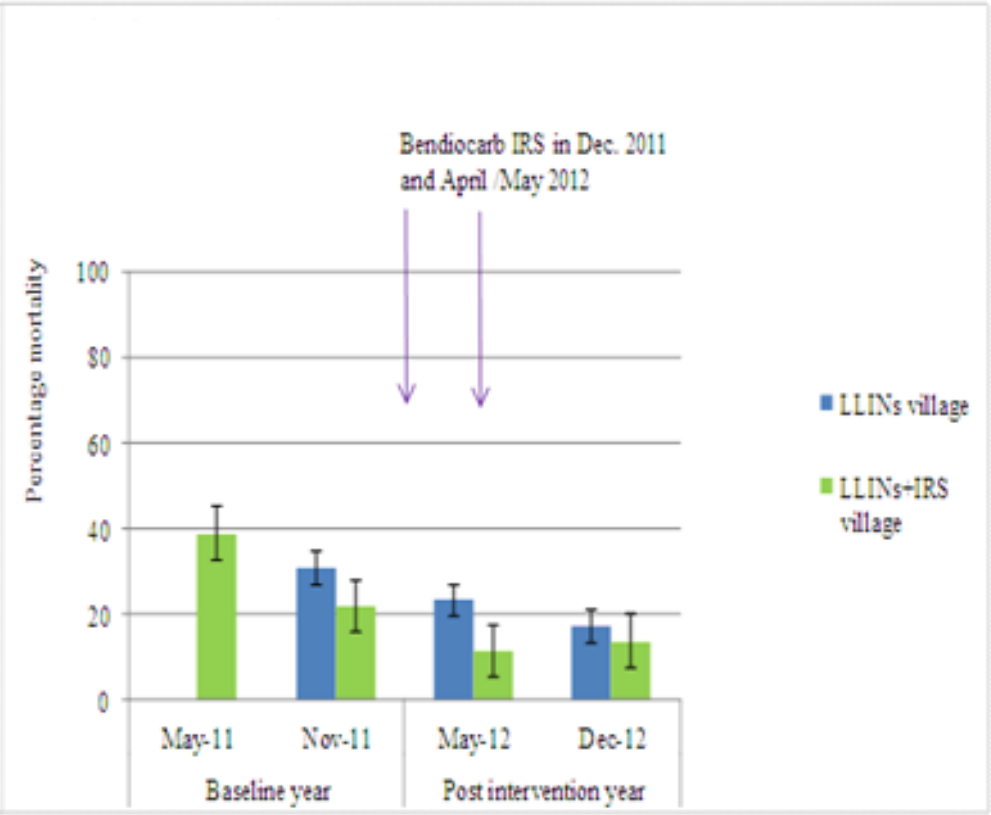
Results …

LLINs/IRS combination

Bendiocarb resistance test



Lambdacyhalothrin resistance test



Trends in the resistance status of Muleba populations exposed to bendiocarb and lambdacyhalothrin; **Evolution of bendiocab resistance**

Discussion

- High pyrethroid and DDT resistance was observed in NW Tanzania with dramatic increase in bendiocarb resistance

- The high pyrethroid resistance, high frequency of *kdr* and development of bendiocarb resistance before it was used as IRS in Muleba might be **a response to selection by recurrent IRS with lambdacyhalothrin**

- High resistance to DDT, pyrethroids and bendiocarb with different mechanisms (metabolic and target-site) is a strong evidence for occurrence of multiple resistance in NW Tanzania

- Multiple resistance may jeopardize the current vector control efforts in the area especially the use of LLINs (ITNs)

Discussion …

- In some West African countries LLINs provide some protection against *kdr* resistant *Anopheles* populations [1,2,3]

- Situation appears to be changing with selection of additional metabolic mechanisms [4,5]
  - Reduced effectiveness of ITNs against vector populations [4,5]

- Selection for metabolic resistance in Muleba *An.gambiae s.l.* may explain the high resistance and persistence as in Kenya [6]

- There is an urgent need to investigate operational impact of observed selection of resistance on the use and effectiveness of LLINs and IRS for malaria control

1,Dabire *et al.*, 2006; 2,Darriet *et al.*, 2000; 3,Henry *et al.*, 2005;4,Asidi *et al.*, 2012; 5,N'guessan *et al.*, 2007;6, Ochomo *et al.*, 2012

Conclusions

- The study has revealed the persistence of pyrethroid and DDT resistance in NW Tanzania.
- Bendiocarb-IRS selected for increased bendiocarb resistance in NW Tanzania
- Bendiocarb-based IRS did not lead to reversion of pyrethroid resistance
- Both target-site insensitivity (*kdr* mutation) and metabolic resistance are involved in pyrethroid and DDT in *An. gambiae s.l.* population of NW Tanzania
- Based on these results, resistance management strategies have been formulated and implemented (e.g. the use of Olyset plus)

Acknowledgements

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