From molecular to spatial approaches for the characterization of malaria vectors in Southeast Asia

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International Malaria Colloquium (IMC 2010) – 02/12/2010
Southeast Asia

- Malaria: public health priority
- 85% of the population leaves in areas with:
  - Active transmission (international borders)
  - Strong epidemic risk
- Major vectors of malaria belong to species complexes, such as *An. dirus*, *An. minimus*, *An. leucosphyrus*, *An. sundaicus*
Vector Control

- To implement efficient vector control programs, characterization of the vectors is necessary as for:
  - Identification of the species,
  - Estimation of the vectorial capacity (pathogen detection),
  - Resistance/susceptibility to insecticides,
  - Spatial characterization of habitats, densities and distribution
Species identification

An efficient vector control must target the correct vector species

• Morphology is still the method of reference for sorting out specimens at the genus or subgenus or complex level

• However, for species complex, molecular assays (PCR) are the only reliable methods for identifying *Anopheles* at the species level
Why molecular approaches for identifying mosquito species?

- Most of the malaria vectors belong to species complexes in which sibling species are morphologically indistinguishable and may present different vectorial capacities and trophic behaviors.

- Precise identification will allow to target the correct vector species in control programs, and help to define the appropriate way to control them in relation to their specific behavior.
High biodiversity in the Oriental Region

- Numerous *Anopheles* species per site
- Each vector belongs to a species complex such as:
  - *An. barbirostris*
  - *An. culicifacies*
  - *An. dirus*
  - *An. fluviatilis*
  - *An. leucosphyrus*
  - *An. philippinensis*
  - *An. maculatus*
  - *An. minimus*
  - *An. subpictus*
  - *An. sundaicus*
Main malaria vectors in Southeast Asia

- *Anopheles dirus*, *An. minimus* and *An. maculatus*: predominant malaria vectors in hilly and forested regions

- *An. sundaicus*: main vector along coastal areas

Vectors of both *P. falciparum* and *P. vivax*
Anopheles dirus complex: one of the world’s most efficient vectors

- Main vectors of *P. f.* and *P. v.* in deep forest
- Highly anthropophilic and exophilic (difficult to control)
- Larval habitats in temporary pools of dense forests, stagnant water, artificial containers
- Complex of 7 species with different roles in malaria transmission (from highly efficient vector to non-vector)
The distribution of the 7 species of the An. dirus complex is as follows:

- **An. dirus**: Main vector
- **An. baimaii**: Main vector
- **An. scanloni**: Local vector
- **An. cracens**: Local vector
- **An. elegans**: Local vector?
- **An. nemophilous**: Non-vector
- **An. takasagoensis**: Non-vector

Manguin et al. 2008
Dirus species identification of samples from Thailand

Molecular assay:

AS-PCR on the ITS2 sequence for the identification of 5 species within the *An. dirus* complex

Walton et al. 1999
Distribution of the *Anopheles dirus* complex (orange) and the *An. leucosphyrus* complex (green)

These 2 complexes are closely related:

- misidentifications possible among species of both complexes
Distribution of the 4 species of the Leucosphyrus complex

No PCR available!!

Major vectors of forest malaria

- *An. latens*
- *An. leucosphyrus*
- *An. balabacensis*
- *An. introlatus*
Frequency of morphological misidentifications at the species level
(On-going Franco-Thai research project)

On 497 *Anopheles* specimens: 75 misidentifications (15% errors)

Frequency of misidentifications depend on the complex (or group):
- 7.3% for the Minimus complex
- 41.5% for the Maculatus group
- 63.2% for the Dirus complex
- 100% for the Leucosphyrus complex

Comparison morphological and molecular identifications

Molecular assays necessary for reliable species identifications
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Plasmodium detection assays

Several methods available:

- Microscopic examination: search for oocysts and sporozoites (WHO gold standard method)

- ELISA* test: detection of circumsporozoites (Wirtz et al. 1985) * ELISA: enzyme-linked-immunosorbent assay

- Nested PCR: standard 18S rRNA PCR using specific primers (Singh et al. 1999), conventional PCR, qPCR

- High-throughput molecular detection: approach based on Dot 18S (18S rRNA) or Cyt-B (mtDNA) (Steenkeste et al. 2009)
Molecular detection tools

- **Advantages:**
  - High sensitivity: detect up to 8 fold *Plasmodium* spp. infections than microscopy
  - High rate of mixed infections detected (>1/3)
  - Automation of the process and objective reading of results by appropriate apparatus
  - Adapted to large-scale epidemiologic studies

(Steenkeste et al. 2009)
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Techniques to detect insecticide resistance?

• **Standard WHO bioassays**

• **Biochemical or enzymatic tests:** metabolic mechanism alterations (oxidases, esterase, GST)*

• **PCR assays:** Target site modifications: *(kdr, Ace.1, Rdl)*

* *kdr*: knockdown resistance; *ACE*: Acetylcholine esterase; *Rdl*: Dieldrin resistance; *GST*: Glutathione S-Transferase
*Kdr* mutations confer cross-resistance to pyrethroids and DDT

- **East African kdr mutation (L1014S)**
  - *An. gambiae* (S-form)
  - *An. arabiensis*
  - *An. culicifacies* (V1010L)

- **West African kdr mutation (L1014F)**
  - *An. gambiae*
  - *An. arabiensis*
  - *An. culicifacies*
  - *An. stephensi*

*Sodium channel*

Soderlund & Knipple 2003; Gayathri & Murthy 2006; Singh et al 2010
Status of insecticide resistance in *Anopheles* species from Asia

- *Anopheles dirus* and *An. minimus*: susceptible to pyrethroids

- *An. epiroticus*: highly resistant to pyrethroids in the Mekong delta (Vietnam)

- *An. culicifacies* B and C, and *An. stephensi*: resistance (*kdr*) in India

PCR assay for *kdr* detection (L1014S mutation)
in *An. culicifacies* B and C from India

Van Bortel et al. 2008

Singh OP et al. 2010
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Spatial tools for the surveillance of malaria vectors

• Localization of vector-borne diseases: largely determined by environmental factors

• Some factors (altitude, temperature, rainfall, vegetation) can be estimated or quantified from satellite image data

• Geo-referenced data of interest (entomology, parasitology, epidemiology, environment, etc.) are integrated into a geographic information system (GIS)
Principal Objectives

Help for field managers to take decision through:

– Surveillance and prediction of the spatio-temporal dynamic of vector populations

– Distribution maps of endemic zones

– Spatial analysis provide information on the determinants of malaria transmission risks

– Possibilities of anticipation and improvement of vector control strategies
GIS with geographical regional background (Malvecasia project)
Study sites in northern and southern Vietnam

Scale: 1/500,000

Study sites for Northern Vietnam

Study sites for Southern Vietnam
Use of Remote sensing

SPOT satellite image (2004)

Topographic map (quite old)

Updated land cover information
Satellite image used for developing a land use layer
Ground proof
Anopheles species association

Anopheles density/species/site

Garros et al. 2008

Northern Vietnam
Ecological associations

High correlation between the main malaria vector, *An. epiroiticus* (Sundaicus complex), and shrimp farm basins

Dusfour et al. 2004
Insecticide resistance

*An. epiroticus* (Sundaicus complex):

- Susceptible to DDT
- Resistant populations to 3 pyrethroids

Van Bortel et al. 2008
Conclusions

- Molecular tools are the most reliable ways to identify sibling species within *Anopheles* complex: the first step towards the characterization of malaria vectors and targeted vector control program.

- Vectorial capacities and resistance to insecticides: important parameters to evaluate and to monitor on a regular basis in malaria endemic foci.

- Spatial approaches: efficient and powerful indicators that help program managers to implement the appropriate control strategy and develop an early warning of the potential for malaria epidemics.
Acknowledgements

• Collaboration through the Franco-Thai research projects (2007-10) with Prof. T. Chareonviriyanaphap (Entomology Research Lab, KU), funding from the French Ministry of Foreign Affairs, Kasetsart University and Thai Research Funds.

• Collaboration through the “Malvecasia” research project (2002-06) P.I.: Prof. M. Coosemans (Institute of Tropical Medicine, Antwerp, Belgium) and teams from SE Asia (4) and Europe (4), funding from the European Commission.
Thank you for your Attention!