1st Symposium on "Research and Innovation for the control of vectors of emerging arboviruses"

# Insecticide resistance in major arbovirus vectors: status, challenges and prospects

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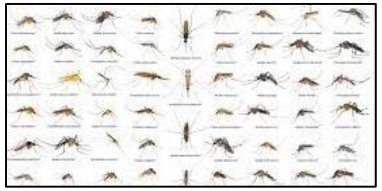
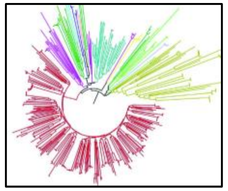
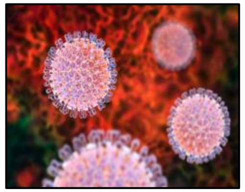
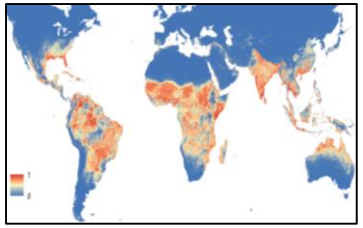
# Insecticide resistance in major arbovirus vectors

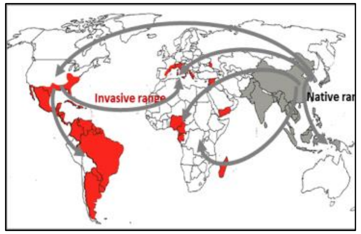


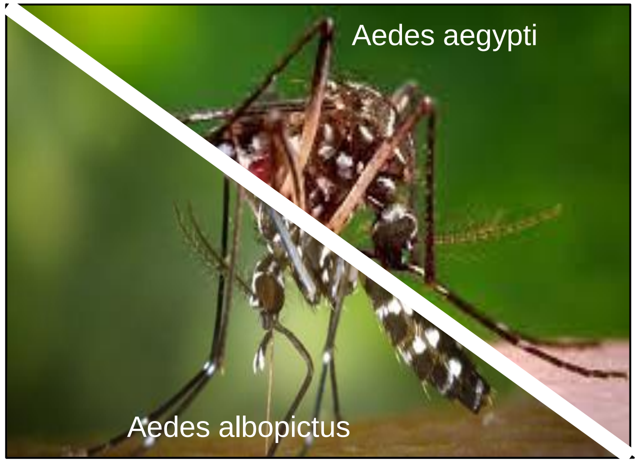




**Vectors of arboviruses…**





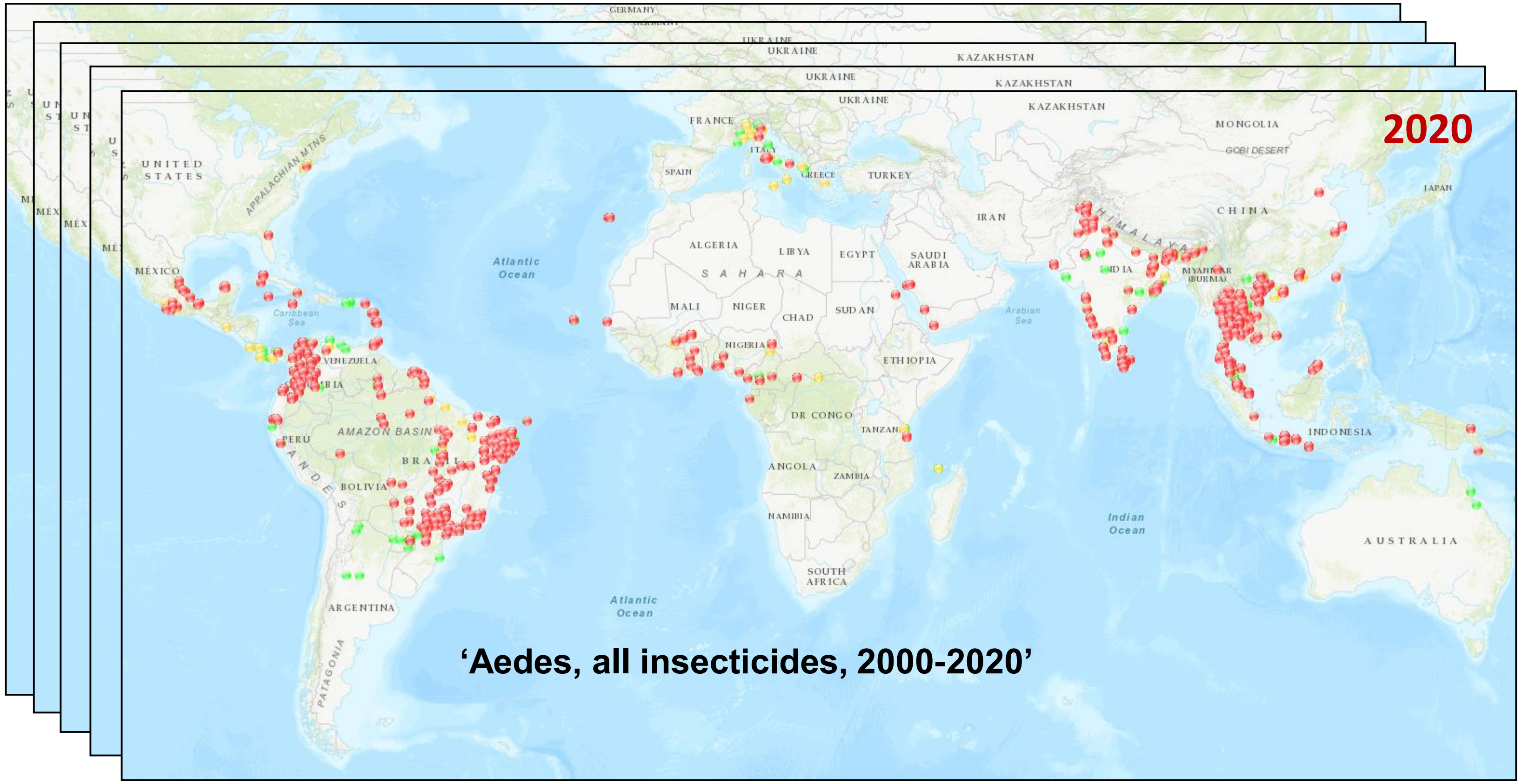

***Ae. albopictus* and *Ae. aegypti* = *key vectors***
Widespread and invasive
Major arbovirus vectors (dengue, chikungunya, zika, …)
Limited vaccine-based strategies (efficiency / availability)

**Arbovirus control mainly relies on vector control**
Multiple integrated control tools … toward greener strategies
But… chemical control still represents a key component of vector control worldwide

**Chemical insecticides for Aedes control**
Multiple chemical classes used since WW2…

Progressive shift to pyrethroids (PYR) for Aedes control since 2000s
+ additional selection pressures (agriculture, at home, private resorts…)

# Dynamics of insecticide resistance in Arbovirus vectors




IR Mapper

WORLDWIDE INSECTICIDE RESISTANCE NETWORK
WIN

**Insecticide Susceptibility**

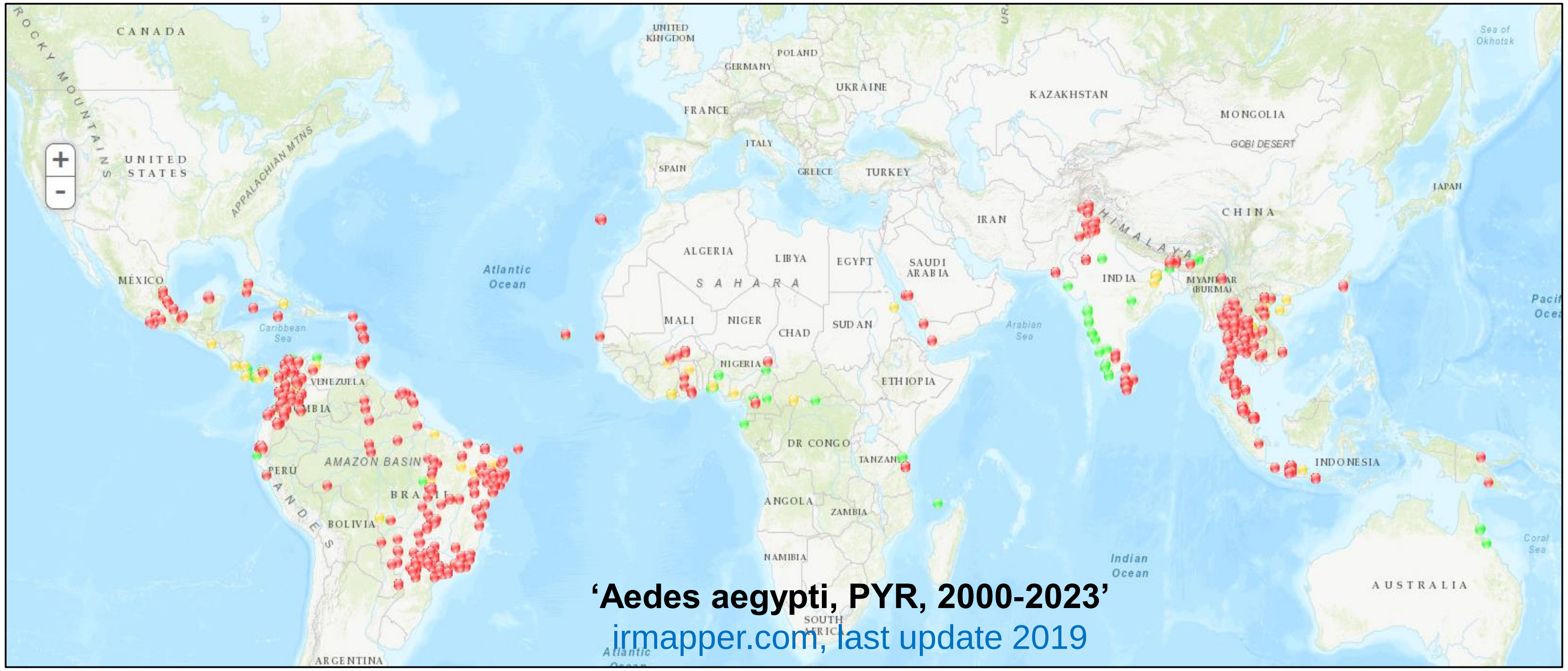
| Confirmed Resistance
| Possible Resistance
| Susceptibility

https://aedes.irmapper.com

- Global rise of insecticide resistance in arbovirus vectors
- Resistance to all insecticides families (OC, OP, Carb, PYR, …)

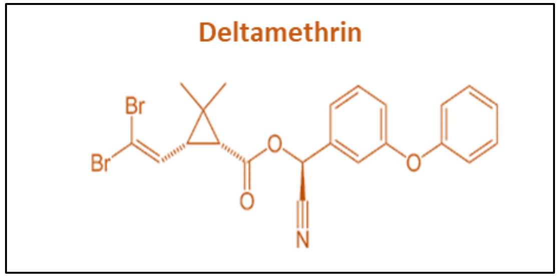
**→ In 2020, resistance to insecticides found in >57 countries at risk of Aedes-borne diseases**

# Status of PYR resistance in *Aedes aegypti*




**'Aedes aegypti, PYR, 2000-2023'**

irmapper.com, last update 2019



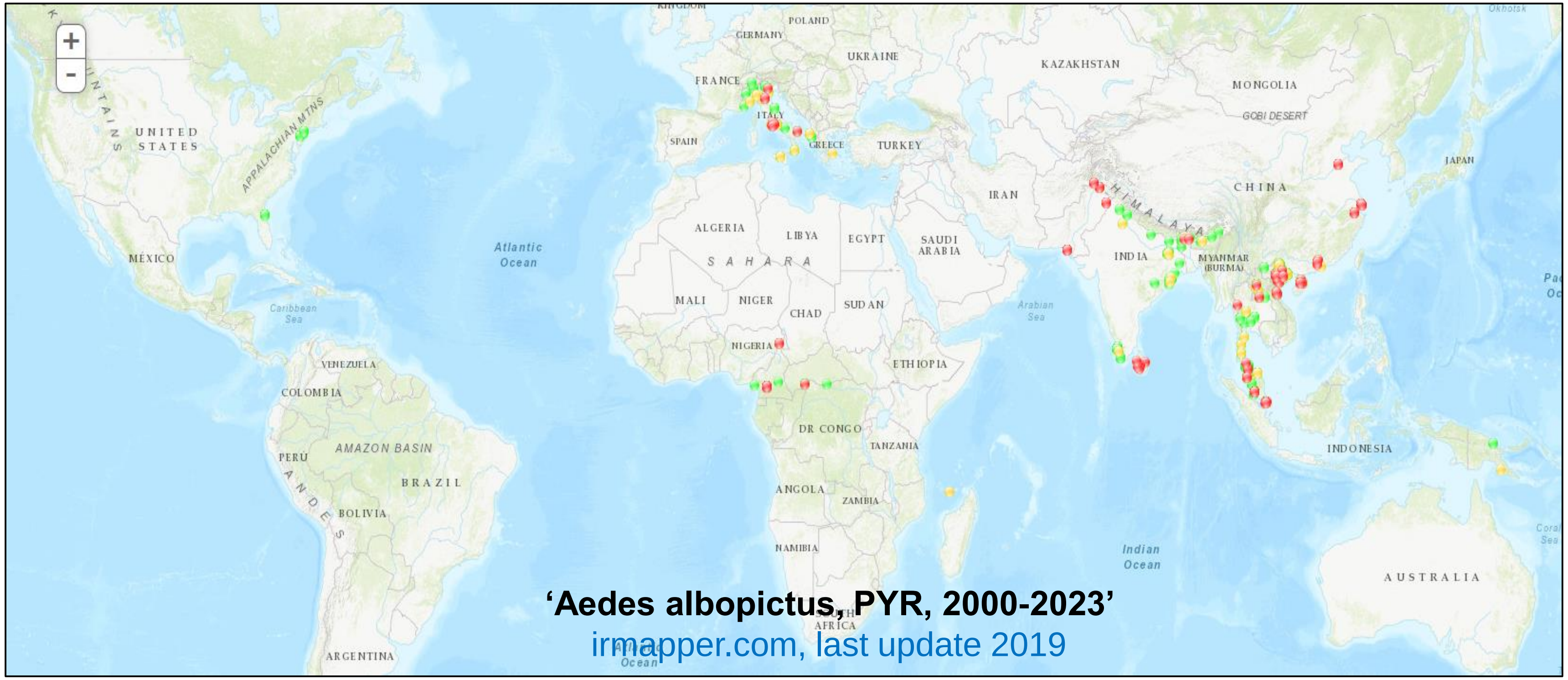

- Pyrethroid resistance occuring worldwide (still few data from Africa)
- High PYR resistance levels in the Americas and Asia

→ can significantly impact vector control efficacy

WORLDWIDE INSECTICIDE RESISTANCE NETWORK WIN

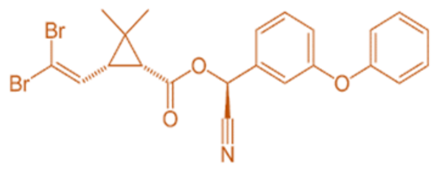
Moyes et al 2017 Plos NTD
Dusfour et al 2019, Plos NTD)

# Status of PYR resistance in *Aedes albopictus*


'Aedes albopictus, PYR, 2000-2023'
irmapper.com, last update 2019

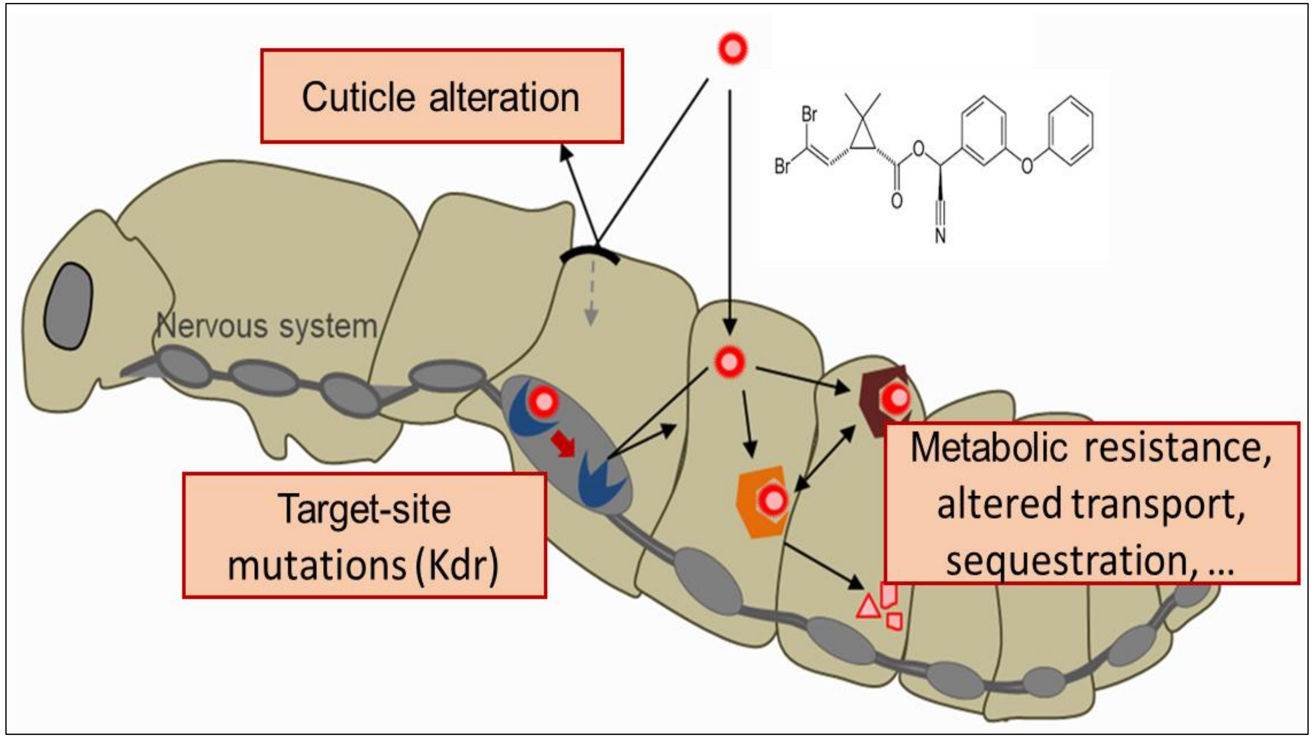
Deltamethrin




- Limited resistance data in public databases (Americas, Africa, …)
- PYR resistance currently rising, in different continents

→ Still time to implement resistance management ?

# Insecticide resistance mechanisms in major Aedes vectors




**Cuticle resistance**
- not well characterized in Aedes

**Target-site resistance**
- PYR and DDT (VGSC gene, Kdr mutations)
- Cyclodienes (Gaba-R gene, Rdl mutation)
- ~~Carb and OP (Ace1 mutation)~~

→ 2 successive mutation events required for G119S mutation (Weill et al. 2004, Curr Biol)

**Metabolic resistance**
Detoxification: P450s, CCEs, GSTs, UDPGTs, …
+ altered transport / sequestration / excretion

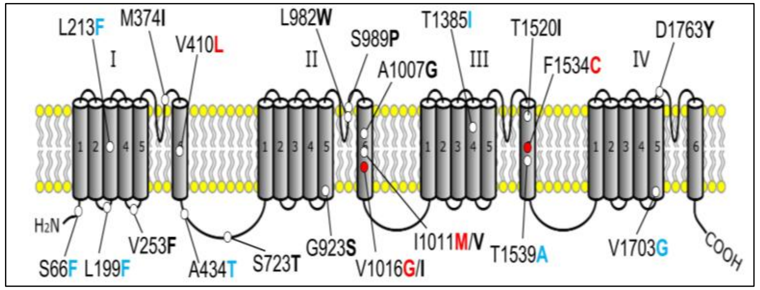
- OP/Carb → mainly detoxification (no ace1 mutation)
- PYR → kdr mutations + detoxification …

What role for cuticle resistance ? (broad spectrum / cross-resistance)

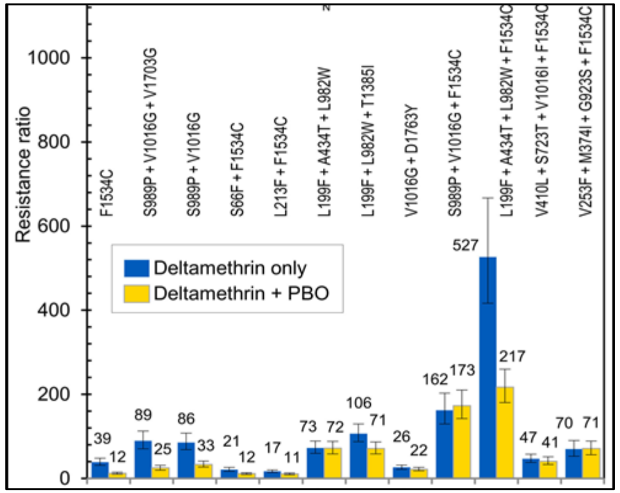
# Molecular markers of insecticide resistance in Aedes

## Target site mutations

| insecticides | mutations | Ae. aegypti | Ae. albopictus |
|---|---|---|---|
| OP /Carb | Ace1 (G119S) | no ? | no ? |
| Cyclodienes | Rdl (A302S) | yes | yes |
| PYR (DDT) | Kdr V410L | yes | ? |
| | Kdr L982W | yes (new) | ? |
| | Kdr S989P | yes | ? |
| | Kdr I1011M | yes | ? |
| | Kdr V1016G/I | yes (G/I) | yes (G) |
| | Kdr F1534C | yes | yes |




- Robust DNA markers
- Need to consider haplotypes



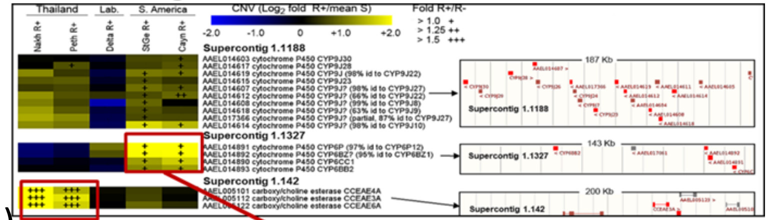

Kasai et al 2022, Sci Advances

## Detoxification enzymes

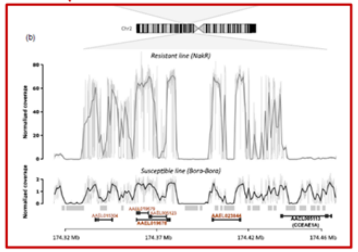
| insecticides | family | gene | Ae. aegypti | Ae. albopictus |
|---|---|---|---|---|
| DDT | GST | GSTE2 | yes | ? |
| OP | CCE | CCEAE3A/6A | yes | yes |
| PYR | P450 | CYP6BB2 | yes | ? |
| | | CYP6P12 | yes | CYP6-like ? |
| | | CYP9M9 | yes | ? |
| | | CYP9J28 | yes | ? |
| Carb | P450 ? | ? | ? | ? |

- Over-transcription (RNA)
- Increased gene copy number (DNA)

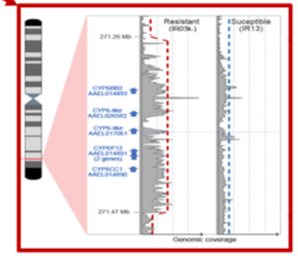
**CNV conferring resistance in Aedes**




Faucon et al., *2015 Genome Research*
Faucon et al 2017 Plos NTD



Cattel et al 2020, *Evolutionary Applications*
Grigoraki et al 2015 and 2017



Bacot et al, *in prep*

**Esterase cluster**
(*aegypti* and. *albopictus*)

**P450 cluster**
(*aegypti*)

→ Good set of markers in *Ae. aegypti*... but very few in *Ae. albopictus*
→ No validated cuticle markers
→ Resistant populations often combine multiple mechanisms !

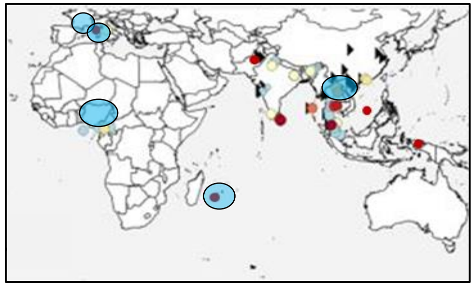
# Tigerisk: Characterizing PYR resistance in *Ae. Albopictus*

Funding: anses, La Région Auvergne-Rhône-Alpes, Université Grenoble Alpes

Partners: cnrs, E.I.D. Rhône-Alpes, Institut Pasteur du Laos, isem, ars

## Main objectives

- Improve PYR resistance surveillance
- Characterize resistance markers (beyond kdr mutations)
- Develop novel molecular diagnostic tools

## Five regions studied

**Europe**
Metropolitan France
EID Méditerranée, Altopictus

**Indian Ocean**
La Réunion
ONE HEALTH OI

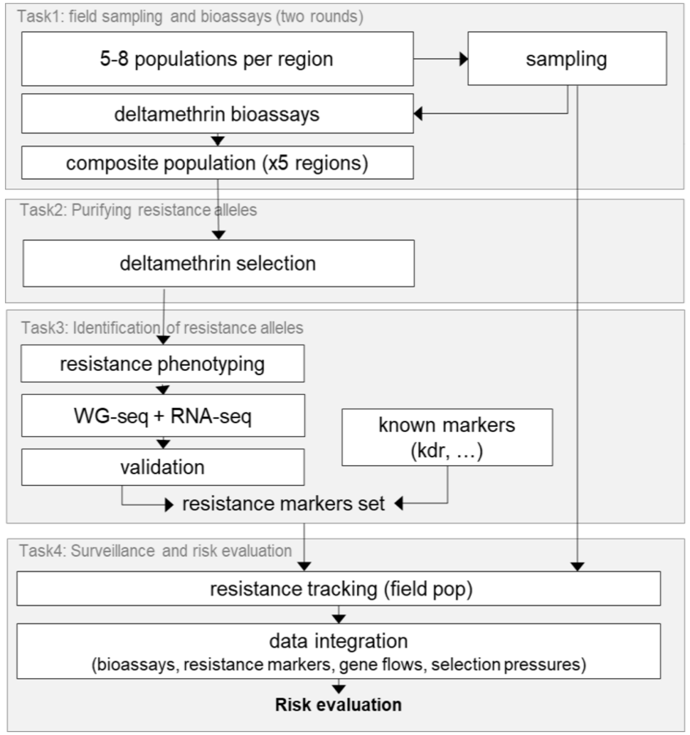
**SE Asia**
Laos/Cambodia
Institut Pasteur du Cambodge

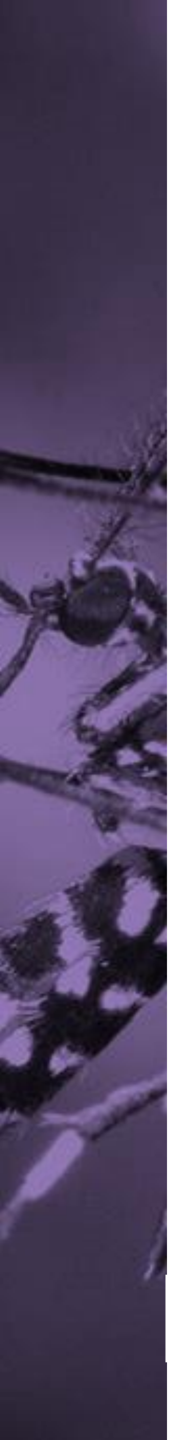
**Europe**
Italy
Sapienza Università di Roma

**Africa**
Gabon/CAR
MIVEGEC

## Four tasks

1 - Perform bioassays (deltamethrin) in sentinel sites form each region
2 - Purify resistance alleles from each region (lab selection)
3 - Identify resistance markers (integrated NGS approach)
4 - Monitor resistance markers in the field…

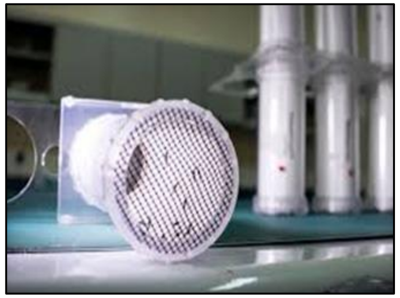




## Task1: Bioassays on sentinel populations

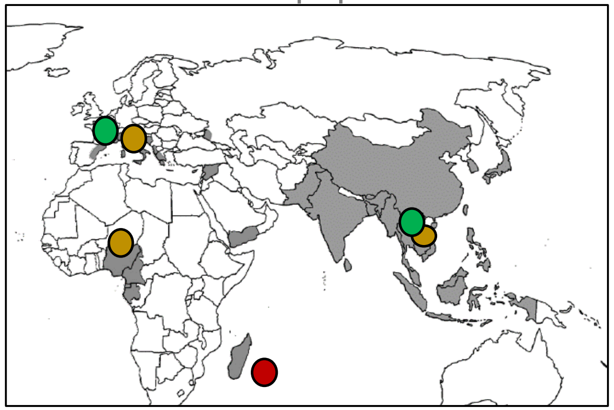
- 6-8 pop / region (France, La Réunion, SE Asia) + published data
- Deltamethrin bioassays

→ No phenotypic resistance in France (metropole) and Laos
→ Moderate resistance in Cambodia (+ Africa, Italy, …)
→ Significant resistance in La Reunion island



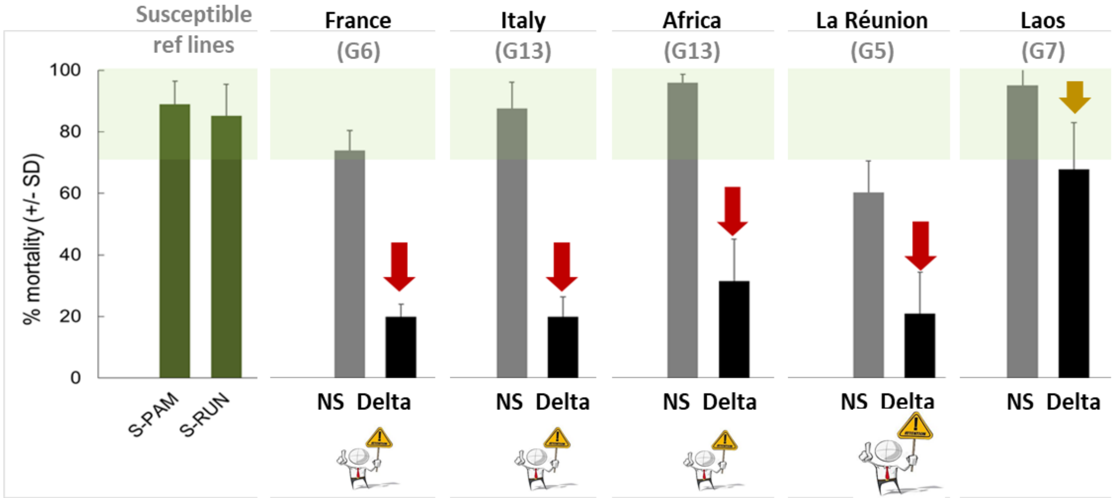
Ngoagouni et al 2016, P & Vectors,
Pichler et al 2015, Pest Man Sci, …

Field populations



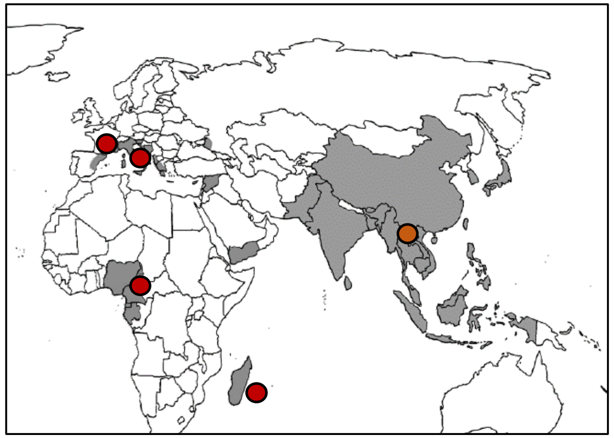
## Task2: purifying resistance alleles by selection

- One composite population per region
- Deltamethrin selection (>50% mortality)
- Comparative bioassays (Non-Sel Vs selected Delta)



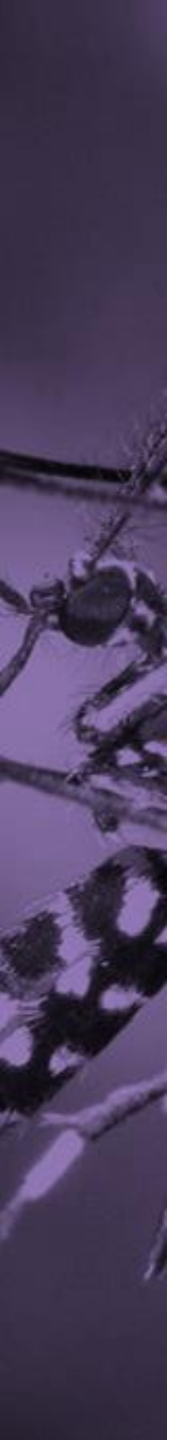

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Kdr | - | - | yes ? | yes | no | ? | no |
| Metabolic | - | - | no ? | no ? | yes ? | yes | ? |

After few generations of selection



Bacot et al, in prep

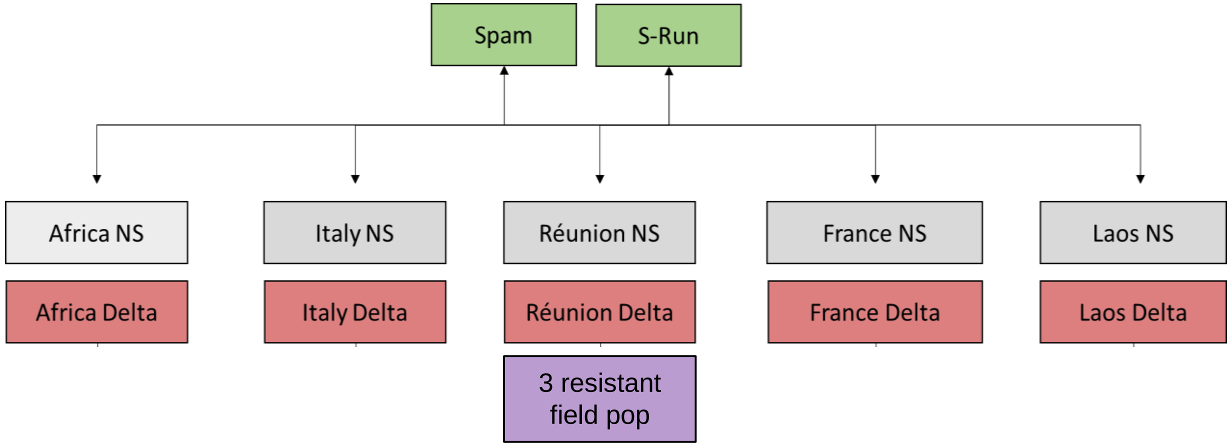
Resistance alleles rapidly selected in most lines
→ resistance alleles already circulating in all continents …?
→ kdr and/or metabolic resistance (region-specific)



# Next step… identifying resistance alleles by NGS

## Experimental design

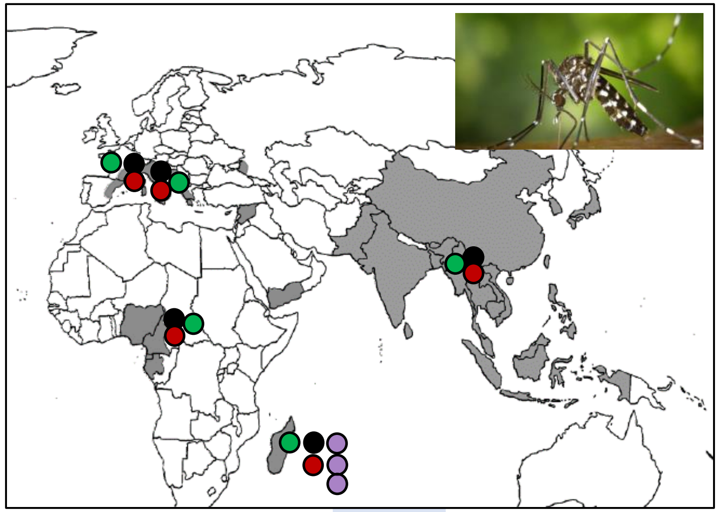
- Susceptible reference lines
- Non-selected lines
- Delta-selected lines
- Field resistant populations (La Réunion)




## Genomics studies

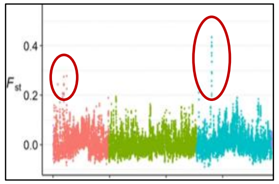
Whole Genome Pool-seq + RNA-seq

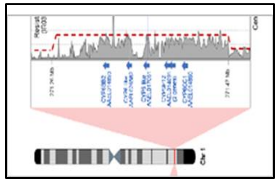
→ NGS data produced, bioinformatics in progress…



**WG sequencing**

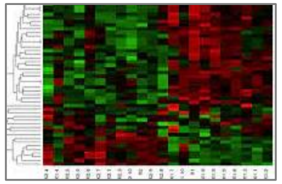
Polymorphisms
CNVs, TEs…

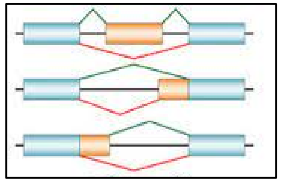


**RNA-seq**

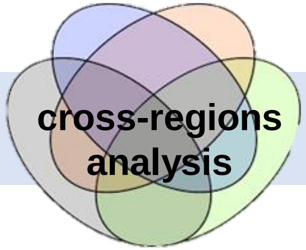
Gene expression levels
Differential splicing





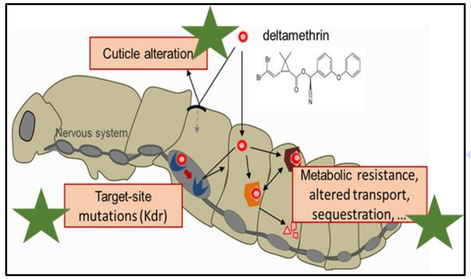
by region

data integration (molecular markers)




data integration (regions)

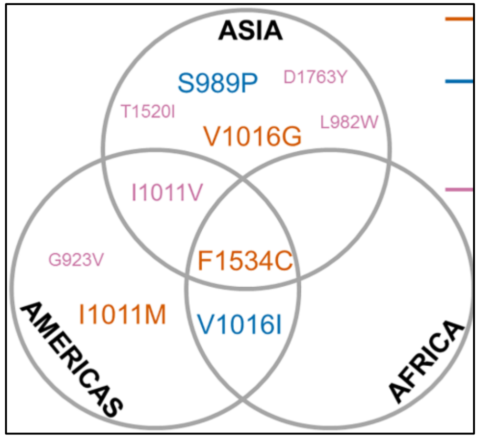
**resistance marker set**



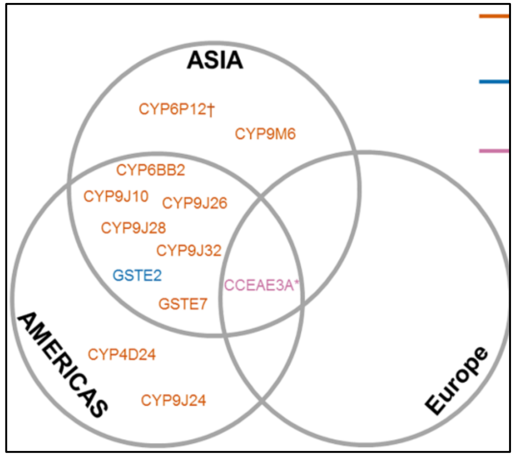
# Conclusions - Next challenges

Basic science

**A robust molecular resistance marker panel in Aedes**
→ Covering all mechanisms ?
→ How many markers to catch the entire resistance phenotype ?
→ toward DNA markers ?




Moyes et al. 2017 Plos NTD (WIN network)




**How resistance affects arbovirus control globally ?**
→ Impact on arbovirus transmission (Immunity, host-virus interactions)
→ Impact on other VC tools (IGRs, Bti, Wolbachia, densoviruses, SIT, ...)

Applied science

**Improve resistance tracking**
→ Integrated surveillance frameworks (robust, standardized, sustainable,, …)
→ Resistance databases (update, maintain, share, …)


**Improve resistance management**
→ Detect resistance earlier
→ better evaluate its impact on arbovirus control
→ Develop alternative (greener) control tools and novel biocides/synergists
→ Improve decision making process (from surveillance to action)

# Aedes resistance - Take-home messages

- Chemical insecticides → still a key component of vector control
- Resistance widespread in *Ae. aegypti* and rising in *Ae. albopictus*
- Resistance can affect vector control efficacy

→ Research still needed ! (surveillance, management, + new tools)
→ All actors working together at a global scale (networking !)

**@thankyou ;)**