Research in *Aedes* mosquito vectors at GHTM/IHMT

João Pinto

Unidade de Parasitologia Médica

Global Health & Tropical Medicine

DESDE 1902
INSTITUTO DE HIGIENE E
MEDICINA TROPICAL
UNIVERSIDADE NOVA DE LISBOA

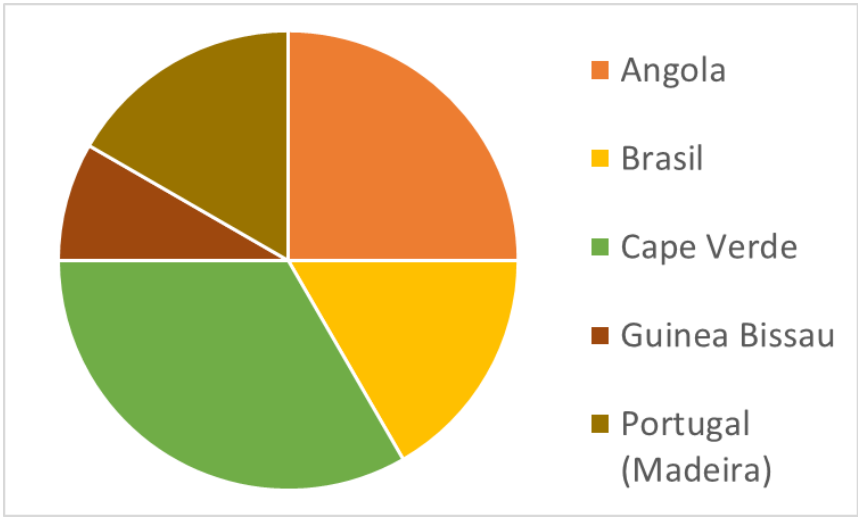
- Academic institution of Universidade NOVA de Lisboa
- Postgraduate training, research and cooperation for health development in Tropical Medicine and Global Health
- Staff: 96 people

Regular Courses

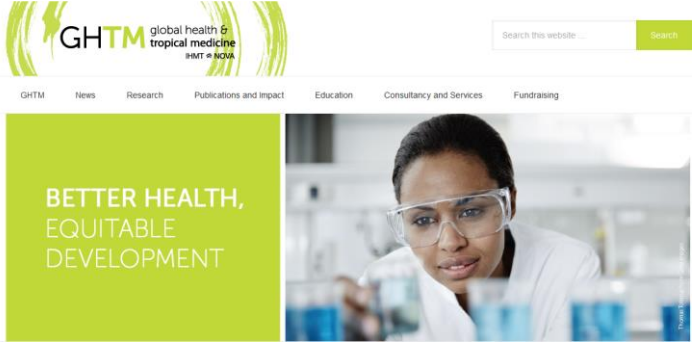
- 5 PhD
  - Biomedical Sciences
  - Tropical Diseases & Global Health
  - Human Genetics ans Infectious Diseases
  - Tropical Medicine
  - International Health
- 6 MSc
  - Biomedical Sciences
  - Medical Parasitology
  - Medical Microbiology
  - Health Statistics
  - Public Health & Development
  - Tropical Health

Teaching in Medical Entomology

12 short courses*, since 2007

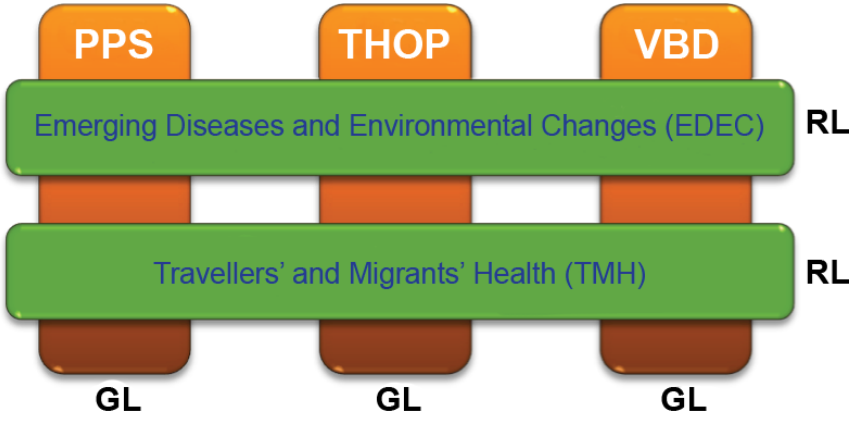


* In vector biology, malaria and arboviruses



Research at IHMT

- Global Health and Tropical Medicine
  - New R&D centre since 2014, rated “excellent” by FCT evaluation



PPS: Population Policy and Services

THOP: TB, HIV and Oportunistic Diseases & Pathogens

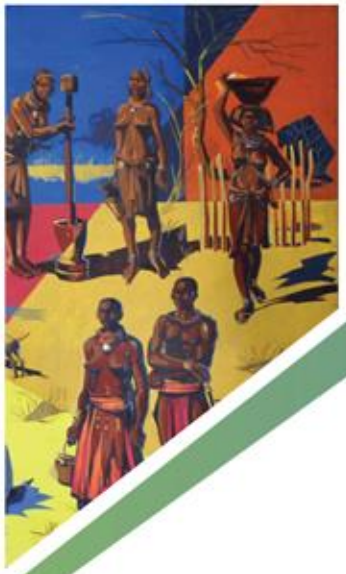
VBD: Vector-Borne Diseases & Pathogens

- Vector-Borne Diseases & Pathogens
  - Vector bioecology and population biology, molecular epidemiology, drug & insecticide resistance, host-pathogen interactions
  - Malaria, leishmaniasis, arboviruses, HAT and TTDs
  - 37 PhD members.

Aedes research at GHTM/IHMT

- Bioecology and vector monitoring
- Population genetics and evolutionary biology
- Mechanisms of insecticide resistance
- New tools for vector control
- Social studies (community awareneness & practice)




The 2012 Madeira Dengue outbreak

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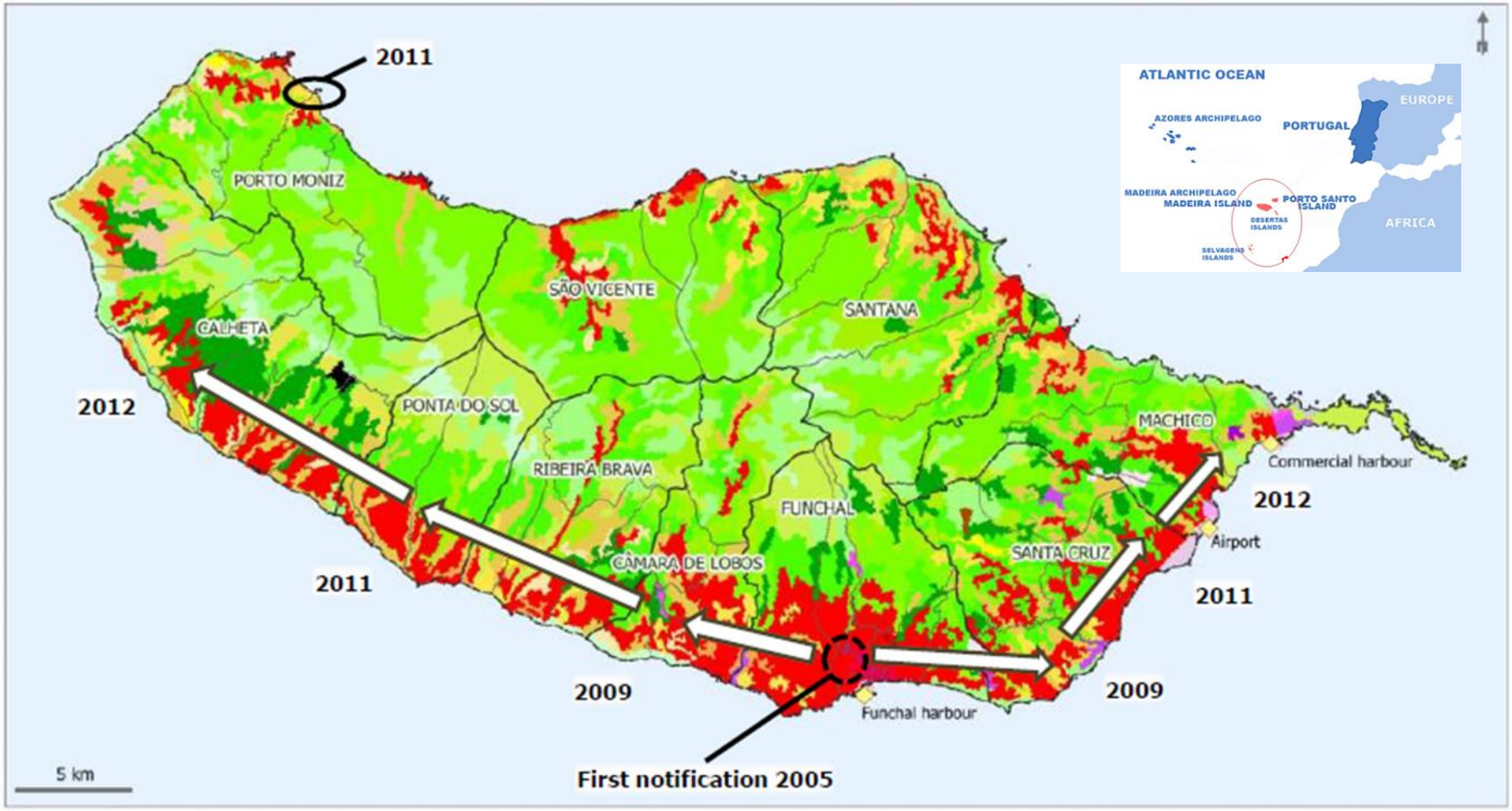
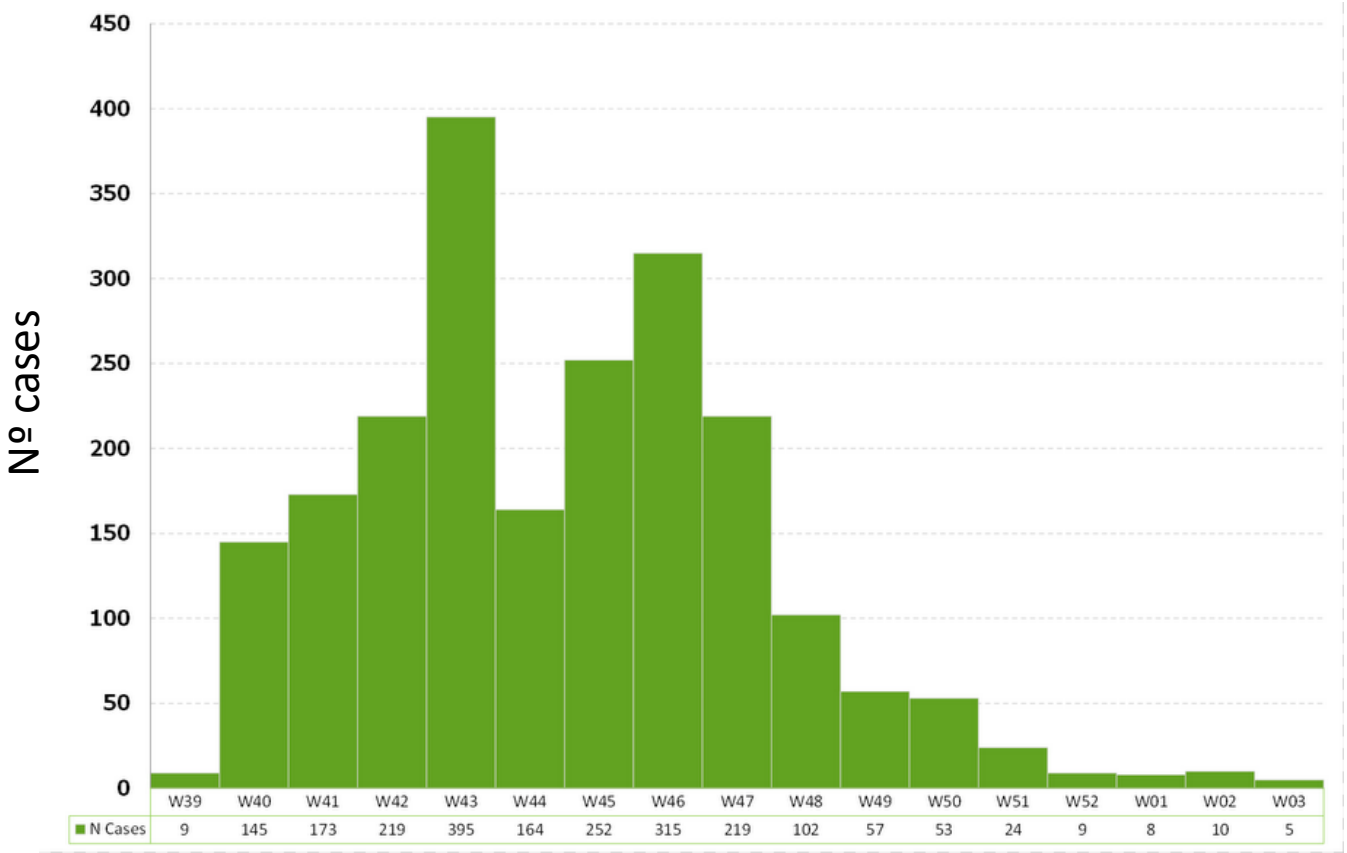
Aedes aegypti in Madeira Island

Ae. aegypti evolution in Madeira between 2005 and 2012



Dengue outbreak in Madeira, 2012-2013

- 2168 notified cases, 128 hospitalizations, no fatalities
  - DENV-1 serotype

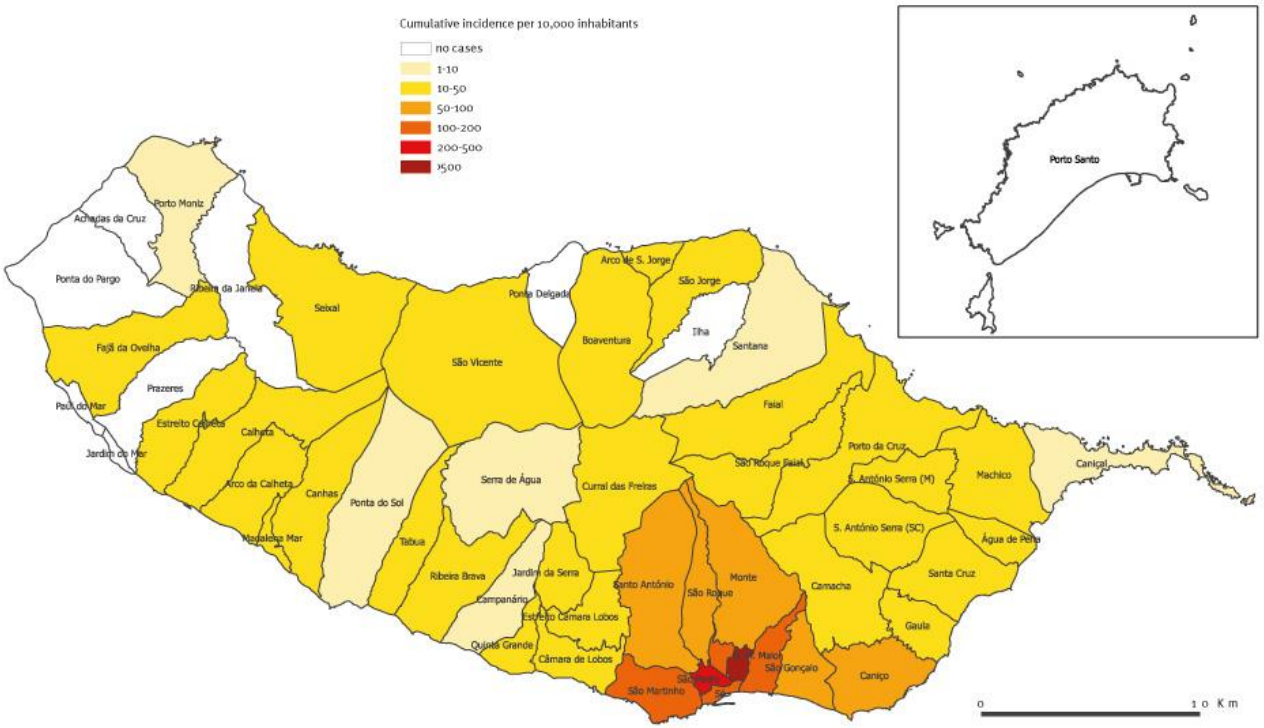


Dengue outbreak in Madeira

- Nearly all municipalities affected but most cases concentrated in the capital Funchal

FIGURE 2

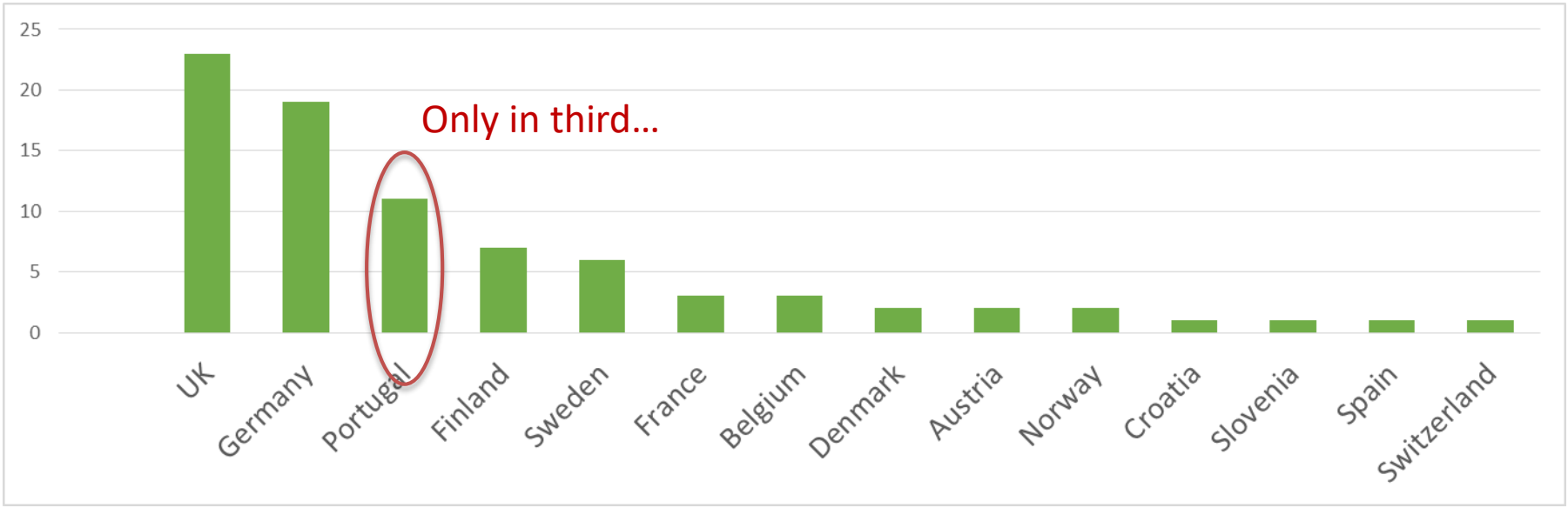
Cumulative incidence of dengue cases by parish, outbreak on Madeira, Portugal, 3 October–25 November 2012



The map was created in Quantum GIS with support from the European Centre for Disease Prevention and Control.

Exported cases

- 82 dengue cases in 14 countries imported from Madeira

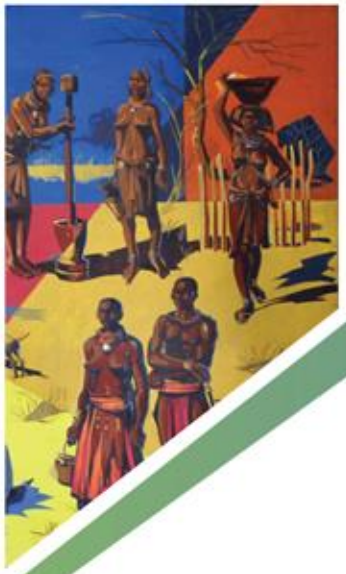


RAPID COMMUNICATIONS

More reasons to dread rain on vacation? Dengue fever in 42 German and United Kingdom Madeira tourists during autumn 2012

C Frank (FrankC@rki.de)[1], M Höhle[1], K Stark[1], J Lawrence[2]




Vector Monitoring

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Vector monitoring

- Infestation indexes (2012 outbreak)

	Funchal	Câmara de Lobos	St Cruz	All municipalities
N. houses surveyed	273	125	22	**420**
N. of containers inspected	1681	1298	431	**3410**
House Index (*HI'*>4)	32.9	12.0	45.5	**27.4**
Container Index (*CI'*>3)	12.8	3.8	3.5	**8.2**

- The problem of Abandoned/closed houses

Vector monitoring

- Storm drains as major breeding sites for *Ae. Aegypti*
  - Absolute breeding index: 28.1

Dry

Negative

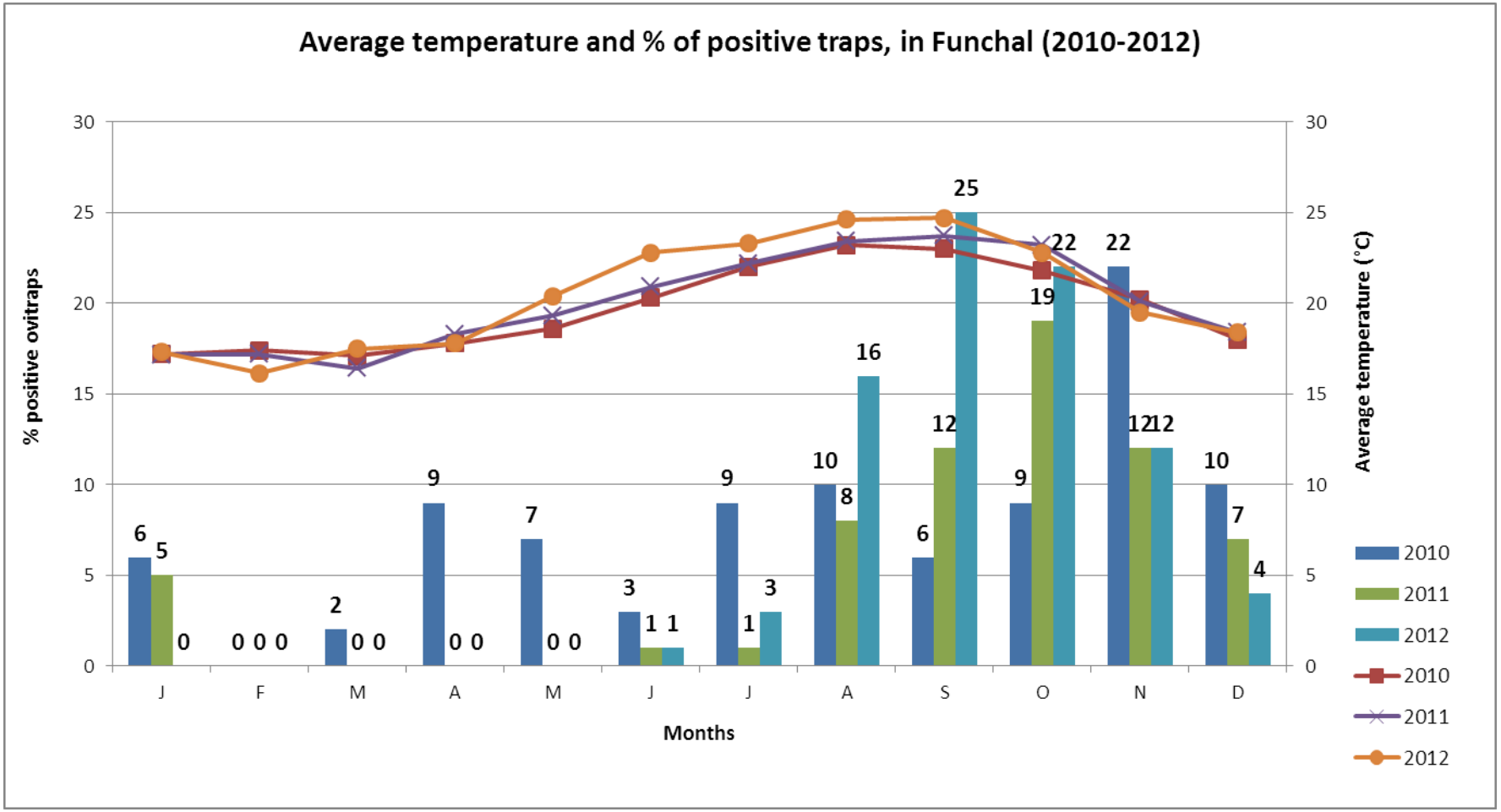
Aedes aegypti

Aedes aegypti & other mosquito spp.

Other mosquito spp.

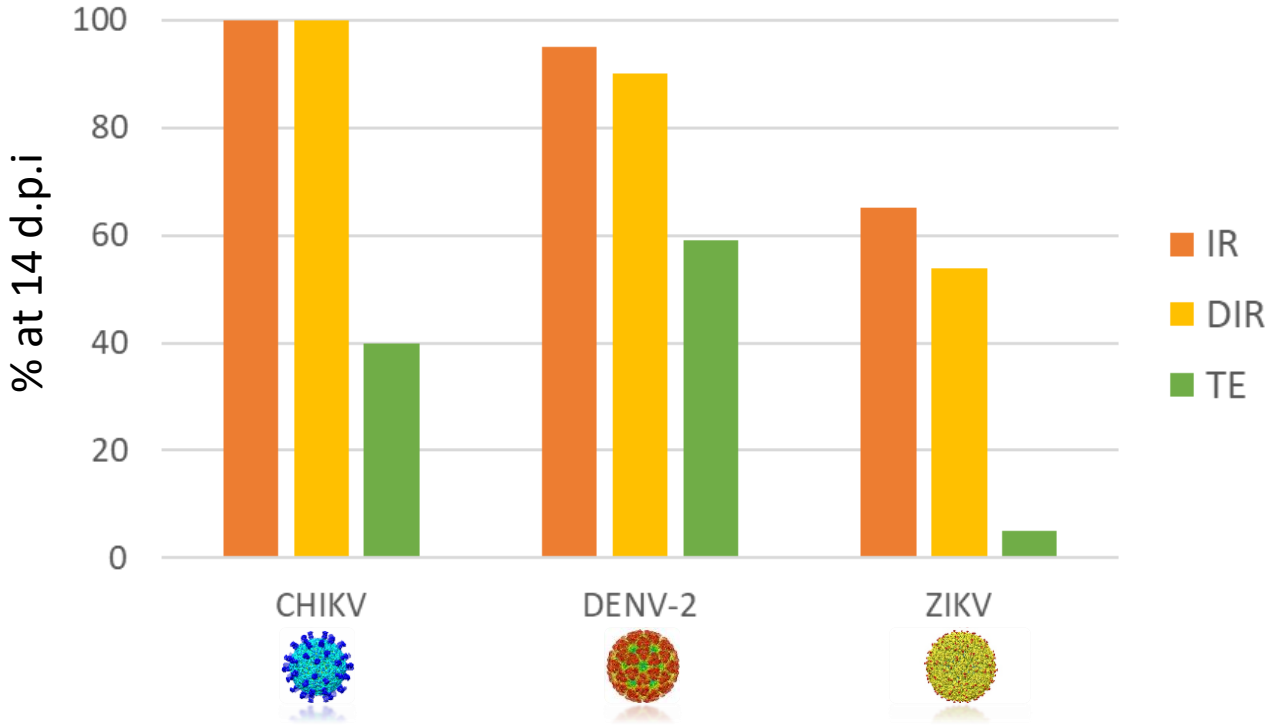
Vector monitoring

- Seasonality pattern of *Aedes aegypti* 2010-2012 (ovitraps)



Vector competence

- Oral infections with CHIKV, DENV-2 and ZIKV (N=20 per exp.)
  - Infection rate (IR)
  - Disseminated infection rate (DIR)
  - Transmission efficiency (TE)



- Higher susceptibility and transmission efficiency to CHIKV and DENV-2
- Lower susceptibility and transmission efficiency to ZIKV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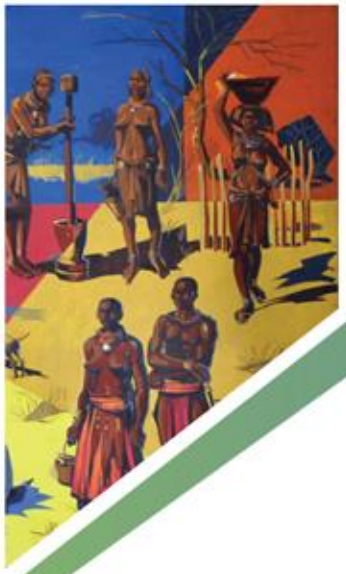
SANITATEM QUAERENS IN TROPICOS
MEDICINA TROPICAL
MAIS DE 100 ANOS
A INOVAR A SAÚDE
NOS TRÓPICOS





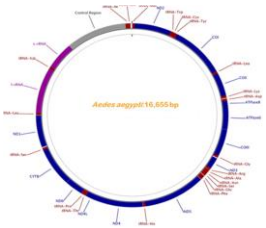

Origins

mtDNA sequencing

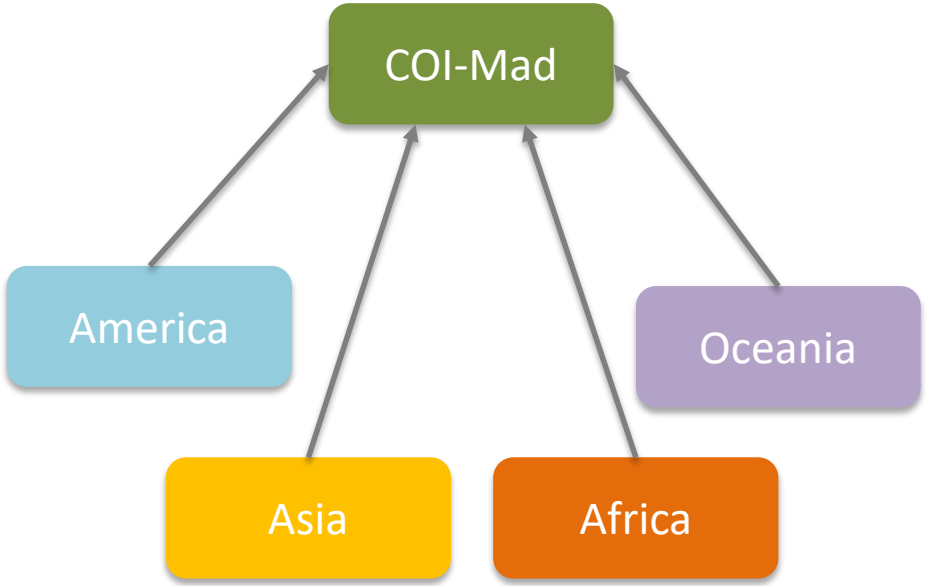
Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. *108*(Suppl. I): 3-10, 2013

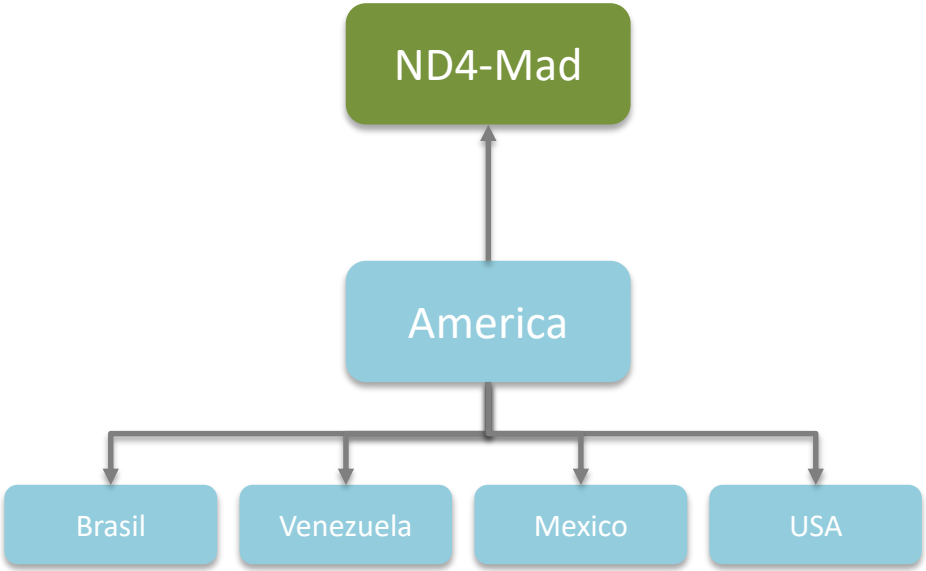
Aedes aegypti on Madeira Island (Portugal): genetic variation of a recently introduced dengue vector

Gonçalo Seixas[1], Patrícia Salgueiro[1,2], Ana Clara Silva[4], Melina Campos[5], Carine Spenassatto[5], Matías Reyes-Lugo[6], Maria Teresa Novo[1,3], Paulo Eduardo Martins Ribolla[5], João Pedro Soares da Silva Pinto[1,2/+], Carla Alexandra Sousa[1,3]



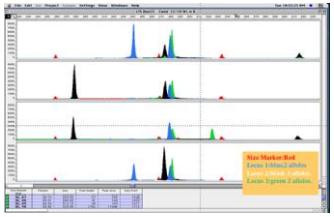
- Single haplotype for both COI and ND4 mtDNA genes





Microsatellites

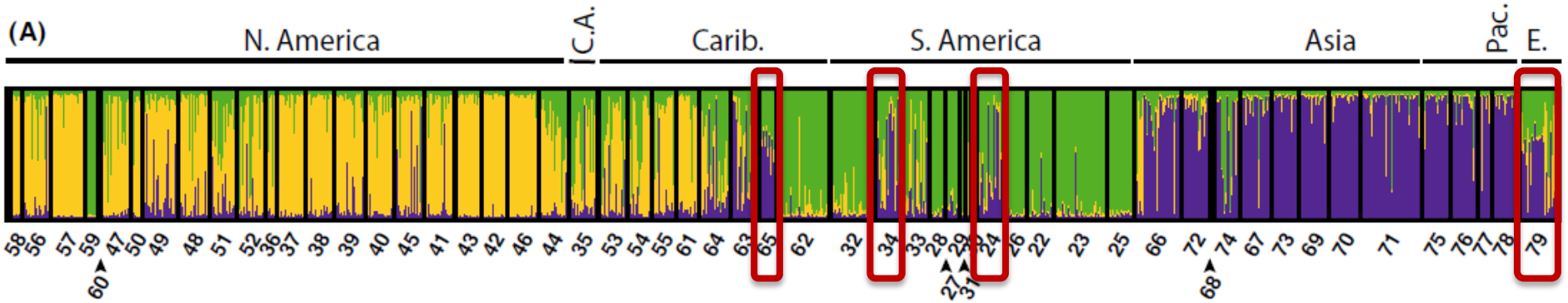
MOLECULAR ECOLOGY
Molecular Ecology (2016)
doi: 10.1111/mec.13866



Global genetic diversity of *Aedes aegypti*

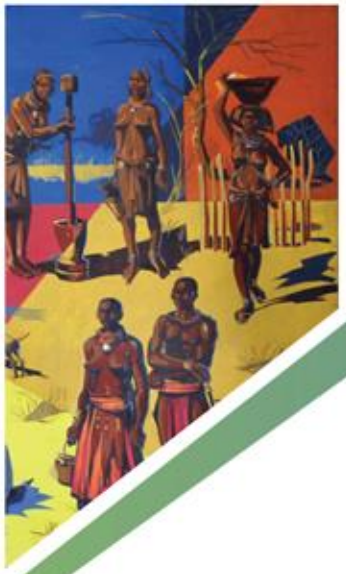
ANDREA GLORIA-SORIA,* DIEGO AYALA,†‡ AMBICADUTT BHEECARRY,§

- 12 loci analised for 79 samples collected worldwide
  - Bayesian clustering analysis (STRUCTURE)



- Genetic ancestry closest to Brasil, Venezuela and Guadeloupe
  - Strong human mobility with Brasil and Venezuela.
  - Further analyses are ongoing




Insecticide resistance

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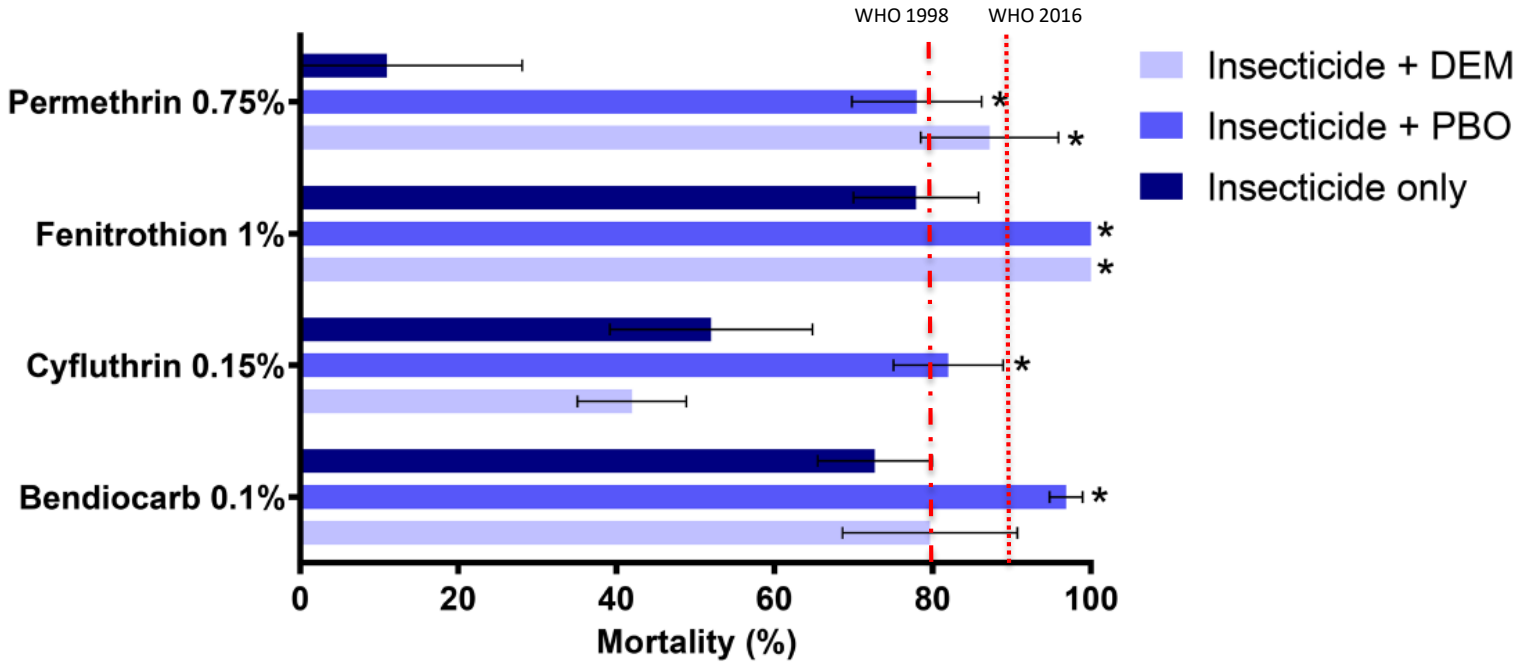
PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Insecticide resistance is mediated by multiple mechanisms in recently introduced *Aedes aegypti* from Madeira Island (Portugal)

Gonçalo Seixas[1], Linda Grigoraki[2], David Weetman[3], José Luís Vicente[1], Ana Clara Silva[4], João Pinto[1], John Vontas[2,5], Carla Alexandra Sousa[1]*

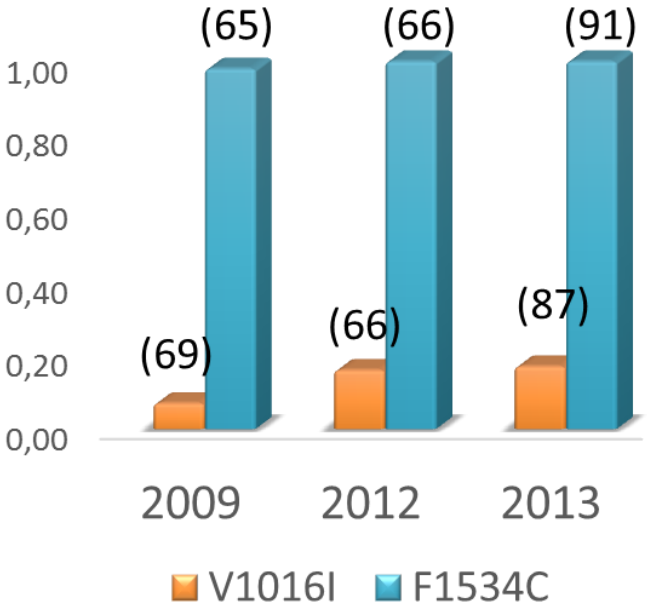
- WHO tests carried out in 2014



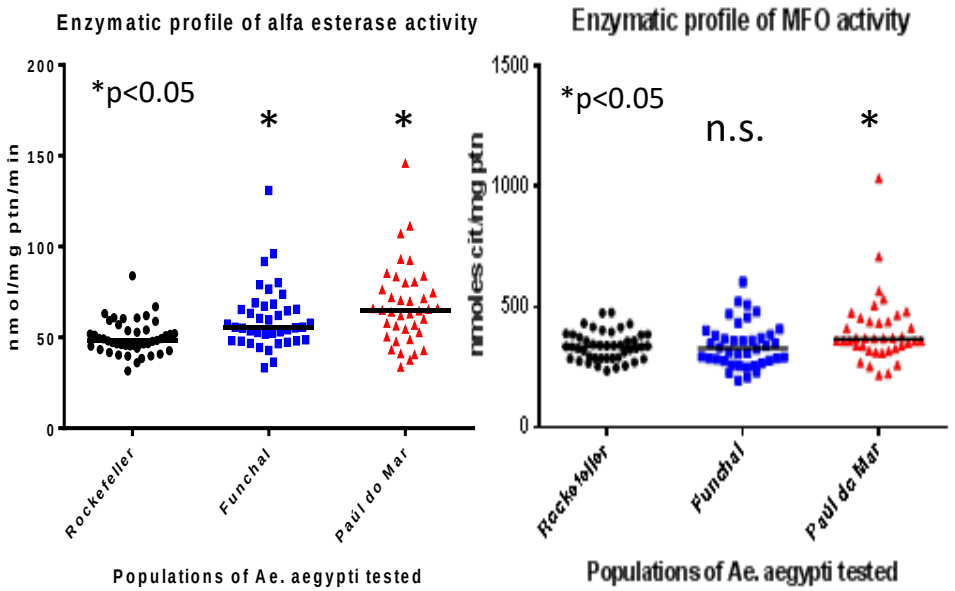
- *Aedes aegypti* is resistant to the three insecticide classes tested

Multiple resistance mechanisms

Knockdown resistance mutations



Metabolic resistance (biochemical assays)



- F1534C mutation is fixed (0.98-1.00)
- V1016I mutation increasing (0.07-0.17)

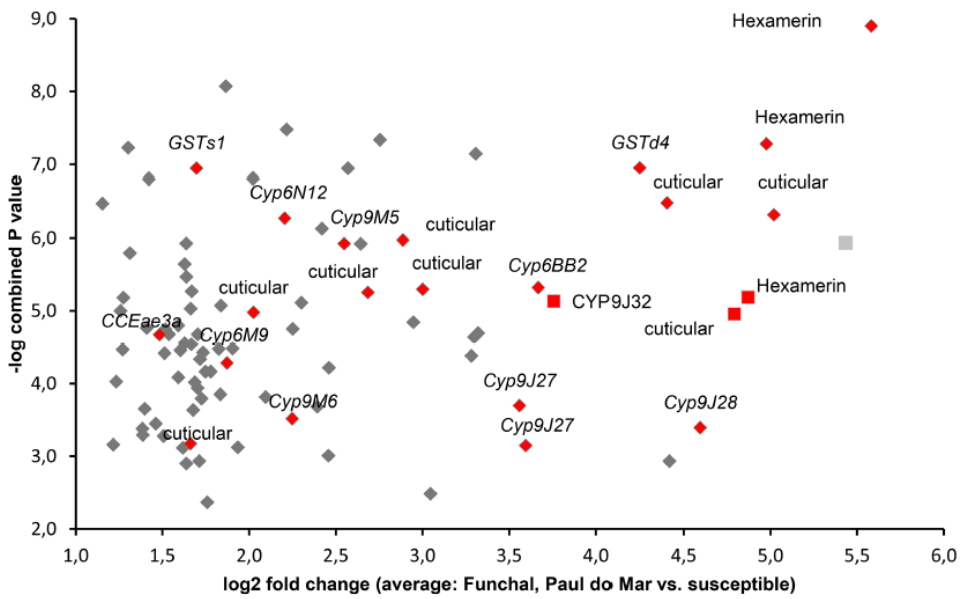
- Overexpression of detoxification enzymes (esterases)

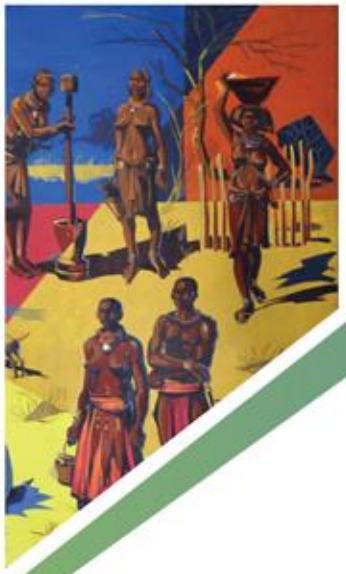
Multiple resistance mechanisms

Detox Chip

- 9 cytochrome P450 oxidases
  - Cyp9J32
  - Cyp9J28
  - Cyp6BB2
  - Cyp9M6

  } Pyrethoid metabolizers
- 1 esterase
  - CCEae3a – temephos metabolizer
- 2 glutathion S-Transferases
- 8 cuticular proteins: resistance through lower insecticide penetration
- 3 hexamerines: involved in cellular trafficking, linked to insecticide resistance.





New tools for vector control

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Alternative methods for larval control

4 pilot-studies

Assessment of storm drains treatment efficacy with marine salt

Evaluation of Vectobac G for larval control in flower pots dishes

Evaluation of Vectobac G for cemeteries treatment

Use of pyriproxyfen as an effective larvicide against *Ae. aegypti*

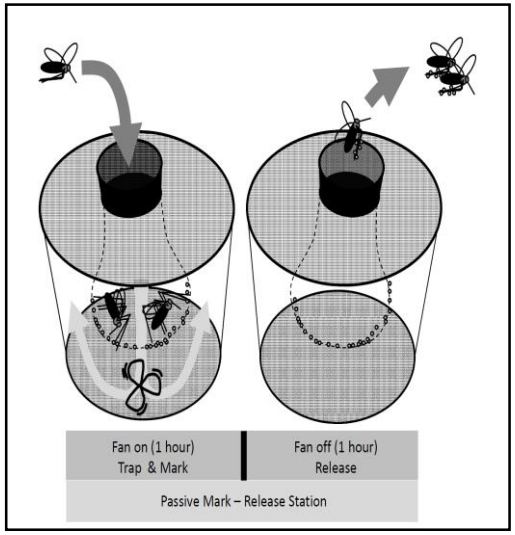
Auto-dissemination of pyriproxyfen (PPF) in Paúl do Mar

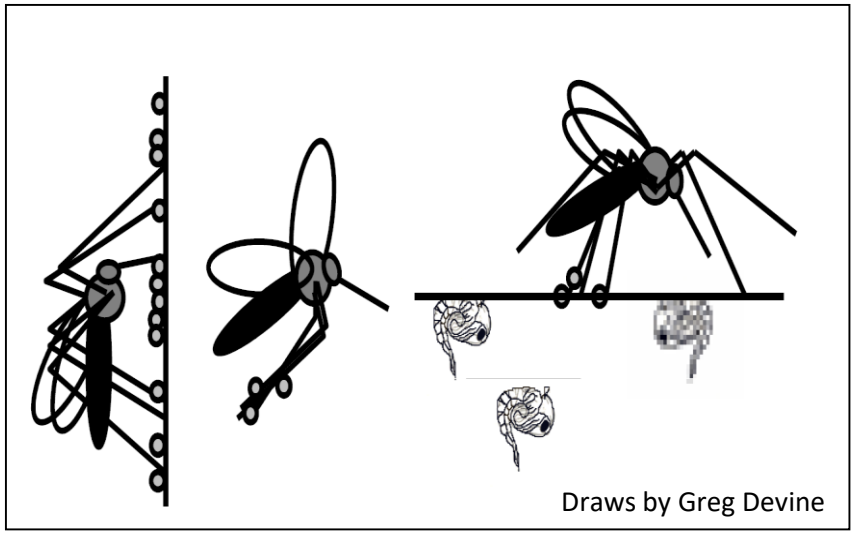
- **Paúl do Mar:** isolated area 40 km off Funchal, accessible by a 2 km tunnel

- *Aedes aegypti was* detected in 2012 and high densities were recorded in 2013

Auto-dissemination of PPF in Paúl do Mar

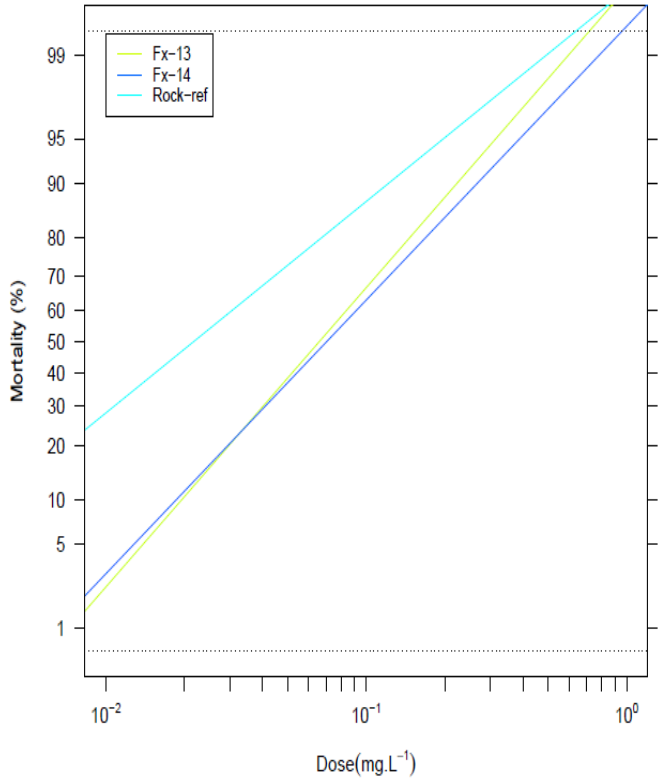
- Pilot-study supported by the FP7/EC **DENFREE Consortium (**Inst. Pasteur, Paris)

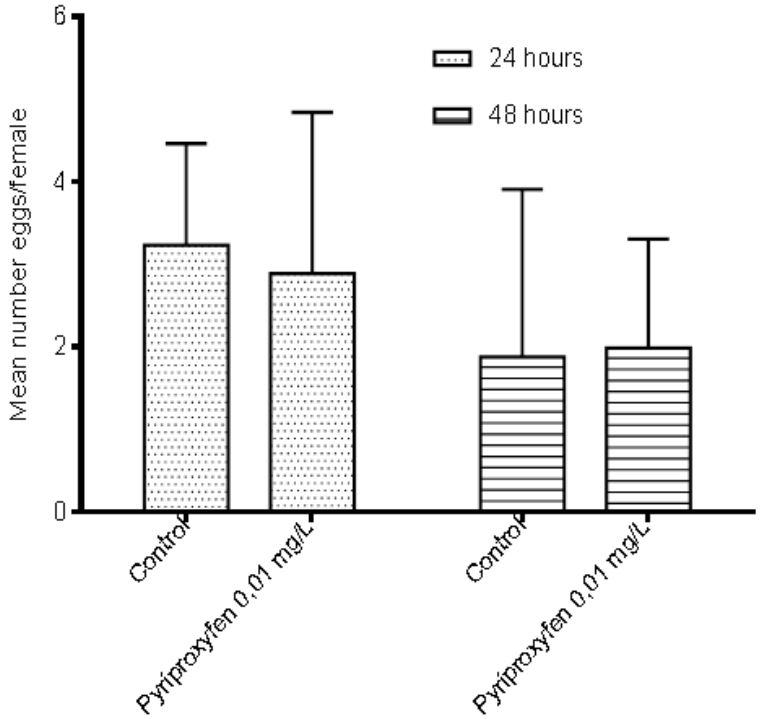




- ***Auto-dissemination process*** *- Mosquitoes resting on PPF-treated surfaces pick up particles on their legs and transport them to their larval habitats where the chemical prevents pupae from emerging as adults.*

Susceptibility of local *Aedes aegypti* to PPF





- Susceptibility of local population to PPF
- No repellent effect in oviposition of *Ae. aegypti*

Auto-dissemination of PPF in Paúl do Mar

- Seven BG-Sentinel traps as PPF dissemination stations
  - Collection bags powedered with PPF (Sumilarv® 0,5G, 20-30 µm particles)
  - One hour On/Off cycles

Modified BG-GAT

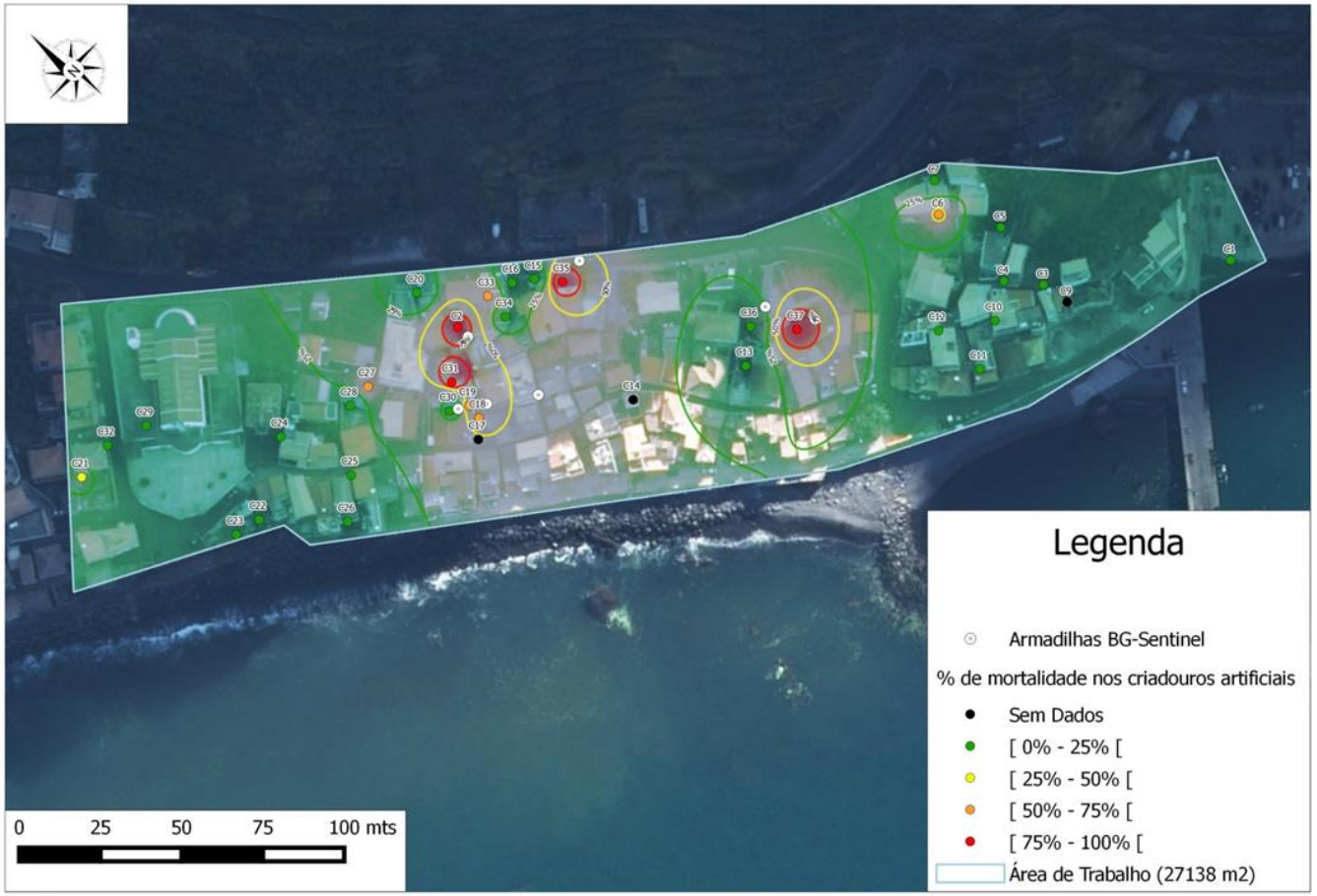
- 37 artificial breeding sites (ABS)
  - 20 3rd instar larvae (strain Funchal)
  - Surveyed in 48h intervals. Pupae collected and reared in lab

- **Pre-treament:** 10 Sept 2014 – 4 Oct 2014
- **Treatment 1:** 4 Oct 2014 – 17 Oct 2014
- **Treatment 2:** 20 Oct 2014 – 17 Nov 2014

Auto-dissemination of PPF in Paúl do Mar

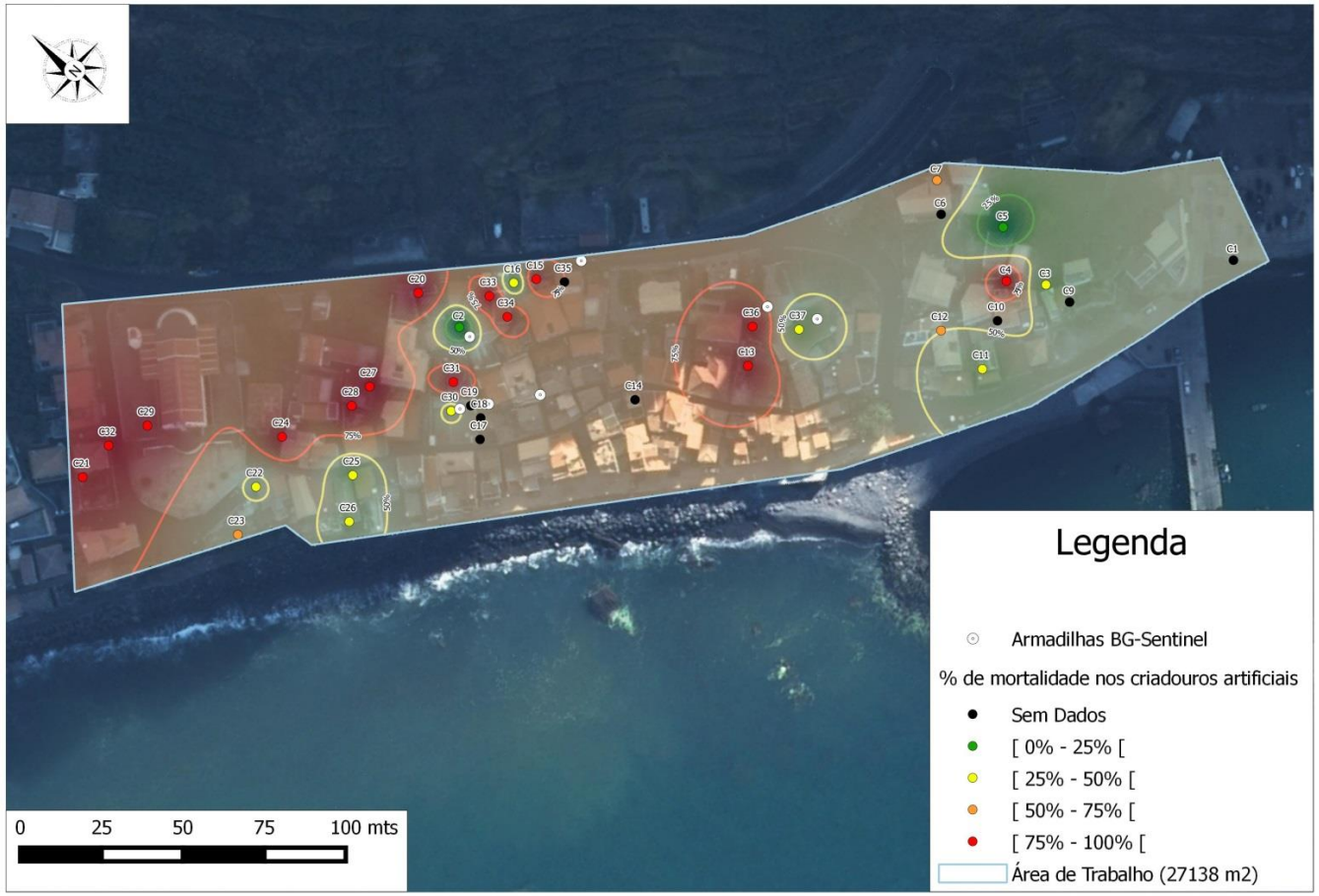
Pre-treatment

Auto-dissemination of PPF in Paúl do Mar



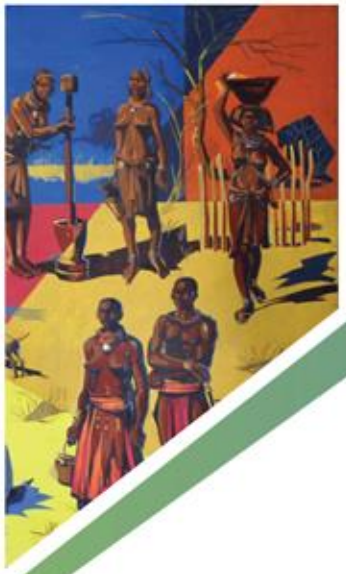
Treatment 1

Auto-dissemination of PPF in Paúl do Mar



Treatment 2




More recently

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The Worldwide Insecticide resistance Network

Ae. albopictus

Ae. aegypti

- 19 partner institutions from 13 countries
  - Identify regions where resistance may chalenge vector control
  - Improve resistance monitoring and implementing new vector control tools

Corbel *et al. Parasites & Vectors* (2017) 10:278
DOI 10.1186/s13071-017-2224-3

Parasites & Vectors

MEETING REPORT — Open Access

International workshop on insecticide resistance in vectors of arboviruses, December 2016, Rio de Janeiro, Brazil

Vincent Corbel[1*], Dina M. Fonseca[2], David Weetman[3], João Pinto[4], Nicole L. Achee[5], Fabrice Chandre[1], Mamadou B. Coulibaly[6], Isabelle Dusfour[7], John Grieco[5], Waraporn Juntarajumnong[8], Audrey Lenhart[9], Ademir J. Martins[10], Catherine Moyes[11], Lee Ching Ng[12], Kamaraju Raghavendra[13], Hassan Vatandoost[14], John Vontas[15,16], Pie Muller[17], Shinji Kasai[18], Florence Fouque[19], Raman Velayudhan[20], Claire Durot[1] and Jean-Philippe David[21*]

160 participants from 30 nationalities
76k online visualizations

- 5 commissioned reviews:
  - *Aedes integrated management*
  - *New tools for vector control*
  - *Current status and mechanisms of insecticide resistance*
  - *Insecticde resistance management*

- Working group to implement WIN/Africa
  - Leader: M. Coulibaly (Malaria Research & Training Center, Mali)

PLOS | NEGLECTED TROPICAL DISEASES

REVIEW

Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans

Catherine L. Moyes[1]*, John Vontas[2,3], Ademir J. Martins[4], Lee Ching Ng[5], Sin Ying Koou[5], Isabelle Dusfour[6], Kamaraju Raghavendra[7], João Pinto[8], Vincent Corbel[9], Jean-Philippe David[10], David Weetman[11]

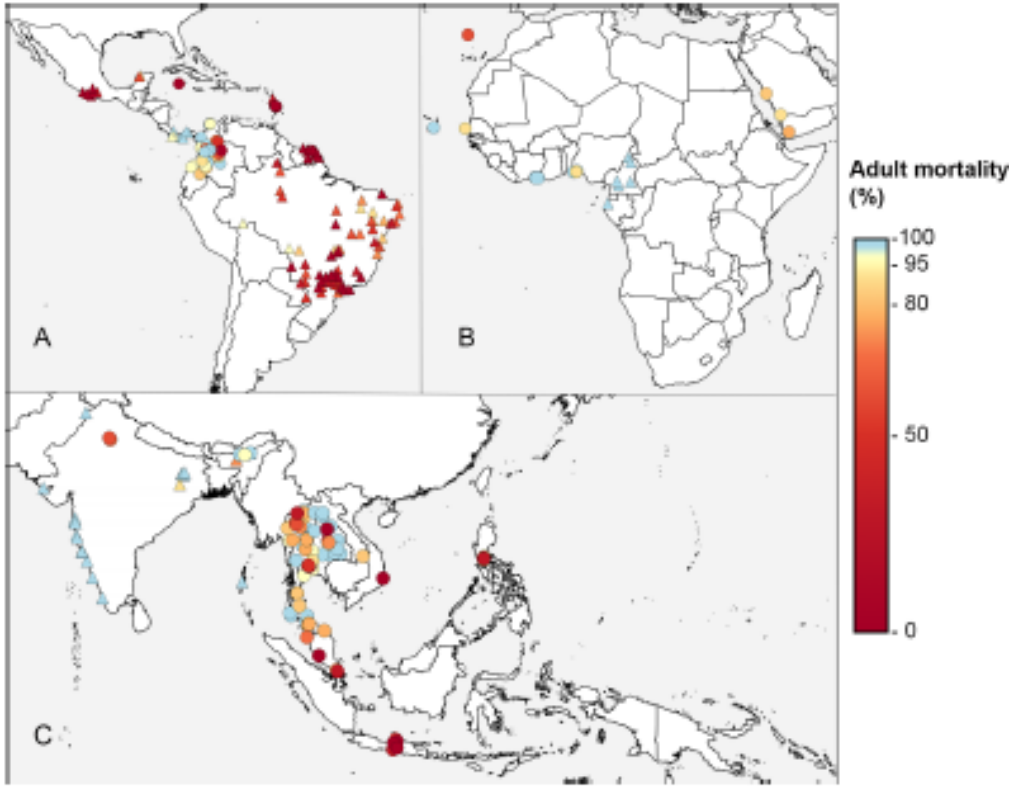

Fig 2. The frequency of resistance to deltamethrin in *Ae. Aegypti*, 2006–2015. Adult bioassays using 0.05% insecticide for 1 hour are denoted as circles and results from non-standard adult bioassays (including different diagnostic doses and exposure periods) are denoted as triangles. The map is zoomed to the 3 regions with data. (A) Americas. (B) Africa/Arabian Peninsula. (C) Asia.

European Commission | Horizon 2020 European Union funding for Research & Innovation

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ZIKAlliance

A Global Alliance for Zika Virus Control and Prevention

A Global Alliance for Zika Virus Control and Prevention

- 56 partners from 21 countries/regions

- Objectives
  - Clarify the impact of Zika infection in pregnancy
  - Understand ZIKV natural history in humans and the environment
  - Establish a collaborative network in Latin America for to boost epidemics preparedness

Work package	Workpackage Title
WP 1	Clinical Science
WP 2	Clinical biology & immunology
WP 3	Virology and antivirals
WP 4	Pathophysiology & animal models
WP 5	Zika virus animal reservoirs
WP 6	Vectors & vector control
WP7	Social sciences
WP8	Communication, Dissemination, and Evaluation (CoDE)
WP9	Management of consortium and exploitation

European Commission
Horizon 2020
European Union funding
for Research & Innovation
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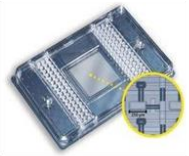
ZIKAlliance
A Global Alliance for Zika Virus Control and Prevention

A Global Alliance for Zika Virus Control and Prevention

- Work Package nº 6: Vectors and Vector Control
  - Identify Zika vectors in Latin America and Caribe
  - Studies on vector competence to ZIKV
  - Importance of coinfections in the mosquito
  - Insecticide resistance and new vector control tools

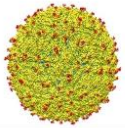
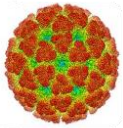
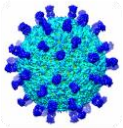


Haemagogous

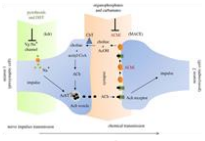

Ae. albopictus

Ae. japonicus

Cx. quinquefasciatus

Ae. aegypti


ZIKV

DENV

CHIKV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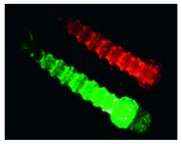
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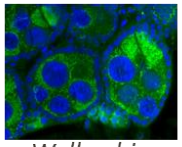
Wolbachia

Team and acknowledgments

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Gonçalo Seixas (PhD student)
- Ecology & Genetics
- Insecticide resistance

Gonçalo Alves (MSc student)
- Vector control tools

Bianca Pires (MSc student)
- Vector control tools

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SRAS
Secretaria Regional dos Assuntos Sociais
Instituto de Administração da Saúde e Assuntos Sociais, IP RAM

Ana Clara Silva (team leader)
Bela Viveiros (mosquito monitoring)
Margarida Clairouin (mosquito monitoring)
Luis Antunes (geography)

Museu de História Natural do Funchal

Manuel Biscoito (team leader)
Ysabel Margarita Gonçalves (mosquito monitoring)
Juan Silva (mosquito monitoring)

DENFREE

Richard Paul
Greg Devine
- PPF experiments

Institut Pasteur

Anna-Bella Failloux
- Vector competence

FORTH
Foundation for Research & Technology - Hellas

Linda Grigoraki
John Vontas
- Metabolic resistance

LSTM
Liverpool School of Tropical Medicine

David Weetman
- Metabolic resistance (Detox chip)

Yale University

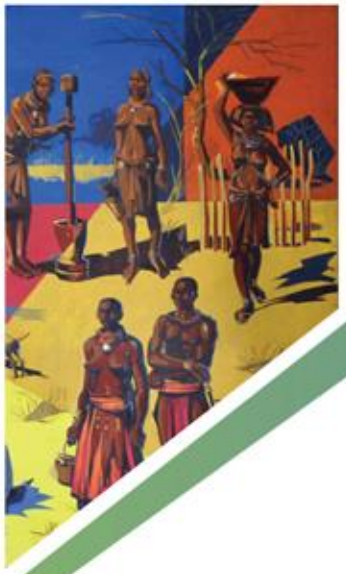
Jeff Powell
- Population genetics

Funding

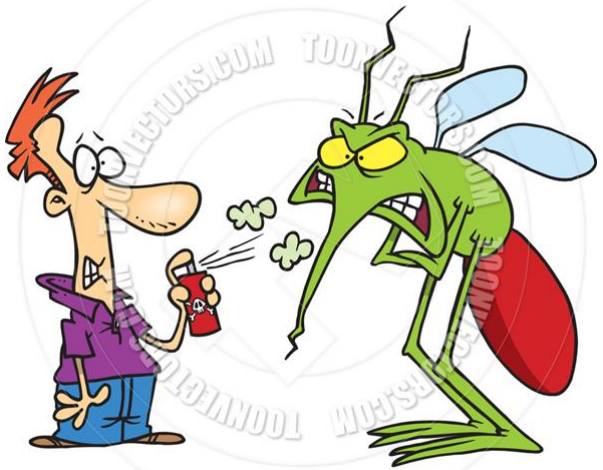
ecdc
European Centre for Disease Prevention and Control

FCT Fundação para a Ciência e a Tecnologia
Ministério da Educação e Ciência

ZIKAlliance
A Global Alliance for Zika Virus Control and Prevention



Thank you!



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