

## 10. Entomology: Molecular entomology

How can population genetics help vector control?

How do we relate to industry requests?



João Pinto et al ([JPinto@ihmt.unl.pt](mailto:JPinto@ihmt.unl.pt))

Molecular entomology at CMDT.LA promotes R&D activities on the bio-ecology, population genetics and molecular evolution of insect vectors of disease. We are interested in the application of genetic tools for monitoring vector control and to assess the evolution and spread of genes of interest. In such context, we are developing three main areas of research:

### 1. Vector bio-ecology and bionomics

Global warming and environmental changes potentially increase the risk of (re)establishment of vector-transmitted diseases. Integrated in a FP6/EC project, (<http://www.eden-fp6project.net/>) bioecological studies are being carried out in Portugal, focusing the former malaria vector *Anopheles atroparvus*. The main objective is to determine the receptivity of these populations for the reestablishment of malaria transmission. Estimates of vectorial capacity and competence were obtained by combination of field surveys and laboratory analyses, including experimental infections with *P. falciparum* strains

### 2. Population structure and history

a) We have studied the origins of the Afrotropical malaria vector *Anopheles gambiae* degree of isolation between forms in sympatric populations from southern Portugal.

in São Tomé and Príncipe (STP) islands by a multi-gene approach comprising microsatellite and DNA sequencing analyses. Genetic data coupled with historical records, pinpoint at least two major colonisation events of this mosquito into the islands, highlighting the importance of man-mediated mosquito dispersal. Microsatellite data was obtained from samples of the islands and from 11 continental African countries. This extensive genotyping is being used to assess levels of gene flow between the islands and mainland. Such information is critical for the potential use of islands as experimental sites for transgenic-based vector control.

More recently we have initiated similar studies on the neotropical malaria vector *Anopheles darlingi* in Brazil.

b) In the mosquito vector *Culex pipiens*, we aim to genetically characterise two behavioural forms of this species (*molestus* and *pipiens*) and relate these data with the potential for transmission of arboviral infections. We are combining ecological, morphometric and microsatellite data in order to determine the

Results point to at least partial genetic/reproductive isolation between molestus and pipiens forms. However, an important proportion of hybrids occur.

### 3. Insecticide resistance

A multi-centre study was undertaken to clarify the molecular evolution of DDT and pyrethroid knockdown resistance (*kdr*) genes in *Anopheles gambiae*. Extensive sequencing analysis of the upstream intron-1 of the sodium-channel gene was carried out in *kdr* genotyped mosquitoes collected from 15 African countries. A minimum of 4 independent mutations giving rise to *kdr* alleles was found.

Traces of selection acting at this genomic region and the heterogeneous geographic distribution of resistance alleles highlight the combined action of gene flow and local insecticide selective pressures in shaping the evolution of this trait.

These hybrids are of great epidemiological relevance since they can act as bridge-vectors for the transmission of arbovirus between birds (natural reservoirs) and humans.

We are also developing population genetic studies focusing on the neotropical malaria vector *Anopheles darlingi*, from Brasil; on the tsetse fly *Glossina palpalis* from three Human African Trypanosomiasis foci of equatorial Guinea (west Africa); and on *Plasmodium falciparum* populations from STP islands in order to infer selective pressures in antimalarial drug resistance associated genes.

Monitoring insecticide resistance is also an important component of our activity. Field surveys have been carried out STP islands (2003-2004) and in Angola (2006) to characterize the susceptibility of mosquito populations mainly to DDT and pyrethroid insecticides, which are the mainstay of malaria vector control.

These surveys were integrated in the anti-vector components of national malaria control programmes of these countries.

### Collaboration with industry and SMEs

Field and laboratory assays have been conducted to evaluate the efficacy of fabrics impregnated with insecticides/repellents by nanoencapsulation, under a contract with Contract with TINAMAR - Tinturaria Têxtil, S.A. (Braga). We are also developing the entomological component of Environmental Impact Studies, with ECOSSISTEMA - Consultores em Engenharia do Ambiente, Lda.

### Selected publications

1. Pinto J, Lynd A, Vicente JL, Santolamazza F, Randle NP, Gentile G, Moreno M, Simard F, Charlwood JD, Rosário VE, Caccone A, della Torre A, Donnelly MJ. 2007. Multiple origins of knockdown resistance mutations in the Afrotropical mosquito vector *Anopheles gambiae*. *PLoS ONE* 11: e1243.
2. Moreno M, Salgueiro P, Vicente JL, Cano J, Berzosa PJ, de Lucio A, Simard F, Caccone A, Rosário VE, Pinto J, Benito A. 2007. Genetic population structure of *Anopheles gambiae* in Equatorial Guinea. *Malaria Journal* 6: 137
3. Pinto J, Lynd A, Elissa N, Donnelly MJ, Costa C, Gentile G, Caccone A, Rosário VE. 2006. Co-occurrence of East and West African *kdr* mutations suggests high levels of resistance to pyrethroid insecticides in *Anopheles gambiae* from Libreville, Gabon. *Medical and Veterinary Entomology* 20: 27-32.
4. Donnelly MJ, Pinto J, Girod R, Besansky NJ, Lehmann T. 2004. Revisiting the role of introgression vs. shared ancestral polymorphisms as key processes shaping genetic diversity in the recently separate sibling species of *Anopheles gambiae* complex. *Heredity* 92: 61-68.

### Team and Main collaborators (2003-2007)

PhD: João Pinto (Investigator), Derek Charlwood (Investigator), Virgílio do Rosário (Investigator), Alexander Yawson (Post-Doc. Fellow).

Non-PhD: Yolanda Kangange (MSc student), Rui Costa e Silva (MSc student), Erika Oliveira (BSc student), André Galvão (BSc student), Andreia Furtado (BSc student), José Vicente (Research Assistant), Suraya Diaz (Research Assistant), Bruno Silva (Research Assistant), Patrícia Salgueiro (Research Assistant).

CEM/Univ. Madeira (M Gouveia), UEI Entomologia Médica/IHMT (AP Almeida, CA Sousa, MT Novo), Centro Nacional de Endemias, STP (MC Ferreira), Instituto Nacional de Saúde, Angola (F Fortes), Universidade Estadual Paulista, Brasil (P Ribolla), OCEAC, Cameroon (F Simard) Inst. Salud Carlos III, Spain (A Benito), Univ. Roma "La Sapienza", Italy (A della Torre), Liverpool School of Tropical Medicine, UK (MJ Donnelly), Dept. of Ecology and Evolutionary Biology, Yale University, USA (A Caccone).



CMDT members and collaborators working in molecular entomology.

*Left-right::* Pedro Cani (Min. Health Angola), João Pinto, Carla Sousa (UEI Medical Entomology/IHMT, EDEN Project), Joana Alves, Bruno Gomes and Sónia Duarte

