Population structure of *Anopheles atroparvus* in Portugal: implications for malaria re-introduction

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State of the art

– MALARIA

  • Major cause of morbidity and mortality in tropical countries
    – 300-500 million cases and more than one million deaths annually

– MALARIA IN EUROPE:

  • Eradication programmes in the 1930’s
    – The European continent considered free of indigenous malaria by 1975
  • In the 1980’s
    – New autochthonous cases
  • In the 1990’s
    – Large scale epidemics in Central Asia and Trans-Caucasian countries
    – 90712 malaria cases reported in the WHO European Region in 1995
State of the art

- **MALARIA IN EUROPE:**
  - Present

  - 35 to 40 million people currently live in areas of varying risk of malaria transmission in the WHO European Region
    - Armenia, Azerbaijan, Georgia, Kyrgyzstan, Tajikistan, Turkey, Turkmenistan and Uzbekistan

10000 annual cases of imported malaria in the European Union

New autochthonous cases (i.e. Russia Spain)

Climate change effect

Human activities

Possibility of malaria re-introduction through all of Europe
State of the art

– MALARIA IN PORTUGAL

• Eradicated since 1973
• Average of 51 annual cases of imported malaria

– Control of vector populations, mostly from the *Anopheles maculipennis* complex

» *Anopheles melanoon* Hackett, 1934
» *Anopheles maculipennis* Meigen, 1918

» *Anopheles atroparvus* van Thiel, 1927

Former main malaria vector in Portugal

Fig.1 - European distribution of *An. atroparvus*. (http://wrbu.si.edu/SpeciesPages_ANO/ANO_A-hab/ANatr_hab.html)
State of the art

Vector population structure studies – Importance

Direct method – Mark Release Recapture analysis

Population size estimates

Effective population size (Ne) estimates

Assess the impact of vector control strategies

Migration estimates

Gene flow estimates

Predict the spread of:
  - Resistance genes
  - Transgenes
  - Malaria
State of the art

Vector population structure studies – Importance

Indirect methods – Genetic analysis

- Effective population size (Ne) estimates
- Demographic equilibrium analysis

Population structure patterns
- Gene flow estimates
- Predict the spread of:
  - Resistance genes
  - Transgenes
  - Malaria

Assess the impact of vector control strategies
Objectives

Main goal

Determine the genetic structure of *An. atroparvus* populations in Portugal

Specific objectives

- To characterize the genetic diversity and demographic history of these populations
- To determine genetic differentiation levels, produce gene flow estimates and relate them with geographic and ecological aspects
- To correlate population structure patterns with the possibility of malaria reintroduction in Portugal
Methodology

Entomological surveys

• Selection of at least 15 sampling points in mainland Portugal
  – Between possible barriers to gene flow
  – Highest historical malaria endemicity

• An. atroparvus bioecology
  – Sampling at summertime, in rural areas
  – Indoor resting mosquitoes, inside houses and animal shelters

• Morphological identification to species or species complex

• Past An. atroparvus collections available for analysis

Fig. 2- Mainland Portugal. (http://geocid-snig.geo.pt/Portugues/)
Methodology

Mark Release Recapture

• Comporta region (Portugal)

  – Mark-Release-Recaptures in five localities on a 10 Km² radius, during summertime

  – Captured mosquitoes marked with a different fluorescent pigment according to sampling point and released

  – Daily recapture attempts at fixed sampling points for at least 15 days

  – Recaptured mosquitoes identified by UV light exposure

Fig 3 – Sampling localities in the Comporta region. (Background image from http://geocid-snig.igeo.pt/Portugues/)
Methodology

Molecular identification of *An. maculipennis* complex species

- DNA extraction of individual female specimens

- PCR – RFLP analysis
  - Amplification of the Internal Transcribed Spacer 2 (ITS2) of the ribosomal DNA
  - Product restriction with enzyme CfoI (Nicolescu *et al.*, 1994) and HpaII (Sousa C.A. pers. comm.)
  - Species-specific fragments for *An. melanoon*, *An. maculipennis* s.s., *An. labranchiae* and *Anopheles atroparvus*.
  - *An. labranchiae* present in Northern Africa

Fig 4 – Example of a PCR-RFLP identification of field collected females of *An. maculipennis* s.l.

1-2: Field collected females; 3: blank; 4: *An. melanoon* (control); 5: *An. maculipennis* s.s. (control); 6: *An. labranchiae* (control); 7: *An. atroparvus* (control); 8: 100 bp marker
Methodology

Microsatellite genotyping of *An. atroparvus*

- **Selection of 15 microsatellite loci**
  - Based on previous work with *An. maculipennis* s.s. (Weill *et al.*, 2003) and *An. sacharovi* (Guillemin *et al.*, 2003)
  - Confirm presence of microsatellite repeat by sequencing, PCR product quality, degree of polymorphism, absence of null alleles

- **Microsatellite genotyping**
  - 45 individual females / sampling point / year of sampling
  - Fluorescent labeled primers (FAM,NED,HEX)
  - Fragment size scored in an automatic sequencer (ABI3730, Applied Biosystems)

Fig 5 – Automatic sequencer ABI3730, (Applied Biosystems).
(http://mbcf.dfci.harvard.edu/docs/mbcfOverview.html)
Methodology

Data analysis – Direct methods

Mark Release Recapture – Past collections

- Frequency of recaptured mosquitoes
- Seasonal abundance data

  - Migration estimates
  - Population size estimates

    - Contemporary gene flow estimates
    - Effective population size (Ne) estimates

  - Population size variation among generations
Methodology

Data analysis – Indirect methods

Microsatellite genotyping

Genetic differentiation index

Global gene flow estimates
(contemporary and historic)

Bayesian and Maximum likelihood Statistics

Effective population size (Ne) estimates

Past collections

Detection of different demographic histories

Demographic equilibrium tests
Methodology

Data analysis – Integration

Direct Methods

Indirect Methods

Geography and ecology data

Migration / dispersion potential of *An. atroparvus* in Portugal

Assess the possible role in the spread of:

- Malaria
- Resistance genes
- Transgenes

Implications for control and monitoring strategies
References

