Culicoides imicola, biting midge species: a recent invader species in the Mediterranean basin?

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The first author: Stéphanie Jacquet, PhD student at Cirad
**Culicoides imicola**, biting midge vector species in expansion?

- Small biting midge (Diptera: Ceratopogonidae), distributed in **Africa (native range)**, India, Middle and Near East

- Well known vector species of several economically important viruses of livestock: bluetongue virus (BTV), African horse sickness virus (AHSV), Epizootic hemorrhagic disease Virus (EHDV)

- The new circulation of BTV serotypes in the northern Mediterranean area from 2000 have revealed the presence of *C. imicola* populations in areas previously suspected as *C. imicola*-free

⇒ Recent colonization vs entomological surveys bias? Population structure and relationships between Mediterranean area and Africa?
Phylogeographic data of *C. imicola* in the Mediterranean basin

- Several studies with single locus (COI) (Dallas et al 2003; Nolan et al 2008; Calvo et al 2009)
- Differentiation between East and West Mediterranean populations, north African populations related to West Mediterranean populations
  ⇒ Coherent with BTV serotypes circulation

- Pattern of rapid and recent expansion populations in West Mediterranean area, not observed in East Mediterranean basin
- Recent development of microsatellite markers (Mardulyn et al 2013)
  ⇒ Scenarios favored long time presence (30 years) of *C. imicola* in Italy and regular migration events
Material and Methods: population set and markers used

- **55 populations for 24 territories**
  - **West Mediterranean basin (WMB)**
    - Portugal, Spain, France and island, Italy and islands, Morocco, Algeria, Tunisia
  - **East Mediterranean basin (EMB)**
    - Greece, Turkey, Israel
  - **West Africa (WA)**
    - Senegal, Mali, Burkina Faso, Benin, Cameroon
  - **South-East Africa (SEA)**
    - Ethiopia, Kenya, Zimbabwe, Mozambique, South Africa, Madagascar, Mauritius, Reunion island

- **3 molecular markers**
  - 8 individuals/site
    - Cytochrome Oxidase subunit I (**COI**, 476bp)
    - Cytochrome B (**CytB**, 635bp)
    - Elongation factor alpha (**Efa**, 556bp)

- **9 microsatellites markers**
  - 32 individuals/site
Results: marked genetic structure within the Mediterranean basin

- Differentiation of two groups: West Mediterranean basin (WMB) vs the others regions
- No shared halootypes between the west (WMB) and east (EMB) Mediterranean basin
- Star-like network in the west Mediterranean basin (WMB) (signature of recent expansion?)
- Same patterns with Cytb, although less informative
Results: marked genetic structure within the Mediterranean basin

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- Star-like network in the west Mediterranean basin (WMB) (signature of recent expansion?)

- Same patterns with Cytb, although less uninformative

- Slight differentiation in the WBM with the nuclear gene

NJ network, Efα polymorphism
Results: one distinct clade in the West Mediterranean basin...

- Populations from EMB genetically related to the populations from SEA or WA
Results: ... and one distinct clade in West Africa!

- Same structuration within the Mediterranean basin
- Differentiation in the sub-Saharan Africa with West Africa (WA) separated from SEA

Tree based on genetic distance of microsatellite polymorphism
Results: clear signature of recent population expansion in 2 areas

- Significant deviation from neutrality (COI+Cytb, Efα) for WA and WMB
- Demographic stability for SEA and EMB, while signature of population growth distribution for WMB and WA

Mismatch analysis based on COI + Cytb

EFα
Results: consistent spatial genetic structure revealed

- Three separate clusters
- Low genetic differentiation between West Mediterranean populations
- High genetic differentiation between West and East Mediterranean populations
- High genetic differentiation between West Mediterranean and African populations

Clusters based on COI polymorphism

<table>
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<tr>
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<th>Southern Europe</th>
<th>WA</th>
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<tr>
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Fst per pair of populations
Results: consistent spatial genetic structure revealed

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Fst per pair of populations

- Low genetic differentiation between West Mediterranean populations
- High genetic differentiation between West and East Mediterranean populations
- High genetic differentiation between West Mediterranean and African populations
Results: consistent spatial genetic structure revealed

- Six separate clusters
- High genetic differentiation between West and East Mediterranean populations
- High genetic differentiation between West Mediterranean and other sub-Saharan populations
- High differentiation between East Mediterranean and other sub-Saharan populations

Clusters based on microsatellite polymorphism

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<td><strong>Greece</strong></td>
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Conclusions: *C. imicola*, invasive species?

- High diversity in Africa and East Mediterranean basin + low diversity in the West Mediterranean basin
- Star-like shape network + signature of population growth/expansion in the West Mediterranean basin

⇒ **Historical range = sub-Saharan Africa**
⇒ “Recent” demographic expansion of West Mediterranean populations, long time presence of *C. imicola* in the East Mediterranean basin

- No shared haplotypes between Sub-Saharan Africa and West Mediterranean basin but West Mediterranean basin genetically closer to West Africa
- East Mediterranean basin genetically closer to South-East Africa
⇒ **Two corridors of expansion from Africa to Med: Atlantic coast and Nile valley**

- No shared haplotypes + high genetic differentiation between West and East Mediterranean basin
⇒ **Limited gene flow between East and West Mediterranean basin**
Conclusions: *C. imicola*, invader species in the West Med. area

- Support one source hypothesis
  - Scenarios to be tested with ABC methods

- In relation to past and current animal movements along the Atlantic coast corridor

![Map of Sub-Saharan Africa with arrow connections between North Africa, Middle East, East Mediterranean basin, and West Mediterranean basin](image)
Acknowledgements to the overseas Cirad colleagues and partners

A Tabbabi
Tunisia

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Many thanks for your attention
Results: high diversity in the native range

<table>
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<tr>
<th>Regions</th>
<th>Haplotypes</th>
<th>Haplotype diversity</th>
<th>Nucleotide diversity</th>
<th>Haplotypes</th>
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<th>Nucleotide diversity</th>
<th>Mean nbr of alleles</th>
<th>Allelic richness</th>
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<tr>
<td>WMB</td>
<td>20</td>
<td>0.491</td>
<td>0.001</td>
<td>26</td>
<td>0.637</td>
<td>0.0019</td>
<td>8.3</td>
<td>6.1</td>
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<tr>
<td>EMB</td>
<td>41</td>
<td>0.721</td>
<td>0.004</td>
<td>7</td>
<td>0.429</td>
<td>0.0009</td>
<td>7.2</td>
<td>6.7</td>
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<tr>
<td>WA + SEA</td>
<td>7</td>
<td>0.930</td>
<td>0.010</td>
<td>25</td>
<td>0.440</td>
<td>0.0011</td>
<td>19.3</td>
<td>12.7</td>
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- With mitochondrial markers, high haplotype diversity but low nucleotide diversity in WMB and EMB.
- With microsatellite markers, diversity higher in Sub-Saharan Africa than in the Mediterranean basin.
Results: consistent spatial genetic structure revealed

- Two separate clusters
- High genetic differentiation between West Mediterranean and African populations
- Low genetic differentiation between East and West Mediterranean populations

Clusters based on EFα polymorphism

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