NEW INSIGHTS ON THE GENETIC DIVERSITY OF THE HONEYBEE PARASITE NOSEMA CERANAE

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**Nosema ceranae**

- *Microsporidia* (fungi)
- Obligate intracellular parasites
- Invade the epithelial cells of honeybee midgut
- Highly resistant extracellular stage: the spore
- Specific infection tool: the Polar Tube
Impact of *Nosema ceranae*?

- High mortality
- No higher mortality
- No symptoms detected

=> Variable virulence <=

(Cox-Foster *et al.*, 2007; Higès *et al.*, 2008; Gisder *et al.*, 2010)
Possible origin of the variable virulence

(Potts et al., 2010)
Are there several variants of *N. ceranae* with different levels of virulence?
Study’s objective

Is it possible to discriminate taxa of *Nosema ceranae* using molecular tools (genotyping)?
Strategy for genotyping

• Genetic marker
  • rDNA – protein encoding genes
  • Multilocus

• Isolate definition
  • One isolate = one bee

• Intra-isolate diversity

(Sagastume et al., 2011; Chaimanee et al., 2011; Hatjina et al., 2011)
Experimental procedure

1 isolate = 1 bee

Isolate A

= 4
N. ceranae populations

Isolate B

4 distant locations

8 to 15 clones sequenced

ATCGGTGATTAGGCTCGATGCA
ATCGGTGATTAGGCTCGATGCA
ATCGGTGATTAGGCTCGATGCA
ATCGGTGATTAGGCTCGATGCA
ATCGGTGATTAGGCTCGATGCA
ATCGGTGATTAGGCTCGATGCA
ATCGGTGATTAGGCTCGATGCA
ATCGGTGATTAGGCTCGATGCA
ATCGGTGATTAGGCTCGATGCA

Intra-isolate A diversity

Inter-isolate diversity

Intra-isolate B diversity

10 genetic markers

116 N. ceranae populations

4 distant locations

8 to 15 clones sequenced
Molecular markers used

Number of polymorphic sites *per* kilobase between pair of sequences

rDNA / Protein encoding genes / Unknown function genetic markers
Genetic diversity

Number of polymorphic sites per kilobase between pair of sequences
Genetic diversity

Number of polymorphic sites per kilobase between pair of sequences

As much diversity within one bee as between two different bees
There are identical alleles in different bees
Phylogenetic analyses

No isolate-specific taxon
Conclusions

- Genetic diversity precludes any variant differentiation
Genetic diversity statistics

Between-isolate diversity has a low weight in the global diversity

=> No divergence: similar population in isolates
Conclusions

- Genetic diversity precludes any variant differentiation
- No divergence between populations from the 4 locations sampled

=> Same population of *N. ceranae* worldwide?
Discussion

- Evolutionary tests

A. cerana                             A. mellifera

Host-jump

(Klee et al., 2007)
Related article

New insights on the genetic diversity of the honeybee parasite *Nosema ceranae* based on *multilocus* sequence analysis

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### Genetic diversity

#### Number of polymorphic sites per kilobase between pair sequences

<table>
<thead>
<tr>
<th>Marker</th>
<th>SSU-rDNA</th>
<th>EnP1B</th>
<th>PTP2</th>
<th>HSWP4</th>
<th>SWP25</th>
<th>SWP30</th>
<th>NCER_100070</th>
<th>NCER_100533</th>
<th>NCER_101600</th>
<th>NCER_101165</th>
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</thead>
<tbody>
<tr>
<td>Nb of sequences</td>
<td>39</td>
<td>41</td>
<td>44</td>
<td>42</td>
<td>39</td>
<td>42</td>
<td>40</td>
<td>37</td>
<td>59</td>
<td>44</td>
</tr>
<tr>
<td>Nb of alleles</td>
<td>37 (95%)</td>
<td>33 (80%)</td>
<td>22 (50%)</td>
<td>13 (31%)</td>
<td>20 (50%)</td>
<td>25 (59%)</td>
<td>22 (55%)</td>
<td>14 (38%)</td>
<td>27 (46%)</td>
<td>25 (57%)</td>
</tr>
<tr>
<td>Variable sites</td>
<td>66</td>
<td>56</td>
<td>26</td>
<td>14</td>
<td>23</td>
<td>35</td>
<td>23</td>
<td>18</td>
<td>48</td>
<td>31</td>
</tr>
<tr>
<td>Singleton variable sites</td>
<td>55 (83%)</td>
<td>24 (43%)</td>
<td>22 (85%)</td>
<td>14 (100%)</td>
<td>22 (96%)</td>
<td>28 (80%)</td>
<td>16 (70%)</td>
<td>17 (94%)</td>
<td>31 (65%)</td>
<td>24 (77%)</td>
</tr>
</tbody>
</table>
Thanks for your attention

Looking for a post-doctoral position!

Apiary in Aubière