Micro-arrays as a molecular identification tool for Culicoides spp.

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Overview

- Problems with identification of *Culicoides*
- Description of the micro-array method
- Development of a micro-array for *Culicoides*
- Results & future plans
- Advantages of micro-arrays

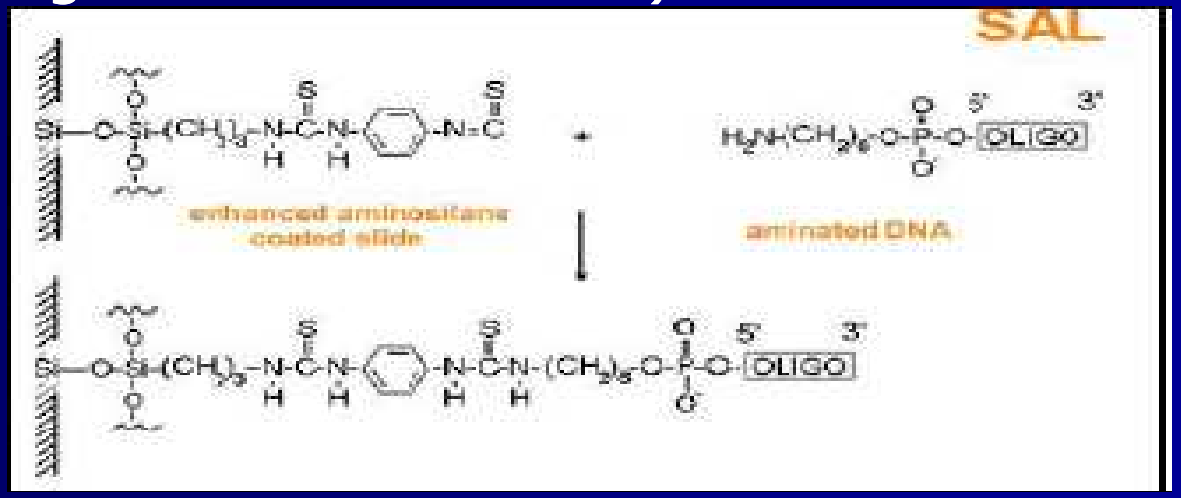
Difficulties with *Culicoides spp* identification

- Morphological identification of adults is labour & time intensive
- Species belonging to complexes are almost impossible to separate on a morphological basis
- Morphological differences between larvae are poorly described in literature
- Specimens used for virus detection (often pooled!) can not be identified afterwards with the traditional methods

→ Need of a biomolecular tool as a possible solution

Micro-arrays
What?

- Micro-arrays: Species specific DNA-segments (probes) arrayed on a solid surface (slide) by covalent attachment to a chemical matrix (aminosilane coating reacting with aminated DNA)



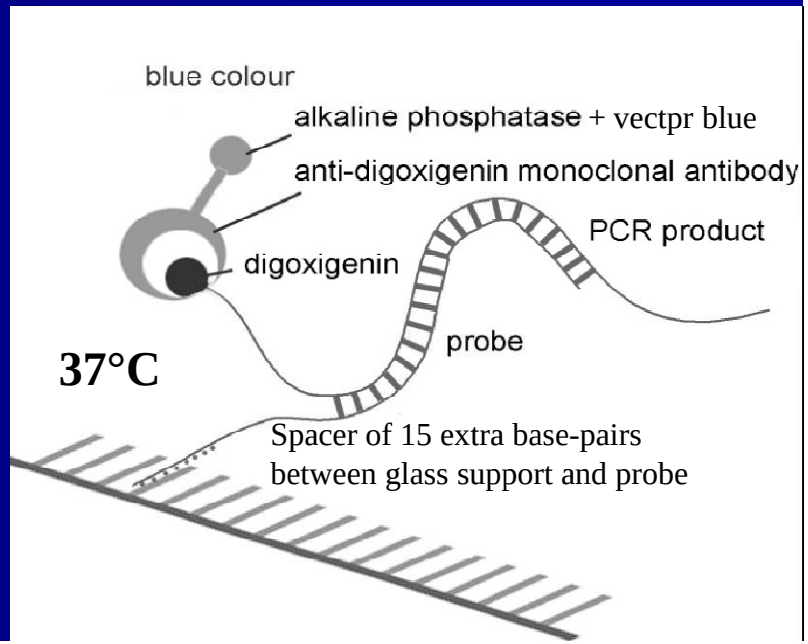
- NA of a Culicoides enriched by PCR is added. When the probe recognises the Culicoides DNA hybridization occurrs and binding is detected by fluorescence or colour

Development of *Culicoides sp* specific micro-arrays

- Search for an adequate genomic region to produce the primers - requirements:
  - Region with high stability
  - Enough inter-species variety
  - (Correct) sequence of certain species already known is an advantage
- --> Internal Transcribed Spacer 1 (ITS1) of rDNA
- Extraction and sequencing of 63 specimen performed
- Apart from the species specific primers a primer pair common to all *Culicoides* was identified

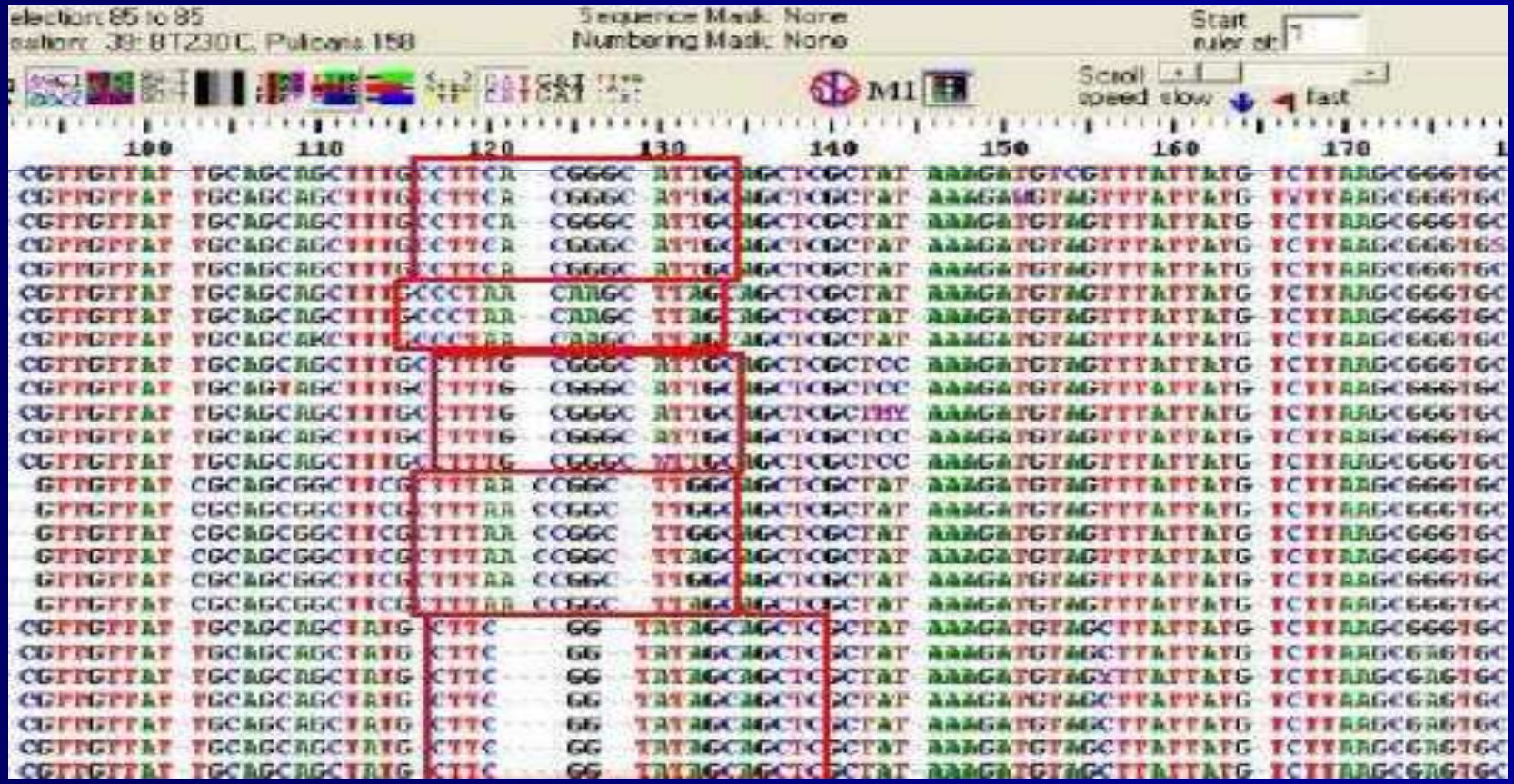
Development of *Culicoides sp* specific micro-arrays

- Asymmetric PCR on the Culicoides DNA so that one strand of the target DNA is amplified preferentially. Hereto the forward primer is used in excess.
- The primer is labeled with Digoxigenine
- Only the PCR-products complementary to the probe's sequence will hybridize
- After the hybridization reaction the plate is rinsed and incubated with anti-digoxenine monoclonal antibodies. Positive samples will color blue trough an enzymatic reaction



Results

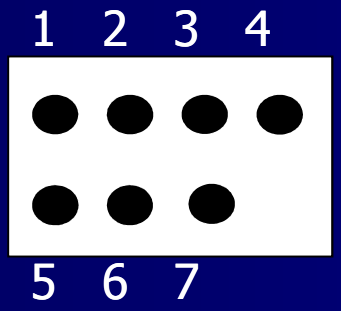
- Currently "perfect" probes developed for 6 *Culicoides* species and 1 common probe for all *Culicoides* species



Results

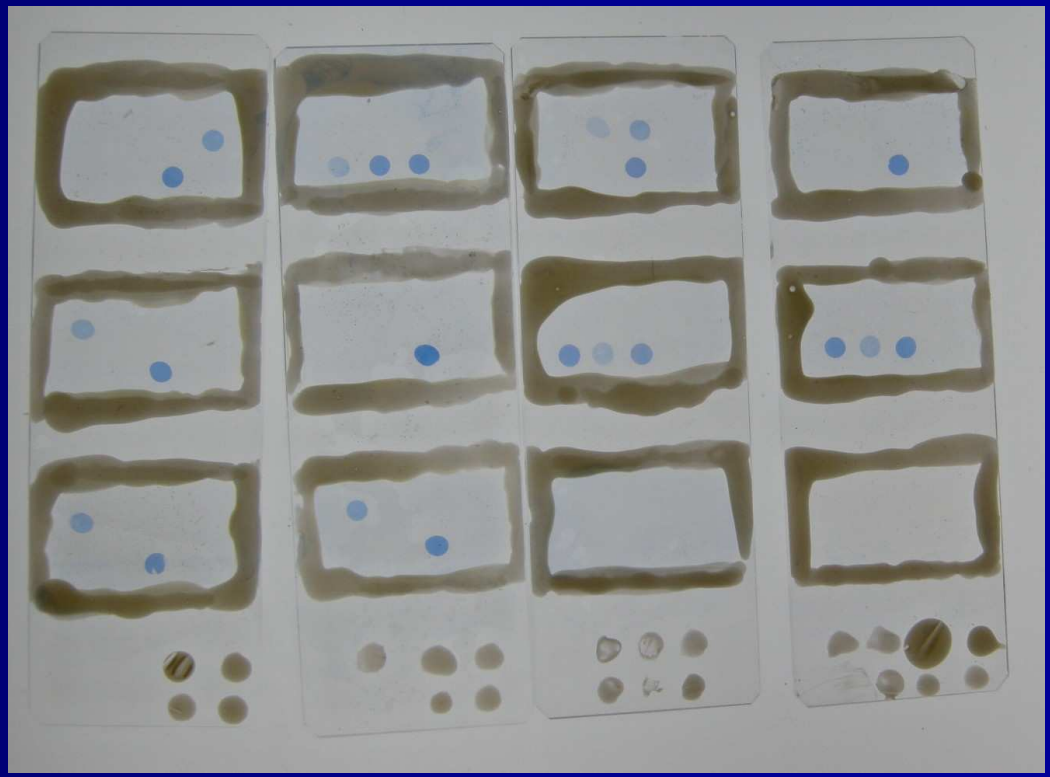
- Probes for *Obsoletus*-group validated trough
  - In house test
  - Medreonet ring trial
- Validated probes for *C. nubeculosus*, *C. impunctatus*
- Cross reactions within the *Pulicaris*-group (e.g. *C.pulicaris* and *C.lupicaris*) possibly due to:
  - Genomic intra-species variation
  - Intermediate species, with morphological aspects of two or more species

Results



Legend:

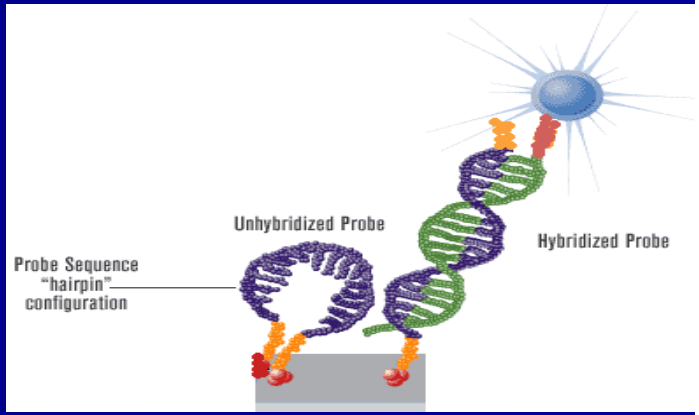
1 *Culicoides obsoletus*
2 *C. scoticus* a
3 *C. scoticus* b
4 *C. dewulfi*
5 *C. chiopterus* a
6 *C. chiopterus* b
7 Common *Culicoides* probe



C. scoticus 2 haplotypes for ITS-1?

Future plans

- Further development and validation of probes for the most important species
- Validation with specimen from different regions of Europe to check on possible regional intra-species varieties
- Identification of larvae and pupae with micro-arrays
  - Ecological studies
- Development of hairpin probes
- Home production of teflon printed slides with more probes per slide



Advantages of Micro-array over PCR and RT PCR

- **Simple PCR with one primer pair**
  - labour intensive and therefore morphological identification up to certain level required
- **Multiplex PCRs**
  - hard to reproduce (non-specific reactions, primer-dimer problems, mutual inhibition of primers)
- **Real time PCR**
  - costly for high numbers of probes
- **Micro-array**
  - No gelelectroforesis needed
  - The color reaction makes an expensive laser-scanning device superfluous.


Thanks for
your attention