SNPs improve population resolution for the harbour porpoise (*Phocoena phocoena*)

Tiedemann, R., Autenrieth, M., Havenstein, K., Lah, L., Hartmann, S., Benke, H., Pawliczka, I., Roos, A., Siebert, U., Dennis, A.B.
**THE HARBOUR PORPOISE IN THE BALTIC SEA**

North Sea - **NOS**
Skagerrak - **SKA**
Kattegat - **KAT**
Belt Sea - **BES**
Inner Baltic Sea - **IBS**

Atlantic Shelf

375,000

40,000

< 500

Baltic harbour porpoise

*(Phocoena phocoena)*
Short Communication

Conservation genetics without knowing what to conserve: the case of the Baltic harbour porpoise.

Oryx 2008

Research Article

Coming of age: Do female harbour porpoises (Phocoena phocoena) from the North Sea and Baltic Sea have sufficient time to reproduce in a human influenced environment?

Tina Kesselring¹, Sacha Viquerat¹, Ralph Brehm², Ursula Siebert¹

Jastarnia Meeting 2018 – Copenhagen
**INDICATION FOR POPULATION STRUCTURING**

- subpopulations arose during end of the last glacial period (~7000yra)
  - North Sea populations recolonized the Baltic Sea

**acoustic data**

**morphometric data**
- NOS-KAT vs. Belt Sea
- Belt Sea vs. IBS

**behavioral data**

**habitat use:**
- food availability
- activity patterns
INDICATION FOR POPULATION STRUCTURING

GENETIC DATA?

• mtDNA, microsatellites:
  • pooled data: structure between population detectable
  • individual level: no clear pattern

• mtDNA haplotypes:
  • inner Baltic Sea private haplotypes (HT)
  • indicative haplotype HT7 for Belt Sea (Wiemann et al. 2010)

**AIM**

further investigate inner Baltic population structure with SNP markers

One panmictic Baltic population \( \text{vs} \) different subpopulations?
INITIAL STUDY - SNP ANALYSIS DETECTING SUBTLE POPULATION STRUCTURE

**main findings**

- SNPs (~1000) outperform microsatellites (~15)

- three main groupings:
  - Black Sea
  - North Atlantic
  - Baltic Sea

- notable distinction between Belt Sea and Inner Baltic Sea

Lah et al. 2016
**STUDY SET UP**

- **samples:** 196 + 37 = 233
  after filtering: 109 (+37)

- **markers:**
  - mtDNA haplotypes
  - 15 microsatellites
  - 2518 new SNP loci
    (+1874 SNPs)
  present in 95% of individuals
  (≥ 10 reads/locus/individual)

- **methods:**
  - PCA and sPCA
  - STRUCTURE
  - association study

- **aim:** **BES** vs. **IBS**

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![Map of study areas](image)
SNP discovery using RADseq

A
RAD sequencing

- Individual 1
- Individual 2
- Rare cut site
- Common cut site
- Genomic interval present in library
- Sequence reads

B
double digest RADseq

- Individual 1
- Individual 2
- a
- b

Challenges:
Sample and DNA quality

Double digest RAD sequencing improves efficiency and robustness while minimizing cost.
SNP discovery using RADseq

233 samples
100 bp Paired End reads
CUTTERS:MspI, PstI
### Fixation Coefficients Based on 2518 SNPs

<table>
<thead>
<tr>
<th></th>
<th>all data (109)</th>
<th>females (74)</th>
<th>reproduction time (74)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>between areas</strong></td>
<td>0.022 ***</td>
<td>0.013*</td>
<td>0.013 *</td>
</tr>
<tr>
<td>NOS vs. SKA</td>
<td>0.018*</td>
<td>0.010</td>
<td>0.012</td>
</tr>
<tr>
<td>SKA vs. KAT</td>
<td>0.024**</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>KAT vs. BES</td>
<td>0.021***</td>
<td>0.011(*)</td>
<td>0.013(*)</td>
</tr>
<tr>
<td>BES vs. IBS</td>
<td>0.019***</td>
<td>0.006</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Significance: (*) p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001

- Differentiation shown in SNPs, also in microsatellites and mtDNA
  (Wiemann et al. 2000, Lah et al. 2016)
STRUCTURE PLOTS

All 5 populations

- All data (n= 109 | 60)
- Only female (n= 74 | 36)
- Only reproduction time (April-September) (n= 74 | 40)

BES vs. IBS
**Spatial Principal Component Analysis**

Complete dataset (5 areas)

- Significant structure only between areas
- No structure within areas detectable

Only BES and IBS

- Significant *between* and *within* areas
- Indicates two subpopulations and seasonal migration
PCA ANALYSIS OF ALL 5 POPULATIONS

SNPs: 1. and 2. PCA-axis
(mean values & standard error)

North Sea - NOS
Skagerrak - SKA
Kattegat - KAT
Belt Sea - BES
Inner Baltic Sea - IBS
**ANALYSIS FOR ONLY IBS -BES**

- analysis of 60 Samples from the Baltic Sea
  - association analysis
  - sPCA analysis

→ two groups can be distinguished
→ Migrants can be detected
## BES-IBS Individual Assignment Based on SNP Data

<table>
<thead>
<tr>
<th>assignment</th>
<th>Occurrence</th>
<th>mitochondrial haplotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BES</td>
<td>IBS</td>
</tr>
<tr>
<td>Alle Daten</td>
<td></td>
<td></td>
</tr>
<tr>
<td>west Baltic subpopulation</td>
<td>39 (49)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>central Baltic subpopulation</td>
<td>0 (0)</td>
<td>14 (17)</td>
</tr>
<tr>
<td>Fisher’s exact test:</td>
<td>p&lt;0.001***</td>
<td>(p&lt;0.001***</td>
</tr>
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<tr>
<td>Only females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>west Baltic subpopulation</td>
<td>24 (34)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>central Baltic subpopulation</td>
<td>0 (0)</td>
<td>8 (10)</td>
</tr>
<tr>
<td>Fisher’s exact test:</td>
<td>p&lt;0.001***</td>
<td>(p&lt;0.001***</td>
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<td></td>
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<tr>
<td>only reproductive season (April-September)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>west Baltic subpopulation</td>
<td>27 (36)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>central Baltic subpopulation</td>
<td>0 (0)</td>
<td>8 (11)</td>
</tr>
<tr>
<td>Fisher’s exact test:</td>
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<td>(p&lt;0.001***</td>
</tr>
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</table>

* Fisher’s exact test: p<0.001***,
* p=0.094(*),
* p=0.084(*),
* p=0.106ns,
* p=0.036*,
* p=0.084(*).
BES-IBS INDIVIDUAL ASSIGNMENT BASED ON SNP DATA

total = #78

**BES**

**IBS**

**INDIVIDUAL ASSIGNMENT BASED ON SNP DATA**

**sampling location**

<table>
<thead>
<tr>
<th>SNP-BES</th>
<th>SNP-IBS</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Pie Chart" /></td>
<td><img src="image2" alt="Pie Chart" /></td>
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</tbody>
</table>

**mtDNA haplotype occurrence**

<table>
<thead>
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<th>SNP-BES</th>
<th>SNP-IBS</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3" alt="Pie Chart" /></td>
<td><img src="image4" alt="Pie Chart" /></td>
</tr>
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</table>

- **all data**
- **only female**
- **only reproduction time (April-September)**

**significance:** (*) p<0.1; * p<0.05; *** p<0.001

**sampled in BES:**

- **IBS:**

**HT 7:**

- **other HT:**

Jastarnia Meeting 2018 – Copenhagen
**NEXT STEPS**

- identify candidate SNPs for SNP panel (for ca. 200+ additional samples)
  - mapping RAD-Seq - per individual
  - design initial Fluidigm’s SNPtype™ assays
  - decide on final SNP panel → apply to new samples

- different ecotypes North Atlantic vs Baltic Sea
  - local adaptation → underlying genes?
    - extract SNP-ID + sequence from STACKS
    - identify coding region and gene identity
    - search for selection → SNP

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Paasche et al. 2015
CONCLUSION

SNP as genetic marker
• superior resolution
  ➢ underscores value of conservation genomics
• provides detailed information for any individual
  ➢ inference of migrants/residents
  ➢ assessment of admixture/genetic exchange

the Baltic harbour porpoise
• splits: Kattegat - Belt Sea - inner Baltic Sea
  • all data
  • only females
  • only reproduction time
  ➢ genetics support Inner Baltic population split
  ➢ conservation measures specific for IBS needed
THANK YOU FOR YOUR ATTENTION …

... QUESTIONS OR COMMENTS?
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