ESTIMATES OF GENETIC VARIABILITY AND INBREEDING IN SELECTED POPULATIONS OF EUROPEAN SEA BASS
INBREEDING AND LOSS OF GENETIC DIVERSITY IN AQUACULTURE

Artificial selection

Reduces genetic variability

Inbreeding

Growth, Morphology, Disease resistance

Physical & developmental abnormalities, metabolic deficiencies ...

Kayim et al. 2010
RESEARCH QUESTIONS

- Does inbreeding increase over a few generations of selective breeding?
- Does genetic diversity within lines decrease over a few generations of selective breeding?
• European sea bass (*Dicentrarchus labrax*)
• IFREMER experimental farm (Palavas-les-Flots, France)
• 2 lines: one selected for growth and one selected for resistance to starvation
SAMPLING

- IFREMER experimental farm (Palavas-les-Flots, France)
- 2 lines: one selected for growth and one selected for resistance to starvation

SELECTED FOR GROWTH

- \( F_0 \): 28 individ – “PA”
- \( F_3 \): 49 individ – “PGL”
SAMPLING

- IFREMER experimental farm (Palavas-les-Flots, France)
- 2 lines: one selected for growth and one selected for resistance to starvation

SELECTED FOR RESISTANCE TO STARVATION

\[ \begin{align*}
F_0: & \quad 19 \text{ individ} - \text{“PM”} \\
F_2: & \quad 28 \text{ individ} - \text{“PSP”} \\
F_2: & \quad 35 \text{ individ} - \text{“PSN”}
\end{align*} \]
Next generation sequencing
  • ddRAD sequencing (2 RE)
  • pooled 144 individuals per library

Bioinformatics: STACKS for SNP discovery
  • after filtering 200 high quality SNPs
    • 1 SNP per tag
    • SNPs present in all pops and 80 % of individuals
    • maf 2.5 %
    • outliers (“under selection”) (Bayescan, Lositan)
• All pairwise $F_{st}$ estimates are significant ($p=0.0000$)
• ATL (growth line) and MED (starvation line) are most differentiated
### INBREEDING

#### INBREEDING ESTIMATES (Fis)

<table>
<thead>
<tr>
<th>GROWTH LINE</th>
<th>STARVATION LINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₀</td>
<td>F₀</td>
</tr>
<tr>
<td>PA (1)</td>
<td>PM (3)</td>
</tr>
<tr>
<td>F₃</td>
<td>F₂ (+)</td>
</tr>
<tr>
<td>PGL (2)</td>
<td>PSP (4)</td>
</tr>
<tr>
<td>F₀</td>
<td>F₂ (-)</td>
</tr>
<tr>
<td>PM (3)</td>
<td>PSN (5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>F₀</th>
<th>F₃</th>
<th>F₀</th>
<th>F₂ (+)</th>
<th>F₂ (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA (1)</td>
<td>0.0682</td>
<td>0.0009</td>
<td>0.3119</td>
<td>0.0447</td>
<td>0.0412</td>
</tr>
<tr>
<td>PGL (2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM (3)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>PSP (4)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>PSN (5)</td>
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</tbody>
</table>

**Graph:**
- **Y-axis:** Inbreeding coefficient
- **X-axis:** Generations (1, 2, 3, 4, 5)
- Average of pairwise relatedness coefficients \( r \) (Queller & Goodnight 1989)
- Increase average \( r \) over generations
- Significant differences between \( F_0 \) and \( F_2 \) or \( F_3 \)
GENETIC DIVERSITY

% polymorphic loci

expected and observed heterozygosities

PA  PGL  PM  PSP  PSN

growth line

starvation line

% polymorphic loci

heterozygosity

Hobs and Hexp per population

Population 1 2 3 4 5

Hobs

Hexp

% polymorphic loci

growth line

starvation line

% polymorphic loci

heterozygosity

Hobs

Hexp

Population 1 2 3 4 5
## EFFECTIVE POPULATION SIZE

<table>
<thead>
<tr>
<th>EFFECTIVE POPULATION SIZE ($N_e$)</th>
<th>LINE 1 (ATL – GROWTH)</th>
<th>LINE 2 (MED – STARVATION)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₀</td>
<td>F₃</td>
<td>F₀</td>
</tr>
<tr>
<td>PA</td>
<td>PGL</td>
<td>PM</td>
</tr>
<tr>
<td>594 (185-∞)</td>
<td>21 (19-23)</td>
<td>423 (117-∞)</td>
</tr>
<tr>
<td>21 (19-23)</td>
<td></td>
<td>16 (15-18)</td>
</tr>
<tr>
<td>26 (23-30)</td>
<td></td>
<td>26 (23-30)</td>
</tr>
</tbody>
</table>

### Graphical Representation

#### Effective Population Size

- **Y-axis:** Effective population size
- **X-axis:** Frequency

### Notes

- **F₀:** Initial generation
- **F₂:** Second generation
- **(+) and (-):** Variation indicates the direction of selection

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**Legend:**

- **PA:** Parental line
- **PGL:** Growth line
- **PM:** Starvation line
- **PSP:** Selection for positive growth line
- **PSN:** Selection for negative growth line
SUMMARY

- Does inbreeding increase over a few generations of selective breeding?
  - NO increase in inbreeding coefficient found
  - Preliminary results
  - Increase in the relatedness & decrease in $N_e$

- Does genetic diversity within lines decrease over a few generations of selective breeding?
  - NO decrease in heterozygosity
  - Decrease in % polymorphic loci in the growth line
THANKS!
High inbreeding values in the broodstock population of line 2 are difficult to explain

- groups (populations) were sequenced in different libraries
- something odd about this library
- no substructure
INTERLIBRARY VARIABILITY

- 3 identical samples were included in all libraries
- PCA: library 4 is an outlier

**Observed levels of homozygosity are library specific and thus an ARTEFACT**
RELATEDNESS

INTRO – M&M – RESULTS – DISCUSSION
GENETIC DIFFERENTIATION

- All pairwise $F_{st}$ estimates are significant ($p=0.0000$)
- ATL (line 1) and MED (line 2) are most differentiated

Roger’s Euclidean genetic distance

<table>
<thead>
<tr>
<th></th>
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<th>PGL</th>
<th>PM</th>
<th>PSP</th>
<th>PSN</th>
</tr>
</thead>
<tbody>
<tr>
<td>line 1</td>
<td>$F_0$</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$F_3$</td>
<td>0.09563048</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>line 2</td>
<td>$F_0$</td>
<td>0.09377498</td>
<td>0.12770844</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$F_2$</td>
<td>0.11530849</td>
<td>0.14531260</td>
<td>0.07295516</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$F_2$</td>
<td>0.10711473</td>
<td>0.14228880</td>
<td>0.08756016</td>
<td>0.077067</td>
</tr>
</tbody>
</table>
• with increasing number of selective breeding programs, it is important to assess inbreeding, genetic diversity and effective population size

• Be careful when considering results of next gen sequencing ➔ library effects! ➔ here clear effect on homozygosity ➔ (or heterozygosity) measures lay at the basis of nearly all population genetic measures ...
**GENETIC DIVERSITY**

### # polymorphic loci

#### INTRO - M&M - RESULTS - DISCUSSION

#### GENETIC DIVERSITY

#### # polymorphic loci

#### Expected and observed heterozygosities

#### Population

### 1

#### 2

#### 3

#### 4

#### 5