European Commission

FIFTH FRAMEWORK PROGRAMME

QUALITY OF LIFE AND MANAGEMENT OF LIVING RESOURCES

Tools for the genetic improvement of sea bass - construction and preliminary application of a medium density linkage and synteny map

BASSMAP

Q5R5-2001-01701

01.11.01-31.12.05

http://www.bassmap.org

Developing genomic tools for European sea bass towards sustainable aquaculture and a better understanding of its evolution
PREAMBLE: basic biology, phylogeny, karyotype and phylogeography

European sea bass
(*Dicentrarchus labrax*)

• Distributed from Norway → Morocco and Spain → Egypt
• Demersal coastal
• Euryhaline & eurythermal
• Seasonal spawning migration
• L < 103 cm; W < 12 kg
• adult: ♂ 2 year; ♀ 3 year
• unspecified sex determination
• game, capture fishery, aquaculture
• > 64,000 ton production at € 5.44/kg

F. Bonhomme, Univ. Montpellier II (France)
The haploid genome of sea bass:
- counts 24 ± equally sized chromosomes
- weighs 0.95 pg
- counts 0.95 Gb
Phylogeography

Two geographical clades: Atlantic and Mediterranean

with an interesting transition zone at the Almeria-Oran Front

Neighbor Joining tree based on Reynolds distances of microsatellite loci (Lemaire et al, 2005)

Research logic and aims of BASSMAP

Aquaculture production of European seabass has grown tremendously in the past 15 years, but selection progress for growth, stress and disease resistance is basic.

A combination of classical (low pressure) and intensive (high pressure) selection should improve quality and economic profitability.
STEP 1: first generation linkage map and large insert library

Research planning

**Tools** → **Data collection** → **Result** → **Demonstration**
Breeding of reference families - CNR

Test panel: family VENEZIA Fbis (2 outbred parents and 50 sibs)
Seabass from the source population (Veneto Agricoltura farm) showed the following genetic values based on 200 microsatellite loci for:

- Heterozygosity: $H_o = 0.718$ (high)
- Polymorphism: $P = 1$ (high)
- Average number of alleles/locus: 7.7
- Inbreeding level: $F_{IS} = 0.022$ (low)
- Confirmed origin: Adriatic Sea

fully outbred fish of wild origin

Construction of a BAC library - IoA + RCGHGP

BAC = Bacterial Artificial Library (whole sea bass genome cut in 164 kb fragments and inserted in 69,000 bacterial plasmid clones)

6x coverage BAC library

Library available in 384 well plates
Spotted on 2 filters of 25,000 clones each
Pools of clones

DNA insert size:
- 200 kb
- 150 kb
- 100 kb
- vector DNA (8.3 kb)
Applications:
✓ BAC library macroarray hybridisation for c/gDNA probe and microsatellite isolation
✓ isolation of candidate genes
✓ isolation of promoters
✓ physical map through BAC end sequencing

In progress at
Max Planck Institute for Molecular Genetics

Microsatellite development and genotyping – KULeuven + HCMR

>149 new type II (noncoding) microsatellite loci have been isolated (Tsigenopoulos et al., 2003; Chistiakov et al., 2004) and genotyped in the test panel
• all 14 previously published microsatellites of sea bass (García de Leon et al., 1995; Castilho and McAndrew, 1998; Ciftci et al., 2002) have been reevaluated
• one microsatellite (SaGT41b) from sea bream, Sparus aurata, has been adopted (Batargias et al. 1999)
• >116 type I (coding) microsatellites derived from EST and gene sequences have been developed (including polymorphic SSRs located within genes: eg PY, CYP19 and RAG-1)

Genotyping of the DLA0253 marker in the test panel (Venezia F bis family) for subsequent mapping; F: female; M: male
226 AFLPs markers have been submitted to the ResSpecies database

<table>
<thead>
<tr>
<th>Primer combination</th>
<th>EcoRI + MseI-A</th>
<th>EcoRI + MseI-C</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFLPs heterozygous in the male parent</td>
<td>48</td>
<td>46</td>
<td>94</td>
</tr>
<tr>
<td>AFLPs heterozygous in the female parent</td>
<td>40</td>
<td>43</td>
<td>83</td>
</tr>
<tr>
<td>AFLPs heterozygous in both parents</td>
<td>16</td>
<td>33</td>
<td>49</td>
</tr>
<tr>
<td>Total</td>
<td>104</td>
<td>122</td>
<td>226</td>
</tr>
</tbody>
</table>

Developing and genotyping AFLP markers - KULeuven

- AFLPs heterozygous in both parents
- AFLPs heterozygous in the male parent
- AFLPs heterozygous in the female parent
- Total

### Public resource

Expressed Sequence Tags – IoA + HCMR

- Initially three cDNA libraries (larvae, adult brain and liver) were constructed (in collaboration with CCMAR)
- **846 ESTs** (557 from the larvae and 289 from the brain/pituitary) have been isolated and sequenced
- among them, 232 larval + 51 brain ESTs showed significant homology with known genes
- larval ESTs are archived in GenBank
- All ESTs have been screened for polymorphic markers (microsatellites & SNPs)

### Public resource
The RESBASS database - part of ResSpecies database (www.resspecies.org) – contains on 24.02.06:

- 287 seabass microsatellites, including
  - 14 previously published
  - 1 marker from sea bream *Sparus aurata* (SaGT41b)

- **226** AFLP markers

- **8** SNPs

**Total: 521** polymorphic markers

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**Mapping and constructing the linkage map – Roslin**

The first *sex-averaged* linkage map of seabass comprises **25 linkage groups** *(n = 24 - haploid karyotype)*
- includes **163 microsatellite loci**, of which **11 type I (coding)** markers
- contains **two mapped genes** encoding peptide Y and cytochrome P450 aromatase
- covers **817.7 cM** of the expected 1500 cM
**Sex-specific linkage maps**

**Male map (567.4 cM)**

**Female map (905.9 cM)**

**Female-to-male (F/M) recombination rate ratio:**

- Human (Gyapay et al. 1994) 1.7:1
- Mouse (Dietrich et al. 1996) 1.7:1
- Dog (Mellersh et al. 1997) 1.67:1
- Pig (Mikawa et al. 1999) 1.55:1
- European seabass 1.6:1
- Zebrafish (Sakamoto et al. 2000) 2.74:1
- Rainbow trout (Singer et al. 2002) 3.25:1
- Atlantic salmon (Moen et al. 2003) 8.26:1
- Japanese flounder (Coimbra et al. 2004) 1:7.4

**Public resource**

**STEP 2: second generation linkage map and pilot QTL analysis**
Towards a second generation linkage map

2nd generation integrated linkage map (microsatellites + AFLPs)

Physical mapping (BAC & RH)
(locating and ordering BAC clones on chromosome linkage maps)

Second generation linkage map – Microsatellites + AFLPs

- 369 markers (205 microsatellite, 164 AFLP)
- 34 linkage groups
- 1506 cM total length
- max. 187.2 cM per LG (LG01)
- 11 microsat - 47 AFLP markers unlinked
- ♀ map 33% longer than ♂
WP 11: QTL pilot project - AUTH/Nireus/HCMR

✓ P: 103 (88) parents from a commercial production broodstock
✓ F₁: 2505 progeny (° 09.03.02; † 13.12.03)
✓ Successful high-throughput parentage assignment through PCR based multiplex genotyping at 6 loci

Parentage analysis – Nireus/HCMR

• Unexpectedly, one female (#48) dominates the progeny
• 24 males contribute to the progeny
• Hence 24 half-sib families qualify for QTL analysis

Frequency distribution of body weight for adult males and females among the F₁ offspring.
Heritability analysis – Nireus/HCMR/AUTH

• Heritability has been identified for 12 production traits and sex (standard length: 0.42; body weight: 0.21)
• Correlations between all 12 production traits and sex were positive and very high (body weight and sex: 0.99)

QTL pilot project – Genome scan of linkage group 1 – Nireus/HCMR/AUTH

• LG01 has been genotyped at 7 loci in 24 parents and 422 progeny
• No significant QTL effect of body weight, although a suggestive linkage of marker DLA0015 with a putative QTL
• The analysis will be expanded for the traits sex, standard length, head length, body length and 6 morphometric measurements
• In progress: family-based selection under commercial conditions

Half sib analysis of LG 01 for body weight
SEA BASS GENETIC RESOURCES as of 01.03.06

- Mapping panel Venezia Fbis (+ others)
- Cell lines (MGE flagship project FICEL)
- BAC library → end sequencing → physical map
- 16 cDNA tissue and several subtractive libraries
- Radiation Hybrid panel → physical map
- Microsatellites → 1st & 2nd generation linkage map
- AFLPs → 2nd generation linkage map
- Polymorphic ESTs → 3rd generation linkage map
- Bioinformatic resources (data base, dedicated software, etc)
- Natural samples
- Aquaculture stocks (Nireus, IFREMER, EILAT, ...)

one of the TEN most genome rich TELEOSTS

The future

**Third** generation linkage map (300 microsatellites, 200 AFLPs and 200 ESTs) will be available by winter 2006

1. Effectiveness of Pedigree Assisted Selection in a commercial setting
2. Genome scan and QTL analysis of 24 linkage groups in a commercial setting
3. BAC library: macroarray hybridisation and gDNA isolation
4. Radiation Hybrid panel under construction (EU-STREP AQUAFIRST)
5. Candidate genes & QTLs for stress under scrutiny (EU-STREP AQUAFIRST) toolbox will be upgraded with mapped ESTs and Radiation Hybrid panel
6. Comparative genomics of perciforms and teleosts (synteny mapping)
7. Functional genomics of larval development, nutrition, sex determination, etc
8. Integration with parallel genomic and genetic projects
9. Adaptive evolution in the natural environment
Our aim: Integrating the sea bass research community

- Metabolism and nutrition
- Sex determination and differentiation
- Linkage, physical & synteny maps
- Endocrinology
- Environmental genomics
- Microbiology and diseases
- Aquaculture
- Fisheries
- Conservation

Thank you