Application of genetic fingerprinting and SNP for parentage assignment in selective breeding programmes

Marc Vandeputte – INRA/Ifremer, France
Not simple: getting family info in fish

- Small size of newly hatched fish: no tagging possible
  Turbot: 2-3 mm  Carp: 5 mm, trout 15 mm, chinook salmon 20 mm
- Earliest tagging claimed at 3 g (usually 10-20g)
- Two possible methods to obtain pedigree info: genotyping and separate rearing of families
Why family information?

• To obtain genetic parameters in mixed families
• Family information allows a better control of inbreeding
• Family information allows the use of family-based genetic evaluation ➔ the most efficient theoretically
  + optimal multi-trait selection, use of sib-selection for lethal traits
  - cost of family information, individual tagging necessary

• However individual selection (based on individual performance without knowledge of genealogy) remains attractive especially for SMEs
Parentage assignment

• For access to family information
• Using highly variable markers (microsats, SNPs)
• An alternative to « classical » separate rearing of families

Fin clip in ethanol 100
How to assign fish?

- **Maximum likelihood** (CERVUS, PAPA,...)
  - Looks for the most likely parental couple
  - Always gives a solution

- **Exclusion** (PROBMAX, VITASSIGN, FAP):
  - Checks compatibility of offspring and parental genotypes with Mendelian inheritance
  - Highly sensitive to genotyping errors, but this can be corrected with high power (>99%) of locus set and allowing for mismatches
  - No match ➔ genotyping error: very secure
Microsatellites or SNPs?

• **Microsatellites**
  - 8-12 markers needed
  - Widely used & effective
  - Some level of genotyping errors
  - Hard to standardize nomenclature between labs
  - May work with medium quality DNA

• **SNPs**
  - 40-100 markers needed
  - Multiplex under development in fish (exists in pig)
  - Claimed low genotyping error rate (0.1-0.5%?)
  - Easy standardization of alleles names
  - High quality DNA needed?
High assignment rates achieved...

<table>
<thead>
<tr>
<th>Species</th>
<th>Cross (# full-sibs families)</th>
<th>Nb µsat</th>
<th>% unique assig.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainbow trout</td>
<td>48 ♂ x 4♀ FF (192 FSF)</td>
<td>10</td>
<td>90 %</td>
<td>Chevassus et al, 2002</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>2 ♂ x 48♀ FF (96 FSF)</td>
<td>14</td>
<td>91.95 %</td>
<td>Fishback et al, 2002</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>38 ♂ x 29♀ FF (?)</td>
<td>8-12</td>
<td>97 %</td>
<td>Wilson et al, 2003</td>
</tr>
<tr>
<td>Atl . salmon</td>
<td>78 ♂ x 149♀ NE (149 FSF)</td>
<td>8</td>
<td>95.8 %</td>
<td>Norris et 2004</td>
</tr>
<tr>
<td>Comm. carp</td>
<td>24 ♂ x 10♀ FF (240 FSF)</td>
<td>7-8</td>
<td>95.3 %</td>
<td>Vandeputte et al, 2004</td>
</tr>
<tr>
<td>Sea bass</td>
<td>41 ♂ x 8♀ FF (40 FSF)</td>
<td>5-6</td>
<td>99.7 %</td>
<td>Launay, comm. pers.</td>
</tr>
<tr>
<td>Sea bass</td>
<td>33 ♂ x 23♀ FS (253 FSF)</td>
<td>6</td>
<td>99.2 %</td>
<td>Vandeputte et al, 2007</td>
</tr>
<tr>
<td>Cod</td>
<td>24 ♂ x 26♀ MS</td>
<td>4</td>
<td>98.6 %</td>
<td>Bekkevold et al 2002</td>
</tr>
<tr>
<td>Cod</td>
<td>24 ♂ x 70♀ MS</td>
<td>5</td>
<td>91.2 %</td>
<td>Wesmajervi et al 2006</td>
</tr>
<tr>
<td>Pacific Oyster</td>
<td>10 ♂ x 3♀ FF (30 FSF)</td>
<td>3</td>
<td>90 %</td>
<td>Taris et al 2006</td>
</tr>
<tr>
<td>Red drum</td>
<td>32 FSF MS</td>
<td>5-16</td>
<td>98.5 %</td>
<td>Saillant et al 2007</td>
</tr>
</tbody>
</table>
...most of the time

<table>
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<tr>
<th>Species</th>
<th>Cross (# full-sibs families)</th>
<th>Nb markers</th>
<th>% unique assig.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comm. carp</td>
<td>147 ♂ x 8♀ FF (1176 FSF)</td>
<td>6-11</td>
<td>75.7%</td>
<td>Mauger, comm. Pers.</td>
</tr>
<tr>
<td>Red sea bream</td>
<td>250 ♂+♀ MS</td>
<td>4</td>
<td>73.5%</td>
<td>Perez-Enriquez et al, 1999</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>45 ♂ x 2♀ FF</td>
<td>5</td>
<td>54%</td>
<td>Vandeputte et al 2006</td>
</tr>
<tr>
<td>Sea bass</td>
<td>45 ♂ x 58♀ MS</td>
<td>6</td>
<td>34.8%</td>
<td>Chatziplis et al 2007</td>
</tr>
</tbody>
</table>

Possible causes:
- genotyping errors (allelic drop-out, stutter bands)
- null alleles,
- DNA quality
- related broodfish,
- not enough markers,
- bad traceability of parents (missing parental DNA)

Problematic for family selection

Less problematic for:
• Inbreeding management
• Estimation of genetic parameters
12 males and 1 female contribute less than half the expected number.

Good family representation…

- Sea bass, 988 families, 2705 offspring, **96.1% reassign**.

76 Related males x 13 wild females. *In vitro* fertilization. 8 loci

<table>
<thead>
<tr>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male 1</td>
<td>Female 1</td>
</tr>
<tr>
<td>Male 2</td>
<td>Female 2</td>
</tr>
<tr>
<td>Male 3</td>
<td>Female 3</td>
</tr>
<tr>
<td>Male 4</td>
<td>Female 4</td>
</tr>
<tr>
<td>Male 5</td>
<td>Female 5</td>
</tr>
<tr>
<td>Male 6</td>
<td>Female 6</td>
</tr>
<tr>
<td>Male 7</td>
<td>Female 7</td>
</tr>
<tr>
<td>Male 8</td>
<td>Female 8</td>
</tr>
<tr>
<td>Male 9</td>
<td>Female 9</td>
</tr>
<tr>
<td>Male 10</td>
<td>Female 10</td>
</tr>
<tr>
<td>Male 11</td>
<td>Female 11</td>
</tr>
<tr>
<td>Male 12</td>
<td>Female 12</td>
</tr>
</tbody>
</table>

2 males and 1 female contribute no offspring.
12 males and 1 female contribute less than half the expected number.
...but not in mass spawnings

• Perez-Enriquez et al, 1999
  (red sea bream)

\[ \begin{align*}
\text{Mating model} \\
\text{Tank 1} \\
\text{Tank 2} \\
\end{align*} \]

\[ \begin{align*}
15 \varnothing/60, 30 \delta/60 \\
\text{Nbreeders} = 120 \\
\Rightarrow \text{effective number of breeders} = 26.4 \\
\end{align*} \]

Chatziplis et al 2007 (sea bass):
Single day mass spawning
\[ \begin{align*}
26 \delta/45, 1\varnothing/58 \ (Nt=103) \\
\Rightarrow \text{effective number of breeders} = 3.4 \\
\end{align*} \]

Good genetics with mass spawnings is difficult, even with genotyping!
Separate rearing for family info

- 100-300 tanks for rearing until tagging size
- Cost lies mainly in family number, not number of fish
  - high investment costs, tank effects (marine and pond species), mating designs and intensity constrained, evaluation in « experimental » conditions
+ slaughtering traits or disease challenge on sibs is « cheap », tagging is now early (10-20g)
Genotyping for family info

- Cost lies in fish number, not number of families
  - genotyping costs (7.5-15 €/fish), assignment rate, indirect criteria necessary, maternal and competition effects?
  + low investment, no constraint on crossing (factorials but also mass spawnings usable), no tank effects, working in production environment possible, prices decrease (20-30 €/fish in 2001), low additional cost of QTL-MAS
How to genotype less fish?

- Parentage assignment of the nucleus in individual selection for minimizing inbreeding (« walkback selection », optimised crosses)
- Parentage assignment of dead/surviving fish after a disease outbreak
- Parentage assignment in a pre-nucleus for family-based selection following individual selection
Estimation of genetic parameters

• Rainbow trout:
  – 2 ♂ x 48 ♀ FF (growth: Fishback et al 2002)
  – 48 ♂ x 4 ♀ FF (growth, quality: Chevassus et al 2002)
  – 38 ♂ x 29 ♀ (growth, spawning time: Wilson et al, 2003)
• Atlantic salmon:
  – 78 ♂ x 149 ♀ NE (flesh colour: Norris et al 2004)
• Common carp:
  – 24 ♂ x 10 ♀ FF (early growth: Vandeputte et al 2004)
  – 92 ♂ x 8 ♀ FF (slaughter weight, morpho and quality: Kocour et al 2007)
• Sea bass:
  – 10 ♂ x 3 ♀ FF (growth, GxE: Saillant et al 2006)
• Other species:
  – 10 ♂ x 10 ♀ FF Hybrid striped bass (Wang et al 2006)
  – 14 ♂ x 6 ♀ FF Tropical abalone (Lucas et al 2006)
  – 32 FSF MS Red drum (Saillant et al 2007)
From individual to family selection...

1) Single-trait individual selection

- Factorial mating: $10 \times (5 \times 5)$
- 200 parents
- Selection on one trait
- 10,000 candidates
- 1 tank/cage
2) Multi-trait individual selection

Factorial mating $10 \times (5 \times 5)$

200 parents

Selection on an index

10,000 candidates

Several individual performances recorded

1 tank/cage

PIT tag
3) Multi-trait individual selection with inbreeding control

200 assignments

200 parents

Selection on an index

10,000 candidates

Factorial mating $10^2(5^2)

10,000 candidates

Several individual performances recorded
From individual to family selection...

4) Multi-trait individual & family selection with pre-selection on one trait

Factorial mating $10^2 (5 \times 5)$

10,000 candidates

200 parents

2,000 pre-selected

Selection on one trait

2,000 assignments

Several individual performances recorded

PIT tag

10,000 candidates

Family selection on an index
From individual to family selection...

5) Multi-trait individual & family selection with pre-selection on one trait + lethal traits on sibs

Factorial mating

10\*(5\*5)

200 parents

Selection on one trait

2,000 pre-selected

Several individual performances recorded

PIT tag

Family selection on an index

4,000 assignments

2,000 slaughtered

10,000 candidates

2,000 pre-selected

10,000 candidates

Ifremer
6) Multi-trait individual & family selection (with lethal traits)

Factorial mating \(10 \times (5 \times 5)\)

200 parents

PIT tag

10,000 assignments

Several individual performances recorded

Family selection on an index

2,000 slaughtered

10,000 candidates

From individual to family selection...
Problems for evaluation of mixed families

• Persistence of maternal effects?

Weight distribution (18 months) of mixed RT offspring from 2 dams with egg weights 53 and 97 mg (Dupont-Nivet et al, unpub.)

But $m^2 < 0.10$ in mixed seabass $m^2 \approx 0$ in carp $\Rightarrow$ species specific
Amplification of family differences through competition?

These problems are of general interest as they also affect the potential success of individual selection.
## Costs for different types of marker use

<table>
<thead>
<tr>
<th></th>
<th>#μsat (€/fish)</th>
<th># Fish genotyped</th>
<th>Cost of genotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inbreeding control</td>
<td>10 (15 €)</td>
<td>300</td>
<td>4,500€</td>
</tr>
<tr>
<td>Genetic parameters estimation</td>
<td>10 (10 €)</td>
<td>1,500</td>
<td>15,000€</td>
</tr>
<tr>
<td>Pre-nucleus genotyping</td>
<td>10 (10 €)</td>
<td>2,000</td>
<td>20,000€</td>
</tr>
<tr>
<td>Pre-nucleus + sibs slaughtered</td>
<td>10 (10€)</td>
<td>4,000</td>
<td>40,000€</td>
</tr>
<tr>
<td>Full genotyping</td>
<td>10 (10 €)</td>
<td>10,000</td>
<td>100,000€</td>
</tr>
</tbody>
</table>

Costs may further decrease with SNPs but are already marginal for 2-3000 ind/yr (rel. to 150k€/yr)
Family selection with genotyping

• Logical evolution from individual selection, not necessarily from family-separate rearing: « small scale » breeding programmes for niche markets/new species ➔ sustainability

• Best use *a priori*: when *in-vitro* fertilisation is possible, but larval rearing results are variable (marine species, pond species)

• Integration of QTL-MAS at marginal cost (tagging, DNA collection and extraction already done)
What remains to be done?

- Optimize methods to lower the number of genotyped fish (not « wasting » family information to increase growth rate)
- Develop indirect individual criteria (correlated traits, QTLs) for lethal or « group » traits (disease resistance, quality traits, feed efficiency)
- Study the role of competition (phenotypic, genetic components) on the observed variability in performance
- Optimize designs to introduce MAS