Breeding for perfection
Journey towards understanding the genetic basis of the perfect pearl

Advanced animal breeding in Aquaculture:
Using genome-wide molecular breeding values for rapid animal improvement in the silver-lipped pearl oyster

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Background

- *Pinctada maxima* is the primary species of Australia and Indonesia’s pearling industry
  - ~40% global pearl production value

- Only oyster to produce highly-sought large silver or gold pearls (>15mm)

- Large variation in pearl quality
  - 5% of pearl > 80-90% revenue

- Advancements in hatchery techniques
  - production of hatchery seed stock
  - initiation of selection breeding programs
**Cultured pearl production complex**

- **Donor oyster**
- **Host oyster**
- **Saibo**
- **Nucleus**

Single pearl = 2 animals/genomes
2 years host + 2 years pearl formation
Pearl trait variability

**PEARL VIRTUE**

Five main quality characteristics (or virtues) associated with pearls:

- **Size**: From 1MM up to 20MM
- **Shape**: Baroque, Circle, Drop, Button, Near round, Round, Semi baroque
- **Colour**: Gold, Silver, Silver rose, White, White gold, White rose
- **Lustre**: Dull, Exceptional, Fine
- **Surface**: Exceptional, Fine, Blemished

**Gold colour quality**

- 12mm Yellow round pearl “A” – US $30
- 12mm Gold round pearl “AAAA” - US $2080
Productivity and Commercial value

- Productivity/profitability in industry linked to
  - Product quality
  - Product uniformity
  - Farm cost reduction

- Achieved through
  - Husbandry/Breeding
  - Nutrition
  - Environmental manipulation
  - Genetic improvement
Productivity and Commercial value

What’s more important?

• Host oyster genotype
• Donor oyster genotype
• Host/Donor genotype interaction
• Environment….

“A” = US$30

“A” = US$3000

Are they heritable, how are they genetically correlated?

What traits?

Colour?
Lustre?
Nuclei Retention?
**P. maxima Program Objectives**

- Understand trait / genetic parameters and environmental (GxE) components
- Unravel donor / host interactions & contributions
- Generate a large genomic sequence and SNP resource for *P. maxima*
- Create dense genetic maps for genome structure and trait association studies
- Integrate genomic selection breeding program based on commercial farm datasets and resources
**P. maxima** genetic parameters

Genetic Parameters – Oyster shell growth traits

Table 1 Heritability ± standard deviation (bold diagonal), genetic (upper diagonal) for oyster shell growth and pearl quality traits. ** indicates the correlation is significant at the 0.01 level (2-tailed), and * indicates the correlation is significant at the 0.05 level (2-tailed).

<table>
<thead>
<tr>
<th>Heritabilities ±SD</th>
<th>Oyster Shell Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length (APM)</td>
</tr>
<tr>
<td>Length (APM)</td>
<td>0.328 ± (0.128)</td>
</tr>
<tr>
<td>Height (DVM)</td>
<td>-</td>
</tr>
<tr>
<td>Width (SW)</td>
<td>-</td>
</tr>
<tr>
<td>Wet weight (WW)</td>
<td>-</td>
</tr>
</tbody>
</table>

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**Height**

**Width**

**Length**
# P. maxima genetic parameters

## Genetic Parameters – Pearl quality traits

Table 2. Heritability ± standard deviation (bold diagonal), genetic (upper diagonal) and phenotypic correlations (below diagonal) for pearl quality traits. ** indicates the correlation is significant at the 0.01 level (2-tailed), and * indicates the correlation is significant at the 0.05 level (2-tailed).

<table>
<thead>
<tr>
<th>Heritabilities ±SD</th>
<th>Pearl Quality</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Size</td>
<td>Weight</td>
<td>Surface</td>
<td>Colour (SW.O.G)</td>
<td>Colour (G.O)</td>
<td>Colour (S.O)</td>
<td>Colour (W.O)</td>
</tr>
<tr>
<td>Size</td>
<td>0.144 ± (0.026)</td>
<td>0.957**</td>
<td>-0.110*</td>
<td>0.044</td>
<td>-0.031</td>
<td>0.014</td>
<td>0.049</td>
</tr>
<tr>
<td>Weight</td>
<td>0.967**</td>
<td>0.161 ± (0.026)</td>
<td>-0.114*</td>
<td>0.017</td>
<td>-0.001</td>
<td>0.002</td>
<td>0.032</td>
</tr>
<tr>
<td>Surface</td>
<td>-0.194**</td>
<td>-0.195**</td>
<td>0.265 ± (0.031)</td>
<td>0.087</td>
<td>-0.055</td>
<td>0.065</td>
<td>0.083</td>
</tr>
<tr>
<td>Colour (SW.O.G)</td>
<td>-0.010</td>
<td>-0.027</td>
<td>0.117*</td>
<td>0.363 ± (0.031)</td>
<td>-0.911**</td>
<td>0.457**</td>
<td>0.789**</td>
</tr>
<tr>
<td>Colour (G.O)</td>
<td>0.012</td>
<td>0.022</td>
<td>-0.090</td>
<td>-0.920**</td>
<td>0.338 ± (0.030)</td>
<td>-0.347**</td>
<td>-0.562**</td>
</tr>
<tr>
<td>Colour (S.O)</td>
<td>0.045</td>
<td>0.017</td>
<td>0.122*</td>
<td>0.454**</td>
<td>-0.366**</td>
<td>0.138 ± (0.026)</td>
<td>0.005</td>
</tr>
<tr>
<td>Colour (W.O)</td>
<td>-0.014</td>
<td>-0.031</td>
<td>0.091</td>
<td>0.799**</td>
<td>-0.593**</td>
<td>0.025</td>
<td>0.235 ± (0.030)</td>
</tr>
<tr>
<td>Colour (SW.O)</td>
<td>-0.005</td>
<td>-0.028</td>
<td>0.131*</td>
<td>0.907**</td>
<td>-0.687**</td>
<td>0.480**</td>
<td>0.879**</td>
</tr>
</tbody>
</table>

S: Snow White; W: White; O: Other; G: Gold
So what is more important: host or donor?

<table>
<thead>
<tr>
<th>P. margaritifera donor</th>
<th>P. maxima donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Black-lip) (N=10)</td>
<td>(Silver-lip) (N=10)</td>
</tr>
<tr>
<td>4 saibo pieces</td>
<td>4 saibo pieces</td>
</tr>
</tbody>
</table>

- P. margaritifera host (N=40) - Allograft Bb
- P. maxima host (N=40) - Xenograft Sb
- P. margaritifera host (N=40) - Xenograft Bs
- P. maxima host (N=40) - Allograft Ss

- Nuclei retention and pearl quality recorded 14 months post operation.
Pearl nacre growth

- Pearl growth strongly influenced by donor oyster (P < 0.001)

![Graph showing mean nacre growth (mm, +/- SE) for different donor and host combinations.]

- Donor: Black, Silver
- Host: Black-lip, Silver-lip

Pearl growth strongly influenced by donor oyster (P < 0.001)
Pearl colour

- Pearl colour was strongly influenced by the donor oyster species (P<0.001).

![Graph showing pearl colour distribution](image)
**P. maxima genetic resources**

Genome-wide sequencing / SNP chip development

- Mantle / pearl sac tissue – RNA to cDNA
- Next-generation sequencing (Roche 454, HiSeq)
- Sequence cleanup & de-novo EST assembly
- Random SNP discovery (MAF & No. reads > 10)
- Custom SNP array designed (many Type I markers)

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**Raw Sequence Data**

- 1.3 million reads, Average length 360bp

**Cleanup / Assembly**

- ~96,000 Contigs
- 5x genome coverage

**SNP Array Design**

- ~21,000 SNPs (MAF > 15%)

**Illumina custom SNP Chip**

- 2,782 SNPs
cM Marker

<table>
<thead>
<tr>
<th>cM</th>
<th>Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>SNP 1431</td>
</tr>
<tr>
<td>0.2</td>
<td>SNP 14627</td>
</tr>
<tr>
<td>2.6</td>
<td>SNP 2018</td>
</tr>
<tr>
<td>0.9</td>
<td>SNP 3082</td>
</tr>
<tr>
<td>0.9</td>
<td>SNP 78645</td>
</tr>
<tr>
<td>0.5</td>
<td>SNP 12403</td>
</tr>
<tr>
<td>0.5</td>
<td>SNP 13568</td>
</tr>
<tr>
<td>1.9</td>
<td>SNP 15261</td>
</tr>
<tr>
<td>1.1</td>
<td>SNP 20839</td>
</tr>
<tr>
<td>0.3</td>
<td>SNP 2270</td>
</tr>
<tr>
<td>0.9</td>
<td>SNP 13540</td>
</tr>
<tr>
<td>0.6</td>
<td>SNP 13942</td>
</tr>
<tr>
<td>1.1</td>
<td>SNP 17630</td>
</tr>
<tr>
<td>0.5</td>
<td>SNP 96</td>
</tr>
</tbody>
</table>

Framework (LOD > 3)

Most likely position
• 924 SNPs mapped
• Total Distance: 1059.2 cM
• Genome Coverage: 96.76%
• Average interval: 1.5 cM
**P. maxima QTL / GWAS analysis**

Markers only explained ~5% of variation
**P. maxima** QTL / GWAS analysis

Markers only explained ~10-15% of genetic variation
Genomic selection (GS)

- **Most traits are complex involving many genes of small effect**
  - Need to simultaneously search for all genes of small effects

- **GS – application**
  - Divide genome into many segments each with many loci
  - Estimate the genetic effect of each segment from a reference population (model training)
  - Genotype target population and sum segment effects to get genomic breeding values for individuals

- **Benefits over traditional selective breeding**
  - Increasing accuracy of selection (~0.7-0.8)
  - Reducing the generation interval
  - Allows within family selection
  - Minimizes inbreeding rates

Meuwissen et al, (2001)
P. maxima GS genetic resources

Genotype-by-Sequencing (GBS) SNPs for GS application

- ddRAD-tag, ~48,000 high quality SNPs identified
- SNPs and Indels separately identified
- High data integrity, >98% replicate repeatability
- Genotype founders with 48K SNPs impute remainder
- Genotype remainder (10,000-15,000 animals) with 5K SNPs @ $20 each

Phenotypic / tissue data for GS application

- Grow-out commercial data 2003-2012 (> 500,000 animals, qualitative only)
- NBC commercial data 2013-ongoing (> 100,000 animals, qualitative + quantitative)

GS program commenced February 2015
Issues Implementing Genomic selection

- Commercial operations ➡️ separate host / donor lines
- Recording reliable phenotypic data (qual + quant)
- Environmental heterogeneity and seasonal variation

**Average Age to Ops for Month of Spawn (2010-2013)**

- Temperature
- Chlorophyll
- Sea surface temperature
- Wind
- Salinity

Eg., Daily MODIS and SeaWIFS satellite data

- All are being measured to increase the accuracy of GS
Quantitative Phenotypic Data

- Host Oyster Growth – automated with GoPro & image analysis
  - Routinely throughout entire production cycle development
Quantitative Phenotypic Data

- Host Oyster Growth – automated with GoPro & image analysis
- Donor Oyster Pearl Quality – spectrophotometry

Distinct profiles and wave lengths

Colour
- Silver
- Silver Rose
- White
- White Rose
- Golden
- White Gold

Lustre
- Excellent
- Very Good
- Good
Final Comments

- Long-term 12+ year research program with industry
- Generated fundamental base-line data
- 1,000’s genetic markers available at relative low cost (~$20 animal)
- Adequate animal records / tissue available 10+ years
- Highly reliable phenotypic & environmental data most important
- Genomic Selection a real option for *P. maxima*, but need to integrate environmental variation effect on models