

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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- *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



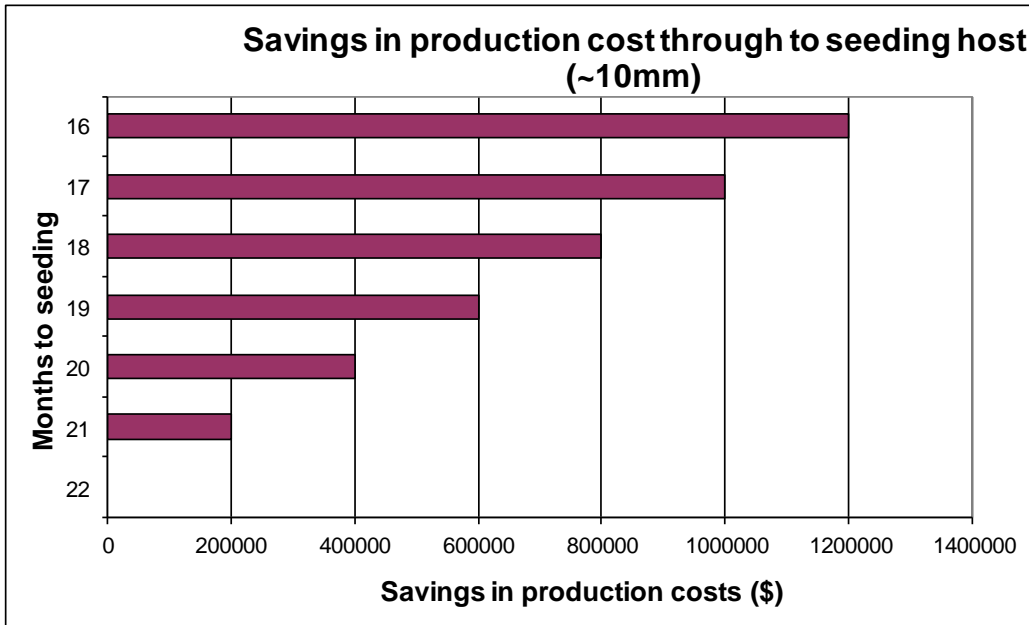
- 12mm Yellow round pearl "A" – US \$30
- 12mm Gold round pearl "AAAA" - US \$2080

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



“A” = US\$30



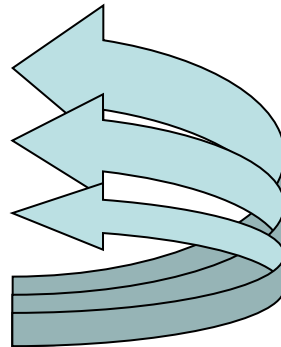
“A” = US\$3000

Are they heritable, how are they genetically correlated?

What's more important?



- Host oyster genotype
- Donor oyster genotype
- Host/Donor genotype interaction
- Environment....

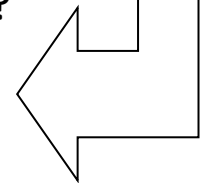


What traits?

Colour?

Lustre?

Nuclei Retention?

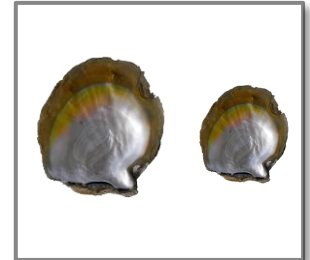


P. maxima Program Objectives

Years

0

- Understand trait / genetic parameters and environmental (GxE) components



3

- Unravel donor / host interactions & contributions

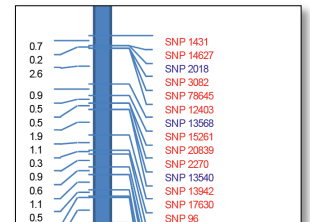


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- Generate a large genomic sequence and SNP resource for *P. maxima*

8

- Create dense genetic maps for genome structure and trait association studies



12

- Integrate genomic selection breeding program based on commercial farm datasets and resources

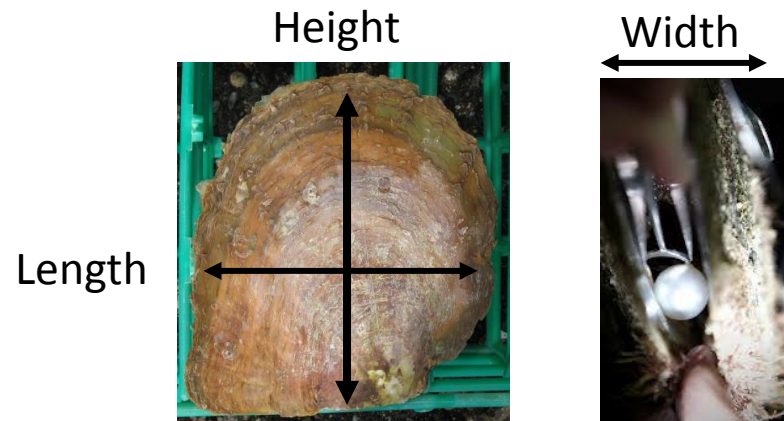


P. maxima genetic parameters

Genetic Parameters – Oyster shell growth traits

Table 1 Heritability \pm 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).

Heritabilities \pm SD	Oyster Shell Growth			
	Length (APM)	Height (DVM)	Width (SW)	Wet weight (WW)
Length (APM)	0.328 \pm (0.128)	0.904**	0.237**	0.852**
Height (DVM)	-	0.286 \pm (0.126)	0.258**	0.882**
Width (SW)	-	-	0.128 \pm (0.081)	0.249**
Wet weight (WW)	-	-	-	0.183 \pm (0.103)

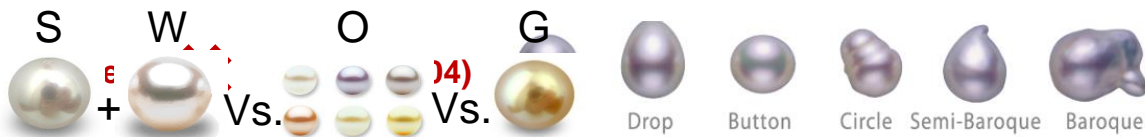


P. maxima genetic parameters

Genetic Parameters – Pearl quality traits

Table 2. Heritability \pm 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).

Heritabilities \pm SD	Pearl Quality							
	Size	Weight	Surface	Colour (SW.O.G)	Colour (G.O)	Colour (S.O)	Colour (W.O)	Colour (SW.O)
Size	0.144 \pm (0.026)	0.957**	-0.110*	0.044	-0.031	0.014	0.049	0.049
Weight	0.967**	0.161 \pm (0.026)	-0.114*	0.017	-0.001	0.002	0.032	0.029
Surface	-0.194**	-0.195**	0.265 \pm (0.031)	0.087	-0.055	0.065	0.083	0.104*
Colour (SW.O.G)	-0.010	-0.027	0.117*	0.363 \pm (0.031)	-0.911**	0.457**	0.789**	0.910**
Colour (G.O)	0.012	0.022	-0.090	-0.920**	0.338 \pm (0.030)	-0.347**	-0.562**	-0.658**
Colour (S.O)	0.045	0.017	0.122*	0.454**	-0.366**	0.138 \pm (0.026)	0.005	0.486**
Colour (W.O)	-0.014	-0.031	0.091	0.799**	-0.593**	0.025	0.235 \pm (0.030)	0.876**
Colour (SW.O)	-0.005	-0.028	0.131*	0.907**	-0.687**	0.480**	0.879**	0.270 \pm (0.029)



So what is more important host or donor?



P. margaritifera donor
(Black-lip) (N=10)



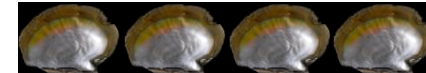
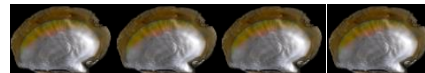
P. maxima donor
(Silver-lip) (N=10)

4 saibo
pieces

4 saibo
pieces

4 saibo
pieces

4 saibo
pieces



P. margaritifera
host (N=40)

P. maxima
host (N=40)

P. margaritifera
host (N=40)

P. maxima host
(N=40)

Allograft

Xenograft

Xenograft

Allograft

Bb

Sb

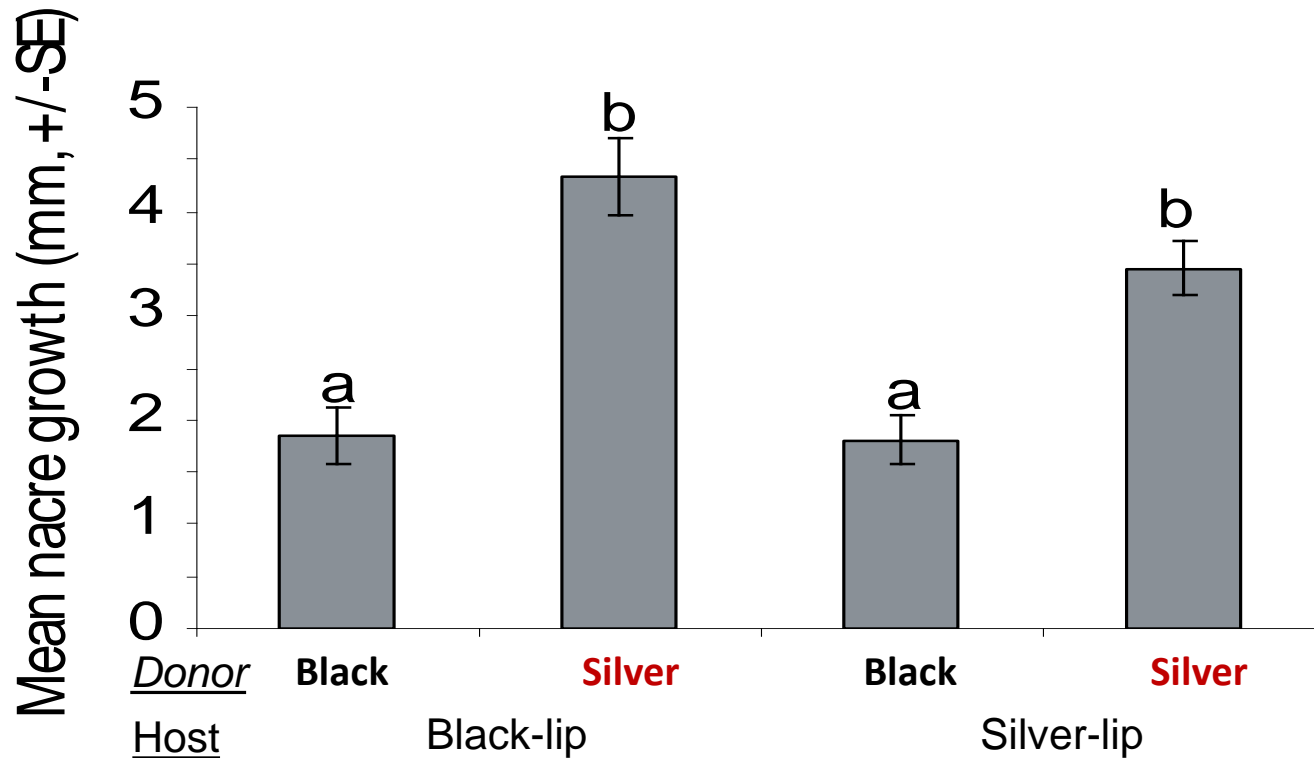
Bs

Ss

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

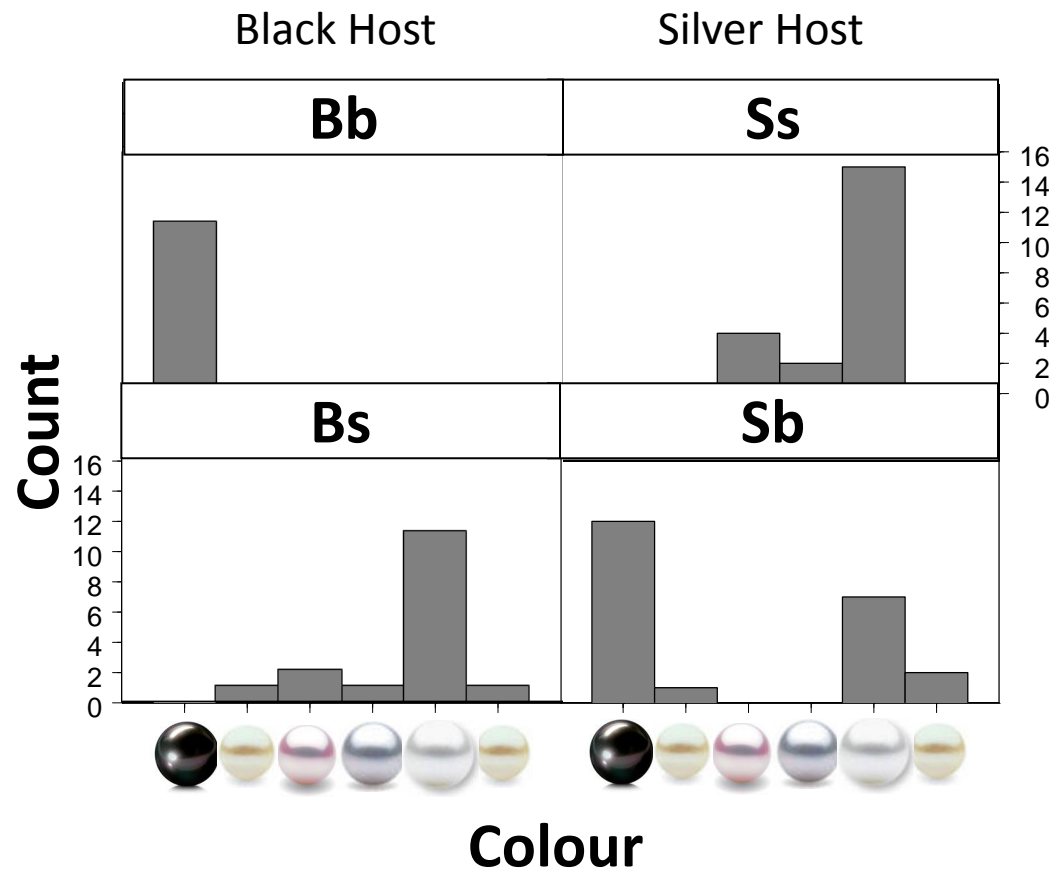
Pearl nacre growth

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



Pearl colour

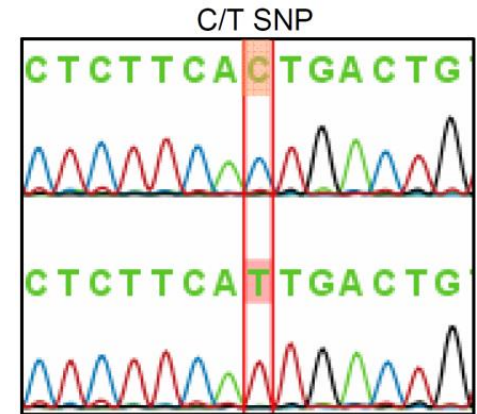
- Pearl colour was strongly influenced by the donor oyster species ($P < 0.001$).



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)

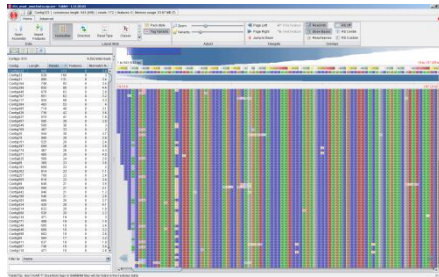


Raw Sequence Data



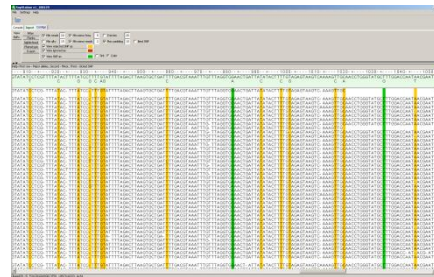
1.3 million reads,
Average length 360bp

Cleanup / Assembly



~96,000 Contigs
5x genome coverage

SNP Array Design



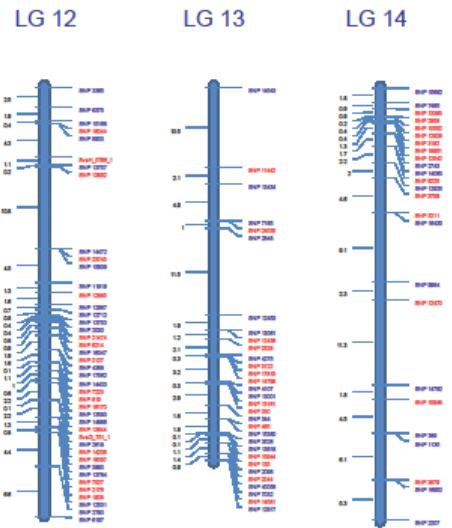
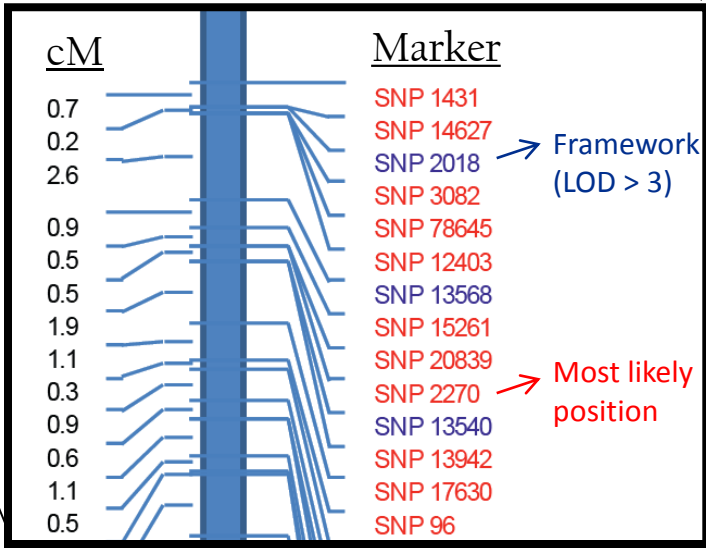
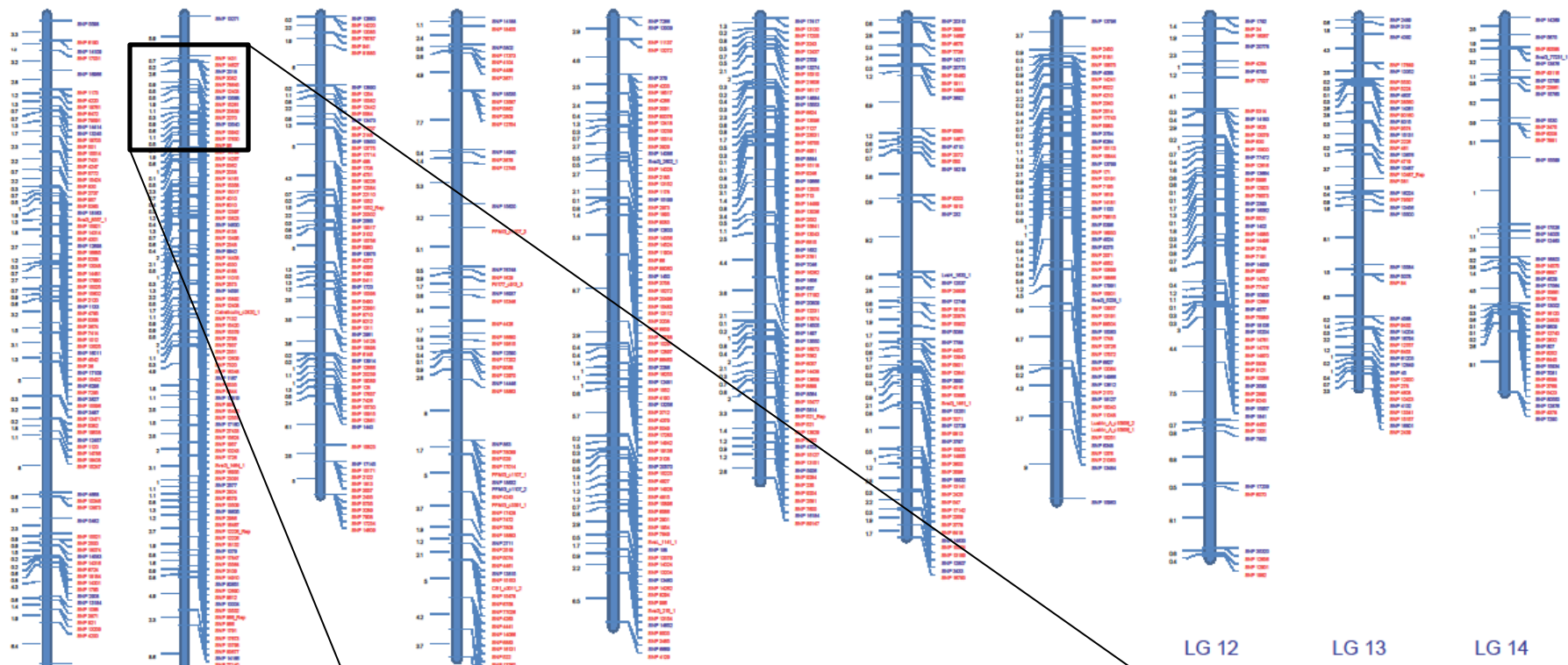
~21,000 SNPs (MAF > 15%)

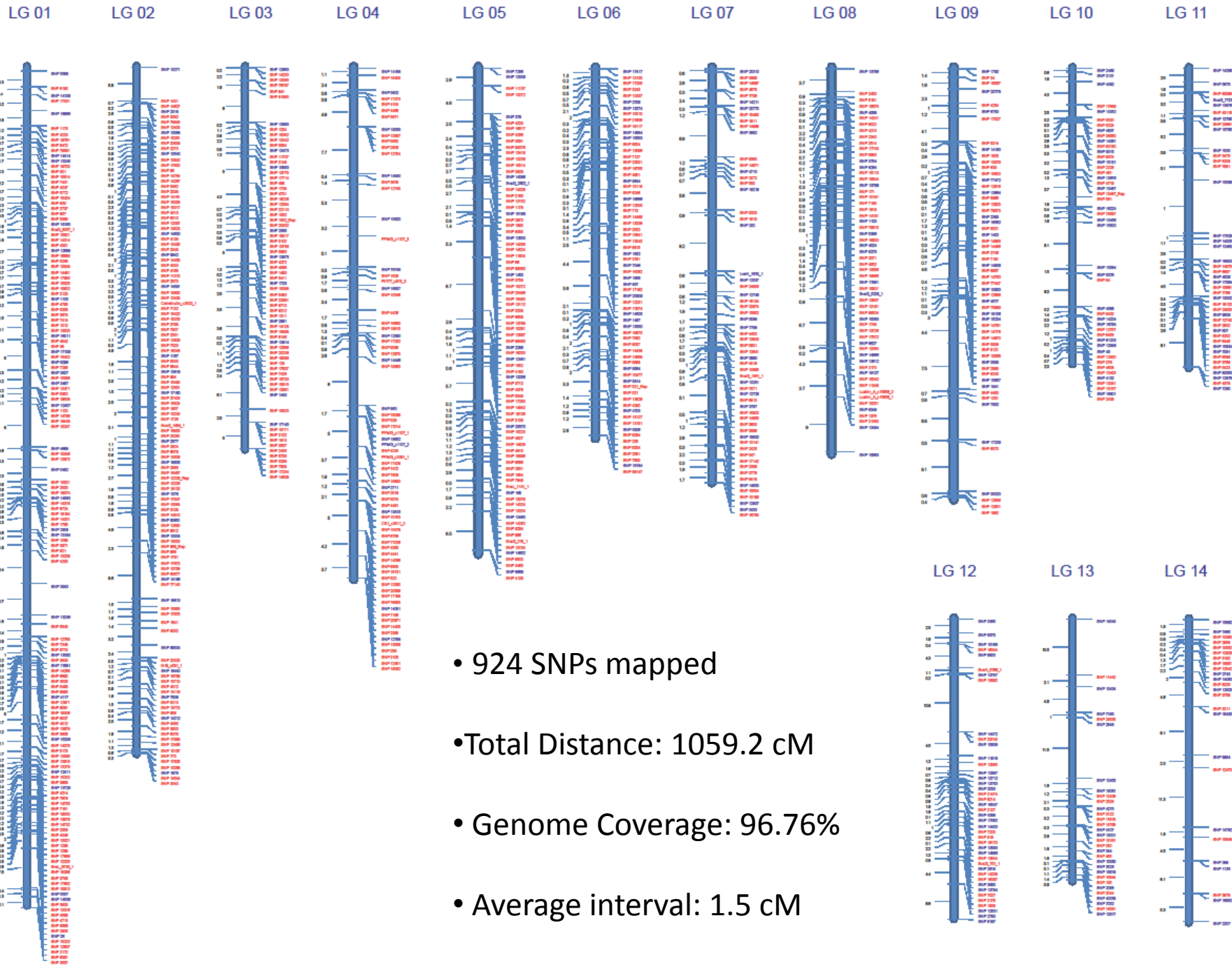
Illumina custom SNP Chip



2,782 SNPs

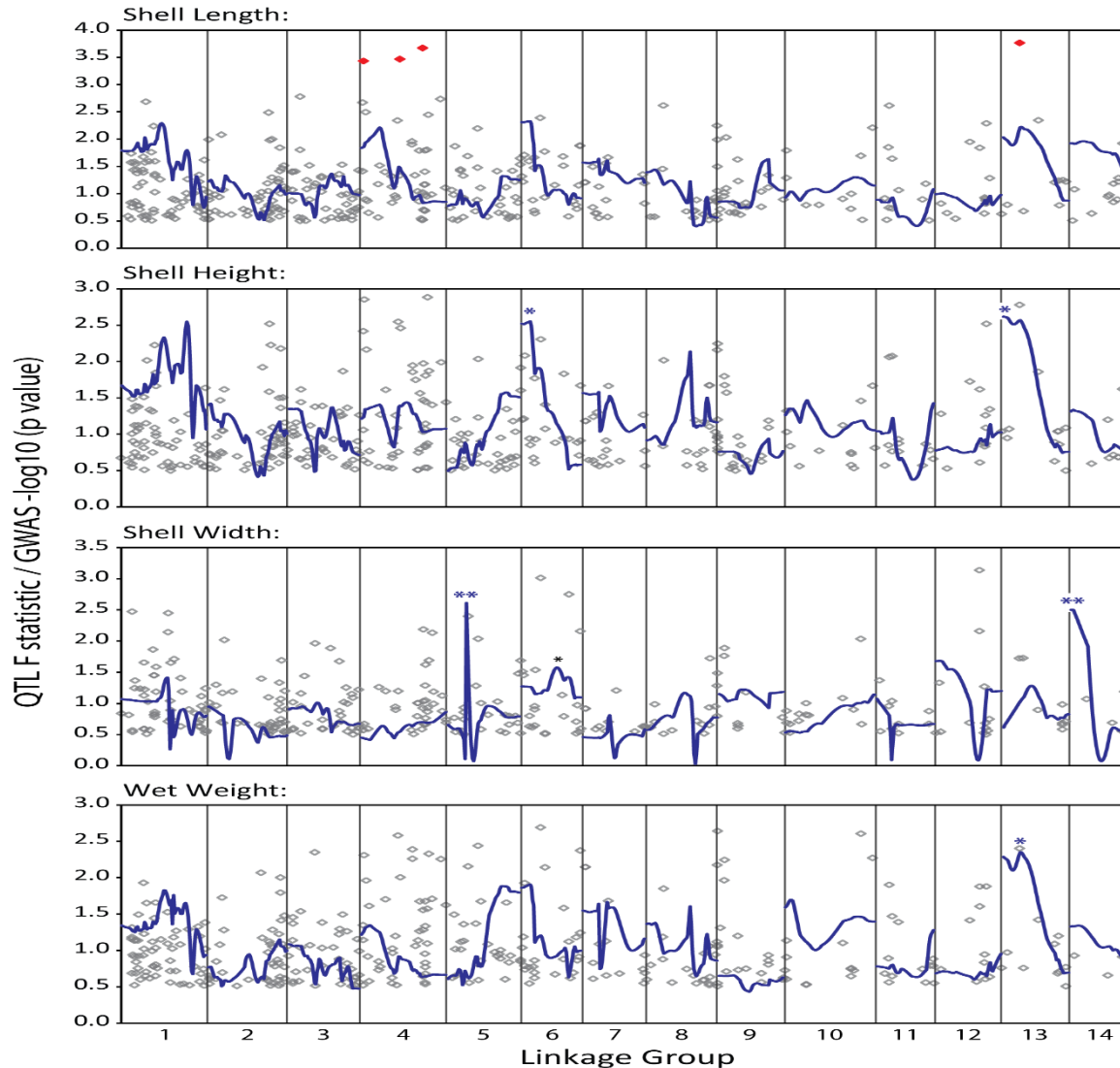
LG 01 LG 02 LG 03 LG 04 LG 05 LG 06 LG 07 LG 08 LG 09 LG 10 LG 11





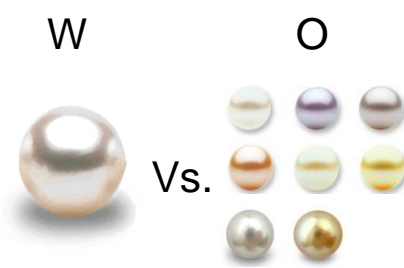
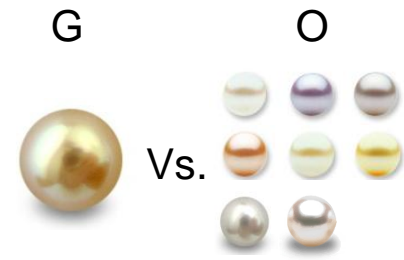
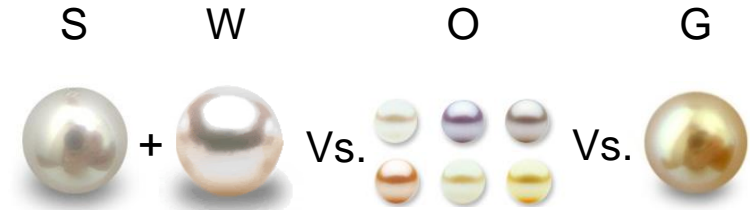
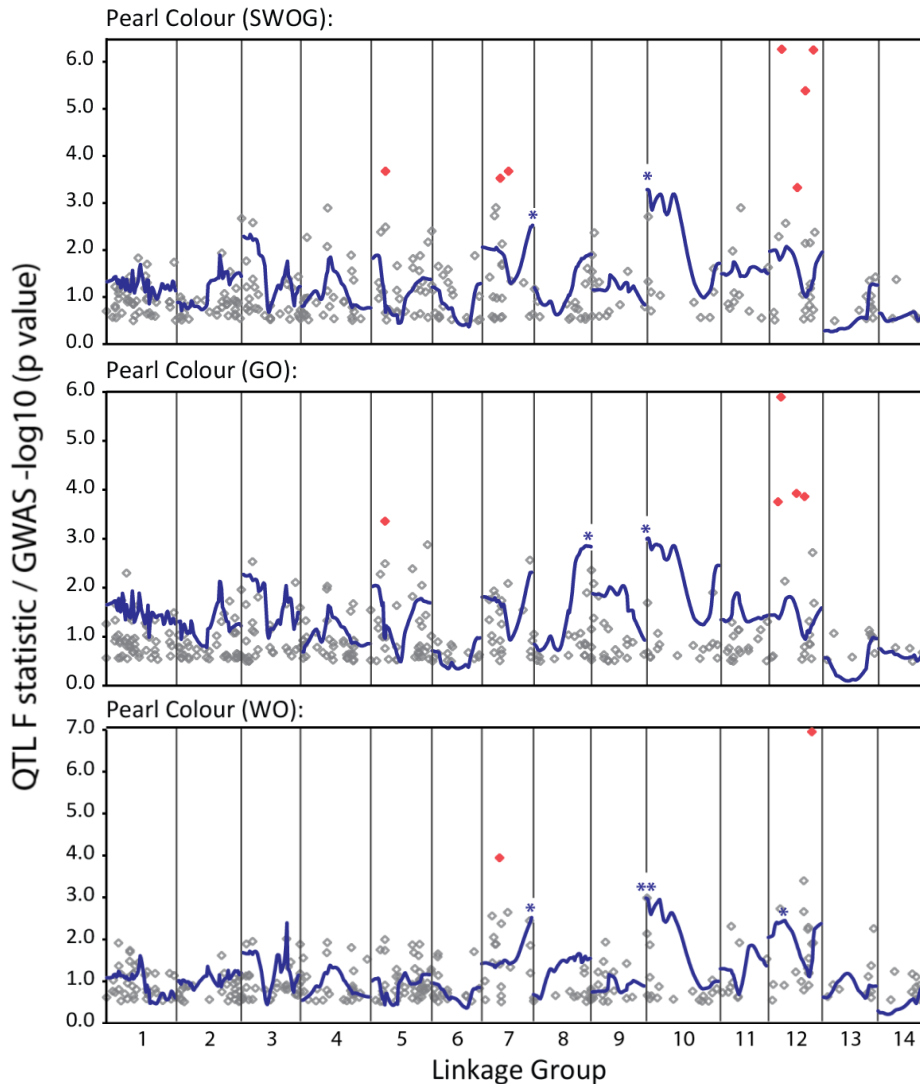
- 924 SNPs mapped
- Total Distance: 1059.2 cM
- Genome Coverage: 96.76%
- Average interval: 1.5 cM

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***

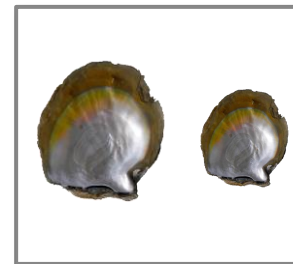
- *Need to simultaneously search for all genes of small effects*

❑ GS – application¹

- 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

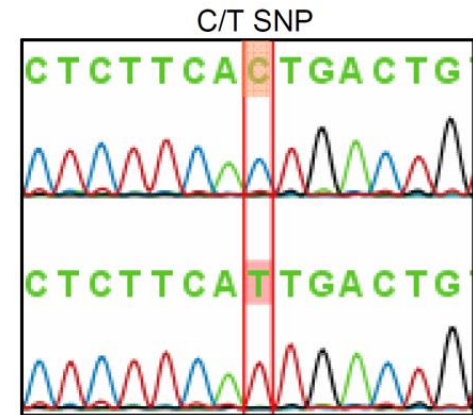
- Increasing accuracy of selection (~0.7-0.8)
- Reducing the generation interval
- Allows within family selection
- Minimizes inbreeding rates



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



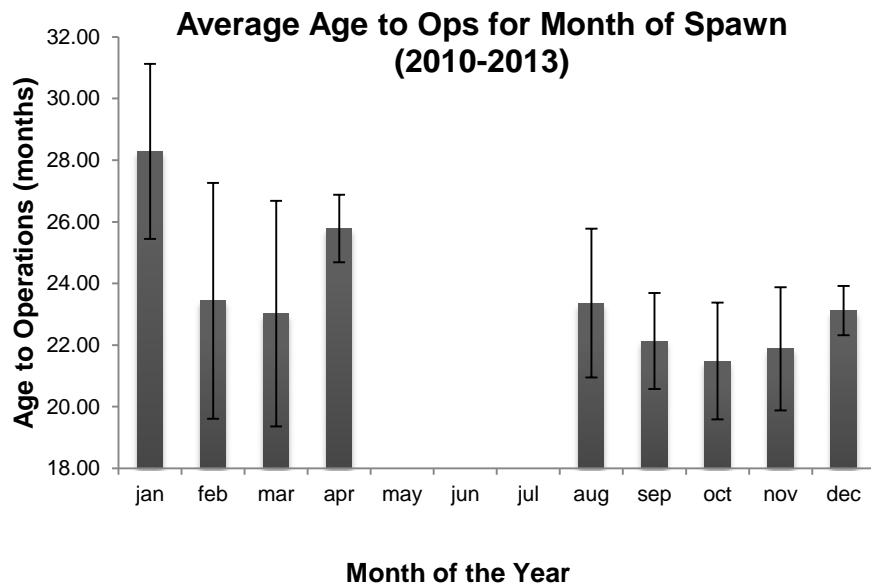
Phenotypic / tissue data for GS application

- 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



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

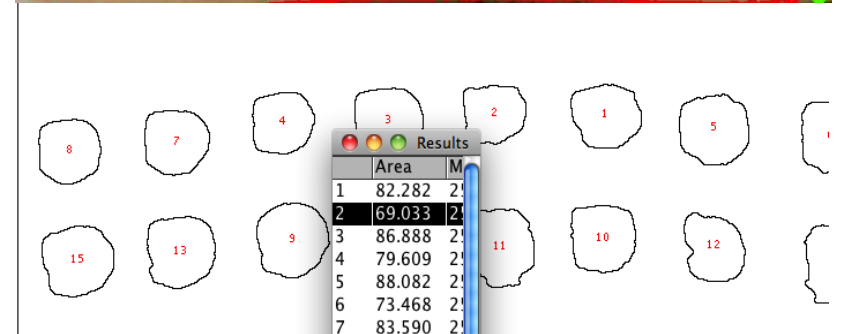
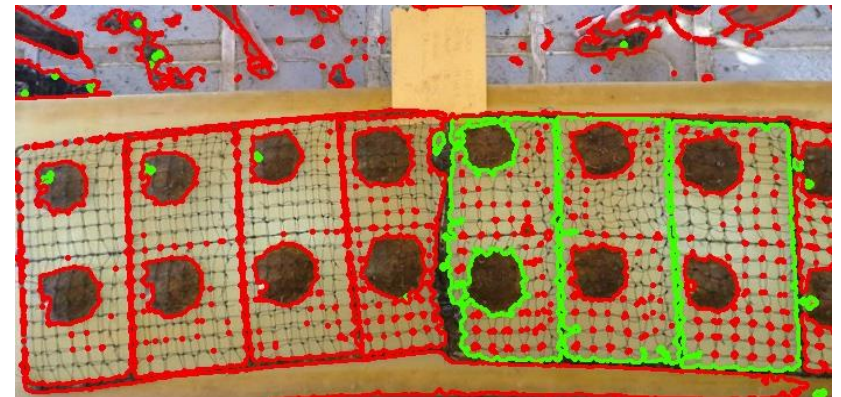


Eg., Daily MODIS and SeaWIFS satellite data

- **All are being measured to increase the accuracy of GS**

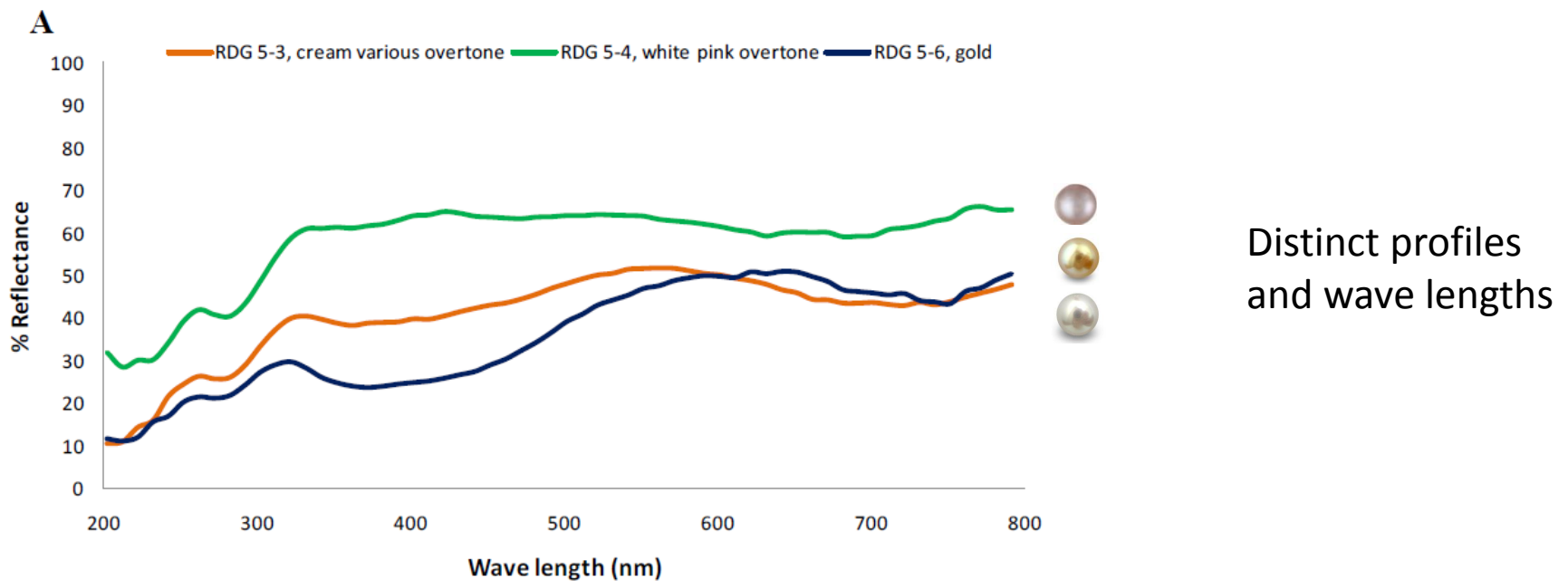
Quantitative Phenotypic Data

- 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



Colour



Lustre



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
-