Mapping QTL for Resistance to Summer Mortality in the Pacific Oyster *Crassostrea gigas*

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Most cultured species – 4.4Mt for a value > to 3 $billions (FAO, 2005)

Large geographic and environmental repartition (except artic zones)

Deliver social benefits
The mortality phenomenon

Significative mortalities were recorded since the mid 70’s (>30%)

Affects spat of oysters (<1yr old) ➔ Economical loss for farmers!

PATHOGENS
Vibrio, Herpes…

ENVIRONMENT
Nutrients, T°C…

GENETICS

Multi-disciplinary research project: MOREST (2001-2006)
The ‘MOREST’ project

GENETICS
Quantitative studies
Genetic basis of resistance to SM

After 3 generations of Selection, Resistant and Sensitive stocks of oysters to summer mortality

Heritability of Spat Survival during summer:
\[ h^2 = 0.70 \pm 0.35 \] 
(Degrémont et al., 2005)

High variance in Spat Survival rates
(Ernande et al., 2004)

Opens the way to selection: MAS
1. Produce F1 R/S hybrid & F2 biological material
2. To challenge F2 families during the summer
3. Development of molecular markers
4. QTL detection in the Pacific oyster
A 3 Generation Experimental Design

Divergent Selection of sensitive and resistant stocks of oysters to summer mortality

2001-04  20 F0 Individuals

2004  10 F1 Individuals

2006  5 F2 families
Phenotyping - Mortality

Kinetic of summer mortality within the 5 F2 families (raceway - July 2006)

6 months old oysters

Daily monitoring and Sampling
High level of infection by Herpes virus detected

Cumulated percentage of mortality within the 5 F2 families (raceway)

Strong correlation: Individual Viral Load ↔ Mortality
OSHV1, the Ostreid Herpes Virus type I is known to be involved in mass mortalities events (Renault et al., 2000)

Individual viral load was quantified by a newly developed Q-PCR assay (Pépin et al., submitted)

Phenotyping was performed on two traits: viral load and survival
SNP Development

- Direct sequencing of partial sequence in a set of 61 EST of known function in the F0 individuals

- Marker characterization
  - Position
  - C / NC
  - Ts / Tv
  - S / NS

- Informativeness in the F0 & F1 individuals
In each EST, 1 SNP was selected according to its informativeness in the F0 & F1 individuals.

Genotyping was performed in the F2 by Good-assay in the MPI.
Molecular Resources

Microsatellites Markers
  • 46 from Hubert & Hedgecock (2004)
  • 3 from Yu & Li (2007)

*In silico* SSR derived from EST
  • 18 from Sauvage et al. (2008, submitted)

SNP markers
  • 47 from Sauvage et al. (2007)

Selective genotyping was performed

3 F2 segregating families
900 individuals
30 % of the distribution

![Graph showing normal distribution with 15% died and 15% survived]
Linkage Mapping – Crimap (Green, 1990)

Consensus Map:
10 LG / Length 1218cM / 82 Markers / average spacing of 13.5 cM/ Coverage=93%

2 point Analysis
Lod>3
900 Ind.
QTL detection – ‘Consensus’ F2 analysis

LGVI
24%
24.3%
63cM
112cM

Mortality
Viral Load

LGV
7.7%
8.6%
98cM

LGVII
9.7%
NS
91cM

LGIX
7.6%
NS
103cM
QTL detection – ‘Consensus’ F2 analysis

V

HA114

Sili29

Sili38

ucdCg150 ucdCg151

ucdCg186

Adrenal_Gland_Protein

ucdCg141

ucdCg173

Glycogen_Phosphorylase

VI

Sodium_Glucoseco-transp.

Amylase_A

Flavin_monooxygenase

Elongation_Factor1

Amylase_B

Serine_Plastin

ucdCg133

Sili4

Cytochrome_P450

ucdCg156

ucdCg156

Sili43

BQ427367

Bcl2

ucdCg197

Laccase

Glutaryl_CoA

Sili50

VII

Sili15

ucdCg149 NADH_Dehydr.

ucdCg210

ucdCg203

ucdCg196

Glutathion_S_transferase

Sili37

IX

Notch3

Tubulin_alpha

ucdCg129

ucdCg171

BMP

ucdCg172

ucdCg189 Vasa-like
QTL detection – F2 Single full-Sib analysis

Family 1
LG V

Family 2
LG VI

Family 3
LG VII

LG IX

Interval Mapping

Var Ratio

Position cm

Interval Mapping

Var Ratio

Position cm

Interval Mapping

Var Ratio

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Interval Mapping

Var Ratio

Position cm
<table>
<thead>
<tr>
<th>Family 1</th>
<th>LGV</th>
<th>LGVI</th>
<th>LGVII</th>
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Fitting QTL effect as a genetic background

To investigate the strength of the QTL

- One QTL a time (e.g. fixing QTL of LGV)
- Several QTL a time (e.g. fixing QTL effect of LG V and IX)
- All QTL at the same time (e.g. fixing QTL effect of the three other LG)

F2 Analysis - LGIV

Lod mortality: 4.666
Lod viral load: 5.939

F2 Analysis in LGIV with all other QTL effect fixed

Lod mortality: 0.771
Lod viral load: 0.595
« False positive QTL »
Fitting one of the phenotype as a cofactor

To investigate the variance in trait

- Fixing Viral load phenotype as cofactor

Not all the variance in the mortality trait is explained by the viral trait.
Sum up of the Results

• First Linkage map that includes type I markers (SNP) in *C. gigas*

• QTL detection was Successful !!!

  - 5 QTLs regions (LG V, VI, VII and IX)
  - robustness of the QTL
  - (too?) large part of the Variance (49%)
  - Differential QTL segregation among F2 families

- However, relatively large 95% IC in QTLs position (≈40cM)
- The variance explained appears over inflated
  1. Selective Genotyping
  2. Bias introduced by segregation distorsion
1. Investigate the genetic architecture of the two traits

2. Add more markers to get a fine scale map
Perspectives ...

Genetic Basis (h²) of Resistance to SM (Degrémont, 2005)

Linkage Mapping & QTL detection

Biological Phenomenon of Mortality

Differentially Expressed Genes (R vs S) (Fleury, 2008, On Line)
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