

A first linkage map for the European flat oyster, *Ostrea edulis* towards the identification of *Bonamia* resistance QTLs

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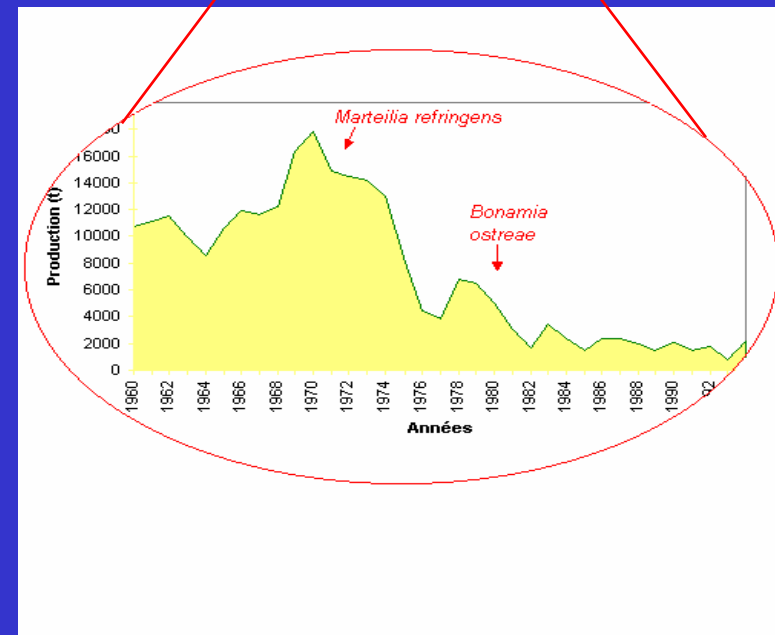
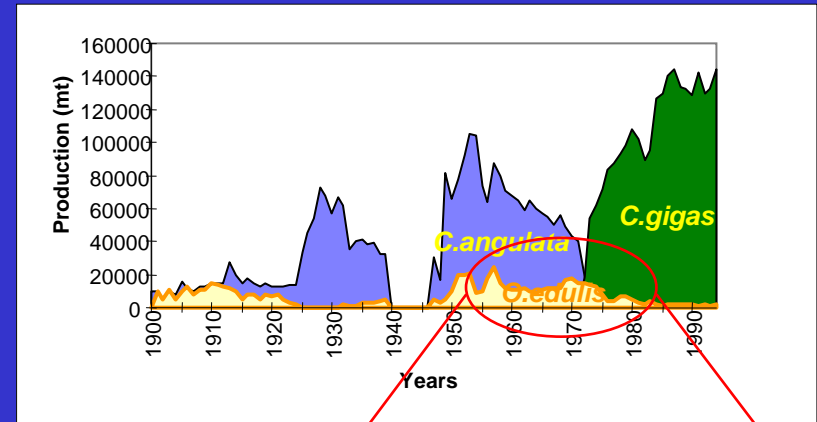
Context of the study

- Aquaculture production of *O. edulis*

Drastic reduction of the French production, from 20,000 tons in 1950s and 1960s to 2,000 tons nowadays

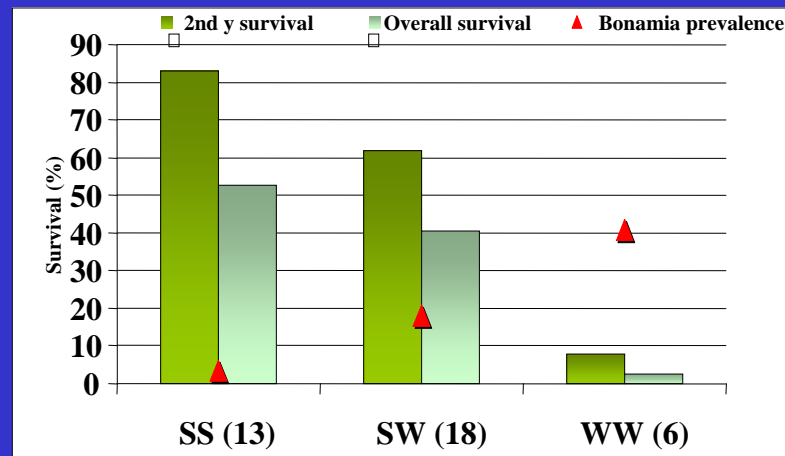
This is due to two parasitic diseases:

- Marteiliosis
- Bonamiosis



Context of the study

- Selective breeding for resistance to *Bonamia*
- First stage: production of improved oyster strains by individual selection in mass spawning populations (Naciri-Graven et al., 1998, Culloty et al., 2001)
 - Low effective population sizes (Launey et al. 2001)
 - Inbreeding depression (Naciri-Graven et al., 2000)
- Second stage: within- FS family selection




Higher survival and lower *Bonamia* prevalence (▲) of selected *versus* hybrid and wild families (Bédier et al., 2001)

In this context, the establishment of a genetic linkage map will provide a foundation for the identification of Quantitative Trait Loci for resistance (or tolerance) to *Bonamia*, with the ultimate objective to implement marker-assisted selection (MAS) in *O. edulis*.

1. Linkage mapping in *Ostrea edulis*

Mapping family

- 2003: Cross between a wild oyster and an oyster from a fifth-generation inbred line
-  F1-L family
- 2004: F2 Cross between 2 F1-L oysters

F0

L002-55 x W102

F1-L

23-31 x 23-32

OE.F2.04.63

F2 progeny (n=92)

Three-generation pedigree



Flat oysters are hermaphrodites and females brood their larvae for about 10 days

 23-31 = "P1"

 23-32 = "P2"

Markers and genotyping

- 20 available microsatellites
- 60 AFLPs primer pairs : 296 segregating markers



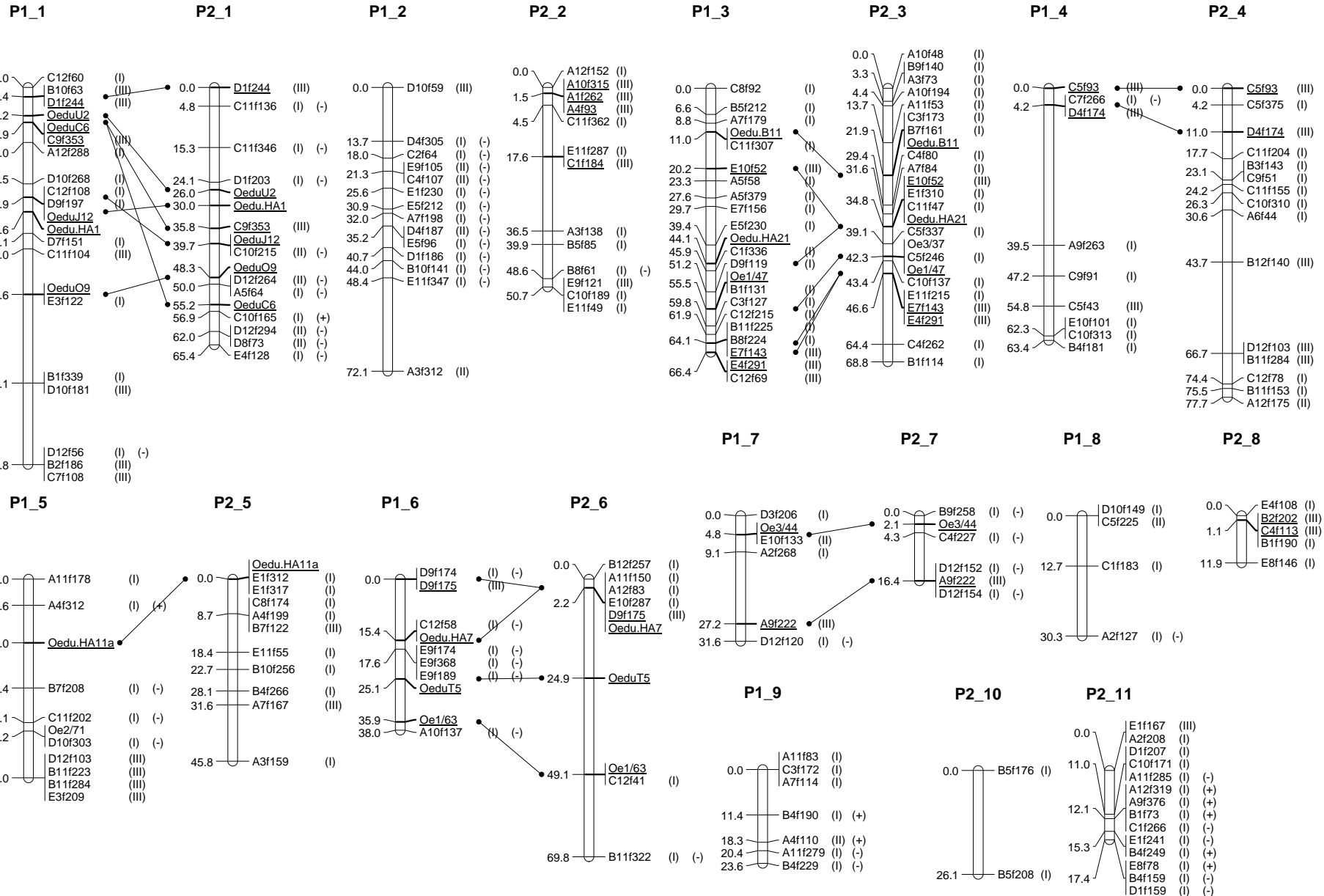
1:1 type

3:1 type



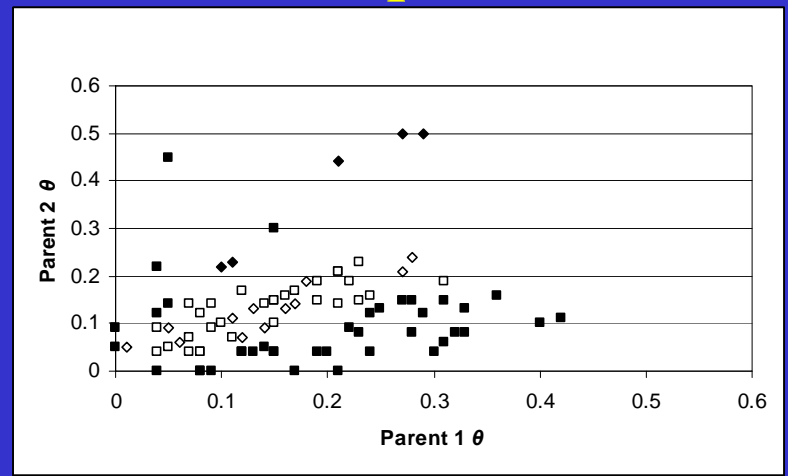
In total, **251** markers used for linkage analysis
(16 microsatellites, 235 AFLPs)

Linkage analysis: CriMap (Green et al., 1990)



- High genetic load in *O. edulis*
 - segregation distortion (32.8% of overall markers): mostly homozygote deficiencies
 - distorted markers tended to cluster on some of the linkage groups
- First genetic linkage map in a flat oyster species, with a relatively good genome coverage
 - P1: 471 cM, average spacing 5 cM, genome coverage 82.4%
 - P2: 450 cM, average spacing 4 cM, genome coverage 84.2%
 - Number of linkage groups matches haploid number of chromosomes (2n=20 in *O. edulis*, P1 with 9 LGs, P2 with 10 LGs)
- Some differences in recombination frequencies between parents

- | | |
|-------------|--------------|
| ◇ Msat/msat | } $p > 0.05$ |
| □ Msat/AFLP | |
| ◆ Msat/msat | } $p < 0.05$ |
| ■ Msat/AFLP | |



A first-generation genetic linkage map of the European flat oyster *Ostrea edulis* (L.) based on AFLP and microsatellite markers

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ANIMAL GENETICS
Immunogenetics, Molecular Genetics and Functional Genomics

2. QTL mapping :
Bonamia challenge experiment

Mapping family

- 2004 : cross between a wild oyster and a selected oyster



F1-S family

- 2005 : Cross between 2 F1-S oysters

F0

98AC703-29 x W31

F1-S

410-7 x 410-8

F2

F2 progeny

Three-generation pedigree

Bonamia challenge



12 raceways (150 l/h).

100 tested oysters / raceway



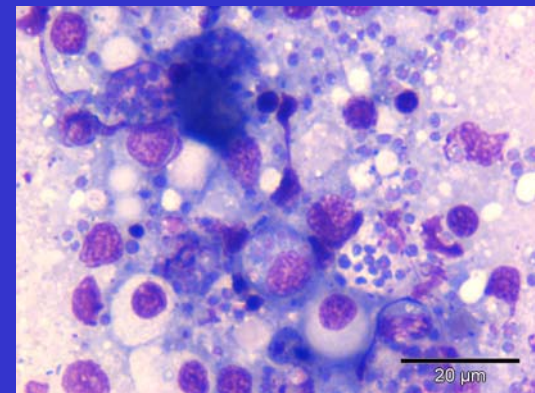
Cohabitation between wild over-infected oysters and our F2 family



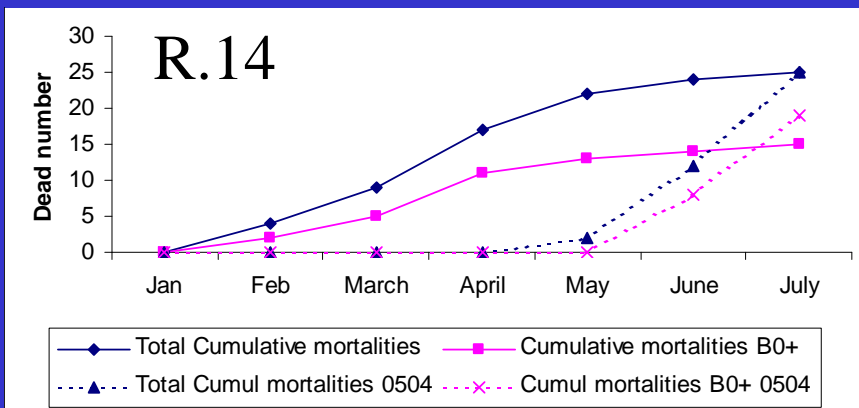
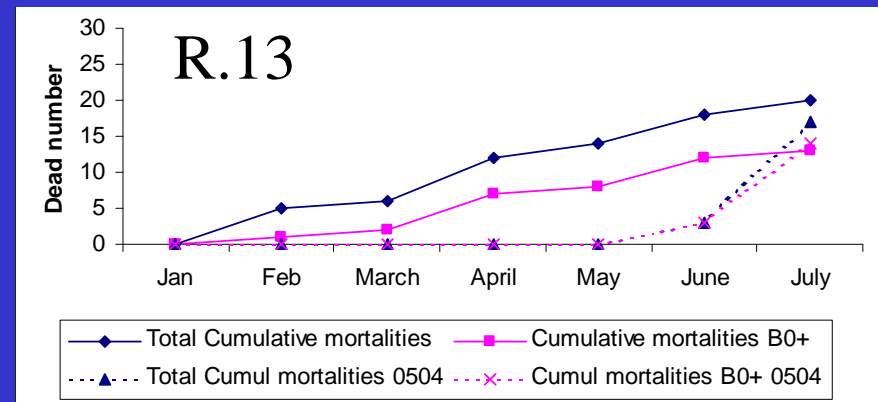
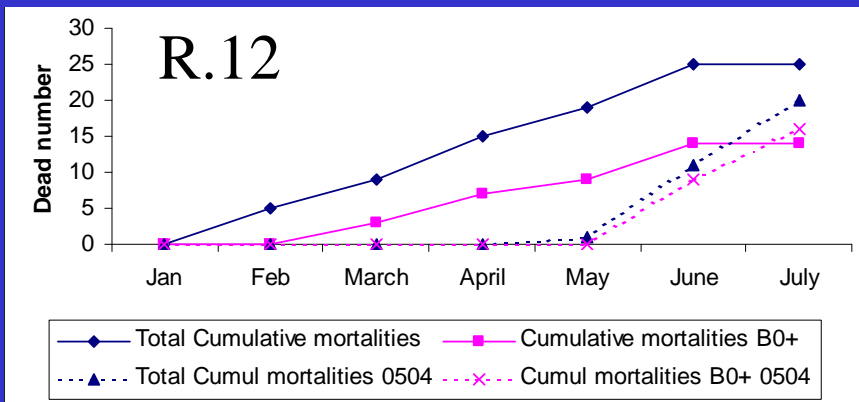
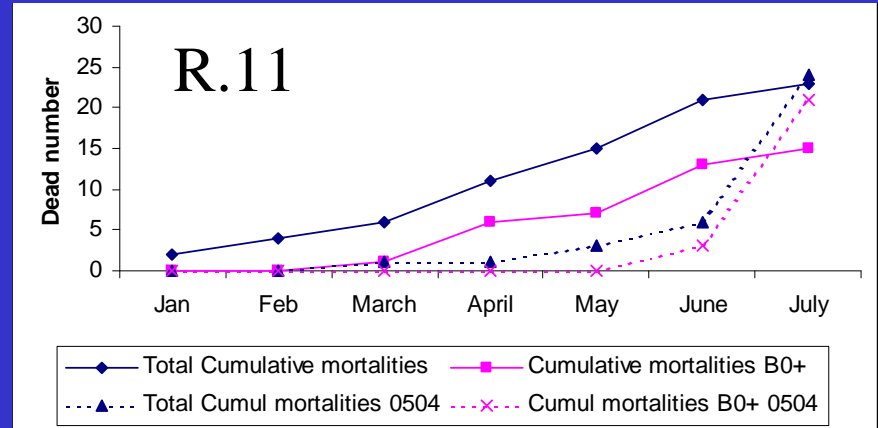
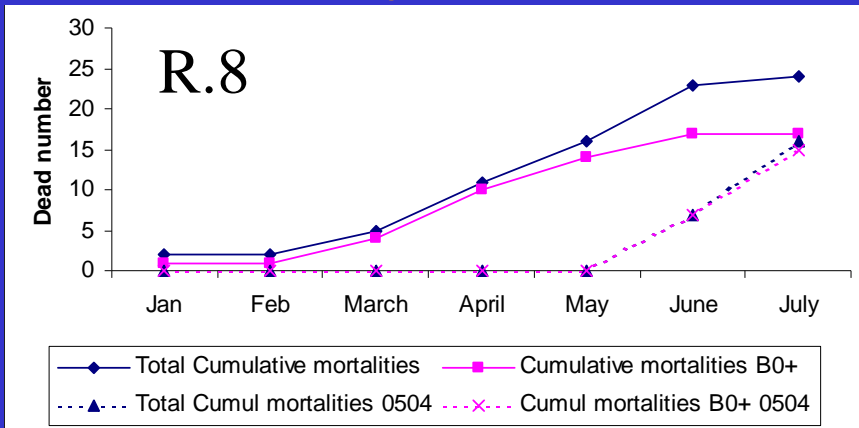
Overinfected wild oysters

F2 family (= tested oysters)

- Mortality checked daily.
- Heart smears on dead oysters to search for *Bonamia*



Mortality (Jan-July 2006)



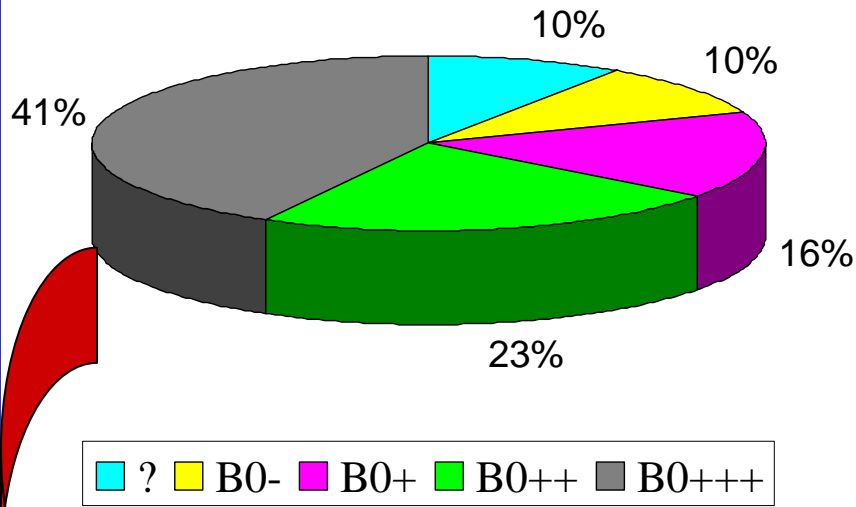
No significant raceway effect,
so data were pooled between
raceways

550 F2 oysters

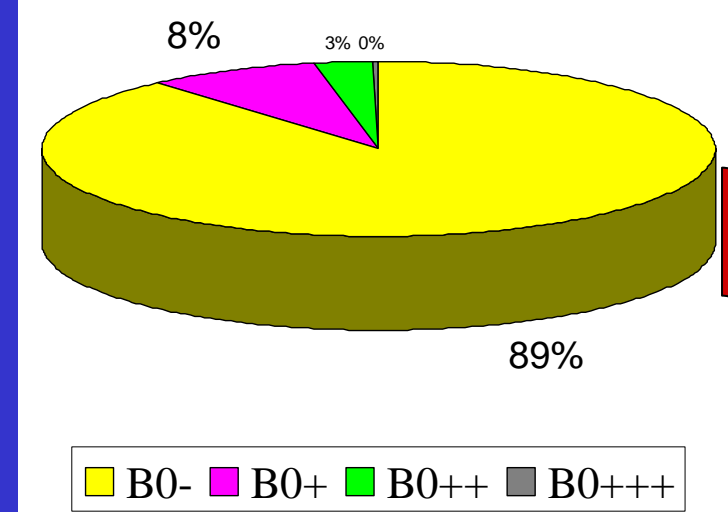
105 dead oysters

444 surviving oysters killed in August 2006 for heart smear

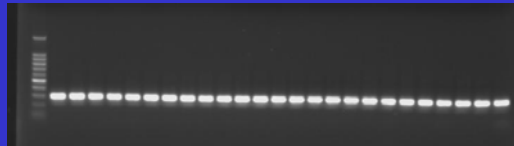
Dead oysters



Surviving oysters

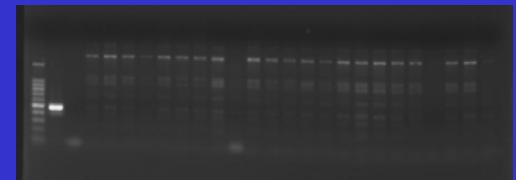


46 dead oysters B0+++



genotyping
microsats and
AFLPs

46 surviving oysters B0-

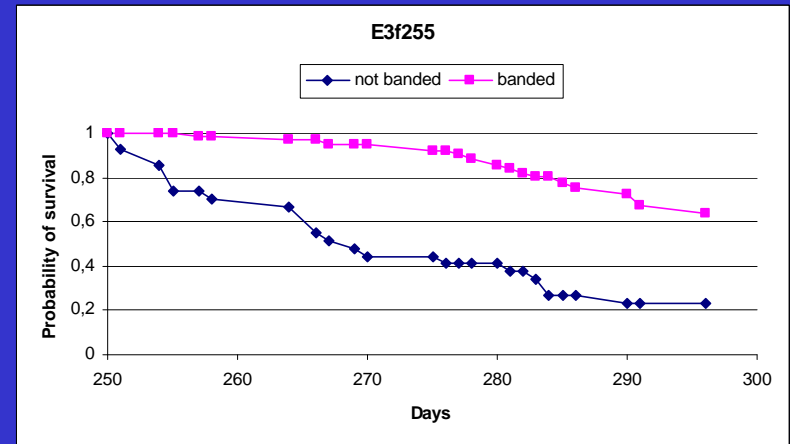
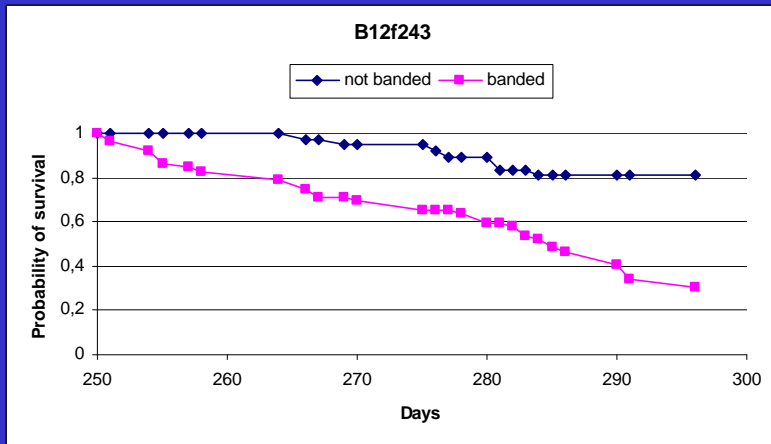
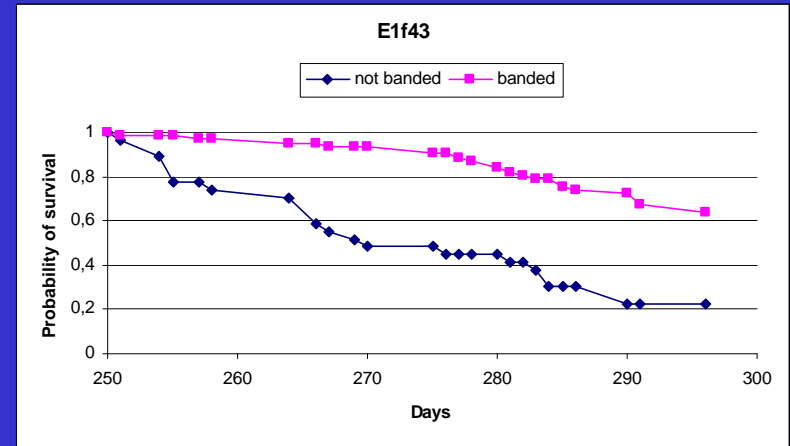
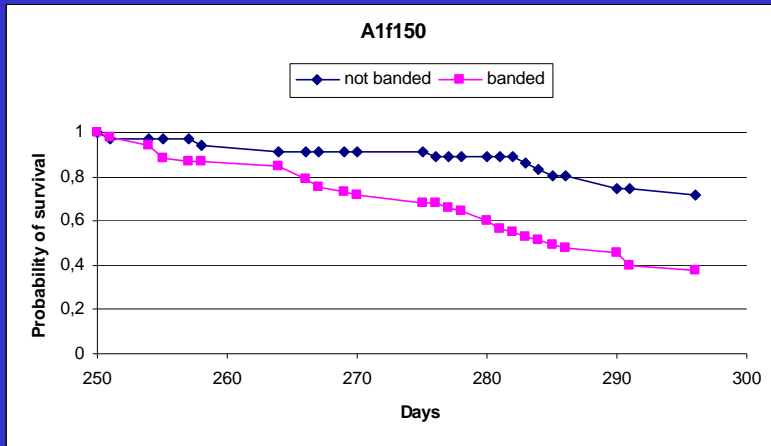


Multistage testing strategy (Moen et al., 2004)

309 AFLPs

6 probable susceptibility markers

9 probable resistance markers



QTL mapping

CriMap (Green et al., 1990) + QTL Express (Seaton et al., 2002)

309 AFLPs + 20 microsatellites

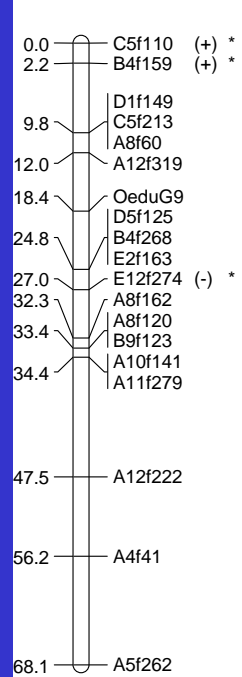
Parent 410_7

- 127 markers mapped
- 3 R + 1 S alleles mapped
- 10 linkage groups
- total length: 465.6 cM
- genome coverage: 84.1%

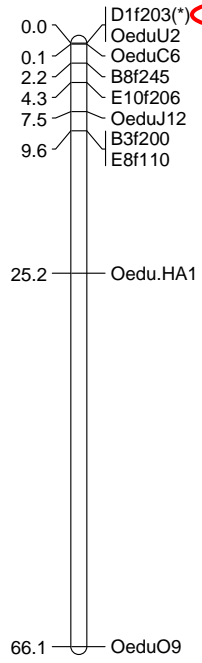
Parent 410_8

- 98 markers mapped
- 8 R + 4 S alleles mapped
- 10 linkage groups
- total length: 386.7 cM
- genome coverage: 69.4%

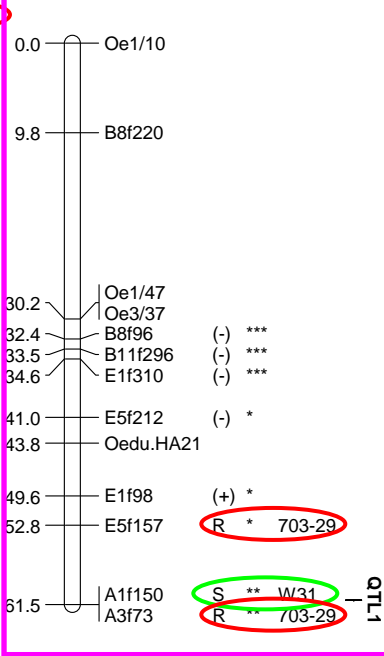
G1_410_8



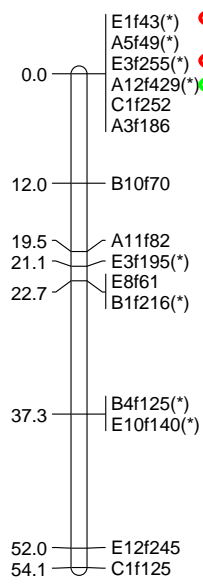
G2_410_8



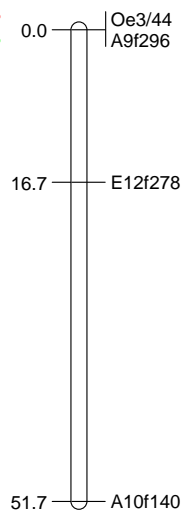
G3_410_8



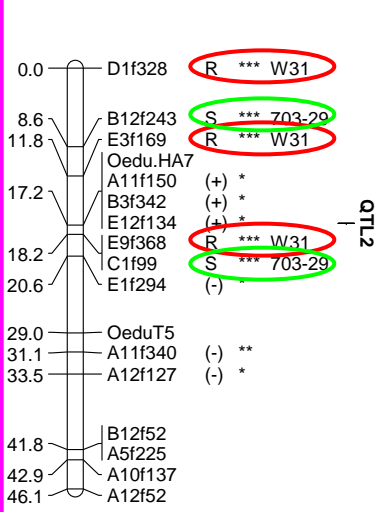
G4_410_8



G5_410_8



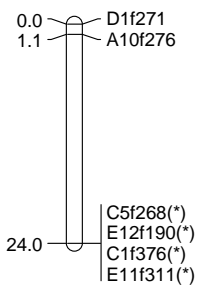
G6_410_8



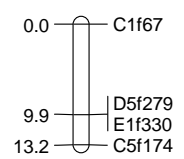
G7_410_8



G8_410_8





G9_410_8

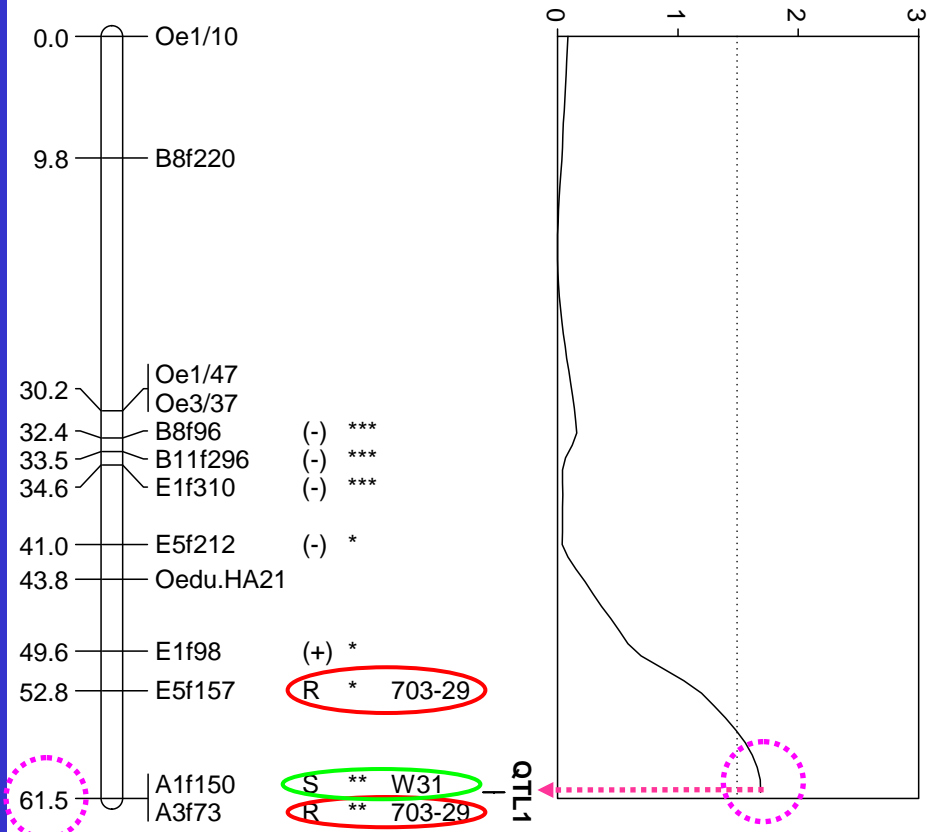


G10_410_8



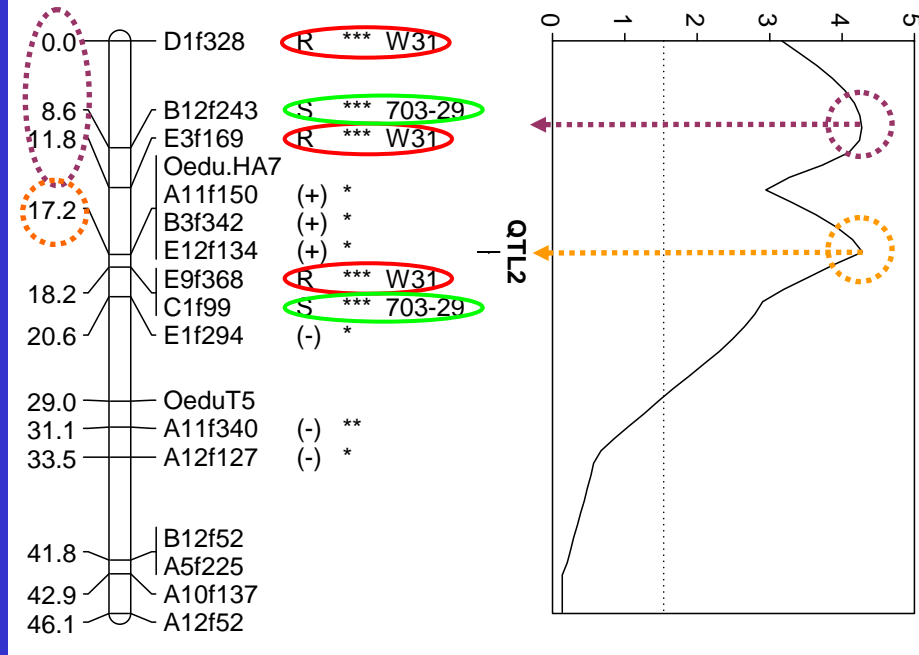
R "resistant allele" 
 S "susceptible allele" 

G3_410_8



61 cM, $p < 0.05$

G6_410_8



17 cM, $p < 0.01$

Coming work

- Development of new microsatellites (ecogenics.ch)
- Increase the density of markers on the linkage map, allowing a more precise localization of QTLs
- *MAS versus non-MAS* breeding experiment
- Improvement of reproductive and hatchery-nursery technology (coll. R. Robert)
- Study of the impact of hatchery-propagated selected oysters on the genetic variability of native stocks

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