

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

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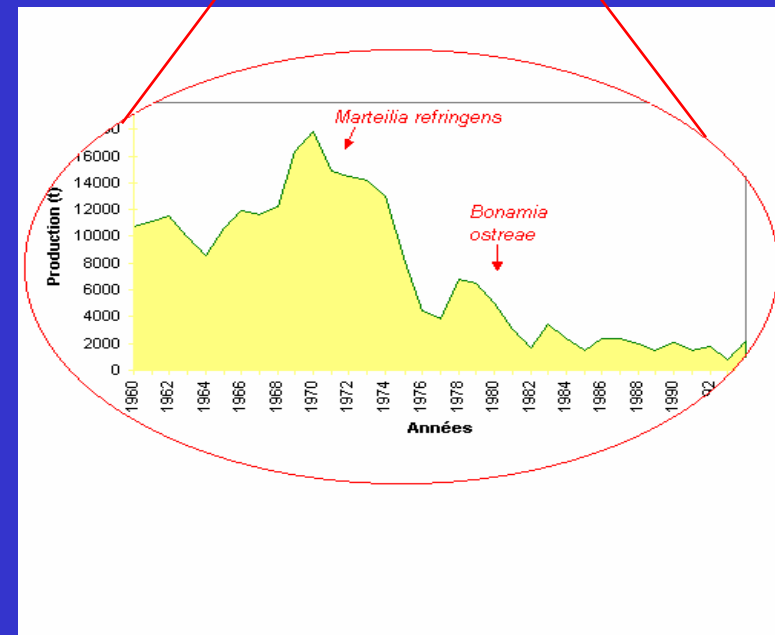
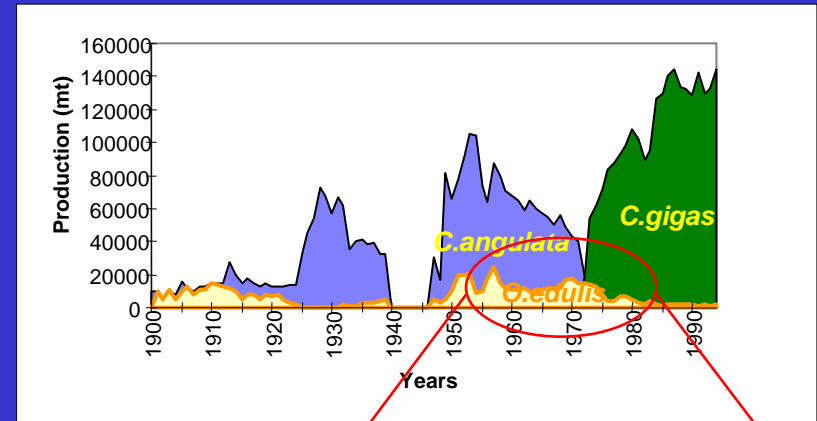
Context of the study

- Aquaculture production of *O. edulis*

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

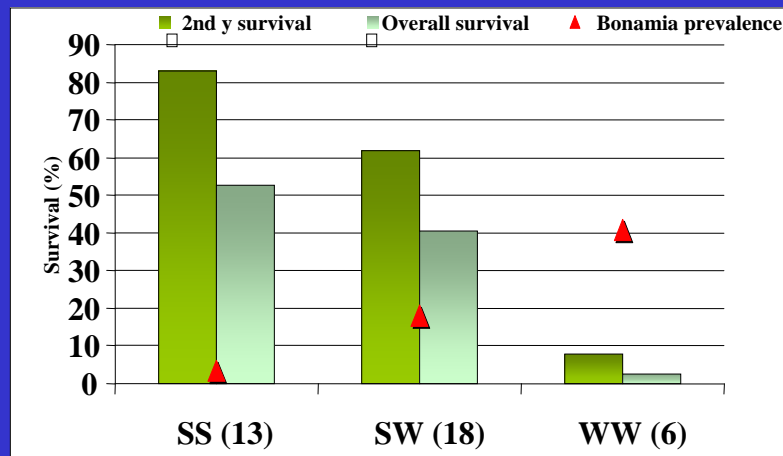
This is due to two parasitic diseases:

- Marteiliasis
- Bonamiasis



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 in *O. edulis*.

Linkage mapping in *Ostrea edulis*

Mapping family

- 2003: Cross between a wild oyster and a selected oyster (fifth-generation inbred line OELL2000 derived from the selected strain S89) → 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



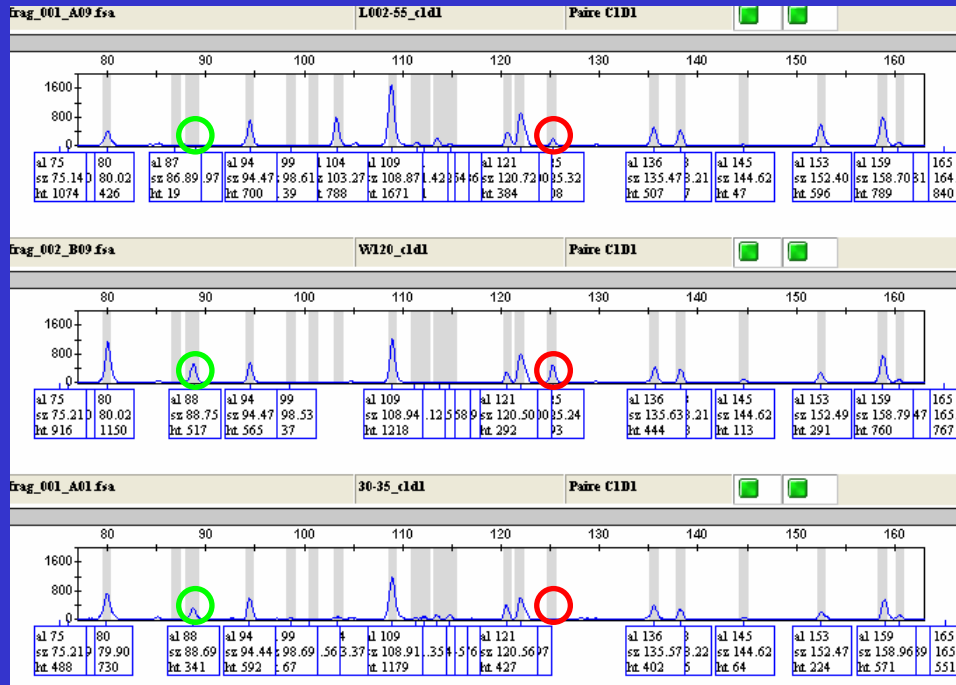
Flat oysters are hermaphrodites and females brood their larvae for about 10 days

→ 23-31 = "P1"

→ 23-32 = "P2"

Markers and genotyping

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



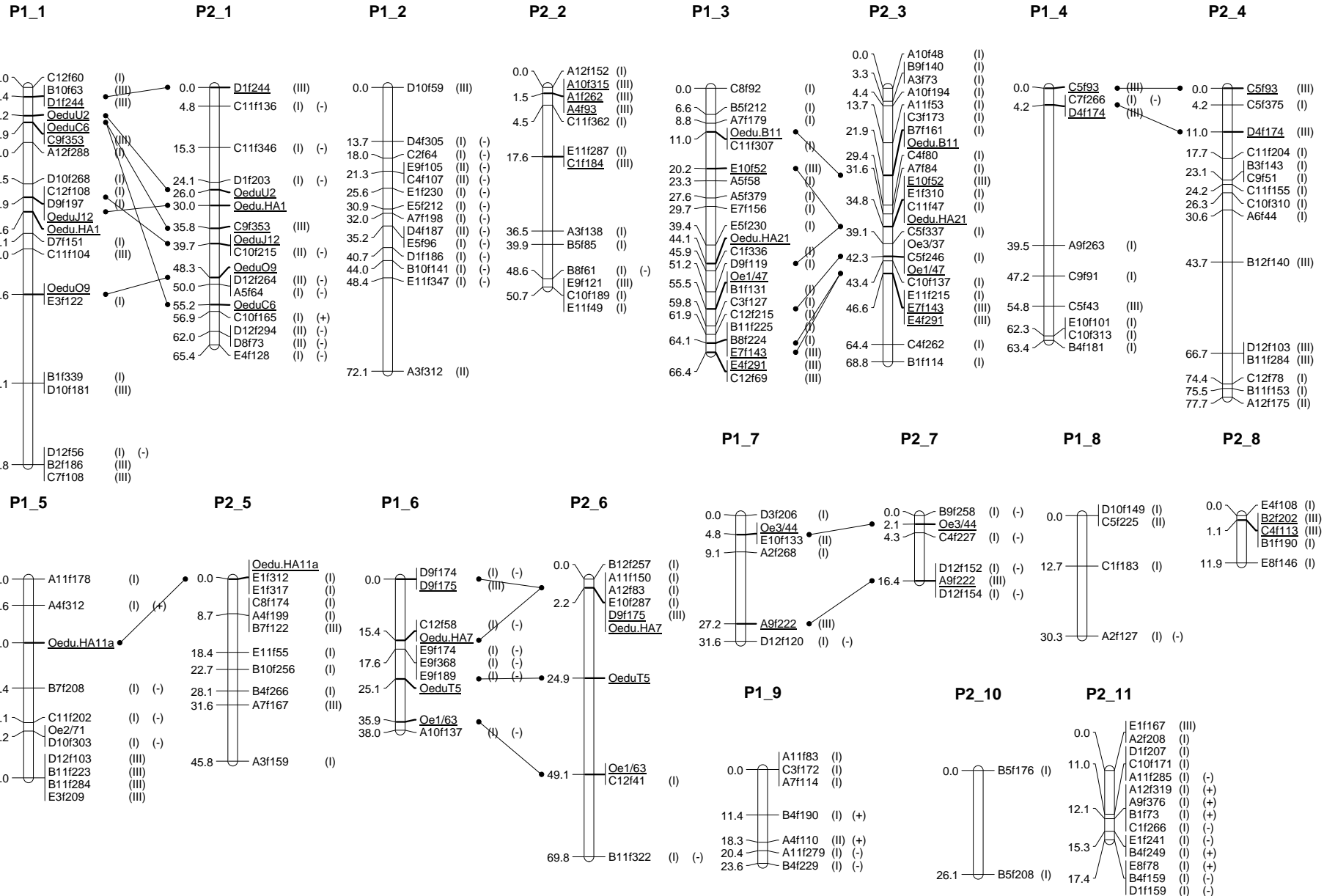
1:1 type

3:1 type



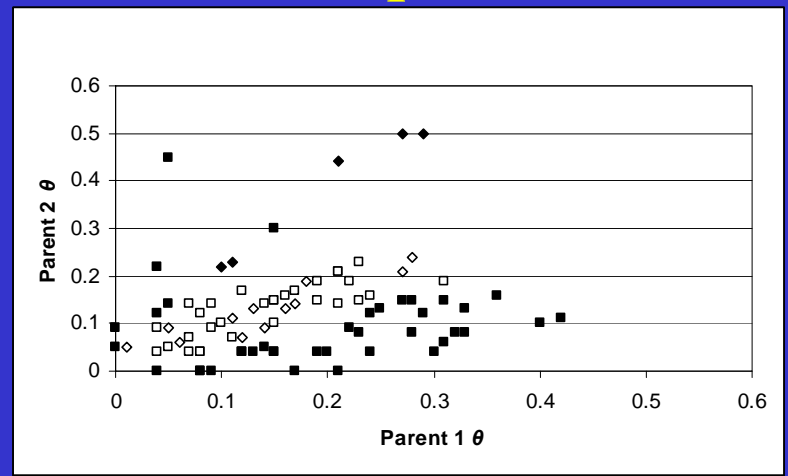
In total, 251 markers used for linkage analysis
(16 microsatellites, 235 AFLPs)

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 | |



Coming work

- Mapping more codominant markers
 - Portability/transferability of the map
 - Increasing accuracy of the map, to find more homology groups (more anchor loci)
 - Investigating more deeply eventual recombination differences between the two parents (more pairwise comparisons)

Towards 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



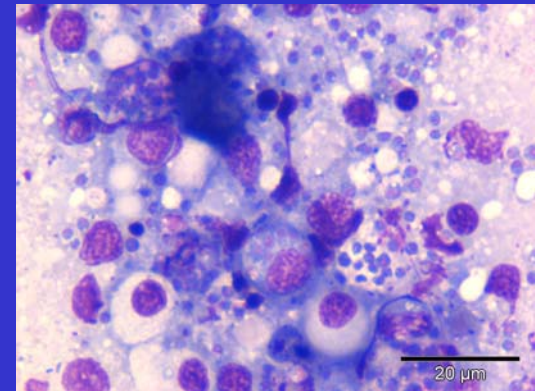
Cohabitation between wild over-infected oysters
and our F2 family



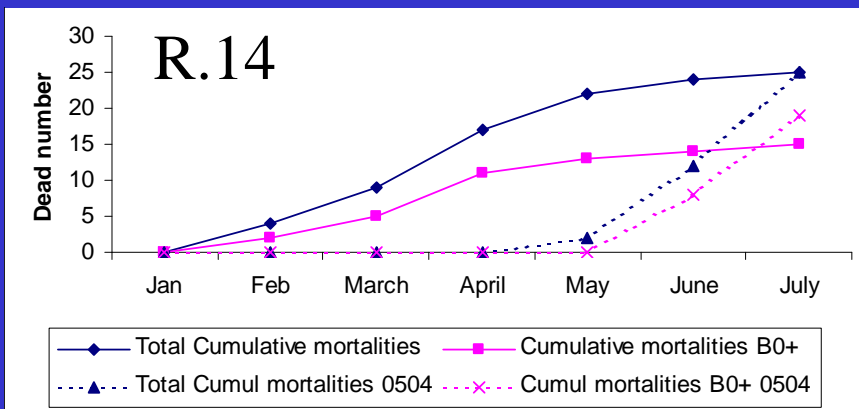
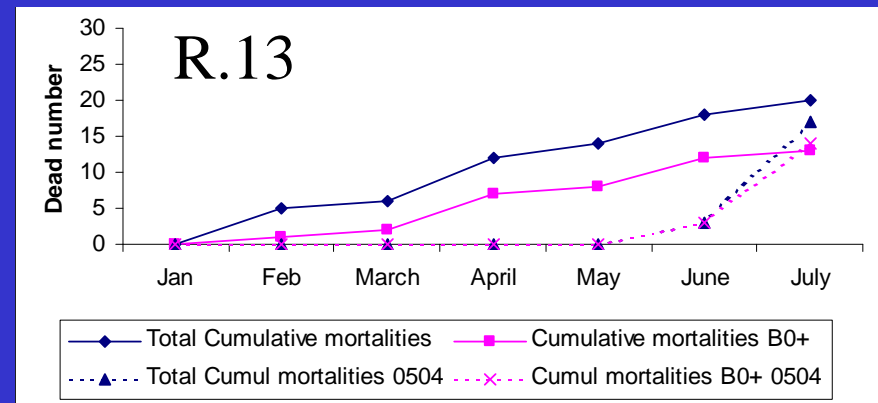
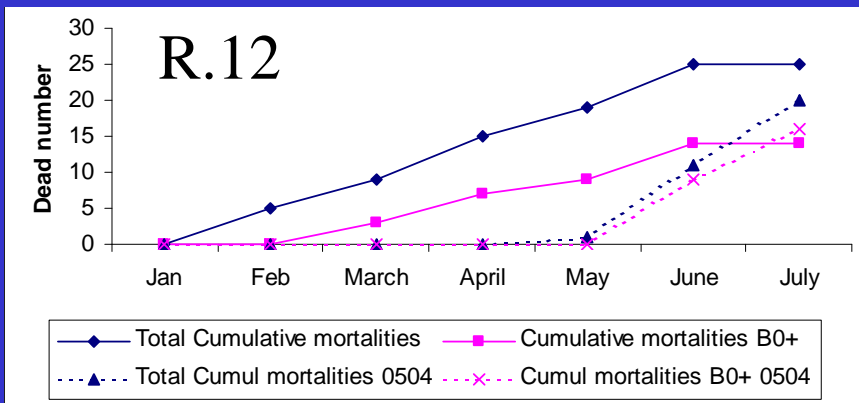
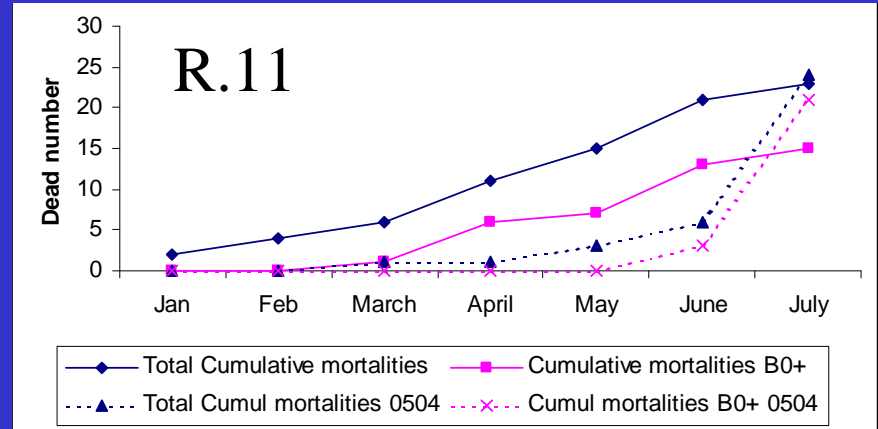
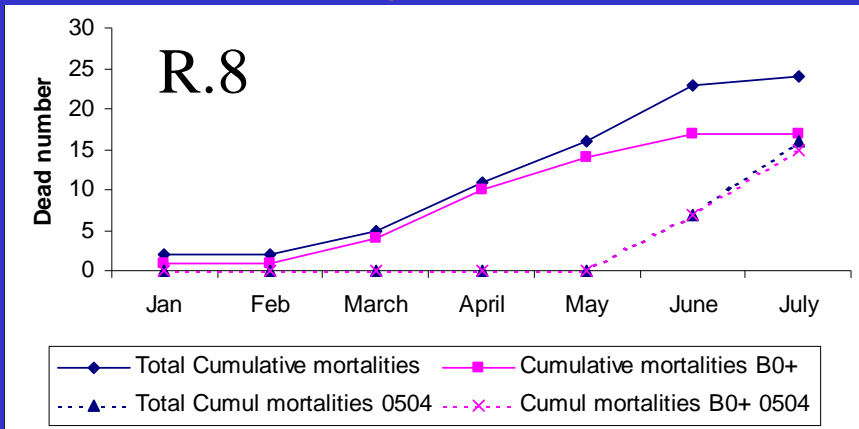
Overinfected wild oysters

F2 family (= tested oysters)

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



Mortality (Jan-July 2006)



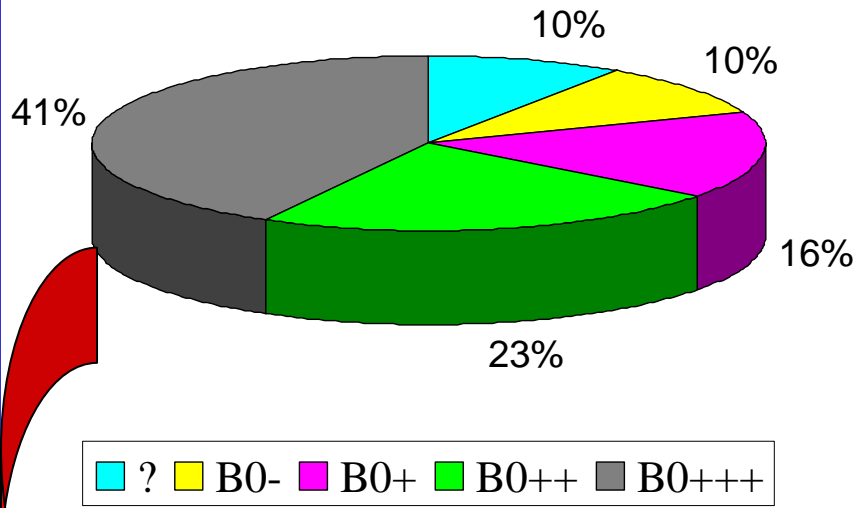
No significant raceway effect,
so data were pooled between
raceways

550 F2 oysters

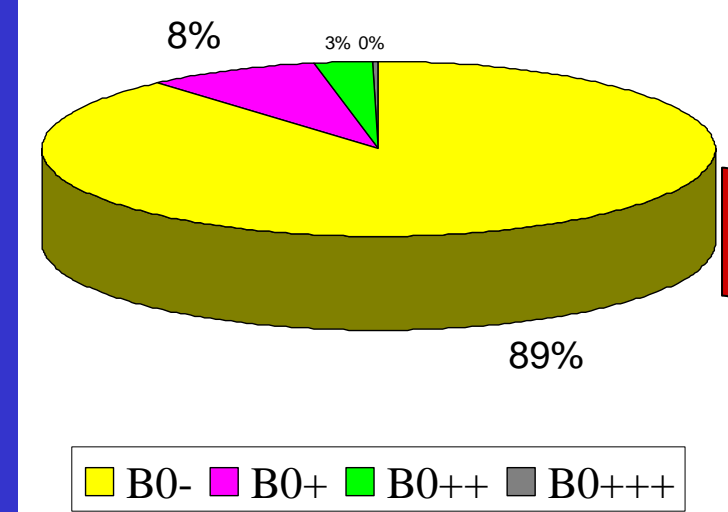
105 dead oysters

444 surviving oysters killed in August 2006 for heart smear

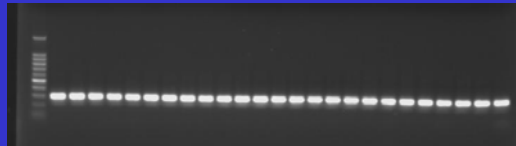
Dead oysters



Surviving oysters

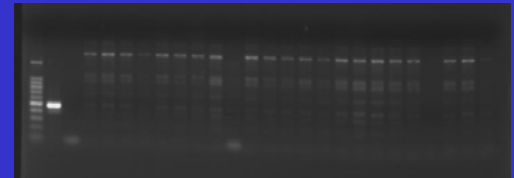


46 dead oysters B0+++



Scoring msats and AFLPs

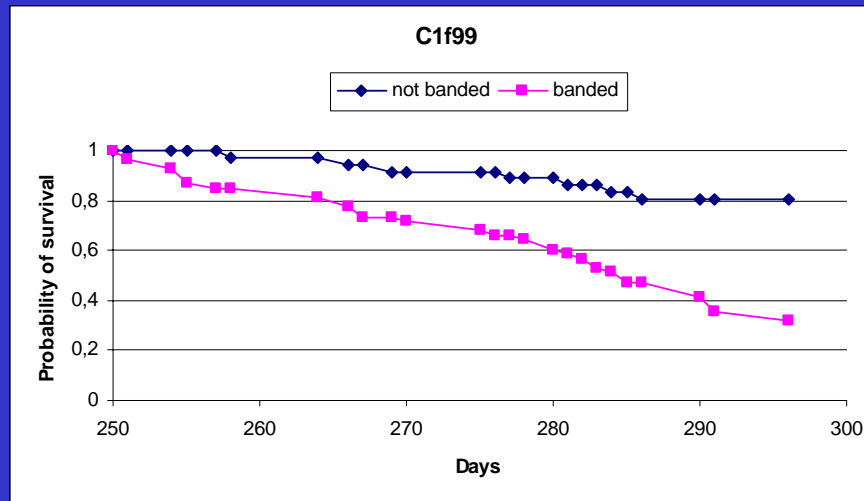
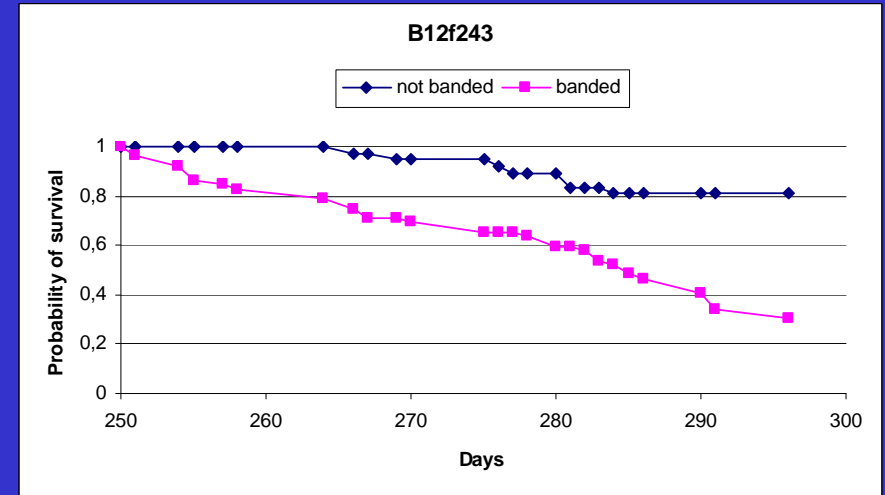
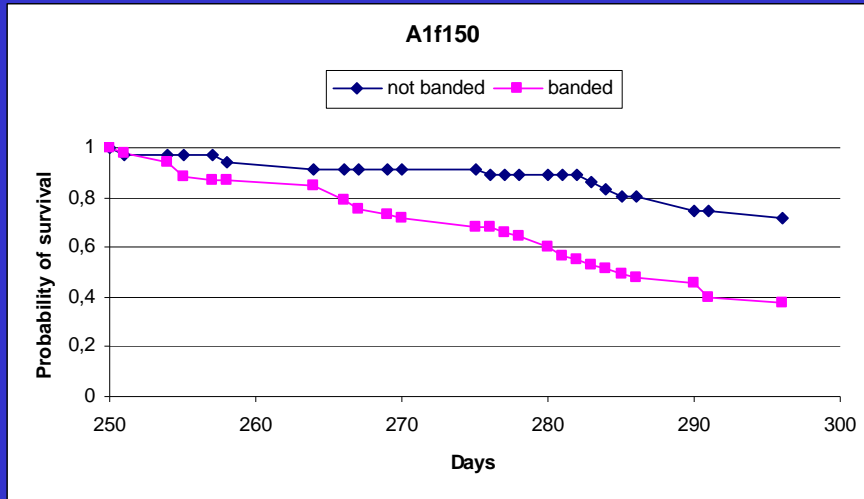
46 surviving oysters B0-



Multistage testing strategy

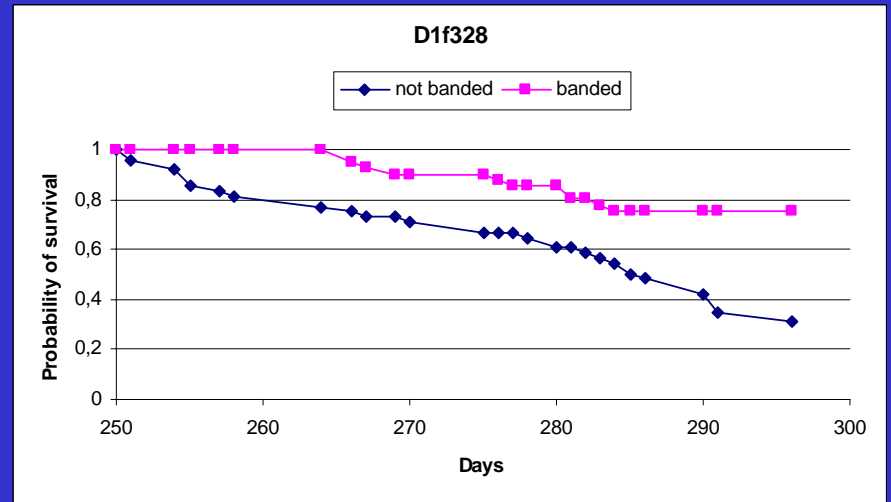
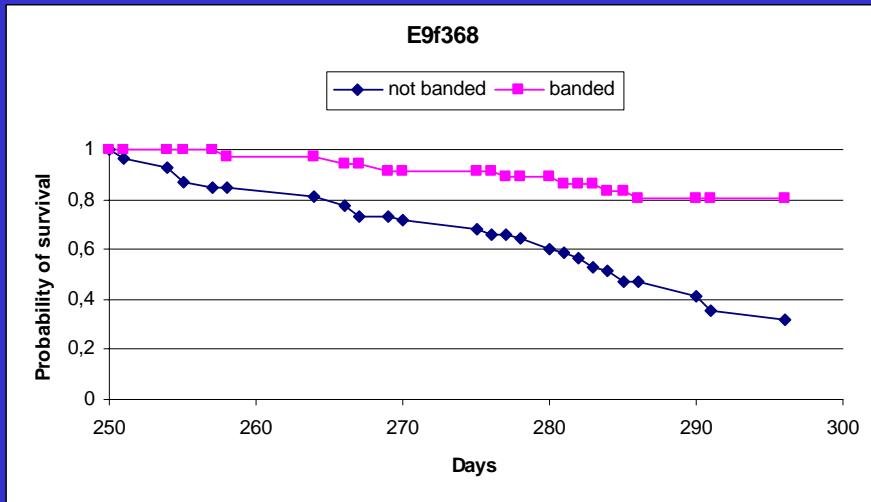
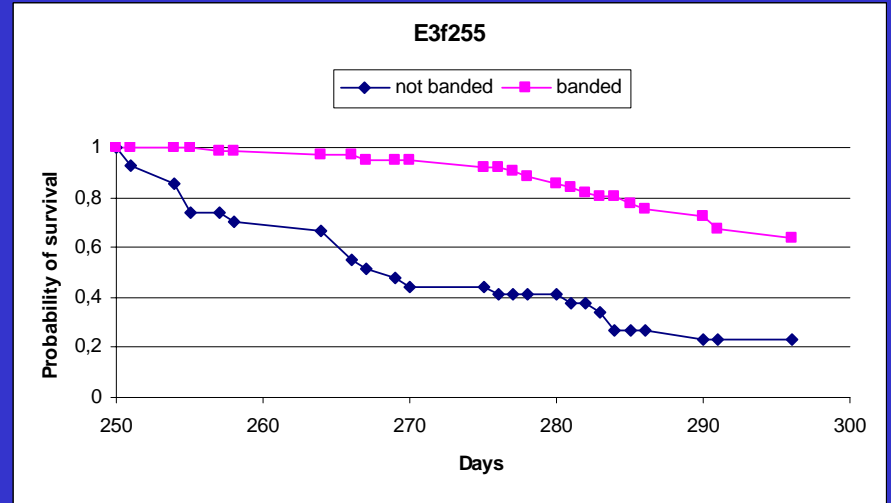
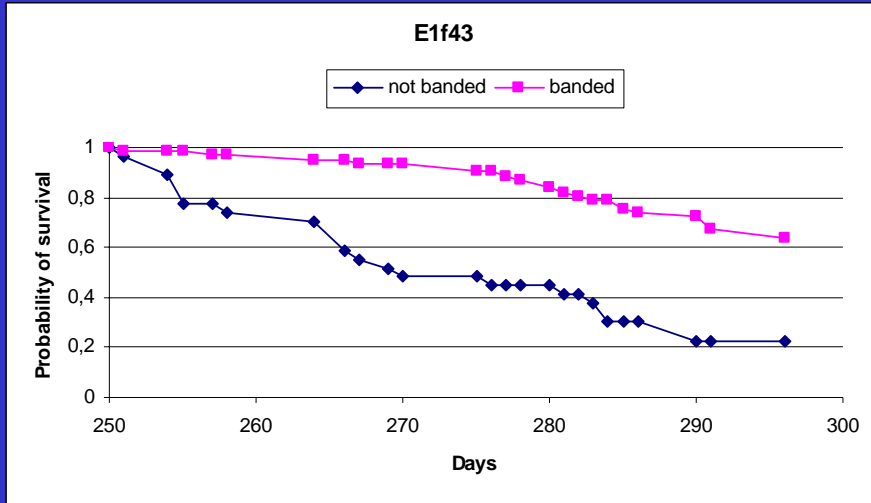
(Moen et al., 2004)

6 probable susceptibility alleles



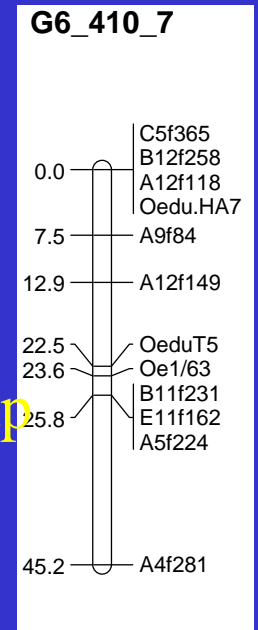
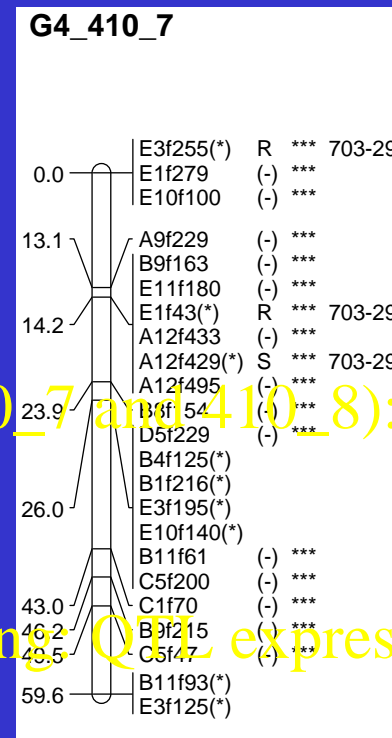
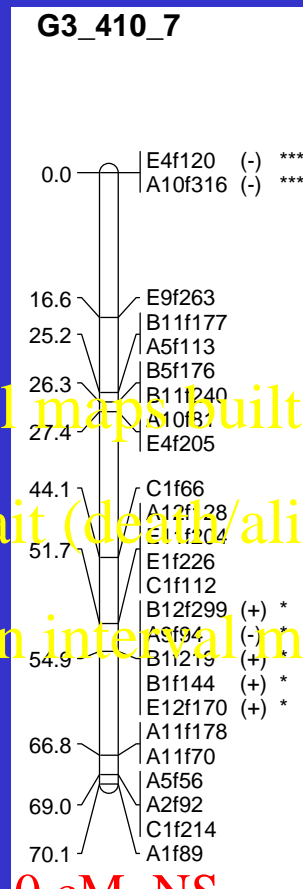
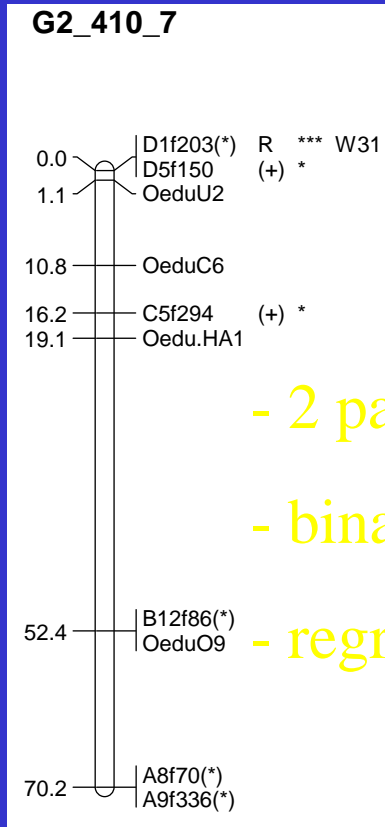
(Kaplan-Meier survival curves)

9 probable resistance alleles



(Kaplan-Meier survival curves)

QTL mapping: CriMap software + QTL express



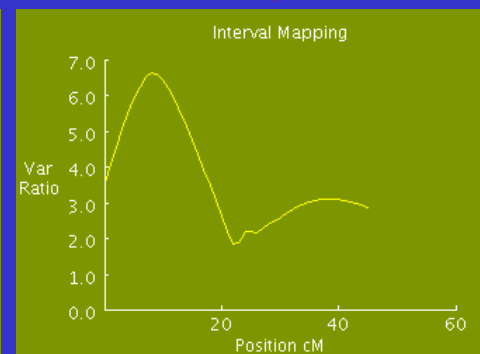
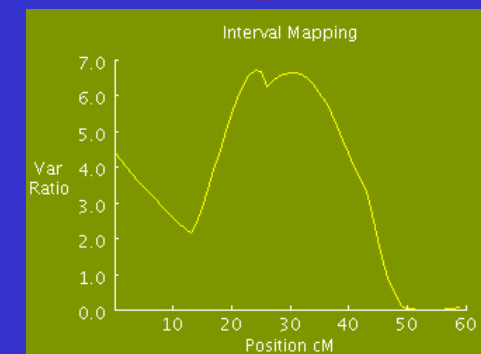
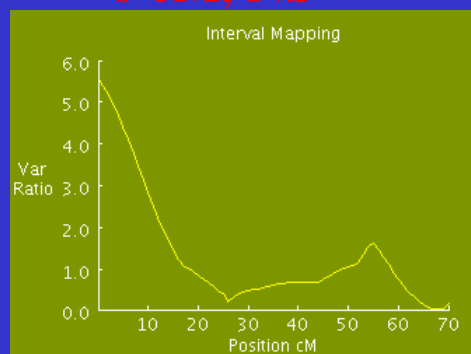
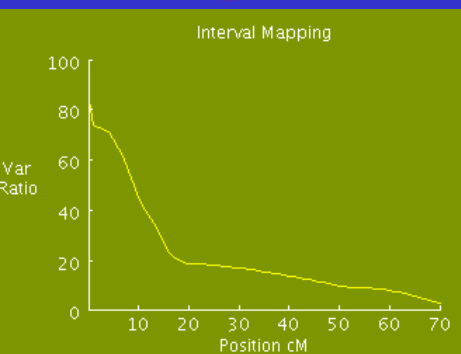
- 2 parental maps built (410_7 and 410_8): CriMap
 - binary trait (death/alive)
 - regression interval mapping QTL express

0 cM, p 0.01

0 cM, NS

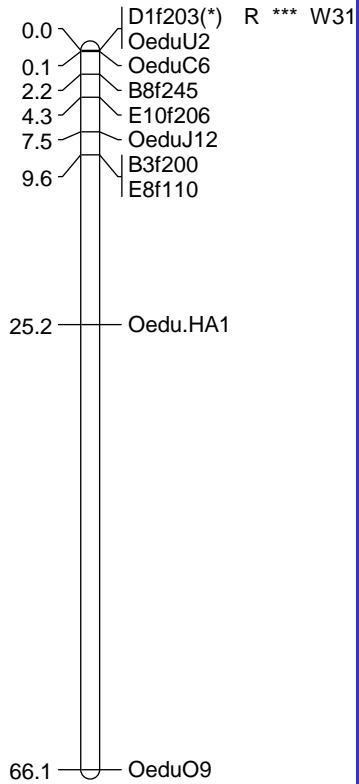
24 cM, p 0.05

8 cM, p 0.05

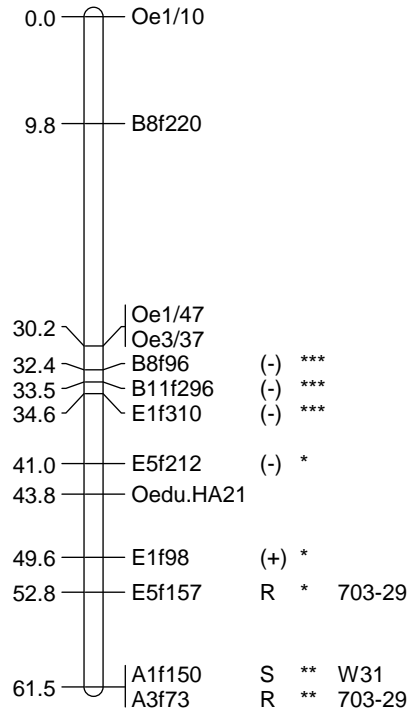


QTL mapping: CriMap software + QTL express

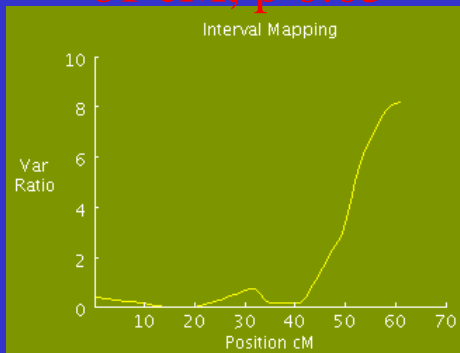
G2_410_8



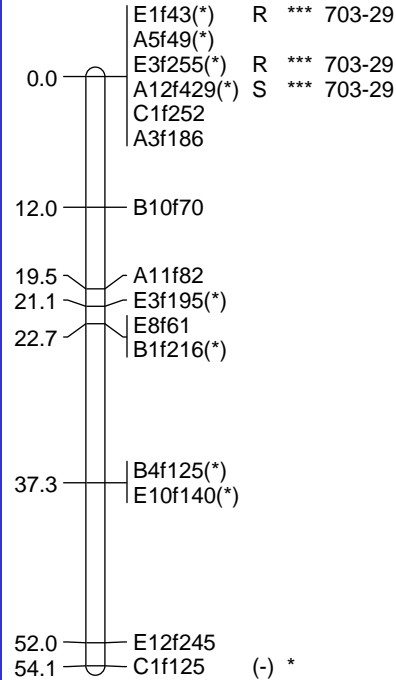
G3_410_8



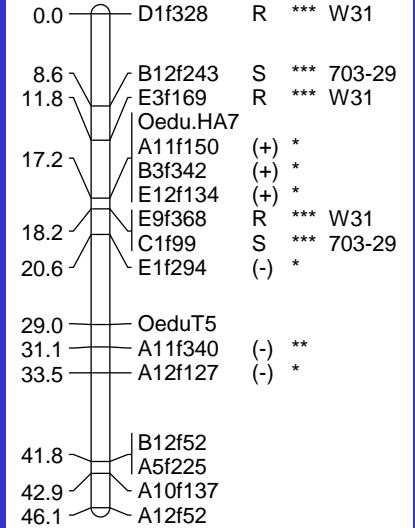
61 cM, p 0.05



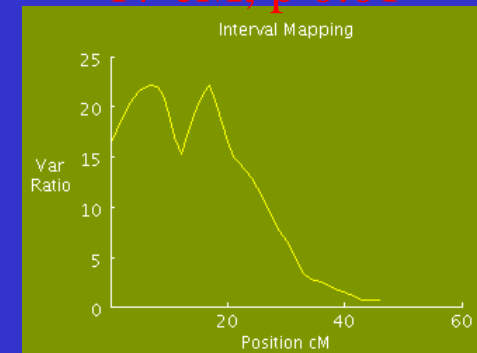
G4_410_8



G6_410_8



17 cM, p 0.01



- Good concordance between multistage strategy, genetic mapping, QTL mapping
- Several QTLs found (resistance, susceptibility), of relatively good effect

Results from fitting a single QTL for the parent 410_7. Threshold p 0.05 and threshold p 0.01 correspond to chromosome-wide significance thresholds at $\alpha=5\%$ and 1% after performing 1000 permutations.

Linkage group	F ratios			Location (cM)	Paternal estimate (standard error)
	Threshold p 0.05	Threshold p 0.01	Observed		
G2_410_7	6.78	9.87	83.65	0	0.3574 (0.039)
G3_410_7	7.97	12.14	5.57	0	-0.134 (0.057)
G4_410_7	6.63	11.72	6.73	24	-0.1601 (0.062)
G6_410_7	6.65	10.65	6.65	8	-0.1329 (0.051)

Results from fitting a single QTL for the parent 410_8. Threshold p 0.05 and threshold p 0.01 correspond to chromosome-wide significance thresholds at $\alpha=5\%$ and 1% after performing 1000 permutations.

Linkage group	F ratios			Location (cM)	Maternal estimate (standard error)
	Threshold p 0.05	Threshold p 0.01	Observed		
G3_410_8	7.22	12.08	8.17	61	-0.149 (0.052)
G6_410_8	7.96	12.14	22.19	17	0.229 (0.049)

Coming work

- Adding codominant markers (microsatellites, SNPs): anchor loci to integrate both maps
- Adding candidate genes from SSH (Benjamin Morga)

Acknowledgements

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