QTL mapping for resistance to bonamiosis in the European flat oyster *Ostrea edulis*

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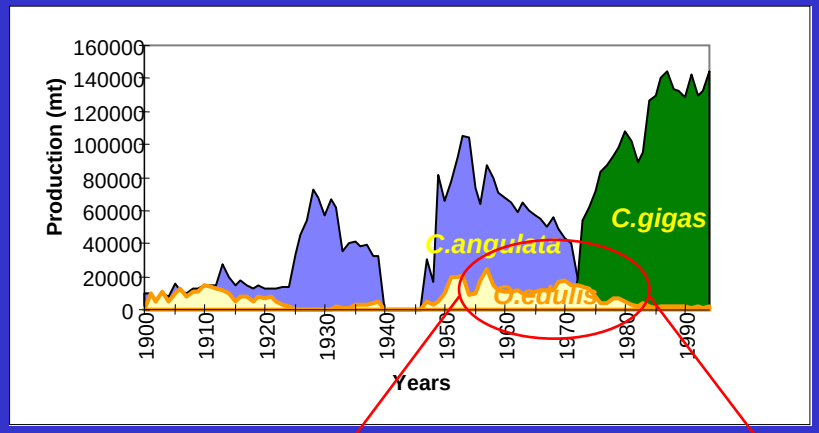
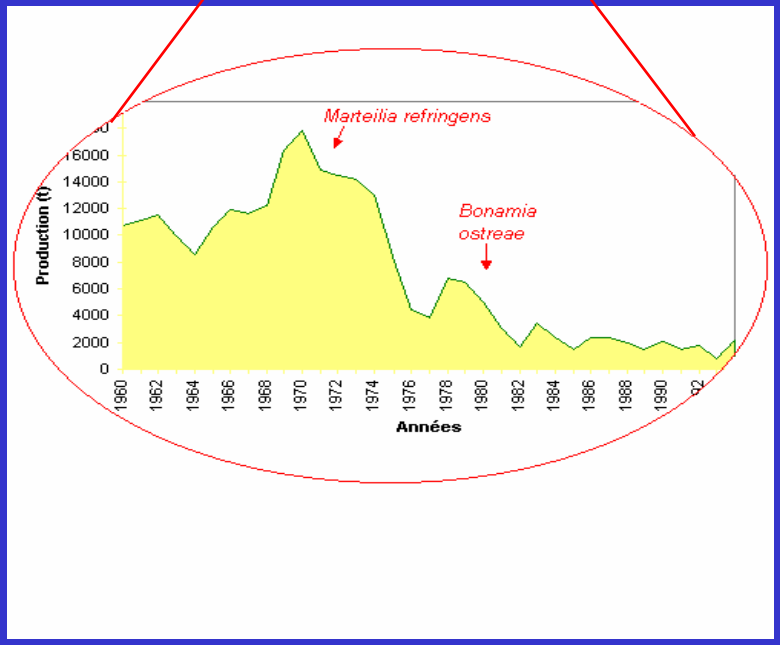
Context of the study

- 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





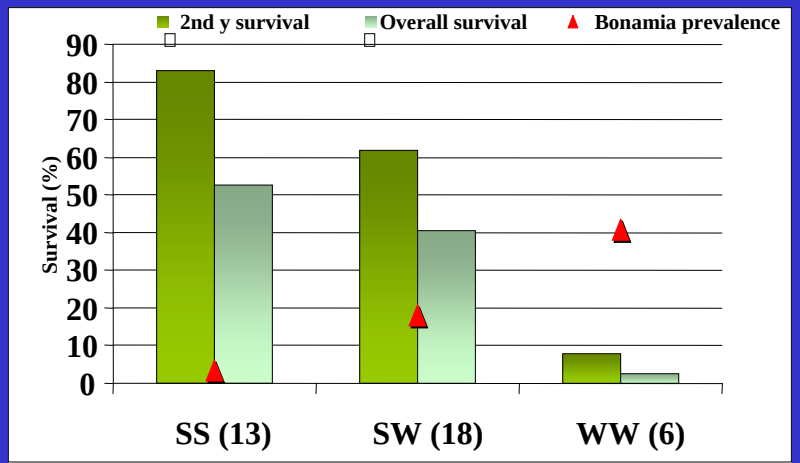
Context of the study

- Selective breeding for resistance to *Bonamia* (France and Ireland)

➢ 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)

Aim of the study

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*.

Mapping family

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

→ F1-S family

- 2005 : Cross between 2 F1-S oysters

F0

F1-S

F2

98AC703-29 x W31

↓

410-7 x 410-8

↓

F2 progeny

→

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

Three-generation pedigree

Bonamia challenge

5 raceways (150 l/h)

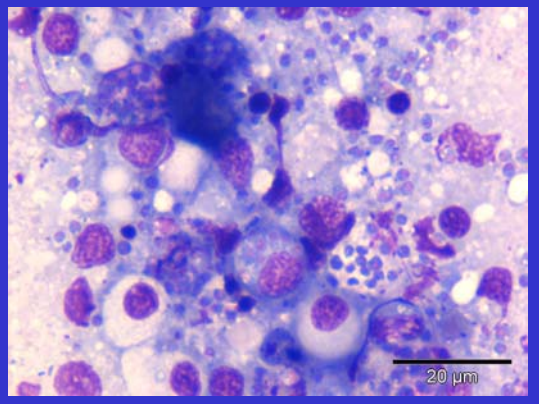
110 tested oysters / raceway

Cohabitation between artificially infected wild oysters and our F2 family

Artificially infected wild oysters

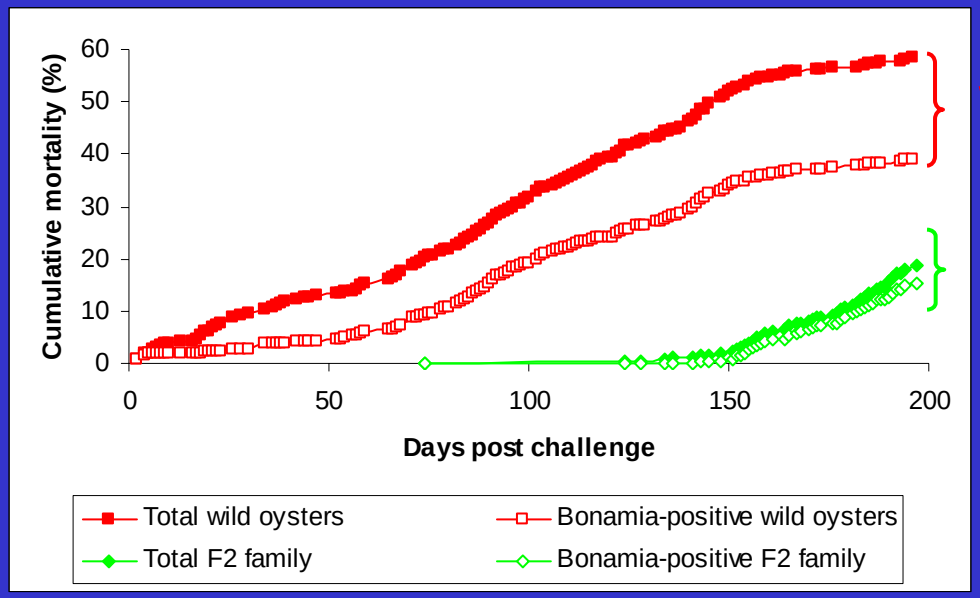
F2 family (= tested oysters)

- 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



Wild oysters

Tested oysters

- 4 months before first infections
- 20% mortality in the tested oysters after 6 months
- scoring of 2 extreme phenotypes (microsatellites + AFLPs):
  - 46 dead oysters (heavily infected)
  - 46 surviving oysters (no parasite detected)

} Heart smear and PCR

Markers and genotyping

- 20 available microsatellites
- 34 AFLPs primer pairs : 309 segregating markers in F2

Parent 410_7

Parent 410_8

Progeny F2

ABI 3100-*Avant*

1:1 type (n=201)

3:1 type (n=108)

98AC703-29 x W31

↓

410-7 x 410-8

↓

F2 progeny

Several steps for the search of QTLs

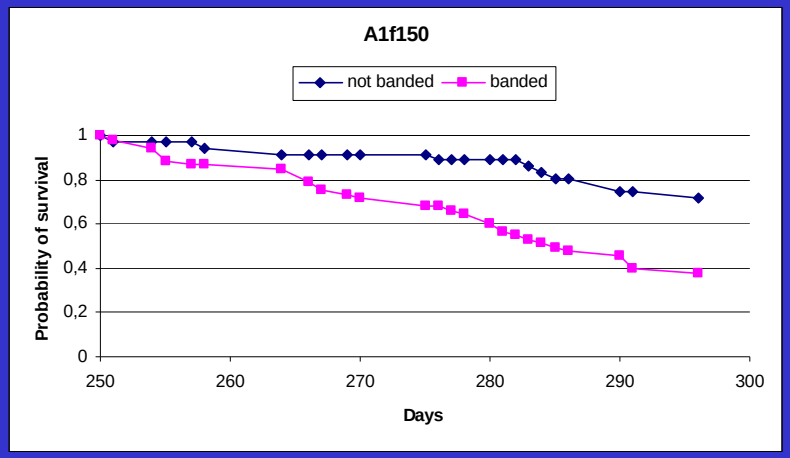
- Identification of potential markers (test marker by marker): multistage testing strategy (Moen et al. 2004)
  - Transmission disequilibrium test (TDT)
  - Mendelian Segregation Test (MST)
  - Survival analysis
- Location of those markers in the genome (genetic mapping)
- QTL mapping approach (uses genetic map and the survival data)

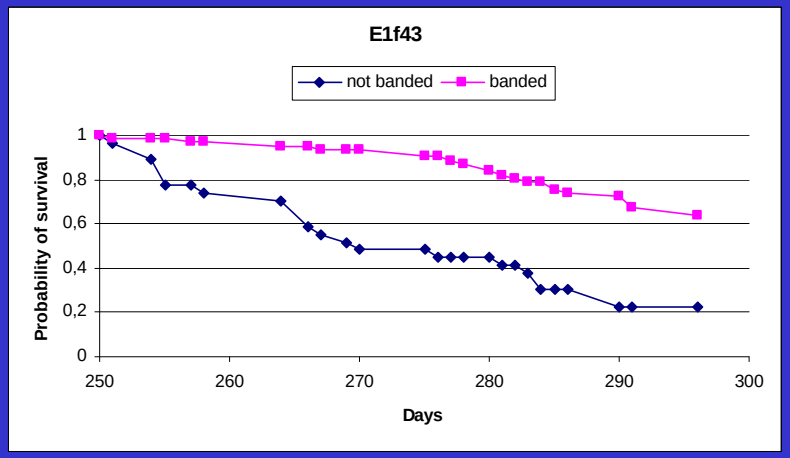
Multistage testing strategy (Moen et al., 2004)

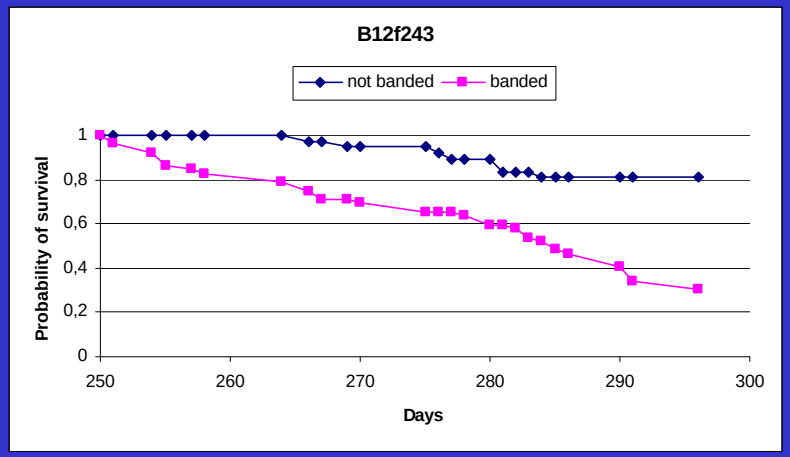
309 AFLPs

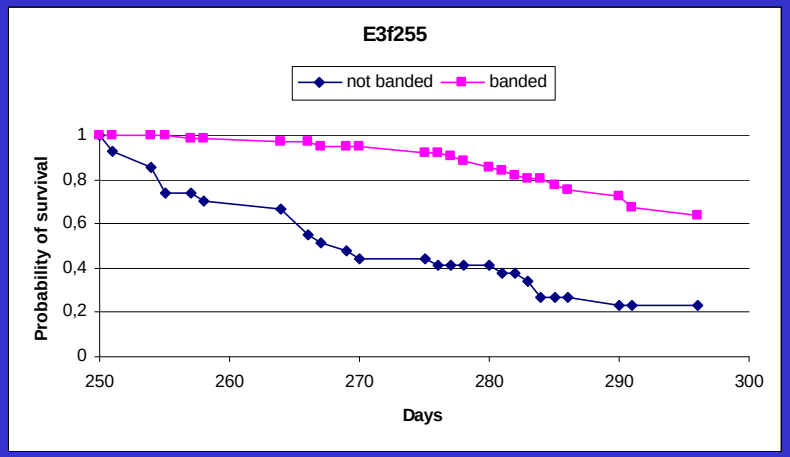
6 probable susceptibility markers

9 probable resistance markers









Several steps for the search of QTLs

- Identification of potential markers (test marker by marker)
- Location of those markers in the genome (genetic mapping)
- QTL mapping approach (uses genetic map and the survival data)

Genetic mapping: CriMap (Green et al., 1990)

309 AFLPs

20 microsatellites

Parent 410_7

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

Parent 410_8

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

Several steps for the search of QTLs

- Identification of potential markers (test marker by marker)

- Location of those markers in the genome (genetic mapping)

- QTL mapping approach (uses genetic map and the survival data)

QTL mapping: CriMap software + QTL express

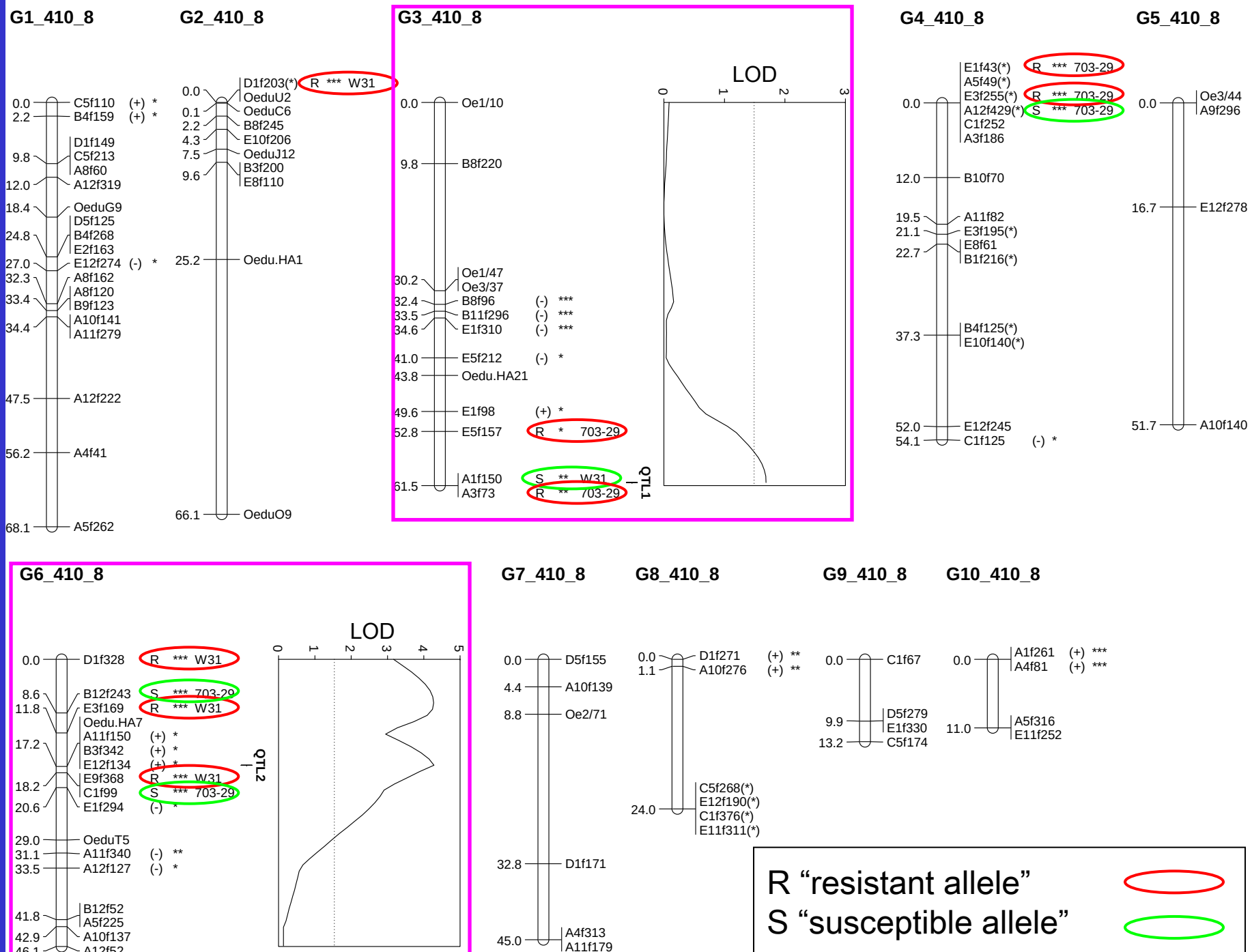
- 2 parental maps built (410_7 and 410_8): CriMap
- binary trait (death/alive)
- regression interval mapping (one-QTL model): QTL express
- large single full-sib family analysis module

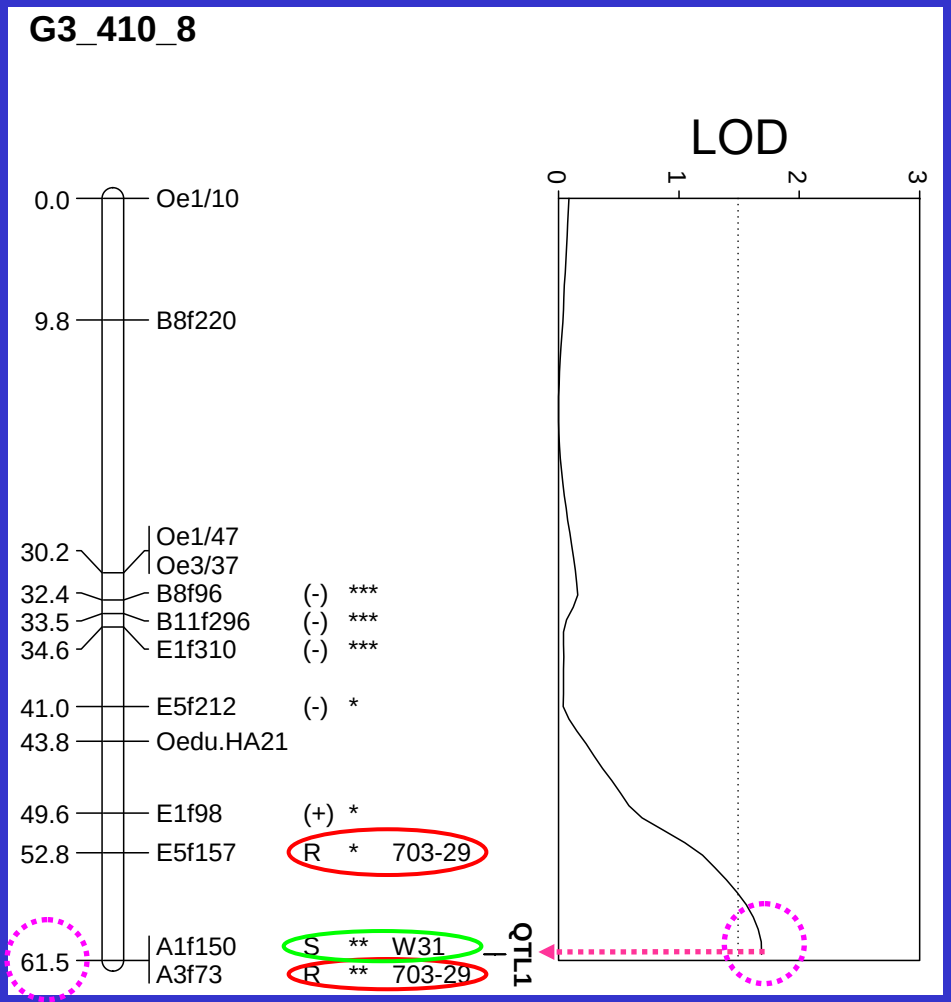
Parent 410_7

- QTL1: 0 cM ($p<0.01$), G2_410_7
- QTL2: 24 cM ($p<0.05$), G4_410_7
- QTL3: 8 cM ($p<0.05$), G6_410_7

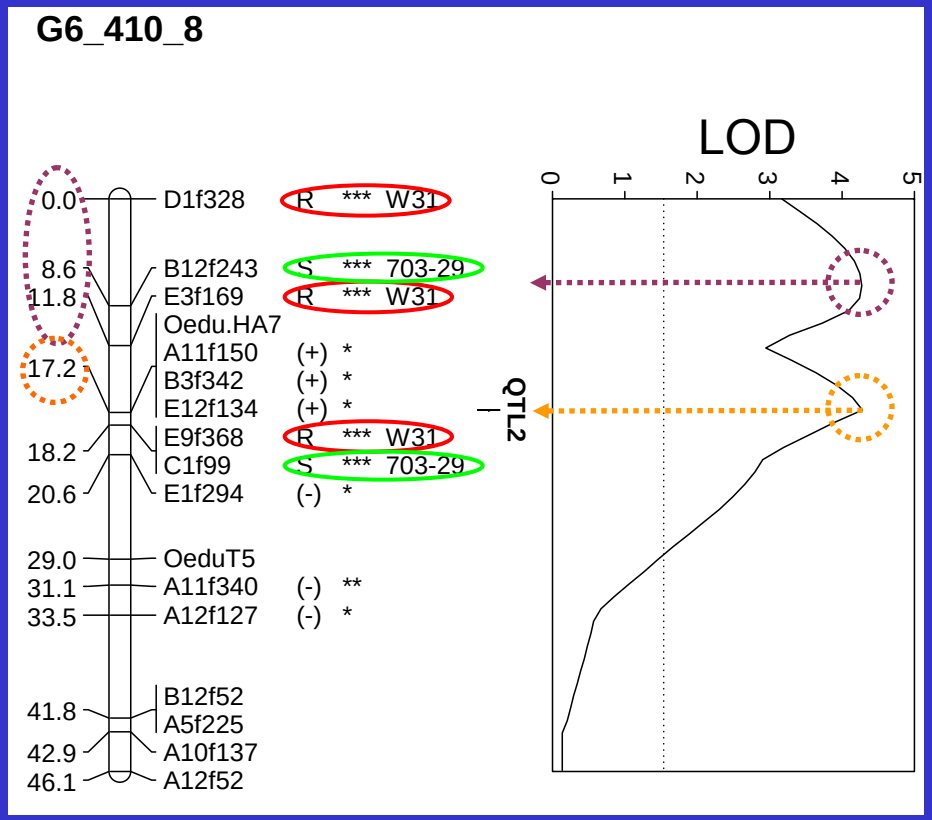
Parent 410_8

- QTL1: 61 cM ($p<0.05$), G3_410_8
- QTL2: 17 cM ($p<0.01$), G6_410_8


G1_410_8
0.0 C5f110 (+) *
2.2 B4f159 (+) *
9.8 D1f149 C5f213 A8f60
12.0 A12f319
18.4 OeduG9
24.8 D5f125 B4f268 E2f163
27.0 E12f274 (-) *
32.3 A8f162
33.4 A8f120 B9f123
34.4 A10f141 A11f279
47.5 A12f222
56.2 A4f41
68.1 A5f262
G2_410_8
0.0 D1f203(*) R *** W31
0.1 OeduU2
2.2 OeduC6
4.3 B8f245
7.5 E10f206
9.6 OeduJ12 B3f200 E8f110
25.2 Oedu.HA1
66.1 OeduO9
G3_410_8
LOD
0 1 2 3
0.0 Oe1/10
9.8 B8f220
30.2 Oe1/47 Oe3/37
32.4 B8f96 (-) ***
33.5 B11f296 (-) ***
34.6 E1f310 (-) ***
41.0 E5f212 (-) *
43.8 Oedu.HA21
49.6 E1f98 (+) *
52.8 E5f157 R * 703-29
61.5 A1f150 S ** W31
A3f73 R ** 703-29
QTL1
G4_410_8
E1f43(*) R *** 703-29
A5f49(*)
E3f255(*) R *** 703-29
A12f429(*) S *** 703-29
C1f252
A3f186
0.0
12.0 B10f70
19.5 A11f82
21.1 E3f195(*)
22.7 E8f61 B1f216(*)
37.3 B4f125(*) E10f140(*)
52.0 E12f245
54.1 C1f125 (-) *
G5_410_8
0.0 Oe3/44 A9f296
16.7 E12f278
51.7 A10f140
G6_410_8
LOD
0 1 2 3 4 5
0.0 D1f328 R *** W31
8.6 B12f243 S *** 703-29
11.8 E3f169 R *** W31
Oedu.HA7
17.2 A11f150 (+) *
B3f342 (+) *
E12f134 (+) *
18.2 E9f368 R *** W31
20.6 C1f99 S *** 703-29
E1f294 (-) *
29.0 OeduT5
31.1 A11f340 (-) **
33.5 A12f127 (-) *
41.8 B12f52 A5f225
42.9 A10f137
46.1 A12f52
QTL2
G7_410_8
0.0 D5f155
4.4 A10f139
8.8 Oe2/71
32.8 D1f171
45.0 A4f313 A11f179
G8_410_8
0.0 D1f271 (+) **
1.1 A10f276 (+) **
24.0 C5f268(*) E12f190(*) C1f376(*) E11f311(*)
G9_410_8
0.0 C1f67
9.9 D5f279 E1f330
13.2 C5f174
G10_410_8
0.0 A1f261 (+) ***
A4f81 (+) ***
11.0 A5f316 E11f252
R “resistant allele”
S “susceptible allele”



61 cM, p<0.05



17 cM, p<0.01

- Good concordance between multistage strategy, genetic mapping, QTL mapping
- Several markers of interest found (resistance, susceptibility)

Very promising results

Coming work

- Development of new microsatellites (ecogenics.ch)
- Increase the density of markers on the linkage map, allowing a more precise localization of QTLs
- SSH library underway (identification of candidate genes for bonamiosis resistance)

Acknowledgements

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