A preliminary genetic linkage map for the European flat oyster *Ostrea edulis* L.

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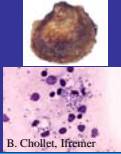[2] IFREMER, Laboratoire de Génétique et Pathologie, La Tremblade, France
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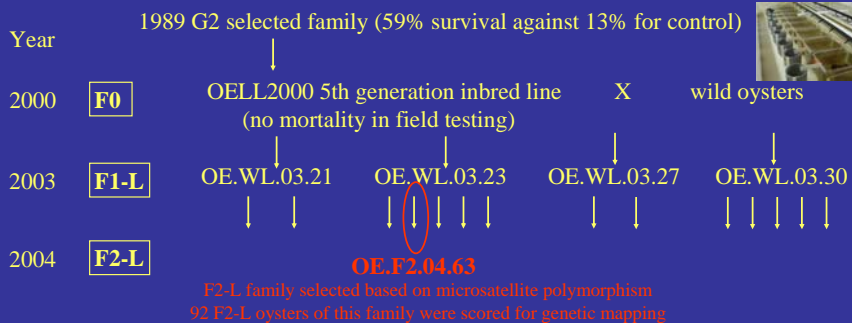
CONTEXT OF THE STUDY

The flat oyster *Ostrea edulis* is the species endemic to European coasts, both Atlantic and Mediterranean. It has been exploited since Roman times, but its aquaculture production decreased from around 20000 tons in the 1950's to 1500 tons today because of two successive diseases due to the intracellular parasites *Marteilia refringens* and *Bonamia ostreae*. Since 1985, Ifremer has been undertaking a breeding program to produce families of oysters tolerant to *Bonamia*. In this context, a further step would be to identify QTLs for resistance to this parasite. Therefore, as a first step a genetic map is now being built.
The establishment of a genetic linkage map will provide a foundation for the mapping of QTLs, with the ultimate objective to implement marker-assisted selection in *O. edulis*.

B. Chollet, Ifremer

PRODUCTION OF SEGREGATING FAMILIES (biparental crosses)

Year				
	1989 G2 selected family (59% survival against 13% for control)			
2000	**F0**	OELL2000 5th generation inbred line (no mortality in field testing)	X wild oysters	
2003	**F1-L**	OE.WL.03.21 OE.WL.03.23 OE.WL.03.27 OE.WL.03.30		
2004	**F2-L**	**OE.F2.04.63**		

F2-L family selected based on microsatellite polymorphism
92 F2-L oysters of this family were scored for genetic mapping
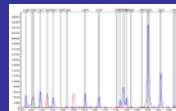
MOLECULAR TOOLS

- 20 microsatellites [1, 2, 3, 4, 5]
- 60 AFLPs primer pairs [6]

Three dyes: FAM, HEX and NED
Selection of good-quality peaks and double-checking of data
(ABI 3100-*Avant* sequencing machine) (GeneMapper® Software 3.7.)

MAP CONSTRUCTION

CRI-MAP v.2.4 (Green *et al.*, 1990)
Two-point linkage was determined at LOD=3.0.
Multipoint linkage analysis was performed using the BUILD and ALL options to determine the highest likelihood order of markers in each linkage group.
The best map was tested against alternative orders using FLIPS3 option.
The CHROMPIC option was used to identify unlikely double crossovers.
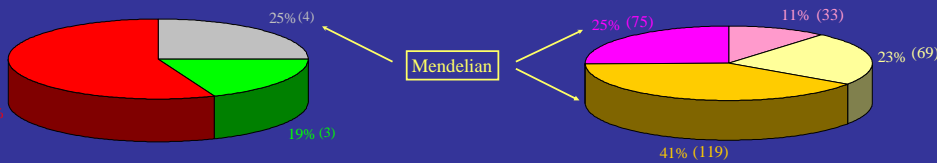
RESULTS

- Segregation distortion was assessed by Chi-square goodness of fit

Microsatellites: NS, p<0.01, p<0.001
AFLPs: 3:1 p<0.05, 1:1 p<0.05, 1:1 NS, 3:1 NS

Microsatellites: 25% (4), 19% (3), 56% (9)

Mendelian

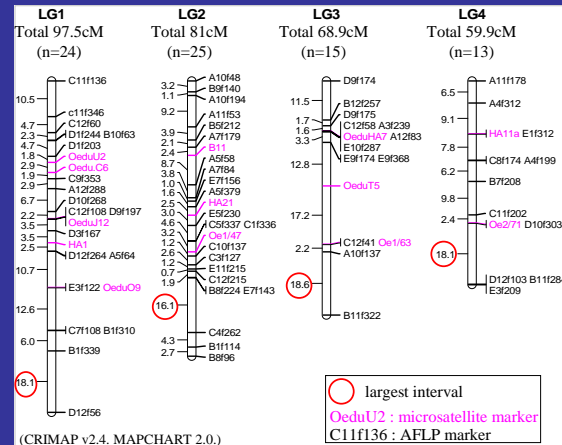AFLPs: 25% (75), 11% (33), 23% (69), 41% (119)

1:1 type : AFLP band present in only one of the 2 F1 parents
3:1 type : AFLP band present in both F1 parents

- Segregation distortion is higher for microsatellites. Nevertheless, all microsatellites were kept for linkage analysis.
  → This is in congruence with high genetic load (Launey & Hedgecock, 2001; Bierne *et al.*, 1998)
- In total, 235 AFLPS were kept for linkage analysis : all the 1:1 markers, and only the Mendelian 3:1 markers.
- 107 AFLPs are specific of grand-parent 1, 114 of grand-parent 2 and 14 are common to both grand-parents

- **Preliminary linkage map**
  - 12 linkage groups with more than 9 markers : 4 with more than 2 microsatellites; 2 with only one microsatellite
  - 5 linkage groups with less than 4 markers (4 dimeres and 1 trimere)
  - 4 sex-average linkage groups were built, containing more than 2 microsatellites which allowed mapping of AFLPs specific to different grand-parents
    - length : from 60cM to 98cM
    - average spacing : from 3.7cM (LG2) to 8.6cM (LG3 and LG4)
    - largest interval : from 16.1cM (LG2) to 18.6cM (LG3)
    - some clusters of AFLPs and clusters AFLPs/microsatellite

LG1 Total 97.5cM (n=24)

	C11f136
10.5	
4.7	c11f346
2.3	C12f60
4.7	D1f244 B10f63
1.8	D1f203
2.9	OeduU2 / Oedu.C6
1.9	C9f353
2.9	A12f288
6.7	D10f268
2.2	C12f108 D9f197
2.3	OeduU12
3.5	D3f167
2.5	HA1
	D12f264 A5f64
10.7	
	E3f122 / OeduO9
12.6	
6.0	C7f108 B1f310
	B1f339
18.1	D12f56

LG2 Total 81cM (n=25)

3.2	A10f48
1.1	B9f140
9.2	A10f194
3.9	A11f53
2.1	B5f212
2.4	A7f179
8.7	B11
3.8	A5f58
1.0	A7f84
1.6	E7f156
2.5	A5f379
3.0	HA21
4.6	E9f230
3.2	C5f337 C1f336
2.6	Oe1/47
1.2	C10f137
1.2	C3f127
1.9	E11f215
	C12f215
16.1	B8f224 E7f143
4.3	C4f262
2.7	B1f114
	B8f96

LG3 Total 68.9cM (n=15)

11.5	D9f174
1.7	B12f257 / D9f175
1.6	C12f58 A3f239
3.3	E10f287
12.8	E9f174 E9f368
	OeduT5
17.2	
2.2	C12f41 Oe1/63
	A10f137
18.6	
	B11f322

LG4 Total 59.9cM (n=13)

6.5	A11f178
9.1	A4f312
	HA11a E1f312
7.8	
6.2	C8f174 A4f199
	B7f208
9.8	
2.4	C11f202
	Oe2/71 D10f303
18.1	
	D12f103 B11f284
	E3f209

(CRIMAP v2.4. MAPCHART 2.0.)

○ largest interval
OeduU2 : microsatellite marker
C11f136 : AFLP marker

PERSPECTIVES

- **Construction of sex-specific genetic maps** in the family OE.F2.04.63 to map all linkage groups (2n=20)
  - Based on 107 AFLPs specific of grand-parent 1; 114 AFLPs specific of grand-parent 2; 16 microsatellites
  - CRIMAP software : "sex-specific" two-point analysis to determine sex-specific groups; then BUILD and FIXED commands to order those markers

- **Adding more codominant markers** (SNPs or microsatellites if more available by cross-amplification) to increase the number of anchor loci to be potentially mapped on a sex-average map and then the number of sex-average LGs

- **QTL mapping of traits of economical importance**
  - growth (follow-up of total length and weight monthly)
  - *Bonamia* "resistance" :
    - Challenge of 550 oysters by cohabitation with over-infected oysters
    - Mortality is recorded daily and heart smears are done to confirm *Bonamia* infection on dead oysters

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