

A PRELIMINARY GENETIC LINKAGE MAP FOR THE EUROPEAN FLAT OYSTER *Ostrea edulis*

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The European flat oyster, *Ostrea edulis*, occurs naturally from Norway to Morocco in the North-Eastern Atlantic and throughout the Mediterranean. The oyster has been a harvested species for at least 6000 years. However, it successively suffered from over harvesting and then from two protozoan diseases during the 1960s and its production has drastically decreased in Europe from that time. Following two generations of selection for tolerance to one of these diseases (*bonamiosis*), our aim is to optimize this selection with the assistance of molecular markers, that is to say to implement marker-assisted selection. This will enhance the efficiency of selection by reducing the generation time, as the selection on sites cannot be made before the oysters are three-years old. The establishment of the genetic linkage map represents the foundation for the identification of QTLs (Quantitative Trait Loci : markers linked to genes involved in quantitative parameters such as growth rate and parasite tolerance). Map building consists of the ordering of genetic marker loci and estimating relative distances between them, using data from individuals in an appropriate family structure. Therefore, we have chosen to follow the segregation of markers in two experimental families.

In 2003, 4 families (F1-L) were produced from biparental crosses between wild oysters and a fifth generation inbred line, obtained from a family from the program of selection for tolerance to this parasite. This inbred line showed no mortality when bred in sites where the parasite was present. Fourteen second generation families (F2-L) were then produced in 2004 (Figure 1). To construct the linkage map, two F2 families have been chosen on the basis of the marker polymorphism present in the grandparents.

Three kinds of molecular markers have been used: microsatellites, that are highly polymorphic, codominant, supposedly neutral, and used as anchor loci; and AFLP (Amplified Fragment Length Polymorphism) loci that require no knowledge of the genome, but are dominant. SNPs (Single Nucleotide Polymorphism) are under development based on information from the EST database available for the Pacific oyster, *Crassostrea gigas*.

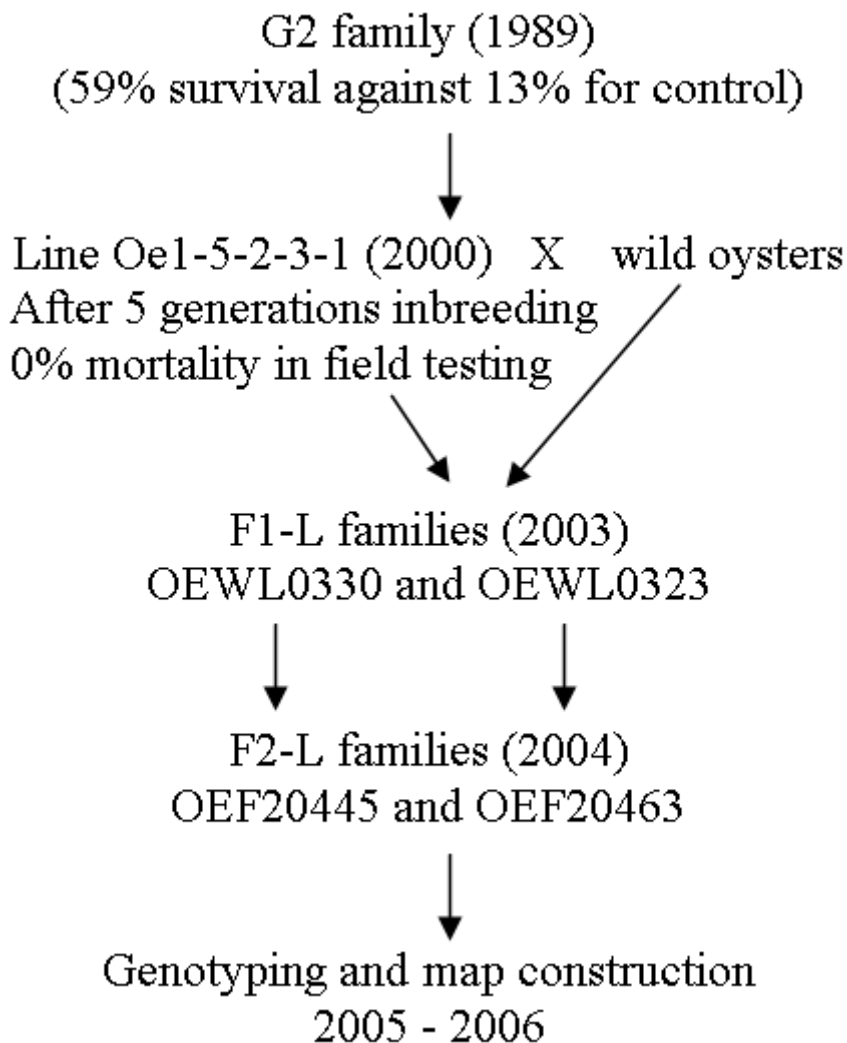


Figure 1. Scheme of the linkage project

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

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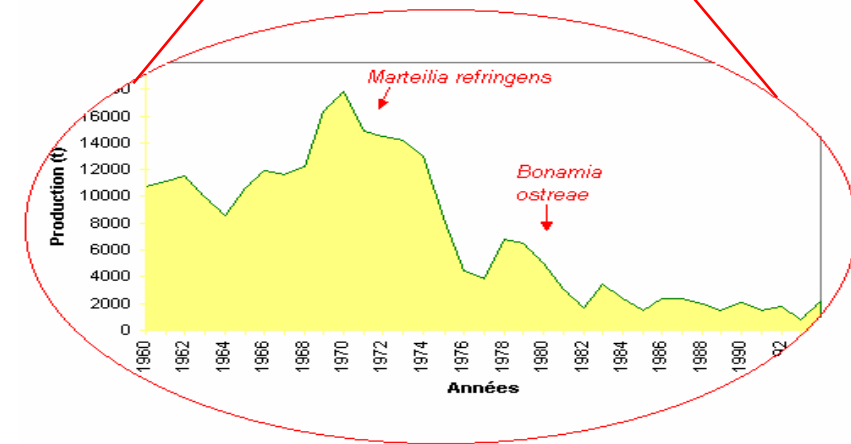
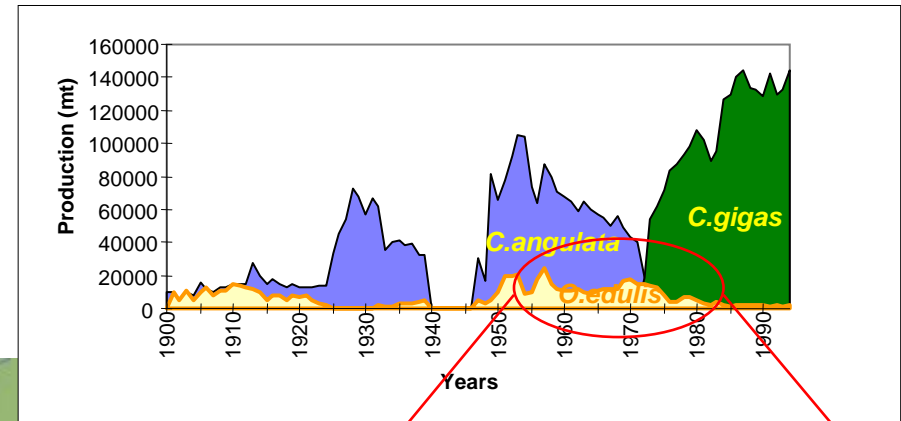


ESPAÑA ESPACIO ATLÁNTICO
FRANCE ESPACE ATLANTIQUE
IRELAND ATLANTIC AREA
PORTUGAL ESPAÇO ATLÁNTICO
U.K. ATLANTIC AREA



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Ostrea edulis the native european flat oyster



Ostrea edulis in 2004 :

World production = 5.100 t/y
 French production = 1.500 t/y

(Jaziri, 1985)



Mass selection : 1985 - 1995

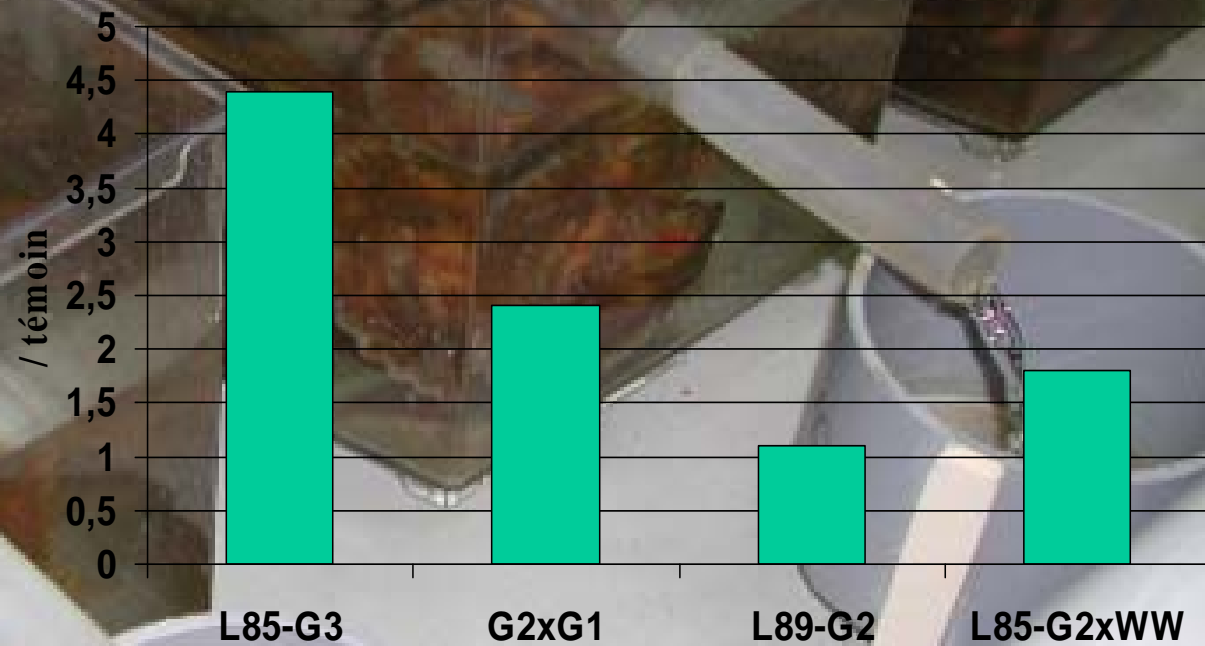
G0 : wild population

Mass spawning

4 years field testing

Survivors are genitors of the next generation

After 1 or 2 generations of mass selection



Genealogical selection

Selected populations

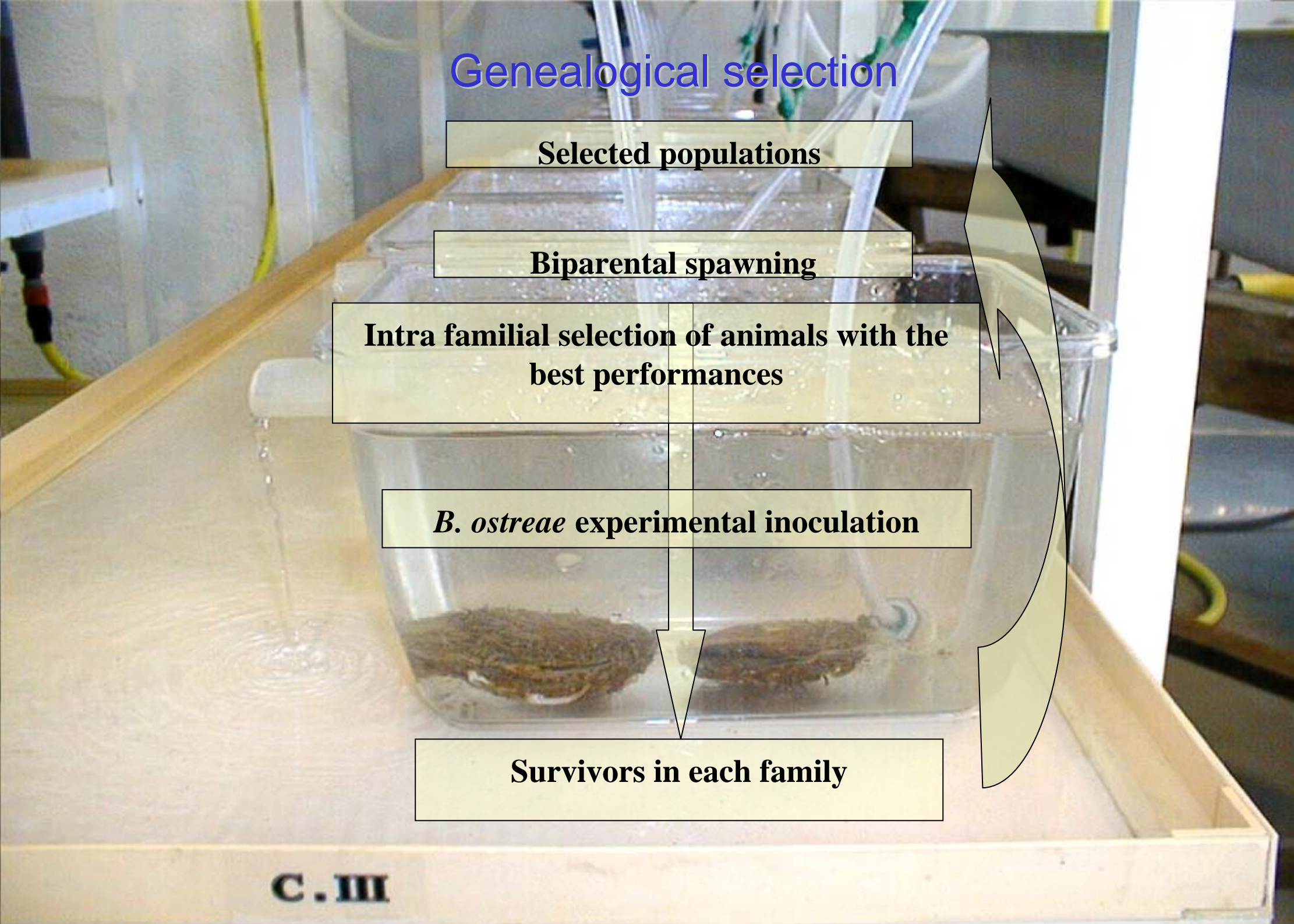
Biparental spawning

Intra familial selection of animals with the best performances

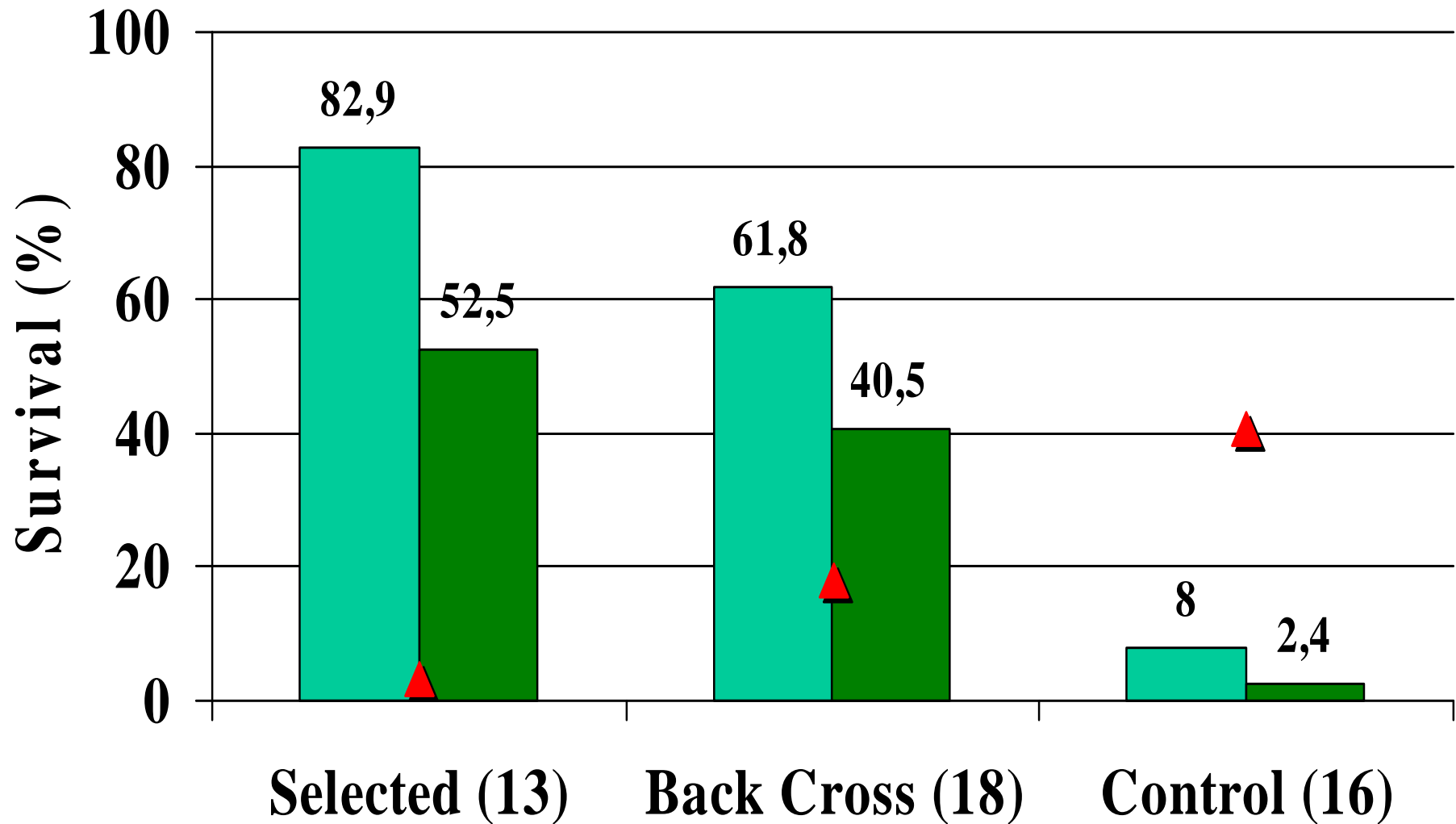
***B. ostreae* experimental inoculation**

Survivors in each family

C. III



Survival in 1998

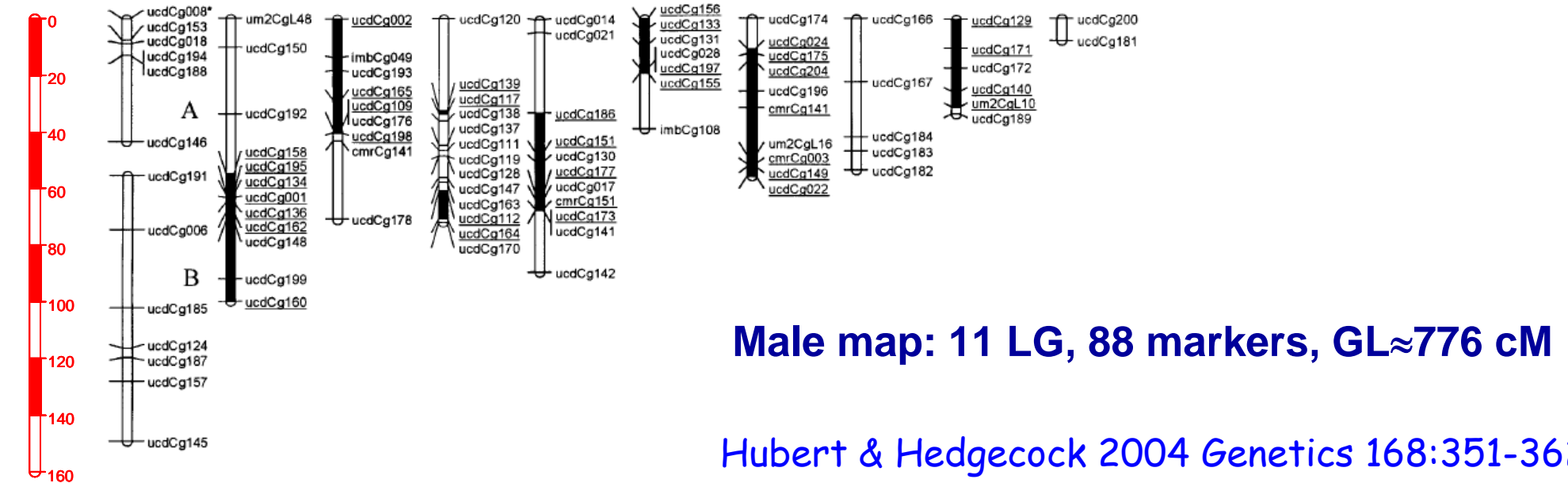
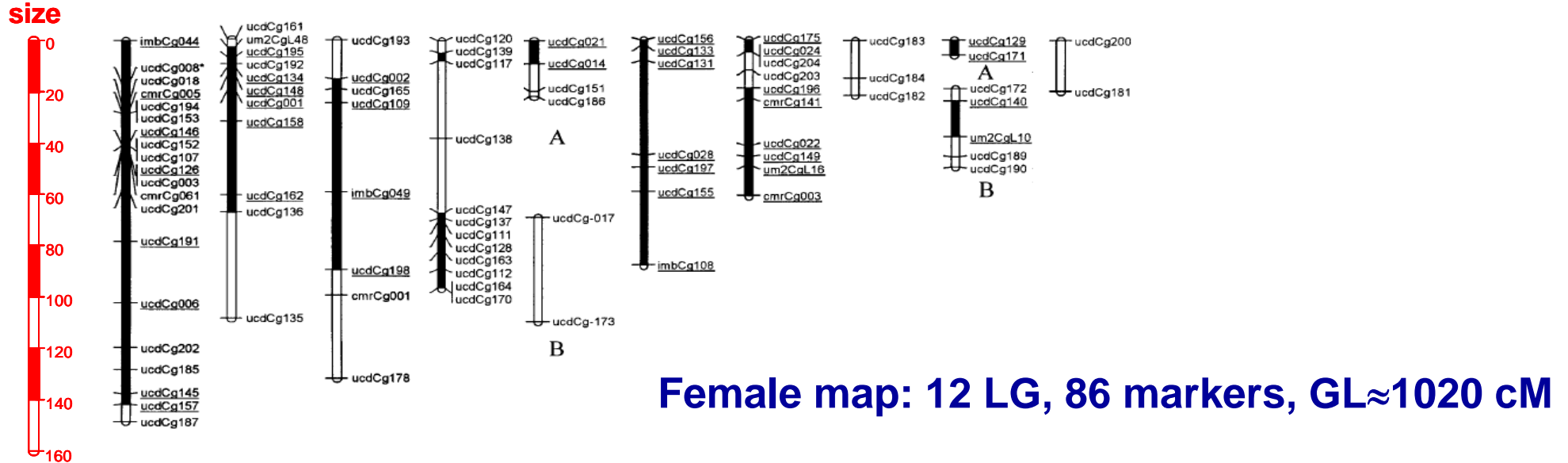


■ 2nd year survival

■ Global survival

▲ Prevalence of Bonamia

What is a genetic map?

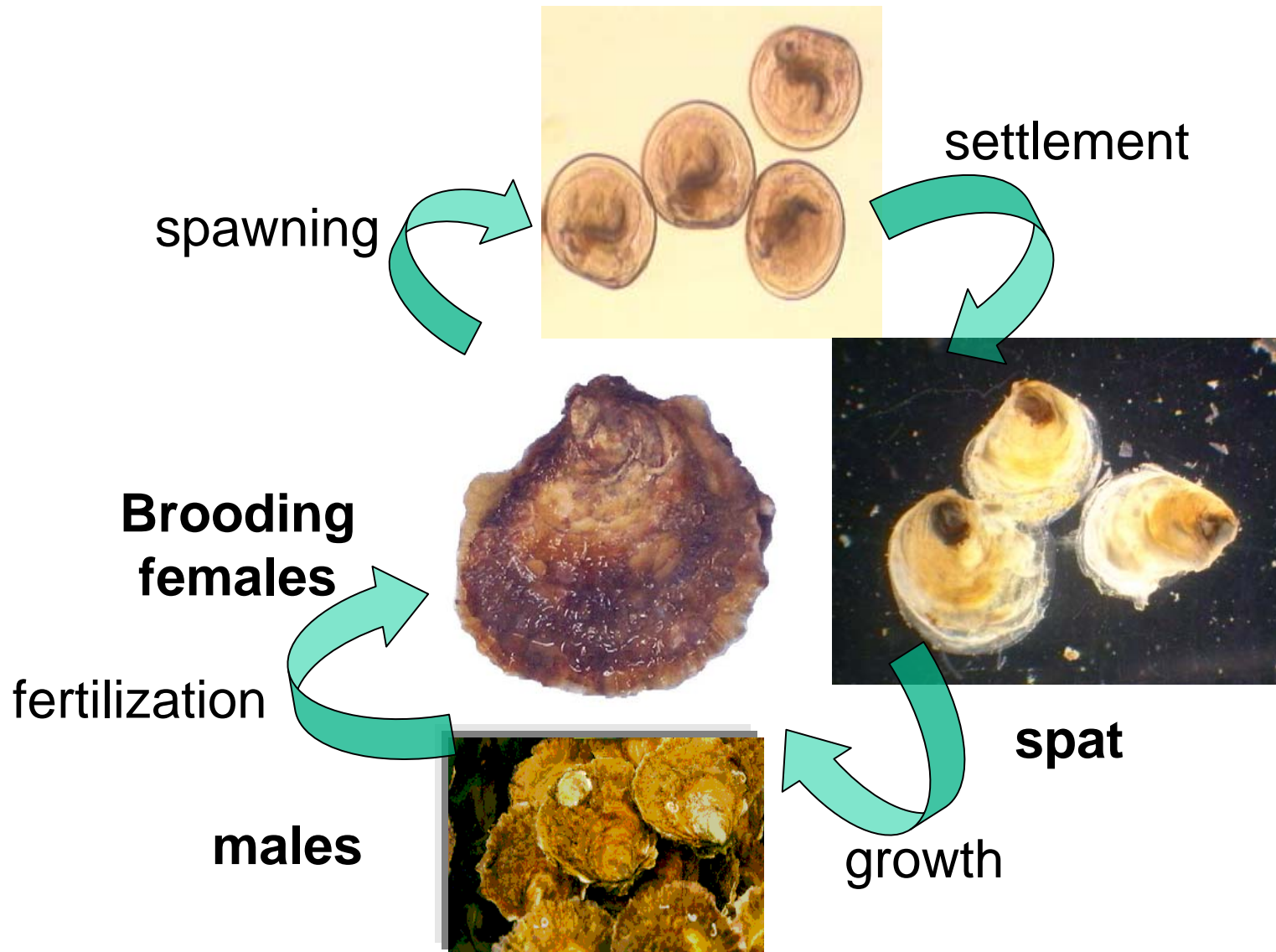


How to establish a genetic map?

- Relies on the relation between recombination frequency (RF) and genetic distance:
 - 2 markers closely linked → no chiasma → no recombinant gametes
 - 2 markers far from each other → observation of recombinant gametes
- To estimate RF, necessity of knowing the parental associations of alleles
 - ➔ requires analysis of segregation of markers among controlled families (parents of known genotype)

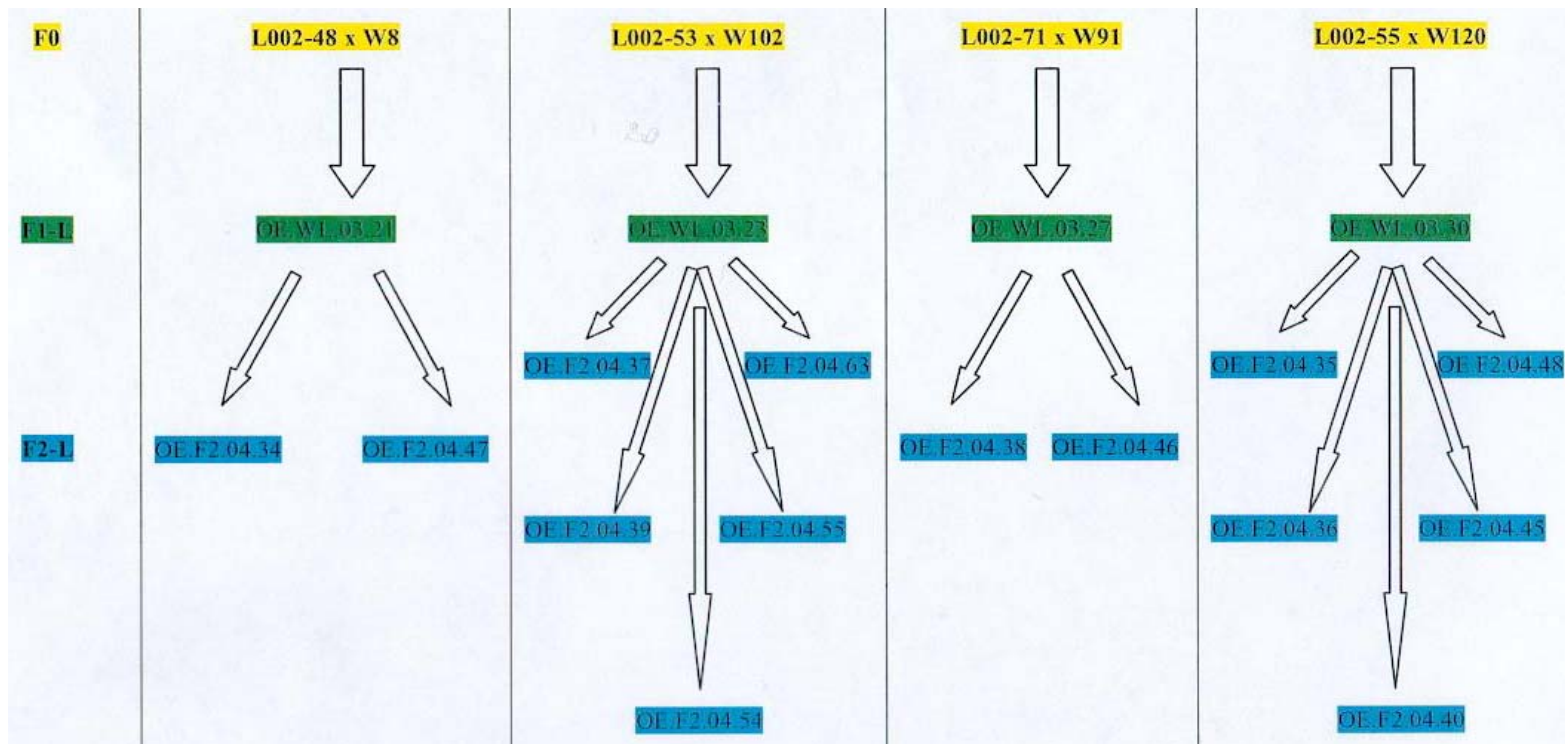
Reproductive cycle

planktonic larvae (± 10 days)



Segregating families

- Production in 2003 of 4 families F1-L from crosses between wild oysters and the inbred line OELL2000-set2
- Production in 2004 of 14 families F2-L from crosses F1-L x F1-L

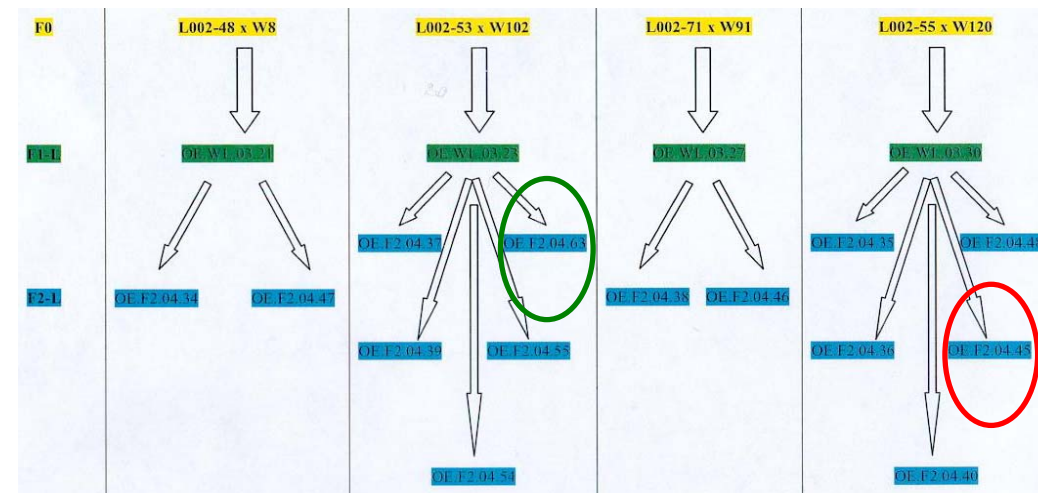


Two F2-L families scored

Family OE.F2.04.45 et OE.F2.04.63

- 20 microsatellites
- 60 AFLPs primer pairs

48 individuals per family



➔ OE.F2.04.45 Self-fertilisation of an F1

➔ OE.F2.04.63 « Real » F2

High segregation distortion

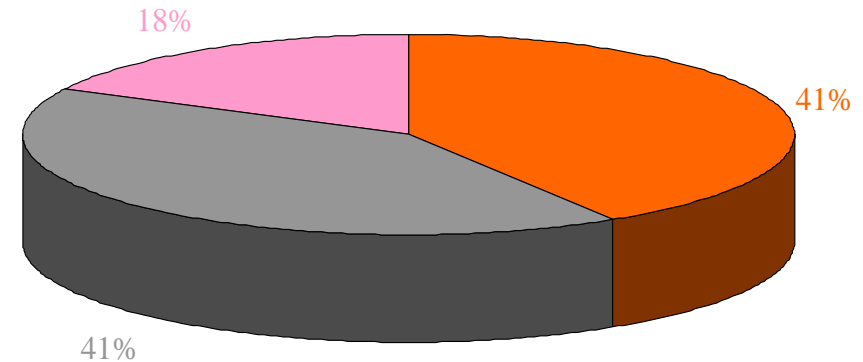
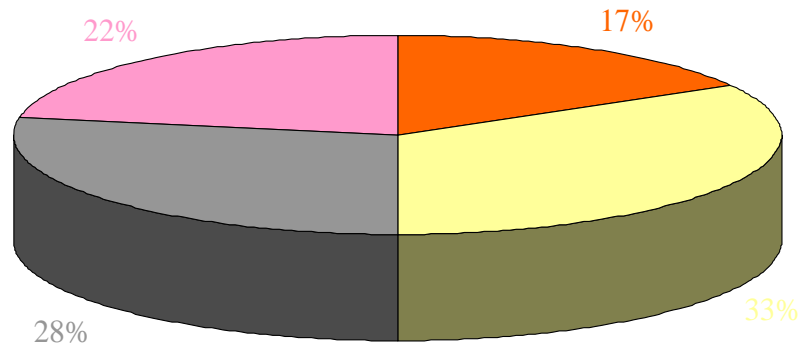
Medelian Segregation

OE.F2.04.45

OE.F2.04.63

■ NS ■ p<0.05 ■ p<0.01 ■ p<0.001

■ NS ■ p<0.05 ■ p<0.01 ■ p<0.001



17 %

41 %

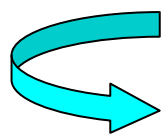
supports the observation of a high genetic load:

- *Ostrea edulis* (Bierne, N., Launey, S., Naciri-Graven, Y. and Bonhomme, F. (1998). Early effect of inbreeding as revealed by microsatellite analyses on *Ostrea edulis* larvae. *Genetics* 148: 1893-1906)
- *Crassostrea gigas* (Launey, S. & Hedgecock, D. (2001). High genetic load in the Pacific oyster *Crassostrea gigas*. *Genetics* 159: 255-265.

Genetic mapping results

Family OE.F2.04.45

- Total markers used (Mendelian only) : 233
- Two-point analysis $LOD > 4.0$.
 - 16 LGs
 - 9 LGs with more than 8 markers
 - 7 LGs with 3 or 2 markers only
- Ordering of microsatellites possible (5 are located into the same LG)
- But clustering of AFLPs: linked but with no recombination. So ordering not possible



Probably due to the structure of the family (self-fertilisation of an F1). Less informative.

Genetic mapping results

Family OE.F2.04.63

- Total markers used (Mendelian only) : 284
- Two-point analysis $LOD > 3.0$.
 - 18 LGs
 - 9 LGs with more than 6 markers
 - 2 LGs with 5 markers; 1 with 4 markers
 - 4 LGs with 3 markers; 2 with 2 markers
- Ordering of markers for the 6 main LGs
 - LG1: 21 markers (on 33). 5 msats. 75cM
 - LG2: 14 markers (on 20). 3 msats. 60cM
 - LG3: 7 markers (on 16). 2 msats. 39cM
 - LG4: 22 markers (on 43). 3 msats. 63cM
 - LG5: 8 markers (on 35). 1 msat. 20cM
 - LG6: 6 markers (on 12). 1 msat. 44cM

Problems encountered

- Clustering of AFLPs
- Difficulty of ordering markers
- For **OE.F2.04.45** due mostly to the family structure (selfing)
- However, family **OE.F2.04.63** looks more promising : some markers can be ordered but lack of information to accurately map them



Score more individuals to increase accuracy and efficiency of ordering markers inside a LG : underway (44 more individuals)

QTL mapping

« *Bonamia* » challenge experiment

Aim : finding QTL (Quantitative Trait Loci) of resistance to the parasite *Bonamia ostreae*. i.e finding markers linked to loci controlling the disease



12 raceways (150 l/h)

100 tested F2 oysters per raceway

QTL mapping

« *Bonamia* » challenge experiment



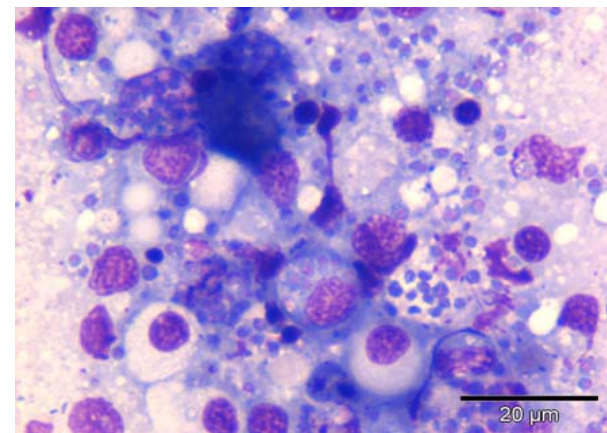
Cohabitation between wild oysters (over-infected with the parasite) and the F2 families produced in the hatchery of La Tremblade



Wild oysters (overinfected)

F2 family (tested oysters)

- Mortality checked daily.
- Smear achieved on dead oysters to detect presence of *Bonamia*



Achievment

- End Scoring F2-L family OE.F2.04.63
- CriMap data analysis
- Preliminary Genetic map for *Ostrea edulis*

- Scoring 94 F1 individuals *Mytilus edulis*
- CriMap data analysis
- Preliminary map of *Mythilus edulis*

- Scoring of one F2-S family
- QTL mapping data analysis
(*Bonamia* challenge experiment)



May 2006



June 2006



November 2006

Thank you for your attention

