A FIRST GENETIC LINKAGE MAP FOR THE BLUE MUSSEL *Mytilus edulis*.

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The blue mussel, *Mytilus edulis*, is widely distributed in the northern hemisphere, from the White Sea to the Atlantic coast of southern France, and is an economically important species (1 million tons per year, FAO, 2002). At present however, little domestication has been achieved with bivalves, in contrast to agricultural crops, livestock and horticultural species like tomatoes, where selection and production is entirely controlled by man. For bivalves, despite their economic importance, no lines with selected characteristics have been taken to the production scale. There is therefore a need to make up for lost time.

Our interest in genetic mapping sprang from some results showing a genetic component to some economically important traits like growth and survival. It was therefore primordial to establish a genetic linkage map in order to better understand the heritability of these traits. The genetic map will provide a framework for the localization of loci linked to the traits, so as to identify QTLs.

The reference family used in this study was an F1 family issued from the single pair mating of one male and one female from the wild. Eighty two of the progeny were used for linkage analysis in this study. The AFLP (Amplified Fragment Length Polymorphism) methodology was chosen, as these molecular markers require no knowledge of the genome and can quickly generate a large number of markers. Thirty-six different primer pairs were used for scoring the AFLPs. Only markers that showed a peak present in one parent, but absent from the other parent, and segregating in the progeny were scored for mapping analysis. Linkage analysis was carried out using MAPMAKER 3.0.

After double-checking the data, the number of segregating markers was 341 in the mother and 295 in the father. Among all segregating markers, χ^2 analysis indicated that 243 markers in the mother (71%) and 209 (71%) in the father segregated in a 1:1 Mendelian ratio. The female and male framework maps consisted of 14 linkage groups (2n=28). Only 4% of the markers were not linked to any other marker. The size of the linkage groups ranged from 40 to 115 cM for the female, with an average marker spacing of 9 cM; and from 25 to 95 cM for the male, with an average marker spacing of 8.5 cM. Some clustering of AFLPs was observed. The three first linkage groups of the female map are shown in Figure 1.

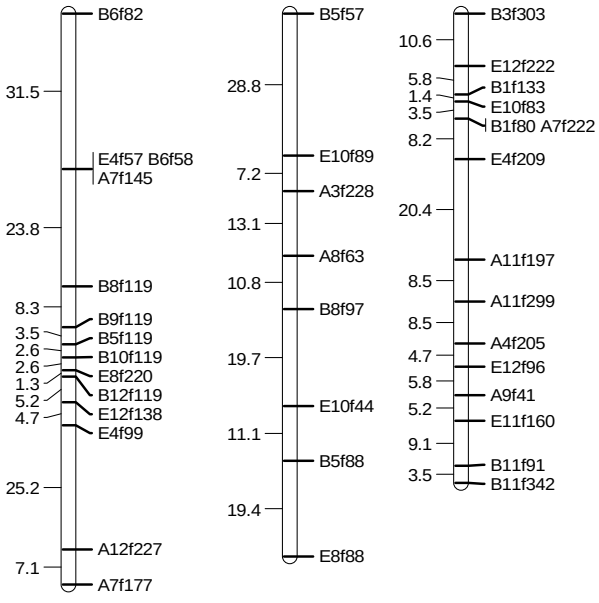

Figure 1. First three linkage groups of the female AFLP linkage map in the blue mussel *Mytilus edulis*.

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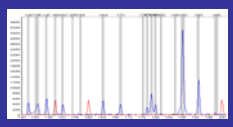
CONTEXT OF THE STUDY

The blue mussel, *Mytilus edulis*, is widely distributed in the northern hemisphere, from the White Sea to the Atlantic coast of southern France. It is a species of economical importance (1 million tons per year, FAO, 2002). Despite their economical importance, bivalves in general are characterized by their lack of domestication. At the present time, no selection of lines with a particular characteristic has been achieved at the production scale. Our interest in genetic mapping comes from the existence of some data reporting a genetic component of some economically important traits such as growth or survival. It is therefore critical to establish a genetic linkage map in order to better understand the heritability of these traits. The genetic map will represent a framework for the localisation of Quantitative Trait Loci (QTLs).

PRODUCTION OF SEGREGATING FAMILIES (bi-parental crosses)

Several full-sib mapping families were produced by bi-parental crosses between two wild mussels collected from the Menai Strait, Wales, UK.
The mapping family was randomly chosen for study and DNA was extracted from the 2 parents and 86 20-month old progeny.

MOLECULAR TOOLS



36 AFLP primer pairs (Vos *et al*. 1995)
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

MapMaker 3.0 software (Lander *et al.* 1987) was used to build sex-specific linkage maps, based on AFLPs that were present in one parent, absent in the other parent and that were segregating in the progeny (1:1 type). Then, a consensus map was built using JoinMap 4.0 software (Van Ooijen 2006) based on AFLPs that were present in both parents (3:1 type).

RESULTS-DISCUSSION

- **Relatively high polymorphism generated by the AFLP primer pairs (33.6%)** → **Efficiency of AFLP methodology** for producing numerous molecular markers

  Of the 2354 markers produced by the 36 AFLP primer pairs, 791 were polymorphic in the progeny, averaging 22 polymorphic markers generated per primer pair

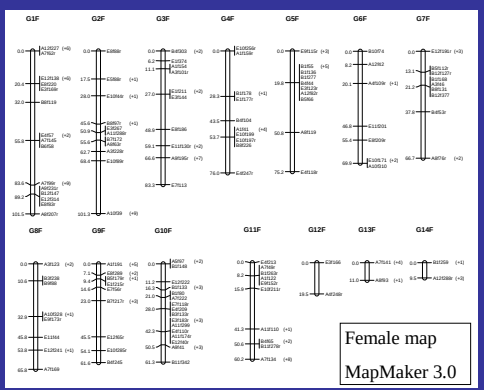
- **High segregation distortion was assessed by Chi-square goodness of fit (29%)** → **High genetic load** in the blue mussel (distorted markers probably linked to deleterious genes)

  Of the 791 polymorphic markers, 565 Mendelian AFLPs were kept for linkage analysis study, 243 segregating through the female parent, 210 through the male parent and 112 through both parents

- **Construction of sex-specific maps (female and male) and a consensus map in the blue mussel (2n=28): first genetic linkage maps built in any mussel species**
  - Female map: 121 framework markers (+ 115 associated markers: linked but not mapped) grouped into 14 linkage groups, spanning 863 cM, with an average marker spacing of 8 cM (Figure 1)
  - Male map: 116 framework markers (+ 88 associated markers) grouped into 14 linkage groups, spanning 825 cM, with an average marker spacing of 8 cM (Figure 1)
  - Consensus map: 12 probable homology groups were found and a consensus map was established for the 9 of them in which there were at least 3 AFLPs (3:1 type) mapped in the two parental maps and exhibiting parallel linkages; consensus map (9 linkage groups) consisted of 308 markers spanning 816 cM (Figure 2)

→ **AFLP methodology coupled with double pseudo-test cross strategy** (Grattapaglia & Sederoff 1994): a powerful tool for the establishment of genetic maps

→ **Good representation of the blue mussel genome**: 14 linkage groups correspond to the haploid number of chromosomes; genome coverage of 86% for both parental maps when associated markers are taken into account



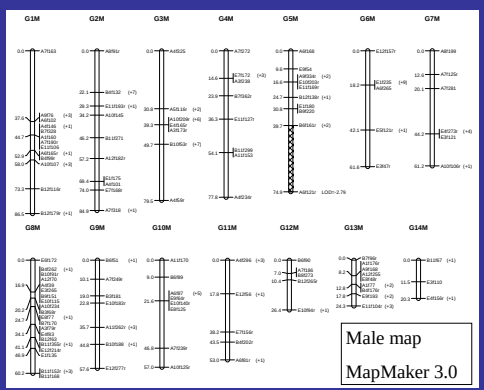


Figure 1. Female and male AFLP linkage maps of the blue mussel *M. edulis* obtained with MapMaker 3.0. AFLP markers are labelled with the primer pair name followed by the letter "F" (for fragment) and a 3-digit fragment size in base pairs. Markers are indicated on the right and absolute position on the left (in Kosambi cM). Numbers in brackets on the right of locus name correspond to number of associated markers (linked but unplaced).

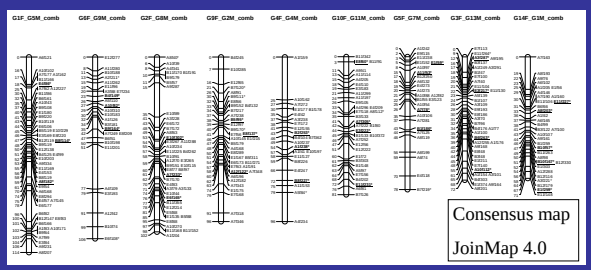


Figure 2. AFLP-based consensus map established in the blue mussel *M. edulis* with JoinMap 4.0. The consensus map presented is based on the finding of 9 homologous pairs of linkage groups based on at least 3 markers of type 3:1 exhibiting multiple and parallel linkages in the two parental maps. Homologous markers are displayed in bold and underlined, ending with an asterisk (*). Markers are indicated on the right and absolute positions on the left (in Kosambi cM).

FUTURE WORK

- **Adding more 3:1 AFLPs and codominant markers (such as microsatellites or Single Nucleotide Polymorphisms)** to increase the accuracy of the consensus map as well as its portability in the context of QTL mapping
- **Investigating eventual sex-specific recombination rate differences** by pairwise comparisons between markers common to female and male maps (serving as anchor loci)

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