Recent advances in EST sequencing in Crassostrea gigas: towards the sequencing of the Pacific oyster genome.

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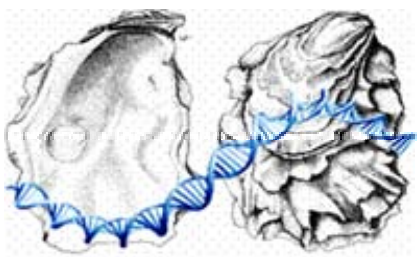
Introduction

Rationale for EST and genome sequencing of the Pacific oyster:
- its membership of the Lophotrochozoa, an little studied branch of the Eukaryotes,
- its worldwide aquacultural importance (4.2 million metric tons, worth 3.5 billion US dollars),
- its high fecundity, with concomitantly high DNA polymorphism,
- its use as a biosensor of coastal pollution.

In 2004, an international community of biologists, the Oyster Genome Consortium (OGC) led by D. Hedgecock (University of Southern California) and uniting 70 participants from 10 countries, first presented the Pacific oyster *Crassostrea gigas* as a genome-sequencing candidate to Joint Genome Institute (JGI, USA) (Hedgecock et al., 2005). Besides this project (that remains to be accepted), several EST sequencing projects have been successfully initiated at JGI, Genoscope (France) and the Max Planck Institute (Germany). These will considerably enlarge the first public database specifically dedicated to *C. gigas* (Gueguen et al., 2003; www.ifremer.fr/GigasBase).



Genome size and characteristics

Consensus haploid genome size is ~0.89 pg or 824 Mb. Genome sizes of 174 mollusks range from 0.43pg (for the limpet *Lottia gigantea*) to 5.88pg (for the whelk *Neobuccinum eatoni*), with a mean of 1.80 ± 0.07pg. Among those, only 23 mollusks have genome sizes smaller than that of the Pacific oyster.

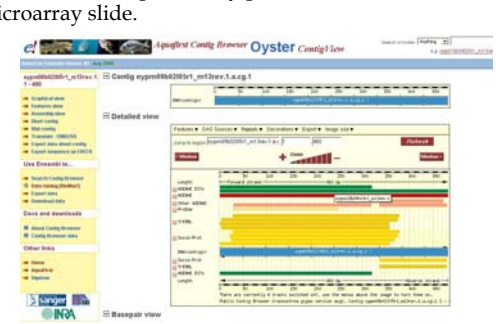
Protein polymorphism of marine bivalves is among the highest for animals. In the Pacific oyster, the average density of SNPs was estimated to be among the highest levels reported to date with one SNP every 61 bp in coding regions and one every 41 bp in non-coding regions (Sauvage et al., submitted). As a result, abundant non-amplifying PCR-null alleles are commonly observed. Because of the enormous fecundity and the very large population sizes in this species, a high genetic load is maintained in wild populations, explaining (1) frequent distorted inheritance ratios in lab-reared progeny of wild parents and (2) correlation of heterozygosity with fitness related traits in natural populations.

The available microsatellite and AFLP-based linkage maps (Hubert and Hedgecock 2004 ; Yu and Guo 2003), have 10 linkage groups, in accord with haploid chromosome number and cover ~80% of the Pacific oyster genome. SNPs are now being mapped and a physical map is being developed using a BAC (Cunningham et al., 2006) fingerprinting approach.

Current EST sequencing projects and their use in gene expression studies

More than 8000 ESTs resulting from 12 subtractive and 3 normalized libraries from 6 tissues have been sequenced by MPI within the framework of the EU funded projects Aquafirst and Marine Genomics Europe. In addition, normalized tissue or stage specific libraries are currently being sequenced by Genoscope and JGI.

A new EST database is hosted by INRA on the Sigenae Information system, bringing together these new ESTs with all previously published ESTs and mRNAs. The 1758 obtained contigs plus 7518 singletons are now being used to construct an European *C. gigas* microarray slide.



Conclusions and Future work

➡ **Past and present work on EST sequencing in Europe and the USA provide a solid basis for gene expression studies, based on SSH (*eg* Huvet et al., 2004) and sequencing of normalized libraries** (Tanguy et al., in prep), **microarrays** (Jenny et al., in press), **SAGE, MPSS** (Hedgecock et al., 2007) **and other approaches** (Saavedra and Bachère, 2006).

➡ **OCG is currently seeking new opportunities to have the Pacific oyster genome sequenced. A conference proposal will soon put forward to the National Research Initiative Animal Genomes (USDA).**

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