marine genomics

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SIXTH FRAMEWORK PROGRAMME

Combined **functional genomic** and **genetic approaches**

in oyster to identify summer mortality-resistant markers

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The oyster *Crassostrea gigas* has global distribution and for the past several years the highest annual production of any freshwater or marine organism. Economic importance of oysters motivates a great deal of biological research, which provides the most immediate scientific rationales for sequencing ESTs and developing genomic tools.

Strong rationales for studying the oyster genome products also come from contrasts to other genomes: membership in the Lophotrochozoa, an understudied branch of the Eukaryotes, high fecundity, with concomitantly high DNA sequence polymorphism. Oysters play also an important, sentinel role, in estuarine and coastal marine habitats, where the majority of humans live, environmental degradation is substantial, and oysters suffer intense mortalities from disease and stress.

Select oysters that are « resistant » or « susceptible » to summer mortalities

The most immediate applications of a wide variety of sequences and genomic tools from *C. gigas* fall under three headings:

- **functional genomics,** in which oysters permit a phylogenetic contrast in studies of genome function, and diversity,
- **comparative genomics,** in which oysters belonging to the Lophotrochozoa help to shed light on mechanisms of evolutionary biology, speciation in the sea and the evolution of sexuality,
- **environmental genomics,** in which oysters are a model for understanding the adaptation through genetic and physiological bases of complex traits (e.g. growth, survival) that are strongly correlated with Darwinian fitness and population responses to environmental change and stresses, such as disease.

Large scale mortalities of *C. gigas* have been reported in all areas of the world where this species is cultivated. Examination of the question of oyster summer mortality in France has suggested that there are complex interactions between the oyster, their environment and opportunistic pathogens. For the oyster, a large genetic basis was shown to exist for observed variation in resistance to summer mortality. This has opened up possibilities of improvement by selection and has in turn allowed us to select oysters that are 'resistant' (R) or 'susceptible' (S) to summer mortalities (Dégremont et al., 2007). As we have demonstrated that the physiological state of the animal plays a major part in this interaction (Huvet et al., 2004; Samain et al., 2007), the selected genetic character is likely to be connected with one or more functions within the oyster that explain the differential survival observed between R and S. >>>

The South Brittany site where oysters were reared

A collaborative work between MGE and Aquafirst

In the framework of the Marine Genomics Europe (MGE) Network of Excellence, a collaborative research of teams shared effort to increase the lack of genomic information in oyster and develop tools of general use, such are cDNA libraries, EST collections and microarray technology. This effort has been joined to the European program "Aquafirst" (coordinator P Prunet, INRA), devoted to genetic and functional genomic approaches for stress and disease resistance marker selection in fishes and oysters, to form a powerful consortium for fish and oyster genomics.

Partners of the "Fish and Shellfish node" of MGE network have produced four cDNA libraries for *C. gigas*, which have been built by the Max Plant Institute (Berlin) and have resulted in a total of 5712 EST sequences (Tanguy et al., 2008). Multiple normalized libraries were constructed from three tissues (gonad, gills and digestive gland) and are still available for further sequencing in order to increase the EST collections. All these sequences have complemented previous ESTs and mRNA available in Genbank (clones provided by all partners for the microarray) and 8064 ESTs produced by subtraction between R and S oyster lines in the Aquafirst program. All together, these sequences were assembled in a unique database to form 9272 contigs (http://www.sigenae.org/aquafirst/), resulting from the collaborative work of the consortium between MGE and Aquafirst.

Consequently, these two European projects met an agreement to construct a cDNA microarray. PCR amplifications of insert clones and spotting were realized by the MPI (Berlin). The slide contains 9059 unigenes spotted in duplicates. In total, 219 slides were produced and divided between the partner for developing functional genomics studies to reach a common goal: characterize summer mortality-resistant markers and identify the biological mechanism(s) involved in summer survival.

Thus, several experiments were analysed using microarray corresponding to R and S tested in different environments: in the field and in laboratory experiments in response to bacterial challenge, hypoxia or sulfoxia, all presumed to interact in summer mortality (Samain et al., 2007). Part of the hybridizations have been processed at the IFR 140-Ouest Genopole platform (Rennes, France) and are currently under statistical treatment. Correlation coefficients of both technical and biological replicates appeared very high which supports that our data are highly reproducible. First results indicate that among the 9059 unigenes, 438 are differentially expressed between R and S in the field. A second batch of hybridization is running to identify regulation processes of gene networks involved in hypoxia stress in digestive gland of R and S.

Identification of markers of resistance

Functional genomics studies allow the characterization of genes that are differentially expressed between R and S progenies. To evaluate these genes as potential candidate markers of resistance to summer mortality, they will be next studied "one by one" in terms of the physiology of these oysters with RNA interference as functional promising tools and also in terms of valuable markers in the search for Quantitative Trait Loci (QTL).

Indeed, genetic markers linked to summer mortality resistance in oyster spat are being sought. This search, using the QTL approach was initiated by the production of F1 'hybrid' families between R and S batches, and then F2 segregating families. The genetic map published by Hubert & Hedgecock (2004) is the basis to be enriched with SNPs (Single Nucleotide Polymorphisms) and new microsatellites detected from EST databases, called "in silico" microsatellites.

The search for SNPs was performed by sequencing some candidate ESTs in the 24 grand-parents (F0) of our families of reference. The first analysis of sequence polymorphism and codon usage bias with a set of 41 nuclear loci revealed a very high level of DNA polymorphism in oysters, in the order of magnitude of the highest levels reported in animals to date. >>>

A total of 290 SNPs were detected, 76 of which being localised in exons and 214 in non-coding regions. Average density of SNPs was estimated to be one SNP every 60 bp in coding regions and one every 40 bp in non-coding regions. Non-synonymous substitutions contributed substantially to the polymorphism observed in coding regions. The non-synonymous to silent diversity ratio was 0.16 on average, which is fairly high compared to the ratio reported in other invertebrate species recognised to display large population sizes. Therefore, purifying selection does not appear to be as strong as could have been expected for a species with a large effective population size. The level of non-synonymous diversity varied greatly from one gene to another, in accordance with varying selective constraints. We also examined codon usage bias and its relationship with DNA polymorphism. A table of optimal codons was deduced from the analysis of an EST dataset, using EST counts as a rough assessment of gene expression. As recently observed in some other taxa, we found a strong and significant negative relationship between codon bias and non-synonymous diversity suggesting correlated selective constraints on synonymous and non-synonymous substitutions. Codon bias as measured by the frequency of optimal codons for expression might therefore provide a useful indicator of the level of

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The Pacific cupped oyster Crassostrea gigas (Thunberg, 1793)

constraint upon proteins in the oyster genome. Complementary studies based on an evolutionary approach of gene polymorphism have been conducted in both *C. gigas* and *C. angulata* with the aim to identify selective effects in functional genes and allowed to identify more SNPs and indels markers (Tanguy et al., unpublished). Furthermore, in silico microsatellites are currently being developed by searching repeat motifs in ESTs from the database. A first set of 18 markers will be soon available (Sauvage et al., unpublished) and other markers are being developed. Microsatellite and SNP markers were then used to construct a genetic map on three F2 families that were also tested for resistance to summer mortality in summer 2006. This experiment combined to the maps will be used to search for QTLs of resistance or susceptibility to summer mortality. ■■■

>>> Bibliography (page 23)

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