

Overview on molecular tools in bivalve shellfish aquaculture

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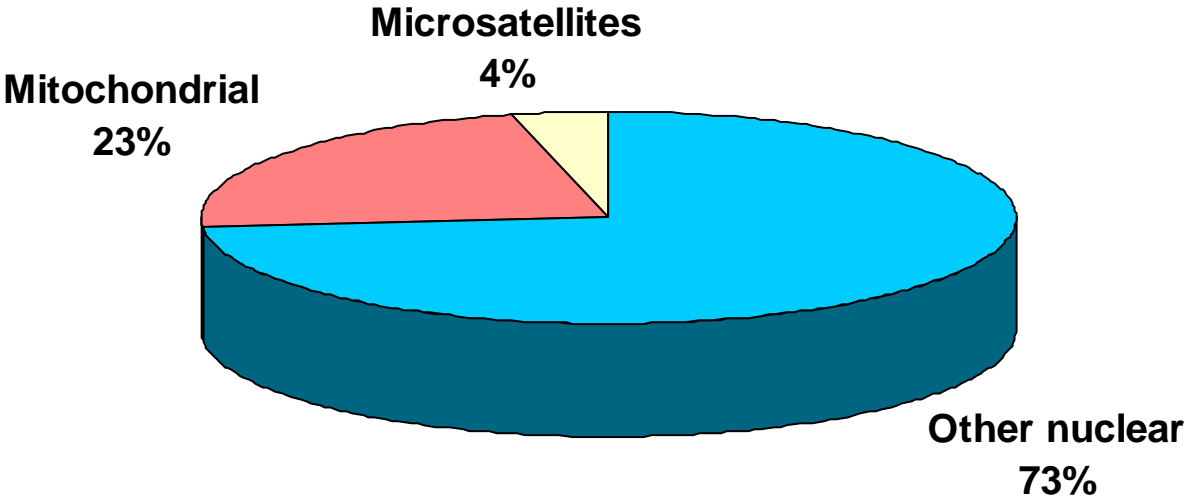
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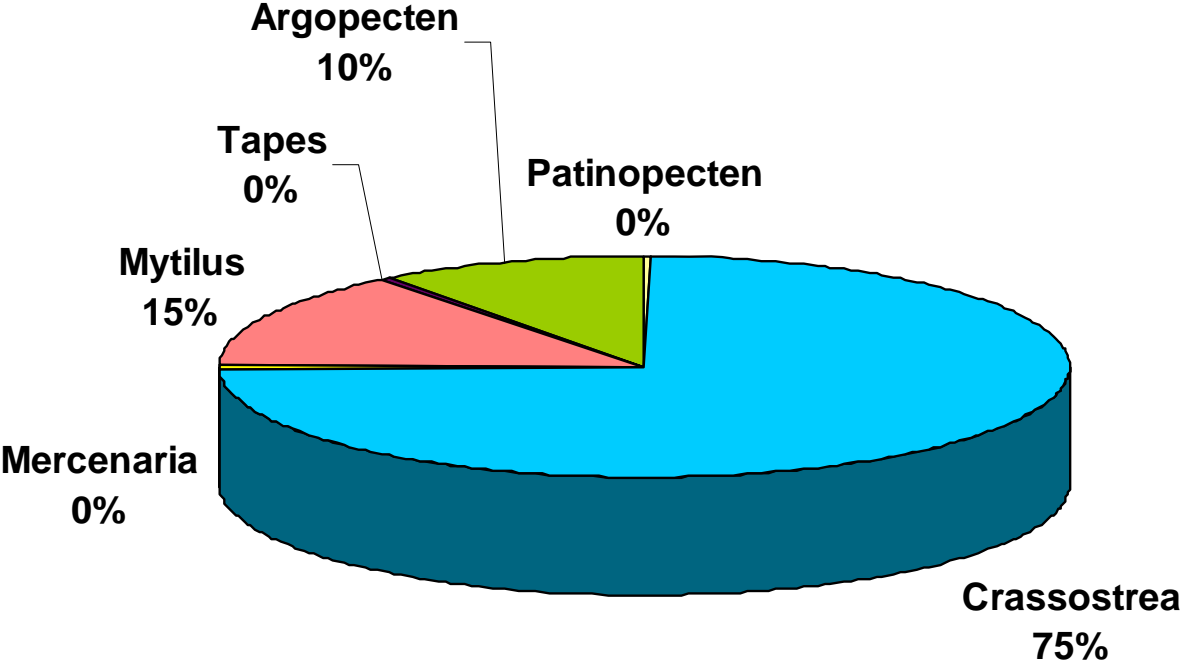


Bivalves in GenBank



Bivalve Nuclear Sequences in GenBank

(excluding microsatellites)



Nuclear Sequences from “single-gene” research

Immunity and disease (oyster, mussel) :

- **Antimicrobial peptides**

Byssus formation (mussel) :

- **Foot adhesive proteins**

Digestion (mussel, oyster) :

- **Amylases, cellulases**

Pollution and stress (mussel, oyster) :

- **Metallothioneins, HSP70**

Shell formation (pearl oyster)

Development (mussel, oyster)

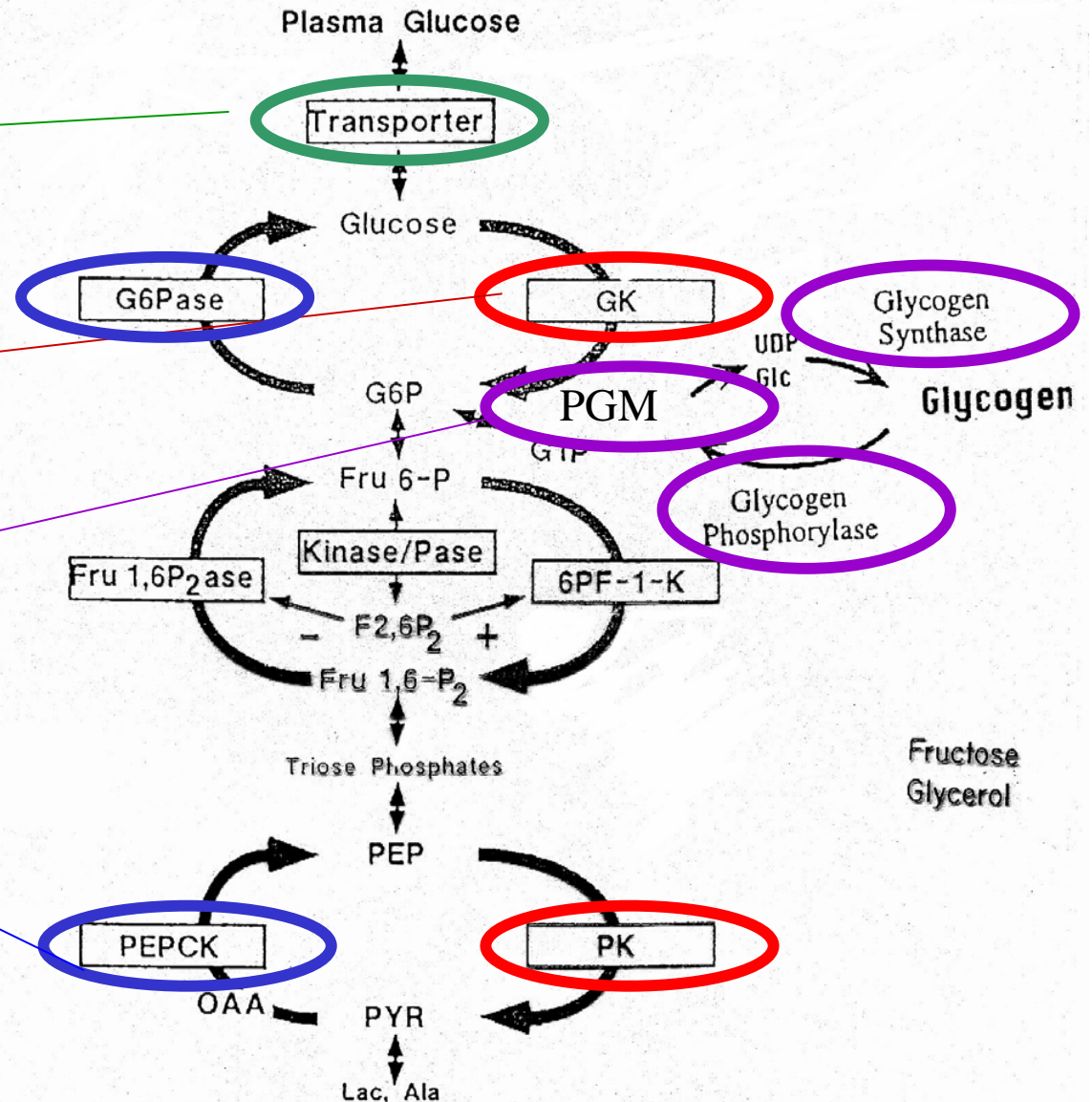
Enzymes in glucose pathway: characterized cDNAs (*C. gigas*)

Glucose transporter
(SGLT)

Glycolysis
(PK, hexokinase)

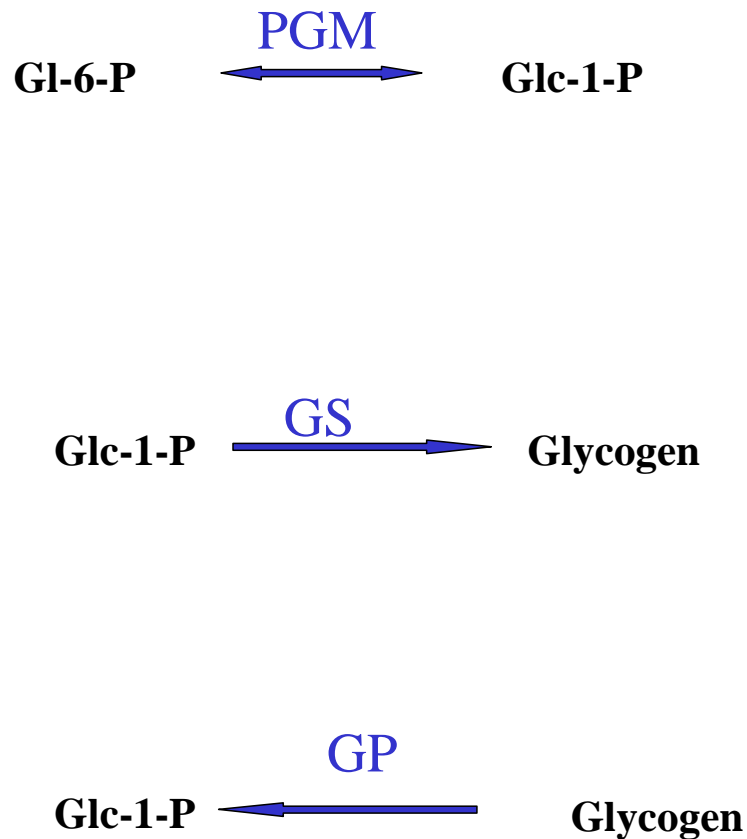
Glycogen metabolism
(GS, GP, PGM)

Gluconeogenesis
(PEPCK, G6Pase)

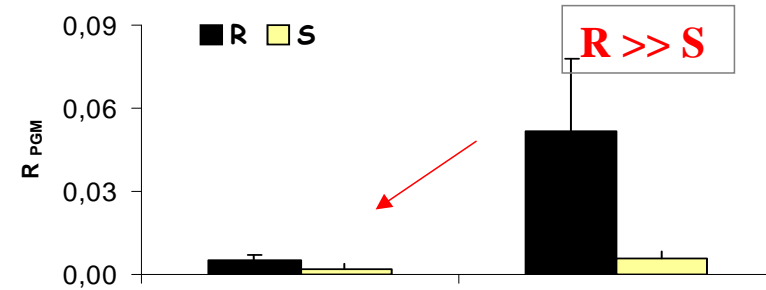


The substrate cycles and enzymes involved in hepatic glucose metabolism. PEP, phosphoenolpyruvate; PYR, pyruvate; OAA, oxaloacetate; Kinase/Pase, 6-PF-2K/Fru-2,6-P₂ase; Lac, lactate.

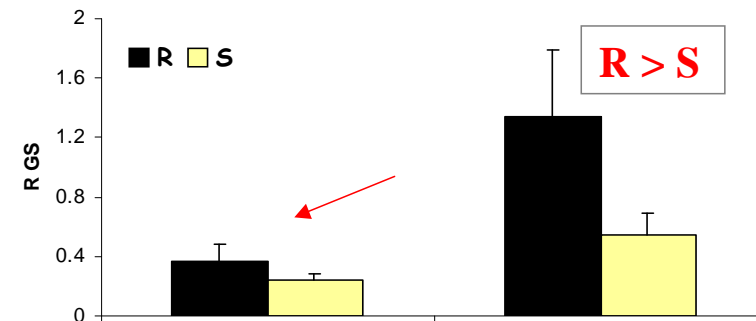
Real-time PCR studies of genes involved in glycogen pathway



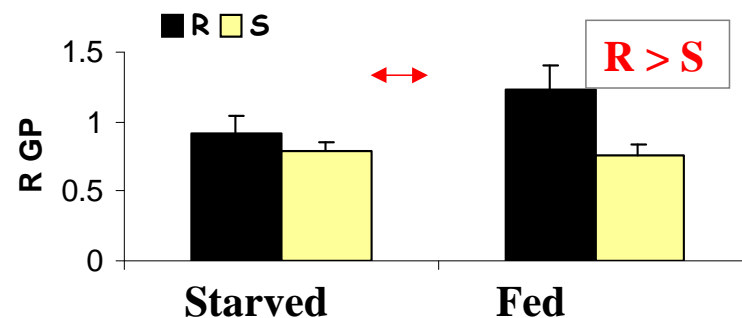
PGM : palpes, CN3, 13 juin



GS : Palpes, CN3, 13 juin



GP : Palpes, CN3, 13 juin



cDNA libraries in Bivalves

<i>Species</i>	<i>Tissue</i>	<i>Author (lab)</i>
Oyster CG	Hemocytes Mantle Larvae Gill	Bachere, Escoubas (Montpellier) Cunningham (Bergen) Favrel (Caen) Rafferty (Galway) Moraga (Brest)
Oyster CV	Hemocytes, embryos Gonad	Chapman (Charleston, SC) Liu (Auburn, AL)
Mussel MG	Whole body Sperm	Venier (Padova) Cornudella (Barcelona)
Scallop AI	Spat	Roberts (Woods Hole MBL)

ESTs in Bivalves

- EST characterization of genes involved in relevant biological processes

Oyster CV – stress response

(Chapman, SC) – 649 ESTs, 29 potential biomarkers

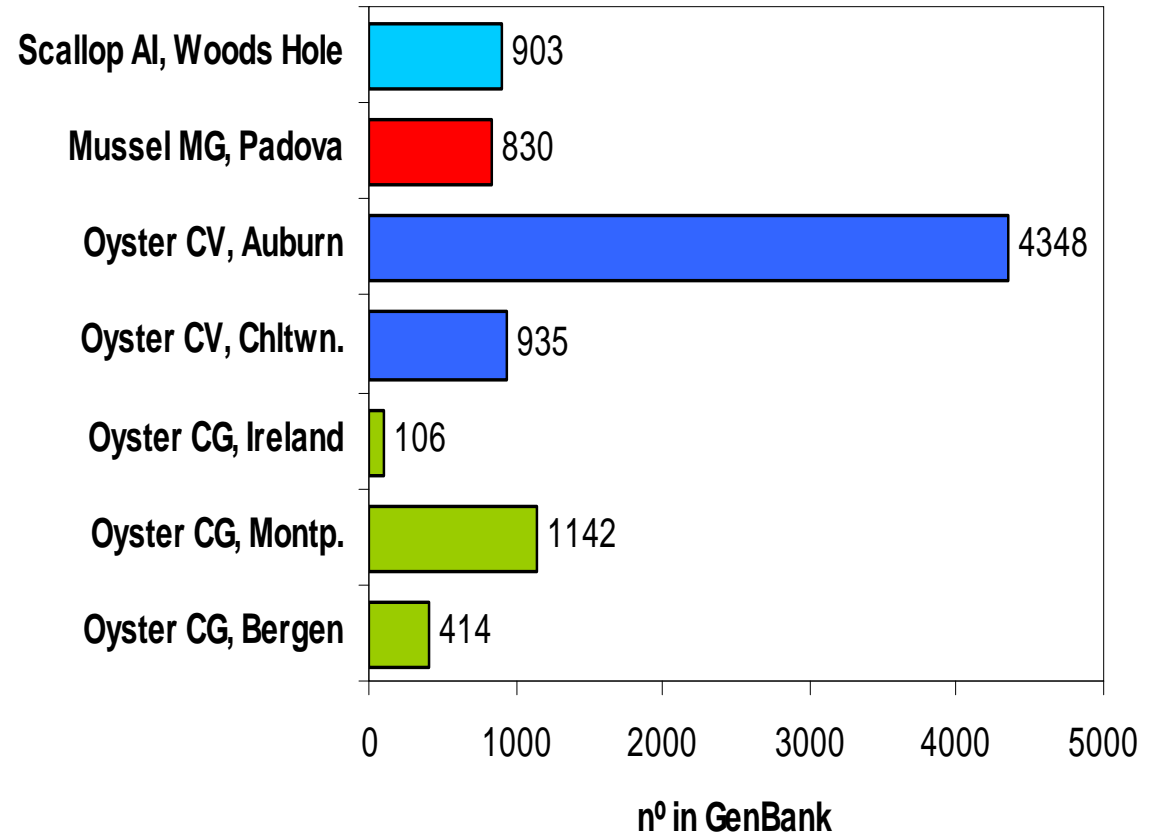
Oyster CG – immune function

(Bachere et al., Montpellier) – 1200 ESTs, 20 immunity genes

– Stress response

(Moraga, et al., Samain et al., Brest)

Mussel MG – genes expressed in normal life conditions vs. polluted – 524 ESTs (Venier; Padova)



http://www.ifremer.fr/GigasBase

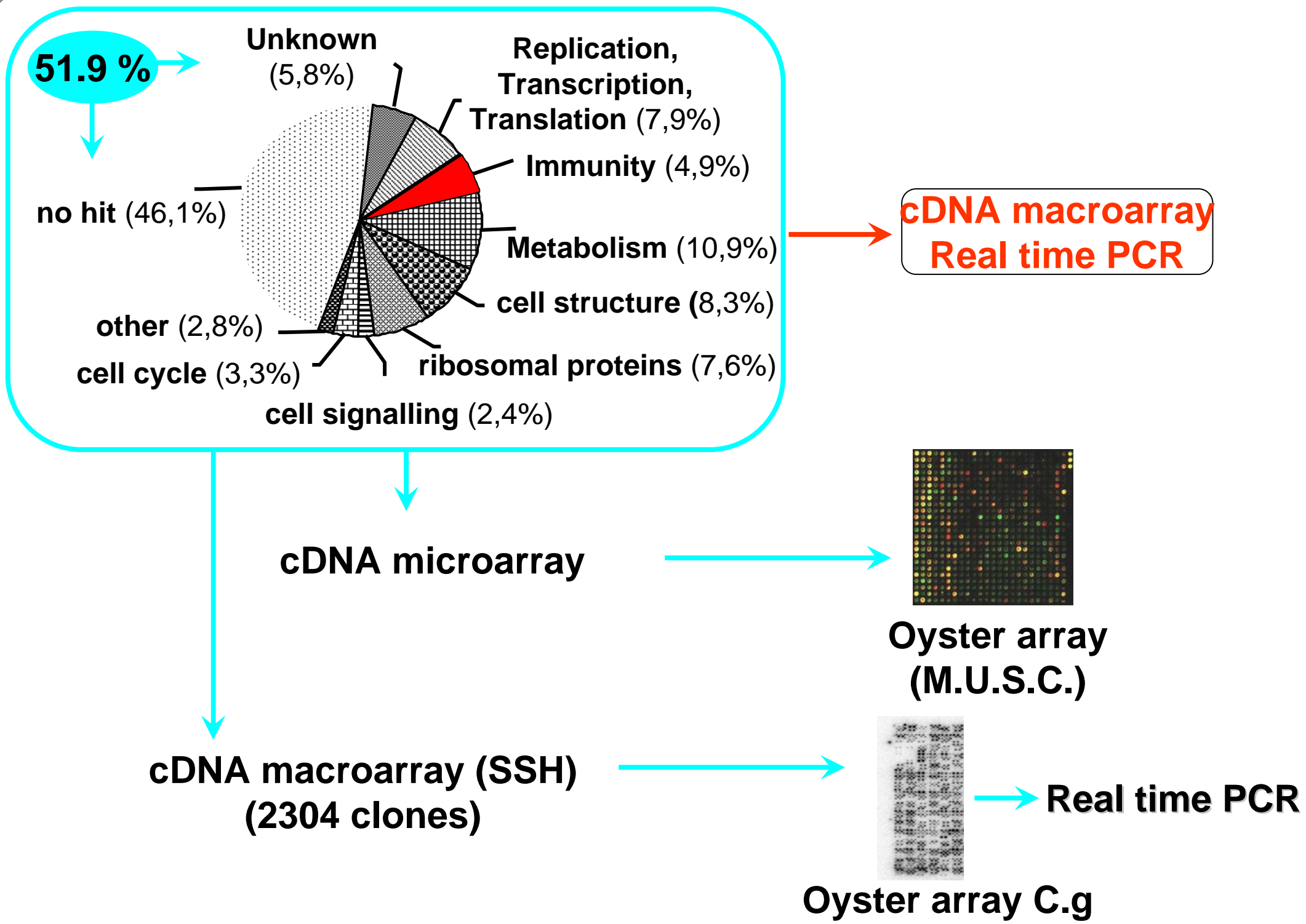
The image shows a composite screenshot of a web browser displaying BLAST results and NCBI sequence viewer for the Cg-TIMP protein. The browser windows are titled "BLAST - Netscape" and "NCBI Sequence Viewer - Netscape".

BLAST - Netscape: The page shows the BLAST results for a query sequence. The title is "BLAST FILE on Contig 53". The query sequence is 734 letters long. The database searched is "genpept" with 1,084,824 sequences. A color key for alignment scores is provided, ranging from <40 to >200. The alignment results show several high-scoring matches.

NCBI Sequence Viewer - Netscape: The page displays the NCBI sequence viewer for the protein AAG42824.1. The protein is identified as "tissue inhibitor of metalloproteinase TIMP (Crassostrea gigas)". The accession number is AAG42824.1. The protein is 221 amino acids long. The source is Crassostrea gigas (Pacific oyster). The authors are Montagnani, C., Le Roux, F., Berthe, F., and Escoubas, J.M. The title is "Cg-TIMP, an inducible tissue inhibitor of metalloproteinase from the Pacific oyster Crassostrea gigas with a potential role in wound healing and defense mechanisms (1)". The journal is FEBS Lett. 500 (1-2), 64-70 (2001). The PubMed ID is 1144223.

Entrez-PubMed - Netscape: The page shows the PubMed entry for the article "Cg-TIMP, an inducible tissue inhibitor of metalloproteinase from the Pacific oyster Crassostrea gigas with a potential role in wound healing and defense mechanisms (1)". The authors are Montagnani C, Le Roux F, Berthe F, Escoubas JM. The journal is FEBS Lett. 2001 Jun 29;500(1-2):64-70. The abstract states: "We have cloned and characterized a cDNA encoding Cg-TIMP, the first tissue inhibitor of metalloproteinase identified in mollusks. The isolated cDNA encodes a protein of 221 residues that has a domain organization similar to that of vertebrate TIMPs including a signal sequence, and the 12 cysteines characteristic of the TIMP signature. Analysis of Cg-TIMP expression in adult oyster tissues, by Northern blot and in situ hybridization, indicates that Cg-TIMP was only expressed in hemocytes which are the key components of defense mechanisms in mollusks. We also observed that Cg-TIMP mRNA accumulated during shell damage and bacterial challenge. This pattern of expression suggests that Cg-TIMP may be an important factor in wound healing and defense mechanisms." The MeSH terms are "Amino Acid Sequence".

InterPro: Tissue inhibitor of metalloproteinase - Netscape: The page shows the InterPro entry for the protein. The protein is identified as "Tissue inhibitor of metalloproteinase". The accession number is IPR001820. The protein is 221 amino acids long. The family is TIMP. The function is "metalloendopeptidase inhibitor activity (GO:0008191)". The component is "extracellular matrix (GO:0005578)". The abstract states: "Tissue inhibitors of metalloproteinases (TIMPs) are a family of proteins [1, 2, 3] that can form complexes with extracellular matrix metalloproteinases (such as collagenases) and irreversibly inactivate them. Members of this family are common in extracellular regions of vertebrate species [4]. TIMPs are proteins of about 200 amino acid residues, 12 of which are cysteines involved in disulfide bonds [5]. The basic structure of such a type of inhibitor is shown in the following schematic representation:"



Studies of Genome Function

➤ cDNA Microarray development

Oyster CV – Environmental genomics – development of gene chips for monitoring environmental pollution (mercury) (Auburn, US)

Oyster CG – For monitoring response to pathogens, to environmental parameters, resistance to summer mortality (France)

Mussel MG - similar approach for hydrocarbon pollution (L. Cornudella; Barcelona)

➤ MSSP (massively parallel signature sequencing)

Oyster CG – Heterosis - patterns of expression in inbred and hybrid larvae (D. Hedgecock, Lynx Therapeutics; CA, USA)

➤ Proteomics

Mussel MG and ME – proteome characterization (G. Alvarez, Spain)



Mitochondrial Genomes in Bivalves

➤ Special mtDNA inheritance – DUI

2 genomes F and M, transmitted through females and males, respectively

Demonstrated in marine Mussels (Mytilidae), freshwater mussels (Unionidae) and clams (Veneridae)

➤ Complete mt Genomes sequenced

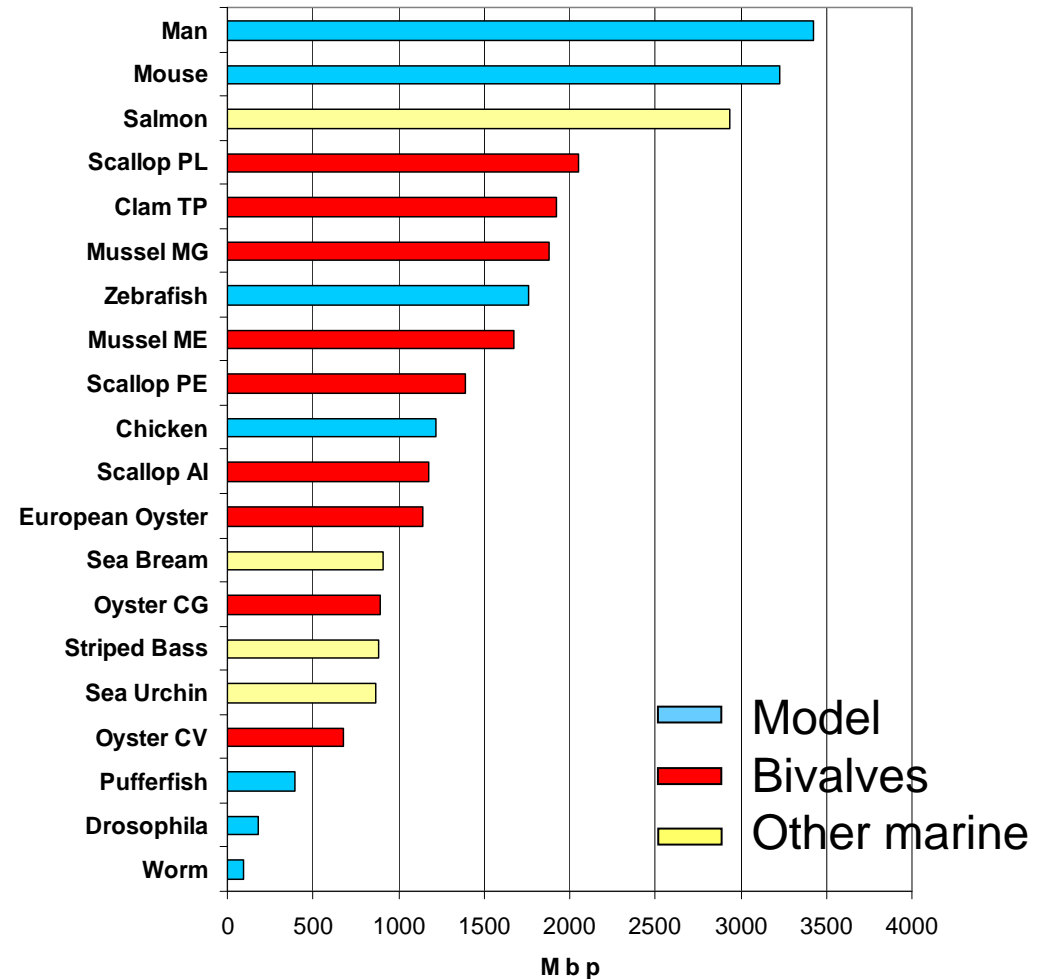
Mussel F and M (Brown, USA; Lunt, Hull; Zouros, Greece)

Clam F and M (Okazaki, Tokyo)

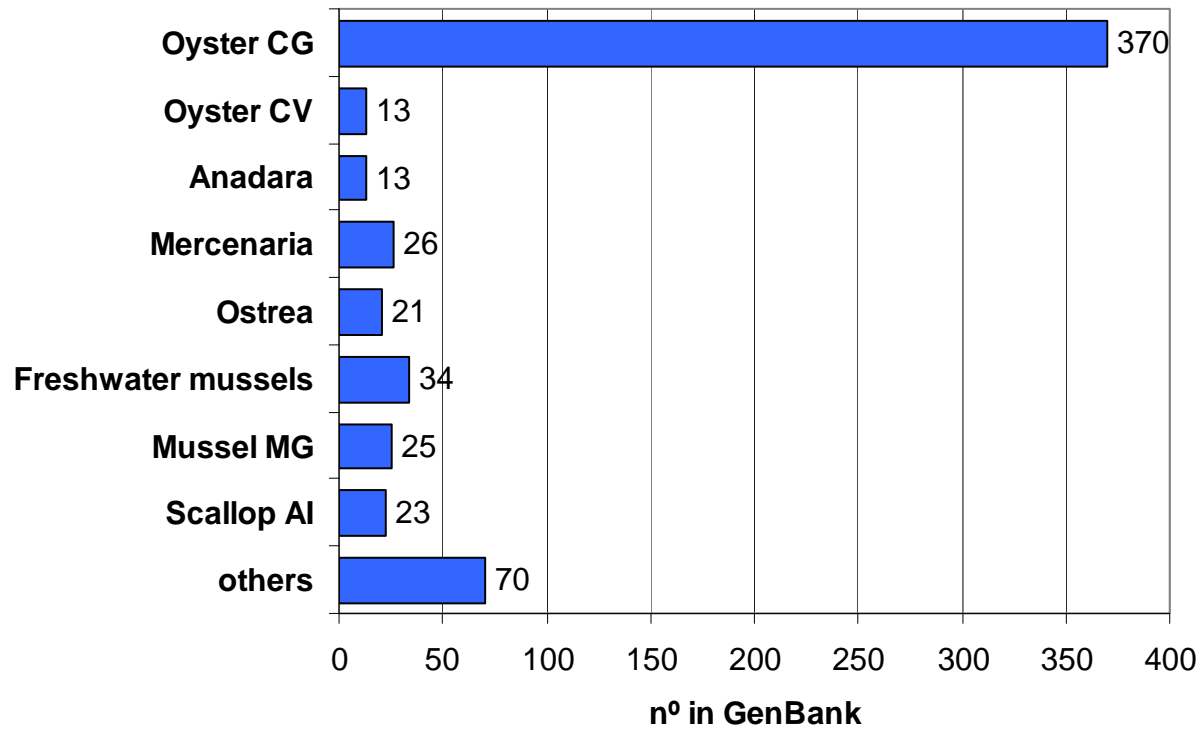
Oyster CG (Kim, S. Korea)

The Oyster Genome Consortium

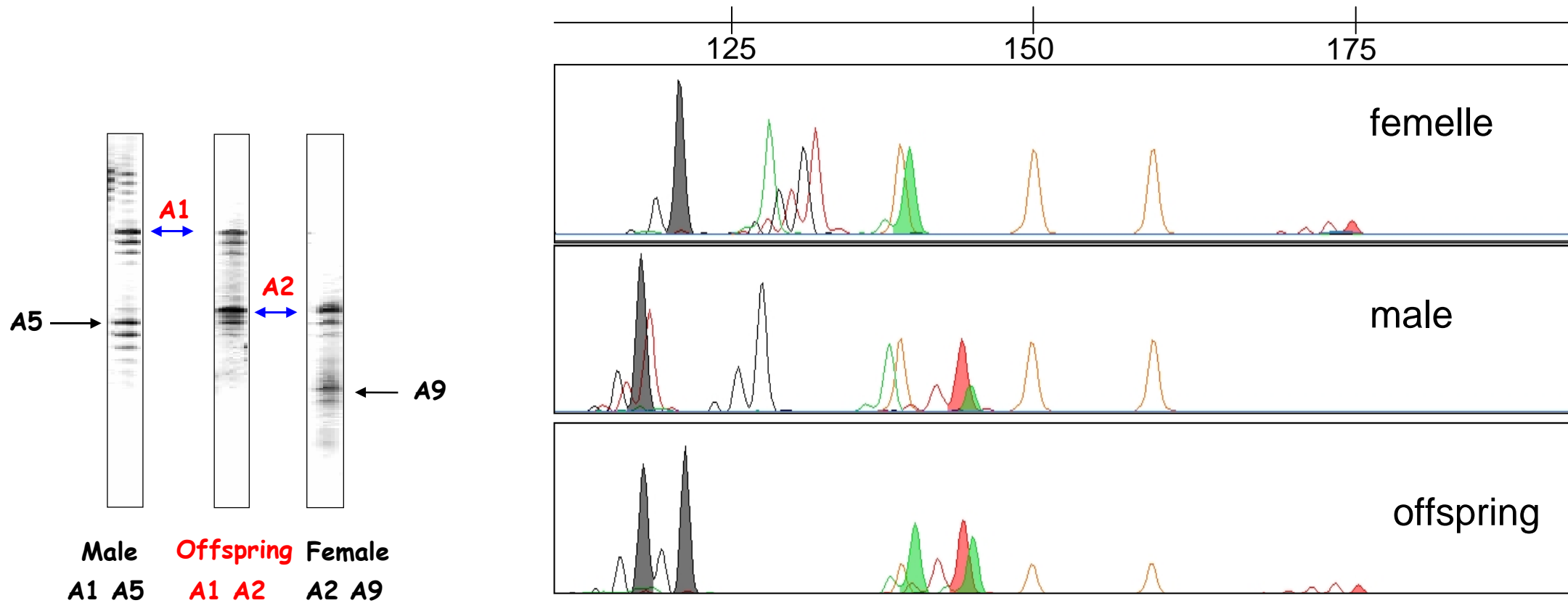
- Constituted in 2003 PAG meeting
- Coordination: Pr. Dennis Hedegeock
- 70 member laboratories
- 10 Countries: 6 European, Australia, Canada, China, Japan, USA
- OGC requested JGI (DOE Join Genome Institute) to sequence *C. gigas* genome.
- This first request was rejected because *C. gigas* genome is too large



Microsatellites in Bivalves



Mixed-family approach using microsatellite-based parentage analysis



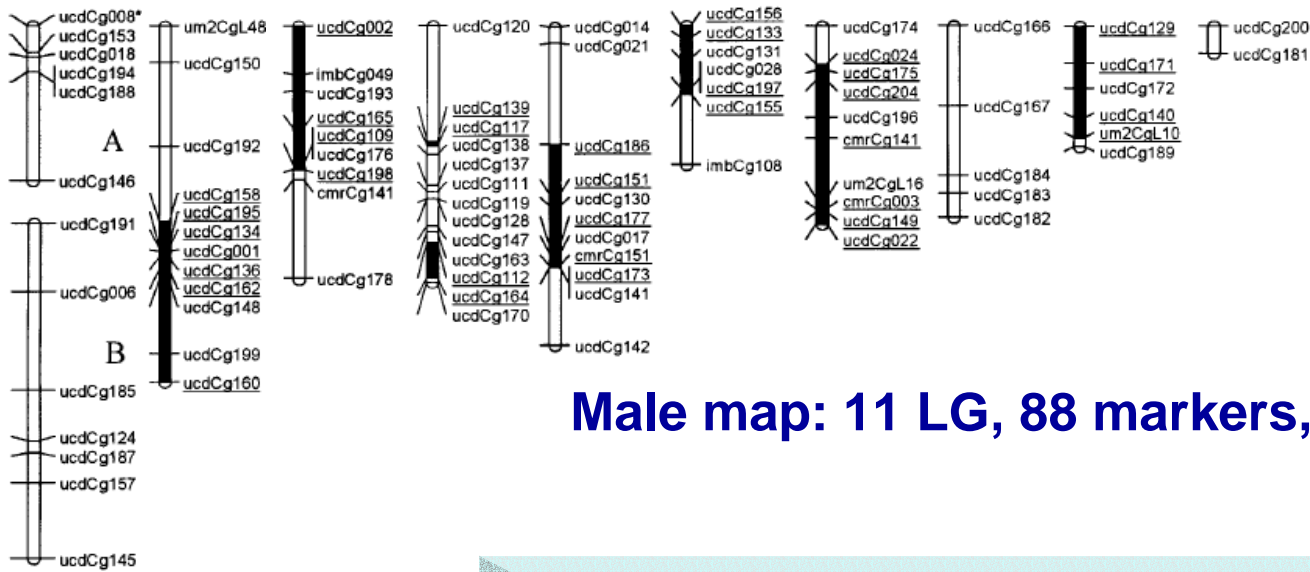
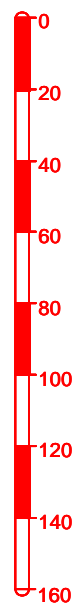
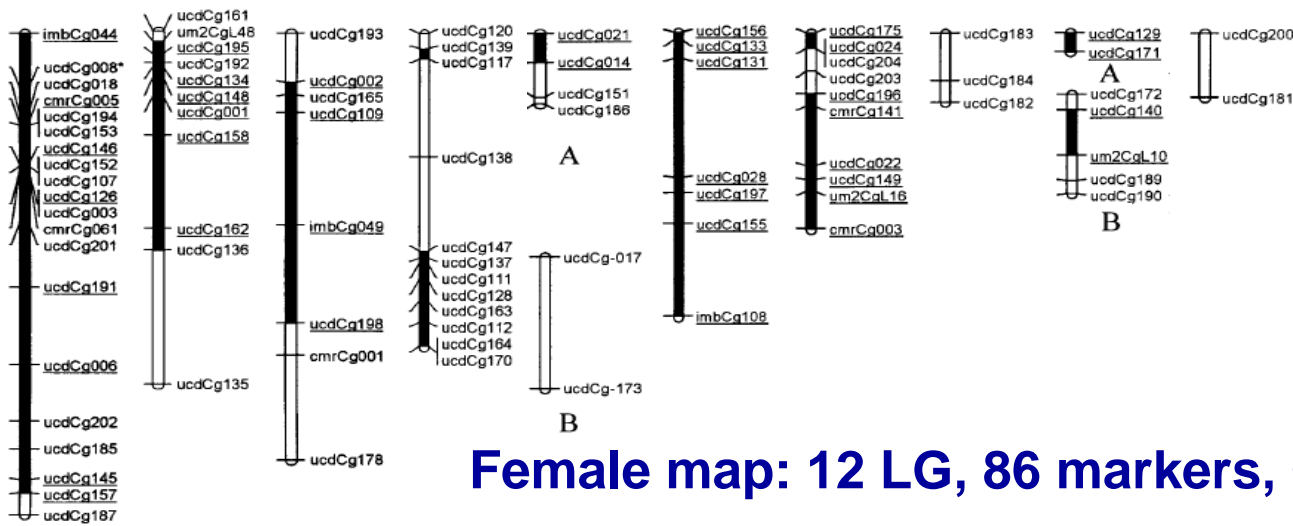
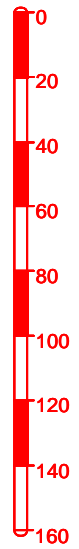
PCR-multiplexed loci (*Taris et al., in press*)

Linkage maps in Bivalves

- Only available in Oyster (n=10)
- *C. virginica* – X. Guo (Haskin SRL, Rutgers)
 - 282 markers: 298 AFLP, 5 microsats, 2 EST
 - 12 linkage groups
 - Physical mapping (FISH) in progress -
- *C. gigas* – D. Hedgecock (USC, CA)
 - 100 microsats, >1100 AFLP
 - 10 linkage groups
 - X. Guo
 - 96 AFLP

Female & Male Linkage Maps of *C. gigas*

size

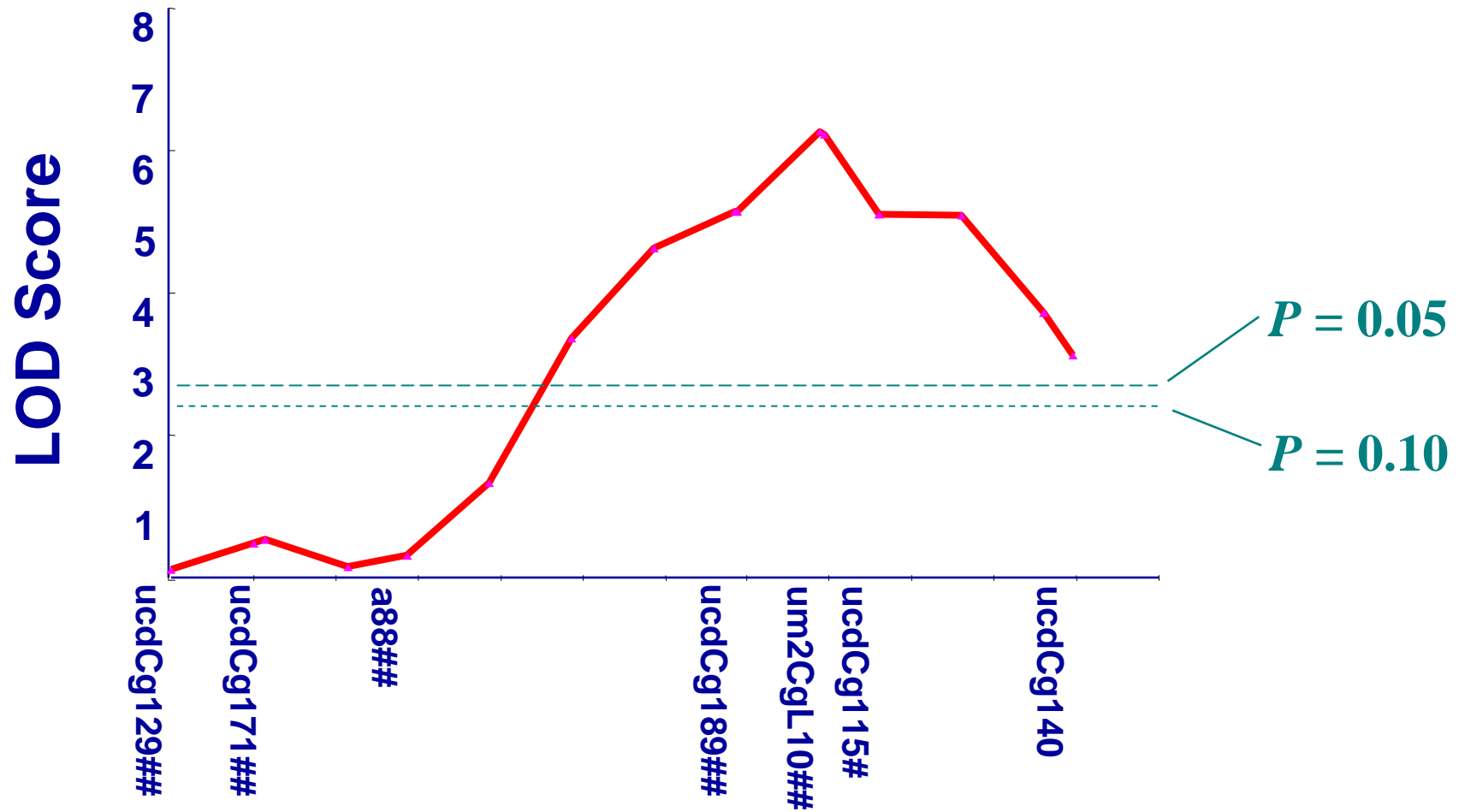


→ Heterogeneity of *r* and gene order!

Tagged F₂: Largest & Smallest



Size QTL on LG IX for 35×51 F₂



Same QTL observed in samples of both indoor- and outdoor-reared, individually tagged juveniles

