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Aspects of Comparative Genomics in the Gilthead Sea Bream, *Sparus auratus*

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Key words: gilthead sea bream, radiation hybrid mapping, linkage mapping, QTL mapping, comparative genomics, co-expressed genes, selective breeding, aquaculture genomics

Extended Abstract

Genome projects for new fish species are found in a transitional period during which resource development and meaningful comparative genomics analysis or use of any other comparative approach must be done in parallel. In new species, full sequencing is still prohibitive due to the high cost of vertebrate genome sequencing and the fact that present technology is time consuming. However, in the case of the few model species whose research is pioneering and where huge progress has been made on developmental, physiological, behavioral, and genomic levels, knowledge integration is in a process of rapid change. These reasons may explain why a general strategy to facilitate the choice of target species and the kind of developments needed has not yet been adopted. In such an environment, BRIDGEMAP, the European gilthead seabream genome project, was completed in December 2005. This was one of the first genome projects for a non-model fish of aquaculture interest.

BRIDGEMAP was carried out in close collaboration with another European project, BASSMAP, for the genetic linkage mapping of the European sea bass, *Dicentrarchus labrax* (http://www.bassmap.org/). Some parallel projects and direct or indirect follow-up projects of BRIDGEMAP, such as AQUAFIRST (http://aquafirst.vitamib.com/), are currently running, while other independent aquaculture genomics projects are on-going in an internationally effervescent genomics environment. This has led to the need for coordination of related aquaculture genomics projects. One is AQUAFUNC (http://ec.europa.eu/research/fp6/ssp/aquafunc_en.htm), the main objective of which is the integration and optimal use of the overall knowledge and functional genomics resources arising from the European Framework programs 5 and 6.

However, despite existing genome sequencing projects, the overall lack of functional information is huge. Therefore, comparative genomics is increasingly applied as a shortcut to link sequence, syntenies, and gene order information, and function. In addition, it constitutes a framework for integration of diverse types of biological data including population genetics, QTL, and expression data. As such, comparative genomics may greatly accelerate the process of linking genes to phenotypes, which may result in a large range of applications.

For organisms in which the complete genome is available, comparative approaches have been very efficient. Nevertheless, more species amenable to such analysis are needed to serve as operational stepping stones between genomes. Economically important fish species are priority candidates due to their

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socio-economic interest and the considerable information produced on aspects of their husbandry, physiology, biology, and pathology. For these species, however, comparative genome studies are difficult due to a huge lack of molecular resources.

**Brief Review of Results**

Gilthead sea bream (*Sparus auratus*), a key species of Mediterranean aquaculture, belongs to the species-rich Sparidae family, Perciformes order. From a phylogenetic viewpoint, this order is close to *Fugu rubripes* and *Tetraodon nigroviridis* which have fully sequenced genomes. It is even closer to medaka (*Oryzias latipes*), whose draft genome sequence can be accessed at http://dolphin.lab.nig.ac.jp/medaka/ and which is a very elegant research model for functional analysis and mutation mapping (Akihiro et al., 2003; Henrich et al., 2004). It is also close to the zebrafish, *Danio rerio* (draft sequence available at http://www.sanger.ac.uk/Projects/D_rerio/), that was the initial target for the comparative genomics of sea bream (Glaser, 2005; Dahm and Geisler, 2006). Contrary to the traditional relationships between model fish species and the Perciformes order such as the gilthead sea bream, Steinke et al. (2006) showed that medaka (Beloniformes), and cichlids (Perciformes) are more closely related to each other than to pufferfish (*Tetraodontiformes*).

The gilthead sea bream resources and information comprise a genetic linkage map based on about 200 markers (Franch et al., 2006), a radiation hybrid panel that allows easy mapping of thousands of markers and genes (Senger et al., 2006), a radiation hybrid map comprising about 1000 molecular markers and genes (Senger et al., 2006; Sarropoulou, unpublished), cDNA libraries and expressed sequence tags (ESTs) of different tissues (Louro and Power, 2005), polymorphic microsatellite markers (Brown et al., 2005; Franch et al., 2006), population genet-ic-genomics analysis of environmental and aquaculture samples at the species range for stock structure analysis and traceability (Tsavaloutas et al., in prep.; Louro and Power, in prep.), optimized protocols for chromosome set manipulation for mito-gynogenesis and mito-androgenesis (Gorshkov et al., 1998), and a high quality 6X coverage BAC library. In addition, a partial QTL project for weight at commercial size is in progress, parallel to a breeding program based on parental identification after mass spawning (Batargias et al., in prep.).

Comparative analysis has been conducted both *in silico* and experimentally. It comprises comparative mapping using the recombination independent radiation hybrid map and the genetic linkage map. The radiation hybrid was mainly anchored to *Tetraodon* on the basis of expressed sequences and revealed considerable synteny conservation with *Tetraodon* where, in some cases, gene order was conserved within long syntenic groups (Senger et al., 2006; Sarropoulou et al., unpublished). Surprisingly, the linkage map could be anchored to the *Tetraodon* map, as a considerable number of microsatellites gave significant hits to the *Tetraodon* genome based on analysis of their flanking regions (Franch et al., 2006), very similar to the case of another Perciformes species, *Cottus gobio* (Stemshorn et al., 2005). For the gilthead sea bream linkage map, males derived recombination events in some linkage groups less frequently than females, while in other chromosomes the reverse occurred, making sex specific maps that differed for different linkage groups. Nevertheless, at the whole genome level, the length of the male and female maps did not differ significantly. Given that sea bream is a sequential hermaphrodite, this result is both surprising and interesting. Comparison of the radiation hybrid map and the linkage map gave fairly consistent results. The distribution of recombination frequency across the genome could be mapped at the level of resolution of the radiation hybrid map, since it is independent of recombination and closer to a physical map (Sarropoulou, unpublished).

An experimental shortcut to comparative mapping has been the use of cross species amplification of mapped microsatellite markers, an approach that may lead to comparative linkage maps. We applied this approach at an
exploratory level with considerable success within Sparid species (Brown et al., 2005; Franch et al., 2006) and between gilthead sea bream and the European sea bass, *D. labrax* (Chistiakov et al., 2005; Tsigenopoulos et al., in prep.).

The use of a comparative genomics framework for integration of different aspects of biological research was exemplified by mapping genes that were co-expressed in microarray analysis on the radiation hybrid map, an approach we are following by increasing the number of mapped genes for genes with available expression analysis (Sarropoulou et al., 2005).

The above results reveal the high potential of combining genetic approaches based on polymorphic markers (such as QTL analysis) with candidate gene approaches (by using SNP information) for the establishment of gene-to-phenotype links and achievement of efficient selective breeding programs. The produced resources make the gilthead sea bream a good target species for genomic applications in aquaculture, as well as for assessing aspects of the development and biology of the species and its adaptation to different environmental conditions including domestication.

**References**


Genetic Basis of Sex Determination in Fishes: Searching for Master Key Regulator Genes in the Sex Determination Pathway of Tilapias*

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Key words: genome mapping, candidate genes, sex determination, tilapia, Oreochromis spp.

Abstract

Recent results show a striking similarity in the dynamics of expression of gonad differentiation regulators in zebrafish and mammals. Candidates for the role of master key regulators (MKRs) in tilapia were selected, based on three concepts established in the literature for non-mammal species. These three concepts are: (a) MKRs are DM-proteins or their close downstream/upstream partners in the sex determination pathway, (b) MKRs are genes working just upstream of aromatase in the sex determination pathway, or the aromatase itself, and (c) MKRs are homologs of mammal genes which are close partners of SRY (“missing” in non-mammal vertebrates), mostly belonging to the SOX family. Coding sequences of putative genes were searched in cichlid (TIGR) and general (NCBI) databases, and in the tilapia gonad EST library (RBEST). Primers in two adjacent exons were designed, based on predicted exon-intron boundaries for each of 11 selected genes. Amplified segments of the targeted genes in two purebred tilapia species were sequenced. Seven SSLP and four SNP-based markers were identified in the candidate genes for MKRs of sex determination and mapped to the tilapia genetic map using genotype data of 76-90 individuals of the F2 mapping family. The mapping positions of the selected genes relative to previously reported QTL regions for sex determination are discussed.

* The full paper: Amh and Dmrt2 Genes Map to Tilapia (Oreochromis spp.) Linkage Group 23 within QTL Regions for Sex Determination, by Andrey Shirak, Eyal Seroussi, Avner Cnaani, Aimee E. Howe, Raisa Domokhovsky, Noam Zilberman, Thomas D. Kocher, Gideon Hulata and Micha Ron has been accepted for publication by Genetics.
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