ADAPTATION TO EXTREME SALINITY VARIATIONS IN TILAPIAS

H. D'Cotta¹, E. Pepey¹, M. Tine², N. Ouattara³, J. F. Baroiller¹, E. Bezaulet¹, J. D. Durand², F. Bonhomme², G. Charmantier³, P. Morissens¹, J. P. Poivey⁴, and B. Chevassus³

¹ CIRAD-EMVT, UPR20 Aquaculture et Gestion des Ressources Aquatiques, Campus International de Baillarguet, TA 30/A, 34398 Montpellier cedex 5, France
² IRD-UR070 & UMR 5171 Génomé, Populations, Interactions, Adaptation, Station Méditerranéenne de l'Environnement Littoral, 1 quai de la Daurade 34200 Sète, France
³ INRA, Laboratory of Fish Genetics, F-78352 Jouy-en-Josas, France
⁴ INRA, CIRAD, Campus International de Baillarguet, TA 30/A, 34398 Montpellier cedex 5, France

There is a growing concern for freshwater availability and accessibility in the near future particularly regarding aquaculture purposes, in view of the competition for freshwater resources. Brackish water farming or expansions to seawater have enormous fish farming potentials. Resistance to salinity has become a major challenge for tilapia culture. Apart from tilapia’s numerous good farming attributes, extensive cultures of tilapia together with prawn in brackish waters have contributed in prawn disease prevention and therefore, saline-tilapia farming could also promote the development of prawn industry in coastal tropical regions. The majority of tilapias live in freshwaters, amongst which figures the major commercial species, Oreochromis niloticus an extremely robust and fast-growing species. Only a few species such as Oreochromis mossambicus and Sarotherodon melanotheron are euryhaline fish, tolerating the salinities found in brackish or higher saline waters. One approach to increase the salinity tolerance of commercially important species is the selection programme of resistant strains. The other, is through physiological studies aimed to search for the physiological mechanisms of salinity adaptation, a complex fish trait.

Within the group of tilapias, various hybrids have been produced either to obtain all-male progenies or to combine the zootechnical characteristics of two parental species. Most of these hybridizations have involved 2 species of a same genus, especially Oreochromis, the major commercially important species. Hybridization can therefore be a way to combine two important aquaculture traits, the good growth-rate of one species with the salinity tolerance of another species. This procedure could be developed in countries where both fish species already exist.

In order to analyze the feasibility of this type of approach, we produced reciprocal hybrids between a species presenting a rapid growth rate O. niloticus (Bouaké strain-Ivory Coast), and an extremely tolerant, euryhaline species Sarotherodon melanotheron (Lagune Ebrié-Ivory Coast) by artificial fertilization and incubation. F1 (O. niloticus x S. melanotheron) and F’1 (O. niloticus x S. melanotheron) hybrids are viable and fertile. Fertilization success and larval survival rates in these hybrids are similar to those of the parental species. Microsatellite analyses showed normal hybrid meiosis, leading to the maintenance of a stable hybrid gene
pool through generations. Cytogenetic analyses using karyotype, fluorescence in situ hybridisation (FISH) and comparative genomic hybridisation (CGH) confirmed the existence of the same diploid chromosome formula (2n=44) in both the hybrids and the pure parental species, as well as the presence of a similar genomic structure between them. Furthermore, hybrids presented an intermediate morphology relative to the parental species, with larger phenotypic variance. Therefore in tilapia hybrids, the genes are combining and associating in similar ways to what they do in pure species.

These tilapia characteristics have allowed us to conduct a selective breeding program (named Molobicus) of interspecific hybrid populations in the Philippines, in order to produce a new strain of tilapia that grows quickly in an elevated salinity environment. The first phase of this project was the hybridisation of *O. niloticus* (GIFT) a rapid growing strain, with *O. mossambicus* which has a high tolerance to salinity, followed by further backcrosses of the hybrids with *O. mossambicus*, until an acceptable resistance to salinity was reached. The second ongoing phase is the selection of the fast growing trait from the interspecific hybrid population. Salinity tolerance of the successive generations of hybrids has been determined through direct and indirect transfer challenges to seawater. After two generations of successive backcrosses with *O. mossambicus*, H2 and H3 hybrids present very similar salinity tolerances to that of *O. mossambicus*. This selection programme is aimed to be combined with prawn farming and thus will help to prevent disease problems.

Some tilapia species have been found to have an enormous plasticity towards different environmental water salinities. In Senegal, several wild populations of *Sarotherodon melanotheron heudelotii* have colonised a wide variety of water environments, ranging from fresh to hypersaline waters. These tilapias show also an extraordinary flexibility in their salinity tolerance related to tidal rhythms and freshwater discharge during the rainy season. The brackish fish can tolerate salinities ranging from 4% to 40% while the hypersaline population tolerates salinities from 56% up to 130%. These four populations (fresh, brackish, marine and hypersaline) are therefore naturally submitted since hundreds of generations to different environmental selective pressures. They present a double interest because their comparisons permit both physiological and genetic approaches to be applied for the analysis of salinity adaptation mechanisms, and therefore these fish constitute ideal models. We have initiated work on the adaptive response of these *S. melanotheron* populations analysing the physiological bases as well as the genetic components of these fish to salinity pressures.
Figure 1. Protocol of a direct transfer to seawater 35% and successively to hypersalinity 70% or re-transferred back to freshwater 0%. Sampling times are shown. They were performed on juvenile S. melanotheron fish. Gills, brain, intestine and kidney were sampled.

Part of the study has been greatly facilitated by performing experimentations under controlled conditions using the two extreme populations living in freshwater 0% (FW), and in the hypersaline waters ranging between 56% to 130% (HSW), which were collected from the wild and transferred to our experimental facilities at Cirad, France. Comparison of their salinity tolerance is being undertaken to search for the molecular mechanisms involved in salinity adaptation, by analysing and identifying the saline differentially expressed genes. From a physiological point of view, we can probably consider that adaptation to salinity really involves two major stages: 1) a period of salt tolerance and 2) a slower process of salinity adaptation.

The first period is a short-term response and rather rapid, which we have named the period of “salt tolerance”. When a fish enters higher salinities, it will be faced with an immediate water loss and passive entrance of salts through the skin and gills. The fish will start drinking to compensate, and salts will then pass across the intestine and to be finally excreted in the gills. This ion influx will result in elevation of plasma osmolarity, to which the fish must respond rapidly. At this stage compensatory adjustments are activation of transport mechanisms already existing and possibly expression of certain genes after ACTH or catecholamine surge. The period which we have called “salinity adaptation” is probably initiated with cellular and endocrine mechanisms of compensation. These will lead to the synthesis of hormones and enzymes known to be enhanced in saltwater as well as the development of new chloride cells, and will ultimately permit fish adaptation. An inadequate osmoregulation when facing changes in salinity will result in mortality but it can also cause stagnant growth, which may not be evident initially.

We are interested in studying both processes in order to better understand the trait of salinity adaptation. We have therefore started, by using under experimental conditions both the FW and HSW populations and have submitted them to salinity challenges (0% to 35% to then to 70% or 0% to 35% and re-transfer to 0%) (Fig. 1). Various organs have been collected in order to study the molecular mechanisms of short term salinity tolerance (after a few hours) and the long-term responses (after 7 days to 45 days of transfer). We have
nevertheless focused our genomic research on the gills since they are the major osmoregulating organ. Total ARN was extracted from the gills of hypersaline individuals to create differentially expressed cDNA libraries between 2 conditions, by using suppressive subtraction hybridisation (SSH). Five cDNA libraries (Table 1) have been constructed and currently 1536 clones have been sequenced from clones randomly picked from four of the SSH libraries (384 clones / library).

Table 1. cDNA libraries produced from the gills of S. melanotheron challenged to different salinities

<table>
<thead>
<tr>
<th>Name of cDNA library</th>
<th>Tissue</th>
<th>Sampling time after transfer to % salinity</th>
<th>Salinity treatment</th>
<th>SSH Driver and tester</th>
</tr>
</thead>
<tbody>
<tr>
<td>SmelSSH-A-0</td>
<td>Gills</td>
<td>45 days in 0% freshwater</td>
<td>Freshwater, no salinity treatment</td>
<td>Tester : FW 0%  Driver : SW 35%</td>
</tr>
<tr>
<td>SmelSSH-A-35</td>
<td>Gills</td>
<td>45 days in 35% saltwater</td>
<td>Direct transfer to 35%</td>
<td>Tester : SW 35%  Driver : FW 0%</td>
</tr>
<tr>
<td>SmelSSH-A-t4-35</td>
<td>Gills</td>
<td>4 hours after transfer to 35%</td>
<td>Direct transfer to 35%</td>
<td>Tester : SW 35% 4h  Driver : FW 0%</td>
</tr>
<tr>
<td>SmelSSH-B-70</td>
<td>Gills</td>
<td>45 days in 70% saltwater</td>
<td>Direct transfer to 35%, 10 days adaptation and direct transfer to 70%</td>
<td>Tester : HSW 70%  Driver : FW 0%</td>
</tr>
<tr>
<td>SmelSSH-B-0</td>
<td>Gills</td>
<td>45 days in 0% freshwater</td>
<td>Direct transfer to 35%, 10 days adaptation and direct transfer to freshwater 0%</td>
<td>Tester : FW 0%  Driver : HSW 70%</td>
</tr>
</tbody>
</table>
Annotation of these ESTs was performed following Gene Ontology assignments.

**Figure 2.** Schematic representation of the functional classification of the *S. melanoatheron* ESTs obtained from the 35% and the 70% SSH cDNA libraries.

Small macroarrays on nylon membranes containing 780 spots (384 clones spotted in duplicates plus some candidate genes and controls) have been produced. They will be used to further select some of the genes involved in salinity tolerance and adaptation to different salinities, by hybridizing the gill RNAs from individuals sampled at the different salinity challenges.

We are simultaneously analysing by real-time PCR the expression profiles and kinetics of some genes identified by EST sequencing. This functional genomic study is coupled to the analysis of changes occurring in the gill morphology, using biochemical and molecular parameters.

**REFERENCE**


