Morphological and genetic differentiation of West African populations of *Sarotherodon melanotheron* Ruppel, 1852 (Teleostei, Cichlidae)

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Introduction

Sarotherodon melanotheron is a tilapia species which lives in lagoons and estuaries from Senegal to Angola. It is also found in the sea, notably off the coast of Dakar or in Guinea. TREWAVAS in 1983 described five subspecies based on morphological characteristics whose distributions follow:

- *S. m. paludinosus*, in some freshwater regions of Dakar
- *S. m. heudelotii*, from Senegal to Guinea
- *S. m. leonensis*, from Sierra Leone to Liberia

- *S. m. melanotheron*, from Côte d'Ivoire to Cameroon
- *S. m. nigripinis*, from the Rio Muni to Zaïre.

This species, because of its good salinity tolerance interests aquaculturists. However, the low (0.45 g/d) daily growth rates observed until now have discouraged its use.

In this context, the research on genetically differentiated populations would be the first step in the quest for populations having zoo-technical aptitudes of interest for aquaculture. In effect, we can hope that an eventual genetic differentiation would be accompanied by physiological type differences and display the latter to their best.

Material and methods

This work was carried out under the auspices of the Genetics progam, in the ichthyology laboratories in Tervuren (Belgium), the genetics lab of the Centre de Recherches Océanologiques in Abidjan (Côte d'Ivoire) and by the Genome and Populations laboratory of Montpellier (France). Twenty-nine samples were analyzed consisting of 911 specimens coming from different West African hydrographic basins (fig.1): In Sénégal, Saint Louis (2 samples), Lake Redba, Hann, Dakar, Somone, Kaolack, Foundiougne, Missirah; in Gambia, Banjul; in Guinea, Diouloulou, Kandiaffara, Koba, Forrecariah; In Ivory Coast, Grand Bérébi, Grand Lahou (2 samples), Tiegba, Adiopodoumé (3 samples), Lake Bakré, Biétry Lagoon (2 samples), Anga (2 samples), Lake Ayamé; in Benin, Cotonou; in Congo, Bas Kouilou.

In the morphological study, 22 measurements were taken on each specimen. These were: (1) total length, (2) standard length, (3) head length, (4) snout length, (5) eye diameter, (6) inter-ocular distance, (7) preorbital bone length, (8) width of the toothed zone of the pharyngial bone, (9) length of the pharyngial bone, (10) body height, (11) caudal peduncle height, (12) cadudal peduncle length, (13) predorsal distance, (14) prepectoral distance, (15) preventral

distance, (16) preanal distance, (17) dorsal fin length, (18) greatest dorsal spine length, (19) pectoral fin length, (20) ventral fin length, (21) anal fin length, (22) third anal spine length (upper, lower and on the caudal) and number of scales around the caudal peduncle.

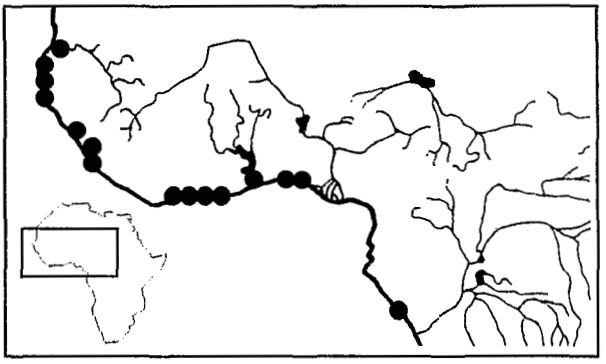

Fig. 1
Collecting sites of *S. melanotheron* samples.

The meristic characteristics analyzed for each specimen follow: number of branchiospines on the lower part of the first branchial arch, number of spines in the dorsal fin, number of branched rays in the dorsal fin, number of spines in the anal fin, number of branched rays in the anal fin, number of scales in the lateral line.

Statistical analyses of the data were carried out using the CSS: Statistica program (Statsoft, version 3.1).

Electrophoretic enzymatic protein analysis and the microsatellite study are the two methods used in the populations' genetics study. Concerning the enzymatic protein electrophoresis, 27 loci were analyzed. The genetic variability was evaluated with the help of two indices: i) the polymorphism rate P which corresponds to the number of polymorphic loci compared to the total number of loci

studied, ii) mean heterozygosity (H) calculated using NEI'S formula (1978).

The genetic divergence among the populations was calculated using the "Neighbor joining UPGMA method" (SAITOU and NEI 1987) with the Neighbor program by J. Felsenstein (Department of Genetics, University of Washington, Seattle, Washington 98195).

The allele frequency table was transformed into an allele present/absent matrix (1/0). This matrix was treated with the parsimony algorithm (ECK and DAYOFF, 1966 ; KLUG and FARRIS, 1969) from J. Felsenstein's Mix program.

The microsatellite study consisted of the analysis of the following 4 loci : 79 M, 79 H, 73 C and locus 28. Mean genetic diversity was estimated using the mean theoretical heterozygosity rate (H). Genetic differentiation among samples was evaluated with WEIR and COCKERHAM'S (1984) unbiased estimator. Reynolds distances, determined using estimators, allowed the construction of a phylogenetic network using the Neighbor joining method of the Phylip program of J. FELSENSTEIN (1989).

Results and discussions

The various types of principal component analyses carried out on the morphometric data (*i. e.* analyses of metric data alone or in association with merisitic data) gave almost identical figures. In this figure (fig. 2), all the populations overlap either partially (the case of Senegal and Guinea) or completely as is the case of the Gambian population being overlapped by those of Côte d'Ivoire, whereas the Congo population is the more isolated.

The Congo population's isolation is due to the fact that the latter clearly distinguishes itself by the combination of the following characteristics: head length, snout length, length/width relationship of the lower pharyngial bone, dorsal fin length, ventral fin length, anal fin length, number of branchiospines on the lower part of the

first branchial arch and number of branched rays in the dorsal fin. This population corresponds to the description of the *S. m. nigripinnis* population given by TREWAVAS (1983). The Côte d'Ivoire populations belong to a single subspecies which according to TREWAVAS, 1983 is the *S. m. melanotheron* subspecies. In the Guinea populations, TREWAVAS' 1983 criteria show that the Bofon population belongs to the subspecies *S. m. leonensis*. The second population is that which is overlapped by those of Senegal and Gambia. According to TREWAVAS' 1983 definition, these Guinea, Senegal and Gambia populations belong *to S. m. heudelotii*.

Finally, the partial overlapping observed between all the populations shows the necessity of refining the notion of subspecies as proposed by TREWAVAS (1983).

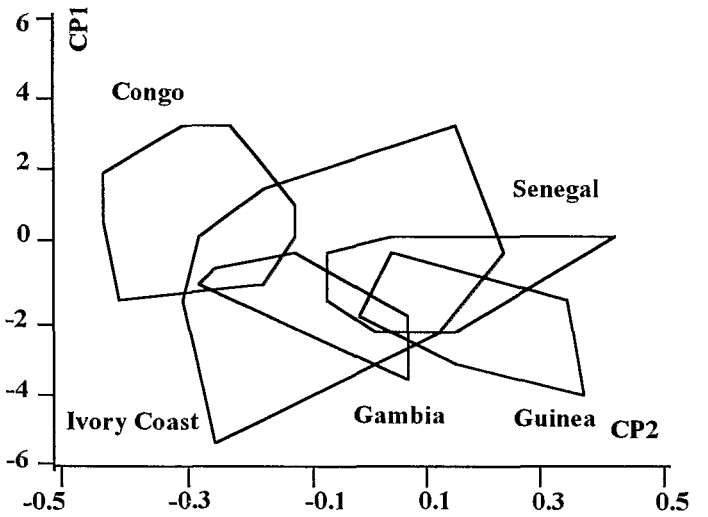

Fig. 2
result of principal component analyses carried out on the morphometric data (*i. e.* analyses of metric data alone or in association with merisitic data).

Seventeen of the 27 loci analyzed by enzymatic protein electrophoresis were polymorphic. Some were expressed by 4 or 5 alleles.

If we observe the allele frequency distribution at the Aat-2, Acp-1 and Gpi-2 loci, in all of the samples analyzed, we notice that the genetic differentiation of *S. melanotheron* presents as a clinal geographic variation (Fig. 3). The three clines observed are all superimposed. The slopes, more or less abrupt, are located in the region between the Guinea-Sierra Leone border (point 13) and the Liberia-Côte d'Ivoire border (point 14). This region represents the transition between the two relatively differentiated forms.

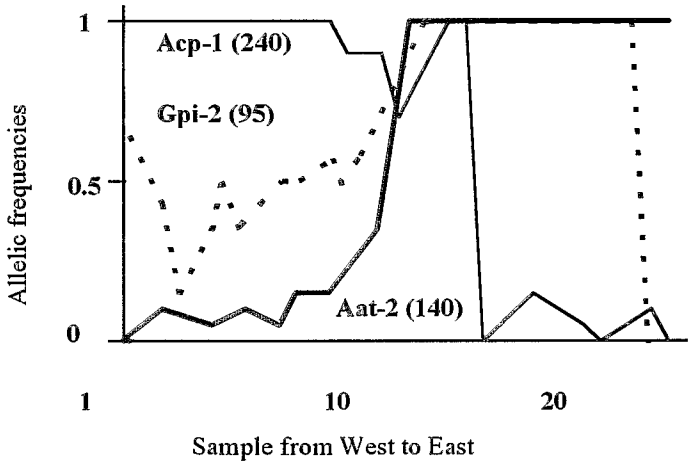

Fig. 3
Clinal geographic variation of the allele frequencies distribution at the Aat-2, Acp-1 and Gpi-2 loci, in all of the samples analyzed.

The mean polymorphism rate is 13.33 %. This rate is lower than that found in *Tilapia guineensis* (16.9 %); but it is higher than that found in *T. zillii*: 3.45 % .

The mean heterozygosity rate is 4.4%. This rate is comparable to those found in *Tilapia guineensis* (6.4%) and *T. zillii* (3.4%). This rate is also close to that estimated by McANDREW and MAJUMDAR (1983) in *S. gallileus* (4.3%) and by ROGNON (1993) in *Tilapia guineensis*.

In table 1 samples were regrouped by region. A relationship exists between the values of these parameters and the geographic location of the populations. In effect, it is in the Western zones of West Africa, that is, Senegal and Guinea, that the genetic variability is the greatest. Contrarily, in the more Eastern regions like Côte d'Ivoire, Benin and Congo, the genetic variability is half as great.

Geographic area	Senegal	Guinea	Ivory Coast	Benin-Congo
Polymorphism (%)	17.15	17.26	9.68	9.25
Heterozygosity	0.06±0.02	0.057±0.02	0.032±0.02	0.026±0.01

Table 1
Geographic variations of polymorphism and heterozygosity in *S. melanotheron* populations.

The geographic distribution of allele frequencies, polymorphism and heterozygosity rates show the presence of two relatively differentiated groups.

From the genetic distance matrix (NEI, 1978), a dendrogram was made using the "Neighbor joining" method. The branch length is proportional to the genetic distance separating the populations. We find the two previously defined groups: the Senegal and Guinea populations on one hand, the Côte d'Ivoire, Benin and Congo populations on the other. A more detailed analysis shows that the Lower Kouilou population from Congo is very differentiated from the other populations from Côte d'Ivoire or Benin. In fact, it forms an independent subgroup.

Finally, a dendrogram (Fig. 4) that was obtained with the parsimony technique using an alleles present/absent matrix allowed observation of the same previously mentioned groupings of the populations.

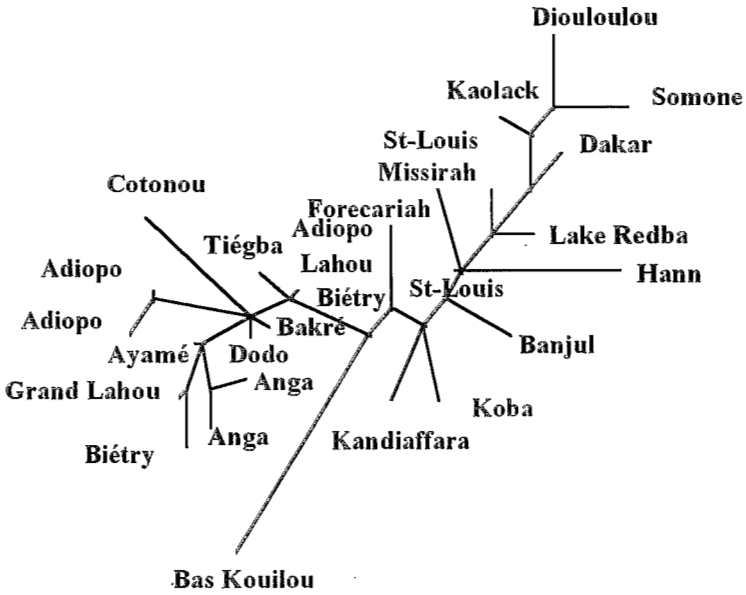

Fig. 4
Dendrogram obtained with the parsimony technique using an alleles presence/absence matrix.

If the different subspecies studied are shown on the figures, we see an affirmation of the groupings based on the morphological systematic and those revealed through enzymatic protein electrophoresis.

- The populations from Senegal and Guinea belong to the subspecies *S. m. heudeulotii*,
- The populations from Côte d'Ivoire and Benin belong to the subspecies *S. m. melanotheron*,

- The population from the Lower Kouilou in Congo belong to the subspecies *S. m. nigripinnis*.

Concerning the microsatellites, two (79M, 79H) of the four loci analyzed were monomorphic in all samples. The loci 28 and 73C had 6 and 26 alleles respectively. The mean theoretical Heterozygosity (H) varies from 32 to 78%. All samples presented relatively elevated value. Only the Cotonou sample where the specimens are homozygous for all loci studied showed a null mean heterozygosity value. The genetic differences are few, of a strictly qualitative order, and concern few of the alleles of which only allele 126 of locus 28 distinguishes the two groups. The first group is made up of samples from Western Côte d'Ivoire. The second includes not only samples from Côte d'Ivoire, but also those from the Eastern regions of this country. These groups are identical to those revealed by enzymatic electrophoresis. These two groups are also found in the phylogenetic network built using genetic distances. However, the microsatellite markers analyzed in this study did not allow identification of the different subspecies described by TREWAVAS (1983) for this region.

Conclusion

The morphological and genetic study of *S. melanotheron* populations has clearly shown that these latter can be placed into three groups, each one corresponding to a subspecies described by TREWAVAS (1983). However, our results show the need for the refinement of the classification proposed by TREWAVAS (1983). These results show that the genetic differentiation between the populations is clinal. It is therefore difficult to apply the concept of subspecies to them. The great genetic diversity observed between the samples may lead to different zoo-technical behaviors. Also, this study may open up new avenues of research into the zoo-technical characteristics of *Sarotherodon melanoteron* populations in the hopes of selecting more performing aquacultural strains.

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