



Assessment of Culture Potentialities of Hybrids from Crosses between *Oreochromis niloticus* and *Sarotherodon melanotheron* Reared in Ebrié Lagoon (Côte d'Ivoire) Using Geometric Morphometric Approach

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Culture potentialities of the hybrids from crosses between *Oreochromis niloticus* (Linnaeus, 1758) and *Sarotherodon melanotheron* (Rüppel, 1852) were assessed in breeding design through morphological features using geometric morphometric approach. The crosses and rearing procedure, that lasted six months, are performed into a concrete pond at Layo Aquaculture Station. The morphological studies were undertaken with morphometric biomarkers such as average body shape and components of developmental homeostasis (canalisation and developmental stability). Average body shape between groups was investigated using Relative Warps Analysis (RWA) and Canonical Variates Analysis (CVA), whereas canalisation and fluctuating asymmetry were evaluated

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from ANOVA Procrustes. The results showed that the body shape of the hybrids is intermediate between those of the two parental species and that the reciprocal hybrids of first generation exhibit heterosis in size. This hybrid vigor appeared more on the hybrids resulting from crosses between *S. melanotheron* males and *O. niloticus* females. In a general way, the morphology of the head of the hybrids is similar to that of the parent *O. niloticus* and their fins resembled to those of the parent *S. melanotheron*. Moreover, fluctuating asymmetry in shape does not vary between parental species and hybrids, contrary to fluctuating asymmetry in size, which is lesser in latter. Canalisation was found to be smaller in the hybrids compared to the parental ones. So, these results revealed that the hybrids could benefit from most of accrued advantages of parental species.

Keywords: Geometric morphometric; hybrids; landmarks; heterosis; body shape.

1. INTRODUCTION

In Côte d'Ivoire, aquaculture is mainly based on the promotion of lagoon fish farming by the development of the culture of several species in general, and tilapia in particular. The large area of Ivorian lagoon network is brackish water. However, among tilapias, neither fresh water species nor the lagoon ones are well suitable for culture in brackish water [1]. For instance, there is hardly at all species that have both rapid growth and ability to withstand marked variations in salinity [2]. Red tilapia, which is hybrids between *Oreochromis mossambicus* or *O. hornorum* and *Oreochromis niloticus* or *O. aureus*, sometimes raised in sea water [3], could be a good candidate to develop culture of tilapia in lagoon and estuarine environments. Hybridisation programs using these species from *Oreochromis* genus are already attempted in Côte d'Ivoire [4]. These programs did not popularise in fish farmers because it was difficult to have the other species of *Oreochromis* genus apart from *O. niloticus* [5]. Among succeeding trials, one involved the crosses between *O. niloticus* and *Sarotherodon melanotheron*, present in Ivorian water body. Moreover, *O. niloticus* is known for its very rustic characteristics, its higher growth rates especially in freshwater system despite a low adaptation in brackish water [6], whereas *S. melanotheron* has a weak potential reproductive but, it is characterised by water salinity tolerance [7]. It's possible to take advantages of both species characteristics by combining valuable traits from them into hybrids, and finally, genotypes well suited to culture performance in brackish water could be obtained. Although several studies have been about the artificial hybridisation between *O. niloticus* and *S. melanotheron* [8,9,10,11,12], the knowledge about culture potentialities of these hybrids is insufficiently investigated. Furthermore, the performances of hybrids are *a priori* sometimes little seeable and require

experimental tests. In this context, the study of phenotypic traits such as biological form could be a straightforward approach, because such a phenotype is in relation with organism functions [13,14]. When based on geometric morphometric methods, study of biological form could provide many findings at a microevolutionary level [15]. One of the strength of geometric morphometric methods is to visualise shape change directly on the structure studied. Hence, morphological traits shared among individuals can be described. This approach has been also proved to be relevant in study of developmental stability (DS) and canalisation [16,15]. These components of developmental homeostasis can be used to evaluate the degree of incompatibility of genetic systems controlling developmental processes [17,18]. It is possible that great developmental incompatibilities between hybridising taxa can induce contrasting consequences on offspring performances. In fishes, this could be particularly true. These organisms experience higher environmental variance during development like all poikilotherms [19,20] and they exhibit intrinsic properties due to a higher sensitivity to genetic stress [18]. Thus, valuable information about hybrids potentialities could be provided, studying the components of developmental homeostasis. The developmental stability refers to the suite of processes through which organisms reduce phenotypic variation resulting from developmental accidents [21]. Canalisation indicates the property of an organism that ensures similar phenotypic expression by buffering developmental against both environmental and genetic perturbation [22]. In populations, canalisation is appraised by estimating interindividual variation and, developmental stability by fluctuating asymmetric (FA) which is defined as small, random deviations of bilateral traits from perfect symmetry [23]. The objective of this survey is to determine culture potentialities of hybrids from

crosses between *O. niloticus* and *S. melanotheron* by morphometric characteristics in order to use them in fish culture.

2. MATERIALS AND METHODS

2.1 Experimental Procedure

S. melanotheron and *O. niloticus* broodstock were a third generation obtained respectively by the crossing of wild parents from the Ebrié lagoon and by the crossing from Bouaké synthetic strain [24]. These fishes are a part of specimens reared in brackish water ponds (salinity 2.43) of Layo Aquaculture Station (5°19'N, 4°19'W; Côte d'Ivoire). The crosses procedure and rearing conditions used to produce specimens of parental species and hybrids of first and second generations is as follow. Purebred and reciprocal hybrids crosses of first generation between *S. melanotheron* (referred as Sm) and *O. niloticus* (referred as On) were carried out into separate concrete pond (2 x 2 x 1 m) at 2:5 ratio (2 males for 5 females) in triplicate. Each concrete pond received renewed brackish water (3 m³/h) through a pumping system from the lagoon. During the period of reproduction that lasted two months, the fish were fed at ration of 5% fresh weight three times a day (8:00, 12:00 and 17:00 hours) with a commercial diet containing 30% crude protein. Hybrids of first generation from crosses ♂Sm x On♀ (referred as F1SmOn) and ♂On x Sm♀ (referred as F1OnSm) are used to produce hybrids of second generation in same breeding design compared to first generation: F1SmOn x F1SmOn (referred as F2SmOn) and F1OnSm x F1OnSm (referred as F2OnSm). At the end of the experiment that lasted about four months, all sampled specimens are adults or sub adults and were collected and counted from each replicate. A total of 182 specimens from six samples sets were used and the number of males and females specimens was maintained similar in each sample in order to avoid the effect of sexual dimorphism on the results. The samples were composed by 36 specimens of On, 34 specimens of Sm, 26 specimens of F1OnSm, 26 specimens of F1SmOn, 28 specimens hybrids of F2OnSm and 28 specimens of F2SmOn.

2.2 Geometric Morphometric Method

The form of an object comprises both its shape and its size. Geometry morphometric methods allow the study of shape independently to size through superimposition procedure which the

most prevalent is Procrustes superimposition. This procedure eliminates mathematically non-shape variation (such as effects of position, orientation, and scale of specimens) and extracts the shape information by scaling to unit centroid size, superimposing the centroids of all configurations, and rotating the configurations. Finally, using superimposition procedure, places the shapes of specimens in a linear tangent space to Kendall's shape space [25], allowing the use of linear multivariate statistical methods [26]. The resulting shape variables are called partial warps scores (PW) or their principal components, named relative warps (RW). Size variable is represented by centroid size which is the measure of scale or overall size used in Procrustes analysis [26]. Centroid size was computed as the square root of the sum of squared distances of each landmark from the centroid of all landmarks of a specimen [27]. This method has now been used in a large spectrum of field of morphometrics, including ontogeny or the study of developmental stability [15]. The knowledge about body form asymmetry is useful to study developmental homeostasis. Antisymmetry (AS) and directional asymmetry (DA) is two other types of asymmetry, apart from fluctuating asymmetry. Both AS and DA occur when one side of a bilateral character is consistently larger than the other, but in the case of DA, the side that is larger is the same in population [28].

2.3 Landmarks Capture and Data Analyses

The whole body form and components of developmental homeostasis were studied by using 19 landmarks (Fig. 1a) and 11 landmarks (Fig. 1b) from the right and left sides, respectively marked with entomological pins. Each specimen was placed on board platform in a horizontal position and lateral image is captured using a digital camera (Samsung, model N°.WB 200F) held parallel to the measuring board at 35 cm. It was noted that the settings were kept constant lighting and magnification.

2.4 Study of Whole Body Form

Captured images were imported into tpsDig software [29] for landmarks digitizing step. Landmarks positions were scored as (x, y) coordinates. Data were submitted at Generalized Procrustes Analysis, a superimposition method available in the tpsRelw software [30]. Partial

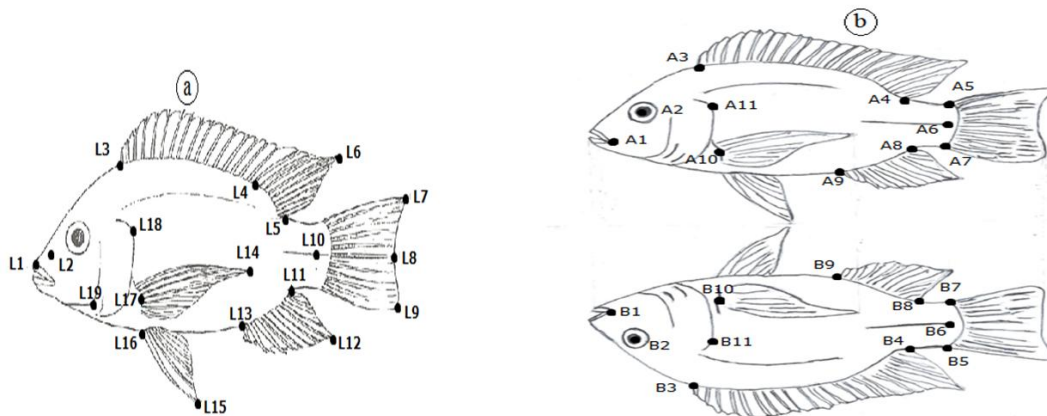


Fig. 1. Position of the landmarks used to study whole body shape (a) and components of developmental homeostasis (b)

L1: Summit of the superior lip; L2: Opening of the nose; L3, A3, B3: Previous insertion of the dorsal fin; L4: Posterior basis of the last ray hard of the dorsal fin; L5, A4, B4: Posterior insertion of the dorsal fin; L6: Summit of the soft rays of the dorsal fin; L7: Previous extremity of the caudal fin; L8: Posterior limit of the caudal fin; L9: Posterior extremity of the caudal fin; L10, A6, B6: Posterior limit of the lateral line; L11, A8, B8: Posterior insertion of the anal fin; L12: Superior extremity of the anal fin; L13, A9, B9: Previous insertion of the anal fin; L14: Superior extremity of the pectoral fin; L15: Lower extremity of the pelvic fin; L16: Previous insertion of the pelvic fin; L17, A10, B10: Lower extremity of the pectoral fin; L18, A11, B11: Most elevated point of the curvature of the opercula; L19, A12, B12: Lowest point of the curvature of the lower jaw, A1, B1: End of mouth opening; A2, B2: centre of the eye; A5, B5: Dorsal insertion of caudal fin; A7, B7: ventral insertion of caudal fin.

warps scores were used as shape variables. Relative warps analyses (RWA), an equivalent of principal component analysis (PCA), was performed in order to get an overall view of the group differences. This ordination is a method to reduce a large set of variables to a few dimensions that represent most of the variation in the data. Canonical variate analysis (CVA) was used to investigate the variation between groups. This ordination is the same than Discriminant Analysis technique for assessing and displaying variation among groups relative to the variation within the groups. The method successively extracts axes (canonical variates, CVs) from multidimensional space that best discriminate among predefined groups by determining the linear combinations of the original variables that display greatest variance between groups relative to within-group variance [26]. Therefore, plots of the first few CVs are optimal displays of differences among groups. These plots are scaled relative to the pooled estimate of within-group variation. The RWA and CVA analyses were generated using the PAST software [31].

The shape change implied by the relative warps and canonical axes was visualised on a deformation grid through thin-plate spline interpolation function [32]. Centroid sizes were calculated and compared between groups of

specimens by using one way ANOVA. Graphical comparison was done through boxplot, using MOG module available in CLIC software [33]. Allometric effects were assessed by multivariate regression of shape variable (partial warps score) on centroid size. Size data was log-transformed in order to reduce dimensionality. Allometry is determined within groups of specimens. It was tested by a permutation test with 10 000 iterations that simulated the null hypothesis of independence between size and shape by randomly exchanging the value for centroid size among individuals. Allometry was assessed using tpsREGR software [34].

2.5 Study of Fluctuating Asymmetric (FA) and Canalisation

The captured images (right and left faces) were digitised using tpsDIG [29] where landmarks coordinates are determined. These images were replicated in order to determine measurement errors. The coordinate data taken from both sides of the bodies of the fishes permitted to study all asymmetry using the software SAGE [35]. Accordingly, shape and size asymmetries were studied through a two-way mixed model analysis of variance following Palmer's procedure [21]. In geometric morphometric context, this procedure is called ANOVA

Procrustes and it is an adaptation made by [16]. This ANOVA is performed on Procrustes residuals, obtained after Procrustes fit which is a geometric transformation that minimises the sum of squared distances between corresponding landmarks of the configurations of fish both symmetric faces. After this step, each fish were described by 22 residuals that are the x and y coordinates of the vectors connecting, at each of the 11 landmarks, every single specimen to an average configuration. ANOVA was calculated for each of 22 residuals and, the sums of squares of potential sources of variation were summed across the 22 variables to obtain the Procrustes sums of squares. Procrustes mean squares were computed by dividing these sums of squares by the relevant degrees of freedom. The sources of variation are „individual“, which represents canalisation or interindividual variation, the “side“ which represents DA, the “interaction (individual x side)“, which represents FA, and “measurement error“. The Procrustes mean squares obtained were used to estimate the population indices of FA (FA10) and interindividual variation (VAR) for size and shape respectively. The FA10 and VAR indices for size and shape were respectively compared between specimens groups using parametric *F*-ratios.

2.6 Bias in the estimation of FA

Allometric effects in size and shape FA, measurement error and the occurrence of 2 other forms of asymmetry (DA and AS) can potentially bias FA estimates. DA and measurement error were determined through ANOVA Procrustes. For size AS, tests for multivariate normality and bimodal distribution were done. The presence of shape AS was investigated by verifying the clustering or no of the scatter plots of the vectors corresponding to the right minus left differences for each landmark. Allometric effects in size FA were assessed by a linear regression of $(R_S - L_S)$ against the mean centroid size $((R_S + L_S)/2)$. Allometric effects in shape FA were tested using multiple regression of the between-side difference vector on centroid size.

3. RESULTS

3.1 Whole Body Form

3.1.1 Parents and hybrids differentiation

Shape difference between parental species and hybrids has been studied by combining observations from all groups for a single, pooled-

group relative warp analysis. Two RWs have been considered. These axes explained 74.18% of total shape variation (RW1=51.12%, RW2=23.06%). The scatter plot shows two specimens cluster in the first RW axis. An upper cluster of scores with positive values that comprises hybrids of first and second generations observations and a lower cluster with negative values that includes parental species (Fig. 2). The description of deformation grids of the shape change explained by the RWs showed the body shape features for each of the two discriminate clusters. The body shape of the parental species was wider than the hybrids one. The deformation grid of the parental species was therefore characterised by a relative fins contraction, particularly strong in the dorso-ventral region. Overall, the parents had a large head and an extended body shape in comparison to the hybrids. In contrast, hybrids morphology showed various degree of anterior-posterior contraction. That compression was more pronounced in the head region, whereas the fins were relatively long. In summary, hybrids had a small head and elongated fins. The highest variability among data is provided by landmarks 18, 19 and landmarks 4, 14, 15, 17 respectively in head and fins parts. In different ordinations, shape areas of parental species (*O. niloticus* and *S. melanotheron*) were overlapped each other, indicating a morphology convergence between *O. niloticus* and *S. melanotheron*. In general way, the shape representative areas of all hybrids showed an important overlap, noticing like this, that the hybrids are morphometrically closer to each other.

3.1.2 Differences among specimens groups

The CVA of the pooled samples also showed similar differences among specimens groups. This analysis indicated considerable differentiation among specimens groups (Wilk's lambda= 0.001824; $P < 0.05$). The percentage of the variance explained by the two first canonical axes which display the greatest separation of the groups relative to within group variance were 91.68%. The percentage of individuals correctly classified in a leave-one-out cross validation procedure was 80%. The inspection of the plot of CV1 against CV2 indicated three clearly distinct groups (Fig. 3). Parental species (*O. niloticus* and *S. melanotheron*) were quite well separated each other along the first canonical axis. The second canonical axis separated out the parental species from the hybrids and indicated that those

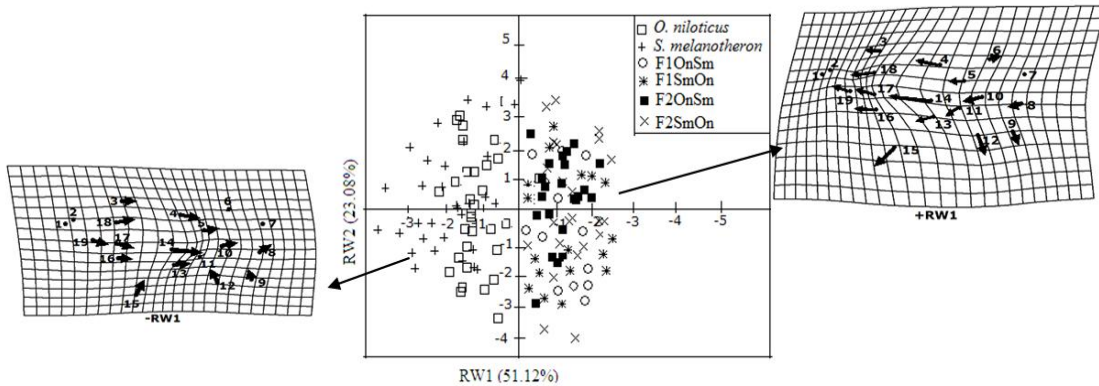


Fig. 2. Scatter plot of two first axes from the relative warp analysis of the whole specimens
Deformation grids showing shape changes implied by RW1 and RW2 axis (-RW and +RW deformations expressed respectively by positive and negative directions)

latter occupied an intermediate morphology position between parental species. Neither of the two canonical axes is able to separate hybrid groups each other (Fig. 3). The scatter of data referring to *O. niloticus* was located in positive area respectively of CV1 (+CV1) and CV2 (+CV2) axis, while *S. melanotheron* was mainly spotted in negative area of CV2 (-CV2) axis and a little bit in CV1 negative area (-CV1). The thin plate deformation grids depicting overall shape differences as captured by CVA axes can be used to assess to organism morphometric features. Accordingly, the negative area (-CV2) was associated with an extension of head and fin contraction pronounced in dorso-ventral region.

These features marked *S. melanotheron* main characterisations. According to (+CV1) and (+CV2), *O. niloticus* was characterised by a short head and extended fin. In (+CV2), hybrids specimens had the similar head shape than the parent *O. niloticus*. The negative area of CV2 (-CV2) axis indicated that the hybrids fins were as similar as those of the parent *S. melanotheron*. In summary, *S. melanotheron* differed from *O. niloticus* in the following respects: the head is markedly large and the fins are relatively short. It noticed that landmarks with higher variability in relative warp analysis are also those that better discriminate between parents and hybrids.

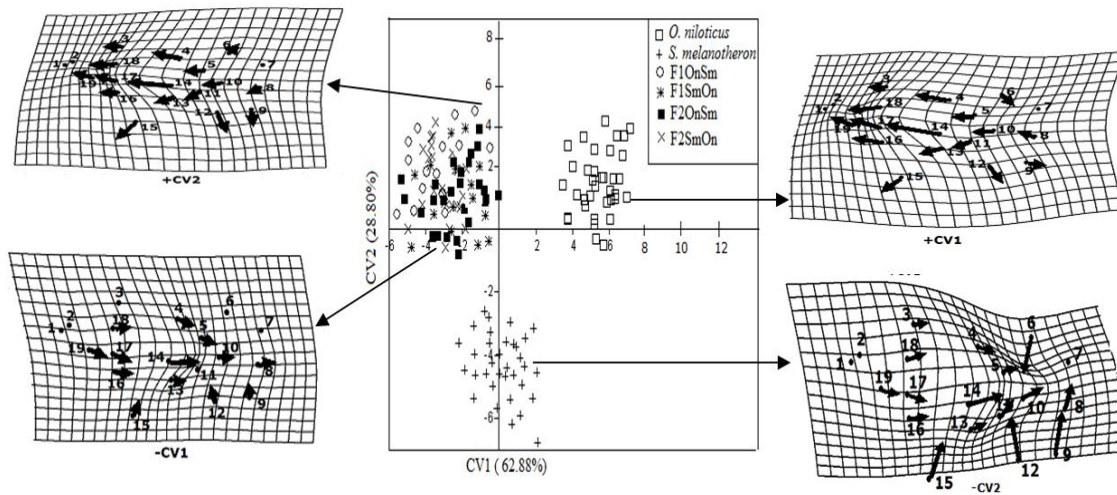


Fig. 3. Scatter plot of two first axes from canonical variate analysis
Deformation grids showing shape change associated to CV1 and CV2 axis (+CV and -CV deformations expressed respectively by positive and negative directions)

3.1.3 Centroid size comparison

The centroid sizes of the reciprocal hybrids of first generation are not significantly different each other, but are significantly different from those of the parental species and of the reciprocal hybrids of second generation (Table 1).

Centroid size in reciprocal hybrids of first generation was larger than the largest parent's centroid size (*Oreochromis niloticus*). Reciprocal hybrids of second generation had the smallest centroid size (Fig. 4).

3.1.4 Allometric effects

Allometric effects were significant ($P < 0.05$) within three groups: *O. niloticus*, *S. melanotheron* and hybrid individuals F1 from crosses *S. melanotheron* x *O. niloticus* and not significant within the three other groups of specimens. The variation of shape non explained by size is high in all groups (Table 2).

3.2 Canalisation and Developmental Stability

3.2.1 Shape

The significance of the side effect in the four Procrustes ANOVA reveals the presence of DA in configurations of all groups (Table 3). No clustering was found in scatter plots of the (R-L) shape vectors (Fig. 5). This result suggests that AS is not present in all groups. No significance was found in multiple regressions of the between-side difference vectors on centroid size: size effect on shape asymmetry is absent (Table 3). In each group, the individual and individual x side-effects were significant. The VAR index is the same in parental groups in one hand and in F1 groups, in another hand. However, VAR index is significantly lower in F1 groups than parental groups (Table 3; Fig. 6). The FA10 index is the same in parental groups and the F1 one.

Table 1. Pairwise comparison of mean centroid sizes of groups

Specimens	On	Sm	F1OnSm	F1SmOn	F2OnSm	F2SmOn
On		0.2070	0.0311*	0.0490*	0.0020*	0.0008*
Sm			0.0104*	0.0250*	0.0620	0.0690
F1OnSm				0.3610	0.0170*	0.0010*
F1SmOn					0.0002*	0.0060*
F2OnSm						0.5560

*: $p < 0,05$

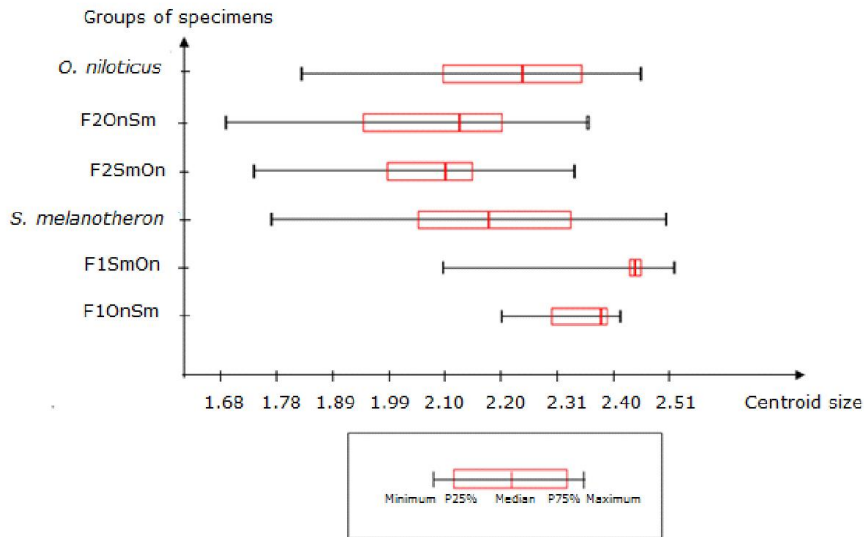


Fig. 4. Boxplot showing centroid size variation between groups

Table 2. Allometric effects within groups

Specimens	F	p	% non explained	d.f
On	2.6900*	0.0002	91.78	34 , 1020
Sm	2.3200*	0.0005	91.62	34 , 884
F1SmOn	2.7230*	0.0001	52.41	34 , 102
F1OnSm	1.1980	0.2310	76.95	34 , 136
F2OnSm	1.1880	0.1280	94.47	34 , 748
F2SmOn	1.2800	0.1320	95.63	34 , 884

p < 0,05: significant allometric effects, d.f : degree of freedom

3.2.2 Size

No evidence of AS was found in the data, as indicated by non-significant p value of Kurtosis ($p > 0.05$) unimodal normal distribution.

Tests for AD have revealed that it is absent in all samples, as indicated by the non-significant value of side effect in the two-way ANOVA. The regressions of |R-L| on (R+L)/2 were not significant in all samples (Table 3). There is independence between size asymmetry and size. The individual and individual x side effects were

significant for all groups (Table 3). Pairwise comparisons of FA10 were all significant ($P > 0.05$), indicating that The FA level were different between samples.

4. DISCUSSION

This study used the variation of average body form and the two components of developmental homeostasis that is developmental stability and canalisation, to appraise culture potentialities of hybrids from crosses between *Sarotherodon melanotheron* and *Oreochromis niloticus*.

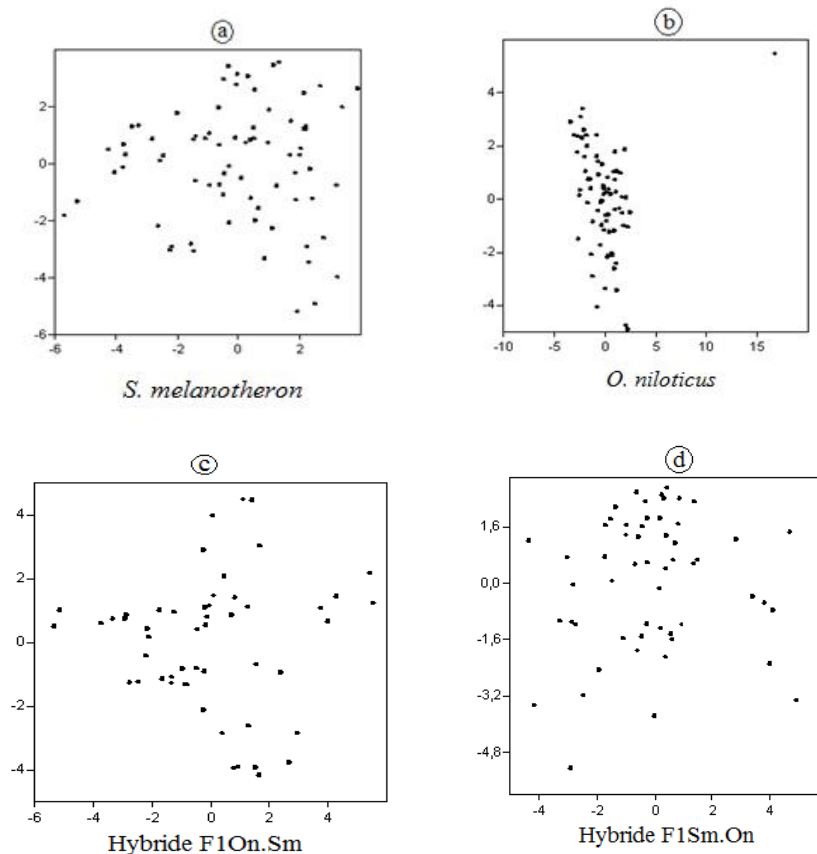


Fig. 5. Scatter plot of right minus Left shape vectors

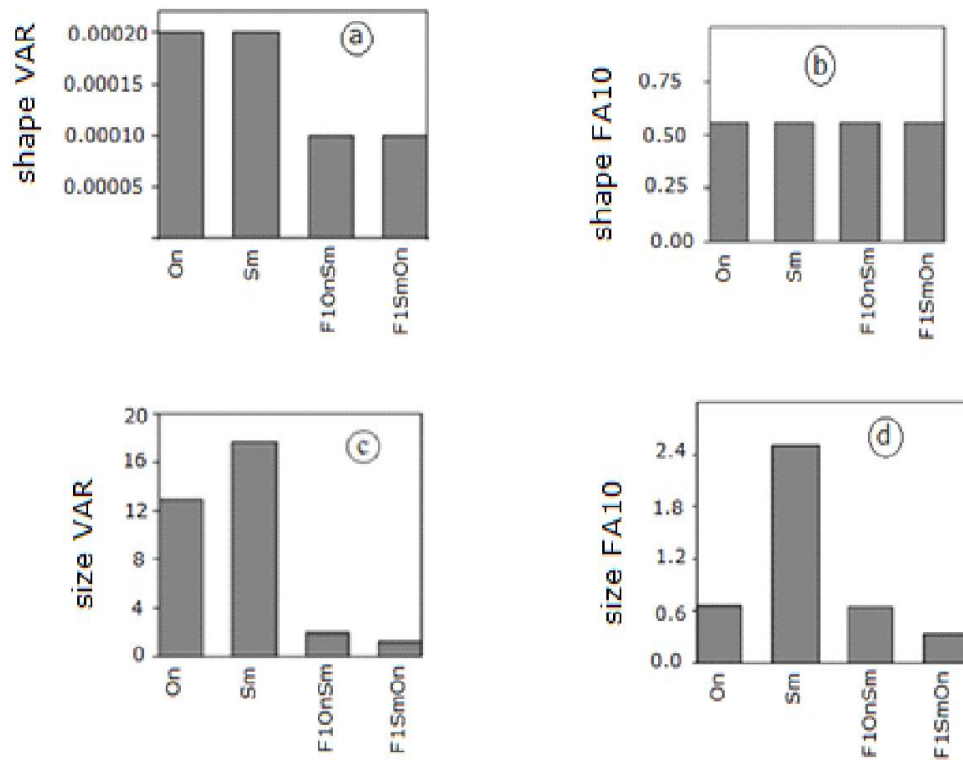


Fig. 6. Variation of shape VAR, size VAR, shape FA10 and size FA10

Preliminary analyses showed that allometric effects explain globally a weak variation of body shape of groups. The body shape of the hybrids is intermediate between the parental ones. This result indicates the fact that the genetic material that underlies the shape of the hybrids comes, for half, of each of the two parental species, suggesting that hybrids express additive genetic effects in body shape [36]. Considering that body shape is linked to organism functions [13,14], these hybrids could combine some functional traits of parental lines. Moreover, an example of mixed functional traits of parental lines into these hybrids could be noticed by the fact that their morphology of the head is similar to that of *O. niloticus* and their fins resembled to those of *S. melanotheron*. Accordingly, in ecomorphological context, the hybrids could inherit the head and fin biomechanical properties from *O. niloticus* and *S. melanotheron* respectively. According to [37], biomechanics is increasingly seen as an essential component in understanding the complex relationship between structure and

function of an organism. Thus, for instance, hybrids could inherit head properties such as female oral incubation, useful for alevin survival rate in *O. niloticus* reproduction and fin properties from *S. melanotheron* such as swimming capacities, useful for displacement in brackish water body. Such an adaptation of morphological features to life environment is particularly important in fish and other aquatic organisms because the rigors of moving in the dense, viscous medium that is water suggest that morphological correlates should be particularly clear [37]. This findings attest certain properties of hybrids which are usually intermediate both phenotically and genetically, because they are a mix of genotypes [38]. Mixed functional traits of parental lines into hybrids are sometime noticed in tilapias. A survey carried out by [39], about the assessment of some physiological features of the hybrids of *O. niloticus* and *O. mossambicus*. These authors proved that the hybrids combine a high growth rate received from the parent *O. niloticus* and a high salinity tolerance inherited from *O. mossambicus*.

Table 3. ANOVA Procrustes of shape and size

	Groups							
	Shape				Size			
	On	Sm	F1OnSm	F1SmOn	On	Sm	F1OnSm	F1SmOn
Var	0.0002	0.0002	0.0001	0.0001	13	17.64	1.90	1.20
FA10	0.564	0.564	0.282	0.282	0.090	2.616	0.657	0.349
Size regression	0.024 ± 0.013	0.016 ± 0.021	0.048 ± 0.031	0.069 ± 0.003	0.029 ± 0.014	0.019 ± 0.013	0.038 ± 0.010	0.052 ± 0.009
F	0.158	0.215	0.008	0.003	0,101	0.120	0.010	0.006
individual								
d.f	700	740	500	540	35	740	500	540
MS	0,0002	0,0002	0,0001	0,0001	13	0,0002	0,0001	0,0001
F	2,495	1,686	1,497	1,205	118,679	1,686	1,497	1,205
Side								
d.f	200	20	20	20	1	20	20	20
MS	0,0074	0,0066	0,0039	0,0034	0,4036	0,0066	0,0039	0,0034
F	101,073	71,255	40,561	34,540	2,335	71,255	40,561	34,540
interaction								
d.f	700	740	500	540	35	740	500	540
MS	0,0001	0,0001	0,0001	0,0001	1,2	0,0001	0,0001	0,0001
F	4,5778	10,1723	1,4972	7,2662	13,640	10,172	7,4452	7,266
error								
d.f	1440	1520	1040	1120	72	1520	1040	1120
MS	0	0	0	0	0,0127	0	0	0
F	-	-	-	-	-	-	-	-

d.f: degree of freedom; MS: mean square; *: $P < 0.05$

Concerning heterosis effect, many studies indicate different genetic mechanisms affecting size and shape, suggesting size as a character prone to show heterosis in case of genetically differentiated parents [40]. Centroid size in each of the reciprocal hybrids of first generation was significantly increased than parental ones. It could suggest that these hybrids are endowed with heterosis. Generally, the hybrids endowed with heterosis present larger centroid size than the largest parent's size [15,41]. Recent study made by [11] on 10 microsatellites markers, together with those of [42] and [43] using different microsatellite markers, showed heterozygote deficiency in both parental species. Hence, hybrid vigor may be mainly due by the masking of deleterious recessive alleles from one parent by alleles from the other parent in hybrids [44]. The observed heterosis is more important in the hybrids of first generation resulting from the crosses " $\text{♂}S. \text{melanotheron} \times O. \text{niloticus}\text{♀}$ ". The intensity of heterosis according to cross direction or parental strain used as dam or sire may be explained by parental effects in one hand and genetic linkage between sex genes and performance genes in other hand. Parental effects are more often observed during the early development of fry [45]. In *Oreochromis* and *Sarotherodon* genus, mouthbrooding system adopted for egg care could be considered as a main parental effect. In interspecific pairing contexts, *O. niloticus* males and females were the most dominant in front of respectively female and male of *S. melanotheron* [11]. These findings suggest that oral egg incubation is made by female *O. niloticus* in the crossing " $\text{♂}S. \text{melanotheron} \times O. \text{niloticus}\text{♀}$ " although male *S. melanotheron* can do it, and by the female *S. melanotheron* in crossing " $\text{♂}O. \text{niloticus} \times S. \text{melanotheron}\text{♀}$ ". According to the dominance and the aggressiveness of *O. niloticus* on *S. melanotheron*, it is supposed that oral egg incubation is carried out badly in crossing " $\text{♂}O. \text{niloticus} \times S. \text{melanotheron}\text{♀}$ " compared to one in crossing " $\text{♂}S. \text{melanotheron} \times O. \text{niloticus}\text{♀}$ ", influencing probably differently offspring performances. Genetic linkage between sex genes and performance genes can result in sex-biased gene expression that may influence the predominance of a specific strain as dam or sire [45]. The intensity of heterosis according to parental strain used as dam or sire has been noticed in many crosses. By example, the hybridisation between mosquito fishes, *Gambusia holbrooki* and *G. affinis* showed that hybrids presented better performance than parents when the male involved in crosses was

G. affinis [46]. Furthermore, the reciprocal hybrids of second generation have the weakest centroid sizes, suggesting thus, a reduction of the hybrid vigor in second generation. These hybrids of second generation result from crosses between closely related conspecifics, producing inbreeding depression [47]. The genetic effect that ensues is an increase of homozygous rate, that decreases the genetic variability and heterosis level is reduced.

About components of developmental homeostasis, fluctuating asymmetry (FA) based on shape were not different between reciprocal hybrids of first generation and parental species, but size FA exhibited a lower significant level for hybrids. Moreover, these hybrids presented a lower significant level size VAR and shape VAR than parental species ones respectively. These results suggest that the development of hybrids benefited, globally, from a heterotic effect. Thus, hybrids don't suffer from outbreeding depression. It's possible that the two hybridising genomes are not divergent to the extent that the genes system controlling development becomes incompatible [18]. Thus, the compatibility between regulatory genes system (which govern developmental homeostasis process) seems to occur despite high dissimilarities between structural genes of both parental species, attested by the low rate of common alleles (13.4%) and F_{ST} value (0.2) [11]. High heterozygosity level could be also observed in these hybrids genome that has been sufficient to buffer against the negative effects of disruption of two coadapted genes, mixed during hybridisation. Outbreeding depression, the negative effects of the breakdown of genomic coadaptation on fitness, is essentially studied in terms of reduction of fertility and survival rate [48]. The reciprocal hybrids of first generation are then supposed to have a good survival rate and to be fertile. A good survival rate has already noticed in many works concerning these hybrids [8,10]. About fertility, the existence of reciprocal hybrids of second generation is the proof of a normal working of the meiosis, and even, the good fertility of the hybrids of first generation. Hence, several premating barriers have been overcome to produce the hybrids of first generation, and in the two senses of crosses. In many cases, the interspecific hybridisation takes place in one sense only, the reciprocal crosses always not succeeding. For example, *O. niloticus* is very often the male parent when it hybridises with *O. mossambicus* [39]. Nevertheless, the aggressiveness of *O. niloticus* when paired with *S. melanotheron*, seems mirror certain pre-

mating barriers between both species, which would affect badly the occurrence of hybridisation [11]. This fact could explain the weak number of reciprocal hybrids of first generation used in this study. It is also attested in previous studies. For instance, [5] used only reciprocal hybrids of second generation because of the same reason.

5. CONCLUSION

The hybrids seem combine parental strain morphological features such as head of *O. niloticus* and fins of *S. melanotheron*. These properties are useful for foraging function, growth and reproduction, contributing meaningfully to fitness. Moreover, through size heterosis, it is possible that many performances of hybrids of first generation outweigh the parental ones. Heterosis effects also seem affect positively fluctuating asymmetry and canalisation levels, which mirror developmental compatibilities between hybridising taxa. This work should continue while trying to characterise the hybrids, especially on the physiological plan. Hence, the growth and tolerance to the salinity of the hybrids could be assessed and compared to those of parents. In addition, to confirm the strong genetic determination of the body form, the relationship between genetic markers and those of morphometric should be valued.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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