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Inheritance and Relationship Between Canalization, Developmental Stability and Morphological Integration in Cichlidae Fish *Oreochromis Niloticus*, *Sarotherodon Melanotheron* and their F1 Reciprocal Hybrids

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ABSTRACT

Morphological integration, canalization and developmental stability are three major processes involved in phenotypic variability. In spite of increasing interests, they have incurred as factors that may contribute to the evolvability, little is known about some of their properties such as inheritance and the relationship between them. This issue was addressed in the present study through geometric morphometrics approach applied to the body shape of fish belonging to Cichlidae. These fish are *Oreochromis niloticus*, *Sarotherodon melanotheron* and their reciprocal hybrids of first generation. Then, the level and morphological patterns of intra et interindividual variation were tackled while taking the relationship between buffering mechanisms into account. As for morphological integration, hypotheses of modularity were tested using 3 statistics. Developmental stability and canalization were found to reflect single mechanistic process according to the congruence of their related morphological patterns between and within groups. Both buffering mechanisms also to act on the same components of shape. An interesting but overlooked observation is that, the congruence between canalization and developmental stability seem to depend on the nature of traits under consideration, specifically those closely related to organism's fitness. Furthermore, in patterns of morphological integration, the hypotheses that the

head represents a relatively integrated unit and the fins of fish are combined in the same module are strongly supported. Finally, it was observed an inheritance of best fitting models together with features of buffering mechanisms which interact mostly with morphological integration.

Keywords: canalization, developmental stability, geometric morphometrics, morphological integration.

INTRODUCTION

The phenotype of individual represents the integration of the different components encoded by the genotype in a given environmental and epigenetic context [1]. Until then, a central goal of biology is to understand the complex interactions that mediate the translation from genotype to phenotype [2]. But, this translation is hardly ever one to one correspondence. However, one of the conceptual framework around which the relationship between the genotype and phenotype can be understood, is the study of variability [2]. Phenotypic variability is defined as the potential of an organism to vary [3]. It informs how developmental systems structure the production of phenotypic variation, which results from the balance between sources of variation, such as genetic mutations, environmental effects and developmental errors; and counteracting regulatory processes buffering against this variation [4, 5]. Thus, many properties of variability may lead to phenotypic variation, but three widely studied ones are emphasized here, in the morphological realm. There are morphological integration, canalization and developmental stability. Morphological integration is a tendency of different traits to vary jointly, in a coordinated manner, throughout a morphological structure or even a whole organism through common functional activities, developmental pathways, or genetic linkages and pleiotropy [5, 7]. Functionally, morphological integration is the way of phenotypic production that biases the direction of variation. It tightly linked to concept of modularity, which exist if integration is concentrated within certain parts or regions of structure, termed modules, but is relatively weak between them [7]. Canalization and developmental stability are components of developmental homeostasis. Both correspond to phenotypic production that minimize variation. The former is viewed as process that buffers against genetic and environmental perturbations avoiding the production of unexpected phenotypes, whereas the latter refers to the suite of process through which organisms reduce phenotypic variation resulting from developmental accidents [8, 9]. In population, the easiest way to appraise canalization may be by estimating interindividual variance and developmental stability by intraindividual variance, which is often estimated by the level of fluctuating asymmetry (FA) in bilaterally symmetrically organisms [10, 8]. All three phenomena may have importance effect on the rate and direction of evolutionary change [4]. They can also be viewed as related relative descriptors of epigenetic system which may include strategies of organisms to respond to intrinsic and/or extrinsic environmental fluctuations. This could hold particular interest in spreading adapted traits in species subject to environmental fluctuations, particularly in aquatic organisms. This because, water is often the ultimate receptacle for most pollutants, these organisms are constantly subjected to a stressful environment from which they cannot escape. Therefore, their inheritance and congruence could lead to robustness of developmental system trans generationally. This paper explores these issues through Cichlid fish as model-system, namely *Oreochromis niloticus*, *Sarotherodon melanotheron* and their reciprocal hybrids of first generation. This was

a favorable situation since developmental stability and canalization have been already studied in the same context by [11]. These authors had shown that F1 hybrids benefited from an heterosis effect, and had lower FA levels and high canalization value than both parental groups. The modular body plans of these fishes offer additional opportunity to study integration among structures. In particular, the method of geometric morphometrics may offer a decisive advantage because it is able to disentangle the effects of morphological integration, canalization and developmental stability [12].

Thus, the following research questions are raised:

1. Is there a relationship between developmental stability and canalization in each group under study?
2. Is there a transgenerational inheritance of putative congruence between developmental stability and canalization as well as developmental integration?
3. Is there a connection between developmental integration and buffering mechanisms?

MATERIAL AND METHODS

Sample

Four groups of fish were used in this work in order to examine the inheritance of canalization, developmental stability and morphological integration. There are two parental strains that are *Sarotherodon melanotheron* and *Oreochromis niloticus*, and two reciprocal hybrids of first generation. *S. melanotheron* (referred as Sm, n= 34) and *O. niloticus* (referred as On, n= 36) 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 [13]. 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 reciprocal hybrids of first generation are individuals resulting from ♂On x Sm♀ crosses (referred as F1OnSm, n=26) and ♂Sm x On♀ crosses (referred as SmOn, n=26). The crosses procedure and rearing conditions used to produce specimens of parental species and hybrids of first generation are detailed in [14].

Geometric Morphometrics

Fluctuating Asymmetric (FA) and Canalization were studied by using 12 landmarks (Figure 1 a & b; table 1) digitized from the right and left sides with tpsDig software [15]. Both sides of the fish were then digitized twice to quantify digitizing error. The coordinate data taken from both sides permitted to study all asymmetry using the software SAGE [16]. Accordingly, shape and size asymmetries were studied through Procrustes ANOVA, a two-way mixed model analysis of variance. Details about intra and inter individual variation for size and shape were previously published in [11]. To analyze morphological integration and hypotheses of modularity, 27 landmarks were placed along fish body (Figure 1 c). Digitized coordinates were superimposed using a General Procrustes Analysis [15]. Tests of a priori models of variational modularity in data have been carried out by Mint ver. 1.61 [16].

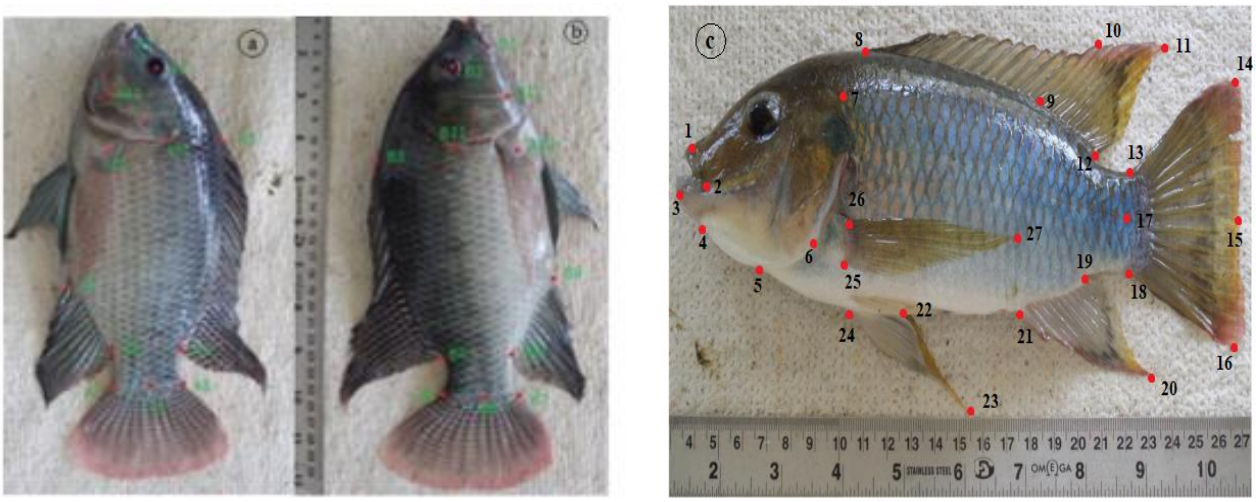


Figure 1: Positions of the landmarks on fish

Table 1: Description of landmark for the analyse of geometry morphometric

Landmarks°	Description of landmarks
1	Summit of superior lip
2, A1, B1	End of the mouth opening
A2, B2	Centre of the eye
3	Summit of inferior lip
4	Ventral limit of inferior lip
5	Anterioventral point of bony opercle
6	Posterior point of the bony opercle
7	Anterior limit of the lateral line
8, A3, B3	Previous insertion of dorsal fin
9	Posterior basis of the last ray hard of the dorsal fin
11	Summit of the soft ray of the dorsal fin
12, A4, B4	Posterior insertion of dorsal fin
13, A5, B5	Dorsal insertion of caudal fin
14	Previous extremity of the caudal fin
15	Posterior limit of the caudal fin
16	Posterior extremity of the caudal fin
17, A6, B6	Posterior limit of the lateral line
18, A7, B7	Ventral insertion of caudal fin
19, A8, B8	Posterior insertion of anal fin
20	superior extremity of anal fin
21, A9, B9	Previous insertion of anal fin
22	Ventral insertion of ventral fin
23	Superior extremity of ventral fin
24	Posterior insertion of ventral fin
25	Dorsal insertion of pectoral fin
26	Ventral insertion of pectoral fin
27	Superior extremity of pectoral fin

Testing for Congruence Between Developmental Stability and Canalization

We use 3 approaches in order to determine whether developmental stability and canalization between and within samples are based on the same biological process. Firstly, the correlation values between inter-individual variation (canalization) and intra-individual variation (fluctuating asymmetry) were established. The correlation was obtained from the variance-covariance matrices (VCV), respectively related to inter-individual variation and intra-individual variation. These matrices are derived from a 2-factor MANOVA (individual, side and individual x side interaction), performed on landmark coordinates after Procrustes superposition. Based on the assumption of complete dissimilarity [17], the correlation between these matrices was tested using permutation tests (10000 repetitions) to determine their level of significance. Next, because correlative patterns of whole shape variation are often difficult to interpret, it would be appropriate to use their principal components [8]. Thus, a principal component analysis was carried out on the VCV matrices corresponding to each effect within and between the groups of specimens. This analysis also depicts the landmarks displacements corresponding to the principal components through the deformation grids. Only the first principal components per effect have been represented.

Finally, the relation between canalization and developmental stability has been analysed through Muldimensional Scaling (MDS) also known as Principal Coordinate Analysis. This is a distance analysis between matrices, which has the advantage of presenting a graphical representation of their similarities and differences. Using this analysis, it is possible to visualize very large multivariate datasets in a synthetic and intuitive way [18]. In this work, MDS was used to verify the effective congruence of intra-individual and inter-individual patterns of variation.

Testing for Integration and Modularity

Twenty modular hypothesis were established, using the combination of main body parts of fish (Table 2). These *a priori* models are based on those proposed for fins modules and cichlid skull [19, 20], as well as functional and topological relationships between regions of fish in general [21]. These hypotheses were tested using goodness of fit procedures where we're interested in assessing whether a pre-defined model is good enough to explain variation in a dataset. For that, a covariance matrix that the data would be expected to produce if the model being tested was true are computed. Practically, tests are carried out by comparing observed and expected covariance matrices using minimum deviance methods. The fit of the hypotheses is assessed through 3 statistics which don't measure the same properties of observed and expected covariation patterns [22]. There is standardized gamma (γ^*) [23], angles (θ) between the subspaces spanned by these covariance matrices [24], and matrix correlation (r_M) [25]. Furthermore, the null hypothesis that the value of these statistics is no greater than expected by chance is evaluated by permutation tests. A parametric approach, Monte Carlo, was implemented to compute the null distribution. The test is accepted ($P > 0.05$) for no differences between observed and expected covariance matrices illustrated by low γ^* or θ values, or large absolute r_M values. It seems worth noting that a discrepancy could be appeared between these 3 statistics. Indeed, the difference in the number of fixed parameters in models may be overly influenced the results. Contrary to (θ) and (r_M), only (γ^*) has been fully standardized to prevent from this problem [22]. Unfortunately, no method is yet available for statistically comparing each of these 3 statistics between groups under study.

Table 2: Description of the 20 a priori hypotheses of modularity

Hypothesis	Description of modular partitions
1	Head (1:7) Dorsal fin (8 :12) + tail (13 :18°) + anal fin (19 :21) + ventral fin (22 :24) + pectoral fin (25 :27) + trunk (7, 8, 9, 12, 13, 17, 18, 19, 21, 22, 23, 24, 25)
2	Head + trunk + tail Dorsal fin + anal fin + ventral fin + pectoral fin
3	Head + dorsal fin + trunk + anal fin + ventral fin + pectoral fin Tail
4	Head + dorsal fin + tail + anal fin + ventral fin + pectoral fin Trunk
5	Head + trunk Dorsal fin + tail + anal fin + ventral fin + pectoral fin
6	Head + dorsal fin + anal fin + ventral fin + pectoral fin Trunk + tail
7	Head + tail Dorsal fin + trunk + anal fin + ventral fin + pectoral fin
8	Head + tail + anal fin + ventral fin + pectoral fin Dorsal fin Trunk
9	Head Dorsal fin Tail + trunk + anal fin + ventral fin + pectoral fin
10	Head + trunk + anal fin + ventral fin + pectoral fin Dorsal fin Tail
11	Head + dorsal fin + anal fin + ventral fin + pectoral fin Tail Trunk
12	Head Trunk Dorsal fin + tail + anal fin + ventral fin + pectoral fin
13	Head Trunk Tail Dorsal fin + anal fin + ventral fin + pectoral fin
14	Head Tail Dorsal fin + trunk + anal fin + ventral fin + pectoral fin
15	Head + tail Dorsal fin trunk anal fin ventral fin pectoral fin
16	Head tail Dorsal fin

	trunk anal fin ventral fin pectoral fin
17	Head + pectoral fin Dorsal fin + tail + trunk Anal fin + ventral fin +
18	Head + trunk Dorsal fin + tail Anal fin + ventral fin + pectoral fin
19	Head + dorsal fin tail + trunk Anal fin + ventral fin + pectoral fin
20	Head Trunk + pectoral fin Anal fin + ventral fin + dorsal fin + tail

RESULT

Analyze of Congruence Between Canalization and Developmental Stability

Permutation tests indicate that the correlations between the respective VCV matrices of canalization, FA and measurement error are significant ($p < 0.05$) within each group of specimens (Table 3). In hybrids, the correlation values remain high compared with the corresponding values in the parental species. Measurement error is also correlated with FA and canalization in each group, but remains lower than the correlation between FA and canalization. On the other hand, the comparison of canalization, FA and measurement error respectively between specimen groups shows values that are all significant ($p < 0.05$) (Table 4).

Comparison of the deformation grids reflecting the first main components of canalization and FA shows almost a similarity between groups, especially in hybrid individuals (Figure 2). Vector displacements at the respective landmarks are almost identical in both direction and sense. These results confirm the strong correlation observed between canalization and FA in their respective VCV matrices respectively. In parental species, similarity exists, but is less perceptible between the deformation grids of the first principal components of canalization and FA.

The results of the correlation analysis show that the canalization and FA, VCV matrices of parental species and first-generation hybrids are significantly correlated in most respective cases. A principal coordinate ordination was therefore carried out to refine these results. The plane presented by the first principal coordinates (PCO1) and (PCO2) shows that the points of the FA matrices are completely merged with those of the canalization matrices (Figure 3). There is no clear-cut separation between the groups of points in these two datasets. The points representing the respective matrices are close to each other, and many of them form small groups of couples, triplets or quadruplets. This principal-coordinate analysis therefore shows that the patterns of variation of the canalization and the FA are similar. However, although there are many groups of AF-canalization points, some points in the respective

matrices remain grouped together or remain alone. This confirms the correlation reported between these two datasets in the parental species.

Table 3: Within group analysis of the correlations between VCV matrices related to intra and interindividual variation.

Group	Effect	Correlation	P
	Ind/FA	0.761	< 0.05
On	Ind/error	0.304	< 0.05
	FA/error	0.286	< 0.05
	Ind/FA	0.783	< 0.05
Sm	Ind/error	0.327	< 0.05
	FA/error	0.394	< 0.05
	Ind/FA	0.807	< 0.05
F1OnSm	Ind/error	0.731	< 0.05
	FA/error	0.690	< 0.05
	Ind/FA	0.719	< 0.05
F1SmOn	Ind/error	0.504	< 0.05
	FA/error	0.704	< 0.05

Table 4: Between group analysis of the correlations between VCV matrices related to intra and interindividual variation.

Effect	Effect		Correlation	P
	On/Sm		0.388	0.01
	On/F1OnSm		0.276	0.02
	On/F1SmOn		0.377	0.01
Individual	Sm/F1OnSm		0.170	0.03
	Sm/F1SmOn		0.342	0.01
	F1OnSm/F1SmOn		0.198	0.02
	On/Sm		0.401	0.01
	On/F1OnSm		0.252	0.02
	On/F1SmOn		0.305	0.01
FA	Sm/F1OnSm		0.301	0.01
	Sm/F1SmOn		0.278	0.01
	F1OnSm/F1SmOn		0.311	0.01
	On/Sm		0.188	0.02
	On/F1OnSm		0.125	0.03
	On/F1SmOn		0.212	0.02
Error	Sm/F1OnSm		0.130	0.03
	Sm/F1SmOn		0.307	0.01
	F1OnSm/F1SmOn		0.206	0.02

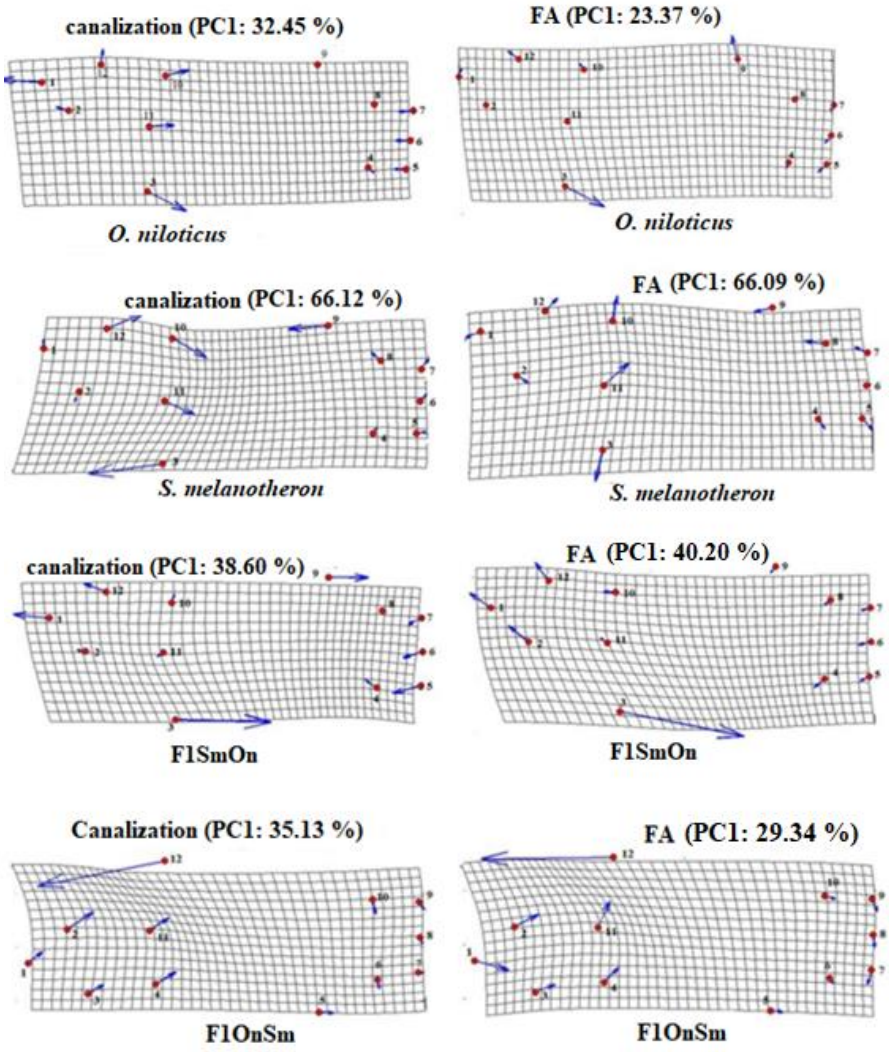
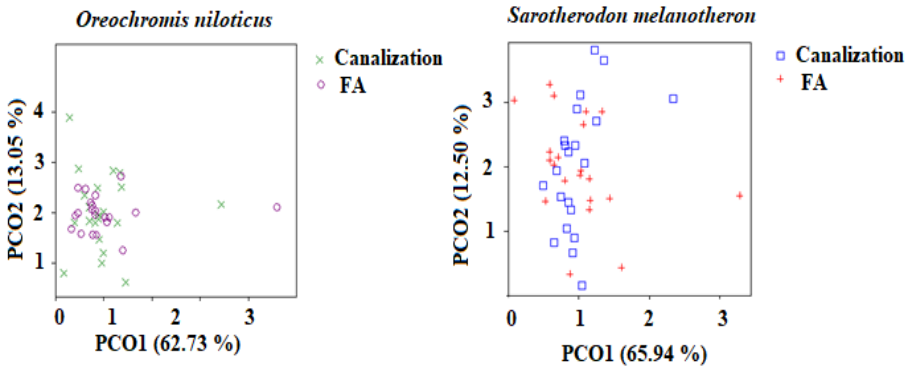


Figure 2: Deformation grids showing vectors of the landmarks displacements relative to the first two axis of principal components analysis of canalization and FA.



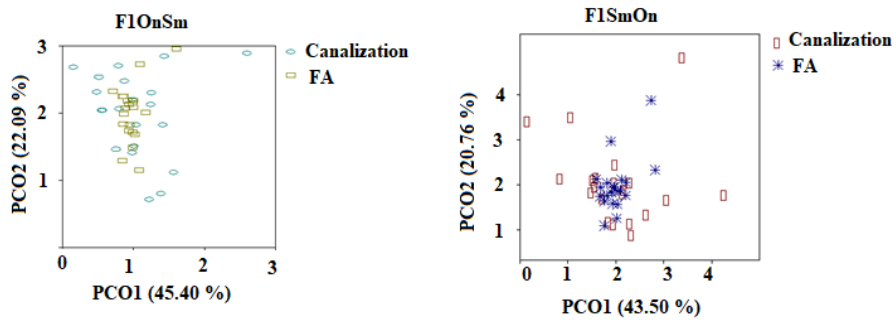


Figure 3: Principal Coordinate Analysis (PCO) relative to FA and canalization matrices in groups under study.

Analysis of Modularity

The best-fitting models are sorted differentially according to the statistic used to test hypotheses of modularity. Thus, in contrast to r_M value, hypotheses are ranked from smallest to largest γ* and θ value (Tables 5; 6; 7; 8). The best first three supported models are similar in parental species, *Oreochromis niloticus* and *Sarotherodon melanotheron.* They are 1; 20; 12 in one hand and in other hand hypothesis 6; 5; 2 or 1 respectively based on γ* and θ, r_M statistics. Model 1 predicts that the head and other parts of fish seem independent of each other. Although, hypothesis 20 and 12, the next best -supported hypothesis, don't differ from the previous but propose that the trunk is integrate with pectoral fin, likewise to the tail and the other all fins. However, according to hypothesis 6; 5 and 2, the head is not completely alone, it might be connected with trunk and tail. Moreover, based on γ*, the best common first four fitting models for hybrids groups, OnSm and SmOn are hypothesis 1; 12; 20; 9. Concerning θ statistics, the best first four fitting models are 6; 5; 2; 1 for SmOn and 5; 1; 12, 20 for OnSm. Concerning r_M statistics, these values are respectively 5; 1; 6; 2 and 5; 1; 3; 6. Compared to the previous parental best-fitting models and independently to the statistic used, only models 3 and 9 appear as new best-fitting ones in hybrids. While model 9 goes in the direction of distinguishing the head as a module, model 3 rather isolates the caudal part. However, surprisingly the worst-fitting hypotheses are as good as the same in groups under study whatever the statistic used. These are hypotheses 10; 15; 16 and 17 among which models 15 and 16 are the most lacking integration. Roughly, these hypotheses don't support the distinct parts of fish as constituting different modules. Furthermore, apart from these general features of modularity, specific ones may be focused on. Hence, hypotheses can be categorized in terms of the number of best-fitting models obtained per group according to the γ*statistic based classification by referring to null hypothesis (Table 9). Thus, the best-fitting models and the worst fitting ones are respectively above and under null hypotheses of absence of modularity. In this, the number of best-fitting models are 12, 11, 10 and 6 respectively for *O. niloticus*, *S. melanotheron*, SmOn and OnSm. Although there is no statistical test to verify the significance of these data, they nevertheless, provide further insight into the modularity of each group. Concerning parental species, most best-fitting hypotheses are similar. Very little ones are therefore different, among which the model 7 is only in *S. melanotheron* as well as models 2 and 19 in *O. niloticus*. Among these models, the relevant difference seem to be the integration between head and dorsal fin in *O. niloticus.* The same applies to hybrid groups, all best supported hypotheses in OnSm hybrid are similar to SmOn, but the models 5; 6; 13 and 14 are specific to the latter. Scrutinizing these hypotheses, the

discrepancy between hybrid group seem result from the modularity of the trunk. In addition, the correspondence of best-fitting models between parental species and hybrids may inform to inherited traits. Firstly, concerning hybrid SmOn and parental species, it should be noted that, apart from models 2 and 11, all the best-fitting models in the parent *O. niloticus* are also included in the SmOn hybrid. Therefore, models 2 and 11 might not to be inherited from *O. niloticus* to SmOn hybrid. With the exception of models 7 and 11, all best-fitting models of *S. melanotheron* are transmitted to hybrid SmOn. Thus, the models 7 and 11 might not to be inherited from this parental species. However, only model 19, included in the SmOn hybrid, is absent in the *S. melanotheron*. Since model 19 is specific to the parental species *O. niloticus*, it could come from the latter. Secondly, as for hybrid OnSm and parental species, it should be noted that, all the best-fitting hypotheses in *O. niloticus* are also included in the hybrid OnSm apart from models 2; 5; 6; 11; 13; 14 which might not to be inherited. All models of *S. melanotheron* are transmitted to hybrid OnSm with the exception of models 5; 6; 7; 11, 13 and 14. So that, they might not be considered inheritable. In contrast to models 2 and 7, which are not inherited only from one parent, *O. niloticus* and *S. melanotheron* respectively, models 5; 6; 7; 11, 13 and 14 are not inherited from both parental species. Overall, the models 2 and 7 respectively specified in *O.niloticus* and *S. melanotheron* are not transmitted to the hybrid group as well as model 11 which is specified in none of parental species.

Table 5: Results of statistical analyses of modularity for *Oreochromis niloticus*

Hypothesis	γ*	P	θ(radian)	P	r_M	P
1	-0.13407	1	0.43125	0.778	0.82497	0.858
2	-0.027665	1	0.23978	0.999	0.88047	1
3	0.048721	1	0.54466	0.461	0.8308	0.914
4	0.021402	1	0.54262	0.476	0.82955	0.874
5	-0.02897	1	0.26396	0.999	0.90378	1
6	-0.08289	1	0.23769	0.998	0.92486	1
7	0.034694	0.821	0.7037	0.147	0.6631	0.105
8	0.046515	0.997	0.46437	0.7	0.77293	0.664
9	-0.057692	0.845	0.58369	0.211	0.67707	0.139
10	0.083818	0.878	0.65527	0.164	0.68007	0.166
11	-0.026366	1	0.55002	0.426	0.80374	0.874
12	-0.10446	1	0.48379	0.634	0.79218	0.82
13	-0.066692	0.566	0.71941	0.117	0.61289	0.09
14	-0.042145	0.716	0.72452	0.108	0.6304	0.058
15	0.10366	0.136	0.83127	0.026	0.44269	0.016
16	0.026977	0.09	0.85108	0.012	0.40697	0.016
17	-0.096161	0.943	0.52598.	0.521	0.73763	0.552
18	0.090366	0.673	0.67632	0.146	0.64253	0.218
19	-0.030936	0.818	0.6059	0.277	0.68862	0.296
20	-0.11533	0.985	0.49056	0.568	0.76153	0.596

Table 6: Results of statistical analyses of modularity for *Sarotherodon melanotheron*

Hypothesis	γ*	P	θ(radian)	P	r_M	P
1	-0.15747	1	0.31919	0.843	0.86243	0.937
2	0.032311	1	0.27951	0.954	0.84375	0.953
3	0.045943	1	0.41368	0.451	0.84547	0.831

4	0.016743	1	0.41552	0.432	0.84518	0.769
5	-0.018048	1	0.2348	0.991	0.90788	1
6	-0.040483	1	0.22325	0.993	0.89807	1
7	-0.0033112	0.928	0.53196	0.282	0.72158	0.145
8	0.086482	0.985	0.52568	0.186	0.75967	0.377
9	-0.0017052	0.65	0.65436	0.034	0.65415	0.039
10	0.095077	0.862	0.59628	0.095	0.69665	0.084
11	-0.02046	1	0.42786	0.517	0.81109	0.72
12	-0.12523	1	0.349	0.815	0.83531	0.938
13	-0.077632	0.507	0.56429	0.258	0.65312	0.066
14	-0.075236	0.798	0.56135	0.209	0.68665	0.062
15	0.14321	0.005	0.86149	0.023	0.42998	0.006
16	0.071529	0.001	0.88755	0.022	0.38749	0.003
17	-0.060646	0.858	0.43172	0.593	0.7432	0.473
18	0.11271	0.563	0.66138	0.106	0.64554	0.095
19	0.011667	0.671	0.60874	0.137	0.68015	0.171
20	-0.12997	0.998	0.3556	0.818	0.80732	0.864

Table 7: Results of statistical analyses of modularity for OnSm hybrid

Hypothesis	γ*	P	θ(radian)	P	r_M	P
1	-0.13117	0.997	0.34519	0.963	0.84333	0.998
2	0.13135	0.796	0.62644	0.711	0.76692	0.972
3	0.083802	0.976	0.56713	0.691	0.83484	0.999
4	0.1151	0.841	0.58143	0.651	0.78339	0.959
5	0.032107	0.999	0.30197	0.987	0.86417	1
6	0.059574	0.962	0.50233	0.808	0.83333	1
7	0.047285	0.344	0.67354	0.588	0.69922	0.797
8	0.022865	0.973	0.57831	0.728	0.82571	1
9	-0.073205	0.57	0.65566	0.566	0.72986	0.809
10	0.13198	0.345	0.7122	0.476	0.67825	0.606
11	0.071435	0.589	0.58047	0.719	0.75787	0.98
12	-0.10148	0.962	0.38977	0.937	0.81076	1
13	0.0045764	0.05	0.72849	0.449	0.59829	0.519
14	0.012094	0.124	0.68423	0.472	0.63394	0.475
15	0.042577	0.024	0.93251	0.47	0.5479	0.378
16	0.0082372	0.001	0.91975	0.378	0.47615	0.203
17	-0.048964	0.565	0.75602	0.631	0.7177	0.844
18	0.082112	0.352	0.78411	0.641	0.67395	0.659
19	-0.05337	0.567	0.66449	0.631	0.74866	0.984
20	-0.06536	0.691	0.46141	0.875	0.74712	0.941

Table 8: Results of statistical analyses of modularity for SmOn hybrid

Hypothesis	γ*	P	θ (radian)	P	r_M	P
1	-0.20396	1	0.27631	0.994	0.89617	1
2	0.01638	1	0.23576	1	0.86339	1
3	0.13325	1	0.47964	0.919	0.78597	0.996
4	0.11194	0.999	0.49406	0.894	0.77527	0.977
5	-0.013266	1	0.21528	1	0.90645	1

6	-0.02465	1	0.21521	1	0.89474	1
7	0.0059282	0.997	0.54547	0.863	0.72023	0.933
8	0.045223	0.998	0.43316	0.951	0.80322	1
9	-0.11785	0.998	0.48631	0.878	0.75328	0.945
10	0.16517	0.956	0.59262	0.789	0.6404	0.764
11	0.081674	0.997	0.50396	0.924	0.73688	0.984
12	-0.16147	1	0.30321	0.996	0.86291	1
13	-0.048211	0.929	0.56879	0.86	0.63364	0.85
14	-0.059387	0.981	0.56221	0.833	0.67972	0.801
15	0.21182	0.538	0.87995	0.465	0.35134	0.378
16	0.14655	0.439	0.89208	0.419	0.2993	0.375
17	-0.039404	0.992	0.52143	0.889	0.71771	0.979
18	0.14747	0.941	0.6246	0.799	0.62946	0.903
19	-0.068008	0.995	0.4987	0.906	0.75195	0.997
20	-0.1461	1	0.35616	0.984	0.81767	1

Table 9: Rank of the best fitting *a priori* hypotheses of modularity according to the three statistics γ*, θ and r_M. For each group, ordered based on the γ*value, best fitting models and worst fitting ones are respectively above and under the null model of absence of modularity.

O. niloticus			***S. melanotheron***			**F1OnSm**			**F1SmOn**		
γ*	θ	r_M	γ*	θ	r_M	γ*	θ	r_M	γ*	θ	r_M
1	6	6	1	6	5	1	5	5	1	6	5
20	2	5	20	5	6	12	1	1	12	5	1
12	5	2	12	2	1	9	12	3	20	2	6
17	1	3	13	1	3	20	20	6	9	1	2
6	8	4	14	12	4	19	6	8	19	12	12
13	12	1	17	20	2	17	3	12	14	20	20
						0					
9	20	11	6	3	12	13	8	4	13	8	8
14	17	12	11	4	11	16	11	2	17	3	3
19	4	8	5	11	20	14	4	11	6	9	4
5	3	20	7	17	8	8	2	19	5	4	9
									0		
2	11	17	9	8	17	5	9	20	7	19	19
			0								
11	9	19	19	7	7	15	19	9	2	11	11
0											
4	19	10	4	14	10	7	7	17	9	17	7
16	10	9	2	13	14	6	14	7	11	7	17
7	18	8	3	10	19	11	10	10	4	14	14
8	7	18	16	19	9	18	13	18	3	13	10
3	13	14	8	9	13	3	17	14	16	10	13
10	14	13	10	18	18	4	18	13	18	18	18
18	15	15	18	15	15	2	17	15	10	15	15
15	16	17	15	16	16	10	15	16	15	16	16

DISCUSSION

Patterns of morphological variation are been investigated through developmental stability, morphological integration and canalization in two species of cichlid fishes and their offsprings for the purpose of assessing their transgenerational inheritance and relationship between buffering components. The question whether canalization and developmental stability represent two distinct processes has then been be tackled by comparing inter-individual variation within a population with intra-individual variation. A significant correlation was found between VCV matrices associated with inter- and intraindividual variation within groups under study. The congruence of these patterns of expression resulting from both sources of morphological variation supports the hypothesis which states that developmental stability and canalization reflect a single mechanistic process. Moreover, the vectors expressing the interinvidual variation within groups were correlated with those of intraindividual variation. This, as well as the relative congruence between vectors of the landmark displacements corresponding to the two axes PCs of the interindividual and intraindividual, indicate that developmental stability and canalization act on the same components of shape. Furthermore, this finding is clarified by muldimensional scaling analyse that noticed the effective congruence of intra-individual and inter-individual patterns of variation. The study of the relationship between canalization and developmental stability has been tackled in a wide range of model organisms but it has seldom, if ever, been applied to fish and other poikilotherms. However, there are many examples in other taxonomic groups, such as insects, mammals. In this sense, these results are in line with those of [17] in insects and in annual herbaceous species plants [26] contrary to those of [8] on the house mouse. In most of these papers, the developmental stability was more likely to have positive correlations with canalization but nonetheless, undoubtedly weak. It is likely that, the congruence between canalization and developmental stability is not related to taxonomic groups, but depends on the nature of the traits, in other words, the functional importance of the symmetry of the characters under consideration. As pointed out in many studies, like [27], the more essential the symmetry of the organs designated by the landmarks is for the organism's fitness, the greater the congruence between developmental stability and canalization. In contrary, for ordinary morphologically character under consideration or traits not closely related to fitness, there is a discrepancy between canalization and developmental stability. For instance, [8] suggested that there is no palpable evidence that the symmetry of the skull of the house mouse is of crucial importance in terms of individual fitness. Moreover, between groups analyse showed that, there is a concordance of the interindividual variation features between all groups. Similarly, there is a significant correlation between the VCV matrices of the interindividual variation. These results suggest that the control of morphological variation by canalization and developmental stability is identical among specimen groups respectively. These components of developmental homeostasis seem to be controlled by similar processes between groups. Furthermore, patterns of morphological integration were investigated for the purpose of identifying congruence in modules between parental species and hybrids. Despite a growing body of evidence of fish modularity has been used to set out the *a priori* models, the best-fitting hypotheses tend to differ across statistics. However, θ and r_M are two of the statistics that yield nearly similar results. However, in all groups, most of the best supported hypotheses do suggest that the head represents a relatively integrated unit. Likewise, they widely don’t support the fins of fish as constituting different modules. These similarities might be a result of common mechanisms such as

underlying developmental pathways, pleiotropy or genetic linkage. This reflects the interplay of factors acting within groups and across homologous organs. This trend points also to molecular mechanisms through same regulator genes or common developmental factors during morphogenesis. More obviously, the molecular cues that underlie the morphological structure of the head or fins within each species seem very close, producing specific shearing patterns among groups under study. This idea is widely reported about regulator genes like Hox, ParaHox, and KCNA gene clusters which suggests, the genomes of cichlids do not seem to differ all that much within and from those of other fishes [28, 29]. For instance, a signaling molecule *bmp4* posited as an attractive candidate for the coordinated evolution of cichlid dentition [30], as well as genes involved during fin formation, such as *Gdf5, bmp2a, bmp2b* [31] could be the same in these disparate groups. In contrast, as for parts which develop weak degree of integration like trunk and tail, molecular cues shaping morphogenesis could be different from an organism to another. These lesser extent in integration might also reflect differences in developmental complexity. In addition, differences in integration are observed in hybrid groups resulting from the same parents, *O. niloticus* and *S. melanotheron*, taken alternatively as sir or dam. Most signaling molecules that regulate more important stages like condensation and differentiation during morphogenesis could be come from sexual chromosomes. Based on γ*statistic, further insights related to evolvability could be found from the number of best-fitting models per groups under study. According to [8], the use of the standardized γ statistic to assess the fit of models to covariance matrices of landmarks implies also evolvability because modules are placed in orthogonal subspaces. As a consequence, a model will fit well only if modules are independent each other [8]. In other words, the number of best-fitting models is linked to the dis-sociability of parts into more discrete modules in contrast to a accrued number of worst-fitting models which can mean strong integration of many traits. The number of best-fitting models are 12, 11, 10 and 6 respectively for *O. niloticus*, *S. melanotheron*, SmOn and OnSm. Although there is no statistical test to verify the significance of these data, this result nevertheless, provides few insights into the evolvability or potential of each population to respond to selection or to undergo nonadaptive evolution by drift [32]. Because large scale integration is argued to constrain variability and inevitably evolvability contrary to integration of subsets of organismal structures which should also increase the likelihood that favorable changes in one module will not have an effect on a separate module and will therefore, be more likely to persist within a population [2]. Integration of parts seem strong in hybrid groups which may lead to a better functionally coordinated but may limit the potential for future variability. By contrast, parental species seem a composite of several subunits that are more free to vary independently from one another, which may be in favor of variability. These results are part in congruence with previous work carried out by [11] where same and low variance of intra and inter individual variation were respectively found in shape and size in hybrids compared to parental species. Generally, increase variance found in hybrids has been attributed to a breakdown of the coadapted genes complexes [2]. Thus, in these hybrids, buffering mechanisms have been too strong to buffer against negative effects of disruption of two coadapted genes complexes mixed during hybridization [11]. Apart from fluctuating asymmetry and canalization; morphological integration could be another process that has contributed to robustness of developmental mechanism in hybrids. Because, it is widely recognized that the inter-dependence between structures is thought to enhance the overall stability of the organism. Hence, this could be one of the evidences that fluctuating

asymmetry, canalization and morphological integration are related and have unique and overlapping relationships with variability [2]. This supports also the idea of a general developmental mechanism governing the structuralist connection between organs and buffering all perturbations independently from their origin, In addition, one could point to basic properties of genetic architecture such as dominance, epistasis and pleiotropy whereby fluctuating asymmetry, canalization and morphological integration may be together influenced organism's variability. Several lines of evidence have made mention of this finding through divers examples in mouse mandibles. For instance, using a quantitative trait loci (QTL) approach, size and shape variation in mouse mandibles were affected by dominance effects [33], which may allow the system to tolerate reduced gene dosage and subsequent gene products required for normal mandibular development [2]. Likewise, epistatic gene interactions which may act on phenotypic variability in mouse mandibles, have been found to affect fluctuating asymmetry of centroid size in inbred mouse mandibles [34]. As for pleiotropy, it has been found to contribute to mandibular morphology of inbred mice particularly between traits that are functionally or developmentally related [35] where it provides a way to coordinate development between structures with similar functions potentially limiting phenotypic variability. Pertaining to best-fitting models after investigating patterns of morphological integration, it's worth noting that there is a high degree of similarity between parental species on the one hand, and between hybrid groups on the other, as well as between parental species and hybrids. Likewise, morphological patterns of both buffering mechanisms exhibit intra and inter population congruence. These results could be explained in parental species in one hand and in hybrids groups in another hand. At parental level, *S. melanotheron* and *O. niloticus* are closely related within the Cichlidae. Therefore, they could share many characters including protective mechanisms for developmental processes. The protective mechanisms for developmental processes inherited from their common ancestor, have been preserved during evolutionary divergence [8]. In addition, it is also possible that there is a preservation of epigenetic mechanisms through gene regulation in both parental species [36]. Thus, the divergence between the parental genomes does not exceed the threshold that could make the gene systems controlling development incompatible in hybrids. For the latter, the mechanisms protecting developmental processes may have been inherited from the parental species with greater fidelity and without fundamental modification. This suggests also that in both parental and hybrid individuals, there are developmental processes that affect structures in similar ways. Therefore, epigenetic states are not erased and reset in hybrids but heritable. It's a evidence that, some epigenetic states are transmitted intergenerationally through and affect the phenotype of offspring's.

CONCLUSION

This work address 3 developmental issues about the patterning of variability related to morphological integration, canalization and developmental stability in cichlidae fish. A relationship is found between buffering mechanisms which are connected with patterns of morphological integration. There is also a transmission of some features of these properties of morphological variability.

References

[1] Peaston, A. E., and Whitelaw, E. Epigenetics and phenotypic variation in mammals. Mammalian Genome, 17(5), 2006. 365–374.

[2] Willmore, K.E, Young N.M., and Richtsmeier, J.T. Phenotypic variability: its components, measurement and underlying developmental processes. Evol Biol.; 2007. 34: 99–120.

[3] Wagner, G. P., and Altenberg, L. Complex adaptations and the evolution of evolvability. Evolution, 1996. 50, 967–976.

[4] Hallgrımsson, B., Willmore, K., and Hall, B.K. Canalization, developmental stability, and morphological integration in primate limbs. American Journal of Physical Anthropology, 2000. 119: 131–158.

[5] Debat V., and David P. Mapping phenotypes: canalization, plasticity and developmental stability. Trends Ecol Evol, 2001. 16: 555–561.

[6] Cheverud, J. M., Routman, E. J., and Irschick, D. J. Pleiotropic effects of individual gene loci on mandibular morphology. Evolution, 1997. 51, 2006–2016.

[7] Klingenberg CP. Studying morphological integration and modularity at multiple levels: concepts and analysis. Phil. Trans. R. Soc. B, 2014. 369: 2013.

[8] Debat, V., Alibert, P., David, P., Paradis, E., and Auffray, J.-C. Independence between developmental stability and canalization in the skull of the house mouse. Proceedings of Royal Society of London, Series B, 2000. 267, 423–430.

[9] Dworkin, I. A study of canalization and developmental stability in the sternopleural bristle system of Dro sophila melanogaster. Evolution, 2005. 59: 1500–1509.

[10] Palmer, A. R., and Strobeck, C. Fluctuating Asymmetry: Measurement, Analysis, Patterns. Annual Review Ecology Systematics, 1986. 17, 391–421.

[11] Bopo, Z.S.O, Sokouri, D.P, Amon, Y.N, Boua, A.C, and Assanvo, N.S.P. 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. Annual Research & Review in Biology, 2018. 29(1): 1-14.

[12] Klingenberg, C.P, Mebus, K, and Aurffay, J.C. Developmental integration in a complexstructure: how distinct are the modulesin the mousemandible? EvolDev, 2003; 5:522–531

[13] Rognon, X., and Guyomard, R.). Large extent of mitochondrial DNA transfert from *Oreochromis aureus* to *O. niloticus* in west Africa. Mol Ecol ; 2003. 12(2) :435-445.

[14] Rohlf, F.J. TpsSeries.Department of EcologyandEvolution, State University of New York, Stony Brook, NewYork. 2003. Available: http://life.bio.sunysb.edu/morph/index.html. Accessed 2024 July 07.

[15] Rohlf, F. J., and Slice, D. Extensions of the Procrustes method for the optimal superimposition of landmarks. Systematic Zoology, 1990. 39(1), 40–59.

[16] Marquez, E. J. Mint: Modularity and integration analysis tool for morphometric data, 2014. Version, 1, 61.

[17] Klingenberg, C. P., and McIntyre, G. S. Geometric Morpho metrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. Evolution, 1998. 52, 1363–1375.

[18] Breno, M., Bots J., and Van Dongen, S. Relationship between canalization and developmental stability of fœtal rabbit limbs in two reproductive toxicological experiments. Bological journal of the linnean Society, 2011. 109(2), 434-440.

[19] Mabee, P. M., Crotwell, P. L., Bird, N. C., and Burke, A. C. Evolution of median fin modules in the axial skeleton of fishes. Journal of Experimental Zoology (Molecular and Developmental Evolution), 294(2), 2002. 77–90.

[20] Parsons, K. J., Cooper, W. J., and Albertson, R. C. Modularity of the Oral Jaws Is Linked to Repeated Changes in the Craniofacial Shape of African Cichlids. International Journal of Evolutionary Biology, 2011.

[21] Larouche, O., Cloutier, R., and Zelditch, M. L. Head, Body and Fins: Patterns of Morphological Integration and Modularity in Fishes. Evolutionary Biology, 2015. 42(3).

[22] Marquez, E. J.). A statistical framework for testing modularity in multidimensional data. Evolution, 62(10), 2008. 2688–2708.

[23] Richtsmeier, J. T., S. R. Lele, and T. M. Cole, III. Landmark morphometrics and the analysis of variation. Pp. 49-69 in B. Hallgrímsson and B. K. Hall, eds. Variation: a central concept in biology, 2005. Elsevier, Amsterdam.

[24] Zelditch, M. L., J. Mezey, H. D. Sheets, B. L. Lundrigan, and T. Garland. Developmental regulation of skull morphology II: ontogenetic dynamics of covariance. Evol. Dev, 2006. 8:46-60.

[25] Dietz, E. J. Permutation tests for association between two distance matrices. Syst. Zool., 1983. 32:21-26.

[26] Shu Wang; and Dao-Wei Zhou. Developmental stability, canalization and phenotypic plasticity in annual herbaceous species under different biotic and abiotic conditions. Research square, 2021. 22 p.

[27] Clarke, G.M. The genetic basis of developmental stability. V. Inter- and intra-individual character variation. Heredity, 1998. 80, 562–567.

[28] Hoegg S, and Meyer, A. Phylogenomic analyses of KCNA gene clusters in vertebrates: why do gene clusters stay intact? BMC Evol Biol, 2007. 7:139

[29] Siegel N, Hoegg S, Salzburger W, Braasch I, Meyer A: Comparative genomics of ParaHox clusters of teleost fishes: gene cluster breakup and the retention of gene sets following whole genome duplications. BMC Genomics 2007, 8:312.

[30] Streelman, J.T, and Albertson, R.C. Genetic and developmental basis of cichlid trophic diversity Evolution of Novelty in the Cichlid Dentition. Heredity, 2006. 97, 211-221.

[31] Crotwell, P. L., Sommervold, A. R., and Mabee, P. M. Expression of bmp2a and bmp2b in late-stage zebrafish median f in development. Gene Expression Patterns, 2004. 5(2), 291–296.

[32] Klingenberg, C.P. Developmental Constraints, Modules, and Evolvability. Pp. 219-247 in B. Hallgrímsson and B. K. Hall Eds. Variation, 2005. Elsevier, Amsterdam.

[33] Klingenberg, C.P., and Leamy, L.J. Quantitative genetics of geometric shape in the mouse mandible. Evolution, 2001. 55, 2342–2352.

[34] Leamy, L. J., Routman, E. J., and Cheverud, J. M. An epistatic genetic basis for fluctuating asymmetry of mandible size in mice. Evolution, 2002. 56, 642–653.

[35] Cheverud, J. M. Developmental integration and the evolution of pleiotropy. American Zoologist, 1996. 36, 44–50.

[36] Alibert, P. De la microévolution à la macroévolution : exemples d'apports de la morphométrie. Habilitation à Diriger des Recherches, Université de Bourgogne, France, 2006. 191 p.