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Genetic identification of hybrids between two autochthonous tilapia species, *Tilapia zillii* and *Tilapia guineensis*, in the man-made lake Ayamé

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Abstract – Tilapia species are able to hybridize in captivity or in natural conditions following introduction of alien species. Here, we report a case of hybridization between two (and perhaps three) autochthonous species, *Tilapia zillii* and *T. guineensis*, resulting from a modification of their environment due to human activities. The man-made dam Ayamé (Côte d'Ivoire) transformed the River Bia into a lake (Lake Ayamé, area: 197 km^2). In this new environment, both tilapia species have hybridized. In order to study hybridization along the Bia System, 25 allozyme loci have been screened in 470 specimens from ten locations. Two loci *LDH-1* and *PGI-2* were diagnostic between the two different species and can be used to identify hybrids in each sampling location of the River Bia basin. The percentage of pure *T. zillii* individuals in Lake Ayamé varied from 18.1% in Ebikro to 4.2% in Kétesso while percentage of pure *T. guineensis* varied from 81.3% in Yaou to 50.0% in Ebikro. The proportions of hybrids varied from 8.3% in Yaou to 31.9% in Ebikro. The existence of private alleles observed in tilapia from Lake Ayamé compared to what has been observed in both reference populations, *T. zillii* and *T. guineensis* led to the hypothesis that a third species could have been engaged in this hybridization. This third species is likely to be *T. busumana*, formerly present in the lake but now rarely observed. If this natural hybridization is due to the dam construction and the correlated ecological changes, then other hybridizations could result since many dams have been constructed within the range of these species in Côte d'Ivoire.

Key words: Hybridization / Introgression / Man-made lake / Tilapia / Allozyme

Résumé – Identification génétique, à l'aide de marqueurs, des hybrides entre *Tilapia zillii* et *T. guineensis*, deux espèces autochtones du lac artificiel d'Ayamé. Les tilapias sont capables de s'hybrider en captivité comme en conditions naturelles à la suite d'introduction d'espèce par exemple. Dans cette étude, nous mettons en évidence l'hybridation entre deux (peut-être trois) espèces autochtones : *Tilapia zillii* et *T. guineensis*, qui a pour origine les modifications de l'environnement dues aux activités humaines. Le barrage hydroélectrique d'Ayamé (Côte d'Ivoire) a transformé la rivière Bia en un lac d'une superficie de 197 km^2. Dans ce nouvel environnement, les deux espèces de tilapia se sont hybridées. Afin d'étudier cette hybridation sur l'ensemble du système hydrographique de la Bia, 25 locus enzymatiques ont été étudiés chez 470 spécimens provenant de 10 stations différentes réparties le long du bassin hydrographique. Deux locus, *LDH-1* et *PGI-2*, se sont révélés diagnostics entre les deux espèces et ont été utilisés pour identifier les hybrides dans chaque localité échantillonnée. Le pourcentage d'individus *T. zillii* non hybridés dans le lac d'Ayamé a varié de 4,2 % (Kétesso) à 18,1 % (Ebikro) alors que la représentation de *T. guineensis* a variée de 50,0 % (Ebikro) à 81,3 % (Yaou). Des hybrides ont été observés dans toutes les stations du réseau hydrographique de la Bia et ont constitués de 31,9 % (Ebikro) à 8,3 % (Yaou) des effectifs des populations. L'observation de 6 allèles privés chez les tilapias du lac d'Ayamé comparativement aux populations de référence prises pour *T. zillii* et *T. guineensis*, laisse penser qu'une troisième espèce de tilapia serait également engagée dans ces hybridations. Cette troisième espèce pourrait être *T. busumana* qui était autrefois présente dans la rivière puis le lac à ses débuts mais qui n'est que très rarement été observée de nos jours. Si cette hybridation naturelle est bien due à la construction du lac de barrage et aux modifications de l'environnement qu'il a apportées, alors il est vraisemblable que d'autres cas d'hybridation devraient avoir lieu entre *T. zillii* et *T. guineensis* car ces barrages sont nombreux dans toute la Côte d'Ivoire et l'Afrique de l'Ouest en général.

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1 Introduction

Tilapia zillii (Gervais 1848) and *T. guineensis* (Bleeker 1862) are two morphologically and genetically related cichlid species belonging to the same subgenus *Coptodon* (Thys van den Audenaerde 1968; Pouyaud and Agnèse 1995). They occur naturally in most West African basins. *Tilapia zillii* is a fresh water species present in many West African rivers including the Sénégal, Niger, Volta, Sassandra and Bandama rivers. It can also be found in Lakes Chad, Albert, Turkana, the Nile River and the Jordan basins (Lévêque et al. 1992). *Tilapia guineensis* is a brackish water species present in all coastal basins from Sénégal to Angola. It can sometimes be present in rivers distant from the sea as in the Gambia and Mono rivers (Teugels and Thys van den Audenaerde 1992). These two species can thereby be considered as parapatric in many rivers where they are both abundant. In Côte d'Ivoire, live specimens of each species are easy to distinguish one from the other using coloration as a criterion. *Tilapia guineensis* displays a typical caudal coloration, the top half grey and the bottom half yellow without any spot. *Tilapia zillii* has a uniform grey caudal fin with white spots (Gourène et al. 1995; Nobah et al. 1996). These two species can also be genetically distinguished using three diagnostic allozyme loci. Pouyaud (1994) analyzed 10 West African populations of *T. zillii* and 23 of *T. guineensis*. *Tilapia guineensis* was characterized by the alleles *EST-1*60*, *LDH-1*100*, *PGI-2*100*, whereas *T. zillii* possessed alleles *EST-1*80*, *LDH-1*250*, *PGI-2*108*.

In Lake Ayamé (at Ayamé), a man-made lake on the Bia River in Côte d'Ivoire, some specimens have been found to display intermediate caudal fin colorations. These fishes were also genetically intermediate showing all the different possible combinations of the diagnostic alleles and have been considered as hybrids (Pouyaud 1994; Pouyaud and Agnèse 1995). Later on, Gourène et al. (1999) and Nobah et al. (2006) confirmed the presence of specimens with intermediate coloration at Ayamé. Until now the range of this hybridization is still unknown. Does this hybridization only occur around Ayamé or in the whole Lake or even in the river? In order to assess the importance of this phenomenon in the whole of the River Bia basin, a survey of different populations has been done in Lake Ayamé, along the River Bia downstream and upstream, in the Aby Lagoon where the river ends as well as in some other river basins in which the populations were considered as control (i.e. pure specimens).

2 Materials and methods

Fish (470 specimens) were sampled using gill nets in 10 locations (Fig. 1): Kétesso (KET), Ebikro (EBI), Bakro (BAK), Yaou (YAO) and Ayamé (AYA) in Lake Ayamé; Bianouan (BIA), on the Bia River upstream of the lake; Aboisso (ABO) on the Bia River downstream of the lake. Reference samples were taken for both species: Aby lagoon (ABY) at Adiake for *T. guinenensis*, Adzopé (ADZ) on the Me River and Bouaké (BOU) in the Bandama basin for *T. zillii*.

After dissection, a piece of muscle, liver and eye were stored in liquid nitrogen until the analysis which was done a few months later. Protocols used for allozyme analyses are described in Pouyaud and Agnèse (1995) and in Agnèse et al. (1997).

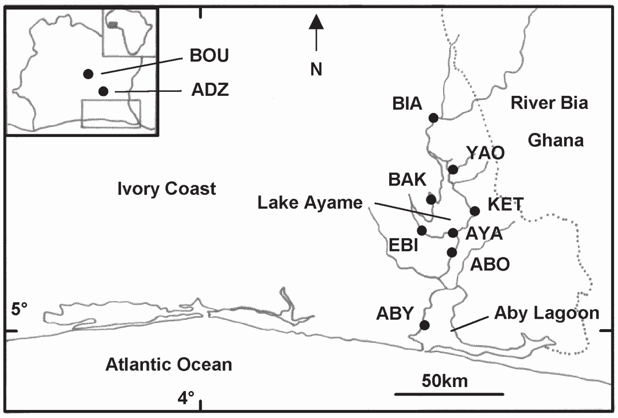

Fig. 1. Sampling locations for *Tilapia zillii*, BOU (Bouaké), ADZ (Adzopé); *T. guineenesis*, ABY (Adiaké, Aby Lagoon), and Hybrid populations from the Bia system, BIA (Bianouan), YAO (Yaou), KET (Kétesso), EBI (Ebikro), BAK (Bakro), AYA (Ayamé), ABO (Aboisso).

Population genetics statistics were computed using the GENETIX software (Belkhir et al. 1996). Genetic diversity within populations was evaluated by computing observed and unbiased expected heterozygosity. Fixation index (F) statistics were evaluated using Weir and Cockerham (1984) estimator. To test the departures from Hardy Weinberg equilibrium proportions, permutations of alleles within each population were realized and the F_{is} ($F_{is} = (\bar{H}_S - H_S) / \bar{H}_S$ where H_S is the expected heterosygosity and H_I the observed heterozygosity) estimator (f) calculated for each data set. This gave a distribution of the F_{is} estimator under the null hypothesis (panmixia i.e. when there is random breeding and free interchange of genes within a population). The F_{is} will be considered significantly different from 0 at $p \leq 0.05$. When two different genomes hybridize, this creates a important genotypic linkage disequilibrium in the hybrid population (when genotypes at one locus are not independent from genotypes at the other locus there is a genotypic disequilibrium between them). Linkage disequilibria were determined for all pairs of loci by the Linkdis algorithm (Black and Krafsur 1985). The existence of genotypic linkage disequilibrium was tested by permuting monolocus genotypes among individuals in the same sample. All tests were realized with 1000 permutations.

3 Results

Twelve enzymatic systems have been screened for polymorphism at 25 loci. The diagnostic Esterase (EST) system (Pouyaud 1994) has not been retained due to difficulties to interpret some patterns obtained. The 10 samples were monoallelic for the same allele at 15 loci: *AAT-1*, *AAT-3*, *AK*, *CK-3*, *FPB-1*, *FBP-2*, *IDH-1*, *LDH-2*, *LDH-3*, *MDH-1*, *MDH-2*, *PGI-1*, *PGM*, *PT-1* and *SOD*. The allelic frequencies observed at polymorphic loci are listed in Table 1. Reference

Table 1. Sample size (*n*), allozyme frequencies, average observed heterozygosity (H obs.), unbiased heterozygosity (H. n.b.) and percentages of polymorphic loci (P_{95}) in ten populations of tilapia from Côte d'Ivoire. See Fig. 1 for population abbreviations, Tz: *Tilapia zillii*, Tg: *T. guineensis*. Diagnostic loci between *T. zillii* and *T. guineensis* are underlined. Private alleles (of unknown origin) only found in the Bia System are in bold.

Locus		Population									
		BOU	ADZ	KET	EBI	BAK	AYA	BIA	YAO	ABO	ABY
		Tz		River Bia System including Lake Ayamé							Tg
AAT-2	(*n*)	*(26)*	*(2–)*	*(47)*	*(70)*	*(64)*	*(70)*	*(22)*	*(48)*	*(48)*	*(28)*
	50			**0.33**	**0.24**	**0.32**	**0.26**	**0.39**	**.034**	**0.01**	
	100	1.00	1.00	0.67	0.76	0.68	0.74	0.61	.066	0.99	1.00
ADH	(*n*)	*(27)*	*(27)*	*(48)*	*(70)*	*(75)*	*(70)*	*(22)*	*(48)*	*(46)*	*(28)*
	50	1.00	1.00	0.27	0.56	0.48	0.39	0.59	0.27	0.92	0.12
	100			0.73	0.44	0.52	0.61	0.41	0.73	0.08	0.88
CK-1	(*n*)	*(27)*	*(27)*	*(48)*	*(72)*	*(75)*	*(75)*	*(22)*	*(48)*	*(48)*	*(28)*
	80			**0.02**		**0.01**					
	100	1.00	1.00	0.98	1.00	0.99	1.00	1.00	1.00	1.00	1.00
CK-2	(*n*)	*(27)*	*(27)*	*(48)*	*(72)*	*(75)*	*(75)*	*(22)*	*(48)*	*(48)*	*(28)*
	80									**0.01**	
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00
IDH-2	(*n*)	*(27)*	*(27)*	*(48)*	*(72)*	*(75)*	*(75)*	*(22)*	*(48)*	*(48)*	*(28)*
	90	0.02									
	100	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
LDH-1	(*n*)	*(27)*	*(27)*	*(48)*	*(72)*	*(75)*	*(73)*	*(22)*	*(48)*	*(48)*	*(28)*
	100			0.84	0.65	0.75	0.73	0.77	0.87	0.11	1.00
	250	1.00	1.00	0.16	0.35	0.25	0.27	0.23	0.13	0.89	
MPI	(*n*)	*(27)*	*(27)*	*(45)*	*(20)*	*(23)*	*(18)*	*(0)*	*(0)*	*(48)*	*(28)*
	80	0.28	0.28	0.11	0.15	0.09	0.28			0.33	
	100	0.72	0.72	0.89	0.85	0.91	0.75			0.67	1.00
PGDH	(*n*)	*(27)*	*(26)*	*(48)*	*(72)*	*(75)*	*(75)*	*(22)*	*(48)*	*(43)*	*(28)*
	80		0.52								
	100	1.00	0.48	1.00	1.00	1.00	1.00	1.00	1.00	0.69	1.00
	105									**0.31**	
PGI-2	(*n*)	*(27)*	*(27)*	*(48)*	*(72)*	*(75)*	*(75)*	*(22)*	*(48)*	*(48)*	*(28)*
	100			0.91	0.69	0.72	0.74	0.80	0.86	0.10	1.00
	108	1.00	1.00	0.09	0.31	0.27	0.26	0.20	0.14	0.90	
PT-2	(*n*)	*(27)*	*(27)*	*(48)*	*(712)*	*(74)*	*(73)*	*(22)*	*(48)*	*(48)*	*(28)*
	80			**0.03**	**0.03**	**0.05**	**0.07**	**0.14**	**0.08**	**0.01**	
	100	1.00	1.00	0.96	0.97	0.95	0.93	0.86	0.92	0.99	1.00
	105			**0.01**							
H obs.%		1.8	2.4	5.0	4.5	4.3	5.0	5.3	4.0	4.5	0.7
H n.b.%		1.8	3.7	6.4	8.3	7.9	8.6	8.0	6.1	5.9	0.9
P_{95}		0.04	0.08	0.20	0.20	0.20	0.24	0.21	0.21	0.20	0.04

populations of *T. zillii* (from Bouaké and Adzopé) and *T. guineensis* (from Aby Lagoon) could be easily distinguished by two diagnostic loci as previously described from all West African populations studied (Pouyaud 1994; Agnèse et al. 1997). All *T. guineensis* specimens were homozygous for the alleles *LDH-1*100* and *PGI-2*100* while all *T. zillii* specimens were homozygous for the alleles *LDH-1*250* and *PGI-2*108* (Table 1). Another locus, *ADH*, even though not diagnostic, was helpful to distinguish the two species: all individuals of *T. zillii* of Bouaké and Adzopé populations were homozygous for the allele *ADH*50* while *T. guineensis* from Aby Lagoon possess two alleles *ADH*50* and *ADH*100*, the latter being more frequent (88%).

As expected populations that contained hybrids were highly polymorphic compared with the populations composed of only one species. The observed heterozygosity varied from 4% in Yaou to 5.3% in Bianouan (hybrids) while never exceeded 2.4% in a pure species population (Adzopé for *T. zillii*). The unbiased heterozygosity and percentage of polymorphism loci (P_{95}) followed the same pattern.

Table 2. Number of individuals of each different possible genotype observed at the diagnostic loci. Individuals homozygous for the alleles *LDH-1*250* and *GPI-2*108* have been considered as pure *T. zillii,* except those which possess at least one allele *ADH*100* and were considered as hybrids and indicated in brackets. Individuals homozygous for the alleles *LDH-1*100* and *GPI-2*100* have been considered as pure *T. guineensis*. All the other individuals have been considered as hybrids. * statistically significant F_{is} value ($p < 0.05$), ** statistically highly significant F_{is} value ($p < 0.001$). Sp. Comp., species composition of the population (taking into account that hybrids proportions are underestimated). See Fig. 1 for population abbreviations.

Population	BOU	ADZ	ABO	KET	EBI	BAK	AYA	BIA	YAO	ABY
Genotype										
LDH-1 GPI-2										
T. zillii										
250/250 108/108	27	27	38$^{(2)}$	2	13	15$^{(6)}$	12$^{(2)}$	3	5	
Hybrids										
100/250 108/108			3		3	1	1			
100/100 108/108				1	5	1	3		1	
250/250 100/108			1		1	1	1			
100/250 100/108			2		1	1	1	1		
100/100 100/108				3	2	7	4	2	1	
250/250 100/100				2	8		2	1		
100/250 100/100				4	3	1	7	1	2	
T. guineensis										
100/100 100/100			4	36	36	48	42	14	39	28
Sp comp. (%) (composition%										
T. zillii	100	100	75.0	4.2	18.1	12.0	13.7	13.9	10.4	
Hybrids			16.7	20.8	31.9	24.0	28.8	22.7	8.3	
T. guineensis			8.3	75.0	50.0	64.0	57.5	63.7	81.3	100
Fis *LDH-1*			0.495**	0.455**	0.790**	0.823**	0.689**	0.751**	0.813**	
Fis *GPI-2*			0.561**	0.638**	0.806**	0.635**	0.760**	0.596*	0.913**	
Fis *ADH*			0.237**	0.166	0.427**	0.577**	0.437**	0.270**	0.271**	0.201
Fis Total	0.000	0.324	0.182**	0.240**	0.539**	0.458**	0.456**	0.340**	0.349**	0.201

Some private alleles have been found in populations from the River Bia system but never in any of the "reference populations": *PT-2*105* in Kétesso (1%), *PGDH*105* in Aboisso (31%), *CK-1*80* in Kétesso (2%) and Bakro (1%), *CK-2*80* in Aboisso (1%), *AAT-2*50* in all populations (from 1% in Aboisso to 39% in Bianouan).

The reference populations (Bouaké and Adzopé for *T. zillii* and Aby for *T. guineensis*) were characterized by non-statistically significant F_{is} values and no genotypic disequilibrium could be found. These populations could be considered as panmictic. In all other populations from Lake Ayamé or from River Bia (upstream or downstream of the lake) both individuals homozygous for *LDH-1*250* and *PGI-2*108* or for *LDH-1*100* and *PGI-2*100* have been found (Table 2). These individuals could be considered as belonging to *T. zillii* or *T. guineensis* respectively. Many heterozygotes at the two diagnostic loci were noted. These individuals have been considered as hybrids between the two species. In Ayamé and Ebikro populations, all possible genotypes have been observed indicating that these hybrids are not only F1 but were also the result of backcrossing. In the other populations (Aboisso, Bakro, Bianouan, Kétesso and Yaou) most of the hybrids are not F1. In three populations (Aboisso, Bakro and Ayamé) individuals homozygous for *LDH-1*250* and *PGI-2*108* i.e. supposed to belong to the species *T. zillii*, possessed at least one allele *ADH-100*. These individuals were also considered as hybrids. In all these populations, highly significant F_{is} values were observed (after a Bonferonni correction, Rice 1989) indicating heterozygote deficiencies (Table 2).

The hybrid populations were also characterized by high genotypic disequilibria (Table 3) mainly between the diagnostic loci (*LDH-1*, *PGI-2* and *ADH*). Linkage disequilibria were highly statistically significant between *LDH-1* and *PGI-2* in all populations from the Ayamé drainage but one (only significant in Kétesso).

Observed proportions of hybrids varied from 8.3% at Yaou to 31.9% at Ebikro. Proportions of the different pure specimens of each species also varied from 4.2% at Kétesso to 75% at Aboisso for *T. zilli*, and from 8.3% at Aboisso to 81.3% at Yaou for *T. guineensis*. Taking into account that these hybrids were not F1, some backcrosses fish could have been considered as pure. Percentages of hybrids observed are then underestimations of their presence in the populations (and consequently, pure species proportions are overestimated).

In the Bia drainage, five loci exhibited six private alleles that have neither been observed in *T. zillii* nor in *T. guineensis* populations: *AAT-2*50, CK-1*80, CK-2*80, PGDH*105, PT-2-*80* and *PT-2*105.* Three of these (*CK-1*80, CK-2*80* and *PT-2*105)* appeared rarely (one or two heterozygotes within approximately 400 individuals studied) and could easily be present in *T. zillii* or *T. guineensis* for which samples sizes were less important (54 and 28 specimens respectively). For the three others alleles (*AAT-2*50, PGDH*105* and *PT-2-*80*), it is not possible to explain their presence taking into account

Table 3. Genotypic disequilibrium observed. * statistically significant value ($p < 0.05$), ** highly statistically significant value ($p < 0.001$). Values for the two diagnostic loci *LDH-1* and *PGI-2* are in bold. See Fig. 1 for population abbreviations.

Population	ADZ	ABO	AYA	BAK	KET	BIA	YAO	EBI
Loci	Genotypic disequilibrium							
AAT-2 / ADH		0.056	0.194	0.135	0.238	0.243	0.348*	0.427**
AAT-2 / LDH-1		0.062	0.099	0.273*	0.095	0.475*	0.310	0.461**
AAT-2 / PGI-2		0.058	0.285*	0.281*	0.350*	0.448	0.370**	0.339**
ADH / LDH-1		0.609**	0.468**	0.120	0.096	0.249	0.647**	0.632**
ADH / MPI		0.111	0.066	0.445	0.260			0.775**
ADH / PGDH		0.428**						
ADH / PGI-2		0.756**	0.525**	0.145	0.486**	0.287	0.809**	0.531**
CK-2 / PT-2		0.100*						
LDH-1 / MPI		0.204	0.625*		0.283			0.230
LDH-1 / PGDH		0.550**						
LDH-1 / PGI-2		**0.914****	**0.674****	**0.852****	**0.339***	**0.756****	**0.847****	**0.531****
MPI / PGDH	0.505*	0.090						
MPI / PGI2		0.176	0.439	0.117	0.630**			0.289
PGDH / PGI2		0.538**						
PGI-2 / PT-2		0.219	0.065	0.314**	0.086	0.366	0.185	0.034

the differences in sampling sizes. These private alleles are either found with high frequencies or found in every populations of the Bia Drainage.

4 Discussion

In museum collections, natural hybrids had never been reported between *T. zillii* and *T. guineensis* (Teugels and Thys van den Audenaerde 1992). In Lake Ayamé, hybridization occurs (these results confirmed and clarify previous observations: Pouyaud 1994; Gourène et al. 1999; Nobah et al. 2006) and hybrids can represent more that 31.9% (underestimated value observed in the Ebikro population; Table 2). Large F_{is} values exist in all Lake Ayamé populations and can be due to prezygotic barriers and/or to a lower fitness of the hybrids and between the two species in a lake environment. This second hypothesis could probably be tested later (e.g. by determining the fertility of F1 and backcrossed individuals). The hybrid populations could also be characterized by high genotypic disequilibria (Table 3). These disequilibria were mainly observed between diagnostic loci as expected when two different genomes hybridize. All these observations indicated that samples were not panmictic or at least that some Wahlund effect (Wahlund 1928; Hartl and Clark 1997) does exist (a subdivided population contains fewer heterozygotes than predicted despite the fact that all subdivisions are in Hardy-Weinberg equilibrium) and confirmed the presence of hybrids all along the Bia System.

We now have clear evidence that *T. zillii* and *T. guineensis* hybridize in Lake Ayamé, but there is also the possibility that a third species could have participated in this hybridization event. Private alleles were observed in all samples from the Bia drainage. There are two alternative hypotheses to explain the presence of such private alleles. In the first hypothesis, one can consider that these alleles were already present in the native pure population of *T. zillii* in the Bia Drainage, this population being somewhat differentiated from the two pure populations (Bouaké and Adzopé) sampled in another drainage. The reciprocal situation for *T. guineensis* is not possible because landlocked *T. guineensis* in Lake Ayamé are supposed to have originated from the Aby Lagoon population. Pouyaud (1994) in a genetic survey of West African populations of *T. zillii* analysed 10 populations from Côte d'Ivoire, Sénégal and Niger. None of these populations exhibited a polymorphism for four of these five loci *CK-1*, *CK-2*, *PGDH* and *PT-2*. Only the locus *AAT-2* was polymorphic in the Shibli population (in the Western part of Côte d'Ivoire) with one private allele *AAT-2*160*. This allele could not be the same as that which we named *AAT-2*50* because the former is faster and the latter is slower than the common *AAT-2*100* (migration buffers and coloration recipes were the same in both studies). Considerate is therefore unlikely that the pure *T. zillii* population that was present in the Bia Drainage could have been so different from the other *T. zillii* populations. A second hypothesis, where these private alleles could have originated from a third tilapia species, could be applicable. The occurrence of hybridization between three tilapia species has been observed downstream of the Koroboué Falls in the Comoé River. This hybridization occurred between *T. zillii*, *T. guineensis* and *T. dageti* Thys van den Audenaerde, 1971 (Pouyaud 1994). These three species hybridized when individuals of *T. dageti*, living normally upstream of the Koroboué Falls, went down and got in contact with the other two tilapia species. In the present case, the third species could have been *T. busumana* (Günther 1903) which was formerly very common in the River Bia before the dam construction but has become very rare nowadays. This hypothesis still needs to be confirmed. In any case, hybridization seems to be due to man-made ecological changes through dam construction. This implies that similar situations will have the same consequences. Since man-made lakes are numerous in West Africa and especially in Côte d'Ivoire, such hybridization may have occurred several times.

Tilapias species are well known for their ability to hybridize in captivity, and a large number of crosses have occurred. Quite often, the F1 individuals are viable and at most times fertile (Trewavas 1983). Under natural conditions, there are numerous cases of hybridization in tilapia

(Agnèse et al. 1998). Most of them following species introduction resulting in one or more introduced species hybridizing with native or previously introduced species. First, some factors lead to the rupture of ethological barriers between species (when they existed), then competition phenomena between the parent species and their hybrids enable the situation to evolve, often in favor of one of the parental species. For example, in Lake Naivasha in Kenya, *Oreochromis spilurus niger* Günther, 1894, was introduced in 1925 followed by *O. leucostictus* (Trewavas 1933) (Elder et al. 1971). In 1959, the first hybrids between the two species were harvested. By 1972, *O. spilurus* had disappeared from the lake (Siddiqui 1977; 1979). In Lake Itasy, in Madagascar, *O. macrochir* (Boulenger 1912) was introduced in 1958 and *O. niloticus* (Linnaeus, 1758) in 1961. In 1965 and 1966 intermediate specimens between these two species were found and named "tilapias trois quarts" (three quarter tilapias) (Daget and Moreau 1981). These hybrid individuals had a noticeable pharyngeal bone resembling that of *O. niloticus* but were morphologically closer to that of *O. macrochir*. Between 1965 and 1969, the hybrid population in the captures increased from 5% to 74%. *O. macrochir* was considered as vanished in 1971 and *O. niloticus* became predominant.

Here we report a case where hybridization is associated with an anthropogenic modification of the environment. *Tilapia zillii* and *T. guineensis,* both autochthonous, are phylogenetically very close (Pouyaud and Agnèse 1995). They belong to the same subgenus *Coptodon* (Thys van den Audenaerde 1968). According to their distribution (Teugels and Thys van den Audenaerde 1992), it appears that both species are able to inhabit rivers, lagoons or lakes. Nevertheless, if the two species are present in the same basin, then *T. zillii* occupies the riverine environment while *T. guineensis* occupies the lagoon or lake environment. For example *T. zillii* is present in Lakes Chad (Chad), Turkana (Kenya), and Manzalla (Egypt) where *T. guineensis* is not present. *Tilapia guineensis* is present all along the rivers Mono (Benin) Cross (Nigeria), Gambia (Gambia, Sénégal and Guinea) and Konkoure (Guinea) where *T. zillii* is absent of these basins. With the construction of the Ayamé Dam in 1959, the upper part of the River Bia became a lake. Both species were likely to have been present in the upper river during the construction of the lake because *T. guineensis*, the lagoon species, is well known to go very far upstream of rivers, even if it is not able to establish a population in these rivers. Some individuals were thus blocked upstream and after the few months necessary to fill the dam, the two species were present in a new environment, the river being transformed into a lake. It is then likely that *T. guineensis* had a greater advantage over *T. zillii* in this new context and as a consequence was regularly observed in fisheries statistics (Gourène et al. 1995). A similar situation has been observed with another Tilapine species inhabiting the Aby lagoon, *Sarotherodon melanotheron* Rüppell, 1852. This is a brackish water species also able to live and reproduce in fresh water (Gilles et al. 1998). In 1974 this species appeared in the fisheries statistics of Lake Ayamé and is now the dominant species in the lake (Gourène et al. 1995). Some extent of prezygotic barriers to prevent hybridization between *T. zillii* and *T. guineensis* could exist because from the previous 33 populations genetically analyzed from rivers or natural lakes and lagoons, no traces of hybridization have been found even in populations coming from the mouth of the rivers where both species were present and sampled (Pouyaud 1994).

Taking into account the present results, a survey of tilapia populations in other man-made lakes in Côte d'Ivoire and in all regions where both species are parapatric is necessary. The future of *Tilapia* species in Lake Ayamé is also questionable. It is very likely that hybrid proportions are not reflecting a stable situation but only a transitory step. How these populations or species will evolve? To address this question, the genetic composition of *Tilapia* populations in Lake Ayamé should be monitored now and in the future.

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