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Enzymatic variation in African clariid catfishes

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Enzymatic polymorphism was examined at 13 protein loci in four African clariid catfish species: *Clarias anguillaris* (Linnaeus, 1758), *C. ebriensis* Pellegrin, 1920, *C. gariepinus* (Burchell, 1822) and *Heterobranchus longifilis* Valenciennes, 1840. The latter appears to be closer to *C. anguillaris* and *C. gariepinus* than *C. ebriensis*. These results correspond with recently published karyological and morphometrical data.

Reproductive compatibility, under laboratory conditions at least, is demonstrated between *C. gariepinus* and *H. longifilis*. The hybrids were shown to be completely intermediate between the parental strains.

Key words: *Clarias*; *Heterobranchus*; hybrids; enzymatic variation.

I. INTRODUCTION

Catfishes of the genus *Clarias* (Clariidae) together with various tilapiine species (Cichlidae) are at present the most important freshwater fishes used in fish culture in Africa. The taxonomy of both groups has for a long time been very confusing and only recently have detailed systematic revisions become available, enabling the correct identification of the species used [Thys van den Audenaerde (1970) and Trewavas (1983) for tilapiine cichlid fishes; Teugels (1986) for *Clarias*].

The foregoing monographs are based on analyses of morphological and osteological characters of large collections of fishes, and may be regarded as a 'classical' taxonomic approach. In some cases, however, problems in identification remain unresolved. Other, more recent techniques, such as cytology and electrophoresis, have lately been applied to good effect in such cases, especially to differentiate tilapiine species. Several authors have reported efficient electrophoretic methods for species identification [see Trewavas & Teugels (1991) for a bibliographic account]. Apart from species identification, these methods prove useful in 'race' or 'strain' identification and also in hybrid recognition. From this, the direct application of these new techniques to tilapiine and other pisciculture is now evident.

Despite the increasing commercial importance of African clariid catfishes in capture fisheries and pond culture, none of these modern techniques have been tested on them until recently. Ozouf-Costaz *et al.* (1990) provided the first account of a karyological study of different strains of *C. gariepinus* (Burchell, 1822).

The present paper deals with the results of a study on enzymatic polymorphism in three species of *Clarias*, *C. anguillaris* (Linnaeus, 1758), *C. gariepinus* (Burchell,

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TABLE I. Collecting data and key characters for populations examined of *Heterobranchus longifilis* (HLO1/HLO2), *Clarias gariepinus* (CGA/CGA2/CGAn), the hybrids between female *H. longifilis* and male *C. gariepinus* (female HLO1 × male CGA1), *C. anguillaris* (CAN1/CAN2/CANn) and *C. ebriensis* (CEB)

Code	Origin	Date	*n*	S.L. (mm)	H.L. (%SL)	LAd (%SL)	GR
HLO1	Layo, Ivory Coast	Apr. 1988	6	262–760	30·4–34·0	27·4–29·7	25–32
HLO2	Layo, culture	Oct. 1989	6	245–530	32·3–33·9	27·9–30·6	24–27
CGA1	Bouake, culture	Apr. 1988	7	335–505	27·0–32·3	Absent	59–88
CGA2	Bouake, culture	Oct. 1989	10	172–272	29·0–32·1	Absent	36–48
CGAn	Seberi, Niger	Sep. 1989	2	176–232	29·4–31·0	Absent	57–78
female HLO1 × male CGA1		Apr. 1988	6	342–490	29·3–32·1	15·8–19·4	32–38
CAN1	Layo, brooks	Apr. 1988	6	220–325	29·6–32·0	Absent	28–31
CAN2	Layo, brooks	Oct. 1989	9	170–400	30·0–31·7	Absent	33–47
CANn	Seberi	Sep. 1989	8	171–256	29·5–32·6	Absent	24–32
CEB	Layo, brooks	Apr. 1988	6	185–275	21·0–24·6	Absent	16–20

n = Sample size; S.L. = standard length; H.L. = head length; LAd = length adipose fin; GR = gill rakers on first branchial arch.

1822) and *C. ebriensis* Pellegrin, 1920, one species of the genus *Heterobranchus*, *H. longifilis* Valenciennes, 1840 and the hybrid of *C. gariepinus* × *H. longifilis*, obtained under artificial conditions.

II. MATERIALS AND METHODS

The material examined is listed in Table I. HLO1 specimens were taken from the domesticated Layo strain; they originally descended from wild stocks that spontaneously colonized the fish ponds at Layo from the neighbouring inundated plains during the 1982 rainy season (Legendre, 1983). Although no evidence is available for confirmation, it is believed that the CGA1 population descends from an interbreeding of two populations of *C. gariepinus*, one originating from Ivory Coast, the other introduced from Central African Republic (see also Discussion). The HYB population descends from an artificial reproduction of the *C. gariepinus* strain from Bouake (CGA1) and the *H. longifilis* strain from Layo (HLO1).

Clarias species were identified using the keys produced by Teugels (1986), while the *Heterobranchus* species was determined following Teugels *et al.* (1990). The specimens examined have all been deposited in the Muséum National d'Histoire Naturelle, Paris (France), except for the Niger specimens that are housed in the Musée Royal de l'Afrique Centrale, Tervuren (Belgium).

Muscle and liver tissue was removed from the specimens at the Layo station (Ivory Coast). Tissues were immediately frozen (−20° C) and sent to the Institut National de Recherches Agronomiques (INRA) at Jouy-en-Josas (France) for electrophoretic analysis. Ten enzyme systems were analysed (Table I). Electrophoretic and staining procedures are described in Guyomard & Krieg (1983) and Krieg & Guyomard (1985). Locus and allele nomenclature follow the general recommendations proposed by Shaklee *et al.* (1990). Heterozygosities and standard genetic distances were calculated according to Nei (1975).

III. RESULTS

The electrophoretic conditions used in this study are given in Table II. The number of loci encoding the different enzyme systems has been assessed from (1)

TABLE II. Electrophoretic conditions used and genetic determinants of the enzyme systems examined in this study

Enzyme	Enzyme number	Tissue	Locus	Migration buffer
Aspartate aminotransferase (AAT)	2.6.1.1	Muscle	*AAT-1**	MC1
			*AAT-2**	
Creatine kinase (CK)	2.7.4.3	Muscle	*CK**	TCB1
Fumarase hydratase (FH)	4.2.1.2	Muscle	*FH**	MC1
Glucose-6-phosphate isomerase (GPI)	5.3.1.9	Muscle	*GPI-1**	TCB1
		Muscle, liver	*GPI-2**	TCB1
Isocitrate dehydrogenase (IDHP)	1.1.1.42	Muscle	*IDHP-1**	T.P.
		Liver	*IDHP-2**	MC2
L-Lactate dehydrogenase (LDH)	1.1.1.27	Muscle	*LDH-1**	TCB1
		Liver	*LDH-2**	
Malate dehydrogenase (MDH)	1.1.1.37	Muscle	*MDH-2**	MC1
		Liver	*MDH-1**	MC2
Phosphogluconate dehydrogenase (PGDH)	1.1.1.44	Muscle	*PGDH**	T.P.
Phosphoglucomutase (PGM)	5.4.2.2	Muscle	*PGM**	TCB1
Superoxyde dismutase (SOD)	1.15.1.1	Muscle, liver	*SOD**	TCB1

MC1 and MC2: morpholine-citrate pH 6·1 (Clayton & Tetriak, 1972); TCB1: Discontinuous system (Ridgway *et al.*, 1972); T.P.: Tris-phosphate pH 7·4; buffers described in detail in Guyomard & Krieg (1983).

the comparison of the phenotypes observed in liver and muscle and (2) the variations among individuals in each of the two tissues; when no variation among individuals was observed within a species, the enzyme was assumed to be encoded by only one locus in this tissue. The different enzyme systems were found to be encoded by the same number of loci in the four species examined. Originally, 15 loci were identified (Table II). Two of them, *AAT-2**, and *IDHP-2**, however, were not adequately resolved in all the samples and therefore were not further taken into account. The electrophoretic polymorphism observed in the 13 remaining loci is shown in Table III. Figure 1 shows the electrophoretic variations seen in *LDH-1** and *LDH-2**, *PGM** and *SOD**. The female *H. longifilis 1* × female *C. gariepinus 1* specimens analysed were all heterozygous at *PGM** and *MDH-2** for the alleles observed in the parental stocks. They also exhibited intermediate frequencies between *C. gariepinus 1* and *H. longifilis 1* at the other loci. These results confirm that the interspecific fertilization between both species resulted in hybrid progenies. The heterozygosity levels are given in Table III. It is noteworthy that the value observed in the ' domesticated ' *H. longifilis* stock is 0·00 *v.* 0·11 in the wild stock.

The largest genetic divergence was between *C. ebriensis v. H. longifilis*, *C. gariepinus* and *C. anguillaris*. Five loci (*AAT-1**, *LDH-1**, *MDH-2**, *PGDH** and *SOD**) were systematically found to be discriminating between *C. ebriensis* and the three other species. An additional locus (*CK**) was fixed for alternate alleles in *C. ebriensis* and *C. anguillaris*. The differentiation between *C. anguillaris*, *C. gariepinus* and *H. longifilis* was much lower. Only two discriminating loci were found between *H. longifilis* and *C. gariepinus* and none between *C. anguillaris* and

TABLE III. Allele frequencies at 13 loci in the 10 samples of clariid catfishes analysed

Loci	Allele	Sample									
		HLO1 ($n=6$)	HLO2 ($n=6$)	CGA1 ($n=7$)	CGA2 ($n=10$)	CGAn ($n=2$)	CAN1 ($n=6$)	CAN2 ($n=9$)	CANn ($n=8$)	HYB ($n=6$)	CEB ($n=5$)
*AAT-1**	100	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	0·0
	140	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	1·0
*CK**	100	1·0	1·0	0·79	0·30	1·0	0·0	0·0	0·0	0·67	1·0
	120	0·0	0·0	0·21	0·70	0·0	1·0	1·0	1·0	0·33	0·0
*FH**	100	1·0	1·0	0·29	0·30	0·0	1·0	1·0	0·94	0·67	1·0
	120	0·0	0·0	0·71	0·70	1·0	0·0	0·0	0·06	0·33	0·0
*IDHP-2**	100	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	0·80
	70	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·20
*LDH-1**	100	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	0·0
	120	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	1·0
*LDH-2**	100	1·0	1·0	0·29	0·50	0·0	0·67	1·0	0·81	0·75	1·0
	50	0·0	0·0	0·71	0·50	1·0	0·33	0·0	0·19	0·25	0·0
*MDH-1**	100	1·00	0·60	0·71	0·80	0·0	0·0	0·11	0·0	0·83	1·0
	75	0·00	0·40	0·29	0·20	1·0	1·0	0·89	1·0	0·17	0·0

*MDH-2**	100	0·00	0·0	1·00	1·0	1·0	1·0	1·0	1·0	0·50	0·0
	105	1·0	1·0	0·0	0·0	0·0	0·0	0·0	0·0	0·50	0·0
	32	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·00	1·0
*PGDH**	100	1·0	1·0	0·14	0·10	0·0	0·50	0·50	0·75	0·86	0·0
	75	0·0	0·0	0·86	0·90	1·0	0·50	0·50	0·25	0·14	0·0
	80	0·0	0·0	0·0	0·0	0·0	0·00	0·00	0·00	0·00	1·0
*PGM**	100	0·0	0·30	1·0	1·0	1·0	1·0	1·0	1·0	0·50	0·80
	85	1·0	0·60	0·0	0·0	0·0	0·0	0·0	0·0	0·50	0·20
	60	0·0	0·10	0·0	0·0	0·0	0·0	0·0	0·0	0·00	0·00
*GPI-1**	100	1·0	1·0	1·0	1·0	0·50	1·0	1·0	1·0	1·0	0·90
	200	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·10
	300	0·0	0·0	0·0	0·0	0·50	0·0	0·0	0·0	0·0	0·00
*GPI-2**	100	1·0	1·0	0·21	0·50	0·75	1·0	1·0	1·0	0·75	1·0
	80	0·0	0·0	0·79	0·50	0·00	0·0	0·0	0·0	0·25	0·0
	110	0·0	0·00	0·00	0·00	0·25	0·0	0·0	0·0	0·00	0·0
*SOD**	100	1·0	0·50	0·93	1·00	1·0	1·0	1·0	0·88	1·0	0·0
	120	0·0	0·00	0·00	0·0	0·0	0·0	0·0	0·00	0·0	1·0
	70	0·0	0·00	0·07	0·0	0·0	0·0	0·0	0·00	0·0	0·0
	50	0·0	0·50	0·00	0·0	0·0	0·0	0·0	0·00	0·0	0·0
	115	0·0	0·00	0·00	0·0	0·0	0·0	0·0	0·12	0·0	0·0
H		0·0	0·11	0·17	0·17	0·06	0·07	0·05	0·07	0·24	0·06

n = Sample size; H = average heterozygosity.

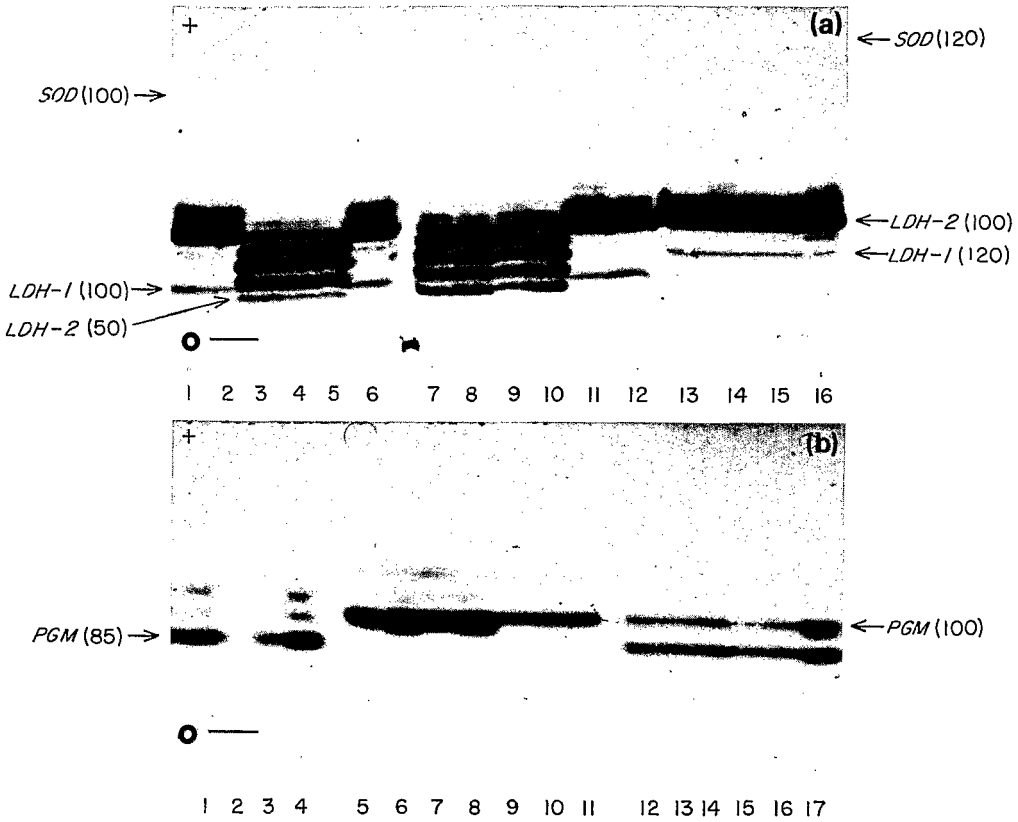

FIG. 1. (a) Polymorphism observed at *LDH-1**, *LDH-2** and *SOD**: 1–6 = female *Heterobranchus longifilis* (*HLO1*) × male *Clarias gariepinus* (*CGA1*); 7–12 = *C. anguillaris* (*CAN1*); 13–16 = *C. ebriensis* (*CEB*). Specimens 3, 4, 5 and 7–10 are heterozygous at *LDH-2** [genotype *LDH-2** (100/50)]; as LDH is a tetrameric enzyme, heterozygotes should exhibit five-banded patterns. Only the bands corresponding to homotetrameres are identified on the figure. The two additional anodal bands migrating in front of the *LDH-2** (100) band are observed in all samples and are artefacts. SOD appears as bleached bands. O = origin. (b) Polymorphism at *PGM**: 1–4 = *H. longifilis* (HLO1); the two bands migrating in front of the *PGM** (85) band are artefacts. 5–11 = *C. gariepinus* (*CGA1*); 12–17 = female *H. longifilis* (*HLO1*) × male *C. gariepinus* (*CGA1*); the two bands migrating in front of the *PGM** (100) band are artefacts.

C. gariepinus. However, large frequency differences between these species were observed at some loci (Table III).

The phenetic relationships inferred from the frequencies reported in Table III, are represented in Fig. 2. *H. longifilis* appears closer to *C. gariepinus* and *C. anguillaris* than *C. ebriensis*.

IV. DISCUSSION

From the results obtained, *C. anguillaris*, *C. gariepinus* and *H. longifilis* seem closer to each other than to *C. ebriensis*. *C. ebriensis* and the other species differ by a large genetic distance (Nei's standard distance = 0·75) and a large number of discriminating loci (five to six, out of 13 analysed). Compared to the range of

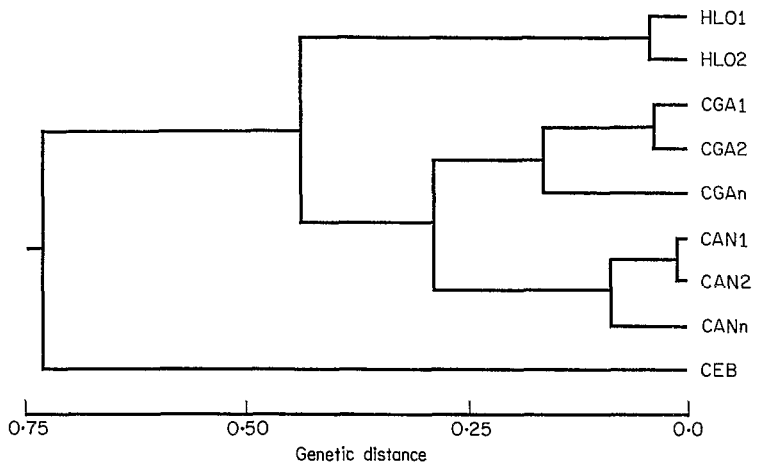

FIG. 2. Dendrogram produced by UPGMA cluster analysis (Sneath & Sokal, 1973) of Nei's (1975) standard genetic distances based on allele frequencies reported in Table III.

genetic differentiation usually found between and within species (Nei, 1975), it is highly improbable that the examination of new samples will modify the topology of the phenetic tree obtained here, at least for the first branching (*C. ebriensis v.* the other species).

The same conclusions resulted from a karyological analysis of the same material as studied in this paper (Ozouf-Costaz *et al.*, 1990; Teugels *et al.*, 1992 and unpublished data): *C. anguillaris* and *C. gariepinus*, both arranged in the subgenus *C. (Clarias)* by Teugels (1986), have the same chromosome number ($2n = 56$) and a nearly identical chromosome formula, while *C. ebriensis*, placed in the subgenus *C. (Anguilloclarias)* by Teugels (1986), has a different number ($2n = 48$); *H. longifilis* has $2n = 52$ chromosomes. The close relationship between *Heterobranchus* species and the species of the subgenus *C. (Clarias)* has also been emphasized by Teugels *et al.* (1990) in a revisionary study using morphological and osteological features. Thus from a phenetic approach, the species from the subgenus *C. (Clarias)*, are more closely related to *Heterobranchus* than to some other species of the genus *Clarias*. However, the phenetic relationships inferred from the allozyme frequencies do not necessarily reflect the true phylogeny of the species.

In a cladistic context, it is a mistake to assume that a phenetic similarity in electrophoretic protein patterns or in karyotypes necessarily implies a close phyletic relationship or that these biochemical and cytogenetical data are, somehow, more profound than morphological data. One could simply postulate, for example, that all clariid catfish share a common, primitive, range of proteins and that some species show specialized departures from this basic inheritance. From this, it would follow that the protein characters of *C. ebriensis* are a unique derivation from the norm, i.e. an autapotypy, of no use in determining wider phyletic relationships. By the same token, *H. longifilis* need not be more closely related to *C. anguillaris* and *C. gariepinus*—the shared similarity in proteins may simply indicate a primitive groundplan, i.e. a symplesiotypy, of no use in determining relationships. Outgroup comparisons are the only means of determining

the absolute phylogenetic values of the characters under investigation. This is the subject of forthcoming research.

Our electrophoretic data support the view of Teugels (1982, 1986) that *C. anguillaris* and *C. gariepinus* form two distinct genetic entities. The calculated genetic distance between both falls within the limits of that for known species (Nei, 1975), but it is rather small. It is noteworthy that no discriminating loci were found between the two species. Gene flow has been frequently observed between closely related species (see Hewitt, 1988 for a review of local hybridization) and, in view of their similar biology, it is not impossible that natural hybrids occur between *C. anguillaris* and *C. gariepinus*. These genetic exchanges could involve particular regions of the genome.

Despite this limited survey, a noticeably higher level of heterozygosity is observed in the CGA1 and CGA2 populations of *C. gariepinus*, compared to that found in the CGAn population. It is believed that the former populations descend from an interbreeding between two populations of *C. gariepinus*, one originating from Ivory Coast and the other introduced from Bangui (Central African Republic) in the 1970s in the fish culture station of Bouake. This hypothesis is supported by the fact that the CGA1 and CGA2 populations have a relatively reduced number of gill rakers on the first branchial arch, in comparison with the CGAn populations: Teugels (1982) demonstrated the existence of a north–south clinal variation in the number of gill rakers on the first branchial arch for *C. gariepinus*. The number as found in the CGA1 and CGA2 populations is intermediate to that occurring in natural populations of the species from Ivory Coast and Central African Republic.

The difference in heterozygosity between HLO1 and HLO2 populations of *H. longifilis* is explained by the fact that all specimens of this species examined in this study descend from a reduced number of brooders.

The electrophoretic results obtained for *C. gariepinus* × *H. longifilis* specimens clearly confirm the hybridization and definitely exclude gynogenesis or androgenesis, conditions known to occur in interspecific reproductions (see Chevassus, 1983). Except for a paper by Hecht & Lublinkhof (1985), who first reported this artificial hybridization, nothing has been published on this hybrid. On several occasions, we successfully crossed the two species (Legendre *et al.*, 1992). The morphotype of the specimens obtained is intermediate between that of the parents. The results of this study confirm the hybrid status of these individuals. It should be noted that the mere fact of hybridization is no evidence for phyletic relationships.

In conclusion, this study on enzymatic polymorphism in some African clariid catfishes revealed interesting data for both fundamental and applied research. Species from the subgenus *C. (Clarias)* showed closer affinity to *H. longifilis* than to a species of the subgenus *C. (Anguilloclarias)*, and support previous results of morphometric and karyological research. The importance of electrophoretic analysis in aquaculture to determine the purity of the species has already been emphasized by other authors (McAndrew & Majumdar, 1983; Allendorf & Leary, 1988; Van der Bank *et al.*, 1989). Finally, electrophoretic evidence for the *C. gariepinus* × *H. longifilis* hybridization is given.

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