Aquat. Living Resour., 1989, 2, 127-134

Evidence for genetic isolation among four morphological species of *Leporinus* (Anostomidae, Pisces) in French Guiana

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Received March 28, 1988; accepted February 15, 1989.

Renno J.-F., R. Guyomard, T. Boujard, C. Bastide. *Aquat. Living Resour.*, 1989, 2, 127-134.

Abstract

Genetic differentiation was studied among *Leporinus friderici*, *L. granti*, *L. lebaili* and *L.* affinis *steyermarki*, neotropical fish of the anostomid family found in French Guiana, using starch-gel protein electrophoresis. Fifteen enzymatic proteins representing twenty loci have been screened. This revealed marked genetic differences between these species, which are hardly morphologically discernable and indicates they are reproductively isolated. Diagnostic loci for unequivocal species identification have been found and will allow establishment of pure breeding stocks for planned aquaculture of *Leporinus* species.

Keywords : South America, French Guiana, anostomid fish, biochemical genetics.

Mise en évidence de l'isolement génétique entre quatre espèces morphologiques de Leporinus *(Anostomidae, Pisces) en Guyane française.*

Résumé

La différenciation génétique a été étudiée entre *Leporinus friderici*, *L. granti*, *L. lebaili* et *L.* affinis *steyermarki*, poissons néotropicaux de la famille des Anostomidés trouvés en Guyane française, en utilisant la technique d'électrophorèse de protéines sur gel d'amidon. Quinze systèmes enzymatiques correspondant à vingt locus ont été analysés. Des différences génétiques prononcées entre ces espèces morphologiquement peu discernables, indiquent qu'elles sont reproductivement isolées. Des locus, diagnostiques pour une identification sans équivoque des espèces, ont été trouvés et permettront l'établissement de souches d'élevage pures pour développer l'aquaculture des espèces de *Leporinus*.

Mots-clés : Amérique du Sud, Guyane, poissons Anostomidés, génétique biochimique.

INTRODUCTION

In the neotropical region approximately 2000 species of freshwater fish have been described, based on their morphology (Géry, 1969). The genus *Leporinus*, represented by 70 species, is widely distributed in South America (Garavello, 1979). *Leporinus friderici* is thought to occur naturally in the Amazon, Parana (Nomura, 1984) and Orinoco (Mago-Leccia, 1970) river basins and in the rivers of the Guyana shield. In French Guiana, *L. friderici* is integrated in a taxonomic complex that Géry (1977) described as "the worst chinese puzzles of characoides systematic" and to which belongs *L. granti*, *L. lebaili* (Géry and Planquette, 1983) and *L.* affinis *steyermarki*, a new species. Juveniles of these species have similar morphologies and markings and are difficult to distinguish. While the adult fish differ in colouration and in some morphomeristic characters, their correct identification can also be difficult.

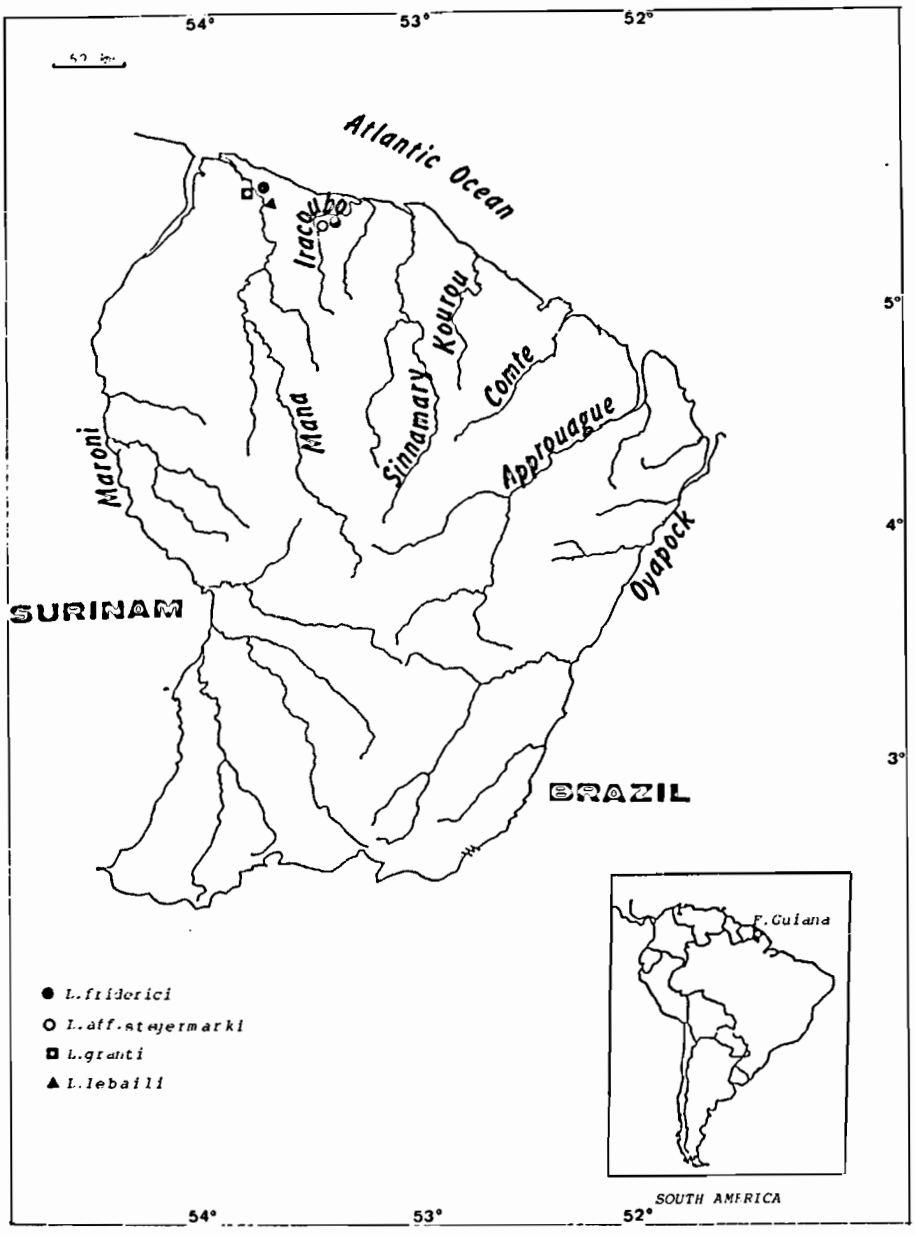

Figure 1. – Sampling points in French Guiana of the four studied species.

Among the aquacultural potential of freshwater fish in French Guiana, *L. friderici* has been selected for experimental breeding on the basis of biological criteria (Planquette and Rojas-Beltran, 1981; Boujard *et al.*, 1988). Establishing a reproductive stock from natural populations requires knowledge of the biological status of the species and its population structure. The artificial mixture of genetically different populations, or of different species, may lead to the appearance of undesirable phenomena such as segregation, hypofertility and the premature death of embryos. Identification of genetically based biochemical differences among species of *Leporinus* complex would clarify the status of a species in a species-complex.

The present study analyses genetic differences among *L. friderici*, *L. granti*, *L. lebaili* and *L.* aff. *steyermarki* from French Guiana, using protein electrophoresis to verify their reproductive isolation and identify diagnostic genetic variants for species identification.

MATERIALS AND METHODS

Samples

The samples were collected between January and June 1987, and always at the same location on the Mana and Iracoubo rivers. Adult fish were taken with 30-60 mm mesh trammel nets, while juveniles were obtained by poisoning, introducing Rotenone

Table 1. — Enzymatic systems and corresponding migration buffers. A: morpholine-citrate buffer (MC2), after Clayton et Tretiak (1972). B: tris-(hydroxymethyl)-aminoethane 0.1 M ajusted to pH 7.4 with NaH_2PO_4. C: discontinued buffer tris-citrate-borate (RID), after Ridway *et al.* (1970). The molecular structure (monomeric, dimeric or tetrameric) is deduced from the phenotype of the heterozygote, (*) according to Allendorf *et al.* (1977). (m): muscle, (l): liver.

Enzymatic systems	Loci	Buf.	"Zygote"	Bands	Molecule	Comments
Aspartate aminotransferase	Aat-1 (m)	A	homo.	1	dimeric*	
	Aat-2 (l)	A	homo.	1	dimeric*	
Alphaglycerophosphate dehydrogenase	Agp	B	homo.	4	dimeric*	phenotypic interpretation
Adenylate kinase	Ak (m)	A	homo.	1	monomeric*	
Creatine phospho kinase	Cpk (m)	C	homo.	1	dimeric*	
Esterase	Est (m)	C	homo.	1	monomeric	
			hetero.	1		
Fumarase	Fum (m)	A	homo.	2	tetrameric	2 times
			hetero.	10		5 merged bands
Isocitrate dehydrogenase	Idh-1 (m)	B	homo.	1	dimeric	
			hetero.	3		
	Idh-2 (l)	A	homo.	1	dimeric	
			hetero.	3		
Lactate dehydrogenase	Ldh-1 (m)	A	homo.	1	tetrameric*	
	Ldh-2 (l)	A	homo.	1	tetrameric	
			hetero.	5		
Malate dehydrogenase	Mdh-1 (l)	A	homo.	2	dimeric*	merged bands
	Mdh-2 (m)	A	homo.	1	dimeric*	
Malic enzyme	Me-1 (l)	A	homo.	1	tetrameric	
			hetero.	5		merged bands
	Me-2 (m)	A	homo.	1	tetrameric	
			hetero.	5		merged bands
6 Phosphogluconate dehydrogenase	GPgdh (l)	A	homo.	1	dimeric	
			hetero.	3		
Phosphoglucose isomerase	Pgi-1 (m)	C	homo.	1	dimeric	
			hetero.	3		
	Pgi-2 (l)	C	homo.	1	dimeric	
			hetero.	3		
Phosphoglucomutase	Pgm (m)	C	homo.	1	monomeric	
			hetero.	1		
Phosphomannose isomerase	Pmi (m)	B	homo.	1	monomeric*	
Superoxide dismutase	Sod (l)	C	homo.	1	dimeric	
			hetero.	3		

into small streams. This protocol was adapted from Boujard and Rojas-Beltran (1988). *L. friderici* was captured along with *L. granti*, *L. lebaili* in the Mana river and along with *L.* aff. *steyermarki* in the Iracoubo river (*fig.* 1).

Fish were identified according to the keys established by Géry (1977) and Le Bail *et al.* (1984); labelled according to the nomenclature of Géry *et al.* (1988). The morphomeristic characters, the number of scales along the maximum vertical, longitudinal axes, around the caudal peduncle and colouration (distribution of black and red marks), have proved to be the most useful. Around thirty morphomeristic data were taken for each specimen for future study. In uncertain cases, the specimens were preserved in 70% alcohol for re-examination after removal of tissue for electrophoretic analyses.

Genetic analysis

Starch-gel electrophoresis of muscle and liver tissue was employed to examine enzymatic variability. The tissue samples were removed from each fish, preserved in liquid nitrogen and then were transferred to the laboratory and stored at −40°C.

After thawing, the samples were homogenized in an equal volume of Tris/EDTA buffer (pH 6.8). The muscle homogenate was then centrifuged at 5000 g for 30 minutes, while the liver was first mixed with an equal volume of carbon tetrachloride, considerably improving the enzyme migration and the pattern definition. The supernatant can be frozen for storage without subsequent alteration. The samples were then processed by routine electrophoresis.

The enzyme examined and the corresponding buffers used are presented in *table* 1. Gels were developed according to Guyomard and Krieg (1983) and Pasteur *et al.* (1987). The nomenclature used to denote the locus and alleles is that proposed by Allendorf and Utter (1979) and May *et al.* (1979). The electromorphs, indicating alleles and characteristic of each system, are numbered in order of increasing electrophoretic mobility. *Leporinus friderici* from the Iracoubo river is considered as the reference population. The most frequent alleles at each locus of this population were arbitrarily designated as 100. The other

Table 2. – The allelic frequences and between parentheses the number of the observed allele in the samples of *L. friderici* from the Iracoubo (FI) and the Mana (FM) rivers, *L. granti* from the Mana (GM) river, *L. lebaili* from the Mana (LM) river and *L.* aff. *steyermarki* from Iracoubo (SI) river.

Loci	All.	FI	FM	GM	LM	SI
Aat-1	090	0	0	1	1	0
	100	1	1	0	0	1
		(80)	(120)	(26)	(16)	(18)
Aat-2	100	1	1	1	1	1
		(20)	(162)	(24)	(16)	(2)
Agp	080	0	0	0	0	1
	100	1	1	1	1	0
		(62)	(202)	(26)	(10)	(18)
Ak	100	1	1	1	1	1
		(80)	(172)	(28)	(20)	(18)
Ck	100	1	1	1	1	1
		(80)	(194)	(28)	(20)	(18)
Est	080	0	0	0.04	0	0
	090	0	0	0.82	1	0
	095	0.10	0.03	0.14	0	0
	100	0.90	0.97	0	0	1
		(76)	(166)	(28)	(20)	(18)
Fum	080	0	0	1	1	0
	100	1	1	0	0	0.64
	160	0	0	0	0	0.36
		(70)	(214)	(28)	(20)	(14)
Idh-1	000	0	0	0	0	0.62
	100	1	1	1	1	0.38
		(80)	(214)	(28)	(20)	(16)
Idh-2	025	0.08	0.02	0	0	0
	050	0	0.02	0.33	0	0
	100	0.89	0.94	0.67	1	1
	150	0.04	0.02	0	0	0
		(26)	(92)	(12)	(16)	(18)
Ldh-1	100	1	1	1	0.90	0
	110	0	0	0	0	1
	175	0	0	0	0.10	0
		(62)	(110)	(28)	(20)	(18)
Ldh-2	100	1	1	1	0.95	0
	130	0	0	0	0.05	1
		(80)	(222)	(28)	(20)	(18)
Mdh-1	000	0	0	0	0.20	0
	100	1	1	1	0.80	1
		(80)	(224)	(28)	(20)	(18)
Mdh-2	080	0	0	1	1	0
	100	1	1	0	0	1
		(80)	(206)	(28)	(20)	(18)
Me-1	100	0.78	0.77	1	1	0
	200	0	0	0	0	1
	300	0.22	0.23	0	0	0
		(50)	(214)	(8)	(20)	(18)
Me-2	100	1	1	?	?	0.19
	110	0	0	?	?	0.81
		(80)	(220)			(16)
Gpgdh	070	0	0	0	0	0.80
	075	0	0	0.18	0	0
	085	0.51	0.38	0.59	0.90	0.20
	100	0.49	0.62	0.18	0.10	0
	110	0	0	0.05	0	0
		(78)	(168)	(22)	(20)	(10)
Pgi-1	000	0.24	0.34	0	0.55	0
	100	0.66	0.57	1	0.05	0.94
	200	0.10	0.09	0	0.40	0
	300	0	0	0	0	0.06
		(80)	(224)	(28)	(20)	(16)
Pgi-2	095	0.08	0.00	0	0.65	0
	100	0.91	0.99	0.96	0.35	1
	110	0.01	0.01	0.04	0	0
		(76)	(224)	(28)	(20)	(18)
Pgm	065	0.03	0.04	0	0	0
	080	0.34	0.52	0	0	0
	100	0.64	0.45	0.96	1	1
	110	0	0	0.04	0	0
		(80)	(220)	(28)	(16)	(18)
Pmi	080	0.07	0	0	0	0
	100	0.93	1	0	1	1
	125	0	0	1	0	0
		(28)	(224)	(28)	(20)	(18)
Sod	060	0	0	0	0	0.22
	090	0	0.04	1	0	0.72
	100	1	0.96	0	1	0.06
		(76)	(184)	(28)	(20)	(18)

alleles are given according to their relative positions after migration.

RESULTS

Twenty one putative loci common to the four species were examined. No difference in genetic determinism of the species examined was found in the 15 systems studied (*table* 1). For αGpdh several bands appear consistently without intraspecific polymorphism. No reference showing several loci in muscle is known in fish, this zymogramme is considered to be a phenotype, without any genetic interpretation.

The allelic frequencies are given in *table* 2 and all the alleles obtained of the four species are represented in *figure* 2. In each species population, the Hardy-Weinberg equilibrium among genotype frequencies is found for each locus, indicating that the population in each case is likely to be panmictic.

The loci for which one or more alleles exist, and of which the total frequencies equal 1 in one group and 0 in another (alternative alleles), are considered as the diagnostic loci of the species. The proportion of diagnostic loci among those loci studied and which loci are diagnostic for separating each species from the others are given in *table* 3.

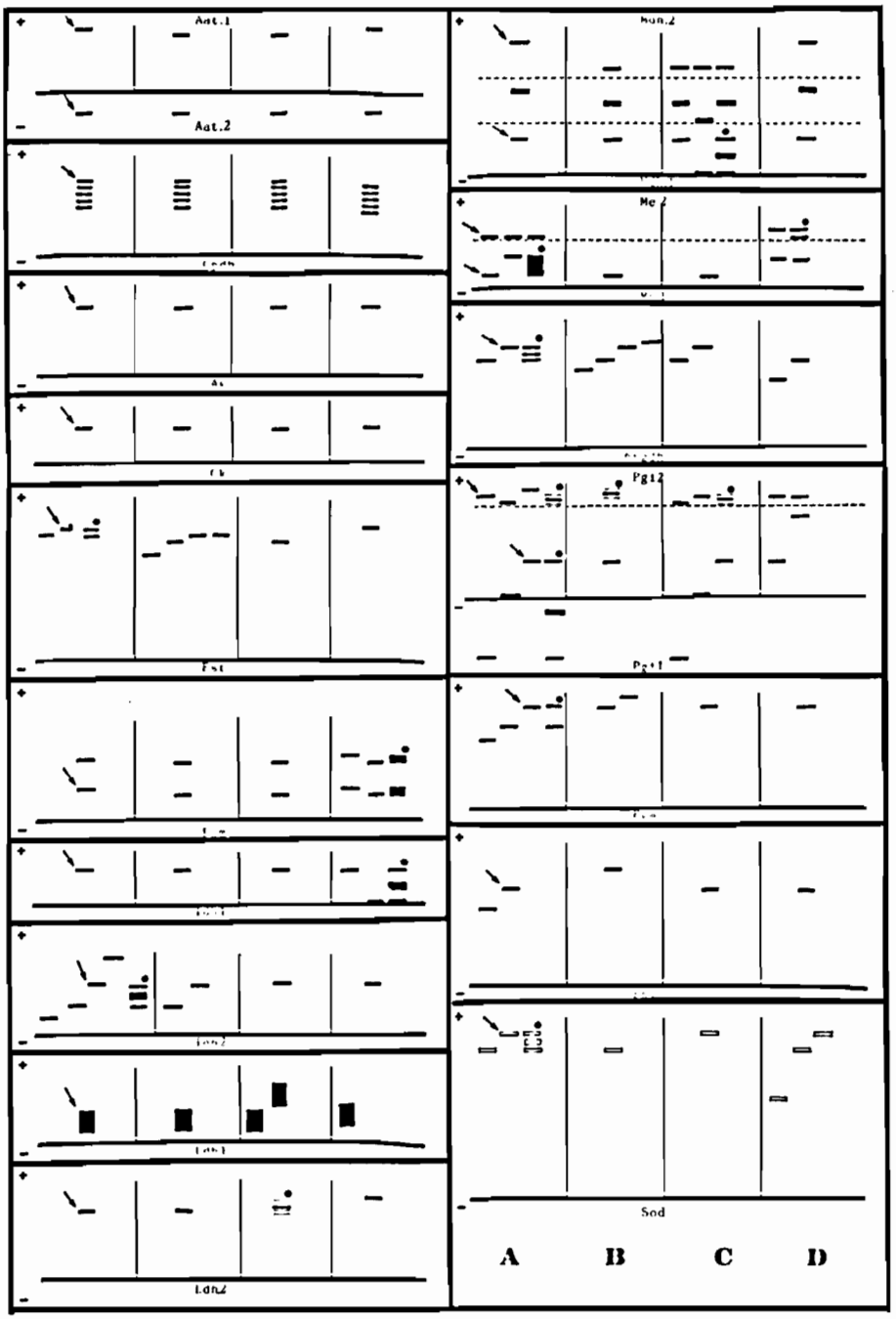

Figure 2. — Representation of all the alleles from different loci, from the four species, placed in the following order: *L. friderici* (A), *L. granti* (B), *L. lebaili* (C), *L.* aff. *steyermarki* (D). The alleles labelled 100 are indicated by an arrow, the heterozygotic phenotypes are indicated by a star (all are not represented).

The genetic variability in each population species is estimated by the proportion of polymorphic loci (P) and by the rates of heterozygosity observed (H) which indicates the mean frequency of heterozygotes in a population. A locus is considered as polymorphic in a population when the common allele has a frequency lower than 0.95. Among the four species (P) varies from 0.21 (*L. granti*) to 0.33 (*L. friderici*, Iracoubo) and (H) from 0.08 (*L. granti*) to 0.12 (*L. friderici*, Iracoubo) (*table* 3).

The Nei genetic distances (1971) between each species are always greater than 0.201, while it is only 0.004 between the two *L. friderici* populations (*fig.* 3 and *table* 4).

Table 3. — Diagnostic loci for separating each species from the others, and percentage. In each species, P: proportion of polymorphic loci (a locus is considered polymorphic when the incidence of the most frequently occurring allele is less than 0.95), H: mean heterozygosity observed in a population (I: Iracoubo river, M: Mana river).

	L. lebaili		*L. aff. steyermarki*		*L. granti*
L. friderici	Aat-1 Est Fum		Agp Ldh-1 Ldh-2		Aat-1 Fum Mdh-2
(I) H=0.12 P=0.33	Mdh-2		Me-1		Pmi
(M) H=0.11 P=0.29		20%		19%	20%
L. granti	Pmi Sod		Aat-1 Agp Est	Ldh-2 Mdh-2 Me-1	
H=0.08			Fum	Pmi	
P=0.21		10%	Ldh-1	45%	
L. aff. steyermarki	Aat-1 Agp Est	Me-1 Ldh-1			
H=0.12	Fum				
P=0.27	Mdh-2	35%			
L. lebaili					
H=0.11					
P=0.26					

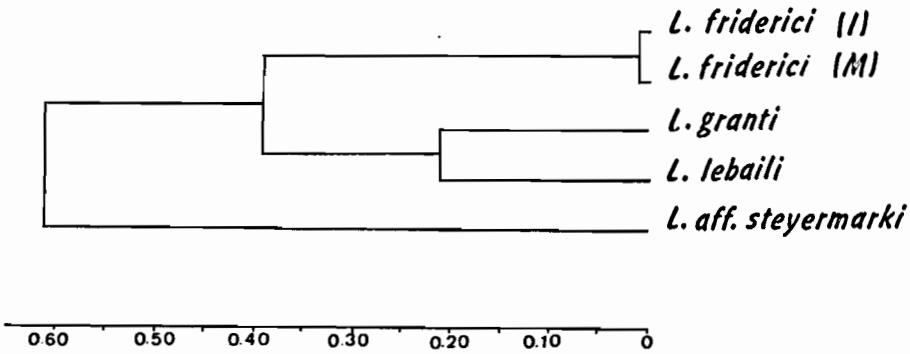

Figure 3. — The dendrogram (UPGMA) deduced from the Nei genetic distances between the four species.

Table 4. — Nei genetic distances between the species; *L. friderici* from the Iracoubo (FI) and the Mana (FM) rivers, *L. granti* from the Mana (GM) river, *L. lebaili* from the Mana (LM) river and *L.* aff. *steyermarki* from Iracoubo (SI) river.

	FM	FI	SM	GM	LM
FM	0	0.004	0.432	0.436	0.373
FI		0	0.423	0.411	0.348
SM			0	0.742	0.860
GM				0	0.201
LM					0

DISCUSSION

The presence of loci with alternative allele, among sympatric populations of *L. friderici*, *L. lebaili*, and *L. granti* from the Mana river, and *L. friderici* *L.*, aff. *steyermarki* from the Iracoubo river is demonstrated. Loci with alternative alleles within two populations, show that there is not any heterozygote resulting from an hybridation. In nature this clearly proves the existence of an isolation in the reproduction. If two populations are both in sympatry and have alternative alleles, it means they belong to two biological species (Mayr, 1974). Loci with alternative alleles can be used to identify the species they characterize (diagnostic loci). *L.* aff. *steyermarki* was not able to be studied in sympatry with *L. granti* and *L. lebaili*. However, its reproductive isolation is indicated by the large number of diagnostic loci observed and by the large genetic distances which separate it from the other species. The four species studied are thus true biological species.

Cytogenetic analysis has been used by Galetti *et al.* (1984) to characterize eight species of Anostomidae.

The slight differences in karyotype found among species belonging to three genera of anostomid fish, including *L. friderici*, indicate an evolution of the karyotype where the number and morphology of the chromosomes is conserved (Galetti *et al.*, 1981). In contrast, the present results reveal well marked interspecific genetic differences with protein loci. Nevertheless a strong chromosomic polymorphism, both inter and intraspecific, has been observed in several different groups of neotropical fishes (Foresti *et al.*, 1984; Scheell *et al.*, 1971).

Given the reproductive isolation of the four species, breeding stocks, starting from genitors caught from the studied geographic zone, can be established without the risk of introducing wild hybrids or using misidentified species. The management of populations crossing within the same species, or of interspecific hybridations, should be done taking in consideration genetic markers of crossed forms in order to identify the exact genetic characteristics of fry obtained during the successive generations. The usefulness of isozyme and protein markers in identifying species and their hybrids was demonstrated by Macaranas *et al.* (1986) with tilapia.

The genetic variability represents the potentialities that could be revealed under various culture conditions (Chourrout *et al.*, 1986). In cold water finfish, for 34 loci studied the average heterozygosity among twenty populations of masu salmon (*Oncorhynchus masou*) was 0.05 (Okasaki, 1986), and the average heterozygosity among 6 populations of brown trout (*Salmo trutta*) was 0.09 (Guyomard and Krieg, 1983). In *L. friderici*, the mean genetic variability intrapopulation reach 0.12 (Iracoubo population) and thus allows us to consider a genetic improvement programme. However, a genetic study of an higher number of populations of *L. friderici* would enable us to appreciate the interpopulation genetic variability and thus to reorientate or raise the polymorphism of breeding stocks. This would be by the addition of spawners from nature, possessing new genetic markers, or those which have disappeared during the breeding process.

Acknowledgements

The study translated by Karen Black, was carried out with the aid of a grant from the research fund of the MRES and supported by the AIP (INRA), "Biologie et Écologie des poissons guyanais d'intérêt aquacole".

REFERENCES

Allendorf F. W., N. Mitchell, N. Ryman, G. Stahl, 1977. Isozymes loci in brown trout (*Salmo trutta* L.): detection and interpretation from population data. *Hereditas*, 86, 179-190.

Allendorf F. W., F. M. Utter, 1979. Population genetics. *In*: D. J. Randall, J. S. Hoar, R. Brett Eds. Fish physiology. Academic press, New York, 407-454.

Boujard T., R. Rojas-Beltran, 1988. Zonation longitudinale du peuplement ichtyque du fleuve Sinnamary (Guyane française). *Rev. Hydrobiol. trop.*, 20, 41-61.

Boujard T., P.-Y. Le Bail, P. Planquette, 1988. Données biologiques sur quelques espèces continentales de Guyane française d'intérêt piscicole. *Aquat. Living Resour.*, 1, 107-113.

Chourrout D., B. Chevassus, R. Guyomard, 1986. L'amélioration génétique des poissons. *Recherche*, 1028-1038.

Clayton J. N., D. N. Tretiak, 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. *J. Fish Res. Board Can.*, 29, 1169-1172.

Foresti F., L. Foresti de Almeida Toledo, S. de Almeida Toledo, 1984. Chromosome studies in *Gymnotus Carapo* and *Gymnotus* sp. (Pisces, Gymnotidae). *Caryologia*, 37, 141-146.

Galetti P. M., F. Foresti, L. A. Bertollo, O. Moriera, 1981. Karyotypic similarity in three genera (*Leporinus*, *Leporellus* and *Schizodon*) of family Anostomidae (Pisces-Teleostei). *Rev. Brasil. Genet.*, 4, 11-15.

Galetti P. M., F. Foresti, L. A. Bertollo, O. Moriera, 1984. Characterisation of eight species of Anostomidae Cypriniformes fish on the basis of nuclear organizing region. *Caryologia*, 37, 401-406.

Garavello J. C., 1979. Revisao taxonomica do género *Leporinus*. *These dr.*, São Paulo (Brasil), 450 p.

Géry J., 1969. The freshwater fishes of South America. *In:* Biogeography and ecology in South America, E. J. Fittkau, G. H. Schwabe, H. Sioli Eds., The Hague, 828-848.

— 1977. Characoids of the world. T. F. H., New York, 672 p.

Géry J., P. Planquette, 1983. Une nouvelle espèce de *Leporinus* (Poissons characoides, Anostomidés) de la Guyane et du Surinam: *L. lebaili* n. sp. *Revue fr. Aquariol.*, 10, 65-70.

Géry J., P. Planquette, P.-Y. Le Bail, 1988. Nomenclature des espèces du groupe *Leporinus maculatus* et formes affines des Guyanes (Pisces, characoidei, Anostomidae). *Rev. Suisse Zool.*, 95, 699-713.

Guyomard R., F. Krieg, 1983. Electrophoretic variation in six populations of brown trout (*Salmo trutta* L.). *Can. J. Genet. Cytol.*, 25, 403-413.

Le Bail P.-Y., P. Planquette, J. Géry, 1984. Clef de détermination des poissons continentaux et côtiers de Guyane. CRAAG (INRA) *Bull. liaison*, 4, 1-97.

Macaranas J. M., N. Taniguchi, M. J. R. Pante, J. B. Capili, R. S. V. Pullin, 1986. Electrophoretic evidence for extensive hybrid gene introgression into commercial

Oreochromis niloticus (L.) stocks in the Philippines. *Aquac. Fish. Manage.*, **17**, 249-258.

Mago-Leccia F., 1970. Lista de los peces de Venezuela. Ministerio de Agricultura – Oficina national de pesca Caracas. Caracas, 283 p.

May B., E. Wright, M. Stoneking, 1979. Joint segregation of biochemical loci in Salmonidae: results from experiments with *Salvinus* and review of the literature on other species. *J. Fish. Res. Board Can.*, **27**, 1617-1625.

Mayr E., 1974. Populations, espèces et évolution, Hermann, Paris, 496 p.

Nei M., 1971. Genetic distance between populations. *Am. Nat.*, **106**, 283-292.

Nomura H., 1984. Dicionario dos peixes do Brasil. Brasilia Editerra, Brasil, 482 p.

Okasaki T., 1986. Genetic variation and population structure in Masu Salmon Oncorhynchus masou of Japan. *Bull. Jap. Soc. Sci. Fish.*, **52**, 1365-1376.

Pasteur N., G. Pasteur, F. Bonhomme, J. Catalan, J. Britton-Davidian, 1987. Manuel technique de génétique par électrophorèse de protéines. Lavoisier ed., Paris, 232 p.

Planquette P., R. Rojas-Beltran, 1981. Hydrobiologie et aquaculture en Guyane: premiers résultats de prospections piscicole. *CRAAG (INRA), Bull. liaison*, 4, 1-24.

Ridway G. J., S. W. Sherburne, R. O. Lewis, 1970. Polymorphismes in esterases of Atlantic herring. *Trans. Am. Fish. Soc.*, **99**, 147-151.

Scheell J. J., V. Simonsen, A. O. Gyldenholm, 1971. The karyotypes and some electrophoretic patterns of fourteen species of the genus *Corydoras*. Sonderdruk aus Z. f. zool. Systematik u. Evolutionsforschung, **10**, 144-152.