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Enzymatic polymorphism study as help for constitution of initial broodstook for a new cultivated finfish species

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Abstract — ***Leporinus friderici*** *is a South American promising species for fish culture. In French Guiana the « morphological species », **L. friderici, L. granti, L. lebaili** and **L. affinis steyermarki** are found. Their identification is sometimes difficult on usual criterion. To constitute an initial broodstook of **L. friderici** from local wild populations, the genetic structure of these species was studied. Fifteen enzyme systems, representing twenty loci, were screened.*

The biological status of the morphological species was investigated. Diagnostic loci for unequivocal species identification and marked genetic differences indicate that these species are reproductively isolated and therefore constitute true biological species.

*On an other hand, two **L. friderici** groups of populations are distinct in French Guiana. In spite of geographical closeness, a genetic distance of 0.016 separates West populations (Sinnamary, Iracoubo and Mana rivers) from east populations (Oyapock, Approuague and Comtè rivers). The western group possesses the allele Me-1 (300) with high frequency (0.022 to 0.26) but not the allele Ldh-2 (130). The eastern group possesses the allele Ldh-2 (130) with high frequency (0.29 to 0.49) but not the allele Me-1 (300). Furthermore, the heterozygoty of the west populations ($0.04 < h < 0.07$) is twice that of those of the east ($0.10 < H < 0.11$).*

*The interspecific differences and the interpopulational differences of **L. friderici** will allow us to establish pure broodstook. Now we have a guide for selecting genetically different populations for comparisons of breeding and rearing performances.*

INTRODUCTION

Given the aquacultural potential of freshwater fish in French Guiana, *L. friderici* has been selected for experimental breeding on the basis of biological criteria (Boujard et *al.*, 1988). Establishing a reproductive stock from wild populations requires knowledge of the biological status of the species and its population structure. The artificial mixture of genetically different populations, or different species, may lead to the appearance of undesirable phenomena such as segregation, hypofertility, and premature death of embryos.

In French Guiana *L. friderici* is integrated in a taxonomic complex that Gèry (1977) qualified as « the worst chinese puzzles of characoïdes systematics ». Juveniles of this species have similar morphologies and markings to those of *L. granti, L. lebailli,* and *L. affinis. steyermarki.* While adult fishes differ in coloration and in some morphomeristic characters, their correct identification can be difficult as well.

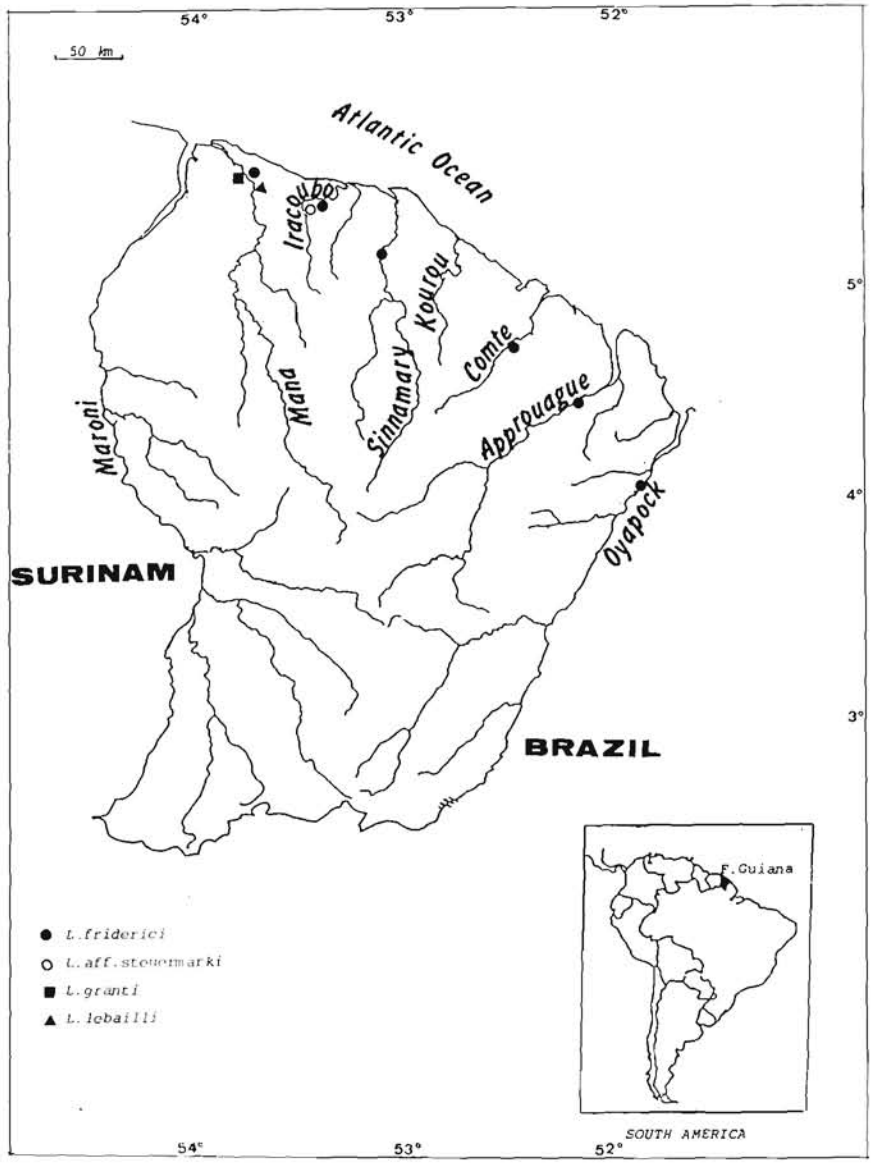

Fig. 1. — Sampling points of *L. friderici, L. granti, L. lebaili* and *L. aff. steyermarki* in French Guiana considered in this study.

In order to provide a guide for the establishment of initial broodstocks, diagnostic genetic variants using protein enzymatic electrophoresis were identified. In this way the reproductive isolation of the different species of *Leporinus* complex was verified. Then the genetic differenciation among six populations of *L. friderici* was described.

MATERIALS AND METHODS

Samples

The samples were collected between January 1987 and June 1988, in the six principal river basins of French Guiana. Adult fish were caught with 30-60 mm net trammel while juveniles were obtained by poisoning.

Fish were identified with morphomeristic characters according to the keys established by Gèry (1977) and Le Bail et *al.*, (1984) and labelled according to the nomenclature of Gèry et *al.*, (1988).

Genetic analysis

Starch-gel electrophoresis of muscle and liver tissues was employed to examine enzymatic variability. Gels were developed according to Guyomard and Krieg (1982) and Pasteur et al., (1987). The electromorphs, indicating alleles and characteristics of each enzymatic system, were numbered in order to increase electrophoretic mobility.

RESULTS

Twenty one putative loci common to the four species were examined. No difference in genetic determinism of species was found in the 15 systems studied. Hardy-Weinberg equilibrium among genotype frequencies was found for each locus, indicating that the population in each case is likely to be panmictic.

Interspecific differenciation

The loci for which there exists one or more alleles, of which the total frequencies equal 1 in one group and 0 in another (alternative alleles), are considered as diagnostic loci of species. The number of such diagnostic loci, separating each species from the others varies from 2 (*L. granti/L. lebailli*) to 9 (*L. granti/L. aff. steyermarki*). Four diagnostic loci (20 %) distinguish *L. friderici* from the other species. The proportion of these diagnostic loci are given in table 1.

The genetic variability in each population is estimated by the proportion of polymorphic loci (P), and by the rate of observed heterozygosity (H). A locus is considered as polymorphic 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*). (H) which indicates the mean frequences of heterozygotes in a population varies from 0.08 (*L. granti*) to 0.12 (*L. friderici*).

Tab. 1. — Diagnostic loci for separating each species from the others, and the corresponding percentage. In each species, P = proportion of polymorphic loci (a locus is considered polymorphic when the incidence of the most frequently occuring allele is less than 0.95), H = mean observed heterozygosity in a population (I = Iracoubo river, M = Mana river).

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

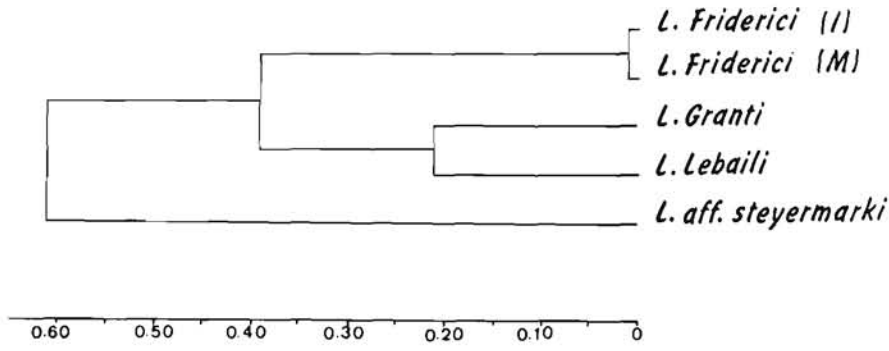

Fig. 2. — Dendrogram (UPGMA) deducted from the Nei genetic distances between the four species, *L. friderici, L. granti, L. lebaili* and *L. aff. steyermarki.*

The Nei (1971) genetic distances between *L. friderici* and the other species are always superior to 0.35.

Intraspecific differenciation

Two distinguishable blocks of *L. friderici* populations are emphasized. The western block, including populations from the Mana, Iracoubo and Sinnamary rivers, possesses the allele Me-1 (300) with a high frequency (0.22 to O.26), but not the allele Ldh- 2 (130). In contrast, the eastern block, including populations from the Comté, Approuague and Oyapock rivers, possesses the allele Ldh-2 (130) with high frequency (0.29 to 0.49) but not Me-1 (300). The heterozygosity rate between populations doubles from east ($0.04 > H > 0.07$) to west ($0.10 > H > 0.11$), and the proportion of polymorphic loci varies from 9.5 % (Approuague, Comté) to 33 % (Iracoubo). The calculated genetic distance between the blocks is 0.016.

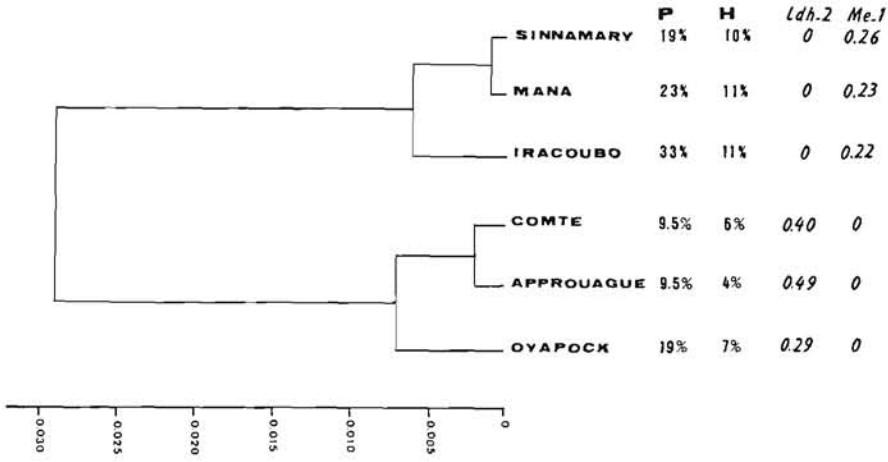

Fig. 3. — Dendrogram (UPGMA) deducted from the Nei distances between the *L. friderici* populations. For each population is indicated : P = polymorphic proportion of loci, H = mean observed heterozygosity, and the frequencies for the Ldh-2 (130), and Me-1 (300) alleles.

DISCUSSION

This study demonstrates the presence of loci with alternative alleles among sympatric populations of *L. friderici* with *L. granti*, *L. lebailli*, and *L. affinis steyermarki*. This indicates that there is not any heterozygote resulting from hybridation and clearly proves the existence of an isolation in reproduction. Thus the *L. friderici* species studied is true biological species.

Given this, broodstocks of *L. friderici* can be established without the risk of introducing wild hybrids or using misidentified species. The management of *Leporinus* populations crossing within the same species,

or interspecific hybridations, should be done taking into consideration genetic markers of crossed forms in order to identify the exact genetic characteristics of successive generations.The usefulness of isozyme and protein in identifying species and their hybrids was demonstrated by Macaranas et *al.* (1986) with *Tilapia* sp.

The genetic variability in *L. friderici* will allow a genetic improvement programme. The mean genetic intrapopulation variability reaches 0.12 (Iracoubo population); this is a high value compared to those reported for bred fish. For example, the average heterozygozity 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, 1982).

The genetic structure of *L. friderici* into blocks could be considered as a help for screening populations in the management of the broodstocks. In a genetic improvement programme based on the comparison of the rearing performances, testing populations of both blocks seems more judicious than working in the dark.

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