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Evolutionary and population genetics of Siluroidei

Filip A. Volckaert [1] and Jean-François Agnèse [2]

[1] *Katholieke Universiteit Leuven, Laboratory of Ecology and Aquaculture, Zoological Institute, Naamsestraat 59, B-3000 Leuven, Belgium.*

[2] *Centre de Recherches Océanographiques, BP V18, Abidjan, Ivory Coast.*

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Abstract

The genetic characterization of catfishes by means of phenotypic markers, karyotyping, protein and DNA polymorphisms contributes to or forms an integral part of the disciplines of systematics, population genetics, quantitative genetics, biochemistry, molecular biology and aquaculture. Judged from the literature, the general approach to research is pragmatic; the Siluroidei do not include model species for fundamental genetic research. The Clariidae and the Ictaluridae represent the best studied families. The systematic status of a number of species and families has been either elucidated or confirmed by genetic approaches. Duplication of ancestral genes occurred in catfishes just as in other vertebrates. The genetic structure of and gene flow among natural populations have been documented in relatively few cases, while the evaluation of strains for aquaculture (especially Ictaluridae and Clariidae) is in progress. The mapping of genetic markers has started in *Ictalurus*.

It appears that a more detailed knowledge of catfish populations is required from two perspectives. First, natural populations which are threatened by habitat loss and interfluvial or intercontinental transfers are poorly characterized at the genetic level. Secondly, the selection of suitable strains for aquaculture should be encouraged. Implementation should pose no problems given the present powerful means, such as DNA characterization combined with protein polymorphisms and phenotyping, to solve the above-mentioned issues.

Keywords: Biodiversity, cytogenetics, DNA polymorphisms, population genetics, protein polymorphisms, *Clarias, Ictalurus*, Siluroidei.

Évolution et génétique des populations des Siluroidei.

Résumé

La caractérisation des poissons-chats à l'aide de marqueurs phénotypiques, caryologiques, protéiques ou à l'aide du polymorphisme de l'ADN, contribue ou fait partie intégrante de disciplines telles que la systématique, la génétique des populations, la génétique quantitative, la biochimie, la biologie moléculaire ou encore l'aquaculture. À en juger par la littérature, l'approche générale des recherches est pragmatique ; il n'y a pas d'espèce modèle pour les recherches fondamentales de génétique. Les Clariidae et les Ictaluridae sont les familles les mieux étudiées. Le statut taxinomique d'un certain nombre d'espèces ou même de familles a été soit élucidé soit confirmé par des approches génétiques. La duplication de gènes ancestraux a lieu chez les poissons-chats comme chez les autres vertébrés. La structure génétique et les flux géniques entre les populations naturelles ont été étudiés dans relativement peu de cas alors que l'évaluation des souches aquacoles (spécialement chez les Ictaluridae et les Clariidae) se fait de plus en plus. La cartographie des marqueurs génétiques a commencé chez *Ictalurus*.

Il apparaît qu'une connaissance plus approfondie des populations de poissons-chats est nécessaire pour deux raisons. D'une part, les populations qui sont menacées par la disparition de leur habitat ou des transferts d'un bassin fluvial ou d'un continent à l'autre, sont trop peu génétiquement caractérisées. D'autre part, la sélection de souches adaptées à l'aquaculture doit être encouragée. Compte tenu de la

capacité des moyens actuels telles que les études de l'ADN combinées avec celles des protéines et des phénotypes, les questions que nous venons de mentionner pourront être résolues.

Mots-clés : Biodiversité, cytogénétique, polymorphismes, ADN, génétique des populations, polymorphismes protéiques, *Clarias*, *Ictalurus*, Siluroidei.

INTRODUCTION

Nothing was known of the genetics of catfishes (Siluroidei) 25 years ago. This is no surprise given that catfishes do not possess any of the virtues which characterize typical model species (short generation time, easy culturing, easy detection and maintenance of mutants) such as zebra fish (*Danio rerio*; Cyprinidae) and medaka (*Oryzias latipes*; Adrianichthyidae). Thus genetic studies in catfishes have with a few exceptions not aimed to further the science of fundamental genetics, but rather the application of genetics to problems related to the biology and aquaculture of the species. They include phylogenetic questions, gene flow, evolutionary questions, selection programmes and the conservation of natural resources. An indication of the rather applied interest in the genetics of Siluroidei is the growing interest since the early 1980s, which corresponds to a growing interest in aquaculture. Since the publication of Gyldenholm and Scheel (1971) on the karyotype of catfishes, there is a clear predominance of papers on karyology and protein-encoding loci.

The aim of this article is to review the genetics of wild and cultured catfish populations and species. Since the authors realize that not all readers might be familiar with the genetic tools used, care has been taken to explain them concisely in a section on the detection of genetic variation in fish. A section then follows on the taxonomic status as studied with genetic tools. The genetic differentiation of populations reflects a central question in genetics and is of major interest to geneticists, wildlife managers and aquaculturists. Further, the growing significance of gene mapping and selection for aquaculture will be addressed from the perspective of catfishes. The evolutionary implications of gene duplication have a general impact on all vertebrates. Recent progress on sex determination in mammals has brought this question to the forefront for fish.

DETECTION OF GENETIC VARIATION

The methods available to detect genetic variation in fish have come in four waves. First came the now classical breeding experiments, then the karyotyping of genomes was developed, followed by protein electrophoresis and the past fifteen years has seen the appearance of molecular biology. If the methodology offers many opportunities for answering questions, time and resources represent a serious limitation.

Several excellent reviews and books on genetic variation in fish are available (Wilkins and Gosling, 1983; Gall and Busack, 1986; Ryman and Utter, 1987; Gjedrem, 1990; Powers, 1991; Gall and Chen, 1993; Avise, 1994; Hochachka and Mommsen, 1993). Useful methodology books on the genetics of vertebrates are, amongst others, Whitmore (1990) and Hoelzel (1992).

Three important levels of genetic variation are distinguished: cytogenetics, protein variation and DNA nucleotide variation.

Cytogenetics

The detailed morphological description of the karyotype, whose preparation has profited from great progress (Blaxhall, 1983), provides a first approach to characterize species genetically. Cytogenetics include various aspects:

– The number of haploid chromosomes (n), their weight (expressed in picogram) and the shape of the chromosomes provide basic information. Chromosomes may be classified according to their centromeric index (CI) in four classes: metacentric (M), submetacentric (SM), subtelocentric (ST) and telocentric (T) (Levan *et al.*, 1964). The number of arms (the fundamental number) is not always unambiguous because certain authors consider subtelocentric chromosomes as having just one arm, while others allocate two arms (indeed they can be observed). One has to be careful when comparing the fundamental number among different authors.

– Chromosome marking techniques (banding patterns) have improved the accuracy of karyotyping. They include the visualization of the Nucleolus Organiser Region (NOR) with silver staining. The NOR represents the genes encoding for 18s and 28s ribosomal RNA in chromosomes. Banding techniques such as C-, Q- (quinacrine) and BrdU-banding, fluorescence staining and restriction enzyme banding are being used increasingly to improve resolution in differentiating among chromosomes. C-banding refers to banding patterns of heterochromatin of mitotic chromosomes; it plays an important role in phylogenetic studies. BrdU/dT-banding involves the selective incorporation of 5-bromodeoxyuridine/deoxythimidine molecules in mitotic DNA, fluorescence staining is the labelling of DNA with various kinds of fluorescent molecules, and restriction enzyme banding refers to the selective digestion of DNA with restriction enzymes. More recently, the physical localization of DNA sequences (so called markers) on the chromosomes

has been realized with fluorescence labelled probes by *in situ* hybridization techniques (also called FISH).

Protein variation

The detection of variation in the electrophoretic mobility of allozymes has provided much information on the genetic variation at the molecular level. Allozymes reflect variations in amino acid sequence of the genes encoding them which lead to a difference in the enzymes' overall ionic charge. Heterozygous monomeric (consisting of a single polypeptide chain) allozymes appear as 2 bands, dimeric allozymes as 3 bands, and tetrameric allozymes as 5 bands. Proteins of interest are separated on a starch, cellulose acetate or polyacrylamide gel and stained with chromogens for general proteins or enzyme-specific reactions. Another method for looking at protein variation is the sequencing of the amino acids.

Variation can be detected at the population level (variation in genotypic and allelic frequencies) and at the specific level (presence of diagnostic fixed alleles).

DNA nucleotide variation

The finest resolution of genetic variation occurs at the level of the coding and non-coding nucleotide sequences of the mitochondrial and nuclear DNA. Coding sequences show indeed considerable variation at the DNA level (the gene) despite the highly conservative nature of functional proteins (mutations often jeopardize functionality). Molecular variability includes deletions and mutations, transpositions, common base substitutions, and nucleotide sequence alterations due to intragenic recombination and gene conversion.

Several methods and approaches are available to look at DNA variation. There is the digestion of DNA with restriction enzymes that cut DNA strands at specific nucleotide sequences. The resulting DNA fragments are called restriction fragment length polymorphisms (RFLP's). For example, the restriction enzyme *AluI* cuts DNA at the four nucleotide sequence AGCT (it is called a four-cutter). Variation at the AGCT site will permit the detection of DNA polymorphisms. Detection occurs after separation on an agarose gel and hybridization with a probe DNA molecule complementary to the nucleotide sequence of interest.

Sequence variation of the maternally inherited DNA of mitochondria (*e.g.* at the cytochrome c gene) has been used for detecting genetic variation within and among populations, and among species. Sequence variation of coding nuclear genes, such as growth hormone and homeobox genes, are of phylogenetic interest.

In addition, allelic variation of non-coding nuclear and mitochondrial DNA sequences has become very important. Minisatellites are highly variable repetitive DNA sequences containing a core sequence of 15 to 40 nucleotides. Microsatellites are very common highly repetitive DNA sequences containing a core sequence of 2 to 4 nucleotides. The advantage of both are their often very high degree of allelic variability. In principle, individual genetic characterizations are possible. In addition, sequencing of non-coding DNA sequences and the detection of RFLP's may also be used to characterize non-coding sequences. Finally, the selective amplification of DNA with the polymerase chain reaction (PCR) has considerably diminished the requirements for large amounts of tissue to detect DNA variation. Moreover, it has opened the way to study genetic variation in recent fossils and museum collections.

TAXONOMIC STATUS AS STUDIED WITH GENETIC TOOLS

So far about 2,598 valid species and 33 valid families of Siluroidei have been described, largely on the basis of morphological and meristic traits (Teugels, 1995). Unfortunately, the taxonomic status of many species and families remains uncertain because of incomplete information and lack of comparative material. Additional information may be obtained from ethology, ecology and genetics. Indeed karyotypes, enzyme/DNA markers, and DNA sequence information do not stand on their own to identify phylogenetic relationships among organisms; morphological and molecular information complement each other (Avise, 1994).

Cytogenetics

Due to the large number of references on the karyotype of Siluroidei, only those which are relevant have been included. Siluroidei show a great diversity in the organization of the genome; this includes the karyotype (number and shape of the chromosomes) as well as the amount of DNA included in each nucleus. For example *Liobagrus andersoni* (Amblycipitidae) has only $2n=28$ chromosomes (Kim *et al.*, 1982), while *Corydoras aeneus* (Callichthydiae) has $2n=132$ chromosomes (Scheel *et al.*, 1972). Each diploid cell of the latter contains 8.8 pg of DNA while the nucleus of *Pimelodella gracilis* (Pimelodidae) only contains 1.76 pg (Hinegardner and Rosen, 1972). Nevertheless, the representative karyotype of the Siluroidei has a diploid number of $2n=54\pm6$ and a fundamental number of $FN=100\pm20$. The average diploid DNA content is 2 to 4 pg.

Without any doubt, the South American species of the genus *Corydoras* have the largest karyotypic variation. The diploid number of chromosomes varies between 44 (*C. paleatus*) and 132 (*C. aeneus*) (Scheel *et al.*, 1972; Hinegardner and Rosen, 1972; Dunham *et al.*, 1980; Oliveira *et al.*, 1988, 1990, 1992). *C. julii* and *C. agassizi* show intermediate values of $2n=92$

and 98. According to Hinegardner and Rosen (1972) the amount of DNA per diploid nucleus varies from 4.6 pg (*C. myersi*) to 8.8 pg (*C. aeneus*). The ancestral genus *Corydoras* probably had a diploid number of 56 to 60 chromosomes and a DNA content of 2.9 pg. At least 5 groups of species sharing similar cytogenetic traits can be distinguished (Oliveira *et al.*, 1992).

The North American species of the genus *Ictalurus* (Ictaluridae) and *Noturus* (Ictaluridae) have been well studied especially by LeGrande and Cavender (1980) and LeGrande (1981). They have $2n=50\pm10$ chromosomes and 64 to 106 chromosome arms. In Europe and Asia, the Siluridae and Bagridae especially have been studied. The chromosome number in Bagridae varies typically between 54 and 58 (Agnèse *et al.*, 1990). The Siluridae (Iliadou and Rackham, 1990) have 58 to 60 chromosomes and 100 to 116 arms; one species (*Parasilurus microdorsalis*) carries microchromosomes (Kim *et al.*, 1982). Remarkable thoroughness has been applied to the study of more than 100 (sometimes 300) mitoses per species (Krasznai and Marian, 1978; Rab, 1981; Kim *et al.*, 1982; Hong and Zhou, 1983; Iliadou and Rackham, 1990).

The family of the Bagridae have received special attention in Asia (Magtoon and Arai, 1988); up to 40 species have been karyotyped so far. The number of chromosomes varies between $2n=44$ (*Coreobagrus brevicorpus*; Bagridae) (Kim *et al.*, 1982) to $2n=60$ (*Mystus macropterus*; Bagridae) (Hong and Zhou, 1984). The number of arms varies between 74 and 102. The representative karyotype seems to be $2n=54\pm6$ and $NF=90\pm12$.

In Africa, only 5 species of Claroteidae (formerly called Bagridae) have been studied. Two carry a karyotype similar to the Bagridae, *Auchenoglanis occidentalis* (Claroteidae) $2n=56$, $NF=104$; *Bagrus docmak* (Bagridae), $2n=54$, $NF=98$). Three other species have a higher number of chromosomes (*Chrysichthys maurus* (Claroteidae) $2n=70$, $NF=102$; *C. auratus*, $2n=72$, $NF=108$; *Clarotes laticeps*, $2n=70$, $NF=102$). In the latter case, the number of arms seems to have been conserved. It is most likely that the evolution of the karyotypes is due to centromeric fissions which transform a chromosome with two arms into two chromosomes with one arm. The karyotype of *Clarias gariepinus* and *Heterobranchus longifilis* (both Clariidae) is respectively $2n=56$ and 52 (Ozouf-Costaz *et al.*, 1990; Teugels *et al.*, 1992*a*). An overview of the chromosome number per continent does not reveal obvious differences (Agnèse *et al.*, 1990) (*table* 1).

Possible chromosome polymorphisms in *Noturus flavus* (LeGrande and Cavender, 1980) and *Pseudobagrus aurantiacus* (Ueno, 1974) have to be confirmed since it is not clear whether variation is population-specific. Within the same perspective, the conflicting karyotypes of for example *Silurus glanis* (Siluridae) might be explained by the techniques used (Rab, 1981; Krasznai and Marian, 1978; Sofradzija, 1982; Vujosevic *et al.*, 1983).

Table 1. – Summary of the number of chromosomes ($2n$) and chromosome arms (FN) of Siluroidei per family.

Family	2N	FN
Bagridae (Asia)	44-60	74-102
Bagridae (Africa)	54-72	98-108
Callichthyidae	44-132	–
Clariidae	52-56	–
Ictaluridae	40-60	64-106
Siluridae	58-60	100-116

In brief, Siluroidei are characterized by a very dynamic history of cytogenetics. Polyploidization and chromosome rearrangements have occurred among and within families on several occasions.

Methods of chromosome banding have not been used in Siluroidei to the same degree as in Salmoniformes. The banding technique most often used is silver staining of the nucleolus organizer region (NOR) (Oberdorff *et al.*, 1990; Ozouf-Costaz *et al.*, 1990; Rab *et al.*, 1991; Oliveira *et al.*, 1992). Since most species have 1 to 3 pairs of chromosomes with NORs, this marking technique is not very informative.

Hybridization among species has been confirmed in a number of cases on the basis of cytogenetic information. Na-Nakorn *et al.*, (1993) found two morphotypes (confirmed by karyotyping) in first generation offspring from the artificial hybridization between *Clarias macrocephalus* and *Pangasius sutchi* (Pangasiidae). First generation artificial hybridization between *C. gariepinus* and *Heterobranchus longifilis* resulted in a karyotype intermediate between *C. gariepinus* ($2n=56$) and *H. longifilis* ($2n=52$) (Teugels *et al.*, 1992*a*). Similarly, the hybrids of *Ictalurus catus* and *I. punctatus* or *I. furcatus* had an intermediate karyotype (LeGrande *et al.*, 1984). Measuring genome size with flow-cytometry in half-sib families of hybrid Ictaluridae gave the same results and makes it thus possible to identify hybrids (Tiersch and Goudie, 1993).

Protein polymorphisms

The identification of strains and species has benefited considerably from the electrophoretic separation of proteins. A number of studies analysing protein-encoding loci in catfishes provide complementary information to the taxonomic status. A second group aims at the identification of populations and gene flow in natural populations, while a third group looks at the potential or realized genetic impact of selection under aquaculture.

At the level of the superorder one can clearly discriminate Ostariophysi (which includes the suborder of the Siluroidei) from others by the number of loci detected at the multilocus isozyme systems of glucose-6-phosphate isomerase (GPI), lactate dehydrogenase

(LDH), soluble malate dehydrogenase (sMDH) and creatine kinase (CK) (review by Coppes *et al.*, 1990). Ostariophysi combine 2 loci for enzyme system GPI, 3 for LDH, 2 for sMDH and 3 to 4 for CK.

The taxonomic status of the species complex *Chrysichthys auratus longifilis* and *C. filamentosus* (Claroteidae) remained dubious. Agnèse (1991) studied allozyme polymorphisms and concluded from the low genetic distance between them that they belonged to one species *C. auratus*. These results complement careful morphological research and a combined study on the population genetics and the associated parasitological fauna by Euzet *et al.*, (1989).

Electrophoretic patterns of haemoglobin in *Ictalurus nebulosus* and *I. melas* (Ictaluridae) seem to be species specific (Basaglia and Callegarini, 1987 *a*). In addition eye lens proteins and LDH permit discrimination among *I. nebulosus marmoratus* and *I. punctatus* (Basaglia and Callegarini, 1987 *b*; Basaglia, 1989).

Malaysian catfishes (*Clarias macrocephalus, C. batrachus, Prophagorus cataractus* (all Clariidae) and *Mystus nemurus* (Siluridae)) clearly differentiate on the basis of 15 isozyme loci as well at the species, genus and family level (Daud *et al.*, 1989; Ismail *et al.*, 1989).

According to Erondu *et al.* (1993) *Clarias gariepinus, Heterobranchus longifilis* and *Chrysichthys nigrodigitatus* can be discriminated by blood group (A, AB, O+ and O–). The homozygote AA is the predominant genotype.

The genetic distance between *Heterobranchus longifilis* and *Clarias gariepinus/C. anguillaris* is shorter than the distance between the mentioned *Clarias* species and *C. ebriensis* (Teugels *et al.*, 1992 *b*). The monophyly of the Clariidae has not been demonstrated yet and Teugels (pers. comm.) suggests that the family is polyphyletic. This is apparently confirmed by the large phenetic distance between the *Clarias* species studied electrophoretically.

DNA variation

In addition, the status of various taxa can be clarified by comparing DNA sequence similarities of nuclear genes, of mitochondrial DNA sequences (especially the D-loop), and of ribosomal RNA sequences.

The taxonomic status of *Arius bilineatus* and *A. thalassinus* (Ariidae) of the Arabian Gulf was dubious, based on morphological criteria. A comparison of the electrophoretic pattern of mitochondrial DNA cut with 3 restriction endonucleases showed species-specific variation (Simsek *et al.*, 1991). Unfortunately no information is given about intra-population variation.

Because the comparative study of genes and their corresponding amino acid sequence is a relatively recent discipline, insufficient data have accumulated for comparative studies within the Siluroidei. The DNA and amino acid sequence of growth hormone (GH) of *Ictalurus punctatus* and *Pangasius sutchi* (Watanabe *et al.*, 1992) is similar to the sequence of *Clarias gariepinus* except for one amino acid substitution at position 3 of the N- terminal end (Lescroart, pers. comm.). Overall, the amino acid sequence of GH of *I. punctatus* is 77 similar to carp.

GENETIC DIFFERENTIATION OF POPULATIONS

Differentiating between geographic populations, hybridizations and gene flow was often difficult before the introduction of protein electrophoresis. At that time, research depended on phenotypic markers such as an autosomal recessive albino strain of *Ictalurus punctatus* (Goudie *et al.*, 1992), a red (actually albino) strain of *Clarias gariepinus* (Prinsloo *et al.*, 1989; 1990) and a recessive albino strain of *C. batrachus* (F.V., pers. obs.). Gene-frequency data have become a standard tool in understanding the genetic structure of fish populations (Ryman and Utter, 1987). With the recent advance of molecular methods, these questions may be addressed even more powerfully.

Relatively few catfish populations have been studied in detail with the exception of the genera *Chrysichthys, Clarias, Ictalurus* and *Synodontis.*

The populations of *Chrysichthys nigrodigitatus* in West Africa show a clear genetic differentiation (based on 5 polymorphic loci) between the coastal plains (Ivory Coast) and the inland Niger basin at Bamako (Mali) (Agnèse *et al.*, 1989). The high genetic identity among some coastal populations suggests extensive gene flow or recent divergence. *C. auratus* populations show in West Africa a low average heterozygosity ($H = 0.024$ based on 5 polymorphic loci) and a high genetic identity (Agnèse, 1991). The reasons for the low average heterozygosity are unknown; they may be due to severe bottle-necking or the small size of isolated populations. Allelic variation at several loci of *C. maurus* allows for the reconstruction of the direction of colonization of the West African basins: from the coastal basins of Ivory Coast to the east and west. A distinctive population is limited to the upstream reaches of the Comoe River beyond the falls south of Route d'Abengourou.

The mean heterozygosity (calculated on 15 polymorphic loci) of *Schilbe mystus* (syn. *S. intermedius*) and *Eutropius niloticus* (syn. *S. mystus*) (both belonging to the Schilbeidae) collected in the Volta Basin of Ghana varied from very low (0.001) in the first species to below average (0.018) in the second species (Abban and Skibinski, 1988). Engelbrecht *et al.* (1994) also observed for *S. intermedius* in southern Africa a low level of polymorphism (0.283) and heterozygosity (0.029). Although both authors suggest various causes for the observed values, the low values cause concern for the future of the species.

The genetics of channel catfish have received particular attention in conjunction with its commercial importance. Nine lines of *Ictalurus punctatus* selected for rapid growth differences in frequencies of alternative protein alleles and in heterozygosity had a lower mean heterozygosity, lower percentages of polymorphic loci (at most 13 of the 28 loci scored) and fewer alleles per locus than control lines. At some loci, selection for rapid growth caused the differences in enzyme allele frequencies between select and control lines (Hallerman *et al.*, 1986).

The number of protein markers was expanded to 69, of which 25 were polymorphic, in a consecutive study. Seventy-seven percent of these loci could be resolved by the sampling of low-risk tissues such as the adipose fin, the caudal fin and the barbels. The advantage of non-invasive sampling and of the induction of low stress to the fish involved are obvious. In addition, thirteen of these loci are fixed between channel catfish and blue catfish (*I. furcatus*) (Carmichael *et al.*, 1992).

Genome size among stocks of *Ictalurus punctatus* was shown by flow-cytometry to be stable (Tiersch *et al.*, 1990) and is thus not useful as marker of populations. It might reflect stabilization of the genome by man or alternatively evolutionary conservation within its genome.

Wild leopard squeaker, *Synodontis leopardinus* (Mochokidae), from the Upper Zambezi River, showed an important degree of heterozygosity. This is remarkable given that 15 polymorphic loci (of the 51 protein loci) were scored (Van der Bank, 1993).

The growing commercial importance of the Clariidae has stimulated a number of population genetic studies. Allozyme variation at 5 polymorphic protein encoding loci (22 were scored) of the African or sharptooth catfish *Clarias gariepinus* was found to be as expected in a wild population from the Limpopo River (South Africa), above average in a cultured population (with possible overcompensation for an expected loss of genetic variation) and below average (actually close to 0) in another cultured population (Van der Bank *et al.*, 1992). When the protein-encoding polymorphisms are compared to an aquacultured population from The Netherlands, the lack of polymorphisms is striking. Only the locus esterase, which was not studied previously, showed polymorphisms. The small founder population, the limited number of parents used in reproduction and genetic drift are possible causes (Van der Walt *et al.*, 1992).

Initially a significant effect of the cryopreservation of semen was observed at the *GPI-2** locus by Van der Bank and Steyn (1992). In a later study where catfish semen was frozen with a similar extender but at a slower freezing rate, the influence of the cryopreservation on the selection for specific alleles was eliminated (Van der Walt *et al.*, 1993 *a*).

Protein-encoding allelic variation at the population level was observed by Daud *et al.* (1989) and Ismail *et al.* (1989) in various *Clarias* spec Teugels *et al.* (1992 *b*) observed small differer within and among populations of wild and cultu *C. gariepinus, C. anguillaris, C. ebriensis* and *He obranchus longifilis*. The level of heterozygosity native *C. anguillaris* and *C. ebriensis* reached ab 6%; wild *C. gariepinus* attain average heterozygosi higher than 10% (Guyomard, pers. comm.). A cl loss of genetic variability was observed in *H. longi* when comparing wild, F_1 and F_4 families (Agn *et al.*, 1995). Inbreeding is a real problem in spec with a high fertility.

Two mitochondrial DNA groupings were revea in the phylogenetic analysis of 11 genotypes hardhead catfish (*Arius felis*) which was based a survey at 10 coastal sites between North Carol and Louisiana (USA) (Avise *et al.*, 1987). Its fem population size (N_f) has been conservatively estima at 10 million individuals annually, but the calcula long-term effective population size ($N_{f(e)}$) amou to 45,000 individuals (a 200-fold discrepancy). Av *et al.* (1988) calculated a mtDNA evolutionary rate 4.5×10^{-5} Substitutions/bp/lineage/Myr which is mu lower than the conventional rate of 1×10^{-2}.

Multiple-locus and single-locus DNA fingerprinti provide new approaches to study populations great detail. The former has been developed fi and is based on DNA probes with highly repetiti DNA sequences. Multiple-locus DNA fingerpri (which refer to banding patterns typical for given probe and species) are most informati to discern parentage (*e.g.* Volckaert *et al.*, 199 Single-locus DNA fingerprinting makes it possible discriminate individuals on the basis of the Mendeli inheritance of alleles. Their interpretation is similar polymorphic enzyme markers, with the difference th the observed allelic variation is usually much high The probes are in principle species-specific, althou cross-hybridization with closely related species a families is possible. A growing number of studi with microsatellite loci on catfishes are in preparati (Morizot *et al.*, 1994; Galbusera, pers. comm.; Zourc pers. comm.).

The above-mentioned studies are simply too few prepare a general picture of catfish population genetic It is not clear whether natural populations constitu geographically isolated groups or whether gene flo occurs and how much. In general the subpopulatic heterozygosity of freshwater fish (such as is the ca with most Siluroidei) is lower than marine fish, whi the average degree of subpopulation differentiation significantly greater (Ward *et al.*, 1994). As expecte in cultured populations, a reduction in genetic variatic has been observed.

A solid characterization facilitates the managemer of native populations and the selection of broodstocl Wild populations need to be managed from a fisherie perspective (to avoid overexploitation of the stocks and the conservation of natural variation (biodiversity

The heavy pressure on their natural habitat by river calibration, logging, pesticides, agriculture, fishing and industry, poses real threats to the survival of catfish populations and species. The translation of knowledge from other freshwater fish (salmonids and cyprinids) should help in designing a strategy. The question is the more urgent since natural populations harbour a genetic resource for the aquaculture of selected strains.

GENE MAPPING, SELECTION AND AQUACULTURE

It has become standard practice in model organisms to evaluate the genetic basis of protein variants (allozymes) by means of breeding, to map allozyme and DNA markers on the chromosomes and to correlate phenotypic traits to these markers. The latter is an important tool for managers to speed up the selection process of desirable production traits such as age-specific size, carcass weight, age at sexual maturity and stress/disease resistance. The principal assumption, that the markers located on the nuclear genome are inherited in a Mendelian fashion, seems to be generally the case (Liu *et al.*, 1992; Morizot *et al.*, 1994).

Two catfishes are the focus of genetic segregation analysis: *Ictalurus punctatus* and *Clarias gariepinus*. As mentioned above, of the 28 allozyme markers scored in *I. punctatus* 13 (46 %) are polymorphic (Hallerman *et al.*, 1986). A more recent study by Carmichael *et al.* (1992) detected at least 69 coding genes of which minimally 17 (25 %) were polymorphic. This is an underestimate because several polymorphic loci detected by Hallerman *et al.* (1986) were not scored. Moreover, 5 polymorphic loci were detected in a single blue catfish (*I. furcatus*) population. Fifty-four of the 69 (77 %) gene products could be satisfactorily resolved from caudal fin and barbel tissue.

Linkage analysis of 24 single-pair matings of channel catfish and 3 interspecific crosses of channel catfish x blue catfish chosen for their profile of polymorphic enzymes, establishes strong evidence for linkage of glutathione reductase (*GR**) and *PGM**. It was designated as *I. punctatus* linkage group I (Morizot *et al.*, 1994). These 5 markers are located at most on four of the 58 pairs of chromosomes. Eight enzyme loci were mapped by means of gynogenetic offspring by Liu *et al.* (1992). Meiogynogenetic progeny allow for crossing-over before the first meiotic division and the impossibility for participation of the paternal genome (which might impair unambiguous scoring). The genome of the progeny combines the haploid chromosome set and the second polar body of the oocyte. Frequency estimates of gene-centromere recombination at six enzyme loci ranged from 0.36 to 1.00. The loci *GPI-B** and sex determining gene (*SDG**) are linked (see later). Linkage group II includes genes coding for *MANA**, *GPI**, *IDH-M** and *MPI** (Morizot, 1994). Linkage among other polymorphic enzyme and DNA markers is presently under study (Morizot, pers. comm.). Simplifications in the procedures to isolate and characterize DNA markers (see Zhang *et al.*, 1994) improves the chances of obtaining a low resolution linkage map reasonably soon. The long term aim is the preparation of a detailed linkage map such that traits important to managers can be identified in the complete genome. From an evolutionary perspective there are good indications that linkage groups are conserved among vertebrates (Morizot, 1994).

Of the 18 protein coding loci scored in domesticated and wild *Clarias gariepinus* 5 (28 %) were polymorphic (Van der Bank *et al.*, 1992). Five of the 20 (25 %) protein coding loci of a domesticated population from the Netherlands were polymorphic (Van der Walt *et al.*, 1992). Among these loci, the locus glucose-6-phosphate isomerase (*GPI-1**) turned out to be correlated to enhanced growth in wild and cultured populations (Van der Walt *et al.*, 1993 *b*). The highest mass at 90 days of age was observed in the progeny of the fish mass- selected during several generations for rapid growth. In addition, locus *LDH** correlates with growth in slow and fast growing groups of African catfish (Grobler *et al.*, 1992). It is expected that more protein-encoding and DNA loci will become known and mapped in the near future.

EVOLUTIONARY IMPLICATIONS OF GENE DUPLICATION

Gene duplication is an important mechanism in the evolution and biochemical diversification of organisms. In fish, the orders of Acipenseriformes, Salmoniformes, Siluriformes, Cypriniformes, Perciformes and Atheriniformes were subjected to multiple events of polyploidization. Realising that polyploidization has been suspected to be the major driving force behind the presence of duplicate genes with similar, related or different actions, this phenomenon merits close attention.

With the tabulation of genome size (the haploid DNA content) and chromosome numbers of Teleostei (including Siluroidei), Gyldenholm and Scheel (1971), and Hinegardner and Rosen (1972) intended to relate genome size, genomic complexity and phylogenetic status. It was assumed that genome size reflected the ploidy status and this could measure polyploidization events during evolution. It turned out that more evolved species did not necessarily have a larger genome size ("C paradox"). The relationship was more apparent than real because DNA mass did not increase with increasing genetic complexity. For example, bony fish have the smallest (< 1 pg) and the largest (> 200 pg) genome of all vertebrates (Wachtel and Tiersch, 1993).

Quite quickly it became clear that several protein-encoding genes occurred at more than one locus. Mo

et al. (1975) characterized both isoforms of glucose-6-phosphate isomerase (*GPI**) in *Ictalurus punctatus* and showed that there was a clear tissue-specificity at each locus. Most tissues contain the more liver-type isozyme, while muscle tissue contains the more basic isozyme.

Dunham *et al.* (1980) looked at variation in cellular DNA content, chromosome number and number of isozyme loci in four species of armoured catfishes (Callichthyidae). The genus *Corydoras* provides a good example of significant differences among species in ploidy level, and thus of gene duplication. The percentage of loci expressed in duplicate is higher in species with a high DNA content, which is in turn related to tetraploidization and regional gene duplication. It is a clear demonstration of the rapid evolutionary radiation of this genus.

When looking at the origin of duplicated genes, the creatine kinase (CK) isozyme loci A and C in teleosts, including *Ictalurus* sp. and *Corydoras* sp., seem to be ancestral, while the B and D loci seem to be the result of polyploidization and regional genome duplication (Fisher and Whitt, 1978). Moreover, more advanced teleosts tend to have tissue-specific expression of the four CK loci. In an even larger study on CK and four other allozymes (*LDH**, *MDH**, *GPI** and glycerol-3-phosphate dehydrogenase) the evolutionary implications of gene duplication were confirmed (Fisher *et al.*, 1980). Most duplicated genes have lost their function, but a small percentage has diversified to different physiological roles. However, the link between gene duplication and genome size (the "C paradox") does not stand the test. The authors concede there are several problems in making evolutionary interpretations from cellular DNA content.

As mentioned above, Coppes *et al.* (1990) reviewed the multilocus enzyme systems of *CK**, *LDH**, *GPI** and *s-MDH**. The polyploidization of these loci permits taxonomic discrimination up to the level of the Ostariophysi, the superorder which includes the catfishes.

Gene duplication is confirmed as a general mechanism in vertebrate evolution in a number of catfishes. Monteiro *et al.* (1991) describe the duplication of MDH in several South American catfishes of the families Pimelodidae and Loricariidae. Shukla and Tripathi (1993) demonstrate electrophoretically the tissue-specificity of the duplicate loci of MDH and LDH in *Clarias batrachus*.

Ngai *et al.* (1993) showed that functional divergence in duplicated genes leading to multi-gene families is a result of positive selection rather than a fortuitous consequence of mutations that are fixed by chance. The transmembrane helical domains of the channel catfish family of olfactory receptors show a higher number of non-synonymous (amino acid altering) nucleotide substitutions than in the remainder of the gene, which supports the hypothesis of positive Darwinian selection.

Duplication of ancestral loci is also evident in channel catfish VH and JH segments of immunoglobulin (Ig) (Hayman *et al.*, 1993). The multiple JH gene segments constitute the structural diversity of the V region of the Ig Heavy chain (IgH locus).

SEX DETERMINATION

The genetic sex of Teleostei varies from macroscopically heterogametic karyotypes (in about 10 % of all species studied) over molecular heterogametic to suspect homogametic karyotypes (Chourrout, 1988). Indeed, few nearly-mammal-like sex chromosomes have been discovered. The phenotypic sex is often labile and might be influenced by temperature (*e.g.* in *Menidia menidia*; Atherinidae) and population structure (*e.g.* in Serranidae). Although the molecular mechanisms of genetic sex determination in amniotes are being elucidated, environmentally controlled sex differentiation (ESD) and genetically controlled sex differentiation (GSD) are observed in cold-blooded vertebrates (fish and reptiles).

Siluroidei are no exception to these observations (*table* 2). The genetic sex has been identified in most cases on the basis of karyotypes. Unfortunately, conclusions are often founded on few mitoses collected from few specimens. Thus, a more detailed investigation is required since the presence/absence of a homogametic/heterogametic karyotype does not preclude the presence of a stable sex ratio. This means resorting to G/C/BrdU-banding, restriction enzyme banding, hybridization with synthetic oligoprobes, breeding of normal and sex manipulated populations, gynogenesis, and if possible, the detection of regulatory sex genes. Studies on the genotypic sex of *Clarias gariepinus* (sex ratio, karyotype and ploidy manipulation) (Ozouf-Costaz *et al.*, 1990; Teugels

Table 2. – Compilation of Siluroidei with heterogametic sex chromosomes. XX-XY: the male genotype is heterogametic; ZW-ZZ: the female genotype is heterogametic.

Species	Sex chromosomes	Reference
Callichrous bimaculatus	X1X1X2X2-X1X1X2	Rishi, 1976
Clarias gariepinus	ZW-ZZ	Ozouf-Costaz *et al.*, 1990
Heterobranchus longifilis	ZW-ZZ	Teugels *et al.*, 1992
Ictalurus punctatus	XX-XY	Davis *et al.*, 1990
Microlepidogaste leucofrenatus	ZW-ZZ	Andreata *et al.*, 1993
Mystus tengara	ZW-ZZ	Rishi, 1973
Noturus taylori	XX-XY	LeGrande, 1981
Plecostomus ancistroides	XX-XY	Michele *et al.*, 1977
Pseudotocinclus tietensis	XX-XY	Andreata *et al.*, 1992
Synodontis sp.	ZW-ZZ	Agnèse *et al.*, 1990

et al., 1992*a*) and *Ictalurus punctatus* (sex ratio, karyotype, ploidy manipulation, mapping) show clear GSD with a stable genetic sex ratio.

Ictalurus punctatus has macroscopically similar sex chromosomes with a similar DNA content in male and female fish (Tiersch *et al.*, 1990) and a sex ratio in outcrossed populations of 1:1 (Davis *et al.*, 1990). Females from all-female populations, produced by treatment with sex hormones and mated to normal males, had a sex ratio different from 1:1, suggesting a model of female homogamety and viability of YY males (Davis *et al.*, 1990). Fish from gynogenetic families produced only female offspring (Goudie *et al.*, 1995). Reproduction between outbred heterozygous males and homozygous gynogenetic female channel catfish results in a stable 1:1 sex ratio (Goudie and Liu, pers. comm.). DNA-DNA hybridizations with the probes *SRY, ZFY, Bkm* and human telomeric repeats did not detect sex-specificity in channel catfish (Tiersch et al., 1992). The Y-specific genes *ZFY* and *SRY* are associated with a particular sex in mammals.

More recently, the allozyme marker glucosephosphate isomerase-B (GPI-B) has been mapped to the sex-determining gene (*SDG**) as *SDG** – centromere – *GPI-B** in *Ictalurus punctatus* (Liu and Goudie, pers. comm.)

CONCLUSION

This review of the genetics of Siluroidei confirms a pragmatic research approach. Catfishes have been studied either because they provide a good model to a specific question or because of their potential for aquaculture. They do not represent a general model for genetics.

Detecting genetic variation has lost its technical limitations; first came breeding, then there was karyotyping, then came protein electrophoresis and now mitochondrial DNA variation, mini- and microsatellite DNA fingerprinting and gene sequencing have spawned fully fledged approaches. The combination of established biological approaches (*e.g.* biometry, quantitative genetics and physiology) with the more recent molecular tools looks very promising. The limit is now one of manpower and resources. Molecular genetics are probably underrepresented in this review because of their recent introduction. It is expected that in the near future many studies on molecular population genetics and DNA structure (genes and non-coding sequences) will be published.

The long-standing interest in karyology has elucidated to a limited degree the taxonomic status of some species, but support to phylogenic questions has been helpful to a lesser degree. Furthermore Siluroidei have made a significant contribution to the understanding of evolution. A definitive answer is premature but important aspects of this puzzle are surfacing. Providing simple genetic tools to environmental and aquaculture managers is an appropriate contribution of researchers. Marker assisted selection is being developed and progress can be fast as judged from the medaka and zebra fish map. Given the high interest in aquaculture of catfishes and the efforts to domesticate selected species, a genetic programme has to become an integral part of such strategy. The chances are too high for ill guided efforts due to indiscriminate stock transfer, hybridization and breeding with limited parental stock. Issues related to the biodiversity of catfishes remain underrepresented in the literature. Several species are threatened by habitat destruction and introductions, but the lack of background data makes it difficult to take appropriate measures. In the same vein, the impact of pollution on the genetic diversity of the 2,598 presently known catfishes remains a guess.

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