Morphological and genetic differentiation of 11 populations of the African catfish *Chrysichthys nigrodigitatus* (Siluroidei; Claroteidae), with consideration of their biogeography

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Abstract: Eleven samples of *Chrysichthys nigrodigitatus* and one sample of *C. maurus* were studied using morphometrics and enzyme polymorphism. Samples from opposite areas in the range of the species were most differentiated morphologically (Dagana in Senegal and Selingue in Mali, on one hand, and Bas Kouilou in Congo, on the other hand). In the sample from Bas Kouilou, most specimens overlap those from Ebrie Lagoon in Côte d'Ivoire. The populations from Côte d'Ivoire are genetically the most variable and have the highest number of alleles in common with the sample of *C. maurus* (ancestral alleles). The samples from the limits of the species' range (Dagana, Bas Kouilou) are those that have the least polymorphism (both monomorphs) and the fewest alleles in common with *C. maurus*. On the basis of these results we present a hypothetical scenario for the colonization of basins by populations of *C. nigrodigitatus* after the last glaciation. Colonization started in the area between Côte d'Ivoire and Benin. From this region of origin, some populations colonized the basins to the west via the coastal area up to Senegal. Independently, other populations colonized the basins to the east via the coastal area up to Zaïre. The colonization of the Niger most likely occurred through its Lower Delta.

Résumé : Deux techniques ont été employées pour étudier onze échantillons de *Chrysichthys nigrodigitatus* et un échantillon de *C. maurus* : l'analyse de la différenciation morphologique et l'analyse du polymorphisme des protéines enzymatiques. Les échantillons représentant les populations en limite d'aire de répartition de l'espèce sont les plus morphologiquement différenciés (Dagana au Sénégal et Sélingué au Mali d'une part et Bas Kouilou au Congo d'autre part). Dans l'échantillon de Bas Kouilou la plupart des spécimens sont superposés à ceux de la lagune Ebrié en Côte d'Ivoire. Les populations de Côte d'Ivoire sont les plus variables génétiquement; elles ont également un plus grand nombre d'allèles en commun avec *C. maurus* que les autres populations (allèles ancestraux). Les échantillons provenant des limites de l'aire de répartition (Dagana, Bas Kouilou) présentent les taux de polymorphisme les plus bas (ils sont tous les deux monomorphes). Ces résultats nous permettent de proposer un modèle hypothétique de colonisation des bassins par les populations de *C. nigrodigitatus* après la dernière période de glaciation. La région de départ de la colonisation serait située entre la Côte d'Ivoire et le Bénin. De cette région d'origine, certaines populations auraient colonisé les bassins à l'ouest jusqu'au Sénégal par la zone côtière. Indépendamment, d'autres populations auraient colonisé les bassins à l'est jusqu'au Zaïre, également par la zone côtière. La colonisation du bassin du Niger a pu se faire à partir de son delta inférieur.

Introduction

The African catfish genus *Chrysichthys* Bleeker, 1858 contains more than 35 species, which are not always easily distinguished morphologically. Risch (1986) revised the taxonomic status of the species. Agnèse (1989, 1991) and Agnèse et al. (1989) confirmed certain synonymies introduced by Risch (1986) using a genetic approach.

Chrysichthys nigrodigitatus (Lacépède, 1803), one of the species alluded to above that is particularly widespread, is found in most coastal basins from the Senegal River to the Zaire River (Risch 1992). It is commercially important and its culture is developing in certain countries such as Côte d'Ivoire (annual production 350–400 t; Otémé 1993). The first study of enzymatic polymorphism of six samples from Côte d'Ivoire and Mali showed that certain populations of this species were genetically very differentiated (Agnèse et al. 1989). This study was undertaken to quantify the variability and the genetic structure of this economically important species.

Agnèse (1989), studying the enzyme polymorphism of 16 populations of *Chrysichthys maurus* (Valenciennes, 1839), demonstrated that the present-day distribution pattern seems to be of recent origin: colonization of basins by this species occurred from east to west (from Côte d'Ivoire to Senegal), probably during the last glaciation (10 000 BP). In this paper we examine a hypothetical model of the colonization of the river basins currently occupied by the different populations of *C. nigrodigitatus*. This model might be applicable to other

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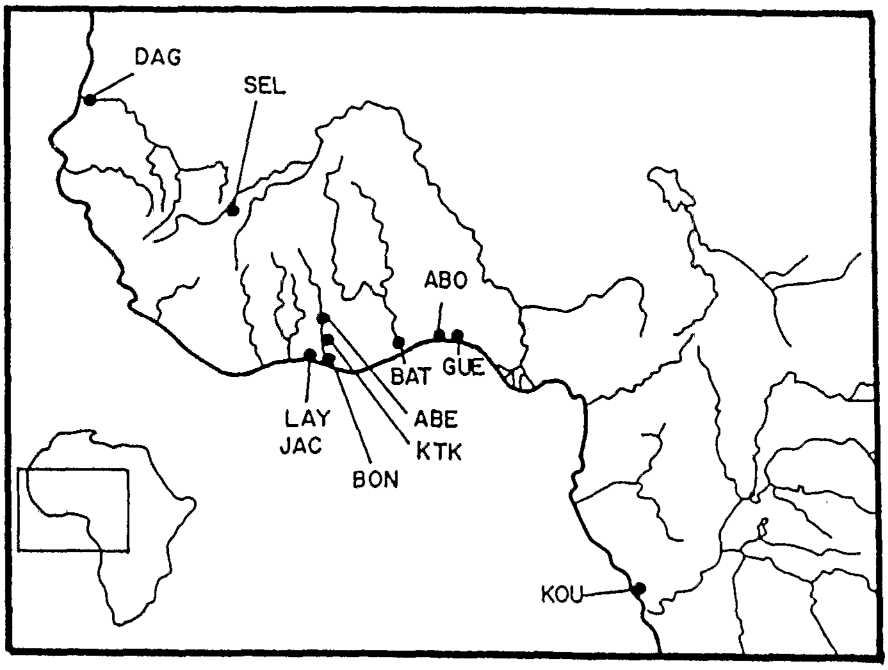
Fig. 1. Sampling sites of *Chrysichthys nigrodigitatus*. DAG, Dagana; SEL, Sélingué; LAY, Layo; JAC, Jacqueville; ABE, Abengourou road; KTK, Koutoukro; BON, Bonoua; BAT, Battor; ABO, Abobo; GUE, Guézin; KOU, Bas Kouilou.



fish groups and contribute to a better knowledge of West African biogeography.

Materials and methods

Eleven samples, including 354 specimens of *C. nigrodigitatus* originating from different coastal basins (Fig. 1) and 1 sample of *C. maurus* (19 specimens), were examined. The same specimens were studied morphologically and genetically; however, owing to problems of preservation, certain specimens were analyzed using only one technique (Table 1). All the specimens are deposited in the Musée Royal de l'Afrique Centrale, Tervuren, Belgium.

Morphology

For each specimen, 15 metric characters were noted (Fig. 2): total length; standard length; head length; snout length; width of premaxillary toothplate; occipital process length; occipital process width; nasal barbel length; predorsal distance; preadipose distance; prepectoral distance; prepelvic distance; preanal distance; distance between dorsal and adipose fins; and dorsal fin length. Eight meristic counts were made per specimen: gill raker number on the epibranchial of the first gill arch; gill raker number on the cerato- and hypo-branchials of the first gill arch; number of soft dorsal fin rays; number of soft pectoral fin rays; number of unbranched pelvic fin rays; number of branched pelvic fin rays; number of unbranched anal fin rays; number of branched anal fin rays. The selection of these morphometric and meristic characteristics was based on their diagnostic importance in the identification key for West African *Chrysichthys* species produced by Risch (1992).

The results obtained were statistically analyzed using the CSS: STATISTICA package (Statsoft, version 3.3).

Genetics

The sample from Jacqueville contained domesticated fish (fifth generation) taken from a fish farm (Société Ivoirienne d'Aquaculture Lagunaire). For the electrophoretic study, samples of liver, eye, and muscle taken from each fish were preserved in liquid nitrogen and stored at −80°C until analysis. The buffers used to study the enzymatic systems have been described by Agnèse (1989, 1991) and Pasteur et al. (1988). Genetic variability was evaluated using three indices: A, the mean number of alleles per locus; P, the level of polymorphism that corresponds to the number of polymorphic loci in relation to the number of loci studied; and H, the mean heterozygosity calculated using Nei's formula (1978).

The tests of allelic frequency deviation in relation to those expected under the equilibrium hypothesis of Hardy−Weinberg were performed using the G test furnished by the program BIOSYS1 (Swoford and Selander 1989).

Results and discussion

A comparison of the morphological data (as a percentage of head length (HL) or standard length (SL)) (Table 2) showed some variation between the populations studied, especially in nasal barbel length, premaxillary toothplate width, and dorsal fin length.

The data obtained (log transformed) for 14 metric characters (excluding total length) were submitted to principal component analysis (Fig. 3). The populations were arranged in four groups based on their country of origin (for each country except Côte d'Ivoire, all specimens were collected from only one drainage basin; the specimens from Côte d'Ivoire were sampled in the Comoe River and Ebrie Lagoon; see below). Nasal barbel length was the most discriminating character on the second axis; dorsal fin length, width of the premaxillary toothplate, and occipital process length were most discriminating on the third axis. The populations from Congo and Côte d'Ivoire are clearly separated from the Senegal and Mali populations. The Congo population, collected in brackish water near the mouth of the Kouilou River, is completely overlapped by the populations from Côte d'Ivoire, particularly, though not exclusively, those originating from the Ebrie Lagoon (Layo, Jacqueville, and Bonoua). The Senegal and Mali populations overlap slightly.

Table 1. Sampling localities and numbers of specimens examined for *Chrysichthys* species studied.

Location	Abbr.	River	Country	Number of specimens studied: morphologically	genetically	in total
			C. nigrodigitatus			
Dagana	DAG	Senegal	Senegal	14	17	17
Selingue	SEL	Niger	Mali	30	67	67
Layo	LAY	Lagoon	Côte d'Ivoire	19	16	19
Jacqueville	JAC	Lagoon	Côte d'Ivoire	6	96	96
Abengourou road	ABE	Comoe	Côte d'Ivoire	5		5
Koutoukro	KTK	Comoe	Côte d'Ivoire	2		2
Bonoua	BON	Comoe	Côte d'Ivoire	10		10
Battor	BAT	Volta	Ghana		30	30
Abobo	ABO	Lagoon	Togo		25	25
Guezin	GUE	Lagoon	Benin		16	16
Bas Kouilou	KOU	Kouilou	Congo	30	67	67
			C. maurus			
Layo	MAU	Lagoon	Côte d'Ivoire		19	19

Table 2. Variations in nasal barbel length, premaxillary toothplate width, and dorsal fin length in different populations of *C. nigrodigitatus*.

Location	*N*	Mean	SD	Min.	Max.
Nasal barbel length (% HL)					
DAG	14	4.7	2.0	1.3	7.9
SEL	30	8.3	1.2	5.5	10.8
ABE	4	11.9	2.2	8.8	14.1
BON	10	15.6	2.6	12.6	19.2
KTK	2	14.3	1.3	13.4	15.2
JAC	6	12.3	1.8	10.0	14.5
LAY	19	12.5	1.9	9.4	18.6
KOU	30	13.0	1.6	10.2	17.3
Premaxillary toothplate width (% HL)					
DAG	14	21.3	1.2	19.3	23.3
SEL	30	19.3	0.7	18.3	21.0
ABE	5	23.3	1.0	21.7	24.4
BON	10	24.4	3.1	18.7	29.3
KTK	2	23.6	2.0	22.1	25.0
JAC	6	22.6	1.5	21.0	25.5
LAY	19	23.8	1.3	21.5	26.0
KOU	29	24.4	1.9	21.8	29.1
Dorsal fin length (% SL)					
DAG	14	27.6	3.4	21.5	35.4
SEL	22	38.0	3.2	34.0	45.8
ABE	4	31.6	4.2	29.2	37.8
BON	10	27.7	3.7	21.7	33.5
KTK	2	28.4	5.3	24.6	32.2
JAC	6	27.9	3.0	22.5	30.5
LAY	18	27.6	2.1	24.2	32.0
KOU	29	27.3	1.5	24.5	30.8

Note: For an explanation of abbreviations of locations see Table 1. *N* is the number of specimens studied.

Concerning the meristic characters, small differences were noted between populations only for the number of gill rakers on the cerato- and hypo-branchials and the number of branched anal fin rays. Fifteen to 18 gill rakers are counted in the Congo population, while this number is lower in the other ones (14–16 in the population from Senegal; 13–16 in the Mali population; 11–16 in the populations from Côte d'Ivoire). The anal branched fin ray number ranges between 8 and 10 in the Senegal population, while it varies between 10 and 12 in the Congo specimens.

The study of enzyme polymorphism was conducted using 19 loci. In the samples of *C. nigrodigitatus* analyzed, 6 of the 19 loci were polymorphic. Frequencies of polymorphic alleles and indices estimating the degree of polymorphism are given in Table 3. The sample from Bas Kouilou (KOU) in Congo was monomorphic at all loci studied. The specimens from Dagana (DAG) in Senegal were also monomorphic at all loci studied. Although the number of individuals analyzed from Dagana (DAG) was limited (16), this sample is probably representative of the genotypic structure of its population; the sample from Layo (LAY), which was of a comparable size (17 specimens), was as polymorphic as the sample of 96 specimens taken from Jacqueville (JAC) and originating from the same population.

Among the samples of *C. nigrodigitatus* studied, those from Layo (LAY) and Jacqueville (JAC) were characterized by the presence of the private alleles *EST-1*A* and *PROT*B*. These different alleles are also present in *C. maurus* (MAU). The sample from Selingue is characterized by the presence of *EST-1*C* (also found in *C. maurus*), *IDH-2*B*, and *IDH-2*C* alleles. Finally, the samples from Abobo (ABO) and Guezin (GUE) were characterized by the presence of the *IDH-2*E* allele, which is also found in *C. maurus*.

In all samples studied, eight loci were represented by a single allele: *AAT-1*, *AK*, *CK*, *IDH-1*, *SOD-2*, *LDH-2*, *MDH-1*, and *MDH-2*.

The distributions of the different genotypes in the polymorphic samples did not differ from those expected under the

Fig. 2. Morphometric characters studied on specimens of *Chrysichthys nigrodigitatus*. 1, total length; 2, standard length; 3, head length; 4, snout length; 5, premaxillary toothplate width; 6, occipital process length; 7, occipital process width; 8, nasal barbel length; 9, predorsal distance; 10, preadipose distance; 11, prepectoral distance; 12, prepelvic distance; 13, preanal distance; 14, distance between dorsal and adipose fins; 15, dorsal fin length.

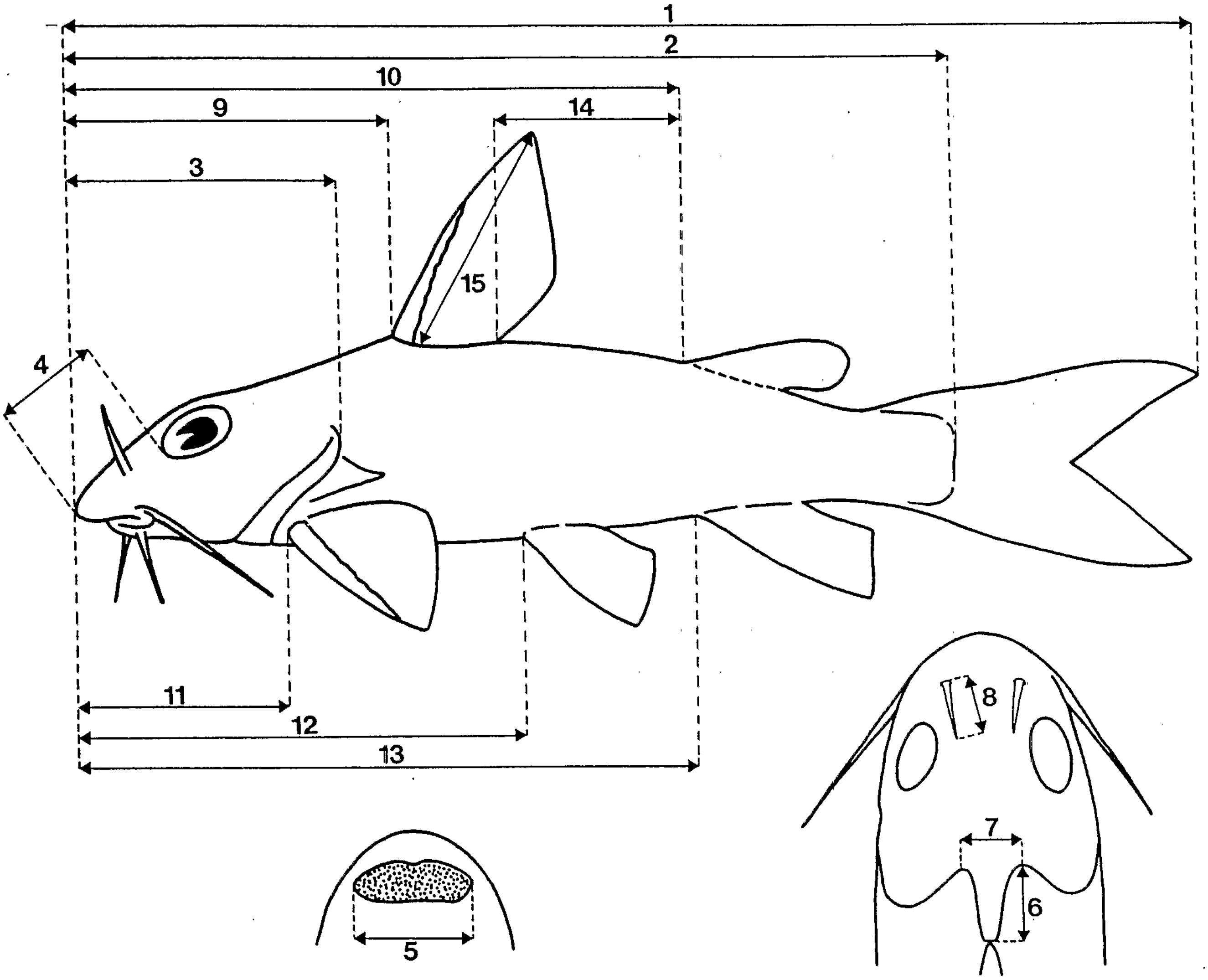


Fig. 3. Principal component analysis using 14 morphometric changes (log transformed) of *Chrysichthys nigrodigitatus* specimens arranged in four groups, based on their country of origin. S, Senegal (DAG); M, Mali (SEL); I, Côte d'Ivoire (ABE, BON, KTK, JAC, LAY); C, Congo (KOU).

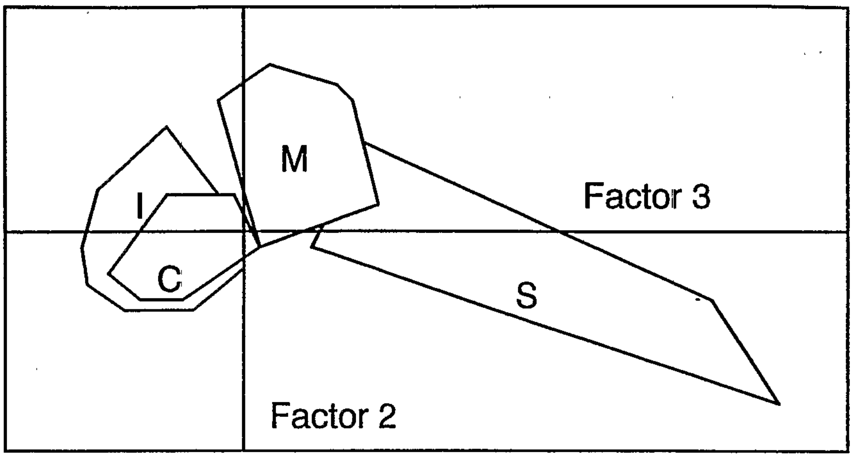


Table 3. Observed allele frequencies in samples from eight populations of *Chrysichthys nigrodigitatus* and one sample of *C. maurus*.

Locus	Allele	DAG	SEL	LAY	JAC	BAT	ABO	GUE	KOU	MAU
AAT-2	*N*	17	67	16	96	30	25	16	64	19
	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	B									1.00
EST-1	*N*	17	67	16	96	30	25	16	64	19
	A			0.97	1.00					0.03
	B	1.00	0.98	0.03		1.00	1.00	1.00	1.00	
	C		0.02							0.97
EST-2	*N*	17	67	16	96	30	25	16	64	19
	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	B									0.95
	C									0.05
G3PDH	*N*	17	67	16	96	30	25	16	65	19
	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
	B								1.00	
GPI	*N*	17	59	16	96	29	25	16	65	18
	A		0.46	1.00	1.00	0.55	0.54	0.97	1.00	0.06
	B	1.00	0.38			0.12	0.40	0.03		
	C		0.16				0.6			
	D					0.33				0.94
IDHP	*N*	17	67	16	96	30	25	16	65	19
	A	1.00	0.54	0.69	0.70	0.23	0.24	0.28	1.00	
	B		0.20							
	C		0.26							
	D			0.31	0.30	0.77	0.74	0.69		0.68
	E						0.02	0.03		0.29
	F									0.03
LDH-1	*N*	17	67	16	96	30	25	16	65	19
	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	B									1.00
MPI	*N*	17	67	16	96	30	25	16	65	19
	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	B									1.00
PGM	*N*	17	67	16	96	30	25	16	65	19
	A			0.75	0.76				1.00	1.00
	B	1.00	1.00	0.25	0.24	1.00	1.00	1.00		
PROT	*N*	17	67	16	96	30	25	16	65	19
	A	1.00	1.00	0.97	0.99	1.00	1.00	1.00	1.00	
	B			0.03	0.01					0.26
	C									0.74
SOD-1	*N*	17	67	16	96	30	25	6	65	19
	A			0.84	0.73			0.17		1.00
	B	1.00	1.00	0.16	0.27	1.00	1.00	0.83	1.00	
	A	1.0	1.3	1.3	1.2	1.2	1.2	1.3	1.0	1.3
	H (%)	0.0	5.8	6.3	6.0	4.5	5.3	4.7	0.0	6.1

Note: For an explanation of abbreviations see Table 1.

Hardy–Weinberg equilibrium hypothesis. The different samples could be considered panmictic. The mean number of alleles per locus is 1.2. This value is of the same order of magnitude as that observed by Agnèse (1989) for populations of *C. nigrodigitatus* from Côte d'Ivoire and Mali (1.4) and for *C. maurus* (1.27), *C auratus* (1.16), and *C. johnelsi* (1.08).

The mean heterozygosity of fish is 5.4% (Avise and Aquadro 1982). Among African catfish, Van der Bank et al. (1992) observed 4.7% for *Clarias gariepinus* (Clariidae), Abban and Skibinski (1988) noted 1.18% for *Schilbe mystus* (Schilbeidae), and Teugels et al. (1992*a*) mentioned 11% for *Heterobranchus longifilis* (Clariidae).

The highest mean heterozygosity rates were observed at Layo (6.3) and Jacqueville (6.2). The Jacqueville population is composed of domesticated individuals (fifth generation in captivity) originating from several hundred broodfish, many of which were taken from the wild each year around Layo. Therefore the loss of genetic variability was limited using these culture techniques.

The results obtained by the two techniques show certain similarities. The different populations of *C. nigrodigitatus* (except for those from Côte d'Ivoire and Congo) are morphologically different. This differentiation is geographically ordained: the most differentiated populations are those that are the farthest apart geographically. Senegal and Mali on one hand and Congo on the other. For genetic differentiation, the most polymorphic populations are those from Côte d'Ivoire (LAY, JAC) and the most monomorphic populations are those at the limits of the species' range, Senegal and Congo (DAG, KOU).

These results are sufficiently congruent to allow the history of basin colonization by populations of *C. nigrodigitatus* to be postulated using three hypotheses.

The first one concerns genetic variability. The populations found at the site of origin of the species have undergone the fewest bottlenecks, events that happen every time a new basin is colonized by a founding population. Consequently, they should have the highest mean number of alleles, while the most distant populations should have the lowest mean number of alleles.

The second hypothesis concerns the fixation of new alleles and the loss of ancestral alleles. Genetic drift, which occurs at each bottleneck, is accompanied by the loss (unpredictable) of certain alleles. New alleles are continuously created by mutation, this renewal leading to the fixation of new alleles. The newly colonizing populations will therefore have more new alleles and fewer ancestral alleles than the populations found at the site of origin of the species. The ancestral alleles being those that are found in closely related species, recently arrived populations will have fewer alleles in common with these species than the populations from the site of origin.

The last hypothesis concerns morphological differentiation. This is also a reflection of the migrations of populations: the closer the origins of the populations, the more morphologically similar they are.

Considering the results obtained and the hypotheses formulated, a model of the colonization of West African basins by populations of *C. nigrodigitatus* can be proposed.

The most recent (see below) site of origin of the species is situated between Côte d'Ivoire and Benin. These populations have the highest mean number of alleles (10.5 = <A = <15.8), the highest number of alleles in common with *C. maurus* (from 3 in Abobo (ABO) in Togo and Battor (BAT) in Ghana to 6 in Layo (LAY) and Jacqueville (JAC) in Côte d'Ivoire), and are morphologically quite different (especially the populations from Côte d'Ivoire).

From this site of origin, certain populations colonized the basins westwards along the whole coast up the Senegal River (*P*95% = 0.00 (DAG)); there is not a single allele in common with *C. maurus* in the sample from Dagana (DAG). Independently, other populations colonized the eastern basins all the way to Congo (*P*95% = 0.00; the sample from Bas Kouilou (KOU) had two alleles in common with *C. maurus*). For the colonization of the Upper Niger (*P*95% = 10.5 (SEL); the sample from Selingue (SEL) had two alleles in common with *C. maurus*, two hypotheses can be proposed: (1) colonization happened during contacts (captures or floods) with the Senegal River; or (2) colonization occurred through the Lower Niger all the way to the upper course. An argument in favour of the former is the high number of species in common between the Senegal and Niger rivers (see Lévêque et al. 1991), both being part of the Nilo–Sudan Ichthyogeographical Province as defined by Roberts (1975). An argument in favour of the latter hypothesis is the present distribution of *C. nigrodigitatus*, which is limited to the lower course of the coastal basins except for the Niger. Several other fish taxa have a similar distribution pattern: the catfish *Arius gigas* Boulenger 1911 (Ariidae) is present almost throughout the Niger and the Volta basins, in contrast to all its congeners, which are confined to coastal waters from Senegal to Angola (Daget 1992); the cichlid genus *Gobiocichla* shows a disjunct distribution, with one species in the Upper and Lower Niger and one in the Cross River, a coastal basin neighbouring the Niger delta (Teugels et al. 1992*b*); the cichlid species *Tylochromis sudanensis* Daget, 1954 is known from the Upper and Lower Niger and the Cross River (remarkably, all other West African *Tylochromis* species are generally confined to lower courses of coastal basins, and *Tylochromis* is absent from the Senegal River; see Stiassny 1989). Results obtained concerning enzymatic variation tend to support the colonization of the Upper Niger through its Lower Delta: if the Senegal population (Dagana) had been the ancestral one, then the Niger population (Selingue) should show a lower level of polymorphism and a lower mean number of allele per locus (because of a bottleneck) than the Senegal population. This contradicts our genetic observations.

The double colonization to the east and the west agrees with the morphological observations: the populations at the two extremes of the species' range (Dagana (DAG) in Senegal and Kouilou (KOU) in Congo) have evolved morphologically in opposite directions.

The distribution of species and their genetic structure depend not only on biological and environmental factors, but also on historical factors. In fact, it is difficult to explain the spatial distribution of populations forming one species without taking these factors into account. Numerous works have shown that from the Pleistocene to our present time, Africa had alternating dry and humid climatic phases that have had a profound influence on the phenomena of expansion and regression of populations as well as speciation (Haffer 1982). The existence of refuge zones seems to be established (Mayr and O'Hara 1986; Livingstone 1982), even if some authors have contested them (Endler 1982) and their delimitations vary from one author to another. These differences are undoubtedly the reflection of the diversity of the organisms studied; birds (Crowe and Crowe 1982), mammals (Grubb 1982), and amphibians (Duellman 1982) show different modes of distribution and are not necessarily reacting the same way to climatic constraints.

All the authors agree that the climatic variations which left the most noticeable mark on the actual distribution of species are those of the last 20 millennia. For Africa the events that succeeded each other are relatively well known (Hamilton 1976; Maley 1987*a*, 1987*b*, 1991). Between 30 000 and 9000 BP, the temperature was several degrees lower than the present one, and evaporation was less. There was tropical mountain vegetation at low altitudes. It was during this period that, around 19 000 years BP, the arid phase reached its peak. This aridity diminished from about 15 000 BP, and between 9000 and 8500 BP the tropical forest suddenly reappeared, occupying a greater surface area than that known today.

Around 20 000 BP, the current species or populations and (or) their ancestors saw their ranges reduced to zones of refuge. For most of these groups, there is a concordance between these refugia and the probable zones where the forest persisted (Myers 1982; Mayr and O'Hara 1986). According to certain authors, these zones of persistent forest are exclusively mountainous (Maley 1987*b*); others state that they are located both on the plains and in the mountains (Hamilton 1976).

We can expect that fish were affected by the cold, dry conditions that seemed to exist 20 000 years ago. Since the forests persisted in places where these conditions were the least unfavourable, there sould be a correlation between the presence of forests and the refugia for fishes. Such refugium–distribution relationships have been demonstrated for Neotropical fish (Weitzman and Weitzman 1982) and African fish (Hugueny 1989; Teugels et al. 1992*b*).

Our results suggest that the region between Côte d'Ivoire and Benin could have been a refuge zone for populations of *C. nigrodigitatus*. However, in view of the absence of certain samples in the study, this colonization model must be verified by studying additional samples: observation of samples from basins in Liberia, Sierra Leone, and Guinea would complete our knowledge of the mechanics of westward colonization; samples from Cameroon and Gabon would confirm or refute the idea of colonization eastward from Benin.

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