

Relative ages of present populations of *Barbus barbatus* and *Barbus meridionalis* (Cyprinidae) in southern France : preliminary considerations

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Received March 19, 1990, accepted July 16, 1990.

Persat H., P. Berrebi. *Aquat. Living Resour.*, 1990, 3, 253-263.

Abstract

The biogeographic history of French freshwater fish is poorly defined, and fishery managers do not currently take into account the only functional level, that of genetic stocks. The aim of the present paper is to provide a contribution for filling this gap. It surveys existing knowledge and previous studies of the genus *Barbus* in France. A first part chronicles the populations of the two French species according to ancient (tertiary) and recent (pre- and post-glacial, and contemporary) history. Thus, the *Barbus meridionalis* lineage seems to have been present in this country for 5 million years at least (Upper Miocene), but the present populations of the Rhône Valley seem to be the result of recent recolonization by populations restricted to a Languedoc-Roussillon sanctuary during the glaciations. *B. barbatus* has its origin in the Danube drainage basin. It is a more recent invader in Western Europe. Its immigration may have occurred during the Pliocene interconnections between the Rhône, the Rhine and the Danube basins (and perhaps even that of the Loire), or even more recently. If the first assumption is correct, the present populations of the Rhône are presumably descended from populations that found a shelter in the southern tributaries of the right bank of the river, or even in the tributaries of the right bank of the River Saône. In the Rhine basin, the sanctuary may have been the tributaries of the left bank, the Meuse and the Mosel Rivers, from which probably descend the populations of Eastern England. The present occurrence of *B. barbatus* in the Garonne catchment and perhaps also the Seine catchment seems to be due to man. The second part of the paper considers the genetic point of view, but the lack of data make it possible to discuss only the status of *B. meridionalis*. A synthesis of the available enzymatic data, compared with the hypotheses expressed in the first part, reveals a good agreement between the two approaches. In particular, the French and Spanish populations are well differentiated from one another, confirming the ancient settlement of the species in these countries. Moreover, the homogeneity of the French populations, and the higher polymorphism of the Roussillon population, agree with the presumption of a survival in this province during the glaciations. For instance, the first results on *B. barbatus* show a very low polymorphism and a very high homogeneity throughout the different catchments examined (Rhône, Meuse, Garonne, Hérault). Based on this work, a historical diagram is proposed, with the aim of ordinating the hypotheses for future research on the genetics of this group.

Keywords : *Barbus barbatus*, *B. meridionalis*, biogeography, history, genetics.

Ancienneté comparée des différentes populations actuelles de Barbus barbus et Barbus meridionalis Cyprinidac du Sud de la France : introduction au problème.

Résumé

L'histoire biogéographique du peuplement ichthyologique de la France est méconnue, et l'échelle de perception des espèces par les gestionnaires n'est pas encore vraiment descendue au seul niveau réellement fonctionnel, à savoir celui de la population, au sens génétique du terme. Le présent travail s'attache à combler quelque peu cette lacune en faisant le point sur les connaissances actuelles et les études en cours sur le genre *Barbus*. Dans une première partie, une chronique des populations des deux espèces présentes en France est établie sur la base de l'histoire ancienne (tertiaire) et récente (pré et post-glaciaire, et contemporaine). Ainsi, la branche évolutive de *Barbus meridionalis* semble installée dans la région étudiée depuis au moins 5 millions d'années (Miocène supérieur), mais les peuplements actuels de la vallée du Rhône proviendraient d'une recolonisation post-glaciaire à partir des populations réfugiées en Languedoc-Roussillon durant les glaciations. *B. barbus* originaire du bassin du Danube, est un immigrant plus récent en Europe occidentale. Son implantation pourrait dater des interconnexions pliocènes entre les réseaux hydrographiques du Rhône, du Rhin et du Danube (et éventuellement de la Loire), mais on ne peut exclure une pénétration encore plus récente. Dans la première hypothèse, les populations actuelles du Rhône pourraient descendre de populations ayant trouvé refuge dans les affluents méridionaux de la rive droite du Rhône, ou même ceux de la rive droite de la Saône. Dans le bassin du Rhin, la zone refuge correspondrait aux affluents rive gauche, Meuse, Moselle, également points de départ de la colonisation de l'Est de l'Angleterre. Sa présence actuelle dans le bassin de la Garonne semble être le fait de l'homme, et sa présence dans la Seine pourrait l'être aussi. La seconde partie examine la question sous l'angle génétique, mais la pénurie de données ne permet de discuter que du cas de *B. meridionalis*. Une synthèse des données enzymatiques disponibles, confrontée aux hypothèses formulées dans la première partie, fait ressortir une bonne concordance entre les deux approches. En particulier, les populations françaises et espagnoles sont bien différenciées, ce qui confirme l'arrivée ancienne de l'espèce dans ces régions. D'autre part l'homogénéité des populations françaises et le polymorphisme plus important de la population du Roussillon est conforme aux hypothèses de survie durant les dernières glaciations. Les tous premiers résultats sur *B. barbus* font apparaître un très faible polymorphisme et une très grande homogénéité pour les différents bassins inventoriés (Rhône, Meuse, Garonne, Hérault). A partir de ce travail, un essai d'organigramme historique est proposé en vue de sérier les hypothèses pour la suite des recherches sur la génétique du groupe.

Mots-clés : *Barbus barbus*, *B. meridionalis*, biogéographie, histoire, génétique.

INTRODUCTION

Scientists and fishermen do not seem to be particularly concerned with the origin and present geographic distribution of freshwater fishes in France, as if the facts were well known. It soon becomes evident that this is not the case, if one seeks precise and comprehensive information on the subject in the literature. Data are exhaustive on only a very small number of species. Other, more common species, are thought to be homogeneous and distributed everywhere.

This phenomenon is found even within the genus *Barbus*:

- Since the species *Barbus meridionalis* has been considered to be somewhat special, its distribution has been studied in considerable detail, e. g. by Dorier (1957), although this study did not extend westward beyond the Hérault river.

- On the other hand, *Barbus barbus* is reported to be "present almost everywhere in France" in Spillman's "Faune de France" (1961) (admitting, however, that it may be absent in the departments of Finistère and Ille-et-Vilaine, and that it is definitely

absent in the Alpes-Maritimes), although in fact there has been no extensive investigation.

Consequently, the present distribution of fishes in France requires further investigation, particularly as it is changing quickly owing to human influence. But still greater is our ignorance concerning the actual processes that have led to the present situation, and the history of colonization in western European waters.

To summarize briefly the works of Banarescu (1960, 1973) and Almaça (1976, 1985a and b), it can be said that the present biogeography of the genus in Europe is the result of several phases of colonization, carried out by several lineages. The first phases were early and probably contemporary with the general penetration of cyprinids into Europe. There is evidence for this in the middle Oligocene in the region of Marseille (Gaudant and Rousset, 1979) and in the Limagnes (subgenus *Paleoleuciscus* in the lacustrine Stampian around Vichy and Clermont-Ferrand) (Gaudant, 1984). These first waves extended to the different Mediterranean peninsulas. In contrast, the most recent wave, which is difficult to place chronologically between the Pliocene and Quaternary, was

excluded from the Mediterranean regions by geographic barriers that had appeared in the meantime (see Pomerol, 1973).

Species found around the Mediterranean, such as *Barbus meridionalis*, originated from the earliest phases of colonization, whereas the most recent colonization was by *Barbus barbatus*, which does not seem to have undergone speciation in the western part of its distribution area (Almaça, 1981). In particular, none of the barbel in the Iberian Peninsula can be considered to be subspecies of *B. barbatus*, even though some authors have recognized *B. barbatus* subspecies.

The biogeographic evidence suggests that in France *Barbus meridionalis* populations are older than those of *B. barbatus*. Nevertheless, the absolute age of a species should be distinguished from the length of time it has inhabited a given location. Although it appears that *B. meridionalis* has been present in western Europe longer than *B. barbatus*, in view of their respective geographic distributions, it is premature to assume that one of the species has evolved more than the other until a detailed morphological study has been carried out to determine which characters in the genus *Barbus* are ancestral and which are derived. The age of the species is a false problem, since by definition they issued from a common ancestor and evolved over the same period of time. At most, it might be possible to determine different evolutionary speeds (e. g. different rates of mutation). Our objective is thus to determine the ages of populations in the area studied.

First of all, it is important to know how long a population of a particular species has been present in a given hydrographic basin, secondly from which other basin and by which tributary the colonization took place, e. g. from which population it probably descended, and lastly what degree of isolation and therefore genetic differentiation has been attained since its establishment.

First, we discuss the case of the two French barbel species on the basis of biogeographic arguments that can be drawn from information on the geological, climatic, and faunistic history of the zone considered; secondly, we examine recently acquired data on the population genetics of these species, to compare theoretical deductions with actual circumstances.

BIOGEOGRAPHIC AND PALEOHISTORIC DATA

Barbus meridionalis

In France, *B. meridionalis* occupies most waterways from the Spanish to the Italian borders, which implies early colonization. A fossil *Barbus* from the layers of lacustrine diatomites in Andance Mountain (Upper Miocene in the Rhône Valley) (Mein *et al.*, 1983)

presents a generally stocky profile, poorly developed denticles on the last unforked ray of the dorsal fin⁽¹⁾, which had a convex form, and probably a low number of vertebrae (40 at most) (fig. 1). As a potential ancestor, this fossil appears to resemble *B. meridionalis* more than *B. barbatus*. That would imply that the *B. meridionalis* lineage has been in the region for at least 5 million years. However, the present distribution of the species in France is limited to the Mediterranean drainages, and does not extend northwards up the Rhône Valley as far as Lyon (fig. 2), suggesting a thermophilic tendency. Nevertheless, in the presence of *B. barbatus*, *B. meridionalis* is confined to the upstream portions of rivers (Dorier, 1957), where the water is rather cool.

To explain this contradiction, Berrebi *et al.* (1988) proposed that there was competition with *B. barbatus*. The latter seems to force *B. meridionalis* to occupy higher altitudes, or to hybridize with it (Berrebi *et al.*, 1987). The only cases in which *B. meridionalis* is found at low altitudes are in the small tributaries of the Hérault (southern France), such as the Boyne and the Dourbie (Le Brun, 1988), which are isolated from the main river by rocky outcrops that prevent the ascent of *B. barbatus*, the small coastal rivers of Roussillon, such as the Têt and the Tech (Berrebi *et al.*, 1988; Philippart and Berrebi, 1990), and in Provence, e. g. in the Argens (Kiener *et al.*, 1981) and the Gapeau (Kiener and Ollier, 1970) from which *B. barbatus* is absent.

Considering how long the *B. meridionalis* lineage may have been present in this part of France, it is strange that it is absent from other French basins, particularly the Garonne⁽²⁾, which is the nearest and has the same latitude. It is possible that it never existed there, or was eradicated during the last ice age, and that only a warmer basin, e. g. that of the Mediterranean, enabled it to resist this climatic event. It is therefore of interest to examine in detail what may have happened in this region. Figure 3 shows the hydrographic profile during the Riss glaciation. The Gulf of Lion was partially exposed because of the lower sea level, and this offered many possibilities for connections between watercourses from the Pyrenees to the basin of Marseille (Debrand-Passard and Courbouleix, 1984). These circumstances may have facilitated the movements of freshwater species along the coast of Languedoc. However, the Rhône and the Durance in this epoch were cataclysmic rivers

⁽¹⁾ Gaudant has recently informed us that *Barbus* with well developed denticles occurred in the same site.

⁽²⁾ A small population exists in the upper course of the Tarn, a tributary of the Garonne, but its restricted area suggests a recent introduction. In addition, individuals from Roussillon have been transplanted to the region of Toulouse, where they have hybridized with *B. barbatus*.

originating from an enormous alpine glacier, which were swept by gigantic floods from massive bodies of water that must have collected and periodically burst

from the glacial ice cap. It can be assumed that *B. meridionalis* could not have survived in such an environment.

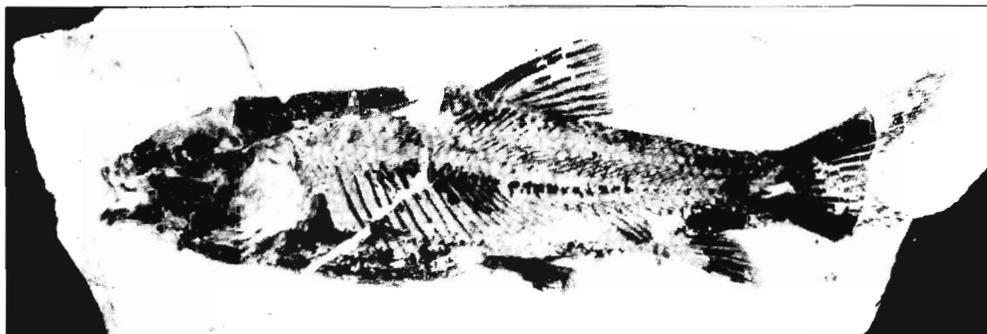


Figure 1. *Barbus* sp. from the Upper Miocene of the Rhône Valley. Collection J. P. Romaggi (Mein *et al.*, 1983) (with the kind permission of the *Nouvelles Archives du Muséum d'Histoire Naturelle de Lyon*).

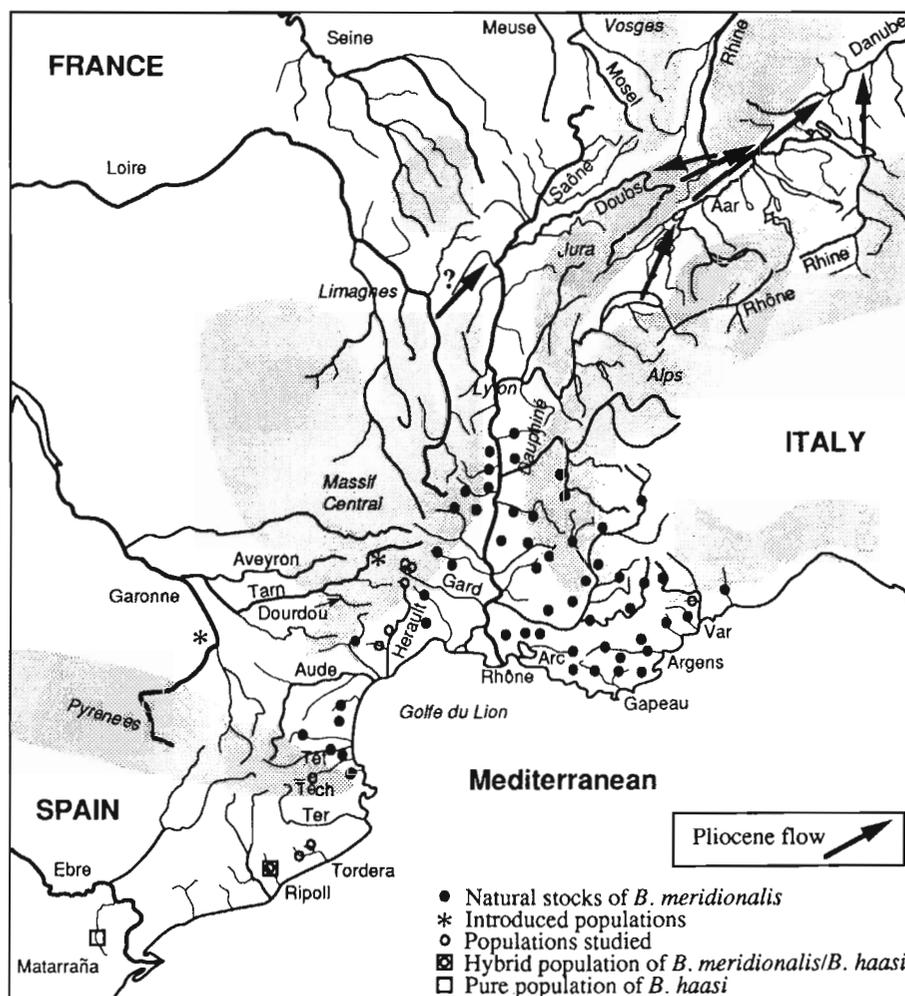


Figure 2. – Distribution of *Barbus meridionalis* in France, according to Dorier, 1957; Olivier, 1970; Berrebi *et al.*, 1988; Machordom *et al.*, 1990; Kiener, 1985.

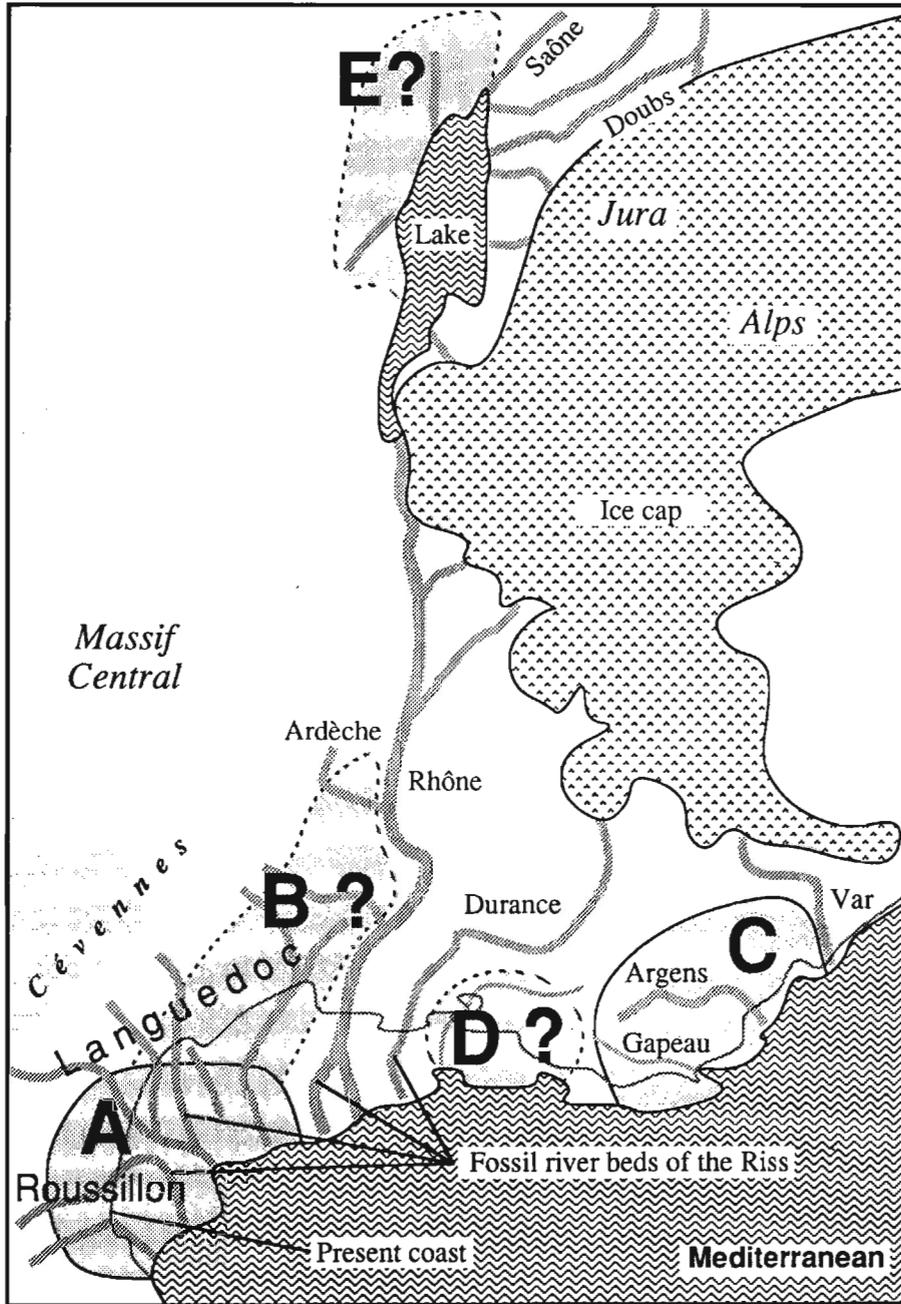


Figure 3.— Structure of the French Mediterranean hydrographic systems during the Riss ice-cap maximum, with the possible refuges for *Barbus meridionalis* A(+B?), C(+D?), and *Barbus barbatus* A–B(+E?).

To the east of the Rhône, the only sectors where species appears to have survived are the Argens and neighbouring watercourses, with the exception probably of the Var itself, which was also fed by large glaciers. To the west of the Rhône, the coastal rivers were more habitable, but because of thermal conditions it is possible that the distribution area of the species was limited to the southernmost rivers

(Roussillon). Since there seems to have been no plausible connection between France and Spain in the Quaternary, it is practically certain that the species survived in the region during the ice ages. In this overall scheme, the present populations in the Rhône Valley seem to be very recent, since they could only have been present for 10,000 years. They must have originated in Languedoc (unless a small remnant

population survived in the basin of Aix-en-Provence), and had to be very similar.

The populations of Languedoc-Roussillon have been present for a much longer time (at least 5 million years), but since they had ample time to merge in the Quaternary when the sea level was lower, and since they probably generated the Rhône populations, it is likely that they have not been genetically isolated from one another and from the Rhône Valley populations for more than 10,000 years. Consequently, there is probably only one large genetic unit from Roussillon to the Dauphiné.

On the other hand, greater differences can be expected with Spanish species and with those of the coast of Var. The only river connections that could have existed between these three geographic units occurred 5 million years ago during the Messinian, when the straits of Gibraltar were closed and the Mediterranean began to dry up. It is possible that the populations on the coast of Var were isolated several million years earlier, since even with the desiccation of the Mediterranean the possibilities of connection remain problematic.

Barbus barbus

The second French species, *Barbus barbus*, is present in all the major river basins except in Roussillon, the coast of Var, and the extremity of Brittany. It is the main representative of the genus in Europe, with the largest range, from the Atlantic to the Black Sea. However, apart from the tributaries of the Gulf of Lion, the species is absent throughout the Mediterranean basin. This distribution, which is characteristic of late dispersal, is similar to those of a large number of central European species that evolved within the vast hydrographic system of the Danube before spreading rather recently throughout central Europe.

The morphological homogeneity of the French and European populations argues against long genetic isolation of populations in different locations. The penetration of the species into western Europe may have occurred in the Pliocene. In the Lower Pliocene, the upper Doubs, the Aar, the Rhine, and the Swiss Rhône formed the upper basin of the Danube (Thienemann, 1950), hence harbouring a Danubian fauna. In the Middle Pliocene, the upper Doubs and the Aar were disconnected from the Danube in favour of the lower Doubs, e. g. the Rhône basin. At the end of the Pliocene, the Aar was diverted to the north by the subsidence of the Alsatian plain, and only at the beginning of the Quaternary was the Rhine in turn removed from the Danube basin by regressive erosion of the Aar from the Alsatian plain (Villinger, 1986).

According to this hypothesis, the separation of the Rhône and Rhine populations occurred in the Middle Pliocene, and the Rhine and Danube populations were separated in the beginning of the Pleistocene.

However, it is possible that later temporary connections appeared between the Rhine and Danube basins and between the Aar (Rhine) and Swiss Rhône basins during the Quaternary, as a result of ice caps and captures of tributaries.

Assuming that *B. barbus* was able to colonize the Rhine and Rhône during the Pliocene, the problem raised above with respect to *B. meridionalis* applies to present *B. barbus* populations, e. g. the impact of the ice ages on the preglacial distribution of the species in the two basins, and thus the origin and age of the present populations. As mentioned above, it can be assumed that glacial watercourses were insuitable for the survival of a permanent population. On the other hand, this species appears to be less thermophilic than *B. meridionalis*. It is thus possible that it survived in right-bank tributaries of the Rhône (the Eyrieux, the Ardèche, the Gard, etc.). Its presence has in fact been reported in the Acheulean of the Ardèche (Desse and Garnier, 1976). A population may also have survived in a sub-basin of the Saône. Both are right-bank tributaries that were not affected by ice caps. Under these conditions, it may have taken advantage of the lower sea level to colonize the rivers of Languedoc, but it does not appear to have reached Roussillon, unless it was eradicated by the dryness of the present climate.

Again assuming that penetration occurred during the Pliocene, during the ice ages the sub-basins of the Meuse and Mosel must have been its refuge in the Rhine basin, and it would have taken advantage of the lower sea level to colonize eastern England. In contrast, it does not appear to have reached the English Channel basin, since, as is the case of all central European species, it is not found under natural conditions in the coastal rivers of southern England (Wheeler, 1977). This is an important observation because it implies that the species was also absent from the whole Seine basin, which merged at that time with the rivers of southern England in the middle of the desiccated Channel.

The case of the Seine raises the problem of the origin of the populations in the three large basins to the west of the Rhine-Rhône axis, e. g. the Seine, the Loire, and the Garonne. From a paleohydrographic point of view, the possible absence of *B. barbus* from the Seine is a logical hypothesis: following the subsidence of the Saône plain in the Miocene, which increased the slope, captures of rivers on the threshold of Burgundy have only occurred eastward. Similarly, in the northeast, the transfers of watersheds appear to have favoured the Meuse and Mosel and not the Seine. The Quaternary fish fauna of the Seine may in fact have been rather sparse until quite recently.

According to Chaput (*in* Henry, 1976), the Loire flowed for a time toward the Saône during the Upper Pliocene, following the route of the present "Canal du Centre". Henry (1976) considers that the distribution of a hypogean asellid (Crustacea, Isopoda) is

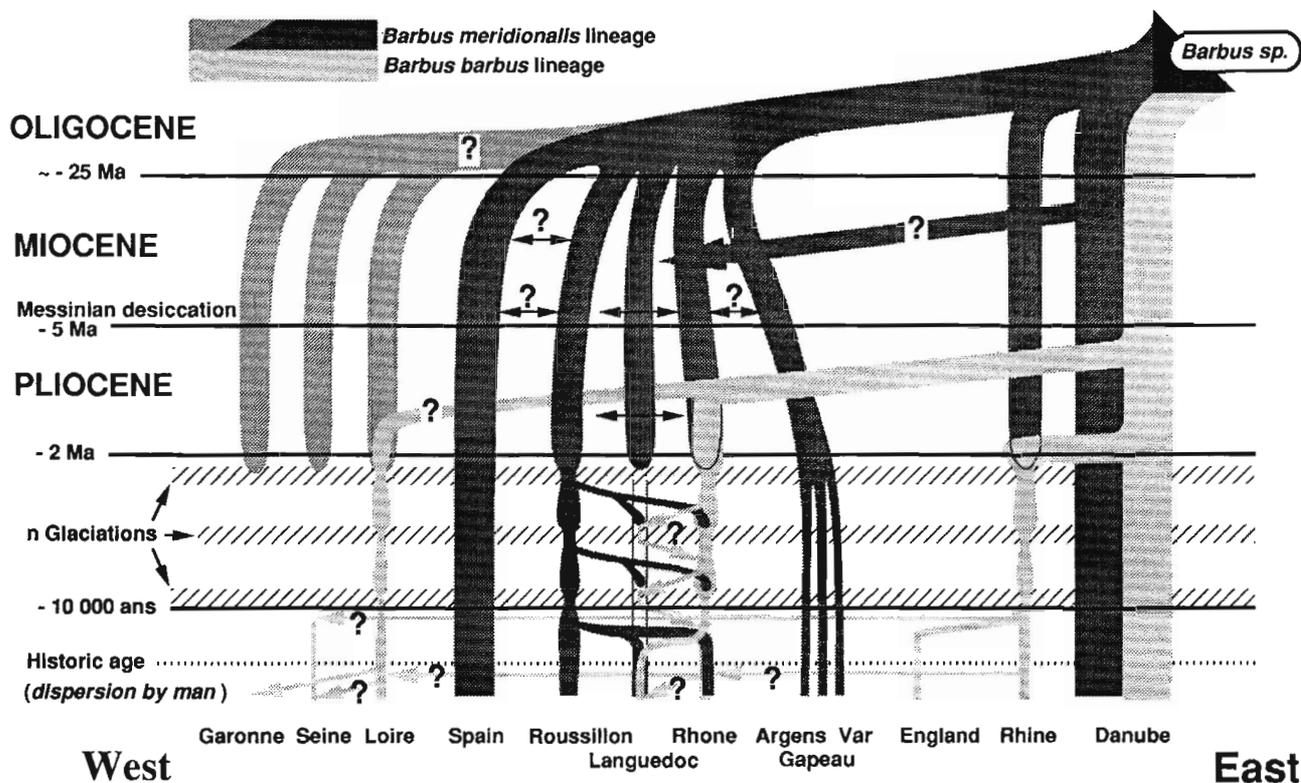


Figure 4. – Chronological hypotheses on the colonization of Western Europe by *Barbus meridionalis* and *B. barbatus*. The width of the lines represents the possible genetic diversity of the different populations.

Table 1. – Recapitulation of the genetic data used in the study. *B. m.*: *Barbus meridionalis*, *B. h.*: *Barbus haasi*.

Locality	Species	Genetic diversity	References
Var (French Alps)	<i>B. m.</i>	H=0	Berrebi <i>et al.</i> (1988)
Cévennes (Massif Central, France)	<i>B. m.</i>	0 < H < 0.018	Berrebi <i>et al.</i> (1988)
Tech (French Pyrenees)	<i>B. m.</i>	H=0.025	Berrebi <i>et al.</i> (1988)
Riera de Arbucias (N-W Spain)	<i>B. m.</i>	H=0.044	Machordom <i>et al.</i> (1990)
Tordera (N-W Spain)	<i>B. m.</i>	H=0.044	Machordom <i>et al.</i> (1990)
Ripoll (Barcelona, Spain)	<i>B. m.</i> x <i>B. h.</i>	H=0.090	Machordom <i>et al.</i> (1990)
Matarraña (Ebre, Spain)	<i>B. h.</i>	H=0.009	Machordom <i>et al.</i> (1990)

Taxons	Genetic Distance	References
<i>B. m.</i> (France) and <i>B. m.</i> (Spain)	0.021 < D < 0.025	Machordom <i>et al.</i> (1990)
<i>B. m.</i> (France) and <i>B. m.</i> (France)	0 < D < 0.009	recalculated
<i>B. m.</i> (Spain) and <i>B. h.</i> (Spain)	0.255 < D < 0.258	Machordom <i>et al.</i> (1990)
<i>B. m.</i> (France) and <i>B. h.</i> (Spain)	0.242	Machordom <i>et al.</i> (1990)

an indication of this. If this is true, *B. barbatus* and accompanying species would have been able to ascend the Loire basin before it resumed its normal course.

On the other hand, since the separation of the lower Loire basin from the Seine basin occurred much earlier, *B. barbatus* would not have had access to the Seine. Moreover, there appear to have been no natural connections between the Loire and Garonne basins since well before the Pliocene. Consequently,

B. barbatus would not have been able to reach it naturally. This seems to be confirmed by the freshwater fish fauna at prehistoric sites in the Aquitanian basin. Le Gall (1984) describes this fauna as characterizing a barbel zone, with the presence of trout, salmon, pike, eel, dace, chub, and bleak, but no barbel has yet been found. Under these conditions, its presence in the Garonne could only be quite recent, and the opinion of Almaça (1981) concerning the irrelevance

of the Garonne subspecies *B. b. gallicus* created by Karaman (1971) seems to be completely justified.

The case of *B. barbuis* in France strongly indicates that the present distribution of freshwater fishes is probably even more artificial than was previously thought. It would appear that *B. barbuis* is native only to the Rhine and Rhône basins. Its presence in the Loire might be natural, but the data are not yet conclusive. Its arrival in the Seine is probably quite recent, and even more recent in the Garonne. Its dispersal to the last two basins is probably related to the construction of canals that began in the 17th century.

In any case, the capacity of earlier human inhabitants to spread certain species should not be underestimated. Religious communities of the Middle Ages, with their obligation to eat fish on Fridays and their propensity to build monasteries in different regions, clearly participated in the dispersal of certain species (see discussion in Batias, 1958). In view of its environmental preferences, *B. barbuis* was probably not the easiest species to transplant, but its size may have made it worth the effort.

GENETIC DATA

Several genetic studies on barbel populations have been carried out in recent years. The data are for the moment very preliminary, but it is of interest to compare them with the hypotheses proposed in Part One.

The scarcity of genetic arguments

One of the important characteristics of these species is the almost complete absence of polymorphism (table 1). Although Stratil *et al.* (1983) reported high polymorphism in the transferrins of these two species in Yugoslavia, this is not at all the case in southern France where *B. meridionalis* shows a genetic diversity (H) ranging from 0 to 0.025 (calculations based on data reported by Berrebi *et al.*, 1988), which is extremely low. The genetic diversity of *B. barbuis* is even lower: except for loci *Pgi-2*, *Pgi-3*, and *Pgi-4*, whose origin remains unknown (Philippart and Berrebi, 1990), no variant has been detected in France (in the Rhône at Lyon and Montélimar, in the Hérault, in the Garonne at Toulouse, the Aveyron, the Dourdou) or in Belgium (the Ourthe) (unpublished data).

Very low enzymatic polymorphism is generally interpreted in two ways: either the species has been subject to one or more "founder effects" (the smaller the founder population, the greater the reduction in polymorphism), or it is a characteristic of the phylogenetic group, and thus arises from an intrinsic cause. It is difficult to favor one of these hypotheses over the other since so little work has been done on barbel.

First, there are no data on a population that has definitely not undergone a drastic reduction, and second, only one work deals with enzymatic polymorphism of another European barbel species. Based on an analysis of 30 enzymatic loci, Machordom *et al.* (1990) found a genetic diversity of $H=0.044$ for Spanish *B. meridionalis*, which is an average value for fishes, and $H=0.009$ (a very low value) for *B. haasi*, a closely related Spanish species.

Apart from its biological significance, which calls for study, this low polymorphism has the disadvantage of providing very few markers for determining the recent history of the two species. In view of the scarcity of data, only the status of *B. meridionalis* can be discussed.

The case of *B. meridionalis*

Data concerning this species have been summarized in two articles. The first (Berrebi *et al.*, 1988) is based on five samples of 36 specimens of French *B. meridionalis*, analyzed at 34 loci. Sampling was done in small watercourses of the three mountain ranges in southern France (Alpes Maritimes, Massif Central, and eastern Pyrenees) (fig. 2). Only the population of the Tech (a small independent Mediterranean watercourse crossing the plain of Roussillon at the foot of the French Pyrenees) showed measurable polymorphism ($H=0.025$). The three populations of the Cevennes (southern Massif Central), captured in small affluents belonging to the basins of the Hérault and the Rhône, and that of the southern Alps, captured in a tributary of the Var, showed practically no polymorphism (0 to 0.018).

The second article (Machordom *et al.*, 1990) concerns the hybridization of *B. meridionalis* and *B. haasi* in Spain, studied by electrophoretic analysis of 30 enzymatic loci. Two *B. meridionalis* populations of northeastern Spain, occupying the same hydrographic basin but separated by dams, were compared with a population of the same species sampled in France, in a tributary of the Hérault in the southern Massif Central. The French population again showed very low polymorphism ($H=0.008$), whereas the two Spanish samples, which were very similar genetically, showed an enzymatic polymorphism close to values usually observed in fishes ($H=0.044$) (see summary in table 1).

Despite the lack of effective markers, these results can form the basis of a hypothesis that is consistent with the arguments developed in the first part. The Nei distances separating the French populations from the Spanish populations (table 2) range from 0.021 to 0.025, whereas the French populations show distances between themselves ranging from 0 to 0.009 units (distances recalculated from primary data). Thus it might seem paradoxical that the Mediterranean slopes of the three French mountain ranges (Alps, Massif Central and Pyrenees), which are separated

from each other by several hundred kilometres, are populated with genetically homogeneous barbel, whereas on either side of the Pyrenees, populations separated by scarcely a few dozen kilometres are genetically well differentiated.

The relatively strong differentiation between French and Spanish barbel reveals the importance of the biogeographic barrier formed by the Pyrenees. This confirms what we have indicated as being the paleohydrographic history of the region, e. g. an absence of recent connections between the French and Spanish populations. The most recent possibility for such contact was the Messinian desiccation 5 million years ago. However, the genetic distance observed cannot be used as a time scale since the low enzymatic polymorphism introduces a large confidence interval into the evaluations.

On the other hand, the homogeneity of the present French populations clearly expresses the recent history of the Languedoc-Roussillon region, which formed a large emerged plain during the ice ages, where connections between watercourses were more numerous than at present. This plain was inhabited by *B. meridionalis* populations that were genetically homogeneous due to the possibilities of gene exchange, with a polymorphism at least as high as that of the present population of the Tech (Roussillon). In this period, *B. meridionalis* must have been confined to the edge of the Mediterranean, at the limit of its ecological tolerance, and probably only the downstream portions of the watercourses were populated, since the upstream portions were too cold. This restricted distribution may have been the origin of its lower genetic polymorphism compared with the Spanish populations.

In the French populations, the higher genetic polymorphism in Roussillon may have to do with the fact that it is only population of the plain that has been examined, but it is quite consistent with the hypothesis of a refuge within this region alone during maximum glaciation. During the last postglacial warming (10,000 years ago), the geographic zone that was ecologically colonizable by *B. meridionalis* increased tenfold, but the ascent to the higher portions of the watercourses was definitely not easy. The three highest altitude populations (450 to 600 m, Massif Central and the Alps) analyzed by Berrebi *et al.* (1988) showed little or no enzymatic polymorphism. It would thus appear that the high altitude populations underwent a "bottleneck" or "founder effect" that strongly reduced their polymorphism. However, it cannot be excluded that survival in extreme conditions itself induced strong selective pressure, which reduced genetic variation in populations that were necessarily of small size, as suggested by Persat (1988) with regard to the high genetic homogeneity in populations of the grayling *Thymallus thymallus* in the upstream portions of the zones it inhabits. It is nevertheless probable that the parent populations were

quite comparable to the population in Roussillon, since the fixed alleles in the high altitude populations are those that are most frequent on the Roussillon plain.

In this general scheme, it remains to be determined how *B. meridionalis* was able to disperse on either side of the Rhône, whereas, as seen above, it is present in the plains only in locations where *B. barbatus* is absent, which suggests competitive exclusion. It is possible that there were phases of rapid dispersal during which certain individuals were able to cross the river systems occupied by *B. barbatus* to reach the upstream portions of other affluents, but a problem remains as to the reciprocal positions of the two species during ice ages. It seems unlikely that they would have cohabited in the same watercourses on the plain of Languedoc, since there appears to be strong introgression in present contact zones (Berrebi *et al.*, 1987). For the moment it is difficult to predict the fate of these introgressed populations. Cyprinids have a remarkable capacity for hybridization, notably in zones degraded by man (pollution, sewage) where the destruction of natural spawning grounds appears to induce strong promiscuity between parents of different species.

According to Berrebi *et al.* (1988), the different cases of hybridization can be classified between two extremes: (i) completely sterile F1s, with hybridization having no effect on the genetic pool of the parent species, and (ii) F1s and subsequent generations that are normally fertile, with a fitness that is the same as, or locally greater than, the parent species, which can ultimately lead to a synthetic species.

The present case is closer to the second condition, since the extant *B. barbatus* populations of Languedoc show uniform introgression by *B. meridionalis* of about 15%. However, even in these circumstances, cases have been reported in which hybrids are counterselected, protecting the parent species from excessive introgression (Dowling and Moore, 1984). Moreover, Philippart and Berrebi (1990) have shown that the F1 males are sterile, which clearly hampers introgression. Lastly, even more than thermal conditions, the relative sizes of adult individuals might be enough to maintain the two forms, since *B. barbatus* parents, in particular the females, which generally do not reach maturity before attaining a length of 35 cm (Philippart, 1987), may be unable to ascend small streams where only smaller barbel, e. g. those of the pure *B. meridionalis* strain, can reproduce.

Thus, the barbel of southern France may be in a locally very strong introgressive situation, arising from two ecotypes that are nevertheless stable, e. g. the apical type (taken in the hydrographic rather than altitudinal sense) *B. meridionalis*, and the basal type *B. barbatus*. The size factor alone may have made it possible for the two species to be in contact, even during the ice ages, while keeping their respective integrity, the small species in small affluents, and the

large species in large rivers. Moreover, cohabitation of the same basin by two barbel species is a rather frequent occurrence, as observed with *B. barbatus* and *B. meridionalis petenyi* in the Danube basin, and with other species in Spain and Greece.

CONCLUSION

The biogeography of West-European fishes is the result of a complex history in which geological, geographic, climatic, hydrological, ecological, and human parameters interfere. Although progress has been made in investigating most of these domains, the human factor is one of the most poorly understood elements. Only data related to occurrences prior to the Roman era, such as those provided by paleontology and particularly archeology, can be used to distinguish between natural geographic distribution and human interferences. However, these data are still too sparse to provide a comprehensive view of the phenomena.

Fortunately, genetic methods now offer an extremely practical complementary means of investigating markers of respective geographic and evolutionary histories within different populations. In the case of French barbel, genetic and biogeographic data enable us to estimate that *B. barbatus* appeared in France much more recently than *B. meridionalis*, and that the plain of Roussillon was probably the center of postglacial dispersal of the latter species towards the Rhône Valley. The chronology and exact details of these events remain to be determined. Figure 4 is an historical flow chart of the different hypotheses formulated in the present study, showing the relative times at which the geographic units now present in France may have become genetically isolated. Genetic studies now in progress should make it possible to confirm or invalidate these hypotheses in the near future.

Since the evolution of freshwater fish species appears to occur very slowly (Turner, 1984), priority should be given to studies of what are thought to be the earliest major isolations. For instance, the Rhône and Danube populations of *B. barbatus* and *B. meridionalis* should be compared to verify whether there are actually greater genetic differences in the latter species, as suggested by biogeographic data. Similarly, within the French populations of *B. meridionalis*, emphasis should be placed on studying the most isolated populations, e. g. those of the Var coast (the Argens, the Gapeau, and the Var). However, the comparison with the nearest Italian populations from both the Riviera and Po sides of the watershed would then be necessary.

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