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Spanish barbel hybridization detected using enzymatic markers: *Barbus meridionalis* Risso × *Barbus haasi* Mertens (Osteichthyes, Cyprinidae)

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Abstract

Five *Barbus* populations were analyzed by enzyme electrophoresis to verify hybridization between two species in Spain, *B. meridionalis* and *B. haasi*. Two populations supposed to be hybrid have been named *B. meridionalis*-2 and *B. haasi*-2 because of their morphological resemblance to the corresponding parent species. 88.9% of *B. haasi*-2 genes are of the "*meridionalis*" type, a value obtained using six loci distinguishing the two species. None of the individuals in this population was identified as an F1 hybrid. On the other hand, *B. meridionalis*-2 showed no sign of introgression and is thus a population of the pure species *B. meridionalis*. The absence of known current contact between the hybrid population and both parent populations and the present genetic results suggest that the hybridization is currently interrupted in the studied locality.

Keywords : Hybridization, enzyme electrophoresis, hybrizymes, *Barbus*.

Hybridation de barbeaux espagnols détectée par des marqueurs enzymatiques : Barbus meridionalis *Risso* × Barbus haasi *Mertens* (*Osteichteyes, Cyprinidae*).

Résumé

Cinq populations de barbeaux ont été analysées par électrophorèse des protéines enzymatiques afin de vérifier la réalité d'une hybridation entre deux espèces d'Espagne: *Barbus meridionalis* et *B. haasi*. Deux populations supposées hybrides ont été nommées *B. meridionalis*-2 et *B. haasi*-2 d'après leur ressemblance morphologique avec l'une ou l'autre des espèces parentales. *B. meridionalis*-2 n'a pas montré de trace d'introgression et constitue donc une population d'espèce pure. Par contre *B. haasi*-2 possède 88,9 % de gènes de type *« meridionalis »*, valeur obtenue grâce à l'utilisation de six locus marqueurs entre les deux espèces. Aucun individu de cette population n'a pu être identifié comme F1. Les résultats génétiques obtenus et l'absence de contact actuel connu entre cette population hybride et les populations parentales suggèrent que l'hybridation est actuellement interrompue dans la localité étudiée.

Mots-clés : Hybridation, électrophorèse enzymatique, hybrizymes, *Barbus*.

INTRODUCTION

The occurrence of localized interactions between the differentiated genomes of two taxa and the apparent stability of many hybrid zones raises problems that are both theoretical (species definitions, reproductive isolation) and evolutionary (homogenization of genomes, speciation). Many authors have discussed the adaptive value or biological significance of hybrids (e.g. Ferguson *et al.*, 1985*a*, Barton and Bengtsson, 1986; Descimon and Geiger, 1988). Studies of hybrid populations in different zoological groups have proliferated, e.g. Hung (1985) and Rand (1988) in the invertebrates, Avise and Saunders (1984), Gollmann *et al.* (1988), and Vanlerberghe *et al.* (1988) in the vertebrates. Different approaches (morphology, karyology, polymorphism of enzymes or mitochondrial DNA) have made it possible to investigate new cases of hybridization or to complement data on previously detected cases. They have also allowed the evaluation of gene flow and the study of hybrid zones, which are "zones of tension" (Key, 1968; Barton and Hewitt 1985, 1989), resulting from an equilibrium between selection and dispersion.

In the fishes, many cases of hybridization or introgression have been detected between individuals or different species (Aspinwall and Tsuyuki, 1968; Avise and Smith, 1974; Wallis and Beardmore, 1980) and more specifically in the cyprinids (Brassington and Ferguson, 1976; Cross, 1978; Bianco, 1982; Collares-Pereira and Coelho, 1983; Crivelli and Dupont, 1987; Berrebi *et al.*, 1989). In the case of *B. meridionalis*, a species examined in the present article, hybridizations with *B. barbus* has been reported in Yugoslavia by Stratil *et al.* (1983) and in France by Berrebi *et al.* (1987).

In Spain, morphological studies suggest hybridization occurs between two tetraploid species, *B. meridionalis* and *B. haasi* (Doadrio, unpub. data). The taxonomic status of *B. haasi* is at present unresolved. It was first considered to be a species on its own (*B. haasi* Mertens, 1924), and then as a subspecies of *B. barbus* (*B. barbus bocagei* Almaça, 1971), *B. capito* (*B. capito haasi* Karaman, 1971), and *B. plebejus* (*B. plebejus haasi* Almaça, 1982). After studying the external morphology and osteology of Spanish *Barbus*, Doadrio (1984) restored *B. haasi* to species status, considering that *B. haasi* had enough characters of its own to distinguish it from *B. barbus*, *B. plebejus*, or *B. capito*. However, he indicated that only minor characters separated *B. haasi* from *B. meridionalis* and that if there was any doubt about the specific status of *B. haasi*, it should be considered to be a subspecies of *B. meridionalis*. This author has described the geographical distribution of the two species. The boundary separating the two species is illustrated in *figure* 1.

The objective of the present study was to verify putative hybridization between *B. meridionalis* and *B. haasi* in five populations by enzyme electrophoresis. This method has been shown to be effective in this type of study, especially when introgression is slight and cannot be detected by morphological markers (Hunt and Selander, 1973). Moreover, exchanges of alleles between species can be quantified by defining the degree of introgression in putative hybrid populations.

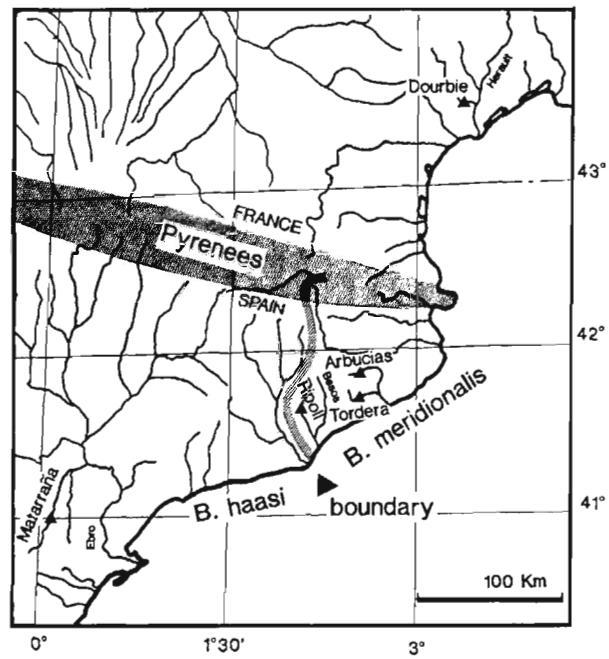

Figure 1. — Distribution of sample populations in France and in Spain.

MATERIALS AND METHODS

Sampling was done at five locations in the Iberian Peninsula and France (*fig.* 1). In the field, taxa were identified by their external morphological characteristics (Doadrio, 1984). Samples were taken from the following rivers, the first four in Spain, the last in France:

- Matarraña: 38 individuals of the species *B. haasi*,
- Ripoll: 27 putative hybrid individuals, morphologically close to *B. haasi*, which we designated as *B. haasi*-2,
- Tordera: 45 individuals of the species *B. meridionalis*,
- Arbucias: 11 putative hybrid individuals, morphologically very similar to *B. meridionalis*, which we designated as *B. meridionalis*-2.
- Dourbie: 17 individuals of *B. meridionalis*.

The *B. haasi* sample from the Matarraña river and the *B. meridionalis* sample from the Tordera river were taken at a distance or nearly 100 km either side

of the interspecific boundary as seen in *figure* 1. Thus, those samples, morphologically typical of each species, are considered as belonging to pure species.

The liver and about 1.5 g of skeletal muscle were taken from each individual and stored in liquid nitrogen for transfer to the laboratory, where they were kept at −70°C until analysis.

Enzyme electrophoresis on horizontal starch gel was carried out according to the method described by Selander *et al.* (1969) and Pasteur *et al.* (1987). Fourteen enzyme systems were studied, corresponding to 29 loci (the nomenclature used is that of Berrebi *et al.* 1988). Electrophoretic data were analyzed by the following methods.

At the individual level, allelic data were coded as 0 when the allele was absent, 1 when it was in the heterozygous state, or 2 when it was in the homozygous state. The resulting tables were processed by Factor Correspondence Analysis (Benzécri, 1973) using the Biomeco program (CEPE [1], 1988). This analysis made it possible to project each individual on a plane according to all its genetic variables, without taking into account its population of origin.

On the population level, the following methods were applied.

– Chi-square analysis was used to verify agreement between the genotype frequencies observed and those predicted according to the Hardy-Weinberg model, applying Yates' correction in the case of small samples.

– Genetic distances of Nei (1972) separating the different populations were calculated from the allele frequencies observed in them. The table of distances was then analyzed by the program "Fitch" which constructs phylogenetic dendrograms (software package "Phylip" by Felsenstein, 1989) using the algorithm of Fitch and Margoliash (1967).

– Phylogenetic dendrograms were also constructed by taking into account the presence or absence of each allele in the populations, according to the Dollo method of parsimony ("Dollop" in "Phylip" by Felsenstein, 1989). This method reveals relationships between groups in terms of the "history" of each allele, whose presence or absence does not merely add to that of the others, but interacts according to the rules of phylogeny and parsimony on which Dollo is based (Farris, 1977). The consistency of the dendrogram obtained (total number of variables analyzed/number of events in the dendrogram) indicates homoplasia (reversions, convergences, parallels) in the data. By applying the program "Bootstrap" (Felsenstein, 1989) the robustness of the branches was also quantified.

– The introgression index "I" was calculated from the frequency of diagnostic alleles (alleles that are fixed in one of the pure species) present in the putative hybrid populations (Hunt and Selander, 1973). This parameter can be used to quantify hybridization. In the present study, "I" was taken to correspond to the percentage of *B. haasi* diagnostic alleles in the genome of the putative hybrids.

RESULTS

Among the 29 loci studied, 16 were monomorphic and expressed the same allele in the 5 populations analyzed. Specifically, these were *Ak*-1, *Ak*-2, *Aat*-3, *Ck*, *Gda*, *Idh*-1, *Ldh*-1, *Ldh*-2, *Ldh*-3, *Mdh*-1, *Mdh*-4, *Mdh*-5, *Pgi*-3, *Sdh*, *Sod*-1, *Sod*-2.

The polymorphic loci (*table* 1) showed the following characteristics:

- Locus 6*Pgd* showed different frequencies for the same alleles in all the Spanish populations, but was fixed for the French sample.
- Three loci expressed alleles characteristic (semi-diagnostic) of a pure species, *i.e.* Aat-1^{90} (*B. haasi*), Fum^{200} (*B. meridionalis*, Spanish distribution), and *Est*-5 (which only showed variants in the French *B. meridionalis* population).
- Two loci expressed, apart from the alleles of the parent species, alleles only found in one of the putative hybrid populations, *i.e.* *Pgi*-1 and *Pgi*-4. In the latter case, there was an almost total disappearance of allele *Pgi*-4^{100}, which was present in the other populations.
- Six loci, which expressed fixed alleles that differed between the Spanish populations of *B. meridionalis* and *B. haasi*, were chosen as markers, *i.e.* *Aat*-2, *Mdh*-2, *Mdh*-3, *Pgi*-2, *Pgm*-1, and *Pgm*-2.
- Locus *Est*-1, which could be considered to be a marker, was not used because of difficulties in interpreting heterozygotes.

Deviations from panmixia

Significant divergences from the genotype frequencies of Hardy-Weinberg (*table* 2) appeared in the following cases. In the "pure" *B. meridionalis* species, the divergences corresponded to the loci *Fum* and *Pgi*-2, expressing alleles that are exclusively those of populations of this species in Spain. There were disequilibria at certains of the loci in *B. haasi*-2 showing introgression, particularly due to the absence of one of the three possible genotypes. Tests were carried out separately for males and females to investigate possible sex-related disequilibrium, but no difference between the sexes was detected in the polymorphic loci.

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Table 1. – Allele frequencies of polymorphic loci. (H = *Barbus haasi*; H2 = *B. haasi*-2; ME = *B. meridionalis* (Spanish sample); ME2 = *B. meridionalis*-2; MF = *B. meridionalis* (French sample)).

Species	H	H2	ME	ME2	MF
n	38	27	45	11	17
Aat-1					
90	0.092				
100	0.908	1.000	1.000	1.000	1.000
Aat-2					
100		0.963	1.000	1.000	1.000
103	1.000	0.037			
Est-1					
98		1.000	1.000	1.000	
100	1.000				1.000
Est-5					
95	1.000	1.000	1.000	1.000	0.912
100					0.088
Fum					
100	1.000	0.648	0.400	0.364	1.000
200		0.352	0.600	0.636	
Mdh-2					
100		0.852	1.000	1.000	1.000
125	1.000	0.148			
Mdh-3					
88	1.000	0.222			
100		0.778	1.000	1.000	1.000
6*Pgd*					
125	0.039	0.333	0.155	0.182	
130	0.961	0.667	0.845	0.818	1.000
Pgi-1					
90		0.352			
100	1.000	0.648	1.000	1.000	1.000
Pgi-2					
100		1.000	0.467	0.636	1.000
109			0.533	0.364	
111	1.000				
Pgi-4					
0		0.962			
100	1.000	0.038	1.000	1.000	1.000
Pgm-1					
85	1.000	0.056			
100		0.944	1.000	1.000	1.000
Pgm-2					
84	1.000	0.204			0.029
100		0.796	1.000	1.000	0.971

Multidimensional analyses

All the allele data were subjected to Factor Correspondence Analysis (*fig.* 2). Populations of "pure" species formed clouds that were well differentiated on the plane defined by factor axes 1 and 2. The first axis (63% inertia) is mainly explained by the alleles Aat-2^{100}, Mdh-3^{88}, Pgi-2^{111}, Pgm-1^{85} and Pgm-2^{84} in the positive direction and by the alleles Aat-2^{100}, Fum^{200} and Pgm-1^{100} in the negative direction of the axis. The second axis (16.5% inertia) is essentially explained by the allele Pgi-4^{100} (positive direction) and the alleles Pgi-1^{90}, Pgi-2^{109} and Pgi-4^{0} (negative direction). The individuals of *B. meridionalis*-2 were included in the Spanish population of *B. meridionalis*. The putative hybrids, *B. haasi*-2, were not at the F1 hybrid position (halfway between the two parent populations), found by Neff and Smith (1979) using Principal Components Analysis, nor did they reflect a "Guttman effect" (individuals distributed along an arc: Volle, 1981). The alleles specific to *B. haasi*-2 (Pgi-1^{90} and Pgi-4^{0}) distinguish them from the other groups, which are in turn differentiated by their characteristic alleles. However, the genetic characteristics of *B. haasi*-2 are mostly of the "*meridionalis*" type, which makes it resemble this species.

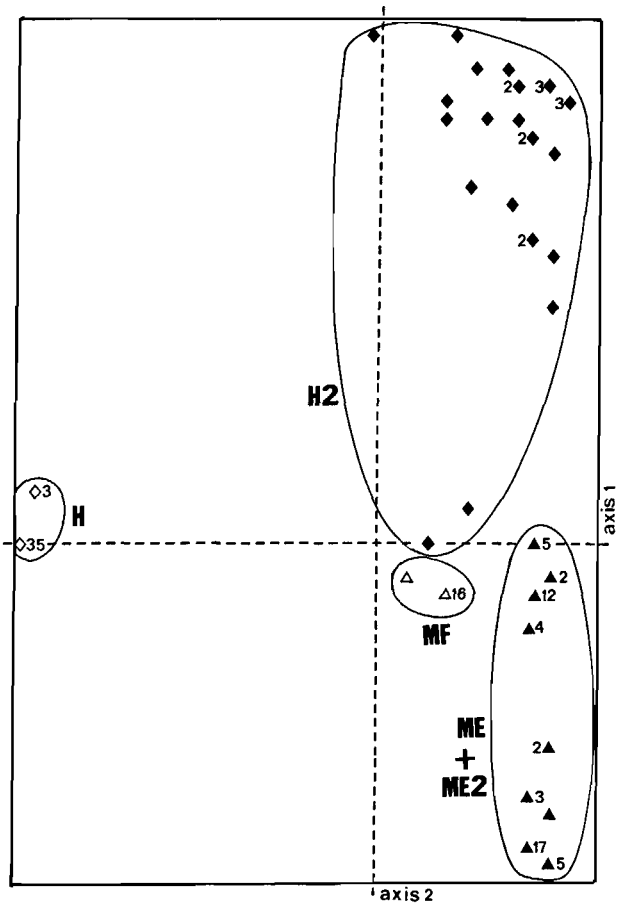

Figure 2. – Projection of individuals on the first plane of factor correspondence analysis (axis 1: 63% inertia; axis 2: 16.5% inertia). The number of superimposed individuals on the same point is signaled by numerals. H = *Barbus haasi;* H2 = *B. haasi*-2; ME = *B. meridionalis* (Spanish sample); ME2 = *B. meridionalis*-2; MF = *B. meridionalis* (French sample).

Genetic distances

Table 3 gives the Nei (1972) genetic distances between populations. The data was analyzed with the Fitch program to construct a dendrogram (*fig.* 3). One of the putative hybrid populations, *B. haasi*-2, is unquestionably similar to the *B. meridionalis* populations, though more similar to the French population than to the Spanish populations.

Table 2. – Test showing significant deviation between the genotype frequencies observed and those predicted by the Hardy-Weinberg law. FO: frequencies observed; EO: effects observed; FP: frequencies predicted; EP: effects predicted. Chi^2c: value of chi-square with Yates correction. ($*p<0.05$; $**p<0.01$; $***p<0.001$.)

Locus	Genotype	FO	EO	FP	EP	CHI^2	ΣCHI^2	CHI^2c	ΣCHI^2c
B. meridionalis (Spain)									
Fum	100100	0.00	0	0.16	7.20	7.20	20.00	6.23	17.95***
	100200	0.80	36	0.48	21.60	9.60		8.84	
	200200	0.20	9	0.36	16.20	3.20		2.77	
Pgi-2	100100	0.47	21	0.22	9.80	12.80	45.00	11.68	42.04***
	100109	0.00	0	0.50	22.40	22.40		21.41	
	109109	0.53	24	0.28	12.80	9.80		8.94	
B. meridionalis-2									
Pgi-2	100100	0.64	7	0.40	4.45	1.45	11.00	0.94	7.96**
	100109	0.00	0	0.46	5.09	5.09		4.14	
	109109	0.36	4	0.13	1.45	4.45		2.88	
B. haasi-2									
Fum	100100	0.30	8	0.42	11.34	0.99	7.96	0.71	6.24*
	100200	0.70	19	0.46	12.31	3.63		3.11	
	200200	0.00	0	0.12	3.34	3.34		2.42	
Mdh-2	100100	0.85	23	0.73	19.59	0.59	27.00	0.43	20.55***
	100125	0.00	0	0.25	6.81	6.81		5.85	
	125125	0.15	4	0.02	0.59	19.59		14.26	
6*Pgd*	125125	0.00	0	0.12	3.00	3.00	6.75	2.08	5.12*
	125130	0.67	18	0.44	12.00	3.00		2.52	
	130130	0.33	9	0.44	12.00	0.75		0.52	
Pgi-1	90090	0.00	0	0.12	3.34	3.34	7.96	2.42	6.24*
	90100	0.70	19	0.46	12.31	3.63		3.11	
	100100	0.30	8	0.42	11.34	0.99		0.71	

Table 3. – Genetic distances of Nei, 1972 (top): H = *Barbus haasi*; H2 = *B. haasi*-2; ME = *B. meridionalis* (Spanish sample); ME2 = *B. meridionalis*-2; MF = *B. meridionalis* (French sample).

Parameters for measuring genetic variability (bottom): A = mean number of alleles; P = rate of polymorphism; Ht = mean theoretical heterozygosity.

Species	H	H2	ME	ME2	MF
B. haasi					
B. haasi-2	0.2570				
B. meridionalis (Spain)	0.2547	0.0606			
B. meridionalis-2	0.2579	0.0549	0.0011		
B. meridionalis (France)	0.2416	0.0530	0.0250	0.0213	

Species	A	P		H_T
		0.95	0.99	
B. haasi	1.071	0.036	0.071	0.009
B. haasi-2	1.321	0.250	0.321	0.090
B. meridionalis (Spain)	1.107	0.107	0.107	0.044
B. meridionalis-2	1.107	0.107	0.107	0.044
B. meridionalis (France)	1.071	0.036	0.071	0.008

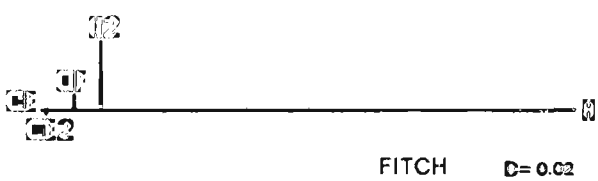

Figure 3. – Fitch dendrogram using Nei's genetic distances. H = *Barbus haasi*; H2 = *B. haasi*-2; ME = *B. meridionalis* (Spanish sample); ME2 = *B. meridionalis*-2; MF = *B. meridionalis* (French sample).

Parsimony

In the Dollo system (*fig.* 4), no character distinguishes the two Spanish *B. meridionalis* populations. The French *B. meridionalis* population is situated between the *B. haasi*-2 hybrids and Spanish *B. meridionalis*. However, there are more events separating *B. meridionalis* (French distribution) and *B. haasi*-2 than *B. meridionalis*/*B. meridionalis*-2 and *B. haasi*. The consistency of this dendrogram, *i.e.* 0.87 (21/24),

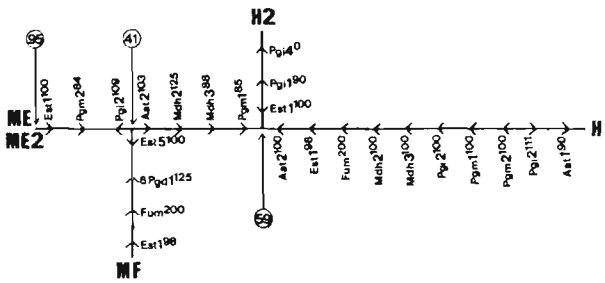

Figure 4. – Dollo dendrogram obtained from a table of presences and absences of alleles. The results of the Bootstrap test (shown in circles), are expressed as percentages of appearance of the branch in 100 random drawings of variables. H = *Barbus haasi*; H2 = *B. haasi*-2; ME = *B. meridionalis* (Spanish sample); ME2 = *B. meridionalis*-2; MF – *B. meridionalis* (French sample).

indicates low homoplasia. The robustness of the branches in the dendrogram, as tested by the program "Bootstrap", shows that the structure is stable.

Introgression index

The index "I" ranged from 0% introgression of *B. haasi* genes into the *B. meridionalis*-2 population, to 11.12% into the *B. haasi*-2 population. *B. meridionalis*-2 is thus a "pure" population that is only distinguished from the Spanish distribution of *B. meridionalis* by small differences in allele frequency at certain loci.

Thus, 88.88% of the alleles of *B. haasi*-2 are of the Spanish "*meridionalis*" type, in contrast with what its morphological characteristics appear to indicate. None of the individuals in this population could be identified as F1 hybrids (heterozygous at all diagnostic loci).

With regard to two of the loci marking introgression, *Aat*-2 and *Mdh*-2, the allele characteristic of *B. haasi* was only present in the homozygous state in the putative hybrid individuals. In the individuals of the *B. haasi*-2 population, locus *Pgi*-2 expressed only allele 100, which is characteristic of Spanish *B. meridionalis*, and was also the only allele expressed by the French population of *B. meridionalis*. None of the putative hybrid individuals showed, even in a heterozygous state, allele 111, which is characteristic of *B. haasi*, nor allele 109 of Spanish *B. meridionalis*. It should also be noted that even in the latter "pure" population of *B. meridionalis*, which expresses two different alleles at nearly equal frequencies, no heterozygote was detected out of 45 individuals studied (*table* 2).

DISCUSSION

Enzymatic data releaved the existence of genetic markers (fixed or alternative alleles) distinguishing pure species of *B. meridionalis* and *B. haasi*. These species can therefore be unambigously identified with the markers. Other alleles showed different frequencies in these two species in Spain (Aat-1^{90} and Fum^{200}).

Fixed alleles revealed the existence of introgression by *B. haasi* genes into a pool of "*meridionalis*" genes in one of the two putative hybrid populations, *i.e. B. haasi*-2. No introgression was found in the other population, *B. meridionalis*-2, at the loci studied. This therefore represents a population of *B. meridionalis* with no characteristics other than those found this species in Spain.

We reported above that, in the *B. haasi*-2 population, no heterozygote was detected at the three diagnostic loci, *Aat*-2, *Mdh*-2 or *Pgi*-2. All the hybrids were homozygous at these loci and expressed mostly a "*meridionalis*" genotype. The loci may have undergone selection or they could be evidence of incompatibility in expressing alleles of the two species simultaneously. Incompatibility between alleles belonging to differentiated genomes, or preferential expression by certain genes of fixed alleles of only one parental population, has been evidenced on several occasions in hybrids (e.g. Herrera, 1979; Pasdar *et al.*, 1984; Bürki, 1985; Hung 1985).

Certain loci of *B. haasi*-2 show significant divergences from frequencies predicted by the Hardy-Weinberg law. The genotypes are hybrid at these loci, or express alleles typical only of the hybrid population. The almost total loss of expression by one of the loci (null allele for *Pgi*-4) and the existence of an allele that was not detected in the other populations analyzed (Pgi-1^{90}) may indicate that the *B. haasi*-2 population has evolved independently, perhapts because of isolation.

In view of the existence of two alleles specific to *B. haasi*-2 (Pgi-1^{90} and Pgi-4^{0}), it is also possible that this is a different group or is the original population of *B. meridionalis* and *B. haasi*. However, in one of the two cases (Pgi-4^{0}), this "characteristic" allele only represents the predominant non-expression of locus *Pgi*-4, as indicated above. This locus is monomorphic and expresses the same allele (Pgi-4^{100}) in a dozen different species of *Barbus* studied in Europe (Berrebi *et al.*, 1988, and unpublished data). Thus, in the case of *B. haasi*-2, it could represent silencing of a duplicate locus, which is common in tetraploids (Allendorf and Thorgaard, 1984). Moreover, high allele frequency in hybrids versus low frequency in the pure species, and the existence of alleles specific to hybrids (named hybrizymes by Woodruff, 1989), are phenomena that have previously been detected. They could be caused by deterioration of selective values, higher mutation rates in the hybrids, intracistronic recombinations, or genetic drift (*see* Gollman *et al.*, 1988). We thus favor the concept that *B. haasi*-2 is of hybrid origin, which appears to be the most probable explanation for the data obtained in this population.

The genetic distance between *B. haasi* and *B. meridionalis* (D = 0.25) is of the same order as that found between other species, such as *Cottus confuses* and *Cottus cognatus*, which hybridize under natural conditions (Zimmerman and Wooten, 1981). The genetic distance separating the hybrids of *B. meridionalis* in Spain is greater than that separating these hybrids from French *B. meridionalis*. This quantitative paradox can be explained by the disappearance of allele Pgi-2^{109} in the hybrids. From a qualitative viewpoint, the existence in *B. haasi*-2 of alleles characteristic of *B. meridionalis* in Spain (Fum^{200} and 6 Pgd^{125}) shows that the hybridization occurred with a population having characteristics rather similar to the Spanish populations analyzed.

None of the individuals in this hybrid population could be identified as an F1 hybrid. Consequently, these individuals clearly arise from many backcrosses, showing an absence of strong reproductive barriers, in contrast with other cases where the hybrid populations apparently only consist of first-generation hybrids (Danzmann and Down, 1982; Avise and Saunders, 1984; Berrebi *et al.*, 1989).

Two opposite predictions can be made about the fate of hybrid populations:

- If the hybrids originated from secondary contact between two species that diverged into allopatry, they will have reduced fitness due to the disturbance of genetic complexes that had coadapted in the parent species, which will reinforce, in a certain way, the mechanisms of reproductive isolation (Dobzhansky, 1970) and progressively reduce their numbers.
- The existence of an ecotone between the preferential habitats of the parent species could give the hybrids increased fitness (Moore, 1977). In addition, a heterosis effect (selective superiority of heterozygotes) could appear in the hybrids (Ferguson, 1980; Ferguson *et al.*, 1985 *a*), which would produce high stability in the hybrid population.

Data on the *B. haasi*-2 hybrid population favor the second hypothesis. No individual of "pure" species identity was found in this locality. Nevertheless, we cannot give evidence for an increase in hybrid fitness. It is not a question of a simple dilution of 11% of *B. haasi* alleles and of 89% of *B. meridionalis* alleles. Thus, the original pool has undergone modifications (drift, selection, appearance of hybrizymes) which explains why we found different specific proportions at each locus. For example, Pgi-2^{111} and Pgi-2^{109} totally disappeared in the hybrid population. It can therefore be assumed that the hybrids have acquired a certain equilibrium or advantage in the environment they occupy, which has allowed them to form a stable introgressed population.

There are two arguments to suggest that the interspecific contact which occurred previously, did not occur this time: (1) there were modifications of the allelic proportions relative to those expected if we had a simple dilution of alleles from two species and (2) according to our field data, there is not, at present, any sympatry between the two pure species in that region (*fig.* 1). This contact can be considered as ancient because of the period of time modifications to allelic proportions need to establish.

Another explanation for the success of the hybrids is based on the tetraploid condition of the species analyzed. Ferguson *et al.* (1985 *b*) reported that the absence of incompatibility in the development of hybrids of different trout species may be due to the large flexibility of salmonids (tetraploids) compared to diploid groups. Moreover, Chevassus *et al.* (1983) carried out experiments on crosses between *Salmo gairdneri* and other salmonids (*Salmo trutta*, *Salvelinus fontinalis*, and *Oncorhynchus kisutch*) which support this hypothesis. The authors induced retention of the second polar body in the ovules of females that were to be inseminated, thereby producing diploid ovules. There was a higher level of viability in the "triploid" hybrid strains obtained than in the "diploid" controls. In our case, geographic or climatic conditions favorable to secondary contact, the absence of strong reproductive barriers, and the tetraploid condition of the species involved, among other conditions, have allowed or even favored the existence and persistence of the hybrid population.

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REFERENCES

Allendorf F. W., G. H. Thorgaard, 1984. Tetraploidy and the Evolution of salmonid fishes. *In*: Evolutionary Genetics in fishes, B. J. Turner ed., Plenum Press, New York, 1-53.

Aspinwall N., H. Tsuyuki, 1968. Inheritance of muscle proteins in hybrids between the redside shiner (*Richardsonius balteatus*) and the peamouth chub (*Mylocheilus caurinum*). *J. Fish. Res. Board Can.*, **25**, 1317-1322.

Avise J. C., M. H. Smith, 1974. Biochemical genetics of sunfish. II. Genic similarity between hybridising species. *Am. Nat.*, **108**, 458-472.

Avise J. C., N. C. Saunders, 1984. Hybridization and introgression among species of sunfish (*Lepomis*): analysis by mitochondrial DNA and allozyme markers. *Genetics*, **108**, 237-255.

Barton N., B. O. Bengtsson, 1986. The barrier to genetic exchange between hybridising populations. *Heredity*, **56**, 357-376.

Barton N. H., G. M. Hewitt, 1985. Analysis of hybrid zones. *Ann. Rev. Ecol. Syst.*, **16**, 113-148.

– 1989. Adaptation, speciation and hybrid zones. *Nature*, **341**, 497-503.

Benzécri J. P., 1973. L'analyse des données. Tome I and Tome II, Dunod, Paris, 615 and 619 p.

Berrebi P., N. Le Brun, F. Renaud, A. Lambert, 1987. Hybridation interspécifique de deux Cyprinidae (genre *Barbus*). Conséquence sur la spécificité parasitaire de *D. gracile* (Monogenea). *In*: Actes du Colloque National du C.N.R.S. « Biologie des populations », I.A.S.B.S.E., ed. Univ. C. Bernard, Lyon.

Berrebi P., G. Lamy, G. Cattaneo-Berrebi, J. -F. Renno, 1988. Variabilité génétique de *Barbus meridionalis* Risso (Cyprinidae): une espèce quasi monomorphe. *Bull. Fr. Pêche Piscic.*, **310**, 77-84.

Berrebi P., F. Dupont, G. Cattaneo-Berrebi, A. J. Crivelli, 1989. An isozyme study of the natural cyprinid hybrid *Alburnus alburnus* × *Rutilus rubilio* in Greece. *J. Fish Biol.*, **34**, 307-313.

Bianco P. G., 1982. Hybridization between *Alburnus albidus* and *Leuciscus cephalus cabeda* in Italy. *J. Fish Biol.*, **21**, 593-604.

Brassington R. A., A. Ferguson, 1976. Electrophoretic identification of roach (*Rutilus rutilus* L.), rudd (*Scardinius erythrophtalmus* L.), bream (*Abramis brama* L.) and their natural hybrids. *J. Fish Biol.*, **9**, 471-477.

Bürki E., 1985. The expression of creatine kinase isozymes in *Xenopus tropicalis*, *Xenopus laevis laevis*, and their viable hybrid. *Biochem. Genet.*, **23**, 73-88.

Chevassus B., R. Guyomard, D. Chourrout, E. Quillet, 1983. Production of viable hybrids in salmonids by triploidization. *Génét. Sél. Evol.*, **15**, 519-532.

Collares-Pereira M. J., M. M. Coelho, 1983. Biometrical analysis of *Chondrostoma polylepis* × *Rutilus arcasi* natural hybrids (Osteichthyes, Cypriniformes, Cyprinidae). *J. Fish Biol.*, **23**, 495-509.

Crivelli A. J., F. Dupont, 1987. Biometrical and biological features of *Alburnus alburnus* × *Rutilus rubilio* natural hybrids from Lake Mikri Prespa, northern Greece. *J. Fish Biol.*, **31**, 721-733.

Cross T. F., 1978. Isozymes of interspecific hybrids of the family Cyprinidae, Proc. *R.I.A.*, **78**, 323-334.

Danzmann R. G , N. E. Down, 1982. Isozyme expression in F_1 hybrids between carp and goldfish. *Biochem. Genet.*, **20**, 1-15.

Descimon H., H. Geiger, 1988. Electrophoretic detection of interspecific hybrid in *Parnassius* (Lepidoptera, Papilionidae). *Génét. Sél. Evol.*, **20**, 435-440.

Doadrio I., 1984. Relaciones filogenéticas y biogeográficas de los barbos (*Barbus*, Cyprinidae) de la Península Ibérica, y aportes corológicos y biogeográficos a su ictiofauna continental. Tesis Doctoral. Fac. Biol. Universidad Complutense, Madrid, 1146 p.

Dobzhansky T., 1970. Genetics of the Evolutionary Process. Columbia University Press, New York.

Farris J. S., 1977. Phylogenetic analysis under Dollo's law. *Syst. Zool.*, 26, 77-88.

Felsenstein J., 1989. PHYLIP, Phylogenetic inference Package, version 3.2, Department of Genetics, SK 50. University of Washington, Seattle, Washington 98195, USA.

Ferguson A. B., 1980. Hybrids and polyploids. *In*: Biochemical systematics and Evolution. Blackie ed., Glasgow, London, 114-130.

Ferguson M. M., R. G. Danzmann, F. W. Allendorf, 1985*a*. Developmental divergence among hatchery strains of rainbow trout (*Salmo gairdneri*). II. Hybrids. *Can. J. Genet. Cytol.*, **27**, 298-307.

Ferguson M. M., R. G. Danzmann, F. W. Allendorf, 1985*b*. Absence of developmental incompatibility in hybrids between rainbow trout and two subspecies of cutthroat trout. *Biochem. Genet.*, **23**, 557-570.

Fitch W. M., E. Margoliash, 1967. Construction of phylogenetic trees. *Science*, **155**, 279-284.

Gollmann G., P. Roth, W. Hödl, 1988. Hybridization between the fire-bellied toads *Bombina bombina* and *Bombina variegata* in the karst regions of Slovakia and Hungary: morphological and allozyme evidence. *J. Evol Biol.*, **1**, 3-14.

Herrera R. J., 1979. Preferential gene expression of an amylase allele in interspecific hybrids of *Xiphophorus* (Pisces: Poeciliidae). *Biochem. Genet.*, **17**, 223-227.

Hung A. C. F., 1985. Isozymes of two fire ant species and their hybrid. *Biochem. Syst. Ecol.*, **13**, 337-339.

Hunt W. G., R. K. Selander, 1973. Biochemical genetics of hybridization in European house mice. *Heredity*, **31**, 11-33.

Key K. H. L., 1968. The concept of stasipatric speciation. *Syst. Zool.*, **17**, 14-22.

Moore W. S., 1977. An evaluation of narrow hybrid zones in vertebrates. *Q. Rev. Biol.*, **52**, 263-277.

Neff N. A., G. R. Smith, 1979. Multivariate analysis of hybrid fishes. *Syst. Zool.*, **28**, 176-196.

Nei M., 1972. Genetic distance between populations. *Am. Nat.*, **106**, 283-292.

Pasdar M., D. P. Philipp, W. A. Mohammad, G. S. Whitt, 1984. Differences in tissue expressions of enzyme activities in interspecific sunfish (Centrarchidae) hybrids and their backcross progeny. *Biochem. Genet.*, **22**, 931-956.

Pasteur N., G. Pasteur, F. Bonhomme, J. Catalan, J. Britton-Davidian, 1987. Manuel technique de génétique par électrophorèse des protéines. Technique et Documentation, Lavoisier, Paris, 217 p.

Rand D. M., 1988. Population genetics of mitochondrial DNA size variation in *Gryllus* crickets. *Genome*, **30**, 329.

Selander R. K., W. G. Hunt, S. Y. Yang, 1969. Protein polymorphism and genetic heterozygosity in two european subspecies of the house mouse. *Evolution*, **23**, 379-390.

Stratil A., P. Bobak, V. Tomasek, M. Valenta, 1983. Transferrins of *Barbus barbus*, *Barbus meridionalis petenyi* and their hybrids. Genetic polymorphism, heterogeneity and partial characterization. *Comp. Biochem. Physiol.*, 76B, 845-850.

Vanlerberghe F., P. Boursot, J. Catalan, S. Gerasimov, F. Bonhomme, B. A. Botev, L. Thaler, 1988. Analyse génétique de la zone d'hybridation entre les deux sous-espèces de souris *Mus musculus domesticus* et *Mus musculus musculus* en Bulgarie. *Genome*, 30, 427-437.

Volle M., 1981. Analyse des données. Ed. Economie, Paris, 317 p.

Wallis G. P., J. A. Beardmore, 1980. Genetic evidence for naturally occurring fertile hybrids between two goby species, *Pomatoschistus minutus* and *P. lozandi* (Pisces, Gobiidae). *Mar. Ecol.*, **3**, 309-315.

Woodruff D. S., 1989. Genetic anomalies associated with *Cerion* hybrid zones: the origin and maintenance of new electrophoretic variants called hybrizymes. *Biol. J. Linn. Soc.*, 36, 281-294.

Zimmermann E. G., M. C. Wooten, 1981. Allozymic variation and natural hybridation in sculpins, *Cottus confuses* and *Cottus cognatus*. *Biochem. Syst. Ecol.*, 9, 341-346.