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Microsatellite markers and management of brown trout *Salmo trutta fario* populations in southwestern France

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Abstract – The brown trout populating the western part of the Pyrenees mountains (southern France) constitute a patchwork of differentiated forms for two main reasons: the region corresponds to the maximum extension of the modern Atlantic form at the expense of the ancestral Atlantic one; stocking is commonly practiced. This situation renders urgent in this region the analysis of the genetic resources of wild origin and the impact of domestic introgression. Because allozymes which are generally used in the Mediterranean region are inefficient in Atlantic drainages in detecting the genetic impact of stocking, we used new kinds of markers, the VNTR microsatellites. Three microsatellite loci, moderately or highly polymorphic, were tested in comparison with three diagnostic allozymic loci. An adapted index of domestic introgression (IPI) is proposed and was tested for five natural populations and three domestic strains. Finally, geographical variation is summarised by a phylogenetic tree. The value of microsatellite markers in the Atlantic drainage and the use of such data for protection and management of brown trout populations are discussed. © Inra/Elsevier, Paris

brown trout / conservation–biodiversity / microsatellites / introgression / stocking

Résumé – Marqueurs microsatellites et gestion des populations de truite commune, *Salmo trutta fario*, dans le sud-ouest de la France. La truite commune peuplant l'ouest des Pyrénées forme un puzzle de formes différenciées dû à deux causes principales : la région constitue le maximum d'extension de la forme atlantique moderne au détriment de la forme atlantique ancestrale, et d'autre part, les pratiques de repeuplement y sont généralement très actives. Cette situation rend urgente l'analyse de la biodiversité naturelle et de l'introgression par les formes domestiques. Parce que les enzymes, généralement employés en zone méditerranéenne, sont quasiment inefficaces pour détecter l'impact génétique des repeuplements sur le versant atlantique, un nouveau type de marqueur VNTR, les microsatellites, est employé ici. Dans cette

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étude, trois locus microsatellites, plus ou moins polymorphes sont testés en comparaison avec trois locus enzymatiques diagnostiques. Un index de l'introgression d'origine domestique (IPI) a été spécialement adapté à ce type de données. Il est appliqué sur cinq populations naturelles et trois échantillons de pisciculture. L'intérêt des marqueurs microsatellites utilisés en zone atlantique et l'exploitation des résultats pour la protection et la gestion de la truite commune sont discutés.

truite commune / conservation–biodiversité / microsatellites / introgression / repeuplements

1. INTRODUCTION

Salmonids constitute one of the most manipulated fish in temperate countries. Moreover, salmonid fish, and especially the brown trout (*Salmo trutta fario* L.), present some interesting biological characteristics for the study of genetic differentiation: they live in the upper part of rivers, and exhibit 'homing' behaviour. Therefore, the different populations are relatively isolated but can be connected by migration (sometimes through the sea). The first genetic studies, using allozymes, confirmed marked genetic differentiation (see e.g. Ferguson, 1989, or Guyomard, 1989, for French populations). Moreover, Hamilton et al. (1989), using the distribution of the different alleles of *LDH5**, proposed the existence of two different brown trout forms: an 'ancestral' form fixed for the *LDH5**100 allele and a modern one characterised by the *LDH5**90 allele. According to this hypothesis, the ancestral form would have been supplanted by the modern one in the northern part of the distribution area; it survived only in some less accessible parts of some head-river systems. This analysis was completed in France using the transferrin locus (Guyomard, 1981), which, along with *LDH5** and *FBP1**, was used to hypothesise that the Atlantic form is divided into ancestral and modern forms named 'modern Atlantic' and 'ancestral Atlantic' distinguishable by particular allozymic markers (the locus *LDH5** is diagnostic; see Poteaux, 1995 and *table I*). Most trout from the French Atlantic basin belong to the modern type (except from some rivers in Brittany [Guyomard, 1989] and, as will be discussed later, some Pyrenean rivers). *LDH5**90 was probably absent from Iberian populations prior to introduction of hatchery fish (Garcia-Marin and Pla, 1996). In the southwest of France, in the Atlantic part of the Pyrenees, the two Atlantic forms seem to coexist (Poteaux, 1995; Berrebi, 1997a). These hypotheses still raise the questions of the origin and the coexistence of these different forms. This complex of populations provides a good example of high biodiversity and should be protected. However, the situation is complicated by stocking practices that can genetically endanger original populations (Berrebi, 1997b). This is why it is useful to evaluate the impact of stocking, and possibly to search for wild populations to protect them as pure sources of genotypes. Domestic trout belong mainly to the modern Atlantic form as shown by enzymatic (Guyomard, 1989) and mitochondrial deoxyribonucleic acid (DNA) studies (Bernatchez et al., 1992). Distinguishing domestic and wild trout is easy in the Mediterranean part of France or where ancestral Atlantic trout is found alone (Taggart and Ferguson, 1986). In the area of wild modern Atlantic populations, however, allozymes are inefficient, which is why new markers, like microsatellites, which are able to discriminate such close taxa, are an important target for scientists. The high variability of

these genetic markers makes them appropriate for different purposes. Firstly, management of brown trout populations is essential, as the definition of purely wild populations for protection or for constitution of new strains; secondly, the microsatellites would be useful for the analysis of natural differentiation, and especially the relationships between modern and ancestral Atlantic trout.

Table I. Characterisation of the different brown trout forms according to their allozymic diagnostic alleles.

Locus	Mediterranean	Ancestral Atlantic	Modern Atlantic
*LDH5**	100	100	90
*TF**	102	100	100
*FBP1**	150	150	100

Because the Pyrenees basins of southwestern France seem to be the limit of the modern Atlantic trout's extension into the territory of the ancestral Atlantic trout, it is also important to know if the two taxa continue their competition or if an equilibrium has now been reached. Marked genetic melting occurs in the boundary area.

The objective of this paper is to test microsatellite loci in such a situation with the help of useful allozymic markers. Value assessment of this new tool in future research in Atlantic drainages is also an important objective with a view to a complete description of the genetic structure of French populations of brown trout.

2. MATERIALS AND METHODS

2.1. Sample sources

Five natural populations were investigated, with a sample size varying from 17 to 28 individuals (the details are given in *table II* and their localisation in *figure 1*). For the Andurentako River, only five individuals were available, due to the small population size. Despite this, they were analysed because of their interesting morphological and enzymatic characteristics (Berrebi, 1997a), but the statistical parameters are given simply for indication. Populations with no or almost no allozymic modern alleles were supposed not to have been stocked with hatchery fish (see *table III*); for the Andurentako and Beherekobentako rivers, angling associations affirmed that these streams had not been restocked for several years. Moreover, three hatchery samples were analysed to compare genetic variability.

2.2. DNA extraction

Total DNA was obtained from muscle or blood kept frozen just after dissection, using the Chelex extraction protocol described in Walsh et al. (1991). DNA was kept frozen at $-20\,^{\circ}C$ until amplification.

Table II. Description of sample origins.

No. (map)	Locality	River	Basin	Altitude	Sample size	Sampling date
	La Canourgue (48)	Hatchery			50	July 93
	Brassac (81)	Hatchery			29	June 93
	Suech (31)	Hatchery			16	March 93
1	Cauterets (67)	Marcadau	Adour	1 410	17	Sept 92
4	Sare (65)	Beherekobentako	Nivelle	75	24	Sept 93
3	Dancharia (65)	Nivelle	Nivelle	65	28	Sept 93
5	Herboure (65)	Andurentako	Untxin	55	5	Sept 93
2	Béhérobie (65)	Nive de Béhérobie	Adour	325	25	Sept 93

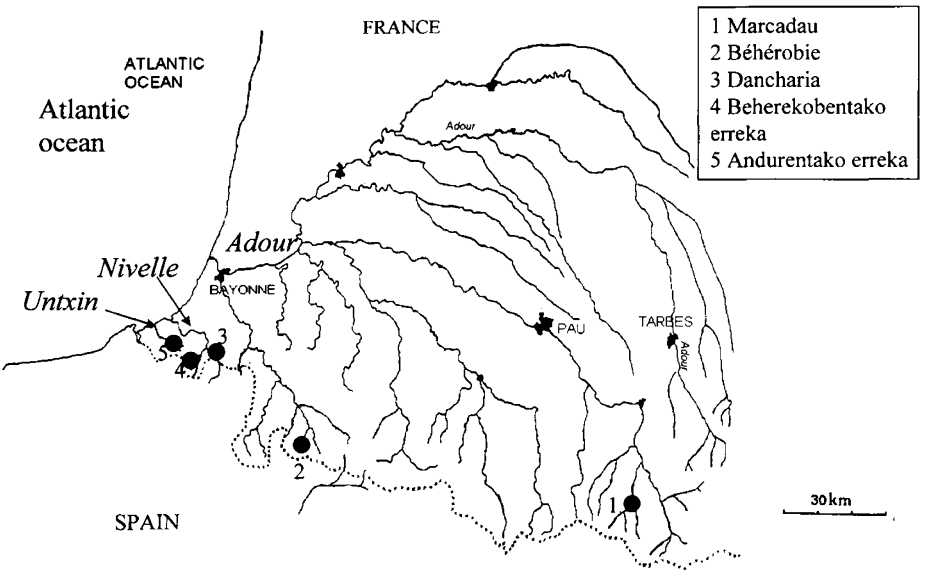

Figure 1. Location of the sampled sites.

2.3. DNA amplification and microsatellite analysis

Three microsatellite loci were used; the primers, repeat, sequences and annealing temperatures are given in *table IV*. *Strutta58* was cloned by Poteaux (1995); *MST73* and *-15* were cloned by Estoup et al. (1993). Polymerase chain reactions (PCR) were performed in 10 µL with 0.2 units of *Taq* polymerase, 2 mM $MgCl_2$, dNTP (100 µM each), 1X reaction buffer and 5 pmol of each primer. One of 5′ ends of the two primers was covalently linked to fluorescein. The typical PCR programme used was: 2 min at 94 °C; 30 cycles comprising 45 s at 92 °C; 45 s at the annealing temperature and 45 s at 72 °C; final step: 1 min at 72 °C.

Table III. Allelic frequencies for enzymatic and microsatellite loci.

Locus	Canourgue	Brassac	Suech	Marcadau	Behereko	Dancharia	Andurentako	Béhérobie
58								
102	0.16	0.14	0.22	0.04	0.28	0.5	0	0
104	0.03	0	0	0	0	0	0	0
108	0.07	0	0.072	0	0	0	0	0
110	0	0	0.072	0	0	0	0	0
112	0	0	0.02	0	0	0	0	0
116	0.04	0	0.02	0.11	0	0	0	0.04
120	0.05	0.04	0.09	0	0	0	0	0
122	0	0.09	0.04	0	0	0	0	0
124	0.18	0.05	0.05	0	0	0	0	0
126	0.06	0.02	0.05	0.25	0.20	0	0	0.1
128	0.11	0.20	0.12	0.14	0	0	0	0.04
130	0.01	0.13	0.04	0	0	0	0	0.34
132	0	0.09	0.02	0	0	0	0	0
134	0.03	0.04	0.05	0	0	0	0	0.04
136	0.02	0.04	0.09	0	0	0	0	0.06
138	0	0.04	0	0.07	0.15	0	0	0.06
140	0	0.02	0	0.04	0.11	0	0	0.04
144	0	0	0.02	0	0.09	0.15	0	0.02
146	0.18	0.09	0.05	0.07	0.02	0.11	0	0.02
148	0.02	0.04	0.04	0.04	0	0	0	0.08
150	0.02	0	0.04	0.11	0	0	0	0.06
152	0	0	0.04	0	0	0	0	0.02
154	0	0	0	0	0	0	0	0.06
156	0	0	0	0	0	0.02	0	0
158	0	0	0	0	0	0.06	0	0
168	0	0	0	0	0.02	0	0	0
170	0	0	0	0	0	0.02	0	0
172	0	0	0	0	0	0.04	0.9	0.02
182	0	0	0	0.04	0.02	0	0.1	0
184	0	0	0	0.07	0.07	0.02	0	0
186	0	0	0	0	0.02	0	0	0
188	0	0	0	0.04	0.02	0.06	0	0
190	0	0	0	0	0	0.04	0	0
73								
141	0.33	0.03	0.06	0.33	0.15	0.1	0	0.08
143	0.02	0	0.02	0	0.02	0.12	0.2	0
145	0.32	0.57	0.31	0.2	0.13	0.46	0.7	0.08
147	0.33	0.40	0.59	0.43	0.69	0.3	0.1	0.66
149	0	0	0.02	0.03	0	0.02	0	0.1
151	0	0	0	0	0.02	0	0	0.08
15								
216	0	0	0	0.07	0	0	0	0
218	0	0	0	0.03	0.10	0.11	0.1	0.02
220	0.21	0.04	0.14	0.3	0.73	0.66	0.9	0.42
222	0.29	0.31	0.14	0.07	0.06	0.09	0	0.18
224	0.33	0.22	0.5	0.2	0.06	0	0	0
226	0.17	0.43	0.20	0.33	0.04	0.07	0	0.38
228	0	0	0.02	0	0	0.07	0	0
*LDH5**								
90	0.95	1	0.99	0.33	0	0	0	0.26
100	0.05	0	0.01	0.67	0.94	0.98	1	0.74
110	0	0	0	0	0.06	0.02	0	0
TF								
80	0	0	0	-	0	0	0	0.02
98	0	0	0.03	-	0	0	0	0
100	1	1	0.97	-	0.77	1	1	0.90
102	0	0	0	-	0.23	0	0	0.08
*FBP1**								
100	0.86	0.87	0.73	0.5	0.23	0.28	0.3	0.54
135	0	0	0.03	0	0	0	0	0
150	0.14	0.13	0.23	0.5	0.77	0.72	0.7	0.46

Table IV. Characteristics of the microsatellite loci analysed.

Name	Origin	Motif	Primers	Nucl. number	Annealing temperature (°C)	Size of the amplified fragment (bp)
Strutta58	Poteaux	(AC)40	R: AACAATGACTTTCTCTGAC	19	54	100 to 192
			F: AAGGACTTGAAGGACGAC	18		
MST73	Inra	(GT)13TTATCT(GT)3	R: CTATTCTGCTTGTAACTAGCCTA	23	58	135 to 151
			F: CCTGGAGATCCTCCAGCAGGA	21		
MST15	Inra	(GT)13	R: AATCCTCTACGTAAGGGATTTGC	23	58	216 to 228
			F: TGCAGGCAGACGGATCAGG	20		

Strutta58 was cloned by Poteaux (1995); *MST73* and *-15* were cloned by Estoup et al. (1993) at the Institut national de la recherche agronomique (Inra).

Amplification products were resolved by electrophoresis on 6 % polyacrylamide denaturing gels in a Pharmacia automated sequencer (ALF).

The results of previous enzymatic analysis of the same samples were also used, especially for the identification of wild and Atlantic forms (see complete results in Berrebi, 1997a). Only diagnostic loci (lactate dehydrogenase *LDH5**, transferrin *TF** and fructose biphosphatase *FBP1**) are taken into account here to calculate a percentage of modern and ancestral fish alleles.

2.4. Data analysis

The distributions of allele frequencies for the three microsatellite loci are given in *figure 2*. For the locus *58*, the high number of alleles (33) compared to the sample sizes (generally < 30) does not allow a statistically significant estimation of allele frequencies. Nevertheless, these frequencies are given for relative comparisons between samples. They have also been used for an introgression estimation, which is not intended to give an absolute value of introgression (see later). Unbiased expected heterozygosities were calculated according to Nei (1978) using the computer programme GENETIX (Belkhir et al., 1996). The Weir and Cockerham (1984) estimators of Wright's indices *Fis* and *Fst* were calculated with the same programme. The statistical significance of the observed values was evaluated using 500 permutations of the original data set. Reynolds genetic distances (Reynolds et al., 1983) were used for the construction of a phylogenetic tree with the PHYLIP 3.0 programme (Felsenstein, 1993) using the 'Fitch' method (Fitch and Margoliash, 1967).

The impact of stocking was evaluated using an introgression index to estimate the percentage of domestic genes in natural populations. Poteaux and Berrebi (1997) used the percentages of *LDH5**90, *TF**100 and *FBP1**100 alleles in enzymatic studies; for microsatellites, Poteaux (1995) proposed a 'maximal introgression index' using alleles shared with domestic stocks. These alleles can have several origins: ancestral polymorphism, homoplasy or introgression. In view of the substantial proportion of shared alleles in domestic and natural samples in our data, we propose here another index, the 'weighted introgression index' or IPI (indice pondéré d'introgression), which is not designed to give an accurate estimation of introgression, but can be used, in relative terms, for management purposes.

This index compares the allele frequencies between rivers and hatcheries. It only retains those supposed to be more informative, i.e. those whose mean frequencies in considered domestic stocks are more than X times the frequency in natural populations (the comparison is done with mean frequencies of natural populations): these alleles are supposed to be domestic. Their presence in natural populations is assumed to be due to introgression rather than homoplasy, for example. For a more sensitive estimation, we should use some populations identified as purely wild. However, even populations with the modern allele of *LDH5** can contain modern wild fish; this is why for this first attempt, we also included these populations. It should be noted that if a mean frequency is calculated over too many river populations, some alleles with a high frequency in one population (not significant for this particular population) could be considered as significant; this means that the more populations that are used, the less sensitive this index could become. For a first attempt, and

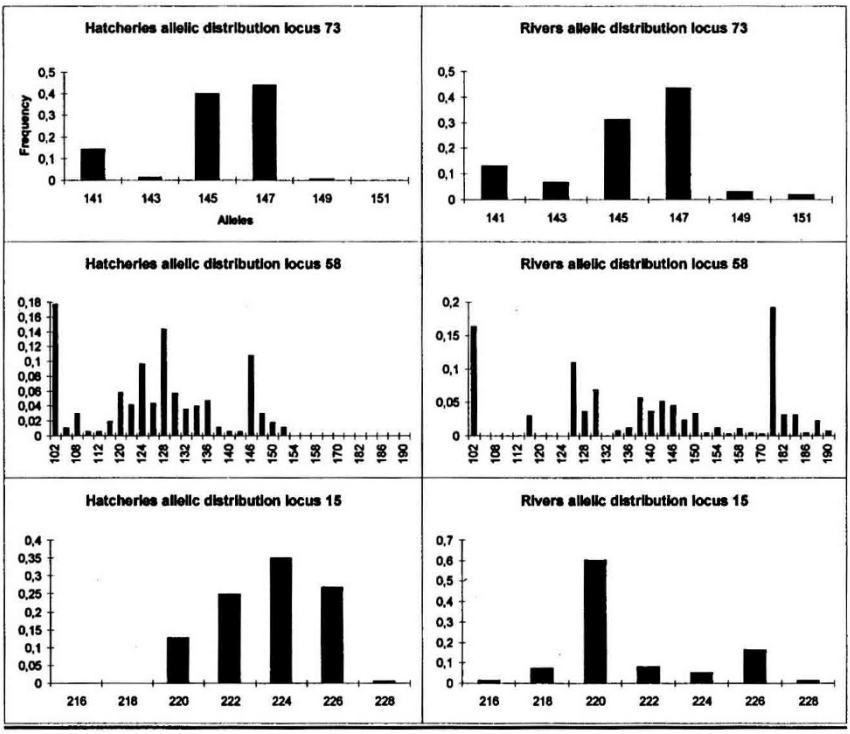

Figure 2. Allelic frequencies for hatchery and natural populations at the three microsatellite loci.

with few populations, however, comparisons between populations agree well with allozyme results, which can be considered as quite good, especially when compared to the Poteaux index (see later). Various X parameters can be tested (IPI_X). The frequencies of these alleles are then added over all loci (ΣD). This first addition does not take into account all the other alleles with a higher frequency in hatcheries but with a ratio lower than X. The addition of the hatchery mean frequencies is made on the same alleles (ΣD_{hs}). For example, with $X = 1.1$, the calculation of the mean frequency of retained domestic alleles gives 0.64 for hatcheries. To reach the theoretical 100 % value, we have to divide these relative values by 0.64. The same weighting is then applied to the sum of domestic allele frequencies in natural populations. X values were tested for 1.1, 1.5, 2 and 3 (*table V*). As mentioned earlier, it is only a first trial, and a more precise analysis of this problem should be performed. The formula is:

$$\mathrm{IPI}_X = \Sigma D/(\Sigma D_{hs})$$

where IPI_X is the IPI index for the chosen X parameter value; ΣD is the sum of frequencies of domestic alleles in the natural population considered (a *domestic allele* is designated as an allele whose frequency in involved hatcheries is X times greater than in natural samples); ΣD_{hs} is the sum of frequencies of these expected domestic alleles in hatchery stocks.

Table V. Introgression indexes (see methods for the calculation).

IPI	Marcadau	Beherekobentako	Dancharia	Andurentako	Béhérobie
$X = 1.1$	0.54	0.16	0.24	0.36	0.47
1.5	0.55	0.12	0.18	0.00	0.54
2	0.39	0.12	0.16	0.00	0.28
3	0.37	0.11	0.08	0.00	0.29
Poteaux index	0.95	0.96	0.89	0.6	0.96
*LDH5**90 frequency	0.33	0	0.02	0	0.27

IPI: weighted introgression index.

3. RESULTS

Allele frequencies are given in *table III* with the three allozymic diagnostic loci for comparison.

3.1. Intrapopulation variability

The total number of alleles by locus is 33 for *Strutta58*, 6 for *MST73* and 7 for *MST15*. As already mentioned, the sample sizes for highly variable loci (here locus *Strutta58*) should be great enough for significant estimations of allele frequencies. The mean unbiased expected heterozygosity obtained for these three loci was generally slightly higher for the hatchery stocks (from 0.70 to 0.77) than for river samples (from 0.60 to 0.68, except Marcadau with 0.78). These values are higher than those of Presa (1995), probably because the studied loci are different. The lowest value was obtained for the Andurentako River (0.30), but the sample size (5) does not allow a firm conclusion to be drawn. A higher variability in domestic stocks had already been found by Poteaux (1995) (both for enzymatic and microsatellite) and by Presa (1995) with more microsatellite loci.

3.2. Departures from Hardy-Weinberg equilibrium

The *Fis* multiloci calculated and the percentages of permutations with values superior and equal to the observed value are given in *table VI*. None of the hatchery populations studied here depart significantly from the Hardy-Weinberg proportions. In contrast, all natural populations (except Andurentako) showed a very significant deficiency in heterozygotes.

Table VI. *Fis* multiloci and their significance tested with 500 permutations.

Population	Canourgue	Brassac	Suech	Marcadau	Beherekobentako	Dancharia	Andurentako	Béhérobie
Fis	0.023	0.089	0.074	0.171	0.327	0.459	0.368	0.250
p	0.30	0.09	0.07	0.01	0.01	0.01	0.09	0.01

p gives the percentage of permutations with a value superior or equal to the observed value.

3.3. Differentiation between populations

The *Fst* values estimated by the θ of Weir and Cockerham (1984), and the associated probabilities, are given in *table VII*. All paired comparisons between populations were significant (at 1 %), but *Fst* values between domestic stocks (from 0.04 to 0.057) were globally lower than between natural populations (from 0.088 to 0.324) or between natural and domestic ones.

Allele frequency distributions for natural and domestic populations (mean for each population type) are given in *figure 2* for the three loci. For the less variable loci (*15* and *73*), there were few differences in terms of presence/absence of alleles. For example, on locus *15*, allele sizes inferior to 220 were only found in natural populations and not in hatcheries. At locus *58*, differences were greater; allele sizes larger than 152 were found only in nature. Conversely, alleles from 120 to 124 were characteristic of hatcheries. Major differences between these distributions were quantitative. The IPI used alleles such as 224 at locus *15*, which is much more frequent in hatcheries than in nature.

The IPI values are given in *table V* with four examples of parameter X values. Because we know that the samples Beherekobentako, Dancharia and Andurentako are pure wild samples (see *LDH5*90* frequencies), it is obvious that the $X = 3$ parameter is the more realistic. This result must be tested by extrapolation to other samples (especially some modern natural populations).

According to the IPI values, the most introgressed populations were Marcadau and Beherobie. Moreover, the *Fst* estimations between the Marcadau population and the three domestic stocks analysed were lower than for the other populations. The lowest introgression indexes were found for Dancharia and Beherekobentako and for Andurentako (as an indication only). This agrees well with the allozyme results (0–2 % of modern allele *LDH5*90*, which is the characteristic of the ancestral Atlantic form), and confirms that these populations mainly comprise wild fish. On the other hand, the values obtained with the Poteaux index are always almost one and probably do not clearly reflect the differences in the introgression rates. This shows that the different forms (wild and domestic modern Atlantic) compared here are closer (sharing a higher number of alleles) than the Mediterranean and Atlantic ones compared in Poteaux's study (1995), and need a more discriminating index.

These results are confirmed by the analysis of the phylogenetic tree constructed with microsatellite allele frequencies (*figure 3*), which clearly separates hatcheries and natural populations: Marcadau is the nearest natural population from domestic stocks, and Beherekobentako and Dancharia are on the other side of the tree. The marked separation of Andurentako from the other populations cannot be taken into account due to the very small size of the sample.

4. DISCUSSION

The first results have shown that the structure given by microsatellites is similar to that given by allozyme markers analysed in Berrebi (1997a). Only three loci were used for this preliminary study; more loci would probably allow a more precise analysis. An average of the information over several loci

Table VII. Weir and Cockerham's θ between populations.

Fst Microsatellites								
%/*Fst*	Canourgue	Brassac	Suech	Marcadau	Beherekobentako	Dancharia	Andurentako	Béhérobie
Can	0	0.057**	0.040**	0.033**	0.143**	0.121**	0.284**	0.120**
Bra		0	0.053**	0.080**	0.218**	0.168**	0.328**	0.123**
Sue			0	0.055**	0.140**	0.152**	0.333**	0.110**
Mar				0	0.088**	0.128**	0.289**	0.054**
Bek					0	0.080**	0.305**	0.094**
Dan						0	0.201**	0.167**
And							0	0.324**
Beh								0

* Significant at 5 %; ** at 1 %; significance was tested with 500 permutations. The first three populations are hatchery samples.

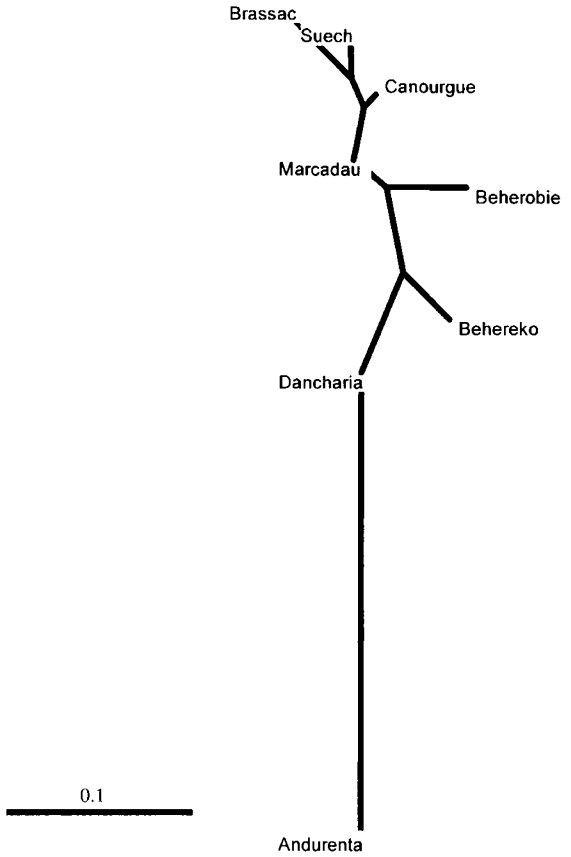

Figure 3. Phylogenetic tree constructed using Reynolds distances and the Fitch algorithm. A scale for branch lengths is given.

would tend to reduce differences due to natural differentiation between wild populations. Nevertheless, a really diagnostic locus between wild and domestic populations would be more useful.

For the intrapopulation analysis, we found a higher intrapopulation variability in domestic stocks than in natural populations. This is probably linked to the breeding practices in aquaculture: fish are exchanged between hatcheries and a single stock can contain alleles with extremely diverse origins (otherwise, genetic drift would tend to reduce heterozygosity as shown by Allendorf and Phelps [1980] for cut-throat trout). Conversely, population sizes and their variations (e.g. bottlenecks) can explain a lower heterozygosity in natural samples. The allozymic marker is generally inefficient in Atlantic basins for discriminating wild and domestic fish. The ancestral Atlantic form area is an exception. *Table III* shows that the populations of Andurentako, Beherekobentako and Dancharia are nearly homozygous for the alleles $LDH5^*100$ (and with a frequency of allele $TF100$ varying from 0.77 to 1, according to Berrebi [1997a]), corresponding to the natural ancestral Atlantic trout. The negligible impact

of the domestic strains is demonstrated by the absence of *LDH5**90 alleles. It is concluded that these populations are composed of only wild trout. The IPI parameter gave a similar result which makes it possible to trust the IPI values for the other populations in which the enzymatic marker is inefficient.

In such a composite genetic environment, the great variability of the locus *58* probably represents an obstacle for the discrimination of these different forms, because some alleles may have been shared several times independently (homoplasy). On the other hand, some less variable loci would perhaps not show enough differences between such close forms. As already mentioned, the most useful locus would be moderately variable but diagnostic.

Nevertheless, even if the microsatellite loci used are not diagnostic markers, the global results given by this new kind of marker and allozymes are similar for the ancestral Atlantic form (where allozymes are efficient). This is why microsatellites are also expected to be efficient for the detection of the wild modern Atlantic form.

Departures from Hardy-Weinberg expectations are constant in nature in the direction of heterozygote deficiency and can have several origins. Technical problems such as the presence of null alleles, even if they cannot completely be rejected, would not have such an impact (because of the too few possible null homozygotes found). Moreover, heterozygote deficiencies were also found with allozymes, where detection of homozygotes of null alleles is efficient. If two different populations are supposed to coexist in the same stream, a 'Wahlund effect' could explain the observed *Fis*. This would be the case, for example, if domestic and wild trout are present in the same sample, without (or before) complete mixing, as already found by Largiadèr and Scholl (1996). In our case, this phenomenon can probably explain the significant *Fis* found for the expected most introgressed populations: Marcadau and Beherobie (see *table VI*). Departures from panmixia demonstrated in pure wild populations (Beherekobentako, Dancharia and Beherobie streams) by significant and even higher values of *Fis* than for the other populations are more difficult to explain. Garcia-Marin and Pla (1996) and Apostolidis et al. (1996) in enzymatic studies, and Presa (1995) with microsatellites, did not find any significant *Fis* in natural populations. Poteaux (1997) also observed such deficiencies in Mediterranean populations. At our sites, a biological mechanism may be the cause: in natural populations, particularly in Atlantic basins, a regular cycle of migration has been observed. Because adults migrate upstream to reproduce (Delacoste, 1995), sympatry of differentiated cohorts can occur, provoking a Walhund effect. However, this deficit could also be partly due to some past stocking practices, which would always be detectable if hybrids between domestic and wild fish were selectively eliminated. This has yet to be demonstrated (this explanation would be more probable for populations where *LDH5**90 is always present, even if it may have been eliminated by drift). This phenomenon alone is probably not sufficient to generate such a deficiency. More likely, it results from a combination of several different mechanisms.

Our results confirm the high natural genetic diversity of brown trout populations. Biodiversity (comprising genetic diversity) is an important challenge for nature conservation, especially in wild species with developed domestic strains in commercial exchanges. The question of the gene flow between the two components of the species is of interest for two reasons: the wild populations as a

reservoir of useful genotypes for future improvement of domestic strains, and the domestic form as possible 'genetic pollution' of wild populations. From a scientific point of view, the interaction between two differentiated genomes is of obvious interest.

In the case of French brown trout, the wild diversity is frequently used to increase domestic polymorphism (which explains in part the higher heterozygosity of hatchery strains). For this purpose, wild males are sometimes crossed with domestic females.

The most important impact of the coexistence of wild and domestic forms of the same species is the genetic impact of domestic trout heavily stocked in rivers inhabited by natural trout. The literature gives some estimations of the stocking effect on French brown trout wild populations. Guyomard and Krieg (1986) gave introgression values from 0 to 50 % in Corsican trout populations, Barbat-Leterrier et al. (1989) found 0 to 40 % in the continental Mediterranean region and Beaudou et al. (1994) estimate that in the Orb basin (south Mediterranean region) only 0.5 % of the stocked juvenile trout reach the reproductive stage, which leads to 10 to 20 % of such limited cumulative introgressions over decades. Berrebi (1995) gave a large set of examples in the Mediterranean basins of the French Pyrenees with an estimated introgression value of 0 to 78 % of domestic alleles in wild populations.

5. CONCLUSION

Knowledge of domestic impact is valuable in various ways: definition of zones of protection, global protection of the genic biodiversity of the species, conservation of local adaptation as proposed by Leary et al. (1995) (this would have to be demonstrated), possible modification of the stocking practices according to its deduced efficiencies in each region, creation of new strains of local origin, conservation of morphologically differentiated entities, reconstitution of the origins of the natural taxonomic groups and of the history of populations (colonisations, migrations).

This kind of development has occurred in France through the description of geographic structures of brown trout established by allozymic studies. The need to continue similar investigations in the Atlantic basins is obvious and the present paper tries to estimate the qualities of the microsatellite markers in such a context. These preliminary results clearly show the utility of microsatellite loci for this purpose. They can help to determine if a population is highly introgressed or not (using an introgression index). In some respects microsatellite loci are also easier to use than allozyme loci and can be obtained without killing the fish (a piece of fin suffices). They are a good tool for the management of natural populations in order to highlight which ones would have to be protected. Here, for example, Behrekobentako or Andurentako could be protected, either by creating reserves or by raising the minimum acceptable size in angling. Microsatellite loci probably represent an original component of brown trout diversity.

Nevertheless, more investigations are necessary to improve this approach. We first intend to test other loci to find less variable and more informative (diagnostic if possible) ones. Comparisons should also be made with populations mainly comprising modern fish to discriminate wild and domestic modern

Atlantic trouts. This constitutes the greatest technical challenge. And from a fundamental point of view, larger scale sampling and a more detailed study will have to be done on natural populations for a better understanding of their evolution and genetic characteristics. A large-scale description by allozymes is nearly finished all along the Pyrenees chain. The complementary description by microsatellites of the Atlantic drainages is planned.

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