Differences between nuclear and mitochondrial introgressions of brown trout populations from a restocked main river and its unrestocked tributary

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The introgression of domestic genes in wild trout populations as a result of intensive restocking has been observed to be low in numerous rivers of the Mediterranean watershed. With the aim of examining the process of introgression and improving our understanding of the reasons for its possible limitations, we analysed the percentage of domestic genes for three protein-coding loci and for mitochondrial haplotypes. Two samples were analysed: a restocked river and a tributary which is no longer stocked. In order to follow the introgression over time, trout were separated into two age groups. The mitochondrial locus showed the same tendencies as those obtained with the nuclear loci, but with generally higher introgression values. A differential introgression among protein-coding loci was also observed. From these results, we discuss the possibility of selective factors acting against the domestic genes in river. The hypothesis of a difference in reproductive success between domestic males and females is also presented to explain the higher introgression of mtDNA.

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ADDITIONAL KEY WORDS:—restocking—allozymes—mtDNA—introgression.

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INTRODUCTION

The broad population subdivisions observed in most salmonids (Krieg & Guyomard, 1985; Ferguson, 1989) has made conservationists aware of the fact that massive and intensive restocking could have negative consequences on the conservation of natural biodiversity. The hatchery introductions have, however, long been justified for the maintenance or rehabilitation of a species threatened by over-exploitation or by the destruction or modification of its habitat (by pollution, dam construction). Beyond ecological disturbances, these introductions result in a global decrease in the genetic variability of these species, by the elimination of local populations (displacement or death due to diseases introduced at the same time as the fish), or by introgression (Leary, Allendorf & Knudsen, 1985; Waples, 1991; Ryman et al., 1993). This awareness of possible genetic impacts on wild populations of numerous salmonids has inspired a number of studies attempting to evaluate the domesticated genetic contribution. With advances in technology, these studies have successively used protein markers (reviews in Guyomard, 1989 and Ferguson, 1989), chromosomes (Garcia-Vasquez, Moran & Pendas, 1991), mitochondrial DNA (Danzmann, Ihssen & Hebert, 1991; Giuffra, Bernatchez & Guyomard, 1994; Hansen et al., 1995) and more recently minisatellites (Beacham, 1996) and microsatellite loci (Presa et al., 1994; Sanchez et al., 1996). However, the aim was the same for each marker: finding one or more diagnostic markers distinguishing wild and introduced forms.

In early studies, the allozymic loci were first used as biochemical markers of hatchery strains. This genetic tagging was carried out with the aid of diagnostic loci discriminating among these forms or by fixing a rare allele present in hatchery stocks (Murphy, Nielsen & Turner, 1983; review in Utter & Seeb, 1990). Other loci recognized as diagnostic between wild and domesticated forms were afterwards used more systematically (Hamilton et al., 1989; Moran et al., 1991; Garcia-Marin & Pla, 1996). Several studies evaluating the genetic contribution of hatchery fish in wild populations were in this way based on several protein-coding loci (Guyomard, 1989; Largiadèr & Scholl, 1996) but only on a unique allozymic marker, Ldh-5*, in the case of western European brown trout (Taggart & Ferguson, 1986; Garcia-Marin et al., 1991; Moran et al., 1991), or Me* for the Atlantic salmon (Garcia de Leaniz, Verspoor & Hawkins, 1989; Verspoor & Jordan, 1989).

Mitochondrial DNA (mtDNA) can also be used to study gene flow. Its matrilineal transmission without recombination and its high rate of mutation permits intraspecific studies, the tracing of introgression events or bottlenecks in female lineages. Numerous investigations have been made of the mtDNA polymorphism in several species of fish, with the aim of finding the haplotypes that would enable distinguishing among the stocks (Knox & Verspoor, 1991; Wirgin et al., 1993; Nielsen, Gan & Thomas, 1994; McVeigh, Hynes & Ferguson, 1995).

The brown trout occurs in two forms in France (Krieg & Guyomard, 1985): a southern form located in the Mediterranean basin, termed the Mediterranean form,
and a trout which occupies the rest of the area of the French distribution of this species, termed the Atlantic form. They are differentiated genetically by allozymes (Barbat-Leterrier, Guyomard & Krieg, 1989; Beaudou, 1993; Beaudou, Cattaneo-Berrebi & Berrebi, 1994; Largiadèr & Scholl, 1996), by microsatellites (Presa et al., 1994; Poteaux et al., subm.) and by a diagnostic mtDNA haplotype (Bernatchez, Guyomard & Bonhomme, 1992). The genetic distance between Atlantic and Mediterranean forms is estimated at 0.1 by allozymes (Nei’s standard genetic distance, in Guyomard, 1989) and the divergence estimate by mtDNA sequence data is more than 1.5 % (Bernatchez et al., 1992). As the domesticated strains all belong to the Atlantic form, it is thus possible to evaluate gene flow of domesticated trout towards the Mediterranean populations.

To estimate the genetic impact of restocking, we analysed and compared three wild Mediterranean populations using different markers. These populations originated from the same watershed (River Orb), but were subjected to different introduction regimes: the populations of the main river and one tributary were both annually restocked, whereas the population of another tributary had not been restocked for some years. First, we conducted a study of allozymes, focusing on the dynamics and the structure of the populations of these rivers, in 1991 (Beaudou et al., 1994) and in 1993 for only two rivers (Poteaux et al., in press). The samples were analysed for their conformity with the Hardy-Weinberg equilibrium. We not only found that the heterozygote deficiency was important, but also the number of significant pairwise linkage disequilibria was high in disturbed populations. Afterwards, we obtained data on microsatellite loci for the two rivers of this basin sampled in 1993, which we reported in another article (Poteaux et al., subm.). These microsatellite data confirmed the subdivision of populations of the main river and its tributary, as well as the genetic disturbance in these samples due to introductions.

In the present article, which corresponds to the third part of this study, we analyse by mitochondrial DNA restriction the domestic introgression in the matrilineal lineages for the two rivers sampled in 1993. We compare it with the indices of nuclear introgression, previously obtained from three diagnostic loci on the Mediterranean basin (Ldh-5*, Fbp-1* and Tf*). The comparison with results obtained by microsatellites was not carried out because of the difficulty in identifying the origin of alleles: microsatellite loci are characterized by a high homoplasy and there were no population references of diagnostic alleles with microsatellite loci. Thus, we compared mtDNA data to introgression based on protein-coding loci only. Our aim was to compare markers having different transmission modes and to find a possible asymmetry of male-female exchanges, in order to better understand the dynamics of introgression.

MATERIAL AND METHODS

Study site and sampling

The natural trout populations originate from the basin of the River Orb. This basin was chosen owing to the absence of restocking in one of its tributaries, the River Tes. This tributary has become a pilot river in the fish-breeding management
Figure 1. Distribution of brown trout size caught in the Orb (A) and Tes (B) rivers. Age groups 0+ and 1++ are indicated on the histogram.

plans of the Conseil Supérieur de la Pêche (CSP): no domesticated trout have been introduced into the Tes since 1988 (B. Reynier, pers. comm.). It was thus possible to compare in the same basin samples from heavily restocked zones with samples from zones that have not been restocked for 5 years. Moreover, the fish managers carry out regular surveys (CSP archives, unpublished), and demographic and ecological data were thus readily available.

During 1993, sampling was carried out in two locations, using electrofishing. The total length of each fish was measured and the scales were removed for determining the age of each fish. In this manner, two age groups were differentiated (Fig. 1): young born in that year (termed cohort 0+) and trout more than 1 year old (age group 1++).

In addition, in order to have frequencies of domesticated trout alleles, samples of 30 individuals were taken from the hatcheries used for at least 25 years for stocking in this basin: La Canourgue (Lozère French department), Mouline (Aveyron) and Brassac (Tarn). Eyes, blood, muscle and liver tissues were removed for enzyme analysis (Poteaux et al., in press). A sample of muscle tissue was taken from the same individuals for the study of mtDNA.

mtDNA RFLPs

Total genomic DNA was extracted by standard phenol/chloroform-isoamyl alcohol protocol, from frozen muscle or total blood preserved in TNES-urea buffer.
(10 mM Tris-HCl, 10 mM EDTA, 2% SDS, 0.3M NaCl, 4M urea, pH = 8). DNA was then quantified by fluorimetry and its purity was estimated after migration on 0.6% agarose gel.

Bernatchez et al. (1992) showed that the polymorphism observed in the control region by sequencing can distinguish among five groups of brown trout, which can be also identified with restriction endonuclease profiles (Bernatchez & Osinov, 1995). Giuffra, Guyomard & Forneris (1996) already used this RFLP marker to analyse the restocking impact of domesticated brown trout on Italian marbled trout populations (S. marmoratus).

The mtDNA control region was amplified by PCR with the primers reported in Bernatchez & Danzmann (1993), LN20 and HN20. Technical procedures of amplification are detailed in Bernatchez et al. (1992). Amplification products were digested with the Asnl enzyme (Appligene) which discriminates the Mediterranean form from the Atlantic form. Resulting fragments were electrophoretically separated on 2% agarose gel, ethidium bromide stained and photographed under UV light.

**Estimation of introgression**

For allozymic data, we reported from Poteaux et al. (in press) the introgression rate (\( T_i \)) for each diagnostic locus (\( Fbp-1^* \), \( Ldh-5^* \) and \( T^* \)), calculated for each sample according to the formula described by Barbat-Leterrier et al. (1989), as the difference between the frequency of domestic alleles in the sample and in pure populations weighted by the difference between their frequency in hatchery stocks and pure populations. For the mitochondrial DNA, the introgression rate corresponds to the frequency of the Atlantic haplotype in the sample under consideration. Studies carried out in several rivers around the Mediterranean that have never been restocked have shown that these loci are fixed for the Mediterranean alleles (Barbat-Leterrier et al., 1989; Presa et al., 1994).

In addition, an individual hybridization index (\( I_{HI} \)) was also computed from the three diagnostic loci according to the method described by Campton & Utter (1985). Each individual was classified according to genotype in an introgression category calculated on the basis of allele frequencies in wild and domesticated populations. This index varies from 0 to 1, with 0 indicating a domesticated type and 1 indicating a pure Mediterranean form.

**Cytonuclear disequilibria**

To estimate cytonuclear disequilibria, we performed a combined test using the three nuclear loci, disregarding possible lack of independence of these loci due to an eventual chromosomal linkage (Forbes & Allendorf, 1991). To do it, we assigned each fish mtDNA in a D (domestic) or a M (Mediterranean) haplotype, and then counted in each of these groups the total number of D or M nuclear alleles they possess, disregarding their homozygote or heterozygote condition. This yielded a 2 x 2 square contingency table on which we performed chi-square tests.
Table 1. Allelic frequencies at diagnostic loci and mitochondrial haplotype frequencies (N=sample sizes; D = domestic haplotypes; M = Mediterranean haplotypes; 'total' = 0 + and 1 ++ groups together).

<table>
<thead>
<tr>
<th>Loci</th>
<th>Orb</th>
<th>Tes</th>
<th>Hatcheries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total</td>
<td>0 ++</td>
<td>1 ++</td>
</tr>
<tr>
<td>N</td>
<td>106</td>
<td>45</td>
<td>65</td>
</tr>
<tr>
<td>Ldh-5*</td>
<td>100</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.87</td>
<td>0.93</td>
</tr>
<tr>
<td>Fbp-I*</td>
<td>100</td>
<td>0.18</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.88</td>
<td>0.90</td>
</tr>
<tr>
<td>mtDNA</td>
<td>D</td>
<td>0.37</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>0.51</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Table 2. mtDNA domestic haplotype frequencies, introgression indices (Ti) at each diagnostic nuclear loci and their mean value (N=sample sizes; 'total' = 0 + and 1 ++ groups together).

<table>
<thead>
<tr>
<th>Locations</th>
<th>Age group</th>
<th>N</th>
<th>mtDNA</th>
<th>Ti&lt;sub&gt;mean&lt;/sub&gt;</th>
<th>Ti&lt;sub&gt;TiB&lt;/sub&gt;</th>
<th>Ti&lt;sub&gt;LDEP&lt;/sub&gt;</th>
<th>Ti&lt;sub&gt;UP&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orb</td>
<td>total</td>
<td>66</td>
<td>0.49</td>
<td>0.24</td>
<td>0.16</td>
<td>0.18</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>0 ++</td>
<td>21</td>
<td>0.33</td>
<td>0.15</td>
<td>0.15</td>
<td>0.07</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>1 ++</td>
<td>51</td>
<td>0.53</td>
<td>0.27</td>
<td>0.16</td>
<td>0.27</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>73</td>
<td>0.20</td>
<td>0.11</td>
<td>0.09</td>
<td>0.08</td>
<td>0.15</td>
</tr>
<tr>
<td>Tes</td>
<td>0 ++</td>
<td>21</td>
<td>0.28</td>
<td>0.13</td>
<td>0.12</td>
<td>0.07</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>1 ++</td>
<td>44</td>
<td>0.13</td>
<td>0.09</td>
<td>0.05</td>
<td>0.09</td>
<td>0.13</td>
</tr>
</tbody>
</table>

RESULTS

Age groups of each sample are shown according to the size distribution of wild fish (Fig. 1). These histograms were compared with those obtained from catch inventories carried out in the area of our study (CSP archives from 1990 to 1994, unpublished). This permitted us to confirm that the sample caught was representative of the population in place. In this way, the decrease in the number of sizes between 120 mm and 170 mm in the histogram of the Tes (Fig. 1), corresponding to an abnormal decrease in the number of 1 ++, could be explained by poor reproduction conditions at the end of 1991, which resulted in low recruitment the following year (CSP archives, unpublished).

Protein-coding loci

We used the allele frequencies reported by Poteaux et al. (in press) for the three diagnostic loci: Ldh-5*, Fbp-I* and Tj* (Table 1). From the introgression indices (Ti) calculated for each locus (Table 2), one can see differences in the behaviour of
each locus, the transferrin locus indicating systematically the highest introgression value. The domestic introgression is also higher in the Orb than in the Tes (significant $F_{ST}$ values $P<0.01$ for all loci, Poteaux et al. [in press]). The differences in the inter-age groups comparisons were significant in the Orb but not in the Tes.

The hybridization index $I_H$ computed for each individual is presented in the form of a histogram for each age group (Fig. 2). This figure shows pure wild individuals or fish with little recombination and one trout in the Orb $1++$ group was apparently of hatchery origin.

**mtDNA**

The major part of our sampling (230/311) was analysed for mitochondrial DNA (Table 1). The mitochondrial DNA transmitted by the domesticated stocks is of the Atlantic form only. The restocking of the Orb introgressed more than in the Tes, at 49% and 20% respectively ($2 \times 2$ contingency chi square test, $\chi^2 = 16.7$, $P<0.001$). Here also, this large haplotype frequency difference is observed when comparing groups $1++$ from the Orb and Tes ($\chi^2 = 19.48$, $P<0.001$). If we compare age groups by river, there is no significant differences of haplotype frequency (Table 1).

The results of cytonuclear disequilibrium tests are given Table 3. The Orb and Tes $1++$ age groups show significant associations at the 5% and 1% level respectively, due to an excess of wild form combinations. In contrast, both $0+$ age groups did not show cytonuclear disequilibrium. This lack of significance may reflect the small value of certain counts in the $0+$ contingency tables.
Table 3. Combined test for cytonuclear disequilibria opposing the mtDNA characters (DNA D = domestic haplotypes, DNA M = Mediterranean haplotypes) to the sum of allozymic alleles (alloz. D = sum of allozymic domestic alleles at three diagnostic loci; alloz. M = Mediterranean alleles). Four $2 \times 2$ contingency tables were obtained, on which we performed chi-square test (chi-square = value obtained; $P$ = probability estimation, NS = not significant; df = 1).

<table>
<thead>
<tr>
<th>Age group</th>
<th>Orb 1+ + DNA D</th>
<th>DNA M</th>
<th>Orb 0+ DNA D</th>
<th>DNA M</th>
<th>Tes 1+ + DNA D</th>
<th>DNA M</th>
<th>Tes 0+ DNA D</th>
<th>DNA M</th>
</tr>
</thead>
<tbody>
<tr>
<td>alloz. D</td>
<td>53</td>
<td>115</td>
<td>2</td>
<td>28</td>
<td>9</td>
<td>27</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>alloz. M</td>
<td>27</td>
<td>117</td>
<td>8</td>
<td>54</td>
<td>13</td>
<td>203</td>
<td>7</td>
<td>53</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>6.6</td>
<td>1.58</td>
<td>13.95</td>
<td>2.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Differences between rivers

In this study, we have shown a difference in the introgression level between the population of the main river, the Orb, which is intensively restocked and that of its tributary which has not been restocked for several years. This difference could be attributed to two main causes:

1. It could be due solely to pre-existing differences. This hypothesis may be realistic, although in the case of the Tes, we have two measures of allozymic introgression, one in 1991 (Beaudou et al., 1994) and one in 1993 (Poteaux et al., in press). The introgression index indicates a decrease of domestic alleles in the Tes during this two year period, which would favour hypothesis (2):

2. Alternatively these two rivers, which were restocked in exactly the same way, could have shown similar introgression levels in the past which would allow us to suggest that introgression began to decline since restocking stopped. In the Tes, 5 years without domestic introductions corresponds to only two or three generations of trout. This time would have been sufficient to increase the difference in the frequencies of domestic alleles for both the allozymes and the mtDNA between the two rivers. This tendency has already been noted in the analysis of the same samples with microsatellite loci (Poteaux et al., subm.).

Frequent migrations of the trout population have been reported and observed in these rivers: (i) ascent of genitors during the reproductive period (Huet & Timmermans, 1979; pers. observ.); (ii) migration of spring juveniles from the Tes to the Orb, showed by a trap experiment carried out in the fish pass of a dam on the Tes during several weeks in the spring of 1992 (Poteaux, 1992) or (iii) other episodic movements. However, even if there are no potential barriers in the river, there are significant differences between the populations of the main river and those of its tributary. These differences probably indicate the existence of population subdivisions along the length of both rivers, and this despite frequent migrations.

Differences between age groups

Differences in the allele frequencies seem too large to be due to stochastic factors alone, since for each river the differences between 0+ and 1+ + go in the same
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direction at each locus. The fact that the introgression indices seem to behave in a different way between age groups in the two rivers is in itself an indication of a complex impact of the restocking maintained in one of the waterways: how could this be explained without disappearance of some genotypes or differential migration of individuals?

Because of the near-total absence of individuals born in hatcheries (Fig. 2), in contrast to some Italian populations (Giuffra et al., 1996), our results cannot be attributed to an unequal sampling of freshly introduced fish. They thus pertain to survival of fish born in the river or to the reproductive success of their parents.

The Orb sample was caught at the end of October, a long time after the introductions at the beginning and the additional introductions during the course of the fishing season, so it would seem that the population in place was typically 'native' in the sense of 'individuals born in the river'. However, the analysis of populations in other Mediterranean rivers (in the eastern Pyrenees) that have been restocked has shown that at the same sampling date, the percentage of domesticated individuals was still quite high (Poteaux & Berrebi, 1997). These hatchery trouts in the Orb have, therefore, either been eliminated (naturally or by fishing) or displaced by competition downstream where a reservoir could provide better survival conditions.

Nuclear introgression

The extent of introgression varies markedly among loci. We can notice that the locus of the transferrin, which is not an enzyme, presents the highest values and the largest interval of variation. The domestic allele at this locus would, therefore, introgress better in nature than the domestic alleles of the two other loci studied.

Selective influences on protein loci have been suggested to explain the spatial pattern of gene frequencies of several species of fish (Verspoor & Jordan, 1989; Powers et al., 1991). It has been shown for poikilotherms that the functional differences among allozymes of numerous loci could result in different adaptive values (Phillip, Childers & Whitt., 1985; Powers et al., 1991; Kirpichnikov, 1992). In the case of brown trout, differences in affinity for the substrate have been found among the genotypes of the locus Ldh-5* by Henry & Ferguson (1985). It has been suggested that these differences could explain the almost complete colonization of the open basins of the Atlantic watershed by the modern Atlantic form (Hamilton et al., 1989).

The difference of introgression rates between the discriminating loci being found in the other Mediterranean introgressed trout populations (Largiadèr & Scholl, 1996; Poteaux & Berrebi, 1997), one could then question the neutrality of these protein-coding loci. These loci could be affected by differential selection pressures, acting either directly on them, or on linked genes or parts of the genome non-neutral vis-à-vis introgression. The higher introgression value of the Tff* locus suggests that this locus is less counter-selected than the others. A differential selection between loci was found in the analysis of numerous hybrid zones, indicating that a force acted against the penetration of alleles of a taxon in the genome of the other taxon, species or sub-species (Hunt & Selander, 1973; Hewitt, 1988; Dod et al., 1993). The case of brown trout may reflect a similar situation; it is, however, not exactly a hybrid zone since there is no geographic confrontation between the two entities but a dilution of a foreign gene pool in a receiving population.
The second notable fact is that mitochondrial introgression is two to three times higher than that estimated from two of the diagnostic allozymic loci (\textit{Fbp-1*} and \textit{Ldh-5*}). The mitochondrial genome is considered to be more sensitive to founder effects than nuclear genes. However, the hypothesis of a founder effect is to be eliminated in this case because (i) no catastrophic mortality event of trout has been recorded in this basin during the last 30 years (B. Reynier, pers. comm.), and (ii) the allozymic variability observed in this study is in conformity with that described for Mediterranean trout in the literature (Barbat-Leterrier et al., 1989; Presa et al., 1994).

The introgression difference observed between the two types of markers could be due either to differential counter-selection as invoked above or to a difference in the reproductive contribution of males and females. The former phenomenon has been often described for naturally occurring hybridization (Powell, 1983; Ferris et al., 1983; Vanlerberghe et al., 1988) and noticeably with fish (Campton, 1987; Dowling, Smith & Brown, 1989; Forbes & Allendorf, 1991; Bernatchez et al., 1995). The latter situation is also plausible if there is a higher participation in reproduction by domesticated females than their wild counterparts. Alternatively, this could also originate from a lower contribution by hatchery males less adapted to competition for females. During the reproductive period, the sex ratio is in general tipped in favour of males (Huet & Timmermans, 1979; Baglinière et al., 1989). On the spawning ground a female is present for several males and only the dominant male will reproduce (Crisp & Carling, 1989). One can then suppose that the domestic males, less aggressive owing to the selection that they have undergone in order to adapt them to the crowding in the hatchery basins, have a lower rate of reproductive success.

Alternatively, although mtDNA was supposed to be selectively neutral, several recent studies suggested that the mtDNA variation pattern is inconsistent with a strictly neutral model (Ballard & Kreitman, 1995). The differential introgression could then reflect the more ‘neutral’ characteristic of mtDNA when compared with nuclear genes.

\textit{Evidence of natural selection?}

The results obtained in this study—the introgression difference between the Tes and the Orb rivers, both for nuclear and mitochondrial markers, plus a differential introgression between the protein loci and perhaps between types of markers—suggest that selection pressures could act to limit hatchery gene flow. We propose that the differential of introgression between upstream and downstream could be the result of differential elimination of selected alleles during the course of the development of individuals, either by death or by differential migration of individuals according to genotype. The persistent cytonuclear disequilibrium is one more indication of some disfunctioning of recombined genotypes, which would be less fit (intrinsic factors, i.e. interaction of genes). In addition, individuals possessing domestic genes in variable proportions would be selectively inferior to individuals genetically closer to the wild state (extrinsic factors, additive effects). For the survival and reproduction of hatchery trout in river, extrinsic factors, such as the type of environment in which
they are introduced, seem to be important. In supplementing natural populations with hatchery stocks of equal quantity, it has been shown that the success rate of introduced stocks (for survival and introgression) is higher in lakes or in reservoirs than in rivers (Spanish reservoirs in Martinez et al., 1993; several Andorran lakes, unpublished data). Nevertheless, an argument seems to emerge in favour of a differential survival of individuals as a function of genotype. Furthermore, an experiment of backcrosses (Poteaux, 1995) showed that segregation distortions were observed for the locus \( Ldh-5^* \) as well as pseudo-linkage for six pairs of loci over 72 analysed. Thus, a part of the selection pressure exerted on the loci could be due to intrinsic factors rendering the theory of the disruption of the coadapted structures attractive. From mtDNA sequence variation, Bernatchez et al. (1992) have estimated that the Atlantic group has diverged from all other trout forms more than 500,000 years ago, which would be a sufficient time for coadaptation of groups of genes to develop.

CONCLUSION

We therefore propose that there is a difference in reproductive success between the genotypes introgressed by domestic genes and the wild type. According to the more likely hypothesis, the percentage of introgression should decrease progressively over time in the tributary that was not restocked. However, this tributary is not isolated from the main river. As the main river is continually restocked, it constitutes a reservoir of hatchery genes that eventually filter into the trout population of the tributary. Therefore, one question concerns whether the decrease in domestic genes in the wild population could continue to the point of complete elimination if restocking is discontinued.

The mitochondrial locus, used for the first time on a large scale to measure inbreeding through the female line, shows the same tendencies as those obtained with the allozymes. The values obtained are, however, higher than with two of three allozymes, which enables us to propose an asymmetry between the male and female reproductive success of introduced fish.

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