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Genetic introgression between wild and stocked brown trout in the Douro River basin, Spain

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The genetic diversity of Spanish brown trout is currently threatened by stocking with exogenous brown trout from Central and Northern Europe. In the Douro River basin 25% of the analysed populations in the present study showed introgression by genes of hatchery origin. The mean introgression estimated by the single locus approach ($\hat{S}$) varied from 0 to 22% among populations, with a mean value of 3%. The hatchery allele markers were absent in populations where stocking ceased in 1993. However, the introgression effect was observed in all populations stocked until 1998. It seems that cessation of stocking is a good measure for restoring native populations. A thorough review of published and present data of genetic interactions between wild and stocked brown trout in Spanish rivers indicates different levels of introgression between basins. The absence of a clear geographical pattern in the introgression level suggests that ecological interactions and local stocking programmes may play an important role in stocking success. Finally, several guidelines are provided for conservation and management of native brown trout populations in Spanish rivers.

Key words: conservation; introgression; management; *Salmo trutta*; Spain; stocking.

INTRODUCTION

The brown trout *Salmo trutta* L. is the most important sport fish in Spain, but its populations are currently overexploited by angling (Almodóvar & Nicola, 1998; Almodovar *et al*., 2001) and their genetic diversity is threatened by stocking with exogenous trout from Central and Northern Europe (Machordom *et al*., 1999, 2000). Over the last 40 years public demand for more fish has focused the management of Spanish rivers on stocking with brown trout irrespective of their origin. However, few efforts have been made to preserve the native stocks. Further, stocking has been generally regarded as beneficial for the native populations, but it may result in the extinction of local, wild gene pools (Ryman *et al*., 1995; Allendorf & Waples, 1996). Therefore, exogenous trout genes are introgressing gene pools of indigenous and possibly locally adapted wild populations. Several studies have reported introgression of genes from stocked brown trout on native populations of brown trout (Taggart & Ferguson, 1986; Largiader & Scholl, 1996; García-Marín *et al*., 1998; Poteaux *et al*., 1998).

On the other hand, genetic studies suggest that a large part of the evolutionary diversity of brown trout corresponds to Southern European countries bordering the Mediterranean (García-Marín *et al*., 1999; Sanz *et al*., 2000). For instance, Spanish brown trout populations display high genetic differentiation among drainage systems, with the Douro River basin having a unique mitochondrial

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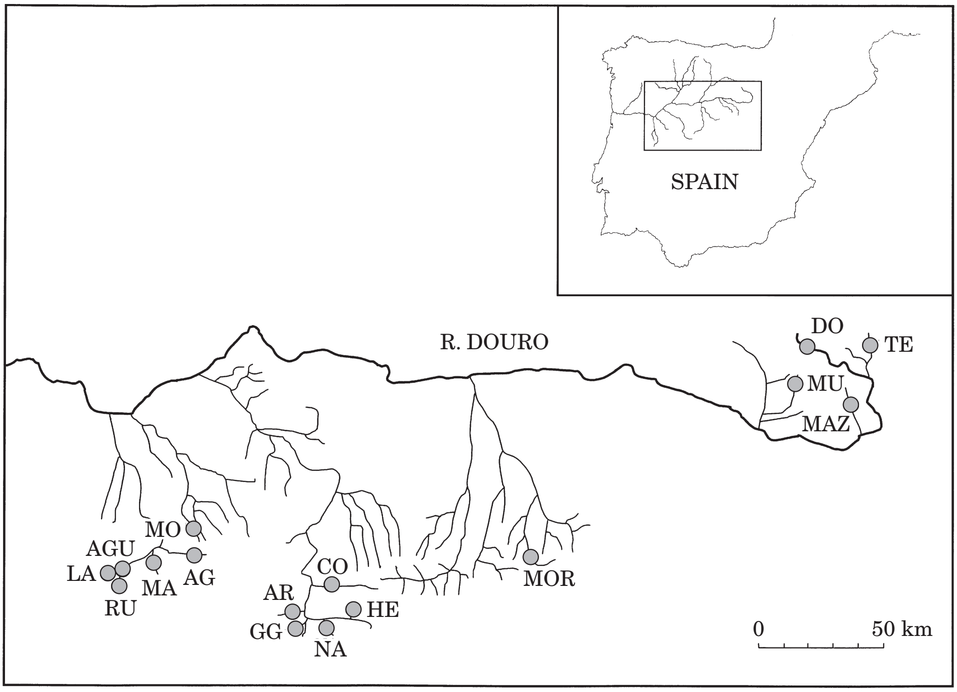

FIG. 1. Geographical location of the sampling sites. AG, R. Agadón; AGU, R. Águeda; AR, R. Aravalle; CO, R. Corneja; DO, R. Douro; GG, R. Galin Gómez; HE, R. Herguijuela; LA, R. Ladrón; MA, R. Mayas; MAZ, R. Mazo; MO, R. Moros; MU, R. Muriel; NA, R. Navamediana; RU, R. Rubioso; TE, R. Teva.

haplotype (Machordom *et al*., 2000). Therefore, it seems crucial to address the conservation genetic status of these genetically unique stocks of brown trout. The aim of this work was to assess the introgression level by allochthonous genes in wild populations from the Douro River basin by means of allozymic markers. This work is part of a project concerning the conservation status of brown trout in Central Spain. Finally, several conservation genetic guidelines and recommendations are provided for the management of native brown trout populations in Spanish rivers.

MATERIALS AND METHODS

Samples of wild brown trout were collected by electrofishing between 1999 and 2000 in 16 streams from the Douro River basin (Fig. 1, Table I). Trout were immediately frozen on dry ice and stored in a $-80°$ C freezer in the laboratory. A total of 294 brown trout between 2+ and 4+ years were analysed. For comparative purposes, hatchery samples from a Northern European stock were also screened. Extracts of eye and muscle tissues were subjected to horizontal starch (11%) gel electrophoresis. Electrophoretic procedures and visualization of enzyme activity combined the traditional methods of Aebersold *et al.* (1987) and Pasteur *et al.* (1987). The occurrence of allochthonous genes was estimated by means of the analysis of the diagnostic *LDH-5** locus (L-lactate dehydrogenase, Enzyme Commission Number 1.1.1.27). The allele *LDH-5*100* characterizes the native Spanish populations, while hatchery stocks are fixed for *LDH-5*90* (García-Marín *et al*., 1991). Two hatchery marker loci were also tested, the glyceraldehyde 3-phosphate dehydrogenase (1.2.1.12, *GAPDH-1*, -2*, -3**) and the malate dehydrogenase (1.1.1.37, *SMDH-A1*,*

Table I. Main characteristics of the sampling sites, number of trout analysed and stocking effort, i.e. approximate number of trout released per year

River	Altitude	Substratum	*n*	Stocking (before 1993)	Stocking (1993–1998)
Agadón	960	Siliceous	15	100 000	0
Agueda	900	Siliceous	25	100 000	0
Aravalle	1080	Siliceous	22	100 000	0
Corneja	1060	Siliceous	6	20 000	0
Douro	1190	Siliceous	28	6000	6000
Galín Gómez	1200	Siliceous	21	100 000	0
Herguijuela	1380	Siliceous	16	100 000	0
Ladrón	900	Siliceous	9	0	0
Mayas	820	Siliceous	12	100 000	0
Mazo	980	Calcareous	22	0	0
Morasverdes	980	Siliceous	15	0	0
Moros	1180	Siliceous	28	—	—
Muriel	1100	Calcareous	26	10 000	10 000
Navamediana	1150	Siliceous	7	100 000	0
Rubioso	1060	Siliceous	20	0	0
Tera	1040	Calcareous	22	12 000	12 000

-*A2*, *B1,2**). Two alleles (*G3PDH-2*50* and *sMDH-A2*120*) were apparently absent in Spanish native populations before introductions of the hatchery-reared fish for Central and Northern Europe (Martínez *et al*., 1993; García-Marín & Pla, 1996). Hardy-Weinberg equilibrium was tested using the exact probability test (Raymond & Rousset, 1995). The introgression ($\hat{S}$) of hatchery genes in each sample was estimated as the average contribution for each three allele markers from the expression: $\hat{S}=(pw-pn)(ps-pn)^{-1}$, where pw, ps and pn are, respectively, the allele frequencies of the wild population, the hatchery stock and the native population before stocking (Taggart & Ferguson, 1986). Since these alleles were not present in native Spanish populations, pn was 0 for all of them (Sanz *et al*., 2000). For each allele ps was estimated as the average value observed among the available data on hatchery stocks.

There are no precise records of stocking programmes in the studied rivers for the last decades (Table I). Four of the rivers (Ladrón, Mazo, Morasverdes and Rubioso) have never been stocked. Eight rivers (Agadón, Águeda, Aravalle, Corneja, Galín Gómez, Herguijuela, Mayas and Navamediana) were highly stocked until stocking was stopped in 1993. Four rivers (Douro, Moros, Muriel and Tera) were moderately stocked until 1998, when stocking ceased. The rivers were stocked with fingerling 0+ year brown trout.

RESULTS

Only 25% of the analysed populations showed introgression by genes of hatchery origin (Table II). The analysis of the *LDH-5** locus in the studied populations revealed three different electromorphs. Two of them (*LDH-5*100* and *LDH-5*110*) indicated native brown trout, while *LDH-5*90* was fixed in all the stocked brown trout. The introgression deduced from the diagnostic *LDH-5*90* allele varied from 0 to 18% among populations, with a mean value of 3%. The hatchery diagnostic *LDH-5*90* allele was not observed in populations where release practices stopped in 1993. The *sMDH-A2*120* allele was found in locations stocked during 1993–1998, except River Moros, showing an average

TABLE II. Mean frequency (%) of the *LDH-5** diagnostic locus and other hatchery markers in the analysed samples and from the available data from Douro River basin (see references in the table). A single locus estimation of hatchery genes introgression ($\hat{S}$) for all populations is also given

River	*LDH-5*90*	*G3PDH-2*50*	*sMDH-A2*120*	$\hat{S}$	Reference
Agadón	0·00	0·00	0·00	0·000	Present study
Agueda	0·00	0·00	0·00	0·000	Present study
Aravalle	0·00	0·00	0·00	0·000	Present study
Corneja	0·00	0·00	0·00	0·000	Present study
Douro	0·02	0·00	0·04	0·070	Present study
Galín Gómez	0·00	0·00	0·00	0·000	Present study
Herguijuela	0·00	0·00	0·00	0·000	Present study
Ladrón	0·00	0·00	0·00	0·000	Present study
Mayas	0·00	0·00	0·00	0·000	Present study
Mazo	0·00	0·00	0·00	0·000	Present study
Morasverdes	0·00	0·00	0.00	0·000	Present study
Moros	0·04	0·00	0·00	0·013	Present study
Muriel	0·17	0·02	0·04	0·172	Present study
Navamediana	0·00	0·00	0·00	0·000	Present study
Rubioso	0·00	0·00	0·00	0·000	Present study
Tera	0·18*	0·02	0·05	0·191	Present study
Aguisejo	0·21	0·00	0·11	0·236	Machordom *et al.* (1999)
Bernesga	0·00	0·00	0·00	0·000	Sanz *et al.* (2000)
Carrión	0·00	0·00	0·00	0·000	García-Marín & Pla (1996)
Eresma	0·02	0·02	0·00	0·059	Machordom *et al.* (1999)
Esla	0·12	0·00	0·00	0·040	Sanz *et al.* (2000)
Millar	0·00	0·00	0·00	0·000	Sanz *et al.* (2000)
Pisuerga	0·00	0·00	0·00	0·000	García-Marín & Pla (1996)
Riaño	0·00	0·00	0·00	0·000	Sanz *et al.* (2000)
Viomar	0·29	0·07	0·02	0·310	Sanz *et al.* (2000)
Yuso	0·00	0·00	0·00	0·000	Sanz *et al.* (2000)
Hatchery average	1·00	0·13	0·21		

*Significant (P<0·01) Hardy–Weinberg disequilibrium.

frequency of 4%. The *G3PDH-2*50* allele occured at stocked Tera and Muriel rivers with a mean frequency of 2%. The average contribution of hatchery fish estimated by the single locus approach ($\hat{S}$) was zero in populations unstocked after 1993, and ranged between 0·013 (River Moros) and 0·191 (River Tera) in populations stocked until 1998. The estimated introgression ($\hat{S}$) in the studied populations (0·032) was similar than that deduced from the diagnostic *LDH-5*90* allele (0·026).

Most of the introgressed populations did not show significant deviations for the Hardy-Weinberg equilibrium (Table II). Only the River Tera presented a disequilibrium at the *LDH-5** locus caused by a heterozygote deficiency. This probably reflects a Wahlund effect due to the presence of some hatchery-release brown trout in the sample, since this locality was stocked until 1998.

When considering all the studied Douro Basin populations the mean estimated introgression was $\hat{S}$=0·042 (Table II), but such value ranged between 0·039 (*G3PDH-2*50*) and 0·047 (*sMDH-A2*120*). The introgression estimated from the diagnostic *LDH-5*90* allele was 0·041, which was equal to the amount deduced from the single locus approach.

DISCUSSION

Despite the enormous stocking effort made over past decades in the Douro River basin, the presence of allozymic exogenous markers was very scarce in the study populations. The cessation of stocking seems to have been a good measure to conserve the native populations. Thus, diagnostic alleles of allochthonous trout were not detected in those populations where stocking was stopped ten years ago. On the contrary, the genetic effects of hatchery stocks appeared in populations stocked during the last decade. This denotes that stocking is only aimed at increasing the immediate anglers' yield. Hatchery-reared trout are probably more susceptible to angling than native brown trout (García-Marín *et al.*, 1998). Therefore, stocking needed to be repeated annually to support the fishery.

The low percentage of allozymic exogenous markers found in the studied populations could also indicate poor interbreeding between wild and stocked trout. This suggests either a lower reproductive performance or a lower survival of hatchery stocks in these locations compared with native brown trout. Previous studies have demonstrated the low viability of brown trout released into the wild. According to McNeil (1991), only 5% of the stocked salmonids in the world (*c.* 5×10^9) survive to adulthood. In particular, it seems that in flowing water hatchery-reared brown trout has a poor performance compared with wild specimens in terms of survival and growth (Jorgensen & Berg, 1991; Fjellheim *et al.*, 1995; Skaala *et al.*, 1996). For instance, Cresswell & Williams (1989) found that released brown trout contributed <1% to the catch in the season after stocking, and lower survival rates are usually found in rivers already inhabited by native populations (Kelly-Quinn & Bracken, 1989; Wiley *et al.*, 1993).

On the other hand, the majority of the introgressed studied populations were at Hardy-Weinberg equilibrium, which indicates that gene flow between non native and wild populations really exists. Therefore, release of allochthonous brown trout should be stopped to avoid negative genetic impacts on wild brown trout and the effects of previous stocking must be evaluated in order to quantify this impact. Stocking should be only viewed as an important measure to restore native populations which are in immediate danger of extinction. However, local broodstock must be used for production of stocking material and there should exist a subsequent monitoring programme to assess the efficiency of stocking.

A thorough review of published and present data on genetic interactions between wild and stocked brown trout in Spanish rivers showed different levels of introgression among basins. Thus, the populations from the Douro basin showed a scarce presence of foreign genes, which resembled that found in the North (mean $\hat{S}$=0·012) and Eastern Pyrenees (mean $\hat{S}$=0·079) basins (Morán *et al.*, 1991, 1995; Martínez *et al.*, 1993; Arias *et al.*, 1995; García-Marín & Pla, 1996; Sanz *et al.*, 2000). Moreover, the genetic introgression in the Douro basin was low compared with the Ebro (mean $\hat{S}$=0·421), Tagus (mean $\hat{S}$=0·233) and Júcar (mean $\hat{S}$=0·109) basins (García-Marín & Pla, 1996; García-Marín *et al.* 1998; Cagigas *et al.*, 1999; Machordom *et al.* 1999). All these Spanish basins have been highly stocked in the past with non-native trout. However, the observed differences in the introgression level among rivers suggest that ecological interactions and local stocking programmes may play an important role in stocking success.

Finally, conservation releases with local trout should only be considered a temporary situation. The factors responsible for the population decline should be identified and alternative methods of enhancement, such as habitat improvement or a sustainable management of native populations, should be first considered.

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