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Allozyme diversity in brown trout (*Salmo trutta*) from Central Spain: Genetic consequences of restocking

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SUMMARY

1. The brown trout (*Salmo trutta*) represents one of the main freshwater resources in Spain, but habitat alterations and overharvesting have contributed to the decline or disappearance of numerous natural populations. In addition, reinforcement programs of wild populations based on releases of hatchery reared fish of exogenous origin compromise the conservation of remnant native trout resources.
2. We present allozymic data from Central Spain trout populations including stocked and unstocked populations. Although the levels of genetic variation observed were low and affected by hatchery releases ($\overline{p}$ = 18.23%, $\overline{Ho}$= 3.39%), they were within the range observed in other European areas.
3. The effective introduction of hatchery reared fish is genetically homogenising the populations in the studied area and disturbing the ancestral pattern of genetic variation that distinguishes the Tajo and Duero basins. Within the eight natural populations analysed, seven had alleles assigned to the foreign trout. The introgression in these populations, following the *LDH-5*90* allele frequency, ranged between 2% and 29.4%, but those values are not in concordance with the respective stocking effort undertaken in each population. Moreover, the release of hatchery-reared fish does not solve the problems related to the reduced size of wild populations and their recruitment instability.

Keywords: brown trout, *Salmo trutta*, genetic variability, restocking, introgression

Introduction

The brown trout (*Salmo trutta* L.) represents one of the main freshwater fish resources in Europe, both for its commercial interest and, especially, for its great sport fishing value. Nevertheless damage to natural habitats and probably overfishing have caused the decline or disappearance of numerous natural populations in Spain (García de Jalón & Schmidt, 1995). The Spanish public administration responsible for the management and conservation of this natural resource has been attempting to maintain salmonid quality and quantity in continental waters for the past 100 years via stocking with hatchery-reared fish of foreign origin. Only recently has attention been focused on the genetic population structure of the native Spanish trout and the genetic consequences of foreign introductions. Genetic variation is an important feature of populations, both for short-term fitness and long-term survival, as it allows adaptation to changing environmental conditions to occur (Ryman, 1981; Allendorf, Ryman & Utter, 1987; Utter, 1991; Dowling & Childs, 1992; Presa *et al*., 1994). Genetic variation is similarly important in farmed populations, allowing selective breeding and preventing loss of fitness due to inbreeding depression (Bartley *et al*., 1992).

Previous studies indicated that native Spanish populations belong to ancient lineages of the species, and that the Iberian gene pools were characterised by: (i) strong individuality based on distinct allele frequencies in both adjacent and distant rivers (ii)

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locally high frequency of rare alleles and (iii) low heterozygosities (García-Marín & Pla, 1996). Although present policies of stocking with hatchery fish of German origin are eroding the genetic resources of the species in many areas of Spain (García-Marín *et al*., 1991; García-Marín, Sanz & Pla, 1998), native genes predominate in most of the study locations (Martínez *et al*., 1993; Morán *et al*., 1995; García-Marín & Pla, 1996).

In this paper, we describe patterns of genetic variation among native populations of brown trout in Central Spain. This area includes large brown trout populations from the Tajo (Tagus) and Duero rivers. The contribution of hatchery and native genotypes in the area is discussed in relation to conservation of native gene pools and to potential management options.

Materials and methods

Samples

We analysed 154 brown trout from eight sampling sites on five streams in the Tajo and Duero basins (Table 1, Fig. 1). Twenty-nine individuals from a German stock from the Uña hatchery were also screened (this stock has been used to reinforce native populations in the sampled area). Details on stocking in the study area are incomplete and largely dependent on personal communications from people involved in the management of the hatcheries or streams in question. Apparently some sampled locations were stocked in the past, but none is currently being directly reinforced.

The primary criterion for collection locations was the likelihood that populations represented descendants of natural reproduction rather than hatchery released fish of exogenous origins. Legal limitations restricted sample sizes to a maximum of twenty-five individuals per collection, and numbers were further restricted by fish availability. Although data interpretations could be limited by these sampling restrictions, early studies of native Iberian brown trout populations with similar sample sizes detected low levels of intrapopulation polymorphism, but great differentiation among populations (Martínez *et al*., 1993; García-Marín & Pla, 1996). The data can be easily evaluated on the basis of its qualitative nature.

Electrophoresis

Liver, skeletal muscle and eye tissues were used. The fish were dissected *in situ* and the tissues maintained in liquid nitrogen before being transported to the laboratory, or were transported whole, frozen in dry ice. The samples were homogenised and then stored at –70 °C before use. Horizontal starch (11%) electrophoretic procedures and visualisation of enzyme activity combined the traditional methods of Aebersold *et al*. (1987) and Pasteur *et al*. (1987), with a few modifications as described in García-Marín *et al*. (1991).

The following thirty-seven enzymes were studied (Commission number in parentheses): Acid phosphatase (3.1.3.2, ACP), Aconitate hydratase (4.2.1.3, AH), Adenylate kinase (2.7.4.3, AK), Alcohol dehydrogenase (1.1.1.1, ADH), Aspartate aminotransferase (2.6.1.1, sAAT), Creatine kinase (2.7.3.2, CK), Diaforase (1.6.4.3, DIA), Esterase (3.1.1.1, EST), Fructose biphosphatase (4.1.2.13, FBP), Fumarate hydratase (4.2.1.2, FH), β-N-acetyl-galactosaminidase (3.2.1.53, βGALA), N-acetyl-β-glucosaminidase (3.2.1.30, βGL UA), Glucose-6-phosphate deshydrogenase (1.1.1.49, G6PDH), Glucose-6-phosphate isomerase (5.3.1.9, GPI), β-Glucuronidase (3.2.1.31, ßGUS), Glutamate dehydrogenase (1.4.1.2, GLUDH), Glutathione reductase (1.6.4.2, GR), Glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12, GAPDH), Glycerol-3-phosphate dehydrogenase (1.1.1.8, G3PDH), Guanine deaminase (3.5.4.3, GDA), Hydroxybutyrate dehydrogenase (3.1.1.31, HBDB), Isocitrate dehydrogenase (1.1.1.42, IDHP), L-Lactate dehydrogenase (1.1.1.27, LDH), Lactoylglutathione lyase (4.4.1.5, LGL), α-Mannosidase (3.2.1.24, αMAN), Malate dehydrogenase (1.1.1.37, sMDH), Malic enzyme-NAD (1.1.1.39, ME), Malic enzyme-NADP (1.1.1.40, MEP), Mannose-6-phosphate isomerase (5.3.1.8, MPI), Peptidase Leucine-tyrosine (3.4.11.-, PEPLT), Proline dipeptidase (phenilalanine-proline substrate, 3.4.13.9, PEPPAP), Tripeptidase (Leucyl-glicyl-glicine substrate, 3.4.11.4, PEPLGG), Phosphoglucomutase (5.4.2.2, PGM), 6-Phosphogluconate dehydrogenase (1.1.1.44, PGDH), Pyruvate kinase (2.7.1.40, PK), Sorbitol dehydrogenase (1.1.1.14, SORD), and Superoxide dismutase (1.15.1.1, SOD). A total of sixty-one loci were resolved; however, detection was not available in all the samples, and so forty-eight loci (including nineteen polymorphic) were used in the analyses. Nomencla-

Table 1 Description of streams where brown trout were sampled

Basin stream	Sample size	Spring density (ind m^{-2})	Spring age mode (Min, Max)	Fishing	Stocking
Tajo					
1. Pelagallinas	17	0,37	0+ (0+, 4+)	No	No
2. Bornova	17	0,05	2+ (l+, 5+)	Yes	Past
3. Palomares	17	0,21	l+ (1+, 5+)	No	No?
4. Guadiela	19	0,06	3+ (l+, 4+)	Yes	Past
5. Dulce	20	0,54	0+ (0+, 4+)	catch-&-release	Past
Duero					
6. Aguisejo	19	0,15	0+ (0+, 4+)	No	Downstream
7. Eresma-1	25	0,42	l+ (0+, 5+)	No	Downstream?
8. Eresma-2	20	0,39	2+ (0+, 4+)	Yes	No?
Hatchery					
9. Uña	29	–	–	–	–

ture for the designation of loci and alleles mainly follows Ferguson (1989) and Shaklee *et al*. (1990).

Most loci were codominantly expressed, permitting direct counts of allele frequencies from gel phenotypes. Allele frequencies for the isoloci *sMDH-3,4** were allocated to individual loci, *sMDH-3** and *sMDH-4**. All variation of the **80* allele was assigned to the *sMDH-3** locus, and frequencies were estimated from the square root of the recessive **100/100* phenotypes, assuming Hardy–Weinberg equilibrium. Frequencies of the **75* allele could be calculated directly from electrophoretic phenotypes and were

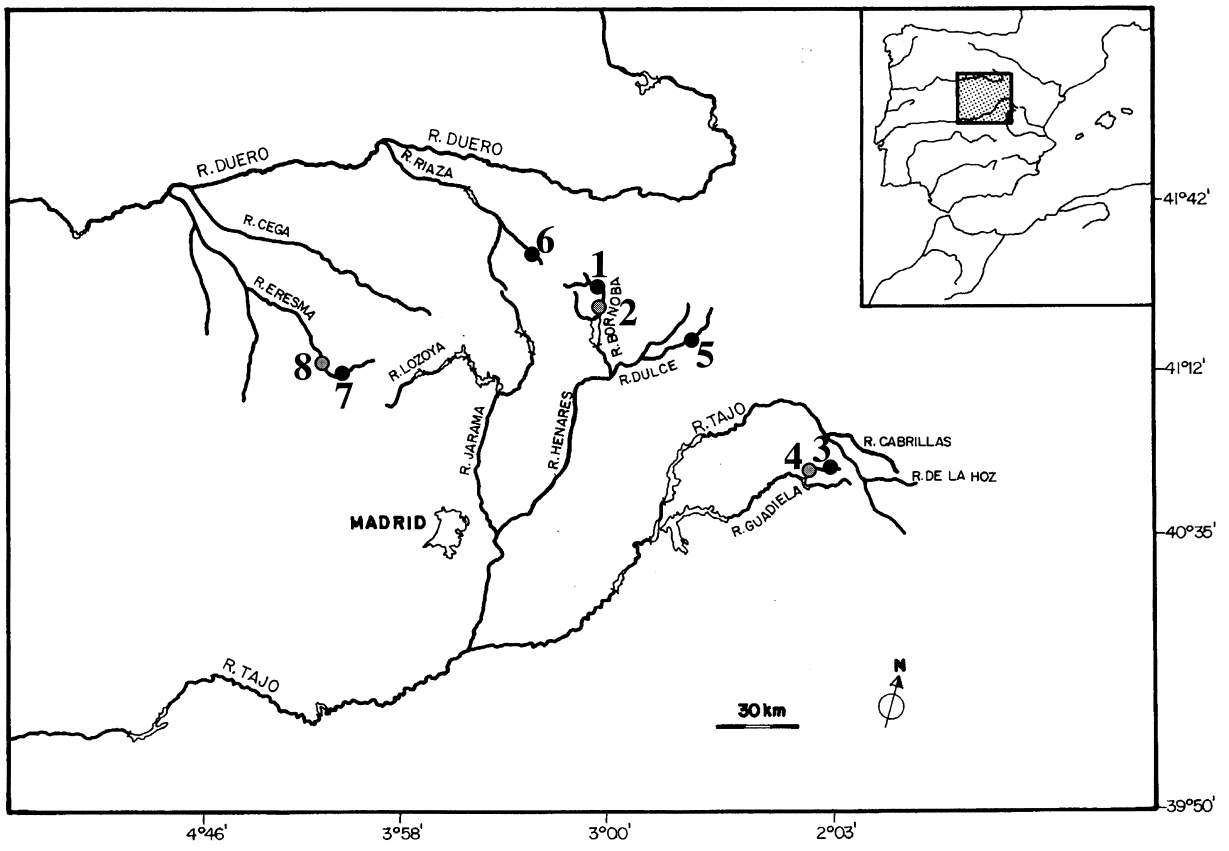

Fig. 1 Sample localities of natural populations analysed. Black dots: preserved fishing areas; grey dots: fenced fishing areas. The references for the populations as in Table 1.

assigned to *sMDH-3** locus as indicated in García-Marín & Pla (1996).

Data analysis

Phenotype distributions of all codominantly expressed loci were tested for agreement with Hardy–Weinberg expectations by X^2 and exact probability tests. The unbiased composite-linkage disequilibrium measure, D (Weir, 1990), examined possible gametic disequilibria for all possible pairs of loci in each population. Wright's *F*-statistics were computed to allocate the genetic variability of brown trout to the total sampled area (T), the locations (S) and individuals (I). The three values F_{IT}, F_{ST} and F_{IS} were calculated according to Weir & Cockerham (1984). Pairwise multilocus comparisons between samples were calculated by Nei's measure of genetic distance (Nei, 1972). Patterns of genetic variation were assessed from the matrix of distances by hierarchical analyses (phenograms using UPGMA) and by a Principal Co-ordinates Analysis. In addition, relationships among populations were cladistically assessed from a matrix of presence/absence of the alleles using Dollo and Wagner methods. Computations were made using BIOSYS-1 (Swofford & Selander, 1989), GENEPOP and its additional software LINKDOS (Raymond & Rousset, 1995), NTSYS-PC (Rohlf, 1993) and DISPAN (Ota, 1993).

Results

Gene diversity within locations

Among the eight natural populations analysed, only Pelagallinas did not exhibit the *LDH-5*90* allele, the primary indicator of hatchery fish (Table 2). This population showed the lowest polymorphism values (H = 0.1%, P = 2.08%, A = 1), which contrasted with values 10 times higher found in the other populations. Low levels of polymorphism typified native unstocked populations of the Duero and Tajo basins analysed in previous studies (García-Marín & Pla, 1996). In general, our values ranged from P = 2.08–29.17% and from H_o = 0.1–5.9% or H_e = 0.1–7.2%. Although these values could be considered relatively low, they are in the range of others found for this species: P = 15–25% (Paaver, 1989), $\overline{p}$ = 26.7%, H = 1.6–8.9% (Skaala & Nævdal, 1989), P = 6–12%, H = 0.4–4.4% (García-Marín *et al.*, 1991), P = 2.9–14.3%, H = 1.2–5.6% (Martínez *et al.*, 1993), H = 0–10.87% (Presa *et al.*, 1994), P = 5.7–25.7%, H_e = 1.93–5.17% (Riffel, Storch & Schreiber, 1995).

Genotype distributions were tested for a total of seventy-five locus/sample combinations (excluding the *sMDH-3** locus) for the Hardy–Weinberg equilibrium. Only six revealed significant deviations (Table 2). This number of rejections is slightly larger than expected at the 5% significance level, and all of them represented a deficit of heterozygotes. Three of the six significant differences occurred in the locus coding for fumarate hydratase in populations Bornova, Guadiela and Eresma-1. Crossbreeding data in brown trout showed that this enzyme system is coded for by two loci (Allendorf *et al.*, 1977) with shared alleles (Krieg & Guyomard, 1985). However, our phenotypes can be explained by the presence of allelic variants at a single locus (*FH**). Due to the tetrameric nature of this enzyme, the zymograms are difficult to interpret (Krieg & Guyomard, 1985). The heterozygote zymograms are usually the most doubtful since they have more bands and less definition than the homozygotes. Consequently, genotypic identification is usually difficult and uncertain. We opted to omit these doubtful samples. This provides a reasonable explanation for the disequilibria observed at the *FH** locus, and therefore we consider the analysed populations as in equilibrium. Significant *D*-values for gametic disequilibrium were observed in 33 (11.6%) of 284 pairs of loci. Most of these disequilibria were a positive association between alleles of hatchery origin or between native ones.

Genic differentiation among locations

The exact probability tests detected significant frequency differences ($P < 0.05$) among the populations in 14 of the 19 polymorphic loci observed in the centre of the Iberian Peninsula. In fact, the *F*-statistics values showed that an important fraction (25%) of the total gene diversity represents interpopulation differences (Table 3). In the Duero basin, the greatest differences were observed at the following loci: *CK-1**, *LDH-5**, *sMDH-2**, *sMDH-3** and *PGM-1**. The differentiation obtained at the first four loci and probably in *AAT-4** and *MPI**, might reflect the different levels of exogenous allele introgression as a result of stocking. The differences detected at the *PGM-1** locus and the

Table 2 Allelic frequencies for the polymorphic loci and parameters of genetic variability (A, P, H). ns, not significant; np, test not possible; *, **, ***, $P < 0.05, 0.01, 0.001$. The references for the populations (POPs) as in Table 1

		TAJO					DUERO			UÑA
LOCI	POPs:	1	2	3	4	5	6	7	8	9
*sAAT-1**	(N)	17	17	17	19	20	19	24	19	2
	100	1.000	0.971	1.000	0.974	0.900	1.000	1.000	1.000	1.000
	105	0.000	0.000	0.000	0.026	0.100	0.000	0.000	0.000	0.000
	130	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	H-W	–	ns	–	ns	ns	–	–	–	–
*sAAT-4**	(N)	17	17	17	18	20	19	25	20	29
	100	1.000	1.000	0.971	0.861	0.975	1.000	0.920	0.800	0.655
	74	0.000	0.000	0.029	0.139	0.025	0.000	0.060	0.125	0.345
	107	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.075	0.000
	H-W	–	–	ns	ns	ns	–	ns	ns	ns
*ADH**	(N)	17	17	17	19	20	19	25	20	29
	100	1.000	1.000	1.000	1.000	0.950	1.000	1.000	1.000	1.000
	40	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000
	H-W	–	–	–	–	ns	–	–	–	–
*AH-2**	(N)	17	17	17	19	20	19	25	20	29
	100	1.000	0.941	1.000	0.974	1.000	1.000	1.000	1.000	1.000
	88	0.000	0.059	0.000	0.026	0.000	0.000	0.000	0.000	0.000
	H-W	–	ns	–	ns	–	–	–	–	–
*CK-1**	(N)	17	17	17	19	20	19	24	20	29
	100	0.000	0.029	0.265	0.263	0.225	0.079	0.708	0.650	0.655
	115	1.000	0.971	0.735	0.737	0.775	0.921	0.292	0.350	0.345
	H-W	–	ns	ns	ns	ns	ns	ns	ns	*
	EXACT									*
*FH**	*(N)*	17	17	16	19	20	19	20	2	29
	100	0.971	0.765	0.781	0.526	0.925	0.974	0.850	1.000	1.000
	84	0.029	0.235	0.094	0.026	0.000	0.000	0.150	0.000	0.000
	124	0.000	0.000	0.125	0.447	0.075	0.026	0.000	0.000	0.000
	H-W	ns	**	ns	*	ns	ns	**	–	–
	EXACT		*		*			*		
*G3PDH**	(N)	17	17	17	19	20	19	25	20	29
	100	1.000	1.000	1.000	0.816	0.925	1.000	0.960	1.000	0.810
	50	0.000	0.000	0.000	0.184	0.075	0.000	0.040	0.000	0.190
	H-W	–	–	–	ns	ns	–	ns	–	ns
βGALA*	(N)	17	17	17	19	20	19	25	18	29
	100	1.000	1.000	0.853	0.974	1.000	0.974	1.000	1.000	0.362
	95	0.000	0.000	0.147	0.026	0.000	0.026	0.000	0.000	0.638
	H-W	–	–	ns	ns	–	ns	–	–	ns
*GPI-2 **	(N)	17	17	17	19	20	19	25	20	29
	100	1.000	1.000	1.000	1.000	0.900	1.000	0.860	0.875	1.000
	130	0.000	0.000	0.000	0.000	0.100	0.000	0.140	0.125	0.000
	H-W	–	–	–	–	ns	–	ns	ns	–
*GPI-3 **	(N)	17	17	17	19	20	19	25	20	29
	100	1.000	1.000	1.000	1.000	1.000	1.000	0.980	0.975	0.966
	110	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.025	0.034
	H-W	–	–	–	–	–	–	ns	ns	ns
*IDHP-1 **	(N)	17	17	17	19	20	19	25	20	29
	100	1.000	1.000	1.000	0.947	1.000	1.000	1.000	1.000	1.000
	85	0.000	0.000	0.000	0.053	0.000	0.000	0.000	0.000	0.000
	H-W	–	–	–	ns	–	–	–	–	–

Table 2. Continued

		TAJO					DUERO			UÑA
LOCI	POPs:	1	2	3	4	5	6	7	8	9
*LDH-5**	(N)	17	17	17	19	20	19	25	20	29
	100	1.000	0.941	0.706	0.763	0.875	0.789	0.980	0.925	0.034
	90	0.000	0.059	0.294	0.237	0.100	0.211	0.020	0.025	0.966
	110	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.050	0.000
	H-W	–	ns	ns	ns	ns	ns	ns	ns	ns
αMAN*	(N)	17	17	15	19	20	19	21	19	29
	100	0.000	0.059	0.833	0.474	0.950	1.000	1.000	1.000	1.000
	90	1.000	0.941	0.167	0.526	0.050	0.000	0.000	0.000	0.000
	H-W	–	ns	ns	ns	***	–	–	–	–
	EXACT					*				
*sMDH-2**	(N)	17	17	17	19	20	19	25	20	29
	100	1.000	1.000	0.971	0.947	0.900	0.895	1.000	1.000	0.862
	152	0.000	0.000	0.029	0.053	0.100	0.105	0.000	0.000	0.138
	H-W	–	–	ns	ns	*	ns	–	–	ns
	EXACT					ns				
*sMDH-3**	(N)	17	17	17	19	20	19	24	20	29
	100	1.000	1.000	1.000	0.816	0.825	0.842	0.896	0.950	0.724
	75	0.000	0.000	0.000	0.000	0.000	0.000	0.104	0.050	0.000
	80	0.000	0.000	0.000	0.184	0.175	0.158	0.000	0.000	0.276
	H-W	–	–	–	np	np	np	ns	ns	np
*MPI**	(N)	17	17	17	19	20	19	24	18	28
	100	1.000	1.000	0.853	0.947	0.800	0.947	0.771	0.722	0.732
	105	0.000	0.000	0.147	0.053	0.200	0.053	0.229	0.278	0.268
	H-W	–	–	ns	ns	ns	ns	**	ns	*
	EXACT							**		ns
*PEP-LT**	(N)	17	17	17	19	20	19	25	13	29
	100	1.000	1.000	1.000	1.000	0.850	0.947	1.000	1.000	1.000
	70	0.000	0.000	0.000	0.000	0.150	0.053	0.000	0.000	0.000
	H-W	–	–	–	–	ns	ns	–	–	–
*PGDH-2**	(N)	17	17	17	19	20	19	25	19	29
	100	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000
	85	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000
	H-W	–	–	–	–	ns	–	–	–	–
*PGM-1**	(N)	17	17	17	19	20	19	25	19	29
	100	1.000	1.000	0.882	0.711	1.000	1.000	0.720	0.842	1.000
	80	0.000	0.000	0.118	0.289	0.000	0.000	0.280	0.158	0.000
	H-W	–	–	ns	ns	–	–	ns	ns	–
	Ho	0.001	0.012	0.043	0.059	0.051	0.027	0.039	0.039	0.051
	He	0.001	0.017	0.047	0.072	0.053	0.026	0.045	0.041	0.059
	He(u)	0.001	0.017	0.049	0.074	0.054	0.026	0.046	0.042	0.060
	P	2.08	12.50	18.75	29.17	29.17	16.67	20.83	16.67	18.75
	P(0.05)	0.00	8.33	14.58	22.92	25.00	12.50	14.58	14.58	14.58
	A	1.0	1.1	1.2	1.3	1.4	1.2	1.2	1.2	1.2

difference obtained at *sMDH-3** and *GPI-2** appear to be associated with a divergence between the indigenous populations of the Eresma and Aguisejo rivers, and to their genetic erosion due to the incorporation of hatchery fish.

In the Tajo basin, the observed differentiation was higher, affecting 13 of the 18 polymorphic loci in this basin. Nevertheless, it seems to be due to similar causes: the divergence among the native populations of these rivers (e.g. *PGM-1**, *FH**), hatchery genes

Table 3 Distribution of electrophoretically detected genetic variation, F_{ST} based, in wild brown trout populations from Central Spain.'sig' indicates significant genic differentation ($P < 0.05$)

Locus	Total F_{ST}	Duero F_{ST}	Tajo F_{ST}	Duero-Tajo (pooled data)
*sAAT-1**	0.04428 sig		0.03475 sig	0.01782
*sAAT-4**	0.05055 sig	0.05673 sig	0.05393 sig	0.01108 sig
*ADH**	0.02513		0.02220	0.00289
*AH-2**	0.01883		0.01269	0.00758
*CK-1**	0.33381 sig	0.38950 sig	0.09329 sig	0.23333 sig
*FH**	0.16073 sig	0.04733	0.17739 sig	0.03815 sig
*G3PDH**	0.08969 sig	0.01409	0.10272 sig	0.01478
β*GALA**	0.08182 sig	0.00332	0.09612 sig	0.00749
*GPI-2**	0.06620 sig	0.04566 sig	0.07398 sig	0.04470 sig
*GPI-3**	– 0.00792	– 0.01344		0.01220
*IDHP-1**	0.02828		0.02563	0.00289
*LDH-5**	0.08100 sig	0.10195 sig	0.08050 sig	0.00630
α*MAN**	0.74303 sig		0.64230 sig	0.47724 sig
*sMDH-2**	0.03184 sig	0.09171 sig	0.01397	– 0.00694
*sMDH-3**	0.06811 sig	0.04044 sig	0.10567 sig	0.00572 sig
*MPI**	0.08022 sig	0.05852 sig	0.08485 sig	0.04365 sig
*PEP-LT**	0.08558 sig	0.02944	0.11605 sig	– 0.00387
*PGDH-2**	– 0.00158		– 0.00364	– 0.00214
*PGM-1**	0.13913 sig	0.12164 sig	0.18569 sig	0.01981 sig
combined	0.25295 sig	0.15052 sig	0.22805 sig	0.15418 sig

introgression (*G3PDH*50*, *CK-1*100*, βGALA-2**95*, *LDH-5*90* and *sMDH-3*80*) and the resulting elimination of autochthonous alleles, especially *aMAN*90*. The Bornova river (samples Pelagallinas and Bornova) appeared to have maintained its ancestral origin, while the Guadiela and Dulce rivers (samples Palomares, Guadiela and Dulce) populations were more affected by stocking with foreign fish.

The genetic distances (Nei, 1972) ranged from 0.001 (between the two samples taken from the Bornova River or the two taken from Eresma River) to 0.068 (between the Pelagallinas population and the exogenous hatchery stock). As expected, the values diminish when we exclude the hatchery stock; the largest distance found between two natural populations was 0.036 between Pelagallinas from the Tajo basin and Eresma-1 from the Duero basin. These values are similar to those detected among populations of geographically comparable separation: 0.006–0.016 (in Northern Ireland, Crozier & Ferguson, 1986), 0.000–0.020 (western Norway, Hindar *et al*., 1991), 0.001–0.042 (north-western Spain, Martínez *et al*., 1993), 0.000–0.037 (Denmark, Møller-Hansen *et al*., 1993), or 0.001–0.029 (south-western Germany, Riffel *et al*., 1995).

Although the greatest similarities in the dendrogram occurred between some paired samples from common rivers (Pelagallinas-Bornova, Eresma-1-Eresma-2), no broad geographic patterns were apparent. Some Tajo basin samples are linked with the Duero basin populations, and only the two Bornova river samples seem to constitute one clearly differentiated branch among the native populations (Fig. 2). When non-hierarchical or semihierarchical forms of genic variation are responsible for geographic genetic structure, hierarchical algorithms are somewhat limited for the study of that structure (Lessa, 1990). This situation probably occurs in our study area, where releases of hatchery fish have superimposed a radial

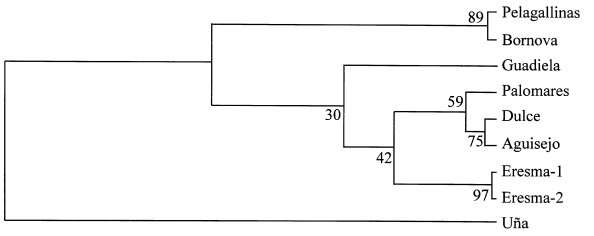

Fig. 2 UPGMA dendrogram of the nine localities based on Nei's distances. Branch numbers represent Bootstrap values (100 repetitions).

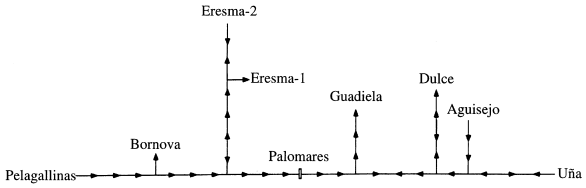

Fig. 3 Wagner tree based on the presence/absence of alleles.

pattern of gene dispersion from a common hatchery origin onto a presumably ancestral hierarchy among the populations associated with river basins. The spread of hatchery genes due to stocking is apparent in the Wagner tree obtained from the matrix of allele presence/absence (Fig. 3), where the populations with higher polymorphism levels (Dulce, Guadiela) or without native Spanish polymorphism (Aguisejo) have high *LDH-5*90* allele frequency (Table 2) and are the most closely related to the German stock in the tree. Thus, the structure due to the release of foreign trout (and the introgression of their characteristic genes) seems stronger than the natural structure found for different fishes in these basins (Machordom, Doadrio & Berrebi, 1995).

Recognition of the limitations of hierarchical algorithms has prompted geneticists to use ordination techniques to analyse non-hierarchical patterns of geographic genetic structure. In this sense, the results of the principal co-ordinate analyses (Fig. 4) are clearer than the dendrogram (Fig. 2). The samples

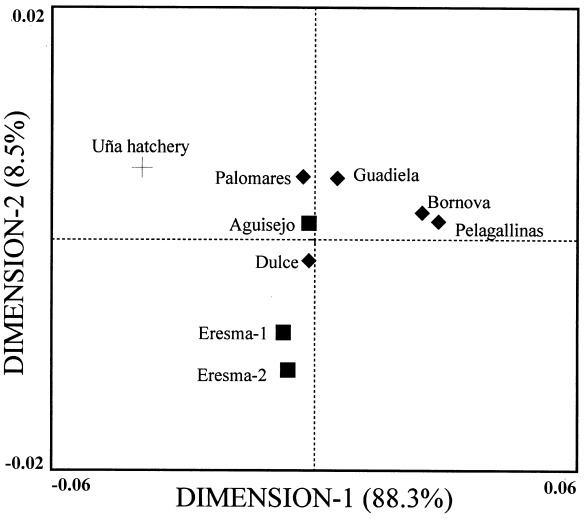

Fig. 4 Principle Co-ordinate analysis of trout populations analysed based on Nei's genetic distances. Populations are projected onto the plane formed by the first two principal co-ordinates axes.

from the Bornova river and Eresma river populations, which are the least affected by stocking in the Tajo and Duero basins, were the most separate in the plane constituted by the two first co-ordinates as a result of the genetic differentiation described for both basins. The principal co-ordinate projections tended to draw together in a central position the four populations from streams where stocking have been more successful (Table 2).

Discussion

Hatchery introductions and native gene diversity

Spanish hatchery stocks originated from brown trout imported from Germany, where subsequent exchanges among hatcheries have resulted in a high degree of genetic homogeneity among stocks (García-Marín *et al.*, 1991; Morán *et al.*, 1995). Previous studies indicated fixed allelic differences for the *LDH-5** locus between pure hatchery (*LDH-5*90*) and pure native (*LDH-5*100*) Iberian populations (García-Marín *et al.*, 1991; Morán *et al.*, 1991; Martínez *et al.*, 1993; García-Marín & Pla, 1996). Nevertheless, in the Uña hatchery stock the Spanish native allele **100* was detected with a frequency of 3.4%. This result could be related to hybridisation between native brown trout and foreign stocks (F. Alonso, personal communication) or to the source of this foreign trout (Hamilton *et al.*, 1989; Riffel *et al.*, 1995). However, this hardly influences the conclusions given below about the role played by stocking in the study area.

All the populations analysed, except Pelagallinas, exhibited the hatchery allele *LDH-5*90*, and other alleles indicating hatchery fish (*G3PDH*50*, *sMDH-2*152*, *sMDH-3*80*, García-Marín *et al.*, 1991; Martínez *et al.*, 1993) were observed in the populations Palomares, Guadiela, Dulce and Aguisejo (Table 2). These results confirm that the populations were affected by the stocking described in Table 1. Moreover, the lack of Hardy–Weinberg disequilibrium suggest that some introduced individuals could survive in the rivers and hybridise with native trout, thereby introducing exogenous genes into the natural populations. However, native alleles (*GPI-2*130*, *aMAN*90*, *MDH-3*75*, *PGM-1*80*), some times in moderate or high frequency, were present in all the locations and still contribute to their differentiation.

The observed gametic disequilibria in populations

apparently under Hardy–Weinberg conditions probably indicate that the studied wild populations have a reduced effective number of individuals randomly reproducing. The larger proportions of significant *D*-values were observed in populations Bornova (2 out of 15), Guadiela (10 out of 78) and Aguisejo (5 out of 21). These populations presented the lowest values of spring density and in Bornova and Guadiela no individuals of the 0+ class were observed (Table l). This fact suggests that some years these populations could suffer serious recruitment problems, and consequently genetic drift might explain the observed gametic disequilibria. As deduced from Table 1, these recruitment failures seem to be associated with fishing activities, with the harvested populations presenting the oldest fish for minimum spring age.

The Duero and Tajo basins have been classified into two separate subgroups of the same area of freshwater fish biogeography of the Iberian Peninsula (Doadrio, 1988). Our results confirm previous genetic differences between these two basins. The absences of the *aMAN*90* allele in the Duero basin and of the *sMDH-3*75* allele in the Tajo basin have been already reported by García-Marín & Pla (1996). However, the latter allele presents lower frequencies than those found by these authors, and was not found in the Aguisejo population. Genic differences in the pooled frequencies between the Duero and Tajo basins, which mask intrabasin differentiation, were observed at 8 out of 19 loci (Table 3). This reduction in the proportion of significant loci, such as those detected in the two intrabasin comparisons, is related to both the existence of an ancient divergence between the two basins (e.g. *aMAN** locus $F_{ST} = 0.477$) and to a similar effect of hatchery transplantations resulting from the common policies and strategies of fish management of these two basins.

Management implications

The above results establish the existence of significant gene diversity within and between the Duero and Tajo basins. However, the stocking carried out with exogenous hatchery trout replaced the Spanish indigenous genetic patrimonies and favoured the homogenisation of the respective gene pools, reducing genetic differentiation among the native populations (as established by Ferguson, 1990). These introductions are the result of constant and growing political pressures to satisfy the demands of sport anglers rather than an alternative to understanding the underlying causes of a population's inability to sustain a fishery. Management, in such cases, has focused on stocking in supposedly underpopulated waters.

In our study area, the lack of juveniles (younger than +2 class) in several populations in spring led us to assume the existence of recruitment problems in Bornova, Guadiela and Eresma-2 locations. These problems seem, *a priori*, to be a good excuse for the re-stocking that has been carried out (Table 1). However, this restocking procedure has involved several accumulated errors that are contrary to the objectives of a good management programme for the area, which should serve to optimise sport fishing while simultaneously permitting the genetic resources of the species to be conserved. These resources provide the raw material for the evolutionary future of the species and for the development of brown trout aquaculture for human consumption, but the use of exogenous hatchery stocks has genetically eroded some locations (e.g. Guadiela). This fact could cause the extinction of this population as an autochthonous genetic pool (Rhymer & Simberloff, 1996). On the other hand, the scarce presence of exogenous genes (e.g. *LDH-5*90*) in two populations stocked in the past (Bornova and Eresma-2) indicates that stocking is not an adequate solution to the recruitment problems since no density increase occurred. Despite the reduced genetic effect of the exogenous stocks on the native gene pools in these locations, fish from these populations are not free from other threats such as introduced diseases or resource competition from stocked fish or reduced native fishes productivity (Leary, Allendorf & Forbes, 1993). According to our data, recruitment problems are observed now when populations are exploited but not stocked. Therefore, we think that harvesting, and more probably overharvesting, is responsible for the observed recruitment failures. In this sense, the population of the Dulce river, with a stocking history similar to the above mentioned populations but in which 'catch-and-release' is practised, does not have these problems, and presents the highest fish density in spring.

There needs to be a change in the current management programme undertaken for these populations, which focuses on exploitation with general criteria, involves broad geographical territories and does not reproduce the differences detected among the trout

populations. The selected programme has to recognise these singularities and the demographic fluctuations in the populations, and to allow exploitation sustained by natural reproduction. This programme undoubtedly needs to involve the cataloguing and constant screening of the existing populations to determine year by year their contributions to the brown trout fishery.

Initially, it would be more prudent to try to increase population size through habitat improvement and restrictive regulations rather than hatchery introductions (Leary *et al*., 1993). If the populations cannot be restored through different 'natural' actions, such as restoring spawning sites and preventing water pollution, other measures could be implemented. As long as the demand for supplementation of recreational fishing through hatchery production persists, we propose the replacement of exogenous hatchery stocks with local native ones. On the basis of our findings, we recommend the founding of at least two new stocks that reproduce the genetic distinction between the Tajo and Duero basins. This would be a major step towards a more efficient output of catchable fish and more effective protection of native gene pools. Nevertheless, even this measure has to be adopted with caution by starting the program with an adequate number of selected breeding individuals. Genetic variation is necessary for organisms to adapt to changing environments, thus making maintenance of genetic diversity the primary objective of genetic management (Templeton, 1990).

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