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Stocking impact and allozyme diversity in brown trout from Mediterranean southern France

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Analysis of trout from 13 stations on the Mediterranean slopes of the French Pyrenees by 31 presumptive enzyme loci demonstrated the major impact of restocking programmes. Although the annual introgression resulting from these introductions was small, the accumulation of genes of Atlantic origin has resulted in a change in allele frequencies. Genetic disequilibria within and between loci exist. Introgression by genes of domestic (hatchery) origin varied from 0 to 77% among stations. Natural Mediterranean populations show no detectable geographical structure. There was a direct relation between the degree of introgression and heterozygosity. However, restocking could not explain all of the observed genetic disequilibria.

Key words: stocking; allozymes; *Salmo trutta*; Mediterranean rivers; introgression.

INTRODUCTION

The brown trout *Salmo trutta* L. is widely distributed in western Europe (Blanc *et al.*, 1971). Because of fishery interest, the species has been cultured for restocking for about 150 years, both within and outside its natural range (Baglinière, 1991). The description of the natural biodiversity of this taxon therefore requires that the genetic contribution of cultured stocks is distinguished from the contribution of the native populations.

Since the middle of the 1970s (Allendorf *et al.*, 1976, 1977) brown trout have been subject to genetic analyses using allozymes, which have demonstrated the existence of several genetically different populations. Many regions within the distribution range have been analysed using these markers including drainages in Scandinavian countries, the British Isles and several southern peninsulas. The first analyses in France date from 1983 (Guyomard & Krieg, 1983). This study, plus others (Krieg & Guyomard, 1985), showed the existence of two forms of trout with different fixed alleles at several diagnostic loci: the populations of the French Mediterranean catchment are characterized by the alleles LDH5*(100), TF*(102) and, with a few exceptions, FBP1*(150), whereas the populations in catchments draining towards the Atlantic, so-called modern Atlantic populations, have LDH5*(90), TF*(100) and FBP1*(100) as fixed alleles. In Brittany and in south-western France (and in the Atlantic catchments of the Iberian Peninsula) a so-called ancestral Atlantic form is characterized in terms of

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enzymes by a single marker LDH5*(100), the markers *TF** and *FBP1** bearing alleles occurring in the rest of the Atlantic region (Guyomard, 1989; Hamilton *et al.*, 1989; Poteaux *et al.*, 1998).

These loci have a high discrimination value, since the great majority of domestic strains belong to the modern Atlantic type. They were derived from northern Atlantic European populations which, in turn, were derived from northern European trout. It is therefore possible to evaluate the domestic gene flow in the Mediterranean populations.

Southern France has been explored relatively little to date; two stations in the Massif Central have been analysed (Barbat-Leterrier *et al.*, 1989; Guyomard, 1989); five in the Languedoc region (Barbat-Leterrier *et al.*, 1989; Guyomard, 1989; Beaudou *et al.*, 1994; Poteaux *et al.*, 1998), three in the southern Alps (Poteaux *et al.*, 1998), two in the eastern Pyrenees (Barbat-Leterrier *et al.*, 1989) and eight in Corsica (Krieg & Guyomard, 1983, 1985; Guyomard, 1989).

The present work is part of a larger study of the biogeographical structure of trout in southern France. To do this, 13 localities were sampled belonging to four river catchments in the French Pyrenees: the Aude, Agly, Têt and the Tech, which flow to the French Mediterranean coast, plus the Ebro, which flows to the Spanish Mediterranean coast (Fig. 1). The aim was to describe the natural genetic diversity of trout from these watercourses by attempting to identify the artificial inputs from restocking.

MATERIALS AND METHODS

DATA ACQUISITION

Electrofishing was conducted in French Mediterranean rivers in April, June and December 1993 and in June 1994 (Table I and Fig. 1). The protein electrophoreses were conducted on horizontal starch gels as in Pasteur *et al.* (1987). The enzyme proteins (in this case 15 systems allowing the detection of 31 presumptive loci) were revealed using the method developed by Krieg (1984) as modified by Beaudou (1993) and Poteaux (1995).

SAMPLE CONSTITUTION

Most stations sampled are regularly restocked. However, very few domestic trout reach adult age and breed in southern France (estimated at <0·5% by Beaudou, 1993). Trout which possessed a typically modern Atlantic multilocus genotype were considered to be artificially introduced and therefore not representative of the native population nor of its future composition because of their low chance of survival. These presumed introduced domestic trout were homozygous for Atlantic alleles at three diagnostic loci (the multilocus genotype LDH5*(90/90), TF*(100/100), FBP1*(100/100)) and were excluded from the analyses. The probability of this domestic multilocus genotype being formed anew by recombination, even in a highly introgressed population, is very low. The sample size after exclusion of presumed domestic trout varied between eight and 30 (Table I).

ESTIMATE OF INTROGRESSION (I)

It was assumed that the genetic composition of natural populations (before interference by man) had fixed diagnostic Mediterranean alleles at *LDH5**, *TF** and *FBP1**. The mean degree of introgression (*I*) was calculated from the mean percentage of Atlantic alleles at these three diagnostic loci. A variable, but limited, occurrence of Mediterranean alleles in the hatchery strains in the region (Beaudou *et al.*, 1994; Poteaux *et al.*, 1998) and the improbable natural occurrence of Atlantic alleles in the wild Mediterranean

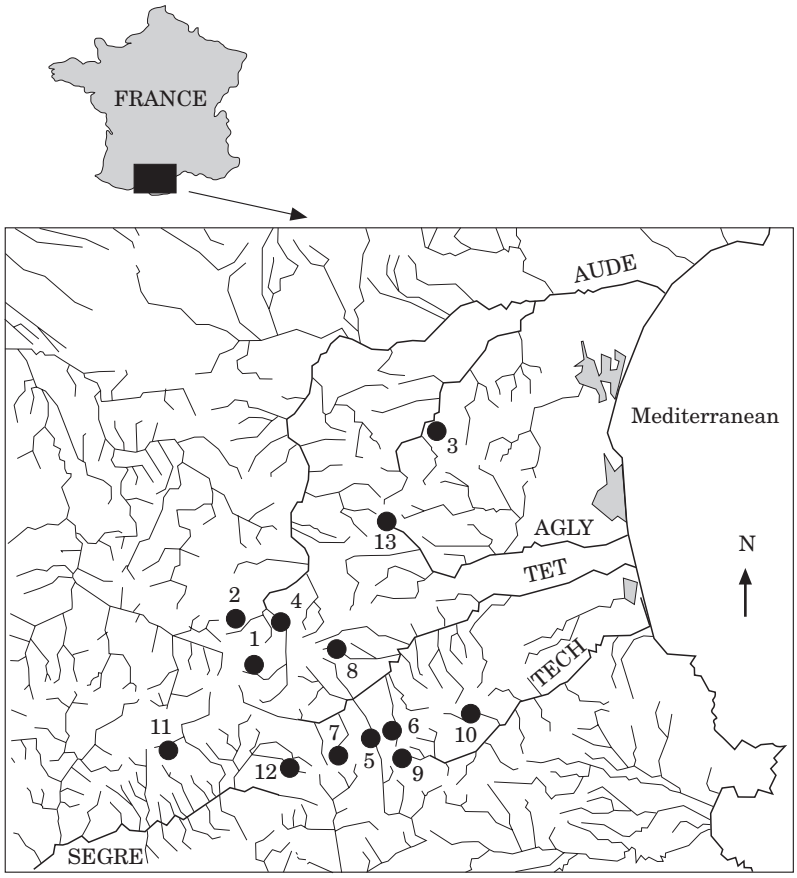

FIG. 1. Map of sampling stations: 1, Lladure; 2, Boutadiol; 3, Orbieu; 4, Aude; 5, Alemany; 6, Campeilles; 7, Carança; 8, Nohèdes; 9, Tech; 10, Riuferrer; 11, Campcardos; 12, Eyne; 13, Agly.

populations were not taken into account in the estimates. Both these biases would tend to underestimate slightly the degree of introgression, especially for the *FBP1** locus.

ESTIMATE OF THE RELATIVE AGE OF INTROGRESSION (WMD)

During the first few years after restocking a given site, the percentage introgression at each diagnostic locus should be identical. With time, through selection, genetic drift or migration, these percentages should evolve and diverge to present different introgression rates. Therefore the mean deviation in introgression rates between the diagnostic markers was considered to be related to the time elapsed since the main introductions. This relationship is probably altered by other local factors such as population size and environmental factors influencing the mechanisms of divergence between loci, but these factors could not be estimated in this study. To take into account the variable degree of introgression among populations, the mean deviation was weighted by the introgression rate I. The formula for the index of introgression age (WMD=weighted mean deviation in introgression) was therefore:

$$\frac{|I_{\mathrm{LDH5*}} - I_{\mathrm{TF*}}| + |I_{\mathrm{LDH5*}} - I_{\mathrm{FBP1*}}| + |I_{\mathrm{TF*}} - I_{\mathrm{FBP1*}}|}{I_{\mathrm{LDH5*}} + I_{\mathrm{TF*}} + I_{\mathrm{FBP1*}}}$$

TABLE I. Description of sampling sites and fish samples

River	Station	Map code	Altitude (m)	Numbers caught	Numbers retained*
Aude	Lladure	1	1770	29	21
	Boutadiol	2	1630	30	25
	Orbieu	3	400	25	22
	Aude	4	915	30	30
Têt	Alemany	5	1550	30	11
	Campeilles	6	1250	8	8
	Carança	7	2189	28	28
	Nohèdes	8	1980	30	16
Tech	Tech	9	1200	28	18
	Riuferrer	10	1000	28	27
Ebre	Campcardos	11	1750	31	22
	Eyne	12	1710	30	24

*Numbers retained after eliminating trout recognized as coming directly from a hatchery (see text).

CALCULATION OF POPULATION GENETIC PARAMETERS

The within-sample deviation from Hardy–Weinberg proportions was tested by Wright's F_{is} (1951). The software program used, GENETIX (Belkhir *et al.*, 1998), calculates the f estimator of Weir & Cockerham (1984). The significance of the result is estimated by making Monte-Carlo type permutations of alleles within each sample: 1000 random permutations without re-inclusion were made, leading to 1000 matrices of fictional data. Then the values of the parameter (f) calculated for each matrix were compared with the original matrix. The proportion of fictional values that were equal to or greater than the observed value was the probability that the observed value could be explained by chance alone.

Linkage disequilibria and associations between alleles belonging to different loci, were calculated from the coefficient of genotypic disequilibrium (Cockerham & Weir, 1979) using the GENETIX software. The ability to detect such disequilibria depends on the allele frequencies and on sample size (Weir, 1979). Therefore loci analysed with <20 individuals and those showing a dominant allele with a frequency of >0·9 were excluded.

Differences between samples were calculated using the value of Wright's F_{ST}. It was estimated from the θ parameter of Weir & Cockerham (1984). The significance of the values obtained was estimated using the permutation method described above. Nei's (1978) genetic distance (D) was calculated between pairs of samples. A phenetic classification was produced from these distances by means of the neighbour joining method (Saitou & Nei, 1987) using the PHYLIP 3.5 package (Felsenstein, 1993). Bonferroni's correction was applied to all multiple tests, using the sequential method of Rice (1989).

RESULTS

POLYMORPHISM

Twelve loci were polymorphic by the 95% criterion (Table II). For *CK1** and *MDH3** not all the genotypes were detectable and the allele frequencies were recalculated from identifiable homozygote genotypes, assuming Hardy–Weinberg proportions. The *GP12** locus had a high frequency of null allele at a single station (station no. 11, Campcardos, Ebro catchment), allowing a similar calculation of the allele frequencies. *MDH1** was only 99% polymorphic while

TABLE II. Allele frequencies at 12 polymorphic loci (95% criterion), expected heterozygosity, F_{is} values estimated from the parameter f and its significance obtained by permutations (P)

Populations	1	2	3	4	5	6	7	8	9	10	11	12
n	*21*	*25*	*21*	*30*	*10*	*8*	*24*	*16*	*17*	*26*	*22*	*24*
*AAT4**												
65	0·12	—	0·1	—	0·25	—	—	0·06	0·12	0·13	0·07	—
100	0·88	1	0·9	1	0·75	1	1	0·94	0·88	0·87	0·93	1
(*CK1**)												
100	1	1	0·63	1	1	1	1	0·57	1	1	1	1
125	—	—	0·37	—	—	—	—	0·43	—	—	—	—
*FBP1**												
100 (A)	0·24	0·20	0·88	0·02	0·45	—	—	0·75	0·39	0·06	0·42	0·14
150 (M)	0·76	0·80	0·12	0·98	0·55	1	1	0·25	0·61	0·94	0·58	0·86
*FH1**												
100	0·95	0·95	0·86	1	0·68	0·81	1	0·66	0·83	0·98	0·82	0·9
135	0·05	0·05	0·14	—	0·32	0·19	—	0·34	0·17	0·02	0·18	0·1
*G3PDH2**												
50	0·02	—	0·11	—	0·05	—	—	0·09	0·03	—	0·02	—
100	0·98	1	0·89	1	0·95	1	1	0·91	0·97	1	0·98	1
*LDH5**												
90 (A)	0·12	0·04	0·32	0·02	0·45	—	—	0·59	0·36	0·23	0·19	0·18
100 (M)	0·86	0·96	0·68	0·98	0·55	1	1	0·41	0·58	0·77	0·81	0·82
110 (M)	0·02	—	—	—	—	—	—	—	0·06	—	—	—
*MDH2**												
100	0·93	1	1	1	0·95	1	1	0·81	0·92	1	0·97	0·9
200	0·07	—	—	—	0·05	—	—	0·19	0·08	—	0·03	0·1
(*MDH3**)												
075	0·05	0·23	0·1	0·02	0·09	—	—	0·29	0·15	0·02	0·1	—
100	0·95	0·77	0·9	0·98	0·91	1	1	0·71	0·85	0·98	0·9	1
*MPI**												
100	1	0·98	0·59	1	0·68	1	1	0·57	1	0·87	0·94	0·77
105	—	0·02	0·41	—	0·32	—	—	0·43	—	0·13	0·06	0·23
(*PGI2**)												
1†	—	—	—	—	—	—	—	—	—	—	0·66	—
100	1	—	1	1	0·8	1	1	0·97	1	1	0·33	1
200	—	—	—	—	0·2	—	—	0·03	—	—	—	—
*SOD1**												
50	—	—	—	—	0·05	—	—	—	—	—	—	—
100	1	1	1	1	0·95	1	1	1	1	1	1	1
*TF**												
100 (A)	0·35	0·36	0·73	—	0·50	—	—	0·97	0·25	0·28	0·58	0·19
102 (M)	0·65	0·64	0·27	1	0·50	1	1	0·03	0·75	0·72	0·42	0·81
H_e	0·055	0·037	0·08	0·002	0·12	0·011	0	0·1	0·07	0·05	0·08	0·056
F_{is}	0·42	0·58	0·09	—	0·42	—	—	0·08	0·45	0·21	0·52	0·32
P	**	**	NS	—	**	—	—	NS	**	NS	**	**

NS, Not significant; **P<0·01; loci in parentheses: allele frequencies calculated from frequency of one homozygote genotype (see text); †null allele; (A), diagnostic allele for Atlantic populations; (M), diagnostic allele for Mediterranean populations.

TABLE III. Degree of introgression by domestic genes at the 12 sampling stations and weighted mean deviation (WMD) between introgression per locus

Station	Map code	% Atlantic alleles			*I*	WMD
		*LDH5**	*TF**	*FBP1**		
Lladure	1	12	35	24	23·7	0·65
Boutadiol	2	4	36	20	20	1·07
Orbieu	3	32	73	87	64	0·57
Aude	4	2	0	2	1	—
Alemany	5	45	50	45	46·7	0·07
Campeilles	6	0	0	0	0	—
Carança	7	0	0	0	0	—
Nohèdes	8	59	97	75	77	0·33
Tech	9	36	25	39	33·3	0·28
Riuferrer	10	23	28	6	19	0·77
Campcardos	11	19	58	42	39·7	0·66
Eyne	12	18	19	14	17	0·20

Samples in which the mean introgression rate (*I*) was <5% were not used in the calculation of WMD.

the following loci were monomorphic: *AAT1**, *AAT2**, *ADH**, *CK2**, *CK3**, *FBP2**, *IDH1**, *IDH2**, *IDH3**, *IDH4**, *LDH1**, *LDH2**, *LDH4**, *MDH4**, *6PGDH**, *GP11**, *GP13**, and *PGM**.

DEGREE OF INTROGRESSION

The introgression rates (Table III) were often higher than those observed in the Orb basin by Beaudou *et al.* (1994) and Poteaux *et al.* (1998) and exceeded 75% at the Nohèdes (no. 8) station.

The indices of introgression age (WMD, Table III, last column) suggest that introductions took place earliest at Boutadiol (2), followed by Riuferrer (10), Lladure (1), Campcardos (11) and Orbieu (3). Some stations had only very slight deviations showing that the introgression was recent, even though the impact could be high in Alemany (5), low or even absent in Aude (4), Carança (7) and Campeilles (6).

The high introgression rates (33–77%) probably result from the earliest introductions. The index of introgression age is a relative one and cannot provide dates. The Alemany station had a combination of high introgression rate and a low (i.e. young) index of introgression.

EFFECT OF RESTOCKING ON GENETIC DIVERSITY

The level of genetic variation, as measured by expected heterozygosity (H_e), varied from 0 to 0·12, which is high compared with values recorded in studies of other Mediterranean populations. There was a clear relationship between the estimated introgression rate and heterozygosity (r^2=0·782; P<0·001; Fig. 2). This can be explained by the fact that Mediterranean populations are always less polymorphic than hatchery strains. This relationship still existed when the

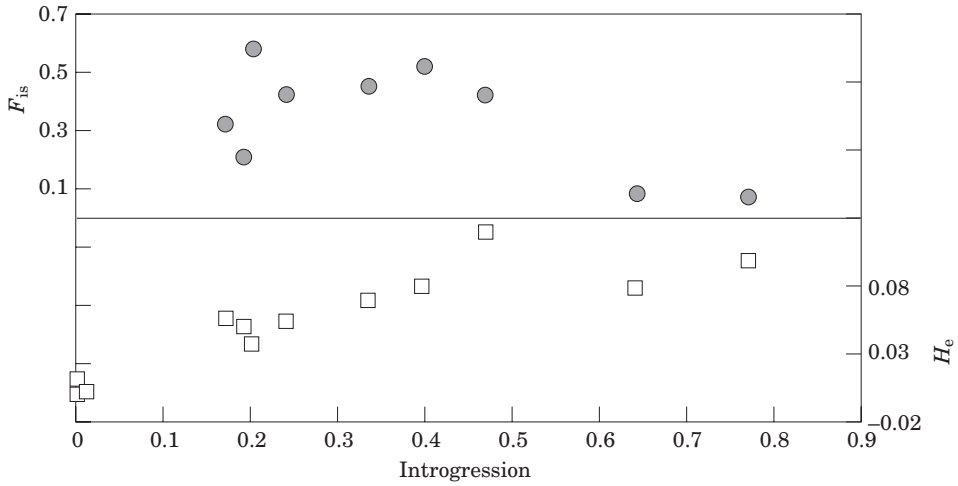

FIG. 2. Squares and right axis: relation between expected heterozygosity (H_e) and introgression rate (r^2=0·782, P<0·001). Circles and left axis: relation between F_{is} values and introgression rates (r^2=0·122, P=0·443).

diagnostic loci were excluded from the calculation of expected heterozygosity (data not shown), indicating that enrichment by hatchery alleles also occurred for non-diagnostic loci.

POPULATION GENETIC DISEQUILIBRIA

F_{is} values and their significances were estimated separately for each sample (Table II). The deficits in heterozygotes were often very high (F_{is} values up to 0·58) and significant. When F_{is} was analysed locus by locus (data not shown), it was found that the diagnostic loci were involved in nearly all of the significant cases. However, there was no obvious relationship between F_{is} and I (Fig. 2) (r^2=0·122, P=0·443). It seems therefore that factors other than introgression could explain the panmictic disequilibria.

Allele frequencies differed between samples, so it was impossible to compare the exact degree of linkage disequilibrium between them. Seven linkage disequilibrium tests out of 36 were significant after applying Bonferroni's correction. Among six tests involving two diagnostic loci, three (50%) were significant. Among the 30 tests involving only one or no diagnostic loci, only four (13%) were significant. The diagnostic loci (and therefore the hybridisation between wild and domestic strains) seemed to be a major cause of linkage disequilibria.

DIFFERENCES BETWEEN POPULATIONS

The NJ phenetic classification based on Nei's (1978) D formed a single line along which all the samples were attached without forming any separate branches (not shown). Therefore it appears that there was a single cause for the differentiation: introgression by domestic alleles. As all the loci were influenced to an extent approximately proportional to the mean introgression rate, the variation was one-dimensional, and so there did not seem to be any natural genetic structure among the Mediterranean trout populations in this region.

DISCUSSION

The results suggest that the main structuring factor in this region was human activity and more precisely restocking. Evidence for this was (1) that heterozygosity was a function of introgression (Fig. 2), (2) that significant F_{is} values (Table II) and linkage disequilibria involved mainly diagnostic loci, and (3) that the same diagnostic loci appeared to be the main factors structuring the populations (NJ phenetic classification).

The estimated introgression rate was very variable (0–77% of domestic alleles). This allowed the stations to be classified into three categories:

(1) Mediterranean populations with little or no introgression: The Carança (7) sample came from an isolated high-altitude population of small size, in the upper reaches of a river, which explains the almost complete absence of any polymorphism (Shaw *et al.*, 1994). Protected by their geographical isolation, these trout were of pure Mediterranean type. The Campeilles (6) stream is not isolated but is very narrow and steep, which restricts habitat availability, and recent heavy rainfall had greatly reduced the size of the population. These factors seem to favour Mediterranean forms. The population from the Aude (4) was also natural, at least at the sampling site. The sampling station was isolated between two nearby dams and sediment release from the upstream dam led to frequent siltation of spawning sites and probably leads to a great reduction in population size. This could explain the low variation observed.

(2) Highly introgressed or almost entirely artificial populations: Nohèdes (8) and Alemany (5) were two highly introgressed stations in the Têt catchment. The Nohèdes station (1980 m altitude) is located downstream of a regularly restocked lake. This lake could play the role of a reservoir of domestic genes and maintain a continuous penetration of Atlantic forms within the river, because hatchery fingerling survival is much higher in lakes at this altitude. Among these two stations, Alemany seems to have been subjected to relatively effective recent restocking (low value of WMD, Table III). The Agly (13) population is entirely artificial since it contained domestic forms almost exclusively. This small catchment lies below an altitude of 500 m and the river water becomes warm in summer. It seems likely that no natural trout population has ever inhabited the sampled portion. The Orbieu (3) is one of the highly introgressed stations (I=64%) and one of the most polymorphic (H_e=0·08) but showing no disequilibrium. Its high WMD value (0·57) seemed to indicate that this state of affairs was the result of ancient introgressions.

(3) The other stations contained populations with moderate introgression whose heterozygosity was in line with their introgression rates, confirming the diversifying effect of restocking. For these stations there was no relation between introgression and altitude, or between introgression and location within a particular river catchment (data not shown).

ALLELE FREQUENCIES AND HETEROZYGOSITY

The main hatchery strain, the so-called commercial strain, was created at the beginning of the century from north Atlantic populations (Krieg & Guyomard, 1985; Presa *et al.*, 1994; Keith, 1998). Since then, each hatchery has incorporated local genes by using variable proportions of wild males for breeding. This

practice, plus exchanges between hatcheries, has probably helped maintain a high polymorphism among domestic strains: generally H_e=0·1 (Krieg & Guyomard, 1985).

The influence of restocking, as measured by the frequencies of diagnostic alleles sometimes exceeded 75% in the Têt catchment (station Nohèdes), this being the most altered catchment. It is not known what was the polymorphism of natural populations before stocking but from a quantitative viewpoint, the effect of introductions led to an increase in heterozygosity (Fig. 2). The pure Mediterranean populations of this study have very low polymorphism because the following features are linked: difficult access to streams for managers/limited stocking/high altitude/small streams/small size of populations/low polymorphism. This has prevented the existence of large-sized non-introgressed Mediterranean populations at the present time.

The heterozygosity levels obtained for the 13 stations analysed in this study were low to medium (0–0·08). For comparison, the analyses conducted on Mediterranean type populations outside the eastern Pyrenees, also gave low heterozygosities. In France, Beaudou *et al.* (1994) measured heterozygosities of 0·033–0·069 in the Orb (southern France) for introgressions estimated to be 8–13%. In Corsica, Guyomard & Krieg (1985) analysed a purely Mediterranean population (Aïtone, $\bar{H}$=0·02) whereas Berrebi (unpubl. data) obtained $\bar{H}$ values of 0·015–0·04 in the Golo. In the Spanish Pyrenees, Pla Zanuy & Garcia-Marin (1990) analysed a population that was about 23% introgressed and found a heterozygosity of 0·032; Garcia-Marin *et al.* (1991) analysed four wild populations in the Ebro (Spain) which showed a low heterozygosity ($\bar{H}$=0·02–0·044).

Present results indicate that, overall, trout populations of the Mediterranean form have a low heterozygosity (<0·05). The highest values (0·05–0·08) were recorded in populations where domestic trout have been introduced either recently, or in the past. The current state of these populations in the Mediterranean region is probably a consequence of their small size. This can fluctuate in correlation with various factors such as isolation, confinement to narrow streams, frequent catastrophic floods, and human influence that is irregular in time and space (Largiadèr & Scholl, 1996).

ANALYSIS OF DISEQUILIBRIA

The analyses showed several intra- and inter-loci disequilibria at the diagnostic loci. The significant allele linkages were always those combining domestic alleles together and Mediterranean alleles together (three cases in three tests). However, a Wahlund effect, due to restocking, could not explain the long maintenance of significant F_{is}, especially since trout that had been introduced recently were excluded from the analysis.

Possible prezygotic barriers (assortive mating, temporal or spatial differences in spawning, differential migration between trout of distinct origins, etc.), can counter the reduction in disequilibrium after stocking and information is lacking on possible behavioural differences between Mediterranean and introduced domestic trout. Largiadèr & Scholl (1996) also reported high linkage disequilibria between allozyme loci in introgressed populations in the river Doubs (northern Rhône catchment, France). They attributed these disequilibria to partial prezygotic isolation (=assortative mating). To a lesser extent,

post-zygotic incompatibility between wild and domestic trout leads to selection pressure against hybrids (Poteaux *et al.*, 2000). The proposed processes alone or in combination could explain the observed disequilibria. They would tend to restrict the gene flow between domestic and wild fish, or between natural sub-units.

The variable success of survival of introduced fish is probably the main factor explaining the different introgression rates observed. This could also explain the indirect effect of restocking on the structure of the restocked populations, also the long-term perturbations caused even if restocking were to cease (Poteaux *et al.*, 1998, 1999, 2000).

The observed disequilibria, which also concern populations composed of purely Mediterranean forms and more or less recombined hybrids, do not always disappear: several samples considered to have been introgressed for a long time (high WMD) can have high and significant F_{is} values. Also deficits observed in the present study had natural causes as well as artificial causes. On the other hand, some highly introgressed populations, such as Orbieu and Nohèdes, showed no deficit. The artificial causes are due to restocking, but there could be other natural causes. Heterozygote deficits in allozymes have been recorded in completely wild populations in Corsica (P. Berrebi, unpubl. data). Largiadèr & Scholl (1996) also observed linkage disequilibria in completely wild populations in the river Doubs. D. Aurelle (unpubl. data) detected the same in wild populations of the Nive and Nivelle catchments (extreme south-western France), between microsatellite loci and between allozyme and microsatellite loci. These disequilibria in natural populations could be explained by factors such as trout migrations, genetic differences between age classes due to ecological changes between years or relatedness. Very small populations could consist of highly related individuals, so that some samples could come from one or very few families, which could result in disequilibria (Hansen *et al.*, 1997).

CONSERVATION OF WILD POPULATIONS

Present data showed no divergence between stations in Mediterranean trout when the stocking influence is removed. Largiadèr & Scholl (1996) also reported such uniformity in Mediterranean populations of the upper Rhône, and Po rivers. From the point of view of the natural biodiversity of the species, the current phase of the restocking process has led to an enrichment in alleles among the populations, to the extent that the differences between stations are almost entirely related to the degree of introgression. If stocking practices continue at the European level, the various natural taxa will end up being replaced by uniform commercial domestic stocks, and the average genetic distances between regions, and therefore the species' genetic diversity will be reduced.

Projects for replacing domestic strains by strains created from wild populations, such as the Carança strain from the Sahorre hatchery (M. Manier, pers. comm.) are being studied to reduce the genetic impact of restocking. However, one of the obstacles to such projects is the very low polymorphism among purely Mediterranean populations. This low polymorphism together with the maintenance of classical breeding in small fish farms (in tanks) is liable to make the wild strains derived difficult to acclimatize to varied habitats.

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