Genetics of brown trout (*Salmo trutta* L.) stocks above and below impassable falls in the Conwy river system, North Wales

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Abstract

Samples of trout (*Salmo trutta* L.) were taken from four rivers in the Conwy system, North Wales: the Roe and the Nant-y-goron, which support sea trout stocks, and the Iwrch and Machno which are situated above impassable waterfalls and contain resident trout. Tissue samples from the fish were used in electrophoretic analysis of enzymes. Fourteen loci were scored of which six were polymorphic (*Pgi-2, Gpd-2, Ldh-5, Mdh-2, Mdh-4* and *Ck-2*). Allele frequency data from these loci were used to test interpopulation heterogeneity, which indicated that the two upstream samples were probably reproductively isolated from each other and also from the downstream samples. Mean heterozygosity was generally lower in the upstream stocks compared with the sea trout populations. UPGMA cluster analysis based on Nei's genetic distance (D) revealed a clear dichotomy between those stocks above, and those below waterfalls. This was best explained by the presence of two strains of trout in the system. One strain, the resident trout occurring above impassable waterfalls, was characterized by high frequencies of the *Ldh-5(100)* allele, the other strain, principally consisting of sea trout, exhibited high frequencies of the *Lhd-5(90)* allele.

Keywords : Trout, *Salmo trutta*, allozymes, genetics, River Conwy.

Génétique des stocks de truite (Salmo trutta L.) de part et d'autre de chutes infranchissables du réseau hydrographique de la Conwy, Nord Pays de Galles.

Résumé

Des truites (*Salmo trutta* L.) ont été échantillonnées et prélevées dans 4 rivières du bassin de la Conwy (Nord du Pays de Galles) : d'une part la « Roe » et la « Nant-y-goron », qui contiennent les stocks de truites marines, et d'autre part la « Iwrch » et la « Machno » qui sont situées au-dessus de chutes infranchissables et contenant des truites résidentes. Des échantillons tissulaires provenant des poissons ont été utilisé en vue d'une analyse électrophorétique des enzymes. Quatorze locus ont été identifiés dont 6 étaient polymorphes. (*Pgi-2, Gpd-2, Ldh-5, Mdh-2, Mdh-4* et *Ck-2*). Les données relatives aux fréquences des allèles de ces locus ont été utilisées en vue de tester l'hétérogénéité entre populations. Celles-ci indiquaient que les deux populations en amont étaient probablement reproductivement isolées l'une de l'autre mais également par rapport aux populations situées en aval. L'hétérozygotie moyenne était généralement plus faible dans les stocks amonts en comparaison avec les populations de truites marines. L'analyse des groupes UPGMA basée sur la distance génétique Nei (D) révèle clairement une dichotomie entre les stocks se situant au-dessus et en dessous des chutes. La présence de deux souches de truites dans le bassin est la meilleure explication. Une souche, les truites résidentes observées au-dessus des chutes infranchissables, a été caractérisée par des fréquences élevées de l'allèle *Ldh-5(100)*, l'autre souche, principalement formée de truites marines, a montré une fréquence élevée de l'allèle *Ldh*-5(90).

Mots-clés : Truite, *Salmo trutta*, allozymes, génétique, la rivière Conwy.

INTRODUCTION

Genetic studies on brown trout (*Salmo trutta* L.) based largely on protein electrophoresis have high-lighted the complex substructuring of their popula-tions into reproductively isolated units or "stocks" (Ferguson and Fleming, 1983; Crozier and Ferguson, 1986; Ferguson, 1989; Hamilton *et al.*, 1989). The technique of electrophoresis identifies separate stocks by revealing significant differences between them in the frequency of alleles at protein encoding loci (Allendorf and Utter, 1979).

The homing behaviour characteristic of the sal-monid family is the major factor in the maintenance of the population structure. Isolation is maintained between neighbouring and even sympatric stocks because mature fish seek out their natal stream in which to spawn.

Physical barriers such as high waterfalls will also cause genetic differentiation in trout by imposing unidirectional gene flow. Downstream movement across falls will occur occasionally (Frost and Brown, 1967), although selection will act strongly against such behaviour since downstream migrants cannot ascend the falls to breed (Jonsson, 1982).

Two forms of *Salmo trutta* can be distinguished on the basis of their life histories; the anadromous sea trout and the freshwater resident brown trout. Anad-romy is not possible in stocks upstream of physical barriers. A river may, therefore, support a fully resi-dent population above an impassable waterfall, but both sea and resident trout populations below it.

The origins of the genetic differences observed between stocks of trout separated by waterfalls are thought to pre-date the creation of the falls. Previous studies have revealed that populations above water-falls are often fixed for, or have a high frequency of the allele *Ldh-5(100)* (previously *Ldh-5(105)*; Fergu-son and Fleming, 1983). This allele has also been recorded in other closely related species like the Atlan-tic salmon (*Salmo salar*), and thus appears to be the ancestral *Salmo* allele. However, the commoner allele at this locus in *S. trutta* is *Ldh-5(90)*. It is predomi-nant in most sea trout populations. Unlike the ance-stral *Ldh-5(100)* allele, *Ldh-5(90)* is unique to *S. trutta* and its presence is seen as representing a more modern form or "strain" of the species.

The two strains may differ in other respects, the older strain having slower growth but greater longev-ity (Fahy and Rudd, 1988). For example, sea trout stocks along the Welsh coast, which do contain the *Ldh-5(100)* allele, are noted for producing specimen fish (Fahy, 1985).

It has been proposed that European rivers were colonised by the ancestral trout strain, following the last ice age. Later, when sea level fell and waterfalls were created by isostatic land uplift, the modern strain invaded the area (Ferguson and Fleming, 1983; Ham-ilton *et al.* 1989). Although the modern strain is

thought to be selectively advantageous (Henry and Ferguson, 1985; Fahy, 1989), it could not infiltrate those ancient stocks which were isolated above impassable waterfalls.

In this study, we explore genetic differentiation of trout populations from four rivers in the Conwy system. Two of the rivers, the Roe and the Nant-y-goron support populations of sea trout and are not isolated from each other by any physical barrier. The two other populations, from the Iwrch and the Machno, are upstream of the Conwy falls which trout cannot ascend and are also isolated from each other by further impassable falls.

MATERIALS AND METHODS

Fifty trout were taken from a single site on each river, except the Roe, by pulsed direct current electro-fishing during June and July, 1989 (*fig.* 1). Twenty five fish were collected from each of two sites on the river Roe (R1 and R2). The trout were killed with benzocaine and stored on ice for transport back to the laboratory. Sample of white muscle, eye, liver, brain and heart tissue were extracted from the fresh fish and stored frozen at $-70°C$. Electrophoresis was carried out on 13% starch gels (Sigma) using either a tris-borate buffer, pH 8.6, of Taggart *et al.* (1981) or a citrate-morpholine buffer, pH 6.2, modified from Clayton and Tretiak (1972). Recipes for staining sol-utions were derived from Taggart *et al.* (1981).

Six enzymes were stained: malate dehydrogenase (MDH, EC 1.1.1.37), glycerol-3-phosphate dehydro-genase (GPD, EC 1.1.1.8), phosphoglucose isomerase (GPI, EC 5.3.1.9), creatine kinase (CK, EC 2.7.3.2), phosphoglucomutase (PGM, EC 2.7.5.1) and lactate dehydrogenase (LDH, EC 1.1.1.27). Fourteen loci were resolved for these enzymes of which six displayed diallelic polymorphisms (Marshall, 1989). Standard nomenclature is employed for these loci; multiple loci are designated by hyphenated numerals in order of increasing anodal migration on the gel and allelic variants are denoted in parentheses by the relative mobility of their homomeric bands.

Where possible, genotype frequencies at the poly-morphic loci were tested for deviations from expecta-tions under the Hardy-Weinberg model using the "G" test (Sokal and Rohlf, 1981). Heterogeneity between population pairs was tested using the Workman and Niswander (1970) chi squared contingency test. Nei's (1972) genetic distance "D" was calculated for all pairwise comparisons between samples based on allele frequencies for all fourteen loci. The relationships between the four sampled populations are illustrated using a dendrogram constructed by the unweighted pair-group arithmetic average (UPGMA) method (Ferguson, 1980). Using Chakraborty's (1980) method, the overall genetic variation was partitioned into that due to variation (*a*) within populations,
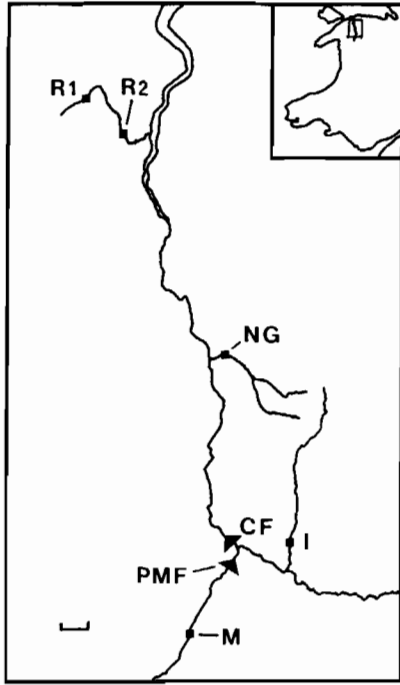
Figure 1. – A simplified map of the Conwy river system showing the sampling sites. R1 and R2: the two river Roe sites; NG: Nant-y-goron; I: river Iwrch; M: river Machno; CF: Conwy Falls; PMF: Pandy Mills Falls. Scale bar: 1 km. Inset: map of Wales showing the area depicted in the Figure.

Table 1. – Allele frequencies at six enzyme loci in samples of *Salmo trutta* from four rivers within the Conwy river system. G: G test of goodness of fit to the Hardy-Weinberg model, ns: not significant. *: $p < 0.05 > 0.01$, **: $p < 0.01 > 0.001$, ***: $p < 0.001$.

Locus	Alleles	Roe	Nant-y-goron	Iwrch	Machno
Pgi-2	100	1.00	0.98	1.00	1.00
	135	0.00	0.02	0.00	0.00
G		–	ns	–	–
Gpd-2	–100	0.69	0.82	0.96	0.90
	–128	0.31	0.18	0.04	0.10
G		ns	ns	ns	ns
Mdh-2	100	0.86	0.74	0.67	0.52
	152	0.14	0.26	0.33	0.48
G		*	ns	ns	ns
Mdh-4	100	0.74	0.75	0.62	0.94
	85	0.26	0.25	0.38	0.06
G		–	–	...	–
Ldh-5	100	0.06	0.12	0.54	0.73
	90	0.94	0.88	0.46	0.27
G		ns	ns	ns	**
Ck-2	100	0.43	0.40	0.07	0.17
	115	0.57	0.60	0.93	0.83
G		–	–		

(b) between populations within the anadromous and resident groups, and (c) between anadromous and resident groups.

RESULTS

Eight of the fourteen loci resolved, were monomorphic for the same allele in all samples *(Mdh-1, Pgi-1, Pgi-3, Ck-3, Pgm-1, Ldh-2, Ldh-3 and Ldh-4)*. The variant alleles at the remaining six loci were *Mdh-2(125), Mdh-4(85), Pgi-2(135), Ck-2(115), Gpd-2(– 128)* and *Ldh-5(90)*. These variants occurred in all samples except for *Pgi-2(135)* which was only found in fish from the Nant-y-goron *(table 1)*. All these variants have previously been described for other populations and their genetic nature has been confirmed by breeding studies (Taggart and Ferguson, 1984; Guyomard and Kreig, 1983).

No significant differences in allele frequencies were observed at any loci between the two samples (R1 and R2) taken from the Roe ($\chi^2 = 3.844$, d.f. 5; $p < 0.7 > 0.5$) so the data from these two samples were pooled in subsequent analyses.

At the *Ck-2* locus, the heterozygote and the common homozygote genotypes could not be readily distinguished from each other, so allele frequencies were calculated by assuming Hardy-Weinberg proportions and taking the square root of the variant homozygote frequency. A similar procedure was adopted for the *Mdh-4* locus, but in this case it was the heterozygote and variant homozygote which could not be distinguished. These loci could therefore not be tested for deviation from Hardy-Weinberg proportions. Of the 13 G tests of goodness of fit to the Hardy-Weinberg model which were performed, two were significant, suggesting a deficiency of heterozygotes at *Ldh-5* in the Machno sample and at *Mdh-2* in the Roe population *(table* 1). However, according to the sequential Bonferroni test (Rice, 1989), only the *Ldh-5* result can be regarded as significant ($p > 0.05$).

Mean expected heterozygosity (He) across all loci, including monomorphic ones, was generally slightly higher in the sea trout populations than in the resident populations (Nant-y-goron, He = 0.128; Roe, He = 0.119; Iwrch, He = 0.116; Machno, He = 0.106).

In the tests for heterogeneity in allele frequencies across all loci between populations, the two sea trout samples (Roe and Nant-y-goron) proved to be different at the 5% level, while all other pairwise comparisons were significantly different at the 0.1% level *(table 2)*.

Table 3 reveals that a substantial proportion (11.9%) of the genetic variation can be attributed to divergence between the anadromous and resident groups. This is well illustrated in the dendrogram for genetic distance *(fig. 2)*.

Table 2. – Results of Workman-Niswander (1970) tests for inter-population heterogeneity between samples of *Salmo trutta* from four rivers within the Conwy system. Above diagonal: summed chi squared values over five or six (') loci. Below diagonal: levels of significance (see *Table 1*).

	Roe	Nant-y-goron	Iwrch	Machno
Roe		13.39'	120.62	161.65
Nant-y-goron	*		81.26'	112.91'
Iwrch	***	***		48.64
Machno	***	***	***	

Table 3. Partitioning of genetic variation (Chakraborty, 1980) within and between populations and between groups of sea and resident trout populations, in the Conwy river system.

Component of variation	Variation	%
Total variation	0.137	100
Within population variation	0.117	85.3
Between population variation (within group)	0.004	2.8
Between group variation	0.016	11.9
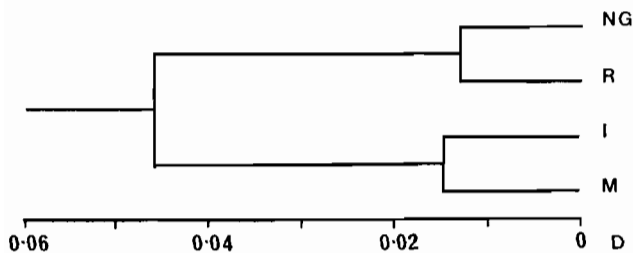
Figure 2. – UPGMA dendrogram constructed from genetic distances, indicating relationships between four stocks of *Salmo trutta* from the Conwy river system. D: genetic distance (Nei, 1972). NG: Nant-y-goron; R: Roe; I: Iwrch; M: Machno.

DISCUSSION

The results of this study are compatible with the hypothesis of an ancestral race of brown trout, characterised by a high frequency of *Ldh-5(100)*, existing largely in high mountain lakes or in upstream areas of rivers isolated from sea trout stocks by impassable waterfalls (Hamilton *et al.*, 1989). This is the common allele observed in both the Iwrch and the Machno, existing at frequencies of 0.54 and 0.73 respectively. In the sea trout stocks of the river Roe and Nant-y-goron however, this allele is rare, occurring at frequencies of 0.06 and 0.12 respectively.

The presence of the *Ldh-5(90)* allele, characteristic of sea trout, in stocks above the waterfalls is possibly due to introgression resulting from occasional stocking events. Stocking in the Iwrch is known to have taken place at various times since 1976 (Hauser *et al.*, 1991), but there are no official records of stocking in

the Machno. Nevertheless, a more extensive genetic study of trout populations in the Conwy river system does indicate the presence of hybrids between sea and resident trout in both these rivers (Hauser *et al.*, 1991). The significant deficiency of heterozygotes found at the *Ldh-5* locus in the Machno sample might be the result of the Wahlund effect; the sampling of a mixture of populations (resident + sea trout), each with significantly different allele frequencies. According to Hauser *et al.* (1991) the Iwrch sample contained a high proportion of hybrids which might explain why there was no significant deficiency of *Ldh-5* heterozygotes in this sample.

The low frequency of the ancestral *Ldh-5(100)* allele recorded in the sea trout populations may be due to intermittent gene flow over the waterfalls during flooding.

Mean expected heterozygosity follows the pattern seen in previous investigations (Ryman and Stahl, 1981; Fleming, 1983) being generally lower in the more isolated stocks above waterfalls. The typically smaller population sizes of headwater stocks are believed to be one reason for this, because the smaller the effective population size, the greater is the chance of random genetic drift reducing heterozygosity. However, for the river system studied here, the greater possibility of gene flow into and out of the sea trout stocks below the falls is also likely to contribute to the greater levels of heterozygosity. Fish may stray between the Roe, Nant-y-goron and many other tributaries of the Conwy. These stocks are also potentially open to anadromous trout from neighbouring drainage systems as well as washdowns from above the falls. In contrast, the rivers Iwrch and Machno are cut off from neighbouring populations by waterfalls or fish traps.

All inter-river tests for heterogeneity were significant, indicating that the samples represented genetically differentiated populations. The lowest significant summed chi squared value came from the comparison of the two sea trout samples ($\chi^2 = 13.39$, $p < 0.05$). All other comparisons involved samples separated by one or two waterfalls and these gave noticeably higher chi squared values all with a probability of < 0.001. The closest interpopulation genetic relationship is therefore the one between the two sea trout populations, followed by that between the two resident populations. The large proportion of the genetic variance (11.9%) which is attributable to divergence between anadromous and resident groups is further evidence of the phylogenetic differences between them (*table* 3).

This dichotomy of stocks into those above and those below waterfalls is shown by the dendrogram for genetic distance estimates (*fig.* 2). Genetic distance values range from 0.013 to 0.065 and are consistent with the samples coming from discrete conspecific stocks. In a survey of 116 British and Irish stocks, Ferguson and Fleming (1983) recorded genetic identities (I, Nei, 1972, where D = ln I) ranging from

0.96 to 0.99. Our distance values correspond to identity values of 0.937 to 0.987.

The proposed construction of a fish pass over the Conwy falls may result in the modern race becoming the dominant one in the upper stretches of the river and in accessible tributaries. Supplemental stocking of the river with sea trout would accelerate this process.

However, due to the presence of a weir and a fish trap, introgression would not occur in the Iwrch. Similarly, due to the presence of the Pandy Mills falls, the Machno would not be affected. A continuing programme of electrophoretic investigations in the Conwy river system will be required to reveal any genetic alterations caused to the various stocks as a result of the building of the fish pass.

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