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ELECTROPHORETIC VARIATION IN FOUR FRENCH POPULATIONS OF DOMESTICATED RAINBOW TROUT (*SALMO GAIRDNERI*)

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Electrophoretic variation of 12 enzyme systems, representing 26 loci in four strains of domesticated rainbow trout (*Salmo gairdneri* Richardson) were studied. The variances among and within strains, estimated from observed frequencies, were compared to data of other studies of biochemical polymorphism carried out in this species. These preliminary results show that a large proportion of the variability in rainbow trout is present in the French hatchery stocks studied and that isolation of these groups for several generations has not caused a significant genetic differentiation as compared to all the domesticated strains studied.

Nous avons examiné les variations électrophorétiques de 12 systèmes enzymatiques, représentant 26 locus chez 4 populations domestiques de Truite Arc-en-ciel (*Salmo gairdneri* Richardson). Les variabilités inter et intra-populations, estimées à partir des fréquences observées, ont été comparées aux données provenant d'autres études de polymorphisme biochimique réalisées sur la même espèce. De cette étude préliminaire, il ressort qu'une large part de la variabilité de l'espèce Truite Arc-en-ciel est présente dans les stocks piscicoles français étudiés et que l'isolement de ces groupes durant plusieurs générations ne s'est pas traduit par une différenciation génétique significative vis-à-vis de l'ensemble des populations domestiques étudiées.

Introduction

The rainbow trout species (*Salmo gairdneri*) poses the following taxonomic problems at diverse levels.

— Its definition as compared to other "evolving lineages" of the genus *Salmo* in northwestern America. The polygenetic relations of the rainbow trout with such forms as *Salmo aguabonita* and *S. apache* are still a controversial subject (Gold, 1977);

— Its genetic structure; at present, rainbow trout seems to be a heterogeneous species, including many geographic (*S. aquilarium*, *S. smaragdus*, *S. regalis*) and ecologic (anadromous, sedentary populations in rivers or lakes) forms. The existence of sympatric lake populations has been reported twice (Behnke, 1972).

All the breeding stocks which, as affected either by selection due to domestication or by random sampling (initial or at each breeding cycle), have differentiated into one or more original sub-units should be added to these natural populations.

Among the various techniques available for phylogenetic studies, those using the genetic variations in proteins have proven particularly useful; they have been widely employed in studies in the genus *Salmo* in northwestern America, indicating the phylogenetic relations within that group (Gold, 1977; Allendorf and Utter, 1974).

Work carried out on the migratory form of rainbow trout (Allendorf, 1975; Utter and Allendorf, 1977) has revealed two genetically differentiation groups corresponding to two distinct geographical areas, one coastal and the other inland. Those authors invalidated the conventional divisions according to seasonal return to the river and reported no genetic diversity between anadromous and resident forms in the same river (Utter and Allendorf, 1977).

The rainbow trout was introduced into Europe some 100 years ago (Mac Crimmon, 1971); we wished to determine what part of the genetic variability of the species was thus introduced and maintained. This paper reports the electrophoretic variants of different enzyme systems in some rainbow trout populations used in French fish culture and attempts to answer three questions:

- 1) what is the proportion of heterozygosity in these populations
- 2) what is their degree of differentiation
- 3) what is their biochemical polymorphism as compared to the main body of the species.

In this article, we present some preliminary results obtained in four of these populations.

Material and Methods

Strains

The four strains studied were taken from three different fish farms and were chosen from a list of farms whose breeding stocks had not been renewed from the outside for at least ten generations. No more data are available about the history of these strains. In one farm, we sampled two different groups, one in 1977 and the other in 1978. The one to two-year old fish were killed and stored at -20°C , and their tissues were tested during the next two weeks. These four strains are named S_1-77 , S_1-78 , S_2 , S_3 and the sizes of samples from these ones are respectively 63, 40, 60 and 40.

Extraction

The extracts were obtained after grinding in a 0.01M phosphate buffer pH 7.4 (weight/volume). The ground heart, eye and muscle were centrifuged at 4000 xg for 30 min, and sometimes re-centrifuged; liver extracts were centrifuged at 26000 xg for 1.5 h. All manipulations were carried out at 4°C .

Electrophoresis

300 ml of 12% Connaught starch gel were poured into $6 \times 170 \times 225$ mm molds. The clear supernatants were individually drawn into 6×6 mm squares of Whatman 3_M paper inserted into the gel. Buffers, electrophoretic conditions and the staining solutions of each system are shown in Table I. The gels were refrigerated during migration with ice packs.

Designation of Alleles

The locus designation adopted here conforms to the conventional nomenclature (Allendorf, 1975; Busack *et al.*, 1979). The most frequent alleles were arbitrarily designated by mobility 1.00.

Measurement of Genetic Distance and Proportion of Polymorphism

We used the formula proposed by Nei (1975). If x_{ij} is the frequency of allele i for a locus in a population j , gene diversity is expressed by $H_j = 1 - \sum_i x_{ij}^2$ which is equivalent to average heterozygosity, assuming Hardy-Weinberg genotype proportions. Genetic distance between populations j and j' is measured by $D_{jj'} = \frac{1}{2} \sum_i (x_{ij} - x_{ij'})^2$. The overall genic diversity of a group of populations is $H_T = 1 - \sum_i x_i^2$ with $x_i = \sum_j w_j x_{ij}$ where w_j is the coefficient of the weight of the population j . In this paper we used the same coefficient for each population ($w_j = \frac{1}{N}$) with N representing the number of populations. Extensions to several loci are immediate.

TABLE I
Electrophoretic and staining techniques

Systems	Examined tissue	Electrophoretic conditions	Revelation
Alcohol dehydrogenase (ADH)	Liver	Ridgway buffer (Ridgway <i>et al.</i> , 1970); 60 Ma, voltage max 350 volts; migration during 3 h	Ethanol 5 ml; NAD 15 mg; NBT 5 mg; PMS 5 mg; 0.05M Tris-HCl, pH 8.00 : 100 ml
Creatine kinase (CK)	Muscle	Ridgway buffer	1) Specific staining : creatine kinase, disodium salt 20 mg; NBT 5 mg; PMS 10 mg; NADP 5 mg; adenosine 5' diphosphate 30 mg; glucose 20 mg; hexokinase 100 units; g-6-pdh 20 units; 0.05M Tris-HCl, pH 8.00 : 100 ml 2) Amidoblack staining
Glutamate oxaloacetate transaminase (GOT)	Heart	Morpholino-citrate buffer (Clayton and Tretiak, 1972) 65mA; 320 volts during 5 h	Aspartic acid : 100 mg; Pyridoxal 5' phosphate 10 mg; Fast blue BB salt 100 mg; α -keto-glutaric acid 80 mg; 0.2M Tris-HCl pH 8.00 : 100 ml
α -glycero-phosphate dehydrogenase (GPDH)	Muscle	0.1M Tris-phosphate pH 7.4 90 MA; 150 volts during 5 h	DL α glycerophosphate, disodium salt 80 mg; PMS 5 mg; NAD 25 mg; NBT 5 mg; EDTA Trisodium salt 16 mg; 0.05M Tris-HCl, pH 8.00 : 100 ml
Isocitrate dehydrogenase (IDH-s)	Liver	1) 0.1M Tris-phosphate pH 7.4 90 MA; 150 volts during 5 h 2) sodium citrate buffer (Cross and Payne, 1977); 100 mA; 130 volts during 9 h	D-L-isocitric acid, trisodium salt 30 mg; NADP 5 mg; NBT 5 mg; PMS 5 mg; MgSO ₄ 40 mg; 0.05M Tris-HCl pH 8.00 : 100 ml
Mitochondrial isocitrate dehydrogenase (IDH-m)	Heart	0.1M Tris-phosphate pH 7.4 90 mA, 150 volts during 5 h	D-L-isocitric acid, trisodium salt 30 mg; NADP 5 mg; NBT 5 mg; PMS 5 mg; MgSO ₄ 40 mg; 0.05M Tris-HCl pH 8.00 : 100 ml
Lactate dehydrogenase (LDH)	Muscle Heart Eye	Ridgway buffer	Lactic acid, lithium salt 200 mg; NAD 15 mg; NBT 5 mg; PMS 5 mg; Tris-HCl pH 8.00 100 ml.
Malate dehydrogenase (MDH)	Liver Muscle Heart	Morpholino-citrate buffer (Clayton and Tretiak, 1972) 65 mA; 320 volts during 5 h	L-Malic acid, monosodium salt 500 mg; NAD 40 mg; NBT 20 mg; PMS 5 mg; 0.15M Triethano-lamine-HCl pH 9.0 : 100 ml
Phosphoglucose isomerase (PGI)	Muscle	0.1M Tris-phosphate pH 7.4 90 mA; 150 volts during 5 h	Fructose-6-phosphate 12.5 mg; NADP 0.8 mg; NBT 1.5 mg; PMS 1.5 mg; G-6-PDH 25 units; 0.05M Tris-HCl pH 8.00 4 ml. 10 $\times$ 22.5 cm rectangle of Whatman n° 1 paper, impregnated with this solution, is applicated on the gel
Phosphogluco-mutase (PGM)	Muscle	0.1M Tris-phosphate pH 7.4 90 mA, 150 volts during 5 h	Same staining procedure; 15 mg of Glucose-1-phosphate instead of F-6-phosphate
Transferrin (Tf)	Serum	Ridgway buffer	Amidoblack
Superoxyde dismutase (S.O.D.)	Liver	Ridgway buffer	Stained in the same time as ADH

Estimation of Allelic Frequencies

An extensive gene duplication was found in salmonid species, confirming that they have been subjected to a tetraploidization during their evolution. These species now have a disomic

mode of inheritance; at the molecular level this means that in some tissues, some enzyme systems are coded by two loci. Since the allelic relations between these four genes and their assignment to each locus could not always be determined on the electrophoregrams, sometimes the genotypes could be only partially ascertained for two loci of a duplicate. We had this problem with the glutamic oxaloacetic transaminase (GOT), malate dehydrogenase (MDH) and isocitrate dehydrogenase (IDH) systems, and therefore adopted the following rule: if n_{ij} was the total observed number of genes of a given allelic type i and n_j the total number of genes sampled, the genetic distance, computed with these two loci was minimal if the frequency $\frac{n_{ij}}{2n_j}$ was assigned to the allele i at each of the two loci. This was the case of

FIG. 1. 1a : GOT in heart; 1b : details of polymorphism corresponding to loci Got1 and 2. FIG. 2. MDH in heart. FIG. 3. IDH-m in heart. FIG. 4. Idh-s 3 and 4; presumed genotypes : 1, 8, 10, 17, 19, 20. Idh 3-4 (1.00/1.00/1.00/0.38); 2 : Idh 3-4 (1.00/1.00/1.00/0.62); 3, 7, 9, 14 : Idh 3-4 (1.00/1.00/0.38/0.38); 4, 12, 13 : Idh 3-4 (1.00/1.00/0.38/1.31); 5 : Idh 3-4 (1.00/1.31/0.69/0.69); 6 : Idh 3-4 (1.00/0.69/0.69/0.38); 11, 15 : Idh 3-4 (1.00/1.00/1.00/1.00); 16 : Idh 3-4 (1.00/1.00/1.00/1.31); 18 : Idh 3-4 (1.00/1.00/0.69/0.69).

FIG. 5. Genetic interpretation of FIG. 1a. 1, 2 and 5 : Got 1-2 (1.00/1.00/1.00/1.17); 3 : Got 1-2 (1.00/1.00/1.00/0.70); 4 : Got 1-2 (1.00/1.00/1.17/1.17).

IDH and MDH. The whole of the values $\frac{n_{ij}}{2n_j}$ ($j = 1, \dots, n$) minimized the mean genetic distance $\frac{1}{N^2} \sum_j \sum_i w_j w_j' D_{jj'}$, where N is the number of populations, but maximized $\sum_j H_j w_j$ as well as the overall genic diversity H_T . The degree of heterogeneity of the populations group $\frac{\sum_j \sum_i w_j w_j' D_{jj'}}{N^2 H_T}$ was also minimum, but the dendrogram of those values did not necessarily correspond to that obtained from the true frequencies. For GOT, we considered the possibility that only one of the two loci might be polymorphic; both hypotheses (one or two polymorphic loci) were tested using the law of Hardy-Weinberg.

Comparison with Results in the Literature

Two papers (Allendorf, 1975; Busack *et al.*, 1979) have furnished data on nine stocks from American fish farms; we have compared these with our results. For the between-population comparisons, we used only the loci common to the three studies. For each one, we accepted the homologies of the alleles most frequently described in the three articles; this was well corroborated by the electrophoretic mobilities of other alleles. For both the MDH and IDH systems, we regrouped some observed alleles which could not be differentiated in the other two studies (see Results).

Construction of Dendrograms

The dendrogram indicating the genetic distances between the 13 populations was constructed using a Fortran program provided by Dr. Neï.

FIG. 6. Genetic interpretation of Fig. 3. 1 : Idh-2 (1.00/1.00); 2 : Idh-2 (1.00/1.20).

Results

Description and Interpretation of Electrophoregrams

We studied 11 enzymatic systems, and the electrophoregrams obtained agreed with the results of the other two authors (Allendorf, 1975; Busack *et al.*, 1979). Table II shows the models currently used in the genetic determinism of those systems and preferentially indicated the references mentioning "genetic tests" carried out on rainbow trout species. The other methods sometimes used are genetic studies in phylogenetically close species and the distribution of enzymatic forms in the various tissues. Figures 1, 2, 3, 4, 5, 6 and 7 illustrate some of the variants found.

The electrophoretic behavior of GOT deserves attention; in Ridgway and Tris-phosphate buffers, the system was monomorphic. This agrees with the observations of Allendorf (1975) and Busack *et al.* (1979). However, at pH 6.1 extreme variations resembling those of MDH-B and suggesting a genetic control of two loci, were found (Figs. 1a and 1b). Table II also presents results on the duplicated or unduplicated state of the different loci studied. Whether or not duplication is found partially depends on technique sensitivity, as illustrated for α -glycerophosphate dehydrogenase (α -GPDH) (Allendorf, 1975). We estimated a total of 26 loci for the 11 enzymatic systems.

Allelic Frequencies

Table III shows the alleles found and their computed frequencies at each locus. For IDH-s and MDH-B, each allele was presumed to have the same frequency at the two loci coding for each system (minimal genetic distance). For GOT according to the test of the two hypotheses (minimization of genetic distance and only one polymorphic locus) using the law of Hardy-Weinberg, we retained the second one in agreement with this law (Table IV).

The distribution of the allelic frequencies in each stock, followed a conventional pattern: at most loci a high frequency allele common to all the populations was found.

We usually observed a larger number of alleles than mentioned in the other two studies of rainbow trout populations (Allendorf, 1975; Busack *et al.*, 1979).

TABLE II
Genetic determinism of studied enzymatic systems

Systems	Enzyme structure	Genetic determinism	Duplication	References
APH	dimeric	1 locus	no	Allendorf (1975): mendelian inheritance studies on rainbow trout
CK	monomeric	2 loci: Cpk-1 variable Cpk-2 fixed	yes, disomic heredity	Allendorf (1975): mendelian inheritance studies on rainbow trout
GOT	dimeric	2 loci: Got-1 fixed Got-2 variable	idem	Allendorf (1975): mendelian inheritance studies on rainbow trout
α -GPDH	idem	2 loci: α gpdh-1 fixed α gpdh-2 variable	idem	Utter <i>et al.</i> (1973); May <i>et al.</i> (1979); mendelian inheritance tests on brook trout (<i>Salvelinus fontinalis</i>) and lake trout (<i>Salvelinus namaycush</i>) $\times$ brook trout
IDH-s	idem	2 loci: Idh-3 and Idh-4 variable	idem	Allendorf and Utter (1973): inheritance studies on rainbow trout
IDH-m	idem	2 loci: Idh-1 fixed, Idh-1 variable	idem	
LDH-M	tetrameric	2 loci: Ldh-1 and Ldh-2 fixed	idem	Bailey <i>et al.</i> (1976)
LDH-H	idem	2 loci: Ldh-3 and Ldh-4 variable	idem	Wright <i>et al.</i> (1975): inheritance studies on rainbow trout
LDH-E	idem	1 locus, Ldh-5 variable	no	Wright <i>et al.</i> (1975): inheritance studies on rainbow trout
MDH-A	dimeric	2 loci: Mdh 1 and 2	yes	Allendorf (1975): inheritance studies on rainbow trout
MDH-B	idem	2 loci: Mdh 3 and 4, variable	yes disomic heredity	Bailey <i>et al.</i> (1970): inheritance studies on rainbow trout
PGI	idem	3 loci: Pgi 1, 2 and 3	idem for Pgi-1 and 2, Pgi-3 not duplicated	Awise and Kitto (1973); Schmidtke <i>et al.</i> (1975); May <i>et al.</i> (1979): inheritance studies on brook trout
PGM	monomeric	1 locus variable	no	Roberts <i>et al.</i> (1979); Utter <i>et al.</i> (1973): inheritance studies on sockeye salmon (<i>Oncorhynchus nerka</i>)
TO	dimeric	idem	idem	Utter <i>et al.</i> (1973): inheritance studies on rainbow trout
Tf	monomeric	idem	idem	idem

FIG. 7. Genetic interpretation of Fig. 5 for anodal variations. Cathodal part should correspond to mitochondrial MDH.

1 : Mdh 3-4 (1.00/1.00/1.00/1.00); 2 : Mdh 3-4 (1.00/1.00/1.00/0.96) ;
3 : Mdh 3-4 (1.00/1.00/1.00/0.79); 4 : Pdh 3-4 (1.00/1.00/1.00/0.70)

This is certainly not related to the sample size of the populations, but to true differences between the frequencies or more likely to the electrophoretic techniques used.

The eight loci, A-gpdh 1 and 2, Idh 3 and 4, Mdh 3 and 4, Pgm and Sod, common to the three studies, were used for subsequent comparisons. These eight loci give highly biased absolute values, but we are mainly interested in relative comparisons. Four relatively variable systems (IDH-m, GOT, CK and p-ALB), either studied under very different electrophoretic conditions or not examined in the overall populations, were discarded.

For IDH-s and MDH-B, we regrouped the following alleles; Idh (1.31-1) and (1.31-2), Idh (0.69) and (0.62), Mdh (1.00) and (0.96), Mdh (0.79) and Mdh (0.70). The allelic frequencies for IDH-s reported by Allendorf (1975) had to be revised, that author having hypothesized a single variable locus for the system (Appendix Table I). Finally, we tested the panmictic deviation at some loci; the χ^2 values obtained are shown in Table IV. The gene diversity (Table V) and genetic distances (Table VI) were computed according to Nei (1975). The distance dendrogram is shown in Fig. 8.

TABLE III
Allelic frequencies

System locus and alleles	strain 1-77 n=63	strain 1-78 n=40	strain ² n=60	strain ³ n=40
ADH Adh	-	fixed	-	fixed
CPK Cpk1 (1.00)	-	0.975	-	0.885
Cpk1 (0.48)	-	0.025	-	0.115
Cpk2	-	fixed	-	fixed
GOT Got-1	-	fixed	-	fixed
Got-2(1.00)	-	0.562 ($X^2_1 = 0.74$)	-	0.650 ($X^2_1 = 0.58$)
Got-2(1.17)	-	0.438	-	0.350
α GPDH α gpdh1	fixed	fixed	fixed	fixed
α gpdh2(a)	0.984	1.000	1.000	0.975
α pdh2(b)	0.016	0.000	0.000	0.025
IDH-s Idh 3 and 4				
Idh (1.00)	0.595	0.587	0.646	0.612
Idh (0.38)	0.230	0.250	0.154	0.244
Idh (0.62)	0.024	0.013	0.071	0.013
(1) Idh (1.31-1)	0.091	0.081	0.046	0.031
(1) Idh (1.31-2)	0.012	0.006	0.054	0.062
Idh (0.69)	0.048	0.063	0.029	0.038
IDH-m Idh1	-	fixed	-	fixed
Idh2(1.00)	-	0.850	-	0.862
Idh2(1.20)	-	0.150	-	0.138
LDH-M Ldh 1 and 2	-	fixed	-	fixed
LDH-H Ldh 3 and 4	-	fixed	-	fixed
LDH-E Ldh 5	-	fixed	-	fixed
MDH-A Mdh 1	-	fixed	-	fixed
MDH-B Mdh 3 and 4 :				
Mdh (1.00)	0.716	0.719	0.916	0.731
Mdh (0.79)	0.156	0.156	0.071	0.160
Mdh (0.70)	0.083	0.081	0.013	0.045
Mdh (0.96)	0.045	0.044	0.000	0.064
PGI Pgi 1,2 and 3	-	fixed	-	fixed
PGM Pgm (1.00)	0.700 ($X^2_1=0.57$)	0.737 ($X^2_1=2.06$)	0.983	0.810
Pgm (0.70)	0.300	0.263	0.017	0.190
TO To (1.00)	0.340 ($X^2_1=0.12$)	0.275 ($X^2_1=1.01$)	0.640 ($X^2_1=9.1^*$)	0.350 ($X^2_1=0.01$)
To (1.61)	0.660	0.712	0.280	0.637
To (0.39)	0.000	0.013	0.080	0.013
TFN Tfn (a)	0.940	0.962	0.992	0.975
Tfn (b)	0.060	0.038	0.008	0.025

Distinction between Idh (1.31-1) and Idh (1.31-2) is based on utilisation of two buffers : 0.1M Tris-phosphate pH 7.4 and 0.04M Sodium Citrate pH 5.

X^2_1 values correspond to tests of Hardy-Weinberg law * $0.001 < p < 0.005$

n = sample size

Discussion

Intrapopulation Variability

The four stocks studied (S_1 -77, S_1 -78, S_2 , S_3) showed a higher mean proportion of polymorphism than other populations. On account of the precautions taken, it would seem that the effect of the electrophoretic techniques can be eliminated, but no more precise reasons can be given for this variability of heterozygosity.

A highly polymorphic domesticated population may result from a mixture of natural populations which are less polymorphic but genetically differentiated or a high polymorphic natural population; this possibility cannot be rejected. Some steel-head populations are very polymorphic (computed from the data of Allendorf, 1975

TABLE IV
Test of Hardy-Weinberg law for GOT under two hypotheses
1) one locus is fixed 2) allele b is present at the two loci with the same frequency

	Observed		Expected (hypothesis 1)		Expected (hypothesis 2)	
	Strain 1-78	Strain 3	Strain 1-78	Strain 3	Strain 1-78	Strain 3
aaaa	14	18	12.5	16.9	3.93	7.14
aaab	17	16	19.7	18.2	12.36	15.38
aabb	9	6	7.8	4.9	14.57	12.42
abbb	0	0	0	0	7.63	4.46
bbbb	0	0	0	0	1.50	0.6
$X^2 =$						
$X_1^2=0.74$ $X_1^2=0.52$ $X_2^2=38.8$ $X_2^2=24.92^*$						

* : <0.0005

for the same subsample of eight loci, $H = 0.31$ in a natural steelhead population, and the resident forms which do not seem to be genetically differentiated from the migratory ones might exhibit the same degree of polymorphism.

Although it is impossible to choose between these two possibilities, we can conclude that introduction and breeding have not led to a reduction of the intra-population genetic variability of these stocks. Such processes, however, seem to operate in some populations, as in the stock of the University of Washington (Allendorf, 1975).

Table V shows the proportion of heterozygosity of stocks S_1 -78 and S_3 computed from the whole of the systems studied. The values in parentheses correspond to monomorphic Mdh_4 and Idh_4 loci, all the variations of the MDH-A and IDH-s systems being reported to the Mdh_3 and Idh_3 loci, respectively.

Allendorf and Utter (1978) have demonstrated that rainbow trout are highly polymorphic as compared to other salmonids. Comparisons with other species entail some corrections; the mean value of intrapopulation variability (0.22) was computed for eight loci, seven of which were very polymorphic in the rainbow trout. Using

TABLE V
Heterozygosity levels

	8 loci	25 loci
S_1 -77	0.348	
S_1 -78	0.336	0.149 (0.125)
S_2	0.241	
S_3	0.324	0.149 (0.134)
Univ. of Washington*	0.086	
Chamber Creek*	0.228	
Mac Creary*	0.210	
West Virginia*	0.088	
RTH**	0.171	
RTHX**	0.174	
RTS**	0.214	
RTW**	0.295	
RTV**	0.150	

*Allendorf (1975).

**Busack *et al.* (1979).

FIG. 8. Dendrogram of genetic distances between 13 populations of domesticated rainbow trout.

some of Allendorf's data (1975) taken from a more representative sample of the whole of the loci generally used for estimating the proportion of heterozygosity, we obtained a correct estimate of $H_c = 0.063$. Other vertebrates (28 species in Fuerst *et al.*, 1977) shown a mean value of 0.038 (SD = 0.033) after elimination of very variable esterases ($H_{\text{esterases}} = 0.134$ for the 28 species) which Allendorf did not study.

Interpopulation Variability

Samples S₁-78 and S₁-77, representing two successive groups from the same fish farms, were almost identical. This frequency stability, which should be followed through several generations, has been reported several times (Altukhov *et al.*, 1975; Busack *et al.*, 1979), and shows the repeatability of the method, the representative nature of the samples and the advantage of this technique as a tool for the genetic description of species and populations.

Table VII gives the analysis of overall genetic variance into mean intrapopulation genic diversity and mean interpopulation genetic distance for the whole of the

TABLE VI
Genetic distances matrix

	S ₁ -77	S ₁ -78	S ₂	S ₃	Univ. of Wash- ington	Chamber Creek	Mac Creary	West Virginia	RTH	RTHX	RTS	RTW
S ₁ -78	0.00074											
S ₂	0.03222	0.03537										
S ₃	0.00199	0.00187	0.02244									
Univ. of Washington	0.08566	0.09356	0.02039	0.07180								
Chamber Creek	0.01723	0.01984	0.02809	0.01586	0.05575							
Mac Creary	0.03041	0.03422	0.01958	0.02421	0.04200	0.01395						
West Virginia	0.07047	0.07624	0.01492	0.05664	0.00402	0.03935	0.02380					
RTH	0.03392	0.02976	0.07363	0.03393	0.14328	0.03085	0.04641	0.10914				
RTHX	0.03601	0.03345	0.08196	0.03914	0.14881	0.02855	0.04777	0.11464	0.00216			
RTS	0.03364	0.03793	0.00318	0.02518	0.01507	0.02115	0.01382	0.00857	0.07179	0.07709		
RTW	0.06608	0.07270	0.03431	0.05959	0.06079	0.09349	0.08266	0.07069	0.06102	0.17174	0.04840	
RTV	0.05003	0.05183	0.03038	0.03834	0.04991	0.02744	0.00831	0.02875	0.05297	0.05878	0.02446	0.11060

TABLE VII

$$\text{Analysis of genic diversity } H = \frac{\sum_j H_j}{N} + \frac{1}{N^2} \sum_{jj'} D_{jj'}$$

Population grouping	Genic diversity intrapopulation $\frac{\sum H_j}{N}$	Average genetic distance $\frac{\sum \sum D_{jj'}}{N^2}$
H_T : (all 13 populations)	0.210	0.048
H_1 : (the 4 French populations)	0.302	0.013
H_2 : (the other 9 populations)	0.179	0.052
H'_T : (French populations and American populations considered as two groups)	0.270	0.006

13 populations (H_T), the four stocks studied (H_1), the other nine populations (H_2), as well as the classification obtained when the French populations and the American populations were considered as two different units (H'_T).

The mean genetic distance within the groups S_1 , S_2 and S_3 is lower (0.013 vs. 0.052), indicating strong homogeneity. However, examination of H'_T does not reveal any significant differentiation between the two groups. This is illustrated by the genetic distribution dendrogram in Fig. 8.

The constitution of French stocks began about 100 years ago with populations introduced from North America. These introductions, which are still in progress either directly or by the intermediary of some European country or other continents, are difficult to trace. However, it would appear from this preliminary study that a large part of the genetic variability of the rainbow trout species is present in the stocks studied and that the isolation of these groups for several generations has not caused significant genetic differentiation, as compared to all the domesticated strains studied.

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APPENDIX TABLE I
Allelic frequencies for comparison of the 13 strains of domesticated rainbow trout

	S ₁ -77	S ₁ -78	S ₂	S ₃	Univ. of Wash- ington	Chamber Creek	Mac Creary	West Virginia	RTH	RTHX	RTS	RTW	RTV
	Locus Agpdh-2												
α -gpdh (b)	0.0160	0.0	0.0	0.0250	0.0	0.0	0.1120	0.0	0.0020	0.0030	0.0	0.0090	0.2510
α -gpdh (a)	0.9840	1.000	1.0000	0.9750	1.0000	1.00000	0.8880	1.0000	0.9980	0.9970	1.0000	0.9910	0.7490
	Locus Idh-3												
Idh-3 (1.31)	0.1030	0.0870	0.1000	0.0930	0.0065	0.0	0.1000	0.0	0.0010	0.0	0.0240	0.3820	0.0
Idh-3 (1.00)	0.5950	0.5870	0.6460	0.6120	0.8125	0.7790	0.8735	0.8750	0.6990	0.7330	0.7440	0.3400	0.9260
Idh-3 (0.69)	0.0720	0.0760	0.1000	0.0510	0.0	0.0	0.0	0.0	0.1730	0.1580	0.1030	0.0750	0.0
Idh-3 (0.38)	0.2300	0.2500	0.1540	0.2440	0.1810	0.2210	0.0165	0.1250	0.1270	0.1080	0.1280	0.2030	0.0740
	Locus Idh-4												
Idh-4 (1.31)	0.1030	0.0870	0.1000	0.0930	0.0065	0.0	0.0	0.0	0.0010	0.0	0.0240	0.3820	0.0
Idh-4 (1.00)	0.5950	0.5870	0.6460	0.6120	0.8125	0.7790	0.8735	0.0	0.6990	0.7330	0.7440	0.3400	0.9260
Idh-4 (0.69)	0.0720	0.0760	0.1000	0.0510	0.0	0.0	0.0	0.8750	0.1730	0.1580	0.1030	0.0750	0.0
Odih-4 (0.38)	0.2300	0.2500	0.1540	0.2440	0.1810	0.2210	0.0165	0.0	0.1270	0.1080	0.1280	0.2030	0.0740
	Locus Mdh-3												
Mdh-3 (1.25)	0.0	0.0	0.0	0.0	0.0	0.0	0.0060	0.0	0.0	0.0	0.0	0.0060	0.0
Mdh-3 (1.00)	0.7610	0.7630	0.9160	0.7950	0.9810	0.9530	0.9380	1.0000	0.9990	1.0000	0.9150	0.7520	1.0000
Mdh-3 (0.79)	0.2390	0.2370	0.0840	0.2050	0.0190	0.0470	0.0570	0.0	0.0010	0.0	0.0850	0.2420	0.0
	Locus Mdh-4												
Mdh-4 (1.25)	0.0	0.0	0.0	0.0	0.0	0.0	0.0060	0.0	0.0	0.0	0.0	0.0060	0.0
Mdh-4 (1.00)	0.7610	0.7630	0.9160	0.7950	0.9810	0.9530	0.9380	1.0000	0.9990	1.0000	0.9150	0.7520	1.0000
Mdh-4 (0.79)	0.2390	0.2370	0.0840	0.2050	0.0190	0.0470	0.0570	0.0	0.0010	0.0	0.0850	0.2420	0.0
	Locus Pgm												
Pgm (1.00)	0.7000	0.7370	0.9830	0.8100	1.0000	0.6390	0.8250	1.0000	0.6840	0.5610	0.9200	1.0000	0.9690
Pgm (0.70)	0.3000	0.2630	0.0170	0.1900	0.0	0.3610	0.1750	0.0	0.3160	0.4390	0.0800	0.0	0.0310
	Locus Sod												
Sod (1.61)	0.6600	0.7120	0.2800	0.6370	0.0	0.5580	0.5000	0.1560	1.000	0.9800	0.2950	0.1170	0.5570
Sod (1.00)	0.3400	0.2725	0.6400	0.3500	1.000	0.4420	0.5000	0.8440	0.0	0.0200	0.7050	0.8830	0.4420
Sod (0.39)	0.0	0.0130	0.0800	0.0130	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0