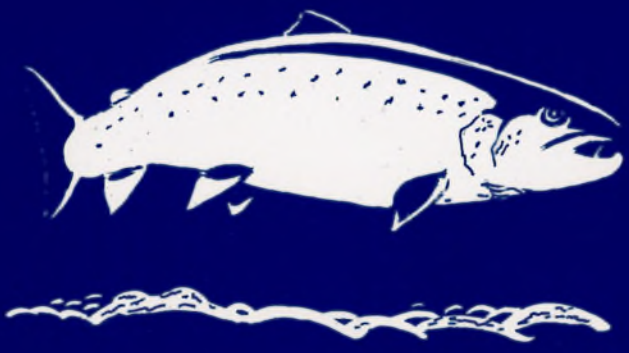
ATLANTIC SALMON TRUST

SALMON STOCKS: A GENETIC PERSPECTIVE

By N. P. WILKINS



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Atlantic Salmon Trust
Moulin, Pitlochry
Perthshire PH16 5JQ

SALMON STOCKS: A GENETIC PERSPECTIVE

N. P. Wilkins,
National University of Ireland,
University College,
Galway.

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FOREWORD.

The objective of the Atlantic Salmon Trust is to promote the conservation and enhancement of Atlantic salmon stocks. We seek to achieve this objective by fostering international agreements, EEC and domestic legislation and by stimulating scientific management and research. In furthering these aims, we liaise with a wide range of other organisations and with scientists in Government, Universities and Water Authorities. We aim to act as a focal point of contact for similar international interests.

It is in the context of the whole of the scientific aspects of the Trust's activities that this excellent monograph by Professor N.P. Wilkins should be seen.

In the scientific and economic fields the aim of the Trust is to stimulate investigations, whose results we see as essential to an understanding of the problems which face those who are concerned to preserve salmon for the benefit of the community and to secure the optimum cropping of this valuable resource. In some cases we are able to help with modest contributions from our limited funds, but in the main, we try to arrange sponsorships for such investigations or to persuade appropriate organisations to undertake them. The Trust also arranges workshops which provide fora where fishery managers, scientists, economists and others can discuss topical problems. The problems of Data Collection, Economic Evaluation of Fisheries and Stock Enhancement have already been discussed at such workshops and from these come recommendations for action by relevant authorities or for investigations of some urgency. Furthermore, we are convinced that we can make a useful contribution by disemminating information about the latest developments in knowledge, which bear importantly upon the problems of preserving salmon stocks. As part of this, we intend to ask experts to give us their views on what is now known about specific topics and their significance for the conservation of salmon and what better start could we have than Professor Wilkins on "A Genetic Perspective"?

With salmon catches in many rivers in decline and with serious depletion of Spring running fish, thoughts have turned to an intensification of stocking as one possible remedy, but stocking would have to be on a much more massive scale than in the past to have any chance of success. This would be likely to involve quite large transfers of eggs or juvenile stock from natal to non-natal rivers. A possible additional source of "foreign" stock is the salmon farming industry. Fish biologists warn of possible long-term dire genetic consequences of such transfers. Some take the view that, whilst ideally such stocking is undesirable in rivers that are in a reasonable state of productivity, conditions are not ideal and that the dire consequences of the more immediate threat are likely to be the decimation of stocks. Stocking may be the only immediate remedy.

The Trust's Honorary Scientific Panel decided that the time was opportune for an authority on the subject to give an account of what is known about the genetics of salmon, so that the debate could be conducted within a background of a better understanding by the layman and the non-specialist. There is a widespread interest in the extent of the genetic variation between stocks of different rivers and why they have developed that way; in the reasons why geneticists see it as important to preserve these; in the part genetics plays in the homing of salmon; in the geneticists' view of the prospects of recreating Spring runs by selective stocking;in possible safeguards against the dangers as seen by the geneticists. Professor Wilkins addresses himself to these and many other questions and the Trust is grateful to him for this valuable monograph.

Sir Ernest Woodroofe, PhD, FInstP, FIChemE.
Chairman, Honorary Scientific Panel.

INTRODUCTION

The question of the number of races of Atlantic salmon that can be distinguished is one that has engaged the foremost salmon experts of many generations. It is one, moreover, on which opposing views have ranged from one extreme to the other. Andrew Young of Invershin, one of the pioneers of modern salmon cultivation, wrote as long ago as 1854:-

> "...each river has its own peculiar race of fish... We have now shown that salmon undoubtedly return to the river where they have spawned, and where they belong to the race of fish that inhabit that particular river. It may be that they know their own race..."

Over one hundred years later, Dr. J.W. Jones, one of the most eminent of salmon biologists, could state:

> "The migratory Atlantic salmon is a single interbreeding and remarkably homogenous species around the enormous periphery of the North Atlantic..."

Which of these opposing views can be correct, or most nearly correct? No one can disagree with Jones that the Atlantic salmon is recognisably the same species throughout its vast range, whereas, for example, Pacific salmon has split into numerous species within its particular area. But this view may overlook real stock or racial structures which are evident only when the populations of individual rivers are carefully compared. The importance of identifying and understanding the population structures of this species must be emphasised. The Atlantic salmon is an invaluable natural resource in every country it inhabits. Rational management, protective legislation, interpretation of catch statistics and, increasingly, commercial development all require an accurate knowledge of the number and the kinds of stocks or races in question.

WHAT IS A RACE?

The long controversy over the racial structure of the salmon was fuelled to some extent by the absence of an agreed definition of a race. As Dr. Huntsman, the famous Canadian fishery biologist, pointed out:

> " There is no definite clarification of the meaning of the word 'race' as applied to salmon"

Depending on the perspective of the observer a "race" or "stock" might, for example, be the output of a specific hatchery, or the population of a single river, or the fish of a well defined region, or the totality of fish inhabiting the rivers and near coastal waters of a single country. This last case is often the meaning when, for example, fish are marketed as "Irish" or "Scotch" salmon, or when statistics of salmon catches are compared between countries. From a biological perspective, a race or stock is a population which differs genetically from other populations of the same species. The important matter is that races differ genetically. In this paper the terms "race" and "stock" are used interchangeably to mean the same thing. The populations of two separate rivers might well be distinguishable, over many generations, by means of visible characteristics and yet be members of the same race, if the distinguishing visible characteristics were not due to genetic differences. Many differences between salmon populations of different rivers may simply reflect the quality of the habitat or environment: poorer habitats may give slower growing fish with different bodily features than richer, optimal habitats. The problem is to distinguish those characteristics which are habitat induced from those which are exclusively or predominantly due to heritable genetic differences. It is only in modern times that this distinction has become relatively easy.

The separation of the genetic composition, or gene pool, of one stock from another which characterises true stocks or races is achieved and maintained by differential mating. Mating occurs regularly and at random between individuals within a stock, but occurs only infrequently or never at all between individuals of separate stocks. When mating between populations is restricted over a long period the gene pools of the separate populations will evolve independently and heritable genetic differences may appear by which the seperate populations, now called stocks or races, can be identified.

IDENTIFICATION OF STOCKS

The size, shape, behaviour and physiology of any organism reflect its genetic constitution (its genotype), although often at a far remove. The life history of each individual - what and how much it has eaten, its age, sex, the temperatures and salinities it has experienced, the quality of its habitat - all influence its final visible form, called its phenotype. The phenotype is not, for this reason always a good reflection of the genotype, and when the phenotypes of salmon in one river differ from those in another it is not correct to infer that their genotypes differ also. To detect genotypic differences between populations we need to look at the genetic material itself, or at some immediate product of the genotype. The immediate products of many genes comprising the genotype are proteins; these can be relatively easily examined by the technique of electrophoresis. It is not appropriate to describe this process in detail here. Interested readers should see Dr. Ferguson's useful and readable book on this, which also deals with much that is of interest in salmon and trout biology. By means of electrophoresis

it is possible, using protein or enzyme* characteristics, to tell the genotype of each individual and the frequency, in each population, of the different genes determining these characteristics. Each individual inherits two genes for each protein, one from its male parent and one from its female parent. If we examine 100 individuals, each with two genes for a particular protein, we will detect 200 genes. Many of the 200 genes will be identical but some may be slightly different. If 100 individuals are examined from each of a number of populations, it is a relatively easy matter to identify and count how many of their genes are identical; to determine whether particular genes occur in all populations or only in some; to work out whether the differences, if any, between populations are statistically trivial or important; finally, to infer whether or not mating between the populations is restricted or occurs freely. Remember that when mating is restricted between populations and they show heritable genetic differences, they can be termed true stocks or races.

*Enzymes are special proteins which act as catalysts in living cells, controlling the chemical reactions which we call the metabolism of the cell.

CONTINENTAL STOCKS OF ATLANTIC SALMON

Two features of the biology of the Atlantic salmon would cause us to suspect that this species is indeed divisable into a number of distinct stocks. These are, firstly, its widespread distribution in temperate and arctic zones of the North Atlantic basin (fig. 1), and secondly, its well marked homing instinct. As to the former, it is virtually axiomatic in animal biology that species with such wide geographic distributions are comprised of distinct, if not identifiably different, spawning stocks. The reason is simple: it is highly unlikely that individuals from different extremes of the range will meet and mate with the same frequency and regularity as close neighbours do; individuals tend to cluster into localised spawning groups rather than mate at random throughout the whole range. Even in species which undergo extensive migrations, the tendency to form separate spawning stocks and for the gene pools of the separate stocks to evolve differently, can be maintained by a homing instinct. The homing instinct is particularly well developed in Atlantic salmon.

If, for the moment, we ignore the widespread distribution of the Atlantic salmon and its very precise homing instinct, we certainly can, I believe, point to some distinct races of this species. In many parts of northern Europe and in North America, populations are known which are unable through geographical constraints, or if able are unwilling through behavioural or physiological constraints, to migrate to sea at any stage of the life cycle. These are the famous landlocked or non-anadromous* populations such as occur, for instance, in Sweden and Finland in the East, and in lakes of Maine and Newfoundland in the West. In these cases the landlocked or non-migratory stocks are well separated, in a genetic or hereditary sense, from the rest of the species, and they constitute races which have long been recognised as separate, and in some instances given sub-specific status e.g. Salmo salar sebago in the U.S.A. We will see later that they also differ significantly from anadromous stocks in the frequency of certain genes.

* Salmon are normally anadromous, that is they spend part of their life in the sea but they return to freshwater to spawn. Some salmon populations spend all their life in freshwater and these are called non-anadromous populations.

If we consider the topography of the salmon's range (fig. 1) we see that it falls into two distinct geographic regions, the North American and the European. This might lead us to suspect (especially if we ignore the shared marine feeding ground at West Greenland, which was unknown prior to 1960) that, as a first approximation, the salmon of the two continents comprise two distinct stocks. However, the discovery of a common feeding ground for European and North American salmon in the Davis strait off West Greenland might suggest otherwise. Here, it appears, is an area where salmon from many rivers meet and intermingle, and from which they might recruit, possibly at random, to both continents. It was precisely the emergence of this fishery, and its possible effects on home water fisheries and their management, which first made it essential that the precise origin of the salmon at West Greenland be accurately known.

It became abundantly clear from early tag returns that not all rivers, or regions, contributed equally to the high seas fishery for salmon at West Greenland. For example, salmon from the Swedish East coast, and other Baltic states, spend their feeding phase predominantly in the southern Baltic Sea, from whence they migrate back to the rivers of their origin. In so far as these fish rarely, if ever, mingle with those of other European areas, they appear to be reproductively separate and could possibly, constitute one or more distinct races. Other tagged fish evidence suggested that the European fish at West Greenland came largely from the westermost countries of Europe i.e. fish from Norway and from S.W. Sweden were not present in the expected concentration at West Greenland. Based on tag returns alone, then, the migratory pattern of Baltic, Norwegian and Western European salmon seemed to be quite different and since this would reduce the amount of intermingling between them during their marine feeding phase it suggested the possibility that they might, in fact, be different stocks. What we needed to determine this was genetic evidence.

The Swedish scientist Lennart Nyman was the first to provide the genetic evidence, based on electrophoretic studies of proteins in the blood serum of individual salmon. He examined 2 year old immature individuals which had been reared, under identical conditions, in Sweden from ova obtained from Canada, from Sweden and from Finland. He also examined some sexually mature Swedish salmon. There existed small, but significant, protein differences between the Canadian and Swedish fish, with the Finnish fish, and others from Ireland, Scotland and Norway, resembling the Swedish. Later on, he showed that these protein differences were also evident in large individuals, and using these differences he could calculate the proportions of North American and European fish in the West Greenland and Labrador fisheries. This was the very first evidence that North American salmon differed genetically from those of European rivers and that they constituted a seperate stock or race.

Very soon other studies were carried out with even more precise results. Dag Moller, a Norwegian working in Canada, undertook the analysis of the blood serum of almost 2,000 salmon from 10 Canadian rivers, and two rivers in Maine. Concentrating, unlike Nyman, on a single, identifiable protein called transferrin, he could distinguish eleven different individual patterns, of which only three patterns were common. The three patterns could be explained by the presence of two, quite different transferrin genes (called TfA and TfC) at the transferrin locus. Individuals which inherited the TfA gene from both the male and female parent had the genotype TfA/TfA;

those which inherited the TfC gene from both parents had the genotype TfC/TfC; those which inherited the TfA gene from one parent and the TfC gene from the other, had the genotype TfA/TfC. From this information it was possible to determine the frequencies of the TfA and the TfC genes in the various populations.* Only one kind of transferrin gene - TfC - occurred in Norwegian fish while three kinds - TfA, TfB and TfC occurred in Canada. This confirmed Nyman's result that North American and European (Norwegian) salmon were genetically distinct stocks, although only very few European salmon were examined by Moller.

This shortcoming on the European side was overcome by Ron Payne and others. They examined almost 10,000 salmon from British and Irish rivers and identified two transferrin genes. One of these - Tf1 - was identical to the TfC gene of Canadian salmon. The second gene - Tf2 - did not occur at all among Canadian fish. Among Canadian salmon they identified three genes. Tf1, Tf3 and Tf4. These were shown to correspond to Moller's TfC, TfB and TfA respectively. So now it appears that Tf2 occurs exclusively in European salmon and Tf3 and Tf4 exclusively in North America. This evidence clearly indicated that the stocks or races of the two continents are genetically distinct, to such an extent, indeed, that Payne and his colleagues suggested they should be recognised as two distinct sub-species. Salmo salar americanaus and Salmo salar europaeus

* Genes are sections of chromosomes which themselves are long molecules of DNA. The section of the chromosome at which the genes for a particular character occur is called the locus for that character. Different characters are controlled by genes at different loci. Transferrin genes occur at a single locus called the transferrin locus.

Individuals who inherit the TfA gene at the locus from both parents are called TfA/TfA homozygotes. Those who inherit two TfC gene are called TfC/TfC homozygotes. Individuals who inherit TfA from one parent, and TfC from the other are called TfA/TfC heterozygotes. Since each TfA/TfA homozygote possesses 2 TfA genes, and each heterozygote possesses one TfA gene, the frequency of the TfA gene in the population is calculated as follows :-

$$\frac{(2 \times \text{No. of TfA/TfA homozygotes}) + (\text{No. of heterozygotes})}{2 \times \text{total number of individuals tested.}}$$

if the gene frequency of TfA is 0.4, then 40% of all the Tf genes in the population are TfA. The remaining 60% will be Tf B,C, etc. There are statistical tests which can then be used to determine the significance of the differences observed between the TfA gene frequencies of different populations. Generally speaking, the greater the number of individuals tested the easier it is to detect small but statistically significant differences between them.

REGIONAL STOCKS OF ATLANTIC SALMON

The genetic distinction observed between the North American and the European races represents a significant split in the Atlantic salmon species but it was not the only important result of the studies of Moller and Payne. Within both races, significant genetic differences were observed between the various river populations of different regions. The North American race is not a single, homogeneous stock, nor is the European.

Table 1 presents the data on Canadian populations obtained by Moller and later extended by Payne. The frequency of the Tf4 (TfA) gene among anadromous populations varied from 0.60 in Maine to 0.07 in Newfoundland. However, when the frequency is plotted against the latitude of each river (fig. 2), it shows a steady decline in value with increasing latitude. In other words, rivers in the Northern region have populations with lower Tf4 values than those at southern latitudes, with no abrupt transition between them.

In the European area, transferrin studies on British and Irish populations indicate that two distinct races of salmon can be distinguished in these islands. Fig.3 shows the distribution of these two races, based on the study of almost 10,000 fish from 25 rivers. The populations of the south-western, Celtic race have a higher Tf2 gene frequency than those of the Boreal race and the boundary between them is sharply defined; there is, for example,a five-fold difference in the Tf2 frequency between the Kenmare river and the river Shannon, whose mouths are only 150Km apart around the coast.

To summarise the macrogeographic variation then, the Atlantic salmon can be divided genetically into a North American race and a European race; neither race is homogeneous, both consisting of subsidiary stocks. Within North America these stocks are related clinally to each other. In Europe there exist at least two races, the Celtic and the Boreal. The Baltic salmon constitute a third distinct European race.

If we anticipate a little the work on individual rivers discussed below it is possible to illustrate diagrammatically the relationship of the different races to one another. This is done by examining the gene frequencies not just of one kind of protein (like transferrin), but of a number of different protein systems, usually enzymes. The small differences in each enzyme system observed between populations can be summed, and a dendrogram, or tree, which divides the populations according to their degree of genetic dissimilarity can be constructed. Fig. 4 shows such a dendrogram constructed by Dr. Tom Cross. Although the data for this tree are derived from only a small number of rivers, and a small number of enzymes thereby producing unusually high values, it illustrates well the continental and regional race structure discussed so far. The greatest degree of genetic distance occurs between the North American and the European races; within both continental races genetically separate stocks can be detected. Within Europe, Swedish (Baltic) salmon are well differentiated genetically from salmon of Atlantic rivers; the Irish salmon (from a river in the Celtic race area) are genetically distinct from Norwegian (in the Boreal race area). Although dendrograms of this form must be approached with critical caution, the picture is reasonably clear.

DIFFERENCES BETWEEN RIVER STOCKS

The differences between regional races are based on the genetic differences observed between different river populations, and it is time now to examine briefly this heirarchical level of stock distinction. Turning again to Moller's and Payne's results in Table 1, we see that each river population has a different frequency of the Tf4 gene. Generally speaking, the greater the distance between rivers the more their Tf4 frequencies differ. Sometimes the differencies are only trivial, for example, rivers 7 and 8. But when sample 6 from the N-W branch of the Miramichi river is compared with sample 5 taken from the S-W branch of the same river, the gene frequencies show a highly significant difference. On this evidence the stocks of the two main branches of this river are genetically distinct. On the other hand, there is almost no difference between the smolts and the grilse taken from the N-W Miramichi (samples 6A, B, C), suggesting that within the stock of this major branch there is little change in gene frequency in different generations. Other examples worth noting are the major difference between the anadromous stock of the Exploits River, Newfoundland (sample 14) and the non-anadromous stock above Grand Falls on the same river (sample 15) or the difference between the anadromous population of White Bear River, Newfoundland (9) and the non-anadromous populations of lakes in that river system (10 and 11)

It is not just in the North American race that the stocks of different rivers are genetically distinct. Nor is transferrin the only protein which can be shown to differ genetically in the various stocks. The most recent electrophoretic studies analyse genetic variation in a number of very precise enzyme systems. This adds extra expense to the analyses, but the range of enzyme systems and the genetic precision with which they can be identified more than compensate for this.

Tom Cross and his colleagues in Ireland examined two enzyme systems in salmon from the rivers Bandon and Munster Blackwater. Both these rivers contain fish of the Celtic race, but there were significant genetic differences between their parr populations when they were examined for genetic variation involving the liver enzymes Asparate aminotransferase and Isocitrate dehydrogenase. Adults taken from the estuary of the Blackwater did not differ from parr in that river. Cross later extended his study to fish of the river Moy and the river Carrowniskey, two rivers in the area of the Boreal race. The Carrowniskey parr were significantly different from those of the other rivers examined, including the Moy which is 125 Km distant around the coast. So far these are the only published data available which compare the stocks of different rivers in the Irish and British area by analysis of enzyme protein variants. Nevertheless they show clearly that within both the Celtic and the Boreal races there exist genetically distinct river stocks of salmon. Further studies on British and Irish populations are urgently needed. The populations of these islands constitute the largest elements of the European stock of salmon and they are under significant threat from over-exploitation. Once wild stocks decline quantitatively, qualitative changes occur in their genetic make-up by a process called randon genetic drift. This phenomenon, discussed later, causes a decrease in genetic variability and can hasten the extinction of wild stocks.

By far the most extensive studies of genetic variation in the enzymes of Atlantic salmon have been those carried out on Swedish Baltic salmon by Nils Ryman and Gunnar Stahl. Stahl was able to analyse up to 45 separate enzyme

systems. Variant genes occurred in 6 of these systems and it was possible to use them in comparing different populations. Samples of 438 wild parr were collected from the rivers Torne, Lainio, Kalix Kaitum, Byske and Logde. The Torne and Lainio form part of a single drainage system, the Kalix and Kaitum are part of a second, nearby, drainage system and the Byske and Logde are two other, quite separate, drainages further south. When the populations of these rivers were compared, there was considerable genetic diversity between them. Not alone were there differences between the drainages, but there were statistically significant differences between populations of rivers within drainages also. A dendrogram of genetic differences (fig. 5) shows that the geographically close populations were more similar than those further apart. The main branching point in the dendrogram is between the two populations from the more southerly rivers Byske and Logde, and the four other populations. The greatest similarity is between the two populations of the Kalix/Kaitum drainage. So, once again, the evidence indicates not only that the salmon stocks of different rivers are genetically distinct but that stocks within common river drainages may also differ genetically.

Summarising these and other studies on 32 populations of salmon from Northern Europe, Nils Ryman tabulated the amount of genetic diversity observed and how it was distributed. Table 2 presents the relevant part of Ryman's tabulation. Columns 2 and 3 give a technical measure of the gene diversity measured over all the data (Col. 2) and the average gene diversity within populations for each enzyme, (Col. 3). Columns 4-8 indicate how the total gene diversity is distributed. Looking at the overall average values, 78.6% of the observed variation occurs within populations and represents differences between individual fish. The remainder of the variation, 21.4% arises from differences between populations of Atlantic and Baltic rivers (12.3%), differences among rivers within the Atlantic and Baltic groups (6.1%) and differences between populations within rivers (2.8%). For comparison, about 7% of the total gene diversity in man arises from differences between the 3 major races.

Thus when we consider all the genetic evidence it appears that the Atlantic salmon is far from a "single interbreeding and remarkably homogeneous" species throughout its whole range. On the contrary, it can be divided into a large number of local spawning populations which differ genetically from one another in small but statistically significant ways. This division, like so many in biology, constitutes an hypothesis which best explains all the information at present available. There are, undoubtedly, many other genetically-controlled characteristics which could be examined and used in the racial analysis of salmon stocks. However, to have uncovered so much genetic diversity without mentioning "spring fish" or "grilse" or "summer fish" is an achievement in itself. Of course it also serves to emphasise that we are really only now coming to understand some of the major biological features of this species and that many facets of its biology are still unknown.

Are we finding out about the racial structure of salmon too late? After all, salmon ova and fry are transferred almost every year between localities; hatchery stocking is widespread and natural populations are declining almost to extinction in many regions. The answer to the question lies in yet another: what do the racial differences mean and how important are they?

THE SIGNIFICANCE OF THE STOCK STRUCTURE

In any species, the pattern of genetic diversity that exists between natural populations results from the operation of three factors. These are migration, natural selection and genetic drift.

Migration of individuals between populations facilitates what is termed "gene flow", and it erodes any differences which might otherwise occur through natural selection or genetic drift. The demonstration that salmon populations differ not only phenotypically but genotypically as well, confirms that there is relatively little migration or "gene flow" between them. One consequence of this is that once a particular river loses its native stock it is not likely to be rapidly recolonised by "strays" from other rivers or populations. For this reason alone, each stock should be treated as a separate unit for management purposes. Stocks from drainages having many rivers flowing into a single estuary, may, of necessity have to be considered together, if only because the greatest fishing effort is likely to be in the common estuary. But at the level of recruitment, and in other critical features, it is the local biological unit which is the most important management unit.

The virtual absence of inter population migration or gene flow can be attributed to the very precise homing of individuals to their natal streams. Experience in Iceland and Ireland suggests that this precision is not simply a matter of smolts picking up and retaining, during their seaward migration, an "imprint" of the waters from which they migrate. This is shown by the following observations: firstly, wild fish, grown naturally in their natal rivers and tagged as downstream smolts, show lower straying rates compared with single-generation hatchery smolts, and compared to wild smolts released in and "imprinted" to non-natal streams. Secondly, the rate of straying in a hatchery reared stock declines over a number of generations when progeny of that hatchery which has successfully returned from the sea is the only broodstock used in each successive generation. These are exactly the observations expected if homing to a particular freshwater locale has a genetically inherited component which can be augmented by selection. They suggest that precise homing to and identification of natal streams is not alone "physiologically imprinted" but may also be "genetically programmed" for every stock. Problems arising from the genetic programme may well explain why stocking with hatchery stock or transplanted wild fish is not universally successful.

What is the benefit to salmon in having so complex a stock structure maintained at least in part, by genetic means? Many biologists believe that species adopt a strategy of maintaining multiple, separate, spawning populations as a way of maximising their exploitation of favourable breeding sites and of reducing the risk of extinction associated with having only a single spawning concentration. This strategy may be particularly important in temperate and sub-arctic regions where the period during which freshwater conditions are suitable for spawning and egg incubation varies seasonally and geographically. Reproductive success in these regions is maximised for a species when different stocks are genetically adapted to the conditions of their natal streams, and homing is precise and reliable. Because the physical, chemical and biological conditions of each river are different, the evolutionary pressure of natural selection will be different for each population and they will diverge genetically over time. In this way, the genetic diversity increases within the species, and this diversity enhances the species' ability to survive.

Even within a single population, diversity of phenotype is exploited to enhance survival. Consider, for instance, a hypothetical population comprised entirely of individuals which smoltify after one year in freshwater and return to spawn once only as grilse. Should an environmental hazard, such as flood, ice etc., destroy all the redds and developing eggs of one year class, this cohort would be wiped out for many years in the future. Every third year from then on, there would be no spawning stock, and it would not be replaced unless strays or hatchery stock were successfully introduced and established. However, in real populations of salmon, individual phenotypic differences in growth rate, age of smoltification and age at sexual maturity help to buffer the population against such transient, but maybe catastrophic, environmental problems. Not all individuals smoltify in the same year; adults return after one, two or more years at sea and some are repeated spawners. It should now come as no surprise to learn that growth rate, smolt age and age of sexual maturity have all been shown to have genetic factors controlling them in a most complex fashion, (see next section) and they exhibit different frequencies in different populations. Diversity of phenotype enhances survival within a generation; diversity of genotype maintains a stock's, and ultimately a species', survival over many generations.

Random genetic drift is the third factor which affects patterns of genetic diversity between stocks. Briefly it means that in every generation small random changes occur in the frequencies of those genes which are not strongly influenced by natural selection. Its ultimate, long term effect is to increase homozgosity and reduce variability within each stock. Because the process is random, different stocks become homozygous for different genes and for genes affecting different characteristics. In this way, genetic differences between populations may accrue without the influence of natural selection. Since this occurs only in genes which are neutral or only weakly responsive to selection, it could normally be overlooked. But random genetic drift, which is ultimately a form of inbreeding increases as population size decreases. It is most important in very small populations. Populations undergoing serious numerical decline may also be undergoing significant genetic change; eventually when population size is very small (say below 100 spawning individuals) random genetic drift and inbreeding may be more important than natural selection in determining gene frequencies, and all genetic characters will be affected. This could be a recipe for final disaster in declining wild stocks. When genetic variability is lost for any reason, it cannot be restored without human help or the slower process of mutation*. A restoration of the population size will not, of itself, restore the lost variability.

* A Mutation is a spontaneous heritable change in a gene. Mutations occur at random, but very infrequently, in all organisms. Mutations introduce new genes into a species genetic make-up but this is a very slow process requiring many generations to build up a significant amount of variability.

GENETICS OF QUANTITATIVE CHARACTERS

The genetic differences in proteins and enzymes which can be detected by electrophoresis are relatively simple when compared with quantitative characters like "growth rate" or "age at first maturity" or "time of return to the river". With a little reflection readers will realise that these latter characters are physiologically complex features and that individuals even within a single population often exhibit a vast array of phenotypes, some shading almost imperceptibly into others. "Like" does not always "breed like"

where these characters are concerned. For instance, when a 2-sea winter female is crossed with a 2-sea winter male, a large proportion of the offspring (perhaps even all of them) may exhibit the grilse (1 sea winter) phenotype. This failure to "breed true" is confusing to the non-expert, who may conclude, erroneously, that inheritance is not important in these characters. In fact, there is abundant evidence from wild fish and from hatchery experiments, that inheritance is a significant determinant of age at first maturity in the salmon. What these studies show is that quantitative traits are governed by genes at a number of different loci and that the effects of these are modified by environmental factors.

Referring back to the transferrin studies (p 9), recall that if there are 2 kinds of genes, say A and C, at a locus there are three possible genotypes, AA, AC. and CC These are conventionally written as $\frac{A}{A}$ and $\frac{A}{C}$ and $\frac{C}{C}$. An individual of genotype AC at one locus and, say, XY at another locus is conventionally written as $\frac{AX}{CY}$. Now consider a hypothetical quantitative trait which is governed by genes at three separate gene loci. Suppose that a + gene at any of the three loci adds one unit to the trait and a - gene adds nothing, and that 4 + genes (4 units) must be present if the trait is to be expressed. There are $3^3 = 27$ possible genotypes ranging from $\frac{+++}{+++}$ to $\frac{---}{---}$ through $\frac{+++}{---}$, $\frac{++-}{--+}$ and so on. Of the 27 possible genotypes, 6 will contain 4 + genes (e.g. $\frac{+++}{+--}$, $\frac{+-+}{-++}$ etc.), 3 will have 5 + genes ($\frac{+++}{++-}$, $\frac{+++}{+-+}$, $\frac{+++}{-++}$) and 1 will have 6 + genes ($\frac{+++}{+++}$). In other words, 10 genotypes will have "sufficient" + genes to express the trait, but each genotype will be different and they will not all be equally capable of producing offspring with 4 + genes. For example, individuals of the genotypes $\frac{+++}{--+}$ and $\frac{-+-}{+++}$ both have 4 + genes each; if they were mated, some of their offspring would have the genotype $\frac{-+-}{--+}$ and therefore would not express the trait as they would not have 4 + genes. Conversely, two adults of the genotype $\frac{+-+}{-+-}$ would not themselves have the 4 + genes to express the trait but some of their offspring, of genotype $\frac{+++}{+++}$, would! In none of these examples would the parents "breed true". Obviously, the greater the number of + genes among the parents the greater the probability that they will breed true for the trait in question.

This model is very much simplified, but it illustrates the complicated genetic background involved in quantitative traits. The inheritance of these traits is complicated even further by environmental factors. Imagine, to continue the above model, that 4 + genes are necessary to express the trait under "normal" conditions, but that 5 + genes, or perhaps even 6 + genes, are necessary at other times. Then the number of genotypes (of the 27 possible) which will express the character at any given time will depend not only on the genotype (number of + genes present) but on the prevailing environmental conditions (determining the number of + genes required). Finally, there are likely to be

many more loci than three involved in these traits and the "Value" of the genes at each locus are unlikely to be equal under all conditions. If 6 loci are involved there are 3^6 =729 different genotypes! Many of these will be of equal value, and many will have different values in different environments.

The complexity of the "age at first maturity" phenotype and other quantitative traits should now, hopefully, be a little clearer: they depend on genotype and environment, but we do not always know the relative importance of each at any particular time or in any particular stock. Regarding the environment, we do know that high winter sea temperatures seem to favour the grilse phenotype. We know little about the number and kinds of genotypes involved except that different local stocks have different genetic capacities to produce grilse or 2-sea winter fish. What can be done to maintain a stock of spring fish, or early returning fish, in a population where we know so little about their genotypes? From our knowledge of quantitative traits in domestic livestock we know that constant mating of like with like may build up a selected line in which the desired genes will accumulate at the many loci governing the selected trait. Thus we should ensure, as far as possible, that the individuals or families that possess the desired characteristic, mate predominantly among themselves. This strategy should be maintained over a number of generations, using as broodstock in each generation individuals which not only express the desired character but are themselves the offspring of parents expressing that character. This strategy may not always be as successful as we might wish (some characters may not respond well to this kind of selection). But the strategy emphasises yet again the importance of maintaining the integrity of the local wild stock: adaptation to local conditions is very likely to be a quantitative trait which is maintained by the mating; in each generation, of locally adapted individuals. Dilution of the local stock with strays or introductions may disrupt the process of local adaptation by disrupting the accumulation of suitable genotypes. On the other hand, by selectively allowing only fish of a desired type to enter the spawning stock (e.g. by permitting only very early or very late returners to spawn) it may be possible, over a number of generations, to accumulate genes for a specific return habit in a specific stock. This, however, will not be easy!

STOCKS AND ARTIFICIAL PROPAGATION

What practical advice can we draw from our present understanding of the stock structure of the Atlantic salmon? It seems clear that our first priority should be preservation, and if possible augmentation, of existing wild stocks before it is too late. Prevention in this matter is better than cure: improved natural spawning is much more desirable than hatchery introductions or transplants. Only wild stocks contain all the genotypic diversity that forms the basis of the flexibility of the species to adapt to varying environmental conditions. The fact that we often do not know the biological significance of the observed diversity is no reason to alter it indiscriminately; in the absence of this information, but in the light of experience with other species under domestication, a conservative approach is the path of wisdom.

Where natural spawning stocks have a chance of recovery, this can be aided by rehabilitating old spawning beds, predator control, pollution abatement and other good resource management activities. In localities where the only runs of fish are those maintained exclusively by hatchery production, then every effort should be made in the hatchery to breed from sea-run progeny of that

hatchery in every generation. In this way, homing precision should improve and the hatchery stock may come to form an identifiable stock in its own right. In Ireland, for example, over 95% of fish returning to the fish counter on the river Lee are hatchery reared. On the river Shannon, where sea-run hatchery-reared fish have been used as broodstock at Parteen hatchery over a long number of years there is no straying of tagged, hatchery reared salmon.

In the light of our knowledge of the genotypic diversity of salmon and the genetic differences between natural stocks, what practical use can be made of hatchery reared juveniles in stocking programmes? For rivers having no native stock their value is obvious: it may be possible to start a run de novo from such artificially propagated fish, as seems to have happened, for example, on the river Lee. For such rivers a strategy like the following might be found suitable:

(1) Rehabilitate natural spawning and nursery areas, or create new ones if necessary. Ensure that nursery areas are properly protected and managed (predator control, pollution prevention etc.)

(2) Stock artificially produced S1* pre-smolts in the lower reaches of the river but above an upstream trap.

(3) Stock artificially produced S2* parr in suitable nursery areas in the upper reaches of the river.

(4) Repeat steps (2) and (3) each year; stock may come from different hatcheries each year, or the S1 and S2 fish can come from different hatcheries in any year, until a run of adults develops.

(5) If and when an adult run to the river develops allow some adults to escape to natural spawning beds; use some of the returned adults as broodstock in a hatchery to produce the fish for further stocking.

(6) Repeat (2) and (3) each year using the offspring of (5) only.

The rationale underlying this strategy is as follows :

The S1 pre-smolts will be "imprinted" to the river in the year of planting and may form the basis of an adult run to the river within one or two years. The S2 parr will provide smolts which have survived river conditions successfully for one year before migration and which therefore have commenced the process of environmental adaptation. The mixture of S1 and S2 fish, and their diverse hatchery origins, will help to provide phenotypic and genotypic diversity. Using progeny of returning adults when a run is established will help to generate a specific stock by selection, especially when individuals stocked as S2 parr and returning from the sea are used as broodstock. This strategy, whatever its faults, may be more successful in establishing a viable spawning run in the long term than an unplanned stocking project.

* S1 juveniles are those which become smolts after one year in freshwater. S2 juveniles are those which do not smoltify until two years have been spent in freshwater.

In rivers where some native stock still exists the use of hatchery-produced fish could upset the precarious balance of the existing wild stock; in this case the appropriate strategy may need to be much more subtle than some existing practices. The indiscriminate transplantation of non-native wild fish, or hatchery reared fish, to such rivers may have, as an early effect,

the interbreeding of native and non-native individuals with consequent alterations in genetic composition and reducing homing precision in the offspring. Serious consideration must always be given to the demographic status of the native stock before the introduction of non-natives is comtemplated. In these rivers a strategy to be preferred might be:

(1) As (1) above.

(2) Take native male and female fish from the river and use them to produce young in the hatchery.

(3) Stock the S1 pre-smolts from these parents in the lower reaches of the river, but above an upstream trap.

(4) Stock the S2 parr from these parents in the upper reaches of the river.

(5) Use the males of (2) to fertilize non-native females in the hatchery, if native stock is too low to provide enough females.

(6) Dispose of the S2 parr of (5); stock the S1 pre-smolts of (5), suitably marked, in the lower reaches, above an upstream trap.

(7) Repeat (2), (3), (4) and (5) each year, using unmarked fish taken from the fishery.

(8) Take marked returned females from the fishery also, for hatchery use if low numbers of unmarked females makes this necessary. These marked females are the offspring from (5) and (6). Fertilize them with unmarked returned males, or with sexually precocious males if necessary. Repeat (6) with the offspring.

(9) Remove all marked adult males from the fishery if possible.

The rationale of this strategy is as follows:- Steps (2) to (4) in the first generation are a compromise: there will be higher survival over the freshwater stage of the lifecycle if the offspring are reared for one or two years in good artificial conditions, although the survivors may be less suitable for eventual release in the wild. The reason for using S1 and S2 fish is as outlined previously. Step (5) utilizes any excess sperm from (2) to provide native x non-native hybrids to increase numbers. Disposal of the S2 hybrid parr ensures that they cannot interbreed with natives through precocious sexual maturity. Return from sea of the hybrid S1 offspring of (5) will augment the return adult run. Step (8) backcrosses these hybrids to the native stock. Removal of male hybrids (step 9) ensures that hybrid to native backcrossing is the maximum degree of non-native mating that can occur in the natural spawning. A strategy like this will help maintain as far as possible the genetic integration of the native stock while endeavouring to increase it numerically.

It should be emphasised that introductions, stocking projects and hatchery production all have a useful and legitimate, even essential, role in salmon management. But where their use displaces or alters a native stock the cure may prove costly in the long run. There are, too, genetic criteria, regarding numbers of sire and dam fish for instance, that must be met in the hatchery and in the wild if restocking is to have a chance of success. The stocking strategies outlined here are not the only ones possible, but they serve to illustrate the kind of approach that artificial production makes possible.

It hardly needs to be stated that no stocking programmes will be successful unless exploitation outside the home water area is sensibly regulated.

Artificial production of salmon, whether for stocking, cage-culture or ranching operations, can be expected to increase in future years. Provided that broodstock numbers are sufficiently large, an increase in the number of hatcheries and a greater spread of their locations, will ultimately be of benefit. Locally produced fry or smolts are more likely to be suitable for local introductions than more distant stock. The increased number of distinct broodstock lines derived from different founder stocks which is implicit in the strategy of multiple dispersed hatcheries, will facilitate the maintainance of genetic diversity which can be exploited through cross breeding. Perhaps it is only as salmon production becomes increasingly harnessed to commercial development that the intrinsic value of the wild salmon stocks and their genetic diversity will be appreciated. Whether there will still be wild stocks in existence by then is entirely a matter of how we act now.

SELECTED READING LIST

The following list includes books and papers which give general information and will be found useful by most readers. Those seeking more detailed information should refer to the reading lists given in these items.

The pamphlet (Item 1) gives helpful tables of the international catches of Atlantic salmon and other statistical information. Item 2 gives a clear, readable description of the electrophoretic technique and its application to salmon and trout. Jones's book (Item 3) remains an invaluable account of the fresh water life cycle and biology of the salmon. Item 4 is a good account of the early Canadian genetic studies and their management implications The Swedish salmon situation and the question of conserving genetic variability is very well treated in Item 5. Homing studies are reviewed in Item 6. The final item (Item 7) contains the most up-to-date collection of papers for experts on genetics in aquaculture, including studies on salmon stocks by Ryman, Stahl and Cross.

1. Atlantic Salmon Trust, 1983. Atlantic Salmon Facts. The Atlantic Salmon Trust, Farnham 16pp.

2. Ferguson, A., 1980. Biochemical systematics and Evolution. Blackie, Glasgow. 194pp.

3. Jones, J.W. 1959. The Salmon. Collins, London 192pp.

4. Moller, D., 1970. Genetic diversity in Atlantic salmon and Salmon Management in relation to Genetic Factors. The International Atlantic Salmon Foundation, St. Andrews, Canada 29pp.

5. Ryman, N. (Ed.), 1981 Fish gene Pools. Preservation of Genetic Resources in relation to Wild Fish Stocks. Ecological Bulletins (Stockholm) 34 111pp.

6. Stabell, O.B., 1984 Homing and olfaction in Salmonids: a critical review with special reference to the Atlantic salmon. Biol. Rev. 59 333 - 388

7. Wilkins, N.P. and Gosling, E.M., (Eds.) 1983. Genetics in Aquaculture. Elsevier Press, Amsterdam. 425pp.

Figure 1

The distribution of natural populations of Atlantic salmon.

Figure 2

The frequency of the Tf4 gene in the salmon populations of various Canadian rivers plotted against the latitude of the rivers. The frequency declines significantly with increasing latitude. The data are based on Moller's and Payne's results and the graph is based on that in Payne's report in the Journal of the Fisheries Research Board of Canada, Vol.31 1037 - 1041. (1974)

Figure 3

The frequency of the Tf2 gene in the salmon populations of various British and Irish rivers, showing the distribution of the Celtic and Boreal races. Map based on results in Child et al., Journal of Fish Biology, Vol.8, 35 - 43. (1976)

Figure 4

Diagrammatic representation of the genetic difference between salmon populations in different areas. The scale is a measure of genetic distance based on electrophoretic analysis of 6 enzymes in samples of salmon from each region. Populations are connected by means of vertical lines, the position of the vertical determining the degree of genetic distance between them. The genetic distance between the sample from Ireland and Norway is less than 0.1 while the genetic distance between the Canadian and European samples is greater than 0.5. Dendrogram reproduced from Tom Cross's report in the Annual Report of the Salmon Research Trust for Ireland for 1982 - No.27.

Figure 5

Dendrogram summarising the genetic difference between the salmon populations of 6 rivers in Sweden. Genetic distance was calculated from gene frequency data for 45 enzymes. Reproduced from Stahl's report in reference 5.

Table 1

The frequency of the Tf4 gene in various Canadian and American salmon populations. Data from Payne's report in the Journal of the Fisheries Research Board of Canada, Vol 31, 1037 - 1041. 1974.

Table 2

The distribution of genetic diversity detected by electrophoresis of seven enzymes systems in 32 populations of Atlantic salmon from Northern Europe. The average value includes data on 30 other enzymes. The values of absolute gene diversity are statistical measures derived from the gene frequencies. The table shows that most of the genetic variability occurs within populations i.e. between individuals. The amount of variability between populations is greatest when the populations are from distant drainages. There is measurable degree of diversity between populations within rivers. Data from Nils Ryman's paper in reference 7.

Fig. 2.

0·6
0·5
0·4
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44
45
46
47
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2
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4
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6
8
qTf4

Fig. 3.

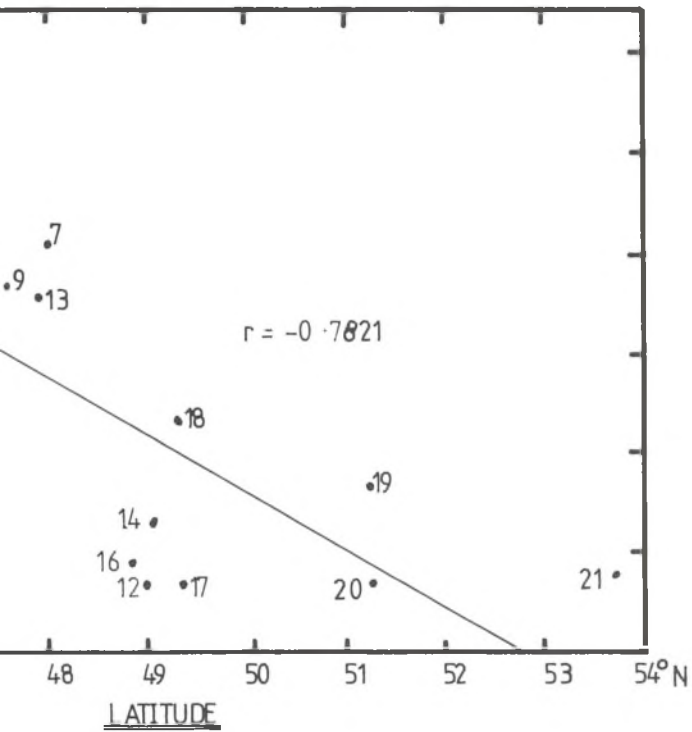
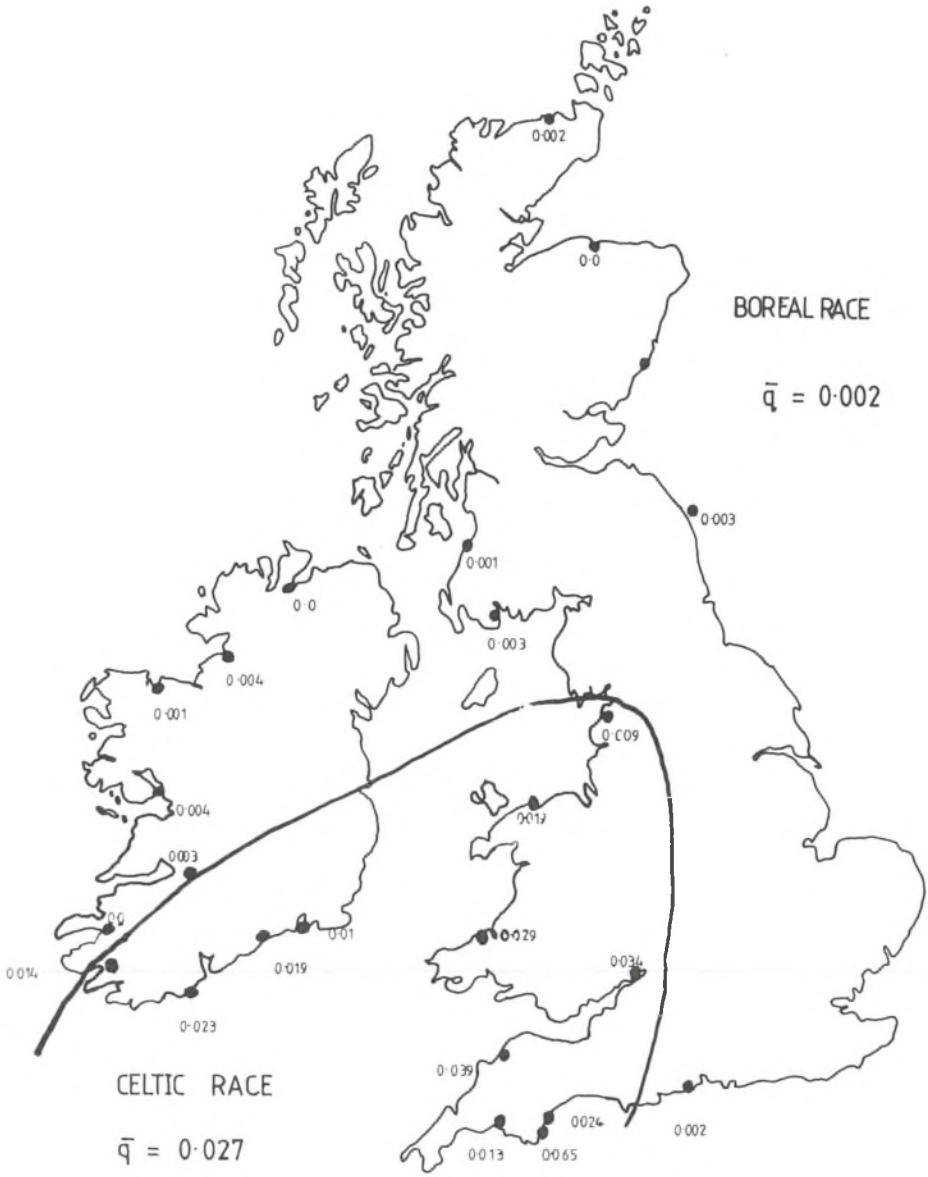


Fig. 1.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BOREAL RACE
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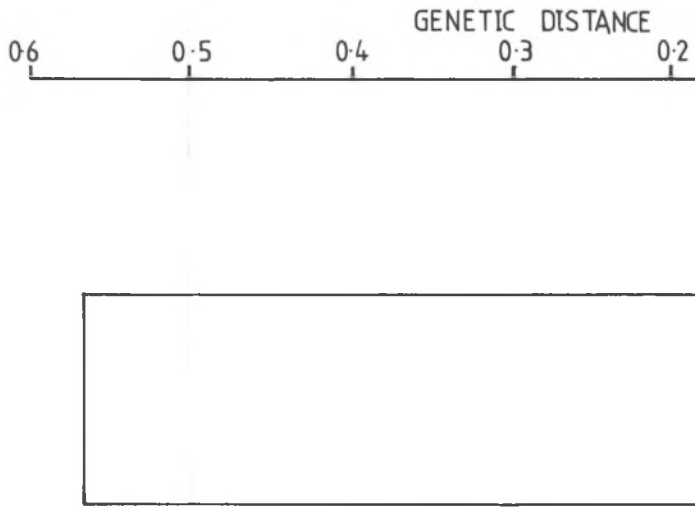
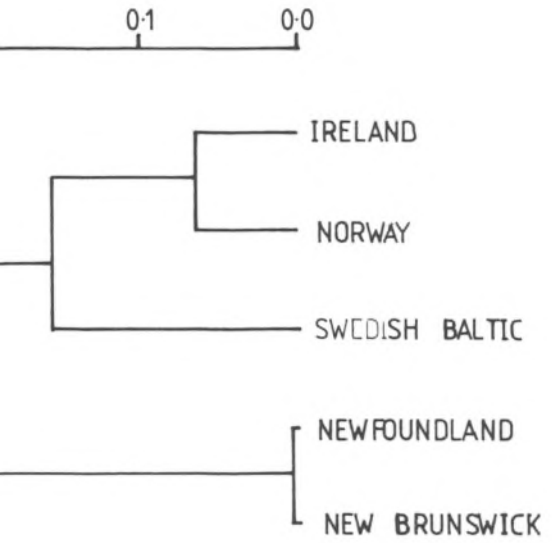
0·019
0·023
0·029
0·034
CELTIC RACE
$\bar{q}$ = 0·027
0·039
0·024
0·002
0·013
0·065

Fig. 4.

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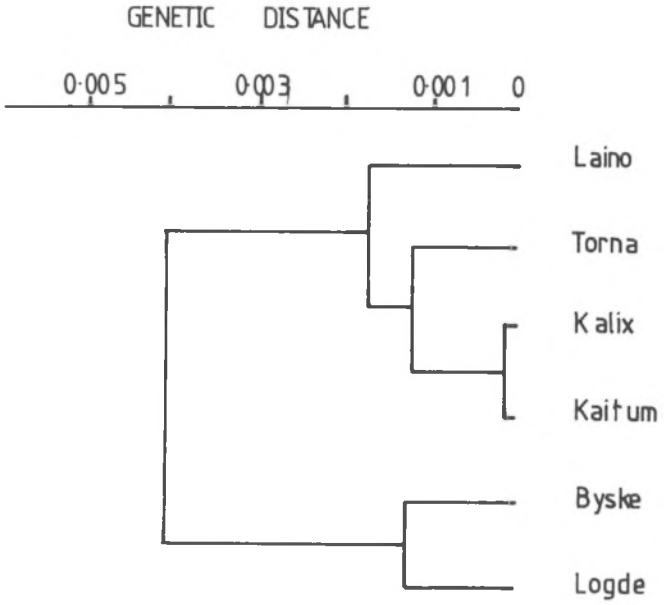
IRELAND
NORWAY
SWEDISH BALTIC
NEWFOUNDLAND
NEW BRUNSWICK

Fig. 5.


GENETIC DISTANCE
0·005
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0
Laino
Torna
Kalix
Kaitum
Byske
Logde

TABLE 1

	Population	Type of Fish	No.	Frequency Tf4
1.	Narraguagas R., Maine	Grilse and adults	24	0.60
2.	Machias R., Maine	Grilse and adults	32	0.59
3.	St. John R., N.B.	Grilse and adults	338	0.26
4.	Philip R., N.S.	-	120	0.32
5.	S.W. Miramichi R., N.B.	Grilse and adults	117	0.22
6A	N.W. Miramichi R., N.B.	Smolt	93	0.35
6B	N.W. Miramichi R., N.B.	Grilse	117	0.36
6C	N.W. Miramichi R. N.B.	Grilse	146	0.39
6.	N.W. Miramichi Subtotal	-	356	0.37
7.	Restigouche R., N.B.	-	240	0.41
8.	Margaree R., N.S.	-	95	0.44
9.	White Bear R., Nfld.	-	95	0.37
10.	White Bear Lake Nfld.	Non-anadromous	27	0.91
11.	Granite Lake, Nfld.	Non-anadromous	37	0.78
12.	Adies Stream, Nfld.	Grilse	112	0.07
13.	Come-By-Chance R., Nfld.	-	196	0.36
14.	Exploits R., Nfld.	-	100	0.13
15.	Exploits R., Nfld.	Non-anadromous	78	0.81
16.	Middle Brook, Nfld.	-	95	0.05
17.	Salmon Brook, Nfld.	-	67	0.07
18.	Dog Bay R., Nfld.	-	91	0.23
19.	St. Genevieve R., Nfld.	-	81	0.17
20.	West R., Nfld.	-	156	0.07
21.	Sand Hill R., Nfld.	Grilse	130	0.08

Table 2

Enzyme	Absolute gene diversity		Relative gene diversity (%)			
	Total (H_T)	Within Populations (H_S)	Within Populations	Between populations within rivers	Between rivers within drainage groups	Between drainage groups (Atlantic and Baltic)
Aat-3	0.291	0.228	78.3	5.0	12.8	3.5
Agp	0.001	0.001	98.7	0	1.2	0
Me-2	0.423	0.277	65.5	1.3	4.6	28.6
Mdh-1	0.013	0.013	93.8	0.6	5.3	0.3
Mdh-3	0.005	0.004	95.6	0.3	3.8	0.3
Pgm-1	0.013	0.011	89.4	5.9	4.1	0.5
Sdh-1	0.494	0.440	89.0	2.9	3.6	4.4
Average	0.034	0.026	78.6	2.8	6.1	12.3
S.E.	0.019	0.015	6.6	0.8	2.1	7.3

SOME BIOGRAPHICAL INFORMATION

Noel Wilkins graduated from University College Cork in 1961. After a short period studying the effects of arterial drainage on the salmon of the River Moy, he joined the Marine Laboratory of the Department of Agriculture and Fisheries for Scotland at Aberdeen. Here he was among the first fishery scientists to study the biochemical genetics of commercially important fish. When the fishery for Atlantic salmon developed off West Greenland, he commenced work on salmon and was part of the Scottish research team at West Greenland during the fishing seasons of 1966 to 1969. In 1970 he was appointed lecturer in Zoology at University College Galway where he commenced research on the genetics of shellfish while continuing research on salmon stocks. In 1979 he was appointed associate Professor of Zoology at Galway where he still carries out research and lectures extensively. He is the author of more than thirty research papers on the genetics of fish and shellfish. He was Chairman of the C.O.S.T. 46 project on mariculture from 1980 to 1983 and is currently a member of the I.C.E.S. Working Party on Genetics. At the present time he is the Dean of the Faculty of Science at University College Galway.

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