GENETIC STOCK DISCRIMINATION OF ATLANTIC SALMON

AT CATCHMENT OR SUB-CATCHMENT LEVELS

A. YOUNGSON

Research Contractor:
Scottish Office Agriculture & Fisheries Dept.

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This Report represents a contemporary review of Genetic Stock Identification practices for Atlantic Salmon. It was commissioned for use as an aid to the production of PIDs for future R & D work on the discrimination of salmon and sea trout stocks and stock components.

It is also intended for use by fisheries scientists who may require guidance on current genetic discrimination techniques.

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Executive Summary

Genetic stock discrimination (GSI) demonstrates the potential practical application of genetics in fisheries management. GSI is of potential importance in the regulation of mixed-stock fisheries for all species that disperse from their juvenile locations before returning there to spawn. If GSI is practicable for Atlantic salmon, it should be possible to use genetic information to discriminate within mixed-stock fisheries, assigning parts of mixed catches to their source genetic populations.

This report consists of a preliminary review of the subject which was carried out at the Scottish Office Agriculture and Fisheries Department in order to assist the Agancy in the definition of the study, prior to a competitive tendering exercise. The findings of the review were as follows:-

1. The principles of GSI are sound, but the technique is some way from being a practical fisheries management technique.

2. The potential sources of error affecting the accuracy and precision of GSI can be identified, estimated and therefore modelled.

3. Modelling of GSI and its application to real situations will have to be developed in the appropriate local context.

It is therefore recommended that the utility of GSI based on DNA variation should be explored further because of its potential importance as a management tool; that a local modelling approach should be adopted on a catchment or catchment group; and that the overall study should consist of a sequence of three elements that (a) models spatial variation in DNA variation and the GSI approach, on the appropriate geographical scale, using existing data from other systems; (b) models spatial genetic structure based on an extensive sampling survey of DNA RFLP variation in the chosen catchment unit and models the GSI exercise using bootstrapping techniques and realistic estimates for sampling errors from all sources; (c) performs GSI on a sample of the fishery and validates this exercise by non-genetic means.

Key words:

Atlantic salmon; stock discrimination; genetics; modelling; fisheries management

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GENETIC STOCK DISCRIMINATION OF ATLANTIC SALMON AT THE CATCHMENT OR SUB-CATCHMENT LEVELS

I. Introduction

Genetic stock identification (GSI) demonstrates the potential practical application of genetics in fisheries management. GSI is of potential importance in the regulation of mixed-stock fisheries for all species that disperse from their juvenile locations before returning there to spawn. If GSI is practicable for Atlantic salmon, it will be possible to use genetic information to discriminate within mixed-stock fisheries, assigning parts of mixed catches to their source genetic populations.

There are a number of contexts in which GSI might prove useful. In the ocean fishery buy-outs, for example, payment might be attributed to continental interests on the basis of GSI. In the same way, mixed-stock fisheries in home-waters and financial support for fisheries management might be linked. However, the compelling argument for GSI is a biological rather than a fiscal one and it centres on a requirement to manage salmon on a local or population level. This approach links pre-fishing abundance with exploitation in competing mixed-stock fisheries (coastal and rod) and with final abundance at spawning. In particular, it aims to conserve populations that are being over-exploited by lowering exploitation or by making compensatory arrangements (eg hatchery rearing) to maintain juvenile production.

GSI might be attempted in a purely geographical context, matching exploitation patterns with patterns of spawning among populations defined on the basis of geographical patterns of adaptively neutral genetic variation. Usually however, the driving force behind attempts to use GSI centres on a desire to conserve populations that contribute in particular ways to the fisheries. Usually, the desire to manage in this way has a genetic management component - incorporating the notion that run-timing, for example, has a driving genetic component based on adaptation. In this case the utility of GSI based on neutral genetic variation rests on parallelism between patterns of distribution of neutral genetic variation (that can be measured) and patterns of distribution in adaptive genetic variation (which cannot yet be directly described). This is considered more fully below.

Absolute precision in GSI (the ability to correctly allocate single fish to membership of one of a number of candidate populations) is not a realistic target for Atlantic salmon. Absolute precision requires that unique genotypes distinguish populations. In the case of Atlantic salmon this cannot be expected, as is discussed below. The allocation of individual fish or portions of catches to any of a number of candidate populations will therefore be probabilistic. The quality of any probability estimate will depend on a number of factors that are also dealt with below. In general, these fall into either of two categories that relate to the form of the genetic structure of the populations in question or to the level of knowledge of their genetic make-up.

Since GSI is emphatically genetic it differs radically from attempts at stock discrimination on the basis of phenotypic characters (MacCrimmon and Claytor, 1985, 1986; De Pontual and Prouzet, 1987). For example, juvenile growth differences reflected in scale increment patterns (and presumable due to differences in environmental temperature) have been used to assign catches of salmon at West Greenland to continent of origin (eg Lear and Sandeman, 1980). Intuitively, GSI appears to carry greater power than phenotypic approaches, although the usefulness of the latter should not be discounted. Phenotypic classification may prove a valuable adjunct to future genetic work - as well as providing an independent check on the accuracy of the genetic approach (eg Lear and Payne, 1975; Reddin *et al.*, 1989). Indeed, the utility of the phenotypic approach is not in doubt. Recently, for example, the relationship between time of river entry and final spawning location has been used to ease fishing pressure on early-running populations of the Aberdeenshire Dee and of the Wye, Usk and Welsh Dee. In the case of GSI, however, questions remain as to whether its potential power can be used for Atlantic salmon and, particularly, whether it can be used for practical management for the species at a catchment or sub-catchment level.

II. Background

A. Historical development

The historical development of the GSI approach is rooted in Pacific North America and based on species of the genus *Oncorhynchus* (Marshall *et al.*, 1991; Shaklee *et al.*, 1991; Utter, 1991). It must be borne in mind that the onchorhynchids may be particularly amenable to GSI since, in general, populations of fish on the Pacific coast have a longer post-glacial history than those on the Atlantic coasts. In particular, the Pacific coast of North America was not so much affected as the eastern Atlantic coasts by the most recent (*ca* 12,000 BP) glaciation. Correspondingly, the salmonids of the Pacific coast have had longer than the European salmonids to diverge genetically and to the develop the pronounced and stable patterns of geographical population structuring that facilitate GSI. Although many of the more southern European catchments (including some of those in southern Britain Isles) remained free of ice, major disturbances will have occurred due to profound climatic change and changed patterns of inter-population competition in both the pre- and post-glacial phases. These events are relevant to a consideration of GSI: they set the starting point for the relatively short period of genetic development (*ca* 2,000 generations) that has resulted in the patterns of genetic structure that are evident today.

The study of genetic variation in Atlantic salmon has been dogged by mismatches between the expectations of fishery managers and the apparent ability of genetic analysis to produce insights of lasting or practical value. Early studies of transferrin polymorphisms led Payne *et al.* (1971) to postulate the existence of Celtic and Boreal races of salmon in the British Isles. The range of the Celtic race was supposed to more-or-less coincide with the area that had remained ice-free during the last glaciation. This extended from the rivers of the southern Irish coast, to those of South Wales, the West Country and the Channel coast. The hypothesis was supported by more extensive work on the same locus carried out by Child *et al.* (1976).

However, the hypothesis has not been supported by later work on other polymorphic protein loci and is now regarded as mistaken (see Jordan, 1992). On the other hand, subsequent study of allozyme variation has demonstrated that geographical population structure does exist - on continental (Verspoor, 1986; Cross *et al.*, unpublished), regional (Ståhl, 1987; Jordan *et al.*, 1992; Wilson *et al.*, 1995) and sub-catchment (McElligott, 1987; Ståhl, 1987; Verspoor and Jordan, 1989; Jordan *et al.*, 1992) scales.

In general, the allozyme techniques offer insufficient resolving power to test the GSI approach. The development of new techniques based on direct analysis of DNA variation has therefore been awaited and these are now available for minisatellites (Taggart and Ferguson, 1990), mtDNA (McVeigh *et al.*, 1995) and microsatellites (McConnell *et al.*, 1995). It remains to be seen whether the additional power of these new techniques is sufficient to offer GSI solutions to practical fisheries problems.

B. Genetic variation

Cross and Ward's landmark paper of 1980, is an inventory of the allozyme variation known to exist in Atlantic salmon at that time. Using a standard suite of allozymes on fish derived from Irish rivers, they identified a number of polymorphic loci and codified the variation observed. Subsequent studies have not altered Cross and Ward's conclusions to any great extent and the paper remains a standard reference. With respect to the allozyme loci, Atlantic salmon appears to be a relatively monomorphic species. Most studies of salmon have been based on a suite of only six polymorphic loci (*mMEP-2** [formerly Me-2], *sAAT-4**, *IDHP-3** [Idh-3], *IDDH-1,2** [formerly Sdh-1,2] and *MDH-3**). In 1992, Jordan reviewed all the available information to that date (including unpublished data) for allozyme variation for salmon in the British Isles. Jordan concluded that statistically significant genetic heterogeneity was present within and among rivers, although the same allozyme variants were widely distributed among sampling sites. Jordan recommended that additional genetic markers should be identified to clarify the observed patterns of genetic differentiation and to test the mechanisms that generate the patterns. Recent studies (Verspoor and Jordan, 1994; Wilson *et al.*, 1995) have added a number of novel, additional protein polymorphisms to the list of informative loci but also confirmed that (with respect to the allozymes) the Atlantic salmon is one of the least genetically variable salmonids.

The major advantage of the use of allozymes has been the relative simplicity and inexpensiveness of performing laboratory analysis. As a result, study of allozymes has been extensive and a substantial volume of interesting and useful information has emerged. Important insights have been gained on the central question of neutrality - whether genotypes at variable protein loci can be regarded as being without effect on relative fitness. The validity of many forms of genetic analysis is conditional on this neutrality and the assumption is often made that neutrality holds. However, the assumption is demonstrably violated for *mMEP-2** (Verspoor and Jordan, 1989; Jordan *et al.*, 1990; Jordan and Youngson, 1991; Verspoor *et al.*, 1991; Jordan *et al.*, submitted) and questions have been raised regarding transferrin and MDH (Verspoor, unpublished). Bearing in mind that *mMEP-2** is one of only a small

polymorphic allozyme set in Atlantic salmon, and the most variable, its non-neutrality is an impediment to the valid use of conventional genetic modelling of allozyme data sets. Non-neutrality will confound GSI techniques if patterns of allele distribution are affected by the effects of natural selection.

Within the last decade, and especially within the last five years, the search for genetic markers has turned from the functional loci represented by allozymes towards polymorphisms in non-coding sequences in nuclear and mitochondrial DNA (Taggart and Ferguson, 1990; McConnell *et al.*, 1995; McVeigh *et al.*, 1995). The technology has advanced rapidly and restriction fragment length polymorphism (RFLP) analysis of DNA is a routine procedure carried out in many laboratories. Minisatellite and microsatellite DNA techniques, especially, provide access to many more, highly variable loci (Taggart *et al.*, 1995). It has been expected that the advent of the new DNA methods would resolve the present impasse on GSI caused by the deficiencies of allozyme data. This may prove to be the case. Alternatively, it may be that Atlantic salmon populations do not conform nicely to genetic patterns that are amenable to GSI analysis. New access to more, variable loci will not resolve difficulties posed for GSI by the behaviour of salmon.

III. Constraints on GSI

A. Population structure

Populations are defined as breeding groups that are wholly or partially reproductively isolated. In general, the behaviour of salmon is consistent with the existence of population structuring. Juvenile salmon are territorial and tend to live their fresh water lives near the locations in which they were spawned, before they leave for the sea. On their return from the ocean, adults are well-known to be capable of homing with considerable precision to their own rivers. Tagging studies have shown that many adults home with substantially greater precision than this. For example, The Girnock Burn comprises about 1% of the Aberdeenshire Dee catchment and it joins the main river about 65 km from the sea. In spite of the stream's smallness and its remoteness, *ca* 50% of the adults reaching the Girnock Burn trap are derived from smolts produced in the stream above it (Youngson *et al.*, 1994). This measure under-estimates the true extent of homing because trapping before tagging is not total. In addition, a single trap is unlikely to enclose a discrete population unit. Thus, some adults probably do not reach the trap in spite of having homed accurately while other homing fish will have originated near to but below the trap.

On the grounds summarised above, *biological* population structure can be stated to exist in Atlantic salmon and fisheries management ought to be approached in the knowledge that homing units (populations) exist at a sub-catchment level. Precise geographical description of population units is not possible. However, treating smolt tagging and adult trapping as a mark-recapture exercise, suggests a numerical dimension for the Girnock unit of *ca* 10,000 smolts.

Whether discrete *genetic* populations exist as a result of this biological organisation is another question that must be considered separately. Indeed, the question has two parts. The first part relates to the behaviour of adaptively neutral alleles. No fishery manager is likely to argue that neutral genetic variation is of conservation value itself. However, the conventional GSI model is based on a detailed description of the distribution of selectively neutral genetic variation within the geographical area of interest.

The second part of the question relates to the genetic basis of the phenotypic heterogeneity among populations (eg run-timing) that is often the principal management target. An important divergence of interests with respect to GSI should be noted here. Population geneticists target neutral variation because non-neutrality disrupts conventional genetic models. Fisheries managers are often most interested in the non-neutral (adaptive) variation that determines how individuals contribute to the fisheries.

B. Neutral versus adaptive genetic variation

To a large extent, the rules that determine how genetic variation is distributed differ with regard to neutral and adaptive variation. In the case of adaptively neutral genetic variation, genotype is not expressed in phenotype and therefore has no effect on fitness. Under these circumstances the development of patterns in the distribution of alleles (and therefore genotypes) among populations is stochastic. Geographical differentiation is driven by population events like founder-effects or bottle-necking or by genetic drift. In the case of adaptive variation - drift, bottle-necking and founder effects may all determine patterns of distribution of genetic variation but, in addition, patterns of environmental variation have important effects.

Mismatches in patterns of neutral and adaptive variation might be expected since the rules that govern the temporal and spatial distribution of variation of the two types are different. In the case of the non-neutral *mMEP-2** locus, for example, patterns of spatial distribution of alleles within (Verspoor and Jordan, 1989) and among catchments (Jordan *et al.*, submitted) differ from those evident for putatively neutral loci. In addition, the distribution of allele combinations (genotypes) differs among individuals with respect to phenotypic characters like juvenile growth (Jordan and Youngson, 1991) and adult sea-age at maturity (Jordan *et al.*, 1990).. It is not possible to estimate the likely extent of geographical mismatches between neutral variation and the different categories of adaptive variation that can be envisaged. Targeting adaptive genetic variation directly for novel GSI models would eliminate difficulties of mismatching. Although this approach is becoming feasible, it is not well-developed.

C. The effects of straying

Genetic analysis of allozyme data shows that alleles are widely shared among populations. Hierarchical analysis of gene diversity in Scottish salmon populations, demonstrated that >98% of all variation was shared among populations and <2% was attributable to differences among catchments (Jordan *et al.*, 1992). So, while genetic exchange is low enough to ensure that genetic population structure is maintained within and among catchments, it is also evident that reproductive isolation is far from complete. GSI is therefore likely to involve the interpretation of relative rather than absolute differences in genotype frequencies. Additionally, geographical and genetic distance tend to be correlated (Jordan, 1990; Elo, 1993; Wilson *et al.*, 1995) suggesting that allele sharing is especially likely to be a problem for GSI at finer geographical scales.

Thus, although homing is observed to be a substantial behavioural effect it is equally evident that straying has exerted substantial effects on the distribution of genetic variation. Present-day distributions of variation are in part a reflection of historical patterns of straying. Since many present salmon populations were founded by strayers from glacial refuges only relatively recently, it would be surprisingly if straying or other exploratory behaviours did not continue to occur. Straying poses two problems for GSI discussed below.

a) Defining Base-line Genetic Data.

Strayers among spawning populations may contribute alleels to the receiving populations, increasing levels of temporal genetic variation.

Straying is known to occur to natural populations. In the River Don in Aberdeenshire, for example, micro-tagged strayers have been procured among brood stock collected at spawning time *ca* 80 km from the sea. Since 1991 proven strayers to the Upper Don (*ca* 150 brood-stock per annum) have been single fish from each of the Girnock Burn in the neighbouring Dee catchment, the Dysynni in Wales and from Kotlafjordur in western Iceland. Genetic models demonstrate that even these low rates of proven straying would be expected to exert major effects on the distribution of alleles. In theory, repeated in each generation, these levels are sufficient to reduce or perhaps eliminate observed levels of neutral genetic differentiation between populations. However, the presence of strayers need not result in a proportionate and permanent genetic contribution to the recipient population. Lower relative fitness in the fish themselves or in their progeny may limit the genetic contribution that strayers are able to make.

From a genetic point of view, stocking is a form of straying and its effects must be considered in the same way. Given the recent history of stocking policy, straying by stocking may be a major effect in some locations. The size of the effect will be dependent on the source of the brood stock, the extent of stocking, the success of the exercise and the breeding protocols involved and to the random effects of genetic drift.

b) Defining Mixed-Stock Fisheries.

Accurate classification of individuals to populations using GSI procedures requires that the catch and the candidate source populations are parts of matching sets. Extraneous catches (strayers) from outwith the defined set of populations may be assigned erroneously by GSI. In some locations this may be a major source of error. Even within rivers, substantial catches of adults originating from distant catchments may occur. For example, tagged fish originating from the Girnock Burn on the Aberdeenshire Dee are regularly reported from fisheries in the nearest major river to the south.

D. Sampling error

On a local level, the real frequency of adaptively neutral genotypes is dependent only on the frequency of alternative alleles for the neutral loci in question. In general, allele (and genotype) frequencies are estimated by sampling the population (or the location) in question. Sampling errors arise from three sources.

a) Sampling Heterogeneous Populations.

In the case of salmon populations, sampling errors are likely to be relatively large: i) because in many cases effective population size is small (hundreds); and ii) because of the strong spatial aspect to the representation of single families. Unless these factors are addressed specifically (Webb *et al.*, 1993) sampling may not be random among all the elements of the population.

Sampling error is likely to be particularly marked when alleles are present at low frequencies. Ultimately, even when rare genotypes have been found only in a single population it is impossible to know from sampling that they are absent from all others.

b) Sampling Population Sets.

Sampling error is possible in compiling the population set on which candidate genetic populations are defined. Although the potential effects of incomplete population sampling are mitigated by the greater genetic relatedness of geographically close populations, the genetic description of study areas should be based on extensive sampling within the range of locations of interest.

c) Temporal Variation.

In all circumstances, the temporal stability of allele frequency estimates among recurring generations of single populations must be established. This has often been neglected in past studies, leading to the possibility of Type I statistical errors by which stochastic, temporal variation is mis-classified as geographical population structuring. Many genetic studies might be criticised in this respect.

Jordan *et al.* (1992) dealt with temporal variation specifically, by sampling from three generations at the same locations. Hierarchical analysis of gene diversity demonstrated that spatial differentiation was present over this (minor) time-scale: temporal effects (1.2% of total variation) were insufficient to explain the observed patterns of variation. Attempts have been made in other studies to make comparisons among year-classes by sampling in sequences of years or between pairs of samples obtained some years apart. In general, allele frequencies are considered to be stable in time (eg McElligott *et al.*, 1987; Hurrell and Price, 1993) although the question of temporal stability has not been given the attention it requires.

IV. The Application of GSI

Accuracy and precision are the parameters that determine the utility of the GSI approach for practical, fisheries management. Absolute definition is not attainable. In particular circumstances, different minimum levels of accuracy and precision may be acceptable. Fisheries managers may define target values for particular applications. The utility of the GSI approach can be assessed in this framework.

A. Theoretical considerations

An authoritative paper by Pella and Milner (1987) is a key reference on the principles and theory of GSI and it makes a number of central points regarding accuracy and precision that are worth listing.

1. The accuracy of GSI estimates is dependent on:

a) the number of populations involved;
b) the extent to which the populations are genetically heterogeneous.

2. The precision of GSI estimates is dependent on:

a) the accuracy of the sampling exercise on which the genetic population analysis that supports the GSI is based;
b) the accuracy with which the catch is sampled;
c) the real composition of the mixed catch group being treated.

B. Application to Atlantic salmon

Recently, Galvin *et al.* (1995) have explored the utility of GSI on the River Shannon catchment, using both allozyme and minisatellite DNA data. They were able to demonstrate that minisatellite data offer greater potential power - greater accuracy and greater precision on smaller sample sizes - than allozyme data. They used composite genotypes for three minisatellite loci and boot-strapping techniques to derive maximum likelihood estimates for the correct allocation of sub-samples (simulated catches) to each of the 10 geographical sample groups from which they had been derived. Precision and, especially, accuracy were high.

It must be recognised however, that - as the authors concede - the model in which the GSI approach to the data was tested is much simpler than any situation likely to be encountered in fisheries practice. In addition, the GSI model:

i) does not include a component describing sampling errors (temporal and Type I) in each of the 10 contributing geographical groups;

ii) assumes that the sampling groups are equivalent to genetic populations and that they represent populations of equal size

iii) does not include consideration of the effects of extraneous fish (ie strayers to the modelled system) occurring among catches.

On the basis of their genetic population model, the authors used the same boot-strapping and maximum likelihood techniques to allocate real catches to the same 10 geographical groups. This exercise is seductive but it incorporates a circularity and, as Galvin *et al.* point out, independent validation is required. In its most telling form, this necessitates the matching of data based on DNA analysis with independent supporting information (eg derived directly from radio-tagging or, by inference, from scale reading, mineral analysis of scales or entry timing) to demonstrate that tagged adults spawn in the locations indicated by GSI. This exercise is crucial but it has not yet been attempted.

V. Conclusions

1. The principles of GSI are sound but the technique is some way from being a practical fisheries management technique;

2. The potential sources of error affecting the accuracy and precision of GSI can be identified, estimated and, therefore, modelled;

3. Modelling of GSI and its application to real situations will have to be developed in the appropriate local context.

VI. Recommendations

1. That the utility of GSI based on DNA variation should be explored further because of its potential importance as a management tool.

2. That a local modelling approach should be adopted on a system (catchment or catchment group unit) that has:

a) a history of study using non-genetic methods;
b) a biologically complex stock structure;
c) a relatively simple geographical structure;
d) is considered not to have been genetically altered in the past by over-management.

Potential catchments for study in England and Wales include the Wye, Usk, Dee, Eden, Lune and Exe.

3. That the overall study should be constructed of a sequence of three elements that:

a) models spatial variation in DNA variation and the GSI approach, on the appropriate geographical scale, using existing genetic data from other systems.

b) i) models spatial genetic structure based on an extensive sampling survey of DNA RFLP variation in the chosen catchment unit;
ii) models the GSI exercise using bootstrapping techniques and realistic estimates for sampling errors from all sources;

c) i) performs GSI on a sample of the fishery;
ii) validates this exercise by non-genetic means.

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