

Chapter 9

MITOCHONDRIAL DNA AND THE ANALYSIS OF FISH POPULATION STRUCTURE

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I. INTRODUCTION

In this chapter I hope to play both the role of a reporter and, to a lesser extent, that of an advocate. I will report on nucleic acids as genetic markers and the inferences about fish population structure that can be made following the study of these genetic tags. I will advocate that fishery managers learn as much about the genetic architecture of fish stocks as they are able. My advocacy results from a conviction that knowledge concerning the population genetics of a fish stock is critical to a basic understanding of that stock. I will not, however, champion the idea that genetic stock identification is always a necessary tool in the year-to-year management of a fishery. Instead, through example and discussion, I hope to provide some insight into the kinds of information that might be anticipated following genetic analysis of fish population structure.

My report will focus on restriction fragment length polymorphism (RFLP) analyses of mitochondrial DNA (mtDNA), a reflection of both my own interests and the enthusiasm with which many population geneticists have embraced this molecule as a tool for studying population subdivision. However, much of my discussion and most of the analyses I cover pertain also to nuclear gene RFLPs or DNA sequence data. The perspective that I will provide is that of a population geneticist and it is important that students in fishery science bear this in mind as they read this chapter. In my mind there can be a difference between a fish population and a fish stock and, at times, this distinction may be important.

What is a stock and is it different from a population — does it matter? Yes and no. In many senses and in some cases the same words can be used to define both a fish population and a fish stock. Most discussions that describe genetic identification of fish stocks begin by providing a definition of a stock. The definitions provided are usually not different from those used by population geneticists to define fish populations. That is, a fish stock or population is a group of conspecific fish that interact more frequently with one another than with individuals belonging to other conspecific populations. The result of this increased likelihood of interaction, at least reproductive interaction, is increased genetic similarity of fish within populations as compared to the genetic identity of fish in different populations. The degree of genetic distinctiveness between fish populations will depend on a number of factors including migration, genetic drift, and selective differences between locally different environments.

Fish population geneticists use the variety of methodologies described in this book to discern genetic relationships within and between populations of fish. The genetic data collected can be placed in a rich comparative framework, including both fish and nonfish studies, that often provide considerable insight into the evolutionary forces responsible for the observed genetic patterns. Often, however, and especially if one studies marine or anadromous fish stocks, weak or nonexistent genetic differences are observed that identify fish stocks. At this point a population geneticist would argue, mostly from an *evolutionary* perspective, that genetic evidence points to the existence not of several populations but to a single population or metapopulation. A fishery resource manager would argue, mostly from an *ecological* perspective, that the genetic methods used are not resolving differences between fish stocks that he/she knows to exist. It is critically important that students of fishery biology understand both viewpoints if they are to make informed decisions concerning the conservation and management of fish stocks.

Initially I pulled together material for this chapter with the feeling that I would review and summarize papers that had used nucleic acid methodologies to study the genetic structure of fish populations. After several false starts I discarded that strategy in favor of the one I present here. Perhaps drawing too heavily on my own work I have selected examples that both demonstrate the sensitivity of molecular techniques and some of the limitations of these methods. (More even-handed discussions of DNA approaches to fish stock identification have recently been published by Avise,¹ Hallerman and Beckmann,² and Ferris and Berg.³) Whether

one chooses to study genetic variation at the level of proteins, RFLPs, or DNA sequence, and there are advantages to each, laboratory bench protocols are available that are straightforward and effective methods for producing raw data. The biochemical analysis of mtDNA is covered elsewhere in this volume⁴ and this chapter focuses on the application of restriction analyses of mtDNA to the study of the genetic structure of fish populations.

II. MITOCHONDRIAL DNA, BACKGROUND

Several recent reviews by Avise et al.,⁵ Wilson et al.,⁶ and Moritz et al.⁷ present a solid foundation of knowledge concerning the molecular and evolutionary dynamics of mtDNA variation in natural populations. As pointed out by Avise et al.⁵ "mtDNA has provided the first extensive and readily accessible data available to evolutionists in a form suitable for strong genealogical inference at the intraspecific level". What are the features of mtDNA that make the molecule such a powerful tool for studying the genetic architecture of populations? First, the mtDNA molecule evolves at a relatively rapid rate, approximately 1% per million years,⁷ and thus easily surveyed differences in mtDNA haplotypes almost always exist within a species. Second, mitochondria are maternally inherited and are nonrecombining. Third, usually only a single mtDNA genotype exists within an individual (however, see Reference 8) and, therefore, most individuals are considered homoplasmic. Finally all animals have mitochondria and these mitochondria have the same function and similar molecular characteristics across most animal taxa studied to date. As a result, mtDNA is a superb genetic system for comparative studies of population subdivision.

There are two aspects of the biology of the mitochondria that I believe should be emphasized in any discussion concerning the use of mtDNA as a genetic tag for the study of fish populations. The first is its uniparental mode of inheritance. Because only females transmit mitochondria to progeny, mtDNA reveals a matriarchal evolutionary history. This fact should be remembered in studies of fish where males and females behave, in an evolutionary sense, differently. For example, if there is asymmetric dispersal by sex, and males disperse much further than females, mtDNA might indicate greater population subdivision than would be inferred through study of biparentally inherited markers such as allozymes or nuclear RFLPs. The second aspect of mitochondria that I would like to emphasize regards its use in comparative studies. Because we are able to make secure, homologous comparisons of mtDNAs across independently evolving lineages, we can study the roles that geologic history, demography, and life history strategies play in the genetic structuring of fish populations with greater confidence that our conclusions will be robust.

III. SOUTHEASTERN FISHES OF THE U.S.

My first example is drawn from a study of population subdivision in several species of freshwater fishes with widespread distributions in the southeastern U.S.⁹ Here, I will describe intraspecific mtDNA polymorphism in only two of the species — the bowfin (*Amia calva*) and the spotted sunfish (*Lepomis punctatus*). The raw data and subsequent analyses for both bowfin and spotted sunfish are straightforward and thus provide a good introduction to mtDNA-based analyses of population structure in fish.

Fourteen restriction enzymes per bowfin and 17 enzymes per spotted sunfish were used to digest aliquots of purified mtDNA isolated from individual fish collected from most of the major rivers in the southeastern U.S. (Figure 1). This study is representative of most restriction enzyme surveys with respect to the number of restriction enzymes used. However, Lynch and Crease¹⁰ warn that most of the variance on estimates of nucleotide polymorphism result from sampling at the nucleotide level. Thus they recommend increasing the number of restriction enzymes used (thereby increasing the sampling of nucleotides). In reality, however, the

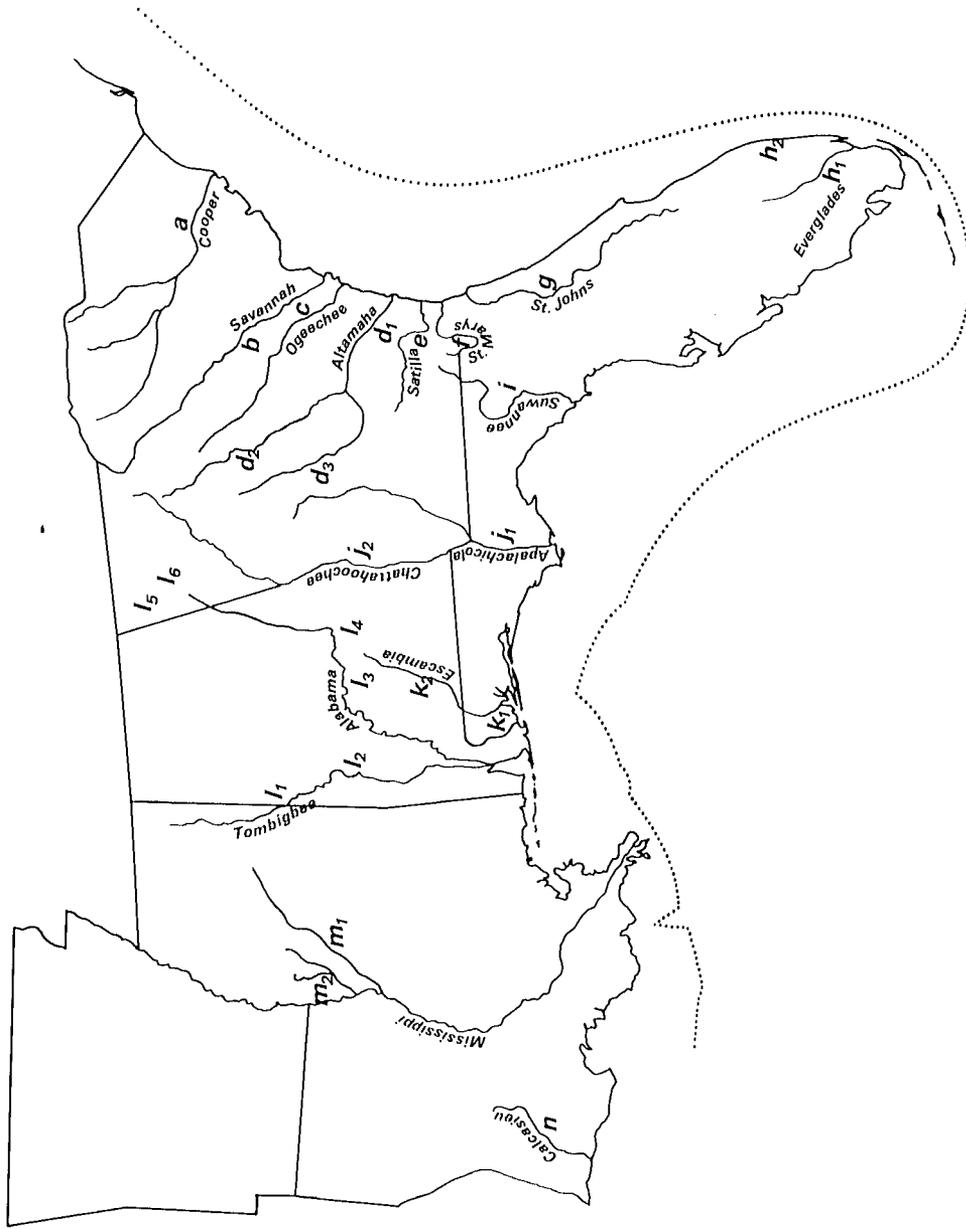


FIGURE 1. Major drainages and collection locales (labeled as in Table 1) in the southeastern United States. From Birmingham, E. and Avise, J. C., *Genetics*, 113, 942, 1986. With permission.

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TABLE 1
Composite Clonal Genotypes and Number of Individual Fish in Each Clone
for Bowfin and Spotted Sunfish

| Clonal designation | Composite clonal genotype | No. of individual fish | Locale |
|--------------------------|---------------------------|------------------------|------------------------------------|
| <i>Amia calva</i> | | | |
| 1 | AAAAAAAAAAAAA | 30 | a—g, i, j, j ₂ |
| 2 | AAAAAAAAABAAAA | 4 | a |
| 4 | AAAAAAAAABAAA | 3 | b, c |
| 5 | AAAAAAAAACAAAA | 10 | d, d, f, g, i |
| 6 | ABAAAAAAAAAAAA | 2 | e |
| 7 | AAAAAABAAAAAA | 1 | e |
| 8 | EAAAAAAAAAAAAAB | 3 | g, h ₂ |
| 9 | BAAAAAAAAAAAAAB | 5 | h, h ₂ , i |
| 10 | CAAABAABAAAAAC | 6 | k ₁ , k ₂ |
| 11 | CAAABAABACAAC | 1 | k ₂ |
| 12 | CCAABAAAAAAAC | 7 | l, l ₂ , l ₃ |
| 13 | CCAABAAAAABAC | 2 | m, m ₂ |
| <i>Lepomis punctatus</i> | | | |
| 1 | AAAAABAAAAAA | 34 | a — i |
| 2 | AAAAAAAAAAAAA | 7 | a |
| 3 | AAAAADBAAAAA | 1 | g |
| 4 | AAAAABBAAAAA | 1 | g |
| 5 | AAAAABAAAAFA | 1 | d ₁ |
| 6 | AAAAABAABAAA | 1 | h ₁ |
| 7 | ABBAABABCABB | 3 | i |
| 8 | ABAABBABDABB | 1 | i |
| 9 | BCCACBCEACE | 9 | j ₁ , l ₃ |
| 10 | BCCACBCEACC | 1 | j ₁ |
| 11 | BCCACBEEAHD | 1 | j ₁ |
| 12 | BCCADCBEADE | 7 | k ₁ |
| 13 | BECADCBEADE | 3 | k ₁ , l ₃ |
| 14 | BCCADCBBHAGE | 1 | k ₁ |
| 15 | DECBECBGAEF | 4 | n |
| 16 | CDBBACBBFAEF | 3 | l ₄ , l ₃ |
| 17 | CECBACBBHAEF | 1 | n |

* Letters, from left to right, refer to digestion profiles produced by the endonucleases listed below (enzymes producing zero or one cut are not included). *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Clal*, *Eco*RI, *Hind*III, *Kpn*I, *Nde*I, *Pst*I, *Pvu*II, *Sac*I, *Stu*I, *Xba*I, *Ava*I, and *Hinc*II

From Bermingham, E. and Avise, J. C., *Genetics*, 113, 944, 1986. With permission.

appropriate number of enzymes to use (or nucleotide bases to sequence), like the appropriate number of individuals to sample and the geographic distribution of those samples, is difficult to determine *a priori*. Sampling strategies will depend on both the history and demographics of the fish stocks being studied and the questions being addressed and are best determined empirically. Preliminary surveys, using several genetic methodologies, often provide an economical means for formulating a testable null hypothesis that can provide the necessary direction for a productive sampling design. I will return to the discussion of sampling several times in this chapter.

The products of restriction enzyme digestion are next size fractionated on gels and the observed restriction fragments constitute the raw data. Considering all restriction enzymes simultaneously, each fish can be assigned a composite letter code that describes its observed mtDNA genotype (see Table 1). It should be noted that there are usually a smaller number of composite genotypes than there are individuals sampled. Thus, individual fish that share a

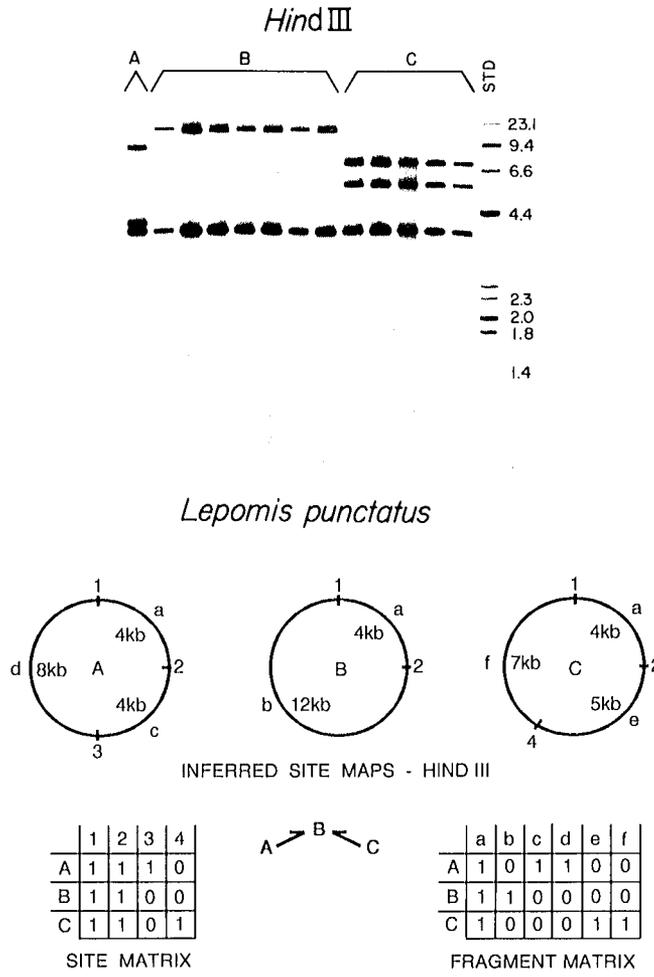


FIGURE 2. Representative *Hind*III digests of mtDNA from *L. punctatus* with restriction site maps for the three *Hind*III genotypes inferred from the restriction fragment patterns pictured in the gel autoradiograph. Below are shown presence/absence matrices for both the restriction site data and the restriction fragment data as revealed by *Hind*III, just one of the restriction endonucleases used in the original survey. The network joining the three *Hind*III genotypes is drawn with arrows indicating the direction of restriction site loss and does not necessarily indicate the direction of evolution.

composite mtDNA genotype, often called a haplotype, are said to belong to the same maternal clone. Increasing the number of nucleotide bases surveyed, either by increasing the number of restriction enzymes used or by DNA sequencing, will increase the number of observed haplotypes. Often, however, the resulting increase in haplotype diversity will not be particularly informative (see beyond).

In most mtDNA studies of fish collected from conspecific populations, it is also possible to use the restriction fragment data to infer restriction site data. Restriction site characters are preferred to restriction fragment characters because fragment data provide redundant information concerning a single evolutionary event (the gain or loss of a restriction site). For example, the loss of a single restriction fragment due to the gain of a restriction site within that fragment is accompanied by the gain of two novel restriction fragments. This difference between fragment and site data is further exemplified in Figure 2, an autoradiograph of spotted sunfish mtDNA fragments accompanied by both a restriction site and restriction fragment matrix.

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Using restriction fragments to infer restriction site maps is only usefully accomplished when all haplotypes analyzed are closely related and when each fragment pattern can be related to another by inferring a single site loss or gain (as is the case in the maps inferred from the fragment patterns pictured in Figure 2). When multiple-site gains or losses are required to transform one fragment pattern to another it is preferable to physically map restriction sites. (In the next section I provide an example of a restriction site map for Atlantic salmon that was determined using a double enzyme digestion procedure.) A physical map that provides the relative position of restriction sites to one another, although not necessary to any data analysis that I am aware of, reduces the possibility of error in the raw data. Whether one physically maps restriction sites, infers sites, or uses fragment data should depend on any apparent ambiguity observed in the data. In my experience, working with conspecific populations and very closely related species, all three classes of data provide very similar estimates of both DNA sequence divergence and phylogenetic relationship.

The bowfin data set is very satisfying in the sense that all individuals can be related to one another in a simple and unambiguous fashion. With respect to genotypes revealed by a single restriction endonuclease, individuals are either identical or differ by a single mutation step (gain or loss of one restriction site) from their closest relative. In the case of bowfin most differences in the composite mtDNA genotypes distinguishing individual fish represent a straightforward accumulation of single-site mutations. As a result few dilemmas result from the phylogenetic interpretation of the data.

In fact the only inconsistency in the cladogram inferred for bowfin is the relationship between taxon D and taxon I (see Figure 3b). The cladogram represents what we posit is the true relationship between D and I and states that there are four changes in character states distinguishing these taxa. Fragment analysis of the composite genotypes for D and I, however, reveals only two restriction site differences distinguishing the two taxa (*Bam*HI and *Hinc*II). We suspect the similarities in the *Bam*HI fragment patterns are convergent and therefore the cause of the homoplasy. In theory it would be possible to resolve this dilemma although it might require sequencing portions of the two mtDNA genomes. That effort would appear unjustified in the present example as reasonable alternative placements of either clone D or clone I on the cladogram do not significantly alter our interpretation of the phylogeny.

Most population surveys of mtDNA polymorphism utilize restriction fragment data or inferred site data to construct matrices that consist of presence-absence information for each restriction site or fragment in each mtDNA haplotype. In turn, these matrices can be used in a variety of analyses. It should be carefully noted that in the analyses I am about to discuss each mtDNA haplotype is considered an operational taxonomic unit (OTU). In other words, I am taking an analytical approach that is more phylogenetic than populational in that each haplotype's frequency in the population is not considered. This approach can be followed in the present examples because there is a strong geographic pattern to the distribution of mtDNA haplotypes. Regional groupings of haplotypes tend to be exclusive; this would be somewhat analogous to observing fixed allele differences between populations that were analyzed using allozymes. These fixed differences provide strong support for the genetic distinctiveness of the populations being analyzed.

Typically the presence-absence matrices are used to calculate estimates of sequence divergence between all pairs of haplotypes. Although indebted to Upholdt,¹¹ Nei and his co-workers are responsible for most of the distance metrics that are in current use and, at least with respect to restriction site data, refine the analytical techniques every few years.¹²⁻¹⁴ The resulting distance data can be joined in a number of ways and I recommend two approaches which have both been well validated for molecular data.¹⁵⁻¹⁷ The first is neighbor-joining¹⁵ and the second is the unweighted pair-group method of analysis (UPGMA).¹⁸ The phenograms relating mtDNA haplotypes sampled for both bowfin and spotted sunfish were generated by UPGMA from sequence divergence estimates (p) based on Nei and Li¹² (Figure 3a and 4a).

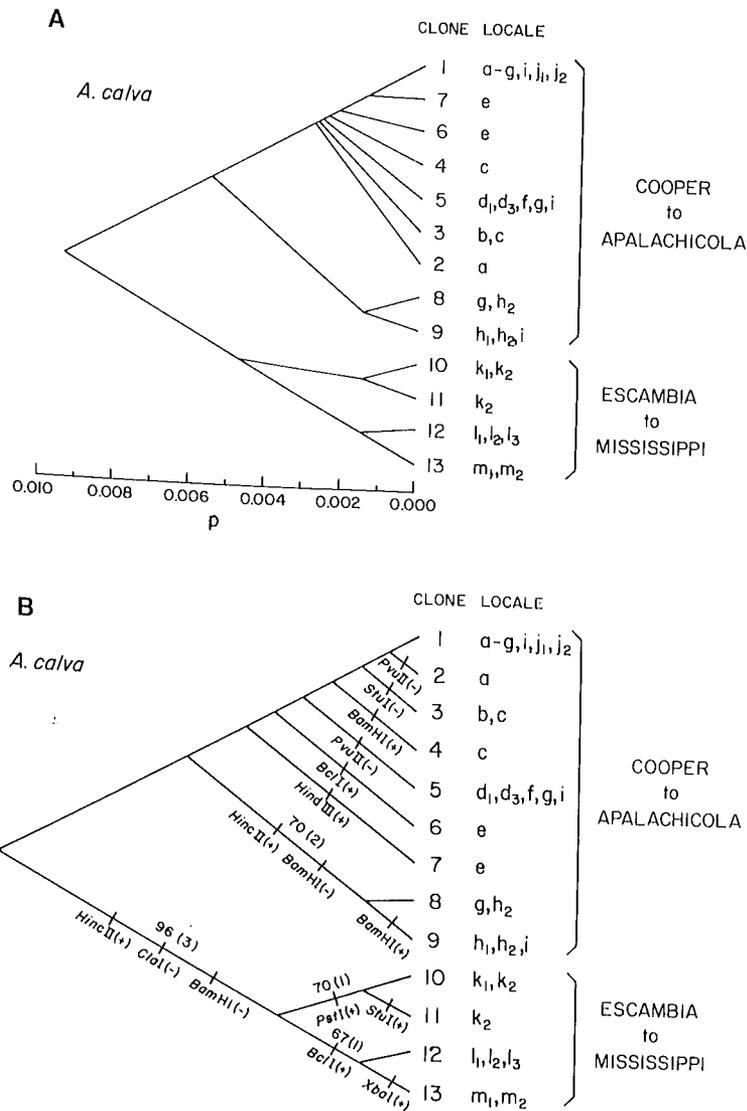


FIGURE 3. A, Phenogram of mtDNA genotypes in *A. calva* generated by UPGMA cluster analysis of nucleotide sequence divergence (p) estimates. mtDNA clones are numbered as in Table 1, and locales are lettered as in Figure 1. B, Wagner parsimony network of mtDNA genotypes in *A. calva* generated from the presence-absence site matrix. mtDNA clones are labeled as in Table 1, and locales are lettered as in Figure 1. First numbers along branches indicate the proportion of times that a group was distinguished in the bootstrap analysis (only proportions greater than 50% are shown). Inferred restriction site changes are shown along all branches. Numbers in parentheses indicate *minimum* numbers of restriction site differences along the path. The network was arbitrarily rooted in a position that facilitates visual comparison with the UPGMA phenogram. (From Bermingham, E. and Avise, J. C., *Genetics*, 113, 946, 1986. With permission.)

Analyses based on molecular distance data have seductive appeal to many investigators due to the concept of the molecular clock first proposed by Zuckerkandl and Pauling.¹⁹ This is the notion that molecules may change at a constant rate over time and, when properly calibrated, can provide information about branching times as well as branching pattern on phylogenetic trees. Notice that the phenograms, in Figures 3 and 4, relating mtDNA haplotypes within both bowfin and spotted sunfish have similar tree topologies but differ markedly with respect to the extent of sequence divergence observed between mtDNA haplotypes within each species. If

FIGURE 3. cluster analysis and Wagner parsimony network of mtDNA genotypes in *A. calva* generated from the presence-absence site matrix. (From *Genetics*, 113, 946, 1986.)

mtDNA evolves as divergent as mtDNA haplotypes recently than is t

Fragment or networks by a number of synapomorphies. a synapomorphic 11 from the Escambia were generated by package distribut

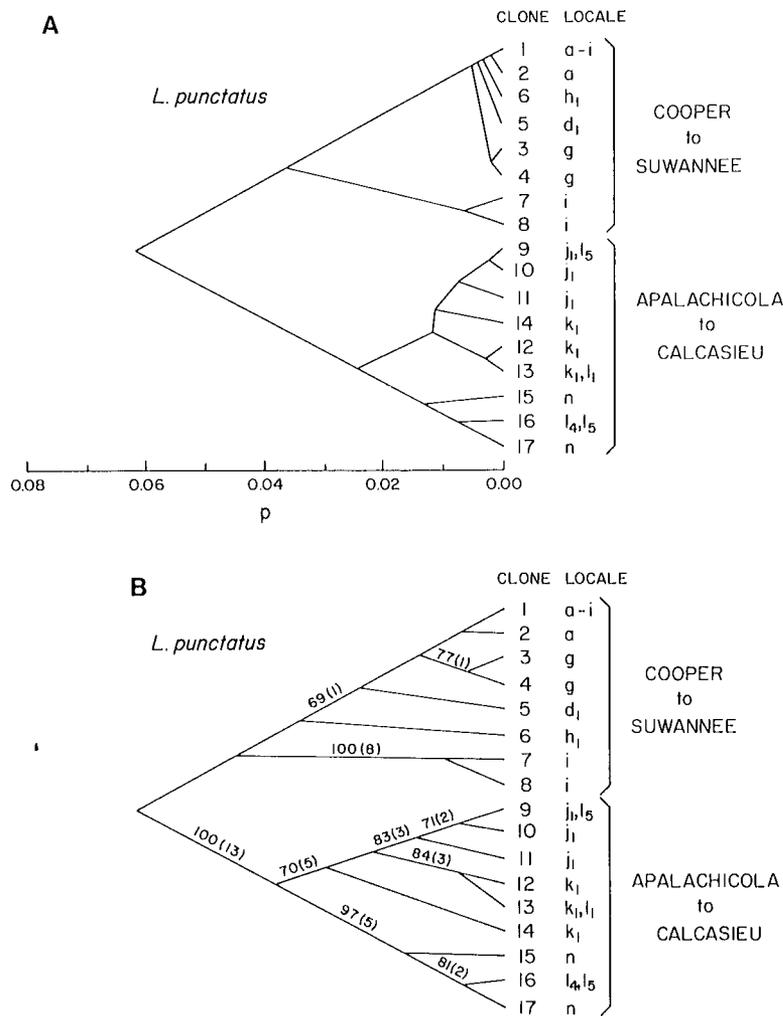


FIGURE 4. A, Phenogram of mtDNA genotypes in *L. punctatus* generated by UPGMA cluster analysis of nucleotide sequence divergence estimates (see legend to Figure 3A). B, Wagner parsimony network of mtDNA genotypes in *L. punctatus* generated from the presence-absence fragment matrix (see legend to Figure 3B). (From Bermingham, E. and Avise, J. C., *Genetics*, 113, 949, 1986. With permission.)

mtDNA evolves in a clock-like fashion and ticks at a more or less constant rate across lineages as divergent as bowfin and spotted sunfish — two open questions — the data indicate that all mtDNA haplotypes observed within bowfin last shared a common ancestor much more recently than is the case for spotted sunfish mtDNA haplotypes.

Fragment or site presence-absence matrices can also be used to generate parsimony networks by a number of algorithms. Whereas the above phenetic analyses link haplotypes by estimating overall similarity, cladistic analyses link taxa using shared derived characters, or synapomorphies. For example, in the cladogram linking bowfin mtDNA haplotypes there is a synapomorphic *Pst*I restriction site that evidences a link between mtDNA haplotypes 10 and 11 from the Escambia River. The parsimony networks pictured for bowfin and spotted sunfish were generated by the Metropolis simulated annealing algorithm in the Phylip phylogenetic package distributed by Joe Felsenstein. David Swofford's Paup algorithm and Steve Ferris'

g86 algorithm are additional parsimony programs that are both excellent and fast. As determined by inspection of Figures 3 and 4, both cladistic and phenetic methods, in present examples, give trees that are highly concordant with one another. For molecular being used to infer genealogical relationship between closely related taxa, I strongly urge investigators to take both approaches.

Confidence estimates on the branches of the parsimony networks were calculated by the boot method (BOOTM in the Phylip package). There is considerable controversy surrounding statistical testing procedures for phylogenetic trees and, in fact, Wiley²⁰ (pp. 114) for a majority when he says, "There are no known models of phylogenies with which a phylogenetic systematist can empirically calculate the chances for either types of errors (Type I and Type II). This forces us to use nonstatistical inference at the '100 percent' level. Parsimony must take its course". Nonetheless I have elected to include a brief description of bootstrapping and refer those interested in the application of this method to phylogenetics to Felsenstein's discussion of this approach.²¹

Basically, the bootstrap method resamples the original data, by drawing data points at random with replacement, to construct a series of fictional sets of data. A phylogeny is inferred from each new data set with the resulting phylogenies summarized by counting the number of times that the bootstrap estimate contained corresponding monophyletic groups. The percentages that a monophyletic assemblage is supported enables one to assess the strength of the phylogenetic hypothesis. Intuitively one can see that the more characters there are supporting a group the more likely it is that a random sampling of characters will include enough information to define that group.

In this study, both restriction site data and restriction fragment data were separately analyzed by the bootstrap method. For both sets of data the input order of taxa was rearranged 100 times and each reordered data set was run 100 times for a total of 500 replicates per data set. Numbers beneath the nodes of the cladogram (Figure 3b) represent the percentage of times the bootstrap estimate contained the corresponding monophyletic group.

The principal conclusion that emerges from these analyses, and analyses of several other species considered in the original paper, is that there are genetically distinct populations, as evidenced by mtDNA polymorphism, in each of the species surveyed.⁹ Bowfin and spotted bass, particularly, share an east-west genetic break that is remarkable for its concordance with these independently evolving lineages. In fact, the generally high level of congruence among populations of population subdivision across all the fish species studied (see Figure 5) led us to suggest a historical biogeographic explanation for our observations. Specifically, we suggest that the cyclical nature of sea-level change during the Pliocene and Pleistocene relative to the generally static continental morphology of the southeastern U.S. caused the observed patterns of phylogenetic relationship between populations. Small differences in the distributions of mtDNA haplotypes within each fish species probably reflect slightly different patterns of dispersal postdating fragmentation or vicariant events. Overall, however, the data on dispersal and gene flow have not been sufficient to override geographic influences on population subdivision.

This study provided an early indication that restriction analyses of mtDNA could be a very effective technique for reconstructing evolutionary relationships between conspecific populations. Somewhat unfortunately, the results obtained in this study and earlier studies on the fish fauna²²⁻²⁵ convinced a number of fisheries organizations that mtDNA was a crystal ball which could be seen the future of genetic identification of fish stocks. This fails to recognize that genetic change between populations is a function of time and distance (or more precisely the lack of gene flow). In the present example, significant population subdivision was observed in river populations of freshwater fishes. Gene flow, at least in fishes with low tolerance to marine conditions, can only occur through river capture and translocation by man and thus is expected to be low between river drainages. In addition, for eastern fishes the temporal scale over which genetic change between geographically

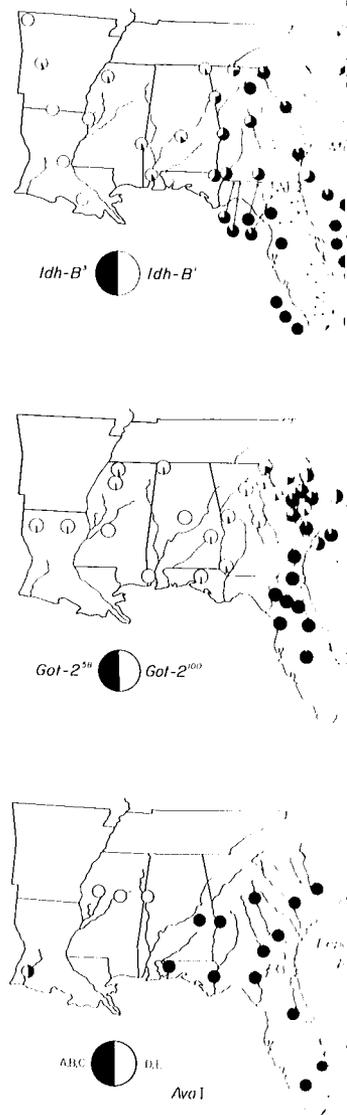


FIGURE 5. Composite showing the geographic distribution of mtDNA haplotypes for three freshwater fish considered in Birmingham, Alabama.

separated populations has occurred. The geographic distribution of freshwater and anadromous fishes and the extent of gene flow between populations and the resulting genetic evidence of population subdivision.

IV. Atlantic Salmon

Atlantic salmon (*Salmo salar*) represents a major fishery in Europe and North America. The management of a mixed stock fishery of Atlantic salmon in North America comprises a major fishery for both Europeans and North Americans, providing a source of income to enhance the success of the

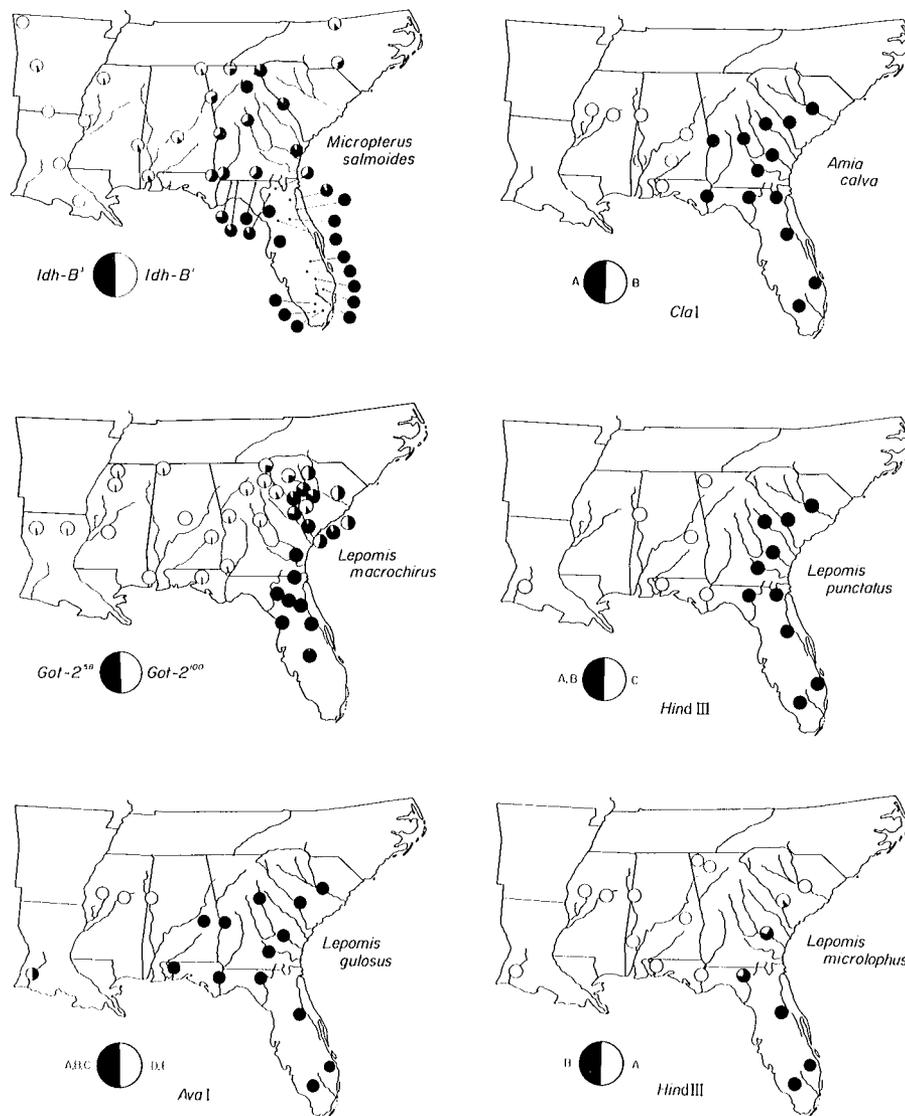


FIGURE 5. Composite showing the geographic distribution of representative genotypes for the six species of freshwater fish considered in Bermingham and Avise's original study.⁹

separated populations has occurred is long compared to that for many north temperate freshwater and anadromous fishes and probably for many marine species as well. When there is gene flow between populations and/or population subdivision is recent, much less dramatic genetic evidence of population substructure should be anticipated.

IV. ATLANTIC SALMON

Atlantic salmon (*Salmo salar*) represent a classic problem in fisheries biology, namely that of a managing a mixed stock fishery. Atlantic salmon spawned in both Europe and North America comprise a major fishery off the west coast of Greenland every summer. Both Europeans and North Americans, primarily through hatchery programs, spend large sums of money to enhance the success of this fishery. For conservation and economic reasons it is

important, on a year to year basis, to determine the relative contributions that different stocks of Atlantic salmon make to the West Greenland fishery. If Atlantic salmon harvested in the West Greenland fishery can be confidently assigned to continent (or river) of origin it will be possible to both enact specific fishery closures designed to protect certain stocks from overfishing and fairly apportion the proceeds from the catch. Towards this end a variety of techniques, genetic and nongenetic, have been applied with a goal of accurately determining continent of origin of Atlantic salmon caught in the West Greenland fishery.²⁶⁻²⁸

Both scale and allozyme analyses have been used, with reasonable success, to estimate the proportions of European and North American Atlantic salmon comprising the West Greenland fishery. For reasons described below, however, managers responsible for this fishery have continued to look for techniques that more accurately distinguish between continent of origin of Atlantic salmon harvested off Greenland. My lab continued this quest and studied NA variation in hatchery stocks of Atlantic salmon representing ten geographically separated stocks in North America and Europe.²⁹ We used restriction analyses of mtDNAs to characterize geographic population structure within Atlantic salmon and to determine whether mtDNA genotypic characters could provide qualitative markers useful for differentiation between salmon from North America and Europe.

Three distinct mtDNA genotypes were observed in 43 Atlantic salmon mtDNAs (representing both pooled and individual fish) surveyed with 20 restriction endonucleases. The three NA haplotypes are strongly patterned geographically and the North American salmon NAAs observed in this study are readily distinguished from mtDNAs isolated from fish of European origin. Maps of most restriction sites (excluding *Ava*II, *Clal*, *Hind*III, and *Stu*I) were constructed indirectly by using measured fragment sizes produced by single- and double-digestion of Atlantic salmon mtDNAs³⁰ (Figure 6). *Bam*HI, *Bcl*I, *Bgl*II, *Bst*EII, *Pst*I, and *Xho*I endonucleases each produce one or two cuts in Atlantic salmon mtDNAs and were mapped. Maps were considered complete when cleavage sites for a new enzyme could be assigned to locations internally consistent with those for the above enzymes. Position 0 of approximately 16,800 base pair mtDNA of *Salmo salar* is an *Eco*RI site that we believe is homologous with a conserved *Eco*RI site located at 7.5 kb on 'Thomas and co-workers' maps of *Onchorhynchus* species.³¹ We estimate that the placement of cleavage sites on the map is accurate to within ± 200 base pairs.

Two mtDNA haplotypes isolated from European fish differ at a minimum of seven restriction sites from the single mtDNA genotype observed in all Atlantic salmon of North American origin (hereafter referred to as the North American type). The minimum estimated sequence divergence observed between the North American salmon mtDNA type and European types is $p = 0.0072$.

Two mtDNA haplotypes obtained from European origin fish differ by only a single restriction site (within the context of the present survey using 20 restriction endonucleases) and the estimated sequence divergence between these two types is $p = 0.0011$. Again, there is a geographic pattern to the distribution of the two mtDNA genotypes observed in Atlantic salmon. Salmon whose origins trace to rivers in Iceland, Scotland, and Norway share a mtDNA haplotype (referred to hereafter as the northeast Atlantic type) that differs from the mtDNA haplotype recovered from fish of Russian origin (Baltic type) by only one *Ava*II restriction site. Both the Baltic and the northeast Atlantic mtDNA haplotypes are, however, observed in salmon derived from the Morrum River stock in Sweden. A major feature, then, of the mtDNA data in Atlantic salmon is the genetic divergence between North American and European forms. A secondary feature of the data is the distinction between the two European mtDNA haplotypes. Figure 7, an autoradiograph showing the results of an *Ava*II digestion of a number of different Atlantic salmon mtDNAs, depicts these features pictorially.

In the second phase of this study we used restriction analysis of mtDNAs to analyze 328

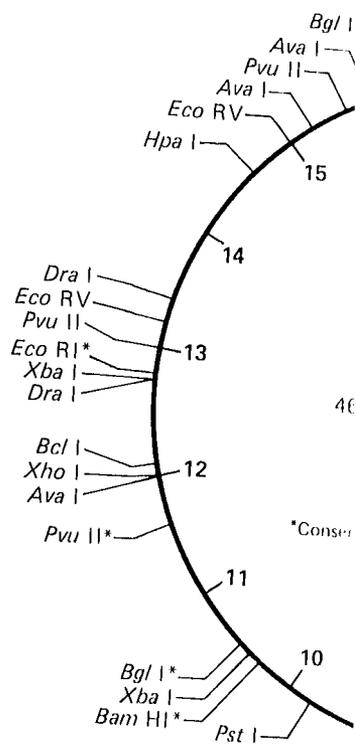


FIGURE 6. Map positions of restriction sites on the mtDNA genome of Atlantic salmon. Sites labeled outside the circle were observed in all "North American" mtDNA genomes. Sites labeled inside the circle were observed in "European" mtDNA genomes of *S. salar*.

Atlantic salmon caught in the Greenland fishery. The enzymes *Dra*I and *Bst*EII, were used to analyze the mtDNA. As a result of these criteria, 74 fish (23%) were identified as North American fish. The remaining 21 fish were identified as European fish. The origin of the remaining 21 fish were recovered from 68 of the 328 fish. Scoring the mtDNA data we identified 48 fish as North American. The high level of concordance between the results of the restriction analyses of mtDNA will be discussed in a later paper. Both scale meristics and protein values are being used to distinguish between Atlantic salmon.

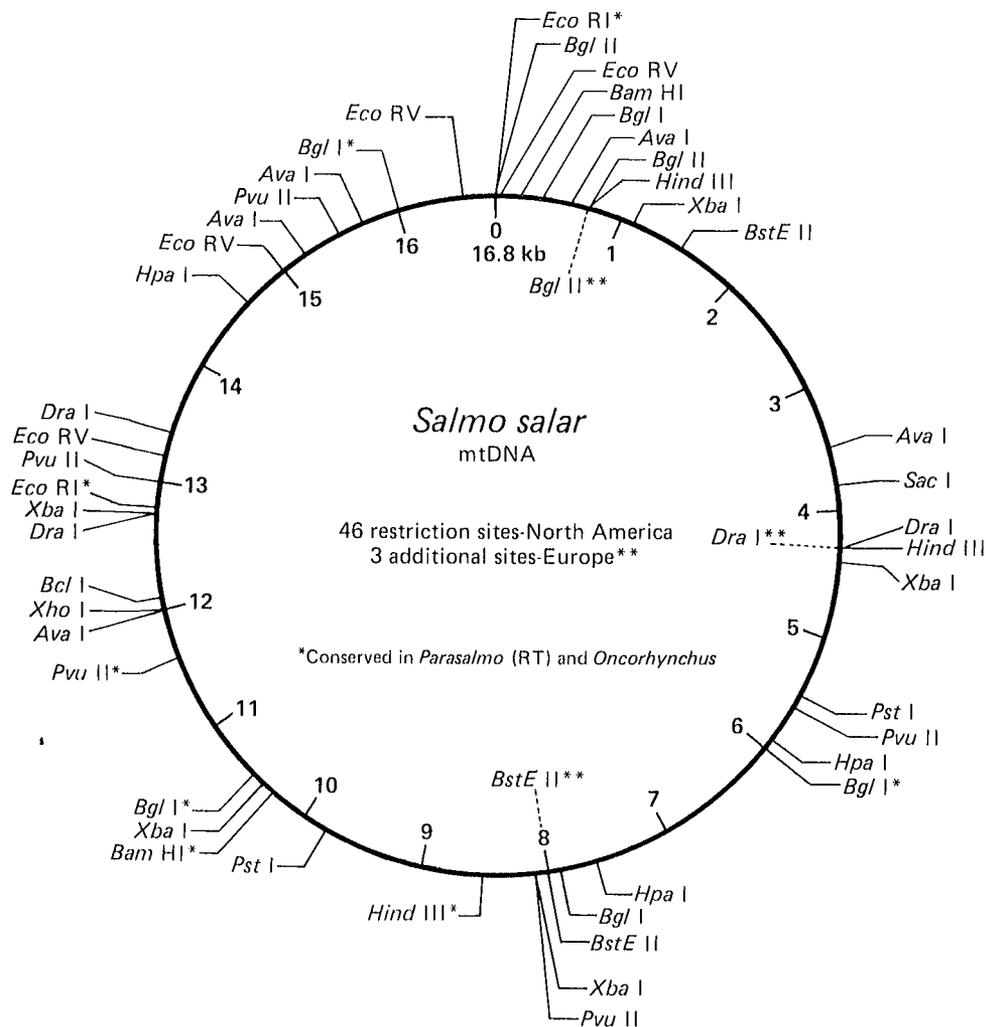


FIGURE 6. Map positions of restriction sites recognized by 16 restriction endonucleases in the "North American" and "European" mtDNA genomes of *S. salar*. Additional restriction site differences between these two genomes were revealed by other restriction enzymes that were not mapped (see text). Restriction enzyme sites labeled outside the circle were observed in all "North American" and "European" mtDNA genomes sampled. The three restriction sites labeled inside the circle were observed only in the "European" genomes sampled.

Atlantic salmon caught in the Greenland fishery. Two of the restriction endonucleases that produce genetic patterns useful for discriminating European from North American salmon, *Dra*I and *BstE*II, were used to analyze mtDNAs isolated from all 328 fish. Using mtDNA criteria, 74 fish (23%) were identified as European origin fish and 254 salmon (77%) were identified as North American fish. Physical tags indicating river, country, and continent of origin were recovered from 68 of these Atlantic salmon and identified 47 North American salmon and 21 European fish. Scoring the mtDNAs of these 68 fish without knowledge of tag data we identified 48 fish as North American in origin and 20 salmon as European in origin. The high level of concordance between mtDNA data and physical tag data suggests that restriction analyses of mtDNA will be a very useful tool for determining continent of origin in Atlantic salmon and, thus, in the management of this species.

Both scale meristics and protein variation have also been shown to be useful in identifying and distinguishing between Atlantic salmon stocks.²⁶⁻²⁸ However, for discriminating between

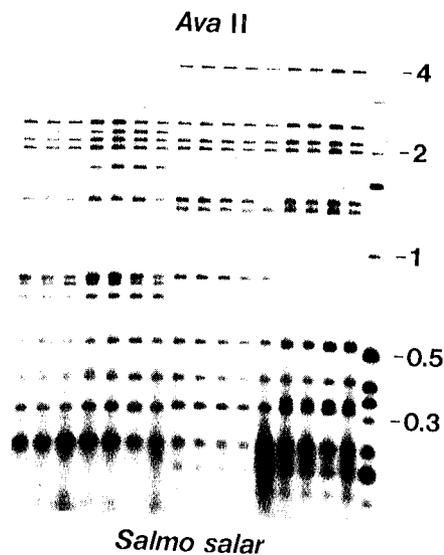


FIGURE 7. Representative *Ava*II digests of mtDNA isolated from *S. salar*. Reading from left to right the first 7 individuals were collected from North American locales, the next 5 individuals were collected from North Atlantic locales including Iceland, and the last 4 individuals were collected from Baltic Sea locales. The right-hand most lane is a molecular weight standard.

salmon of North American and European origin, restriction analysis of mtDNA to have advantages over both these techniques. The primary advantage that mtDNA has compared to scale analysis is that it is a genetic technique, and the mtDNA's (restriction sites or fragments) are inherited and thus are likely to be stable over time. Variation in scale meristic characters is largely determined by environmental factors such as temperature and thus are unlikely to be stable over time. A case in point is made by Birt and Birstein who demonstrated that scale circuli counts of the first sea year for European salmon varied substantially between a 1968 to 1970 database³³ and a 1980 database.³² Use of the 1970 database in the analysis of scales from Atlantic salmon caught in the West Greenland fishery in recent years underestimated the proportion of European salmon caught in the fishery. Birstein has updated both the scale database and statistical technique used to classify salmon from West Greenland but recommends that testing with new scale samples be carried out every 2 years.²⁶

Restriction analysis of mtDNA also has advantages compared to protein electrophoresis in determining the continent of origin of Atlantic salmon caught in the West Greenland fishery. The basic unit of mtDNA analyses is the individual, in contrast to allozyme analyses which are typically based on population allele frequencies. In other words, mtDNA RFLPs are markers which identify the continent of origin of each Atlantic salmon sampled. Qualitative markers, such as mtDNA RFLPs, share many of the attributes that have made allozyme analyses so useful in fisheries management and have the added benefit that all individuals carry a tag and thus are considerably more cost effective than physical tags. A second advantage that mtDNA analyses have compared to allozyme analyses is in the area of sample availability and preservation. The power of population analyses based on allele frequencies is a function of the

number of polymorphic protein loci available for study in the tissue (e.g., muscle, and eye) and the adequate preservation of the tissue in liquid nitrogen with subsequent storage. Tissue preservation can be problematic in remote areas and the potential for use in the West Greenland fishery is limited. Tissue necessary for determining protein electrophoresis preservation are significantly reduced. Only 10-20 mg of either liver or skeletal muscle is necessary for 30 digestions with restriction enzymes. Tissue preservation is a necessary consideration in species discrimination. In addition, tissue preservation appears to be a disadvantage compared to starch gel electrophoresis. The samples were collected on dry ice and maintained at -80°C. The weight of the DNA from tissues preserved in liquid nitrogen is a significant weight.

In terms of both stock discrimination and genetic diversity of mtDNA appears likely to be a valuable tool. The previous example of freshwater salmonids regarding these analyses need to be considered and the fact that preliminary sampling is not fully representative of the diversity of mtDNA in North America and Europe. This is a preliminary sample was obtained from southern Canada. In fact, Birt and Birstein identified a European mtDNA haplotype in fish from southern Canada. Possible explanations account for the presence of this haplotype in fact not solely European origin. It may be between European and North American haplotypes. It may indicate that if this is the case there may be a genetic exchange and that it is completely replaced by the European haplotype. The second explanation is that transport of the European haplotype in some North American fish. It is to note that in three independent surveys of Atlantic salmon that no North American haplotypes were found.

In any event it is clear that a more detailed analysis, especially in North America, is required to provide an enhanced picture of the distribution of mtDNA haplotypes. It is particularly important to a determination of the continent of origin in distinguishing between continent of origin. This is especially important in the West Greenland fishery where it occurs naturally in North American salmon. A more refined look at these genotypes. For example, the recognition sequences or directly sequencing the DNA is possible to find additional nucleic acid markers which identify European and North American origin.

A second caveat concerning mtDNA analyses deserves consideration regarding further studies in the West Greenland river drainages. I have indicated that a major concern in the West Greenland fishery is determining continent of origin. If this is true, there is obviously also concern regarding the presence of different river stocks. Restriction analysis

number of polymorphic protein loci that can be reliably scored. Maximizing the number of protein loci available for study in turn depends on the availability of tissues (typically liver, muscle, and eye) and the adequate preservation of those tissues (samples frozen on dry ice or liquid nitrogen with subsequent storage at -70°C ³⁴). Both tissue collection and preservation can be problematic in remote areas and as noted by Verspoor "... protein variation has limited potential for use in the West Greenland fishery due to difficulties in obtaining samples of liver tissue necessary for determining protein genotypes"²⁸. The problems of tissue availability and preservation are significantly reduced when nucleic acid methodologies are used. Using 100 mg of either liver or skeletal muscle yields sufficient high molecular weight DNA for 20 to 30 digestions with restriction enzymes. The amount and source of tissues sampled is a necessary consideration in species for which whole fish market condition is important. In addition, tissue preservation appears to be less critical in many nucleic acid methodologies in comparison to starch gel electrophoresis of proteins. Although in the present study tissues were collected on dry ice and maintained at -70°C , our lab routinely isolates high molecular weight DNAs from tissues preserved in 70 to 90% ethanol.

In terms of both stock discrimination and sample preservation, restriction enzyme analyses of mtDNA appears likely to be a valuable tool in the management of Atlantic salmon. As in the previous example of freshwater fishes in the southeastern U.S., however, certain caveats regarding these analyses need to be discussed. The first caveat regards geographic sampling and the fact that preliminary sampling of Atlantic salmon mtDNA genomes was probably not fully representative of the diversity and distribution of mtDNA haplotypes in the rivers of North America and Europe. This is particularly true in North America where all of our preliminary samples were obtained from a group of geographically related rivers in Maine and southern Canada. In fact, Birt and Davidson⁴⁶ have recently observed what I termed the European mtDNA haplotype in fish sampled from several rivers in Newfoundland. Two possible explanations account for their observation. The first is that the European mtDNA haplotype is in fact not solely European and represents an ancestral polymorphism shared between European and North American Atlantic salmon populations. Preliminary data indicate that if this is the case there may be a clinal distribution of the European mtDNA haplotype and that it is completely replaced by the North American type in southern Canada and the U.S. The second explanation is that transplantation of European fish accounts for the presence of the European haplotype in some North American stocks of Atlantic salmon. It is interesting to note that in three independent surveys of both wild and hatchery populations of European Atlantic salmon that no North American haplotype has yet been observed.^{47,48}

In any event it is clear that a more complete geographic sampling of Atlantic salmon, especially in North America, is required. The outcome of such a survey will provide an enhanced picture of the distribution of mtDNA haplotypes in Atlantic salmon. This is particularly important to a determination of the utility of mtDNA as a genetic marker useful for distinguishing between continent of origin. If it turns out that the so called European haplotype occurs naturally in North American stocks of Atlantic salmon it will be necessary to take a refined look at these genotypes. For example, using restriction enzymes that cut at four base recognition sequences or directly sequencing portions of the mtDNA genome, it may be possible to find additional nucleic acid markers that unequivocally distinguish between fish of European and North American origin.

A second caveat concerning mtDNA analyses of Atlantic salmon stock structure that deserves consideration regards further documentation of genetic differences between different river drainages. I have indicated that a primary concern of managers responsible for Atlantic salmon is determining continent of origin of salmon harvested in a mixed fishery. Although this is true, there is obviously also considerable interest in being able to distinguish fish from different river stocks. Restriction analyses of mtDNAs isolated from Atlantic salmon collected

number of polymorphic protein loci that can be reliably scored. Maximizing the number of protein loci available for study in turn depends on the availability of tissues (typically liver, muscle, and eye) and the adequate preservation of those tissues (samples frozen on dry ice or liquid nitrogen with subsequent storage at -70°C ³⁴). Both tissue collection and preservation can be problematic in remote areas and as noted by Verspoor "... protein variation has limited potential for use in the West Greenland fishery due to difficulties in obtaining samples of liver tissue necessary for determining protein genotypes".²⁸ The problems of tissue availability and preservation are significantly reduced when nucleic acid methodologies are used. Using 100 mg of either liver or skeletal muscle yields sufficient high molecular weight DNA for 20 to 30 digestions with restriction enzymes. The amount and source of tissues sampled is a necessary consideration in species for which whole fish market condition is important. In addition, tissue preservation appears to be less critical in many nucleic acid methodologies in comparison to starch gel electrophoresis of proteins. Although in the present study tissues were collected on dry ice and maintained at -70°C , our lab routinely isolates high molecular weight DNAs from tissues preserved in 70 to 90% ethanol.

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from different drainages within Europe or within North America has not provided as much information on the genetic architecture of these stocks as studies of allozyme polymorphism have.^{27,28} It is somewhat unlikely, moreover, that analysis of mtDNA alone is likely to improve knowledge of regional stock structure in Atlantic salmon. I say this only because these rivers have only been recolonized within the last 8000 to 10,000 years and, thus, not much time has transpired for the accumulation of genetic differences likely to qualitatively distinguish local river stocks. In the absence of river stock specific mtDNA markers, allozymes representing unlinked protein coding loci or nuclear RFLPs are likely to provide more efficient assays of population subdivision. This is because the power of test for population heterogeneity increases with the addition of polymorphic loci.

I am highly optimistic that additional genetic analyses, utilizing both mtDNA RFLPs (or sequence data) and nuclear RFLPs, will provide additional markers useful for the analysis of stock structure in Atlantic salmon. I will also predict that if the so-called European mtDNA haplotype observed in some Newfoundland Atlantic salmon populations represents a historical North American polymorphism, as opposed to an introduced one, that further study will reveal mtDNA characters that permit it to be differentiated from the mtDNA haplotypes of European Atlantic salmon.

V. SKIPJACK TUNA, ATLANTIC HERRING, PACIFIC SALMONIDS, LAKE TROUT, AND WALLEYE

My final illustrations are drawn from a variety of sources and certainly do not represent an exhaustive list of possible fish examples. They were chosen as a representative sampling of marine, anadromous, and freshwater mtDNA-based studies of fish undertaken to address questions of genetic stock identification important to the management of fisheries. Furthermore they all share a characteristic that I wish to emphasize in the final section of this chapter; namely shared mtDNA haplotypes between previously characterized fish stocks or geographically separated samples. The interpretation of these data, with respect to fisheries analysis, is considerably less straightforward than interpretation of data presented in the earlier two sections. Do the data provide evidence that we are dealing with a single population and that any previous notions that we had about distinct fish stocks are incorrect? In a sense, perhaps more ecological than evolutionary, the answer to this question surely has to be "no" with respect to the freshwater fish I will discuss in this section. After all, at least since the recession of the glaciers and recolonization of north temperate lakes, some of the walleye and lake trout populations studied have been reproductively isolated from one another (excepting, of course, any transplantation by man). The degree of reproductive isolation between stocks of either marine fish or anadromous fish is less clear and provides the principal reason for studying the genetic architecture of these populations.

What we would like to determine is how we can use changes in the frequency of mtDNA haplotypes between stocks of fish to infer levels of gene flow, or the lack thereof, between these stocks. Unfortunately, none of the studies that I am about to report have been analyzed in such a way to permit any clear conclusions about levels of gene flow between the samples of fish analyzed. For the most part, this is because the development of statistical methodologies for analyzing mtDNA haplotype data in a population genetic, as opposed to systematic, sense has lagged behind the production of mtDNA haplotype data. However, several years ago Takahata and Palumbi³⁵ published a *Gst* statistic that has not been widely used. Recently Lynch and Crease¹⁰ have published a method for using mtDNA haplotype data to determine *Fst* values, the traditional statistical measure of inbreeding. Slatkin³⁶ recently published a paper in which he uses the method of coalecscents to provide a test for small amounts of gene flow between populations. Both papers have been published so recently that they provide the only examples of their respective methods. As use of these tests become more widespread and

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more statistical tests are developed, fisheries managers should enjoy more sophisticated measures of gene flow between stocks of fish. I expect that one outcome of more rigorous statistical analysis of population subdivision based on molecular characters will be a requirement for larger sample sizes.

In probably the first published application of restriction enzyme analysis of a fish stock, Graves and co-workers studied population structure of skipjack tuna (*Katsuwonus pelamis*) collected from both the Atlantic and Pacific Oceans.³⁸ Previous studies of meristic, morphometric, and allozyme variation had detected little differentiation between individual skipjack sampled in the two oceans and analysis of mtDNA haplotypes uncovered no evidence for genetic differentiation between Atlantic and Pacific stocks of skipjack tuna. As reviewed by Graves and his collaborators, skipjack do not have discrete spawning areas and their pelagic larvae are found circumtropically.³⁸ In addition, physical tag data provide evidence that skipjack can travel thousands of kilometers. Taken together the data suggest that gene flow between ocean basins has been sufficient to prevent any measurable genetic differentiation. Due to the small numbers of skipjacks assayed and the small number of restriction enzymes used in this survey, however, it might be premature to determine that Atlantic and Pacific skipjack represent a single stock.

In another study of mtDNA polymorphism in a marine species, Kornfield and Bogdanowicz analyzed 69 Atlantic herring (*Clupea harengus*) representing two discrete fall-spawning stocks and a spring-spawning stock.³⁹ mtDNAs isolated from each fish were digested with 16 six-base restriction enzymes and 26 distinct mtDNA haplotypes were observed. As Kornfield and Bogdanowicz discuss, herring stock recognition is based on meristic characters, spawning time, and spawning location and this fishery is managed as if stocks represent discrete genetic groups.³⁹ However, neither earlier studies of allozyme variation nor the present study of mtDNA restriction site polymorphism can distinguish between these stocks. The five most frequent mtDNA haplotypes were observed in all three herring stocks studied and most of the other haplotypes were unique (see Table 2). In three out of seven instances the most probable precursor mtDNA haplotype for a unique haplotype was observed in a different spawning stock (see Figure 8). Kornfield and Bogdanowicz concluded that their results were not consistent with the idea that separate genetic stocks of herring exist in the Gulf of Maine.³⁹ They also point out, however, that their data do not permit a rigorous analysis of gene flow between herring stocks.

I turn next to the king (or chinook) of anadromous salmonid species, *Onchorhynchus tshawytscha*, which has a life history that might be expected to produce more evident genetic stock structure. Specifically, chinook salmon return to their natal stream to spawn and then die. Straying between streams is relatively rare and thus salmon in one river are expected to be reproductively isolated from fish in other rivers.^{40,41} Tremendous effort has been directed over the last 20 years, primarily by Utter's group at the Northwest and Alaska Fishery Center (see Utter et al.⁴² for a recent summary), towards developing protein electrophoretic techniques for genetic identification of chinook salmon stocks. Although allozyme methods are generally useful for distinguishing between regional stocks of chinook salmon, managers of salmonid fisheries continue to look for methods that will permit them to more finely resolve stock differences. Similar to the case for Atlantic salmon described earlier, stock-specific genetic markers would provide managers the opportunity to close fisheries to protect overfished stocks and would allow catches to be fairly apportioned.

As a result Wilson and his co-workers have studied population subdivision in chinook salmon using mtDNA RFLP markers.⁴³ Seventy-six salmon were collected from seven sites in British Columbia and Canada and their mtDNAs were analyzed with 14 five- and six-base restriction enzymes. Six different mtDNA haplotypes were revealed by restriction analysis, however three of the haplotypes were unique (Table 3). None of the three other mtDNA haplotypes were stock specific, but there was regional differentiation (Figure 9). One hap-

TABLE 2
Distribution of mtDNA Composite Clonal Genotypes in Samples
of Atlantic Herring

| Clonal designation | Composite clonal genotype | Jeffries Ledge | Trinity Ledge | | St. Lawrence |
|--------------------|---------------------------|----------------|---------------|------|--------------|
| | | | 1984 | 1985 | |
| 1 | AAAAAAA | 5 | | 2 | 5 |
| 2 | AABAAAA | 2 | 2 | 2 | 6 |
| 3 | BAAAAAA | 2 | | 3 | 6 |
| 4 | AABAAAB | 1 | 1 | 3 | 1 |
| 5 | ABAABAA | 1 | 1 | 2 | 1 |
| 6 | BABAAAA | 1 | | | |
| 7 | AABACAA | | | 1 | 1 |
| 8 | AAAADAB | 1 | | | |
| 9 | IAAAAAA | | | | 2 |
| 10 | DAAAAAA | | | | 1 |
| 11 | CAAAAAB | | | | 1 |
| 12 | BAEAAAA | | | | 1 |
| 13 | HABAAAA | | | | 1 |
| 14 | AAAAABB | | 1 | | |
| 15 | CAAAAAA | | 1 | | |
| 16 | BABAAAB | | 1 | | |
| 17 | ABABBAA | | 1 | | |
| 18 | ABBACAA | | | 1 | |
| 19 | AAACCAA | | | 1 | |
| 20 | IABAAAA | | | 1 | |
| 21 | AACAAAA | | | 1 | |
| 22 | BADAAAA | | | 1 | |
| 23 | CABAAAA | | | 1 | |
| 24 | AADAAAA | | | 1 | |
| 25 | ACDAAAA | | | 1 | |
| 26 | ADDABAA | | | 1 | |
| Total | | 13 | 8 | 22 | 26 |

Note: Letters (from left to right) are digestion patterns for *ApaI*, *BglII*, *BstEII*, *EcoRI*, *EcoRV*, *KpnI*, and *XhoI*.

From Kornfield, I. and Bogdanowicz, S. M., *Fish. Bull.*, 85(3), 564, 1987. With permission.

lotype (A) was observed only in the five British Columbia stocks sampled while another haplotype (C) was observed only in the two Alaska populations. Haplotype B was observed in both Alaska and British Columbia populations. Wilson and his co-workers' results are provocative and suggest that mtDNA might be a useful tool in the management of chinook salmon.⁴³ We are left wondering, however, whether or not differences in mtDNA haplotype frequencies between salmon stocks will provide additional information on population subdivision in chinook salmon that is not obtainable with allozyme analyses.

Grewe and Hebert used 18 four-, five-, and six-base enzymes to analyze mtDNA haplotype variation in 126 lake trout (*Salvelinus namaycush*) sampled from seven different hatchery stocks and two introduced lake populations.⁴⁴ An additional 55 fish, representing two of the above brood stocks and two natural populations, were assayed solely by *BamHI* and *HindIII*. Thirteen mtDNA haplotypes were observed and fell into three major clusters that are distinguished from one another by a minimum estimate of sequence divergence equal to 1.4% (Table 4 and Figure 10). As in the marine and anadromous species of fish discussed above, no stock-specific mtDNA haplotypes were observed (Figure 10). In fact, any mtDNA hap-

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FIGURE 8. C...
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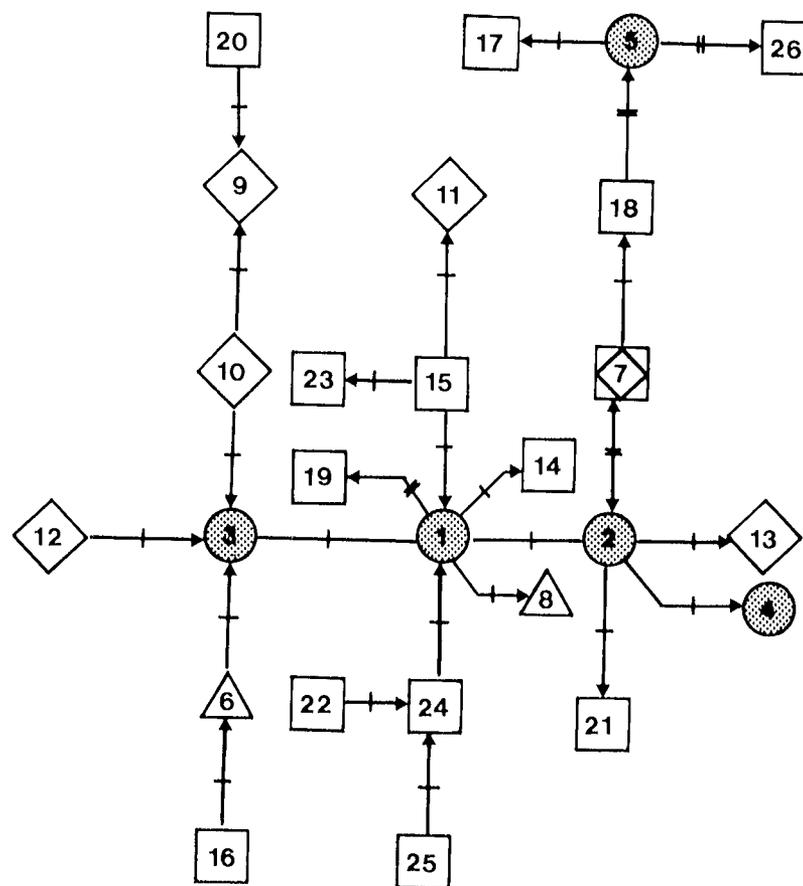


FIGURE 8. Cladistic relationships of 26 composite cleavage patterns of Atlantic herring mtDNA. Composites are connected parsimoniously to minimize the number of restriction site changes required. Shaded numbers refer to composites observed at all three spawning locations. Crossbars on connecting lines indicate minimum number of site changes required to connect adjacent composites; arrows indicate direction of site losses. Locality symbols: square — Trinity Ledge; triangle — Jeffries Ledge; diamond — St. Lawrence. (From Komfield, I. and Bogdanowicz, S. M., *Fish. Bull.*, 85, 564, 1987. With permission.)

lotype that was surveyed in more than one fish was found in two or more stocks of lake trout. Grewe and Hebert suggest that patterns of mtDNA haplotype distribution between brood stocks may reflect post-glacial colonization of temperate lakes by lake trout previously isolated in three distinct refugia (Figure 11).⁴⁴ This scenario is somewhat difficult to evaluate, however, because most of the sampling effort was directed at brood stocks which have population histories that are likely to be very different from the histories of natural populations. Nonetheless, the authors indicate that differences in mtDNA haplotype frequency between brood stocks might prove useful in the management of lake trout.⁴⁴

The final study I will report on is similar, in many aspects, to the study of lake trout discussed above. Walleye (*Stizostedion vitreum*), like lake trout, is an economically important resource in the Great Lakes region, and has been intensively managed. Billington and Hebert investigated mtDNA polymorphism in walleye sampled from ten spawning stocks that are distinguished largely on the basis of tagging studies.⁴⁵ Nine restriction enzymes (22 enzymes for a subset of 65 fish) were used to digest mtDNAs isolated from 141 walleye. Two mtDNA haplotypes accounted for over 90% of the nine haplotypes observed. These two haplotypes

TABLE 3
Polymorphic Restriction Sites In Chinook Salmon

| Enzyme | Clonal designation | | | | | |
|----------------------|--------------------|---|---|---|---|---|
| | A | B | C | D | E | F |
| <i>Bgl</i> I, site 1 | + | + | + | | | + |
| <i>Bgl</i> I, site 2 | + | | | | | |
| <i>Xba</i> I | + | | | | + | |
| <i>Hind</i> III | | | + | | + | + |
| <i>Ava</i> II | | + | + | | | + |

Note: + indicates the presence of the cut site.

From Wilson et al., *Can. J. Fish. Aquat. Sci.*, 44, 1303, 1987. With permission.

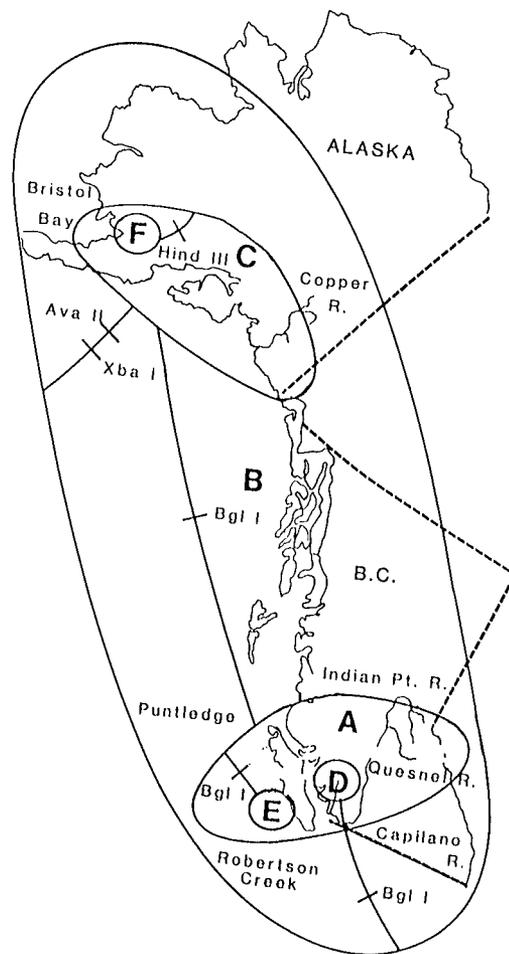


FIGURE 9. Parsimony network linking the six mitochondrial genotypes of *O. tshawytscha* detected in this study. Geographic distribution of mtDNA clones A, B, and C is shown. Mitochondrial genotypes D, E, and F were detected in single fish. (From Wilson, G. M., Thomas, W. K., and Beckenbach, A. T., *Can. J. Fish. Aquat. Sci.*, 45, 1304, 1987. With permission.)

TABLE 4 -
Composite Clonal Genotypes of the 13 mtDNA Clones Resolved by
10 Polymorphic Restriction Enzymes and Their Abundances Among
9 Brood Stocks of Lake Trout

| Clone | No. of individual fish | AvaI | BamHI | BstEII | HindIII | NcoI | SmaI | HinfI | NciI | HpaII | TaqI |
|-------|------------------------|------|-------|--------|---------|------|------|-------|------|-------|------|
| A1 | 40 | A | A | A | A | A | A | A | A | A | A |
| A2 | 18 | A | A | A | A | A | A | D | A | A | D |
| A3 | 5 | A | A | A | A | A | A | A | C | C | A |
| A4 | 1 | A | A | B | A | A | A | A | A | A | A |
| A5 | 1 | A | D | A | A | A | A | D | A | A | D |
| A6 | 1 | A | A | A | A | A | A | A | D | A | A |
| A7 | 1 | A | A | A | A | A | A | D | A | A | A |
| A8 | 1 | A | A | A | A | A | A | D | A | A | E |
| A9 | 1 | A | A | A | B | A | A | D | A | A | D |
| B1 | 41 | B | B | A | A | A | A | B | B | B | B |
| C1 | 13 | C | C | A | A | A | A | C | B | B | C |
| C2 | 2 | D | C | A | A | A | B | C | B | B | C |
| C3 | 1 | C | C | A | A | B | A | C | B | B | C |
| Total | 126 | | | | | | | | | | |

From Grewe, P. M. and Herbert, P. D. N., *Can. J. Fish. Aquat. Sci.*, 45(12), 2117, 1988. With permission.

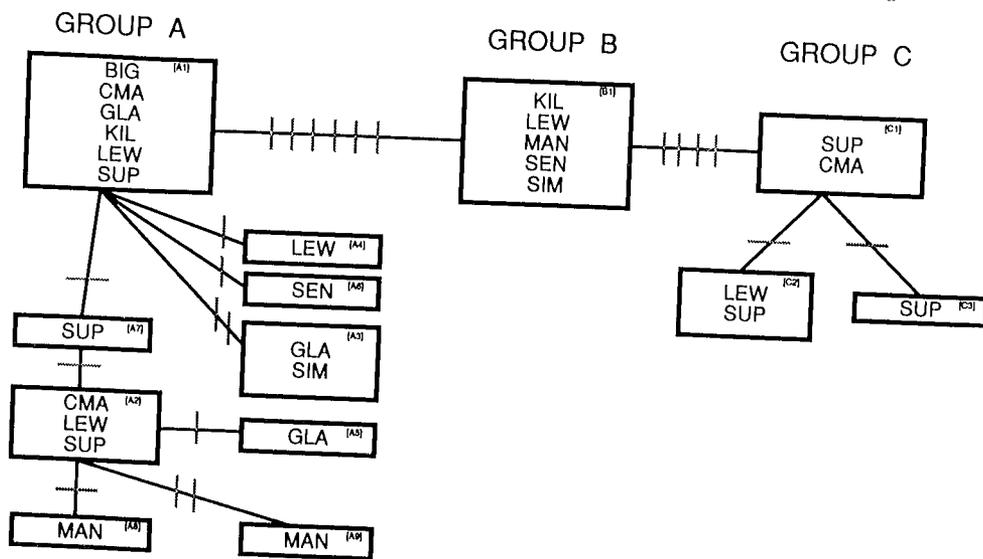


FIGURE 10. Parsimony network of the 13 lake trout mitochondrial DNA clones recognized by the ten polymorphic restriction enzymes. Clonal designations are indicated by the square brackets and dotted lines represent the number of restriction endonucleases differentiating each clone. The three-letter abbreviations indicate the stocks containing each clone. (From Grewe, P. M. and Herbert, P. D. N., *Can. J. Fish. Aquat. Sci.*, 45(12), 2118, 1988. With permission.)

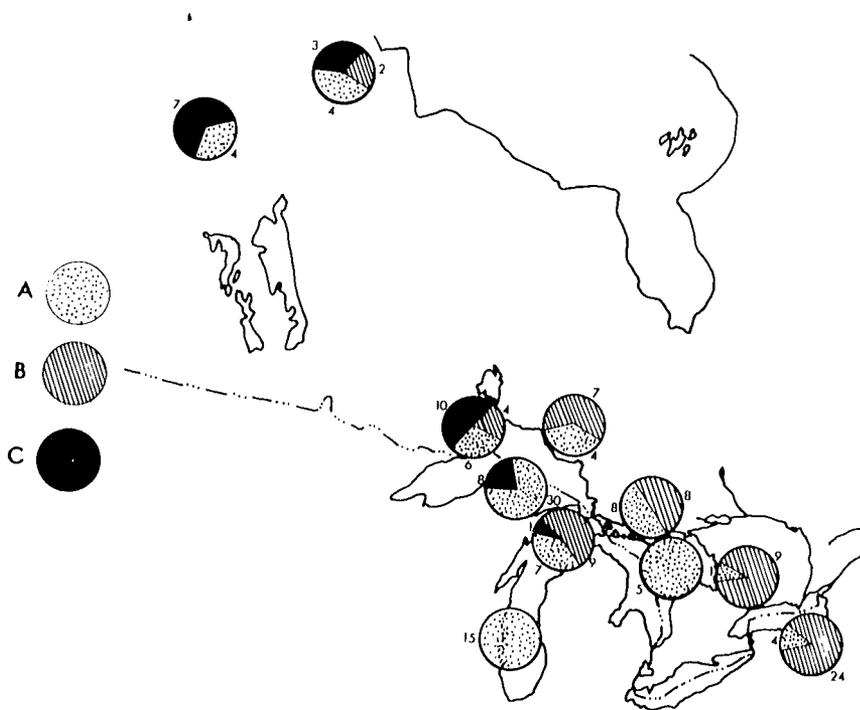


FIGURE 11. Distribution of the A, B, and C mtDNA clonal groups based on a total sample size of 181 lake trout from the 11 populations surveyed. (From Grewe, P. M. and Herbert, P. D. N., *Can. J. Fish. Aquat. Sci.*, 45(12), 2120, 1988. With permission.)

could be distinguished. The estimated sequence of mtDNA haplotypes in one population that predominated in glacial refugia may be different from the mtDNA polymorphic genotypes observed

I have attempted to do this, particularly as they are the study of bowfin populations chosen because they are managers. In most cases, as I indicated earlier, as statistical techniques are indicated. Sometimes we use DNA markers that are, however, I suspect that the in haplotype frequencies

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I am grateful to you for your discourse on the lack of

My research has been done at the Fisheries Service, and

could be distinguished on the basis of their *ScaI* and *NcoI* fragment patterns and have an estimated sequence divergence of .47%. Although most of the stocks sampled contained both mtDNA haplotypes, one haplotype predominated in the western Great Lakes while the other one predominated in the eastern Great lakes. Billington and Hebert posit that two distinct glacial refugia may have existed for walleye as evidenced by the geographic pattern of mtDNA polymorphism observed. In addition, they suggest that several of the rare mtDNA genotypes observed may be useful in creating genetically tagged brood stock.⁴⁵

VI. CONCLUSIONS

I have attempted to provide a reasonable cross section through mtDNA studies of fish, particularly as they are relevant to a discussion of genetic identification of fish stocks. Except the study of bowfin and spotted sunfish mtDNA polymorphism, all other examples were chosen because they were studies undertaken to answer questions posed by fisheries resource managers. In most cases, particularly in the final section, direct answers were not forthcoming. As I indicated earlier, the answers that geneticists can give fisheries managers will improve as statistical techniques for the analysis of haplotype frequency data become more sophisticated. Sometimes we will be lucky, as may be the case for Atlantic salmon, and we will find DNA markers that permit qualitative genetic identification of a fish stock. More often, however, I suspect the search for stock-specific genetic tags will elude us and only differences in haplotype frequencies may predominate.

I think this for two reasons. First, most of the fish stocks that have been historically studied are temperate and thus, even for marine species, have had demographies recently influenced by the Pleistocene glacial periods. As a result, I believe, little time has transpired for the accumulation of fixed genetic differences that would indicate that stocks are reproductively isolated. Second, and with respect to anadromous and marine species, I doubt that most fish stocks are completely reproductively isolated. A very small number of migrants between populations can prevent genetic differentiation. Furthermore, fish do not need to migrate between populations every generation. Local population crashes due to disease or environmental perturbation followed by colonization of genotypes from the outside will maintain gene flow between populations as surely as a steady trickle of migrants each generation.

If, in fact, stocks are reproductively isolated, it will most likely be possible, with time and money, to sooner or later find a stock-specific genetic marker. Be forewarned, however, that unless stocks have been isolated for a very long period of time (typically many thousands of generations) a long and expensive look may be required. If, however, we suspect that there is gene flow between stocks, we will ultimately be defeated in our search for a stock-specific genetic tag. Nevertheless we will have learned much about processes governing the evolution of fish populations and this can only help us in the management of fish stocks.

ACKNOWLEDGMENTS

I am grateful to an anonymous reviewer for her/his comments and particularly for a lively discourse on the lack of communication between fishery biologists and population geneticists.

My research has been funded by the National Science Foundation, the National Marine Fisheries Service, and the National Research Council.

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