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February 15, 1991

Dr. Irving Kornfield
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Dear Irv:

Many thanks for a copy of your paper assessing char by mtDNA.

Enclosed is a paper written by a student several years ago for a research project. This is my only copy, but you are welcome to xerox it before returning. I believe we fairly well documented that Sawtooth Lake was stocked in 1925 with char from Sunapee L., NH (this was an exchange between states, the federal Bur. Fish was not involved) and with brook trout in 1941 (from Hagerman, ID, federal hatchery). I have no reasonable doubt that the present hybrids in Sawtooth L. are brook trout x Sunapee. Photos and specimens of Sawtooth char I've seen in recent years indicate that hybridization has become more prevalent compared to the 1978 collection (when was your sample collected?) I suspect that your failure to associate the hybrid mtDNA in your specimens with S. fontinalis may relate to the incomplete knowledge of the range of fontinalis mtDNA types throughout the range of the species. The hybrid characters discussed in the paper could only come from fontinalis.

Also enclosed is a copy of a letter to Louis Bernatchez which I hope may stimulate your interest to apply mtDNA to Maine sympatric smelt populations and see if the results for whitefish might be duplicated. One paper mentioned was published in Evolution 1990 44(5):1263-71, the other is "in press" in Evolution. They nicely demonstrate the sensitivity of mtDNA for detecting monophyletic lineages where other methods have failed. Perhaps you have already seen this letter via Ann Baker's network.

Have you made a "breakthrough" by attaining a 10% sampling of the mtDNA genome? The 2-3% samples on which most other studies are based, has been a limiting factor on sensitivity.

I call your attention to my comments on the danger that I foresee in the use mtDNA or any quantitative characteristic of genetic identification as the ultimate criterion for an endangered species preservation program. The most significant intraspecific life history - ecological distinctions are convergent traits such

as anadromy vs. nonadromy, timing of anadromous runs, etc. A waterfowl hunter would find it ridiculous if he were told that a chihuahua could be substituted for a Labrador retriever because of their proven genetic identity.

Sincerely,

Robert Behnke

RB/nt

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February 11, 1991

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Dear Bob,

I have enclosed a final report on a recent study that is nearly completed on the status of the Sunapee char of Maine. The report will form the basis for a manuscript to be prepared later this year, most probably for the Canadian Journal of Fisheries and Aquatic Sciences; Seifu Seyoum and Fred Kirchais will be co-authors. I think you will find this particular study of some interest, not only because of its conclusions with respect to the status of the Sunapee and its origins, but also because of the sensitivity of the mtDNA analyses: I estimate that we sampled approximately 10% of the entire mitochondrial genome! There is one more facet of this investigation that has yet to be explored and I am in the process of obtaining material to finish it. According to very old fishery records, German freshwater char ("Saibling") were introduced into New England in the late 1800's. I want to examine these fish to exclude the possibility that the distinctive mitochondrial genome characteristic of true Sunapee char are really of European origin. After that test has been concluded (I hope by the end of March) this iteration of the Floods Pond story will be finished.

We have recently shifted from doing restriction studies of mtDNA to direct sequencing. Part of this change has been motivated by the need to have greater acuity in making deeper taxonomic comparisons.

I have been in extensive contact with Ann Baker about molecular genetics. She has kindly kept me informed of some of your activities. I trust that we may have an opportunity to talk at some point in the near future.

Best regards,

Irv Kornfield, PhD
Professor of Zoology

Enc.

IK/mcd

FINAL REPORT

Genetic characterization of Maine populations of Arctic char (*Salvelinus alpinus oquassa*): uniqueness and affinities of mitochondrial DNA

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When engaged in scientific study that may impact public policy, it is most important to remain as objective as possible, particularly when presented with competing social perspectives. We have endeavored to do so in the conduct of this research. In synthesizing the information presented here, we have discussed portions of research and their implications with colleagues and students. However, the views expressed are entirely our own and are not intended to reflect the position of any individual, group or agency.

SUMMARY

Samples of Arctic char from five isolated lakes in New England and eastern Canada were genetically examined to estimate relationships and establish their taxonomic status. Of particular interest was the status of Sunapee char, the native char from Floods Pond, Maine. Floods Pond is the source of water for towns served by the Bangor Water District; water usage coupled with variation in annual precipitation may directly impact the long-term survival of this geographically isolated population.

Fish were characterized by restriction endonuclease analysis of mitochondrial DNA, a sensitive molecular technique widely applied in systematic studies of fishes. Characterization of North American char and comparisons with lake trout and samples of char from Scandinavia support the following conclusions:

(1) None of the studied North American populations merits separate subspecific status. Arctic char of Floods Pond have no formal taxonomic standing and should not be recognized as a distinct taxonomic entity.

(2) Arctic char of Eastern North America should be considered members of the *Salvelinus alpinus oquassa* subspecies.

(3) All specimens from Floods Pond possessed a unique DNA banding pattern for one restriction enzyme that was not observed in fish from any other sampled locality. This slight, but consistent difference delineates a distinct lineage of Arctic char. Preservation of this lineage is warranted on genetic grounds.

(4) By contrast, unique banding patterns did not characterize any other studied char population, including blueback char and the Quebec red trout. One composite banding pattern was common to fish from all eastern locations (excluding Floods Pond). Fish from these waters may be viewed as genetically homogeneous; based on genetics, none of these other populations warrant protection.

(5) Descendants of fish from Sunapee Lake, New Hampshire, which were introduced in the early 1900s to remote lakes in Idaho, displayed four mitochondrial DNA banding patterns. One was the pattern of eastern North American chars while the others were significantly different. The presence of very different mitochondrial DNAs in the Idaho fish suggests that the original introduction included both "true" Sunapee char as well as other New England char.

(6) Since the mitochondrial DNA from Floods Pond fish is different from that of Idaho fish, Floods Pond char are not "true" Sunapee char, but represent another, separate and as yet unnamed form. Because of the differences in mitochondrial DNA, it would not be appropriate to stock Floods Pond with char from Idaho. Further, because hybridization has occurred between the eastern char and "Sunapee char" in the Idaho lakes, it is highly improbable that any genetically pure Sunapee char still exist.

INTRODUCTION

The Arctic char, *Salvelinus alpinus*, is a taxonomically complex group of salmonid fishes widely distributed around the world at high northern latitudes. Populations in several places over the range of the species possess distinctive morphologies, particularly color patterns, that distinguish them from other local char. Of particular interest is the Sunapee char of Floods Pond, Maine, the last natural population of a rare landlocked fish characterized by unique color variation (Kircheis, 1989).

Like much of the Arctic char species complex (Behnke, 1984, 1989), the taxonomic status of the fish in Floods Pond has been the subject of considerable debate which has not been resolved. Floods Pond provides the water source for the Bangor Water District, a public organization which services communities in the greater Bangor area. Water usage coupled with natural variation in annual precipitation has impacted the substrate available for spawning by the Sunapee. Because future demands for water may impact the breeding habitat and influence the long-term survival of the Sunapee in Floods Pond, it is important to clarify the uniqueness of this fish. The principal goal of the study reported here was to investigate the genetic distinctiveness of the char from Floods Pond in order to resolve their formal taxonomic status.

Several genetic studies have been conducted to clarify the systematics of Arctic char and the Sunapee char by conventional electrophoretic analysis (Kornfield et al., 1981; Anderson et al., 1983; Magnusson and Ferguson, 1987; Partington and Mills, 1988). While some have convincingly demonstrated genetic isolation of sympatric char morphs, the status of allopatric populations such as the Sunapee of Floods Pond has not been resolved. One reason such studies have been of limited value is that conventional electrophoresis does not have sufficient sensitivity to discriminate very closely related lineages (see Gyllensten, 1985). Since these earlier studies, new techniques have become available to investigate problems in population biology. In particular, restriction analysis of mitochondrial DNA (mtDNA) permits characterization of rapidly evolving genetic material and may reveal genetic events in the recent history of evolving taxa (Hillis and Moritz, 1990).

Restriction endonuclease analysis of mtDNA has proved to be an excellent system for probing relationships among populations and among species at early stages of divergence (Avise et al., 1987; Moritz et al., 1987). In fact, a number of acute systematic problems in ichthyology that could not be resolved with conventional electrophoresis have been unambiguously settled using mtDNA (e.g, Avise et al., 1986; Gonzalez-Villasenor and Powers, 1990). Given that the endemic fishes of Floods Pond are of recent origin, analysis of mitochondrial DNA would appear to be the method of choice for population characterization.

Mitochondrial DNA is a small (16 - 17,000 base pair), circular piece of DNA that is strictly maternally inherited. Because of this unisexual inheritance, mtDNA can be used to determine the direction of mating in hybrids (e.g., Herke et al., 1990); unlike nuclear DNA which is altered by recombination during sexual reproduction in each generation, mtDNA is passed on to offspring unchanged. Variations in the nucleic acid sequence of mitochondrial DNA can be characterized by the use of restriction endonucleases. These enzymes recognize specific, short (4-6 base) sequences of DNA bases and cut (or break) the DNA molecule wherever such sequences are encountered. Thus, exposing mtDNA to restriction enzymes produces a number of smaller pieces of DNA, restriction fragments, which reflect the number of sites on the DNA molecule that possess the restriction sequence. Restriction fragments are separated by electrophoresis (migration in an electric field) and are visualized with radioactive labels. The result is a pattern of bands that is similar to a bar code. A series of mathematical operations is used to convert the number of bands observed into estimates of similarity among patterns. Additional numerical operations are used to characterize the relationships among banding patterns or phenotypes.

Comparisons of species in the genus *Salvelinus* using mtDNA have been conducted by Grewe et al. (1990). In their work, landlocked populations of Arctic char could be clearly distinguished from anadromous populations. Their preliminary comparisons of Arctic char from Great Britain with samples from Floods Pond revealed extremely high similarities.

METHODOLOGY

Tissue samples were obtained from fish collected from five populations in eastern North America including three Maine locations: Blueback char (Big Reed Pond, Gardner Lake, and Walton Lake), Sunapee char (Floods Pond), and Quebec Red trout (Lac Godin). On the basis of traditional taxonomy, fish from this New England/eastern Canada region are all considered to represent the Arctic char subspecies *Salvelinus alpinus oquassa* (Behnke, 1989). Samples of *S. a. alpinus*, the western European subspecies, were obtained from a landlocked population in Norway. Samples of lake trout, *S. namaycush*, a species which is closely related to, but distinct from Arctic char, were obtained from a State of Maine fish hatchery. In addition, tissue samples were obtained for char which are the descendants of fish from Sunapee Lake, New Hampshire. The parental population of these fish was stocked into Sawtooth Lake, Idaho, early in the 1900s. The char population native to Sunapee Lake subsequently vanished due to competition with other introduced fishes. Details of specimen collection are provided in Table 1.

Tissues from all specimens were dissected from live material and transported frozen on dry ice or liquid nitrogen to the University of Maine. Specimens were kept frozen at -80^{o}C for up to four months prior to processing.

Mitochondrial DNAs were extracted from liver and egg tissues by standard protocols and purified by cesium-chloride ultracentrifugation (Lansman et al., 1981; Dowling et al., 1990). Purified samples were dialyzed and stored in an ultracold freezer prior to use. Aliquots of purified mtDNA were digested with a battery of 18 informative restriction endonucleases (Kornfield and Bogdanowicz, 1987). Most of these enzymes had recognition sequences of four base pairs which resulted in the production of large number of cleavage fragments (Table 2). Resulting restriction fragments were end-labeled with ^{32}P-NTPs and separated by agarose electrophoresis on gels containing molecular weight standards (Maniatias et al., 1982). Restriction banding patterns were visualized by autoradiography. For a number of restriction enzymes, sample sizes were reduced when banding patterns were identical among all North American samples (Table 1).

Banding patterns were recorded and restriction fragment phenotypes were assigned to all individuals. Intrapopulation variation and interpopulation differentiation in restriction profiles was quantified by standard mathematical procedures (Upholt, 1977; Nei and Li, 1979; Nei, 1987). Fragment data and estimates of sequence divergence were summarized using phenetic (Sneath and Sokal, 1973; Rohlf, 1988), cladistic (Swofford, 1990) and maximum-likelihood techniques (Felsenstein, 1988, 1989).

RESULTS

A total of 230 cleavage fragments were visualized by digestion with the 18 restriction enzymes. Given an approximate size of 16,800 base pairs for the mitochondrial DNA of *Salvelinus* (Grewe et al., 1990), about 5.9% of mtDNA genome of these samples was examined.

In all, 13 mitochondrial DNA banding patterns or phenotypes were observed among the samples of Arctic char (Table 3). For the North American localities, three of the populations (Big Reed Pond, Lac Godin, and Walton Lake) exhibited a single phenotype which was identical for all individuals; the Quebec red troout had the same phenotype as the blueback char. Fish with this phenotype were also common in the Gardner Lake and Sawtooth Lake samples. However, these two localities also possessed additional banding phenotypes which did not occur in the other samples. The three additional phenotypes from Gardner Lake occurred in single individuals and each differed from the common pattern by a single band.

All fish examined from Floods Pond had identical phenotypes which differed from those of all other Arctic char for banding produced by the enzyme *Sty*I (Table 4). This unique pattern, "A", possessed one additional band (=cleavage site) which was not present in the "B" phenotype that was common to all other eastern populations (Figure 1). The banding phenotypes for Floods Pond char produced by all other restriction enzymes were identical to the common patterns observed in other eastern population samples.

Sawtooth Lake, Idaho, which contained char that had the common eastern phenotype (SL-1), also had fish which exhibited three additional banding phenotypes. One of these (SL-2) was similar to the common form while the others (SL-3, SL-4) had patterns which differed from the eastern form for 4 restriction enzymes (Table 3).

Sequence divergence estimates based on shared banding patterns were calculated for all composite mitochondrial DNA phenotypes (Table 5). Percent nucleotide sequence divergence (p) among phenotypes for char from New England and eastern Canada were very small (p= 0.02 % - 0.12 %). Despite having a unique cleavage pattern for *Sty*I, Sunapee char from Floods Pond exhibited very low sequence divergence with the cleavage phenotype for blueback char and Quebec red trout samples (p= 0.03%). By contrast, sequence divergence between these eastern cleavage phenotypes and those found in Sawtooth Lake char was much greater (maximum p = 0.39%). Sequence divergence between the North American subspecies of Arctic char, *S. a. oquassa*, and the Scandinavian subspecies, *S. a. alpinus*, was sizable (p= 0.42 % - 0.78 %). Sequence divergence between Arctic char and lake trout averaged 3.55 %.

Relationships among char samples were visualized by clustering (UPGMA; Sneath and Sokal, 1973) the estimates of sequence divergence among mtDNA banding phenotypes (Table 5). The resultant phenogram (Figure 2) depicts the low divergence among eastern North American samples of char. The population from Floods Pond is embedded within a cluster of eastern phenotypes, though because of the high similarity among these fish, the ordering of groups within this cluster is largely arbitrary. By contrast, two mitochondrial banding phenotypes from Sawtooth Lake char (SL-3, SL-4) are divergent from the eastern samples and form a distinctive group of their own. However, these Sawtooth char are clearly related to the eastern fish and distinct from the samples representing the subspecies from Scandinavia. Finally, all Arctic char are genetically well separated from lake trout.

Phylogenetic associations among char mitochondrial DNAs were statistically examined by bootstrapping of banding patterns (Felsenstein, 1988). Lake trout were used to infer the direction of DNA changes. The resulting maximum likelihood network (Figure 3) was similar to the phenogram produced from estimates of sequence divergence. This analysis showed the existence of three groups of char which were statistically distinct from each other. One group included all studied char from eastern North America as well as one of the chars from Sawtooth Lake (SL-1). None of these fishes, including the char from Floods Pond, were sufficiently divergent that they could be statistically distinguished by this analysis. In contrast, two of the phenotypes from Sawtooth Lake (SL-3, SL-4) formed a group which was significantly distinct from all other char. One additional char from Sawtooth Lake (SL-2) could not be placed in either group with statistical confidence. All char from Norway were included in a cluster that was significantly distinct from the North American char.

DISCUSSION AND IMPLICATIONS

The results obtained here must be interpreted within the context of: (1) the limitations and assumptions involved in the application of restriction analysis of mitochondrial DNA (Avise et al., 1988; Pamilo and Nei, 1988; Hillis and Moritz, 1990), (2) previous mtDNA studies on fishes (e.g., Avise et al., 1987; Moritz et al., 1987; Grewe et al., 1988; Bentzen et al., 1989; Gonzales-Villasenor and Powers, 1990; Grewe et al., 1990; Stephenson and Kornfield, 1990), (3) principles of population genetics (Wright, 1978; Lacy, 1987; Nei, 1987) and (4) the procedures and principles of systematic zoology.

The choice of restriction enzymes used in this study was designed to maximize the detection of differences among char samples; the use of more conservative (6-base) enzymes was limited. The number of bases assayed here was more than four times that in the study of Grewe et al. (1990); this increase enhanced the sensitivity of the assays.

It is clear from the mitochondrial DNA analyses conducted here and from the work of Grewe et al. (1990) that the populations of Arctic char from eastern North America are of recent origin. Using a conventional calibration for the mitochondrial DNA molecular clock (Moritz et al., 1987; Hillis and Moritz, 1990), the estimated percent sequence divergence among these samples ($p < 0.12$ %) suggests that they last shared a common ancestor 60,000 years ago. Given the caveats associated with such estimates, this number is compatible with origination after glacial withdrawal less than 15,000 years ago. The results from earlier electrophoretic studies are consistent with this idea (Kornfield et al., 1981; Anderson et al., 1983; Magnusson and Ferguson, 1987; Partington and Mills, 1988).

By all characterizations, the samples of char from eastern North America are very closely related to each other. The low levels of estimated sequence divergence and the absence of stable or significant clustering (Figs. 2, 3) are similar to many observations made among conspecific populations in a diversity of fishes including other salmonids (see Avise et al. [1987] and Gonzalez-Villasenor and Powers [1990] for reviews). None of these populations should be distinguished by subspecific or specific rank. Formal taxonomic status is not warranted for the char of Floods Pond, for blueback char or the red trout of Quebec.

As a group, the fishes from eastern North America are readily distinguishable from the other subspecies in this study represented by specimens of landlocked char from Norway. The average sequence divergence between the char from eastern North America (Sunapee, Blueback and Quebec Red) and Norway was $p = 0.50\%$ (range 0.40% - 0.62%). This level of divergence is consistent with that observed between geographically isolated populations of freshwater fishes, including subspecies (Gonzalez-Villasenor and Powers, 1990; Meyer et al., 1990). Grewe et al. (1990) had

previously compared samples of these two subspecies (Floods Pond versus Lake Windermere, England) and had recorded lower divergence between them (p = 0.22 %). However, their estimate was probably conservative in view of the number of sites assayed. Indeed, their estimate of sequence divergence between lake trout and char from Floods Pond (mean p = 2.91 %) is lower than that obtained here for the same taxa (p = 3.49%).

In maximum likelihood analyses (Fig. 3), samples from the two subspecies formed groups which were statistically distinguishable. This pattern was also seen in conventional (Swofford, 1990) cladistic analyses. The extent and pattern of differentiation between these groups strongly supports their recognition as representatives of discrete subspecies. Landlocked Arctic char from eastern North America (including all fish in Maine and eastern Canada) should be taxonomically recognized as belonging to the subspecies, *Salvelinus alpinus oquassa*, distinct from *S. a. alpinus*, the subspecies that occurs in Norway (and elsewhere).

Despite their overall genetic similarity to neighboring populations, the char of Floods Pond possess a unique mitochondrial DNA banding pattern for one restriction enzyme. Such a difference is equivalent to a change in one base pair (bp) among the estimated 1000 bases examined. Simple extrapolation to the entire mitochondrial DNA sequence of 16,800 bp would suggest the existence of 15 additional base changes. While mitochondrial DNA is generally used as a proxy to infer the extent of genetic differences that occur in nuclear DNA, it is not realistic to numerically extrapolate to this class of DNA. Regardless, differentiation of mitochondrial DNA implies parallel differentiation at the nuclear level.

Quebec red trout from Lac Godin and Blueback char from Big Reed Pond, Gardner Lake, and Walton Pond had no distinctive mitochondrial DNA banding patterns. From the perspective of evolutionary genetics, the absence of DNA phenotypes which could distinguish any of these populations means that there is no compelling reason for preserving the genetic material of any particular lake. That is, it does not matter exactly which populations are conserved as long as the genetic lineage of Arctic char which is common to all of these populations is preserved. By this reasoning, if the mitochondrial DNA from Floods Pond char were indistinguishable from that of the other populations, there would be no genetic justification for preservation of the population as distinct from other char in the region. However, the presence of a mitochondrial DNA banding phenotype in all individuals from Floods Pond (n = 22) and its absence from all other North American fishes studied (n = 52) justifies the conclusion that these fish represent a distinct genetic lineage of North American Arctic char. This lineage is not present in the descendants of char from Sunapee Lake which occur in Idaho (see below). Thus, protecting this lineage of Arctic char is warranted on genetic grounds.

A surprising result of the mitochondrial DNA comparisons was the diversity present in the char from Sawtooth Lake, Idaho. These fish are the direct descendants of char introduced as eggs and fingerlings from Sunapee Lake, New Hampshire in the late 1800s and early 1900s (Kircheis, 1989). The presence of four mitochondrial DNA banding patterns, including the common eastern form and three unique forms, among the 17 individuals examined suggests that the original stocking involved a large number of fish. The divergence between the eastern phenotype and the unique Sawtooth Lake forms is substantial (Table 5) and a significant feature of North American char evolution (Fig. 3).

Sawtooth history

What is the origin of these genetically distinct mitochondrial DNAs? The answer to this question turns out to be quite interesting. The Idaho fish have been isolated for too short a period of time to have evolved these differences within the lake. Brook trout are also present and occasionally hybridize with the char. However, since mitochondrial DNA is maternally transmitted, it can not become mixed between these two species; distinctive brook trout mtDNA phenotypes (Gyllensten and Wilson, 1987) are not present in the char. Thus, all observed types of char mtDNA must have been introduced together when the lake was stocked. Since the parents originated from Sunapee Lake, these forms would have been present there at this time. Indeed, the older fisheries literature suggests that char not native to Sunapee Lake had been introduced to augment production (Kircheis, pers. comm.). Thus, the divergent mitochondrial DNAs present in Sawtooth Lake represent the "true" Sunapee trout and the introduced char. Since two of the mitochondrial types present in Sawtooth Lake are either identical (SL-1) or very similar (SL-2) to the form common among eastern populations, the divergent types (SL-3, SL-4) are probably that of the "true" Sunapee char. Because these latter mitochondrial DNAs are quite divergent from that of the char in Floods Pond ($p = 0.3$ %, Fig. 3), the name "Sunapee" has been used for two distinct forms of char. The fish in Floods Pond are not "true" Sunapee char, but represent another as yet unnamed Arctic char lineage. It would not be appropriate to stock Floods Pond or other Maine waters with fish from Idaho.

Because mitochondrial DNA is maternally inherited and does not recombine, phenotypes can persist unchanged in populations for long periods of time (Avise et al., 1987). Such is the case for the divergent mtDNA phenotypes in Sawtooth Lake; it does not matter whether individuals possessing different phenotypes mate with each other because the resulting offspring will carry only the mother's mtDNA. Thus different mtDNAs can be maintained in a population despite extensive hybridization. However, this is not the case for nuclear DNA. When chars with divergent nuclear DNAs cross, their offspring possess characteristics of both parents. When these offspring themselves reproduce, their progeny will contain DNAs which have become mixed by recombination; the uniqueness of the nuclear DNAs that originally distinguished the parents has disappeared. In the confines of Sawtooth Lake, the chars that were originally introduced from New Hampshire have been interbreeding over the last 80--100 years. While their

mtDNAs have remained divergent, the distinctiveness of their nuclear DNAs has vanished. It is improbable that any genetically pure Sunapee trout still exist. It may thus be ill advised to "reintroduce" these fish back to their "native" waters.

ACKNOWLEDGMENTS

Paul Moran, University of Maine, assisted with autoradiography late in the study. Fred Kircheis, Maine Dept. of Inland Fish and Wildlife, coordinated the acquisition of char samples and provided background literature. Peter Grewe, Cornell University, provided unpublished data on his analysis of *Salvelinus* and provided helpful comments on some of the findings.

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Table 1. Collection localities for samples of fish used in mitochondrial DNA comparisons.

	Symbol	Mean N*	Latitude	Longitude
Arctic Char				
(Salvelinus alpinus oquassa)				
Big Reed Pond, Maine, USA	BR	6.6	46° 21'N	69° 03'W
Floods Pond, Maine, USA	FP	13.3	44° 43'N	68° 28'W
Gardiner Lake, Maine, USA	GL	7.2	46° 58'N	68° 53'W
Lac Godin, Quebec, Canada	LG	5.0	47° 57'N	71° 20'W
Sawtooth Lake, Idaho, USA	SL	7.8	44° 13'N	115° 03'W
Walton Lake, New Brunswick, Canada	WL	4.5	45° 30'N	65° 20'W
(Salvelinus alpinus alpinus)				
Jonsvann, Norway	NN	6.7	63° 22'N	10° 37'E
Lake Trout				
(Salvelinus namaycush)				
Maine Dept. of Inland Fish and Wildlife hatchery stock	LT	1.0	-	-

* Samples sizes were reduced for a number of restriction enzymes which produced identical banding patterns among North American samples.

Table 2. Restriction endonucleases used to characterize fish mitochondrial DNA. Restriction phenotypes from samples of *Salvelinus alpinus oquassa*, the subspecies which occurs in New England and Eastern Canada (NE), were compared to *S. alpinus alpinus*, the landlocked subspecies sampled from Norway. Note the number of restriction phenotypes which differed between the two subspecies.

Enzyme	Recognition Sequence (bp)	Pooled Sample Size for NE Localities	Total Number of Phenotypes Seen in NE	Are Phenotypes of Subspecies Equal?
*Alu*I	4	32	1	no
*Ava*I	4.6	19	1	no
*Ava*II	4.6	64	1	yes
*Bst*EII	6	64	1	no
*Cfo*I	4	64	1	yes
*Dde*I	4	60	2	no
*Eco*RII	4.6	64	1	yes
*Hae*III	4	64	1	yes
*Hinc*II	5.3	34	1	no
*Hinf*I	4	51	2	no
*Mbo*I	4	30	3	no
*Msp*I	4	51	2	no
*Nci*I	4.6	64	2	no
*Nco*I	6	19	1	no
*Rsa*I	4	61	1	no
*Sau*96I	4	64	1	no
*Sty*I	5.3	57	3	no
*Taq*I	4	25	1	no

Table 3. Mitochondrial DNA restriction fragment phenotypes observed for all sampled localities. Note (1) the high relative similarity of phenotypes among localities in New England, (2) the presence of a single phenotype for all fish from Floods Pond, and (3) the presence of multiple phenotypes in samples from Gardiner Lake, Sawtooth Lake and Norway. Enzymes composing the composite phenotypes are listed sequentially as in Table 2.

Taxon	Locality	Composite Phenotype
S. alpinus oquassa	Floods Pond	AAAAAAAAAAAAAAAAAA
"	Big Reed Pond	AAAAAAAAAAAAAAAACA
"	Gardiner Lake-1	"
"	Lac Godin	"
"	Sawtooth Lake-1	"
"	Walton Lake	"
"	Gardiner Lake-2	AAAAAAAAAADAAAAACA
"	Gardiner Lake-3	AAAAAAAAABAAAAAACA
"	Gardiner Lake-4	AAAAAAAAAABAAAAACA
"	Sawtooth Lake-2	AAAAAAAAAAABDAAACA
"	Sawtooth Lake-3	AAAAAAAAAAABDAAADA
"	Sawtooth Lake-4	AAAAABAAAAABDAAADA
S. alpinus alpinus	Norway-1	BBAAAAAAAAAAAABBBB
"	Norway-2	BDAAAAAABAAAAAABBB
"	Norway-3	BDAAAAAAAAACDAABBB
"	Norway-4	BDAAAAAAAAAAAAABBB
"	Norway-5	BDAAAAAAAAAECAABBB
S. namaycush	Maine	CCBBBCBBCCCDEACCEC

Table 4. Discrimination of char from Floods Pond with the restriction enzyme *Sty*I. All char from Floods Pond possess the mitochondrial DNA cleavage phenotype "A" which was not observed in fish from any other locality.

Locality	sample size	phenotype
Salvelinus alpinus oquassa		
Floods Pond	22	A
Big Reed Pond	10	B
Gardiner Lake	12	B
Lac Godin	5	B
Walton Lake	8	B
Sawtooth Lake	7	B
	10	C
Salvelinus alpinus alpinus		
Norway	8	D
Salvelinus namaycush		
Maine	1	E

Table 5. Genetic divergence (percent nucleotide sequence divergence, p) among mitochondrial DNA phenotypes. Note the low levels of divergence among the samples from New England, the higher level of divergence between samples from different subspecies, and the highest level of divergence between species. Symbols as in Table 1; numbers after symbols refer to phenotypes given in Table 3.

	FP	SL3	SL4	BW*	SL2	NN1	NN2	NN3	NN4	NN5	GL2	GL3	GL4	LT
FP	--													
SL3	0.31	--												
SL4	0.29	0.02	--											
BW	0.03	0.31	0.29	--										
SL2	0.09	0.25	0.23	0.06	--									
NN1	0.47	0.74	0.73	0.43	0.50	--								
NN2	0.49	0.78	0.76	0.46	0.52	0.14	--							
NN3	0.53	0.70	0.68	0.50	0.56	0.18	0.14	--						
NN4	0.57	0.74	0.72	0.54	0.60	0.22	0.18	0.04	--					
NN5	0.44	0.72	0.70	0.40	0.47	0.09	0.05	0.09	0.12	--				
GL2	0.07	0.34	0.33	0.04	0.10	0.47	0.50	0.54	0.58	0.44	--			
GL3	0.11	0.39	0.37	0.08	0.14	0.52	0.54	0.58	0.62	0.49	0.12	--		
GL4	0.05	0.33	0.31	0.02	0.08	0.45	0.48	0.52	0.56	0.42	0.06	0.10	--	
LT	3.49	3.36	3.33	3.49	3.53	3.59	3.72	3.68	3.68	3.64	3.47	3.65	3.51	--

* BW is the common composite phenotype found in Big Reed Pond, Gardiner Lake (GL-1), Lac Godin, Sawtooth Lake (SL-1) and Walton Lake.

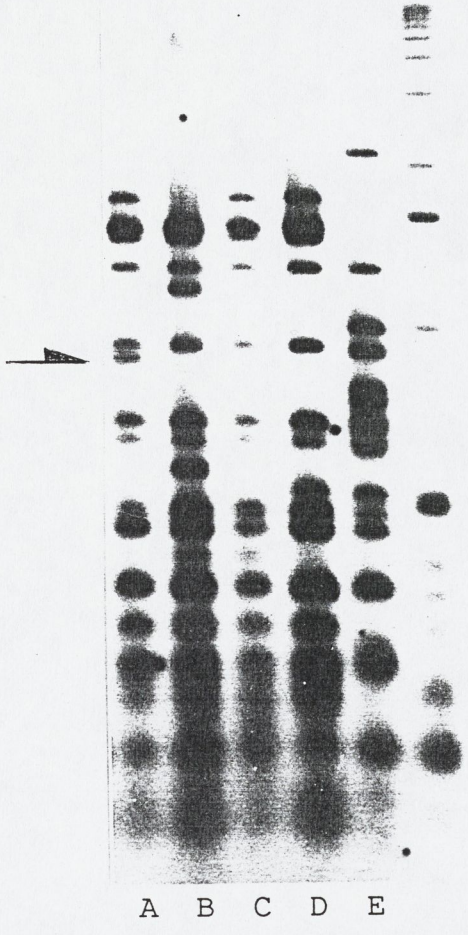

Figure 1. Autoradiograph of mitochondrial DNA from char populations digested with the restriction enzyme *Sty*I. This enzyme revealed a unique banding pattern, "A", for fish from Floods Pond. Note the band (arrow) that is present in "A" but absent from all other patterns. Geographic distribution of banding patterns (phenotypes) is given in Table 4. Banding pattern in the far right lane is a molecular weight size standard.

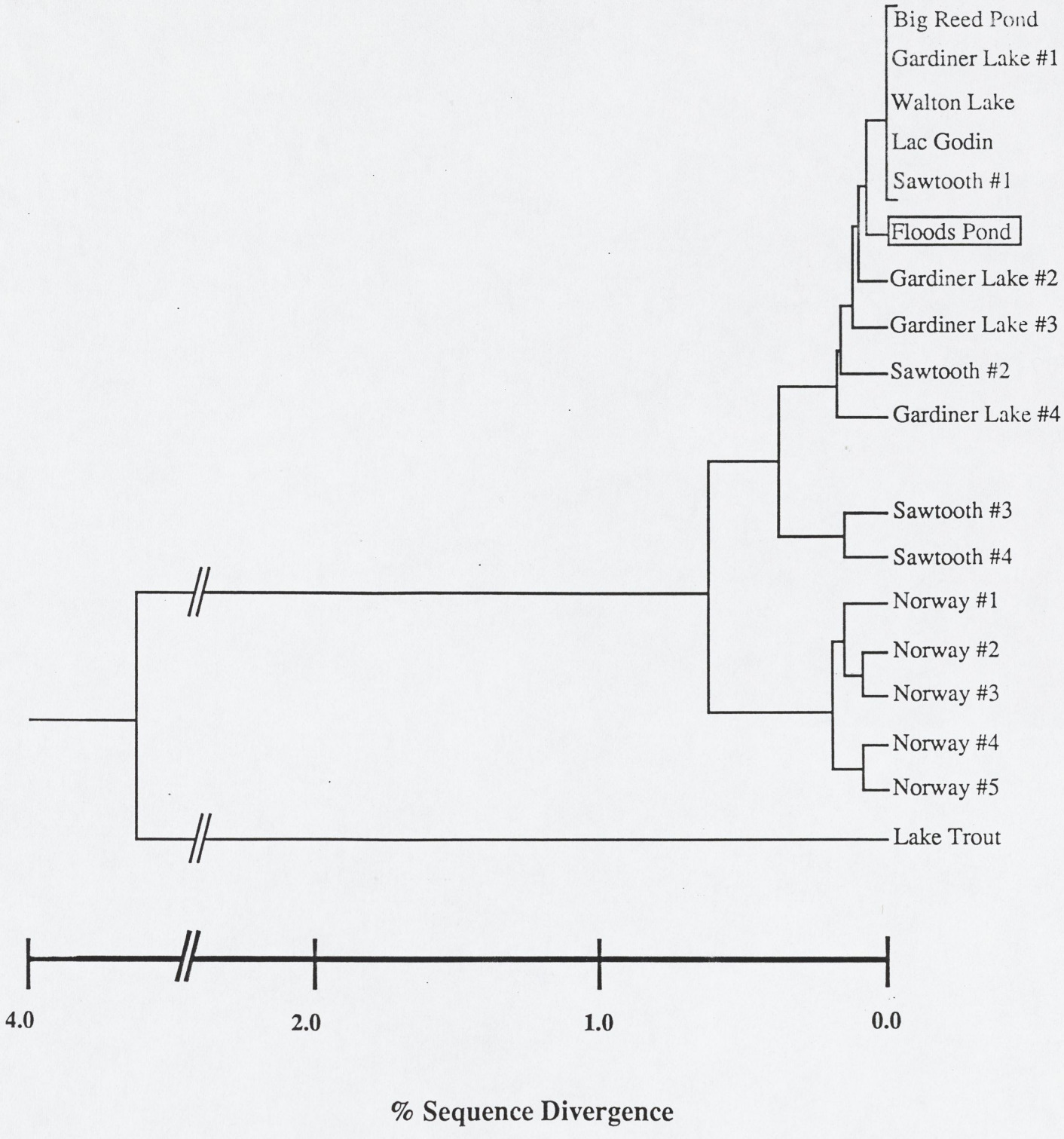

Figure 2. Relationships among char populations based on observed mitochondrial DNA banding patterns. Note that the char from Floods Pond are very similar to the other samples of the New England subspecies, *Salvelinus alpinus oquassa*; the sequence divergence of the Floods Pond population is small relative to the level observed between subspecies from New England and Norway. This clustering diagram is based on the estimates of genetic divergence obtained from mtDNA banding patterns (Table 5).

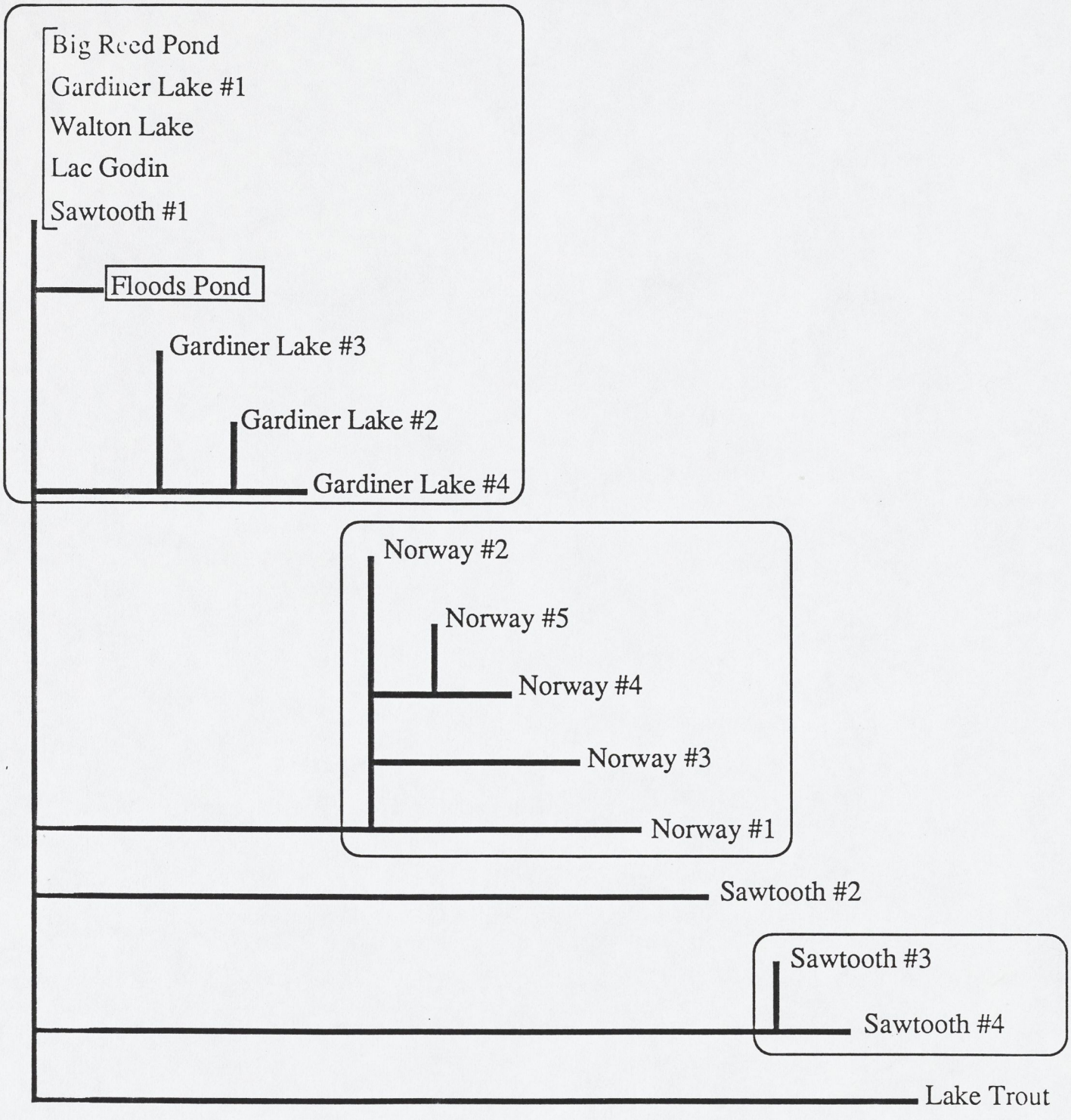

Figure 3. Significant associations among mitochondrial DNA banding patterns for char populations. Three statistically significant groups (enclosed in large boxes) were observed including one which comprises two mtDNA phenotypes among the introduced char of Sawtooth Lake, Idaho. The ordering of samples with boxes is not significant. Fish from Floods Pond, Big Reed Pond, Gardiner Lake, Lac Gogin and Walton Lake do not form separate groups isolated from other samples of New England char.

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