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Mitochondrial DNA Variation in Steelhead Trout (*Oncorhynchus mykiss*): Comparison of Collections from the Kodiak National Wildlife Refuge, Alaska

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Abstract: The purpose of this study was to genetically compare steelhead trout (*Oncorhynchus mykiss*) among three streams in the Kodiak National Wildlife Refuge (NWR) to determine whether separate spawning populations exist. Mitochondrial DNA variation was analyzed in steelhead (anadromous) collected during the spring spawning season from three refuge streams that had river mouths along 30 km of island coastline. Large genetic differences existed among the three Kodiak NWR collections ($P < 0.001$). The genetic variation among the Kodiak steelhead was also compared to two rainbow trout (resident) collections made from the Kisaralik River (Yukon Delta NWR). Large differences were detected among the five collections based on an overall G test ($P < 0.001$). In pair-wise comparisons, each of the Kodiak steelhead collections were different from the Yukon Delta NWR collections ($P < 0.01$). This study provided preliminary evidence of a population structure for steelhead trout on Kodiak Island organized among major river drainages. To conserve the natural diversity of steelhead trout, populations should be the unit of focus for refuge management. Future studies should further characterize the population structure within and among each major river system in the refuge.

Introduction

The Kodiak National Wildlife Refuge (NWR) is located on a large island area managed by the U.S. Fish and Wildlife Service, and is well-known for its brown bears (*Ursus arctos*) and prodigious runs of Pacific salmon (*Oncorhynchus* sp.). The Kodiak NWR is located about 435 km southwest of Anchorage, Alaska and encompasses 755,000 ha, larger than the state of Delaware (U.S. Fish and Wildlife Service 1987). The refuge occupies approximately two-thirds of Kodiak Island plus a portion of northwestern Afognak Island, with some of the lands within the refuge boundary being privately owned. The island is deeply indented by glacially formed fjords, such that no place is more than 25 km from the ocean. Most rivers are short, with low flow, and steep gradient. However, a few watersheds in the southwestern portion are larger, have more wetlands (Ayakulik River), and in some cases drain large post-glacial lakes (Karluk River). Portions of these watersheds appear to have been unglaciated during the most recent glaciation and may have served as a refugium for some aquatic species (Karlstrom and Ball 1969; Mann and Peteet 1994). Highest discharges in the rivers coincide with snowmelt and with late summer and fall rains. Large lakes, like Red Lake (Ayakulik drainage), serve as important habitats for fish and are formed by glacial moraines that act as dams at their outlets.

The Kodiak NWR contains self-sustaining fish populations of five species of Pacific salmon, rainbow trout (*Oncorhynchus mykiss*) including the anadromous steelhead form, Arctic char (*Salvelinus alpinus*), and Dolly Varden char (*S. malma*; U.S. Fish and Wildlife Service 1990). The annual cycle of the transport of marine-origin nutrients and energy via Pacific salmon is a dominant characteristic of these systems. The nutrients and energy from salmon are

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critically important inputs that sustain the productivity of the freshwater aquatic ecosystem (e.g., Levy 1997). These inputs also promote the growth of riparian vegetation and serve as food for terrestrial animals such as bears.

Steelhead trout populations occur primarily on the west and south portions of Kodiak Island and on Afognak Island. Most steelhead populations of Kodiak Island are located in the southwestern corner between Alitak and Uyak bays; the Ayakulik and Karluk rivers have the largest runs (U.S. Fish and Wildlife Service 1990). In the Karluk River, 7,252 steelhead were estimated to be present in the spring of 1996 and 10,377 fish in 1997 based on mark-recapture estimates (Begich 1997, 1998). Adult steelhead return to the river systems of Kodiak Island starting in late August, overwinter, spawn from late April through May, and then return to the ocean from late May through July (Chatto 1987). Steelhead fry emerge from spawning gravel in late summer and may spend from one to four years in freshwater as juveniles before smolting and moving to the ocean. Once in the ocean, these fish feed and grow for one to four years. Upon maturity they return to rivers to spawn and complete the life cycle (U.S. Fish and Wildlife Service 1990). Individual fish may spawn up to three or more times, though typically runs are composed predominately of first time spawners. Fork length of the Karluk River adults varies from 500 mm for females that have spent two years in the ocean to multi-spawning males with a marine age of six years and a length of 830 mm (Begich 1997).

Steelhead populations contribute to important sport and subsistence fisheries, and are incidentally caught in commercial fisheries. Sport fisheries for steelhead typically occur from September through November and can have a high rate of catch and release fishing at some locations. For example, anglers contacted from September 29 through November 5, 1995 at the Portage area of the Karluk River released 2,466 steelhead and only harvested 32 of the fish (Begich 1997). Steelhead are also traditionally harvested in subsistence fisheries by residents of the villages of Karluk and Larsen Bay. In addition, commercial gillnet and purse seine fisheries catch some steelhead incidental to salmon fisheries. An estimate from the Karluk marine study area (from Sturgeon River to Little River) indicated that in 1995 the total commercial catch was 203 steelhead between August 15 and September 30 (Begich 1997). Harvest regulations for the fisheries are established by the State of Alaska. Commercial sport fishing guides within the refuge are regulated through a permit system by the U.S. Fish and Wildlife and provide guided float trip fishing, daily fly-in fishing, and drop-off, non-guided float-trip fishing (U.S. Fish and Wildlife Service 1993).

Kodiak National Wildlife Refuge (Kodiak NWR) was established by Executive Order 8857 on August 19, 1941 due to its significance as a "natural feeding and breeding ground for brown bears and other wildlife". In December 1980, Congress enacted the Alaska National Interest Lands Conservation Act (ANILCA) which redesignated the Kodiak refuge, added some new lands to the refuge, and defined refuge purposes. Conservation of the natural diversity of fish and wildlife is a primary goal for the Kodiak NWR and is illustrated by the first purpose listed for the refuge (from ANILCA):

> *...to conserve fish and wildlife populations and habitats in their natural diversity including, but not limited to, Kodiak brown bears, salmonids, sea otters, sea lions and other marine mammals and migratory birds* (Section 303 (5) (B) (i))

Supporting this purpose, the goal of public use management at the Kodiak National Wildlife Refuge is "to provide high quality fish and wildlife oriented recreation, interpretive and educational opportunities consistent with the refuge's resource oriented purposes" (U.S. Fish and Wildlife Service 1993).

Populations are an important organizational unit of natural diversity because they can accumulate and maintain genetically-coded adaptations that are specific for survival in their particular environments due to their semi-reproductive isolation from other populations. The term population here means a local inter-breeding group of animals of the same species. The sub-division of a species into populations requires random mating within a population and reproductive isolating mechanisms to separate populations to reduce interbreeding. Isolating mechanisms include homing to natal areas and physical barriers to fish passage (e.g., waterfalls). Homing capabilities in rainbow trout have been well documented (e.g., Lindsey et al. 1959) and may include the use of

olfactory cues (e.g., Cooper and Scholz 1976; Scholz et al. 1978). Homing can allow genetic differences to develop among steelhead populations, causing different populations to contain different portions of the natural genetic diversity of the species.

Genetic differentiation among steelhead populations has been shown for wild populations from Canada but has been variably exhibited among U.S. populations where stocking has been a common practice. Allozyme data from British Columbia steelhead showed genetic differences between populations in adjacent streams and that populations were regionally organized into three major groups (Parkinson 1984). In contrast, no statistical differences were reported for steelhead from northern California and Oregon (Reisenbichler et al. 1992) nor among streams from the north coast of Washington (Reisenbichler and Phelps 1989). Stocking and subsequent interbreeding between hatchery and wild fish may have caused the apparent genetic homogeneity among steelhead. However, more recently, differentiation of steelhead populations in Washington was reported by Phelps et al. (1994) using similar data. In this case, some collections were genetically different even though they came from streams that had a history of stocking. Thus, the effects of hatchery fish and the level of interbreeding probably have been variable among populations within this region. The geographic pattern of genetic differences reported by Phelps et al. (1994) was comparable to the pattern of genetic variation reported by Parkinson (1984) from more-pristine steelhead populations further north. The lack of genetic data prior to stocking can hinder the ability to detect effects on genetic diversity.

The purpose of this study was to genetically compare steelhead trout among streams in the Kodiak NWR. The primary question to be answered was, "Do steelhead trout randomly spawn with each other in refuge waters or are they subdivided into separate spawning populations?" Mitochondrial DNA variation was analyzed in rainbow trout collected from three refuge streams. The refuge waters and their steelhead populations are viewed as being pristine with relatively few serious adverse human activities. The relative level of genetic differentiation observed among the three Kodiak collections was then compared to two out-group collections from the Kisaralik River (Yukon Delta NWR). This study was viewed as a preliminary step to determine whether a more detailed investigation of steelhead population structure would be warranted on Kodiak NWR.

Methods

Collections

Fin samples (1 cm^2) from adult steelhead were collected from 29 to 78 fish caught from each of three rivers in Kodiak NWR (Table 1; Figure 1). Angling and weirs were used to capture fish. Fins were stored in 70 –100% ethanol until analyzed. Steelhead were separated from resident rainbow trout based on the large size of steelhead, and the presence of steelhead during the spring and fall in the main stem of each of the rivers when the smaller resident rainbow are uncommon. The three collections were made from rivers hydrogeographically unrelated that empty into Shelikof Strait of the Pacific Ocean. The mouth of the Ayakulik River is about 20 km from the mouth of the Sturgeon River which is approximately 7 km from the mouth of the Karluk River.

Outgroup collections were included in the analyses and comprised resident rainbow trout caught by angling in August 1997 at two locations separated by 35 km in the Kisaralik River (Yukon Delta NWR; Table 1). One collection was designated as "upper" (from Golden Gate Falls to Quartz Creek) and the other as "lower" (from a 9 km stretch beginning 35 km downstream of Quartz Creek). The Kisaralik River is located 530 km northwest of Kodiak Island in mainland Alaska.

mtDNA analysis

Nucleic acids were extracted from about 25 mg of fin tissue. DNA from fin tissue was isolated by using Puregene™[2]. Tissues were placed in 500 µl of cell lysis buffer and 30 µl proteinase K (10mg/ml), then incubated overnight at 65°C. Three µl of RNAase A solution (4 mg/ml) was added to cell lysate and incubated for an additional 30 min at 37°C, cooled to room temperature, and 200 µl protein precipitation buffer added and vortexed vigorously. The solution was then placed on ice for 60 min and centrifuged at 13,800 RCF for 3 min. Supernatant was poured into 1.5 ml tubes and 600 µl of isopropanol (2-propanol)

[2] Mention of a commercial product does not represent an endorsement.

Table 1. Location, dates, and sample sizes (N) of collections of steelhead trout from the Kodiak National Wildlife Refuge and rainbow trout from Yukon Delta National Wildlife Refuge (NWR), Alaska.

Collection	Approximate Location (°N, °W)	Date	N
Kodiak NWR			
Ayakulik River	57° 11′ 26″N, 154° 31′ 45″W	June 4 – July 9, 1997	69
Sturgeon River	57° 26′ 34″N, 154° 30′ 49″W	October 30, 1996	9
	57° 30′ 02″N, 154° 30′ 49″W	May 25 – July 4, 1998	20
Karluk River	57° 31′ 42″N, 154° 09′ 49″W	April 18 – 22, 1997	78
Yukon Delta NWR			
Kisaralik-Upper	60° 30′N, 160° 15′W	August 5 – 7, 1997	44
Kisaralik-Lower	60° 45′N, 160° 30′W	August 14 – 15, 1997	48

added. Tubes were inverted several times to mix alcohol and the supernatant then centrifuged at 13,800 RCF for 1 min. Supernatant was poured off and the pellet washed with 600 µl 70% ethanol, and then centrifuged for 1 min at 13,800 RCF. The supernatant was decanted and the pellet air dried at ambient temperature. The DNA pellet was then hydrated in 100 µl Tris-EDTA buffer (pH 7.4) as described above.

To assess DNA quantity and quality, DNA samples (5 µl) were electrophoresed in 1.2% agarose gels cast in TBE buffer (Sambrook et al. 1989), stained with ethidium bromide, and photographed with Polaroid™667[2] film on an ultraviolet light table.

One mitochondrial DNA (mtDNA) segment was amplified using the polymerase chain reaction (PCR) with the following primer:

cytochrome–B (*cyt*B; Bickham et al. 1995) ;
LGL765 5'-GAAAAACCAYCGTTGTWATTCAACT-3'
LGL766 5'-GTTTAATTAGAATYTYAGCTTTGGG-3'

Each PCR reaction comprised 3 µl (~150 ng) total genomic DNA, 2.5 µl of 10X buffer (Sigma or Perkin Elmer 500 mM KCl, 100 mM Tris [pH 9.5 at 25°C]), 1.5 µl $MgCl_2$ (25 mM), 2.5 µl of dNTP mix (2 mM each of dATP, dTTP, dCTP, dGTP in 10 mM Tris-HCL [pH 8.0]), 0.5 µl of a 10 mM solution of each of two primers, 1.5 units of *Taq* polymerase, and deionized H_2O added for a final volume of 25 µl. The amplification cycle for *cyt*B consisted of 95°C for 3 min for 1 cycle; 95°C for 45 sec, 50°C for 50 sec, 70°C for 2 min 30 sec for 32 cycles; 70°C for 5 min for 1 cycle.

Three restriction enzymes (*Dde*I, *Dpn*II, and *Msp*I) were used to identify different mtDNA genotypes (sometimes called haplotypes). Each restriction enzyme recognizes a unique sequence of four or five bases and cuts the DNA at that site. Restriction digests consisted of five units of a restriction enzyme (*Dde*I, 5 base; *Dpn*II, 4 base; or *Msp*I, 4 base), 5 µl of amplified product, 1.5 µl of each enzyme's 10X buffer, and deionized H_20 added to a final volume of 15 µl. Digests were electrophoresed on 2.5% agarose gels, stained with ethidium bromide, and photographed under UV light. Sizes of restriction fragments were estimated by comparison to a 100 base pair (bp) ladder. Restriction fragment patterns were visually identified from gels and photographs. Each fish was assigned a composite genotype based on the genotypes observed from the three restriction enzymes.

Data analysis

Counts of composite genotypes (= haplotypes) were used to genetically characterize each collection and to make comparisons among collections. Differences among collections were determined by two approaches. The first approach compared the presence and absence of composite genotypes among collections, and identified the most common and most rare genotypes. The second approach used statistical tests to compare mtDNA genotype frequencies among collections, calculate summary measures of genotype variation (h and F_{st}), and calculate measures of genetic distance between collections.

Statistical comparisons of genotypic data used the log-likelihood ratio G-test (Sokal and Rohlf 1981)

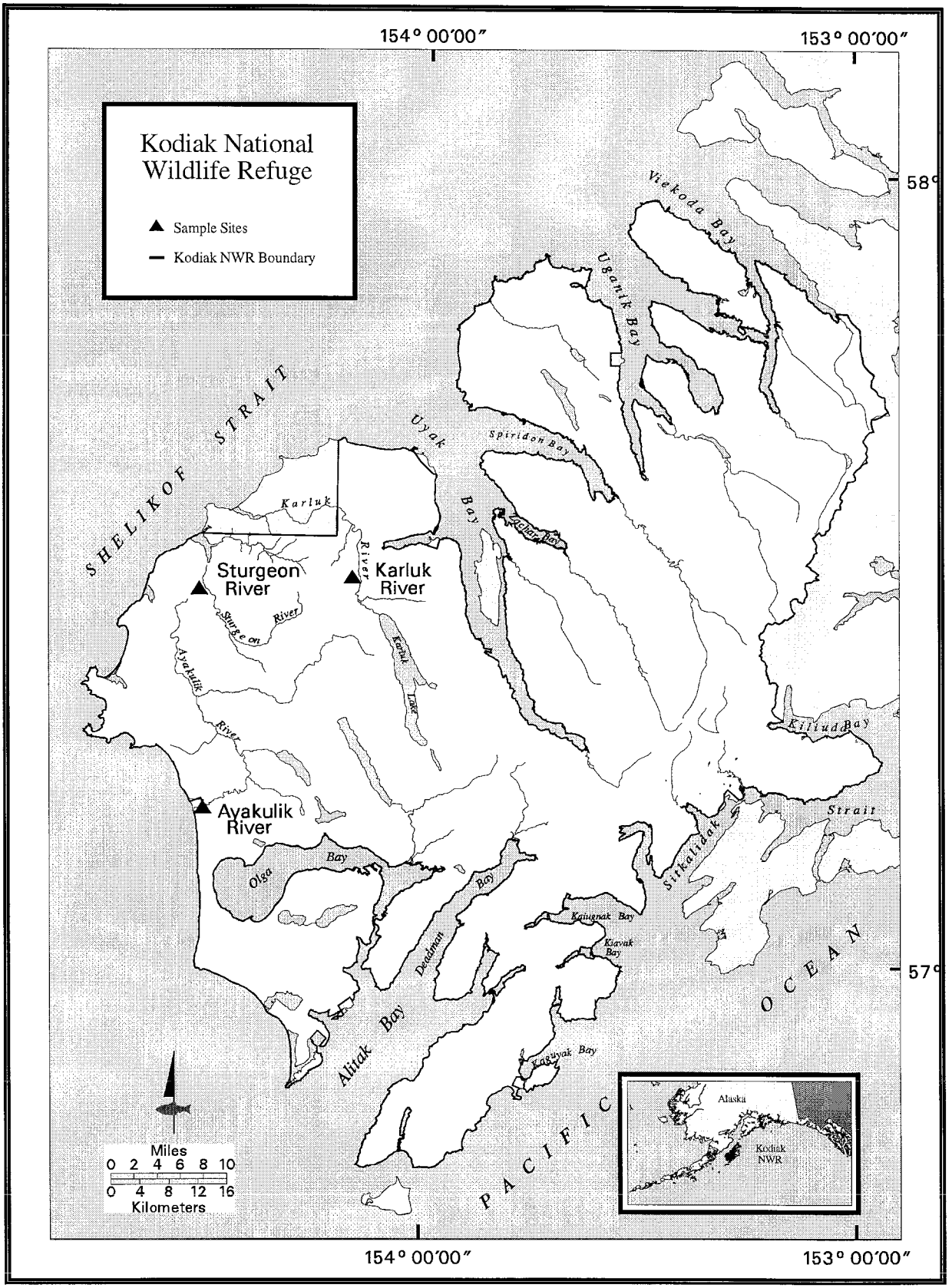

Figure 1. Map of the Kodiak National Wildlife Refuge showing locations of collections of steelhead trout from the Ayakulik, Sturgeon, and Karluk rivers.

compared to a χ^2 distribtution. The null hypothesis of no evidence of differences (homogeneity) among genotypic frequencies was rejected at $P < 0.05$. The critical values used in the pair-wise *G*-test comparisons were modified to account for the increase in Type-I errors when multiple tests of the same hyphothesis were made (Cooper 1968). Rare genotypes (those with frequencies < 0.05) were pooled into the BBA genotype for *G*-tests. Measurement of mtDNA variability within collections used nucleon diversity *h* (Nei and Tajima 1981). This measure is mathematically equivalent to expected heterozygosity at nuclear loci, and uses the frequency of composite mtDNA genotypes in its calculation. Nucleon diversity varies between zero (low) and one (high), and provides a relative measure of the amount of mtDNA variation observed within each collection.

Genotype variability among collections was partitioned with F_{st} (Wright 1965; Nei 1977). F_{st} measures the amount of total variation observed that is attributable to differences among collections. F_{st} varies between zero (no variation among collections) and one (all variation exists among collections; all fish in each collection have the same unique genotype, a genotype not observed in any other collection).

Genetic distances were calculated between each pair of collections (Cavalli-Sforza and Edwards 1967). These distances were then subjected to unweighted pair-group-method cluster analysis based on arithmetic averages (Sneath and Sokal 1973) to generate a dendrogram to assess differences among populations. Distance measures of zero indicate no differences and larger distances indicate larger genetic differences. The longer the horizontal lines in a dendrogram, the greater genetic distance between collections.

Analyses of the data (data input modified because of their haploid character) were performed with "Genes in Populations" version 2.1 designed by B. May and C. Krueger and written in C by W. Eng and E. Paul. Calculation of genetic distances and UPGMA cluster analysis were performed using PHYLIP 3.57c (Felsenstein 1995).

Results

Fragment pattern variation

Genetic variation among individuals was revealed by the fragment patterns of *cyt*B for *Dde*I (three genotypes A, B, and C), *Dpn*II (two genotypes A and B), and *Msp*I (two genotypes A and B; see Appendix 1). These genotypes occurred in eight combinations (AAA, AAB, ABB, ABA, BBA, BBB, CBB, and CBA) to provide the composite genotypes used in subsequent analyses (Table 2). The uncut *cyt*B fragment was 1300 base pairs (bp) long and corresponded approximately to the sum of fragments yielded by *Dpn*II and *Msp*I (Appendix 1). However, the sum of the fragments for each *Dde*I genotype was consistently less than 1300 bp by approximately 300 bp. This difference was possibly due to small fragments (< 50 bp) not observed on the agarose gels.

Genotypic frequencies

Each steelhead collection from Kodiak NWR had a different genotype that was most abundant but none of the genotypes exceeded 0.5 in frequency in any collection (Table 2). The only similarity among the three collections was that the ABA genotype was not rare (> 0.17 frequency). Among fish from the Ayakulik River, the ABA genotype had the highest frequency (0.39) with most of the balance of the variation comprised of genotypes ABB and AAA (approximately 0.51). These genotypes also occurred in fish from the Karluk and Sturgeon rivers. In Karluk River steelhead, the most frequent genotype was ABB (0.45) and the next most common genotype was (ABA). Sturgeon River steelhead had the AAA genotype as most frequent (0.41). The CBA genotype was prevalent among Sturgeon River fish (0.31) but was rare in Ayakulik River (0.06) and absent from the Karluk River collection. The rare AAB genotype was observed only in fish from the Ayakulik River. The rare BBB and CBB genotypes were observed only in fish from the Karluk River. Nucleon diversity (*h*) was similar among the three Kodiak NWR rivers (approximately 0.7; Table 2). The two Yukon Delta collections had high frequencies of the AAA genotype (1.00 and 0.96) and the ABA genotype was absent or rare (0.00 and 0.04; Table 2). In contrast, the ABA genotype was comparatively common among Kodiak NWR steelhead. Nucleon diversity was much lower in Kisaralik fish (0.0 to 0.08) when compared to Kodiak steelhead (range 0.67 to 0.71).

Differences among collections

Large genetic differences were detected among

Table 2. Mitochondrial DNA genotype frequencies, nucleon diversity (h), and sample sizes (N) of collections of steelhead and rainbow trout from rivers in the Kodiak and Yukon Delta National Wildlife Refuges (NWR), Alaska. Upper (-U) and lower (-L) reaches of the Kisaralik River were sampled.

	Composite mtDNA Genotype									
Location	AAA	AAB	ABB	ABA	BBA	BBB	CBB	CBA	h	N
Kodiak NWR										
Ayakulik	0.275	0.014	0.232	0.391	0.029	--	--	0.058	0.713	69
Sturgeon	0.414	--	0.034	0.172	0.069	--	--	0.310	0.697	29
Karluk	0.064	--	0.449	0.321	0.141	0.013	0.013	--	0.672	78
Yukon Delta NWR										
Kisaralik-U	1.000	--	--	--	--	--	--	--	0.000	44
Kisaralik-L	0.958	--	--	0.042	--	--	--	--	0.080	48

the three collections from the Kodiak NWR ($P < 0.001$; Table 3). Differences in mtDNA genotypic frequencies occurred between all paired comparisons of Ayakulik, Sturgeon, and Karluk collections (Table 4). Large genotypic frequency differences were also detected among the five collections from Kodiak and Yukon Delta NWRs based on the total G test ($P < 0.001$; Table 3). Most (72%) of the balance of the total G value (176 of 246) was due to the difference between the Kodiak and Yukon Delta NWR fish (Table 3). All Kodiak NWR collections were different from the Yukon Delta NWR collections in pair-wise comparisons (Table 4; $P < 0.01$). A within-drainage difference was not observed between the upper and lower Kisaralik River collections from the Yukon Delta NWR ($P > 0.30$).

Diversity analysis of Kodiak NWR fish (F_{st}; used to partition the total variation) indicated that 10% of the variation observed was due to differences among collections (Table 3). The balance (90%) was due to differences among individuals within collections.

Cluster analysis of genetic distances clearly organized the collections into the two refuge groups (Table 5; Figure 2). The first group contained the three collections of steelhead from the Kodiak NWR. Within this group, Sturgeon River fish were the most divergent of the three collections due to a high frequency of the CBA genotype (Table 2). Average distance between the Kodiak NWR collections was 0.12 (range 0.072 to 0.22). The second group included the upper and lower Kisaralik River samples from the Yukon Delta NWR that were separated by a distance of 0.012.

Table 3. Mitochondrial DNA genotype differentiation (F_{st}) and heterogeneity tests (G; Sokal and Rohlf 1981) among collections of steelhead trout from the Kodiak National Wildlife Refuge and rainbow trout from Yukon Delta National Wildlife Refuge, Alaska. The total G test for all collections is subdivided into G values contributed by genotype variation within and among refuges. Probability values are given for the H_o that no differences in genotype frequencies were detected among collections.

Comparison	F_{st}	G	df	P
Kodiak National Wildlife Refuge				
Total Kodiak (3 collections)	0.10	67.1	8	<0.001
Yukon Delta National Wildlife Refuge				
Kisaralik River Upper vs. Lower	0.021	2.64	4	>0.5
Between Refuge Analysis				
Kodiak pooled vs.Yukon Delta pooled	0.33	176	4	<0.001
Total				
All collections	0.33	246	16	<0.001

Table 4. Pair-wise G tests and probability values (P) for heterogeneity of mitochondrial DNA genotype frequencies within steelhead and rainbow trout collections from the Kodiak and Yukon Delta National Wildlife Refuges, Alaska. Upper (-U) and lower (-L) reaches of the Kisaralik River were sampled. ** indicates $P < 0.01$ for the H_0 that the two collections are not different from each other. Probabilities were adjusted to account for multiple comparisons.

	Kodiak NWR		Yukon Dela NWR	
	Sturgeon	Karluk	Kisaralik-U	Kisaralik-L
Kodiak NWR				
Ayakulik	19.5**	27.8**	73.9**	65.3**
Sturgeon	—	56.5**	39.9**	34.5**
Karluk	—	—	127**	120**
Yukon Delta NWR				
Kisaralik-U	—	—	—	2.64

Discussion

Population structure

All three steelhead trout collections from the Kodiak NWR were genetically different from one another, indicating the presence of multiple populations in the refuge. These collections of steelhead came from rivers that all empty into the ocean along approximately 30 km of coastline in northwestern Kodiak Island. Ten river systems on Kodiak Island and six rivers on Afognak Island are known to contain steelhead (Begich 1997). Based on the results of this study, these rivers probably each contain separate steelhead populations and together represent the critical spawning and juvenile rearing habitats essential for the maintenance of the natural diversity of this species within the archipelago. Of these systems, eight of these rivers exist within the Kodiak NWR. This diversity is likely organized not only among rivers but also within rivers.

The steelhead populations of Kodiak Island probably differ from populations in other regions. Steelhead from Alaska (Karluk River) were shown to have large allelic frequency differences at minisatellite loci from steelhead in British Columbia rivers (Taylor 1995). Large differences among regions have been noted in allozyme, minisatellite, and microsatellite allele frequencies, and mtDNA genotype frequencies in studies that examined steelhead populations over broad geographic areas (Parkinson 1984; Nielsen et al. 1994; Taylor 1995).

More than one population may exist within some of the drainage systems on Kodiak Island. No collections were made or analyses performed to compare steelhead from different spawning areas within a river system. Two spawning areas, separated by 20 km of stream, are known to exist within the Ayakulik River. The upstream area includes the lower 10 km of the East Fork, located approximately 30 km upstream from the ocean. The downstream area is in the lower main-stem river, within 10 km of the ocean.

Table 5. Genetic distances (chord; Cavalii-Sforza and Edwards 1967) between collections of steelhead trout from the Kodiak National Wildlife Refuge and rainbow trout from the Yukon Delta National Wildlife Refuge, Alaska based on mtDNA frequencies. Upper (-U) and lower (-L) reaches of the Kisaralik River were sampled.

	Kodiak NWR		Yukon Dela NWR	
	Sturgeon	Karluk	Kisaralik-U	Kisaralik-L
Kodiak NWR				
Ayakulik	0.077	0.072	0.272	0.205
Sturgeon	—	0.217	0.204	0.163
Karluk	—	—	0.427	0.364
Yukon Delta NWR				
Kisaralik-U	—	—	—	0.012

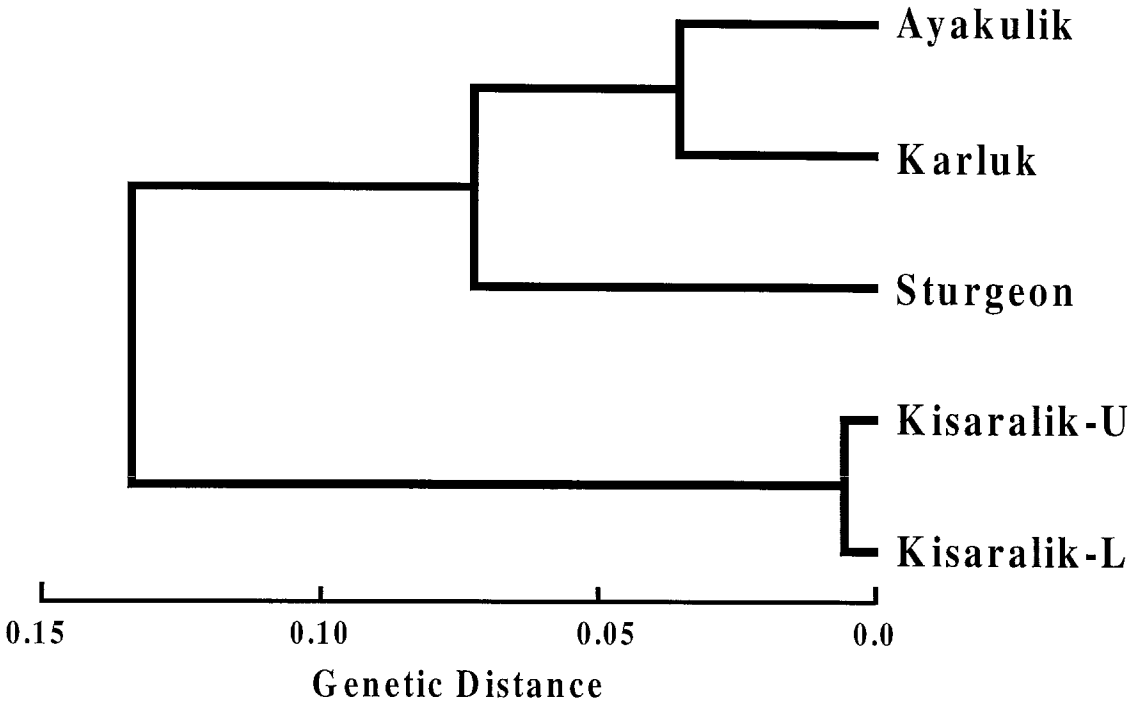

Figure 2. Dendrogram generated by cluster analysis of genetic distance (chord distance; Cavalii-Sforza and Edwards 1967) between steelhead trout collections from Kodiak National Wildlife Refuge. Kisaralik River collections were of freshwater resident rainbow trout from Yukon Delta National Wildlife Refuge and were used as outgroups.

If more than one population uses some of these systems, population diversity would be greater than represented simply by the sixteen river systems in the archipelago as described above. Genetic and ecological data have been used to detect multiple populations within a drainage for steelhead from coastal areas of British Columbia (Parkinson 1984), resident rainbow trout in lakes (Lindsey et al. 1959), and for rainbow trout above and below waterfalls (Northcote et al. 1970; Currens et al. 1990).

More than one population could occupy the Kisaralik River system (Yukon Flats NWR) even though no evidence was provided by the mtDNA genotypic frequencies (Tables 3 and 4). The lack of mtDNA genotype variation and the timing of these collections reduce the ability to detect the presence of multiple populations in this river. Both collections showed little genotypic diversity (Table 2). Also, both collections were made in August after the spawning season and may represent mixtures of several populations that had dispersed after spawning to summer feeding areas. Future studies of this system would require collections from spawning areas during the spawning season that would be analyzed for genetic markers different than those used in this study (see Krueger et al. 1999 for further discussion).

Management implications

Genetic data indicated that steelhead trout in the Kodiak NWR were not one large random-mating population but that several populations exist, geographically defined by river system. Such differences in mtDNA genotypic frequencies cannot exist if steelhead that were born in the Karluk River regularly returned and spawned in the either the Sturgeon or Ayakulik rivers or vice versa. Homing to natal areas is the most likely stock-isolating mechanism that genetically separates populations by river (by preventing interbreeding and hence gene flow) and maintains the differences in mtDNA genotypic frequencies.

To conserve the natural diversity of steelhead within the Kodiak NWR, populations should be the unit of focus for management. Two reasons support this approach. First, populations often contain genetically-encoded adaptations that are required for survival and reproduction. These adaptations are products of natural selection and accumulate within

populations over time through reproductive isolation from other populations. Loss of a population means the loss of potentially specialized adaptations required for the maintenance of the natural diversity of this species. Restoration of genetic diversity through natural selection would require a long period of time. Second, each population may have different population dynamics (e.g., mortality, natality, and growth rates). Thus, monitoring and assessment studies of steelhead should not combine data across populations. Populations, geographically organized within watersheds, function as separate ecological units from one another during the spring spawning season and during early life stages prior to smoltification. These populations may respond differently to environmental events such as floods or sustain different levels of natural and fishing mortality. Geographically localized mortality or habitat loss could severely affect one population but not others. Detection of a population in trouble requires that refuge studies analyze and interpret data by population. Thus, knowledge of the population structure of steelhead trout within each major watershed is essential for the design of future studies. Information about population structure should form the basis for the development of management plans, the regulation of harvests, and the protection of habitat. This information should be a high priority for future investigations.

Description of the population structure of steelhead trout from the 16 Kodiak river systems should be the next focus of investigation for this species. Future design for a genetic study should incorporate the five considerations that follow:

1) use of several genetic characters including mtDNA,
2) ensure that collections (N=60 fish) are from spawning groups,
3) the study should investigate within and among drainage genetic variation,
4) the project design should allow the comparison of collections made in different years from the same location, and
5) resident (non-anadromous) rainbow trout from Kodiak Island should be included as a part of the study.

Additional genetic characters, such as from allozyme and microsatellite loci, will increase the sensitivity of a study to detect populations and reduce the chance for an erroneous conclusion that no differences exist between collections when they actually do. Collections must come from groups of fish actively engaged in spawning so that collections will represent single populations and not mixtures of populations (see Allendorf and Phelps 1981 for further discussion). Collections should include fish from the 16 known steelhead watersheds and also include spawning fish caught at different locations within a drainage such as from the separate spawning areas (e.g., within the Ayakulik River). These collections are essential if variation within drainages is to be assessed. For example, waterfalls or rapids that restrict fish movement, tributaries that contain geographically localized spawning areas, and patchiness in spawning habitat can cause reproductive isolation, resulting in multiple populations within a watershed. These features can help identify locations from which to make collections. Repeating collections at the sites used in the present study as well as repeating future collections over time will assess temporal stability of genetic markers and help confirm that the population structure described is a stable characteristic of the species. Last, collections of resident rainbow trout should be included in the study to provide a complete description of the natural diversity of this species, regardless of the life history.

Movement patterns during the spawning season, which can be detected with radio telemetry, can help verify and interpret patterns of genetic variation. The integration of genetic and movement data can be powerful because they both relate directly to the patterns of gene flow that defines population structure. Information about spawning movements of steelhead will also help identify the locations of important reproductive habitats, habitat that should be monitored and protected. Information about the population structure will allow the managers of Kodiak NWR to effectively monitor populations and to assess threats to steelhead. This information will provide valuable input to decision making to conserve the natural diversity of populations, and thus better accomplish the purpose for which the refuge was established.

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References

Allendorf, F. W., and S. R. Phelps. 1981. Use of allelic frequencies to describe population structure. Canadian Journal of Fisheries and Aquatic Sciences 38:1507-1514.

Begich, R. N. 1997. Assessment of the 1995 return of steelhead to the Karluk River, Alaska. Fishery Data Series No. 97-6. Alaska Department of Fish and Game, Anchorage.

Begich, R. N. 1998. Population ecology of adult steelhead trout (*Oncorhynchus mykiss*) of the Karluk River, Alaska. M.S. thesis. University of Idaho, Moscow.

Bickham, J.W., C.C. Wood, and J.C. Patton. 1995. Biogeographic implications of cytochrome b sequences and allozymes in sockeye salmon (*Onchorhynchus nerka*). Journal of Heredity 86: 140-144.

Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 21:550-570.

Chatto, T. 1987. Instream movements and distribution of fall run steelhead in the Karluk River 1982 – 1984. U.S. Fish and Wildlife Service, Kodiak National Wildlife Refuge. Kodiak, Alaska.

Cooper, D. W. 1968. The significance level in multiple tests made simultaneously. Heredity 23:614-617.

Cooper, J. C., and A. T. Scholz. 1976. Homing of artificially imprinted steelhead (rainbow trout), *Salmo gairdneri*. Journal of the Fisheries Board of Canada 33:826-829.

Currens, K. P., C. B. Schreck, and H. W. Li. 1990. Allozyme and morphological divergence of rainbow trout (*Oncorhynchus mykiss*) above and below waterfalls in the Deschutes River, Oregon. Copeia 1990(3):730-746.

Felsenstein, J. 1995. PHYLIP (Phylogeny Inference Package) Version 3.57c. University of Washington, Seattle.

Karlstrom, T. N. V., and G. E. Ball (editors). 1969. The Kodiak Island refugium: its geology, flora, fauna and history. The Boreal Institute, University of Alberta. The Ryerson Press, Toronto.

Krueger, C. C., M. Lisac, S. J. Miller, and W. J. Spearman. 1999. Genetic differentiation of rainbow trout (*Oncorhynchus mykiss*) in the Togiak National Wildlife Refuge, Alaska. U.S. Fish and Wildlife Service, Alaska Fisheries Technical Report Number 55, Region 7, Anchorage.

Levy, S. 1997. Pacific salmon bring it all home. Bio Science 47:657-660.

Lindsey, C. C., T. G. Northcote, and G. F. Hartman. 1959. Homing of rainbow trout to inlet and outlet spawning streams at Loon Lake, British Columbia. Journal of the Fisheries Research Board of Canada 16:695-719.

Mann, D. H., and D. M. Peteet. 1994. Extent and timing of the last glacial maximum in

southwestern Alaska. Quaternary Research 42:136-148.

Nei, M. 1977. F-statistics and analysis of gene diversity in subdivided populations. Annals of Human Genetics 41:225-233.

Nei, M., and F. Tajima. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97:145-163.

Nielsen, J. L., C. A. Gan, J. M. Wright, D. B. Morris, and W. K. Thomas. 1994. Biogeographic distributions of mitochondrial and nuclear markers for southern steelhead. Molecular Marine Biology and Biotechnology 3:281-293.

Northcote, T. G., S. N. Williscroft, and H. Tsuyuki. 1970. Meristic and lactate dehydrogenase genotype differences in stream populations of rainbow trout below and above a waterfall. Journal of the Fisheries Research Board of Canada 27:1987-1995.

Parkinson, E. A. 1984. Genetic variation in populations of steelhead trout (*Salmo gairdneri*) in British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 41:1412-1420.

Phelps, S. R., B. M. Baker, P. L. Hulett, and S. A. Leider. 1994. Genetic analysis of Washington steelhead: initial electrophoretic analysis of wild and hatchery steelhead and rainbow trout. Report #94-9, Fisheries Management Program. Washington Department of Fish and Wildlife, Olympia.

Reisenbichler, R. R., J. D. McIntyre, M. F. Solazzi, and S. W. Landino. 1992. Genetic variation in steelhead of Oregon and northern California. Transactions of the American Fisheries Society 121:158-169.

Reisenbichler, R. R., and S. R. Phelps. 1989. Genetic variation in steelhead (*Salmo gairdneri*) from the north coast of Washington. Canadian Journal of Fisheries and Aquatic Sciences 46:66-73.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning, a laboratory manual. 2nd edition, Cold Spring Harbor, NY.

Scholz, A. T., C. K. Gosse, J. C. Cooper, R. M. Horrall, A. D. Hasler, R. I. Daly, and R. J. Poff. 1978. Homing of rainbow trout transplanted in Lake Michigan: a comparison of three procedures used for imprinting and stocking. Transactions of the American Fisheries Society 107:439-443.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry, 2nd edition. San Francisco, Freeman.

Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy. San Francisco: Freeman.

Taylor, E. B. 1995. Genetic variation at minisatellite DNA loci among north Pacific populations of steelhead and rainbow trout *(Oncorhynchus mykiss)*. Journal of Heredity 86:354-363.

U.S. Fish and Wildlife Service. 1987. Kodiak National Wildlife Refuge Final Comprehensive Conservation Plan. Region 7, Anchorage, Alaska.

U.S. Fish and Wildlife Service. 1990. Fishery management plan Kodiak National Wildlife Refuge. Kodiak National Wildlife Refuge, Kodiak, Alaska.

U.S. Fish and Wildlife Service. 1993. Kodiak National Wildlife Refuge final public use management plan and environmental assessment. Kodiak, Alaska.

Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19:395-420.

Appendix 1. Restriction fragment patterns for the mitochondrial DNA cytochrome–B fragment in steelhead trout from the Kodiak National Wildlife Refuge. The numbers in the table are fragment sizes in nucleotide basepairs.

Fragment Size	*cyt*B							
		*Dde*I			*Dpn*II		*Msp*I	
	Uncut	A	B	C	A	B	A	B
1300	+	—	—	—	—	—	—	—
1048	—	—	—	—	—	—	+	+
667	—	—	—	—	+	+	—	—
387	—	—	—	—	+	+	—	—
346	—	+	+	+	—	—	—	—
282	—	+	+	—	—	—	—	—
262	—	+	—	++	—	—	—	—
215	—	—	—	—	+	—	—	—
208	—	—	+	—	—	—	—	—
182	—	—	—	—	—	—	+	—
167	—	—	—	—	—	+	—	—
122	—	+	+	+	—	—	—	—
108	—	—	—	—	—	—	—	+
96	—	—	—	—	—	—	+	+
82	—	—	—	—	—	—	—	+
80	—	—	—	—	+	+	—	—
62	—	—	—	—	—	+	—	—
50	—	+	+	+	—	—	—	—
Total	1300	1062	1008	1042	1349	1363	1326	1334

Erratum

Alaska Fisheries Technical Report Number 54

Mitochondrial DNA Variation in Steelhead Trout (*Oncorhynchus mykiss*): Comparison of Collections from the Kodiak National Wildlife Refuge, Alaska

p. 9, para. 2, line 2:
"Yukon Flats NWR" should be "Yukon Delta NWR"

Please post this to the inside-center of the back cover of your report.