AN ABSTRACT OF THE DISSERTATION OF

Kitty E. Griswold for the degree of Doctor of Philosophy in Fisheries Science presented on June 13, 2002.
Title: Genetic Diversity in Coastal Cutthroat Trout and Dolly Varden in Prince William Sound, Alaska.

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Gordon H. Reeves

Genetic diversity of two salmonid species, Dolly Varden (*Salvelinus malma*) and coastal cutthroat trout (*Oncorhynchus clarki clarki*) in Prince William Sound, Alaska were examined at multiple spatial scales with three molecular markers. Pleistocene glaciers covered what is now Prince William Sound 8,000-12,000 years ago and both species colonized the region subsequently. Because these species have different migratory behavior and historic ranges I expected to see different patterns of genetic diversity within Prince William Sound. Haplotype frequency and nucleotide diversity in sixteen mitochondrial DNA (mtDNA) restriction fragment enzymes combinations in Prince William Sound were higher in Dolly Varden than in coastal cutthroat trout, which were close to fixation. Low estimates of these measures in coastal cutthroat trout may reflect a founder effect resulting from colonization of this region from a single glacial refuge (Cascadia) and low dispersal capacity. To examine if genetic diversity in coastal cutthroat trout was higher in areas of glacial refuge, three additional locations from

throughout their distributional range were examined with the same restriction enzymes. Haplotype frequency and nucleotide diversity were lower in areas that were glaciated than estimates of the same measures in a glacial refuge. Again, founder effects during colonization of the three glaciated sites may account for these results. Genetic variation of Dolly Varden and coastal cutthroat trout was also examined within and among populations in Prince William Sound with microsatellites and allozymes. The pattern of genetic variation in coastal cutthroat trout was complex. There was lower genetic diversity within populations that were recently colonized following recent de-glaciation (150-350 ybp). Genetic diversity among some coastal cutthroat trout populations was high, which possibly reflects restricted migration. In other trout populations there was low diversity among populations, possible reflecting historic gene flow. In Dolly Varden, genetic variation among anadromous populations in Prince William Sound was low. There were large differences among resident and anadromous populations. These data provide information for the management for both species, which may reduce the risk of the loss of genetic diversity within local populations.

Genetic Diversity in Coastal Cutthroat Trout and Dolly Varden in Prince William Sound, Alaska.

By
Kitty E. Griswold

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Dean of the Graduate School

I understand that my dissertation will become a part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Kitty E. Griswold

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Genetic Diversity in Coastal Cutthroat Trout and Dolly Varden in Prince William Sound, Alaska

CHAPTER 1

INTRODUCTION

"We are trying to conserve organisms, not molecules" F.W. Allendorf 1995

One of the most challenging problems facing conservation biologists today is determining the criteria for managing species within the context of their evolutionary history. Biological variation is a part of the evolutionary history of a species and is essential to their continued persistence in changing environments. With the advent of readily available and relatively inexpensive molecular genetic tools, it has become easier to describe neutral genetic variation within and among species, populations, and individuals. This information is useful for identifying management units. Genetic variation, however, rarely reflects a one-to-one correspondence to the observed life history variation of fishes (Hedgecock et al. 1995) or other organisms. Natural selection may lead to locally adaptive differences, but the population genetic structure of a species also reflects historic distribution, gene flow, founder effects or random genetic drift in small populations, and mutation.

Understanding the patterns of colonization and gene flow, historical changes in abundance, and constraints of different life histories on current distribution and genetic population structure is a key to solving this challenge.

Recently colonized populations, for example, may have reduced genetic diversity because of genetic drift (Mayr 1963). The effect of these processes on genetic structure is varied and depends on the size and age of the population as well as other factors (Lande 1988, Avise 1994). In western pond turtles (*Clemmys marmorata*) reduced genetic diversity has been detected in the more northern populations in Washington State suggesting limited historic gene flow with more southerly populations in California (Gray 1995). An American bison (*Bison bison*) herd in South Dakota that was founded with a few individuals had low allozymes diversity (McClenaghan et al. 1990). In salmonids, areas of glacial refuge as well as colonization routes shaped the population genetic structure of salmonid species at the range-wide (Wood 1994) and regional scale (Cronin et al. 1993, Seeb and Crane 1999), as well as the watershed scale (Currens et al. 1990).

In this dissertation, I describe genetic diversity at multiple spatial scales in Dolly Varden (*Salvelinus malma*) and coastal cutthroat trout (*Oncorhynchus clarki clarki*), two species of salmonid fishes affected by Pleistocene glaciation. In many areas, where they currently occur together, they have similar life histories. Based on their historic distributions during the most recent Pleistocene glaciation, however, I hypothesize that they will have unique patterns of genetic diversity as a result of different potential evolutionary forces. I describe these differences and how these forces may affect the population genetic structure of these species across large and small spatial scales.

A wealth of molecular data exists on the genetic structure of Pacific salmonids (*Oncorhynchus* spp.) (Altukov and Salmenkova 1991, Wood 1994, Sanz et al. 2000, Smith et al. 2001, Bernatchez et al. 2001). Fisheries biologists use molecular genetic data to define a biologically meaningful entity at a local or regional level for the management of a fishery or to identify conservation units. The National Marine Fisheries Service uses molecular tools to implement the Endangered Species Act for salmon, where the least inclusive biological group entitled to protection is an "evolutionary significant unit" (ESU) (Waples 1991). The ESU definition implies that the group represents an important evolutionary legacy of the species and assumes that important adaptive traits are preserved when genetic variation is preserved (Waples 1991), although Dimmick et al. (2001) suggests that this approach will underestimate biodiversity. Genetic structure within species is commonly used to identify ESU's, although whether this is appropriate is the subject of ongoing debate (Dizon et al. 1992, Moritz 1994, and Nielson 1995, Dimmick et al. 2001). Other information regarding life history and demographics of populations is important to successfully implement long-term management and conservation efforts (Lande 1988).

Perhaps the most important feature of the salmonid family, including the species examined in this dissertation, is their diverse life history. Salmon and trout differ in life history strategies and adaptive traits, such as tendency for anadromy, disease resistance, run timing, fecundity, and size among and within river basins (see Taylor 1991 for review). One of the most striking life history features of

fishes in the salmonid family is anadromy, the migration between freshwater and marine habitats. Anadromous fishes are born in freshwater, enter the marine environment for feeding and sexual development, then complete their life cycle by returning to freshwater to reproduce. Anadromy is costly. Fishes must undergo physiological transformations to tolerate saltwater, and, in the marine environment, they encounter high rates of predation (Gross 1986). In general, fitness benefits conferred by the opportunity of feeding in a rich marine environment offset the costs, and anadromy is an evolved trait in northern temperate latitudes (Gross 1986). Examination of anadromy in the context of salmonid phylogeny suggests that the trait has evolved independently in major branches of salmonid genera (Stearly 1992).

The extent and duration of the marine phase varies among and within anadromous salmonids. Some species, such as sockeye (*O. nerka*) and chinook salmon (*O. tshawytscha*), make extended migrations to the high seas that last for several years (Groot and Margolis 1991), other species, such as coastal cutthroat trout and Dolly Varden (Sumner 1953, Armstrong and Morrow 1980) migrate to estuaries during summer months, and the majority of the population overwinter in freshwater environments. However, in some Dolly Varden populations there is evidence that they undergo more extensive migrations and may overwinter at sea (DeCicco 1992, Bernard et al. 1995).

Understanding the extent, timing, and duration of marine migrations is important scientifically as well as important for the management of anadromous

fishes. For this work, the extent and duration of the marine phase may have implications for the rate of colonization. Dolly Varden, which may travel great distances may have a higher dispersal or colonization rate than coastal cutthroat trout. Because of limited migration in cutthroat trout, they may be slow to recolonize newly developed habitat, or they may occupy new habitat in small numbers.

Following their marine migration, a portion of Pacific salmon and trout populations generally return to their natal streams to spawn. Of those fish that survive, return rates vary from 80-99 % in coho salmon (*O. kisutch*) (Sandercock 1991), to 86% in chum salmon (*O. keta*) (Burgner 1991), and to 97.6% in chinook salmon (Healy 1991). Those fishes that disperse, or spawn in streams other than their natal stream, are referred to as species, of those strays.

Determining management units for salmonid fishes depends, in part, on knowledge of homing and straying rates of populations. Direct measurements such as tagging and tracking of individuals can be used to gain this knowledge. Genetic data, wherein gene flow is inferred, is also used to obtain this knowledge (Ryman and Utter 1987). Because of the relatively high rate of homing, salmonids and trout tend to have a high degree of genetic structure, wherein genetic variation is partitioned at various spatial scales. However, among these species the genetic structure is variable. Fishes with high homing fidelity and limited dispersal, such as coastal cutthroat trout, reflect higher levels of genetic variation at finer scales

such as among river basins (Wenberg and Bentzen 2001) and within river basins (Griswold 1996).

Several species, such as rainbow trout (*O. mykiss*) (Northcote 1992), brown trout (*Salmo trutta*) (Hindar et al. 1991), Arctic char (*S. alpinus*) (Nordeng 1983), sockeye salmon (Ricker 1938), coastal cutthroat trout (Northcote 1992), and Dolly Varden (Savvaitova 1960), have anadromous and non-migratory, referred to as resident, life history types that occur sympatrically within river basins. Some of these life history types are considered dwarf phenotypes, such as the kokanee (*O. nerka*) and dwarf Arctic char (Nordeng 1983). These fishes may be physically isolated from their anadromous counterparts by waterfall barriers. Thus, genetic variation between resident and anadromous fishes may reflect geographic variation or, potentially, adaptive features (Northcote 1992).

The prevalence of the phenomenon of resident and anadromous life history forms among the different genera of salmonids has led to a variety of hypotheses regarding its origin. The primary competing hypotheses are that the different life history types represent ecophenotypes or, conversely, they are reproductively isolated and represent separate gene pools. Thorpe (1987) suggested that environmental variation might lead to sympatric anadromous and resident life history types. Fishes afforded opportunities for fast growth may reach sexual maturity more rapidly than slow growing fish. Thus, assuming there is a trade-off between reproductive development and migration, fast growing fish mature as

residents; slow growing fish delay sexual maturation, undergo smoltification, and become anadromous.

Others have suggested that genetics play a larger role. Jonsson (1985) suggested that anadromy is inherited, as the proportion of anadromous offspring is higher in fishes with anadromous parents. Zimmerman and Reeves (2000) found evidence that rainbow trout and steelhead are reproductively isolated in the Deschutes River, Oregon, but not in the Babine River, British Columbia. Vuorinen and Berg (1993) detected differences among different life history types of Atlantic salmon (*S. salar*) suggesting they are reproductively isolated. McVeigh et al. (1995) found haplotype variation among sympatric life history types of brown trout. Other studies of brown trout, however, suggest that genetic differences represent geographic variation and not variation among life history types (Elliott 1994). Similar results have been suggested for coastal cutthroat trout (Griswold 1996) and sockeye salmon (Foote et al. 1991). Whether the varying strategies represent an array of ecophenotypes or represent separate gene pools remains a question of interest for scientists and managers (Northcote 1992, Waples 1991). Thus, the degree of variation in some salmonid species appears to be a continuum, creating complex problems for their conservation and management.

In this dissertation, I approach the issue of life history variation from the perspective of the colonization of new habitats, some from clear cases of founding events due to recently opened habitats and some inferred by the presence of resident fish restricted above migratory barriers. I have organized this dissertation

into three main chapters. In Chapter Two I describe patterns of genetic diversity for coastal cutthroat trout and Dolly Varden in Prince William Sound, Alaska based on two hypothetical models of colonization following the retreat of Pleistocene glaciers. I also present data for cutthroat trout in more southern portions of their distributional range, including one location that was not glaciated. This unglaciated area may represent the historic center of the range of coastal cutthroat trout, and accordingly, may have higher genetic diversity. Chapter Two sets the stage for Chapter Three and Four, in which I examine more recent colonization of watersheds within Prince William Sound by coastal cutthroat trout and Dolly Varden, respectively.

In Chapter Two I introduce two models of colonization that may affect the genetic diversity in the two different species. The historic distributional range of coastal cutthroat trout (Chapter 2, Fig. 1) allows expansion into northern habitat from only one direction- from the south to the north of the Pacific coast refuge (Cascadia) (Behnke 1992). This may have occurred slowly as this species is characterized by small population sizes (potentially low effective size, Ne, or the hypothetical number of breeding individuals in a population) and relatively low dispersal rates (Sumner 1953, Jones and Siefert 1997). Dolly Varden could have expanded into new habitat from two directions, from southern refuges on the Olympic Penninsula or Cascadia or the northern Alaskan coastal refuge know as Beringia (Haas and McPhail 1991) (Chapter 2 Fig. 1). Expansion could occur more rapidly in Dolly Varden as this species has higher dispersal rates and larger

population sizes and are early colonizers of deglaciated streams (Armstrong 1979, Bernard et al. 1989, Milner et al. 1999). I have presented these two models in a simplified form below:

Coastal cutthroat trout

Low Ne + Limited → Low potential for colonization = Low genetic diversity
Single source
dispersal capacity

Dolly Varden

High Ne + High → High potential for colonization = High genetic diversity
Multiple sources
dispersal capacity

I expect different outcomes for these two models. For coastal cutthroat trout I expect the genetic diversity to be low in newly colonized habitat, perhaps resulting from loss of variation due to founder effects. I would expect genetic diversity to be higher for Dolly Varden because the newly founded population could consist of mixtures from the two regions that acted as refuges during the Pleistocene and founding events may have occurred repeatedly because of their dispersal capacity.

In Chapter Three I present a study of coastal cutthroat trout in Prince William Sound, Alaska. This study examines the potential genetic effects of recent colonization by comparing two watersheds that were recently colonized (150 to 350 ybp) with other sites in Prince William Sound that were colonized earlier. The pattern of glacial activity in Prince William Sound provides a unique opportunity to test the colonization of new habitat and the resulting genetic diversity at the

watershed scale. Resident fish that occur above waterfall barriers are included in this analysis.

In Chapter Four I present a study of the genetic diversity of Dolly Varden in Prince William Sound, Alaska. In this study, the genetic diversity in single recently colonized site is compared with sites not recently colonized. In addition, resident fish located in physically isolated sites are compared to anadromous fish with access to marine environments.

I use different molecular tools in each chapter. In Chapter Two I use mtDNA to examine the genetic variation of both species within Prince William Sound and cutthroat trout from three additional locations. Because of its low effective size and susceptibility to genetic drift mtDNA is sensitive to bottlenecks over long time frames (Davies et al. 1999). Because Chapter Two focuses on recolonization after the retreat of glaciers, mtDNA is the most appropriate tool (Avise 1994). In Chapter Three and Four I use microsatellites and allozymes. One of the potential outcomes following a bottleneck is the rapid expansion of populations. This may offset the genetic effects of bottlenecks leading to rapid genetic divergence (Reusch et al. 2001). It is more likely that the genetic results of rapid expansion would be detected with more variable molecular tools, such as these (Davies et al. 1999, Reusch et al. 2001).

Knowledge regarding the population genetic structure and the processes that may affect these species will contribute to the general knowledge of these species. It may provide insight to their potential recovery from natural and human-caused

disturbance as well as identify areas that potentially have high genetic variation, which may be critical for developing conservation and management units, such as ESU's. This work is timely, as the numbers of Pacific salmon and trout in western North America have declined due to overharvest, loss of habitat, and introgression with hatchery and non-native fishes (Nehlson 1989). In combination or alone, these factors threaten the long and, in some cases, short-term persistence of these fishes. The value of salmon and trout, and thus, their continued persistence in this region, is significant whether measured by economics, cultural values, or ecological importance (Thomas et al. 1993).

CHAPTER 2

COMPARISON OF MITOCHONDRIAL DNA DIVERSITY IN DOLLY VARDEN AND COASTAL CUTTHROAT TROUT IN PRINCE WILLIAM SOUND, ALASKA

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Introduction

Colonization can be broadly defined as the "establishment of a population of a species in a geographical or ecological space not occupied by that species" (Lewontin 1965). Factors that may affect the rate of colonization include life history traits such as fecundity, longevity, and dispersal (Lewontin 1965, Slatkin 1997). Colonization may lead to rapid evolution (Sterns 1992). Newly colonized populations may expand rapidly because they may be free from density-dependent competition (Lewontin 1965) and may have opportunities to exploit new niches (Slatkin 1997).

Colonization may also affect the genetic variation within species. For instance, if habitats are occupied by small numbers of individuals they may be subject to the loss of genetic diversity due to stochastic processes. The effects of this may be short-lived, as the expansion of populations following a founding event may lead to an expansion of genetic diversity (Cornuet and Luikart 1996). However, in some cases, evidence of a founder effect due to colonization may persist for decades (Hedrick et al. 2001) or longer (McCusker et al. 2000). The endpoint of these processes may be speciation, such as the examples of sympatric speciation of fishes in post-glacial lakes (Schluter 1996) or rapid divergence in morphology and behavior within lineages of fishes (Schluter and McPhail 1993, Bernatchez et al. 1996, Hendry et al. 2000).

Salmonid fishes are perhaps better known for their homing behavior but they can colonize new habitats when they become available. Homing rates, the percentage of fish that return to natal streams, vary among and within species from 80-99% in coho salmon (*Oncorhynchus kisutch*) (Sandercock 1991), to 86% in chum salmon (*O. keta*) (Burgner 1991), and to 97.6% in chinook salmon (*O. tshawytscha*) (Healy 1991). Straying, when individuals spawn in non-natal streams, is a mechanism of colonization and dispersal for salmonids. For example, in the eastern region of the Pacific Ocean areas of glacial refuge, Beringia and Cascadia, provided refugia for salmonids and other fishes during the Pleistocene (Fig 1). From these areas, salmonids colonized newly opened habitat following the retreat of glaciers (Behnke 1992). Examination of the genetic structure of sockeye salmon (*O. nerka*) populations from this region has provided insight to the colonization history of present day populations (Wood 1995). In some cases, reduced genetic variation, possibly due to population bottlenecks following colonization, exists in the northern distributional range of whitefish (*Coregonus* sp) (Bernatchez and Dodson 1994), and rainbow trout (*O. mykiss*) (McCusker et al. 2000).

Studies encompassing a large geographic range have used mitochondrial DNA (mtDNA) to describe genetic variation (see Avise 1994 for discussion). MtDNA is maternally inherited and does not undergo recombination. These features constrain the passing of mtDNA genes from generation to generation, essentially reducing the effective size of the population (the hypothetical number of

breeding individuals) to one-quarter of that for nuclear genes (Hartl and Clark 1997). As a result, mtDNA is more susceptible to genetic drift and is more likely to show the effects of small population size (Avise 1994). Recently, mtDNA Restriction fragment length polymorphism's (RFLP's) have been used to examine genetic diversity in areas colonized by rainbow trout (McCusker et al. 2000) and coho salmon (Smith et al. 2001) following the retreat of glaciers in North America, and brown trout (*Salmo trutta*) in Spain (Machordom et al. 2000).

Coastal cutthroat trout (*O. clarki clarki*) and Dolly Varden (*Salvelinus malma*) are members of the salmonid family. A portion of both species' extant range were effected by Pleistocene glaciation. Currently, both species are found throughout Prince William Sound, Alaska (Mills 1988), which was glaciated 8,000-12,000 years ago (Lethcoe 1990). Prince William Sound (Fig. 1) is the northern extent of the distributional range of coastal cutthroat trout however, whereas it is the center of the range for Dolly Varden, which are distributed around the Pacific Rim to Sakelin Island, Russia. Coastal cutthroat trout may have colonized Prince William Sound from the Pleistocene refuge Cascadia (Behnke 1992) whereas Dolly Varden may have colonized the region from the Beringia or Cascadia refuge (McPhail and Lindsey 1970) (Figure 2).

Dolly Varden and cutthroat trout have several similar life history traits with important differences. Both undergo marine migrations after spending 2-4 years in freshwater, and they usually return to freshwater for winter (Sumner 1953,

Figure 1. Current distributional range of coastal cutthroat trout and sampling locations for range-wide study are presented (A). Pie charts show haplotype frequencies detected at each site. Sampling locations for Dolly Varden (B) and coastal cutthroat trout (C) in Prince William Sound. Pie chart show haplotype frequencies detected at each site.

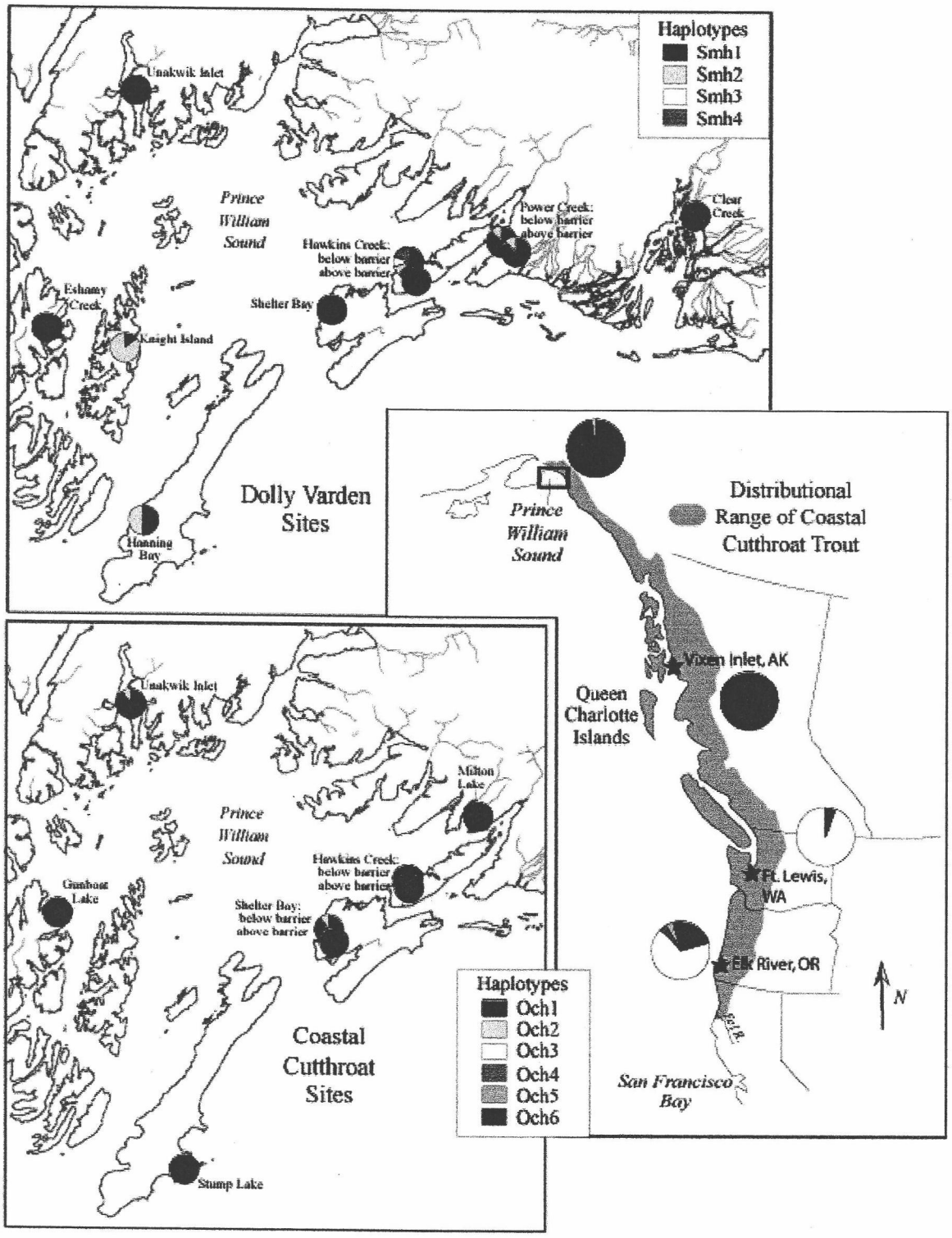
Haplotypes
Smh1
Smh2
Smh3
Smh4
Unakwik Inlet
Prince William Sound
Power Creek: below barrier above barrier
Clear Creek
Hawkins Creek: below barrier above barrier
Shelter Bay
Eshamy Creek
Knight Island
Hanning Bay
Dolly Varden Sites
Distributional Range of Coastal Cutthroat Trout
Prince William Sound
Vixen Inlet, AK
Queen Charlotte Islands
Ft. Lewis, WA
Elk River, OR
N
San Francisco Bay
Unakwik Inlet
Milton Lake
Prince William Sound
Hawkins Creek: below barrier above barrier
Gunboat Lake
Shelter Bay: below barrier above barrier
Haplotypes
Och1
Och2
Och3
Och4
Och5
Och6
Coastal Cutthroat Sites
Stump Lake

Figure 1.

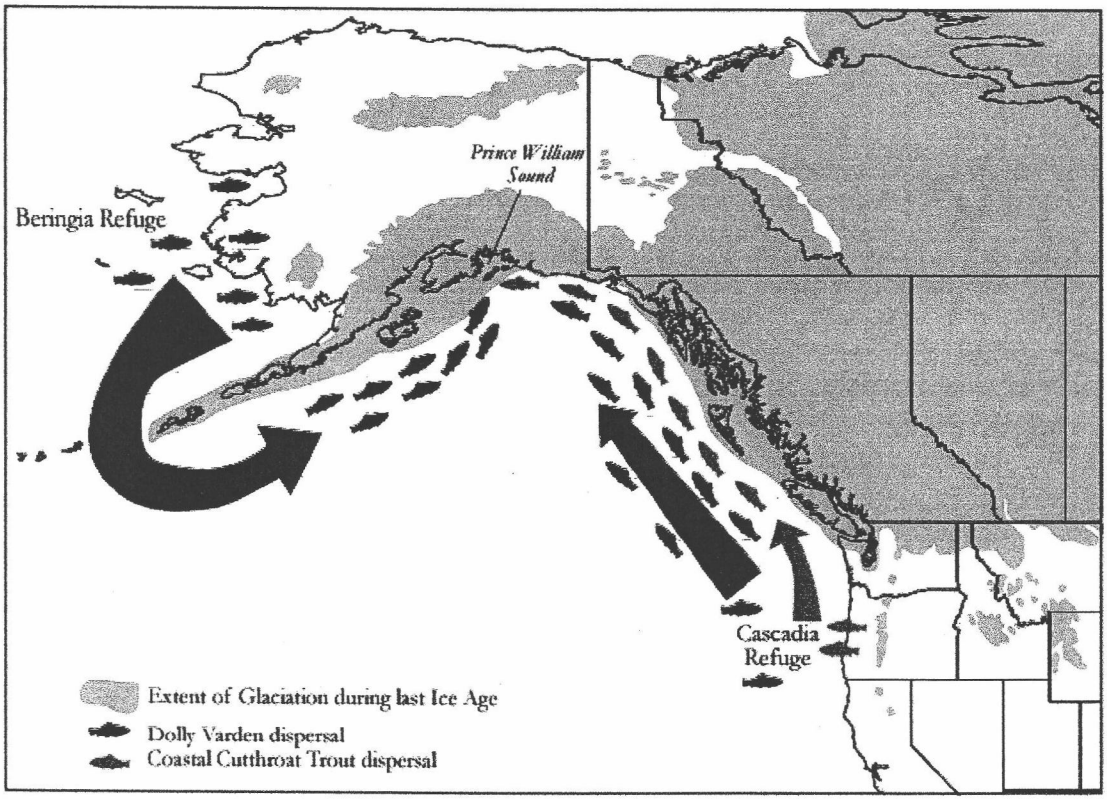

Figure 2. Extent of Pleistocene glaciation (gray) in western North America. Hypothesized Dolly Varden refugia include the Beringia and Cascadia Refuge, with potential post-pleistocene dispersal routes into glaciated areas shown with black fish. Hypothesized coastal cutthroat trout refugia includes the Cascadia refuge, and potential dispersal routes shown with gray fish. Arrow widths represent relative dispersal potential.

Armstrong 1971). In Prince William Sound, Dolly Varden travel extensively during their marine migration (Bernard et al. 1995).

Fishes that were recaptured in Prince William Sound streams tended to have high homing precision (98%) (Bernard et al.1995). However, a large proportion (14-58%) of the population that was marked at the time of outmigration was never recaptured. This led the authors to suggest that these fish spent the winter at sea. However, it is possible that these fishes dispersed to other sites. Dolly Varden in other regions are known to undergo extensive migration (DeCicco 1992). In contrast, tagging evidence suggest that coastal cutthroat trout movement in Prince William Sound is much more restricted (McCarron and Hoffmann 1993). Tagging and genetic data suggest that coastal cutthroat trout movement in the marine environment is limited in Puget Sound as well (Campton and Utter 1987, Wenburg and Bentzen 2001).

The goal of this study is to describe the pattern of genetic diversity of coastal cutthroat trout and Dolly Varden in Prince William Sound, and for trout, in three additional locations. Genetic variation following colonization after the retreat of Pleistocene glaciers has not been examined in these species. Instead, recent genetic investigations of these species have focused on clarifying taxonomic relationships and population structure in Dolly Varden (Taylor et al. 2001, Krueger et al. 1999) and population structure at the regional scale in coastal cutthroat trout (Wenburg and Bentzen 2001). I have two questions of interest for this study. First,

is there lower genetic diversity in coastal cutthroat trout from locations that were colonized after the retreat of glaciers relative to an area that was not glaciated? To examine this question I examine four locations from throughout the distributional range of coastal cutthroat trout including one site that is thought to have been free of glaciers during the Pleistocene (Fig. 1 and Fig. 2). That site, Elk River, Oregon may be part of the historic center of the distributional range, and as such may reflect higher levels of genetic diversity than the other sites. Second, how do differences in historic distribution and life history affect the genetic diversity of Dolly Varden and coastal cutthroat trout following colonization in Prince William Sound? To examine this, I present and compare mtDNA RFLP data from Dolly Varden and coastal cutthroat trout from a number of locations within Prince William Sound. Based on potential colonization history and dispersal capacity of Dolly Varden I expect to see relatively high genetic diversity inthis species in Prince William Sound. In coastal cutthroat trout, colonization comes from one area and this species has a low dispersal capacity which should result in relatively low diversity in the new habitat.

Methods

Study sites and collection

Coastal cutthroat used to examine genetic variation in glaciated and non-glaciated areas were collected from locations in Oregon, Washington, and Alaska

(Table 1, Fig. 1). Prince William Sound, Vixen Inlet, and Ft. Lewis were glaciated during the Pleistocene while Elk River remained ice-free (McPhail and Lindsey 1970). Fish from Elk River, Oregon, and Vixen Inlet, Alaska, were collected in 1992 (Griswold 1996). Fish from Ft. Lewis, Washington, were collected in 1993 (Zimmerman et al. 1997). Samples from Prince William Sound, Alaska were collected in 1996 from eight locations throughout the Sound. A portion of the cutthroat trout collected in the Elk River and two collections in Prince William Sound were made above waterfall barriers. Sample sizes ranged from 8-79 (Table 1).

To examine genetic variation among aggregations within Prince William Sound, coastal cutthroat trout and Dolly Varden were collected in 1996. Cutthroat trout were collected from eight locations and Dolly Varden were collected from ten locations (Table 1, Fig. 1). Sample sizes at individual sites ranged from 3-15 (Table 1) and are within the range or higher than those from similar studies (McCusker et al. 2000, Nielsen et al. 1994).

In all field collections there was an attempt to collect individuals from across the size range of each species. To do this, a variety of sampling techniques were used. Collections were made in fresh water near the estuary or in the estuary near fresh water with baited minnow traps, hook and line, or seines. Juveniles were collected with baited minnow traps from several sampling locations along a stream to avoid collecting full siblings from a single mated pair.

Table 1. Location, haplotype diversity (±SE), nucleotide diversity (±SE) (Nei 1987) and sample size for Dolly Varden and coastal cutthroat trout.

Location	Haplotype diversity (h)	Nucleotide diversity (π)	N
Coastal cutthroat trout range-wide			
Prince William Sound, AK (glaciated)	0.050 (0.034)	0.0028 (0.004)	79
Vixen Inlet, AK (glaciated)	0.000	0.0000	8
Ft Lewis, WA (glaciated)	0.087 (0.078)	0.0037 (0.0047)	23
Elk River, OR (un-glaciated)	0.529 (0.104)	0.0440 (0.0264)	24
Coastal cutthroat trout within PWS			
Unakwik Inlet	0.200 (0.154)	0.0113 (0.010)	10
Gunboat Lake	0.000	0.0000	10
Stump Lake	0.000	0.0000	9
Shelter Bay-anadromous	0.200 (0.154)	0.0113 (0.010)	10
Shelter Bay-resident	0.000	0.0000	10
Hawkins Creek-anadromous	0.000	0.0000	10
Hawkins Creek-resident	0.000	0.0000	10
Milton Lake	0.000	0.0000	10
Dolly Varden within PWS			
Unakwik Inlet	0.000	0.0000	7
Eshamy Creek	0.000	0.0000	6
Hanning Bay	0.571 (0.095)	0.286 (0.170)	8
Shelter Bay	0.000	0.0000	8
Hawkins Creek anadromous	0.644 (0.152)	0.223 (0.132)	10
Hawkins Creek-resident	0.000	0.0000	15
Power Creek-anadromous	0.250 (0.180)	0.125(0.082)	8
Power Creek-resident	0.286 (0.196)	0.143 (0.094)	7
Clear Creek	0.000	0.0000	3
Knight Island	0.286 (0.196)	0.143 (0.094)	7

Adult coastal cutthroat trout were sampled in July when they returned to fresh water from the estuary to over-winter or spawn. Adult Dolly Varden were collected in September, when they spawn. Individual fish were given a lethal dose of MS-222, weighed (to the nearest 0.1 g), fork length measured (to the nearest mm), and tagged with an identification number. Muscle samples from fish > 250 mm in length were removed immediately, placed in plastic tubes, labeled with the identification number of the fish and put on dry ice. Fish < 250 mm were wrapped in plastic wrap and immediately frozen on dry ice. Whole fish and muscle samples were stored at -80° C until they were transported on dry ice to the Oregon Cooperative Fish Research Unit (OCFRU) laboratory in Corvallis, Oregon. Muscle samples were removed from the frozen whole fish in the lab and transferred, while still frozen, to 1.7 ml microcentrifuge tubes and stored at -80° C. Tissue samples from these fish were stored at -80° C until they were analysed.

DNA extraction and Amplification

DNA was extracted from 50-75 mg of muscle with a phenol:choroform:isoamyl alcohol extraction procedure (Sambrook et al. 1989). After extraction, samples were suspended in TE buffer and stored at 2° C. Three fragments of mtDNA, (ND-1, ND-2, and D-loop) were amplified using polymerize chain reaction (PCR) following methods of Cronin et al. (1993). Primers used were developed by LGL Genetics, Inc. The three segments were amplified in a 50:1 reaction consisting of 1.0-5.0 µl extracted DNA, 5.0 µl LGL *Taq* buffer, 1.0 µl

forward primer, 1.0 µl reverse primer, 0.4 µl dNTP (0.1µl each), 0.5 µl *Taq* polymerase, and 41.1-37.1 µl pure water (supplied by LGL Genetics). Eleven restriction enzymes were examined; *Alu* I, *Ava* II, *Bgl* II, *Bst* UI, *Dpn* II, *Hae* III, *Hha* I, *Hind* III, *Mse*I, *Msp* I, *Taq* I (supplied by NE Biolabs). Amplified DNA was digested with restriction enzymes following the manufacturer's instructions. Sixteen fragment-restriction enzyme combinations were chosen because they are known to show variation in other closely related salmonid species (Cronin et al. 1993). ND-1 was digested with *Alu* I, *Ava* II, *Bgl* II, *Bst* UI, *Dpn* II, *Hae* III, *Hind* III, *Msp* I, *Taq* I; ND-2 was digested with *Alu* I, *Hind* III, *Mse* I and; D-loop was digested with *Bgl* II, *Dpn* II, *Hha* I, *Mse* I. Digested fragments were run on 2.8% agarose gels. Variation in ethidium bromide stained fragment patterns was examined under ultraviolet light and photographed. Fragment length was estimated by comparison with a standard 1 kb DNA ladder.

Statistical analysis

Each restriction enzyme fragment pattern was assigned a different letter code and used to construct a composite sixteen-letter haplotype. Composite haplotypes were assigned a reference code based on the abbreviation of the latin name of each species and the composite haplotype number. Relative frequencies of composite haplotypes were calculated for cutthroat trout and Dolly Varden from each sampling location. Two measures of DNA polymorphism- nucleotide diversity and haplotype diversity (Nei 1987)- were used to estimate genetic

diversity within collections of fish from different location using Arlequin (Scheider et al. 2000). Nucleotide diversity estimates the average number of nucleotide substitutions in a segment of DNA, whereas haplotype diversity, which is analogous to average heterozygosity, estimates the average restriction fragment variation within a sample (Nei 1987). Estimates of evolutionary distance (Nei 1987) between haplotypes were estimated with the computer program REAP (McElroy et al. 1992). Among site homogeneity was tested for each species within Prince William Sound using exact tests of haplotype frequencies and Fst values (Raymond and Rousset 1995) using the computer software program Arlequin (Schneider et al. 2000).

The haplotype frequency among coastal cutthroat trout from throughout their range was compared with a variety statistical tests. For these analyses, the eight sites in Prince William Sound were lumped together to represent a single site with a sample size of 79. Data from sites within regions were pooled to test for differences in levels of genetic diversity after testing for among-site homogeneity using exact tests of haplotype frequencies and Fst values (Raymond and Rousset 1995) using the computer software program Arlequin (Schneider et al. 2000). Markov chain parameters were set at 1000 for both of these tests. Pairwise Fst values and their P-values were calculated with the computer software program Arlequin (Schneider et al. 2000). For all tests involving multiple comparisons, Bonferroni corrections were applied to significance levels (Rice 1989).

Results

Coastal cutthroat trout range comparison

Nine polymorphic restriction fragment-enzyme combinations were identified in coastal cutthroat trout in the four sampling sites (Table 2A). Six unique composite haplotypes were identified for coastal cutthroat trout when all 16 restriction enzymes were combined (Table 2B). All samples had the haplotype Och1 which was common in Prince William Sound (95%) and Vixen Inlet (100%), but at low to moderate frequencies in Ft. Lewis and Elk River sites (4% and 21% respectively) (Fig 1, Table 2B). The composite haplotype (Och2) was unique to two individuals in Prince William Sound. Och3 was the most common haplotype in cutthroat trout from Ft. Lewis, Washington, and the Elk River, occurring in 96% and 67% of the sampled fish, respectively, but it was not detected in either Prince William Sound of Vixen Inlet. In Elk River, coastal cutthroat trout had three additional unique composite haplotypes (Och4, Och5, and Och6) that occurred at low frequencies. Two of these haplotypes (Och5 and Och6) occurred in trout collected above a waterfall barrier.

Haplotype diversity within the four sites ranged from zero (Vixen Inlet) to 0.529 (Elk River) (Table 1). Nucleotide diversity ranged from 0.00 to 0.040 (Table 1). Nei's (1987) estimate of evolutionary distance between haplotypes ranged from

Table 2. A) Presence-absence matrix of nine polymorphic restriction sites based on mtDNA restriction enzymes in coastal cutthroat trout from four locations throughout their distributional range. Letter code assigned to fragment pattern in shown in bold. B) Reference code, composite haplotypes for coastal cutthroat trout from four locations throughout their distributional range, location of occurrence are presented.

A)

Fragment-enzyme combination

ND-I					ND-2		D-loop	
Ava II	*Bstu* I	*Dpn* II	*Hae* III	*Taq* I	*Hind* III	*Mse* I	*Dpn*II	*Hha* I
A 01001	**A** 100	**A** 001	**A** 01	**A** 010111	**A** 011	**A** 00111	**A** 01	**A** 01
B 00111	**B** 011	**B** 110	**B** 10	**B** 101000	**B** 100	**B** 11000	**B** 10	**B** 10
C 10000								

B)

Reference code	Composite haplotype	Location
Haplotype 1 (Och1)	AAAAAAAAAAAAAAA	(Prince William Sound, Vixen Inlet, Ft. Lewis, Elk River)
Haplotype 2 (Och2)	AAAAAAAAAAAAABBA	(Prince William Sound)
Haplotype 3 (Och3)	ABAAAAAAAAAAAAA	(Ft. Lewis, Elk River)
Haplotype 4 (Och4)	ACABBBAABAAAAAA	(Elk River)
Haplotype 5 (Och5)	ABAAAAAAAABAAAA	(Elk River)
Haplotype 6 (Och6)	ABAAAAAAAAABAAA	(Elk River)

0.0025 to 0.0189 (Table 3). Haplotype Och4 had unique RFLP fragments patterns in 5 of the 16 restriction enzymes that led to extreme divergence from the other haplotypes. A global test (exact test) for differences among the four regions was statistically significant ($P < 0.0001$). Pairwise differences between regions were detected using exact tests in four of the six comparisons after corrections for multiple tests were conducted ($P < 0.008$; Table 4). Fst values ranged from -0.082 to 0.928 (Table 4). Using both exact tests and pairwise Fst values Prince William Sound haplotypes were significantly different from Ft. Lewis and Elk River ($P < 0.0001$), and Vixen Inlet differed from Ft. Lewis and Elk River ($P < 0.0001$) (Table 4).

Genetic diversity within Prince William Sound

There were four fragment-enzyme combinations detected in Dolly Varden from Prince William Sound (Table 5). At many sites the most common haplotype (Smh1) occurred in all of the samples (Fig. 1). However, the distribution of haplotype frequencies was variable in Prince William Sound (Fig. 1).

Nei's haplotype diversity for Dolly Varden ranged from 0.000 to 0.644 (Table 1). Nucleotide diversity ranged from 0.000 to 0.286 (Table 1). Haplotype diversity for coastal cutthroat trout within Prince William Sound was

Table 3. Estimates of evolutionary distance (Nei 1987) of the composite haplotypes based on mtDNA restriction fragment length polymorphism's in coastal cutthroat trout populations from four locations throughout their range (Fig 1).

	Och1	Och 2	Och 3	Och 4	Och 5
Och 2	0.0028				
Och 3	0.0025	0.0053			
Och 4	0.0134	0.0169	0.0143		
Och 5	0.0050	0.0078	0.0025	0.0170	
Och 6	0.0060	0.0091	0.0035	0.0189	0.0061

Table 4. Pairwise Fst values based on haplotype frequencies between coastal cutthroat trout from four locations throughout their range are presented above the diagonal. Significance levels (+ = P < 0.0001, NS = not significant) are presented below the diagonal). Locations are Prince William Sound, Alaska (PWS), Vixen Inlet, Alaska (VI); Ft Lewis, Washington (FL); and Elk River, Oregon (ELK).

	PWS	VI	FL	ELK
PWS		-0.082	0.928	0.373
VI	NS			
FL	+	+		0.045
ELK	+	+	NS	

Table 5. A) Presence-absence matrix of three polymorphic restriction sites in Dolly Varden from Prince William Sound, Alaska, based on mtDNA restriction enzymes. Only variable sites are presented. Letter code assigned to fragment pattern shown in bold. B) Reference code, composite haplotypes for Dolly Varden from Prince William Sound, Alaska. Each letter represents a fragment pattern for 16 mtDNA restriction enzyme combinations.

A)

Fragment-enzyme combination		
NDI		
Bstu I	*Hae* III	*Msp* I
A 011	**A** 00101101	**A** 100
B 100	**B** 01010010	**B** 011
	C 10010000	

B)

Reference code	Composite haplotype
Haplotype 1 (Smh1)	AAAAAAAAAAAAAAAA
Haplotype 2 (Smh2)	AAABABBAAAAAAAAA
Haplotype 3 (Smh3)	AAAAABBAAAAAAAAA
Haplotype 4 (Smh4)	AAAAACAAAAAAAAAA

0.000 to 0.200; the highest estimate was one-third lower than that observed for Dolly Varden (Table 1). Nucleotide diversity in cutthroat trout ranged from 0.000 to 0.0113, the highest estimate being one-half of that observed for Dolly Varden. Estimates of evolutionary distance (Nei 1987) between haplotypes ranged from 0.0071 to 0.0373 for Dolly Varden (Table 6). The estimate of evolutionary distance (Nei 1987) between haplotype Och1 and Och2 was 0.0028 for coastal cutthroat trout, approximately one-half the lowest estimate for Dolly Varden. Finally, there was a significant difference among Dolly Varden samples within Prince William Sound using exact tests ($P < 0.0001$) while there was no difference detected among coastal cutthroat trout samples within Prince William Sound ($P > 0.05$).

Table 6. Estimates of evolutionary distance (Nei 1987) of the composite haplotypes based on mtDNA RFLP's in Dolly Varden populations from Prince William Sound, Alaska.

	Smh1	Smh 2	Smh 3
Smh 2	0.0373		
Smh 3	0.0154	0.0254	
Smh 4	0.0261	0.0071	0.0154

Discussion

Coastal cutthroat trout range comparison

There were more haplotypes detected in the non-glaciated site, Elk River, than in the sites that were glaciated. The populations in the glaciated sites, Puget Sound, Vixen Inlet, and Prince William Sound were all characterized by low haplotype diversity relative to the Elk River. This pattern is consistent with that observed for other salmonids in western North America (McCusker et al. 2001, Smith et al. 2001) and Eurasia (Bernatchez and Dodson 1994). As species colonize new areas they may undergo founder effects or population bottlenecks (Hartl and Clark 1997). These events may result in genetic drift and the loss of genetic diversity. Thus, as fishes founded new populations following the retreat of glaciers they may have undergone repeated bottlenecks with each subsequent founding event. Based on the results of this study, it is possible that as coastal cutthroat trout founded new populations during the retreat of glaciers there was a reduction in female effective population size leading to population bottlenecks.

It is generally expected that a species' genetic diversity is greatest at the center of their range (Scudder 1989). These areas may have high levels of genetic diversity because they may consist of multiple lineages that have persisted for extended periods of time. However, because expansion and contraction of distributional ranges occur over time around a central location (Scudder 1989), the center of the historic range may not necessarily be the geographic center of the current distributional range. Elk River had the highest haplotype diversity of the sampling locations in this study. Although the Elk River is close to the southern edge of the current geographic range of coastal cutthroat trout, it may be close to, or may be the historic center of the range. Favorable habitat for coastal cutthroat trout may have been available for longer periods of time there than in the central and northern regions of their current distributional range. It is also possible that haplotype diversity in Elk River represents genetic diversity from multiple invasions. The Elk River area may represent an important component of the genetic diversity in this species.

Resident populations may represent an important component of genetic diversity in coastal cutthroat trout. In the Elk River, two unique haplotypes were detected from above a waterfall barrier. The unique haplotypes in the Elk River may represent relicts from an original founder, recent mutation, selection, or introgression with rainbow trout. Meristic counts and allozyme data, however, suggested no evidence of introgression in these individuals (Griswold 1996). It

appears that in some cases the populations in above barrier sites may retain haplotype diversity or may persist long enough to develop haplotype diversity. However, no variation was detected in above-barrier sites in Prince William Sound, suggesting they are similar to the founding population. Thus, it is difficult to generalize that all resident fish will be unique, instead, haplotype variation probably depends on local conditions.

Coastal cutthroat trout in the Oregon coast are considered a single Evolutionary Significant Unit (ESU) (Johnson et al. 1999) and are currently listed as a candidate species under the Endangered Species Act (ESA). This research suggests that coastal cutthroat trout from a single watershed has higher genetic diversity than the three other sites combined. Further work which includes other watersheds may be necessary to determine if the current scale of ESU for coastal cutthroat trout in coastal Oregon is appropriate.

Genetic diversity within Prince William Sound

Genetic diversity based on mtDNA haplotype frequency and evolutionary distance was relatively higher in Dolly Varden than in coastal cutthroat trout in Prince William Sound, Alaska. Differences in colonization sources could account, at least in part, for these differences. Based on their current distribution from the Olympic Peninsula, Washington around the Pacific Rim to Sakelin Island, Dolly Varden had two potential areas of refuge Beringia and Cascadia during the Pleistocene glaciation (Crane et al. 1994). Therefore, Dolly Varden could have re-

colonized Prince William Sound from these two regions. Coastal cutthroat trout were probably not present in the Bering Sea and could only colonize Prince William Sound from the south (Behnke 1992). Other researchers have observed high genetic diversity in salmonids from other regions in Alaska that may have resulted from colonization by multiple sources. For instance, the north side of the Alaska Inlet is an area of high allozyme diversity in chum salmon, which may represent a contact zone for two lineages (Seeb and Crane 1999). High mtDNA diversity in chum and sockeye salmon in the Yukon drainage led researchers to suggest that a central Alaska refuge as well as Pacific Coast refuge may have supported these species during the Pleistocene (Cronin et al. 1993). In sockeye salmon from Russia, Alaska, Canada, and Washington, genetic diversity is highly structured among lakes and within watersheds, creating a mosaic pattern of diversity, however, there are major groupings which suggest that that these populations derived from Cascade and Bering refuges (Wood 1995). Thompson et al. (1997) attributed high genetic diversity of threespine stickleback (*Gasterosteus aculeatus*) in British Columbia to recolonization from the same refuges. In that case lake-stream pairs of threespine stickleback within a lake system were divergent, suggesting that stream types consist of a unique lineage and lake types consist of a unique lineage, and resulted from multiple invasions.

Differences in dispersal capacity and population size between Dolly Varden and coastal cutthroat trout also may have affected observed genetic differences between the two species. Dolly Varden has a relatively high dispersal capacity

(DeCicco 1992), and are thought to be the first colonizers of new habitat (Milner et al. 2000). Relative to coastal cutthroat trout, Dolly Varden have larger population sizes in Prince William Sound and elsewhere (McCarron and Hoffmann 1993, Armstrong 1971). On the other hand, coastal cutthroat trout have low dispersal capacity and small population sizes (Jones and Seifert 1997, Campton and Utter 1987). As a result, they may colonize new habitats more slowly than other salmonids, including Dolly Varden, and, as previously noted, if a small number of individuals colonize an area they may undergo a founder effect or bottleneck Bottlenecks may lead to low genetic diversity, particularly, allelic diversity, due to genetic drift (Leberg 1992). Conversely, if a large number of individuals colonize a new habitat they are less likely to undergo a population bottleneck and the resulting loss of genetic diversity. In addition, if there are continual inputs a persistent bottleneck is unlikely. Finally, greater variation would be expected if there were inputs from multiple sources that are genetically diverse.

Prince William Sound has been de-glaciated for a relatively short period (8,000-12,000 years)(Lethcoe 1990) and was colonized by both species subsequently. As a result, the number of haplotypes compared to other salmonids for both species in Prince William Sound may be expected to be low relative to other salmonids. McCusker et al. (2000), using 14 restriction enzymes researchers detected 32 haplotypes in rainbow trout throughout their range. Seven haplotypes were detected using seven restriction enzymes in chum salmon from the Yukon River drainage (Scribner et al. 1998). However, both these studies were conducted

at larger spatial scales. However, haplotype diversity in Dolly Varden in Prince William Sound was higher than that reported for coho salmon in Alaska (Smith et al. 2001). Smith et al. (2001) suggested that low mtDNA variation in Alaskan coho salmonpopulations is evidence that early Pleistocene glaciation eliminated coho salmon from this region.

Other researchers have suggested that two clades of Dolly Varden exist, one which includes evidence of historic introgression with bull trout (*S. confluentus*), a species closely related to Dolly Varden. Taylor et al. (1999) found that one group of mtDNA haplotypes dominated interior populations of bull trout and another group dominated coastal populations of bull trout (This led the authors to conclude that the two groups derived from separate refugia (Columbia and Chehalis) (Taylor et al 1999). They revised this interpretation when they examined mtDNA and nuclear DNA variation in Dolly Varden throughout their distributional range of Russia, Alaska, British Columbia, and the Continental United States and compared it to the genetic variation in bull trout (Taylor et al. 2001). Significant divergence in mtDNA region ND-1 detected with restriction enzymes (*Hae* III, and *Hinf* I) led them authors to separate Dolly Varden into two major clades, one that included groups from the Kuril Islands, north coast British Columbia, and the Yukon River drainage, and a second clade consisting of more southern populations of the British Columbia coast and Washington State. MtDNA haplotypes in the southern group were more similar to bull trout haplotypes. Nuclear DNA, on the other hand, suggested clear distinctions between the species. Thus, they suggested that the

southern Dolly Varden group may represent historic introgression between bull trout and Dolly Varden. However, these researchers were examining the species throughout their range and their sample sizes within regions were small (1-10), thus within-region variation may have been undetected. The haplotype diversity I observed within Prince William Sound was variable and suggests that the region may have been colonized from two areas of refugia, whether some of these haplotypes represents historic introgression with bull trout is unknown.

I detected high evolutionary distance between Dolly Varden from two sites in Prince William Sound, Knight Island and Hanning Bay. Other researchers have detected haplotype diversity at similarly small spatial scales between early and late runs of chinook salmon (Adams et al. 1994). High homing rates of Dolly Varden in Prince William Sound, as suggested by Bernard et al. (1995) may maintain these haplotypes at high frequencies at the watershed level. Alternatively, a few individuals with these haplotypes that dispersed a great distance may have recently invaded these locations and were disproportionately included in the sample.

Summary

The results presented here are consistent with the idea that colonization may lead to founder effects or bottlenecks resulting in reduced genetic diversity introduced by Mayr (1942). Coastal cutthroat had higher haplotype frequencies in Elk River which occurs at the southern edge of their range. Dolly Varden had higher haplotype frequency within Prince William Sound. This is consistent with

the historic refuge of Dolly Varden allowing them to colonize Prince William Sound from two regions, the Bering and Cascade Refuges. The pattern of genetic diversity also may reflect higher dispersal capacity of Dolly Varden and opportunities for multiple founding events.

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CHAPTER 3

GENETIC VARIATION OF COASTAL CUTTHROAT TROUT IN PRINCE WILLIAM SOUND, ALASKA: POTENTIAL INFLUENCE OF COLONIZATION HISTORY

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Introduction

Small populations colonizing new habitats may undergo a rapid reduction in effective population size (number of breeding individuals in a population) (Wright 1978). Such populations may be susceptible to genetic drift, or the random change in allele frequencies. Consequently, if an area is colonized by a small number of individuals the population may undergo a loss of genetic variation relative to the source population. If genetic variation is reduced in founded populations there may be increased risk of extinction or lowered fitness and the template for future adaptations may be lost (Hedrick et al. 2001). Because of these risks, understanding what happens to genetic variation in recently founded populations has important implications for management and conservation.

The genetic effects of a founding event may be transient (Cornuet and Luikart 1996). If populations expand after a bottleneck or founding event, genetic variation may increase rapidly (Wakely 2000). There can be a rapid ecological response as well. Fishes that are introduced to new habitats may undergo an adaptive response that is remarkable fast, such as the response observed in body shape, age and sexual maturity in sockeye salmon (*Oncorhynchus nerka*) 40 years after they were introduced to Lake Washington (Hendry et al. 2000). In Chinook salmon (*O. tshawytscha*) introducted to New Zealand rapid responses have been observed in fecundity and egg size (Kinneson et al. 1999). Other researchers emphasize that knowledge of life history variation is critical for successful introductions of sockeye salmon (Burger et al. 2000) as there appears to be fidelity

to important traits such as beach spawning. Colonization by fishes in natural habitats in short-time frames has focused on the succession of colonizers following de-glaciation (Milner et al. 2000). Recent work has suggested that resident rainbow trout (*O. mykiss*) introduced to Argentina are genetically similar to anadromous forms that arose following the introduction, suggesting the two forms are not reproductively isolated (Pascual 2001). However, there has not been work that examines the genetic effects of natural and recent founding events in salmonids at small spatial scales.

During the Pleistocene much of what is the current northern distributional range for coastal cutthroat trout (*O. clarki clarki*) was covered with glacial ice (McPhail and Lindsey 1970). Prince William Sound, Alaska, the northernmost region of their distribution, was de-glaciated approximately 8,000-12,000 years ago (Lethcoe 1990), and coastal cutthroat trout colonized the region from coastal locations after the retreat of glaciers (Behnke 1992). Within Prince William Sound there are active glacial advances and retreats that in some cases only recently (150-350 years ago) has created stream habitat for coastal cutthroat trout (Ken Hodges, Cordova Ranger Station, Cordova, Alaska). This unique geologic history provides an opportunity to examine the genetic variation in recently founded populations of coastal cutthroat trout.

The goal of this study is to examine the genetic variation of coastal cutthroat trout in newly colonized habitat. The specific objectives are to examine the effects of colonization by coastal cutthroat trout into new habitats, and examine

the population genetic structure within and among populations of coastal cutthroat trout in Prince William Sound. Understanding genetic diversity in recently founded populations may provide an important framework for understanding ecological divergence and provide insight as to how the genetic diversity of newly founded populations compares with other potential source populations. Given that Pacific salmon (*Onchorhynchus* spp.) occur in a dynamic environment where re-colonization occurs following habitat disturbance (Reeves et al. 1995), this work will have important mangagement implications.

Two tools for genetic analysis, allozymes, a protein-coding genetic marker, and microsatellites, a nuclear DNA marker, were used in this study. There is wide array of molecular tools available that are appropriate for examining genetic variation in wild populations. They vary in terms of their mode of inheritance and mutation rate. As a result, some tools are more applicable than others depending on the study's scope of inference. Highly diverse nuclear markers, such as microsatellites retain diversity when other markers, when markers such as mitochondrial DNA, because of its' mode of inheritance, is sensitive to population bottlenecks and may have low diversity (Davies et al. 1999). Use of highly variable markers is appropriate because coastal cutthroat trout populations examined in this study are at the northern extent of their range. However, examples from the literature suggest that using a single genetic tool may lead to erroneous conclusions (Avise 1994) because of the varying influence of selection, drift, or other evolutionary forces on different genetic markers. Therefore, different classes

of markers may lead to different results (although see Allendorf et al. 2000 for an exception). Using multiple markers may provide a more complete understanding of the variation within amd among populations.

Allozymes are protein-coding loci that may vary in biochemical structure without affecting the function of the enzymes (although see Karl and Avise 1992). Allozyme variability has been an essential tool in fisheries genetics for many decades. Relative to newly developed DNA tools, such as microsatellites, allozymes have low levels of variation (Hedrick 1999). However, they are still commonly used and extensive baseline data exists on salmonid populations. They are useful for detecting mid-scale, and fine-scaled population structure in coastal cutthroat trout (Campton and Utter 1987, and Griswold 1996). However, they can be homogenous, which in some cases led to incomplete results when compared with other tools (Avise 1994).

Microsatellites are a class of generic markers that have variable repeated nuclear DNA sequences. They are highly variable and have a rapid mutation rate. There is considerable debate in the literature as to the model of mutation that microsatellites undergo (O'Connell et al. 1997). However, it is clear that they are suited for small-scale population genetic studies and family and pedigree analysis (Blouin et al. 1996, Letcher and King 2000), and are being applied to fisheries problems at an increasing rate. In this study, they provided a promising tool to examine genetic variation at the regional scale. They may be sensitive to the effects of population bottlenecks within populations because of their high number of

alleles. However, if populations have undergone bottlenecks it may result in large genetic distances among populations as measured by standard analytical tools, especially when compared with less variable markers (Hedrick 1999). This has led to a cautionary warning regarding the interpretation of these highly variable markers (Hedrick 1999). Because coastal cutthroat trout examined in this study are at the northern extent of their distributional range and may have undergone recent bottlenecks during colonization of recently de-glaciated watersheds microsatellites are an appropriate tool for examining their genetic variation.

Methods

Study sites and collection

Coastal cutthroat trout were collected from twelve locations throughout Prince William Sound and one on the Copper River Delta (Fig. 1 and Table1). Two of these sites, Columbia Bay and Unakwik Inlet were deglaciated approximately 150 and 350 ybp, respectively. Prince William Sound was deglaciated approximately 8,000-12,000 ybp; the exact timeframe of deglaciation of individual sites, other than Unakwik and Columbia Bay is unknown. Eleven sites had access to the marine environment and populations in them were considered anadromous (ie. migratory) (Table 1). Two sites were above waterfall barriers and the populations were considered resident (ie. non-migratory) (Table 1).

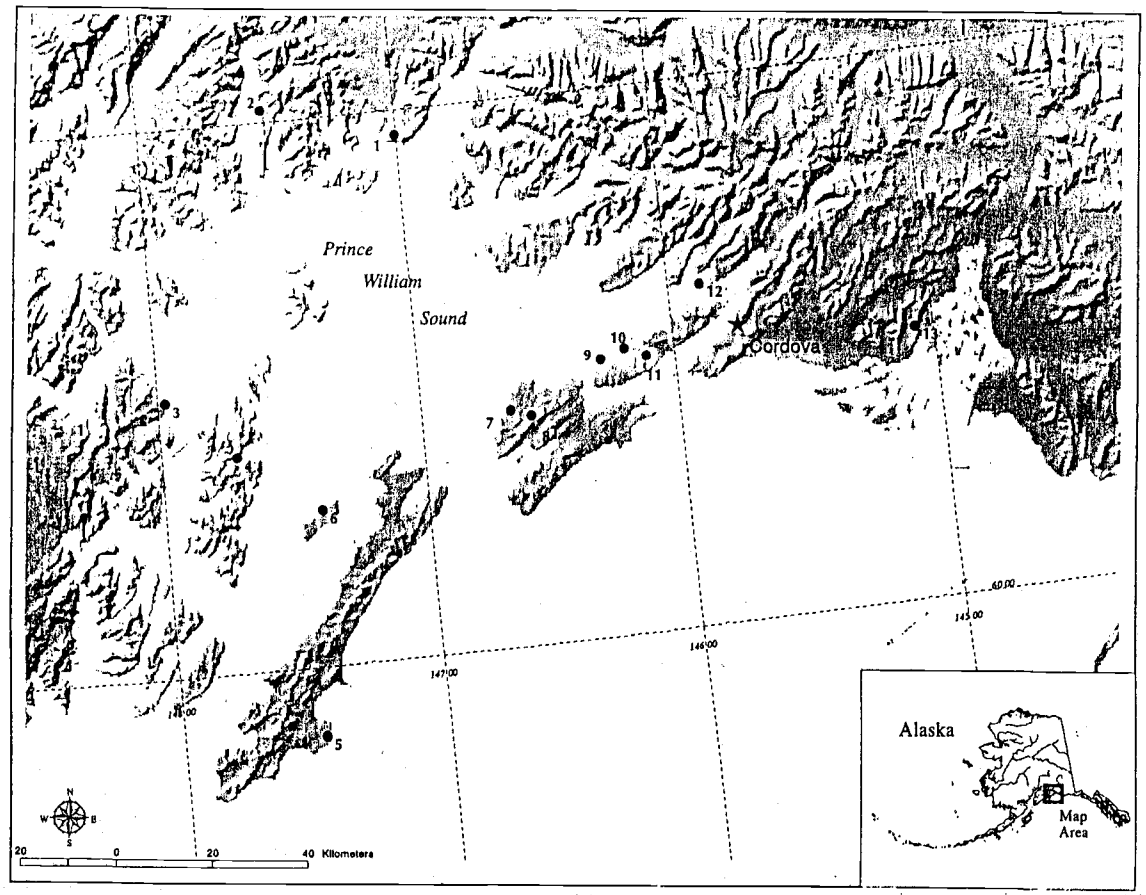

Figure 1. Sampling locations of coastal cutthroat trout collected in 1996 and 1997 in Prince William Sound, Alaska. Locations are as follows: (1) Columbia Bay; (2) Unakwik Inlet; (3) Gunboat Lakes; (4) West Arm, Bay of Isles; (5) Stump Lake; (6) Green Island; (7) Shelter Bay-anadromous; (8) Shelter Bay-resident; (9) Makaka Creek; (10) Hawkins Creek-anadromous; (11) Hawkins Creek-resident; (12) Milton Lake; and (13) 18 Mile Creek.

Table1. Location, sample size (N), of 1996 and 1997 collections of coastal cutthroat trout in Prince William Sound, Alaska. Sites used for allozymes analysis are marked with an "*". Site number refers to location in Figure 1.

Location (site number)	N (1996)	N (1997)
Columbia Bay (1)	not sampled	20
*Unakwik Inlet (2)	20	20
*Gunboat Lake (3)	20	20
West Arm, Bay of Isles (4)	not sampled	20
*Stump Lake (5)	20	20
Green Island (6)	4	13
*Shelter Bay-anadromous (7)	20	20
*Shelter Bay-resident (8)	30	27
Makaka Creek (fin clips only) (9)	20	20
*Hawkins Creek-anadromous (10)	20	20
Hawkins Creek-resident (11)	25	12
*Milton Lake (12)	20	23
18 Mile Creek, Copper River Delta, (13)	20	not sampled

Anadromous coastal cutthroat trout were collected primarily in July, 1996 and 1997. Collections were made in freshwater near the estuary or in the estuary near freshwater. Sampling at these times increased the likelihood of collecting individuals that represent the local population. Resident fish were collected from the stream or lake. Fish were collected with baited minnow traps, seines, electroshockers, and hook and line. Sample sizes ranged from 4-27 individuals per site per year. This is within the range of those reported in recent publications for this species (Wenburg and Bentzen 2001).

At all sites other than 18 Mile and Makaka Creeks, fish were given a lethal dose of MS-222, weighed (to the nearest 0.1 g), fork length measured (to the nearest one mm), and tagged with an identification number. From fish >250 mm, muscle, eye, liver, and heart tissues were removed immediately. The tissues were placed in individual plastic tubes labeled with an identification number, and transported on dry ice to an -80° C freezer at the Alaska Department of Fish and Game office in Cordova, Alaska until they were transported again on dry ice to the Oregon Cooperative Fish Research Unit (OCFRU) laboratory in Corvallis, Oregon. There tissues were removed from the frozen whole fish, transferred while still frozen to 1.7 ml microcentrifuge tubes, and stored at -80° C.

At 18 Mile and Makaka Creeks (Fig. 1 and Table 1), only fin clips were collected. A small piece of the caudal fin (mean size 3 mm^2) was removed from each fish and the fish was released. The clip was immediately placed on dry ice or put in 95% ethanol. Fin clips were used in the DNA analyses only. Coastal

cutthroat trout from archived samples from Ft. Lewis, Washington (Zimmerman et al. 1997) were included in the microsatellite survey as an outgroup.

Microsatellites

DNA was extracted from 50-75 mg of muscle with a phenol:choroform:isoamyl alcohol extraction procedure (Sambrook et al. 1989). Analysis of nuclear DNA microsatellite regions followed the methods of Wenburg et al. (1996), optimized to OCFRU's laboratory conditions. $MgCl_2$ concentrations, primer concentration, and annealing temperature for polymerase chain reactions (PCR) were varied experimentally to ensure that stutter bands had been minimized or eliminated (Wenburg et al. 1996). DNA was amplified using PCR with nine primer pairs (synthesized by Research Genetics, Huntsville, Alabama), in three multiplexing sets (Sets 1a, 1b and 2, Table 2). Multiplexing was used following the protocol outlined in Wenburg et al. (1996).

Total volume of the multiplex set 1a, 1b, and 2 reactions was 20 µl. A reaction cocktail was made consisting of 2 µl 10X buffer, 0.16 µl (0.04 each) dNTP's, 2.0 µl $MgCl_2$, 0.2 µl Amplitaq gold, and 6.64 µl pure water (LGL genetics) for a total volume of 11 µl/sample. Next, a cocktail was made of the target primers in appropriate concentrations consisting of the forward and reverse primer and pure water for each given set. Because primer concentrations vary, thevolume of water was varied to result in a total volume of 8 µl /sample. Respective volumes per individual sample of the two cocktails were pipetted into a

Table 2. Microsatellite primers, multiplexing and amplification protocols for coastal cutthroat trout collected in Prince William Sound, Alaska. See Olson et al. (1996) for primer sequences, references, and source species.

Multiplex Set	Primer	Forward primer label	Concentration	Annealing Temperature
Set 1a	*Sfo*8	Fam	0.20 μM	56° C
	*Ssa*85	Tet	0.30 μM	56° C
	*Omy*77	Hex	0.15 μM	56° C
Set 1b	*One* μ11	Fam	0.10 μM	56° C
	*Ots*1	Tet	0.50 μM	56° C
	*One*μ14	Hex	0.40μM	56° C
Set 2	*One*μ2	Fam	0.60 μM	52° C
	*Ssa*14	Tet	0.55 μM	52° C
	*Omy*325	Hex	0.40 μM	52° C

96 well tray for PCR amplification and 1 μl DNA of was added for each sample. A control was run on each gel to ensure there was no contamination in the cocktail mixture. In addition, a single individual was run on each gel to ensure consistently among samples. Approximately 5% of the total sample was rerun to ensure that results were consistent.

Amplification reactions for PCR products from these primer sets were separated on a denatured polyacrylamide gel using a Perkin Elmer Applied Biosystems, Inc. (ABI) 377 automated sequencer and analyzed using ABI GeneScan 672, analysis software, version 2.0.2. A tamra 350 internal lane standard was used for each individual sample. Genotypes were scored from pherograms with basepair sizing generated by Genotyper software.

Allozymes

Allozyme analysis of coastal cutthroat trout followed the methods of Aebersold et al. (1987). Twenty enzymes encoding 48 loci (Table 3) were examined in coastal cutthroat trout from seven locations sampled in July of 1996 (Table 1). Because there was low variation within sites allozymes analysis was not conducted for samples collected in 1997. Allele designations of the cutthroat trout were determined relative to the mobility of the common allele in a rainbow trout.

Table 3. Enzymes and loci examined with starch gel electrophoresis in coastal cutthroat trout from Prince William Sound, Alaska. Enzyme names are from the International Union of Biochemistry (IUB). Tissues were: M - Muscle, L - liver, E - Eye, and H - Heart. Buffer systems were: TBCLE - a tris-citrate gel buffer and lithium hydroxide borate tray buffer (Ridgeway et al. 1970), TG - tris-glycine ph 8.5, and ACE - an amine-citrate-EDTA gel and tray buffer (Clayton and Tretiak 1972).

I.U.B. Enzyme Name	Locus	Tissue	Buffer
Aspartate aminotranferase (2.6.1.1)	mAAT-1*	E	ACE
	mAAT-2*	E	ACE
	sAAT-1,2*	M	ACE
	sAAT-3*	E	ACE.
Alcohol dehydrogenase (1.1.1.1)	ADH*	L	TBCLE
Adenylate kinase	AK-1*	E	ACE
	AK-2*	E	ACE
Aconitate hydratase (4.2.1.3)	sAH*	L	ACE
Creatine kinase (2.7.3.2)	CK-A1*	M	TBCLE
	CK-A2*	M	TBCLE
	CK-B*	E	ACE
	CK-C1*	E	ACE
	CK-C2*	E	ACE
Fructose-biphosphate aldolase (4.1.1.13)	FBALD-1*	E	TG
	FBALD-2*	E	TG
Glyceraldehyde-3-phosphate dyhydrogenase (1.2.1.12)	GAPDH-2*	H	ACE
	GAPDH-3*	H	ACE
	GAPDH-4*	H	ACE
	GAPDH-5*	H	ACE
Guanine deminase (3.5.4.3)	GDA-1*	L	TBCLE
	GDA-2*	L	TBCLE
Glycerol-3-phosphate dehydrogenase (1.1.1.8)	G3PDH*	M	ACE

Table 3. Continued.

I.U.B. Enzyme Name	Locus	Tissue	Buffer
Glucose-6-phosphate isomerase (5.3.1.9)	GPI-A*	M	TBCLE
	GPI-B1*	M	TBCLE
	GPI-B2*	M	TBCLE
Isocitrate dehydrogenase ($NADP^+$) (1.1.1.42)	sIDH-1,2*	H, L	ACE
	mIDHP-1*	H	ACE
	mIDHP-2*	H	ACE
l-Lactate dehydrogenase (1.1.1.27)	LDH-A1*	M	TBCLE
	LDH-A2*	M	TBCLE
	LDH-B1*	E	TG
	LDH-B2*	E, L	TBCLE
	LDH-C*	E	TG
Malate dehydrogenase (1.1.1.37)	sMDHA-1,2*	H, L	ACE
	sMDHB-1,2*	H, L	ACE
Malic enzyme (1.1.1.40)	MEP-1*	M	ACE
	sMEP-1*	M	ACE
	sMEP-2*	L	ACE
Dipeptidase (3.4.13.18)	PEP-A*	M	TG
Proline dipeptidase (3.4.13.9)	PEP-D*	E	ACE
Phosphogluconate dehydrogenase (1.1.1.44)	PGDH*	M	ACE
Phosphoglucomutase (5.4.2.2)	PGM-1*	L	ACE
	PGM-2*	L	ACE
Superoxide dismutase (1.15.1.1)	sSOD-1*	L	TBCLE
Triose-phosphate isomerase (5.3.1.1)	TPI-1*	M	TG
	TPI-2*	M	TG
	TPI-3*	E	ACE
	TPI-4*	E	ACE

Statistical analysis

Gene diversity estimates were calculated for microsatellite data to examine variation within sampling sites. Differences between the two sampling years were tested for genotypic differentiation for each population pair at each locus (Goudet et al. 1995). Data were tested for departures from Hardy-Weinberg equilibrium at each locus for each population with probability exact tests (Haldane 1954) using the computer program BIOSYS (1.7), which allows for the pooling of rare alleles, those that occur at low frequencies.

Microsatellites are hypervariable and can have many alleles at a locus (Hedrick 1999). In moderate sample sizes, it may not be possible to collect individuals with all possible genotype combinations. Alleles that occur in low frequencies can result in low expected values, therefore pooling of alleles with low expected values may be warranted in these cases (see Weir 1996 for discussion).

Populations that have been recently colonized may have undergone population bottlenecks. During population bottlenecks, populations are not in drift-migration equilibrium and rare alleles are lost at a higher rate than alleles present in intermediate frequencies (Cornuet and Luikart 1996). The loss of rare alleles has little effect on heterozygosity. Thus, bottlenecked populations may generate a heterozygote "excess" relative to the number of alleles sampled. Tests for signatures of bottlenecks were conducted with the computer software program BOTTLENECK (Cornuet and Luikart 1996). A two-phased model of mutation

(TPM) suggested by Cornuet and Luikart (1996) and a Wilcoxon test for statistical significance was used. In addition, a descriptive technique was used which relies on a mode-shift of allele distributions. To test for genetic differences within and among sites that had undergone recent colonization samples consisting of two groups, those from recently colonized sites (Columbia Bay and Unakwik Inlet) and those not recently colonized (which consisted of all samples excluding Ft. Lewis, WA) were created. Goodness of fit tests for significant differences of the following estimates were made: 1) within sample gene diversity (Hs); 2) observed proportion of heterozygotes (Ho); and 3) an estimate of allelic richness corrected for sample size using a rarefaction method (El Mousadik and Petit 1996) using the software program FSTAT 2.9.3.2 (Goudet 1995). For all tests, significance levels were determined by 1000 re-sampling permutations.

For microsatellites, differences among collection sites with sampling years pooled were examined by testing the null hypothesis that genotypic distributions were identical in three arrays: 1) across all loci at all populations: 2) at individual loci over all populations: and 3) for all pairs of populations using a log-likelihood (G) based exact test (Raymond and Rousset 1995a). These tests were done on pooled data from the two sampling years. All of the pairwise comparisons were made with the computer program GENEPOP 3.1c. (Raymond and Rousset 1995b). Markov chain parameters were used to estimate p-values with dememorization number, batch number, and iterations set at 1000, 50 and 1000 respectively.

Sequential Bonferonni corrections to significance values were applied when multiple comparisons were made (Rice 1989).

A number of tests were performed to examine the variance in microsatellite gene frequencies among populations. Weir and Cockerman's θ, an analogue of Wright's F_{st} was calculated. Jacknifing over all loci produced a mean and standard error for overall θ values. To test if θ values were significantly different than zero, sampling with replacement using bootstrapping over all loci was conducted and a 95% confidence interval was estimated. G_{st} estimates were calculated for populations (Nei 1987) with the exception of Ft. Lewis which had been included as an outgroup. These tests were performed using the statistical package FSTAT for windows v2.8 (Goudet 1995). Interpretation of the range of G_{st} values (low 0-0.05, moderate 0.05-0.15 moderate, high 0.15-0.25) follow those recommended by Hartl and Clark (1997). Estimates of gene flow were made using a private allele model (Slatkin 1985).

Patterns of geographical genetic similarity of microsatellites were identified by constructing UPGMA phenograms from cluster analyses of pair-wise estimates of similarity between samples using the modified Cavalli-Sforza distance (Nei et al. 1983). This method is unweighted and requires equal rate of evolution among the taxonomic units being compared. This assumption is probably reasonable when making comparisons within a species. In this study, recently colonized sites may have undergone genetic drift, stretching this assumption. Therefore, multidimensional scaling based on modified Cavalli-Sforza distance (Nei et al.

1983) was also used to examine spatial relationships for the microsatellite data. To test for correlation between geographic distance and pairwise Gst estimates (isolation by distance) a mantel test was used (Mantel 1967) using the computer program GENEPOP 3.1c. (Raymond and Rousset 1995b). Migration distances were estimated by following shoreline distances from USGS quadrangle maps.

To examine allozyme variation within sampling sites, expected average heterozygosity (percent variation at a locus) and mean number of alleles per locus and percent polymorphic loci were calculated. Data were tested for departures from Hardy-Weinberg equilibrium at each locus for each population with probability exact tests (Haldane 1954) using the computer program BIOSYS (1.7). Tests for bottlenecks were conducted using the same methods as for microsatellites.

Differences among samples were examined in allozymes by testing the null hypothesis that genotypic distributions were identical in three arrays: 1) across all loci at all populations: 2) at individual loci over all populations: and 3) for all pairs of populations using a log-likelihood (G) based exact test (Raymond and Rousset 1995a). All of these comparisons were made with the computer program GENEPOP 3.1c (Raymond and Rousset 1995b). Markov chain parameters were used to estimate P-values with dememorization number, batch number, and iterations set at 1000, 50 and 1000 respectively. Sequential Bonferonni corrections to significance values were applied when multiple comparisons were made (Rice 1989).

A number of tests were performed to examine the variance in allozyme gene frequencies among populations. Weir and Cockerman's θ, an analogue of Wright's F_{st} was calculated. Jacknifing over all loci produced a mean and standard error for overall theta values. To test if θ values were significantly different than zero, sampling with replacement using bootstrapping over all loci was conducted and a 95% confidence interval was estimated. These tests were performed using the statistical package FSTAT for windows v2.8 (Goudet 1995). Interpretation of the range of G_{st} values (low 0-0.05, moderate 0.05-0.15 moderate, high 0.15-0.25) follows that recommended by Hartl and Clark (1997).

For allozymes, patterns of geographical genetic similarity were identified by constructing (unweighted pair group method using arithmetic averages) UPGMA phenograms from cluster analyses of pair-wise estimates of similarity between samples using the modified Cavalli-Sforza distance (Nei et al. 1983).

Results

Microsatellites

Within populations

Five microsatellite loci, *Omy*325, *Ssa*14, *Oneμ*2, *Ssa*85, and *Sfo*8 were scored in coastal cutthroat trout from 13 locations in Prince William Sound and Ft. Lewis, Washington. A significant difference between sampling years was detected

at one site with one locus (Gunboat Lake, *Ssa*14). However, because this occurred in only one instance for the remainder of the analysis, sampling years were pooled.

In general, microsatellite loci were highly polymorphic in cutthroat trout populations. The number of alleles at each locus ranged from 19 to 36 (Table 4). New alleles were observed in all loci that increased the range of base pair sizes observed by Wenberg et al. (1996). Estimates of gene diversity for each locus at each population ranged from 0.053 to 0.956 and (Table 4). Loci *Omy*325 and *Sfo*8 were nearly fixed for a single allele in the cutthroat trout from Columbia Bay, a site recently de-glaciated, leading to low estimates of gene diversity. Genetic variation was generally lower in recently colonized sites relative to other sites in Prince William Sound. Estimates of observed heterozygosity, expected heterozygosity, and allelic richness were statistically lower in recently colonized sites than in other Prince William Sound sites ($P < 0.05$) (Table 5). These estimates suggest that allelic diversity as well as heterozygosity is reduced in these sites. Deviations from Hardy-Weinberg equilibrium were detected in 24 of the 70 tests that were conducted after corrections for multiple tests (initial $P < 0.05$; Table 4). Deviations between observed and expected heterozygosity were in all cases due to a deficiency of heterozygotes. Because the deviations were not restricted to a single locus or population, it was assumed that the deviations were not due to null alleles and statistical analysis that require Hardy-Weinberg equilibrium were conducted.

Bottlenecks were detected in some populations of coastal cutthroat trout in Prince William Sound. A mode-shift of allele frequency distributions was detected

Table 4. Estimated gene diversity per locus per site and averaged over all loci, G_{st} values for each locus in coastal cutthroat trout microsatellites from Prince William Sound, Alaska. An outgroup from Ft. Lewis, Washington is included. Allele size ranges are in parentheses. "*" depict deviations from Hardy-Weinberg equilibrium. Site numbers refer to locations in Figure 1.

Site (site number)	*Omy*325 (98-194)	*Ssa*14 (106-136)	*One*_2 (197-253)	*Sfo*8 (187-239)	*Ssa*85 (98-194)
Columbia Bay (1)	0.095	*0.458	0.900	0.053	0.757
Unakwik Inlet (2)	*0.856	*0.567	0.768	0.250	0.767
Gunboat Lakes (3)	*0.125	*0.905	*0.860	0.917	0.956
West Arm (4)	*0.440	0.625	0.673	0.838	0.840
Stump Lake (5)	*0.806	0.771	*0.803	0.791	*0.920
Green Island (6)	0.808	0.747	0.892	0.759	0.929
Shelter Bay, anadromous (7)	*0.898	0.779	*0.876	0.839	0.923
Shelter Bay, resident (8)	0.918	*0.644	*0.812	0.863	0.944
Makaka Creek (9)	*0.933	0.740	0.902	0.801	0.882
Hawkins Creek, anadromous (10)	0.933	*0.652	0.853	0.830	0.943
Hawkins Creek, resident (11)	0.813	*0.869	*0.815	0.852	0.913
Milton Lake (12)	0.833	*0.816	*0.838	0.818	0.942
18 Mile Creek (13)	0.874	*0.788	*0.640	0.606	*0.712
Ft. Lewis, WA	*0.603	0.831	0.853	0.872	0.904
Nei's G_{st}	0.229	0.075	0.079	0.142	0.060

Table 5. Comparisons of allelic richness, observed heterozygosity (Ho), gene diversity (Hs) based on coastal cutthroat trout microsatellites between groups of recently colonized sites (Columbia Bay, Unakwik Inlet) with sites not recently colonized (all remaining Prince William Sound sites, see Fig.1) in Prince William Sound, Alaska. Significance levels were ** $P < 0.01$ and * $P < 0.05$.

	Recently colonized	Not Recently colonized
Allelic richness	** 3.327	5.347
Ho	* 0.225	0.444
Hs	* 0.559	0.807

in the Green Island and West Arm populations, which is consistent with a recent bottleneck event. Using the Wilcoxon test, an excess of heterozygosity relative to that expected under drift-mutation equilibrium was detected in 18 Mile Creek, Hawkins Creek resident, and West Arm populations ($P < 0.05$), which suggests that these sites have undergone a recent and severe bottleneck. This test did not identify a bottleneck in Green Island. However, a small sample size in three of the five loci (N=14) at Green Island may have biased this test.

Evidence of population expansion was detected in two sites. A deficiency of heterozygotes relative to drift-mutation equilibrium was detected in Shelter Bay anadromous and Unakwik Inlet trout populations ($P < 0.05$). These results suggest that these populations have recently received an influx of rare alleles.

Among populations

Significant differences were detected among 65 of 78 pair-wise comparisons of populations of cutthroat trout (Table 6) following corrections for multiple tests (initial $P < 0.05$). The number of loci in which significant differences (initial $P > 0.05$) were detected ranged from one to five. There is a general trend that anadromous populations in the east side of the Prince William Sound (Milton Lake, Makaka, Hawkins Creek, and Shelter Bay) were not significantly different (initial $P > 0.05$) from one another. The exception being that the Shelter Bay anadromous population and Makaka Creek are significantly (initial $P > 0.05$)

different from one another (Table 6). Resident sites were not statistically different from their anadromous counterparts (Table 6).

Using UPGMA, Milton Lake, Makaka Creek, Shelter Bay anadromous and resident, and Hawkins Creek anadromous and resident clustered together with a bootstrap value of 91% (Fig. 2). Multidimensional scaling (MDS) analysis (Fig. 3) shows a tight cluster of the sites Milton Lake, Makaka Creek, Shelter Bay anadromous and resident, and Hawkins Creek anadromous and resident, which is similar to the cluster depicted in UPGMA. Other sites, including Columbia Bay and Unakwik Inlet, were loosely scattered around the plot (Fig 3).

There were moderate genetic differences estimated with microsatellites among populations of coastal cutthroat trout in Prince William Sound. Weir and Cockerman's θ estimated from all of the loci over all populations, excluding Ft. Lewis, was 0.110. The range of the 95% confidence interval was above zero. Over all loci, G_{st} values were 0.118, very close to the estimate given by θ, both of which are similar to what was observed with allozymes. Gst values per locus ranged from 0.060-0.229 (Table 4). The estimated number of migrants per generation, Nm, was 5.4 using the private allele method (Slatkin 1985). There was no evidence of isolation by distance ($F > 0.05$).

Location (site number)	Unakwik Inlet (2)	Gunboat Lake (3)	West Arm (4)	Stump Lake (5)	Green Island (6)	Shelter Bay, anadromous (7)	Shelter Bay, resident (8)	Makaka Creek (9)	Hawkins Creek, anadromous (10)	Hawkins Creek, resident (11)	Milton Lake (12)	18 Mile Creek (13)
Columbia Bay (1)	***	***	**	***	***	**	***	***	***	***	***	****
Unakwik Inlet (2)		****	****	*****	****	**	**	**	****	***	***	****
Gunboat Lake (3)			**** *	****	****	***	****	***	****	****	****	*****
West Arm (4)				***	***	**	**	*	***	**	**	***
Stump Lake (5)					*	**	***	*	***	****	**	**
Green Island (6)						*	*	*	*	***	*	*
Shelter Bay, anadromous (7)							NS	*	NS	*	NS	*
Shelter Bay, Resident (8)								NS	NS	**	NS	***
Makaka Creek (9)									NS	NS	NS	NS
Hawkins Creek, anadromous (10)										NS	NS	**
Hawkins Creek, resident (11)											NS	*
Milton Lake (12)												***

Table 6. Pairwise differences among coastal cutthroat trout populations in Prince William Sound, Alaska based on five microsatellite loci: *Omy 325, Ssa 14, Sfo8, One_2, Ssa85.* "*" refers to number of loci significantly different. Significant levels adjusted for multiple test following Rice (1989) initial P value < 0.05. NS–not significant. Site numbers refer to locations in Fig. 1.

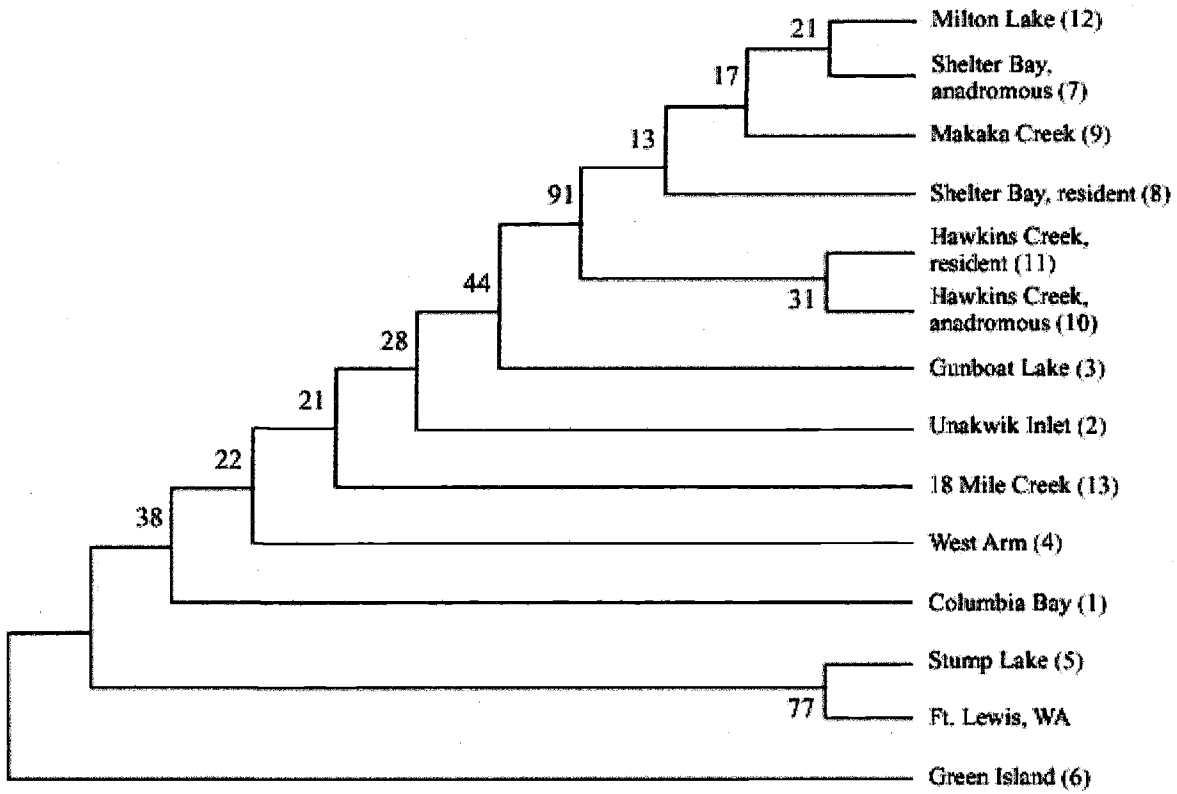

Figure 2. Genetic similarity of coastal cutthroat trout from 13 locations in Prince William Sound, Alaska and one outgroup from Ft. Lewis, WA based on microsatellite DNA variation. Numbers at nodes are bootstrap values (%) based on 1000 simulations. Number in parentheses refers locations in Figure 1.

Figure 3. Multidimensional scaling of microsatellite loci based on Cavalli-Sforza genetic distance measures in coastal cutthroat trout from 13 locations in Prince William Sound, Alaska and one outgroup site from Ft. Lewis, WA.

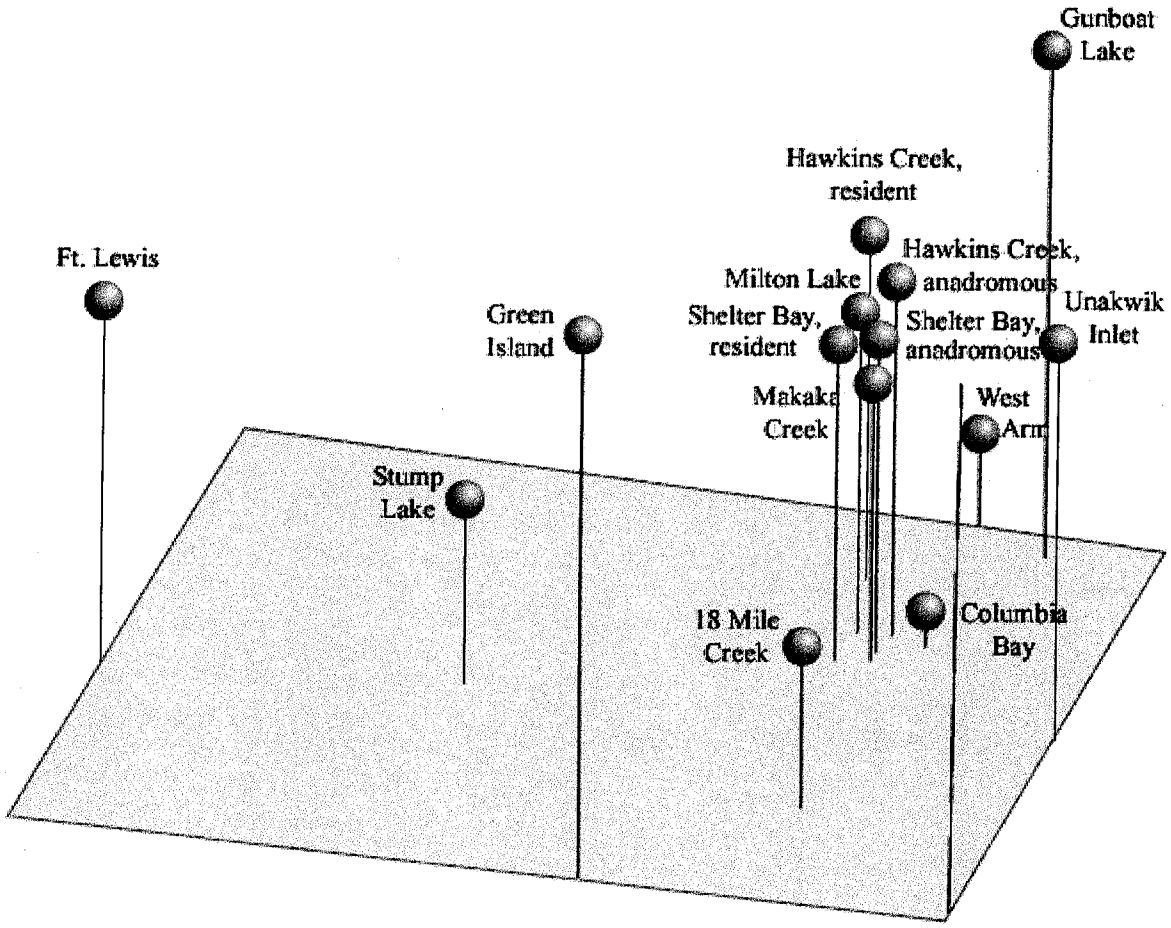
Gunboat Lake
Hawkins Creek, resident
Hawkins Creek, anadromous
Ft. Lewis
Milton Lake
Unakwik Inlet
Green Island
Shelter Bay, resident
Shelter Bay, anadromous
Makaka Creek
West Arm
Stump Lake
18 Mile Creek
Columbia Bay

Figure 3.

Allozymes

Within populations

Eleven of the 40 allozyme loci examined in coastal cutthroat trout were polymorphic at the 0.99 criterion level. Average heterozygosity ranged from 0.039-0.065, polymorphic loci ranged from 10-20%, and mean number of alleles per loci ranged from 1.07-1.3 (Table 7). These estimates are consistent with estimates observed in Washington (Campton and Utter 1987) and Oregon (Griswold 1996). No deviations from Hardy-Weinberg equilibrium were detected using exact tests following corrections for multiple tests (initial P value < 0.05). A mode shift in the distribution of allele frequencies was detected in Unakwik Inlet, Milton Lake, and Shelter Bay resident populations. However, Wilcoxon tests for bottlenecks were not significant in these sites ($P > 0.05$). Unakwik Inlet, which was recently de-glaciated, had low average heterozygosity as well as a low number of mean alleles. The average heterozygosity (0.039) in the Shelter Bay resident population was the lowest we documented, but the mean number of alleles per locus (1.23) and the percentage of polymorphic loci (0.175) were greater than most anadromous populations (Table 7).

Table 7. Average heterozygosity, mean number of alleles per locus, and percent polymorphic loci (95% criterion) at allozyme loci for coastal cutthroat trout collected in July, 1996 in Prince William Sound, Alaska. Number in parentheses refer to locations in Fig. 1.

Location	Average heterozygosity	Mean number alleles/locus	% polymorphic loci
Unakwik Inlet (2)	0.040	1.07	10
Gunboat Lake (3)	0.055	1.17	15
Stump Lake (5)	0.051	1.20	15
Shelter Bay, anadromous (7)	0.055	1.17	15
Shelter Bay, resident (8)	0.039	1.23	17.5
Hawkins Creek anadromous (10)	0.051	1.27	17.5
Milton Lake (12)	0.065	1.3	20

Among populations

There were significant differences of genotypes ($P < 0.0001$) over all populations when loci were pooled. The following individual loci were significantly different ($P < 0.005$) in all populations: AK-1, CKC2, GDA-1, GDA-2, and sMEP-1. Significant differences (initial $P < 0.05$) in 11 of 21 pairwise tests in the seven populations of coastal cutthroat trout were detected (Table 8). At most, two alleles were significant in any given comparison (initial $P < 0.05$). Moderate genetic differences were detected with allozymes among populations in Prince William Sound. Weir and Cockerman's θ estimated from all of the polymorphic loci over all populations was 0.095, suggesting. The range of the 95% confidence interval of this estimate was above zero. Gst was 0.12. Both estimates suggest moderate genetic differences among groups.

There appeared to be no detectable pattern relative to geography using UPGMA, with the exception that the anadromous population at Shelter Bay appeared to be separated from all other populations in UPGMA analysis (Fig. 4). This is different from what was observed with microsatellites in that the Shelter Bay resident and anadromous fish clustered closely together.

Table 8. Genetic differences at allozyme loci among coastal cutthroat trout collected in July 1996 in Prince William Sound, Alaska. Significant levels adjusted for multiple test following Rice (1987) initial P value < 0.05. NS denotes no significant difference. Codes for loci are: a = AK-1*; b = CK-C2*; c = GDA-2*; and d = sSOD-1*. Number in parentheses refers to location in Fig. 1.

Location	Unakwik Inlet (2)	Gunboat Lake (3)	Stump Lake (5)	Shelter Bay, anadromous (7)	Shelter Bay, resident (8)	Hawkins Creek, anadromous (10)	Milton Lake (12)
Unakwik Inlet (2)		NS	b.d	a,c	NS	NS	NS
Gunboat Lake (3)			B	a,c	NS	NS	NS
Stump Lake (5)				b,c	NS	B	NS
Shelter Bay, anadromous (7)					c	A	a,c
Shelter Bay, Resident (8)						C	NS
Hawkins Creek, anadromous (10)							c

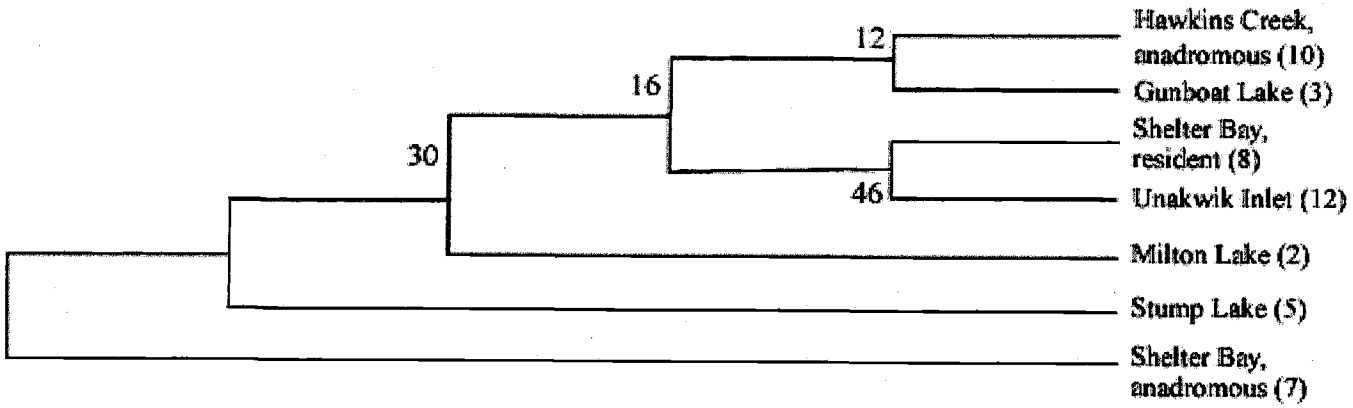

Figure 4. Genetic similarity based on modified Cavalli-Sforza distance estimates of coastal cutthroat trout from seven locations collected in July, 1996 in Prince William Sound, Alaska, using allozymes. Number in parentheses refers to location in Figure 1.

Discussion

Colonization and genetic diversity

This study presents evidence that coastal cutthroat trout from recently colonized sites (Columbia Bay and Unakwik Inlet) have low genetic variation relative to other sites in the Prince William Sound, Alaska region. These results are a possible consequence of a founder effect from a small number of individuals colonizing these sites, time since initial colonization, and low dispersal capacities of coastal cutthroat trout. Genetic diversity may be reduced when a small number of individuals colonize an area (Hedrick et al. 2001). Coastal cutthroat trout may be especially susceptible to founder effects because of their limited migratory capacity and small population sizes (Jones and Seifert 1997, Wenburg and Bentzen 2001) which may lead to small numbers of individuals founding new populations. Alternatively, populations could have lost genetic diversity more recently due to genetic drift, if there was a reduction of population effective size due to a catastrophic event. The results in this study differ from those observed in sockeye salmon (Whithler et al. 2000). These researchers found no evidence of reduced genetic diversity in sockeye salmon in the Fraser River following a severe reduction in effective population size following a rock slide that barred migration

of returning adults. The researchers suggested the inability to detect a bottleneck could have resulted from gene flow following the rockslide, a larger effective size than suspected, or rapid expansion of population size following the recovery of these fish (Whithler et al. 2000).

Although coastal cutthroat trout in Columbia Bay and Unakwik Inlet had low levels of heterozyosity and allelic richness, there was no evidence of recent population bottlenecks in either of these sites. In bottlenecked populations allelic diversity is lost more rapidly than heterozygosity (Leberg 1992). In Columbia Bay, two loci, *Sfo8* and *Omy325,* had lower levels of gene diversity, or heterozygosity, (0.053, 0.095 respectively) than any other site in Prince William Sound. These estimates were also lower than the lowest estimate (0.16) observed in Puget Sound by Wenburg and Bentzen (2001). It is possible that because there were estimates of low heterozygosity as well as low allelic diversity in Columbia Bay the Wilcoxon and mode-shift tests were unable to detect a bottleneck in Columbia Bay (Cornuet and Luikart 1996).

In Unakwik Inlet, a recent influx of rare alleles was detected providing evidence that the site has recently expanded or has undergone gene flow. Theory predicts that, in some cases, after a founding event populations will rapidly increase in size dampening the reduction in genetic variation (Wakely 2000). It is also possible that rare alleles from other populations were introduced to Unakwik Inlet via gene flow. However, pairwise tests and clustering tools suggest that Unakwik

is genetically distinct from other sites in Prince William Sound, which suggests gene flow may be limited between this and other sites in Prince William Sound.

Heterozygote deficiencies were observed in microsatellites that resulted in deviations from Hardy-Weinberg equilibrium. (To clarify, heterozygote deficiency used to test for bottlenecks in populations is based on drift-equilibrium conditions, not Hardy Weinberg equilibrium, the random union of gametes each generation). Other studies have observed similar deviation from Hardy-Weinberg equilibrium in rainbow trout (Nielsen et al. 1994) cutthroat trout (Wenburg and Bentzen 2001) and Atlantic salmon (*S. salar*) (Letcher and King 2001). Deviation from Hardy –Weinberg equilibrium could reflect either a sampling bias or it could accurately reflect a deficiency in heterozygotes in some loci in some populations. Sampling bias could come from the analysis of the microsatellites or the collection of the samples. The microsatellite loci chosen for this study were based on the polymorphic loci and multiplexing design as suggested by Wenburg et al. (1996). Null alleles were not detected in these loci in any previous studies. In this study, small sample sizes relative to the number of rare alleles could have resulted in a sampling bias.

Finally, a deficiency of heterozygotes could have resulted from sampling bias of collecting mixed populations (Hartl and Clark 1997). This is unlikely as sampling was designed to target mixed age class individuals as they were returning to river mouths for spawning migrations, however, a portion of coastal cutthroat trout may have returned to overwinter in non-natal streams and could have been

included in the collection. Hardy-Weinberg equilibrium tests the null hypothesis that the genotypes in a sample result from random mating. Random mating is based on several assumptions including no mutation, migration, genetic drift, selection, or mutation. Half of the tests that detected deviations from Hardy-Weinberg equilibrium were from sites that have undergone recent bottlenecks (Green Island, West Arm, 18 Mile Creek, and Hawkins Creek, resident) or relatively recent colonization (Columbia Bay and Unakwik Inlet). The effects of these events may lead to non-equilibrium conditions. This assumes that there was no sampling bias, and is therefore tentative, however, it is consistent with biological knowledge of this species and theoretical predictions (Santos 1994).

Genetic diversity among populations

Moderate levels of genetic variation among coastal cutthroat trout sites based on allozymes (9.5%) and microsatellites (11%) was detected using G_{st} and θ values. These estimates of genetic variation among populations in this study were greater than those observed in coastal cutthroat trout by Campton and Utter (1987) in Puget Sound (6% using allozymes) and by Wenburg and Bentzen (2001) in Washington (8.8% among 22 streams using microsatellites). Recent colonization may lead to genetic drift which, in turn, can increase variation among sites (Hartl and Clark 1997). Thus, while the levels of variation among populations detected in this study may be higher than those observed by others, some of this variation may result from sites that may have been recently founded. Bottlenecks were detected

in three populations. These bottlenecks also may have contributed to the differences among sites observed in this study.

Hedrick (1999) warns that microsatellites, because of their high variability may result in large Fst values (similar to Gst) that resulted from random events, like bottlenecks, and argues that these estimates may not have biological meaning (Hedrick 1999). In this study, however, lower genetic variation in recently founded populations and bottlenecked populations may represent important features of these populations. Probability of extinction and lowered fitness, which may result from low genetic diversity within populations, is an issue of concern for managers. Therefore, the genetic effects of bottlenecks and founding events in coastal cutthroat trout is important biologically, and may effect their genetic structure at the landscape level.

In two sites where bottlenecks were detected, Green Island and West Arm, populations of coastal cutthroat trout may have undergone a severe reduction in size during the 1989 Exxon Valdez Oil spill that occurred in Prince William Sound (McCarron and Hoffmann 1993). These locations were in the direct path of the oil spill. The extent and duration of the damage is unknown, however. There are no known causes for potential bottlenecks in 18 Mile Creek. While speculative, it is possible that coastal cutthroat trout underwent a population bottleneck during the resulting uplift from the 1964 in the Copper River Delta.

In Prince William Sound the pattern observed in the genetic diversity among sites was complex. Estimates of genetic distance and pair-wise differences

in allele frequencies suggest that populations of anadromous coastal cutthroat trout were, in many cases, highly divergent from one another and in other cases, similar to one another. Physical features of Prince William Sound and limited movement of coastal cutthroat trout in salt water were likely responsible for this pattern. In Prince William Sound Alaska, coastal cutthroat trout have limited marine migrations, average distance of recaptured coastal cutthroat from an original tagging site was 2 km (Bernard et al 1995). Also, coastal cutthroat trout move along shorelines and estuaries (Jones and Seifert 1997). In western Prince William Sound, populations of coastal cutthroat trout are isolated from one another by deep water, which may limit exchange among these among anadromous coastal cutthroat trout populations in Prince William Sound. Trout populations in eastern Prince William Sound are relatively close to each other and the areas between them are relatively shallow possible facilitating exchange among populations. This may partially explain the tight clustering of Makaka Creek, Hawkins Creek, Shelter Bay, and Milton Lake trout populations using UPGMA and MDS. The complex spatial structure of tight clustering of some sites and large genetic distance with little spatial structuring between other sites may have resulted in the lack of correlation between genetic distance and geographic distance.

Some features such as proximity and water depth may provide afford opportunities for gene flow among coastal cutthroat trout populations in Prince William Sound. In general, the genetic variation among populations within a species is a consequence of gene flow and the opposing force of genetic drift. Gene

flow, the movement of genetic material among populations, results in the homogenization of populations (Avise 1994). Small isolated populations may be susceptible to genetic drift, which in turn, may result in differentiation among populations (Slatkin 1995). In some populations of coastal cutthroat trout in Prince William Sound, gene flow appears to be restricted among populations. In addition, it is suspected that some of these populations are small (McCarron and Hoffmann 1993). Genetic drift may occur in cutthroat trout when populations are isolated by physical habitat such as islands and waterfalls, or when new habitats are colonized. Estimates of number of migrants per generation (Nm) for coastal cutthroat trout in Prince William Sound were relatively low. The estimate is in the low range of what is thought to mitigate the effects of genetic drift and in turn maintain genetic diversity in conservation applications (Mills and Allendorf 1996).
Small populations that are isolated such as these may be susceptible to stochastic events (Lande 1988). Such populations are at increased risk of local declines and local extinction (Soulé 1983). The high degree of isolation may be an issue of concern for management of these species.

There appears to be no significant differences in estimates of genetic diversity (allelic richness, Ho, Hs) between resident and anadromous pairs ($P > 0.05$). In addition, using microsatellites, resident populations (Hawkins and Shelter) appear to be similar to their anadromous counterparts based on clustering and ordination tools as well as pairwise tests. This suggests that these sites have been recently isolated or that there is one-way gene flow. In resident fish in

Hawkins Creek, a bottleneck was detected suggesting that when these became isolated they experienced a loss of genetic diversity. In other studies differences between resident and anadromous forms of coastal cutthroat trout appear to be associated with local conditions. Unique mtDNA RFLP haplotypes were detected in resident coastal cutthroat trout in Elk River, Oregon (Griswold 2002). However, using allozymes (Griswold 1996) found that in some case genetic differences were detected between above and below barrier pairs, and in other cases, no differences were detected.

The degree of genetic differences between resident and anadromous populations depends on the extent to which the upstream movement of anadromous fishes is restricted, the possibility of one-way gene flow (from above to below the barrier), and age and persistence of the barrier. It appears in this study that the barriers are not controlling either the upstream or downstream movement of trout, leading to genetic similarities of the forms.

Comparison of allozymes and microsatellites

Microsatellite and allozymes markers led to similar results in some, but not all estimates. Overall, the microsatellites had higher estimates of heterozygosity and allelic diversity than allozymes. Both resulted in comparable estimates of Gst and θ, which suggested moderate genetic differences among populations. These results differ from Allendorf and Seeb (2000) who detected lower Fst values for sockeye salmon with allozymes than with microsatellites. High allelic diversity of

microsatellites may bias estimates of Fst (Hedrick 1999) particularly when compared with allozymes, but this was not apparent in this study. Microsatellites and allozymes were effective at detecting pairwise differences, although in specific cases, there was no concordance between the tools. For instance, Shelter Bay resident and anadromous pairs were not significantly different with microsatellites but were with allozymes. This may be a result of random chance, as a bottleneck was detected in the Shelter Bay resident site that would increase the likelihood of genetic drift. It is also possible that an allozymes locus is under selection (Allendorf and Seeb 2000). Allozymes are protein-coding and while they are considered neutral markers, they may in some case be under selection or linked to traits under selection (Avise 1994).

Summary

This study suggests that in streams recently founded by coastal cutthroat trout there are low measures of within population genetic diversity relative to other populations that were not recently founded. Local conditions may lead to genetic structure in coastal cutthroat trout. Populations of coastal cutthroat trout in eastern Prince William Sound are more genetically similar to one another than populations in western Prince William Sound because western populations are isolated, perhaps by water depth, which may have restricted movement among populations. The genetic structure of coastal cutthroat trout in some sites in Prince William Sound

may reflect the constraints of isolation due to colonization, geographic isolation, and small population size, and in some sites bottlenecks were detected. The relatively low genetic diversity in these sites may suggest these populations may be at increased risk of extripation and stochastic forces.

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CHAPTER 4

DISPERSAL AND GENETIC DIVERSITY IN DOLLY VARDEN IN PRINCE WILLIAM SOUND, ALASKA

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Introduction

Understanding genetic structure, or the partitioning of variation within and among populations within a species, is a critical scientific and management need (Avise 1994). Genetic structure, in general, is assumed to reflect historic processes of gene flow and genetic drift (Ryman and Utter 1987). Because of the relatively high rate of homing in salmon and trout (*Oncorhynchus* spp), genetic variation tends to be high among watersheds (Altukov and Salmenkova 1991). Straying, or dispersal, may lead to genetic similarity among watersheds. In salmonids, dispersal may be an important mechanism for recovery in dynamic environments (Reeves et al. 1995), as well as for colonization of newly available habitats (Milner et al. 2000).

Dolly Varden (*Salvelinus malma*) are noted for their complicated life history, which may consist of resident and anadromous forms. The anadromous form has a complex migration cycle that is reliant on suitable freshwater overwintering habitat (Armstrong and Morrow 1984, Bernard et al. 1995). Individuals spend up to four years in freshwater before they smolt and migrate to marine environments where they are capable of extended migrations of up to hundreds of kilometers (DeCicco 1997). Returning adults may home to natal streams for overwintering or spawning or they may overwinter in lakes (Armstrong and Morrow 1980). In Prince William Sound (PWS), Alaska, Dolly Varden travel extensively during their marine migration (Bernard et al. 1995). Bernard et al.

(1995) found that fishes that were recaptured in streams tended to have high homing precision (98%). However, a large proportion (14-58%) of the population that were marked at the time of outmigration were never recaptured. Whether the surviving fish return to natal streams for spawning after overwintering in saltwater or non-natal lakes, or disperse to other stream to spawn is unknown.

Dispersal capability of Dolly Varden may influence genetic variation within and among populations of Dolly Varden. In some species, such as coastal cutthroat trout (*O. clarki clarki*),which have limited dispersal, a relatively large degree of genetic variation among populations has been detected (Wenburg and Bentzen 2001). In the Beaufort Sea, using mixed stock analysis, Everett et al. (1998a) found mixed collections of Dolly Varden from sites up to 350 km apart. Consistent with high rates of dispersal, Milner et al. (2000) found that Dolly Varden colonize new habitats more rapidly than other fishes. In contrast to this, there is some evidence that the genetic structure of Dolly Varden reflects some degree of homing. In the Beaufort Sea, 8% of the genetic variation observed among populations was attributed to differences among local populations using allozymes. This suggests that homing behavior in Dolly Varden may lead to genetic differences among watersheds (Everett et al. 1998b). Further examination of the genetic structure of Dolly Varden may clarify the issue of homing and dispersal within this species as well as the effects of colonization on the genetic variation within populations of this species.

There are a number of molecular tools used to examine the genetic diversity within and among species at a variety of spatial scales. Allozymes are protein-coding loci that may vary in biochemical structure without affecting the function of the enzymes (although see Karl and Avise 1992). Allozyme variability has been an essential tool in fisheries genetics for many decades. Relative to newly developed DNA tools, such as microsatellites, allozymes have low levels of variation (Hedrick 1999) which can be a disadavantage when trying to discern patterns at a small scale. However, they are still commonly used and extensive baseline data exists on salmonid populations.

Microsatellites are a class of genetic markers that have variable repeated nuclear DNA sequences. They are highly variable and have a rapid mutation rate. There is considerable debate in the literature as to the model of mutation that microsatellites undergo (O'Connell et al. 1997). It is clear that they are suited for small-scale population genetic studies and family and pedigree analysis (Blouin et al. 1996, Letcher and King 2000), and are being applied to fisheries problems at an increasing rate. In this study they provided a promising tool to examine genetic variation in Dolly Varden at the regional and local scale. In addition, because microsatellites have high allelic diversity they are sensitive to the effects of population bottlenecks (Hedrick 1999). Population bottlenecks in Dolly Varden, may occur when new habitats are colonized.

The goal of this study was to examine genetic variation of Dolly Varden populations in Prince William Sound, Alaska. There were two objectives: 1) to

examine the genetic variation within and among anadromous and resident populations and, 2) to examine the genetic variation within a relatively recently founded population of Dolly Varden in PWS. Prince William Sound was de-glaciated approximately 8,000-12,000 years ago (Lethcoe 1990). Within PWS there are active glacial advances and retreats that in some cases only recently (350 years ago) created stream habitat for Dolly Varden (Ken Hodges, Cordova Ranger Station, Cordova, Alaska.) This provides an opportunity to examine the genetic variation in the Dolly Varden that recently founded this watershed.

Methods

Study sites and collection

Dolly Varden were collected from 15 locations throughout PWS and one on the Copper River Delta (Fig. 1 and Table 1). The entire region was de-glaciated 8,000-12,000 years ago (Lethcoe 1990). However, there are still active glaciers in the region, and one site, Unakwik Inlet (Fig. 1), was only deglaciated approximately 350 years ago. Three of these sites, Shelter Bay, Power Creek, Hawkins Creek had resident and anadromous populations (Fig. 1 and Table 1). Presumed resident populations were above migratory barriers to anadromous populations and assumed to be isolated from anadromous populations. The barriers at Power Creek and Hawkins Creek were approximately 40 and 10 m high,

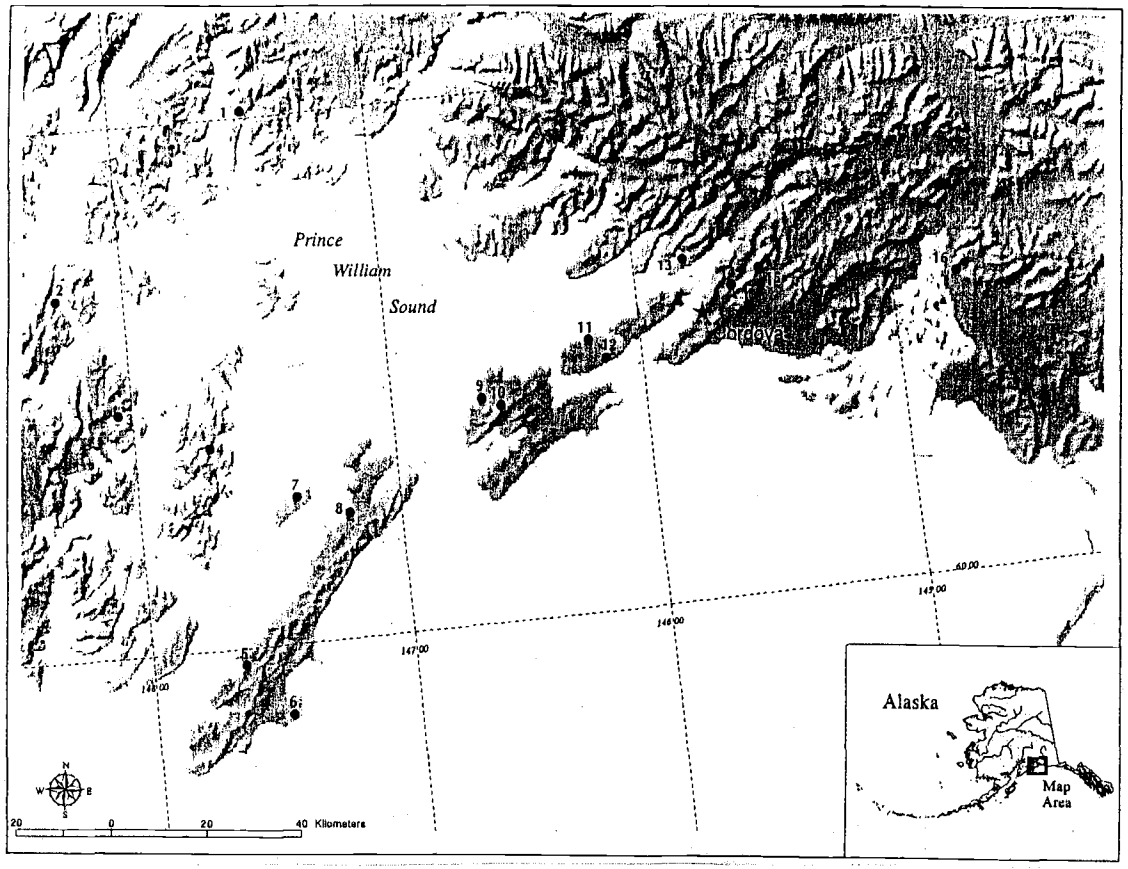


Figure 1. Sampling locations of Dolly Varden collected in 1996 and 1997 in Prince William Sound, Alaska. Locations are numbered as follows: (1) Unakwik Inlet; (2) Shrode Lake; (3) Eshamy Bay; (4) West Arm; (5) Hanning Creek; (6) Stump Lake; (7) Green Island; (8) Port Chalmers; (9) Shelter Bay, anadromous; (10) Shelter Bay, resident; (11) Hawkins Creek, anadromous; (12) Hawkins Creek, resident; (13) Milton Lake; (14) Power Creek, anadromous; (15) Power Creek, resident; and (16) Clear Creek.

Table1. Location, sample size (N), and latitude and longitude of 1996 and 1997 collections of Dolly Varden in Prince William Sound, Alaska. Site number refers to location in Figure 1.

Location (site number)	N (1996)	N (1997)	Latitude Longitude
Unakwik Inlet (1)	19	40	61.014 147.485
Shrode Lake (2)	not sampled	37	60.685 148.259
Eshamy Bay (3)	18	38	60.462 148.054
West Arm, Bay of Isles (4)	25	41	60.388 147.715
Hanning Creek (5)	40	32	59.970 147.653
Stump Lake (6)	40	37	59.868 147.488
Green Island (7)	13	0	60.283 147.400
Port Chalmers (8)	not sampled	19	60.244 147.202
Shelter Bay, anadromous (9)	40	40	60.429 146.652
Shelter Bay, resident (10)	not sampled	29	60.412 146.578
Hawkins Creek, anadromous (11)	32	22	60.515 146.206
Hawkins Creek, resident (12)	31	28	60.479 146.142
Milton Lake (13)	not sampled	37	60.644 145.802
Power Creek, anadromous (14)	32	not sampled	60.583 145.622
Power Creek, resident (15)	23	not sampled	60.611 145.509
Clear Creek (16)	22	25	60.559 144.749

respectively. The barrier at Shelter Bay was not considered a waterfall, but instead consisted of a step-down series of cascades. Resident Dolly Varden from this site were collected from a lake. An additional population was collected from Prince of Wales Island, Alaska to serve as an outgrouroup (N=30).

Dolly Varden were collected in September 1996 and 1997 when they returned to freshwater to over-winter or spawn. Collections of anadromous populations were made in freshwater near the estuary or in the estuary near freshwater. Sampling at these times increased the likelihood of collecting individuals that represented the local population. Fish were collected with baited minnow traps, seines, electroshockers, and hook and line. Sample sizes range from 13-40 individuals per site per year (Table 1).

At all sites, fish were given a lethal dose of MS-222, weighed (to the nearest 0.1 g), fork length measured (to the nearest mm), and tagged with an identification number. Fish <250 mm were wrapped in plastic wrap and frozen on dry ice. For fish >250 mm, muscle, eye, liver, and heart tissues were removed immediately. The tissues were placed in individual plastic tubes labeled with an identification number, and placed on dry ice. Both whole fish and tissues were transported on dry ice to an -80° C freezer at the ADFG office in Cordova, Alaska. Fish and tissues were stored until they were transported, on dry ice, to the Oregon Cooperative Fish Research Unit (OCFRU) laboratory in Corvallis, Oregon. There, tissues were removed from the frozen whole fish and transferred, while still frozen, to 1.7 ml microcentrifuge tubes and stored at -80° C.

Allozymes

Allozyme analysis of Dolly Varden followed the starch–gel electrophoresis methods of Aebersold et al. (1987). Twenty-eight enzymes encoding 51 loci were examined in Dolly Varden (Table 2). Gel and tray buffers and Ph for each enzyme locus combination are listed in Table 2. Migration of variable alleles were estimated relative to the most common alleles detected.

Microsatellites

DNA was extracted from 50-75 mg of muscle with a phenol:choroform:isoamyl alcohol extraction procedure (Sambrook et al. 1989). Analysis of nuclear DNA microsatellite regions followed the methods of Wenburg et al. (1996), optimized to conditions in our laboratory. Sixteen primers were initially screened, failure of amplification or low quality amplifications were detected in 14 (*Omy*77, *One*μ11, *Ots*1, *One*μ14, *One*μ2, *Ssa*14, *Omy*325, *Ots*4, *One*μ8, *Sfo*12, and *Sfo*23). The remaining two loci *Sfo*8 and *Ssa*85 were of high quality and represented two base pair repeats. PCR for these two primer pairs (synthesized by Research Genetics Huntsville, Alabama), was conducted by multiplexing annealing temperatures are listed in Table 3. Total volume of the reactions was 20 μl. A reaction cocktail was made consisting of 2 μl 10X

Table 2. Enzymes and loci examined with starch gel electrophoresis in Dolly Varden from Prince William Sound, Alaska. Enzyme names are from the International Union of Biochemistry (IUB). Tissues were: M – muscle, L - liver, E - eye, and H - heart. Buffer systems: TG - a tris glycine buffer pH 8.5, TBCLE- a tris-citrate gel buffer pH 8.2 and lithium hydroxide borate acid tray buffer (Ridgeway et al. 1970), ACE - an amine-citrate-EDTA gel and tray buffer pH 6.8 (Clayton and Tretiak 1972) ACE 6.1 - a citrate amine pH 6.1., and TC4 - a tris-citrate buffer pH 5.95 (Schaal and Anderson 1974). All gel buffers tested are shown.

I.U.B. Enzyme Name	Locus	Tissue	Buffer
Aspartate aminotranferase (2.6.1.1)	sAAT-1,2*	M,H	TBCLE, ACE 6.8, AC 6.1, TC4
Adenosine deaminase (3.5.4.4)	ADA-1*	M	TG, ACE 6.8
	ADA-2*	M	TG, ACE 6.8
Alcohol dehydrogenase (1.1.1.1)	ADH*	L	TBCLE, ACE 6.8
Aconitate hydratase (4.2.1.3)	sAH*	L	TBCLE
	mAH-1*	M,E,H	ACE 6.8, TC4
	mAH-2*	M,E,H	ACE 6.8, TC4
Alanine aminotransferase (2.6.1.2)	ALAT*	M	TG
Creatine kinase (2.7.3.2)	CK-A1*	M	TG
	CK-A2*	M	TG
Esterase-D (methylumbelliferyl) (3.1.1.*)	ESTD*	M	TBCLE
Formaldehyde dehydrogenase (1.2.1.1)	FDHG*	M,L	TG
Fumerate hydratase (4.2.1.2)	FH*	M,L,E,H	ACE 6.8, AC 6.1, TC4

Table 2. Continued.

I.U.B. Enzyme Name	Locus	Tissue	Buffer
N-Acetyl-b-glucosaminidase (3.2.1.52)	bGLUA*	L	TBCLE, ACE 6.8
Glucose-6-phosphate isomerase (5.3.1.9)	GPI-A*	M, E	TG, TBCLE
	GPI-B1*	M	TG, TBCLE
	GPI-B2*	M	TG, TBCLE
Glutathione reductase (1.6.4.2)	GR*	M,E	ACE 6.8, TG, AC 6.1
Glycerol-3-phosphate dehydrogenase (1.1.1.8)	G3PDH-1*	M,L,H	TBCLE, AC 6.1, ACE 6.8
	G3PDH-2*	M,L,H	TBCLE, AC 6.1, ACE 6.8
l-Iditol 2-dehydrogenase (1.1.1.14)	IDDH-1*	L	TBCLE, TG
	IDDH-2*	L	TBCLE, TG
Isocitrate dehydrogenase (NADP) (1.1.1.42)	mIDHP-1*	M,H	ACE 6.8
	mIDHP-2*	M,H	ACE 6.8
	sIDHP-1,2*	M,L,E,H	ACE 6.8, AC 6.1, TC4
l-Lactate dehydrogenase (1.1.1.27)	LDH-A1*	M	TBCLE
	LDH-A2*	M	TBCLE
	LDH-B1*	M,E	TBLCE, TG ACE 6.8
	LDH-B2*	M,E	TBLCE, TG ACE 6.8
	LDH-C*	E	TG
Malate dehydrogenase (1.1.1.37)	sMDHA-1,2*	L,E,H	ACE 6.8, AC 6.1, TC4
	sMDHB-1,2*	M,H	ACE 6.8, AC 6.1, TC4
Mannose-6-phosphate isomerase (5.3.1.8)	MPI*	M,L,E	TG

Table 2. Continued.

I.U.B. Enzyme Name	Locus	Tissue	Buffer
Nucleoside-trisphosphate pyrophosphate (3.6.1.19)	NTP*	M	TBCLE
Dipeptidase (3.4.13.18)	PEP-A*	M,L,E	TBCLE, TG
Tripeptide aminopeptidase (3.4.11.4)	PEP-B1*	M,L,E,H	TBCLE, TG
Proline dipeptidase (3.4.13.9)	PEP-D1*	M,L,H	ACE 6.8, AC 6.1, TC4
	PEP-D2*	M,H	ACE 6.8,
Leucyl-l-tyrosine peptidase (3.4.-.-)	PEP-LT*	M,E,H	TG, TC4
Phosphogluconate dehydrogenase (1.1.1.44)	PDGH*	M,L,E	ACE 6.8
Phosphoglycerate kinase (2.7.2.3)	PGK-1*	M,H	ACE 6.8
	PGK-2*	M,H	ACE 6.8
Phosphoglucomutase (5.4.2.2)	PGM-1*	M,L	ACE 6.8 ,TG
	PGM-2*	M,L	ACE 6.8 ,TG, AC 6.1
Superoxide dismutase (1.15.1.1)	sSOD-1*	M,L,H	TBCLE, TG
Triose-phosphate isomerase (5.3.1.1)	TPI-1*	E	TG
	TPI-2*	E	TG
	TPI-3*	E	TG
	TPI-4*	E	TG

Table 3. Microsatellite primers, multiplexing and amplification protocols for Dolly Varden collected from Prince William Sound, Alaska. See Olson et al. (1996) for primer sequences, references, and source species.

Multiplex Set	Primer	Forward primer label	Concentration	Annealing Temperature
Set 1	*Sfo8*	Fam	0.20 μM	56° C
	Ssa85	Tet	0.30 μM	56° C
	Omy77	Hex	0.15 μM	56° C

buffer, 0.16 μl (0.04 each) dNTP's, 2.4 μl $MgCl_2$, 0.2 μl Amplitaq gold, and 4.24 μl pure water (supplied by LGL genetics) for a total volume of 9 μl. Next a cocktail was made of the target primers in appropriate concentrations consisting of the forward and reverse primer and pure water. Because primer concentrations vary (Table 3), the volume of water was varied to result in a total volume of 10 μl. Respective volumes per individual sample of the two cocktails were pipetted into a 96 well tray for PCR amplification, and 1 μl of extracted DNA was added for each sample. Amplification reactions for PCR products from these primer sets were separated on a denatured polyacrylamide gel using a Perkin Elmer Applied Biosystems, Inc. (ABI) 377 automated sequencer and analyzed using ABI GeneScan 672, analysis software, version 2.0.2. A tamra 350 internal lane standard was used for each individual sample. Genotypes were scored from pherograms with basepair sizing generated by Genotyper software.

Statistical analysis

To examine variation of allozymes within sampling sites, expected average heterozygosity (percent genotypic variation at a locus) and number of alleles was calculated for each locus and averaged over all loci. Tests for departures from Hardy-Weinberg equilibrium (i.e. the random union of gametes) at each locus for each population with probability exact tests (Haldane 1954) using the computer

program BIOSYS (1.7). These tests were conducted for sampling years combined and pooled.

Populations that are recently colonized may have undergone population bottlenecks. During bottlenecks rare alleles are lost at a higher rate than heterozygosity (Cornuet and Luikart 1996). Bottlenecked populations may generate a heterozygote "excess" relative to the number of alleles detected. The computer software program BOTTLENECK (Cornuet and Luikart 1996) was used to test if populations of Dolly Varden had undergone bottlenecks in Prince William Sound. For allozymes the infinite allele model of mutation was used as suggested by Cornuet and Luikart (1996) and a Wilcoxon test for statistical significance was used. In addition, a descriptive tool was used which relies on a mode-shift of allele frequencies using the program BOTTLENECK (Cornuet and Luikart 1996).

Difference between sampling years for each collection site was tested for population differentiation by testing the null hypothesis that genotypic distributions were identical across all loci at for each population (in this case sampling year per collection location), at individual loci over all populations, and for all pairs of populations using a log-likelihood (G) based exact test (Raymond and Rousset 1995a). These tests were conducted with the computer software program GENEPOP (Raymond and Rousset 1995b). The same tests were done with sampling years combined. Sequential Bonferonni corrections to significance levels were applied when multiple comparisons were made (Rice 1989).

Genetic variation among populations was calculated with G-statisics using the software program FSTAT Version 2.8 (Goudet 1995) with all sites included and also without sites above waterfall barriers. Range divergence of G_{st} values (low 0-0.05, moderate 0.05-0.15 moderate, high 0.15-0.25) follow those recommended by Hartl and Clark (1997).

Patterns of geographical genetic similarity were identified by constructing p henograms from cluster analyses of pair-wise estimates of similarity between samples using the modified Cavalli-Sforza distance (Nei et al. 1983) for allozyme variation. The unweighted pair-group method with arithmetic averages (UPGMA) clustering algorithm was used for all cluster analyses.

Estimates of gene diversity for the two microsatellite loci were calculated for Dolly Varden to examine variation collected at each sampling site. Data were tested for departures from Hardy-Weinberg equilibrium at each locus, probability exact tests (Haldane 1954) using the computer program BIOSYS (1.7). Because only two microsatellites were scored tests for bottlenecks were not conducted.

Population differentiation was examined by testing the null hypothesis that genic distributions of microsatellites were identical across all populations with sampling years combined using the program GENEPOP 3.1c (Raymond and Rousset 1995b). Sequential Bonferonni corrections to significance levels were applied when multiple comparisons were made (Rice 1989).

Estimates of genetic variation among populations were made in several ways. Weir and Cockerman's θ, an analogue of Wright's Fst was calculated. To

test if values were significantly different from zero, sampling with replacement using bootstrapping over all loci was conducted and a 95% confidence interval was estimated. G_{st} values (Nei 1987) were calculated with all sites included and also without sites above waterfall barriers using the computer software program FSTAT (Goudet 1995). Interpretation of the range of G_{st} values (low 0-0.05, moderate 0.05-0.15 moderate, high 0.15-0.25) follow those recommended by Hartl and Clark (1997).

Patterns of geographical genetic similarity used for unplanned comparisons were identified by constructing phenograms from cluster analyses of pair-wise estimates of similarity between samples using the modified Cavalli-Sforza distance (Nei et al. 1983) of microsatellites. The unweighted pair-group method with arithmetic averages (UPGMA) clustering algorithm was used for cluster analyses. Multidimensional scaling based on Cavalli-Sforza distance (Nei et al. 1983) was also used to examine spatial relationships for the microsatellite data. Estimates of gene flow were made with the private allele method (Slatkin 1985). A significance level of 0.05 was used for all statistical tests unless otherwise stated.

Results

Allozymes

Fifty loci were scored in Dolly Varden from 16 sampling locations in Prince William Sound, and one outgroup from Prince of Wales Island, Alaska. One loci,

FH* was excluded because of uncertainty in scoring. When sampling years were separated and combined we detected no deviation from Hardy-Weinberg equilibrium (initial $P > 0.05$). Of the 50 loci, 28 were polymorphic at the 0.99 level. Average heterozygosity ranged from 0.013-0.067, mean number of alleles per locus ranged from 1.09-1.56 and percent polymorphic loci ranged from 4-28 (Table 4). Average heterozygosity and mean number of alleles per locus, were resident populations from Hawkins Creek (0.013, 1.09) and Power Creek (0.037, 1.13), respectively, than any other populations in Prince William Sound (Table 4), although this was not determined statistically. In addition, resident Dolly Varden in Hawkins Creek were fixed for a variant allele at both sAH* and sSOD*.

Significant differences (initial $P < 0.05$) were detected in 74 of the 120 pair-wise tests between Dolly Varden populations in Prince William Sound (Table 5). In general, the differences were attributed to differences at four locations, Hawkins Creek resident, Power Creek resident, Unakwik Inlet and Clear Creek (Table 5). Significant differences ranged from one to eight loci.

There was no heterozygote excess detected in Unakwik Inlet based on the Wilcoxon test ($P > 0.05$), suggesting this site has not undergone a recent bottleneck. However, significant deficiencies ($P < 0.05$) in heterozygotes were detected in Eshamy Bay, Green Island, Hanning Bay, West Arm, Milton Lake, Shrode Lake and Stump Lake, suggesting these populations have been recently expanding or are undergoing gene flow.

Table 4. Average heterozygosity, mean number of alleles per locus, percent polymorphic loci (95% criterion) for allozyme loci in Dolly Varden from Prince William Sound, Alaska. Site number refers to location in Figure 1.

Location (site number)	Average heterozygosity	Mean number alleles/locus	% polymorphic
Unakwik Inlet (1)	0.064	1.31	17
Shrode Lake (2)	0.058	1.37	20
Eshamy Lake (3)	0.057	1.41	17
West Arm (4)	0.050	1.50	17
Hanning Bay (5)	0.057	1.48	19
Stump Lake (6)	0.058	1.50	19
Green Island (7)	0.055	1.30	15
Port Chalmers (8)	0.055	1.33	19
Shelter Bay, anadromous (9)	0.063	1.50	20
Shelter Bay, resident (10)	0.061	1.35	19
Hawkins Creek, anadromous (11)	0.067	1.56	22
Hawkins Creek, resident (12)	0.013	1.09	4
Milton Lake (13)	0.066	1.50	22
Power Creek, anadromous (14)	0.058	1.35	19
Power Creek, resident (15)	0.037	1.13	13
Clear Creek (16)	0.083	1.46	28
Prince of Wales Island, Alaska	0.038	1.13	10

Table 5. Genetic differences ($P < 0.0004$) at allozyme loci from sixteen collection sites of Dolly Varden from Prince William Sound, Alaska. Site number refers to location in Figure 2. Loci are: a = sAAT4*, b = sAH*; c = mAH2*; d = LDHC*; e = NTP*; f = PEPB1*; g = PEPD1*; h = PEPLT*; i = PGDH*; j = PGM1*; k = sSOD1*, l = MPI* and m = sMDA-2*. NS denotes no significant differences.

Locations (site numbers)	Shrode Lake (2)	Eshamy Bay (3)	West Arm (4)	Hanning Creek (5)	Stump Lake (6)	Green Island (7)	Port Chalmers (8)	Shelter Bay anadromous (9)	Shelter Bay resident (10)	Hawkins anadromous (11)	Hawkins resident (12)	Milton Lake (13)	Power Creek anadromous (14)	Power Creek resident (15)	Clear Creek (16)
Unakwik Inlet (1)	g	g, h	F, g, h	f, h, k	f, h	NS	f	f. g, h, m	h, m	h	b, d, f, g, h, k	f, g	d, h	a, b, d, e, g, h, k	b, g, j, h
Shrode Lake (2)		NS	b, j	NS	d, j	NS	NS	d	d	d, f	b, f, k	d	d, j	a, b, d	d, e, j
Eshamy Bay (3)			B	NS	NS	NS	NS	e	NS	NS	b, d, f, k	NS	NS	a, d, k	b, e
West Arm (4)				B, k	b, k	NS	b	a, b	b	a, b	b, d, f, k	b	a, b, d, k	a, b, d, k	a, b, i j, l
Hanning Creek (5)					h	NS	NS	e	NS	NS	b, d, f, g, k, m	NS	NS	a, d	a, b, e, i, l
Stump Lake (6)						NS	NS	e, h	NS	NS	b, d, f, g, h, k	NS	d	a, d	b, i, j, l
Green Island (7)							NS	NS	NS	NS	b, d, f, g, k	NS	NS	d	NS
Port Chalmers (8)								NS	NS	NS	b, e, f, k	NS	NS	b, d, k	NS
Shelter Bay anadromous (9)									NS	NS	b, d, f, g, k,m	NS	b, e	a, d, e, f, k	b, i, j, l
Shelter Bay resident (10)										NS	b, d, f, k, m	NS	NS	a, d, e	b, i, j
Hawkins Anadromous (11)											b, d, f, g, k	NS	b	a, b, d, g, k	b, i, j
Hawkins resident (12)												b, d, f, k	a, b, d, f, g, k	a, b, d, f, k	a, b, d, e, f, i, j, k
Milton Lake (13)													NS	a, b, d, k	b, i
Power Creek Anadromous (14)														d, k	b, d, e, h, i, j
Power Creek resident(15)															b, d, j, i

There were moderate levels of genetic variation among locations. The estimate of Weir and Cockermans's θ was 0.116. Estimates of G_{st} was 0.109. When resident populations at Power and Hawkins Creek were removed, estimates of G_{st} and Weir and Cockermans's θ was 0.04 suggesting low genetic divergence among the remaining anadromous populations. The range of the 95% confidence interval for all estimates of θ was above zero.

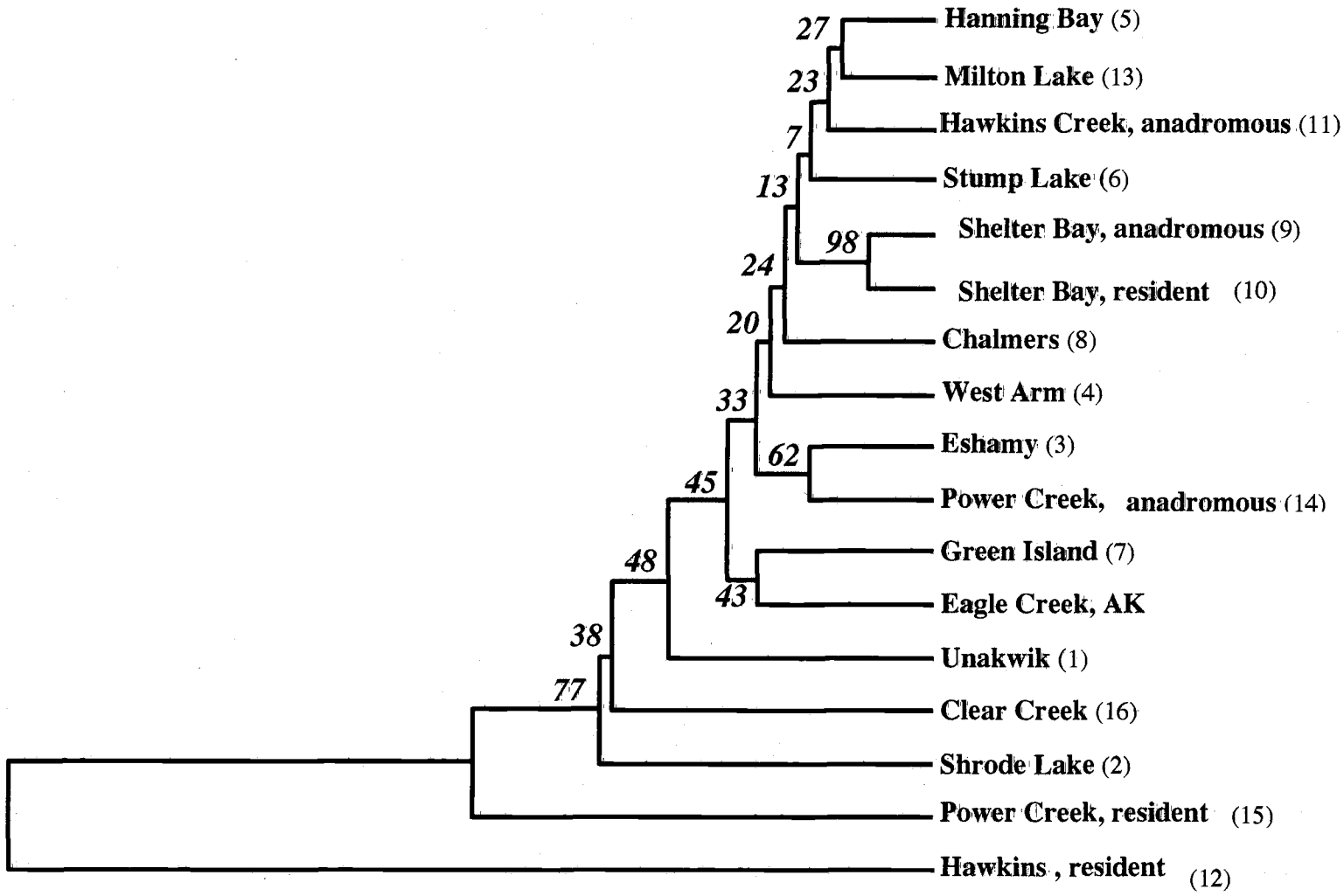
Clustering tools also showed evidence that resident populations at Power Creek and Hawkins Creek were different from all other sites. Based on UPGMA there was greater separation between the two resident sites than all other sites (Fig 2). There was little evidence of geographical structuring among anadromous populations (Fig. 2). Estimate of number of migrants per generation (Nm) using private allele method after correction for sample size, was 2.9.

In contrast to the significant differences between resident and anadromous populations in Hawkins Creek and Power Creek, no difference were detected between the resident and anadromous site at Shelter Bay (initial $P > 0.05$) (Table 5). Geographic structuring depicted by a UPGMA dendrogram suggest that resident and anadromous populations in Shelter Bay cluster closely (Fig. 2).

Microsatellites

Microsatellite loci *Sfo*8 and *Ssa*85 were highly polymorphic in Dolly Varden from twelve locations in Prince William Sound. We were unable to eliminate stutter bands in the remaining samples (Shrode Lake, Port Chalmers,

Figure 2. Genetic similarity of Dolly Varden from 16 locations in Prince William Sound, Alaska, and one outgroup from southeast Alaska (Eagle Creek) based on allozyme variation. Numbers at nodes are bootstrap values (%) based on 1000 replications. Numbers in parentheses refer to location in Figure 2.


27
23
7
13
98
24
20
33
62
45
48
43
38
77
Hanning Bay (5)
Milton Lake (13)
Hawkins Creek, anadromous (11)
Stump Lake (6)
Shelter Bay, anadromous (9)
Shelter Bay, resident (10)
Chalmers (8)
West Arm (4)
Eshamy (3)
Power Creek, anadromous (14)
Green Island (7)
Eagle Creek, AK
Unakwik (1)
Clear Creek (16)
Shrode Lake (2)
Power Creek, resident (15)
Hawkins , resident (12)

Shelter Bay-resident, and Milton Lake) to ensure consistent scoring with bins of two basepair differences therefore these sites were eliminated from further analysis. Allele numbers for *Sfo*8and *Ssa*85 are 31 and 30, respectively.

Gene diversity estimates ranged from 0.0 to 0.951 and are presented in Table 6. A fixed allele was detected in the Power Creek resident populations at *Ssa*85. We detected a deficiency of heterozygotes leading to deviation from Hardy-Weinberg equilibrium in four tests of the 24 that were conducted (initial $P < 0.05$; Table 6). Significant differences (initial $P < 0.05$) were detected among 30 of the 66 pair-wise tests in Dolly Varden in Prince William Sound (Table 7). Of these, 19 resulted from differences between the resident populations in Power Creek and Hawkins Creek. Similar to the allozyme data, we detected significant differences (initial $P < 0.05$) between resident and anadromous populations in Power Creek (Table 7).

Estimates of Weir and Cockerman's θ over both loci at all sites was 0.073. Gst estimates were 0.105. The 95% confidence interval for the estimate of θ over all populations was above zero. Nm based on private alleles was 1.2. As with the differentiation at allozyme data, there was no obvious pattern of geographical structure to the genetic differences (Fig. 3, and Fig. 4), except that both analyses

Locus	Unakwik Inlet (1)	Eshamy Lake ((3)	West Arm (4)	Hanning Bay (5)	Stump Lake (6)	Green Island ((7)	Shelter Bay, (Anad) (9)	Hawkins Creek, (Res) (12)	Hawkins Creek, (Anad) (13)	Power Creek, (Anad) (14)	Power Creek, (Res) (15)	Clear Creek (16)
Sfo8	0.700	0.767	*0.887	0.914	0.897	0.905	0.926	0.896	0.951	0.891	0.736	0.712
Ssa85	0.679	0.733	*0.904	0.940	0.943	0.920	0.949	*0.827	0.932	*0.882	0.000	0.934

Table 6. Estimates of gene diversity per locus per site at two microsatellite loci in Dolly Varden from Prince William Sound, Alaska. '*' refers to deviation from Hardy-Weinberg equilibrium. Number in parentheses refer to location in Figure 1.

Location (site number)	Eshamy Bay (3)	West Arm (4)	Hanning Creek (5)	Stump Lake (6)	Green Island (7)	Shelter Bay, anadromous (9)	Hawkins Creek, anadromous (11)	Hawkins Creek, resident (12)	Power Creek, anadromous (14)	Power Creek, resident (15)	Clear Creek (16)
Unakwik (1)	NS	NS	b	b	B	NS	NS	b	NS	b	NS
Eshamy Bay (3)		a	a	a	B	A	a	a	A	a, b	a
West Arm (4)			NS	b	NS	NS	NS	b	NS	a, b	NS
Hanning Creek (5)				NS	NS	NS	NS	NS	NS	b	NS
Stump Lake (6)					NS	NS	NS	b	NS	b	NS
Green Island (7)						NS	NS	b	NS	b	NS
Shelter Bay anadromous (9)							NS	NS	NS	b	NS
Hawkins Creek, anadromous (11)								NS	NS	b	NS
Hawkins Creek, resident (12)									B	b	a
Power Creek anadromous (14)										b	NS
Power Creek, resident (15)											a, b

Table 7. Genotypic differences ($P < 0.0008$, adjusted Bonferroni correction for multiple tests) at two microsatellite loci in Dolly Varden, from Prince William Sound, Alaska. NS denotes no significant difference. Loci are: a = *Sfo*8 and b = *Ssa*85. Numbers refer to locations on Figure 1.

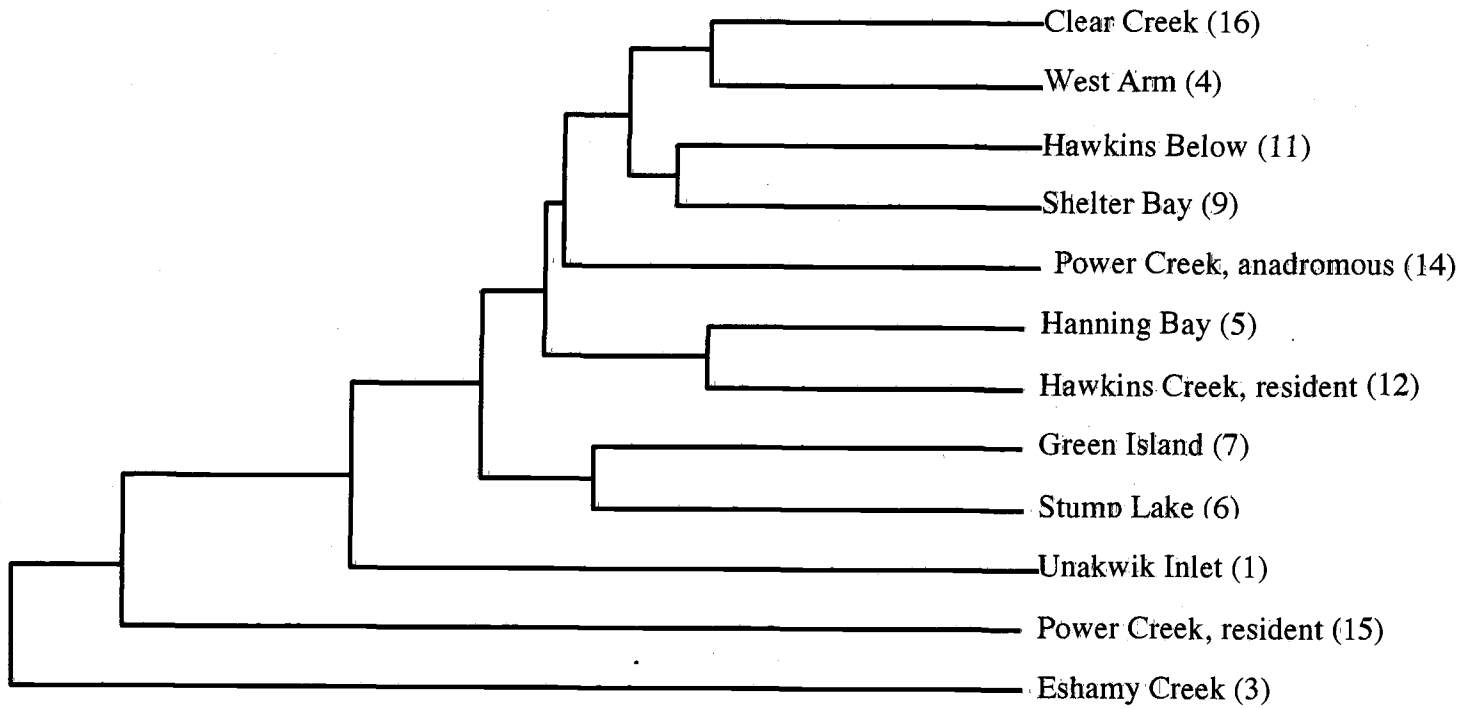


Figure 3. Genetic similarity of Dolly Varden from 12 locations in Prince William Sound, Alaska based on two microsatellite loci. Numbers at nodes are bootstrap values (%) based on 1000 simulations. Numbers in parentheses refer to locations in Figure 1.

Figure 4. Multidimensional scaling based on Cavalli-Sforza distance estimates from microsatellite DNA variation among Dolly Varden in 12 sites from PWS, Alaska. Number in parentheses refer to location in Figure 1.

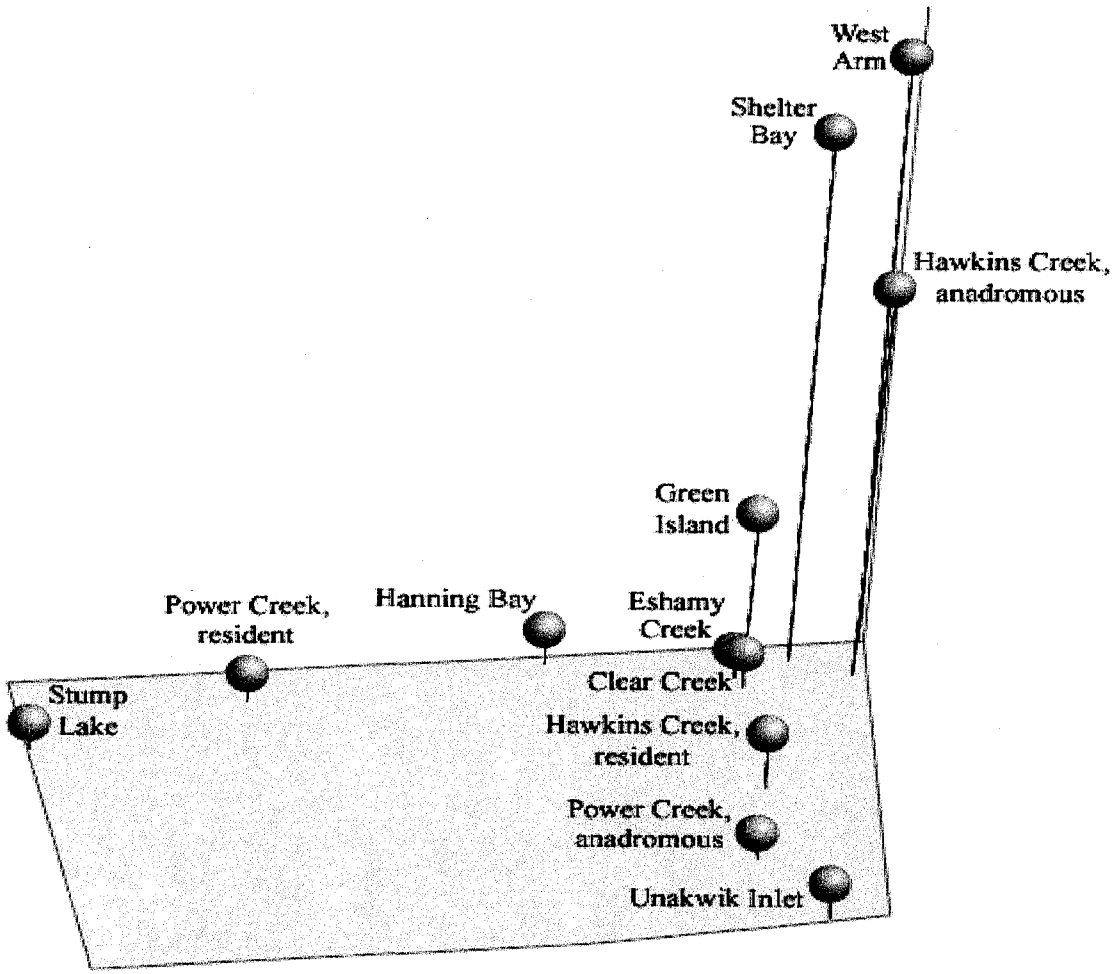

West Arm
Shelter Bay
Hawkins Creek, anadromous
Green Island
Power Creek, resident
Hanning Bay
Eshamy Creek
Clear Creek
Stump Lake
Hawkins Creek, resident
Power Creek, anadromous
Unakwik Inlet

Figure 4.

identified the resident population in Power Creek and the anadromous population in Unakwik Inlet as different from all other sites.

Discussion

There was a moderate amount of genetic variation within and among Dolly Varden populations in Prince William Sound, Alaska detected with allozymes and microsatellites. Estimates of allozyme heterozygosity were comparable to those detected in Dolly Varden by Leary and Allendorf in Washington and Alaska (1997) and Everett et al. (1998a) in Alaska. Heterozygosity of microsatellites in Dolly Varden in Prince William Sound was high, which is typical of this molecular marker and comparable to other species of salmonids (Wenburg and Bentzen 2001).

Fixation of alleles in above barrier populations was detected with both allozymes and microsatellites. The detection of fixed alleles in these sites resulted in pairwise genetic distances between sites. This could have resulted from genetic drift, wherein random events led to the loss of all other alleles. It is also possible that when this site was initially founded there was only allele present. However, given that allelic is high in other microsatellite and allozymes loci this is less likely.

There was no evidence of bottlenecks in the recently colonized site, Unakwik Inlet. This could be because of ongoing gene flow from other sites to this watershed, or, it is possible because there was no population bottleneck when the site was colonized. If there were continued inputs from source populations the

effects of a bottleneck would be mitigated. It is also possible that if there was a bottleneck the population has recovered as the effects are transient (Cornuet and Luikart 1996). There was evidence that populations of Dolly Varden at Eshamy Bay, Green Island, Hanning Bay, West Arm Milton Lake, Shrode Lake and Stump Lake, suggesting these sites have recently expanded or that there has been an influx of alleles from other sites suggesting that there gene flow among anadromous populations. In contrast to these results, bottlenecks were detected in four coastal cutthroat trout populations in Prince William Sound, including West Arm and Green Island (Griswold 2002). West Arm and Green Island were in the direct path of the *Exxon Valdez* oil spill in 1989 (Hepler et al. 1993). In Dolly Varden, recovery from any population declines related to the oil spill (Hepler et al. 1993) would likely be more rapid than that of coastal cutthroat trout because of their dispersal capacity, migratory behavior and relatively large population size (McCarron and Hoffmann 1993).

Estimates of variation among populations of anadromous Dolly Varden was low (4%), which suggests that there was historic gene flow among these populations in Prince William Sound. Tagging studies in Prince William Sound suggested 14-58% of tagged Dolly Varden were not recaptured in their natal streams (Bernard et al. 1995). The authors suggested these fish overwintered at sea. The low estimates of genetic variation detected in this study may suggest that a portion of these fish may disperse to other sites. This contrasts findings for coastal cutthroat trout from Prince William Sound. Griswold (2002) suggested that

there is restricted gene flow in this region. This was attributed in part to their limited dispersal capacity in Prince William Sound (McCarron and Hoffmann 1993).

The relation between resident and anadromous populations within basins varied. In two cases, significant genetic differences were observed and in one case no differences were detected. It is likely that the waterfall barriers severely restrict genetic exchange between the resident and anadromous populations at Power Creek and Hawkins Creek. Waterfall barriers at both Power Creek and Hawkins Creek are high enough that one-way gene flow from above to below the barrier is probably very limited because fishes that pass over these barriers probably have low survival rates.

There were no statistically significant differences in the third resident and anadromous populations, Shelter Bay. Genetic similarity in this case is likely due to gene flow. The barrier at this site has a step down configuration that may allow one-way gene flow from resident to anadromous fish at this site, or alternatively may provide upstream access under certain flow conditions. The different patterns we observed in Dolly Varden resident populations suggest that the pattern of genetic variation may not be predictable on the landscape. This is similar to results observed in coastal cutthroat trout in Oregon and Alaska where in some, but not all, cases barriers led to genetic differentiation (Griswold 1996). The configuration, age, and persistence of barriers may provide variable opportunities for gene flow.

There was no evidence of population bottlenecks in resident Dolly Varden in Power Creek and Hawkins Creek, but both sites had low allozymes allelic diversity and mean number of alleles. Fixed allozymes alleles in Hawkins Creek and microsatellite alleles at microsatellites may reflect the effects of genetic drift. Isolated resident populations may be particularly susceptible to genetic drift, wherein genetic variation is lost due to chance events (Hartl and Clark 1997). Resident populations may be small (Northcote 1992). In addition, resident populations that are isolated from upstream movement such as those in Power Creek and Hawkins Creek have no opportunity for gene flow from other populations. Thus, there is no input of new alleles to offset the effects of genetic drift. The outcome of drift is not predictable; hence, genetic differences may be great among isolated populations due to fixation of alleles

This study suggests that may be some gene flow among anadromous populations. Relative to coastal cutthroat trout in the same region, Dolly Varden probably have a higher likelihood of colonizing new sites without the detectable effects of low genetic diversity. However, in sites isolated above waterfalls, there appears to be some evidence of genetic drift that may lead to high levels of differences among sites.

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CHAPTER 5

CONCLUSIONS

"...before humans walked across the Bering Strait and into the Pacific Northwest; before glaciers gouged out Puget Sound; before the Oregon Coast migrated away from Idaho; before all this, there were Salmon" Jim Lichatowich, Salmon without Rivers

In this dissertation I examined genetic variation within and among populations of coastal cutthroat trout and of Dolly Varden. The focus of these studies is on the genetic variation of these species in Prince William Sound, Alaska, and, for coastal cutthroat trout, in three other locations throughout their distributional range. To do this, I used multiple genetic markers, which, because of their properties and inheritance modes, allows for inferences to be made about patterns and processes at multiple spatial scales.

I found that Dolly Varden and coastal cutthroat trout have different patterns of mtDNA haplotype variation within Prince William Sound (Chapter 2). Dolly Varden are genetically diverse suggesting that, unlike coastal cutthroat trout, these populations did not undergo a reduction in female effective population size when this region was colonized following the retreat of Pleistocene glaciers (8,000-12,000 years ago). Coastal cutthroat trout had low haplotype diversity suggesting these populations have undergone a reduction in female effective population size. The differences in these patterns may reflect differences in historical refuges, dispersal capacity, and historic population sizes. Dolly Varden could have founded

populations in Prince William Sound from Beringia and Cascadia, whereas trout could have only come from Cascadia. Dolly Varden are capable of extensive migrations (DeCicco 1995), whereas cutthroat trout have restricted migrations (Wenberg and Bentzen 2001).

Coastal cutthroat trout from glaciated sites in Prince William Sound, Vixen Inlet, and Ft. Lewis, Washington had lower haplotype variation relative to a non-glaciated site, Elk River, Oregon (Chapter 2). This suggests that genetic diversity was reduced in the glaciated sites when they were colonized. It is unknown if high genetic diversity is unique to the Elk River, or if coastal cutthroat trout on the Oregon Coast are genetically diverse in general. High genetic diversity has been reported in the southern distributional range of rainbow trout (Nielsen et al. 1994) and coho salmon (Smith et al. 2001). Further examination if coastal cutthroat trout have high genetic diversity in their southern distributional range would make an important contribution to the management of this species, as populations of coastal cutthroat trout in this region have undergone a loss of habitat and declining populations (Johnson et al. 1999).

Coastal cutthroat trout are also vulnerable to the loss of genetic variation when they colonize watersheds at the local scale (Chapter 3). Watersheds that were recently colonized (150-350 years ago) in Prince William Sound have lower estimates of microsatellite diversity than sites than sites not recently colonized. Variation within and among coastal cutthroat trout was detected with allozymes and microsatellites. Coastal cutthroat trout in western Prince William Sound were

in general different from one another using pairwise tests and clustering tools. Deep channels and fjords that may have led to isolation among sites dominate this area. Similar results were reported by Campton and Utter (1987) and Wenburg and Bentzen (2001) for coastal cutthroat trout from Puget Sound, Washington. In eastern Prince William Sound there were few significant differences and populations clustered together. This area is characterized by shallow water and alluvial fans, which may have facilitated movement among populations.

The genetic structure of Dolly Varden within Prince William Sound differs from of coastal cutthroat trout (Chapters 3 and 4). In general, there were few genetic differences among Dolly Varden populations, other than among isolated resident fishes. Dolly Varden migratory behavior differs from coastal cutthroat trout in that they undergo extensive migrations (DeCicco 1992) and may have higher level of stray rates. Dolly Varden populations that are physically isolated in Prince William Sound appear to be genetically similar, which may reflect their migratory behavior (Chapter 4). Thus, anadromous Dolly Varden may colonized new sites without an accompanying loss of genetic diversity and may have more exchange among populations. Management risks at the watershed level may be less critical for Dolly Varden than for coastal cutthroat trout.

In this dissertation, I used multiple genetic markers. The use of mtDNA allowed me to examine potential genetic differences at large spatial scales that reflect patterns over long time frames. If I had used this tool alone I would have underestimated the genetic diversity of the coastal cutthroat trout in Prince William

Sound that was detected with allozymes and microsatellites. Within Prince William Sound the high allelic diversity and heterozygosity of microsatellites allowed me to test if recently colonized populations had low genetic diversity relative to other sites. Both allozymes and microsatellites detected fixed loci in resident populations of Dolly Varden, suggesting that these populations have been subject to genetic drift.

These results have important implications for the management of these species. Loss of genetic variation when habitats are colonized or re-colonized may be an important process that structures the genetic variation of coastal cutthroat trout. In the Pacific Northwest, re-establishment of local populations after habitat disturbance may be important for the long-term persistence of salmonid species at the landscape scale (Reeves et al. 1995). If coastal cutthroat trout lose genetic variation when they re-establish new sites, there may be a gradual decline in genetic diversity within populations across the landscape. Loss of genetic diversity within populations may lead to increase risks of stochastic processes and local extinction (Allendorf 1986). In contrast, it appears that anadromous Dolly Varden may have opportunities for continued input of genetic diversity, which could offset the effects of genetic drift and the associated risks. Both coastal cutthroat trout and Dolly Varden were affected by the Exxon Valdez oil spill in 1989 in Prince William Sound (Hepler et al 1993). However, recovery for coastal cutthroat trout resulting from "rescue" from nearby populations might be more limited in some regions of the Sound than for Dolly Varden.

This dissertation suggests that restricted migration of coastal cutthroat trout may lead to low genetic diversity at multiple spatial scales when new habitats are colonized. Given the restricted migration of coastal cutthroat trout at the local scale detected in this study and others (Campton and Utter 1987, Wenburg and Bentzen 2001) it is likely that the genetic diversity in Oregon is partitioned at the watershed scale. Johnson et al. (1999) found that there is 12.6% of the genetic variation within coastal cutthroat trout ESU's is attributed to differences among rivers, within rivers, and between above and below barrier fish in Oregon and Washington. Other Pacific salmonids have less genetic variation within ESU's (Johnson et a. 1999). In coho salmon, which have the second highest estimate relative to cutthroat trout, 3.5% of the genetic variation is attributed to variation within ESU's. The ESU's currently established (Johnson et al. 1999) may not be at a small enough scale to protect the genetic and life history diversity that is present.

One of the greatest risks to coastal cutthroat trout is the potential loss of the anadromous life history form (Johnson et al. 1999). Without the migratory behavior of the anadromous form interactions between populations may be further decreased and small populations may become increasingly isolated. In a dynamic environment such as the Pacific Northwest, populations may function as metapopulations where extinction and re-colonizations occur through time (Reeves et al. 1995). The anadromous form may be critical for re-colonizing habitats where these local extinctions occurred and for maintaining genetic diversity in extant populations through low levels of gene flow. If anadromous forms continue to

decline populations of coastal cutthroat trout may subject to genetic drift and increased risk of extinction (Allendorf 1983). Further efforts to maintain genetic variation in coastal cutthroat trout should focus on and maintaining the anadromous form. Because coastal cutthroat trout are an important sport fish one approach is to protect the anadromous form would be through the regulation of harvest. In addition, efforts could be made to maintain high quality freshwater and estuary habitat.

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