Transactions of the American Fisheries Society 136:926–942, 2007

DOI: 10.1577/T06-214.1

[Article]

Genetic Investigation of Natural Hybridization between Rainbow and Coastal Cutthroat Trout in the Copper River Delta, Alaska

IAN WILLIAMS
10220 Lone Tree Drive, Anchorage, Alaska 99507, USA

GORDON H. REEVES
U.S. Forest Service, Pacific Northwest Research Station, 3200 Southwest Jefferson Way, Corvallis, Oregon 97331, USA

SARA L. GRAZIANO AND JENNIFER L. NIELSEN*
U.S. Geological Survey, Alaska Science Center, 1011 East Tudor Road, Anchorage, Alaska 99503, USA

Abstract.—Molecular genetic methods were used to quantify natural hybridization between rainbow trout *Oncorhynchus mykiss* or steelhead (anadromous rainbow trout) and coastal cutthroat trout *O. clarkii clarkii* collected in the Copper River delta, Southeast Alaska. Eleven locations were sampled to determine the extent of hybridization and the distribution of hybrids. Four diagnostic nuclear microsatellite loci and four species-specific simple sequence repeat markers were used in combination with restriction fragment length polymorphism analyses of NADH dehydrogenase 5/6 (*ND5/6*) mitochondrial DNA (mtDNA) to investigate the genetic structure of trout from both species and identify putative interspecific hybrids. Hybrids were found in 7 of the 11 streams sampled in the Copper River delta, the extent of hybridization across all streams varying from 0% to 58%. Hybrid trout distribution appeared to be nonrandom, most individuals of mixed taxonomic ancestry being detected in streams containing rainbow trout rather than in streams containing coastal cutthroat trout. Genotypic disequilibrium was observed among microsatellite loci in populations with high levels of hybridization. We found no significant correlation between unique stream channel process groups and the number of hybrid fish sampled. Eighty-eight percent of fish identified as first-generation hybrids (F_1) in two populations contained coastal cutthroat trout mtDNA, suggesting directionality in hybridization. However, dominance of coastal cutthroat trout mtDNA was not observed at a third location containing F_1 hybrids, indicating that interspecific mating behavior varied among locations. Backcrossed individuals were found in drainages lacking F_1 hybrids and in populations previously thought to contain a single species. The extent and distribution of backcrossed individuals suggested that at least some hybrids are reproductively viable and backcrossed hybrid offspring move throughout the system.

The evolutionary significance of hybridization continues to be a matter for debate (Arnold 1997; Dowling and Secor 1997; Seehausen 2004). Natural hybridization is recognized as an evolutionary force among plants, but it has often been viewed as unusual and aberrant among animals (Harrison 1993; Seehausen 2004; Mallet 2005). Since reproductive isolation among groups is a main tenet of the biological species concept, hybridization can be viewed as "unnatural" because it challenges the idea that species are strictly divergent (Epifanio and Nielsen 2000). However, in some animals, including fish, natural hybridization has been seen as a key evolutionary process affecting the development of taxa and their adaptation to new environments (Lewontin and Birch 1966; Arnold 1997; Dowling and Secor 1997). The outcome of natural hybridization can be highly variable, depending on nonexclusive effects of pre- and postmating reproductive barriers, parental type, hybrid dispersal, selection against hybrid or recombinant genotypes, and positive assortative mating (Harrison and Bogdanowicz 1997; Epifanio and Philipp 2000; Albert et al. 2006; Nolte et al. 2006).

The idea that barriers to gene flow are required for groups to diverge (within this context pre- and postmating barriers are evolutionarily significant) remains inconsistent with the assumption that the mixing of distinct genetic backgrounds can promote unique adaptive strategies and allow individuals of mixed ancestry to have increased fitness, especially in novel or peripheral environments (Grant and Grant 1992; Seehausen 2004). The debate about the relevance of natural hybridization is focused on this apparent contradiction.

Detection of molecular data consistent with hypotheses of historical hybridization suggests that natural

* Corresponding author: jennifer_nielsen@usgs.gov

Received September 20, 2006; accepted February 14, 2007
Published online June 7, 2007

hybridization has played a role in the evolution of many animals (Arnold 1992, 1997), including salmonid fishes (Allendorf and Leary 1988; Glémet et al. 1998; Baker et al. 2002; Brown et al. 2004). Genetic data provide a window into the ongoing precarious balance between species persistence in sympatric populations and the breakdown of barriers to reproduction between relatively young or closely related species (Gow et al. 2006). It is often unclear whether areas of natural hybridization form as a response to endogenous or exogenous factors (Nolte et al. 2006).

Understanding the role of hybridization in the genetic makeup of taxa and how reproductive barriers originate and are maintained is important in the definition of species complexes. This is especially relevant to salmonids, for which numerous species and subspecies have been identified (Nelson 1994). Species relationships within the genus *Oncorhynchus* have been the subject of considerable discussion, especially in the case of Pacific trout (see review in Utter 2000). Species-specific relationships have been assessed by means of various characteristics, including morphological (Behnke 1992), karyotypic (reviewed by Behnke 1992 and Johnson et al. 1999), and molecular-genetic ones (Leary et al. 1987; Shedlock et al. 1992; Nielsen et al. 1997). Although inconsistencies exist (see Allendorf and Leary 1988), the current consensus clusters subspecies of cutthroat trout *O. clarkii* and rainbow trout *O. mykiss* into separate but closely related monophyletic groups (Utter and Allendorf 1994; Phillips and Oakley 1997; Crespi and Fulton 2004). Behnke (1997) proposed that these sister groups diverged from a common ancestor around 2 million years ago.

Hybridization between these two species was thought to be uncommon, and formal biological criteria for the identification of cutthroat trout–rainbow trout hybrids did not appear until Utter (1981). Since then, considerable attention has been paid to the management implications of hybridization among Pacific trout species (Leary et al. 1987; Allendorf and Leary 1988; Behnke 1992; Baker et al. 2002; Campbell et al. 2002; Weigel et al. 2003; Peacock and Kirchoff 2004). Introgressive hybridization when hybrids are fertile and readily backcross with parental taxa is a concern because it can alter the genetic structure of co-adaptive gene complexes for sensitive populations and lead to the extinction of native genotypes (Rhymer and Simberloff 1996; Allendorf et al. 2001; Weigel et al. 2003; Peacock and Kirchoff 2004).

The natural distributions of rainbow and cutthroat trout suggest that they evolved in sympatry and that various isolating mechanisms restricted gene flow between them (Young et al. 2001; Utter 2000, 2004). There is extensive overlap in their distributional ranges in western North America (Berra 2001; Quinn 2005). Natural populations of coastal cutthroat trout *O. c. clarkii* are found from the Eel River in northern California to the eastern side of the Kenai Peninsula, Alaska (Behnke 1992; Trotter 1997). North American coastal rainbow trout are found from Baja California to Alaska (McCusker et al. 2000), but this species has been introduced throughout the world (Behnke 1992). Both species occur naturally in the Russian Far East, primarily on the Kamchatka Peninsula (Behnke 1996; Pavlov et al. 2001). In areas where the two species overlap, differences in spawn timing are thought to limit gene flow between them (Hendry et al. 1999; Hendry and Day 2005).

Coastal cutthroat and rainbow trout are iteroparous and exhibit a diversity of life history traits, including age at reproduction, spawn timing, and time of freshwater residency (Withler 1966; Hendry et al. 2004). Anadromous and freshwater obligate life histories are common to both species (Trotter 1989; Baker et al. 2002; Quinn 2005). Spawning coastal cutthroat trout and steelhead (anadromous rainbow trout) are generally separated both temporally and spatially, but the two species have been observed spawning at the same time and place in some streams (Campton 1981). There are often large differences in size between the two species at different life stages that might contribute to assortative mating even under conditions of overlap (Foote and Larkin 1988; Hawkins 1997). Unless otherwise stated, the fish in this study will be referred to as either coastal cutthroat trout or rainbow trout regardless of life history or life stage.

Early research generally concluded that temporal and spatial variation during spawning maintained the integrity of *Oncorhynchus* species, limiting opportunities for interbreeding between sympatric populations (Busack and Gall 1981; Campton and Utter 1985; Heggberget et al. 1988; Trotter 1989). Other studies have recently challenged the paradigm of strict genetic isolation among Pacific salmon and trout, persistent bidirectional hybridization being documented in many localities (Campton and Utter 1985; Neillands 1990; Hawkins 1997; Baker et al. 2002; Rubidge and Taylor 2004, 2005). Hybridization between coastal cutthroat and rainbow trout is now known to be relatively widespread along the coast of western North America (Johnson et al. 1999), and low levels of introgression have been detected in many areas where these two species co-occur (Campton and Utter 1985; Hawkins 1997; Wenburg et al. 1998; Young et al. 2001; Baker et al. 2002; Ostberg et al. 2004).

Molecular methodologies provide reliable means for

identifying hybrids (reviewed by Rieseberg 1998) and have been used to document hybridization among many species of salmonids (Bernatchez et al. 1995; Baxter et al. 1997; Glémet et al. 1998; Rosenfield et al. 2000). Linkage disequilibrium (i.e., nonrandom associations of alleles between loci) is expected in hybrid populations with recent introgression of relatively pure taxa (Harrison and Bogdanowicz 1997; Hitt et al. 2003). First-generation (F_1) hybrids are expected to yield strong linkage disequilibrium, with a tendency for alleles to equilibrate over many generations. However, recent or frequent introgression between taxa will maintain a nonrandom distribution of parental nuclear DNA fragments within a sample population and can be used to infer patterns of hybridization (Epifanio and Philipp 1997; Anderson and Thompson 2002).

Combining maternally inherited mitochondrial DNA (mtDNA) and codominant makers (such as microsatellites) can reveal trends and patterns of mating behavior between species (Asmussen et al. 1987; Avise et al. 1990; Forbes and Allendorf 1991; Avise 2000). Instances of both unidirectional (occurring exclusively between the female of one species and the male of another) and reciprocal (between both genders of both species) hybridization have been reported in various salmonid species (Bernatchez et al. 1995; Leary et al. 1995; Baxter et al. 1997; Rosenfield et al. 2000; Porath and Nielsen 2003; Rubidge and Taylor 2004).

Coastal cutthroat trout occur throughout the southeastern coastal region of Alaska, including the study area on the Copper River (Currens et al. 2003). Steelhead migrate up the Copper River and associated tributaries and putatively pure resident rainbow trout populations occur in headwater regions, many beyond the distributional range of coastal cutthroat trout (Wuttig et al. 2004). The spatial distributions of the juveniles and young adults of both species have been shown to overlap in the lower Copper River (Currens et al. 2003; Wuttig et al. 2004); the temporal overlap among spawning adults in this drainage, however, remains undocumented.

Spawning habitat preferences differ among species, rainbow trout and steelhead preferring sites with higher gradients and greater bottom velocities (Heggenes et al. 1991; Quinn 2005). In streams, coastal cutthroat trout utilize a wider range of habitats when sympatric with steelhead (Bisson et al. 1988; Hawkins 1997). Female rainbow trout tend to occupy different breeding sites based on body size, but smaller males will breed with larger females regardless of spawning location or life history (Zimmerman and Reeves 2000; Kuligowski et al. 2005; Quinn 2005). Males vary more in size and age at maturity than females for most salmonid species (Groot and Margolis 1991; Roni 1992). Smaller males tend to "sneak" copulations with larger females rather than compete with larger males (Quinn 2005). Directional hybridization would be expected to occur if rainbow trout males sneak copulations with larger coastal cutthroat trout females. This may be especially true if rainbow trout males are breeding in sympatric habitats more commonly used by coastal cutthroat trout females than by steelhead (i.e., low-gradient habitats).

The structure and composition of fish communities can be strongly influenced by channel geomorphology and habitat (Schlosser 1995; Poff 1997). Hybridization among fishes has been associated with habitat structure, fragmentation, and ecological transitions from small streams to large rivers (Weigel et al. 2003; Peacock and Kirchoff 2004; Nolte et al. 2006). The hypotheses that resident reproductively mature male rainbow trout or precocial steelhead parr can or will breed with spawning coastal cutthroat trout females and that this occurrence is related to habitat conditions are untested.

This study investigated the natural hybridization between rainbow and coastal cutthroat trout on the Copper River delta, Alaska, a more pristine system than sites depicted in other rainbow trout–cutthroat trout hybrid studies. Molecular protocols were developed to reliably identify hybrid individuals and to assess their extent and distribution throughout the Copper River delta. Molecular data were used to investigate the directionality of hybridization between males and females of both species under different conditions. Molecular and habitat data were correlated to test the potential influence of habitat on natural hybridization between these two species.

Methods

Sample collections and channel type.—The Copper River delta encompasses 318,000 ha on the lower portion of the Copper River in south-central Alaska (Figure 1). The area provides a range of aquatic habitats, including intertidal sloughs and glacier-fed streams (Kruger and Tyler 1995). In 2001 and 2002, U.S. Forest Service personnel collected caudal fin tissue from trout within selected streams on the Copper River delta and in nearby Prince William Sound (Figure 1). Adult fish were collected from pool habitats by hook and line from May to September 2001–2002 (Table 1). In 2001, samples were collected from a fishweir on 18 Mile Creek. Samples were collected from a wide geographical area and all available pool habitats. Upon capture, fish were anesthetized with MS-222 (buffered tricaine methanesulfonate) and fork length was measured to the nearest millimeter. A small piece of tissue was removed from the caudal fin of

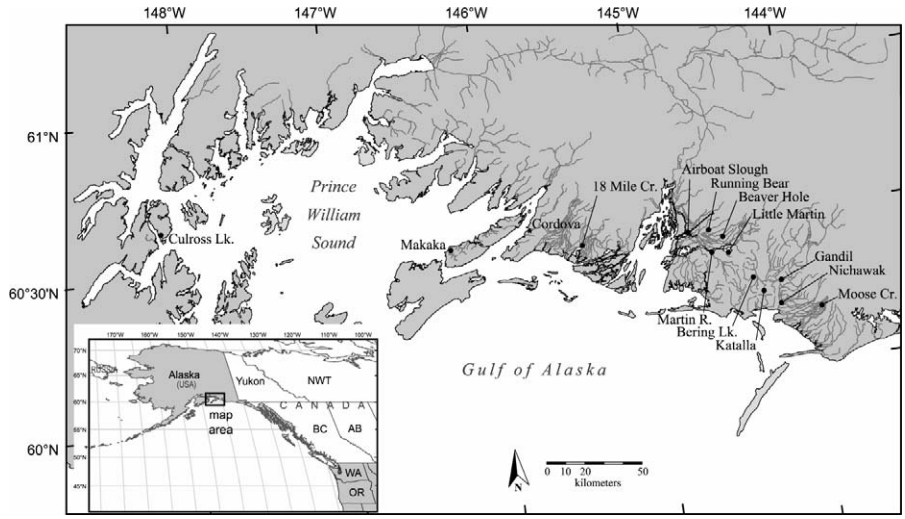

FIGURE 1.—Map of Southeast Alaska near the town of Cordova showing the study locations in the Copper River delta from 18 Mile Creek east to Moose Creek. Two control populations were collected from the Prince William Sound area, namely, rainbow trout from Culross Lake and coastal cutthroat trout from Makaka Creek.

each fish and stored in 100% ethanol for genetic analysis. Each fish was assigned a taxonomic classification in the field based on a set of diagnostic phenotypic criteria derived from characteristics presented in Behnke (1992; Table 2). The physical characteristics used to classify fish to individual taxa were similar to those presented in Weigel et al. (2002), including the intensity of gill slash color, spotting pattern and shape of spots, and position of the end of the maxillary relative to that of the eye; however, no quantitative categorical phenotypic characteristics were recorded for this study. Individuals exhibiting phenotypic traits from both species were classified in the field as hybrids. Several individuals that could not be

TABLE 1.—Collection locations and dates, number of fish, and fish lengths for samples collected from the Copper River delta for this study of natural hybridization.

Location	Year	Collection dates	Fish sampled	Mean fork length (SD [mm])
Martin River	2001	Apr 30, Jun 25, Jul 16	41	408.9 (92.1)
	2002	May 12, 14, Jul 10	70	436.1 (86.5)
Little Martin River	2001	Jul 16	42	267.2 (88.0)
	2002	Jul 10, 12	60	245.4 (112.7)
18 Mile Creek	2001	May 2–Jun 7	40	228.3 (61.9)
	2002	Jul 11	60	305.1 (51.8)
Airboat Slough	2001	Jul 17	40	337.1 (47.4)
	2002	Jul 9	38	324.1 (58.6)
Makaka Creek	2001	Jul 24	40	235.3 (55.2)
	2002	Jul 8	40	288.5 (62.8)
Beaver Hole Creek	2001	Jul 22	40	268.5 (71.9)
	2002	Jul 19	40	271.4 (74.6)
Running Bear Creek	2001	Jul 22	40	268.5 (71.9)
	2002	Jul 16, 18	40	259.4 (65.7)
Fish Creek (Gandil River)	2001	Sep 15	26	281.5 (56.5)
	2002	Sep 12	30	276.6 (54.0)
Moose Creek	2001	Sep 10	35	304.7 (53.9)
	2002	Sep 11, 16	26	324.3 (66.3)
Bering Lake	2001	Sep 11, 15	10	356.9 (28.0)
	2002	Sep 8	40	319.5 (50.6)
Nichawak River	2001	Sep 12	40	293.4 (57.1)
	2002	Sep 9, 10	60	269.6 (73.0)
Culross Lake	2002	Aug 7	41	168.0 (45.2)
Katalla River	2001	Jul 21	8	Not taken

TABLE 2.—Physical features used to classify trout species in the Copper River delta, 2001–2002.

Fish type	Physical features
Coastal cutthroat trout	Red–orange slashes under jaw Maxillary extending beyond eye Spotting, heavy beyond midline of body
Rainbow trout	No red–orange slashes under jaw Maxillary not extending beyond eye Spotting below midline light or absent
Hybrid	Combination of coastal cutthroat and rainbow trout features

classified by the given criteria were listed as unknowns.

Fish from two sample locations in Prince William Sound, Makaka Creek ($n = 77$) and Culross Lake ($n = 41$; Figure 1), represented putatively pure coastal cutthroat and rainbow trout populations, respectively. The Culross Lake population was collected in 2001 by the Alaska Department of Fish and Game. Both of these systems occur outside the putative hybrid zone on the Copper River delta and have no documented history of supplementation or hatchery introductions of the other species. These populations served as controls for the verification of regional allelic variation in molecular markers.

The percent of selected channel process groups (Table 3; U.S. Forest Service 1992) was used to determine possible relationships between the degree of hybridization and geomorphic features of the sampled watershed. The channel process groups included floodplain, high-gradient-constrained, and palustrine channels and were representative of the three major types of channels used by coastal cutthroat and rainbow trout in this area. The channel classification integrates the relations between landform relief, geology, and glacial and tidal influences on fluvial and depositional processes (U.S. Forest Service 1992). Data on the percent of the selected channel process types in many of the sampled streams were obtained from the U.S. Forest Service, Cordova Ranger District, in Cordova, Alaska. Statistical correlations between the percentage of the watershed in any given channel type and the percentage of hybrid fish sampled were determined.

Microsatellite analysis.—Genomic DNA was extracted from caudal fin tissue using the Puregene DNA isolation system (Gentra Systems, Minneapolis, Minnesota) and quantified using a Hoefer DyNA Quant 200 fluorometer. Microsatellite markers developed for use in various salmonid species were screened for bimodal allelic range distributions between rainbow and coastal cutthroat trout. Two markers, *Ots1* (Banks et al. 1999) and *Sfo8* (Angers et al. 1995), had previously been reported as diagnostic species markers for rainbow and cutthroat trout (Wenburg et al. 1998). Two additional markers revealed complete separation in species-specific allele ranges: *One*μ*8* (Scribner et al. 1996) and *Omm1389* (Rodriguez et al. 2003). All four microsatellite markers were amplified independently in 10-μL reactions. Before amplification, the forward (F) primers of *Ots1*, *Sfo8*, and *One*μ*8* were directly labeled with a fluorescent IRD 700 or IRD 800 dye (LI-COR, Lincoln, Nebraska) for visualization of the polymerase chain reaction (PCR) product. Amplification of *Omm1389* used an M13F (29) tail (Steffens et al. 1993; Oetting et al. 1995) synthesized onto the 5′ end of the forward primer. A complementary labeled M13F sequence was added to the PCR reaction mixture and subsequently incorporated into the product for detection and visualization.

The reaction mix consisted of approximately 50 ng of genomic DNA; 10 mM tris-HCl (pH 8.3); 1.5 mM $MgCl_2$; 50 mM KCl; 0.01% each of gelatin, NP-40, and Triton X-100; 0.2 mM each of 2′-deoxyadenosine 5′-triphosphate (dATP), 2′-deoxycytidine 5′-triphosphate (dCTP), 2′-deoxyguanosine 5′-triphosphate (dGTP), and 2′-deoxythymidine 5′-triphosphate (dTTP); and 0.5 units of *Taq* DNA polymerase (Promega, Madison, Wisconsin). For *Ots1*, 0.2 pmol of direct label and 3.8 pmol of unlabeled forward primer were used. *Sfo8* required 0.15 pmol of direct label and 3.85 pmol of unlabeled forward primer. *One*μ*8* required 0.3 pmol of direct label and 1.0 pmol of unlabeled forward primer. *Omm1389* was amplified with the addition of 5.0 pmol each of unlabeled reverse (R) and M13F tailed F primer, *Omm1389* primer, and 1.5 pmol of labeled M13F primer.

Amplification of *One*μ*8* and *Sfo8* was carried out on Robocycler (Stratagene) thermocyclers. An initial 2-min denaturing cycle was followed by 30 cycles of denaturing at 94°C for 60 s, annealing at 54°C for 60 s,

TABLE 3.—Channel process groups (U.S. Forest Service 1992) used in the study.

Process group	Description
Floodplain	Low-gradient channels (<2%) with large amounts of deposition of various-sized sediments; generally lowland and valley bottom streams; some degree of floodplain development
High-gradient constrained	Mountain slope streams, first- and second-order headwater channels characterized by primary sediment sources; substrates are large, small cobble to bedrock
Palustrine	Channels are very low gradient (<1%) associated with low relief landforms and wetlands; water movement is slow and sediment transport low; channel banks are usually stable and floodplain depositional features, such as gravel bars, are absent

and extension at 72°C for 60 s. Amplification of *Ots1* and *Omm1389* was carried out on MJ Research DNA engine thermocyclers with an initial 2-min denaturing cycle followed by 35 cycles of denaturing at 94°C for 15 s, annealing at 52°C for 30 s, and extension at 72°C for 30 s. Gel electrophoresis and visualization of microsatellite alleles was performed using LI-COR Model 4200 LR and IR2 automated fluorescent DNA sequencers. Sizing and scoring was performed using Gene ImagIR version 3.00 (LI-COR). Allele sizes (including primers) for *Sfo8*, *Omm1389*, and *One*μ*8* were determined in relation to an M13 sequence ladder. Allele sizes for *Ots1* were calibrated to individuals with allelic scores determined from genetic analyses performed in the laboratory independently of this study. To verify the accuracy of scoring, approximately 10% of individuals were reamplified.

GENEPOP (version 3.4; Raymond and Rousset 1995) was used to test for genotypic disequilibrium among pairwise comparisons of the four microsatellite loci by population.

Mitochondrial DNA.—Restriction fragment length polymorphism (RFLP) analysis of mtDNA was performed on all individuals from populations with more than 10% hybrids as indicated by microsatellite analysis. All individuals (regardless of the population of origin) displaying hybrid microsatellite signatures were screened to determine the taxonomic origin of their mtDNA using RFLP analyses. The product of a PCR amplification of a fragment of the NADH dehydrogenase 5/6 (*ND5/6*) gene was subsequently digested with *Dde*-1 restriction enzyme to provide species-specific signatures. This protocol was previously shown to be diagnostic between rainbow trout and both westslope cutthroat trout *O. c. lewisii* and Yellowstone cutthroat trout (*O. c. bouvierii* (Mays 2001).

The *ND5/6* mtDNA gene region was amplified in a 30-μL reaction that consisted of approximately 150 ng of genomic DNA, 10 mM tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% each of gelatin, NP-40, and Triton X-100, 2.5 pmol of each *ND5/6* primer, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, and 1.5 units of *Taq* DNA polymerase. The PCR amplification was conducted on Robocycler (Stratagene) thermocyclers. The amplification protocol consisted of an initial 2-min denaturing cycle followed by 30 cycles at 94°C for 45 s, 50°C for 30 s, and 72°C for 2 min 30 s. The amplification products were digested with *Dde*-1 restriction enzyme, fractionated in 2% agarose gels for 2 h at 100 V next to a 100 base-pair (bp) ladder, stained with 5% ethidium bromide, and photographed under ultraviolet light. Primer sequences for the *ND5/6* fragment are reported in Nielsen et al. (1998).

Dominant species-specific simple sequence repeats analysis.—One category of the group of molecular markers called simple sequence repeats (SSRs) contains a class of DNA markers specifically designed to study hybrid zones in western trout (Ostberg and Rodriguez 2002). Simple sequence repeat primer pairs are developed from species-specific sequences that are derived from single primer amplifications of genomic DNA (Rieseberg 1998). These markers have a dominant inheritance pattern, each primer pair being designed to amplify product in the target species only (Ostberg and Rodriguez 2002, 2004). Nonhybrid individuals will display PCR amplification products at species-specific markers only, while F_1 hybrids will have genetic material of mixed taxonomic ancestry and will generate products at all markers specific to either parent species. We made no effort to classify hybrids to later-generation categories (i.e., backcrosses) using these markers.

Populations displaying microsatellite hybrid signatures were subsequently screened with four species-specific SSR markers. Ostberg and Rodriguez (2002) developed a suite of 26 markers to distinguish between rainbow trout and various subspecies of cutthroat trout. Two markers specific to coastal cutthroat trout (*Occ1* and *Occ12*) and two specific to rainbow trout (*Om1* and *Om27*) were chosen for ease and clarity of amplification after screening the nine markers applicable to these species (Ostberg and Rodriguez 2002). Ostberg and Rodriguez (2002) reported that *Om1* and *Occ1* amplified 300-bp products in rainbow trout and coastal cutthroat trout, respectively. *Occ12* reportedly produces a 150-bp product in coastal cutthroat trout and *Om27* a 200-bp product in rainbow trout.

M13R tails were added to the 5′ ends of one primer for each of the primer pairs specific to coastal cutthroat trout (*Occ1* and *Occ12*) and rainbow trout (*Om1* and *Om27*). All SSR markers were amplified in 10-μL reactions consisting of 50 ng of genomic DNA, 10 mM tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1 μL of Promega 10× thermophilic DNA polymerase buffer, 1.5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, and 0.5 units of *Taq* DNA polymerase. *Occ1* and *Occ12* were multiplexed by adding 1 μM of each unlabeled *Occ1* primer, 0.5 μM of each *Occ12* unlabeled primer, and 0.15 μM of labeled M13R primer per reaction. *Om1* and *Om27* were multiplexed by adding 0.5 μM per reaction of each pair of unlabeled primers and 0.1 μM of labeled M13F tail.

Determination criteria for hybrid classification.—The diagnostic genetic variation found in control populations for microsatellite and species-specific SSR loci was used to categorize individuals as rainbow trout–steelhead, coastal cutthroat trout, or F_1 hybrids.

TABLE 4.—Microsatellite and species-specific simple sequence repeat (SSR) loci genotype criteria used to categorize individuals as rainbow trout, coastal cutthroat troat, or first-generation hybrids (F_1). Owing to the lack of resolution provided by four SSR markers (two specific to each species), backcross individuals were categorized based on microsatellite information only (Boecklen and Howard 1997).

Category	Microsatellite criteria	SSR criteria
Rainbow trout	Only alleles within the diagnostic range for rainbow trout (homozygous for rainbow trout)	Product at rainbow trout–specific markers only
Coastal cutthroat trout	Only alleles within the diagnostic range (homozygous for coastal cutthroat trout)	Product at coastal cutthroat trout–specific markers only
F_1 hybrid	Heterozygous, with one rainbow trout and one coastal cutthroat trout allele at each locus for all loci	Product at all species-specific markers
Rainbow trout backcross	Homozygous for diagnostic rainbow trout alleles for at least one marker; heterozygous with one rainbow trout and one coastal cutthroat trout allele at all other loci	Not diagnostic
Coastal cutthroat trout backcross	Homozygous for diagnostic coastal cutthroat trout alleles for at least one marker; heterozygous with one rainbow trout and one coastal cutthroat trout allele at all other loci	Not diagnostic
Later-generation hybrids	Homozygous for both species for at least one locus; heterozygous at other loci	Not diagnostic

Lack of resolution from species-specific SSR markers did not allow the use of these markers to categorize backcrossed or later-generation hybrids (Table 4). Putatively pure individuals will have alleles within a species-specific range for all diagnostic markers, while putative F_1 individuals will be "hybrid" at all loci, having one allele within each species-specific range.

Results

Microsatellite and Species-Specific SSR Markers

Three microsatellite markers (*One*μ*8*, *Ots1*, and *Omm1389*) displayed discrete species-specific allele distributions in control populations (Table 5). At study site locations, alleles beyond these distributions were observed at *One*μ*8* (152 and 158) and *Omm1389* (180 and 194). These alleles were given species-specific assignment in accordance with assumptions made by the stepwise mutation model of microsatellite evolution, which assumes that microsatellite mutation occurs in repeat-unit increments. Species-specific assignments were made in parsimony with this assumption. All *Ots1* alleles fell within the species-specific boundaries detected in control populations. An overlap in allele distributions was detected at a fourth diagnostic locus (*Sfo8*), the 241 allele (assumed to be identical by state) occurring in both species. Fish with this allele could not be identified as hybrid at this locus, so the results from this locus were excluded from analyses. The 197 allele at *Sfo8* occurred outside the lower boundary established by the control distribution but was assigned to coastal cutthroat trout for the reason stated above.

The SSR markers specific to coastal cutthroat trout (*Occ1* and *Occ12*) did not amplify product in the rainbow trout control population (Culross Lake), and the SSR markers specific to rainbow trout (*Om1* and *Om27*) did not amplify product in the coastal cutthroat trout control population (Makaka Creek), confirming the rigor of these markers for F_1 hybrid identification.

Direction, Distribution, and Extent of Hybridization

The trout populations in the Copper River delta were categorized based on genetic data into one of three general groups: (1) groups with putatively pure parental individuals of both species and hybrids; (2) groups with putatively pure coastal cutthroat trout and hybrids; and (3) populations with coastal cutthroat trout only. Hybrids and putatively pure individuals of both species were detected in the 2001 and 2002 sample sets at the Little Martin, Martin, and Nichawak rivers, hybrids being found in relatively equal proportions across years (Table 6). Fifty-eight percent ($n = 52$) and 26% ($n = 23$) of the individuals analyzed from the Little Martin and Martin River systems, respectively, displayed DNA signatures consistent with mixed taxonomic origin. Based on a priori criteria (Table 4), molecular analyses detected 15 F_1 hybrids among the trout sampled from the Martin River (Table 6). Only 5 of these individuals were classified as hybrids based on nonquantitative phenotypic criteria observed in the field. Congruence between molecular results and field-based classifications of F_1 hybrids was observed in 65% of all trout from the Little Martin River over 2 years ($n = 89$). Rainbow trout were present but not abundant in the Nichawak River ($n = 4$; 4%). Approximately 8% of individuals from the Nichawak River region had DNA of mixed taxonomic origin.

TABLE 5.—Allele frequencies detected in Makaka Creek cutthroat trout (CCT) and Culross Lake rainbow trout (RBT) control populations at four diagnostically informative loci, *One*μ*8*, *Ots1*, *Omm1389*, *Sfo8*.

*One*μ*8*			*Ots1*			*Omm1389*			*Sfo8*		
Allele	CCT	RBT	Allele	CCT	RBT	Allele	CCT	RBT	Allele	CCT	RBT
152			166		59.21	180			197		
158			170		1.32	184		9.21	201	20.15	
160		36.59	172			190		90.79	203	0.75	
162			174		23.68	194			205		
164		10.98	237			208	6.94		207	0.75	
168			239			210	20.14		209	14.93	
170			241		10.53	212	11.81		215	30.60	
172		42.68	243		5.26	222	0.69		217	11.94	
178		9.76	251			224	31.94		221	13.43	
184			253			226	11.11		225	2.99	
188	6.43		255	0.71		228	11.81		229		
190			261			232	4.17		241	2.99	3.13
192	11.43		263			236	1.39		243	1.49	
194	7.14		265	21.43					267		
196	2.86		267	2.86					271		
198	3.57		269	22.86					275		21.88
200			271	0.71					277		
202			273	17.14					279		1.56
204	4.29		275						283		
206	15.71		277	1.43					285		
208			279	30.00					287		39.06
218			281	0.71					289		28.13
220	4.29		283	2.14					293		
222									295		
226									299		6.25
228											
232	0.71										
234											
236	1.43										
238	1.43										
240											
242	0.71										
244	1.43										
246	7.14										
250	3.57										
252	1.43										
258	3.57										
260	2.14										
262	0.71										
264	9.29										
266	1.43										
268	3.57										
270	0.71										
272											
274											
276											
278	0.71										
280											
284	2.86										
288	1.43										

Significant genotypic disequilibrium ($P < 0.003$) was found in all possible comparisons of microsatellite loci in the Martin River trout population and in four of the six comparisons on the Little Martin River. Only comparisons between *Sfo8* and *One*μ*8* ($P = 0.369$) and *Sfo8* and *Omm1389* ($P = 0.698$) showed no significant genotypic disequilibrium among the trout on the Little Martin River. Rainbow trout collected from Culross Lake as a control population showed genotypic disequilibrium at one pairwise comparison, *One*μ*8* and *Omm1389* ($P = 0.02$). There was no evidence of genotypic disequilibrium among microsatellite loci in the coastal cutthroat trout control population (Makaka Creek; $P > 0.14$ in all cases). Significant genotypic disequilibrium for at least one comparison was also found in trout from 18 Mile Creek (4% hybrids identified), Katalla River (13% hybrids), and Moose Creek (9% hybrids), but not in any of the other sample populations. Two locations with fish classified as hybrids based on molecular analyses, Airboat Slough

TABLE 6.—Numbers of putative rainbow trout (RBT), coastal cutthroat trout (CCT), first-generation hybrids (F_1), and backcrossed hybrids (BC) analyzed in 2001 and 2002. The geographic area for each sample site is also given.

Watershed	Area (ha)	Year	Total *N*	F_1	BC	RBT	CCT	Proportion hybrid[a]
Airboat Slough	36,096	2001	39	0	0	0	39	
		2002	33	1	0	0	32	0.01
Beaver Hole Creek	1,070	2001	40	0	0	0	40	
		2002	39	0	0	0	39	
Bering Lake	106,792	2001	10	0	0	0	10	
		2002	40	0	0	0	40	
Culross Lake	3,934	2001	41	0	0	41	0	
		2002	0	0	0	0	0	
18 Mile Creek	2,303	2001	39	0	2	0	37	
		2002	46	1	0	0	45	0.04
Fish Creek (Gandil River)	12,922	2001	26	0	0	0	26	
		2002	30	0	0	0	30	
Katalla River	12,294	2001	8	0	1	0	7	
		2002	0	0	0	0	0	0.13
Little Martin River	1,976	2001	42	9	19	7	7	
		2002	47	8	16	19	4	0.58
Martin River	9,185	2001	41	8	4	17	12	
		2002	47	7	4	32	4	0.26
Makaka Creek	2,382	2001	39	0	0	0	39	
		2002	38	0	0	0	38	
Moose Creek	14,907	2001	35	0	3	0	32	
		2002	24	0	2	0	22	0.09
Nichawak River	3,046	2001	39	2	1	1	35	
		2002	52	3	1	3	45	0.08
Running Bear Creek	2,722	2001	40	0	0	0	40	
		2002	40	0	1	0	39	0.01

[a] 2001 and 2002 combined when available.

and the Nichawak River, did not exhibit genotypic disequilibrium ($P > 0.26$ and $P > 0.12$ for all comparisons, respectively).

No putatively pure rainbow trout were found in Airboat Slough, Running Bear Creek, 18 Mile Creek, Moose Creek, and the Katalla River (Table 6). Single hybrid individuals were detected in each of the Airboat Slough and Running Bear Creek samples from 2002. The Airboat Slough individual was a putative F_1 (with rainbow trout mtDNA; Table 7); the Running Bear individual had two rainbow trout microsatellite alleles (*Sfo8* [295] and *One*μ*8* [162]) and cutthroat trout mtDNA. Five hybrid individuals were genotyped from the Moose Creek samples; three putative hybrids were detected in 2001 and two in 2002 (Table 6). Only two rainbow trout alleles were detected in the Moose Creek population (*Sfo8* [285] and *Omm1389* [190]). No putative hybrid individual had more than one rainbow trout allele, and all five fish carried coastal cutthroat trout mtDNA (Table 7).

Two hybrids were detected at 18 Mile Creek in 2001 and one hybrid was detected in 2002. Microsatellite analyses of the four diagnostic loci revealed that six of the possible eight alleles were of rainbow trout origin in both 2001 hybrids. Both individuals had rainbow trout mtDNA. The 2002 hybrid was a first generation hybrid (F_1) and carried coastal cutthroat trout mtDNA. The Katalla River was only sampled in 2001 ($n = 8$). One putative hybrid with a single rainbow trout allele at *Sfo8* (285) was detected. This individual had coastal cutthroat trout mtDNA. No rainbow trout molecular signatures were detected at Beaver Hole Creek, Bering Lake, or Fish Creek (Gandil River).

Thirty-one of 37 (84%) F_1 hybrids had coastal cutthroat trout mtDNA (Table 7). Unidirectional hybridization was not apparent for the five F_1 hybrids at the Nichawak River; three of these F_1 hybrids carried rainbow trout mtDNA. Two backcrossed hybrids from

TABLE 7.—Species-specific mitochondrial DNA restriction fragment length polymorphism patterns (numbers of occurrences) at *ND5/6* found in F_1 and backcrossed hybrids collected from eight sample locations in the Copper River delta, Alaska.

Hybrid type	Location	Total *N*	RBT	CCT
F_1	Airboat Slough	1	1	0
	Little Martin River	17	1	16
	Martin River	15[a]	1	12
	18 Mile Creek	1	0	1
	Nichawak River	5	3	2
Backcrossed	Little Martin River	35	6	29
	Martin River	8	1	7
	18 Mile Creek	2	2	0
	Nichawak River	2	0	2
	Katalla River	1	0	1
	Running Bear Creek	1	0	1
	Moose Creek	5	0	5

[a] Data not available for two fish.

the Nichawak River carried coastal cutthroat trout mtDNA. Seventy-six of the 91 F_1 and backcrossed hybrids (84%) detected in this study contained coastal cutthroat trout mtDNA.

Hybridization and Channel Process Groups

Correlations between the U.S. Department of Agriculture channel process groups and the percent of hybridization were not statistically significant ($P > 0.05$). The correlation coefficients (r) for the floodplain and high-gradient stream process groups were 0.194 and 0.516, respectively. The correlation coefficient for the palustrine group was −0.490.

Discussion

More than 670 microsatellite loci have been developed from species within the Salmonidae family (Peacock et al. 2004). Primers designed for use in one species frequently are successfully amplified in fish from closely related groups (Scribner et al. 1996; Nielsen and Sage 2002). In addition, relatively few markers (2–4) can be used to give probabilistic arguments about the hybrid status of putative parental, F_1, and post-F_1 hybrid individuals (Baverstock and Moritz 1996; Boecklen and Howard 1997). Putatively pure individuals will have alleles within a species-specific range at all diagnostic microsatellite markers, while putative F_1 individuals will be "hybrid" at all loci, having one allele within each species-specific range (Boecklen and Howard 1997).

Young et al. (2001) argued that microsatellite loci may have limited utility in hybridization studies because markers displaying exact bimodal allele distributions would be hard to find. Further, molecular markers that demonstrate interspecies diagnostic ranges in one region may not do so in another owing to biogeographic variation in allelic structure (Landry and Bernatchez 2001). It is important to screen putatively pure populations from the study area to acquire regional allelic baseline ranges for each species (Baverstock and Moritz 1996). Isolated trout populations with no known species overlap and no history of introductions or interspecific supplementation provided the species-specific baseline allelic structure for this study. The species-specific allelic ranges found in these populations supported the use of selected microsatellite markers for assessing hybridization.

Four codominant microsatellite markers were used to assess hybridization. Two markers (*Ots1* and *Sfo8*) had previously demonstrated species-specific resolution for coastal cutthroat trout in Washington State (Wenburg et al. 1998) and for Lahontan cutthroat trout (*O. c. henshawii*) along the Nevada–Oregon border (Peacock and Kirchoff 2004). This study demonstrated the utility of *Ots1* and *Sfo8* for detecting trout hybrids over a greater geographic range.

Categorizing hybrids based on morphological characters assessed in the field can be unreliable, particularly after multiple-generation backcrosses (Allendorf et al. 2004). Juveniles can be difficult to classify, and F_1 hybrids do not necessarily have intermediate phenotypes (Baxter et al. 1997; Weigel et al. 2002). In past studies, field identification was most accurate for coastal cutthroat trout, followed by hybrids and then steelhead (Campton and Utter 1985; Hawkins 1997). Salmonid phenotypes are known to be plastic, making identification in the field (even to the species level) difficult at times, whereas species-specific molecular markers can resolve conflicting morphological assessments (Bartley et al. 1990; Baker et al. 2002; Nielsen et al. 2003).

Our results suggest that there is gene flow and directional hybridization in natural populations of rainbow and coastal cutthroat trout on the Copper River, Alaska. Hybridization between rainbow and coastal cutthroat trout has been recorded at several locations in Washington (Campton and Utter 1985; Hawkins 1997; Johnson et al. 1999; Young et al. 2001; Ostberg et al. 2004), Oregon (Griswold 1996; Johnson et al. 1999), northern California (Neillands 1990; Johnson et al. 1999), and Vancouver Island, British Columbia (Bettles et al. 2005). These reports depict trout populations in areas impacted by artificial habitat conditions, nonnative fish introductions, or both. Habitat disturbance and other human influences have been shown to disrupt aquatic ecosystems and facilitate hybridization between previously isolated groups of fish (Anderson 1948; Hubbs 1955; Dowling and Secor 1997; Rosenfield et al. 2000; Hitt et al. 2003). Distinguishing between natural hybridization and hybridization influenced by human activity (through introductions or habitat modifications) can be difficult (Allendorf et al. 2001). This study looked at natural hybridization between sympatric coastal cutthroat and rainbow trout in a relatively pristine environment with no history of introductions of nonnative or hatchery trout. A growing body of work has shown that hybridization can occur naturally among recently diverged (1–2 million years) taxa (reviewed in Mallet 2005), suggesting that divergent evolution and limited gene flow may not be incompatible in coastal cutthroat and rainbow trout. However, understanding the intrinsic mechanisms leading to natural hybridization and the factors sustaining divergent evolution in areas where natural hybridization has occurred is more difficult.

Stream ecology, hydrogeographic history, and habitat structure have been shown to influence patterns

of hybridization (Glémet et al. 1998; Wilson and Bernatchez 1998; McKinnon et al. 2004; Carson and Dowling 2006; Gow et al. 2006). Coastal cutthroat trout utilize a wide range of habitat types and probably display the broadest range of migratory behaviors within the salmonid group (Northcote 1997). Molecular methods detected coastal cutthroat trout throughout the Copper River delta study area, while rainbow trout were found only at the Martin, Little Martin, and Nichawak rivers. Possible explanations for the limited distribution of rainbow trout are (1) rainbow trout have only recently colonized the Copper River delta and have had limited time to expand their range; (2) coastal cutthroat trout outcompete rainbow trout, thereby limiting the distribution of the latter; and (3) the rainbow trout distribution is limited by the scarcity of favorable habitat in the Copper River delta.

Previous studies of sympatric rainbow and coastal cutthroat trout populations have shown that rainbow trout and their hybrids are likely to competitively exclude coastal cutthroat trout since they grow faster and have a larger size at age (Peterson et al. 1990; Campbell et al. 2002; Meyer et al. 2003). Rainbow trout, including steelhead, generally prefer streams with coarse substrates, such as cobble and larger-sized gravel (Reeves et al. 2002). These types of substrate were limited in the study area (G.H.R., personal observation). Therefore, we suggest that the distribution of rainbow trout demonstrated in this study is best explained by the lack of favorable spawning habitat in the Copper River delta. However, we found no significant statistical correlation between stream channel process and the percent of trout hybrids at any sample locality, suggesting that available habitat was not the determining factor for hybridization in this area.

Documentation of the structure of hybrid zones can lend inference to the processes that generated this structure (Jiggins and Mallet 2000). The distribution of hybrids on the Copper River delta was found to be nonrandom with respect to species distribution; most individuals of mixed taxonomic ancestry were detected in streams with large numbers of rainbow trout. Genotypic disequilibrium was demonstrated to be highest in populations with the highest numbers of hybrids, suggesting recent hybridization events. Backcrossed individuals, however, were located throughout the study area, indicating that at least some of these hybrids were viable and contributing to the distribution of alleles across sample locations. The subsequent dispersal and straying of backcrossed offspring into nonnatal streams has been used to explain the distribution of hybrids over a broader geographic range (Hitt et al. 2003; Rubidge and Taylor 2004).

Mitochondrial DNA markers showed a high degree of gender-based bias in mating behavior at two locations (the Martin and Little Martin rivers), hybridization primarily occurring between female coastal cutthroat trout and male rainbow trout. Gender-based unidirectional hybridization is common among animals. Wirtz (1999) examined 80 studies that analyzed the mtDNA from at least five hybrid individuals and found that 50 reported that all hybrids had obtained their maternal contributions from one species exclusively. Other studies of hybridization between rainbow and coastal cutthroat trout have reported both unidirectional and reciprocal spawning (Hawkins 1997; Young et al. 2001; Ostberg et al. 2004). Wirtz (1999) outlined numerous scenarios that could have resulted in such asymmetry. The simplest explanation for the exclusive presence of one mtDNA haplotype is that a single hybridization event occurred. However, an independent hybridization event does not easily explain the dominance of coastal cutthroat trout mtDNA at the Martin and Little Martin rivers across two sampling years.

Smaller, reproductively mature males of many fish species obtain mating opportunities by sneaking behavior and releasing sperm close to larger spawning pairs (Taborsky 1994; Quinn 2005). Such behavior has been documented within *Oncorhynchus* spp. (Baxter et al. 1997; Quinn 2005). In rainbow trout, it has been shown that anadromous and nonanadromous life histories within the same drainage are derived from one gene pool (Pascual et al. 2001; Thrower et al. 2004; Olsen et al. 2006). Ostberg et al. (2004) reported hybridization between rainbow trout and coastal cutthroat trout on the Olympic Peninsula, Washington. In their study, sneaking behavior by male cutthroat trout may have explained the exclusive occurrence of rainbow trout mtDNA in F_1 hybrids. In our study, sneaking behavior by male rainbow trout remains a possible explanation for the predominance of coastal cutthroat trout mtDNA observed at the Martin and Little Martin rivers. Why this behavior would succeed in creating hybrids in one species but not the other at different localities is unclear if males from both species practice sneak spawning. In contrast to the situation at the Martin and Little Martin River systems, our data suggest that interspecific spawning resulting in F_1 hybrids was reciprocal in the Nichawak River despite the small number of F_1 hybrids found at that location. Therefore, even in the small, relatively pristine geographic area that was the focus of this study reciprocal interspecific matings occur and produce hybrids.

Hybrids present complex issues for the conservation and management of fish species (Rhymer and Simberloff 1996; Allendorf et al. 2001, 2004; Seehausen

2004). The U.S. Endangered Species Act (ESA) currently has no provision for dealing with hybrids (Allendorf et al. 2001, 2004), and considerable controversy has developed over the treatment of hybrids in protected populations (Moritz 1994; Stone 2000; Peacock and Kirchoff 2004). Although trout in the Copper River are not considered threatened under the ESA, regulations prohibit the retention of harvestable trout on the eastern side of the Copper River delta. Since simple morphology characteristics determined in the field have not been proven to be a reliable indicator of hybridization, rainbow and coastal cutthroat trout are managed collectively elsewhere in the region (Matt Miller, Alaska Department of Fish and Game, personal communications). Natural hybridization between native coastal cutthroat and rainbow trout confounds the management strategy in the Copper River delta.

Ecological conditions, variation in life history and behavior, and natural colonization contribute to the suite of processes and outcomes we classify as hybridization (Kearney 2005; Rubidge and Taylor 2005; Schmeller et al. 2005). Introgressive hybridization between rainbow trout and other native trout species has a long documented history through translocations and stock transfers with subsequent reductions in natural population productivity (Avise 2000; Utter 2000; Hitt et al. 2003; Rubidge and Taylor 2004). This study helps to demonstrate that hybridization among trout species is not a unitary process with singular outcomes. Hybridization in a natural system with no artificial introductions, such as the Copper River delta, highlights the complexity of the role of natural hybridization in Pacific trout.

Many interspecific salmonid hybrids remain viable and reproduce (Simon and Noble 1968; Bartley et al. 1990; Hendry et al. 2000; Rosenfield et al. 2000; Scribner et al. 2000). Molecular signatures from hybridization events can leave their mark for many generations, especially through mtDNA (Bartley et al. 1990; Bernatchez et al. 1995; Glémet et al. 1998; Thrower et al. 2004); however, if the signal from natural hybridization is swamped by extensive backcrossing, we would never detect it. Natural hybridization between coastal cutthroat trout and rainbow trout shows a disjunctive distribution across their range (Young et al. 2001). This study documented F_1 and backcrossed hybrids in a putatively pristine aquatic system with a complex pattern of hybrid distribution and multiple pathways leading to hybridization between these species. However, we have no idea just how long natural hybridization has been taking place in the Copper River delta or what mechanisms are in place to sustain taxonomic divergence between the two species.

In many parts of the western United States, hybridization between rainbow trout and cutthroat trout has led to discussions of genetic criteria for the determination of hybrids and conservation and management guidelines to deal with these populations (Allendorf et al. 2001). One risk associated with hybridization is the disruption or loss of "native genomes," which can lead to the loss of key adaptive factors in native trout (Leary et al. 1987; Allendorf et al. 2001). Some of the conservation guidelines, however, appear radical and unnecessary if both species can coexist without significant disruption to native genomes (Peacock and Kirchoff 2004).

The discovery of a complex temporal and spatial distribution of natural hybridization in a relatively pristine aquatic system such as the Copper River delta warrants a precautionary approach to the questions how and why hybridization may result in negative effects on native genomes. Unlike in the study by Weigel et al. (2003), the lack of significant correlation among stream channel process types and demonstrated patterns of hybridization found in this study suggests that selection for specific habitats by different species may not be the determining factor in hybridization in the Copper River delta. While our study of natural hybridization between rainbow and coastal cutthroat trout has led to more questions than answers, it is clear the further knowledge of the basic biological and ecological factors leading to natural hybridization among trout species will help us to define more comprehensive and effective conservation and management objectives without unnecessarily compromising the evolutionary potential of any species.

Acknowledgments

The U.S. Geological Survey, Alaska Science Center, and the Aquatic and Land Interaction Program of the U.S. Forest Service, Pacific Northwest Research Station, provided funding for this study. This paper was greatly improved based on reviews by Jason Dunham and Sandy Talbot. Scott Pavey provided laboratory and technical assistance. Deyna Kuntzsch, Merlyn Schelske, Sean Stash, and Sam Greenwood of the Cordova Ranger District and Michael and Zack Reeves, Lance Campbell, Kelly Christiansen, Terry Roelofs, David Hankin, and Matt Sloat helped with sampling. George Coval provided invaluable information on potential study streams. Mention of trade names does not imply U.S. Government endorsement.

References

Albert, V., B. Jonsson, and L. Bernatchez. 2006. Natural hybrids in Atlantic eels (*Anguilla anguilla*, *A. rostrata*): evidence for successful reproduction and fluctuating

abundance in space and time. Molecular Ecology 15:1903–1916.

Allendorf, F. W., and R. F. Leary. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. Conservation Biology 2:170–184.

Allendorf, F. W., R. F. Leary, N. P. Hitt, K. L. Knudsen, L. L. Lundquist, and P. Spruell. 2004. Intercrosses and the U.S. Endangered Species Act: should hybridized populations be included as westslope cutthroat trout? Conservation Biology 18:1203–1213.

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. Trends in Ecology and Evolution 16:613–622.

Anderson, E. 1948. Hybridization of the habitat. Evolution 2:1–9.

Anderson, E. C., and E. A. Thompson. 2002. A model-based method for identifying species hybrids using multilocus genetic data. Genetics 160:1217–1229.

Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for brook char reveal strong population subdivision on a microgeographic scale. Journal of Fish Biology 47(Supplement A):177–185.

Arnold, M. L. 1992. Natural hybridization as an evolutionary process. Annual Review of Ecology and Systematics 23:237–261.

Arnold, M. L. 1997. Natural hybridization and evolution. Oxford University Press, New York.

Asmussen, M. A., J. Arnold, and J. C. Avise. 1987. Definition and properties of disequilibrium statistics for associations between nuclear and cytoplasmic genotypes. Genetics 115:755–768.

Avise, J. C. 2000. Cytonuclear genetic signatures of hybridization phenomena: rationale, utility, and empirical examples from fishes and other aquatic animals. Reviews in Fish Biology and Fisheries 10:253–263.

Avise, J. C., W. S. Nelson, J. Arnold, R. K. Koehn, G. C. Williams, and V. Thorsteinsson. 1990. The evolutionary genetic status of Icelandic eels. Evolution 44:1254–1262.

Baker, J., P. Bentzen, and P. Moran. 2002. Molecular markers distinguish coastal cutthroat trout from coastal rainbow trout and their hybrids. Transactions of the American Fisheries Society 131:404–417.

Banks, M. A., M. S. Blouin, B. B. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in Chinook salmon (*Oncorhynchus tshawytscha*). Journal of Heredity 90:281–288.

Bartley, D. M., G. A. E. Gall, and B. Bentley. 1990. Biochemical genetic detection of natural and artificial hybridization of Chinook and coho salmon in northern California. Transactions of the American Fisheries Society 119:431–437.

Baverstock, P. R., and C. Moritz. 1996. Sampling. Pages 17–27 *in* D. M. Hillis, C. Moritz, and B. K. Mable, editors. Molecular systematics, 2nd edition. Sinuaer Associates, Sunderland, Massachusetts.

Baxter, J. S., E. B. Taylor, R. H. Delvin, J. Hagen, and J. D. McPhail. 1997. Evidence for natural hybridization between Dolly Varden (*Salvelinus malma*) and bull trout (*S. confluentus*) in a northwestern British Columbia watershed. Canadian Journal of Fisheries and Aquatic Sciences 54:421–429.

Behnke, R. J. 1992. Native trout of western North America. American Fisheries Society, Monograph 6, Bethesda, Maryland.

Behnke, R. J. 1996. Kamchatkan cutthroats? Trout 37(3):40–41.

Behnke, R. J. 1997. Evolution, systematics, and structure of *Oncorhynchus clarki clarki*. Pages 3–6 *in* D. Hall, P. A. Bisson, and R. E. Gresswell, editors. Sea-run cutthroat trout: biology, management, and future conservation. American Fisheries Society, Oregon Chapter, Corvallis.

Bernatchez, L., H. Glémet, C. C. Wilson, and R. G. Danzman. 1995. Introgression and fixation of Arctic char (*Salvelinus alpinus*) mitochondrial genome in an allopatric population of brook trout (*S. fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences 52:179–185.

Berra, T. M. 2001. Freshwater fish distribution. Academic Press, San Diego, California.

Bettles, C. M., M. F. Docker, B. Dufour, and D. D. Heath. 2005. Hybridization dynamics between sympatric species of trout: loss of reproductive isolation. Journal of Evolutionary Biology 18:1220–1233.

Bisson, P. A., K. Sullivan, and J. L. Nielsen. 1988. Channel hydraulics, habitat use, and body form of juvenile coho salmon, steelhead, and cutthroat trout in streams. Transactions of the American Fisheries Society 117:262–273.

Boecklen, W. J., and D. J. Howard. 1997. Genetic analysis of hybrid zones: numbers of markers and power of resolution. Ecology 78:2611–2616.

Brown, K. H., S. J. Patton, K. E. Martin, K. M. Nichols, R. Amstrong, and G. H. Thorgaard. 2004. Genetic analysis of interior Pacific Northwest *Oncorhynchus mykiss* reveals apparent ancient hybridization with westslope cutthroat trout. Transactions of the American Fisheries Society 133:1078–1088.

Busack, C. A., and G. A. E. Gall. 1981. Introgressive hybridization in populations of Paiute cutthroat trout (*Salmo clarki seleniris*). Canadian Journal of Fisheries and Aquatic Sciences 38:939–951.

Campbell, M. R., J. Dillon, and M. S. Powell. 2002. Hybridization and introgression in a managed native population of Yellowstone cutthroat trout: genetic detection and management implications. Transactions of the American Fisheries Society 131:364–375.

Campton, D. E. 1981. Genetic structure of sea-run cutthroat trout (*Salmo clarki clarki*) populations in the Puget Sound area. Master's thesis. University of Washington, Seattle.

Campton, D. E., and F. M. Utter. 1985. Natural hybridization between steelhead trout (*Salmo gairdneri*) and coastal cutthroat trout (*Salmo clarki*) in two Puget Sound streams. Canadian Journal of Fisheries and Aquatic Sciences 42:110–119.

Carson, E. W., and T. E. Dowling. 2006. Influence of hydrogeographic history and hybridization on the distribution of genetic variation in the pupfishes *Cyprinodon atrorus* and *C. bifasciatus*. Molecular Ecology 15:667–679.

Crespi, B. J., and M. J. Fulton. 2004. Molecular systematics of Salmonidae: combined nuclear data yields a robust

phylogeny. Molecular Phylogenetics and Evolution 31:658–679.

Currens, K. P., K. E. Griswold, and G. H. Reeves. 2003. Relations between Dolly Varden populations and between coastal cutthroat trout populations in Prince William Sound. *Exxon Valdez* Oil Spill Restoration Project 98145, USDA Forest Service, Pacific Northwest Research Station, Corvallis, Oregon.

Dowling, T. E., and C. L. Secor. 1997. The role of hybridization and introgression in the diversification of animals. Annual Review of Ecology and Systematics 28:593–619.

Epifanio, J., and J. L. Nielsen. 2000. The role of hybridization in the distribution, conservation, and management of aquatic species. Reviews in Fish Biology and Fisheries 10:245–251.

Epifanio, J., and D. Philipp. 2000. Simulating the extinction of parental lineages from introgressive hybridization: the effects of fitness, initial proportions of parental taxa, and mate choice. Reviews in Fish Biology and Fisheries 10:339–354.

Epifanio, J. M., and D. P. Philipp. 1997. Sources for misclassifying genealogical origins in mixed hybrid populations. Journal of Heredity 88:62–65.

Foote, C. J., and P. A. Larkin. 1988. The role of male choice in the assortative mating of anadromous and nonanadromous sockeye salmon (*Oncorhynchus nerka*). Behaviour 106:43–62.

Forbes, S. H., and F. W. Allendorf. 1991. Associations between mitochondrial and nuclear genotypes in cutthroat trout hybrid swarms. Evolution 45:1332–1349.

Glémet, H., P. Blier, and L. Bernatchez. 1998. Geographical extent of the Arctic char (*Salvelinus alpinus*) mtDNA introgression in brook char populations (*S. fontinalis*) from eastern Quebec, Canada. Molecular Ecology 7:1655–1662.

Gow, J. L., C. L. Peichel, and E. B. Taylor. 2006. Contrasting hybridization rates between sympatric three-spined sticklebacks highlight the fragility of reproductive barriers between evolutionarily young species. Molecular Ecology 15:739–752.

Grant, P. R., and B. R. Grant. 1992. Hybridization in bird species. Science 256:193–197.

Griswold, K. E. 1996. Genetic and meristic relationships of coastal cutthroat trout (*Oncorhynchus clarki clarki*) residing above and below barriers in two coastal basins. Master's thesis. Oregon State University, Corvallis.

Groot, G., and L. Margolis, editors. 1991. Pacific salmon life histories. UBC Press, Vancouver.

Harrison, R. G. 1993. Hybrids and hybrid zones: historical perspective. Pages 3–12 *in* R. G. Harrison, editor. Hybrid zones and the evolutionary process. Oxford University Press, New York.

Harrison, R. G., and S. M. Bogdanowicz. 1997. Patterns of variation and linkage disequilibrium in a field cricket hybrid zone. Evolution 51:493–505.

Hawkins, D. K. 1997. Hybridization between coastal cutthroat trout (*Oncorhynchus clarki clarki*) and steelhead (*O. mykiss*). Doctoral dissertation. University of Washington, Seattle.

Heggberget, T. G., T. Haukebø, J. Mork, and G. Stahl. 1988. Temporal and spatial segregation of spawning in sympatric populations of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta* L.). Journal of Fish Biology 33:347–356.

Heggenes, J., T. G. Northcote, and A. Peter. 1991. Seasonal habitat selection and preferences by cutthroat trout (*Oncorhynchus clarki*) in a small, coastal stream. Canadian Journal of Fisheries and Aquatic Sciences 48:785–790.

Hendry, A. P., O. K. Berg, and T. P. Quinn. 1999. Condition dependence and adaptation by time: breeding date, life history, and energy allocation within a population of salmon. Oikos 85:499–514.

Hendry, A. P., and T. Day. 2005. Population structure attributable to reproductive time: isolation by time and adaptation by time. Molecular Ecology 14:901–916.

Hendry, A. P., Y. E. Morbey, O. K. Berg, and J. K. Wenburg. 2004. Adaptive variation in senescence: reproductive lifespan in a wild salmon population. Proceedings of the Royal Society of London B 271:259–266.

Hendry, A. P., S. M. Vamosi, S. J. Latham, J. C. Heilbuth, and T. Day. 2000. Questioning species realities. Conservation Genetics 1:67–76.

Hitt, N. P., C. A. Frissell, C. C. Muhlfeld, and F. W. Allendorf. 2003. Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarki lewisi*, and nonnative rainbow trout, *Oncorhynchus mykiss*. Canadian Journal of Fisheries and Aquatic Sciences 60:1440–1451.

Hubbs, C. L. 1955. Hybridization between fish species in nature. Systematic Zoology 4:1–20.

Jiggins, C. D., and J. Mallet. 2000. Bimodal hybrid zones and speciation. Trends in Ecology and Evolution 15:250–255.

Johnson, O. W., M. H. Ruckelshaus, W. S. Grant, F. W. Waknitz, A. M. Garrett, G. J. Bryant, K. Neely, and J. J. Hard. 1999. Status review of coastal cutthroat from Washington, Oregon, and California. NOAA Technical Memorandum NMFS-NWFSC-37.

Kearney, M. 2005. Hybridization, glaciation, and geographical parthenogenesis. Trends in Ecology and Evolution 20:495–502.

Kruger, L. E., and C. B. Tyler. 1995. Management needs assessment for the Copper River delta, Alaska. U.S. Forest Service Technical Report PNW-GTR-356.

Kuligowski, D. R., M. J. Ford, and B. A. Berejikian. 2005. Breeding structure of steelhead inferred from patterns of genetic relatedness among nests. Transactions of the American Fisheries Society 134:1202–1212.

Landry, C., and L. Bernatchez. 2001. Comparative analysis of population structure across environments and geographical scales at major histocompatibility complex and microsatellite loci in Atlantic salmon (*Salmo salar*). Molecular Ecology 10:2525–2539.

Leary, R. F., F. W. Allendorf, K. L. Knudsen, and S. R. Phelps. 1987. Genetic divergence and identification of seven cutthroat subspecies and rainbow trout. Transactions of the American Fisheries Society 116:580–587.

Leary, R. F., F. W. Allendorf, and G. K. Sage. 1995. Hybridization and introgression between introduced and native fish. Pages 91–101 *in* H. L. Schramm, Jr., and R. G. Piper, editors. Uses and effects of cultured fishes in

aquatic ecosystems. American Fisheries Society, Symposium 15, Bethesda, Maryland.

Lewontin, R. C., and L. C. Birch. 1966. Hybridization as a source of variation for adaptation to new environments. Evolution 20:315–336.

Mallet, J. 2005. Hybridization as an invasion of the genome. Trends in Ecology and Evolution 20:229–237.

Mays, J. D. 2001. RAPD and mitochondrial DNA analyses of cutthroat trout from four streams in the Salmon River breaks, Idaho. Master's thesis. University of Idaho, Moscow.

McCusker, M. R., E. Parkinson, and E. B. Taylor. 2000. Mitochondrial DNA variation in rainbow trout (*Oncorhynchus mykiss*) across its native range: testing biogeographical hypotheses and their relevance to conservation. Molecular Ecology 9:2089–2108.

McKinnon, J. S., S. Mori, B. K. Blackman, L. David, D. M. Kingsley, L. Jamieson, J. Chou, and D. Schluter. 2004. Evidence for ecology's role in speciation. Nature (London) 429:294–298.

Meyer, K. A., D. J. Schill, F. S. Elle, and W. C. Schrader. 2003. A long-term comparison of Yellowstone cutthroat trout abundance and size structure in their historical range in Idaho. North American Journal of Fisheries Management 23:149–162.

Moritz, C. 1994. Applications of mitochondrial DNA analysis to conservation: a critical review. Molecular Ecology 3:401–411.

Neillands, W. G. 1990. Natural hybridization between coastal cutthroat trout (*Oncorhynchus clarki*) and steelhead trout (*Oncorhynchus mykiss*) within Redwood Creek, California. Master's thesis. Humboldt State University, Arcata, California.

Nelson, J. S. 1994. Fishes of the world, 3rd edition. Wiley, New York.

Nielsen, E. E., M. M. Hansen, and K. L. D. Mensberg. 1998. Improved primer sequences for the mitochondrial *ND1*, *ND3/4*, and *ND5/6* segments in salmonid fishes: application to RFLP analysis of Atlantic salmon. Journal of Fish Biology 53:216–220.

Nielsen, J. L., M. C. Fountain, and J. M. Wright. 1997. Biogeographic analysis of Pacific trout (*Oncorhynchus mykiss*) in California and Mexico based on mitochondrial DNA and nuclear microsatellites. Pages 53–74 *in* T. D. Kocher and C. A. Stepien, editors. Molecular systematics of fishes. Academic Press, San Diego, California.

Nielsen, J. L., and G. K. Sage. 2002. Population genetic structure in Lahontan cutthroat trout. Transactions of the American Fisheries Society 131:376–388.

Nielsen, J. L., I. Williams, G. K. Sage, and C. E. Zimmerman. 2003. The importance of genetic verification for determination of Atlantic salmon in North Pacific waters. Journal of Fish Biology 62:871–878.

Nolte, A. W., J. Freyhof, and D. Tautz. 2006. When invaders meet locally adapted types: rapid moulding of hybrid zones between sculpins (*Cottus*, Pisces) in the Rhine system. Molecular Ecology 15:1983–1993.

Northcote, T. G. 1997. Why sea-run? Pages 20–26 *in* D. Hall, P. A. Bisson, and R. E. Gresswell, editors. Sea-run cutthroat trout: biology, management, and future conservation. American Fisheries Society, Oregon Chapter, Corvallis.

Oetting, W. S., H. K. Lee, D. J. Flanders, G. L. Wiesner, T. A. Sellers, and R. A. King. 1995. Linkage analysis with multiplexed short-repeat polymorphisms using infrared fluorescence and M13 tailed primers. Genomics 30:450–458.

Olsen, J. B., K. Wutting, D. Fleming, E. J. Kretschmer, and J. Wenburg. 2006. Eviddence of partial anadromy and resident-form dispersal bias on a fine scale in populations of *Oncorhynchus mykiss* Conservation Genetics. 7:613–619.

Ostberg, C. O., and R. J. Rodriguez. 2002. Novel molecular markers differentiate *Oncorhynchus mykiss* (rainbow trout and steelhead) and the *O. clarki* (cutthroat trout) subspecies. Molecular Ecology Notes 2:197–202.

Ostberg, C. O., and R. J. Rodriguez. 2004. Biparentally inherited species-specific markers identify hybridization between rainbow trout and cutthroat trout subspecies. Molecular Ecology Notes 4:26–29.

Ostberg, C. O., S. L. Slatton, and R. J. Rodriguez. 2004. Spatial partitioning and asymmetric hybridization among sympatric coastal steelhead trout (*Oncorhynchus mykiss irideus*), coastal cutthroat trout (*O. clarki clarki*), and interspecific hybrids. Molecular Ecology 13:2773–2788.

Pascual, M., P. Bentzen, C. R. Rossi, G. Mackey, M. T. Kinnison, and R. Walker. 2001. First documented case of anadromy in a population of introduced rainbow trout in Patagonia, Argentina. Transactions of the American Fisheries Society 130:53–67.

Peacock, M. M., and V. Kirchoff. 2004. Assessing the conservation value of hybridized cutthroat trout populations in the Quinn River drainage, Nevada. Transactions of the American Fisheries Society 133:309–325.

Peacock, M. M., H. Neville, and V. S. Kirchoff. 2004. Ten species-specific microsatellite loci for the Lahontan cutthroat trout, *Oncorhynchus clarki henshawi*. Molecular Ecology Notes 4:557–559.

Peterson, J. H., M. G. Mesa, J. Hall-Griswold, W. C. Schrader, G. W. Short, and T. P. Poe. 1990. Magnitude and dynamics of predation on juvenile salmonids in Columbia and Snake River reservoirs. U.S. Department of Energy, Technical Report DOE/BP/91964–1. Portland, Oregon.

Phillips, R. B., and T. H. Oakley. 1997. Phylogenetic relationships among salmoninae based on nuclear and mitochondrial DNA sequences. Pages 145–162 *in* T. D. Kocher and C. A. Stepien, editors. Molecular systematics of fishes. Academic Press, San Diego, California.

Poff, N. L. 1997. Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. Journal of the North American Benthological Society 16:391–409.

Porath, M. T., and J. L. Nielsen. 2003. Evidence of sexually dimorphic introgression in Pinaleno Mountain apache trout. North American Journal of Fisheries Management 23:172–180.

Quinn, T. P. 2005. The behavior and ecology of Pacific salmon and trout. American Fisheries Society, Bethesda, Maryland.

Raymond, M., and F. Rousset. 1995. GENEPOP population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

Reeves, G. H., K. M. Burnett, and S. V. Gregory. 2002. Fish

and aquatic communities in the Oregon Coast Range. Pages 68–98 *in* S. D. Hobb, J. P. Hayes, R. L. Johnson, G. H. Reeves, T. A. Spies, C. P. Tappeiner, and G. E. Wells, editors. Forest and stream management in the Oregon Coast Range. Oregon State University Press, Corvallis.

Rhymer, J. M., and D. Simberloff. 1996. Extinction by introgression and hybridization. Annual Reviews in Ecology and Systematics 27:83–109.

Rieseberg, L. H. 1998. Molecular ecology of hybridization. Pages 243–265 *in* G. R. Carvalho, editor. Advances in molecular ecology. IOS Press, Amsterdam.

Rodriguez, F., C. E. Rexroad, III and Y. Palati. 2003. Characterization of twenty-four microsatellite markers for rainbow trout (*Oncorhynchus mykiss*). Molecular Ecology Notes 3:619–622.

Roni, P. 1992. Life history and spawning habitat in four stocks of large-bodied Chinook salmon (*Oncorhynchus tshawytscha*). Master's thesis. University of Washington, Seattle.

Rosenfield, J. A., T. Todd, and R. Griel. 2000. Asymmetric hybridization and introgression between pink salmon and Chinook salmon in the Laurentian Great Lakes. Transactions of the American Fisheries Society 129:670–679.

Rubidge, E. M., and E. B. Taylor. 2004. Hybrid zone structure and the potential role of selection in hybridizing populations of native westslope cutthroat (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*). Molecular Ecology 13:3735–3749.

Rubidge, E. M., and E. B. Taylor. 2005. An analysis of spatial and environmental factors influencing hybridization between native westslope cutthroat trout (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*) in the upper Kootenay River drainage, British Columbia. Conservation Genetics 6:369–384.

Schlosser, I. J. 1995. Critical landscape attributes influence fish population dynamics in headwater streams. Hydrobiologia 303:71–81.

Schmeller, D. S., A. Seitz, A. Crivelli, and M. Veith. 2005. Crossing species' range borders: interspecies gene exchange mediated by hybridogenesis. Proceedings of the Royal Society of London B 272:1625–1631.

Scribner, K. T., J. R. Gust, and R. L. Fields. 1996. Isolation and characterization of novel microsatellite loci: cross-species amplification and population genetic applications. Canadian Journal of Fisheries and Aquatic Sciences 53:833–841.

Scribner, K. T., K. S. Page, and M. L. Bartron. 2000. Hybridization in freshwater fishes: a review of case studies and cytonuclear methods of biological inference. Reviews in Fish Biology and Fisheries 10:293–323.

Seehausen, O. 2004. Hybridization and adaptive radiation. Trends in Ecology and Evolution 19:198–207.

Shedlock, A. M., J. D. Parker, D. A. Crispin, T. W. Pietsch, and G. C. Burmer. 1992. Evolution of the salmonid mitochondrial control region. Molecular Phylogenetics and Evolution 1:179–192.

Simon, R. C., and R. E. Noble. 1968. Hybridization in *Oncorhynchus* (Salmonidae), I. Viability and inheritance in artificial crosses of chum and pink salmon. Transactions of the American Fisheries Society 97:109–118.

Steffens, D. L., S. L. Sutter, and S. C. Roemer. 1993. An alternative universal forward primer for improved automated DNA sequencing for M13. Biotechniques 15:580–581.

Stone, G. 2000. Phylogeography, hybridization, and speciation. Trends in Ecology and Evolution 15:354–355.

Taborsky, M. 1994. Sneakers, satellites, and helpers: parasitic and cooperative behaviour in fish reproduction. Advances in the Study of Behaviour 23:1–100.

Thrower, F., C. Guthrie III, J. L. Nielsen, and J. Joyce. 2004. A comparison of genetic variation between an anadromous steelhead, *Oncorhynchus mykiss*, population and seven derived populations sequestered in freshwater for 70 years. Environmental Biology of Fishes 69:111–125.

Trotter, P. C. 1989. Coastal cutthroat trout: a life history compendium. Transactions of the American Fisheries Society 118:463–473.

Trotter, P. C. 1997. Sea-run cutthroat trout: life history profile. Pages 7–15 *in* D. Hall, P. A. Bisson, and R. E. Gresswell, editors. Sea-run cutthroat trout: biology, management, and future conservation. American Fisheries Society, Oregon Chapter, Corvallis.

U.S. Forest Service. 1992. A channel type user's guide for the Tongass National Forest, Southeast Alaska. U.S. Forest Service, Technical Paper 26, Juneau, Alaska.

Utter, F. 2000. Patterns of subspecific anthropogenic introgression in two salmonid genera. Reviews in Fish Biology and Fisheries 10:265–279.

Utter, F. 2004. Population genetics, conservation, and evolution in salmonids and other widely cultured fishes: some perspectives over six decades. Reviews in Fish Biology and Fisheries 14:125–144.

Utter, F. M. 1981. Biological criteria for the definition of species and distinct intraspecific populations of anadromous salmonids under the U.S. Endangered Species Act of 1973. Canadian Journal of Fisheries and Aquatic Sciences 38:1626–1635.

Utter, F. M., and F. W. Allendorf. 1994. Phylogenetic relationships among species of *Oncorhynchus*: a consensus view. Conservation Biology 8:864–867.

Weigel, D. E., J. T. Peterson, and P. Spruell. 2002. A model using phenotypic characteristics to detect introgressive hybridization in wild westslope cutthroat trout and rainbow trout. Transactions of the American Fisheries Society 131:389–403.

Weigel, D. E., J. T. Peterson, and P. Spruell. 2003. Introgressive hybridization between native cutthroat trout and introduced rainbow trout. Ecological Applications 13:38–50.

Wenburg, J. K., P. Bentzen, and C. J. Foote. 1998. Microsatellite analysis of genetic population structure in an endangered salmonid: the coastal cutthroat trout (*Oncorhynchus clarki clarki*). Molecular Ecology 7:733–749.

Wilson, C. C., and L. K. Bernatchez. 1998. The ghosts of hybridization past: fixation of arctic char (*Salvelinus alpinus*) mitochondrial DNA in an introgressed population of lake trout (*S. namaycush*). Molecular Ecology 7:127–132.

Wirtz, P. 1999. Mother species–father species: unidirectional hybridization in animals with female choice. Animal Behaviour 58:1–12.

Withler, R. E. 1966. Variability in life history characteristics

of steelhead trout (*Salmo gairdneri*) along the Pacific coast of North America. Journal of the Fisheries Research Board of Canada 23:365–393.

Wuttig, K., D. Fleming, and J. Olsen. 2004. Stock status and population biology of the Copper River steelhead. Alaska Department of Fish and Game, Fishery Data Series 04-18, Anchorage.

Young, W. P., C. O. Ostberg, P. Keim, and G. H. Thorgaard. 2001. Genetic characterization of hybridization and introgression between anadromous rainbow trout (*Oncorhynchus mykiss irideus*) and coastal cutthroat trout (*O. clarki clarki*). Molecular Ecology 10:921–930.

Zimmerman, C. E., and G. H. Reeves. 2000. Population structure of sympatric anadromous and nonanadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. Canadian Journal of Fisheries and Aquatic Sciences 57:2152–2162.