

# Intercrosses and the U.S. Endangered Species Act: Should Hybridized Populations be Included as Westslope Cutthroat Trout?

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**Abstract:** *There are currently no policy guidelines for treating hybrids under the U.S. Endangered Species Act (ESA). We considered the scientific basis for determining whether hybridized populations should be included as part of the westslope cutthroat trout (*Oncorhynchus clarki lewisi*) unit considered for listing under the ESA. Westslope cutthroat trout are threatened by genomic extinction because of widespread introgressive hybridization with introduced rainbow trout (*O. mykiss*) and Yellowstone cutthroat trout (*O. c. bouvieri*). Experimental results suggest that first-generation hybrids between westslope cutthroat trout and rainbow trout have reduced fitness. However, hybridization may spread even when hybrids have severely reduced fitness because the production of hybrids is unidirectional—that is, all the progeny of a hybrid will be hybrids. In addition, heterosis resulting from the sheltering of deleterious recessive alleles in early-generation hybrids may increase the effective rate of introgression. However, such short-term increases in fitness may disrupt important long-term adaptations of native populations. The loss of these adaptations will be difficult to detect because some local adaptations might only be apparent during periodic episodes of extreme environmental conditions, such as winter storms, drought, or fire. Thus, rapid spread of hybridization could result in the loss of local adaptations in native populations of westslope cutthroat trout and decrease their probability of long-term persistence. Protection of populations with some admixture would protect sources of spreading hybridization. Treatment of hybrids in conservation planning depends primarily on the amount of evolutionary divergence between the hybridizing taxa and the geographical extent of introgression. We recommend that only nonhybridized populations be included as westslope cutthroat trout in the unit to be considered for listing. Populations of unknown status should be protected until more information about these populations becomes available.*

**Key Words:** admixture, genomic extinction, heterosis, hybridization, inbreeding depression, introgression, outbreeding depression

Entrecruzas y el Acta de Especies en Peligro de E. U. A.: Inclusión de Poblaciones Híbridas de *Oncorhynchus clarki lewisi*

**Resumen:** *Actualmente no hay lineamientos políticos para el tratamiento de híbridos bajo el Acta de Especies en Peligro. Consideramos las bases científicas para determinar si las poblaciones híbridas deben ser incluidas como parte de la unidad de *Oncorhynchus clarki lewisi* considerada para ser enlistada en el Acta de Especies en Peligro. *Oncorhynchus clarki lewisi* está amenazada de extinción genómica debido a hibridación introgresiva generalizada con la trucha arco iris introducida (*O. mykiss*) y *O. c. bouvieri*. Resultados experimentales sugieren que los híbridos de primera generación entre *O. clarki lewisi* y *O. mykiss* tienen adaptabilidad reducida. Sin embargo, la hibridación puede extenderse aun cuando los híbridos tienen adaptabilidad severamente reducida, porque la producción de híbridos es unidireccional (esto es, toda la progenie de un híbrido serán híbridos). Adicionalmente, la heterosis resultante del resguardo de alelos recesivos deletéreos en las primeras generaciones de híbridos puede incrementar la tasa efectiva de introgresión. Sin embargo, tales incrementos*

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Paper submitted July 7, 2003; revised manuscript accepted January 22, 2004.

de corto plazo en la adaptabilidad pueden alterar importantes adaptaciones de largo plazo en poblaciones nativas. La pérdida de estas adaptaciones será difícil de detectar porque algunas adaptaciones locales sólo pueden ser aparentes durante episodios periódicos de condiciones ambientales extremas (e.g. tormentas invernales, sequía o fuego). Por tanto, la rápida expansión de la hibridación pudiera resultar en la pérdida de adaptaciones locales en poblaciones nativas de *O. clarki lewisi* y disminuir su probabilidad de persistencia a largo plazo. La protección de poblaciones con cierta mezcla protegería fuentes de expansión de hibridación. El tratamiento de híbridos en la planeación de conservación depende primariamente de la proporción de divergencia evolutiva entre los taxa hibridizantes y la extensión geográfica de la introgresión. Recomendamos que sólo se incluya a poblaciones no híbridas como *O. clarki lewisi* en la unidad a considerar para enlistar. Se debe proteger a las poblaciones de estatus desconocido hasta que haya más información disponible sobre estas poblaciones.

**Palabras Clave:** depresión por endogamia, depresión por exogamia, extinción genómica, hibridación, introgresión, heterosis, mezcla

## Introduction

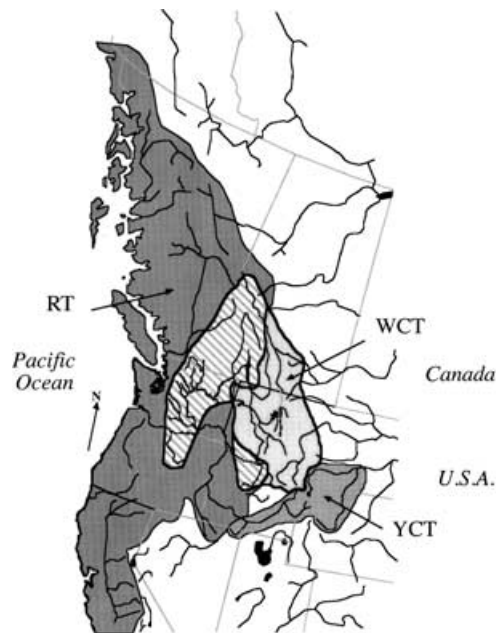
Hybridization presents a difficult set of problems for defining appropriate units to be protected by conservation efforts. An early series of interpretations of the U.S. Endangered Species Act (ESA) by the Department of the Interior, Office of the Solicitor, concluded that hybrids should not receive protection under the ESA because protection of hybrids would not help recover a listed species and could jeopardize the continued existence of that species (U.S. Fish and Wildlife Service [USFWS] & National Oceanic and Atmospheric Administration [NOAA] 1996; see O'Brien & Mayr 1991). This "hybrid policy" was withdrawn in December 1990, however, because "New scientific information concerning genetic introgression has convinced us that the rigid standards set out in those previous opinions should be revisited" (USFWS & NOAA 1996). A proposed policy on intercrosses was published in 1996 (USFWS & NOAA 1996); the term *intercross* was used because of negative connotations often associated with hybrids. This proposed intercross policy was scheduled to be finalized 1 year later but has still not been finalized. Thus, no official policy provides guidelines for dealing with hybrids under the ESA.

Hybridization is generally considered to be interbreeding of parental individuals from genetically distinct populations, regardless of the taxonomic status of populations (for a general consideration of hybrids in conservation and definitions of terms used in this paper, see Allendorf et al. 2001). The parental individuals may be from different populations or subspecies (intraspecific hybridization) or they may be from different species (interspecific hybridization). Under the ESA, intercrosses would include progeny produced by matings between a listed "species" and other taxa. Species, subspecies, or distinct population segments may be listed as species under the ESA (Waples 1995). In this paper, we use the term *hybrid* to refer to any individual that is either a first-generation hybrid or whose recent ancestry (within the last 100 years or so) includes at least one first-generation hybrid individual.

Treatment of hybridized populations has been especially problematic for westslope cutthroat trout (WCT, *Oncorhynchus clarki lewisi*). The USFWS received a formal petition in 1997 to list the WCT as threatened throughout its range (USFWS 2002). The USFWS concluded that listing WCT as a threatened species was not warranted because of the widespread distribution and current status of the overall WCT population (Anonymous 1999a). However, a subsequent lawsuit argued that this finding was incorrect because it included hybridized populations in the WCT population considered for listing (USFWS 2002). The court ruled that the listing determination for the WCT was not based on the best available science and ordered the USFWS to reconsider whether to list WCT as threatened after taking into account the prevalence of hybridization (USFWS 2002).

The WCT is one of four major subspecies of cutthroat trout (Allendorf & Leary 1988; Behnke 2002). The geographical range of WCT is the largest of all cutthroat trout subspecies and includes the Columbia, Fraser, Missouri, and Hudson Bay drainages of the United States and Canada (Fig. 1; Behnke 2002). The WCT is genetically highly divergent at both nuclear and mitochondrial genes from the three other major subspecies of cutthroat trout: the coastal (*O. c. clarki*), Yellowstone (*O. c. bouvieri*), and Lahontan (*O. c. henshawi*) (Gyllensten & Wilson 1987; Allendorf & Leary 1988). For example, 10 of 46 nuclear allozyme loci are diagnostic—fixed or nearly fixed for different alleles—between WCT and Yellowstone cutthroat trout (YCT; Allendorf & Leary 1988). This amount of divergence between WCT and YCT is beyond that usually seen within a single species. For example, it is greater than the divergence at allozyme loci between some species of Pacific salmon (Utter et al. 1973).

We have previously suggested that a small amount of natural introgression has occurred historically between WCT and rainbow trout (*Oncorhynchus mykiss*; RT) in regions where they naturally co-occur (Allendorf & Leary 1988). The WCT exists in sympatry with both resident and anadromous steelhead forms of Columbia River RT



**Figure 1.** Historic distribution of westslope cutthroat trout (WCT) and Yellowstone cutthroat trout (YCT) (modified from Behnke 2002). The area where WCT and rainbow trout (RT) naturally co-occur is indicated by diagonal lines. The range of RT extends north and south of the area shown.

(*O. m. gairdneri*) in many drainages throughout the western portion of their range (Fig. 1). Nevertheless, the presence of the same fixed genetic differences between WCT and RT in regions of sympatry, as in the rest of the distribution of the WCT, indicates that any natural introgression in these regions of sympatry has been extremely rare. Our genetic analyses of WCT over the last 30 years indicate that natural hybridization between WCT and RT is restricted to the occasional first-generation (F1) hybrid individual and rare backcross individuals (Leary et al. 1995). This is similar to patterns of hybridization observed among species of centrarchid fishes that are naturally sympatric (Konkle & Philipp 1992; Epifanio & Philipp 2001).

The WCT are threatened by widespread genomic extinction. Epifanio and Philipp (2001) have defined *genomic extinction* as loss of a lineage (such as WCT) by introgression with another taxon (RT or YCT in this case) or by displacement by a taxon introduced by humans. We suggest that genomic extinction be restricted to the situation where extinction is caused by loss of monophyletic genotypic combinations by introgression. Others have used the term *genetic extinction* for this process (Rhymer & Simberloff 1996). However, *genomic* is more appropriate than *genetic*: it is not genes or single locus genotypes that are lost by hybridization. Rather, it is combinations of genotypes over the entire genome that are irretrievably lost. Genomic extinction results in the loss of the legacy

of an evolutionary lineage; that is, the genome-wide combination of alleles and genotypes that have evolved over evolutionary time will be lost by introgression with another lineage.

The loss of native cutthroat trout by hybridization with introduced RT has been recognized as a major threat to native cutthroat trout since the 1930s (Madsen 1936). Introgressive hybridization with introduced RT, and with YCT to a lesser extent, is widespread throughout the range of the WCT (Allendorf & Leary 1988; Leary et al. 1995). Hybridization of WCT with both RT and YCT generally results in the formation of random-mating populations in which all individuals are hybrids by varying numbers of generations of backcrossing with parental types and mating among hybrids (i.e., hybrid swarms; Gyllensten et al. 1985). Hanzel (1960) provides one of the first reports of hybridization between WCT and RT and concludes that hybridization has occurred in “practically all drainages where rainbow trout were introduced.”

Estimates of the current distribution of WCT are highly variable. Liknes and Graham (1988) estimated that nonhybridized WCT populations remained only within 2.5% of their native range in Montana (U.S.A.). The status review of the WCT (Anonymous 1999a) concluded that WCT populations exist in 20% of the stream miles of their historic range. Thurow et al. (1997) suggest that WCT still exist in a much larger proportion of their range. The primary reason for these differences is that the authors used different criteria to identify WCT. Liknes and Graham (1988) included only nonhybridized populations. Thurow et al. (1997) are not clear in the criteria they used, but it seems they included all populations that appear to be WCT based on morphology. The WCT status review (Anonymous 1999a) relied on classification systems used by state agencies that differed from state to state.

Here we consider the scientific basis for determining whether or not introgressed populations and populations of unknown hybridization status should be included as part of the units considered for listing under the ESA. We consider the WCT an exemplar taxonomic unit for this general problem in conservation. We evaluated the power of morphological and molecular methods to detect hybridization. In addition, we reviewed the literature that examines the fitness of hybrids between the WCT and the two primary taxa with which it hybridizes (RT and YCT). We conclude with a consideration of three possible alternatives for treating hybridized populations of WCT under the ESA.

## Detection of Hybrids and Hybridization

Until the mid-1960s, the detection of hybrid individuals relied mainly on morphological characteristics (Allendorf et al. 2001). Not all morphological variation has a genetic

basis, however, and the amount of natural morphological variation within and among populations is substantial. The detection of hybrids using morphological characters generally assumes that hybrid individuals will be phenotypically intermediate to parental individuals. This is often not the case because hybrids can express a mosaic of parental phenotypes (Leary et al. 1995). Furthermore, individuals from hybrid swarms that receive most of their genes from one of the parental taxa are often morphologically indistinguishable from that parental taxon. Morphological characters generally do not allow determination of whether an individual is a first-generation hybrid (F1), a backcross, or a later-generation hybrid (Leary et al. 1995; Boecklen & Howard 1997).

### Molecular Detection

The use of molecular genetic markers greatly simplifies identification and description of hybridized populations. Molecular identification of hybridization began with protein electrophoresis (allozymes) in the mid-1960s. Recent advances in molecular techniques, especially development of the polymerase chain reaction (PCR), have greatly increased the number of loci that can be used to detect hybridization. In addition, these techniques are more applicable to small populations threatened with extinction because sampling can be nonlethal.

Molecular identification is based on diagnostic loci (Ayala & Powell 1972) that are fixed or nearly fixed for different alleles in taxa suspected to be hybridizing. Nonhybridized populations can be identified by the absence of alleles diagnostic for the suspected hybridizing taxa at these diagnostic loci. Hybridized populations can be identified by the presence of alleles diagnostic for the native taxon and the suspected hybridizing taxa at these diagnostic loci.

Loci that appear to be diagnostic may not be diagnostic for all populations of a taxon because two alleles may have the same electrophoretic mobility resulting from separate mutations or from an incomplete lineage sorting in which some populations maintain an ancestral polymorphism. A shared character state that has arisen separately in two taxa rather than being inherited from a common ancestor is called a "homoplasy." For example, a mutation at a diagnostic locus may occur within a WCT population that produces an allozyme allele with the same electrophoretic mobility as the allele generally considered diagnostic for RT. Such parallel mutations may also occur at PCR-based DNA markers in which alleles are identified on the basis of electrophoretic mobility (e.g., microsatellites).

The presence of such an allele might suggest the occurrence of hybridization in a population in which there has been no hybridization. However, it is possible to detect such parallel mutations by examination of many diagnostic loci. Hybridization should result in approximately equal frequencies of admixture at all diagnostic

loci (Forbes & Allendorf 1991a). Thus, parallel mutations may be identified by the discovery of a high frequency of apparent introgression at one locus, whereas other diagnostic loci do not show any evidence of hybridization. It is important to examine many diagnostic loci to be able to detect parallel mutations.

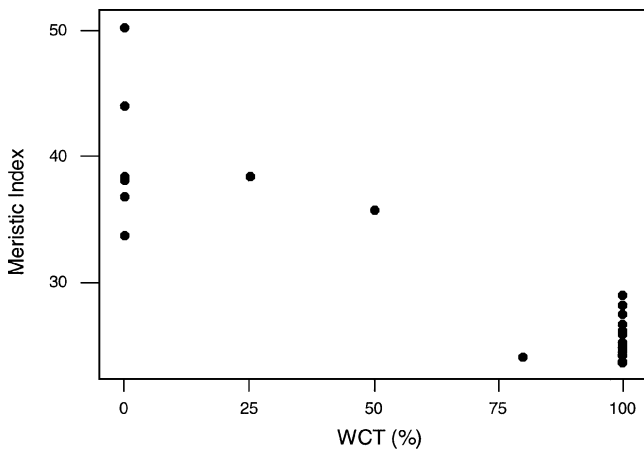
Molecular data should be interpreted at both the individual and population level to elucidate the history of hybridization in populations (Barton & Gale 1993). Parental and F1 hybrids can be identified reliably if many diagnostic loci are examined. Parental individuals will be homozygous at all diagnostic loci for alleles diagnostic of the parental taxon. The F1 hybrids will be heterozygous for all alleles characteristic of both hybridizing taxa at all diagnostic loci. Later-generation hybrids (F2, backcrosses, etc.) will be heterozygous at some diagnostic loci and homozygous for different alleles at others. Individual genotypes will be highly variable among later-generation hybrids.

### Morphological and Phenotypic Detection

Many attempts have been made to identify hybridized and nonhybridized populations of WCT through a variety of meristic and morphological traits. Leary et al. (1984) examined diagnostic allozyme loci and eight meristic characters in two WCT populations and four WCT  $\times$  RT introgressed populations in the Clark Fork River drainage and two coastal RT populations (*O. m. irideus*). Rainbow trout and WCT differed consistently at two of eight characters. Meristics alone suggest that only the hybridized population with 85% RT admixture were not WCT. The other three hybridized populations had <20% rainbow admixture and were indistinguishable from WCT based on meristics. Leary et al. (1984) concluded that the reduced power of meristic comparisons is due to the large amount of intraspecific variability among and similarity between these species for those meristic characters.

Marnell et al. (1987) described 24 cutthroat trout populations in Glacier National Park (U.S.A.) with a combination of meristics and allozyme markers. The meristic index—the sum of the mean counts of gill rakers and basibranchial teeth—distinguished nonhybridized populations of WCT and YCT. However, the meristic index was not useful for identifying hybrid populations (Fig. 2). Hybridized populations that were at least 50% YCT admixture had meristic indexes within the range of YCT, whereas a population with 80% WCT had a meristic index within the range of WCT. None of the hybrid populations had an intermediate meristic index.

Behnke (1992) considered basibranchial teeth to be one of the most useful morphological characters for distinguishing between WCT, rainbow, and hybridized populations. Leary et al. (1996) tested the reliability of the presence of basibranchial teeth for identifying hybridized populations. In five rainbow populations, a complete absence



**Figure 2.** Mean meristic index of 23 cutthroat trout populations from Glacier National Park (Marnell et al. 1987). The meristic index is the sum of the counts of the three meristic characters that best distinguish between Yellowstone cutthroat trout and westslope cutthroat trout (WCT): anterior gill rakers, posterior gill rakers, and basibranchial teeth. The percent WCT admixture of each population was determined by examination of seven diagnostic protein loci.

of basibranchial teeth in the populations reliably indicates the absence of hybridization. However, variability in the percentage of individuals with basibranchial teeth in nonhybridized WCT populations is so large that nonhybridized and moderately admixed populations of WCT were indistinguishable from each other. The authors concluded that the presence of basibranchial teeth is not a reliable indicator of introgression from RT into populations of WCT.

Weigel et al. (2002) used visual identification and a classification model based on phenotypic characteristics measurable in the field to identify hybridized populations of WCT and RT in the Clearwater River drainage of northern Idaho. They concluded that a hybridized population has to contain at least 50% admixture from RT to be identified reliably in the field.

### Summary

Molecular methods provide a powerful and sensitive technique for detecting hybridization in WCT populations. However, it is necessary to examine many diagnostic loci to exclude the possibility of parallel mutations, describe the pattern of introgression on the basis of multiple-locus genotypes, and increase the power to detect a small amount of introgression. We suggest that at least five diagnostic loci should be examined in a sample of at least 30 individuals (Boecklen & Howard 1997). This will provide a 95% probability of detecting as little as 1% admixture in a hybrid swarm where there is little linkage disequilibrium. In contrast, identification of hybridized populations

of WCT on the basis of morphological analysis is not reliable.

### Hybridization and Fitness

Hybridization may result in an increase or a decrease, or it may have no effect on fitness (Edmunds & Timmerman 2003). Outbreeding depression occurs when hybrids have lower fitness than the parental types. Outbreeding depression occurs in a number of animal and plant species and observed decreases in fitness are sometimes substantial (Dobzhansky 1970; Allendorf et al. 2001). Outbreeding depression may result from genic or chromosomal interactions (intrinsic outbreeding depression) or interactions between hybrid individuals and their environment (extrinsic outbreeding depression). For example, intrinsic outbreeding depression results from mispairing of chromosomes during meiosis or disruption of co-adapted gene complexes (Dobzhansky 1970), and extrinsic outbreeding depression results from loss of local adaptations. Extrinsic outbreeding depression depends on the environment, which determines the forces of natural selection acting on the varying phenotypes.

Hybrid vigor, or heterosis, occurs when the hybrid has greater fitness than the parental types (Rhymer & Simberloff 1996). Heterosis is not necessary for introgression to spread and cause genomic extinction. In fact, the model of Epifanio and Philipp (2001) suggests that introgression may spread even when hybrids have severely reduced fitness (e.g., just 10% that of the parental taxa). This occurs because the production of hybrids is unidirectional, a sort of genomic ratchet. That is, all of the progeny of a hybrid will be hybrids. Thus, the frequency of hybrids within a local population may increase even when up to 90% of the hybrid progeny do not survive. The increase in the proportion of hybrid individuals in the population may occur even when the proportion of admixture in the population—the proportion of alleles in a hybrid swarm that come from each of the hybridizing taxa—is constant.

### Laboratory Studies

Leary and coworkers performed a series of reciprocal crosses between WCT and RT from strains at the Creston National Fish Hatchery to measure survival and growth of F1 hybrids under controlled conditions (Allendorf & Leary 1988; Leary et al. 1995). There was no overlap in the spawning times of females from the parental taxa, so they could not compare a complete set of reciprocal crosses.

In the first experiment, the survival and growth rates of WCT × WCT progeny were compared with hybrid progeny between the same female WCT used in the pure crosses and male RT (WCT × RT). Eggs from 24 female

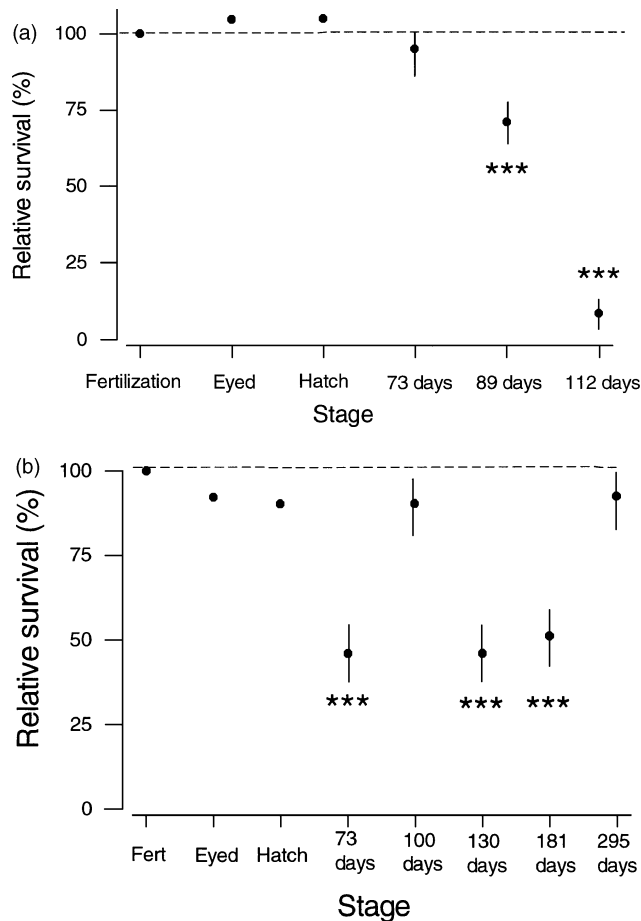


Figure 3. Relative survival of hybrids of rainbow trout (RT) and westslope cutthroat trout (WCT) compared with the survival of the parental types (Leary et al. 1995). Survival at eyed stage and at hatch is based on direct counts of approximately 4000 initial eggs in each cross. Survival at other stages is based on the frequency of the two crosses in a sample of approximately 100 fish raised in a common tank after hatching. In (a) female WCT were mated with male WCT and RT, and in (b) female RT were mated with male RT and WCT. The bars indicate 95% CI (\*\*\*)  $p < 0.001$ .

WCT were subdivided, and half of them were fertilized with male WCT and half with RT from the Eagle Lake strain. The eggs were incubated in separate compartments of a hatchery incubator. The percentage of eggs surviving to the eyed stage tended to be higher in hybrids than in matings between WCT (Fig. 3a). Eleven of 24 comparisons between half-sib families were significant, and hybrids had a higher percentage of eyed eggs in 9 of these 11 comparisons. Similar results were found for hatching success. Nine of the half-sib comparisons were significantly different, and in seven of these the hybrids were more successful. The hybrids had overall significantly greater success at both of these stages.

Table 1. Mean length of parental types and hybrids between female westslope cutthroat trout (WCT) mated with male WCT or rainbow trout (RT) (Allendorf & Leary 1988).

Age (days)	Length (mm)	
	WCT × WCT	WCT × RT
73	22.7	22.3
89	29.0	27.2*
112	40.1	33.6*

\* $p < 0.001$ .

The hybrids, however, performed much poorer after hatching when all fish were pooled into a common tank and identified genetically after sampling. The hybrids had a relative survival of only 71% at 89 days after fertilization, and a relative survival of only 7% at 112 days after fertilization (Fig. 3a). Hatchery personnel were concerned about substantial daily mortalities and sent samples of the mortalities to the USFWS Bozeman Fish Technology Center for analysis. No evidence of disease was detected. In addition, the hybrids were significantly smaller at both 89 days (29.0 vs. 27.2 mm;  $p < 0.05$ ) and 112 days (40.1 vs. 33.6 mm;  $p < 0.05$ ; Table 1). The higher survival of hybrids during early stages of development may have resulted from sheltering of deleterious recessive alleles. Nevertheless, developmental genetic incompatibilities between the two parental genomes apparently resulted in poor performance of hybrids after hatching.

In the second experiment, the survival and growth rate of RT (RT × RT) were compared with those of hybrids produced by the same RT female and male WCT (RT × WCT). Approximately 400 eggs from each of 24 RT were pooled and subdivided into two groups. One of these groups was fertilized from sperm pooled from 31 RT, and the other half was fertilized from sperm pooled from 20 WCT.

Hybrids had slightly reduced survival to the eyed stage of development (Fig. 3b). The fish were raised in a common tank after hatching and were sampled and identified by genetic analysis five times over the next 9 months. The hybrids were significantly smaller in four of the five samples (Table 2). The hybrids had significantly reduced

Table 2. Mean length of parental types and hybrids between female rainbow trout (RT) mated with male RT or westslope cutthroat trout (WCT) (Allendorf & Leary 1988).

Age (days)	Length (mm)	
	RT × RT	RT × WCT
72	25.3	24.5 <sup>a</sup>
100	33.4	31.2 <sup>a</sup>
130	46.3	46.3
181	69.6	62.7 <sup>b</sup>
295	82.6	73.7 <sup>b</sup>

<sup>a</sup> $p < 0.01$ .

<sup>b</sup> $p < 0.001$ .

survival in three of those samples ( $p < 0.001$ ) and slightly reduced survival in the other two samples. The significant ( $p < 0.05$ ) difference in the proportion of hybrids within these five samples is puzzling. Hatchery personnel were not aware of the substantial mortalities that these data seem to suggest. Rather, it appears that these differences among samples represent nonrandom sampling by hatchery personnel of the experimental fish because of either size or behavior. Regardless, the hybrids had significantly reduced survival.

### Studies of Wild Populations

Sage et al. (1986) found that mice in a hybrid zone had greater parasite loads than mice from either of the two parental subspecies outside the hybrid zone. They suggest that coadapted gene complexes responsible for parasite resistance were broken down in the hybrids. Sage (1993) tested this suggestion in WCT by examining macroparasite loads in populations of native WCT and hybrid swarms from 15 lakes from the South Fork of the Flathead River, Montana. He found no evidence that hybrid individuals or hybrid populations had greater parasite loads.

Forbes and Allendorf (1991a, 1991b) examined 12 diagnostic allozyme loci, mitochondrial DNA (mtDNA), and a series of meristic traits in three hybrid swarms of WCT and YCT. All three swarms were determined to be fairly old (perhaps 10 or more generations) on the basis of very little linkage disequilibrium between unlinked allozyme loci. They could not compare the fitness of hybrids with parental types because the parental types no longer occurred in these hybrid swarm populations. Rather, the purpose of these two studies was to search for effects of natural selection on genes originating in either the WCT or YCT genome.

Forbes and Allendorf (1991a) found different amounts of admixture in the three populations. However, allele frequencies at all diagnostic nuclear loci were similar within each of the hybrid swarms. Natural selection affecting the chromosomal segments marked by these loci would have increased or decreased the amount of admixture measured by different loci. Therefore, there was no evidence of natural selection affecting the chromosomal segment marked by allozyme loci from either subspecies. Forbes and Allendorf did find, however, that the WCT mtDNA genotype exceeded the frequency of WCT nuclear alleles in all three swarms, and they suggest that this pattern may have resulted from greater fitness of individuals with WCT mtDNA. Alternatively, this pattern may have resulted from differences in the frequency of reciprocal matings of the original hybrids or from differences in the viability, fertility, or sex ratio of the early hybrid generations.

Rapid spread of introgression from RT into WCT populations may suggest a fitness advantage in hybrids. Rubidge et al. (2001) compared WCT populations in the tributaries of the Kootenay River of British Columbia sam-

pled in 1986–1987 and in 2000. Although only 1 of 9 tributaries contained hybrids in the early samples, 7 of 11 tributaries (including 7 of those previously sampled) contained hybrids <15 years later. Thus, the movement of introgression and admixture from RT was rapid. Rubidge et al. (2001) suggest, however, that their findings may be the result of continued stocking of RT in British Columbia.

Hitt et al. (2003) also found evidence of the continued spread of introgression. They found that RT introgression has spread dramatically over approximately five WCT generations in the Flathead River system in Montana. They also determined that RT introgression is advancing in an upstream direction and does not appear to be restricted by environmental factors (Hitt et al. 2003). Spread of RT introgression in wild populations demonstrates that WCT  $\times$  RT hybrid swarms can maintain some level of survival and reproduction over the short term. However, the long-term fitness consequences of introgression in wild populations are unknown.

### Summary

The results of laboratory studies suggest that first-generation hybrids between WCT and RT often have reduced fitness due to genetic incompatibilities. In contrast, hybridized populations of WCT and RT appear to be extremely common throughout the range of WCT, and introgression with RT appears to be spreading rapidly. These results seem to be in conflict with each other. That is, if hybrids have reduced fitness, why do they appear to be so common in the wild?

One explanation is the ratchet effect within hybrid swarms (Epifanio & Philipp 2001) that can lead to an increase of hybrids in spite of a heavy fitness penalty for hybrids. Fitness differences between parental types and hybrids eventually become irrelevant in hybrid swarms because all individuals are of hybrid origin. Natural selection will subsequently increase the fitness of individuals within hybrid swarms, and these populations can serve as a source of migrant hybrid individuals.

Another explanation for this result is that the experimental results compare the performance of first-generation hybrids to their parental types but that the source of the spreading introgression in the wild is later-generation hybrids of WCT and RT that have existed for many generations. Genetic incompatibilities between the hybridizing taxa may be reduced or eliminated by natural selection. Results from laboratory experiments show that in *Drosophila* this type of outbreeding depression is sometimes temporary because new combinations of genes with fitness equal or superior to the parental taxa may evolve in just a few generations by natural selection (Templeton et al. 1976).

Another possible explanation for the continued rapid spread of introgression from RT is that hybridization may

be a stimulus for “invasiveness,” as proposed by Ellstrand and Schierenbeck (2000). Native populations of WCT have extreme genetic divergence, even between adjacent tributaries, suggesting that movement and gene flow has been extremely low (Allendorf & Leary 1988; Leary et al. 1988). The admixture from RT appears to be increasing the rate of dispersal and thereby causing the spread of introgression. The spread of RT introgression observed by Hitt et al. (2003) is much greater than that expected from the straying rate for WCT populations.

Recent theoretical work suggests that hybrids may have a short-term fitness advantage because of the sheltering of deleterious recessive alleles with small effects. Natural selection will not be effective in removing slightly deleterious alleles from small populations because of the influence of genetic drift. These recessive alleles will be sheltered in hybrids that will be heterozygous for the deleterious recessive allele and the dominant allele carried by migrants. This heterosis may increase the effective rate of gene flow into a population even if native populations have local adaptations that are lost as a result of gene flow (Ingvarsson & Whitlock 2000; Whitlock et al. 2000; Morgan 2002).

Moreover, increases in short-term fitness may disrupt important long-term adaptations. Loss of these adaptations is difficult to detect because some local adaptations of native populations might only be essential during periodic episodes of extreme environmental conditions, such as winter storms, drought, or fire. Weins (1977) argues that short-term studies of fitness and other population characteristics are of limited value because of the importance of “ecological crunches” in variable environments. For example, Rieman and Clayton (1997) suggest that the complex life histories (e.g., mixed migratory behaviors) of bull trout (*Salvelinus confluentus*) are adaptations to periodic disturbances such as fire that may affect populations only every 25–100 years.

Spread of introgression throughout the range of WCT populations is a major threat to the continued existence of native WCT, regardless of the underlying explanation. The ongoing rapid spread of introgression from RT could result in the loss of local adaptations in native populations of WCT and decrease their probability of long-term persistence.

### Should Hybridized Populations be Included in the WCT Unit Considered for Listing?

We present three alternative criteria for including populations in the WCT unit to be considered for listing. Alternatives 1 and 3 are two extreme options for including or not including populations with admixture. Alternative 2 is an intermediate alternative that has been used in several recent management plans. We consider the potential

strengths and weaknesses of each of these alternatives on the basis of available evidence.

#### Alternative 1: Include only Nonhybridized WCT

In the first alternative, only populations that do not show evidence of hybridization from other taxa are included in the WCT unit considered for listing. It is often hard to distinguish between a small proportion of admixture (e.g., <1%) and natural polymorphisms that might exist in some populations. Therefore, finding rare alleles at a single marker that suggest hybridization should not be taken as evidence that a population is hybridized. As discussed earlier, it is important to examine multiple diagnostic loci. This alternative includes all populations that do not show evidence of hybridization at multiple diagnostic markers.

This alternative recognizes that the WCT represents a monophyletic evolutionary lineage isolated from other evolutionary lineages for perhaps 1–2 million years (Allendorf & Leary 1988). This is the only alternative that protects the historical evolutionary legacy of WCT. This alternative also protects the local adaptations important for long-term persistence that may be lost through hybridization. Hybrid populations that pose a threat to WCT populations are not eligible for listing and therefore could be managed to reduce ongoing and spreading hybridization.

The disadvantages of including only nonhybridized populations of WCT are associated mainly with small, isolated populations. Many nonhybridized populations of WCT remain, but many of them are isolated headwater populations protected from introgression by a barrier preventing upstream movement (e.g., waterfalls or degraded habitat) and do not have a lake upstream that could support a population of introduced RT or YCT. Extinction threats to these populations are primarily loss of connectivity and potential inbreeding depression (Shepard et al. 1997). Increasing and improving available habitat increases population sizes and thus decreases threats associated with small, isolated populations (Novinger & Rahel 2003). Opportunities to accomplish this at large scales are minimal, however, because of the widespread presence of hybrid swarms throughout the range of WCT.

#### Alternative 2: Include WCT Populations with Less Than 10% Admixture

The second alternative allows the inclusion of populations that contain up to 10% admixture from RT or YCT in the WCT unit for listing. The value of 10% is arbitrary, but it has been used in recent considerations of this problem. The USFWS status review for WCT (Anonymous 1999a) used 10% in its estimation of the current distribution of WCT. In addition, a cutthroat trout management paper developed by an interstate committee of management agencies (Anonymous 2000) considered two management components: nonhybridized populations and



populations that have the phenotypic attributes of WCT and unique genetic, ecological, or behavioral traits, although they are “slightly” introgressed (up to 10% admixture). This document (Anonymous 2000) also points out that the value of 10% is arbitrary and states that populations with more than 10% admixture could also be included in this category. A conservation agreement for WCT in Montana also recommends protecting only populations with limited introgression (up to 10%) because they indicate suitable habitat for WCT or because they may indicate the presence of nonhybridized WCT nearby (Anonymous 1999b).

This alternative greatly increases the number of populations considered part of the WCT unit for possible listing. It allows greater connectivity and reduces problems associated with small, isolated populations and inbreeding depression.

Under this alternative, however, populations that do not belong to the evolutionary lineage of WCT are part of the unit to be considered for listing. If WCT are listed, this alternative would protect hybridized populations that serve as a potential source of continued introgression into nonhybridized populations. Allowing genes from YCT or RT to be incorporated into WCT could disrupt coadapted gene complexes that are the product of thousands of generations of selection (outbreeding depression) and could result in the loss of local adaptations in many cases. The observed increase in RT admixture (Hitt et al. 2003) suggests that the amount of admixture in slightly hybridized populations is increasing. Thus, this alternative could protect many populations that will not be considered as WCT in the near future and therefore would protect sources of further introgression.

### Alternative 3: Include all Populations that Retain the Morphological Attributes of WCT

The third alternative allows inclusion in the unit to be considered for listing of all populations that appear to retain phenotypic attributes characteristic of WCT, regardless of their genetic composition. Connectivity among populations is greatest with this alternative. The number of populations included in the unit to be considered for listing increases dramatically.

This alternative has all of the disadvantages of alternative 2. The WCT evolutionary lineage is poorly preserved because many introgressed populations may be protected. Many populations will be misidentified if molecular examination is not used for analysis. Some populations of nonhybridized WCT and slightly introgressed WCT may be excluded if they do not have the “correct” phenotype. Some populations that contain very little genetic influence from WCT may be protected. Populations may look like WCT but may not function in the ecosystem as WCT. Local adaptations crucial to long-term survival—

such as disease resistance, behavior, and physiology—will likely be lost.

The best evidence that hybridized populations do not function as WCT is the continued spread of hybridization (Hitt et al. 2003). Native WCT populations demonstrate strong genetic differentiation over even short geographical distances, which indicates very little natural gene flow among populations (Allendorf & Leary 1988; Taylor et al. 2003). The rapid spread of introgression, however, indicates a high rate of dispersal and gene flow from hybridized populations.

## Recommendations

Rates of hybridization and introgression are increasing dramatically worldwide because of translocations of organisms and habitat modifications by humans (Allendorf et al. 2001). Hybridization has contributed to extinction of many species through direct and indirect means (Rhymer & Simberloff 1996). Policies should be designed to reduce anthropogenic hybridization.

Any policy that deals with hybrids must be flexible and account for the fact that nearly every situation is different so that general rules are not likely to be effective. There are situations in which it is appropriate to include hybrids as part of the unit to be considered for listing and protection under the ESA. Taxa that have arisen through natural hybridization should be eligible for protection (Allendorf et al. 2001). In addition, intentional hybridization is an appropriate tool to consider for recovery when hybridization would restore historical connectivity among populations (e.g., the Florida panther [*Puma concolor coryi*], Land & Lacy 2000).

General consideration of the treatment of hybrids depends on two primary criteria: the amount of evolutionary divergence between the hybridizing taxa and the geographical extent of introgression. Absolute reproductive isolation is generally not considered necessary for the recognition of a population as a distinct population segment under the ESA (Waples 1995). Hybridization between populations within a species is much less likely to have detrimental effects than hybridization between species that have been genetically isolated for millions of years. Hybridization between intraspecific populations can play an important role in restoring populations that have lost substantial genetic variation through genetic drift and in which the detrimental effects of inbreeding depression are apparent. In these situations, a small amount of introgression might sufficiently counteract the effects of reduced genetic variation and inbreeding depression without disrupting local adaptations (Ingvarsson 2001).

The other key consideration is how many nonhybridized populations of the taxon remain. The smaller the number of pure populations, the greater the conservation

and restoration value of any hybridized populations. In addition, if there are no nonhybridized populations extant, then hybridized populations cannot serve as a source of continued spread of hybridization as they can in the case of WCT. In some species, such as the New Zealand Gray Duck (*Anas superciliosa*), all populations have apparently become genetic admixtures (Rhymer et al. 1994). In spite of widespread hybridization, this species represents a distinctive gene pool that is an appropriate object of continuing conservation efforts. Although any remaining nonhybridized introgressed populations should be given priority in this case, remaining hybrids should be protected in the hope that they will fill the ecological role of the native taxon.

It is a difficult decision. On the one hand, recognizing only nonhybridized populations as WCT could lead to increased fragmentation and protection of many small, isolated populations. This would increase the probability of demographic extinction for these populations. On the other hand, considering hybridized populations to be WCT would extend protection to populations that are not part of the evolutionary lineage of WCT. This action would also protect sources of future hybridization and would increase the probability of genomic extinction of WCT.

A systematic description of the amount of introgression detected throughout the range of the WCT is required for its management and conservation, regardless of which alternative is used. This description requires the use of molecular markers because of difficulties associated with the morphological identification of hybrids. Under alternatives 1 and 2, the inclusion of a population in the WCT unit depends on its level of admixture. Populations of unknown status can be protected under the "similarity of appearance" criterion of section 4(e) of the ESA until genetic information becomes available, but they should not be included in the unit considered for listing. Under alternative 3, populations that do not contain any admixture from RT or YCT should receive higher priority for protection because they are more likely to maintain important local adaptations that may be lost in hybridized populations.

We believe that only nonhybridized populations should be included as WCT in the unit to be considered for listing under the ESA. Westslope cutthroat trout are a monophyletic lineage that has been evolutionarily isolated from other taxa for 1–2 million years (Allendorf & Leary 1988). This time of isolation and the amount of genetic divergence corresponds to that usually seen between congeneric species of fish. Only nonhybridized populations that still contain the WCT genome that has evolved in isolation are likely to possess the local adaptations important for long-term persistence. In addition, there is no scientific basis to justify a 10% admixture in a population to be considered WCT for purposes of listing, but not one of 15% (or 25% or 50%).

## Acknowledgments

We thank D. Jergens, S. Haig, the Break Five, L. Kaeding, R. Waples, and several anonymous reviewers for their helpful comments. We thank the Montana Department of Fish, Wildlife and Parks for their support over the last 25 years. Writing of this paper was partially supported by the U.S. Fish and Wildlife Service.

## Literature Cited

- 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, P. Spruell, and J. K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. *Trends in Ecology & Evolution* 16:613–622.
- Anonymous. 1999a. Status review for westslope cutthroat trout in the United States. U.S. Fish and Wildlife Service, Region 1, Portland, Oregon, and Region 6, Denver, Colorado.
- Anonymous. 1999b. Memorandum of understanding and conservation agreement for westslope cutthroat trout (*Oncorhynchus clarki lewisi*) in Montana. Montana Department of Fish, Wildlife and Parks, Helena.
- Anonymous. 2000. Cutthroat trout management: a position paper. Genetic considerations associated with cutthroat trout management. Publication 00–26. Utah Division of Resources, Salt Lake City.
- Ayala, F. J., and J. R. Powell. 1972. Allozymes as diagnostic characters of sibling species of *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America* 69:1094–1096.
- Barton, N. H., and K. S. Gale. 1993. Genetic analysis of hybrid zones. Pages 13–45 in R. G. Harrison, editor. *Hybrid zones and the evolutionary process*. Oxford University Press, Oxford, United Kingdom.
- Behnke, R. J. 1992. *Native trout of western North America*. Monograph 6. American Fisheries Society, Bethesda, Maryland.
- Behnke, R. J. 2002. *Trout and salmon of North America*. Simon and Schuster, New York.
- Boecklen, W. J., and D. J. Howard. 1997. Genetic analysis of hybrid zones: numbers of markers and power of resolution. *Ecology* 78:2611–2616.
- Dobzhansky, T. 1970. *Genetics of the evolutionary process*. Columbia University Press, New York.
- Edmands, S., and C. C. Timmerman. 2003. Modeling factors affecting the severity of outbreeding depression. *Conservation Biology* 17:883–892.
- Ellstrand, N. C., and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences of the United States of America* 97:7043–7050.
- Epifanio, J., and D. Philipp. 2001. 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.
- Forbes, S. H., and F. W. Allendorf. 1991a. Associations between mitochondrial and nuclear genotypes in cutthroat trout hybrid swarms. *Evolution* 45:1332–1349.
- Forbes, S. H., and F. W. Allendorf. 1991b. Mitochondrial genotypes have no detectable effects on meristic traits in cutthroat trout hybrid swarms. *Evolution* 45:1350–1359.
- Gyllensten, U., and A. C. Wilson. 1987. Mitochondrial DNA of salmonids: inter- and intra-specific variability detected with restriction enzymes. Pages 301–317 in N. Ryman and F. Utter, editors. *Population genetics and fishery management*. University of Washington Press, Seattle.
- Gyllensten, U., R. F. Leary, F. W. Allendorf, and A. C. Wilson. 1985. Introgression between two cutthroat subspecies with substantial

- karyotypic, nuclear, and mitochondrial genomic divergence. *Genetics* **111**:905-915.
- Hanzel, D.A. 1960. The distribution of the cutthroat trout (*Salmo clarki*) in Montana. *Proceedings of the Montana Academy of Sciences* **19**:32-71.
- 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.
- Ingvarsson, P. K. 2001. Restoration of genetic variation lost: the genetic rescue hypothesis. *Trends in Ecology & Evolution* **16**:62-63.
- Ingvarsson, P. K., and M. C. Whitlock. 2000. Heterosis increases the effective migration rate. *Proceedings of the Royal Society of London Series B* **267**:1321-1326.
- Konkle, B. R., and D. P. Philipp. 1992. Asymmetric hybridization between two species of sunfishes (Lepomis: Centrarchidae). *Molecular Ecology* **1**:215-222.
- Land, E. D., and R. C. Lacy. 2000. Introgression level achieved through Florida panther genetic restoration. *Endangered Species Update* **17**:100-105.
- Leary, R. F., F. W. Allendorf, S. R. Phelps, and K. L. Knudsen. 1984. Introgression between westslope cutthroat and rainbow trout in the Clark Fork River drainage, Montana. *Proceedings of the Montana Academy of Sciences* **43**:1-18.
- Leary, R. F., F. W. Allendorf, S. R. Phelps, and K. L. Knudsen. 1988. Population genetic structure of westslope cutthroat trout: genetic variation within and among populations. *Proceedings of the Montana Academy of Sciences* **48**:57-70.
- Leary, R. F., F. W. Allendorf, and G. K. Sage. 1995. Hybridization and introgression between introduced and native fish. *American Fisheries Society Symposium* **15**:91-101.
- Leary, R. F., W. R. Gould, and G. K. Sage. 1996. Success of basibranchial teeth in indicating pure populations of rainbow trout and failure to indicate pure populations of westslope cutthroat trout. *North American Journal of Fisheries Management* **16**:210-213.
- Liknes, G. A., and P. J. Graham. 1988. Westslope cutthroat trout in Montana: life history, status, and management. *American Fisheries Society Symposium* **4**:53-60.
- Madsen, D. H. 1936. Protection of native fishes in the national parks. *Transactions of the American Fisheries Society* **66**:395-397.
- Marnell, L. F., R. J. Behnke, and F. W. Allendorf. 1987. Genetic identification of cutthroat trout, *Salmo clarki*, in Glacier National Park, Montana. *Canadian Journal of Fisheries and Aquatic Sciences* **44**:1830-1839.
- Morgan, M. T. 2002. Genome-wide deleterious mutation favors dispersal and species integrity. *Heredity* **89**:253-257.
- Novinger, D. C., and F. J. Rahel. 2003. Isolation management with artificial barriers as a conservation strategy for cutthroat trout in headwater streams. *Conservation Biology* **17**:772-781.
- O'Brien, S. J., and E. Mayr. 1991. Bureaucratic mischief: recognizing endangered species and subspecies. *Science* **251**:1187-1188.
- Rieman, B., and J. Clayton. 1997. Wildlife and native fish: issues of forest health and conservation of sensitive species. *Fisheries* **22**:6-15.
- Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics* **27**:83-109.
- Rhymer, J. M., M. J. Williams, and M. J. Braun. 1994. Mitochondrial analysis of gene flow between New Zealand mallards (*Anas platyrhynchos*) and grey ducks (*A. superciliosa*). *Auk* **111**:970-978.
- Rubidge, E., P. Corbett, and E. B. Taylor. 2001. A molecular analysis of hybridization between native westslope cutthroat trout and introduced rainbow trout in southeastern British Columbia, Canada. *Journal of Fish Biology* **59**(supplement a):42-54.
- Sage, G. K. 1993. Population genetic analysis of westslope cutthroat trout in the Bob Marshall Wilderness. M.A. thesis. University of Montana, Missoula.
- Sage, R. D., D. Heyneman, K-C. Lim, and A. C. Wilson. 1986. Wormy mice in a hybrid zone. *Nature* **324**:60-63.
- Shepard, B. B., B. Sanborn, L. Ulmer, and D. C. Lee. 1997. Status and risk of extinction for westslope cutthroat trout in the upper Missouri River basin, Montana. *North American Journal of Fisheries Management* **17**:1158-1172.
- Taylor, E. B., M. D. Stamford, and J. S. Baxter. 2003. Population subdivision in westslope cutthroat trout (*Oncorhynchus clarki lewisi*) at the northern periphery of its range: evolutionary inferences and conservation implications. *Molecular Ecology* **12**:2609-2622.
- Templeton, A. R., C. F. Sing, and B. Brokaw. 1976. The unit of selection in *Drosophila mercatorum*. I. The interaction of selection and meiosis in parthenogenetic strains. *Genetics* **82**:349-376.
- Thurrow, R. F., D. C. Lee, and B. E. Rieman. 1997. Distribution and status of seven native salmonids in the interior Columbia River Basin and portions of the Klamath River and Great Basins. *North American Journal of Fisheries Management* **17**:1094-1110.
- U.S. Fish and Wildlife Service and National Oceanic and Atmospheric Administration. 1996. Endangered and threatened wildlife and plants; proposed policy and proposed rule on the treatment of intercrosses and intercross progeny (the issue of "hybridization"); request for public comment. *Federal Register* **61**(26):4710-4713.
- U.S. Fish and Wildlife Service. 2002. Endangered and threatened wildlife and plants: notice of intent to prepare a status review for the westslope cutthroat trout. *Federal Register* **67**(170):56257-56259.
- Utter, F. M., F. W. Allendorf, and H. O. Hodgins. 1973. Genetic variability and relationships in Pacific salmon and related trout based on protein variations. *Systematic Zoology* **22**:257-270.
- Waples, R. S. 1995. Evolutionary significant units and the conservation of biological diversity under the Endangered Species Act. *American Fisheries Society Symposium* **17**:8-27.
- 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.
- Weins, J. A. 1977. On competition and variable environments. *American Scientist* **65**:591-597.
- Whitlock, M. C., P. K. Ingvarsson, and T. Hatfield. 2000. Local drift load and the heterosis of interconnected populations. *Heredity* **84**:452-457.

