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CONSERVATION AND DISTRIBUTION OF GENETIC VARIATION  
IN A POLYTYPIC SPECIES, THE CUTTHROAT TROUT

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CONTENTS

INTRODUCTION .....	1
CUTTHROAT TROUT .....	3
GENETIC DIVERGENCE AMONG SUBSPECIES .....	4
INTERBREEDING BETWEEN TAXA .....	8
Phenotypic Characteristics of Hybrids .....	8
Methods of Detection .....	10
Occurrence of Hybridization .....	13
POPULATION GENETIC STRUCTURE OF SUBSPECIES .....	15
HATCHERY POPULATIONS .....	18
CONSERVATION ISSUES .....	21
Subspecies Problem .....	21
Local Populations .....	22
Introgression .....	23

**ABSTRACT:** The cutthroat trout (Salmo clarki) presents a series of unusual and difficult problems in conservation biology. As many as 16 subspecies have been recognized in the recent literature. The genetic distance between subspecies based upon 46 enzyme loci ranges from that usually seen between congeneric species to virtual genetic identity. Subspecies from the western portion of the range of the cutthroat trout are genetically more similar to rainbow trout (Salmo gairdneri) than they are to the other subspecies of cutthroat trout. In addition, much of the genetic variation within the westslope cutthroat trout (S. c. lewisi) results from alleles that are found in only one or two local populations, but are often at high frequencies in those populations. Thus, preserving the genetic variation in westslope cutthroat trout entails the preservation of as many local populations as possible.

Captive populations of cutthroat trout present a series of opportunities and genetic problems. A number of management agencies are using captive populations to supplement and reestablish natural populations. Basic genetic principles must be understood and followed in the establishment and maintenance of captive populations. We describe examples of unsuccessful and successful efforts by management agencies to develop captive populations.

The greatest danger to the conservation of the cutthroat trout is introgressive hybridization among subspecies and with rainbow trout. Several factors make salmonid fishes especially susceptible to problems associated with introgressive hybridization. We conclude that biochemical analysis provides a more reliable and informative means of detecting interbreeding than morphological characters. Interbreeding between

westslope and Yellowstone cutthroat trout and non-native Salmo appears to be common and widespread throughout the natural range of these subspecies.

## INTRODUCTION

The primary genetic goal of a conservation program is to ensure the maintenance of existing genetic variation. This genetic variation is the result of some three billion years of evolution and represents the evolutionary legacy of a species. More importantly, loss of genetic variation has a variety of harmful effects on characteristics of individuals that are important to the continued existence of a species: growth, survival, fertility, developmental rate, and the ability of individuals to develop properly (reviewed by Mitton & Grant 1984, Allendorf & Leary 1986, Palmer & Strobeck 1986, Zouros & Foltz 1987). Furthermore, the loss of variation is expected to reduce the ability of populations to adapt to changing environmental conditions and to increase their susceptibility to epizootics (Fisher 1930; Ayala 1965, 1969; Frankham 1980; O'Brien et al. 1985). Thus, the loss of genetic variation is generally expected to increase the probability of extinction.

The total amount of genetic variation within a species usually has a hierarchical geographic structure that commonly is referred to as its population genetic structure. For example, a certain proportion of the total genetic variation in a species may be attributable to genetic differences among populations inhabiting particular regions, among populations within regions, and finally within local populations. The distribution of genetic variation among these levels is the result of long and complex interaction among four evolutionary forces; mutation, natural selection, genetic drift, and migration (i. e., exchange of genes between geographical areas).

The relative importance of these evolutionary factors is likely to differ among species and, therefore, the population genetic structure may be quite different, even among closely related species. An understanding of the population genetic structure of a species will aid in formulating a genetically rational conservation program. For example, when substantial divergence exists among geographic groups of populations, maintenance of genetic diversity requires continued existence of populations in each region. This is especially true when alleles are restricted to particular regions but are common where they occur. In contrast, when little genetic divergence exists between regions and most alleles are ubiquitously distributed throughout the range of the species, then a conservation program should place less emphasis on geographical considerations alone.

In this paper, we provide an overview of current conservation genetic efforts with a polytypic species, the cutthroat trout Salmo clarki. This species presents some unusual problems to conservation biologists. The first problem is the enigmatic pattern of genetic divergence among some 15 or so recognized subspecies. Hybridization and introgression resulting from introductions by humans of cutthroat trout subspecies and other trout species outside of their native range presents a second unusual problem. Finally, the role of hatcheries in providing fish for recreation and to reestablish native cutthroat trout populations also creates some unusual problems and challenges. Reintroduction of animals raised in captivity is becoming increasingly important in many conservation projects. The long history of fish hatcheries and introductions provide useful experience for the development of such programs with other animals.

## CUTTHROAT TROUT

"There is no other group of fishes which offers so many difficulties to the ichthyologist, with regard to the distinction of the species, as well as to certain points in their life history, as this genus" (page 484, Jordan & Evermann 1896).

We believe that current workers would not disagree with this judgement about western trout of the genus Salmo in North America. The cutthroat trout is a polytypic species with a wide geographic range that includes the coastal and interior waters of most of the western United States and Canada, from Alaska to the Pecos River of New Mexico (Fig. 1). Despite over a century of intensive systematic investigation, the amount and distribution of genetic diversity among the subspecies remains poorly understood. This confusion largely arises from extreme genetic, morphological, and ecological variation within and among subspecies.

At least 16 subspecies of cutthroat trout have been recognized in the recent literature (Behnke 1979, Johnson 1987). Eight of these are 'major' subspecies that are native to large geographic regions (Figure 1 and Table 1). Another eight 'minor' subspecies are either undescribed, are native to a very small geographic area (e.g., a single lake), or both (Table 1). It is becoming increasingly important to obtain a reliable understanding of the amount of genetic divergence that exists within and among the subspecies of cutthroat trout. Many of the subspecies are threatened by human alteration of their habitat and by the human introduction of brook trout (Salvelinus

fontinalis), rainbow trout (Salmo gairdneri), and subspecies of cutthroat trout into waters outside their native range (Behnke 1972, Behnke & Zarn 1976). Eleven subspecies currently have protected legal status in one or more states, and two minor subspecies apparently are extinct (Table 1).

The greatest danger to the trout of the American west is the introduction of non-native species. Introduced taxa often have serious harmful effects on native taxa through competition or the introduction of pathogenic agents. In cutthroat trout, however, the main impact of introductions has been interbreeding between native and introduced fishes (Busack & Gall 1981, Leary et al. 1984b, Campton & Utter 1985, Gyllenstein et al. 1985, Martin et al. 1985). Hybrid progeny from these matings are often fertile and capable of interbreeding with each other and the parental taxa. This situation destroys the genetic integrity of native populations and results in introgressed populations or hybrid swarms. That is, populations in which genes from the parental types are randomly distributed among individuals so that no individual is likely to be a 'genetically pure' representative of either parental taxon.

## GENETIC DIVERGENCE AMONG SUBSPECIES

We have used allozymes to obtain estimates of genetic divergence among seven cutthroat trout subspecies and rainbow trout. We used horizontal starch gel electrophoresis to assay genetic variation at 46 loci encoding for proteins present in muscle, liver, or eye tissue. Sample locations, sample sizes, loci assayed, and allele frequencies are given by Leary et al. (1987).

We used two approaches to assess electrophoretic divergence among taxa: Nei's measure of standard genetic distance (Nei 1975) and principal components analysis of the arcsine transformation of the square root of allele frequencies. Genetic distance estimates have heuristic value because they are available between populations of various taxonomic rank for a diversity of fishes. Comparison of our estimates with others allows us to make qualitative judgments about the amount of divergence observed among subspecies of cutthroat trout. Combining all allele frequency information into a single distance metric (i.e., Nei's genetic distance) is valuable in depicting similarities among taxa. Nevertheless, collapsing this information into a single dimension causes the loss of information and may oversimplify patterns of similarity. Principal components analysis provides a powerful means of pictorially representing patterns of allele frequency variation among taxa in multivariate space.

A highly variable pattern of genetic divergence exists among the seven subspecies (Table 2). Very little genetic divergence exists among Colorado, Snake River, greenback, and Yellowstone cutthroat trout. Nei's genetic distance between these subspecies are typical of those reported for conspecific populations in a diversity of freshwater and anadromous fishes (Avice 1974, Avice & Smith 1977, Buth & Burr 1978, Loudenslager & Gall 1980, Buth et al. 1984). In contrast, substantial biochemical genetic divergence exists between coastal, Lahontan, and westslope cutthroat trout and between these fishes and the other four subspecies. These genetic distances are truly exceptional for conspecific populations; they are similar to or larger than values observed between many species of fish (Johnson 1975, Avice & Ayala 1976, Buth & Burr 1978, Phelps & Allendorf 1983, Yates et al. 1984).

Surprisingly, the coastal, Lahontan, and westslope cutthroat trout are as similar or more similar to the rainbow trout than they are to the other subspecies. This pattern of genetic divergence is summarized in Figure 2.

Principal components analysis provides a concordant view of genetic divergence among these taxa. The first principal component, which accounts for 50% of the variance in allele frequencies, separates the cutthroat trout subspecies and rainbow trout into three groups: Colorado, Snake River, greenback, and Yellowstone cutthroat trout; coastal and Lahontan cutthroat trout; westslope cutthroat and rainbow trout (Figure 3). The second principal component, which accounts for an additional 25% of the variation, separates the westslope cutthroat from the rainbow trout and increases the separation of the coastal, Lahontan, and westslope cutthroat trout from each other and the other four subspecies (Figure 3). The coastal, Lahontan, and westslope cutthroat trout are all genetically as similar or more similar to rainbow trout than they are to the other four subspecies analyzed, which are all genetically very similar to each other.

We obtained 26 Rio Grande cutthroat trout from two samples from the Rio Grande River drainage after the first version of this manuscript was submitted. These fish were examined for the 46 allozyme loci analyzed in rainbow trout and other cutthroat trout subspecies. We have not reanalyzed the data with these samples included. However, a comparison of diagnostic loci indicates that the Rio Grande cutthroat trout is similar to the Yellowstone group of subspecies, but it is distinct from the Yellowstone, Snake River, greenback, and Colorado cutthroat trout at one allozyme locus (Me3) and nearly so at another (Gp13) (Table 2).

(Leary et al. 1987). This and other possibilities are considered in detail by Leary et al. (1987).

#### INTERBREEDING BETWEEN TAXA

Natural hybridization is much more common among freshwater fishes than in other vertebrates (Hubbs 1955, Campton 1987). Hybridization is more likely to occur between fish taxa for a variety of reasons, e.g., external fertilization, weak ethological isolating mechanisms, and competition for limited spawning habitat (Campton 1987). In addition, hybrids between distantly related fish species are sometimes viable, suggesting that fishes have less severe developmental incompatibilities than other vertebrate species with comparable levels of genetic divergence (Thorgaard & Allendorf 1987). This appears to be especially true for salmonids in which hybrids appear to be developmentally more compatible than other fishes and hybrids between distantly related taxa are often fertile (Ferguson et al. 1985b). These authors have suggested that this may result from the polyploid ancestry of salmonid fishes (Allendorf & Thorgaard 1984).

#### Phenotypic Characteristics of Hybrids

We are not aware of any comprehensive investigations of natural populations that compare hybridized and native populations of cutthroat trout. Most available information comes from experiments performed in hatcheries, and generally suggests that hybrids have lower fitness than the parental taxa.

Salmonid hybrids often have meristic counts identical to the parental type with the higher count or higher than the parentals for characters that

These data present a discordant view of genetic divergence among cutthroat trout subspecies. Some subspecies show little or no evidence of genetic divergence at isozyme loci and are well within the range of genetic divergence seen between geographically continuous conspecific populations. Similarly, Busack (1977) found that the Paiute is allozymically identical to the Lahontan cutthroat trout, and Loudenslager and Gall (1980) found that the Bonneville is very similar to the Yellowstone cutthroat trout. On the other hand, the amount of genetic divergence between westslope cutthroat trout and the other subspecies far surpasses that reported for other conspecific groups of fish. Coastal and Lahontan cutthroat trout also show extreme genetic divergence from the other subspecies.

The data from 46 allozyme loci suggest that cutthroat trout may be polyphyletic (Figure 2). However, morphological, karyotypic, and mtDNA data all suggest that the cutthroat trout subspecies are more similar to each other than they are to rainbow trout (Gold 1977, Leary et al. 1984b, Gold et al. 1977, Loudenslager & Thorgaard 1979, Thorgaard 1983, Gyllensten & Wilson 1987). The conventional view of the evolutionary relationship of these taxa is that cutthroat trout subspecies are members of a single phylogenetic divergence from rainbow trout.

The discordance in patterns of similarity indicates that evolutionary divergence of the protein loci and these other attributes have proceeded at different rates among these fishes. There are several hypotheses that can account for this difference. For example, we have suggested that secondary contact between the rainbow trout and some cutthroat trout subspecies could have resulted in temporary periods of limited introgression in the past

report some evidence for increased growth in westslope-Yellowstone cutthroat trout hybrids.

#### Methods of Detection

Historically, detection of hybridization and introgression in salmonid fishes has relied upon morphological characters. There are two fundamental problems with this approach. First, these comparisons often assume that hybrid individuals will be morphologically intermediate to the parental types. We have already discussed experimental results that show that this is not a good assumption.

Secondly, a small genetic contribution of one taxon to a mixed population or hybrid swarm may not be morphologically detectable. For instance, the presence of less than 10% genes from rainbow trout in introgressed populations with westslope cutthroat does not detectably alter the counts of meristic characters from distributions characteristic of pure westslope cutthroat trout (Leary et al. 1984b). Thus, the reliance on morphological characters can seriously underestimate the extent of hybridization and introgression between native and introduced salmonid fishes. This can provide misleading information about the genetic status of individuals and populations.

The presence of fixed allelic differences at several loci between taxa provides a means of identifying samples and of detecting interbreeding. Because of this attribute, such loci are commonly referred to as diagnostic loci (Ayala & Powell 1972). We did not detect any diagnostic loci among the Colorado River, Snake River, greenback, and Yellowstone cutthroat trout; we found only one diagnostic locus between these subspecies and Rio Grande

do not differ between them (Leary et al. 1983, 1985b; Ferguson & Danzmann 1987). Interspecific hybrids of other fishes have also been found not to be morphologically intermediate to the parental taxa (Neff & Smith 1979, Joswiak et al. 1982, Campton 1987). Lack of meristic intermediacy results from developmental genetic incompatibility between the parental genomes (see discussion in Ferguson & Danzmann 1987).

Other developmental and morphological characteristics demonstrate similar results. For example, hybrids between hatchery strains of rainbow trout tend to have a slower developmental rate than the parental strains (Ferguson et al. 1985a). Hybrids between rainbow trout and three different subspecies of cutthroat trout all have decreased developmental stability (Leary et al. 1985b). Hybrids between westslope and Yellowstone cutthroat trout also have reduced developmental stability (Ferguson et al. in press).

We produced experimental hybrids between rainbow trout and westslope cutthroat trout to test for differences in growth rate. The hybrids were produced by crossing hatchery strains of both parental taxa at the Creston National Fish Hatchery of the United States Fish and Wildlife Service in 1982 and 1983. The experiment was done in two parts because there was no overlap in time of spawning between females from the two strains. On 22 March 1983, 20 westslope cutthroat trout males and rainbow trout (24 females and 31 males) were mated together by first pooling together approximately equal numbers of gametes. The reciprocal matings were done on 14 May 1982, with 24 rainbow trout males and 24 westslope cutthroat trout of both sexes. Both hybrids had slower growth rates than the parental taxa under these hatchery conditions (Table 4). In contrast, Ferguson et al. (in press)

cutthroat trout (Table 2; Figure 3). Thus, electrophoresis is not a reliable means of detecting interbreeding between these taxa. In contrast, we found two or more diagnostic loci between coastal cutthroat, Lahontan cutthroat, westslope cutthroat, and rainbow trout populations, as well as between all these fishes and the other five subspecies of cutthroat analyzed (Table 2). Thus, electrophoresis can be used to detect interbreeding between all of the subspecies of cutthroat trout we analyzed and rainbow trout, as well as between many pairs of cutthroat trout subspecies.

All individuals in samples obtained from genetically pure populations will possess genotypes of only that taxon at all diagnostic loci. In contrast, first generation hybrids will be heterozygous for alleles characteristic of both parental taxa at all diagnostic loci between them. Matings between parental types and hybrids will produce individuals that are homozygous at some diagnostic loci and heterozygous at others. The multiple locus genotype will be highly variable in mixed populations when the alleles characteristic of the parental taxa are randomly distributed among individuals. Table 3 shows the multiple locus genotypes of 15 individuals from such a hybrid swarm of westslope and Yellowstone cutthroat from Forest Lake, Montana (Gyllensten et al. 1985).

Analysis of mitochondrial DNA (mtDNA) has revealed a great deal of intra- and inter-specific variability in fishes (Avisé 1986, Avisé et al. 1984, Bermingham & Avisé 1986, Gyllensten & Wilson 1987). mtDNA is inherited maternally, and thus provides information about the sexes of the parental types involved in hybrid matings. For example, if only males of a taxon participate in hybrid matings, then the mtDNA of this taxon will not be present in the hybridized population. However, if both sexes of both

taxa make equal genetic contributions, then the proportion of mtDNA from each parental taxon is expected to be similar to the proportional genetic contribution estimated from diagnostic nuclear loci. The limited data available from salmonids is consistent with the latter situation in introgressed populations (Gyllensten et al. 1985).

Certain properties of mtDNA diminish its value as a sole criterion to determine the genetic status of individuals or populations. An individual generally contains only one type of mtDNA. Thus, it is not possible to distinguish hybrids from the parental types (Avisé et al. 1984, Gyllensten et al. 1985, Avisé & Vrijenhoek 1987). Furthermore, if only males of a taxon participate in hybrid matings then the mtDNA of this taxon will be absent from hybrid populations. The effective population size of mtDNA is smaller than nuclear DNA; thus mtDNA frequencies will be more strongly affected by genetic drift than nuclear gene frequencies. When the proportional contribution of a taxon to a hybrid swarm is small, the less frequent mtDNA may be lost by genetic drift. Furthermore, since there is no recombination between mtDNA molecules, the whole mtDNA genome is in terms of identification only a single gene; in contrast, there are many unlinked nuclear genes that can be used for identification purposes.

The analysis of proteins encoded by multiple diagnostic nuclear loci, therefore, is the most sensitive and reliable method available to identify genetically pure populations of cutthroat trout taxa that have diagnostic differences. Busack and Gall (1981) came to a similar conclusion in their study of Paiute cutthroat trout. Studies with other groups of fish also support this conclusion (Whitmore 1983, Buth et al. 1987, Joswiak et al. 1982).



### Occurrence of Hybridization

We began a project approximately five years ago, in cooperation with Montana Department of Fish, Wildlife, and Parks (MDFWP), to assess the extent of hybridization in native populations of westslope cutthroat trout. Approximately one-half of the populations of westslope cutthroat trout in the state at that time were classified, on the basis of morphology, as being hybridized with either rainbow trout or Yellowstone cutthroat trout. We now have analyzed 46 enzyme loci from samples of over 125 populations of cutthroat trout in Montana.

Our results indicate that hybridized populations of westslope cutthroat trout are much more common than previously thought. Thirty-two out of 80 samples (40%) from populations classified as 'genetically pure' westslope cutthroat trout showed evidence of hybridization with either rainbow trout or Yellowstone cutthroat trout, or both of these fishes. All samples from streams classified as containing hybrid populations did show evidence of hybridization. Similar results from samples taken from Glacier National Park have been reported by Marnell et al. (1987).

An interesting and dangerous pattern has emerged from the analysis of samples from the South Fork of the Flathead River drainage. This region is considered to be one of the last remaining 'strongholds' of native westslope cutthroat trout in Montana (Hanzel 1959). Only 2 of 19 headwater lakes sampled contained pure populations of westslope cutthroat trout. Analysis of streams in this drainage indicates that hybridized populations in headwater lakes are 'leaking' into downstream populations. This movement and gene flow is often unidirectional because of barrier waterfalls. Thus,

the stream populations are expected to become genetically more and more similar to the lake populations through time.

The constant spread of non-native fishes from headwater lakes into downstream populations is not unique to this area. Many headwater lakes throughout the west did not contain native trout populations because of recent glaciation and barriers to upstream migration. Six headwater lakes in Glacier National Park that were previously barren of fish now have populations of apparently pure non-native Yellowstone cutthroat trout (Marnell et al. 1987). The Camas Creek drainage provides a clear example of the effects of these headwater populations. The two highest lakes (Evangeline and Camas) have populations of pure Yellowstone cutthroat trout. The next highest lake in the drainage (Arrow) had a hybridized population consisting of approximately 90% westslope genes and 10% Yellowstone cutthroat trout genes. The bottom lake in the drainage (Trout) contained a population of apparently pure westslope cutthroat trout.

Introgression also appears to be common in the native Yellowstone cutthroat trout of the Yellowstone River drainage. Sixteen samples were collected without prior information as to purity from Yellowstone River tributaries in Montana. Thus, these samples should be a reliable indicator of the proportion of hybridized populations in this drainage. Evidence of hybridization and introgression between the Yellowstone cutthroat and rainbow trout was detected in half of the samples.

These results suggest that the introduction of non-native fishes is likely to be responsible for the loss of many native populations of cutthroat trout throughout its range. This conclusion is supported by results with other cutthroat trout subspecies. For example, Martin et al.

(1985) found evidence of introgression from rainbow trout in 7 of 39 populations of cutthroat trout sampled in Utah. Introgressed populations of Paiute and coastal cutthroat trout with rainbow trout have also been described (Busack & Gall 1981, Campton & Utter 1985).

#### POPULATION GENETIC STRUCTURE OF SUBSPECIES

Until this point, we have taken a typological viewpoint of subspecies of cutthroat trout. That is, we have treated each subspecies as being genetically homogeneous. We now abandon this view and address the amount and pattern of genetic diversity within the westslope and Yellowstone cutthroat trout. The westslope cutthroat trout represents a situation of extreme genetic divergence among populations, while there appears to be little genetic divergence among Yellowstone cutthroat trout populations.

The population genetic structure of westslope cutthroat trout is based upon analysis of proteins encoded by 29 loci (see Leary et al. 1985<sub>e</sub> for procedures and loci) in samples obtained from 103 populations throughout most of its natural range. Sample locations are available on request. We analyzed the data using the hierarchical gene diversity analysis of Chakraborty (1980). The total amount of genetic diversity among samples ( $H=0.0287$ ) is partitioned into the proportion due to allele frequency differences among 10 drainages (Table 5,  $H=0.0048$ , 16.7%), allele frequency differences among populations within drainages ( $H=0.0045$ , 15.7%), and genetic variation within local populations ( $H=0.0194$ , 67.6%). Thus, each local population contains only about two thirds of the genetic variation that exists in the taxon. This is a relatively high amount of genetic divergence among conspecific populations but is not unusual among

conspecific populations of salmonids inhabiting interior drainages (reviewed by Gyllenstein 1985).

The pattern in which alleles are distributed among populations of westslope cutthroat trout reveals two interesting features. First, a large number of alleles (29, 43.9%) exist in all populations at high average frequency (i.e., greater than 0.95). These ubiquitously distributed alleles represent the common allele at all the loci examined. Secondly, a high proportion (37.8%) of the remaining alleles were detected in only one or two populations. Furthermore, a number of these geographically 'rare' alleles occur at a relatively high frequency within their respective local populations. Thus, high genetic divergence among westslope cutthroat trout is largely due to numerous alleles, often at high frequencies, with a narrow geographic distribution. Conservation of the genetic diversity in westslope cutthroat trout, therefore, requires ensuring the continued existence of many populations throughout its range.

Limited electrophoretic data indicate that the Yellowstone cutthroat trout in the Yellowstone River drainage has a markedly different population genetic structure than the westslope cutthroat trout. We detected evidence of genetic variation at only 5 of 46 protein loci (see Leary et al. 1987 for details) examined (Table 6). Genetic variation at 4 of the 5 polymorphic loci was restricted to a rare allele ( $<0.05$ ) found in only one of the eight samples. Genetic variation at Aat-3,4 is present in all of the populations, and the allele frequencies are highly heterogeneous ( $P<0.001$ ). Allele frequency differences among samples, however, account for only 3.7% of the total amount of genetic variation detected, the remainder is attributable to genetic variation within local populations. The former value is

substantially lower than that detected among populations of westslope cutthroat trout (Table 5).

Analysis of the population genetic structure of other salmonid species suggests that the differences in the amount of divergence among populations largely reflects different amounts of gene flow (Gyllenstein 1985). Anadromous salmonids usually have relatively little genetic divergence among populations (Table 5). In contrast, more sedentary freshwater fishes generally exhibit substantial divergence among populations (Table 5). The simplest explanation for this dichotomy is that the high mobility of the former fishes increases gene flow among populations and hinders accumulation of genetic differences through natural selection and genetic drift (Gyllenstein 1985).

Ryman (1983) has discussed the significance of the large divergence among local populations of brown trout (*Salmo trutta*) in Europe for fishery management and conservation biology. The westslope and Lahontan cutthroat trout have a genetic population structure that is similar to that of brown trout (Table 5). The Yellowstone cutthroat trout is exceptional as the only non-anadromous species in Table 5 that shows relatively little genetic divergence among local populations. These results are supported by Loudenslager and Gall (1980); they estimated that only 8% of the genetic variation in 10 Yellowstone cutthroat trout populations sampled from a broader geographic area than the populations in Table 5 was due to divergence among them.

#### HATCHERY POPULATIONS

Captive populations can play an integral role in conservation programs. These populations serve two major purposes. First, they can represent a captive source of some of the genetic variability in a taxon; i.e., they serve as a 'gene bank'. Captive populations also represent a readily accessible source of individuals for reestablishing extinct populations or for establishing new populations in suitable habitat within the taxon's natural range but unoccupied because of barriers to dispersal. Loss of genetic variation in hatchery populations can be a serious problem. Allendorf and Ryman (1987) have considered the genetic management of hatchery populations of salmonids and have reviewed the published results demonstrating examples of loss of genetic variation in hatchery populations.

A hatchery broodstock of westslope cutthroat trout was founded by the MDFWP from about 15 males and 15 females collected from a population in Hungry Horse Creek, Flathead County, Montana during June of 1965 and 1967. Electrophoretic analysis of the proteins encoded by 35 loci indicated that by 1976 the hatchery population contained substantially less genetic variation than fish from Hungry Horse Creek (Allendorf & Phelps 1980). Furthermore, significant heterogeneity in allele frequencies was observed among the 1971 to 1976 hatchery year-classes. Loss of variation and temporal instability of allele frequencies in this population were considered to result from two major factors: (1) founding the population from a small number of individuals, all of which probably were not reproductively successful, and (2) perpetuating the population from individuals that were mature during only a small proportion of the reproductive season.

By 1983, there was compelling evidence that loss of genetic variation in this hatchery population was having deleterious effects on a variety of phenotypic characters. An unusually high proportion of individuals in this year class had obvious morphological deformities, such as abnormal vertebral columns and missing fins. Individuals in this hatchery population also had an unusually high amount of asymmetry in the counts of five bilateral meristic characters compared to individuals from natural populations of westslope cutthroat trout, including Hungry Horse Creek (Leary et al. 1985a). The high proportion of deformed fish and levels of asymmetry are indicative of a reduction in the ability of individuals to develop precisely along genetically determined pathways.

This reduction in developmental stability was considered a consequence of the loss of genetic variation. Previous studies of salmonids revealed a negative association between heterozygosity at protein loci and asymmetry among individuals in populations (Leary et al. 1984a, 1985c). Highly inbred individuals also have unusually high levels of asymmetry that are comparable to that observed in the hatchery population (Leary et al. 1985d). Reduced developmental stability, poor fertility, and poor survival led the MDFWP to abandon this broodstock in 1986. They are now establishing a new broodstock with a procedure similar to the one described below.

A hatchery population of Yellowstone cutthroat trout was founded by the MDFWP from an unknown number of males and females collected from McBride Lake, Yellowstone National Park in 1969. Since then, gametes have been obtained periodically from individuals collected from McBride Lake. The progeny from these gametes have been raised at the hatchery and are mated with hatchery fish. This broodstock, therefore, has periodically received

infusions of genes from a wild population. Recent data indicate that this policy has effectively incorporated and maintained the genetic variability in the natural fish in this hatchery population.

We obtained samples (N=50) from the 1985 hatchery year-class and progeny from 11 female and 22 male fish collected from McBride Lake in 1985. The genotype of each fish was determined at 71 loci coding for 31 enzymes (details are available on request). The two samples have significant allele frequency differences at three of seven polymorphic loci (Table 7). The hatchery fish also appear to have a higher level of heterozygosity than the progeny from the wild fish. Although this difference is not statistically significant (Wilcoxon two-sample test;  $P=0.10$ ), the allele frequency differences suggest it is biologically meaningful. In addition, fish from this hatchery stock have a low amount of developmental instability (Leary et al. 1984a, 1985a) and they have high fertility and survival in the hatchery (Thurston Dotson, MDFWP, personal communication).

These results demonstrate the value of maintaining genetic variation by founding a hatchery population from a large number of individuals and by periodically introducing genes from individuals collected from natural populations into the hatchery population. These actions will also reduce undesirable genetic changes (Allendorf & Ryman 1987) that may occur due to adaptation to domestic conditions. Lacey (1987) has considered in detail the genetics of managed populations and the importance of infusions of genes from natural populations.

## CONSERVATION ISSUES

### Subspecies Problem

The classification of such genetically diverged taxa into a single species, cutthroat trout, creates a problem in the conservation of these fishes. This classification implies that the subspecies are distinguishable only by minor and recently evolved genetic differences. Protein data, however, indicate that some groups of cutthroat trout have been separate evolutionary units for long intervals of time. The relatively ancient separation of cutthroat lineages is supported by other considerations. R. J. Behnke (personal communication) has estimated, on the basis of historical biogeographic considerations, that the westslope and Yellowstone cutthroat trout have been isolated for 1-2 million years. Also, the amount of differences in mtDNA molecules (Gyllenstein and Wilson 1987) correspond to a divergence time of about one million years (Wilson et al. 1985). These subspecies also have diverged in many other characteristics (e.g., behavior, habitat preference, etc.). Thus, morphological evolution in cutthroat trout apparently has been very slow so that these subspecies remain morphologically similar, even after a long time of evolutionary independence.

We feel that taxonomic revision of cutthroat trout is warranted in order to portray accurately their evolutionary history. Furthermore, such reclassification would be especially helpful to those involved with the management and conservation of cutthroat trout. For example, we believe that the westslope cutthroat trout should be recognized as a distinct species. A taxonomic revision of cutthroat trout is beyond the purview of

this paper but we believe it is necessary in order to develop a sound conservation program for these fishes.

### Local Populations

Conservation programs should be concerned with the conservation of alleles, rather than just allele frequencies. Allele frequencies are an ephemeral characteristic of populations that can be changed by genetic drift or natural selection. No specific allele frequency now or in the future should be the goal of a conservation program. However, loss of an allele represents a permanent decrease in genetic diversity. Once an allele is lost it can be recovered only by mutation, the probability of which is minuscule. The loss of alleles, therefore, permanently reduces the ability of populations to make adaptive responses to altered environmental conditions and can also reduce their resistance to disease (Allendorf 1986).

Allelic variation in the westslope cutthroat trout is composed largely of alleles that have a narrow geographic distribution, but occur at relatively high frequencies in local populations. Maintenance of the allelic diversity of this subspecies, therefore, requires the continued existence of many populations throughout its range. Limited data suggest that this conclusion is also true for Lahontan cutthroat trout.

The available data from Yellowstone cutthroat trout demonstrates relatively little allelic diversity between local populations. The alleles that occur at appreciable frequencies exist in practically all populations; the other alleles are rare everywhere. We do not feel that conservation biologists need to be concerned about preserving rare alleles. The loss of rare alleles is not likely to be harmful to a population (Hedrick et al.

1986). Thus, allelic diversity of the enzymes detected with electrophoresis in Yellowstone cutthroat trout might essentially be conserved by ensuring the continued existence of a few populations.

Nevertheless, low genetic divergence among populations at isozyme loci does not indicate the absence of important genetic differences between populations. Some populations may be adapted to unusual local conditions, e.g., warmer or more saline water. These populations may possess genetic adaptations for existence under these circumstances that will not be indicated by allelic divergence at isozyme loci. Ensuring the continued existence of these potentially valuable populations should be a concern of conservation programs.

#### Introgression

Introgression is the most important factor responsible for the loss of native cutthroat trout populations. The presence of numerous introgressed populations throughout the range of cutthroat trout threatens the remaining native populations. If this condition persists, the only remaining native populations will be those isolated by dispersal barriers. This fragmentation into a number of small, isolated refuges will increase the chances of extinction (Wilcove et al. 1986, Quinn & Hastings 1987). Furthermore, the lack of gene flow among refuges will accelerate the loss of genetic variation by genetic drift.

Conservation of cutthroat trout, therefore, may sometimes require the destruction of introgressed populations. Poisons have often been used in such attempts (e.g. Rinne & Hanson 1981). This practice, however, is not without problems. Concentrations of poisons that are likely to extirpate a

trout population will also affect many other forms of aquatic life. In addition, many introgressed or non-native populations occur in remote areas or waters that are too large for poisoning to be effective.

The State of Montana has initiated a restoration policy for westslope cutthroat trout that includes extirpation of non-native populations (Joe Huston, MDFWP, personal communication). Populations in the South Fork of the Flathead River drainage that contain a substantial proportion of non-native genes may be poisoned when this is likely to be effective. After the poison has detoxified, westslope cutthroat trout from a hatchery population will be introduced periodically. These fish are expected to spawn among themselves and with any non-native trout that survived the poison. The continued introduction of hatchery fish should reduce the frequency of non-native genes. When poisoning is not practical, stocking is the sole means of restoration. The efficacy of this program depends on having hatchery fish capable of surviving and reproducing in the wild.

Although the above program may not eliminate all non-native genes from a population, it can reduce their frequency so that the result can be viewed as effective restoration. There is likely to be no universal criterion of when restoration has been achieved effectively. We suggest that a level of 1% or less foreign genes may suffice for two reasons. Levels of introgression this low are often difficult to detect conclusively. Furthermore, this amount of introgression is unlikely to alter the biological characteristics of the fishes from those of genetically pure westslope cutthroat trout.

The problem of introgression among western trout raises questions about what genetic resources we should be trying to preserve in conservation



biology. Populations of 'trout' are not lost by introgression; trout remain in the rivers, streams, and lakes of the western United States.

Furthermore, alleles are not lost directly through introgression. Rather, the native alleles are simply diluted by the stocking of trout from other sources. It has been suggested that natural selection will act to preserve those genetic types that are important for local adaptation so that the native type will reevolve in a reasonably short length of time. Perhaps, therefore, we should consider the position that the introduction of new genetic variation through widespread introgression with non-native fishes may actually be beneficial, especially from the perspective of those responsible for managing these populations as a recreational fisheries resource.

There are several compelling reasons why we should be concerned about the potential harmful effects of widespread introgression. The eventual outcome of widespread introgression and continued introduction of hatchery rainbow trout is the homogenization of western North American trout into a single taxon (Salmo ubiquiti?). Thus, we would exchange all of the diversity within and between many separate lineages, produced by millions of years of evolution, consisting of taxa capable of existing from the Arctic to the desert, for a single new mongrel species. This would be a great loss of biological diversity - and of recreational fisheries resources. Sport-fishing groups are active in many conservation programs to preserve local native fishes. The loss of these local native trout would reduce the quality of these resources for many persons.

One danger of homogenization is the loss of valuable locally adapted populations. This danger seems especially important because of the

tendency for salmonid species to evolve genetically discrete, ecologically specialized populations (reviewed by Behnke 1972). The loss of the Lahontan cutthroat trout native to Pyramid Lake is a spectacular example of the value of locally adapted populations. Evolution of these trout in a continuous lake environment for 50,000-100,000 years with an abundant prey species (tui chub, Gila bicolor) resulted in the world's largest cutthroat trout, up to 20 kg (Sigler et al. 1983). The cutthroat trout native to Pyramid Lake disappeared in the late 1930's primarily due to loss of spawning habitat.

Another danger of widespread hybridization is outbreeding depression. That is, hybrids between genetically differentiated populations often have reduced fitness (Templeton 1986). This reduction in fitness may occur either in the first generation hybrids or may be delayed until the backcross or later generations. Outbreeding depression is caused by the existence of coadapted genic or chromosomal complexes. For example, the production of gametes through meiosis usually requires two matched sets of chromosomes. If the two sets of chromosomes differ in number or structure, however, abnormal meiosis can produce gametes that will not produce viable progeny. Hybrids between populations of animal species that have chromosomal differences often have reduced fertility because of meiotic problems (Templeton 1986).

The available evidence indicates that hybrids between the chromosomally differentiated rainbow and cutthroat trout subspecies (Allendorf & Thorgaard 1984) do not have meiotic problems. The common existence in nature of hybrid swarms demonstrates that these hybrids do not have severely reduced fertility.

Outbreeding depression can also result because coadapted gene complexes may depend on the interaction between specific gene loci. That is, the genes in an evolutionary lineage (population or species) have been selected to interact with each other within the internal genetic and biochemical environment. For example, Sage et al. (1986) have shown that mouse interspecific hybrids are more susceptible to parasites than either parental species. The concept of coadapted gene complexes was first used in 1948 to explain reduced fitness in hybrids between populations of the fruit fly Drosophila pseudoobscura (Dobzhansky 1948, 1970). However, experiments with Drosophila mercatorum indicate that this type of outbreeding depression may be only a temporary problem (Templeton et al. 1976). New coadapted gene complexes with fitnesses equal or superior to the parental strains quickly evolved through natural selection.

The phenotypic characteristics of hybrids described earlier in this paper suggest that outbreeding depression and coadapted gene interactions do occur in Salmo. Overall, hybrids do not perform as well as either parental type for a wide variety of phenotypic measures. Nevertheless, the widespread success of hybrid trout populations suggests that outbreeding depression is not a serious problem. The major danger of introgression is the homogenization of the many divergent evolutionary lineages and the loss of important and potentially valuable locally adapted populations.

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Table 1. Subspecies of cutthroat trout and their protection status (Behnke 1979, Williams 1987).

Common Name	Subspecies	Abbrev.	Legal Protection
Bonneville	<i>S. c. utah</i>	BO	US ID NV <u>UT</u> WY
Coastal	<i>S. c. clarki</i>	CC	--
Colorado	<i>S. c. pleuriticus</i>	CO	US CO <u>UT</u> WY
Greenback	<i>S. c. stomias</i>	GR	US CO
Lahontan	<i>S. c. henshawi</i>	LA	US CA <u>UT</u>
Rio Grande	<i>S. c. virginalis</i>	RG	CO NM
Westslope	<i>S. c. lewisi</i>	WS	ID MT
Yellowstone	<i>S. c. bouvieri</i>	YS	ID MT
Alvord	<i>S. c. subsp.</i>	AL	extinct
Bear Lake	<i>S. c. subsp.</i>	BL	ID
Humboldt	<i>S. c. subsp.</i>	HU	--
Mountain	<i>S. c. alpestris</i>	MO	--
Paiute	<i>S. c. seleniris</i>	PA	US CA
Snake River	<i>S. c. subsp.</i>	SR	ID
Willow/Whitehorse	<i>S. c. subsp.</i>	WW	US
Yellowfin	<i>S. c. macdonaldi</i>	YF	extinct

The eight subspecies in the top group are the major subspecies which are endemic to large geographical areas (see Figure 1). Italicized abbreviations represent legal protection and unitalicized abbreviations represent fishes of Special Concern according to Johnson (1987). US=United States; CA=California; CO=Colorado; ID=Idaho; MT=Montana; NM=New Mexico; NV=Neveda; UT=Utah; WY=Wyoming.

Table 2. Nei's genetic distance between seven subspecies of cutthroat trout and the rainbow trout (RT) below the diagonal and number of diagnostic loci between the taxa above the diagonal.

	RT	Cutthroat Trout Subspecies							
		WS	CC	LA	YS	SR	GR	CO	RG
Rainbow	—	6	5	6	10	10	10	10	10
Westslope	0.130	—	7	7	10	10	10	10	10
Ogastal	0.099	0.164	—	2	7	7	7	7	7
Lahontan	0.138	0.175	0.077	—	6	6	6	6	6
Yellowstone	0.246	0.295	0.191	0.164	—	0	0	0	1
Snake River	0.247	0.297	0.192	0.165	0.006	—	0	0	1
Greenback	0.229	0.268	0.194	0.151	0.022	0.025	—	0	1
Colorado	0.223	0.280	0.193	0.150	0.012	0.023	0.005	—	1

Table 3. Allozyme genotypes at 8 nuclear loci and mtDNA genotypes in a hybrid swarm of westslope and Yellowstone cutthroat trout in Forest Lake, Montana.

No.	mtDNA	Nuclear encoded loci							
		Aat1	Qp13	Idh1	Igg	Me1	Me3	Me4	Sdh
1	YS	W	W	WY	W	W	W	W	Y
2	YS	W	WY	WY	WY	Y	W	WY	Y
3	WS	WY	Y	Y	W	Y	WY	Y	WY
4	WS	Y	W	WY	WY	W	Y	W	WY
5	YS	Y	Y	Y	WY	WY	WY	Y	Y
6	YS	WY	Y	W	WY	W	W	W	Y
7	WS	WY	WY	Y	W	WY	W	W	W
8	WS	WY	Y	WY	WY	Y	W	Y	Y
9	WS	Y	Y	WY	WY	W	WY	WY	W
10	WS	WY	Y	WY	WY	WY	Y	W	Y
11	YS	Y	W	W	WY	W	Y	W	Y
12	WS	W	WY	Y	WY	W	WY	WY	Y
13	YS	W	Y	W	Y	W	WY	W	W
14	YS	Y	Y	WY	WY	WY	WY	WY	W
15	WS	WY	Y	WY	Y	W	Y	WY	W

W = homozygous for westslope allele; Y = homozygous for Yellowstone allele; WY = heterozygous for westslope and Yellowstone alleles.

Table 4. Mean length of parental types and hybrids between westslope cutthroat trout and rainbow trout.

Age (days)	Length (mm)			Age (days)	Length (mm)		
	WS	WS x RT	Prob.		RT	RT x WS	Prob.
73	22.7	22.3	NS	72	25.3	24.5	**
89	29.0	27.2	***	100	33.4	31.2	**
112	40.1	33.6	***	130	46.3	46.3	NS
				181	69.6	62.7	***

The maternal species is listed first. NS = not significant; \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$ .

Table 5. Analysis of genic diversity and degree of differentiation in eight taxa of salmonid fishes.

Taxa	No. Pop.	No. Loci	$H_T$	$H_S$	Relative gene diversity		
					Between drainages	Between samples within drainages	Within samples
Cutthroat trout							
CC	21	31	0.101	0.095	2.2	3.6	94.2
LA	15	35	0.065	0.036	—	44.5*	55.5
WS	103	29	0.029	0.019	16.7	15.7*	67.6
YS	8	46	0.014	0.013	—	3.7*	96.3
Atlantic salmon	53	38	0.040	0.023	37.4	3.6	59.0
Brown trout	38	35	0.040	0.025	7.5	29.2*	63.3
Rainbow trout	38	16	0.069	0.058	7.3	7.7	85.0
Sockeye salmon	18	26	0.046	0.044	2.5	3.1	94.4

$H_T$  = total gene diversity;  $H_S$  = average gene diversity within local populations. The data are from the following sources: Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), rainbow trout, and sockeye salmon (*Oncorhynchus nerka*) from Ryman (1983); Lahontan cutthroat trout from Loudenslager and Gall (1980); coastal cutthroat trout from Campton and Utter (1987). \* = samples obtained from non-anadromous populations.

Table 6. Allele frequencies in eight populations of Yellowstone cutthroat trout from the Yellowstone River drainage, Montana.

Samples and Allele Frequencies									
Locus	Alleles	Mill Fork							
		Anderson	Billman	Mission	Miner	Rock	Six Mile	Tom Miner	Turkey
Ast-3,4	100	0.590	0.697	0.488	0.634	0.530	0.850	0.580	0.712
	110	0.260	0.105	0.179	0.250	0.280	0.090	0.250	0.173
	90	0.150	0.197	0.333	0.116	0.190	0.060	0.170	0.115
Adh	-100	1.000	1.000	0.952	1.000	1.000	1.000	1.000	1.000
	0	-	-	0.048	-	-	-	-	-
Gl-1	101	1.000	1.000	0.982	1.000	1.000	1.000	1.000	1.000
	88	-	-	0.018	-	-	-	-	-
Leg	135	1.000	1.000	1.000	0.982	1.000	1.000	1.000	1.000
	100	-	-	-	0.018	-	-	-	-
Sdh-1,2	100	1.000	1.000	1.000	0.982	1.000	1.000	1.000	1.000
	200	-	-	-	0.018	-	-	-	-

Table 7. Allele frequencies in the 1985 year class of hatchery Yellowstone cutthroat trout and the progeny of fish from the wild population from which the hatchery population was founded.

Locus	Alleles	Hatchery	Wild	Probability
Ast-3,4	100	0.655	0.685	*
	110	0.125	0.195	
	90	0.220	0.120	
Acp-1	-100	0.920	1.000	**
	-33	0.080	-	
Pum-1	95	0.900	0.880	NS
	81	0.100	0.120	
A-glu	70	0.867	0.917	NS
	100	0.133	0.083	
Hex	100	0.760	0.900	**
	72	0.240	0.100	
Mdh-1,2	100	0.995	0.995	NS
	44	0.005	0.005	
Tpi-3,4	100	0.995	1.000	NS
	93	0.005	-	
Average observed heterozygosity		0.027	0.022	

NS = not significant; \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ .



#### FIGURE LEGENDS

Figure 1. Range of eight major subspecies of cutthroat trout in western North America (based on Behnke 1979).

Figure 2. Dendrogram (unweighted average linkage method) based on Nei's genetic distance for seven subspecies of cutthroat trout and the rainbow trout.

Figure 3. Plot of first two principal component scores of allele frequencies at 16 diagnostic loci among seven subspecies of cutthroat trout and the rainbow trout.

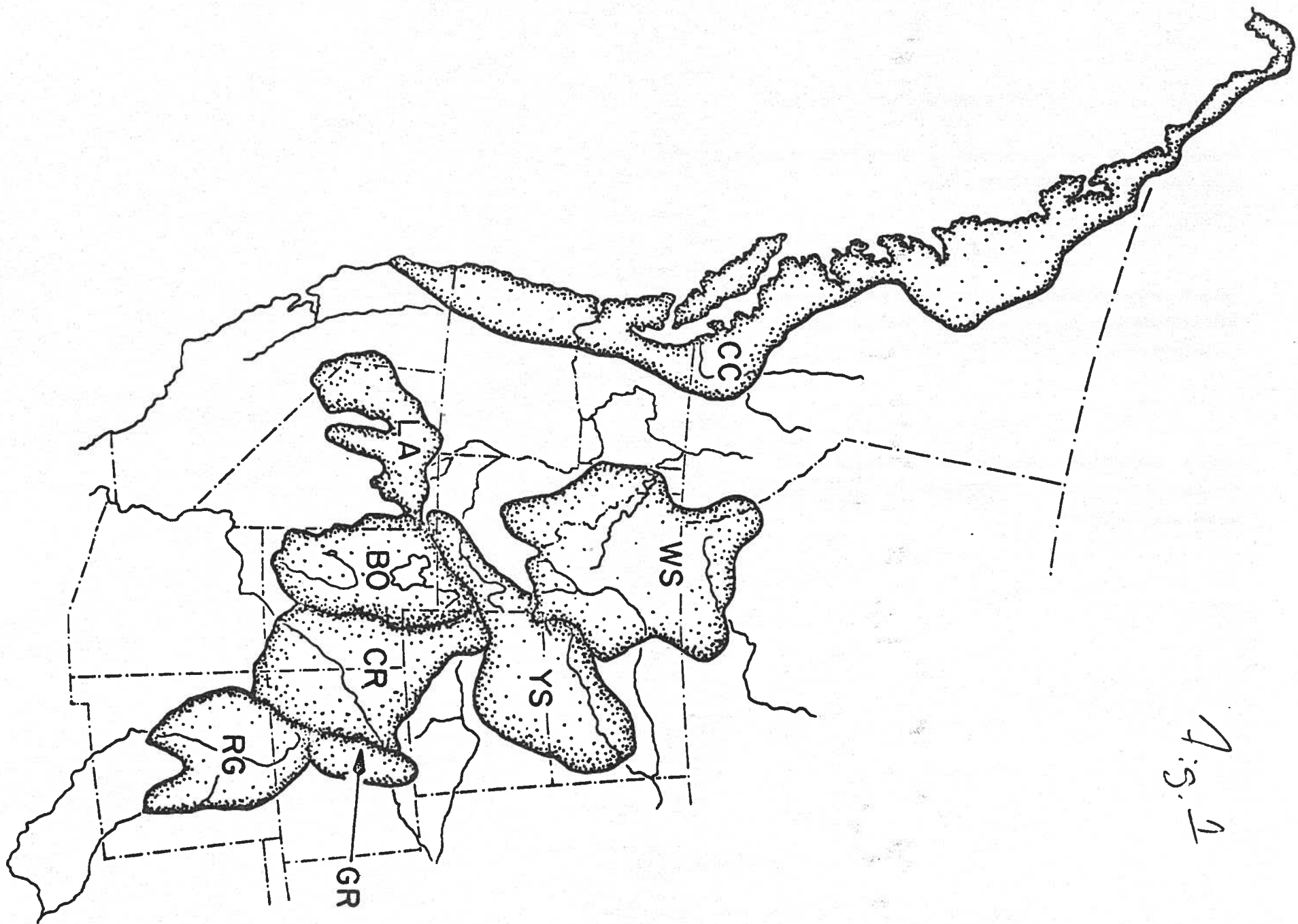
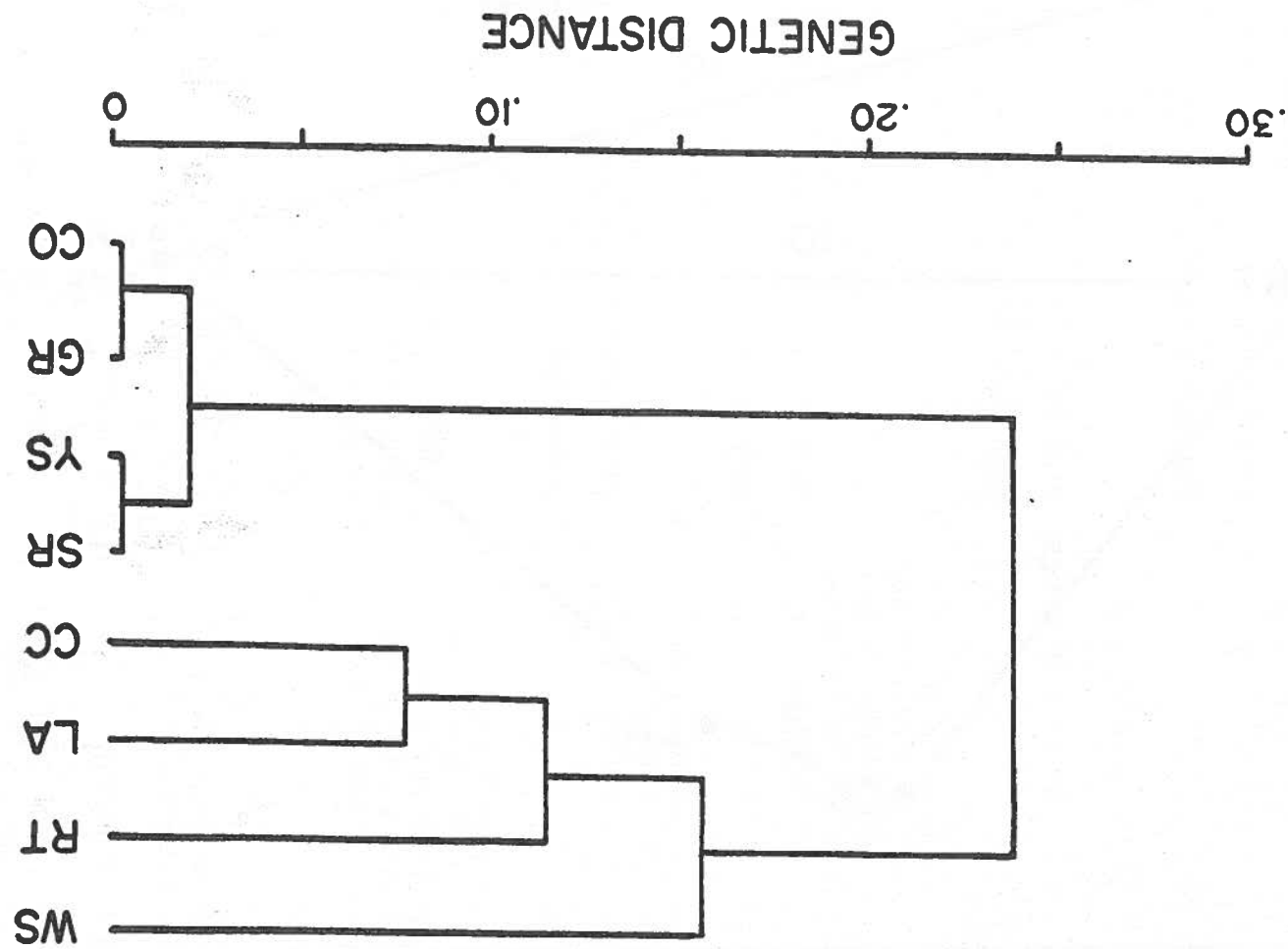


Fig. 1

Fig. 2



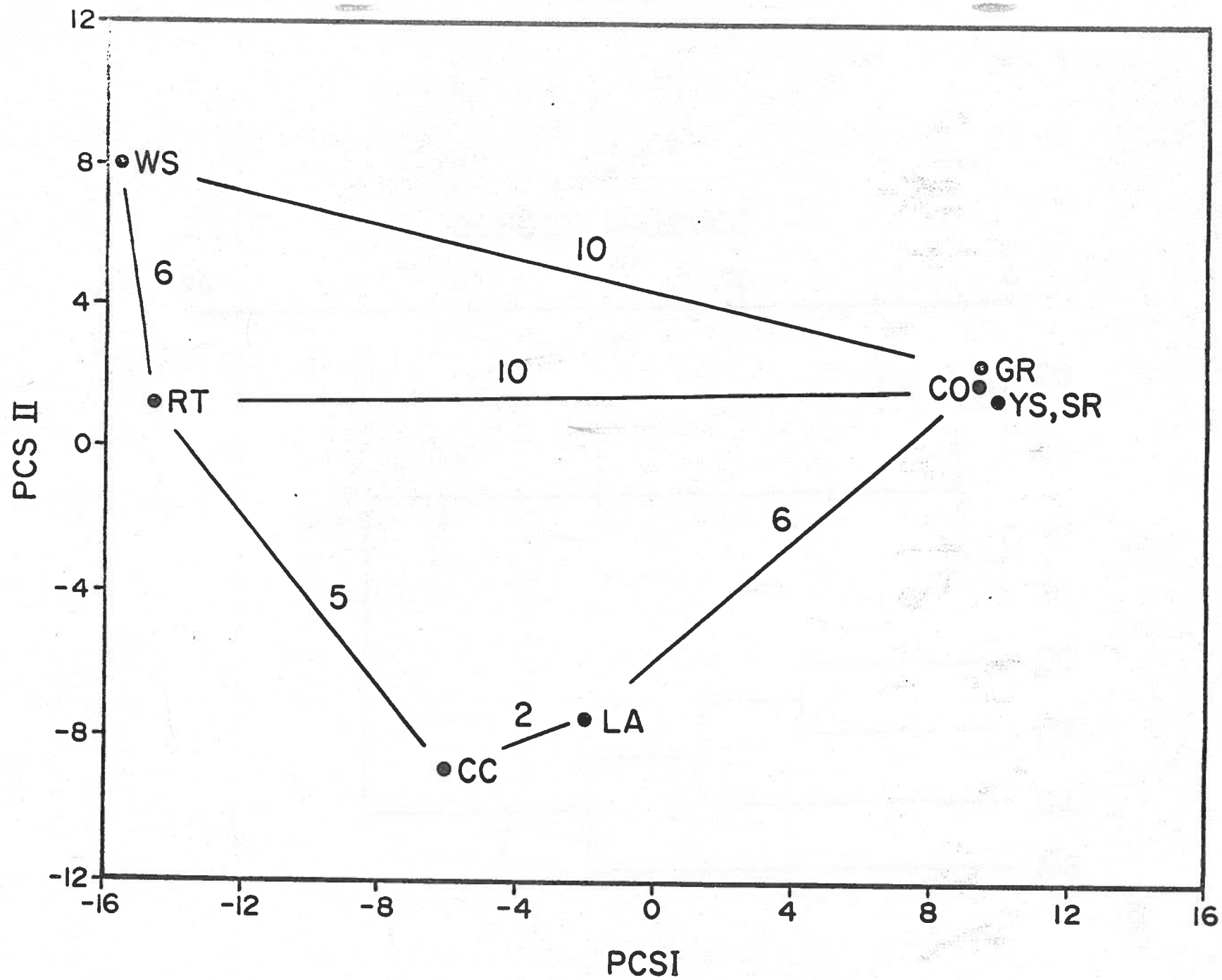


Fig. 3