Conserving Genetic Diversity in Rainbow Trout

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ABSTRACT

Conserving genetic diversity is a major concern for north temperate fauna which are species poor but rich in genetic diversity. The problem is believed to be especially serious for freshwater fish where populations are isolated by barriers to migration. Isolation and low species diversity have been hypothesized to be the key ingredients in an adaptive radiation that has taken place in British Columbia fish since the last glaciation. Our work documents this variation and produces a within-species taxonomy of rainbow trout as a template for other species. Our work also incorporates both molecular data (presumably selectively neutral) as well as morphological and life history data (presumably adaptive). Molecular data are being used to identify ancient subdivisions within the species and patterns of gene flow among interconnected populations. Morphological and life history data will be used to group isolated populations into common adaptive ecotypes. The output of this analysis will be linked to an electronic atlas of British Columbia and will be used to predict the locations and characteristics of rare and threatened genotypes.

Key words: genetic diversity, genotype, population structure, rainbow trout, taxonomy.

In species-rich situations, conservation efforts have typically focused on species or communities of species as the elements that need to be conserved. However, many species vary substantially among populations, in addition to species or subspecies with Linnean nomenclature. The problem is particularly acute in freshwater fish where barriers to movement can subdivide species into hundreds or thousands of populations, commonly called stocks in the fisheries literature (Larkin 1972). These stocks range from semi-isolated populations with independent population dynamics, but little genetic differentiation, to completely isolated populations that are uniquely adapted to local environmental conditions (Taylor 1991). In north temperate areas such as British Columbia, the problem is particularly acute because barriers to movement have combined with climatic and geological diversity to produce ideal conditions for speciation among the relatively few fish species that invaded these areas following the last glaciation. Many examples exist of local adaptations that verge on species-level differences which are confined to a single watershed or habitat type (Taylor 1991). These subdivisions do not fit the traditional concepts of subspecies in terms of the number of different phenotypes represented and the lack of phylogenetic and geographic contiguity for populations with similar phenotypes.

This issue is acknowledged in the U.S. Endangered Species Act which recognizes the status of both subspecies and "distinct population units." Consequently, the concept of an Evolutionarily Significant Unit (ESU) was developed to describe "distinct population units" in Pacific salmon (Waples 1995). The practical problem in conservation is to determine the number of ESUs and then to assess their geographic location, distinctiveness, rarity, and risk of extinction. Conservation efforts can then be directed to sites where distinctive, rare ecotypes are at high risk of extinction.

Several approaches to defining ESUs are available. Individual populations can be assessed to determine if they meet the criteria in the original definition (Waples 1995). Usually this approach is applied to populations that are perceived to be of adaptive significance and appear to be endangered. However, unique populations without obvious distinguishing characteristics can easily be overlooked. Another approach is to divide the entire species into contiguous geographic blocks based on molecular genetic affiliations and general life history characteristics. This approach is based on the assumption that these blocks represent

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monophyletic groups with little internal genetic variation. A third approach, and the one we propose to pursue, involves using molecular genetics to define geographically contiguous groups that are presumably monophyletic. Within these monophyletic groups, however, each population is assigned to a particular ESU based on a suite of habitat and phenotypic characteristics in a system similar to that proposed by Bernatchez (1995). Our knowledge will be imperfect and we will make errors in classifying individual populations. However, the status of each population will be systematically assessed against the diversity found in the entire species to produce the best possible picture using currently available data. Information concerning the reproductive isolation of populations is important in this approach, as well as the others, because reproductive isolation is a basic criteria for defining an ESU.

Our assessment of the diversity within species consists of 3 basic tasks: 1) defining the smallest possible groups of monophyletic populations; 2) developing rules to define the population units within the monophyletic groups in terms of their reproductive isolation; and 3) classifying isolated (or semi-isolated) populations within monophyletic groups based on presumed adaptive differentiation to assess the biological uniqueness of each population. This methodology is being applied to define the structure of rainbow trout as a prototype for other species. Rainbow trout are clearly polytypic and an ample body of ecological knowledge as well as data on molecular and phenotypic variation exists. In addition, all legal fish introductions are recorded in a well-organized database, and, for large areas of British Columbia, there have been few official introductions and little possibility of illegal introduction.

The output of the within-species taxonomy will be tied to the British Columbia Watershed Atlas (http://www.elp.gov.bc.ca/fsh/ids/gis/). The Watershed Atlas is a geographic information system (GIS) at 1:50,000 scale which has stream reaches and lakes as its basic elements (macroreaches). Stock characteristics will be assigned to >1 million macroreaches. Reaches that share a common stock will share a common stock number; those that share a common phylogeny will share a common phylogenetic code; and those that share a common ecotype will share a common ecotype code. Preliminary estimates suggest that there are thousands of stocks, <10 phylogenetic groups, and <100 ecotypes. The output can be displayed in a database or in a map. Revisions in the system will result in new GIS layers for the Watershed Atlas with older classification systems retained for comparison.

DEFINING MONOPHYLETIC GROUPS WITHIN SPECIES

A monophyletic group of populations shares a common ancestral population. A species can be envisioned as a hierarchy of monophyletic groups where upper levels in the hierarchy represent older subdivisions within the species. Monophyletic groups can be recognized by the similarity of molecular markers among isolated, but geographically contiguous populations. This technique has a well-established theoretical basis (e.g., Bernatchez 1995), but the number of phylogenetic groups, their boundaries, and the number of hierarchical levels within these phylogenies has not been established for any fish species in British Columbia.

In British Columbia our main interest is in identifying phylogenetic groups that invaded the province from different refugia following glaciation. This type of split is evident in British Columbia fish species. Rainbow trout are clearly distinguished into an interior and a coastal form by isozyme frequency differences (Parkinson 1984). In addition, some evidence indicates a north–south split on the coast. Other species (longnose sucker, longnose dace) also appear to have coastal and interior forms suggesting that differentiation in glacial refugia is a general phenomenon that forms an important component of the diversity within many British Columbia fish species.

From our understanding of the number of glacial refugia and invasion routes following glaciation, the number of groups originating from distinct glacial refugia must be <10. The key indicators of phylogenetic grouping are molecular genetic characteristics that are uniform within, but differ among, large geographic areas. As a result, a few samples covering a large geographic area can outline the phylogenetic structure. Additional samples taken from boundary areas provide information on the location and sharpness of boundaries. Even with this simplification, a substantial effort is required to adequately document the structure of each species. In extending this concept to other species, an expedient approach is to use whatever information is available until more definitive genetic data are collected. Useful data include phylogenetic boundaries of other species, barriers to fish dispersal, biogeographic patterns in fish communities (which indicate ancient dispersal barriers), glacial history, and morphology data.

Although this methodology is well established, there are obvious difficulties attempting to quantify genetic variation by sampling every population of a species in an area the size of British Columbia. Usually, the pattern of variation must be inferred from incomplete information. Our current understanding of the phylogenetic structure in rainbow trout is outlined in Fig. 1.

DEFINING POPULATION STRUCTURE WITHIN PHYLOGENETIC GROUPS

Populations rarely exist in isolation (i.e., total lack of gene flow from each other). Many fish, and salmonids in particular, are known for their long distance migrations and occasional straying. Even populations totally isolated above impassable barriers have a temporal element; the population may not have been isolated long enough for genetic differentiation to occur. For cultured species such as rainbow trout, an introduction in a single lake may introduce foreign genes into a variety of populations within an interconnected watershed. An accurate picture of the population structure in a species such as rainbow trout must reflect the variation in genetic exchange rates among proposed populations.

In theory, 3 regimes can be envisioned. At very high rates of gene flow, even very strong selective forces do not induce genetic differentiation. At intermediate rates, lower rates enable increasingly weak selective forces to induce differentiation. At very low rates, neutral characters diverge over time because no selection is needed to induce differentiation. Molecular genetic data can only capture the lower end of this continuum. Evidence of population structuring in less isolated populations will be forced to rely on other evidence such as movement patterns or phenotypic differences.

Population structuring is probably related to the geographic structure of the environment. Some populations are isolated by physical barriers. Populations that are farther apart are more isolated. Migration and dispersal patterns differ among populations in different environments. If strays are more successful in similar habitats, then populations in dissimilar habitats should experience lower rates of gene exchange. Consequently, the genetic structure of the species will be related to the geographic structure of the habitat that it occupies.

Linkages between populations occupying interconnected water bodies can be assessed using data on molecular variation combined with data on the patterns of fish movement. Because a species can consist of thousands of populations, patterns of movement and genetic variation can be observed only in representative situations. However, if habitat data related to barriers to dispersal are available, then the likelihood

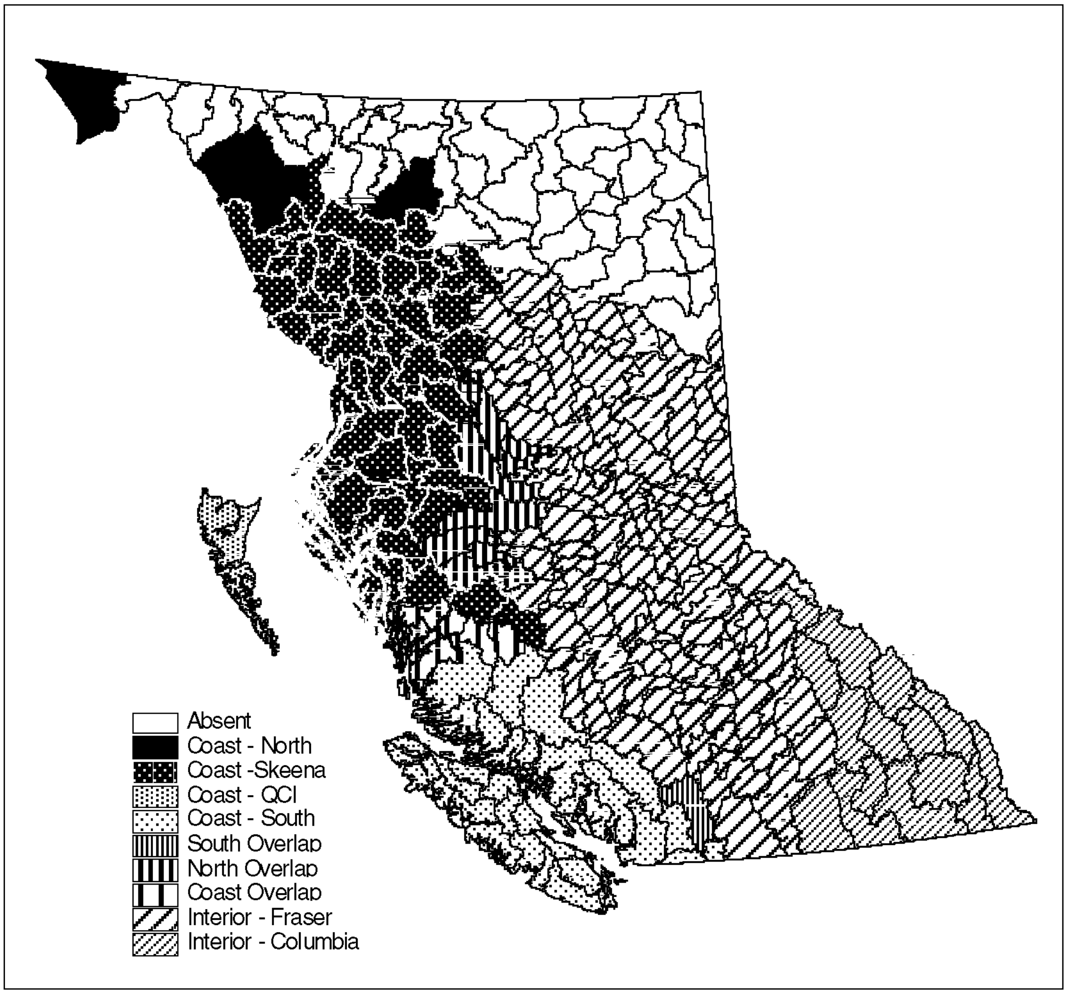

Figure 1. Current picture of rainbow/steelhead trout phylogenetic structure as inferred from molecular genetic data.

of genetic isolation can be inferred from habitat data alone. For example, molecular data from populations upstream and downstream of barriers suggest that little gene flow occurs downstream over barriers. Molecular data from steelhead suggest that there is little gene flow among adjacent watersheds. Quantitative genetic studies suggest that populations in inlet and outlet streams of lakes are often differentiated and that big lakes often contain more than 1 population of rainbow trout. Limited data suggest that there is little movement between nearby lakes but that some ecotypes associated with large lake/stream systems can have extensive migrations. These rules can be combined with the rules for species distributions to show the distribution and genetic structure of a species by map-based habitat characteristics. These data will be stored as a table in an ACCESS database which can be displayed as a layer on a GIS.

Steelhead on Vancouver Island provide a simple example of how these rules can be applied. Isozyme data (Parkinson 1984) suggest that steelhead stocks in adjacent streams are genetically distinct. In addition, ecological data (Hartman and Gill 1968) suggest that steelhead do not occupy very small watersheds. A simple rule that defines an independent population for every stream above magnitude 7 that enters the ocean directly produces a map of probable locations of all steelhead stocks on Vancouver Island (Fig. 2)

DEFINING COMMON ADAPTIVE PHENOTYPES (ECOTYPES)

Attempts to objectively characterize diversity or uniqueness of populations within species usually involve molecular genetic studies using polymorphic allozymes (e.g., Allendorf and Leary 1988, Leary et al. 1993); mitochondrial DNA (e.g., Perkins et al. 1993); or micro-satellite loci (Angers et al. 1995). Most molecular traits seem to be selectively neutral and are therefore ideal for defining monophyletic groups and populations that result from current or past geographic isolation. However, evolutionary significance depends strongly on adaptive traits and therefore, for conservation, the populations that share common adaptive traits must be identified. An important corollary is that successful dispersal or reintroduction to specific habitats is much more likely for populations that share common adaptive traits. Because molecular variation is not strongly coupled to the adaptive differentiation (e.g., Clayton 1981), the pattern of molecular variation is a poor tool for mapping patterns of adaptive differentiation. Others have recognized the importance of evaluating both molecular and adaptive variation in assessing differentiation among populations (MacLean and Evans 1981, Utter 1981, Waples 1991).

Unfortunately, most adaptive traits have an environmental component with inheritance controlled quantitatively by multiple genes. Many populations cannot be surveyed genetically because laborious breeding experiments are required to measure the genetic component of variation in each trait in each population. A convenient alternative is to simply use raw phenotypic variation as a surrogate for underlying genetic variation. Adaptive phenotypic traits, such as body shape or migration timing, are obvious candidates, especially if a genetic component is in the variation. However, if the goal is to classify all populations of a wide-ranging species, this simplification may not be enough. Data on habitats and biological communities, especially for characteristics (e.g., biogeoclimatic zone, stream gradient) that can be estimated remotely, offer more surrogate information for classifying populations into adaptive groups.

This approach is difficult to defend and will lead to a classification system that is prone to error. Variation in morphology, ecology, behaviour, or life history strategies typically have both genetic and environmental components (Kornfield et al. 1982, Vrijenhoek et al. 1987, Carvahlo 1993). Variation among populations in some characteristics (e.g., growth, size at maturity) are obviously dominated by environmental effects. Most conservation strategies explicitly restrict their emphasis and priorities to the conservation of genetic diversity, and implicitly or explicitly discount diversity that results from developmental plasticity (e.g., Meffe and Vrijenhoek 1988). Habitat similarity is unlikely to map on adaptive similarity if some habitat characteristics that exert powerful selective forces are omitted. Therefore, is a dependence on habitat differences as a surrogate for genetic differences justified?

Sometimes this dependence is the case. Length of freshwater migration in Atlantic salmon is associated with a number of differences among stocks that appear to be adaptations to more rigorous migration conditions (Shaffer and Elson 1975). The longer fins and fusiform bodies in Atlantic salmon parr in colder, higher gradient streams have a genetic basis and are probably adaptations to local environmental conditions (Riddell and Leggett 1981). A stock of cutthroat trout from a stream was more surface oriented in its feeding behaviour than a lake stock of cutthroat trout, when compared in a common environment (Trojnar and Behnke 1974). More generally, most phenotypic characters seem to have a genetic component and, sometimes (e.g., spawning migration time of steelhead) different populations are in a common environment when the trait is expressed. However, for most habitat variables in most species, the link between differences in habitat and differences in adaptive characteristics has not been verified quantitatively.

The immediate need for a comprehensive classification system has led us to adopt a pragmatic approach which will produce what we believe to be the most accurate picture given our current knowledge. We expect and will encourage revisions which more accurately reflect observed patterns. Each of the 3 components will be tested for its critical assumptions and refinement of the rules. Population structure rules, for example, will be tested by comparing modeling results with actual population structure as inferred from molecular data.

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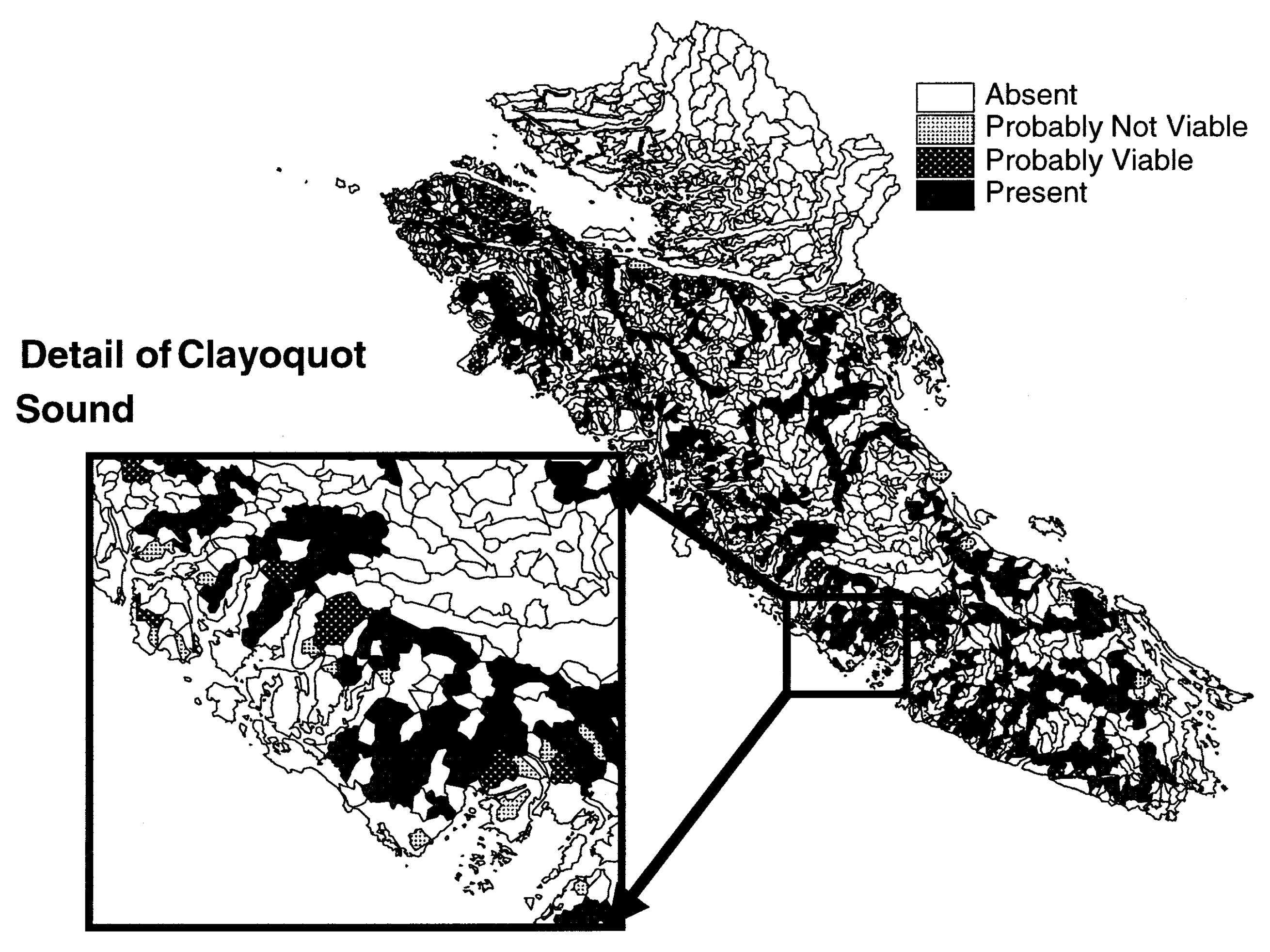

Figure 2. Model of steelhead population structure on Vancouver Island using the Watershed Atlas. The rule that generates this structure is that the stream enters the ocean directly and has a magnitude <6 (probably unviable) or >6 (probably viable) with steelhead observed (present).

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