Population Genetics of Boise Basin Bull Trout (*Salvelinus confluentus*)

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Abstract

We analyzed the population genetic structure of bull trout (*Salvelinus confluentus*) in the Boise River Basin, Idaho. We determined the influence of contemporary (including anthropogenic) and historic factors on genetic structure, taking into account existing data on bull trout habitat patches in this basin. We tested three models of the organization of genetic structure in this system, where genetic structure would: a) parallel the stream hierarchy, b) correspond to habitat patch structure, or c) follow a pattern of isolation by distance. We found strongest support for the isolation by distance model. In addition, we found weak population differentiation within the Boise system (F_{ST} = 0.064), relative to other similarly scaled systems containing bull trout. Frequent disturbance may be responsible for the strong isolation by distance yet weak overall levels of population subdivision in this system. In addition, we found that the South Fork was a genetic outlier from the remainder of the Boise system and may have been colonized at a separate time than the Middle and North Forks. At least one dam (Kirby Dam) has noticeably reduced levels of gene flow. These results show that distinct patterns of genetic structure may occur in separate portions of a species' range, especially at the range extremes. Based on these results, we suggest the South Fork should be treated separately from the remainder of the basin. Bull trout in Mores Creek should be protected but our results suggest that this population may be comprised of adults entrained by Arrowrock Dam and unable to return to natal streams to spawn and are thus spawning in the only available habitat. Connectivity should be restored whenever possible in cases where human activities have eliminated migratory corridors.

Introduction

Theory suggests that most biological systems are structured in a hierarchical manner. Populations of fishes distributed across river basins are likely no exception. We anticipate, for example, that within a typical river basin (100 km^2) individuals will occur within local populations that are part of a larger network, or metapopulation, linked by occasional dispersal and gene flow (Rieman and Dunham 2000). Understanding the distribution of genetic and phenotypic variation associated with this structure can be an important first step toward managing and conserving the biological diversity associated with native fishes. Since the advent of biochemical genetic markers, the description of genetic variation across river basins has become increasingly well understood (e.g. Allendorf and Leary 1988, Taylor et al. 1999, Spruell et al. 2003). With the development of non-lethal and more highly variable markers, more fine-scale examination has become possible. Recently, several authors have attempted to overlay the pattern of genetic variation on the physical characteristics of the environment, thereby increasing our understanding of the ecology of these organisms and helping us understand the factors that cause the observed patterns of genetic structure within individual river basins (e.g. Angers et al. 1999, Castric et al. 2001, Costello et al. 2003).

This hierarchical organization of stream networks may be an important template for the distribution of diversity for fishes within these systems. Within North America, the distribution of fishes is highly confined by recent glacial history. As basins became deglaciated, colonization by a subset of the existing species led to genetic differentiation at a broad scale (Bernatchez and Wilson 1998). Within basins, streams are necessarily hierarchical due to the branching system of tributaries and their associated watersheds. We might expect fish populations to be subdivided in a manner mirroring the structure of the basin they inhabit since gene flow among neighboring populations should be constrained by the linear nature of riverine systems (Meffe and Vrijenhoek 1988). This could lead to further subdivision at the subbasin scale corresponding to metapopulations, or groups of interacting populations. Finally, within populations, there may be structure related to differences in life-histories associated with the nature of migration (Waples 1995), or trophic specialization and the use of distinctive habitats (Skulason and Smith 1995, Robinson and Schluter 2000).

Numerous studies have examined the role that geological history may play in the distribution and among-basin relationships of fish species. These have focused on defining groups of populations that share a common ancestry based on range expansions after glaciation. A general goal of this work has been to define "Distinct Population Segments (DPS's) or "Evolutionarily Significant Units (ESU's)" at a broad scale across the landscape (Waples 1991, Moritz 1994, Waples 1995, Crandall et al. 2000).

Within a single basin, many factors, both historic and contemporary, may alter the distribution of genetic and phenotypic variation (Costello et al. 2003). Generalizing findings from a single system to others in the range of a species, however, will be problematic until we gain a better understanding of the primary forces structuring populations in the systems of interest. For example, Castric et al. (2001) found that altitude (as a surrogate for physical isolation) was highly correlated with genetic divergence among brook trout (*Salvelinus fontinalis*) populations in one stream but isolation-by-distance was responsible for shaping population differentiation in

another. Costello et al. (2003) found evidence of both contemporary (e.g. barriers) and historical (e.g. post glacial retreat) effects in the distribution of genetic variation in bull trout in two systems in British Columbia. The systems described by Costello et al. (2003) were recolonized relatively late following the most recent continental glaciation and it is not clear whether they are representative for bull trout populations of critical conservation interest in more southerly portions of the range.

Management of many remnant populations often recognizes the hierarchical nature of aquatic systems. Conservation actions are generally prioritized among individual sites within a larger network of streams. Collections of relatively small watersheds (10^3-10^4 ha) nested within larger river basins (10^5-10^6 ha) represent primary management units, for example within the interior Columbia River Basin in the United States (Quigley and Arbelbide 1997). Several species of interior salmonids seem to be organized as groups of local populations within a larger networks of populations or metapopulations at these approximate scales (Dunham and Rieman 1999, Spruell et al. 1999, Rieman and Dunham 2000).

We sought to understand the effects of genetic drift and gene flow on the distribution of genetic variation in bull trout at scales relevant for local conservation and management. We focused on bull trout in the Boise River basin with the hopes of clarifying genetic variation in a system where structure of available habitats is relatively well understood (e.g. Rieman and McIntyre 1995, Dunham and Rieman 1999).

Earlier work has documented high differentiation across even limited geographic scales for bull trout (Spruell et al. 1999, Neraas and Spruell 2001, Costello et al. 2003). Most of the fine-scale work on the genetic structure of bull trout populations, however, has focused on populations near central portions of the current species' range. The Pend Oreille/Clark Fork system (Spruell et al. 1999, Neraas and Spruell 2001) and the Canadian systems studied by Costello et al. (2003), for example, were strongly influenced by continental glaciation. The Pend Oreille/Clark Fork system is likely close to a glacial refugium, whereas the systems considered by Costello et al. (2003) are much further north and were subsequently recolonized from a more distant source.

The Boise Basin represents one of the largest networks of interconnected bull trout habitats on the extreme southern limits of the species' range. Because of its southern location, habitats for bull trout were influenced only by alpine glaciation where glacial retreat and subsequent colonization of currently occupied headwater habitats probably occurred earlier than in the more northern populations. The large-scale genetic associations for this region (Spruell et al. 2003) suggests that colonization probably occurred from a refuge associated with the nexus of the Boise, Snake, and Malheur Rivers. The extreme southern location of the Boise system also means that habitats are likely to be more strongly structured by thermal constraints on the distribution of fishes producing a discontinuity of suitable habitats that are more patchy or naturally fragmented than those to the north (Rieman and McIntyre 1995, Dunham and Rieman 1999, Dunham et al. 2001). In addition, the Boise system has been influenced by the construction of four dams between approximately 50 and 100 years ago, which have created impassable barriers to upstream movements of bull trout. The Boise system thus represents an opportunity to examine both the contemporary and historical effects of gene flow on the genetic structure of bull trout that provides both a useful comparison and contrast to earlier work.

Structure of Genetic Variation
From current theory, we hypothesized three generalized distributions of genetic variation that might be expected for stream-dwelling fishes (Figure 1).

First, we considered the alternative in which the genetic structure is defined by the physical template of the stream system (Figure 1a). In this case, all spawning aggregates within any major branch of the stream should be more genetically similar to all other populations within that catchment than to any population outside of the basin.

Several authors have suggested that the genetic population structure of fishes should mirror the physical template of the watersheds in which they live (Meffe and Vrijenhoek 1988). Salmonids may be particularly prone to such a structure based on their tendency to home to natal tributaries to spawn. Within any basin, the confluence of two tributaries represents a decision node at which a migrating fish must choose the "correct" path to return to its natal stream. This migration pattern could produce population genetic structure corresponding to the branching stream system as each "correct" decision increases the similarity of all individuals within a subbasin. In Figure 1a for example, once an individual has moved into subbasin S, they are more likely to spawn in sites S1, S2, or S3 than in any site within subbasins M or N.

Second, we considered the alternative where genetic population structure would correspond to the ecologically defined "patches" of Rieman and Dunham (1999), in which case discontinuities in suitable habitats would serve as barriers to gene flow and thereby define reproductively isolated units. We assumed that under this scenario genetic variation would parallel the patch-based structure for bull trout in the Boise River basin predicted by Dunham and Rieman (1999). If the habitat patch geometry useful for predicting the occurrence of bull trout is the basis for population structure in this system we can make two predictions. First, genetic similarities should show a discontinuity at the same scale as the predicted discontinuities in habitat. Second, gene flow should be much stronger within than among patches. This scenario is illustrated in Figure 1b in which sites within patch 1 are more genetically similar to each other than to sites within patch 2.

Finally, we considered the genetic structure that might emerge from the drift-gene flow interactions characterized by Hutchison & Templeton (1999), and first tested for bull trout by Costello et al. (2003). Hutchison & Templeton (1999) propose four models of isolation by distance in which genetic similarity among pairs of populations varies with geographic distance depending on the progress toward drift-gene flow equilibrium. In the case where equilibrium has been established and dispersal follows a "stepping stone" process (Kimura 1953, Kimura and Weiss 1964), genetic distance should increase monotonically with increasing geographic distances separating the individual populations. In addition, the degree of scatter in the regression of genetic distance on geographic distance should increase with geographic distance, reflecting the relative strength of drift relative to gene flow (Hutchison and Templeton 1999). In general, the prediction would be that drift should become more important as populations are further isolated from each other by distance. Hutchison and Templeton (1999) also define three non-equilibrium "cases" that may be expected as populations progress from genetic homogeneity following founding.

Dunham and Rieman (1999) showed that the probability of occurrence for bull trout in any single patch of suitable habitat in the Boise system was strongly associated with the distance to the nearest occupied patch, implying that dispersal among nearby patches may be an important process in the maintenance of these populations. Thus, we expect to find a pattern of isolation by distance in this system that would be consistent with more frequent demographic support among adjacent populations. As illustrated in Figure 1c genetic differentiation in this model does not necessarily correspond to the physical structure of the stream. Rather, gene flow is determined strictly by the stream distance separating populations such that the downstream samples (N1, M1, and S1) are more similar to each other than to upstream samples from the corresponding subbasin. We can also compare patterns of isolation by distance in the Boise system to the models of Hutchison and Templeton (1999) to investigate the interaction between gene flow and genetic drift.

Anthropogenic alteration

Human caused barriers may also disrupt the patterns of gene flow, thereby confounding or altering the genetic structure of the basin that would ordinarily emerge under natural conditions. Development of the Boise River Basin has included mining, road construction, logging, and perhaps most importantly for our interests, the construction of four dams that are impassible to the upstream migration of bull trout. We hypothesized that if recent barriers had disrupted important patterns of gene flow, populations isolated by those barriers would diverge significantly from the structure implied for the other populations as a whole.

Given these hypotheses, the primary objectives of this work were twofold. The first was to describe the hierarchical structure of genetic variation and use this description to consider the underlying processes leading to that structure (e.g. basin structure, colonization, and subsequent progress toward a gene flow-drift equilibrium) among bull trout populations within the Boise River Basin. Our second goal was to investigate the effects of recent dams on the amount of genetic variation within populations and the genetic divergence among populations. We also consider potential conservation management units for the Boise River Basin network of bull trout populations.

Methods

Sampling

We used the predicted habitat patches from Dunham and Rieman (1999) to identify the stream networks that might define local populations across the basin (Figure 2). We collected tissue samples from small (<150 mm) bull trout distributed among a subset of patches in each of the subbasins of the Boise from the existing archives (samples collected from 1993-1998) and from additional sampling conducted by electrofishing in 1999 and 2000. We restricted the sample to small fish to insure that the sample represented individuals in their natal stream. Care was taken to minimize the occurrence of siblings or the representation of single cohorts in the sample. In general the samples were distributed across at least three age classes and multiple sites distributed throughout the streams of interest. In some cases samples were pooled from collections made across several years. Most samples were pooled from sites throughout a patch,

but additional sampling was conducted to represent bull trout associated with distinct streams within several patches. The final sample represented 21 samples from 20 streams (Table 1) and 15 habitat patches distributed across the basin (Table 2, Figure 2). Several samples were pooled due to small sample size. The Yuba sample (total N=30) contained individuals collected from the Yuba River (N=10), Grouse Creek (N=4), Kirby Creek (N=7), Sawmill Creek (N=4), and Decker Creek (N=5). All of these samples were within eight kilometers. The Bear sample (N=31) contained individuals collected in the Bear River (N=17) and Bear Creek (N=14). Finally, the Skeleton sample contained a sample taken from the East (N=24) and West Fork (N=12) of Skeleton Creek.

Microsatellites

All methods are described in Spruell et al. (1999) and Neraas and Spruell (2001). Briefly, DNA was extracted from each fin clip by standard methods. PCR amplification of each of the eight microsatellite loci was performed in an MJ thermal cycler. The nine loci used: *SSA311*, *SSA456*, *OTS101*, *FGT3*, *SCO19*, *OGO2*, *BT73*, *SFO18* and *ONEμ7* are described in Spruell et al. (1999) and Neraas and Spruell (2001). Fluorescently labeled PCR products were visualized on acrylamide gels. Individual fish of known genotypes from other bull trout populations were used as standards for scoring.

Data Analysis

Allele frequencies, deviations from Hardy-Weinberg expectations, genotypic linkage disequilibrium, observed and expected heterozygosities, pairwise exact tests for genic differentiation, *F*-statistics and pairwise F_{ST}'s were calculated using GENEPOP ver. 3.2c (Raymond and Rousset 1995) and FSTAT ver. 1.2 (Goudet et al. 1996). We adjusted the results from the pairwise exact tests for genic differentiation, which tests the null hypothesis that allele frequency distributions are the same between populations, for multiple tests using the sequential Bonferroni procedure as described in Rice (1989). We used PHYLIP (Felsenstein 1992) to calculate Cavalli-Sforza and Edwards' (1967) genetic distance (CSE) with the GENDIST module and to construct a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram using the NEIGHBOR module.

Tests of Genetic Structure

We tested different hierarchical arrangements of population samples using analysis of molecular variance (AMOVA, Excoffier et al. 1992) performed with ARLEQUIN ver. 2.001. (Schnieder et al. 2000). We performed this test using F_{ST} because R_{ST} estimates may be biased for recently diverged populations (Gaggiotti et al. 1999). We tested three geographical arrangements of populations (Table 2). For our first arrangement we pooled samples into three groups each corresponding to the three forks of the Boise River: South, Middle, and North. The second arrangement consisted of two groups: the first group contained all the samples from the South Fork and the second arrangement had all the remaining samples from the North and Middle Forks. The third arrangement had 15 groups where groups consisted of the *a priori* defined patches.

Isolation by Distance and Mantel tests

We used Mantel tests to test for isolation by distance for both CSE and pairwise F_{ST} using the program Isolation By Distance (IBD, Bohonak 2003). We estimated the distance among all

possible pairs of streams in the sample using a geographic information system (GIS). The distance between any two patches was calculated as the distance along the stream network between the mouths or lower boundaries of the patch as defined by Dunham and Rieman (1999). The distances between samples within patches was defined as that between the lower bounds of the stream reaches where the sampling occurred.

If populations are in drift/gene flow equilibrium, degree of scatter should increase with geographic distance due to the increasing affect of drift (Hutchison and Templeton 1999). To determine the significance of the relationship between the degree of scatter and geographic distance, we obtained residuals from a standard regression of either CSE or pairwise F_{ST} on geographic distance and performed a second Mantel Test using the absolute value of these residuals (Hutchison and Templeton 1999, Costello et al. 2003).

Patch Area versus Genetic Variation

The area of the polygon defining the watershed boundaries of the stream network encompassed in the patch was used as a measure of patch size (Table 2), which we estimated directly as outlined in Dunham and Rieman (1999). We regressed genetic variation (H_E and total number of alleles) on patch area. For patches with more than one sample we averaged both H_E and total number of alleles.

Results

Within Population Analyses

We analyzed 21 population samples and a total of 677 bull trout (Table 1). Sample sizes ranged from 16 to 51. Heterozygosities range from 0.019 to 0.335. The low value of 0.019 was from Emma Creek. The highest heterozygosity was observed in Mores Creek (H_E = 0.335). We found the fewest alleles in Emma Creek and the most in the Yuba River sample.

Our final analysis was performed with five polymorphic microsatellite loci. Three of the loci used (*OGO2, SSA456,* and *SFO18*) were monomorphic in all samples. One locus, *SCO19,* amplified inconsistently due to problems with DNA yield. These problems were due to the use of denatured ethanol containing ketones for preservation of fin clips. Inconsistent amplification substantially reduced sample sizes for *SCO19,* but did not reduce sample sizes substantially for the remaining five variable loci (Appendix 1). We did not include *SCO19* in our final analysis (e.g. estimates of *F*-statistics, isolation by distance) but included the raw allele frequency data from this locus along with the other five variable loci in Appendix 1.

Bull trout will hybridize with brook trout (*Salvelinus fontinalis*) and brook trout do occur in the Boise River Basin. Five individuals (one in the Crooked River sample and four in the Bear River sample) contained microsatellite alleles indicative of brook trout (P. Spruell, unpublished data). We confirmed that these individuals were hybrids using Paired Interspersed Nuclear Element (PINE) PCR (Spruell et al. 2001). These five fish appeared to be first generation hybrids and were excluded from subsequent analysis.

Four of 63 exact tests for deviation from Hardy-Weinberg proportions were statistically significant ($P < 0.05$). Three significant tests were expected by chance alone. There was no pattern either for certain loci or for certain populations to yield significant p-values. Only one test was significant when multiple comparisons were taken into account (Lodgepole Creek at *ONEμ7*; P = 0.002). F_{IS} was –0.550 at this locus, indicating an excess of heterozygotes. The exact test for Skeleton Creek at *FGT3* had a p-value of 0.033. This result was not significant when the sequential Bonferroni method was used and was likely due to the fact that the two copies of the *FGT3*175* allele found in this population occurred in a single homozygous individual.

Two samples appeared to contain mostly progeny from a limited number of spawning adults. The Emma Creek sample contained individuals from three stream reaches. One of these reaches (N = 13) had an extreme heterozygote excess at two loci (*SSA311* and *ONEμ7*) while the other three loci were monomorphic. At *ONEμ7* this portion of the Emma Creek sample deviated from Hardy-Weinberg proportions significantly (P = 0.024) and F_{IS} = -0.625. At *SSA311* this partial sample did not deviate significantly from Hardy-Weinberg proportions (P = 0.056) and F_{IS} = -0.529. All of the fish except one from this section were from one size (age) class. Big Silver Creek (upper North Fork, not included in Figure 2) also appeared to contain closely related individuals. This sample only had one variable locus, *SSA311*, and it significantly deviated from HW proportions (P = 0.046, F_{IS} = -0.385). The size range of all individuals was 80-110mm, suggesting that they came from the same age class. Multiple individuals were collected from relatively small areas in Emma Creek and Big Silver Creek; it is possible that multiple siblings were analyzed from these samples.

We did not find evidence that we pooled genetically divergent population samples from the Bear (Creek and River) sample and the Skeleton Creek (East and West Fork) sample. No significant heterozygote deficit was detected in either case. The Yuba River sample deviated significantly from Hardy-Weinberg expectations at *SSA311* (P = 0.025; one-tailed test for heterozygote deficit) and F_{IS} = 0.436 at this locus, indicating a deficit of heterozygotes. *OTS101* also had a positive F_{IS} value (0.360) in this sample, but the one-tailed p-value for the exact test for Hardy-Weinberg proportions was not significant (0.165). The other three loci had negative F_{IS} (heterozygote excess) values for this sample. One site within the Yuba River sample (Grouse Creek) did have a disproportionately high occurrence of the *SSA311*112* allele. When those five fish were removed from the analysis, the sample conformed to Hardy-Weinberg proportions and allele frequencies changed only slightly (data not shown). The result from *SSA311* in the Yuba River sample does suggest that some population subdivision may be present upstream from Kirby Dam but the affect on our analysis should be slight because removing the Grouse Creek sample had such a small effect on allele frequencies.

No consistent patterns of linkage disequilibrium were observed either among any particular loci or within any given sample. Of a total of 110 exact tests performed, three had p-values less than 0.05. All three involved comparisons with *ONEμ7*, but with three different loci in three different populations. Two comparisons (*ONEμ7* and *BT73* in Roaring Creek; P = 0.0019 and *ONEμ7* and *FGT3* in Boardman Creek; P = 0.0014) showed evidence of significant linkage disequilibrium after sequential Bonferroni correction (Rice 1989). Since there is not an obvious pattern for these three significant results and we expect to see at least this many significant

results by chance alone, we conclude that there is no significant evidence for non-random association of alleles in this study.

Divergence Among Populations

General patterns of genetic divergence were apparent from the distribution of alleles in this system (Appendix 1). *FGT3 *175* and **183* only occurred in the South Fork. In addition, *OTS *100* and *BT73 *138* only occurred in the Middle Fork, the North Fork, and Mores Creek. Several alleles were only found in the Middle Fork, the North Fork, and one or two South Fork samples. *SCO19 *200* occurred throughout the North and Middle Forks and in Rattlesnake Creek and Big Smoky Creek in the South Fork. *BT73 *140* occurred in Rattlesnake Creek and Skeleton Creek in the South Fork and the Roaring River and Yuba Creek in the Middle Fork. *FGT3 *163* occurred in Rattlesnake Creek and Skeleton Creek in the South Fork and throughout the Middle and North Forks. Within the upper South Fork there was evidence of further population structure. The *FGT3 *183* allele only occurred in the main stem of Big Smoky Creek where it was at moderate frequency (Appendix 1). This allele did not occur elsewhere in the Boise River Basin and did not occur in the West Fork of Big Smoky Creek.

Figure 2 shows a UPGMA dendrogram based on Cavalli Sforza-Edwards' (1967) genetic distance (CSE; Table 3). Results from pairwise exact tests for genic differentiation are shown above the diagonal in Table 3. Most of the upper South Fork samples (15, 16, 18, 19, 20, and 21) formed a separate group from the lower South Fork, Middle Fork, and North Fork samples. The three lower South Fork samples (12,13, and 14; Figure 3) clustered with the Middle and North Fork samples. The Middle Fork samples did not form a consistent group, but rather clustered with the North Fork and lower South Fork samples. The Mores, Yuba, and Emma samples were the most genetically divergent (Table 3; Figure 3).

The level of genetic differentiation among samples differed. Samples from the upper South Fork (15-21) were more highly differentiated (F_{ST} = 0.086) than samples collected from the upper North Fork (7-11; F_{ST} = 0.001) despite the fact that a similar geographic area is represented by both groups of samples (Figure 1). This result is also apparent when the number of loci differentiating populations and the pairwise CSE values within basins are compared. The average CSE among South Fork samples is 0.067, as compared to 0.037 for comparisons among North Fork samples (Table 3). This is reflected in the resulting dendrogram that clusters samples 7-11 at much shorter branch lengths than those observed for South Fork samples (15-21; Figure 3).

Table 4 shows the AMOVA results from pooling samples into different hierarchical arrangements. The best-supported genetic structure is expected to maximize the amount of variation found among groups and to minimize the amount of variation found among samples within groups. The arrangement with two groups (the Middle and North Forks as one group and the South Fork as the other) gave the most highly significant among group variance component ($P < 0.0001$).

Isolation by distance

Tests for isolation by distance in the entire Boise River Basin showed a highly significant association between genetic and geographic distance for both Cavalli-Sforza and Edwards'

(1967) genetic distance (r = .46, P < 0.001; Figure 4a) and pairwise F_{ST} (r = 0.17, P = 0.004; data not shown). When above barrier populations were excluded results were still highly significant for CSE (r = 0.51, P < 0.001; Figure 4b) and F_{ST} (r = 0.22, P = 0.004). For the Middle and North Forks considered separately we also found a significant relationship between genetic and geographic distance for CSE (r = 0.58, P = 0.005; Figure 4c) and F_{ST} (r = 0.47, P = 0.006). When we removed the above barrier Yuba sample, results were still significant for CSE (r = 0.52, P = 0.006) and F_{ST} (r = 0.37, P = 0.046). We did not find evidence for an association between genetic and geographic distance in the South Fork for CSE or F_{ST} when all samples were considered (Figure 4d). We showed comparisons with Emma Creek as shaded circles in Figure 4d because this sample appeared to be an outlier in this analysis, which, together with its reduced genetic variation (Table 1), suggested that it might be isolated. Pairwise F_{ST} values in the South Fork that contain Emma Creek showed this sample to be an outlier as well(data not shown).

Scatter, as measured by the residuals from standard regressions of CSE and pairwise F_{ST} values on geographic distance did not increase with increasing geographic distance for the basin overall or for the South Fork (P > 0.05). Scatter did increase in the Middle and North Fork comparison for CSE (P = 0.07) but not for pairwise F_{ST} values (P = 0.52).

Patch Area versus Genetic Variation

We found no significant relationship between patch area and either expected heterozygosity or total number of alleles (P > 0.05; Figure 5). There was a slight trend for larger patches to have greater levels of genetic variation, but the relationship was non-significant.

Discussion

Our results suggest that both historical processes of colonization and gene flow, constrained by geomorphology, and more contemporary anthropogenic fragmentation have had an important influence on the genetic structure of bull trout in the Boise River Basin. We found strong evidence of structuring consistent with the stepping stone models of colonization or subsequent gene flow-drift interaction suggested by others (Hutchison and Templeton 1999, Costello et al. 2003), but the patterns were not consistent across the basin or with the hierarchical organization of the river-stream network. We also found evidence that two of three dams constructed in the last 100 years had important but disparate effects on dispersal and gene flow.

Within Population Analyses

The Boise system has lower overall genetic variation than other groups of bull trout populations (summarized by Costello et al. 2003). Mean levels of both expected heterozygosity and number of alleles in the Boise system are among the lowest for any of the river basins analyzed to date (data from Spruell et al. 1999, Neraas and Spruell 2001, Costello et al. 2003, Spruell et al. 2003). This observation might be consistent with small population sizes and perhaps population bottlenecks associated with frequent disturbance. However, because bull trout in the Boise River Basin may have originated from a more southern glacial refugia than bull trout populations analyzed thus far (Spruell et al. 2003), the differences in the time of colonization and source of colonizers might also be responsible for the observed differences.

Patterns of Genetic Divergence

Our understanding of how genetic variation is distributed among populations at small spatial scales has increased immensely in the last few decades (Avise 1994). Several studies have determined how genetic variation is distributed within river basins for bull trout (Spruell et al. 1999, Neraas and Spruell 2001, Costello et al. 2003). Spruell et al. (1999) tested the alternative hypotheses that metapopulation dynamics or formerly large, but presently smaller populations that currently experience reduced gene flow were responsible for the genetic structure observed in a tributary to Lake Pend Oreille, Idaho. Neraas and Spruell (2001) focused on the effect of a dam on the genetic structure of bull trout in the Clark Fork River and Lake Pend Oreille. Costello et al. (2003) tested hypotheses regarding the roles of postglacial dispersal and current landscape features on the structuring of genetic variation of bull trout populations in British Columbia and Alberta. These three studies used all of the same, or a subset of, the microsatellites used in the present study.

We were able to use detailed ecological information about habitat characteristics combined with allele frequency data to make additional predictions about bull trout genetic structure and to draw more informed inferences about factors shaping the distribution of genetic variation. We were able the to test the predictions from the hypothesis that genetic structure corresponds to habitat patch structure. Bull trout in the Boise River system inhabit habitat patches characterized primarily by water temperature (Dunham and Rieman 1999). We predicted that gene flow would be greater within patches and reduced among patches, leading to greater genetic similarity of populations within patches and genetic differentiation of populations located in separate patches. In addition, by comparing our results to previous studies, we were also able to determine if the genetic structure of bull trout populations is similar in similarly scaled basins in different portions of this species' range.

We did not find evidence that the genetic structure in the Boise system parallels the stream hierarchy, as proposed by Hypothesis 1. Each fork did not contain populations that were genetically most similar to each other and differentiated from populations in the other two forks. Populations from the South, Middle, and North Forks all clustered together towards the bottom of Figure 3. There was some geographic clustering of populations from the upper South Fork and the upper North Fork, but overall our genetic results did not match the expectations of the stream hierarchy model.

We did find some suggestion that gene flow is greater within patches than among patches (Figure 6), however the number of within-patch comparisons was too small to draw any firm conclusions. Under our second hypothesis, we also expected to see reduced genetic variation within smaller patches and genetic differentiation among patches. We did not find evidence for either of these expectations. We found no relationship between patch area and genetic variation (Figure 5) and our AMOVA results indicate that a non-significant amount of genetic variation is partitioned among the 15 habitat patches ($P = 0.11$; Table 4). Thus, we did not find strong evidence supporting the hypothesis that the patch structure of this basin shapes the distribution of genetic variation.

The distribution of genetic variation in the South Fork of the Boise River differs from the remainder of the basin in several ways. The pattern of isolation by distance observed for the basin as a whole and in the Middle and North Forks was not found in the South Fork and there was greater genetic differentiation among South Fork tributaries than elsewhere in the basin.

A genetic discontinuity in the South Fork divides the upstream tributaries that form a single cluster (Figure 3) from the three downstream-most tributaries. Surprisingly, the genetic discontinuity does not coincide with Anderson Ranch Dam, rather it occurs downstream from and including Skeleton Creek (Figure 2) where there is no apparent corresponding discontinuity in the stream network. The three downstream-most tributaries clustered with Middle and North Fork tributaries in the dendrogram because these tributaries were missing alleles found in upper South Fork samples, or they had alleles found in Middle and North Fork samples, but not in upper South Fork tributaries (Appendix 1).

We suggest two hypotheses for why the upper South Fork is genetically unlike the North and Middle Forks:

1) The South Fork was colonized at a different time or from a different source. It is possible that the South Fork was founded by bull trout either earlier or later than the rest of the Boise River Basin and these historic differences have been maintained. Headwater capture with the Salmon River basin is one possible mechanism for a separate founding event. The headwaters of the Big Smoky Creek watershed (one of the most upstream South Fork tributaries) are in close proximity to headwater streams of the Salmon River and show evidence of headwater capture and potential for drainage in either direction (B. Rieman unpublished data). The presence of two alleles at the *FGT3* locus (*FGT3*175* and *183*) found in the upper South Fork but not in the remainder of the Basin provide some evidence for this hypothesis. We sampled bull trout from the upper Salmon River Basin to see if either the *FGT3*175* or **183* alleles also occur there. The *FGT3*175* allele does occur at high frequency (0.88) in the upper Salmon River. Gene flow between the upper South Fork and the Salmon River does appear to be a possibility.

2) The South Fork may have been founded along with the rest of the Boise River Basin, but subsequently isolated from the rest of the system for an extended period of time. Meyer and Leidecker (1999) document that major landslides dammed the Salmon River for multiple decades in recent geologic time (14,000 and 1,400 BP). Others have found evidence of similar events in the Boise, which has the same geomorphology and climate as the Salmon basin (B. Rieman unpublished data). We are currently trying to determine if a major isolating event occurred in this system that would support this hypothesis.

Isolation by Distance

A pattern of isolation by distance (IBD) has been observed previously in bull trout (Costello et al. 2003) and other salmonids at similar scales (Wenburg et al. 1998, Carlsson et al. 1999, Carlsson and Nilsson 2000, Castric et al. 2001, Castric and Bernatchez 2003). The processes of colonization and the subsequent interaction of gene flow and drift have been used to explain these patterns.

Our observation of IBD within the Boise system occurs in a distinctly different system from those considered by Costello et al. (2003). In the Boise, either colonization or subsequent interaction of gene flow and drift may have been important. The pattern of IBD we observed, for example, may reflect the progression of colonization by bull trout following glacial retreat. Central Idaho was influenced only by alpine glaciation during the last glacial maximum. Most of the streams that now support bull trout were glaciated during that period, although the lower main stem Boise River and Snake River into which the Boise flows, were not. If the upper Boise tributaries were colonized as alpine glaciers retreated, habitats in the lower portions of the drainage might have become accessible first. Colonization could have progressed in stepwise fashion moving up the basin with time. A better characterization of timing of glacial retreat will be needed to determine whether a progressive lower-basin-to-upper basin retreat actually occurred.

Alternatively, glacial retreat may have occurred relatively quickly with colonization of headwater streams following from a primary refugia in the lower reaches of the basin more or less simultaneously. Populations may have been genetically similar initially, while the subsequent effects of drift and gene flow could then have given rise to the IBD we observed. Hutchison and Templeton (1999) predicted a significant increase in scatter of the pairwise genetic distances with geographic distance for systems that have had the time to approach a gene flow-drift equilibrium (Case IV in Hutchison and Templeton 1999). Costello et al. (2003) following from Hutchison and Templeton (1999) predicted that bull trout populations colonizing river basins that were exposed first following continental glacial retreat (i.e. those further south) should have advanced further toward such an equilibrium (e.g. stronger differentiation and increasing scatter with geographic distance). River basins founded earlier have presumably had more time to establish an equilibrium, provided the basin in question was founded only once and that all basins were colonized from the same refugia.

The populations that Costello et al. (2003) considered were able to colonize available habitat in a northward progression through a well-documented series of connections among extant rivers following the retreat of the Cordilleran Ice Sheet (McPhail and Lindsey 1986, Costello et al. 2003). Costello et al. (2003) predicted that the pattern of IBD would be stronger in the more southern upper Kootenay River than in the more northern Pine River and their data supported that assertion. For samples from the Middle and North Fork Boise, scatter about the regression of genetic distance on geographic distance increased with geographic distance although the pattern was not highly significant ($P = 0.07$). Our data are consistent with the hypothesis that the Middle and North Fork groups are approaching a similar drift-gene flow equilibrium. If colonization and subsequent drift were the only important processes the scatter resulting from drift would be expected to be uniform with distance.

Our relationship of IBD ($r = 0.45$, $P < 0.001$) also appeared to be stronger than that observed by Costello et al. (2003; Pine River, $r = 0.33$, $P = 0.03$, upper Kootenay River, $r = 0.33$. $P = 0.008$). The time scale required to establish or strengthen such a relationship remains unclear, but Costello et al. (2003) and Hutchison and Templeton (1999) are clearly drawing inferences about processes extending over millenia. Kinnison et al. (2002) however, showed that similar patterns may emerge over much shorter time scales (approximately 30 generations) for chinook salmon (*Oncorhynchus tschawytscha*) in New Zealand. If the strength of the relationship is informative,

the implication would be that the Boise has progressed further in time since colonization than the more northern systems. Because of the more limited extent of glaciation in the Boise this could be the case, but further work on the patterns and timing of glacial retreat in each location will be necessary to clarify this interpretation.

Although our data only hint at the differences in IBD between the northern populations of bull trout and the Boise populations, we found a striking difference in overall levels of genetic differentiation. Our estimated F_{ST} was 0.064 for the entire Boise system; Costello et al. (2003) estimated F_{ST} values of 0.24 for the Pine River and 0.23 for the upper Kootenay River. Our F_{ST} was also substantially lower than has been found for other bull trout populations in the central portions of the range (Spruell et al. 1999, Neraas and Spruell 2001). The geographic scale (~10^2 km) of the populations considered is similar in all these river basins. Thus, in the Boise system, we found evidence for stronger patterns of isolation by distance than populations further north and we found evidence for higher overall levels of gene flow.

We suggest these combined patterns can be explained in three ways. First, habitats in southern extremes of the species range in the Boise River Basin are more highly constrained by water temperatures and may be more patchy and dynamic than populations in central or northern portions of the range (Rieman and McIntyre 1995, Rieman et al. 1997, Dunham and Rieman 1999). Second, effective gene flow, or the rate at which migrant alleles are actually incorporated into a subpopulation, may be greater than what we predict based on neutral expectations due to a rescue from inbreeding effects associated with small populations (Ingvarsson and Whitlock 2000). Finally, the effective migration rate may be higher in this system because levels of local adaptation and demographic resistance are reduced, allowing gene flow without the associated outbreeding depression that is often suggested as a barrier to migration.

Small Populations- The Boise is on the extreme southern limits of the species' range. Habitats are more highly constrained by water temperatures and thus may be more patchy and dynamic than populations in central or northern portions of the range (Rieman and McIntyre 1995, Rieman et al. 1997, Dunham and Rieman 1999). Fires, floods, and debris flows have been common on decadal to centennial time scales and are likely to cause dramatic fluctuation in population size and perhaps even local extinctions (Rieman et al. 1997). Small populations may be less resistant to gene flow, and perhaps more prone to straying (Quinn et al. 2001). Mass failures associated with large landslides have dammed whole channels (e.g. Meyer and Leidecker 1999; J. McKean RMRS Boise, personal communication) and could have forced dispersal similar to the effects suggested for some human constructed dams (see Neraas and Spruell 2001). The small population hypothesis is consistent with the patterns of habitat patch occupation described in Boise River bull trout populations by Dunham and Rieman (1999) where occurrence was more likely in large patches and in patches closer to other occupied sites. They inferred a metapopulaton process where extinction was more frequent in smaller patches and recolonization or demographic support more likely in less isolated patches. The genetic data offer additional support for the metapopulation/small population model.

Effective Gene Flow-It is also possible that migrant alleles have a selective advantage (or are closely linked to alleles with a selective advantage) and this may be partly responsible for the high levels of gene flow we observed. Since bull trout populations in the Boise basin are likely

small, slightly deleterious alleles will be effectively neutral. These slightly deleterious alleles may drift to high frequency or be fixed (Wright 1937), with different alleles likely drifting to high frequency in different populations. Because individuals from different populations are less likely to carry the same deleterious alleles as resident individuals, hybrids may have increased fitness (heterosis; Ingvarsson and Whitlock 2000). This process can allow migrant alleles to increase in frequency in a population to a greater extend that expected under neutral expectations even if these migrant alleles are not directly selected upon because linked alleles can hitchhike to greater frequency (Ingvarrson and Whitlock 2000). This heterosis could be partly responsible for high levels of gene flow in the Boise system because the selective advantage of migrant alleles may increase the effective migration rate of these alleles.

Local Adaptation- Heterosis will not occur if the offspring from a mating between migrant and resident individuals suffer from outbreeding depression (Ingvarsson and Whitlock 2000). Extrinsic outbreeding depression would result from the mating between two locally adapted individuals where the offspring reside in the habitat to which one individual is locally adapted but not the other. Local adaptation may be inhibited by gene flow (Endler 1977, Allendorf 1983, Hendry et al. 2001) and the overall higher levels of gene flow in the Boise Basin may have inhibited local adaptation of bull trout. Moreover, populations of bull trout in the Boise system may have high enough turnover rates that local adaptations have not had time to evolve. While several recent studies have found evidence for rapid evolution in salmonid populations (Hendry et al. 2000, Quinn et al. 2000), it may take longer for local adaptation to evolve in some cases. Either of these scenarios would lead to offspring of matings between migrants and residents that do not have lower fitness than residents, or may even have greater fitness than residents (heterosis) and there may be little barrier to the introgression of migrant alleles. Thus, if local adaptation is not occurring in this system, then the effective migration rate may be even higher than a system where outbreeding depression can reduce heterosis, and the processes described in the previous paragraph may be even more important.

Under some circumstances, we would predict that F_{ST} would be high in a system where extinction and recolonization rates are high and populations are founded from a nearby source (Whitlock and McCauley 1990, Whitlock 1992, Pannell and Charlesworth 2000). However, in the Boise system, the complex life history and long generation length of bull trout may prevent populations from going extinct after major disturbances. Migratory fish may return to their natal stream within a few years following a disturbance and any additional forced dispersers would bring new genes into an already existing population, instead of founding a new population. When this type of process occurs over thousands of years, the result would be reduced genetic differentiation, similar to that observed in this study.

Anthropogenic Effects

Four dams have been constructed in the upper Boise River Basin over the last century that may have dramatically altered the potential patterns of migration and dispersal in bull trout populations. Bull trout are noted for extended movements among spawning, rearing, or refuge habitats that may encompass hundreds of kilometers of interconnected rivers and streams (Swanberg 1997, Rieman and Dunham 2000). Radio tracking studies in the Boise River Basin have demonstrated that bull trout still move throughout the system (T. Salow, USBOR unpublished data). Although bull trout cannot move upstream past any of the four dams without

human assistance, those dams are not necessarily barriers to downstream movements. Downstream entrainment of fish at Arrowrock Dam with subsequent survival in the reservoir above Lucky Peak Dam has been observed and adult bull trout have been collected at the face of Arrowrock Dam during the typical period of adult upstream spawning migration. Recent upstream movements of adult bull trout over a fish ladder constructed at Kirby Dam in the 1990s suggest that a similar downstream entrainment but upstream blockage has occurred there as well.

Fragmentation of habitat networks by dams and the associated constraints on migration, dispersal, and gene flow can have profound effects on the demographic and genetic characteristics of populations. Fragmentation may dramatically reduce the productivity of populations by constraining the expression of migratory life histories (and the occurrence of large fecund adults) and ultimately increasing the risks of extinction through demographic processes (e.g. Dunham and Rieman 1999, Morita and Yamamoto 2002). Isolation may also increase the potential for genetic drift, loss of genetic variation and inbreeding effects (Lande 1988, Williams et al. 2003).

It is not clear whether the disruption of migration has significantly altered life history patterns in Boise River bull trout or not. Morita et al. (2002) demonstrated extreme life history shifts in white spotted char (*Salvelinus leucomaenis*) following the construction of sediment dams that blocked movements of populations in headwater streams. In the Boise and other similar systems, dams have constrained movements within much larger stream networks (Arrowrock and Anderson Ranch ~ 10^3-10^4 km^2 *vs*. Japan 10^{-1}- 10^1 km^2). In most cases bull trout have apparently adapted to a lacustrine life style characteristic of populations associated with large lake systems and that may be the case in the larger reservoirs (Arrowrock, Anderson Ranch) of the Boise system. The maintenance of the migratory life history associated with these reservoirs may not have dramatically altered the productivity and diversity of many of the remnant populations. That may not be the case above Lucky Peak and Kirby Dams, however, where the reservoir and associated stream networks are relatively small (~10^1-10^2 km^2).

Despite the ability of fish to move widely and even gain substantial growth associated with the reservoir environment, analogous to that in natural river-lake systems, the dams have had an important influence on potential dispersal and gene flow. Three of the four dams are associated with notable discontinuities in the genetic patterns that suggest these effects have been significant.

Kirby Dam-The pairwise genetic distances between the Yuba River sample above Kirby Dam constructed in 1906, and all other streams are outliers on the upper range of the relationship characterizing isolation by distance (Figure 4). The strength of the relationship between genetic distance and stream network distance among all samples suggests that the balance between drift and gene flow has been an important force structuring populations across the basin. Isolation of the Yuba River nearly 100 years ago altered that balance by eliminating any gene flow into the system. As a result drift has predominated and may have been accelerated by the isolation.

The local bull trout population above Kirby Dam appears to be relatively small. We do not have actual population estimates but fish occur in samples far less frequently than in other streams. In most streams supporting bull trout it was possible to collect the sample necessary for genetic

analysis in one to at most a few days effort in the field. Biologists have sampled the streams above the dam repeatedly over several years and have observed only the 30 fish used in this analysis. The stream network above Kirby Dam that is potentially available to bull trout is actually roughly twice the size of that associated with the Yuba River habitat patch. No bull trout have been found in repeated sampling in the northern half of this network despite the fact that it lies within wilderness and supports almost pristine habitat. The reservoir behind Kirby Dam is also quite small and offers essentially no lacustrine habitat that might provide a growth advantage to migratory fish. It is conceivable that migratory life histories have been severely constrained in this system by the dam sharply reducing the demographic resilience and size of the population in a manner similar to the effect of sediment dams on white spotted char in Japan (Morita et al. 2000).

Pooling of samples from several sites may have influenced the allele frequencies we estimated for the Yuba River sample. The sample sites were separated by as much as eight kilometers and we may have pooled among independent populations, thereby confounding our results. *SSA311* appeared to be the only locus potentially influenced by population substructure above Kirby Dam. The exclusion of the Grouse Creek fish caused the Yuba sample as a whole to conform to Hardy-Weinberg proportions and only slightly changed allele frequencies. Excluding the Grouse Creek fish did not influence our isolation by distance results or the UPGMA dendrogram. It is possible that some structure exists within the Yuba, but we believe it is limited to that observed in other habitat patches of similar size and that it was not an important influence on the overall evidence of departure of this sample from other populations in the Boise River Basin.

We believe that isolation has had an important influence on the genetic characteristics of the fish above Kirby Dam. The evidence for accelerated drift in the last 100 years (~20 generations) indicates that the pattern of isolation-by-distance observed across the system in general is at least partially due to the patterns of contemporary gene flow and not simply historical patterns of colonization. We found no evidence of reduced genetic diversity in the sample above the dam (Table 1), but that may well occur if the isolation is prolonged.

In the mid 1990's a fish ladder was constructed to allow summer movement over the dam. Some bull trout have moved upstream past the dam (T. Salow unpublished results). Maintenance of that passage and the restoration of both a migratory life history and gene flow from other patches may well be important to the persistence of bull trout in the Yuba River.

Arrowrock and Lucky Peak Dams- Mores Creek was an outlier in our genetic analyses much like the Yuba River sample. Mores Creek is a tributary between Arrowrock (constructed in 1915) and Lucky Peak (constructed in 1954) Dams and flows directly into Luck Peak Reservoir. Mores Creek represents the only potential habitat for bull trout accessible to fish in the reservoir and was likely the lowest entering tributary offering habitat to bull trout prior to human development in the Boise River Basin.

The genetic departure of the Mores Creek sample might be the result of isolation and drift as we suggest for the Yuba River sample. The population is and probably has been quite small relative

to others in the basin with the smallest estimated habitat patch area in our sample (Table 2). Drift could have been pronounced especially if the dams have reduced gene flow from other streams.

It is also possible that the departure of the Mores Creek sample is actually the result of increased movement of fish into the system following construction of the dams. Based on patch size and apparent isolation, empirical models developed in the Boise River Basin predict a very low probability of bull trout presence (see Dunham and Rieman 1999). Until recently we did not believe bull trout actually occurred in this stream. Bull trout have been collected only sporadically in recent samples and over three years only the 16 fish used in this analysis have been observed. We speculate that fish in Mores Creek are actually the progeny of bull trout that were entrained at Arrowrock Dam and were unable to return upstream at maturity. With Mores Creek as the only available habitat, fish were forced to use it even if the population could not persist there. In essence, the Mores Creek population could be a demographic "sink" (*sensu* Pulliam 1988) maintained by forced dispersal from a source of fish upstream of Arrowrock Dam. Neraas and Spruell (2001) found similar evidence of a population maintained by fish entrained at an upstream dam in the Clark Fork River. Interestingly, the Mores Creek sample had the highest H_E observed in any of the samples, but no departure from Hardy-Weinberg proportions; patterns that would not be expected in a very small population that had been isolated for an extended period, but would be consistent with a population supported by forced dispersal from multiple upstream populations.

Anderson Ranch Dam-We found no evidence that the construction of Anderson Ranch Dam in 1950 has had any important influence on genetic structure in the basin. There is an important discontinuity in genetic structure associated with the South Fork, but as we discussed, this discontinuity occurs among streams upstream of rather than simply above and below the dam. It is likely that the interconnected system above Anderson Ranch Dam is large enough or has been isolated for a short enough period that drift has not had an observable influence in the system.

Conclusions

Geomorphology and human disruption have had important effects on the genetic structure of the bull trout in the Boise system. Geomorphology appears to have limited gene flow in some cases and thus contributed to genetic differentiation. In other cases, geomorphology appears to have contributed to gene flow, for example through forced dispersal following disturbance or by head water capture events. The anthropogenic effects of dams have shaped, in part, the distribution of genetic variation of bull trout in the Boise River Basin.

Gene flow among populations has an important impact on genetic structure on ecological time scales. We observed divergence within 100 years in the absence of gene flow in the upper Middle Fork. It appears that Emma Creek is also isolated and has differentiated genetically due to drift. In the extreme cases (e.g. Emma Creek), we can expect the loss of genetic variation, but gene flow may also be an important force maintaining the system. The pattern of IBD and high levels of gene flow observed in this system suggest that dispersal connectivity could follow classic metapopulation processes (Harrison and Taylor 1997).

We found striking differences between the results of Costello et al. (2003) for bull trout populations further north and the Boise system. Historical impacts on genetic structure are clearly important to consider and may be responsible for the striking differences observed. One historical factor that we need to understand better is timing and pattern of glacial retreat (i.e. was the Boise in fact open to colonization earlier than other populations to the north?). Not only are bull trout in different portions of the species range highly genetically differentiated (Spruell et al. 2003), but the genetic structure of populations in different portions of the species range also varies, suggesting that either colonization or post colonization processes have been very different as well.

We recommend the following conservation units within the Boise system. The upper South Fork should be treated as a separate genetic unit. The rest of the system (Middle and North Forks) should also be treated as a separate genetic unit, recognizing that genetic differentiation occurs among populations within these two forks. Mores Creek, if our hypothesis is correct, is likely made up of fish that are entrained by dams and unable to return to their natal streams to spawn. This population should be protected because this hypothesis needs to be tested. However, we suggest that efforts should be made to restore connectivity in this system, at risk of imperiling the Mores population. Bull trout in the Yuba River and adjacent tributary streams should not be considered a distinct genetic group, because the divergence looks to be the direct result of isolation by Kirby Dam and the population may be at risk. Maintenance of gene flow with the rest of the system (and restoration of migratory life histories) could be key for the persistence of bull trout above Kirby Dam.

The origin of the genetic differences among groups in this system is important to unravel. Some questions that follow from our work are: Is the upper South Fork unique because it has been isolated or because of it was founded from a different source? Data from bull trout captured in the upper Salmon support the latter hypothesis but additional samples should be analyzed before any conclusions are drawn. Another question that warrants further attention: Is the Mores Creek population genetically unique (the U.S. Fish and Wildlife Service has designated this creek as critical habitat) or is the observed genetic differentiation simply an artifact of the dams?

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Literature Cited

Allendorf, F. W. 1983. Isolation, gene flow, and genetic differentiation among populations. Pages 51-65 *in* C. Schonewald-Cox, S. Chambers, B. MacBryde, and L. Thomas, editors. Benjamin and Cummings, Menlo Park, CA.

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.

Angers, B., P. Magnan, M. Plante, and L. Bernatchez. 1999. Canonical correspondence analysis for estimating spatial and environmental effects on microsatellite gene diversity in brook charr (*Salvelinus fontinalis*). Molecular Ecology **8**:1043-1053.

Avise, J. C. 1994. Molecular markers, natural history, and evolution. Chapman & Hall, New York.

Bernatchez, L., and C. C. Wilson. 1998. Comparative phylogeography of nearctic and palearctic fishes. Molecular Ecology **7**:431-452.

Bohonak, A. J. 2003. IBD (Isolation By Distance): A program for analyses of isolation by distance. Journal of Heredity **92**:153-154.

Carlsson, J., and J. Nilsson. 2000. Population genetic structure of brown trout (*Salmo trutta* L.) within a northern boreal forest stream. Hereditas **132**:173-181.

Carlsson, J., K. H. Olsen, J. Nilsson, O. Overli, and O. B. Stabell. 1999. Microsatellites reveal fine-scale genetic structure in stream-living brown trout. Journal of Fish Biology **55**:1290-1303.

Castric, V., and L. Bernatchez. 2003. The rise and fall of isolation by distance in anadromous brook charr (*Salvelinus fontinalis* Mitchill). Genetics **165**:983-996.

Castric, V., F. Bonney, and L. Bernatchez. 2001. Landscape structure and hierarchical genetic diversity in the brook charr, *Salvelinus fontinalis*. Evolution **55**:1016-1028.

Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution **21**:550-570.

Costello, A. B., T. E. Down, S. M. Pollard, C. J. Pacas, and E. B. Taylor. 2003. The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: An examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus* (Pisces: Salmonidae). Evolution **57**:328-344.

Crandall, K. A., O. R. P. Binindaemonds, G. M. Mace, and R. K. Wayne. 2000. Considering evolutionary processes in conservation biology. Trends in Ecology & Evolution **15**:290-295.

Dunham, J. B., and B. E. Rieman. 1999. Metapopulation structure of bull trout: Influences of physical, biotic, and geometrical landscape characteristics. Ecological Applications **9**:642-655.

Dunham, J. B., B. E. Rieman, and G. L. Chandler. 2001. Development of field-based models of suitable thermal regimes for interior Columbia Basin salmonids. Final Report Interagency Agreement, U. S. Environmental Protection Agency, Seattle, WA.

Endler, J. A. 1977. Geographic Variation, Speciation, and Clines. Princeton University Press, Princeton, NJ.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics **131**:479-491.

Felsenstein, J. 1992. PHYLIP (*Phylogeny Inference Package*) version 3.5c. Seattle, WA.

Gaggiotti, O. E., O. Lange, K. Rassmann, and C. Gliddon. 1999. A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. Molecular Ecology **8**:1513-1520.

Goudet, J., M. Raymond, T. Demeeus, and F. Rousset. 1996. Testing differentiation in diploid populations. Genetics **144**:1933-1940.

Harrison, S., and A. D. Taylor. 1997. Empirical evidence for metapopulation dynamics. Pages 27-42 *in* I. Hanski and M. E. Gilpin, editors. Metapopulation Biology. Academic Press Inc., San Diego, CA.

Hendry, A. P., T. Day, and E. B. Taylor. 2001. Population mixing and the adaptive divergence of quantitative traits in discrete populations: A theoretical framework for empirical tests. Evolution **55**:459-466.

Hendry, A. P., J. K. Wenburg, P. Bentzen, E. C. Volk, and T. P. Quinn. 2000. Rapid evolution of reproductive isolation in the wild: Evidence from introduced salmon. Science **290**:516-518.

Hutchison, D. W., and A. R. Templeton. 1999. Correlation of pairwise genetic and geographic distance measures: Inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution **53**:1898-1914.

Ingvarsson, P. K., and M. C. Whitlock. 2000. Heterosis increases effective migration rate. Proceedings of the Royal Society of London Series B - Biological Sciences **267**:1321-1326.

Kimura, M. 1953. "Stepping-stone" model of population. Annual Report of the National Institute of Genetics **3**:62-63.

Kimura, M., and G. H. Weiss. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics **49**:561-576.

Kinnison, M. T., P. Bentzen, M. J. Unwin, and T. P. Quinn. 2002. Reconstructing recent divergence: Evaluating nonequilibrium population structure in New Zealand chinook salmon. Molecular Ecology **11**:739-754.

Lande, R. 1988. Genetics and demography in biological conservation. Science **241**:1455-1460.

McPhail, J. D., and C. C. Lindsey. 1986. Zoogeography of freshwater fishes of Cascadia (the Columbia System and rivers north to the Stikine). *in* C. H. Hocutt and E. O. Wiley, editors. The Zoogeography of North American Freshwater Fishes. John Wiley and Sons, New York, NY.

Meffe, G. K., and R. C. Vrijenhoek. 1988. Conservation genetics in the management of desert fishes. Conservation Biology **2**:157-169.

Meyer, G. A., and M. E. Leidecker. 1999. Fluvial terraces among the Middle Fork Salmon River, Idaho, and their relation to glaciation, landslide dams, and incision rates: A preliminary analysis and river-mile guide. Pages 219-235 *in* S. S. Hughes and G. D. Thackray, editors. Guidebook to the Geology of Eastern Idaho. Idaho Museum of Natural History, Idaho.

Morita, K., and S. Yamamoto. 2002. Effects of habitat fragmentation by damming on the persistence of stream-dwelling charr populations. Conservation Biology **16**:1318-1323.

Morita, K., S. Yamamoto, and N. Hoshino. 2000. Extreme life history change of white-spotted char (*Salvelinus leucomaenis*) after damming. Canadian Journal of Fisheries and Aquatic Sciences **57**:1300-1306.

Moritz, C. 1994. Defining 'Evolutionarily Significant Units' for conservation. Trends in Ecology & Evolution **9**:373-375.

Neraas, L. P., and P. Spruell. 2001. Fragmentation of riverine systems: The genetic effects of dams on bull trout (*Salvelinus confluentus*) in the Clark Fork River system. Molecular Ecology **10**:1153-1164.

Pannell, J. R., and B. Charlesworth. 2000. Effects of metapopulation processes on measures of genetic diversity. Philosophical Transactions of the Royal Society of London Series B - Biological Sciences **355**:1851-1864.

Pulliam, H. R. 1988. Sources, sinks, and population regulation. American Naturalist **132**:652-661.

Quigley, T. M., and S. J. Arbelbide. 1997. An assessment of ecostystem components in the interior Columbia Basin and portions of the Klamath and Great Basins: Vols. 1-4. U. S. Forest Service General Technical Report, U. S. Forest Service.

Quinn, T. P., M. T. Kinnison, and M. J. Unwin. 2001. Evolution of chinook salmon (*Oncorhynchus tshawytscha*) populations in New Zealand: Pattern, rate, and process. Genetica **112**:493-513.

Quinn, T. P., M. J. Unwin, and M. T. Kinnison. 2000. Evolution of temporal isolation in the wild: Genetic divergence in timing of migration and breeding by introduced chinook salmon populations. Evolution **54**:1372-1385.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 3.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity **86**:248-249.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution **43**:223-225.

Rieman, B. E., and J. B. Dunham. 2000. Metapopulations and salmonids: A synthesis of life history patterns and empirical observations. Ecology of Freshwater Fish **9**:51-64.

Rieman, B. E., D. C. Lee, and R. F. Thurow. 1997. Distribution, status, and likely future trends of bull trout within the Columbia River and Klamath basins. North American Journal of Fisheries Management **17**:1111-1125.

Rieman, B. E., and J. D. McIntyre. 1995. Occurrence of bull trout in naturally fragmented habitat patches of varied size. Transactions of the American Fisheries Society **124**:285-296.

Robinson, B. W., and D. Schluter. 2000. Natural selection and the evolution of adaptive genetic variation in northern freshwater fishes. Pages 65-94 *in* T. A. Mousseau, B. Sinervo, and J. A. Endler, editors. Adaptive Genetic Variation in the Wild. Oxford University Press, New York.

Schnieder, S., D. Roessli, and L. Excoffier. 2000. Arlequin ver. 2.001: A software for population genetic data analysis. Geneva, Switzerland.

Skulason, S., and T. B. Smith. 1995. Resource polymorphisms in vertebrates. Trends in Ecology & Evolution **10**:366-370.

Spruell, P., M. L. Bartron, N. Kanda, and F. W. Allendorf. 2001. Detection of hybrids between bull trout (*Salvelinus confluentus*) and brook trout (*Salvelinus fontinalis*) using PCR primers complementary to interspersed nuclear elements. Copeia:1093-1099.

Spruell, P., A. R. Hemmingsen, P. J. Howell, N. Kanda, and F. W. Allendorf. 2003. Conservation genetics of bull trout: Geographic distribution of variation at microsatellite loci. Conservation Genetics **4**:17-29.

Spruell, P., B. E. Rieman, K. L. Knudsen, F. M. Utter, and F. W. Allendorf. 1999. Genetic population structure within streams: Microsatellite analysis of bull trout populations. Ecology of Freshwater Fish **8**:114-121.

Swanberg, T. R. 1997. Movements of and habitat use by fluvial bull trout in the Blackfoot River, Montana. Transactions of the American Fisheries Society **126**:735-746.

Taylor, E. B., S. Pollard, and D. Louie. 1999. Mitochondrial DNA variation in bull trout (*Salvelinus confluentus*) from northwestern North America: Implications for zoogeography and conservation. Molecular Ecology **8**:1155-1170.

Waples, R. S. 1991. Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. Marine Fisheries Review **53**:11-22.

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.

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

Whitlock, M. C. 1992. Nonequilibrium population structure in forked fungus beetles: Extinction, colonization, and the genetic variance among populations. American Naturalist **139**:952-970.

Whitlock, M. C., and D. E. McCauley. 1990. Some population genetic consequences of colony formation and extinction: Genetic correlations within founding groups. Evolution **44**:1717-1724.

Williams, B. L., J. D. Brawn, and K. N. Paige. 2003. Landscape scale genetic effects of habitat fragmentation on a high gene flow species: *Speyeria idalia* (Nymphalidae). Molecular Ecology **12**:11-20.

Wright, S. 1937. The distribution of gene frequencies in populations. Proceedings of the National Academy of Science **23**:307-320.

Appendix 1. Allele frequencies at six polymorphic loci for 21 bull trout sample locations from the Boise River Basin. Sample sizes (n) represent the number of successfully amplified individuals for each sample at each locus.

	Locus											
Location	*Oneμ7*			*Bt73*				*Fgt3*				
	**218*	**244*	n	**138*	**140*	**144*	n	**157*	**163*	**175*	**183*	n
Mores Cr.	0.469	0.531	16	0.094	--	0.906	16	0.833	0.167	--	--	15
Middle Fork												
Sheep Cr.	0.609	0.391	23	0.071	--	0.929	23	0.975	0.025	--	--	20
E.F. Sheep Cr.	0.580	0.420	25	0.080	--	0.920	25	0.980	0.020	--	--	25
Roaring R.	0.500	0.500	39	--	0.179	0.821	39	0.949	0.051	--	--	39
Yuba R.	0.740	0.260	25	0.023	0.159	0.818	25	0.923	0.077	--	--	26
North Fork												
Crooked R.	0.500	0.500	34	--	--	1.000	34	0.985	0.015	--	--	34
Bear Cr./R.	0.537	0.463	27	0.083	--	0.917	27	0.840	0.160	--	--	25
Lodgepole Cr.	0.538	0.463	40	--	--	1.000	40	0.909	0.091	--	--	33
Johnson Cr.	0.455	0.545	22	0.023	--	0.977	22	0.917	0.083	--	--	18
Ballentyne Cr.	0.516	0.484	32	0.033	--	0.967	32	0.845	0.155	--	--	29
McLeod Cr.	0.568	0.432	37	0.051	--	0.949	37	0.946	0.054	--	--	37
South Fork												
Rattlesnake Cr.	0.513	0.487	39	--	0.053	0.947	39	0.986	0.014	--	--	36
Elk Cr.	0.550	0.450	20	--	--	1.000	20	1.000	--	--	--	28
Skeleton Cr.	0.361	0.639	36	--	0.015	0.985	36	0.879	0.091	0.030	--	33
Boardman Cr.	0.207	0.793	29	--	--	1.000	29	0.817	--	0.183	--	30
Smoky Dome Cr.	0.542	0.458	24	--	--	1.000	24	0.826	--	0.174	--	23
Emma Cr.	0.048	0.952	21	--	--	1.000	21	1.000	--	--	--	20
Johnson Cr.	0.469	0.531	32	--	--	1.000	32	0.848	--	0.152	--	33
Big Smoky Cr.	0.333	0.667	27	--	--	1.000	27	0.845	--	0.017	0.138	29
Upper Big Smoky Cr.	0.528	0.472	35	--	--	1.000	35	0.811	--	--	0.189	33
W.F. Big Smoky Cr.	0.471	0.529	36	--	--	1.000	36	0.985	--	0.015	--	37

Appendix 1 continued.

	Locus									
Location	*Sco19*				*Ssa311*			*Ots101*		
	**200*	**204*	**206*	n	**112*	**120*	n	**100*	**112*	n
Mores Cr.	--	1.000	--	14	0.250	0.750	16	0.188	0.813	16
Middle Fork										
Sheep Cr.	0.067	0.633	0.300	15	0.040	0.960	25	--	1.000	25
E.F. Sheep Cr.	0.250	0.386	0.364	22	0.083	0.917	24	--	1.000	24
Roaring R.	0.013	0.934	0.053	38	0.026	0.974	39	--	1.000	39
Yuba R.	0.079	0.763	0.158	19	0.283	0.717	30	0.083	0.917	30
North Fork										
Crooked R.	--	0.980	0.020	25	--	1.000	33	--	1.000	33
Bear Cr./R.	0.059	0.735	0.206	17	0.019	0.981	27	0.019	0.981	27
Lodgepole Cr.	0.045	0.750	0.205	22	0.021	0.979	47	0.021	0.979	47
Johnson Cr.	0.100	0.650	0.250	10	0.071	0.929	28	0.017	0.983	30
Ballentyne Cr.	0.109	0.717	0.174	23	0.076	0.924	33	0.029	0.971	34
McLeod Cr.	0.275	0.650	0.075	20	0.077	0.923	39	0.013	0.988	40
South Fork										
Rattlesnake Cr.	0.145	0.605	0.250	38	0.014	0.986	37	--	1.000	37
Elk Cr.	--	0.937	0.063	8	0.017	0.983	29	--	1.000	29
Skeleton Cr.	--	0.554	0.446	28	--	1.000	36	--	1.000	36
Boardman Cr.	--	0.853	0.147	17	0.161	0.839	28	--	1.000	29
Smoky Dome Cr.	--	0.833	0.167	18	0.056	0.944	27	--	1.000	28
Emma Cr.	--	0.857	0.143	14	--	1.000	21	--	1.000	22
Johnson Cr.	--	0.786	0.214	28	0.242	0.758	33	--	1.000	33
Big Smoky Cr.	0.036	0.785	0.179	28	0.052	0.948	29	--	1.000	29
Upper Big Smoky Cr.	--	0.609	0.391	33	0.095	0.905	35	--	1.000	35
W.F. Big Smoky Cr.	--	0.636	0.364	32	0.129	0.871	37	--	1.000	37

Table 1. Sample information (sample numbers, names, and sizes), expected heterozygosities (H_E), and total number of alleles for samples of bull trout from the Boise River Basin.

Sample Number	Location	N	Expected Heterozygosity (H_E)	Number of Alleles
1	Mores Creek	16	0.335	10
Middle Fork				
2	Sheep Creek	26	0.150	9
3	E. F. Sheep Creek	25	0.169	9
4	Roaring River	39	0.191	9
5*	Yuba River	30	0.265	11
North Fork				
6	Crooked River	38	0.107	7
7*	Bear Creek/River	27	0.202	10
8	Lodgepole Creek	51	0.177	9
9	Johnson Creek	30	0.182	10
10	Ballentyne Creek	38	0.208	10
11	McLeod Creek	42	0.174	10
South Fork				
12	Rattlesnake Creek	39	0.132	9
13	Elk Creek	29	0.108	7
14*	Skeleton Creek	36	0.144	9
15	Boardman Creek	30	0.183	8
16	Smoky Dome Creek	28	0.182	8
17	Emma Creek	23	0.019	6
18	Johnson Creek	33	0.228	8
19	Big Smoky Creek	29	0.147	9
20	Upper Big Smoky Creek	35	0.153	8
21	W. F. Big Smoky Creek	37	0.198	8

*Samples from two or more locations pooled for analysis

Table 2. Patch number, tributaries contained within patches, and patch sizes for the Boise Basin. Numbers in parentheses correspond to Table 1 and Figure 2. The area of the polygon defining the watershed boundaries of the stream network contained within a patch was used as a measure of patch size (Dunham and Rieman 1999).

Patch Number	Tributaries within Patches	Area (km^2)
1	Mores Cr. (1)	1,000
2	Sheep Cr. (2)/ E.F. Sheep Cr. (3)	4,065
3	Roaring R. (4)	5,482
4	Yuba R. (5)	12,108
5	Crooked R. (6)	6,941
6	Bear Cr./R. (7)	2,965
7	Lodgepole Cr. (8)/ Johnson Cr. (North Fork; 9)	6,904
8	Ballentyne Cr. (10)/ McLeod Cr. (11)	13,500
9	Rattlesnake Cr. (12)	2,375
10	Elk Cr. (13)	3,542
11	Skeleton Cr. (14)	5,377
12	Boardman Cr. (15)/ Smoky Dome Cr. (16)	5,127
13	Emma Cr. (17)	2,737
14	Johnson Cr. (South Fork; 18)	13,714
15	Big Smoky (19, 20)/ W.F. Big Smoky (21)	22,637

Table 3 . Number of loci for which significant genic differentiation was found (above the diagonal) and Cavalli-Sforza and Edwards' (1967) genetic distance (below the diagnal). The Sequential Bonferroni method (Rice 1989) was used to correct for multiple tests to determine the number of significant loci where we corrected based on the 5 loci analyzed in each population. Asterisks indicate the probability that the allelic distribution is identical between populations when all loci are combined (**P < 0.01, ***P < 0.001).

	Sample Site																				
Sample Site		Middle Fork				North Fork						South Fork									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1		2**	1**	3***	0**	3***	2**	2***	1	0	1**	4***	3***	2	2***	3***	4***	2***	3***	2***	2***
2	0.099		0	1**	2***	0	0	0	0	0	0	0	0	0**	2***	1	1***	2***	1**	0	1**
3	0.079	0.048		1***	2***	0	0	0	0	0	0	0	0	0	2***	1	1***	1***	1**	0	1**
4	0.074	0.052	0.013		3***	1	1**	1**	1	1***	1**	1	1	1**	4***	2***	2***	3***	2**	1	2***
5	0.102	0.030	0.066	0.069		4***	2***	2***	3***	2***	2***	0	1***	4***	3***	3***	3***	4***	4***	2***	4***
6	0.071	0.082	0.083	0.079	0.073		1	0	0	1	0	2***	0	0	3***	1***	1***	2***	1***	1	1***
7	0.103	0.033	0.045	0.053	0.060	0.104		0	0	0	0	0	1	0	3***	1**	1***	2***	1***	1***	1***
8	0.064	0.063	0.039	0.044	0.072	0.085	0.058		0	0	0	0	0	0	3***	1**	1***	2***	1***	1	1***
9	0.075	0.043	0.045	0.050	0.059	0.083	0.036	0.040		0	0	0	0	0	2**	1	1**	1**	1	0	1
10	0.058	0.049	0.035	0.035	0.063	0.077	0.048	0.031	0.027		0	1**	1	0	2***	1**	2***	1	1***	1	1***
11	0.050	0.060	0.043	0.043	0.069	0.073	0.060	0.023	0.032	0.018		0	0	0**	2***	1**	1***	2***	2***	0	1***
12	0.060	0.051	0.021	0.020	0.066	0.072	0.051	0.031	0.035	0.019	0.025		0	0	3***	1**	1***	2***	1***	1	1***
13	0.101	0.033	0.042	0.046	0.063	0.097	0.024	0.066	0.043	0.050	0.063	0.048		0	3***	1	1***	2***	1**	0	1**
14	0.101	0.043	0.063	0.068	0.054	0.105	0.040	0.058	0.044	0.052	0.060	0.062	0.056		2***	1**	1***	2***	1**	1**	1***
15	0.109	0.085	0.091	0.086	0.098	0.119	0.087	0.102	0.089	0.081	0.092	0.087	0.081	0.077		1**	2***	1	1***	2***	2***
16	0.108	0.065	0.068	0.068	0.083	0.105	0.064	0.085	0.069	0.071	0.080	0.070	0.056	0.064	0.050		2***	1	1**	1	1***
17	0.128	0.081	0.097	0.098	0.097	0.145	0.073	0.102	0.089	0.087	0.101	0.097	0.079	0.072	0.083	0.098		3***	1***	1***	2***
18	0.100	0.079	0.078	0.072	0.092	0.098	0.084	0.095	0.081	0.074	0.083	0.074	0.071	0.083	0.038	0.037	0.106		2***	1	1***
19	0.108	0.067	0.074	0.073	0.084	0.113	0.064	0.087	0.072	0.069	0.082	0.074	0.060	0.070	0.067	0.065	0.077	0.070		1**	0
20	0.091	0.049	0.051	0.047	0.070	0.089	0.052	0.074	0.054	0.049	0.063	0.049	0.035	0.065	0.055	0.043	0.083	0.040	0.054		1**
21	0.106	0.070	0.071	0.070	0.086	0.103	0.071	0.089	0.073	0.072	0.081	0.072	0.061	0.086	0.090	0.077	0.102	0.079	0.033	0.060	

Table 4. Results from analysis of molecular variance (AMOVA). The three groups in geographical arrangement 1 correspond to each of the three forks of the Boise River. The Middle Fork and North Fork are combined into one group and the South Fork is the second of two groups in geographical arrangement 2. For geographical arrangement 3, each patch (see Table 2) corresponded to a group, for a total of 15 groups.

Geographical Arrangement	Number of Groups	Variance Component	Percentage of Variation	p-value
1) Three Forks	3	Among groups	1.98	0.013
		Among samples	3.79	<0.001
		Within samples	94.23	<0.001
2) Middle Fork and North Fork versus South Fork	2	Among groups	2.61	<0.001
		Among samples	3.71	<0.001
		Within samples	93.67	<0.001
3) Patches	15	Among groups	2.96	0.114
		Among samples	2.24	<0.001
		Within samples	94.81	<0.001

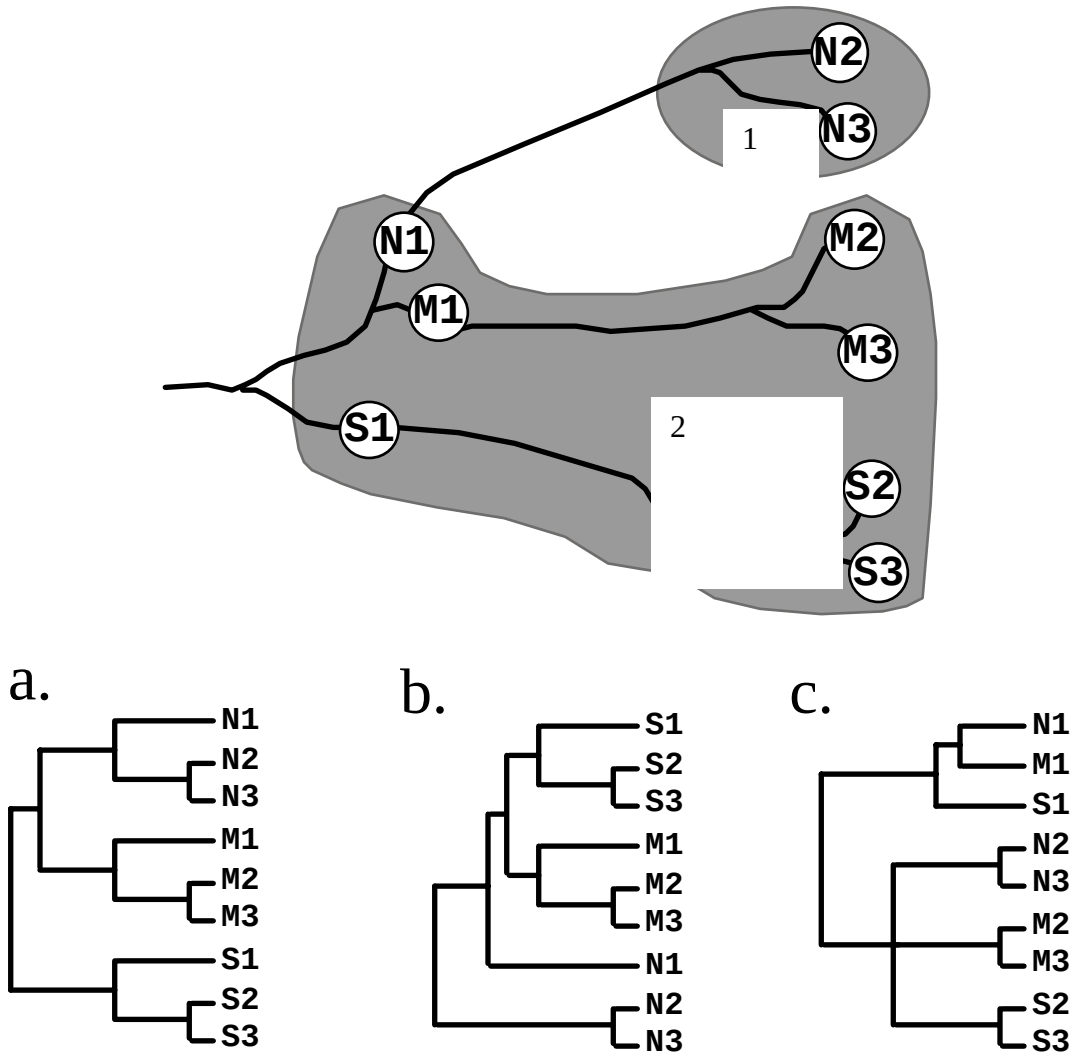

Figure 1. Possible associations between the geographic distribution of populations and their genetic relationships. A hypothetical drainage is diagramed in which there are three major subbasins, N, M, and S. Within each subbasin, three populations exist as represented by white circles. This subbasin is also divided by ecological factors into two patches (1 and 2). In panel a, population genetic structure mirrors the physical structure of the watershed. In panel b, genetic structure is determined primarily by the ecologically defined patch network within the watershed. In panel c, genetic similarity increases with decreasing geographic distance. This is comparable to the isolation by distance (IBD) model of Hutchison and Templeton (1999).

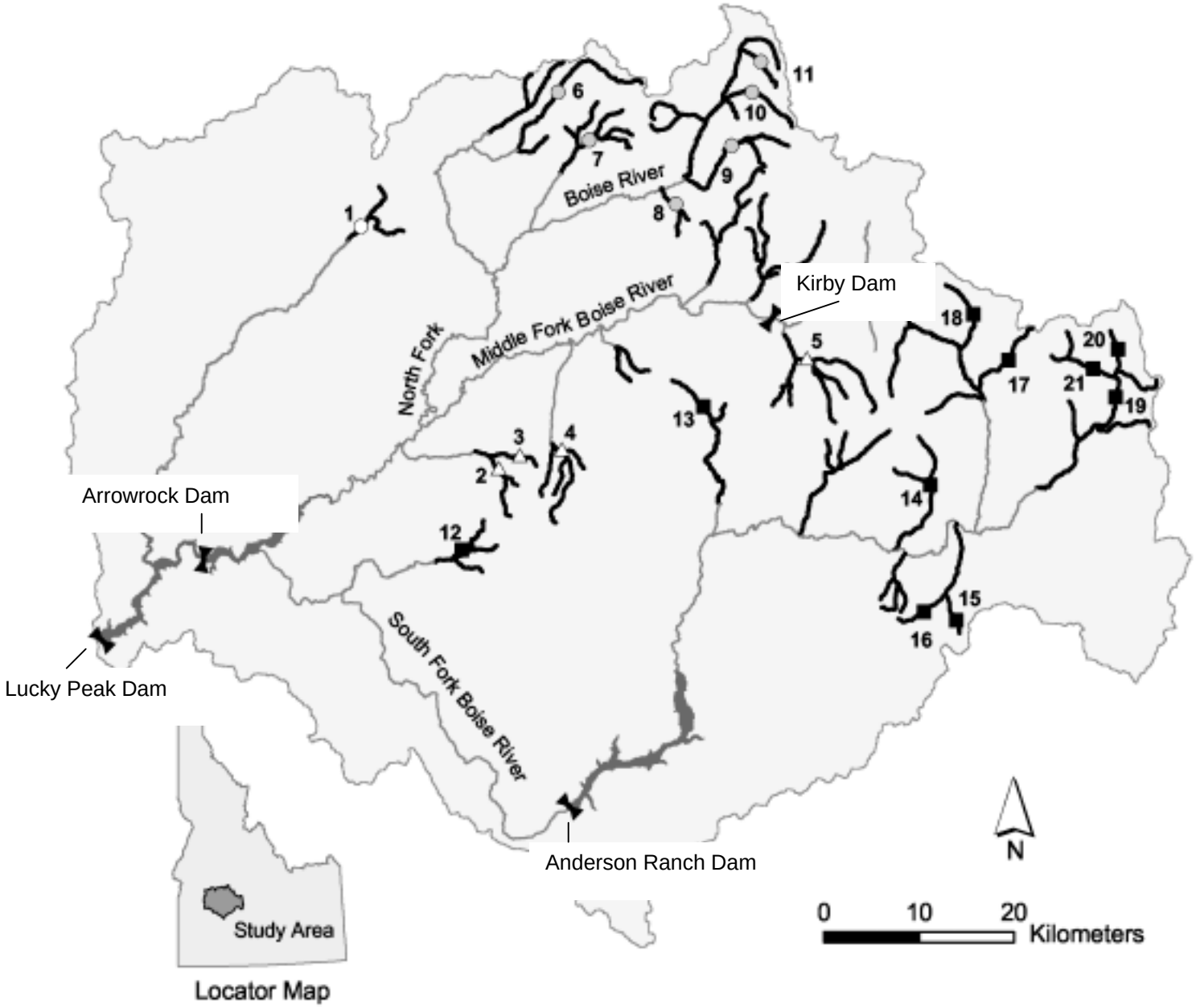

Figure 2. Map of the Boise River Basin. Sample numbers are given in Table 1. Sample sites are marked with open circles (Mores Creek), shaded circles (North Fork), open triangles (Middle Fork), or black squares (South Fork). Symbols correspond to Figure 3. Shaded stream segments are known to contain bull trout (Dunham and Rieman 1999). The four dams in this river system are shown.

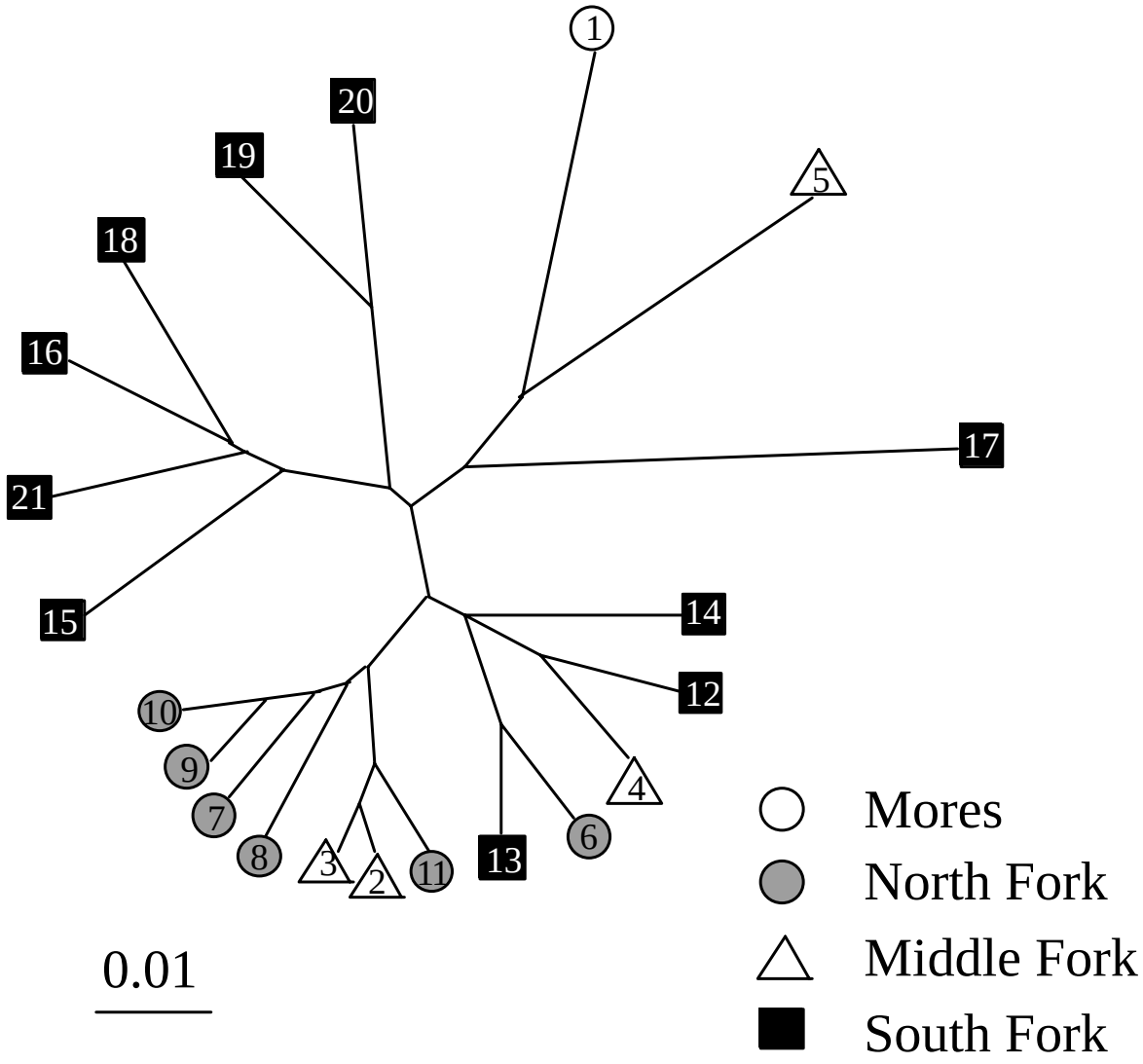

Figure 3. UPGMA dendrogram based on Cavalli-Sforza and Edwards' (1967) genetic distance. Sample numbers and symbols correspond to Table 1 and Figure 2.

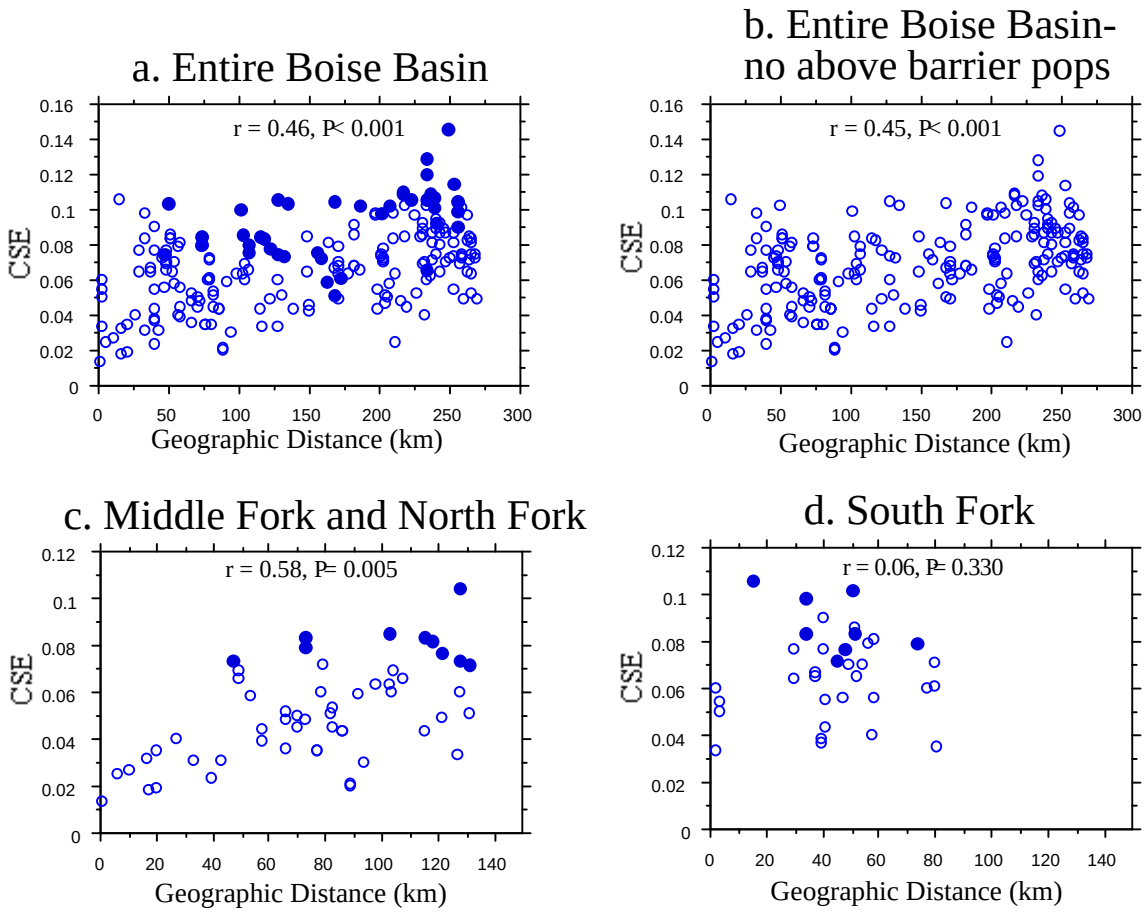

Figure 4. Isolation by distance analysis for the Boise River Basin. Pairwise Cavalli-Sforza and Edwards' (1967) genetic distances are plotted against pairwise geographic distances. Panel a shows all populations in the Boise River Basin. The Mores Creek and Yuba River samples are shown as filled circles in panel a and removed from panel b because they are separated from the rest of the populations by dams. Panel c shows Middle and North Fork populations where comparisons with the Yuba River sample are again shown as filled circles. Panel d shows South Fork populations where comparisons with the Emma Creek sample are shown as filled circles because this population appears to be an outlier and may be separated by a barrier. The results of Mantel Tests are shown for each panel.

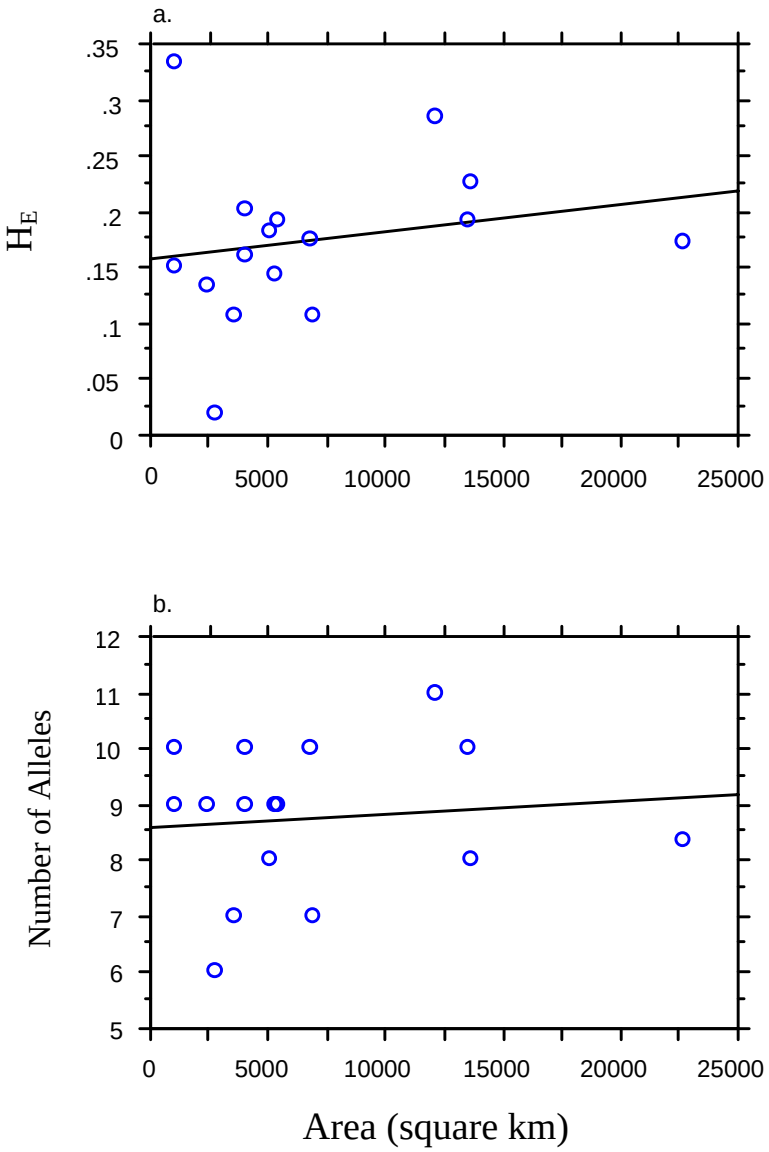

Figure 5. Relationship between patch area and (a) expected heterozygosity or (b) total number of alleles for the Boise River Basin. Neither of these relationships was significant.

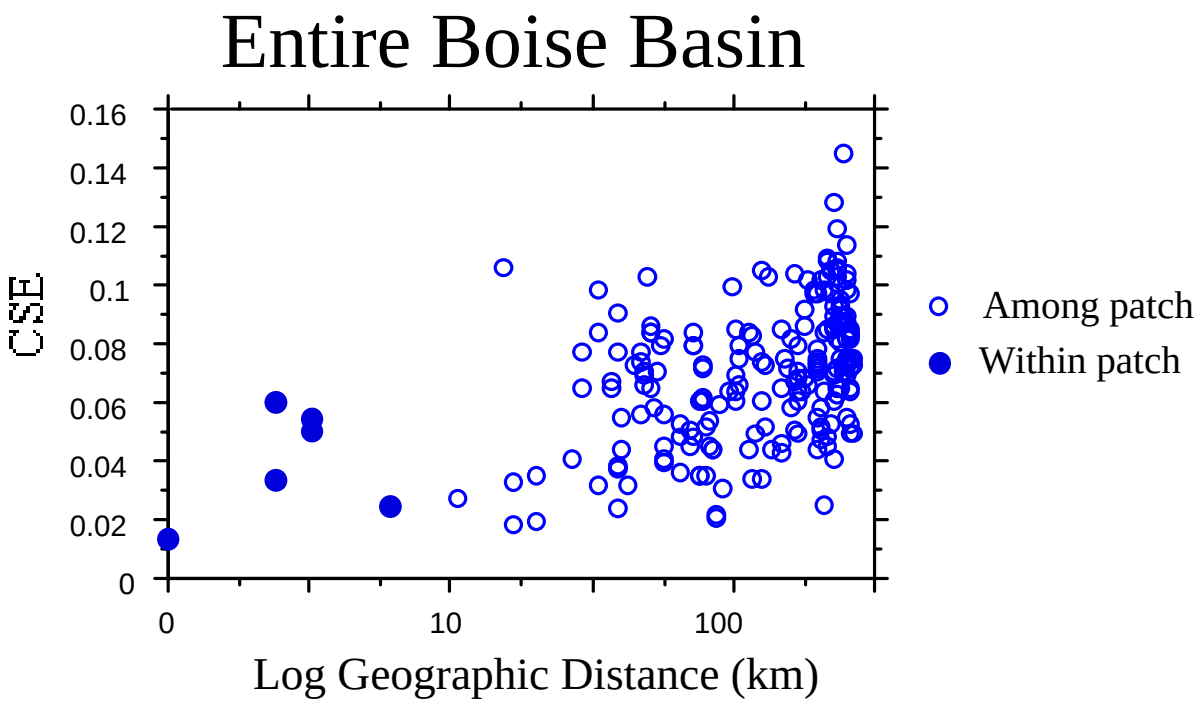

Figure 6. Isolation by distance (IBD) analysis using log-transformed geographic distance for all pairwise comparisons in the Boise system. Pairwise Cavalli-Sforza and Edwards' (1967) genetic distance is plotted against log-transformed geographic distance. This is the same relationship shown in Figure 3a except for the log-transformation of geographic distance. Shaded circles are the six within-patch comparisons (Table 2; Figure 2). Open circles are comparisons of populations in separate patches.