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SCALE DEPENDENT GENETIC STRUCTURE OF IDAHO GIANT SALAMANDERS (*DICAMPTODON ATERRIMUS*) IN STREAM NETWORKS

By

LINDY BETH MULLEN

B.S., Central Washington University, Ellensburg, WA, 2006

Thesis

presented in partial fulfillment of the requirements
for the degree of

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in Organismal Biology and Ecology

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ABSTRACT

Mullen, Lindy, M.S., Spring 2009 Division of Biological Sciences

SCALE DEPENDENT GENETIC STRUCTURE OF IDAHO GIANT SALAMANDERS (*DICAMPTODON ATERRIMUS*) IN STREAM NETWORKS

Co-Chairperson: Dr. Winsor H. Lowe

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Stream network structure constrains population processes of freshwater organisms, with individual, population and community level consequences. This consistent structure provides a framework in which examination of life-history influences on population genetic structure may provide general insight. I examined how stream network structure affects gene flow and genetic structure of the facultatively paedomorphic Idaho Giant salamander, *Dicamptodon aterrimus* in Idaho and Montana, USA. I used microsatellite data to test population structure models by (i) examining hierarchical partitioning of genetic variation in stream networks and (ii) testing for genetic isolation by distance along stream corridors versus overland pathways. Replicated sampling of streams within catchments within three river basins revealed that stream hierarchical scales had strong effects on gene flow and genetic structure. AMOVA identified significant structure among all hierarchical levels (among streams, among catchments, among basins), and divergence among catchments had the greatest structural influence. Isolation by distance was detected within catchments, and in-stream distance was a strong predictor of genetic divergence. Patterns of genetic divergence suggest that differentiation among streams within catchments was driven by limited migration according to the stream hierarchy model, but divergence among catchments and among basins was due to genetic drift, consistent with the death valley model of population structure (Meffe and Vrijenhoek 1988). These results show the strong influence of stream networks on population structure and genetic divergence of a salamander with contrasting effects at different hierarchical scales.

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Introduction

Many species occur in spatially structured sub-populations linked by dispersal and gene flow (Wright 1951, Hanski and Gilpin 1997). While dispersal has important ecological and evolutionary consequences for populations (Hanski and Gilpin 1997, Dieckmann et al. 1999), direct measurements capturing both short and long distance dispersal are difficult to obtain. However, dispersal is closely related to gene flow (Bohonak 1999), which is a primary force that influences genetic differentiation among natural populations (Wright 1951). This genetic structure of populations can be assessed with molecular methods (Bohonak 1999) and used to infer the relative importance of dispersal and gene flow. Much interest has focused on measuring how landscape barriers affect dispersal and gene flow (Manel et al. 2003) . However, results of these studies are often species and system-specific (Hitchings and Beebee 1997, Keyghobadi et al. 1999, Funk et al. 2005, Cushman et al. 2006, Wang et al. 2009). General insight on what controls gene flow and genetic differentiation may best be gained in systems that impose consistent structure across spatial scales, such as dendritic systems of rivers and streams (Grant et al. 2007).

Streams and rivers occur in hierarchical networks where smaller stream channels join to form larger ones in a dendritic pattern that resembles branches on a tree. Rivers and streams also have a fractal-like structure that is highly consistent across scales (Horton 1945). This consistent network architecture of rivers and streams can constrain evolutionary, demographic, and ecological processes, making dendritic ecological networks useful for understanding spatial processes (Lowe et al. 2006a, Grant et al. 2007).

Recent studies show that stream network architecture interacts with physiology, dispersal, and life-history to influence patterns of gene flow and genetic structure in freshwater organisms (Castric et al. 2001, Heath et al. 2001, Costello et al. 2003, Whiteley et al. 2004, Cook et al. 2007). The genetic structure of freshwater organisms has been described by four models (Figure 1; Finn et al. 2007; Meffe and Vrijenhoek 1988). i) Organisms with strictly aquatic life-histories may be characterized by Meffe and Vrijenhoek's (1988) stream hierarchy model of genetic structure (SHM). This model predicts genetic variation to be partitioned by drainage structure, with in-stream dispersal explaining patterns of genetic variation (Meffe and Vrijenhoek 1988). ii) Other strictly aquatic organisms that are isolated in the headwaters by ecological barriers may be characterized by Meffe and Vrijenhoek's (1988) death valley model of genetic structure (DVM). The DVM predicts that populations of habitat specialists will show strong genetic differentiation with no relationship to landscape structure and no isolation by distance—IBD (Meffe and Vrijenhoek 1988, Preziosi and Fairbairn 1992, Finn et al. 2006). iii) Organisms that do not disperse in streams but only overland are characterized by the headwater model of genetic structure (HM), which predicts genetic variation is partitioned in headwater islands (Finn et al. 2007). iv) Lastly, organisms with high gene flow among localities are characterized by the null model which predicts no divergence among sites (Steele et al. 2009).

While useful for characterizing population structure in stream networks, these models do not address an equally important aspect of genetic structure: how patterns of population divergence change with hierarchical scale (Hutchison and Templeton 1999). Stream networks themselves are fractal in structure, with self-similarity across spatial

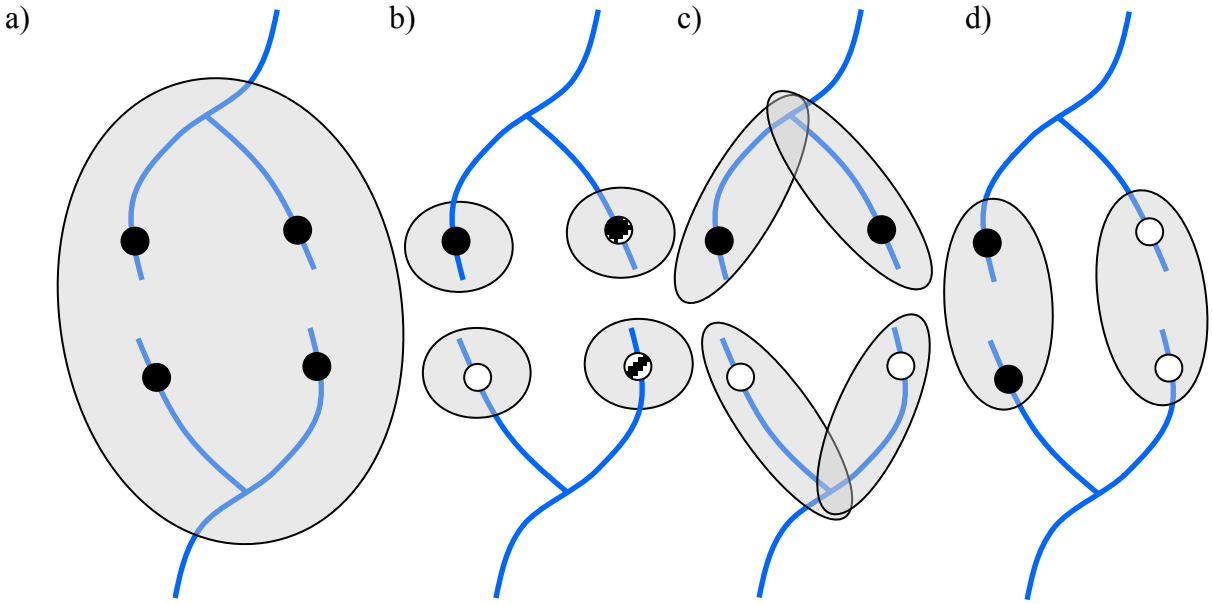

Figure 1. Diagrams of conceptual models for patterns of movement and genetic structure in headwater streams organisms; where a) is the Null Model, b) the Death Valley Model, c) the Stream Hierarchy Model, and d) the Headwater Model. Grey areas show pathways of dispersal in each model. Open, closed, and patterned circles indicate the genetic similarity of localities (Finn et al. 2007; Meffe and Vrijenhoek 1988).

scales. Therefore, population structure may also be scale-specific. To understand how patterns of gene flow and population structure can change across hierarchical scales, sampling must allow for analysis at multiple scales (Fausch et al. 2002, Lowe et al. 2006b). A lack of systematic hierarchical sampling has limited the ability of previous studies to address both the effect of network structure on population structure and the scaling of this effect. By applying a consistent sampling design that encompassed three stream hierarchical scales (streams, catchments, basins; Figure 2), my goal was to explore the effect of network structure on population structure. I used the Idaho Giant salamander, *Dicamptodon aterrimus*, to measure how stream network structure, life-history, and dispersal interact to influence genetic population structure across river network scales.

I examined genetic variation of microsatellite loci to investigate the genetic population structure of *D. aterrimus* in river networks of Idaho and Montana. Using microsatellite data, I tested Meffe and Vrijenhoek's (1988) and Finn et al.'s (2007) models of population structure by: (i) examining hierarchical partitioning of genetic variation at multiple spatial scales in stream networks, and (ii) testing for isolation by distance (along stream corridors and overland) to examine the relative influence of within-stream and overland gene flow on population structure. My results suggest both that stream network structure strongly affects population structure of *D. aterrimus*, and that population processes differ depending on hierarchical scale.

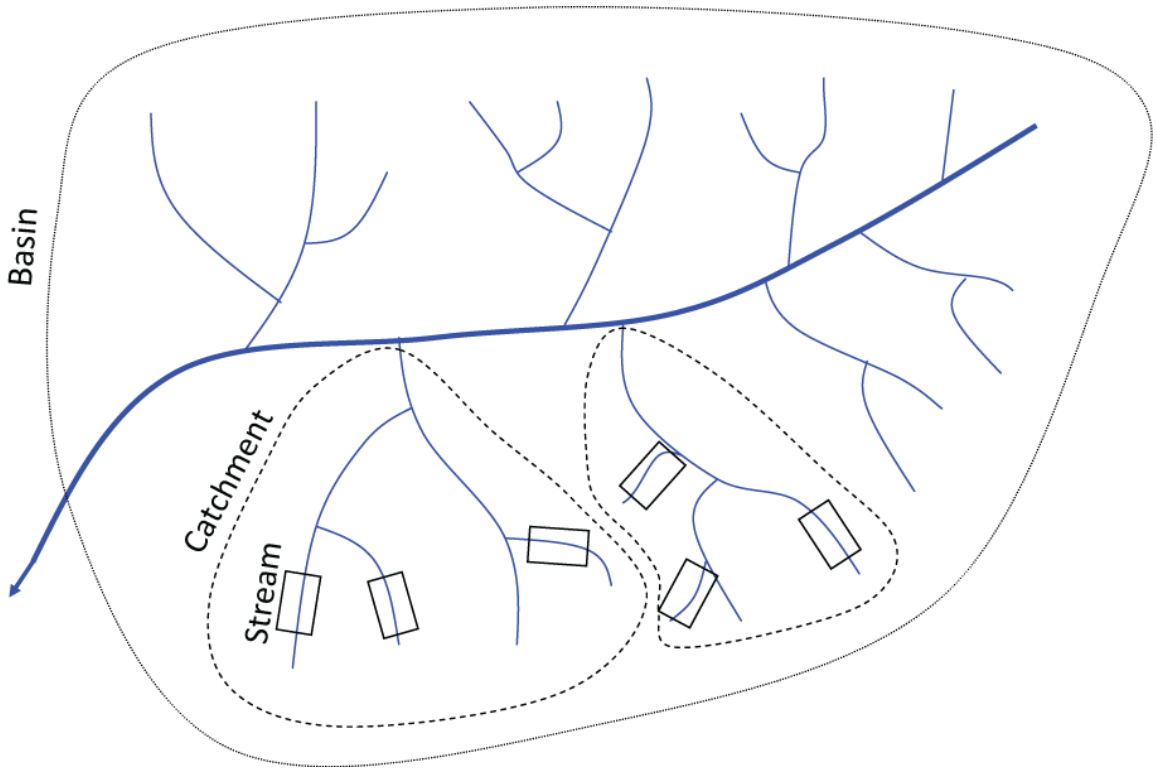

Figure 2. Sampling design showing hierarchical scales; streams nested within catchments within basins. Three streams were sampled within each catchment, survey reaches of streams are indicated by rectangles.

Materials and Methods

Species and Site

The Idaho Giant salamander, *Dicamptodon aterrimus* occurs in mesic forests of northern Idaho and western Montana, USA. This species was isolated from other *Dicamptodon* between 2-5 million years ago due to the xerification of the Columbia basin following the orogeny of the Cascade Mountains (Carstens et al. 2005a). Mitochondrial DNA analysis supports a single refugial population in the south fork of the Salmon River of Idaho during the last glacial maximum (Carstens et al. 2005b), with range expansion and colonization of habitats most likely occurring northward as glaciers receded. The current distribution extends from the south fork of the Salmon River in Idaho to the northernmost peripheral populations in the St. Regis drainage of Montana. While its current distribution is patchy (Carstens et al. 2005b), we know occurrence of *D. atterrimus* is influenced by landscape scale factors including roads, stream isolation, and old growth forest density, and abundance is positively related to the proportion of fine substrate (Sepulveda and Lowe 2009).

D. aterrimus is facultatively paedomorphic: larvae develop in streams and reach maturation after several years as either terrestrial or aquatic forms (Nussbaum et al. 1983). While no empirical data on overland dispersal exists for *D. aterrimus,* Richardson and Neill (1998) showed that its facultatively paedomorphic sister species, *D. tenebrosus*, can move several hundred meters overland in a few days. Direct measures of in-stream dispersal by *D. aterrimus* show that short-distance movement of *D. aterrimus* (5-50m) within streams is common, but movements > 100m are rare (Sepulveda and Lowe, in prep). However, we lack information on the frequency and scale of dispersal beyond

individual streams, and on the relative importance of movements along the stream corridor versus overland pathways.

Sampling Design

To examine the spatial extent of gene flow and population structure in *D. aterrimus*, I applied a consistent sampling design that encompassed three hierarchical levels: streams, catchments, and basins. I sampled individuals in 1st-order streams which were nested within catchments of confluent streams draining into a mainstem river (Figure 2). Catchments were nested within basins of three major rivers: the Lochsa (four catchments), St. Joe (two catchments) and St. Regis (two catchments). I collected 15 *D. aterrimus* from three 1st-order streams within each catchment (Appendix A, Figure 3). Catchments were selected in basins so that they were separated by a common ridge running approximately perpendicular to the mainstem river. This orientation allowed me to test for in-stream and overland gene flow within and among adjacent catchments.

In each stream, I used an LR-20 backpack electrofisher (Smith-Root Inc., Vancouver, WA) to collect salamanders from stream reaches beginning at least 25 m upstream of the confluence with a higher-order stream. Survey reaches ranged from 125-391 m in length (mean survey length± 1 SD: 220 m ± 72.7). Longer survey reaches were required to capture the minimum number of individuals used for analyses. In two streams (LWWF and LPEF; Appendix A), I sampled three 30 m reaches separated by approximately 15 m. I clipped a small section of tail tissue from captured salamanders and stored it in 95% ethanol. Both juveniles and adult salamanders were sampled.

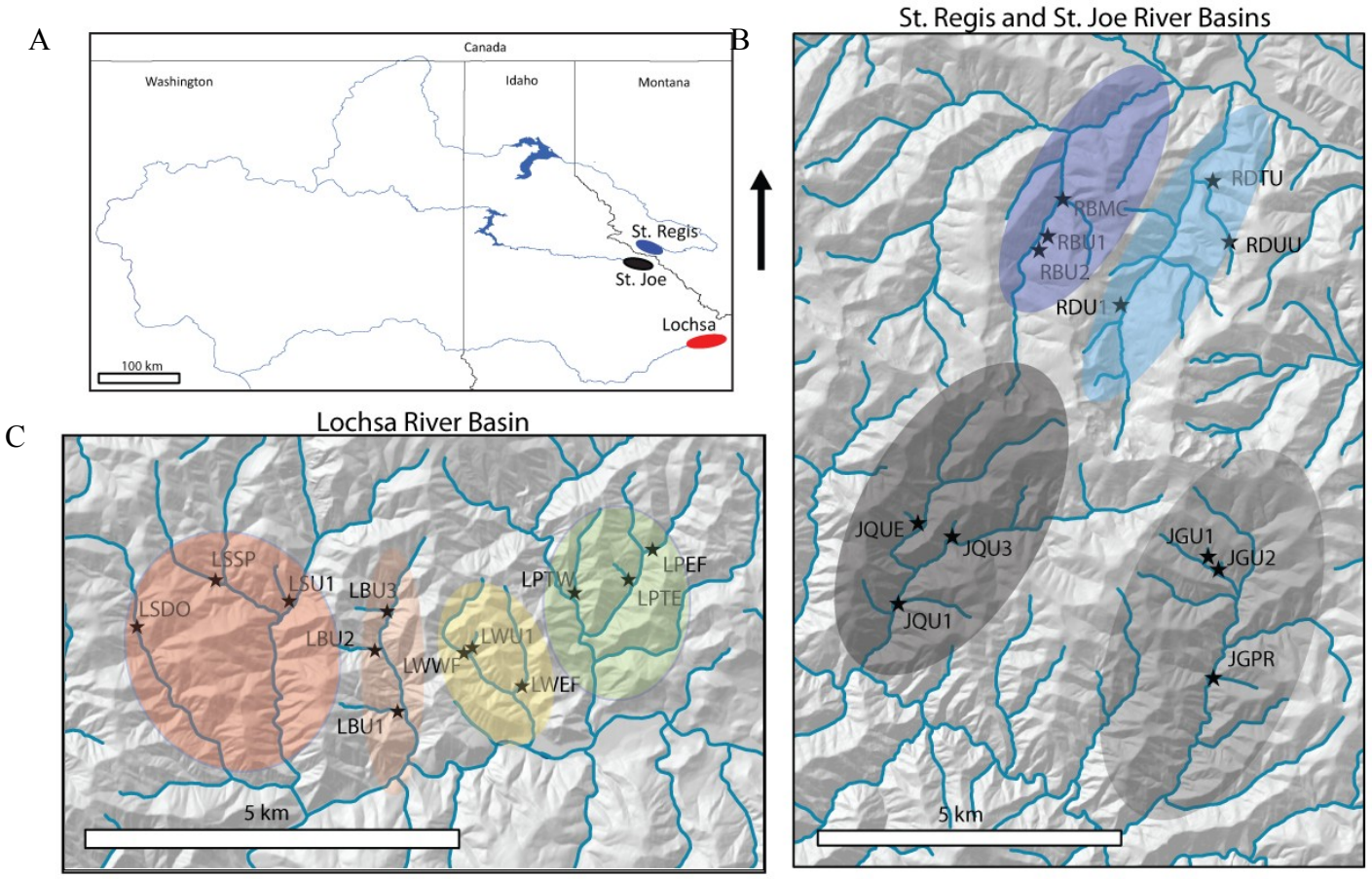

Figure 3. Location of streams sampled for *D. aterrimus* in the Lochsa and St. Joe basins of Idaho and the St. Regis basin of Montana, USA. Stream labels correspond to Appendix A. A) Regional map showing river connections among basins. B) Streams sampled in the St. Regis and St. Joe basins. In the St. Regis basin, Big Cr. catchment is highlighted in dark blue, and Deer Cr. network is highlighted in light blue. In the St. Joe basin, Quartz Cr. network is highlighted in dark grey and Gold Cr. network in light grey C) Streams sampled in the Lochsa basin. Squaw Cr. network is red, Badger is orange, Wendover is yellow, and Papoose Cr. network is in green.

Snout-vent lengths of sampled animals ranged from 22mm-160mm and weights ranged from <1g to 130 g. All sampling took place in July – October of 2008, except for five samples from one stream that were collected in July of 2007 (LSSP; Appendix A).

Microsatellite amplification and scoring

Fifteen salamanders from each stream were genotyped at 14 microsatellite loci (Appendix B) developed for *Dicamptodon tenebrosus* and *D. copei* (Curtis and Taylor 2000, Steele et al. 2008). To extract DNA, I digested tissues with protease in a detergent based cell lysis buffer, then precipitated proteins with an ammonium acetate solution and DNA with isopropyl alcohol. Isolated DNA was re-suspended in 100µl TE buffer and diluted 1:10 for polymerase chain reaction (PCR) amplification in a PTC-100 thermocycler (MJ Research Inc., Waltham, MA) with a total volume of 10µL. Multiplex reactions were setup with QIAGEN multimix, following the QIAGEN microsatellite protocol (QIAGEN Inc., Valencia, CA). I used a single PCR touchdown profile for multiplexed markers, primer annealing started at 67°C and dropped 0.5°C for 20 cycles, followed by 25 cycles with a 57°C annealing temperature. Microsatellite markers *Dte5*, *D04*, *D24*, and *D18* were PCR amplified individually following QIAGEN microsatellite protocols with separate PCR annealing temperatures (Appendix B). Following individual PCR, these markers were pooled with multiplexed markers for fragment analysis. PCR products were visualized on an ABI3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) in the Murdock DNA Sequencing Facility at the University of Montana, Missoula, USA. Allele sizes were determined using the ABI GS600LIZ ladder (Applied

Biosystems Inc.) and allele sizes were called with GeneMapper version 3.7 (Applied Biosystems Inc.).

Genetic Analyses

I tested for significant departures from Hardy-Weinberg proportions and for non-random association of pairs of loci across populations (linkage disequilibrium) using exact tests implemented in GENEPOP version 4.0 (Raymond and Rousset 1995). Loci that deviated from HW proportions in each population were removed from further analyses. After removal of a single locus (*Dte11*) that deviated from HW proportions in all populations where it was not fixed for a particular allele, I calculated genetic diversity within-streams and examined subdivision among hierarchical levels.

Genetic diversity within streams was calculated as allelic richness (A_S), the number of alleles observed in populations (N_A), and expected and observed heterozygosity (H_E, H_O). I then tested for differentiation among streams and the extent of gene flow with pairwise F_{ST}, a measure of genetic divergence based on the number of different alleles, calculated in Arlequin version 3.1 (Excoffier et al. 2005). The inbreeding coefficient, F_{IS}, was calculated for every locus in each stream to detect significant heterozygote deficit or excess in streams (GENEPOP; Raymond and Rousset 1995).

I examined pairwise F_{ST} values to assess levels of divergence occurring among streams. Then I used a hierarchical analysis of genetic variation (AMOVA implemented in the hierfstat package in R v 2.8.1, Goudet, 2005) to partition genetic variance within and among hierarchical scales. Initially I used the entire data set to assess patterns of genetic variance throughout the entire sampling area (Figure 3). Specifically, I tested for

structure at four levels: among-basins, among catchments within basins, among streams within catchments, and within streams. To test for influence of local genetic structure on overall patterns, I performed two additional AMOVAs: (i) within the Lochsa basin, and (ii) within and among the St. Joe and St. Regis basins. The AMOVAs generated hierarchical F-statistics (Yang 1998) in which F_{BT} was divergence among basins, F_{CB} was divergence among catchments within basins, F_{SC} was divergence among streams within catchments, F_{IS} was the inbreeding coefficient of streams, and F_{ST} was the global divergence among streams.

Genetic structure was also visually interpreted using principal components analysis (PCA) which reduces dimensions in a multivariate dataset such that the first principal component (PC1) explains as much of the variance in allele frequencies as possible (Reich et al. 2008). To maintain quasi-independence of the data set, I removed the highest frequency allele of each microsatellite locus and performed the PCA on remaining allele frequencies (Leary et al. 1993). Plots of PC1 against PC2 and of PC1 against PC3 were examined to assess the similarity of allele frequencies among streams within catchments, among catchments within basins and among basins.

I used partial Bayesian individual assignment tests (Rannala and Mountain 1997) to classify individuals to populations based on the expected frequency of an individual's multilocus genotype in each population (basins, catchments, and streams; GENECLASS2; Piry et al. 2004). Those individuals most likely to originate from a population other than their sampling origin were examined with a partial Bayesian exclustion test for a measure of confidence associated with assignment. Individuals with lower than 95% probability of originating in the sampled population were also tested with

exclusion methods. Leaving the individual to be assigned out, distributions of genotypic likelihoods that would occur in sampled populations were approximated with 10,000 Monte Carlo simulations. The likelihoods calculated for genotypes of sampled individuals were compared to that distribution, and if the genotype likelihood was below the $\alpha = 0.01$ threshold, the population was excluded as an origin (Cornuet et al. 1999, Paetkau et al. 2004, Piry et al. 2004). Assignments of individuals to populations other than their collection location were interpreted as migration events when genotypes were unlikely to occur from a random combination of alleles ($p \geq 0.95$). This method can accurately identify migrants especially when genetic differentiation is substantial and many loci are used (Berry et al. 2004, Paetkau et al. 2004). I performed three assignment tests with the above standards: (i) assignment of individuals to basins with basins as reference populations, (ii) assignment of individuals to catchments with catchments as reference populations, and (iii) assignment of individuals to streams with streams as reference populations.

To understand the role of gene flow by in-stream versus overland pathways, I tested alternative hypotheses of *D. aterrimus* gene flow resulting in isolation by distance (IBD). IBD is detected by testing for correlations among matrices of genetic distance (F_{ST}) and geographic distance with Mantel tests that correct non-independence of pairwise points (Mantel 1967). I used two measures of pairwise distance between midpoints of survey reaches to test alternate pathways of gene flow with FSTAT version 2.9.3.2 (Goudet 1995).

To test the hypothesis that *D. aterrimus* gene flow occurs primarily along stream corridors (isolation by stream distance IBSD), I estimated the correlation between F_{ST} and

stream distance in each basin. Stream distance was the shortest pathway along streams connecting two points (ArcMap 9.2, ESRI, Redlands, CA). Second, I tested the hypothesis that gene flow in *D. aterrimus* occurs primarily overland (isolation by Euclidean distance IBED) by estimating the correlation between F_{ST} and surface distance in each basin. Surface distance was the Euclidean distance connecting two points that corrects for changes in elevation along the path (ArcMap 9.2, ESRI, Redlands, CA). Significance of correlations in all Mantel tests were assessed with 10,000 matrix randomizations. Basins were tested separately for IBSD and IBED to detect regional differences in the scale and strength of IBD due to in-stream versus overland gene flow. Pairwise stream and surface distances were significantly correlated ($r = 0.88$, $p < 0.001$). Therefore, the strength of correlations of genetic distance with stream distance versus surface distance were used to assess the relative importance of in-stream versus overland gene flow.

Results

361 individuals from 24 streams (Appendix B, Figure 3) were genotyped at 14 microsatellite loci. Four microsatellite loci were monomorphic and were therefore discarded (Appendix B). Another locus, *Dte11*, deviated significantly from HW proportions in 3 of the 6 streams exhibiting polymorphism before correction for multiple significance tests. Moreover, the inbreeding coefficient for *Dte11* indicated a deficit of heterozygotes and suggested the presence of a null allele. Because *Dte11* was not highly polymorphic and did not conform to HW expectations it was removed from further analyses. No other locus had significant departures from HW proportions in more than

three streams after correcting for multiple significance tests with sequential Bonferroni corrections (Rice 1989). Two of 24 streams deviated from HW proportions with only a single locus out of HW proportions (Appendix C). After sequential Bonferroni correction, no populations deviated significantly from HW proportions. Of the 707 tests for linkage disequilibrium, 5.1% were significant ($p < 0.05$), just slightly more than expected by chance with multiple tests. No pairs of loci were non-randomly associated in more than four of 24 streams, and no comparisons were significant after sequential Bonferroni correction for multiple testing (Rice 1989).

Overall, genetic variation was low and in most streams at least one locus was fixed for a particular allele (Appendix C). Six F_{IS} values were significantly different from zero before correcting for multiple tests, none were significant after sequential Bonferroni correction (Rice 1989), and no population had more than two loci showing either heterozygote excess or deficit. Pairwise genetic distances (F_{ST}) among streams exhibited a wide range of values with the lowest divergence between streams within catchments. Overall, divergence among streams tended to be high (median $F_{ST} = 0.39$; Appendix D). Five pairwise F_{ST} values were not significantly different from zero and all non significant tests corresponded to pairs of streams in the same catchment.

The global AMOVA indicated significant structure at all levels (Table 1). Most genetic variation occurred among individuals within streams, and the greatest proportion of structural genetic variation was due to differences among catchments within basins. While there was significant variation due to differences among streams within catchments, this level explained a small proportion of variation in the data (<10%). The within-Lochsa basin AMOVA resulted in the same patterns as the global AMOVA.

Table 1. Results of hierarchical analysis of molecular variance, A. Global AMOVA, B. Within Lochsa AMOVA, C. St. Joe - St. Regis AMOVA. Significant P values are bolded.

	Source of Variation	df	Variance components	Percentage of variation	F statistics	p
A	Among basins	1	0.725	13.0	F_{BT} = 0.130	**0.0022**
	Among catchments within basins	2	1.285	23.1	F_{CB} = 0.266	**<0.001**
	Among streams within catchments	5	0.310	5.6	F_{SC} = 0.087	**<0.001**
	Within streams	353	3.236	58.2	F_{IS} = -0.024	
	Total	361	5.556		F_{ST} = 0.418	
B	Among catchments within basins	1	1.117	24.3	F_{CB} = 0.243	**<0.001**
	Among streams within catchments	3	0.335	7.3	F_{SC} = 0.096	**<0.001**
	Within streams	176	3.147	68.4	F_{IS} = -0.012	
	Total	180	4.599		F_{ST} = 0.316	
C	Among basins	1	0.044	0.8	F_{BT} = 0.009	0.1685
	Among catchments within basins	1	1.533	29.6	F_{CB} = 0.298	**0.0039**
	Among streams within catchments	2	0.285	5.5	F_{SC} = 0.079	**<0.001**
	Within streams	177	3.324	64.1	F_{IS} = -0.035	
	Total	181	5.187		F_{ST} = 0.359	

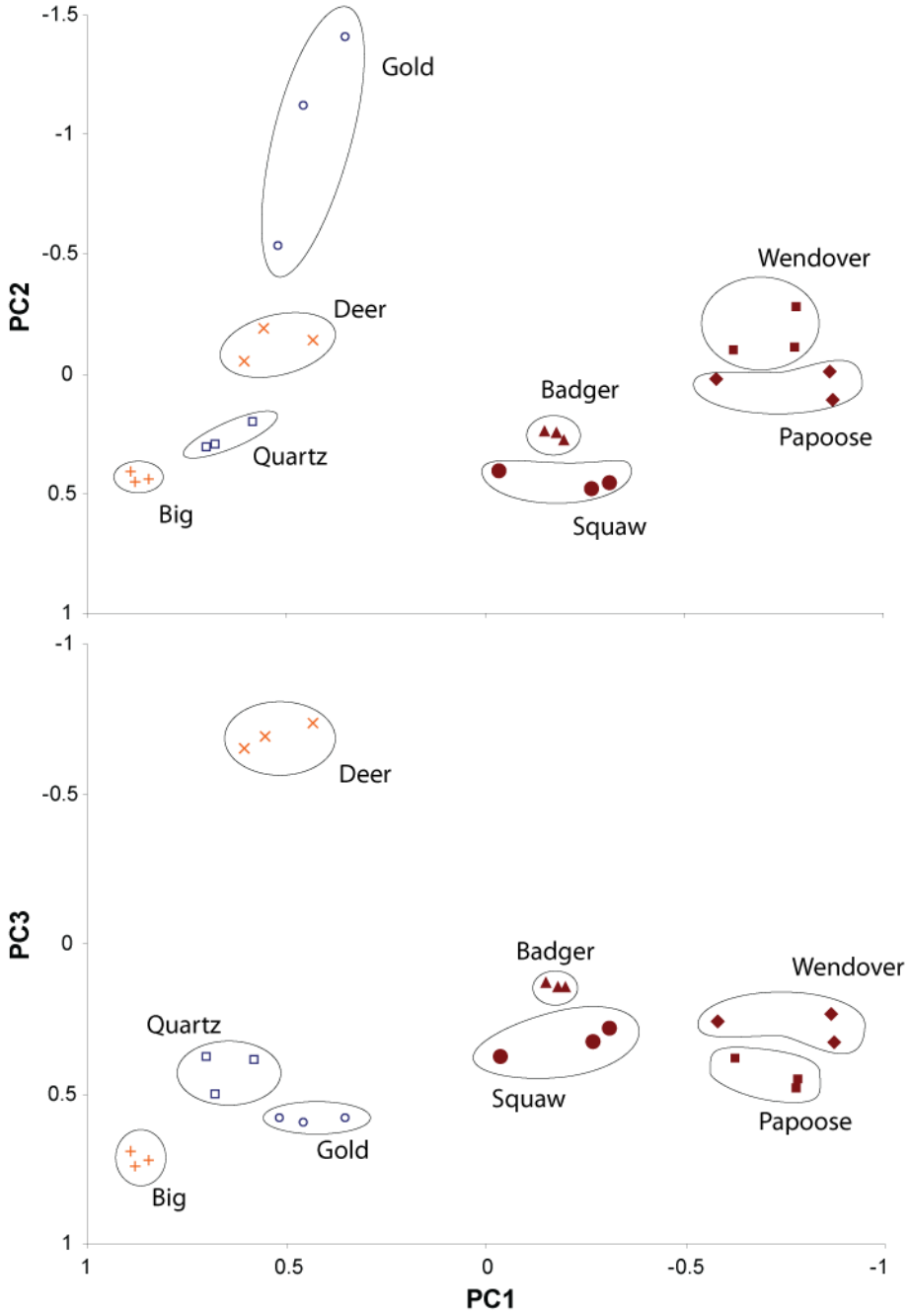

Figure 4. Plots of the first three principal component scores of allele frequencies of 9 microsatellite loci among streams sampled from basins and catchments in Idaho and Montana. Points corresponding to streams within catchments are circled, catchments are labeled. Streams sampled in the Lochsa basin are red, streams from the St. Joe basin are blue, streams from the St. Regis basin are orange.

Conversely, the St. Joe-St. Regis basins AMOVA indicated that variation due to differences among basins was not significant, accounting for less than 1% of the total genetic variation. However, variation among catchments in the St. Joe / St. Regis complex was highly significant, accounting for 29.5% of the total genetic variation (Table 1).

Principal components analysis showed concordant patterns of genetic divergence across hierarchical river network scales. The first principal component (PC1), which accounted for 30% of the variance in allele frequencies, separated catchments into three groups consisting of (i) St. Regis and St. Joe catchments, (ii) Papoose Cr. and Wendover Cr. catchments in the Lochsa, and (iii) Badger Cr. and Squaw Cr. catchments in the Lochsa (Figure 4). The second principal component (PC2), which accounted for an additional 18% of the variation, and the third principal component (PC3), which accounted for an additional 14% of the variation, separated catchments in the St. Regis and St. Joe basins but did not group catchments from basins together.

Individual assignment tests (Rannala and Mountain 1997) supported patterns of genetic structure shown in AMOVA and PCA. The majority of individuals were assigned to the basin (99.4%) and catchment (98.9%) where they were sampled. However assignment of individuals to the stream where they were sampled was much lower (67.1%). Individuals most likely to originate from a population other than their sampling origin ($n = 119$) and those assigned to their sampling origin with $p < 0.95$ ($n = 147$) were evaluated with exclusion methods for a measure of confidence associated with assignment (Paetkau et al. 2004).

The partially Bayesian exclusion test identified no potential migrants among basins, one potential migrant among catchments in the Lochsa, and five potential migrants among streams within catchments in the Lochsa and St. Regis basins. Exclusion tests identified 156 individuals that had the highest likelihood of occurring in another stream. Two of those were assigned to an unsampled stream ($p < 0.01$). Six had the highest likelihood or originating in a stream from a neighboring catchment in the Lochsa basin (2 individuals $p > 0.90$, 4 individuals $p < 0.7$). The remaining 148 individuals had the highest likelihood of occurring in another stream within the catchment. Although only 5 were considered potential migrants ($p \geq 0.95$), 67 individuals had a high likelihood of originating from another stream within the catchment ($p > 0.7$). These individuals may be descendants of immigrants from several generations back. Collectively, individual assignments identified more migrants among streams within catchments than among catchments or among basins.

The hierarchical analysis of genetic variation (AMOVA) identified subdivision due to restricted gene flow across catchment boundaries. This pattern suggests that genetic exchange is more frequent within than between catchments. Therefore, if gene flow is limited by geographic distance, this should be manifested as isolation by distance within catchments. Plots of pairwise genetic and geographic distances in basins show a positive relationship between F_{ST} and stream distance among pairs of streams within catchments (Figure 5). However no relationship was apparent for pairs of streams that were not in the same catchment. The change in the relationship between F_{ST} and geographic distance suggests a major shift in the relative influences of gene flow and drift that occurred in my data according to catchment boundaries. As streams became more

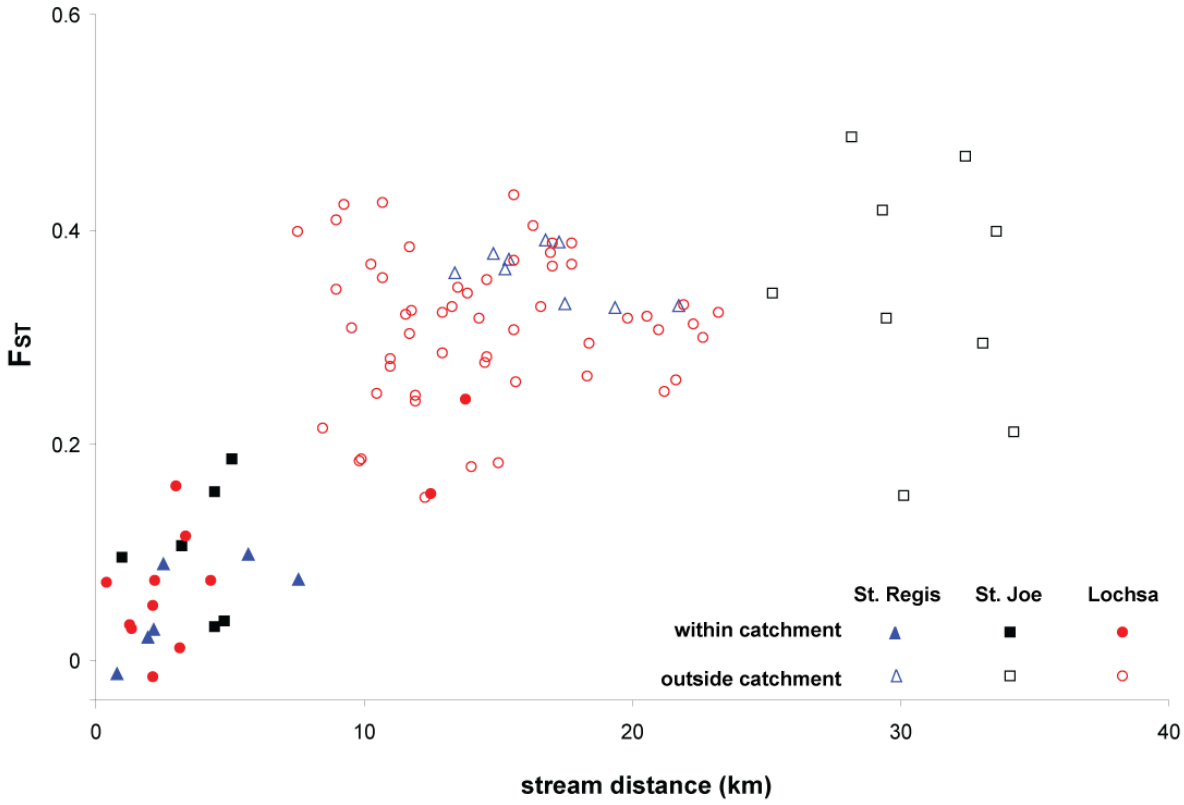

Figure 5. Scatter plot of F_{ST} and stream distance for pairs of streams within the same basin. Pairs in the St. Joe basin are black, in the St. Regis basin are blue, in the Lochsa basin are red. Pairs of streams that are located within the same catchment are distinguished from those that are not within the same catchment.

geographically distant, genetic distance (F_{ST}) increased, suggesting IBD within catchments. However, pairs of streams that were separated by catchment boundaries, showed no indication of IBD.

Using pairwise genetic distances between streams, there was a significant, positive correlation between stream distance and F_{ST} (IBSD) in the Lochsa basin (Mantel; $r = 0.63$, $p < 0.001$), in the St. Regis basin ($r = 0.93$, $p < 0.001$), and in the St. Joe basin ($r = 0.83$, $p < 0.001$). There were significant but weaker positive correlations between surface distance and F_{ST} (IBED) in the Lochsa basin ($r = 0.42$, $p < 0.001$), in the St. Regis basin ($r = 0.80$, $p < 0.001$), and in the St. Joe basin ($r = 0.72$, $p < 0.01$). All Mantel tests were significant after sequential Bonferroni adjustment. Because of the limited number of streams sampled within catchments, I could not test correlations within individual catchments. In sum, correlations of F_{ST} and geographic distance were higher for stream distance than surface distance, and analyses of IBD within basins reveal that there is IBD among streams within catchments.

Discussion

Evolution in stream networks

My microsatellite data show that hierarchical scale is important for microevolution of freshwater organisms in stream networks. Consistent sampling across three hierarchical scales (streams, catchments, basins) provided a framework to test for the influence of stream network structure on genetic structure. Although differences among streams, among catchments, and among basins all contributed to genetic structure, structure was clearly dominated by two patterns: isolation and high divergence between

adjacent catchments but low divergence among streams within catchments. These data suggest that among-catchment structure is driven by genetic drift, which is consistent with the death valley model of population structure (Meffe and Vrijenhoek 1988). They also suggest that within-catchment structure is driven by gene flow among streams, consistent with the stream hierarchy model (Meffe and Vrijenhoek 1988). These results show that dispersal patterns at small and intermediate spatial scales were very different than dispersal patterns on a larger spatial scale.

Divergence among catchments due to genetic drift had a large effect on *D. aterrimus* population structure (global $F_{CB} = 0.27$). There was also evidence for significant divergence among streams (global $F_{SC} = 0.09$), but to a much lower degree than among catchments. While gene flow can explain the moderate divergence among streams, distinguishing between contemporary and historical gene flow is difficult (Peakall et al. 2003). Two lines of evidence point to contemporary gene flow as the cause of this pattern, including observations in the field that suggest small population sizes and the identification of potential migrants with individual assignment tests.

Although I did not estimate population sizes directly, my field surveys suggest that streams were occupied by a small number of individuals: significant effort was required to collect just 15 individuals from many sites (up to 2 hours shock time). Because effective population sizes (N_e), are often only 10% of census population sizes (N_C) in wildlife populations (Frankham 1995), and estimates of N_e are generally lower than N_C for salamanders (Gill 1978, Jehle et al. 2005), my field surveys suggest N_e of *D. aterrimus* was small. Since divergence among populations is a function of N_e and time (t) according to the equation: $[F_{ST} = 1-(1-1/2(N_e))^t]$, F_{ST} rapidly increases over short

periods of time when N_e is small (Wright 1969, Nei and Chakravarti 1977). Genetic divergence would also be elevated by variable N_e among streams (Whitlock 1992). Therefore, it appears likely that migration was important in minimizing divergence among streams. Individual assignment tests provide further support for contemporary migration among streams, identifying few potential migrants among basins and catchments, but many among streams within catchments.

During the most recent glacial maximum (18,000 ybp), the Cordilleran ice sheet extended into northern Idaho (Richmond et al. 1965), forcing organisms into southern refugia that provided climatic insulation (Daubenmire 1975). Genetic data have identified the locations of refugia for codistributed amphibians: the Coeur d' Alene salamander (*Plethodon idahoensis*) in the Clearwater drainage of Idaho (Carstens et al. 2004), and the Rocky Mountain tailed frog (*Ascaphus montanus*) in the Clearwater drainage as well as the South Fork of the Salmon River in Idaho (Nielson et al. 2001, Nielson et al. 2006). Results of Carstens et al.'s (2005a) coalescent simulations suggests that a single refugial population of *D. aterrimus* subsisted in the South Fork Salmon River, Idaho during the most recent glacial maximum.

This putative refuge is situated at the southern end of *D. aterrimus*' current range, suggesting that the population expanded northward as glaciers receded. Northward expansion appears to have left a signature in my genetic data as well; PC1 identified more divergence in allele frequencies among catchments in the Lochsa basin compared to the St. Joe and the St. Regis basins. According to David A. Good's model in Slatkin (1993), a gradual stepwise range expansion from a single refugial population would result in greater genetic divergence among earlier founded populations than among more

recently founded ones regardless of geographic distance among them. Assuming a similar scenario of range expansion from the South Fork Salmon River northward, my results suggest that streams in the Lochsa basin were founded earlier than streams in the St. Joe or St. Regis basins.

This pattern of historical range expansion was also apparent in AMOVA. Divergence among basins was significant in the global test (among St. Regis, St. Joe, and Lochsa basins), but not between the St. Regis and St. Joe basins. Because the Lochsa basin was likely colonized first, greater genetic divergence has accumulated between the Lochsa basin and the St. Regis and St. Joe basins. Conversely, basins separated by minimal distances (i.e., St. Regis and St. Joe), with shorter divergence time, were not structured at the among basin level. Rather, in the St. Regis and St. Joe basins, the structure imposed by differences among catchments was so strong that the relative effect of basin structure was minimal.

Pathways of gene flow

Among pairs of streams in each basin, genetic divergence (F_{ST}) and in-stream distance were strongly correlated, consistent with an increased likelihood of genetic exchange among nearby populations compared to distant populations (Wright 1945). Hutchison and Templeton (1999) described how patterns of isolation by distance (IBD) can change with spatial scale due to the shifting influences of gene flow and drift with distance. My results were consistent with what they described as localized dispersal between nearby populations, and divergence between more distant populations due to drift (Hutchison and Templeton 1999). However, my results suggest that the major shift

in relative influences of gene flow and drift was not due to geographical distance, rather, it occurred because of hierarchical catchment boundaries. IBD was apparent only at some spatial scales – in particular, among streams within catchments, signifying limited dispersal extent within catchments and strong dispersal barriers among catchments.

Studies with other species of *Dicamptodon* in Washington state suggest that genetic structure is strongly affected by life history (Steele et al. 2009). *D. copei* has a primarily aquatic life-history (non-metamorphosing) and a pattern of IBSD, whereas *D. tenebrosus* is a facultative paedomorph (metamorphosing) with no apparent IBSD or IBED among sites separated by a maximum of 20km. Steele (2009) concluded that overland dispersal by terrestrial *D. tenebrosus* adults was an important influence on genetic structure. My results with *D. aterrimus*, however, indicated that genetic structure was more consistent with gene flow along stream corridors than by overland gene flow of metamorphosing *D. aterrimus*. F_{ST} was more strongly correlated with stream distance than with surface distance. However, because the two measures of distance were themselves correlated, I was unable to definitively rule out the influence of overland gene flow based solely on IBD. High divergence between adjacent catchments, however, provided further indication of the limited influence of overland gene flow on population structure.

Steele's (2009) measures of overall genetic divergence in *D. copei* ($\theta = 0.079$) and *D. tenebrosus* ($\theta = 0.031$) were much lower than my results of *D. aterrimus* within basins (Lochsa $\theta = 0.28$, St. Regis $\theta = 0.26$, St. Joe $\theta = 0.27$). Although *D. aterrimus* and *D. tenebrosus* are both facultative paedomorphs, ecological differences, differences in abundance, and/or differences in the intervening habitat of these species have resulted in

dissimilar patterns of genetic structure and evolution. Therefore, the use of empirical data and results collected with *D. tenebrosus* may not be valid for use in conservation of *D. aterrimus*. Predictions of the stream hierarchy model (Meffe and Vrijenhoek 1988), which appears to explain *D. aterrimus* population structure within catchments, suggest that *D. aterrimus* are not ecologically isolated headwater specialists (Nussbaum and Clothier 1973). Rather, they appear to use catchment mainstems as corridors for dispersal and potentially for habitat as well.

This study highlights the importance of stream network structure on population processes of freshwater organisms. Ecological differences between *D. aterrimus* and other *Dicamptodon* species have contributed to much higher genetic divergence among populations of inland salamanders. While populations of *D. aterrimus* are structured by dispersal along stream channels within catchments, at larger spatial scales, catchments are isolated from one another resulting in strong lineage divergence over small geographic scales. Long-term persistence of *D. aterrimus* will depend in part on the maintenance of genetic variation within catchments via dispersal among streams, enabling adaptation in response to shifting environmental conditions.

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Appendix A

Dicamptodon aterrimus sampling reaches. Map datum WGS84 was used for GPS coordinates. 15 individuals were collected from throughout the length of the survey reach, with the exception of RBU1 with 16. Sampling streams are mapped in Figure 3.

Sub-basin	Network	Stream	Length (m)	Coordinates		Elevation (m)	N
St. Joe	Gold	JGPR	360	47.2012	-115.3636	1198	15
		JGU1	224	47.2370	-115.3669	1442	15
		JGU2	201	47.2332	-115.3623	1334	15
	Quartz	JQU1	230	47.2213	-115.5013	1062	15
		JQU3	179	47.2415	-115.4784	1205	15
		JQUE	391	47.2452	-115.4935	1249	15
Lochsa	Badger	LBU1	148	46.5168	-114.8278	1085	15
		LBU2	343	46.5307	-114.8356	1170	15
		LBU3	167	46.5397	-114.8315	1233	15
	Papoose	LPEF	171	46.5549	-114.7430	1246	15
		LPTE	195	46.5477	-114.7509	1222	15
		LPTW	185	46.5445	-114.7688	1127	15
	Squaw	LSDO	281	46.5356	-114.9155	1351	15
		LSSP	170	46.5467	-114.8890	1294	15
		LSU1	163	46.5419	-114.8646	1203	15
	Wendover	LWEF	210	46.5227	-114.7860	1102	15
		LWU1	316	46.5317	-114.8029	1237	15
		LWWF	125	46.5306	-114.8056	1207	15
St. Regis	Big	RBMC	161	47.3422	-115.4327	1070	15
		RBU1	200	47.3312	-115.4392	1161	16
		RBU2	190	47.3269	-115.4431	1201	15
	Deer	RDTU	204	47.3483	-115.3676	1111	15
		RDU1	302	47.3106	-115.4070	1277	15
		RDUU	160	47.3305	-115.3599	1431	15

Appendix B

Microsatellite loci used to genotype *Dicamptodon aterrimus* (Steele et al. 2008; Curtis and Taylor 2000). Primer sequences are given with fluorescent marker applied to forward primers, including additional base pairs added as "pig tails" where required (Brownstein et al. 1996). Repeat units of microsatellites are listed, N_A is the number of alleles per locus, Length refers to the size range of products, T_A is the annealing temperature used for PCR amplification, temperature ranges are given for touchdown profiles used to amplify multiplexes or single PCRs.

Locus	Primer Sequence (5'–3')	Repeat Unit	N_A	Length	T_A
D04	F: (HEX)GAAACTATTTTATCAAAAGCATGC	$(TATC)_{18}(TGTC)_{14}$	4	154-166	54
	R: GTGTCTTTCTAAATATGTGTATGGGTGTATAAG(tail)				
D06	F: (6FAM)GACAAATGGATAGCTGCATAGC	$(GATA)_2GACA(GATA)_3$ $GACA(GATA)_9$	8	199-227	67-57
	R: GCCTTCTGAATTGGGTGAAG				
D08	F: (PET)TGTGCACGGACTACACTTTAGG	$(TATC)_{16}$	6	124-144	67-57
	R: GTGTCTTCCAAGATGCCTCTTTTGGTG(tail)				
D13	F: (NED)CAGGGCAATATGACCTAGTCG	$(CTAT)_{11}$	7	114-138	67-57
	R: GTGTCTTTGGGGGTAACCTGCAACAG(tail)				
D14	F: (HEX)TGCTTCTGAGCAATTATTGTGG	$(CTAT)_{17}$	7	173-197	67-57
	R: AGATTGGTGTGTAGGTGGTTG				
D15	F: (NED)GTGTGTCTGAAGTGGCAAGG	$(CTAT)_{13}CTGT(CTAT)_5$ $CTAC(CTAT)_2$	8	178-206	67-57
	R: AGCCCACTGATTCTACGAGAG				
D18	F: (PET)AAGGCTGGAAGGTTTTATGC	$(CTAT)_{15}$	6	168-188	52
	R: GTGTCTTTGCTAACCGCTCAGATTCAC(tail)				
D24	F: (6FAM)CAACATAATACTGATGGTGTTTGC	$(CTAT)_{24}$	3	154-162	54
	R: GTGTCTTAGAATAAATGGCCGTTTTGG(tail)				
Dte4	F: (6FAM)TGCTTCTGCCACCATAGCC	$GT(GC)_3(GT)_3(GCGT)_2GTGG$ $(GCGT)GC(GCGT)_2(GT)_8$	1	159	67-57
	R: AGAGCCAGCCTTTGTTGCG				
Dte5	F: (HEX)GGAGGAGTTTTTGAAGTTG	$(AG)_3CG(AG)_4AA(AG)_{17}$	1	215	56.5
	R: ATTCTCCAAACATTCTCCC				
Dte6	F: (NED)GGTAGTCATGGTGATGCTG	$CACTA(CA)_{18}$	7	183-195	67-57
	R: GTGTCTTCACTCCCCTATTCTCCCTAC(tail)				
Dte8	F: (PET)CTGCATACATTGCATCTCCG	$(CT)_{16}$	1	161	67-57
	R: GTGTCTTCCGCAAGGTCATCTTCACTAAC(tail)				
Dte11	F: (6FAM)ACACATGGTTGCTCACTC	$(GT)_3(CA)_6CG(CA)_4$ $CG(CA)_5(CT)_3$	4	118-126	67-57
	R: GTGTCTTTAGTGTGTGGCATTAAGGG(tail)				
Dte14	F: (PET)AGGAGTGAGACAGGGTGAGC	$(GA)_{17}$	1	125	67-57
	R: CACCTCTCCTCCTCTTCCAG				

Appendix C

Genetic diversity of each stream where A_S is allelic richness, N_A is the total number of alleles observed in the stream, H_O is observed heterozygosity, H_E is expected heterozygosity, and F_{IS} is provided for all 9 loci when polymorphic. F_{IS} values that are significantly different from zero are bolded, as well as the two streams with significant departures from HW. After correcting for multiple tests, however, none of the F_{IS} values were significantly different from zero, and no populations had significant deviations from HW proportions however. Fifteen individuals were genotyped in each stream with the exception of RBU1 with 16 individuals.

Streams	A_S	N_A	H_O	H_E	Fis								
					D14	***D18***	***Dte6***	***D06***	***D15***	***D13***	***D04***	***D24***	***D08***
JGPR	2.89	26	0.4889	0.4779	-0.222	-0.084	--	0.364	0.24	-0.073	-0.167	-0.12	-0.156
JGU1	2.56	23	0.4074	0.4015	0.306	0.271	-0.189	-0.077	-0.128	-0.032	-0.2	-0.167	--
JGU2	2.44	22	0.3778	0.3295	-0.328	-0.037	--	-0.217	-0.258	-0.114	0.213	-0.12	--
JQU1	3.44	31	0.5333	0.5083	-0.033	-0.08	-0.037	-0.037	-0.155	-0.116	0.2	-0.037	-0.072
JQU3	2.11	19	0.3185	0.3034	-0.023	-0.125	--	-0.167	-0.308	-0.273	0.548	--	--
JQUE	2.56	23	0.4741	0.4148	0.219	-0.011	--	-0.077	-0.235	-0.26	-0.359	-0.037	-0.387
LBU1	2.78	25	0.3481	0.3632	-0.167	-0.067	0.325	0.65	0.364	-0.363	-0.162	-0.037	-0.05
LBU2	2.44	22	0.3407	0.3213	-0.225	-0.050	-0.280	-0.077	0.352	-0.172	-0.069	--	--
LBU3	2.56	23	0.3926	0.3663	0.012	-0.191	0.080	-0.037	-0.474	-0.111	0.155	--	-0.120
LPEF	2.78	25	0.4148	0.3499	-0.363	-0.023	-0.037	-0.077	-0.241	-0.400	-0.037	--	-0.134
LPTE	2.44	22	0.3185	0.3479	0.200	0.051	0.200	0.092	0.333	--	-0.114	--	-0.011
LPTW	2.67	24	0.3556	0.3451	-0.315	0.133	-0.185	--	0.122	0.103	-0.037	--	-0.077
LSDO	3.11	28	0.5111	0.4994	0.000	-0.176	-0.340	-0.081	-0.050	-0.188	0.011	-0.077	***0.531***
LSSP	2.89	26	0.3556	0.4059	0.104	0.011	0.228	0.467	***0.154***	0.491	0.157	--	-0.407
LSU1	2.22	20	0.3111	0.3257	-0.278	0.031	--	0.084	-0.077	0.104	-0.077	--	***0.479***
LWEF	2.11	19	0.237	0.248	0.247	-0.191	--	--	0.228	-0.077	--	--	--
LWU1	2.11	19	0.3481	0.2978	-0.179	-0.081	--	-0.167	-0.162	-0.308	--	--	--
LWWF	2.78	25	0.3111	0.3267	***-0.037***	0.053	0.650	-0.018	0.214	-0.407	0.317	--	--
RBMC	2.67	24	0.4222	0.4079	-0.233	-0.037	--	0.092	-0.217	0.034	0.187	-0.037	-0.167
RBU1	2.56	23	0.375	0.4178	-0.034	0.097	-0.200	0.057	0.306	0.016	0.159	0.455	0.153
RBU2	2.56	23	0.4074	0.4483	0.364	-0.141	-0.333	-0.043	-0.077	-0.037	0.314	0.349	0.352
RDTU	2.11	19	0.2	0.187	-0.151	-0.018	--	-0.273	--	0.192	--	-0.037	-0.037
RDU1	2.22	20	0.4074	0.3144	-0.468	-0.260	-0.167	***-0.389***	--	-0.273	-0.167	--	--
RDUU	2.11	19	0.1778	0.2235	***0.517***	-0.023	--	0.211	--	0.102	--	--	--

Appendix D

Pairwise F_{ST} among all streams. Values that are not significantly different from zero are bold italicized, pairs of streams within the same catchment are highlighted in grey. Significance testing of F_{ST} was based on 10,000 permutations.

	Gold			Quartz			Badger			Papoose			Squaw			Wendover			Big			Deer		
	JGPR	JGU1	JGU2	JQU1	JQU3	JQUE	LBU1	LBU2	LBU3	LPEF	LPTE	LPTW	LSDO	LSSP	LSU1	LWEF	LWU1	LWWF	RBMC	RBU1	RBU2	RDTU	RDU1	RDUU
JGPR	0.00																							
JGU1	0.19	0.00																						
JGU2	0.16	0.10	0.00																					
JQU1	0.15	0.32	0.34	0.00																				
JQU3	0.29	0.47	0.49	0.11	0.00																			
JQUE	0.21	0.40	0.42	0.03	0.04	0.00																		
LBU1	0.38	0.47	0.46	0.35	0.45	0.42	0.00																	
LBU2	0.38	0.48	0.46	0.35	0.44	0.42	***-0.02***	0.00																
LBU3	0.37	0.47	0.46	0.34	0.44	0.39	***0.01***	*0.03*	0.00															
LPEF	0.38	0.41	0.44	0.42	0.53	0.49	0.34	0.37	0.33	0.00														
LPTE	0.40	0.42	0.45	0.42	0.54	0.49	0.32	0.35	0.31	0.03	0.00													
LPTW	0.39	0.45	0.49	0.39	0.49	0.44	0.32	0.34	0.28	0.07	0.11	0.00												
LSDO	0.33	0.40	0.38	0.29	0.41	0.36	0.15	0.18	0.18	0.30	0.26	0.32	0.00											
LSSP	0.31	0.44	0.42	0.23	0.34	0.29	0.28	0.28	0.26	0.32	0.31	0.25	0.24	0.00										
LSU1	0.36	0.48	0.45	0.32	0.42	0.37	0.32	0.33	0.32	0.33	0.31	0.32	0.15	0.16	0.00									
LWEF	0.48	0.55	0.60	0.45	0.57	0.52	0.40	0.42	0.37	0.25	0.31	0.22	0.40	0.38	0.43	0.00								
LWU1	0.46	0.52	0.58	0.39	0.51	0.46	0.41	0.43	0.38	0.24	0.27	0.19	0.39	0.29	0.39	0.07	0.00							
LWWF	0.43	0.49	0.54	0.37	0.48	0.44	0.34	0.35	0.30	0.24	0.28	0.18	0.37	0.26	0.37	0.05	0.07	0.00						
RBMC	0.22	0.38	0.34	0.19	0.34	0.26	0.38	0.39	0.39	0.44	0.43	0.45	0.28	0.31	0.31	0.53	0.50	0.45	0.00					
RBU1	0.26	0.40	0.37	0.22	0.33	0.27	0.36	0.36	0.36	0.44	0.41	0.43	0.28	0.30	0.33	0.52	0.49	0.44	***0.02***	0.00				
RBU2	0.25	0.39	0.35	0.23	0.36	0.30	0.35	0.36	0.35	0.42	0.40	0.42	0.26	0.30	0.32	0.51	0.48	0.43	***0.03***	***-0.01***	0.00			
RDTU	0.39	0.52	0.48	0.37	0.49	0.46	0.47	0.47	0.47	0.53	0.53	0.54	0.41	0.45	0.45	0.60	0.59	0.53	0.36	0.38	0.36	0.00		
RDU1	0.34	0.41	0.41	0.29	0.38	0.38	0.39	0.38	0.40	0.46	0.46	0.45	0.34	0.36	0.37	0.53	0.50	0.45	0.33	0.33	0.33	0.10	0.00	
RDUU	0.39	0.49	0.46	0.35	0.46	0.44	0.45	0.44	0.45	0.51	0.51	0.50	0.39	0.40	0.40	0.57	0.55	0.49	0.37	0.39	0.39	0.09	0.07	0.00