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Scale-dependent genetic structure of the Idaho giant salamander (*Dicamptodon aterrimus*) in stream networks

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Abstract

The network architecture of streams and rivers constrains evolutionary, demographic and ecological processes of freshwater organisms. This consistent architecture also makes stream networks useful for testing general models of population genetic structure and the scaling of gene flow. We examined genetic structure and gene flow in the facultatively paedomorphic Idaho giant salamander, *Dicamptodon aterrimus*, in stream networks of Idaho and Montana, USA. We 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 vs. overland pathways. Replicated sampling of streams within catchments within three river basins revealed that hierarchical scale had strong effects on genetic structure and gene flow. AMOVA **identified significant structure at all hierarchical scales (among streams, among catchments, among basins), but 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, consistent with a stream hierarchy model of population structure. However, there was no evidence of migration among catchments within basins, or among basins, indicating that gene flow only counters the effects of genetic drift at smaller scales (within rather than among catchments). 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.**

Keywords: death valley model, *Dicamptodon aterrimus*, genetic structure, scale dependence, stream hierarchy model

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Introduction

Many species occur in spatially structured sub-populations linked by dispersal and gene flow, and these spatial processes can strongly influence evolutionary, demographic and ecological dynamics at multiple scales (Wright 1951; MacArthur & Wilson 1967; Hanski & Gilpin 1997). While many studies have measured how landscape barriers affect dispersal and gene flow (Manel *et al.* 2003), results of these studies have often been species and scale-specific (e.g. Keyghobadi *et al.* 1999; Funk *et al.* 2005; Wang *et al.* 2009). General insight on what controls gene flow and genetic differentiation may best be gained in systems that impose consistent structure at multiple spatial scales, where it is possible to assess patterns of genetic structure within scales and how those patterns change across scales (Levin 1992; Schneider 2001; Halley *et al.* 2004).

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.

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Rivers and streams are also fractal-like, with the same dendritic branching pattern occurring across scales (Horton 1945). This consistent network architecture can constrain evolutionary, demographic and ecological processes in aquatic organisms (e.g. Finn *et al.* 2006; Muneepeerakul *et al.* 2008; Grant *et al.* 2009). It also makes these dendritic networks useful for testing general models of landscape-scale population structure, and for understanding the scaling of dispersal and gene flow (Lowe *et al.* 2006; Grant *et al.* 2007).

Patterns of population genetic structure in stream and river networks have been described by four general models (Fig. 1; Meffe & Vrijenhoek 1988; Finn *et al.* 2007; Hughes *et al.* 2009). The null model (Fig. 1a) characterizes organisms with high gene flow among all localities by both stream and overland pathways of dispersal. Meffe & Vrijenhoek's (1988) death valley model (DVM; Fig. 1b) characterizes strictly aquatic organisms that are isolated in headwater reaches by ecological barriers (abiotic and/or biotic). The DVM predicts that all populations show strong genetic differentiation, but with no relationship to drainage patterns (e.g. Preziosi & Fairbairn 1992). More mobile and ecologically tolerant aquatic organisms may be characterized by Meffe & Vrijenhoek's (1988) stream hierarchy model (Fig. 1c), which predicts genetic variation to be partitioned by drainages (e.g. Wishart & Hughes 2003). Lastly, the headwater model (Fig. 1d) characterizes organisms that are ecologically isolated to headwater reaches and disperse only by overland pathways. This model predicts genetic variation to be partitioned in headwater islands, irrespective of drainage patterns (e.g. Finn *et al.* 2007).

While useful for characterizing scale-specific genetic structure, these models do not address how patterns of gene flow and divergence change with hierarchical scale. If dispersal patterns of freshwater organisms are influenced by the hierarchical, fractal structure of stream networks, some freshwater organisms may have a scale-dependent genetic structure. Scale dependence could result from differential ability of the organisms to disperse at different hierarchical scales. For example, rates of gene flow at one hierarchical scale (i.e. among streams) may differ from those at another hierarchical scale (i.e. among catchments or among basins; Fig. 2). To understand how patterns of gene flow and population structure change across hierarchical scales, sampling must allow for analysis at multiple scales (Fausch *et al.* 2002; Lowe *et al.* 2006). A lack of systematic hierarchical sampling has prevented previous studies from addressing both the effect of network architecture on population structure and the scaling of this effect.

By applying a consistent sampling design that encompassed three hierarchical scales (streams, catchments, basins; Fig. 2), we explored the effects of both network architecture and spatial scale on population genetic structure of the Idaho Giant salamander, *Dicamptodon aterrimus*. *D. aterrimus* is facultatively paedomorphic, and has the potential to disperse by stream and overland pathways. We examined genetic variation of microsatellite loci to investigate the genetic structure of *D. aterrimus* populations in river networks of Idaho and Montana, USA. Using microsatellite data, we tested Meffe & 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 to assess the relative influence of within-stream and overland gene flow on population genetic structure.

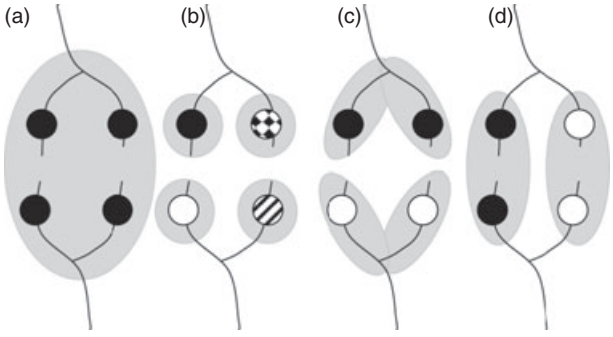

Fig. 1 Diagrams of conceptual models for patterns of movement and genetic structure in stream organisms: (a) the Null Model, (b) the Death Valley Model, (c) the Stream Hierarchy Model, and (d) the Headwater Model. Grey areas show pathways of dispersal in each model. Open, closed and patterned circles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

Materials and methods

Study species and sites

The Idaho giant salamander, *Dicamptodon aterrimus*, occurs in mesic forests of northern Idaho and western

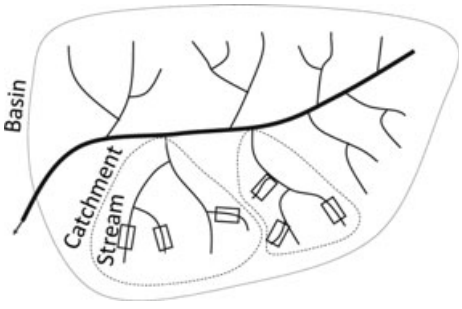

Fig. 2 Sampling design showing hierarchical scales of sampling. Three streams were sampled within each of two adjacent catchments. Survey reaches of streams are indicated by rectangles.

Montana, USA. This species was isolated from other *Dicamptodon* between 2 and 5 Ma due to the xerification of the Columbia river 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 (Sepulveda & Lowe 2009).

Dicamptodon aterrimus is facultatively paedomorphic: larvae develop in streams and reach maturation after several years as either terrestrial or aquatic forms (Nussbaum *et al.* 1983). Our observations in the field suggest that *D. aterrimus* are present in headwater and higher-order reaches. While no data on overland dispersal exists for *D. aterrimus,* Richardson & 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 movements (5–50 m) are common, but movements >100 m are rare. However, we lack information on the frequency and scale of dispersal beyond individual streams, and on the relative importance of movements along stream corridors vs. overland pathways. Testing for support of models of genetic structure may provide insight into both the importance of overland vs. in-stream gene flow, and how stream network architecture influences population structure.

Sampling design

To examine the spatial extent of gene flow and population structure in *D. aterrimus*, we applied a consistent sampling design that encompassed three hierarchical scales: streams, catchments and basins. We sampled individuals in first-order streams which were nested within catchments of confluent streams draining into a mainstream river (Fig. 2). Catchments were nested within basins of three major rivers: the Lochsa (four catchments), the St. Joe (two catchments) and the St. Regis (two catchments). We collected 15 *D. aterrimus* adults (both aquatic and terrestrial) and juveniles from three first-order streams within each catchment (Table S1, Fig. 3). Catchments were selected in basins so that they were separated by a common ridge running approximately perpendicular to the mainstream river. This orientation allowed us to test for in-stream and overland gene flow within and among adjacent catchments.

In each stream, we used an LR-20 backpack electrofisher (Smith-Root Inc.) 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 to 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 we sampled three 30 m reaches separated by approximately 15 m (LWWF and LPEF; Table S1).

A small section of tail tissue was clipped from captured salamanders and stored it in 95% ethanol. Both juvenile and adult salamanders were sampled. Snout-vent lengths of sampled animals ranged from 22 to 160 mm and weights ranged from <1 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; Table S1).

Microsatellite amplification and scoring

Fifteen salamanders from each stream were genotyped at 14 microsatellite loci developed for *Dicamptodon tenebrosus* and *D. copei* (Table S2; Curtis & Taylor 2000; Steele *et al.* 2008). To extract DNA, we 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 100 μL TE buffer and diluted 1:10 for polymerase chain reaction (PCR) amplification in a PTC-100 thermocycler (MJ Research Inc.) with a total volume of 10 μL. Multiplex reactions were set up with QIAGEN multimix, following the QIAGEN microsatellite protocol (QIAGEN Inc.). We 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 (Table S2). Following individual PCRs, these markers were pooled with multiplexed markers for fragment analysis. PCR products were visualized on an ABI3130xl Genetic Analyzer (Applied Biosystems Inc.) in the Murdock DNA Sequencing Facility at the University of Montana, Missoula, MT, USA. Allele sizes were determined using the ABI GS600LIZ ladder (Applied Biosystems Inc.) and alleles were called with GENEMAPPER version 3.7 and verified manually (Applied Biosystems Inc.).

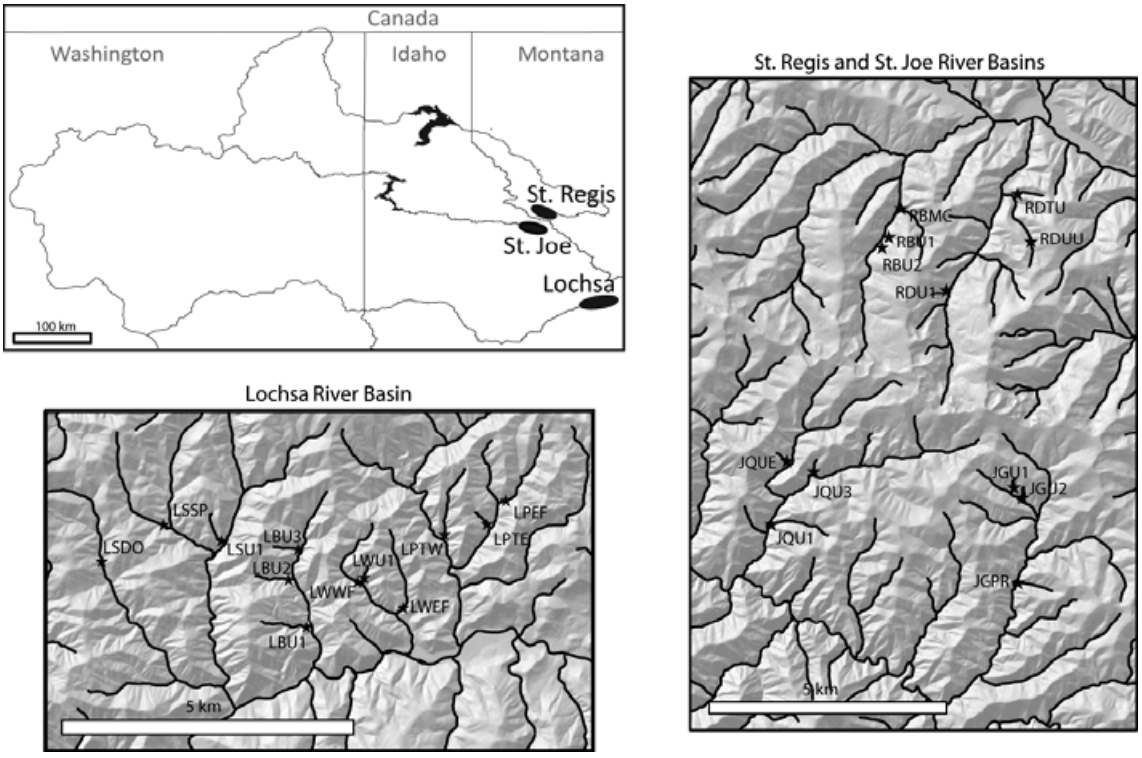

Fig. 3 Map of sampling streams in the St. Regis, St. Joe and Lochsa river basins of Idaho and Montana in northwestern USA. Centre points of stream survey reaches are marked with stars, and four letter stream codes are indicated. Four catchments were sampled from the Lochsa river basin: Squaw Ck. (streams: LSDO, LSSP, LSU1), Badger Ck. (streams: LBU1, LBU2, LBU3), Wendover Ck. (streams: LWEF, LWWF, LWU1) and Papoose Ck. (streams: LPTW, LPTE, LPEF). Two catchments were sampled from the St. Regis river basin: Big Ck. (streams: RBMC, RBU1, RBU2), and Deer Ck. (streams: RDTU, RDUU, RDU1). Two catchments were sampled from the St. Joe river basin: Quartz Ck. (streams: JQU1, JQUE, JQU3) and Gold Ck. (streams: JGPR, JGU1, JGU2).

Analyses

We tested for significant departures from Hardy-Weinberg (HW) proportions and for non-random association of pairs of loci across populations (gametic disequilibrium) using exact tests implemented in GENEPOP version 4.0 (Raymond & Rousset 1995). Loci that deviated from HW proportions in each population were removed from further analyses. 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 and H_O). We then calculated genetic differentiation among streams with pairwise F_{ST} using ARLEQUIN version 3.1 (Excoffier *et al.* 2005). The inbreeding coefficient, F_{IS}, was calculated for each locus in streams to detect significant heterozygote deficit or excess in streams (GENEPOP; Raymond & Rousset 1995).

We examined pairwise F_{ST} values to assess levels of divergence occurring among streams. To partition genetic variance within and among hierarchical scales, we used a hierarchical analysis of genetic variation (AMOVA implemented in the HIERFSTAT package in R v 2.8.1; Goudet 1995). Specifically, we 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, we performed two additional AMOVAS: (i) within the Lochsa river basin; and (ii) within and between the adjacent St. Joe and St. Regis river basins. These two additional AMOVAS were chosen because of the proximity of the basins; we had no samples from a basin adjacent to the Lochsa river basin, but the St. Regis and St. Joe river basins are adjacent and share boundaries. 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. To understand how levels of genetic divergence were influenced by effective population sizes (N_e), we used the linkage disequilibrium method (Bartley *et al.* 1992) to estimate N_e of each stream we sampled with N_e Estimator (Peel *et al.* 2004).

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, we removed the highest frequency allele of each 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.

We used partial Bayesian individual assignment tests (Rannala & 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 exclusion test for a measure of confidence associated with assignment (Paetkau *et al.* 2004). 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 then compared to the distribution of genotype likelihoods, 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 ($P \geq 0.95$). Identification of migrants using this method has been possible especially when genetic differentiation is substantial and many loci are used (Berry *et al.* 2004; Paetkau *et al.* 2004). We 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 vs. overland pathways, we tested alternative hypotheses of *D. aterrimus* gene flow resulting in isolation by distance. Isolation by distance is detected by testing for correlations among matrices of genetic distance (F_{ST}) and geographic distance with Mantel tests that correct for non-independence of pairwise points (Mantel 1967). We 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)], we 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). Second, we 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). 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 isolation by distance due to in-stream vs. overland gene flow. Pairwise stream and surface distances were significantly correlated ($r = 0.88$, $P < 0.001$). Therefore, the strengths of correlations of genetic distance with stream distance vs. surface distance were used to assess the relative importance of in-stream vs. overland gene flow. Plots of pairwise F_{ST} and stream distance were analyzed to detect shifts in the relationship due to hierarchical scale.

Results

We genotyped 361 individuals from 24 streams at 14 microsatellite loci (Table S2). Four microsatellite loci were monomorphic (*Dte4*, *Dte5*, *Dte8* and *Dte14*) and were therefore discarded. Another locus, *Dte11*, deviated significantly from HW proportions in three of the six 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 (Table S3). 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 the 24 streams, and no comparisons were significant after Bonferroni correction.

Overall, genetic variation was low (A_S mean: 2.54, range: 2.11–3.44; H_E mean: 0.359, range: 0.187–0.508) and in most streams at least one locus was fixed for a particular allele (Table S3). There were no significant correlations between genetic diversity (A_S, N_A, H_E) and either date or stream survey length ($P > 0.05$). Six F_{IS} values were significantly different from zero before correcting for multiple tests, none were significant after sequential Bonferroni correction, 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 occurring between streams within catchments. Overall, divergence among streams tended to be high (median $F_{ST} = 0.39$; Table S4). 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 (58.2%) occurred among individuals within streams, and the greatest proportion of structural genetic variation (23.1%) 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 (5.6%). The within-Lochsa river basin AMOVA resulted in the same patterns as the global AMOVA. Conversely, the St. Joe-St. Regis river basins AMOVA indicated that variation due to differences among basins was not significant, accounting for only 0.8% of total genetic variation. However, variation among catchments in the St. Joe-St. Regis complex was highly significant, accounting for 29.5% of total genetic variation (Table 1). Our estimates of N_e (Table 2) show that the N_e of streams is variable, with large 95% confidence intervals around these estimates. Confidence intervals around N_e estimates using the linkage disequilibrium method often include infinity (e.g. Bartley *et al*. 1992; Fraser *et al*. 2007).

Principal components analysis showed concordant patterns of genetic divergence across hierarchical network scales. PC1 accounted for 30% of the variance in allele frequencies and 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 (Fig. 4). PC2 accounted for an additional 18% of the variation in allele frequencies and PC3 accounted for an additional 14% of the variation. PC2 and PC3 separated catchments in the St. Regis and St. Joe river basins but did not group catchments from basins together.

Individual assignment tests 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

Table 1 Results of hierarchical analysis of molecular variance: (a) Global AMOVA, (b) Within Lochsa AMOVA, (c) St. Joe–St. Regis AMOVA

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		$F_{ST} = 0.359$	

Significant *P*-values are in bold.

Table 2 Estimates of effective population size and 95% confidence intervals

Basin	Catchment	Stream	N_e	95% CI	
St. Regis	Big	RBMC	∞	38.4	∞
		RBU1	163.3	21.4	∞
		RBU2	66.7	16.6	∞
	Deer	RDTU	8.8	4.8	20.9
		RDU1	16.1	7.3	103.1
		RDUU	∞	12.3	∞
St. Joe	Gold	JGPR	16.4	9.2	42.4
		JGU1	25.8	10.9	∞
		JGU2	11.4	6.3	28.6
	Quartz	JQU1	53.0	19.4	∞
		JQU3	17.2	7.3	249.0
		JQUE	20.9	9.7	153.3
Lochsa	Badger	LBU1	∞	29.1	∞
		LBU2	∞	38.1	∞
		LBU3	29.8	11.7	∞
	Papoose	LPEF	37.4	13.8	∞
		LPTE	14.9	7.7	48.4
		LPTW	33.6	12.7	∞
	Squaw	LSDO	∞	30.7	∞
		LSSP	11.4	7.0	22.4
		LSU1	17.1	7.6	145.9
	Wendover	LWEF	22.0	7.8	∞
		LWU1	688.3	12.9	∞
		LWWF	2.3	1.7	3.0

confidence associated with assignment (Paetkau *et al.* 2004).

The partial Bayesian exclusion test identified no potential migrants among basins, one potential migrant among catchments in the Lochsa river basin, and five potential migrants among streams within catchments in the Lochsa and St. Regis river basins. Exclusion tests identified 156 individuals that had the highest likelihood of occurring in another stream. Two of those were excluded from all sampled streams ($P < 0.01$). Six had the highest likelihood of originating in a stream from a neighbouring catchment in the Lochsa river basin ($P > 0.90$ for two individuals, $P > 0.70$ for four individuals). The remaining 148 individuals had the highest likelihood of occurring in another stream within their catchment. Although only five were considered potential migrants ($P \geq 0.95$), 67 individuals had a high likelihood of originating from another stream within the catchment ($P > 0.7$); five of these were terrestrial adults. These individuals may be descendants of immigrants from previous generations. Collectively, individual assignments identified more migrants among streams within catchments than among catchments or among basins.

There was a significant, positive correlation between stream distance and F_{ST} (IBSD) in the Lochsa river basin (Mantel; $r = 0.63$, $P < 0.001$), in the St. Regis river basin ($r = 0.93$, $P < 0.001$), and in the St. Joe river basin ($r = 0.83$, $P < 0.001$). There were significant but weaker positive correlations between surface distance and F_{ST} (IBED) in the Lochsa river basin ($r = 0.42$, $P < 0.001$), in the St. Regis river basin ($r = 0.80$, $P < 0.001$), and in the St. Joe river basin ($r = 0.72$, $P < 0.01$). All Mantel tests were significant after sequential Bonferroni adjustment.

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, and that if gene flow is limited by geographic distance, isolation by distance should be apparent within catchments but not between catchments of a particular basin. Mantel tests indicate that correlations of F_{ST} and geographic distance were higher for stream distance than surface distance. Plots of pairwise genetic and geographic distances in basins showed a positive relationship between F_{ST} and distance among pairs of streams within catchments (Fig. 5). However, no relationship was apparent for pairs of streams that were not in the same catchment. This change in the relationship between F_{ST} and geographic distance suggests a major shift in the relative influences of gene flow and drift due to hierarchical scale and catchment boundaries. Because of the limited number of streams sampled within catchments, we could not test correlations within individual catchments.

Discussion

Evolution in stream networks

Our 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 the influence of stream network architecture on genetic structure (Fig. 2). Differences in hierarchical scales at the among-stream, among-catchment, and among-basin levels all contributed to the genetic structure of *D. aterrimus*, but structure was clearly dominated by two patterns: isolation and high divergence between adjacent catchments in a basin, and lower 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 (Fig. 1b; Meffe & Vrijenhoek 1988). They also suggest that within-catchment structure is driven by a different force, which is gene flow among streams, supporting the stream hierarchy model (Fig. 1c; Meffe & Vrijenhoek 1988).

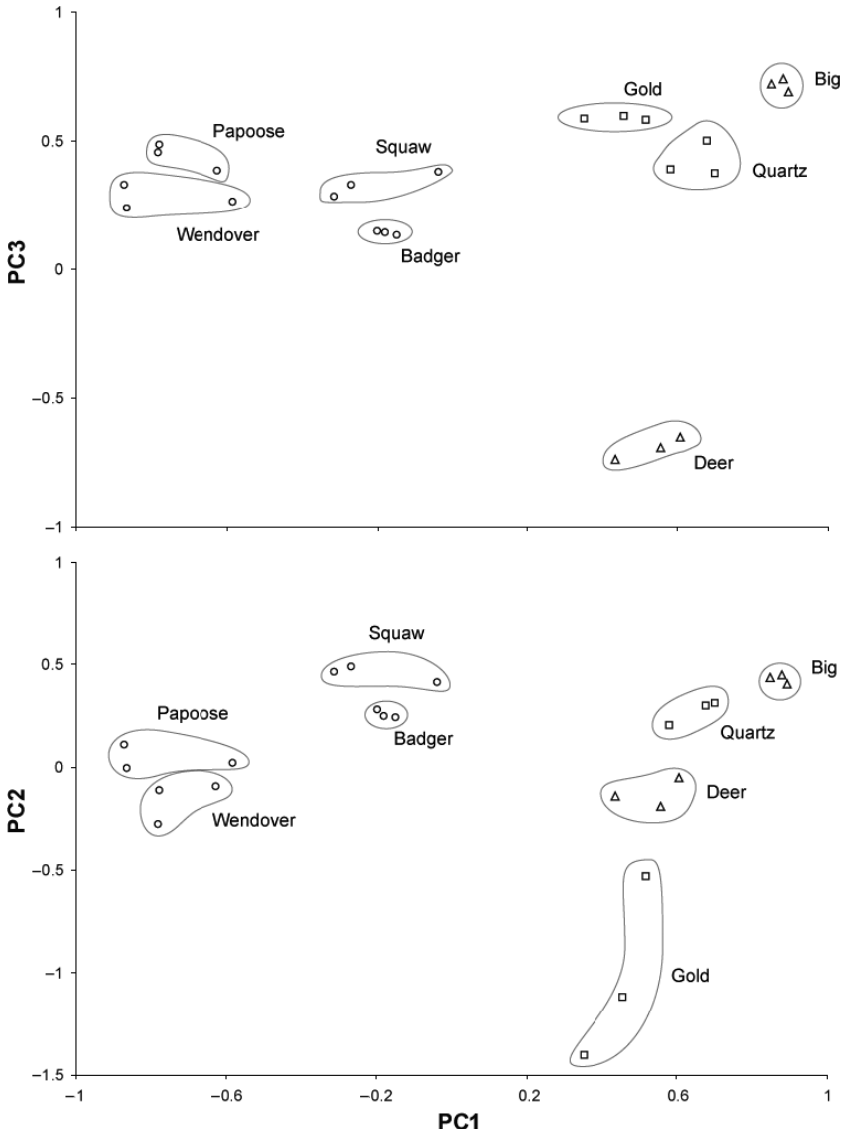

Fig. 4 Plots of the first three principal component scores of allele frequencies of nine microsatellite loci among streams sampled from basins and catchments in Idaho and Montana. Points corresponding to streams within catchments are circled and catchments are labelled. Streams sampled in the Lochsa river basin are circles, streams from the St. Joe river basin are squares, and streams from the St. Regis river basin are triangles.

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, both contemporary and historical patterns influence genetic structure, and distinguishing between current 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 (i) field observations that suggest small population sizes; (ii) small N_e estimates; and (iii) the identification of potential migrants with individual assignment tests.

Up to 2 h of shock time (10–12 h surveying) was required to collect just 15 individuals from many sites. 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), these survey results suggest N_e of *D. aterrimus* was small. Our estimates of N_e using the linkage disequilibrium method (Bartley *et al.* 1992) also provide evidence for small and variable N_e in streams (Table 2).

Divergence among populations is a function of N_e and time (t) according to the following equation:

$$F_{ST} = 1 - \left(1 - \frac{1}{2(N_e)}\right)^t$$

Therefore, F_{ST} increases rapidly over short periods of time when N_e is small (Wright 1969; Nei & Chakravarti

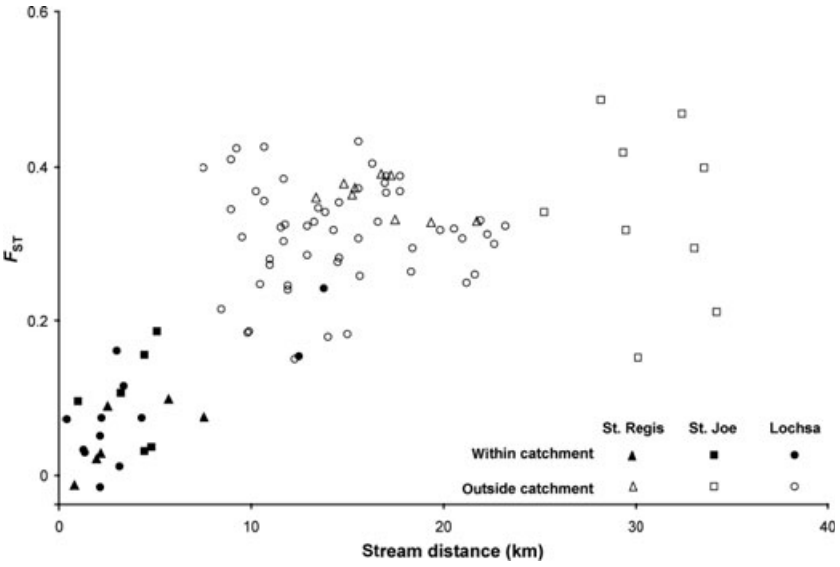

Fig. 5 Scatter plot of F_{ST} and stream distance for pairs of streams within the same basin. Pairs in the St. Joe river basin are squares, in the St. Regis river basin are triangles, in the Lochsa river basin are circles. Pairs of streams that are located within the same catchment (solid) are distinguished from those that are not within the same catchment (open).

1977). In the absence of migration, the observed variation among streams in N_e (Table 2) should also increase genetic divergence (Whitlock 1992). In light of our field observations and N_e estimates, it appears likely that migration was important in minimizing divergence among streams within catchments. 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.

Levels of genetic divergence of *D. aterrimus* at the among catchment level are high compared to those seen in terrestrial mammals (e.g. Schwartz *et al.* 2002; F_{ST} 0.00–0.07), some populations of pond-breeding amphibians (e.g. Spear *et al.* 2005; F_{ST} 0.010–0.479) and stream associated frogs (e.g. Spear & Storfer 2008; F_{ST} 0.00–0.38), but are similar to estimates for some freshwater fish (e.g. Whiteley *et al.* 2004; F_{ST} = 0.304). Bulltrout (*Salvelinus confluentus*) have high levels of genetic divergence due to small N_e, habitat fragmentation, and other ecological and life-history related factors (Whiteley *et al.* 2004). Similarly, the high levels of genetic divergence at among-catchment and among-basin levels in *D. aterrimus* appear to be driven by genetic drift due to small N_e, and limited dispersal at these larger hierarchical scales.

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). Carstens *et al.*'s (2005a) coalescent simulations suggest that a single refugial population of *D. aterrimus* subsisted in the south fork of the Salmon River, Idaho during this period. 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 our data as well: PC1 identified more divergence in allele frequencies among catchments in the Lochsa river basin compared to the St. Joe and the St. Regis river basins (Fig. 4). These results are consistent with Good's model in Slatkin (1993) which predicts that stepwise range expansion from a single refugial population will result in greater genetic divergence among earlier founded populations than among more recently founded populations, regardless of geographic distances among populations.

This pattern of historical range expansion was also apparent in the AMOVA (Table 1). Divergence among basins was significant in the global test (among St. Regis, St. Joe and Lochsa river basins), but not between the St. Regis and St. Joe river basins. Because the Lochsa river basin was likely colonized first, greater genetic divergence has accumulated between the Lochsa river basin and the St. Regis and St. Joe river 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, the structure imposed by differences among catchments in the St. Regis and St. Joe river basins was so strong that the relative effect of basin structure was minimal.

Pathways of gene flow

Genetic divergence (F_{ST}) and in-stream distance were strongly correlated (Fig. 5) among pairs of streams in each basin, consistent with increased likelihood of genetic exchange among nearby populations and divergence among more distant populations due to drift

(Wright 1945; Hutchison & Templeton 1999). However, plots of F_{ST} and in-stream distance show a major shift in the relative influences of gene flow vs. drift that was not due to geographical distance. Instead, this shift occurred because of hierarchical catchment boundaries and scale dependency in patterns of gene flow. Isolation by distance was apparent only among streams within catchments, signifying that gene flow is more important within catchments than between catchments, and that drift overrules gene flow among catchments (Fig. 5).

Studies of 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 isolation by stream distance (IBSD), whereas *D. tenebrosus* is a facultative paedomorph (metamorphosing) with no apparent isolation by stream or Euclidean distances among sites separated by a maximum of 20 km. Steele *et al.* (2009) concluded that overland dispersal by terrestrial *D. tenebrosus* adults was an important influence on genetic structure. Although *D. aterrimus* can metamorphose, F_{ST} was more strongly correlated with stream distance than with surface distance, suggesting that gene flow occurs primarily along stream corridors. High divergence between adjacent catchments (Table 1) is further evidence of limited overland gene flow, but because the two measures of distance were themselves correlated we cannot rule it out. Consistent with the stream hierarchy model (Meffe & Vrijenhoek 1988), *D. aterrimus* appears to use catchment mainstreams as corridors for dispersal and potentially as habitat as well, suggesting that it is not an ecologically isolated headwater specialist (Nussbaum & Clothier 1973).

This study highlights the importance of stream network structure in controlling population processes of freshwater organisms. While populations of *D. aterrimus* are structured by dispersal along stream channels at the within-catchment hierarchical scale, the among-catchment scale shows isolation, resulting in high 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. However, our data also suggest that recolonization of catchments would be very slow, making this species especially vulnerable to disturbances that affect entire catchments, such as road networks, wildfires, and environmental impacts of climate change.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 *Dicamptodon aterrimus* sampling reaches. Map datum WGS84 was used for GPS coordinates. Fifteen individuals were sampled from throughout the length of each survey reach, with the exception of RBU1 where 16 individuals were sampled. Sampling streams are mapped in Fig. 3

Table S2 Microsatellite loci used to genotype *Dicamptodon aterrimus* (Curtis & Taylor 2000; Steele *et al*. 2008). Primer sequences are given with fluorescent marker applied to forward primers, including additional base pairs added as ''pig tails'' where required. Repeat units of microsatellites are listed, N_A is the number of alleles per locus, length refers to the size range of products, and T_A is the annealing temperature used for PCR amplification. Temperature ranges are given for touchdown profiles used to amplify multiplexes or single PCRs

Table S3 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 in bold, as well as the two streams with significant departures from Hardy-Weinberg (HW) proportions. 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. Fifteen individuals were genotyped in each stream with the exception of RBU1 with 16 individuals

Table S4 Pairwise F_{ST} among all streams. Values that are not significantly different from zero are in bold. Pairs of streams within the same catchment are highlighted in grey. Significance testing of F_{ST} was based on 10,000 permutations

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