Rangewide landscape genetics of an endemic Pacific northwestern salamander

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Abstract
A species’ genetic structure often varies in response to ecological and landscape processes that differ throughout the species’ geographic range, yet landscape genetics studies are rarely spatially replicated. The Cope’s giant salamander (Dicamptodon copei) is a neotenic, dispersal-limited amphibian with a restricted geographic range in the Pacific northwestern USA. We investigated which landscape factors affect D. copei gene flow in three regions spanning the species’ range, which vary in climate, landcover and degree of anthropogenic disturbance. Least cost paths and Circuitscape resistance analyses revealed that gene flow patterns vary across the species’ range, with unique combinations of landscape variables affecting gene flow in different regions. Populations in the northern coastal portions of the range had relatively high gene flow, largely facilitated by stream and river networks. Near the southeastern edge of the species’ range, gene flow was more restricted overall, with relatively less facilitation by streams and more limitation by heat load index and fragmented forest cover. These results suggested that the landscape is more difficult for individuals to disperse through at the southeastern edge of the species’ range, with terrestrial habitat desiccation factors becoming more limiting to gene flow. We suggest that caution be used when attempting to extrapolate landscape genetic models and conservation measures from one portion of a species’ range to another.

Keywords: circuitscape resistance, conservation, Dicamptodon copei, geographic range limit, least cost path, spatial replication

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Introduction
The field of landscape genetics combines tools from population genetics, spatial statistics and landscape ecology, enabling researchers to test the relative influence of various landscape characteristics on population connectivity and gene flow (Manel et al. 2003; Storfer et al. 2007). However, most landscape genetics studies suffer from a lack of replication across a study species’ geographic range (Holderegger and Wagner 2008; Segelbacher et al. 2010; but see Short Bull et al. 2011). Such a replication is important, as landscape processes may affect gene flow differently in different parts of a species’ range. Particularly for dispersal-limited species, different evolutionary histories may result in geographic variation in genetic structure across the species’ range (Bohonak 1999; Vucetich & Waite 2003; Willing et al. 2010). Variation in genetic structure can also be due to ecological differences among populations, such as varying interactions with the abiotic environment, species interactions or sexual selection (Rundle & Nosil 2005). On the other hand, differences could simply be due to variation in the landscape and not the biology of the species per se (Short Bull et al. 2011). That is, a landscape feature that limits gene flow in one area, such as fragmented forest cover, may be more continuous and thus not limiting in another area.

Replicated landscape genetics studies can contribute to a better understanding of the evolutionary ecology of
a species, such as the ecology and evolution of species’ range limits (Kawecki 2008; Sexton et al. 2009). Variation in gene flow is thought to be a key component in the formation and maintenance of species’ range edges (Kirkpatrick & Barton 1997) in addition to other processes such as ecological interactions with predators and competitors (Case & Taper 2000) or evolution of a species’ environmental niche (Holt & Gomulkiewicz 1997). Physiological constraints, such as temperature and moisture limitations, can restrict species distributions in more extreme environments, which are often found at range edges (Kawecki 2008; Lee et al. 2009; Trumbo et al. 2011, 2012). Replicated landscape genetics studies at varying distances from a species’ range edge may detect limited gene flow at the range edge relative to the core, suggesting edge populations to be genetically isolated and lack the genetic variability to adapt (Blows & Hoffmann 2005). Conversely, gene flow may be high across the whole range, suggesting gene flow from core-adapted populations may limit local adaptation of populations occupying different habitats at the edge (Kirkpatrick & Barton 1997). Indeed, rangewide gene flow studies can help to discover areas where genetic isolation of populations may potentially lead to divergence and microevolution (Bohonak 1999; Willing et al. 2010).

Lack of spatial replication in landscape genetic studies can also impede the conservation of biological diversity. When genetic data are limited to a small portion of a species’ range, and the results are then extrapolated throughout the entire range to guide management, conservation measures might be ineffective (Waples 1991; Avise 1992; Short Bull et al. 2011). For example, amphibian population connectivity can be limited by forest cover in highly fragmented areas, whereas availability of water bodies for breeding may be limiting in drier portions of a species’ range (Cushman 2006; Murphy et al. 2010). Therefore, conservation measures such as forest or breeding site restoration could be beneficial to gene flow in one part of the species’ range but not in another. Moreover, many ecological and landscape factors are likely to change with future projected changes in climate and human land use (Lannoo 2005; EPA 2011). An understanding of how these factors affect gene flow in portions of a species’ range where they are already limiting, such as in marginal habitats near range edges (Kawecki 2008), may help managers to plan conservation measures that are effective under future climate change scenarios.

Endemic species frequently become targets of conservation and management because they are sensitive to habitat disturbances and are most threatened by range contraction (Mittermeier et al. 2003). Amphibians are particularly vulnerable, as they are undergoing global declines (Stuart et al. 2004, Lannoo 2005) and many are dispersal-limited, often unable to track shifts in suitable habitat resulting from anthropogenic influences (Collins & Storfer 2003). One such species is Cope’s giant salamander (Dicamptodon copei), a dispersal-limited amphibian endemic to the Pacific northwestern USA (Nussbaum 1970). Its geographic range (Fig. 1; Jones et al. 2005; Lannoo 2005) is restricted to the mountains of the Olympic Peninsula in Washington (Olympic region), the Willapa Hills of western Washington and northwestern Oregon (Willapa region), and the western slopes of the Cascade Mountains in southern Washington and northern Oregon (Cascade region).

The ecology and evolutionary history of D. copei probably contribute to restricted gene flow observed in the species (Steele et al. 2009) and possibly to its limited geographic range. Phylogeographic data suggest that D. copei was isolated from other Dicamptodontid populations and began to speciate in the late Pliocene or early Pleistocene, likely due to glacial advancement and recession (Steele et al. 2005). Dicamptodon copei is currently a habitat specialist residing in forested, cool, headwater streams in mountainous or hilly terrain (Jones et al. 2005; Lannoo 2005). In the pluvial, forested environment of coastal Washington, D. copei evolved nearly obligate neoteny, whereby adults retain aquatic phenotypic traits from the larval stage such as external gills (Nussbaum 1970; Steele et al. 2005). Therefore, dispersal and gene flow in the species is thought to be restricted to waterways. As predicted, D. copei showed restricted gene flow in the southern Cascade Mountains portion of its range relative to its sympatric congener Dicamptodon tenebrosus, which commonly metamorphoses to terrestrial adults (Steele et al. 2009). These studies suggest that neoteny and poor overland dispersal abilities may limit D. copei dispersal and gene flow throughout its range.

In this study, we investigated the genetic structure of D. copei across a large portion of its geographic range to test for differences in landscape influences on gene flow. We tested three main hypotheses regarding the landscape genetics of D. copei: (i) stream networks facilitate gene flow throughout the species’ range due to high levels of adult neoteny; (ii) altered landcover, reduced canopy cover, and higher heat loads restrict gene flow more in the Willapa and Cascade regions than in the Olympic region due to higher levels of human disturbance; (iii) more extreme temperatures and lower precipitation levels are a greater constraint to gene flow in the Cascade region, located near the southern interior edge of the species’ geographic range, compared to the Olympic and Willapa regions located further north and closer to the coast.
Materials and methods

Study area

The entire range of *Dicamptodon copei* can generally be characterized as having a wet, cool climate (CEC 2011; WRCC 2011). However, elevational and latitudinal gradients, as well as varying climatic influences of the Pacific Ocean on coastal vs. inland sites, may result in differences in temperature and precipitation regimes experienced by the study species. For example, the Olympic and Cascade regions are more mountainous than the Willapa region, with steep elevational gradients of mild temperatures and high rainfall/snowfall ratios at coastal, low elevations up to alpine or even glaciated conditions at high elevations (CEC 2011). The maritime temperate rainforest habitats found on western slopes of the Olympic Mountains receive some of the highest precipitation levels in the continental USA (up to 4.6 m/year recorded; WRCC 2011). The coastal lowland areas of the Olympic and Willapa regions generally receive high annual precipitation (1.8–2.5 m/year), while the more inland Willapa and Cascade regions tend to receive slightly less precipitation (1.0–2.3 m/year).

In terms of landcover, the Olympic region consists of a large core area of protected, largely old growth forest (Olympic National Park, established in 1909) surrounded by a border of forests managed for timber production. Human developments are mostly along the coast and Puget Sound, with a very high percentage of perennial streams and rivers throughout the region (EcoRegions 7.1.8 and 6.2.5, CEC 2011). The Willapa and Cascade regions are composed of large tracts of forest that are managed for timber production, with scattered low-density human developments. Inland portions of these regions may contain higher percentages of ephemeral streams than the Olympic and coastal Willapa regions (EcoRegions 7.1.7, 7.1.9, and 6.2.7, CEC 2011). *Dicamptodon copei* gene flow is expected to be negatively affected by logging (Blaustein *et al.* 1995; Lannoo 2005) due to siltation of streams and reduction of forest cover, which can reduce oxygen levels, alter critical microhabitats necessary for breeding and shelter, and increase heat loads. Ecological studies have documented the negative impacts of logging on
the distribution of the closely related *Dicamptodon tenebrosus* in British Columbia (Johnston & Frid 2002; Curtis and Taylor 2003), Oregon (Hawkins *et al.* 1983; Corn & Bury 1989), and California (Welsh & Lind 2002; Ashton *et al.* 2006). In addition, gene flow of the coastal tailed frog (*Ascaphus truei*), which has similar habitat requirements and occurs sympatrically with *D. copei* (Jones *et al.* 2005; Lannoo 2005), was recently shown to be fragmented by logging in the Olympic Mountains region (Spear & Storfer 2008).

Population sampling

*Dicamptodon copei* tissue samples were collected in 2006–2008 from the Olympic, Willapa, and Cascade regions (Fig. 1), which span the geographic range of the species. In total, 29 streams containing *D. copei* were sampled; consisting of 12 streams from the Olympic region, 10 streams from the Willapa region, and seven streams from the Cascade region. We sampled multiple individuals (adults and juveniles) from a localized reach of each stream (usually a single 50–200-m-long sampling area per stream; Table 1). Because *D. copei* and *D. tenebrosus* larval individuals are difficult to distinguish morphologically in the field, we sampled all *Dicamptodon* individuals and used genetic markers to determine species. Most locations consisted of second- or third-order streams, with widths rarely >1.5 m. Once a location was confirmed for salamander presence, samples were obtained by kick-netting or by flipping large rocks and other potential cover objects. In collaboration with the Washington Department of Fish and Wildlife (WDFW), tail tips were collected from individuals (5 mm of tissue) and stored in 95% non-denatured ethanol, after which salamanders were released.

Population genetic analyses

DNA was extracted using DNeasy tissue kits (QIAGEN) according to the manufacturer’s protocols. Eleven microsatellite loci were amplified by PCR, using fluorescently labelled primers and conditions from Steele *et al.* (2008) (Table S1, Supporting information). Microsatellite products were run on an ABI 3730XL automated sequencer (Applied Biosystems) at the Washington State University LBB1 core facility and genotyped using ABI GeneMapper 3.7. To eliminate *D. tenebrosus* larvae and any hybrid individuals from the *D. copei* data set, we used NEWHYBRIDS (Anderson & Thompson 2002). We only included individuals with probabilities >95% of being *D. copei* in our analyses. We also used a maximum-likelihood algorithm employed in COLONY (Wang 2004) to identify full siblings, which was advisable given the high site fidelity of Dicamptodontid larvae and the large number of larvae in each stream sample. If full siblings were detected in any stream, we removed all but one sibling from the data set (Goldberg & Waits 2010). After species verification and elimination of full siblings, there were 740 total individuals included in our data set (range 6–81 and mean 24 individuals/site); including 237 individuals (mean 20/site) from the Olympic region, 310 individuals (mean 31/site) from the Willapa region and 193 individuals (mean 28/site) from the Cascade region (Table 1).

We used Genepop 3.4 (Raymond & Rousset 1995) to test whether loci and sites were in Hardy–Weinberg equilibrium (HWE) and to test for linkage disequilibrium (LD) among locus pairs. FREENA 2.0 g (Chapuis & Estoup 2007) was used to estimate the occurrence of null alleles at each locus. We also calculated summary statistics of genetic diversity for each sampled site using GDA (Lewis & Zaykin 2001). Specifically, we estimated

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*Significant *F* at α = 0.05
†Significant *F* at Bonferroni-adjusted α value = 0.003
allelic richness, observed and expected heterozygosity, and inbreeding coefficients ($F_{IS}$). Allelic richness represents the number of alleles present per locus per site, corrected for the variance in sample sizes among sites. Inbreeding coefficients reflect whether each site shows significant levels of inbreeding or outbreeding ($F_{IS} > 0$ or $F_{IS} < 0$, respectively).

Before conducting separate genetic analyses by region, we assessed whether the regions we assigned based on locations in distinct mountain ranges or lowland areas (i.e. Olympic, Willapa, and Cascade regions; Fig. 1) actually consisted of separate genetic populations. We used Structure 2.3.1 (Pritchard et al. 2000) to cluster individuals into genetic populations. We first ran Structure using all populations across the entire study area and then iteratively ran Structure on subpopulations until $K = 1$ had the strongest support (Evanno et al. 2005; Bray et al. 2009; Pires et al. 2009). We conducted all Structure runs for $K = 1$–6, with a burn-in period of 100 000 and 1 000 000 replicates using the admixture model. We used the second-order rate of change in log likelihood for each $K$ to assess the most likely value of $K$ (Evanno et al. 2005). This method cannot identify $K = 1$ because a change in log likelihood cannot be calculated for this value (Evanno et al. 2005). Therefore, we assumed $K = 1$ if its log likelihood value was highest, and complemented this with a visual assessment of the distribution of ancestry values. For instance, if $K = 1$, then a run of $K = 2$ should assign individuals equally to each cluster as there is no structure.

We used two techniques to estimate relative levels of genetic differentiation among populations: proportion of shared alleles ($D_{PS}$ Bowcock et al. 1994) and $G'_{ST}$ (Weir & Cockerham 1984; Hedrick 2005). $D_{PS}$ is not subject to the equilibrium assumptions inherent in the $G'_{ST}$ measure and thus may be more appropriate for measuring genetic connectivity among populations subject to recent disturbance (Bowcock et al. 1994). However, $G'_{ST}$ and other $F_{ST}$ analogues have been more commonly used and are therefore useful to allow comparison with other landscape genetic studies.

**Landscape resistance surfaces**

For each region, we developed six landscape resistance surfaces in ARCGIS (ESRI 2010) based on variables that we hypothesized a priori to affect *D. copei* gene flow (Fig. 1). Positive correlations with gene flow were expected for areas with more interconnected stream networks (USGS 2001; Steele et al. 2009), higher canopy cover (Homer et al. 2004), longer frost-free periods (Rehfelddt 2006), more growing season precipitation (Rehfelddt 2006), and forested landcover types (Homer et al. 2004). Negative correlations with gene flow were expected for nonforest ed and particularly human-developed landcover types (Homer et al. 2004) and areas with high heat load indices (McCune & Keon 2002). Four of six of these cost surfaces consisted of continuous variables, with canopy cover ranging from 0% to 100%, frost-free period ranging from 137 to 302 days/year, growing season precipitation ranging from 726 to 1278 mm/year, and heat load index ranging from 0 to 1 (dimensionless units reflecting the amount of solar radiation received per year; based on latitude, slope and aspect of a landscape; McCune & Keon 2002). The two remaining surfaces were categorical. Stream surfaces were divided into stream (low-cost) and nonstream or upland (high-cost) categories. Landcover surfaces consisted of forested natural habitat (lowest cost), nonforested natural habitat (intermediate cost), and human-developed habitat (highest cost). Cost surfaces were developed at 30-m spatial resolution, except for the temperature and precipitation surfaces, which were at a resolution of 750 m.

We tested multiple hypotheses for landscape relationships to gene flow by assigning multiple resistance costs (Spear et al. 2010). Stream cost surfaces were assigned 10:1 and 100:1 resistance costs for moving outside a stream compared to moving within it. We also tested stream cost surfaces both with and without major rivers, as large rivers may function as either dispersal corridors or barriers to *D. copei*, given the differences in microhabitats and potential predators present in large rivers vs. streams. We tested both linear responses of gene flow to continuous landscape variables, as well as two nonlinear responses (Balkenhol et al. 2009). The first nonlinear response ($T1$) transformed the landscape surface such that the transformed surface $T1 = 100^T$ (original cost surface). This represents a hypothesis of little effect of the landscape on gene flow at low values of the cost surface, but exponentially larger effects at high values. The second nonlinear response ($T2$) transformed the landscape surface such that the transformed surface $T2 = 100 − 100^T(1 −$ original cost surface). Contrary to $T1$, $T2$ represents a hypothesis of a large effect of the landscape variable on gene flow at low values, with little to no additional effects at high values. Graphs depicting the nonlinear response curves across a range of values are included in Fig. S1 (Supporting information).

Landscape distances between populations were estimated using two different techniques: least cost path (LCP) and Circuitscape resistance (Circuitscape) analyses (Fig. 2). LCPs were calculated through each landscape cost surface separately using the ARCGIS costdistance and costpath tools (ESRI 2010). Straight (Euclidean) line paths between sites were also calculated to represent a null model of isolation by Euclidian
distance (IBD; Wright 1942) or no effect of the landscape variables on gene flow. Sets of LCPs developed between all sites in a region represent hypotheses that gene flow is enhanced by increasing stream connectedness, canopy cover, length of the frost-free period, precipitation during the growing season, and forested habitat between sites; as well as minimizing heat load and movement through nonforested and human-developed habitats (Figs 1 and 2). In addition, for each LCP we calculated the weighted averages of all other landscape variables along that path to compare with the LCP distances between sites (Spear et al. 2005). Thus, multiple hypotheses for the most important predictor variables explaining variance in gene flow were compared together (Fig. 2a), as gene flow is expected to be affected by multiple landscape variables simultaneously. Finally, all LCP and straight line paths were corrected for topography, as actual path lengths will be longer than two-dimensional path lengths in areas with high topographic relief, such as the mountainous Olympic and Cascade regions.

Second, we estimated landscape distances using Circuitscape resistance analysis calculated with CIRCUITSCAPE (McRae 2006). This method treats gene flow like electrical current flowing through a resistor, with landscape resistance surfaces analogous to different resistors (McRae 2006). Unlike LCPs, Circuitscape analysis considers all possible pathways for gene flow between a pair of sites simultaneously and thus is theoretically a good compliment to LCPs that consider only a single idealized pathway (McRae 2006; McRae et al. 2008; Fig. 2). We calculated the average resistance between all pairs of sites in a region using an eight-neighbour cell connection scheme. Circuitscape analyses were performed with the same cost surfaces and at the same spatial resolution as the LCP analyses. In addition, we created null IBD models using a Circuitscape surface where all grid cells had a cost value of 1 (i.e. equal cost of movement in any direction) and compared these to our alternative landscape resistance hypotheses, as in the LCP analyses.

To assess potential differences in the distributions of landscape variables among the three regions, we calculated the mean and standard deviation of each variable, within areas that would be available to the species for movement. To estimate available movement areas, we used ARCGIS to dissolve all LCP polylines in a region into one polygon. For continuous variables, we used zonal statistics in ARCGIS to calculate the mean and standard deviation of each variable within the areas available for movement. For the categorical streams and landcover variables, we calculated the correlation length (or area-weighted mean radius of gyration; Keitt et al. 1997) within available area polygons using FRAGSTATS 4.0 (McGarigal et al. 2012). For landcover, a correlation length measures the average distance a salamander can move within a habitat patch before encountering the patch boundary given a random starting point. For streams, it represents the average distance a salamander can move through upland habitat before encountering a stream. Thus, it is a useful biological measure of the average connectedness or traversability of the landscape through a habitat of interest in a landscape genetics context (Short Bull et al. 2011; Cushman et al. 2012). As spatial data are not independent and we did not have the raw correlation length data, we assessed whether there

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Fig. 2 Two methods were used to estimate landscape resistance distances: (a) least cost paths (LCP) and (b) Circuitscape resistances. The landscape distances between sites JB1 (north) and 1236 (south) in the Olympic region are shown, measured through the heat load index cost surface (black to white = low to high resistances). LCPs (a) include canopy cover (dark green), frost-free period (white), growing season precipitation (purple), heat load index (red), landcover (light green), streams at 10:1 cost (light blue), and streams at 100:1 cost (dark blue). The Circuitscape resistance surface shown (b) is for the heat load index (blue to red = low to high circuit current, or movement potential).
were significant differences between regions by examining the extent that the 95% confidence intervals overlapped for each habitat metric (Schenker & Gentleman 2001). No overlap in confidence intervals was considered to be significantly different at $\alpha = 0.01$, and less than half of the confidence intervals overlapping was considered to be significant at $\alpha = 0.05$. Although Schenker & Gentleman (2001) point out that this method may not be as precise as a formal statistical test, it is a conservative estimate, and for the present study it is used as a guide to determine whether there were large differences in habitat variable distributions among regions.

Statistical analyses comparing genetic and landscape distances

The relationships between *D. copei* gene flow and landscape features were assessed using two methods: multiple linear regression on distance matrices (MRDM) using PERMUTE 3.4 alpha 9 (Legendre et al. 1994; Balkenhol et al. 2009) and the best subset of environmental variables with maximum rank correlation (Bioenv) using the VEGAN package (Clarke & Ainsworth 1993; Spear et al. 2005) in R (R Development Core Team 2011). MRDM is a nonparametric procedure that calculates the significance of independent distance matrix variables by randomly permuting rows and columns of the dependent matrix in the manner of a Mantel test and then compares standardized regression coefficients calculated for the dependent matrix to the null distribution of coefficients from random permutations (Legendre et al. 1994). For our MRDM runs, we performed a forward selection followed by a backward elimination of the selected variables. Variables were included in models at Bonferroni-corrected $P$-to-enter and $P$-to-remove values of 0.05, using 1000 random permutations of the dependent distance matrix per step. This procedure only allows independent variables that contribute significantly to the explanation of the dependent variable to enter the model at each step. Therefore, it is resistant to allowing strongly collinear variables that do not explain additional residual variance to enter the model (Legendre et al. 1994). This technique was shown by Balkenhol et al. (2009) to provide a good balance between type-1 error and power in a landscape genetics context, and to perform better than Mantel tests with variables that show some collinearity.

As it is unclear how model selection procedures such as Akaikes Information Criterion perform on distance matrices (Burnham & Anderson 2002), we also assessed landscape genetic models using a Bioenv correlation procedure (Clarke & Ainsworth 1993). Bioenv tests all possible combinations of independent variables (i.e. landscape distances) to find the subset of scaled variables that have the highest correlation with the dependent variable (i.e. genetic distance). For the Bioenv procedure, we calculated a weighted Spearman rank correlation ($p_w$) between the genetic distance matrices and all possible sets of landscape variables for each region to find the subsets of variables that had the strongest correlation with gene flow. We then removed strongly collinear variables and reran models until no sets of correlated variables were included in the most-supported models. Although Bioenv is an exploratory procedure that cannot calculate significance or regression coefficients of explanatory variables, it is suitable for use with distance matrices and allows for an independent analysis of consistency with the most-supported models from the MRDM procedure (Spear et al. 2005).

For both MRDM and Bioenv analyses, pairwise genetic distance ($D_{PS}$ or $G_{ST}$) between sites was the dependent (response) variable and landscape distances calculated for each cost surface hypothesis were the independent (explanatory) variables. Multiple models were tested for LCP analyses, as each model consisted of a LCP primarily correlated with genetic distance as well as the weighted averages of all other variables that were also significantly correlated with gene flow along that LCP (Fig. 2a). For the Circuitscape analyses, we tested only one model for each cost surface per region, as each Circuitscape model consisted of the average resistance through a given cost surface of all possible pathways between sites (Fig. 2b). Therefore, we report the top three LCP models explaining most of the variance ($R^2$ or $p_w$) in gene flow for each region, as well as the Circuitscape resistance variables significantly correlated with gene flow. To quantitatively assess whether there were differences in model performance among regions, we ran each of the best MRDM and Bioenv models for each region in the other two regions. We then calculated the change in $R^2$ or $p_w$ from the best model in the region of interest compared to the top three models from each of the other two regions.

Results

Population genetic analyses

Summary genetic diversity statistics were similar across study sites and regions, except that allelic richness was slightly lower in the Olympic region compared to the other two regions (Table 1). There was no evidence for null alleles at any locus for any region. There was significant inbreeding at some sites, particularly in the Willapa region (Table 1), so these sites may be out of HWE. In addition, at site 6000 in the Cascade region, five loci were out of HWE, and evidence of LD was found among several locus pairs, suggesting
nonrandom mating at this site. However, given that most loci were in HWE, and we used a genetic distance metric ($D_{PS}$) that minimizes equilibrium assumptions, all 11 loci were used for further landscape genetic analyses, but site 6000 was removed.

Structure analyses revealed differences in population genetic clustering from our initially hypothesized geographic regions (Fig. 3). The first Structure run using all sites across the study area revealed two main genetic subdivisions (i.e. $K = 2$). The first cluster included all sites in the Olympic region and, contrary to our predictions, the two northern-most sites in the Willapa region (sites 2260 and 2468). The other cluster consisted of the remaining Willapa region sites and all Cascade region sites (Fig. 3a). When we ran a second clustering analysis individually on the two clusters found in the first round (Fig. 3b), all the Olympic sites separated in a cluster from a different cluster containing the two northern Willapa sites. The remaining Willapa sites and Cascade sites were divided into three new clusters. The Willapa sites grouped into one cluster, while the Cascade sites were further separated into two more clusters, with one cluster containing only two sites (sites 5378 and 6000) spatially located within the larger cluster containing the rest of the Cascade sites (Fig. 3b).

Further rounds of clustering revealed that all regions could be subdivided until only one to four sites were assigned to each cluster ($K = 1$), congruent with the high overall levels of genetic differentiation found in a previous study of *Dicamptodon copei* in the southern Cascade region (Steele et al. 2009). For the purposes of the present study, we used the second cluster run to group sites into regions for landscape genetic analyses (Fig. 3b). These sites clustered both genetically and geographically while retaining sufficient samples sizes for statistical analyses. We removed sites 2260, 2468, 5378, and 6000 from further landscape genetic analyses because they were found to represent two separate genetic clusters with only two sites in each.

Pairwise measures of genetic differentiation (Table S2, Supporting information) were consistent with the findings of Structure analyses (Fig. 3). The Olympic region had the greatest overall similarity based on $D_{PS}$ (average pairwise value = 0.39) and, during the second round of Structure analyses, showed clustering of all sites across the peninsula. The Willapa region had the greatest similarity of pairwise $G'_{ST}$ values (average value = 0.26) and (with the exception of the two northern sites removed from further analyses) clustered into one group during the second division. The Cascade Mountains, the region with the smallest area and the fewest study sites, had the highest level of differentiation (average $D_{PS} = 0.51$, average $G'_{ST} = 0.45$) and was split into two groups during the second round of clustering.

Regional landscape variation

Overall, landscape variable distributions were similar among regions, with the exception of growing season precipitation and connectedness of landcover types.
Table 2 Landscape variation within areas available to salamander movement, calculated using ARCGIS and FRAGSTATS 4.0 for three study regions (Olympic, Willapa, and Cascade)

<table>
<thead>
<tr>
<th>Continuous variables</th>
<th>Olympic region</th>
<th>Willapa region</th>
<th>Cascade region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Elevation (m)</td>
<td>460.91</td>
<td>312.12</td>
<td>280.47</td>
</tr>
<tr>
<td>Canopy cover (%)</td>
<td>80.97</td>
<td>26.46</td>
<td>74.64</td>
</tr>
<tr>
<td>Frost-free period</td>
<td>169.13</td>
<td>17.32</td>
<td>179.05</td>
</tr>
<tr>
<td>Growing season</td>
<td>694.76*</td>
<td>40.11</td>
<td>554.18</td>
</tr>
<tr>
<td>Heat load index</td>
<td>0.98</td>
<td>0.16</td>
<td>0.79</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Categorical variables</th>
<th>Olympic region</th>
<th>Willapa region</th>
<th>Cascade region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streams</td>
<td>15277.22</td>
<td>4946.88</td>
<td>8169.69</td>
</tr>
<tr>
<td>Forested land</td>
<td>13451.95†</td>
<td>2574.33</td>
<td>5806.36</td>
</tr>
<tr>
<td>Nonforested, natural</td>
<td>74.79</td>
<td>34.31</td>
<td>105.49</td>
</tr>
<tr>
<td>Human-developed land</td>
<td>220.76*</td>
<td>57.96</td>
<td>23.24</td>
</tr>
</tbody>
</table>

Available areas were calculated by dissolving all least cost path polylines within a region into a polygon. For continuous landscape variables (raster data), means and standard deviations were calculated. For categorical landscape variables (vector data), correlation lengths (or average distance in m to nearest the nearest edge from random point within habitat) were calculated, as well as standard deviations. Significant differences among regions were estimated by examining whether 95% confidence intervals overlapped. *Regional values are significantly higher than values from both other regions at \( \alpha = 0.01 \). †Regional values are significantly higher than values from both other regions at \( \alpha = 0.05 \).

(Table 2). The Olympic region had significantly more growing season precipitation than either the Willapa or Cascade regions, as expected given that some of the western slopes of the Olympic Mountains receive some of the highest recorded rainfall levels in the continental USA (WRCC 2011). In terms of landcover, the Olympic region had more contiguous forest cover than the other two regions. The Cascade region had significantly higher connectedness of nonforested, natural land than the other two regions, suggesting that forested landcover was more patchy and fragmented, likely due to logging in the area. Finally, the Olympic region had significantly higher connectedness of human-developed land than the other two regions. This was likely attributable to a single residential and agricultural development area near the centre of the region associated with the town of Humptulips (Fig. 1e).

Statistical analyses comparing genetic and landscape distances

The null IBD model was only selected in the two Bioenv Circuitscape resistance analyses for the Cascade region (Table 5), while all other models selected consisted of one to several landscape variables, revealing that the landscape factors chosen for our study were important to gene flow in D. copei. Overall, models for the Cascade region explained more variance \( (R^2 = 0.79–1.00) \) and were more strongly correlated with gene flow \( (\rho_W = 0.75–0.94) \) than models for the Olympic and Willapa regions \( (R^2 = 0.58–0.76 \) and \( 0.33–0.71, \rho_W = 0.30–0.41 \) and \( 0.32–0.49, \) respectively, Tables 3–5, Table S6, Supporting information). Several of the nonlinear transformations were chosen as better models than simple linear relationships with gene flow. There were also some differences within regions in landscape variables \( (D_{PS} \) or \( G_{ST}^0 \)), landscape distance \( (LCP \) or Circuitscape), and statistical association \( (R^2 \) or \( q_W \)).

For the Olympic region (Table 3), models that maximized stream-based movement had the highest support in all but one of the LCP models, as well as in one Circuitscape model. Thus, streams were the best-supported landscape factor influencing gene flow in this region. Both 10:1 and 100:1 costs of movement outside of streams were supported in different models, and most stream-based models included major rivers, suggesting that they were used as dispersal corridors in this region. Other models with high support in the Olympic region included high growing season precipitation, canopy cover, and forested landcover types; as well as a low heat load index. LCP models tended to be complex in this region, with one to four weighted average variables supported along each of the most-supported LCP models.

In the Willapa region (Table 4), stream-based models were less important in explaining gene flow than in the Olympic region, although they were still selected as the best models in two Circuitscape analyses and four LCP models. As in the Olympic region, the stream models selected were a mix of 10:1 and 100:1 cost of movement hypotheses. However, contrary to the Olympic region the most-supported stream-based models in the
Willapa region tended not to include major rivers, suggesting rivers were barriers to dispersal in this region. Models that maximized growing season precipitation, length of frost-free period, and canopy cover, while minimizing the heat load index were also included in the most-supported models. In general, highly supported LCP models were simple in this region, with only one or two weighted average variables supported per model.

In terms of regional differences, the most-supported Olympic region models tended to perform much better than the top three models from either the Willapa or Cascade regions that were run in the Olympics, particularly for LCP models (change in $R^2 = +0.1256 - 0.5075$, change in $\rho_W = +0.3397 - 0.3879$; Table 3, Table S3, Table 5).

<table>
<thead>
<tr>
<th>Variables supported along path†‡</th>
<th>$R^2$</th>
<th>Regional comparison‡</th>
<th>Circuitscape surfaces⋆†</th>
<th>$\rho_W$</th>
<th>Regional comparison‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCP*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STR10</td>
<td>0.6925</td>
<td>+0.1256</td>
<td>GSP1</td>
<td>0.3879</td>
<td>+0.3442</td>
</tr>
<tr>
<td>Dist</td>
<td>0.6879</td>
<td>+0.5075</td>
<td>Dist</td>
<td>0.3432</td>
<td>+0.2855</td>
</tr>
<tr>
<td>HLI2(–)</td>
<td>0.6638</td>
<td>+0.3557</td>
<td>Dist</td>
<td>0.3397</td>
<td>+0.2154</td>
</tr>
<tr>
<td>Dist</td>
<td>0.7617</td>
<td>+0.2319</td>
<td>Dist</td>
<td>0.3644</td>
<td>+0.3190</td>
</tr>
<tr>
<td>HLI2(–)</td>
<td>0.7419</td>
<td>+0.2083</td>
<td>Dist</td>
<td>0.3644</td>
<td>+0.3352</td>
</tr>
<tr>
<td>Dist</td>
<td>0.7441</td>
<td>+0.3060</td>
<td>Dist</td>
<td>0.3559</td>
<td>+0.3066</td>
</tr>
</tbody>
</table>

*Abbreviated letters represent landscape resistance surfaces, and numbers (if any) represent nonlinear transformations of the cost surfaces (transformations 1 and 2 are exponentially increasing and decreasing functions, respectively), except for streams where numbers represent cost of moving outside of streams (10 = 10:1 cost, 100 = 100:1 cost). Supported variables include Dist., topographically corrected distance along LCP; CC, canopy cover; FFP, frost-free period; HLI, heat load index; LC, landcover; STR10, streams (including major rivers, 10:1 cost of movement outside streams); STR100NO, streams (excluding major rivers, 100:1 cost of movement outside streams).

†Negative signs in parentheses (–) represent effects of landscape variables on genetic distances that were opposite from initially hypothesized relationships, as evidenced from standardized regression $\hat{\beta}$ weights. Variables are ordered by relative influence, that is, top to bottom = largest to smallest $\hat{\beta}$ weight absolute values (see Table S6, Supporting information, for all model beta weights).

‡Change in $R^2$ or Bioenv correlation ($\rho_W$) from running the best Willapa or Cascade models in the Olympic region (see Table S3, Supporting information, for model details).

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Table 4 Willapa region comparison of landscape resistance distances [calculated by least cost paths (LCP) and Circuitscape resistances] and genetic distances ($D_{PS}$ and $G_{ST}$) using multiple regression on distance matrices by permutation (MRDM) and Bioenv best subset of environmental variables with maximum rank correlation ($p_W$)

<table>
<thead>
<tr>
<th>Variables supported along path*†</th>
<th>$D_{PS}$</th>
<th>$G_{ST}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRDM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCP*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variables supported along path*†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R^2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regional comparison‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Circuitscape surfaces*†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R^2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regional comparison‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioenv correlations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCP*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variables supported along path*†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p_W$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regional comparison‡</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviated letters represent landscape resistance surfaces, and numbers (if any) represent nonlinear transformations of the cost surfaces (transformations 1 and 2 are exponentially increasing and decreasing functions, respectively), except for streams where numbers represent cost of moving outside of streams (10 = 10:1 cost, 100 = 100:1 cost). Supported variables include Dist., topographically corrected distance along LCP; CC, canopy cover; FFP, frost-free period; HLI, heat load index; LC, landcover; STR10, streams (including major rivers, 10:1 cost of movement outside streams); STR10NO, streams (excluding major rivers, 100:1 cost of movement outside streams).

†Negative signs in parentheses (−) represent effects of landscape variables on genetic distances that were opposite from initially hypothesized relationships, as evidenced from standardized regression $\beta$ weights. Variables are ordered by relative influence, that is, top to bottom = largest to smallest $\beta$ weight absolute values (see Table S6, Supporting information, for all model $\beta$ weights).

‡Change in $R^2$ or Bioenv correlation ($p_W$) from running the best Cascade or Olympic models in the Willapa region (see Table S4, Supporting information, for model details).

Supporting information). In contrast, the most-supported models in the Willapa and Cascade regions performed only slightly better that the most-supported models from the other regions (change in $R^2 = +0.0136 – 0.3168$, change in $p_W = +0.0119 – 0.2638$; Tables 4 and 5, Tables S4 and S5, Supporting information). For all three regions, the most-supported Circuitscape models performed only slightly better than the models from other regions, in comparison with larger differences found among LCP models (Tables 3–5, Tables S3–S5, Supporting information).

Discussion

Landscape genetic studies are rarely replicated across a species’ range (Holderegger and Wagner 2008; Segelbacher et al. 2010), yet patterns of genetic structure are likely to vary due to spatial variation in landscape variables (Short Bull et al. 2011), as well as variation in the biology of a study species (Bohonak 1999; Vucetich & Waite 2003). We sampled populations from across the range of Dicamptodon copei, a dispersal-limited species with a restricted geographic range (Fig. 1), and showed that gene flow is affected by different landscape variables in portions of the range that vary in precipitation, landcover, and degree of anthropogenic disturbance (Tables 2–5). These findings support different management recommendations in different areas for D. copei, a species that is sensitive to anthropogenic disturbance that varies across the species’ range. Thus, our study supports prior recommendations that caution should be used when attempting to extrapolate landscape genetic findings and conservation measures from one portion of a species’ range to another (Waples 1991; Avise 1992; Short Bull et al. 2011).

Landscape genetics of Dicamptodon copei

Dicamptodon copei is a neotenic, dispersal-limited, habitat specialist salamander for which gene flow is likely limited by both its evolutionary history and current ecology. We found restricted gene flow using population and landscape genetic analyses, which were largely consistent with prior reports suggesting limited overland dispersal and high genetic structuring (e.g. Nussbaum
We identified five genetically distinct population clusters in our study area (three of those clusters retaining sufficient sample sizes to perform landscape genetic analyses) that spanned the geographic range of *D. copei*. Among the three study regions, we found higher levels of genetic differentiation in the southeast Cascade region relative to the more coastal and northerly Willapa and Olympic regions (Fig. 3, Table S2, Supporting information). This was somewhat surprising given that the Olympic and Willapa regions cover larger spatial extents than the Cascade region (Fig. 1) with longer average distances between sampling sites (Olympics = 32 km, Willapas = 21 km, Cascades = 15 km), and more population structure was expected across larger areas in a dispersal-limited species (Bohonak 1999). However, the high genetic structure in the Cascades was consistent with prior genetic work on *D. copei* in this region (Steele & Storfer 2007; Steele et al. 2009).

The coastal Willapa and Olympic regions, as well as portions of the Columbia River basin to the south, are thought to have served as refugia for *D. copei* during the glaciation cycles of the late Pliocene and Pleistocene epochs (Nussbaum 1970; Steele et al. 2005; Steele & Storfer 2007). In a likely adaptive response to these fluvial habitats, *D. copei* evolved a high degree of neoteny relative to other Dicamptodontid species (Nussbaum 1970; Steele et al. 2005). As the glaciers receded, neotenic *D. copei* individuals have expanded into the currently inhabited inland and higher-elevation portions of the Olympic, Willapa, and Cascade regions (Steele et al.).
2005; Steele & Storfer 2007). We hypothesize that historical emigration from multiple refugia may play a role in explaining the unexpected clustering of the two northern Willapa region sites with the geographically distant Olympic region sites (Fig. 3a), with the Willapa River and Bay potentially acting as a barrier to gene flow after secondary contact of these populations.

Interregional comparisons also revealed variation in the combinations of landscape factors affecting gene flow among regions (Tables 3–5, Tables S3–S5, Supporting information). In addition to more overall genetic structure, *D. copei* gene flow in the Cascade region was more restricted by factors that cause microhabitat desiccation, particularly high heat load indices and low forest canopy cover (Table 5). This is likely due to the predominance of less suitable habitat for survival and dispersal in southern portions of the range. The most-supported Olympic models performed much better than the Willapa or Cascade models that were run in that region (Tables 3–5, Tables S3–S5, Supporting information), suggesting that landscape genetic relationships in the Olympics are different from those in the other two regions. This could be attributed to the Olympic region having wetter, more contiguous forests than the other two regions (Table 2), as well as a large core protected area (Olympic National Park) which the other regions lack. The Cascade region also has more extreme summer and winter temperatures and receives less growing season precipitation (Table 2), which may result in a higher ratio of ephemeral streams (CEC 2011; WRCC 2011) than more coastal and northerly portions of the species’ range. Finally, the Cascade region has more fragmented forest cover relative to the Willapa and Olympic regions (Table 2) likely attributable to higher logging intensities. Therefore, differences in landscape genetic relationships and restricted gene flow in the Cascade region probably reflect the higher logging intensities and overall drier conditions that occur there. It should be noted that rigorous quantitative assessment of ecological and genetic differences among divergent landscapes remains a challenging issue in the field. One potential future improvement may be to develop methods to compare landscape genetic models to a random distribution of neutral (or null) landscapes with similar spatial properties (e.g. Pearson & Gardner 1997; Beale et al. 2009).

Streams were important for predicting gene flow in the Olympic and Willapa regions, but were surprisingly not as important in the Cascade region (Tables 3–5). In addition, stream models that included major rivers had higher support in the Olympic region relative to the Willapas or Cascades, suggesting rivers may be barriers to dispersal in the southern and inland portions of the range. Large rivers could limit *D. copei* dispersal for many reasons, including unsuitable microhabitats associated with higher streamflow volumes, high levels of predation by large fish (Blaustein et al. 1995), and/or hydrologic alterations of river habitat such as dams. The reason stream networks were less correlated with gene flow in the Cascade region is not clear. One hypothesis is that more overland dispersal occurs in the Cascade region, possibly due to higher rates of metamorphosis into terrestrial adults than previously thought and/or more overland movement during occasional flooding events. Increased overland dispersal could be a response to lower stream quality and more frequent stream drying due to logging, which results in silting and higher heat loads, respectively. Recent anecdotal findings of metamorphosed *D. copei* adults have been reported in the southern portion of the range, although most such reports have come from the southern Willapa Hills rather than the Cascade Mountains (Jones et al. 2005). However, two lines of evidence contradict this hypothesis. First, more overland dispersal would also be expected to increase connectivity among *D. copei* streams, yet we found lower overall gene flow in the Cascades relative to the other two regions (Fig. 3, Table S2, Supporting information). Second, Steele et al. (2009) found evidence of stream-based gene flow in the Cascade region when sampling at a finer spatial scale and testing fewer landscape variables than the present study. An alternative hypothesis is that the apparent decreased reliance on streams in the Cascades is actually an effect of spatial scale, given the more restricted scale of genetic connectivity found in the region (Fig. 3b, Table S2, Supporting information) and that different landscape processes can be important to gene flow at different spatial scales (Anderson et al. 2010; Murphy et al. 2010). Indeed, the Cascade region was the only region where IBD models were highly supported (Table 5), which could also occur when the spatial scale of sampling is large relative to the dispersal abilities of organisms (Waples 1998; Anderson et al. 2010).

The use of different modelling techniques, namely LCP vs. Circuitscape analyses, *D*$_{TS}$ vs. *G*$_{CT}$ genetic distances, and linear vs. nonlinear landscape variables, yielded broadly similar results but also showed some discrepancies within regions (Tables 3–5). We detected a greater number of significant landscape variables and higher model support using LCP analyses, while significant Circuitscape models included fewer landscape variables but occasionally revealed correlations not detected by LCPs. In addition, the differences between the best-supported models for the region of interest compared to the other two regions were lower for Circuitscape analyses than for LCP analyses for all three regions (Tables 3–5, Tables S3–S5, Supporting information).
Discrepancies were not unexpected, as landscape distances between sites are calculated differently and have different assumptions in Circuitscape vs. LCP analyses (see Materials and methods, Fig. 2). Circuitscape analysis is theoretically a good compliment to LCPs because it accounts for the possibility that redundancy of movement pathways between sites can enhance gene flow, as opposed to inferring a single idealized movement pathway in LCPs (McRae 2006; McRae et al. 2008). However, there was a high degree of multicollinearity among Circuitscape landscape variables in our study, apparently due to correlations of Circuitscape resistances to distances between study sites. These results are reflective of Circuitscape analyses in general (Spear et al. 2010) and likely contributed to fewer significant landscape variables in the Circuitscape models than in LCP models, as well as higher similarities between the best models in a region and best models from other regions.

Different genetic distance measurements ($D_{PS}$ vs. $G_{ST}$) also resulted in some discrepancies between significant landscape variables chosen within regions. These may be attributed to relaxed equilibrium assumptions in $D_{PS}$ relative to $G_{ST}$ for estimating gene flow, particularly as several sites had significant $F_{IS}$ values and may have been out of equilibrium (Table 1). For example, $D_{PS}$ may explain more variance in gene flow due to recent disturbances such as human alterations of forest cover while $G_{ST}$ may be more influenced by historical forest patterns as it takes time to reach equilibrium (Bowcock et al. 1994). The instability of models for different genetic distance measures in the Willapa and Cascade regions in particular may also be due to the relatively small differences between those regions’ most-supported models and the best models from other regions (Tables 3–5, Tables S3–S5, Supporting information). Our study also highlights the importance of considering nonlinear relationships of landscape variables to gene flow (Balkenhol et al. 2009), as we found that nonlinear transformations of some landscape variables explained more variance in gene flow than simple linear relationships. For example transformation 1 (exponentially increasing effect) of the canopy cover variable was preferred in the LCP models for Willapa and Cascade regions relative to a linear model. This suggested a threshold effect, whereby forest canopy cover has little effect on gene flow patterns at high cover amounts (i.e. low cost of movement values) but has exponentially larger effects when canopy cover exceeds a certain threshold of openness.

The ecological literature on $D. copei$ reports highly restricted overland dispersal (Nussbaum 1970; Jones et al. 2005; Lannoo 2005) and gene flow (Steele et al. 2009) due to its nearly obligate neoteny and habitat specialization. Our landscape genetic results from sites spanning the majority of the species’ range largely supported these findings, but also revealed new insights into the evolutionary ecology of $D. copei$. $D. copei$ appears to disperse less effectively near the southeast edge of its geographic range, with gene flow becoming more constrained by terrestrial factors that cause microhabitat desiccation (Tables 3–5, Table S2, Supporting information). Contrary to initial hypotheses, gene flow did not appear to be more limited by macroclimate temperature and precipitation factors near the edge of the species’ range (Lee et al. 2009; Trumbo et al. 2011, 2012). However, other microhabitat factors that affect temperature and water regulation in amphibians were relatively more limiting to gene flow at this range edge, such as the heat load index and canopy cover (Table 5). Individual susceptibility to moisture loss and extreme temperatures may be more intense in the inland Cascade region, as a wet, cool macroclimate may be more prevalent in more northerly and coastal regions (CEC 2011; WRCC 2011). These results have implications for the evolution of species’ range edges (Kawecki 2008; Sexton et al. 2009), as reduced gene flow at the edge of the range suggests populations are more isolated than those nearer to the centre of the range. It may be that isolation and reduced genetic variability limit evolution of traits that would allow $D. copei$ to expand its range further south and east (Holt & Gomulkiewicz 1997; Blows & Hoffmann 2005).

Conservation

Effective species conservation and management depends on accurate information regarding variation in dispersal and gene flow across a species’ geographic range. $D. copei$ is currently not a threatened or endangered species, but it is on the Sensitive Vertebrate list in Oregon (ODFW 2011) and is a State Monitor species on the list of Species of Special Concern in Washington (WDFW 2011). With projected future climate change and human population growth in the Pacific northwestern USA (Campbell 1996; Lannoo 2005; EPA 2011), habitats may become less suitable for $D. copei$. Larger human populations may result in increased development practices such as logging that reduce forest cover outside of fully protected areas. Projected warmer temperatures may also cause $D. copei$ range contractions in the southern portion of the range, as well as allow other stream-dwelling species such as $D. tenebrosus$ to expand their ranges further north, potentially resulting in increased competitive or predatory interactions with $D. copei$ (Blaustein et al. 1995; Lannoo 2005). Finally, there are extensive saltwater barriers surrounding the Olympic peninsula (Fig. 1) preventing further northward migration of $D. copei$, potentially resulting in range contractions if southern populations decline.
Our results suggested that conservation measures for *D. copei* should be tailored to specific geographic regions. The Cascade region should probably be prioritized for management, given the already fragmented forest cover (Table 2), restricted gene flow (Fig. 3, Table S2, Supporting information; Steele et al. 2009), and high likelihood of impacts from logging and climate change (Campbell 1996; Lannoo 2005; EPA 2011). Management should focus on conserving high-quality, upland, forested habitats in this region because habitat desiccating factors have a relatively large effect on both stream and upland gene flow in the region (Table 5). However, maintaining high-quality stream networks should not be neglected given that they are critical for breeding (Nussbaum 1970) as well as important for gene flow (Steele et al. 2009; Table 5). Isolated populations in the southern portion of the species’ range could potentially be adapted to slightly drier conditions than populations further north (Bohonak 1999; Willing et al. 2010). If so, maintaining gene flow and spread of potentially adaptive genetic variation with the Willapa region to the northwest could become particularly important if northern regions become warmer and drier. Finally, further surveys and studies to determine the degree to which metamorphosis and upland dispersal occurs in southern portions of the range could help inform managers as to the extent of upland forests necessary to maintain genetic connectivity.

Management directed towards maintaining a continuous network of high-quality stream and river dispersal corridors should be a priority for *D. copei* populations in the Olympic and northern Willapa regions, which appear highly dependent on stream and river-based dispersal (Tables 3 and 4). In addition, maintaining high-quality forests in upland areas around these stream corridors will help limit siltation loads, which is beneficial for the high oxygen demands and microhabitat requirements of *D. copei*. Finally, macroclimatic precipitation and temperature gradients were important for facilitating gene flow throughout the species’ range (Tables 3–5). Hence, it will be important to determine whether future climate change causes contractions (or expansions) of the species’ geographic range. The previously mentioned variation in management priorities among regions, due to variation in landscape genetic patterns across the species’ range, demonstrates the importance of spatial replication in landscape genetics studies.

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### Data accessibility


### Supporting information

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

**Table S1** Multiplex PCR conditions for *Dicamptodon*.

**Table S2** Pairwise *Dps* and *Gst* values for each region.

**Table S3** Comparison of Willapa and Cascade models within the Olympic region.

**Table S4** Comparison of Cascade and Olympic models within the Willapa region.

**Table S5** Comparison of Olympic and Willapa models within the Cascade region.

**Table S6** Standardized beta coefficients for best three models from the Olympic, Willapa, and Cascade regions.

**Fig. S1** Nonlinear response curves for continuous landscape variables.

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