Phylogeographic incongruence of codistributed amphibian species based on small differences in geographic distribution

Craig A. Steele *, Andrew Storfer

School of Biological Sciences, Washington State University, Pullman, WA 99164-4236, USA

Received 10 April 2006; revised 20 September 2006; accepted 9 October 2006

Abstract

Codistributed species may display either congruent phylogeographic patterns, indicating similar responses to a series of shared climatic and geologic events, or discordant patterns, indicating independent responses. This study compares the phylogeographic patterns of two similarly distributed salamander species within the Pacific Northwest of the United States: Cope’s giant salamander (Dicamptodon copei) and Van Dyke’s salamander (Plethodon vandykei). Previous studies of P. vandykei support two reciprocally monophyletic lineages corresponding to coastal populations, located from the Olympic Mountains to the mouth of the Columbia River, and inland populations within the Cascade Mountains. We hypothesized that D. copei would have a congruent phylogeographic pattern to P. vandykei due to similarity in distribution and dependence upon similar stream and stream-side habitats. We test this hypothesis by estimating the phylogeny of D. copei using >1800 bp of mitochondrial DNA and comparing it to that of P. vandykei. Sympatric populations of D. copei and of P. vandykei display an identical phylogeographic pattern, suggesting similar responses within their shared distribution. Populations of D. copei occurring outside the range of P. vandykei displayed high levels of genetic divergence from those sympatric to P. vandykei. Overall, phylogeographic patterns between the two species were ultimately incongruent due to the high divergence of these allopatric populations. These results provide an example of codistributed species displaying overall incongruent phylogeographic patterns while simultaneously displaying congruent patterns within portions of their shared geographic distribution. This pattern demonstrates that a simple dichotomy of congruent and incongruent phylogeographic patterns of codistributed species may be too simplistic and that more complex intermediate patterns can result even from minor differences in species’ ranges.

Keywords: AU test; Bayesian hypothesis testing; Biogeography; Columbia River; Comparative phylogeography; Dicamptodon; Pacific Northwest parametric bootstrap; Plethodon; SH test

1. Introduction

A central objective of comparative phylogeography is to test codistributed species for concordant phylogeographic patterns (Bermingham and Moritz, 1998; Schneider et al., 1998; Avise, 2000; Argobast and Kenagy, 2001; Zink, 2002). Studies that reveal concordance among codistributed biota often provide evidence that a shared series of past events similarly shaped their genetic patterns. Comparative phylogeography enhances our understanding about the role of climatic, geological, and ecological forces in shaping the geographic distribution and intraspecific variation of species comprising an ecosystem. While a variety of studies have demonstrated phylogeographic congruence among codistributed taxa (Avise, 1992; Schneider et al., 1998; Riddle et al., 2000), a comparable number have also revealed incongruence (Zink, 1996; Taberlet et al., 1998; Hewitt, 1999). Discovery of incongruent phylogenies among codistributed species may suggest independent responses to shared evolutionary events due to different ecologies, life histories, or post-glacial expansion routes (Bowen and Avise, 1990; Taberlet et al., 1998; Michaux et al., 2005; Rocha et al., 2005), or may result from “pseudo-incongruence” in which codistributed species respond independently...
to different evolutionary events occurring at different times (Donoghue and Moore, 2003). Incongruence among population-level phylogenies may also be due to variation in the microevolutionary processes, such as lineage sorting or effective population size, that are responsible for generating the patterns of population-level divergence (Mason-Gamer and Kellogg, 1996). Additionally, underlying concordance in phylogenetic topologies may be masked by variation in the geographic patterns of population processes such as extinction, dispersal, sympatric diversification, or a lack of evolutionary response to vicariance (van Veller et al., 1999; Crisci et al., 2003). Phylogeographic incongruence among codistributed species suggests that evolution of biotic communities is often neither a synchronized nor a concerted event (Hewitt, 1999; Sullivan et al., 2000; Brunsfeld et al., 2001; Carstens et al., 2005a). Phylogeographic patterns of codistributed species may also be incongruent while simultaneously displaying patterns of shared responses to past climatic or geologic events (Sullivan et al., 2000). The combination of these findings indicate that phylogeographic patterns of codistributed species are often influenced by differing ecologies, life history traits and population processes.

The temperate rainforests within the Pacific Northwest of the United States are a well-known example of an area with codistributed species known for their high endemism (Brunsfeld et al., 2001). Considerable effort has gone into constructing a regional perspective on the phylogeographic patterns of these endemic organisms (Brunsfeld et al., 2001; Soltis et al., 1997; Carstens et al., 2005a). The codistributed amphibian assemblage within the mesic forest ecosystem of the Pacific Northwest provides an ideal opportunity to test for concerted responses to past climatic and geologic events (Carstens et al., 2005a). This assemblage includes diverse and distantly related amphibian species such as tailed frogs (Ascaphus truei, A. montanus), Pacific giant salamanders (Dicamptodon spp.) and plethodontid salamanders (Plethodon idahoensis, P. vandykei) (Carstens et al., 2005a). Previous studies on this assemblage have demonstrated a concordant response to the uplift of the Cascade Mountains ~2 to 5 mya, resulting in reciprocally monophyletic lineages corresponding to coastal populations and disjunct interior populations within the northern Rocky Mountains (Nielson et al., 2001; Carstens et al., 2004; Steele et al., 2005; Carstens et al., 2005b). The disjunct populations of amphibians found within the Rocky Mountains share similar geographic distributions and intraspecific studies reveal a common pattern of shallow phylogenetic structuring in these species, suggesting recent colonization events within the Rocky Mountains (Nielson et al., 2001; Carstens et al., 2004; Carstens et al., 2005b). Coastal lineages of this amphibian assemblage also share similar geographic distributions. However, detailed comparative studies have not yet been conducted on these coastal populations to test for concerted responses to past climatic or geologic events.

Studies of codistributed amphibian assemblages in the Pacific Northwest have primarily focused on broad scale phylogeography within a genus and the deep genetic divergence between coastal and disjunct inland lineages. In contrast, this study adds a new dimension by focusing on the comparative phylogeography of species that share small fragmented distributions restricted to coastal temperate rainforest. Two of the codistributed amphibians within this mesic forest ecosystem are the Cope’s giant salamander (Dicamptodon copei) and the Van Dyke’s salamander (Plethodon vandykei). These species are endemic to the Pacific Northwest of the United States, have similarly fragmented distributions, and are similarly dependent upon stream and stream-side habitats. The geographic distribution of each species is split into three mountainous regions within the Pacific Northwest: Olympic Mountains, Willapa Hills, and Cascades Mountains (Fig. 1). The Cope’s giant salamander is a neotenic species and usually remains in an aquatic form throughout its life (Nussbaum, 1976), while the Van Dyke’s salamander is considered to be the most aquatic Plethodon species in North America and lives in seeps and streamside splash zones (Brodie, 1970; Petranka, 1998). Several other species of salamander that are strongly associated with streams also occur in the study area (Rhyacotriton spp.) but each has a distribution restricted to just one of these mountainous regions (Good and Wake, 1992). The combination of a similarly fragmented distribution and shared dependence on stream habitat makes D. copei and P. vandykei ideal for testing hypotheses of concerted or independent responses to past climatic and geologic events. Because other mesic forest amphibians show similar responses to past geologic events in the Pacific Northwest, (Carstens et al., 2005a) it is reasonable to predict that these two species should also have concordant phylogeographic topologies.

Results from two previous studies on P. vandykei provide a clear phylogeographic hypothesis (Howard et al., 1993; Wilson and Larsen, 1999) which is used to test the phylogeographic topology of D. copei. Both electrophoretic (Howard et al., 1993) and morphological (Wilson and Larsen, 1999) studies consistently revealed two reciprocally monophyletic lineages corresponding to coastal populations, located in the Olympic Mountains and the Willapa Hills, and inland populations within the Cascade Mountains (Fig. 1). Populations within these two areas are thought to have been isolated since the late Pleistocene (Wilson and Larsen, 1999) and are separated by lowland areas of glacial and alluvial deposits that appear to limit dispersal (Wilson et al., 1995). Both studies also reveal that populations within the Olympic Mountains are indistinguishable from those in the Willapa Hills, indicating recent expansion of P. vandykei into the Olympic Mountains. To test the hypothesis that D. copei has a similar phylogeographic history, mitochondrial DNA is used to estimate a phylogeny and elucidate past demographic patterns within D. copei. The resulting phylogeny is tested for concordance with that of P. vandykei using a variety of phylogenetic comparison tests.
2. Material and methods

2.1. Sample collection and DNA amplification

Tissue samples from tail clips of 80 specimens of *D. copei* were obtained from 24 localities throughout its range. (Fig. 1). DNA was extracted using standard phenol/chloroform extractions (Sambrook et al., 1989). Approximately 1100 bp of the cytochrome *b* gene (cyt *b*) were obtained using the primer sets from Carstens et al. (2005b). In addition, approximately 750 bp of the mitochondrial control region (CR) were amplified and sequenced using a modified 007 primer (5'-gcacccaaagccaaattttca-3') and the 651 primer from Shaffer and McKnight (1996). Amplicons were...
purified using centrifugal filters (Millipore; Bedford, MA) and sequencing reactions were performed using BigDye Kit version 3.1 (Applied Biosystems; Foster City, CA) with 20-40 ng of PCR product in 10 ul reaction volumes. Sequencing reactions for cyt b and CR were performed in both 5'- and 3'-directions, purified with a 70% isopropanol wash, and run on either an ABI 377 or ABI 3730 automated sequencer. Homologous sequences of the three remaining members of the genus (D. aterrimus, D. ensatus, and D. copei) were used as outgroups (Steele et al., 2005). Sequences were aligned and edited with Sequencer 4.1 (Gene Codes; Ann Arbor, MI). Sequences generated from this study are deposited in Genbank (Appendix 1).

2.2. Phylogeny reconstruction

Sequences data were analyzed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses. Redundant haplotypes were removed and DT-ModSel (Minin et al., 2003) was used to select a model of evolution. The CR and cyt b data sets were tested for congruence with a partition homogeneity test in PAUP* ver.4.0b10 (Swofford, 2002) and resulted in a non-significant value of $P = 0.39$. The two datasets were subsequently combined for all analyses. Maximum parsimony and ML analyses were performed with PAUP* using a heuristic search with TBR and 10 random-addition replicates. For the MP analysis, all sites were weighted equally and gaps treated as missing data. The HKY+I+G model of evolution was the best fit for the cyt b and CR data as well as the combined data. The ML analysis was performed under this model where $I = 0.7096$, $G = 0.8779$, transition/transversion ratio $= 3.0749$, and the following equilibrium base frequencies: $A = 0.3252$, $C = 0.1761$, $G = 0.1474$, $T = 0.3513$. Branch support of MP and ML analyses were assessed from 200 non-parametric bootstrap replicates.

To estimate Bayesian posterior probabilities of nodes, MrBayes 3.1 (Huelserbeck and Ronquist, 2001; Ronquist and Huelserbeck, 2003) was used to conduct $5 \times 10^6$ generations of a Bayesian run under an HKY+I+G model with the default flat priors and sampling every 100th generation. Although DT-ModSel selected the HKY+I+G model of evolution for both the cyt b and CR data sets, the values for transition/transversion ratios, proportion of variable sites, and among site rate heterogeneity differed for each gene. Therefore, the cyt b and CR sequences were partitioned and the data sets unlinked, thereby allowing these parameters to vary across the two data sets during analysis. Two independent runs were performed simultaneously on the data with each run using one cold and three heated chains. Examination of the posterior probability distributions suggested the Markov chain reached stationarity within 200,000 generations but the first 25% of the samples (1,750,000 generations) was discarded as ‘burn in’ to ensure stationarity. The average standard deviation of split frequencies between the two independent runs at completion was 0.0036, indicating convergence of the two runs on a stationary distribution.

2.3. Testing topologies

In order to test the hypothesis of congruent phylogeographic patterns between the codistributed species, a constrained phylogeny was created, whereby relationships among populations of D. copei are forced to display the same topological pattern displayed by the codistributed P. vandykei (Fig. 1). This constrained topology comprises a sister relationship between two main lineages corresponding to coastal populations, located in the Olympic Mountains and Willapa Hills (1–11), and inland populations, located in the Cascade Mountains (12–24) (Fig. 1). Topological congruence between the unconstrained phylogeny of D. copei (Fig. 2) and the constrained phylogeny (Fig. 1, inset) was tested using four different methods: Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa, 1999), Approximately Unbiased (AU) test (Shimodaira, 2002), parametric bootstrap (Huelsenbeck et al., 1996), and Bayesian posterior probabilities (Huelsenbeck et al., 2002). These tests are commonly used in topological comparisons of phylogenies but differ in their intrinsic statistical qualities. Testing for phylogenetic concordance using this suite of tests allows one to more easily determine the degree of confidence to place on the resulting $P$-values.

2.3.1. Shimodaira–Hasegawa test

The SH test (Shimodaira and Hasegawa, 1999) is a modified version of the Kishino–Hasegawa test (Kishino and Hasegawa, 1989) and is often preferred because of its ability to compare an $a$ posteriori topology (e.g., a ML topology derived from the dataset) to a topology of interest (Goldman et al., 2000). Even though the SH test is capable of simultaneously testing among many alternative topologies, we used the minimum number of two topologies. This allows for a more direct comparison of the results with other topological tests which can only test between two topologies at a time (Buckley, 2002). The best constrained and unconstrained ML trees were compared in PAUP* using 1000 bootstrap replicates and the RELL resampling criteria.

2.3.2. Approximately unbiased test

While the SH test is generally considered an appropriate test for comparing tree topologies, it has been noted that it may be too conservative of a test (i.e. less likely to reject alternative topologies under consideration) (Shimodaira, 2002; Buckley, 2002). For this reason, the AU test was developed to reduce the potential bias of the SH test (Shimodaira, 2002). We conducted the AU test in Consel (Shimodaira and Hasegawa, 2001) using the site-wise log-likelihood values from the best ML tree obtained from the data and a ML tree constrained a topology consistent with P. vandykei.

2.3.3. Parametric bootstrap

Phylogenetic concordance was also tested using a parametric bootstrap (Goldman, 1993; Huelsenbeck and Bull, 1996). The model of sequence evolution selected by
DT-ModSel (HKY+I+G) was used to simulate 100 datasets on the constrained topology using Seq-Gen (Rambaut and Grassly, 1997). Constrained and unconstrained ML searches were conducted on each simulated dataset in PAUP* and the null distribution of the test statistic was generated by calculating the difference of log likelihood scores from each dataset ($\delta = \ln L_{\text{constrained}} - \ln L_{\text{unconstrained}}$). This same difference in log likelihood scores of the observed sequence data is used as the test statistic to evaluate phylogenetic concordance between the constrained and unconstrained ML trees.

### 2.3.4. Bayesian posterior probabilities

While the parametric bootstrap assesses topological uncertainty by generating a null distribution of the test statistic using simulated data under the chosen model of evolution, Bayesian hypothesis testing generates a distribution of trees given the data, prior probabilities, and model of evolution. Two independent Bayesian runs were performed simultaneously on the data for $5 \times 10^6$ generations with topologies sampled every 100th generation. After discarding the first 25% of samples as ‘burn in’ the remaining 37,500 topologies from each run were imported into PAUP*. This posterior distribution of topologies was then filtered with the constrained topology. The proportion of trees in the distribution consistent with the constrained topology is the Bayesian conditional probability that the constrained topology is correct (Huelsenbeck et al., 2002).

### 2.4. Nested clad analysis

To test for significant association of haplotypes with geography, we performed a Nested Clade Analysis (NCA) (Templeton et al., 1987, 1995). A minimum spanning...
network was constructed using TCS 1.18 (Clement et al., 2000) and haplotypes were nested using rules of Templeton et al. (1987) and Templeton and Sing (1993). Geographical localities for each population were calculated using latitude and longitude. GeoDis 2.4 (Posada et al., 2000) was used to test for significant association of haplotypes and geography. Although there is some controversy surrounding the validity of NCA (Knowles and Maddison, 2002), we followed the inference key that was revised to deal with past criticisms (Templeton, 2004) for clades with significant geographical associations.

3. Results

3.1. Summary of DNA sequences

We sequenced 1830 nucleotides of mitochondrial DNA: 1135 bases of partial cyt b sequence and 695 bases of partial CR sequence. We found 28 haplotypes from 80 individuals; 14 haplotypes were found in multiple individuals and 14 haplotypes were represented by single individuals. The most frequently sampled haplotype, designated as ‘SD1’ in the phylogeny (Fig. 2), was found in 16 of the 80 individuals (20.0%) and was present in 6 of the 24 localities, all of which are located in the Olympic Mountains.

3.2. Phylogenetic analyses

There were 112 parsimony-informative sites in the complete data set and the MP analysis found eight equally parsimonious MP trees with a tree length of 272 steps. Topology of bootstrapped MP trees and the single best ML tree (−ln 4071.6256) with branch support over 50% were identical to the Bayesian topology (Fig. 2).

The phylogeny of D. copei reveals a well-supported lineage corresponding to populations in a small geographic area along the southern edge of the Columbia River (Fig. 2). There is also support for a sister relationship between the coastal populations and inland populations occurring in the Cascade Mountains north of the Columbia River. Populations in the Cascade Mountains restricted to south of the Columbia River form a separate clade that is sister to lineages along the coastal and in the northern Cascade Mountains. The phylogeographic pattern is suggestive of a sister relationship between monophyletic lineages along the coastal and in the Cascade Mountains, but only when considering populations occurring within the range of Plethodon vandykei. Populations of D. copei that occur outside the range of P. vandykei, namely populations in the Cascade Mountains occurring south of the Columbia River, display a high degree of divergence from the remainder of the D. copei populations (Table 1).

3.3. Phylogenetic concordance

The combined results of the SH, AU, parametric bootstrap and Bayesian hypothesis tests confirm that the topology of the D. copei phylogeny is not concordant with the P. vandykei phylogeny (Table 2). All tests resulted in significant P-values, including the most conservative SH test (P = 0.045). The parametric bootstrap and Bayesian hypothesis test easily rejected phylogenetic concordance (Buckley, 2002). The AU test, which was developed to reduce conservative bias in the SH test (Shimodaira, 2002), still had a highly significant P-value (0.007) that was intermediate between the SH test and Bayesian and parametric bootstrap tests.

3.4. Nested clade analysis

The minimum spanning haplotype network consisted of three main clades corresponding to coastal populations within the Olympic Mountains and the Willapa Hills, populations with a distribution restricted to several tributaries along the southern banks of the Columbia River, and the remaining populations found within the Cascade Mountains on either side of the Columbia River (Fig. 3). Some loops are present in the network but were resolved using nesting procedures from Templeton et al. (1992) and Templeton and Sing (1993). These loops ultimately do not affect nesting design or conclusions inferred from the analysis. Seven nested haplotype networks had a signifi-

Table 1

<table>
<thead>
<tr>
<th>Genetic distances among main lineages of Dicamptodon copei</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>Columbia valley</td>
</tr>
<tr>
<td>S. Cascade Mtns</td>
</tr>
<tr>
<td>N. Cascade Mtns</td>
</tr>
<tr>
<td>Coastal</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
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<th>Results for tests of phylogenetic concordance between D. copei and P. vandykei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ln likelihood scores</td>
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<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Constrained</td>
</tr>
<tr>
<td>4079.7863</td>
</tr>
</tbody>
</table>

significant association with geography and conclusive inferences (Table 2). The overall pattern of the spanning network is consistent with phylogeographic results and indicates that genetic structure of populations north of the Columbia River share a genetic pattern similar to that of *P. vandykei* while populations south of the Columbia River represent divergent lineages. The major inferences from the nested clade analysis are northward expansion of populations in the Cascade Mountains, colonization of the Olympic Mountains from the Willapa Hills, and restricted overall gene flow among the fragmented populations (Table 3, Fig. 4).

4. Discussion

The comparison of phylogeographic patterns between the Cope's giant salamander (*D. copei*) and the Van Dyke's salamander (*P. vandykei*) demonstrates an overall pattern of incongruence while sympatric populations simultaneously exhibit identical and congruent patterns. This result provides evidence that similarly distributed organisms can demonstrate concordant phylogenies within their shared distribution, but that allopatric populations may display significant levels of phylogenetic signal and effectively obscure any congruent phylogeographic pattern.

Fig. 3. Minimum spanning haplotype network for *Dicamptodon copei*. Lines indicate a connection between haplotypes. Missing haplotypes are shown as black dots. Sampled haplotypes are designated with abbreviations and correspond to those in the Appendix. One-step clades are shown in white, two-step clades in light gray, three-step clades in medium gray, four-step clades in dark gray and five-step clades in black. Numbers in parentheses indicated clade numbers and are shown only for clades with significant association with geography.
4.1. Phylogeography of the Cope’s giant salamander

The deepest phylogenetic divergence among *D. copei* populations is the separation of several populations found along the Columbia River from the remainder of all other populations. These divergent populations are geographically restricted to several short tributaries that drain directly into the Columbia River and are not joined to the large interconnected network of headwater streams that run throughout the region. Because *D. copei* rarely metamorphose and remain primarily in an aquatic phase, the lack of connection with other watersheds seems to have prevented stream-based dispersal into and out of this population. The Columbia River Valley has been identified as a Pleistocene refugium for a variety of fishes (Brown et al., 1992; Bickham et al., 1995; Taylor et al., 1999; McCusker et al., 2000; Haas and McPhail, 2001) and for other salamander species (Wagner et al., 2005; Steele and Storfer, 2006). These divergent populations of *D. copei* appear to have been restricted into several streams within this glacial

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**Table 3**

Results and inferences of nested clade analysis

<table>
<thead>
<tr>
<th>Clade</th>
<th>( \chi^2 ) statistic</th>
<th>Probability</th>
<th>Inference chain</th>
<th>Inferred pattern*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–1</td>
<td>13.36</td>
<td>0.012</td>
<td>1–2–11–12–No</td>
<td>Contiguous northward range expansion of northern most populations in Cascade Mtns</td>
</tr>
<tr>
<td>1–5</td>
<td>1.0</td>
<td>0.000</td>
<td>1–2–3–5–6–7–Yes</td>
<td>RGF with some long distance dispersal within Olympic Mtns</td>
</tr>
<tr>
<td>2–7</td>
<td>27.00</td>
<td>0.001</td>
<td>1–2–3–5–6–7–8–Yes</td>
<td>Past gene flow from Willapa Hills into Olympic Mtns followed by extinction of intermediate populations</td>
</tr>
<tr>
<td>4–3</td>
<td>16.00</td>
<td>0.017</td>
<td>1–2–11–17–4–No</td>
<td>RGF within Cascade populations north of Columbia River</td>
</tr>
<tr>
<td>5–1</td>
<td>66.07</td>
<td>0.000</td>
<td>1–2–3–5–6–13–Yes</td>
<td>Long distance colonization of Cascade populations across Columbia River coupled with subsequent fragmentation</td>
</tr>
<tr>
<td>5–2</td>
<td>24.15</td>
<td>0.016</td>
<td>1–2–11–Yes</td>
<td>Southward range expansion across Columbia River of populations in Willapa Hills</td>
</tr>
<tr>
<td>6–1</td>
<td>1.0</td>
<td>0.000</td>
<td>1–2–3–5–6–7–Yes</td>
<td>RGF with some long distance dispersal among Cascade, Coastal, and Columbia populations</td>
</tr>
</tbody>
</table>

Haplotype networks without significant geographical associations or significant networks with inconclusive inferences are not listed.

* RGF, restricted gene flow.

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**Fig. 4.** Historical demographic patterns for *Dicamptodon copei* as inferred by the nested clade analysis. A black dot represents the location of the divergent populations along the Columbia River. (1) Populations colonize areas north of Columbia River, (2) Restricted western gene flow established coastal populations, (3) Coastal populations expand north into Olympic Mountains and south across Columbia River, (4) Cascade populations expand northward into current distribution of species.
refugium and have subsequently remained isolated within the Columbia River Valley.

The Columbia River appears to be a fairly strong barrier to gene flow for this species as there is no geographic overlap of haplotypes found north and south of the Columbia River despite the species’ distribution encompassing both sides of the river (Fig. 1). The Fox Creek population (locality #11), which occurs south of the Columbia River in the Willapa Hills region of Oregon, is not as phylogenetically distinct as populations occurring south of the Columbia in the Cascades; however, this population appears to have been separated long enough to accumulate a high number of mutations between haplotypes therein and the nearest haplotype occurring north of the river (Fig. 3).

The Columbia River seems to be a barrier of varying degrees of penetrability for different amphibian species. Similar to *D. copei*, the Larch Mountain salamander (*P. larselli*) has recently expanded northwards across the river into its current range (Wagner et al., 2005). However, the river separates highly divergent northern and southern lineages of the Pacific giant salamander (*D. tenebrosus*) (Steele and Storfer, 2006) and appears to have prevented further northward expansion of the Oregon slender salamander (*Battacoseps wrightii*) (Miller et al., 2005).

4.2. *Dicamptodon copei* and *P. vandykei*: Same but different

Despite *D. copei* and *P. vandykei* being distantly related salamander species, it was expected that these two species would have responded concordantly to past geologic and climatic events due to dependence on stream and streamside habitats, similarly fragmented geographic distributions and evidence of congruent phylogeographic patterns in other stream-associated amphibians (Carstens et al., 2005a). Phylogeographic topologies of the two species were indeed similar, but only when considering populations in sympathy. The removal of allopatric populations from the dataset results in a phylogenetic topology identical to that of *P. vandykei* (not shown) and sympatric populations of *D. copei* display a sister relationship between coastal populations and populations in the north Cascade Mountains. Long term separation between coastal and Cascade populations of *P. vandykei* has been inferred by molecular and morphological evidence (Howard et al., 1993; Wilson and Larsen, 1999). This separation, as first mentioned by Wilson and Larsen (1999), is in agreement with the fossil pollen record (Baker, 1983; Barnosky, 1981; Barnosky et al., 1987) which indicates an uninhabitable xeric environment in the lowlands separating the coastal and Cascade populations of *P. vandykei* during late Pleistocene through much of the Holocene. Presumably, these dry lowlands had a comparable effect on sympatric *D. copei* populations, resulting in phylogeographic patterns similar to that of *P. vandykei*. Additionally, both species appeared to have responded similarly to the availability of a post-glacial environment in the Olympic Mountains (Crandell, 1965; Easterbrook, 1976) by expanding there from the Willapa Hills. The presence of a widespread haplotype in the Olympic Mountains (SD1) indicates recent expansion into this region by *D. copei*. This expansion is also corroborated by the results of the nested clade analysis (Clade 2-7; Table 3). *P. vandykei* also appears to have recently expanded into the Olympic Mountains from the Willapa Hills (Howard et al., 1993; Wilson and Larsen, 1999). Thus, in the areas where the two species have overlapping ranges, they have responded concordantly to past climatic and geologic events, resulting in similar phylogeographic topologies.

Phylogenetic concordance among sympatric populations of *D. copei* and *P. vandykei* represents the extent of any phylogeographic similarity. The range of *D. copei* within the Cascade Mountains is slightly larger than that of *P. vandykei* and extends ~75 km southward across the Columbia River into the Cascade Mountains of Oregon. These allopatric populations of *D. copei* are distinct from populations north of the Columbia River (Fig. 2). Topological incongruence between the *P. vandykei* phylogeny and the *D. copei* phylogeny is driven by these genetically divergent *D. copei* populations. Although the geographic distribution of *D. copei* is only slightly larger than the range of *P. vandykei*, it encompasses a geographical barrier (i.e. Columbia River) capable of producing significant phylogenetic signal. While there is evidence of shared responses to past climatic or geologic events by some populations, the phylogenetic topologies of all populations, taken together, for the two species display significant discordance.

4.3. Comparative phylogeography

Since its origin, the field of comparative biogeography has searched for congruent patterns of speciation or diversification between codistributed taxa using area cladograms (Nelson, 1974; Rosen, 1978; Nelson and Platnick, 1981). However, perfect congruence of area cladograms among all codistributed species is extremely unlikely and discordant phylogeographic patterns could occur under a variety of scenarios such as dispersal or extinction. Other reasons for discordant phylogeographic patterns could be due to “pseudo-incongruence” (Cunningham and Collins, 1994; Donoghue and Moore, 2003) in which codistributed species either display different evolutionary responses to the same series of historical events or to events that occurred in different time periods. Additionally, incongruence of area cladograms can also occur through redundant distributions (when the same geographic area contains several divergent lineages) or in cases of missing areas (when an organism does not occur in a geographic area of interest) (Lomolino et al., 2006). The pattern of incongruence observed in this study appears to be driven by a combination of these two scenarios. The geographic area of the Cascade Mountains contains three distinct lineages for *D. copei* compared to just one lineage for *P. vandykei*, illustrating the potential for incongruence due to redundant distributions. Additionally, *P. vandykei* does not occur south of the Columbia River because it may have never occurred in this area or
may have become locally extinct. Regardless of the reason, its absence from this region illustrates a reason for incongruence due to the slight differences in species’ distribution.

A phylogeographic comparison of *P. vandykei* and *D. copei* provides an example of two codistributed species that are dissimilar in their phylogenetic topologies but nonetheless show some concordance in their past responses to evolutionary events. A variety of comparative phylogeographic studies have demonstrated concordant responses of codistributed taxa to either past climatic events (Avise, 1992; Avise, 1996) or geologic events (Nielsen et al., 2001; Carstens et al., 2004; Steele et al., 2005), while other studies reveal discordant topologies due to independent responses to past climatic and geologic events (Sullivan et al., 2000; Carstens et al., 2005a; Donovan et al., 2000). As more comparative studies are completed, it will likely become clear that the two alternative hypotheses of concordant and independent responses for codistributed taxa represent a false dichotomy (Sullivan et al., 2000). The two alternative hypotheses of concerted and independent responses of codistributed taxa are not always mutually exclusive and topologies may simultaneously display characteristics predicted by both hypotheses (Sullivan et al., 2000). Thus, codistributed species are likely to have a combination of concordant and dissimilar patterns in their phylogenies indicating some degree of a shared history but not complete phylogeographic concordance.

**Acknowledgments**

We thank the Washington and Oregon Departments of Fish and Wildlife for issuing the permits necessary for the collection of samples in the field. We thank A. Hanson for assistance in collecting samples. M.F. Ortega also provided assistance in the field and in the creation of Fig. 3. Special thanks go to M. Patterson for guiding CAS to the hard-to-reach Fox Creek locality in Northwest Oregon. We also thank two anonymous reviewers for constructive comments that improved the overall quality of this paper. Funding for C. Steele and A. Storfer was provided by WDFW Contract No. 04-1305. Funding for A. Storfer was provided by NSF IBN-0213851.

**Appendix A**

**Locality information for *Dicamptodon copei* samples used in this study**

<table>
<thead>
<tr>
<th>Locality number</th>
<th>Number sequenced</th>
<th>Name of haplotypes sampled (abbreviated haplotype name)</th>
<th>Locality information and GenBank Accession Nos.</th>
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<tr>
<td>4</td>
<td>2</td>
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(continued on next page)
Appendix A (continued)

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<th>Number sequenced</th>
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<th>Locality information and GenBank Accession Nos.</th>
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Aligned data matrix is deposited in TreeBase (accession number SN3055). Unique haplotype sequences are deposited in GenBank. Accession numbers refer to cyt b and control region, respectively.

References


