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INVESTIGATING THE EVOLUTIONARY HISTORY OF THE PACIFIC NORTHWEST MESIC FOREST ECOSYSTEM: HYPOTHESIS TESTING WITHIN A COMPARATIVE PHYLOGEOGRAPHIC FRAMEWORK

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Abstract.—We examine the evolution of mesic forest ecosystems in the Pacific Northwest of North America using a statistical phylogeography approach in four animal and two plant lineages. Three a priori hypotheses, which explain the disjunction in the mesic forest ecosystem with either recent dispersal or ancient vicariance, are tested with phylogenetic and coalescent methods. We find strong support in three amphibian lineages (*Ascaphus* spp., and *Dicampton* spp., and *Plethodon vandykei* and *P. idahoensis*) for deep divergence between coastal and inland populations, as predicted by the ancient vicariance hypothesis. Unlike the amphibians, the disjunction in other Pacific Northwest lineages is likely due to recent dispersal along a northern route. Topological and population divergence tests support the northern dispersal hypothesis in the water vole (*Microtus richardsoni*) and northern dispersal has some support in both the dusky willow (*Salix melanopsis*) and whitebark pine (*Pinus albicaulis*). These analyses demonstrate that genetic data sampled from across an ecosystem can provide insight into the evolution of ecological communities and suggest that the advantages of a statistical phylogeographic approach are most pronounced in comparisons across multiple taxa in a particular ecosystem. Genetic patterns in organisms as diverse as willows and salamanders can be used to test general regional hypotheses, providing a consistent metric for comparison among members of an ecosystem with disparate life-history traits.

Key words.—Bayesian hypothesis testing, community genetics, comparative phylogeography, ecosystem evolutionary history.

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Although studies in evolutionary biology often focus on particular ecological interactions (e.g., Pellmyr and Leebens-Mack 1999), evolutionary biologists have seldom directed investigations into the evolutionary history of an ecosystem itself. Ecosystem studies can be expanded into evolutionary time by considering abiotic factors that act on a deeper time scale, such as orogeny and climatic fluctuations, as well as the collection of data that are influenced by these deeper events. As community ecologists engage in studies across more extensive spatial and temporal scales (e.g., Ricklefs 1987) and focus on the roles of historical and geographical circumstances in community construction (e.g., Schluter and Ricklefs 1993), the field of comparative phylogeography is poised to contribute fundamental data on the formation and evolutionary history of ecosystems (e.g., Arbogast and Kenagy 2001; Wares 2002).

Previous regional syntheses of phylogeographic patterns (e.g., Avise 2000), have focused on identifying genealogical concordance among taxa. Such congruence of genealogies has been considered to be evidence that similar environmental processes have structured the genetic diversity within a region. This approach is best suited for comparisons among closely related organisms, where factors that influence the shape of genealogies, such as mutation rates and effective population size, are likely to be similar. Phylogeographic comparisons across more distantly related organisms require methodologies that can accommodate the stochastic processes associated with the gene coalescence as well as differences in organismal life-history characteristics. Unlike descriptive approaches to phylogeography, these methods are primarily concerned with estimating parameters that quantify certain aspects of genetic variation and using these parameter estimates to test a priori hypotheses erected on the basis of independent data. Testing the same set of hypotheses with data gathered from codistributed taxa can lead to a better understanding of the formation of ecological communities (e.g., Zink 1996; Sullivan et al. 2000) and allows for the expansion of the temporal and spatial scale studied to match the scale of the processes responsible for the evolution of ecosystems (Schluter and Ricklefs 1993).

Here we investigate the evolutionary history of the mesic forest ecosystem in the Pacific Northwest (PNW) of North America by gathering genetic data from six taxa endemic to the ecosystem and using these data to test three a priori biogeographic hypotheses. The PNW (40–52°N, 113–126°W) includes the greatest extent of temperate mesic coniferous forests in the world (Brunsfeld et al. 2001) and is characterized by both the late-successional dominance of western hemlock (*Tsuga heterophylla*) and western redcedar (*Thuja plicata*). Mesic forests grow in two disjunct areas in the PNW (Fig. 1). The most extensive tract is in the coastal and Cascades ranges from northern California to Alaska, and the smaller is in the northern Rocky Mountains (NRM) west of

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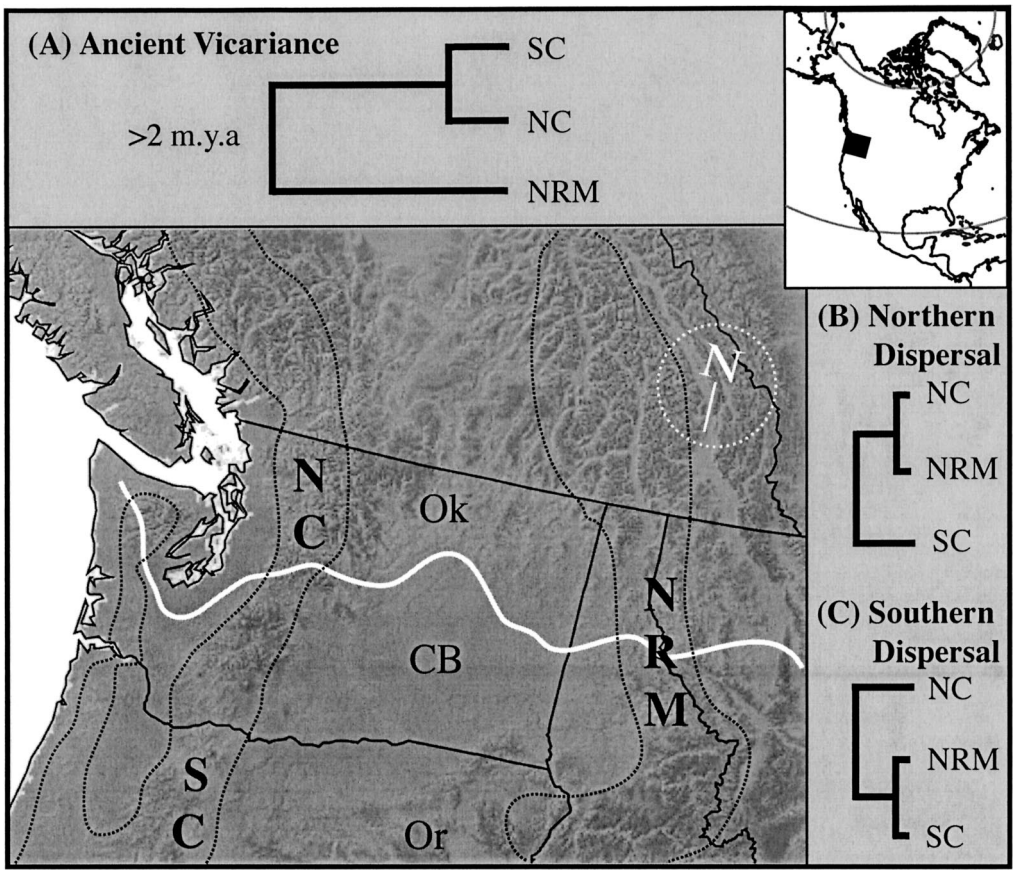

FIG. 1. The small inset shows the position of the Pacific Northwest on a map of North America. The large inset shows the Pacific Northwest, with approximate boundaries of the coastal and inland mesic forest ecosystem shown with dotted lines. The extent of the Cordilleran glaciation is shown with a thick white line. Geographical regions mentioned in the text are abbreviated as follows: North Cascades (NC), South Cascades (SC), Northern Rocky Mountains (NRM), Okanogan Highlands (Ok), Columbia Basin (CB), Oregon Highlands (Or). Also shown are the population histories predicted by (A) the ancient vicariance (AV), (B) the inland dispersal north (IDN), and (C) the inland dispersal south (IDS) hypotheses.

the Continental Divide. Our research has two goals, to infer the process responsible for the formation of the disjunct distribution in each lineage and to assess the degree of congruence among members of the ecosystem.

Three hypotheses have been proposed to explain the formation of the disjunction in the PNW mesic forest ecosystem. First, the ancient vicariance (AV) hypothesis posits, based on paleobotanical studies (e.g., Graham 1999), that a formerly continuous mesic ecosystem was split by the Pliocene xerification of the Columbia Basin associated with Cascadian orogeny. This hypothesis suggests the persistence of NRM taxa in refugia throughout the Pleistocene glacial cycles (Brunsfeld et al. 2001) and predicts relatively deep divergence (e.g., pre-Pleistocene or >1.8–2.6 million years ago) between coastal and inland populations (Fig. 1A). It is likely that sufficient time has elapsed since the separation of coastal and inland populations for lineage sorting to occur (Arbogast et al. 2002; Rosenberg 2003), therefore genealogies within these populations should be reciprocally monophyletic. Second, the inland dispersal north (IDN) hypothesis implies that mesic forests did not persist in the NRM throughout the Pleistocene, and instead posits the establishment of mesic forests in this region by post-Pleistocene dispersal from the Cascades (Mack et al. 1978) along northern routes at the margin of retreating glaciers. This hypothesis is based on recent age (<3000 years) of hemlock in pollen cores from inland areas (Mack et al. 1978; Whitlock 1992). It predicts shallow (post-Pleistocene) divergence between north Cascades (NC) and inland populations, absence of reciprocal monophyly, and little genetic diversity within and among inland populations (Fig. 1B). Finally, the inland dispersal south (IDS) hypothesis, proposes that establishment of inland mesic forest ecosystems could have also occurred via dispersal through the central Oregon highlands, and is based on cpDNA data from *Alnus rubra* (Strenge 1994) and morphology in tailed frogs (*Ascaphus*; e.g., Metter and Pauken 1969). This hypothesis makes similar predictions as the IDN hypothesis, but predicts haplotypes from the NRM to nest within clades from the southern Cascades (SC) due to the different source populations (Fig. 1C).

These hypotheses can be applied to more than 150 plant, animal, and fungal lineages that are known to have disjunct populations in both coastal and inland mesic forests of the PNW (Nielson et al. 2001). In this study, we used molecular data from six plant and animal lineages with disjunct distributions (Table 1) to test the three biogeographic hypotheses.

TABLE 1. Taxon-specific predictions of biogeographic hypotheses. For each lineage examined, the microhabitat, biogeographic prediction, and the basis for the prediction are given.

Taxon	Microhabitat	Prediction	Basis
Ascaphus	riparian	ancient vicariance	mtDNA[1]
Dicamptodon	aquatic/riparian	ancient vicariance	allozymes[2]
Plethodon	terrestrial seeps	ancient vicariance	allozymes[3]
Microtus	riparian	recent dispersal	present distribution/subspecies borders[4]
Salix	riparian	none	no data
Pinus	subalpine	inland dispersal north	RFLP[5]

[1] Nielson et al. (2001).
[2] Good and Wake (1992); Steele et al. (2005).
[3] Howard et al. (1993); Carstens et al. (2004).
[4] Summarized in Ludwig (1984).
[5] Richardson et al. (2002).

Previous phylogeographic studies of three of these lineages have identified patterns of genetic variation that are consistent with the AV hypothesis: tailed frogs (*Ascaphus truei*, *A. montanus*; hereafter *Ascaphus*; Fig. 2A; Nielson et al. 2001), Pacific giant salamanders (Dicamptodontidae; *Dicamptodon aterrimus*, *D. copei*, *D. ensatus*, and *D. tenebrosus*; hereafter *Dicamptodon*; Fig. 2B; Steele et al. 2005), and a plethodontid salamander species complex (*Plethodon vandykei*, *P. idahoensis*; hereafter *Plethodon*; Fig. 2C; Carstens et al. 2004). While these amphibian lineages appear to have exhibited a similar response to geological events and climatic fluctuations, the degree to which congruence is expected across other members of the mesic forest ecosystem is unknown. Paleontological investigations have suggested that ecological communities in temperate regions are not stable during times of climatic fluctuation (e.g., DiMichele et al. 2001), and data from two woody plants (the mesic race of the dusky willow, *Salix melanopsis*; hereafter *Salix*; Fig. 2D; Miller 2004), and the whitebark pine (*Pinus albicaulis* hereafter *Pinus*; Fig. 2E; Richardson et al. 2002), suggest that post-Pleistocene dis-

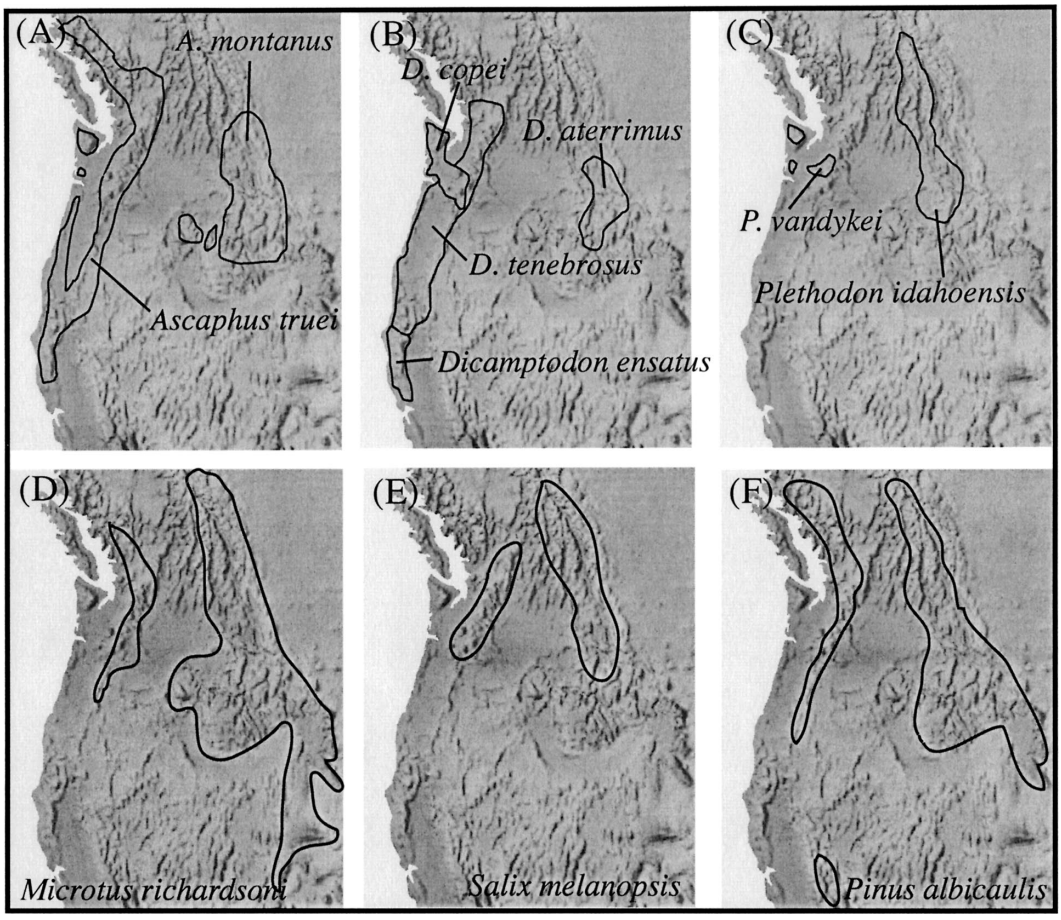

FIG. 2. Current distributions of lineages included in this analysis. The distributions of *Ascaphus* (A), *Dicamptodon* (B), *Plethodon* (C), *Microtus* (D), *Salix* (E), and *Pinus* (F) are shown.

persal may explain the disjunct range in these species. In addition, the water vole (*Microtus richardsoni* hereafter *Microtus*; Fig. 2F) has also been postulated to have responded to Late Pleistocene events with more recent dispersal events tracking Cordilleran ice-sheet retraction (summarized in Ludwig 1984). Post-Pleistocene dispersal may also be important in the formation of a proposed suture zone in the NRM (Remington 1968; Swenson and Howard 2004).

Materials and Methods

Data

We used a combination of data from previous studies and newly generated sequence data to test hypotheses related to the NRM mesic forest ecosystem formation. Data from previous studies included: *Ascaphus* 1559 bp of cytochrome *b* (cyt *b*) and NADH-2 (GenBank accession nos. AF277324–AF277352 and AF277370-AF277353; Nielson et al. 2001; $n = 80$), *Dicamptodon* 1140 bp of cyt *b* (AY734570–AY734627, AY728902, AY728906, AY728907, AY728909, AY728912; Steele et al. 2005; $n = 92$), *Plethodon* 669 bp of cyt *b* (AY572039–AY572107; Carstens et al. 2004; $n = 229$), *Pinus* 252 bp of mtDNA (*nad*5a and *nad*5d) sequence data (AF434844; Richardson et al. 2002; $n = 115$), *Salix* 1159 bp of cpDNA *mat*K gene and *rpl*16 spacer (DQ060264–DQ060269, DQ060271–DQ060276; Miller 2004; $n = 89$). Data generated for this study included, in the *Plethodon* lineage, five *P. vandykei* samples from a previously unsampled population in the Willapa Hills along the Pacific coast and five *P. vandykei* samples from the Olympic Peninsula (Appendix 1). We extracted DNA and sequenced 669 bp of cyt *b* following Carstens et al. (2004). New data were also generated for *Microtus*; we sequenced 747 bp of cyt *b* from 57 water vole samples, collected either from fieldwork ($n = 18$) or tissue loans ($n = 39$). A previously published sequence was also included (AF163905; Conroy and Cook 2000). Total genomic DNA was extracted from *Microtus* tissues using a DTAB/CTAB protocol or using a DNeasy Tissue Kit (Qiagen, Valencia, CA) following Demboski and Sullivan (2003). Polymerase chain reaction (PCR) amplifications were conducted with primers cyt *b* A (5′-GATATGAAAAACCATC GTTG-3′) and the *Microtus*-specific cyt *b* primer V_{MR770} (5′-GGCAAATAGGAATATCATT-3′). Double-stranded PCR products were purified with a 20% PEG/2.5 M NaCl solution, sequenced on an ABI377 automated sequencer (Applied Biosystems, Foster City, CA), and edited, compiled, and aligned using Sequencer 3.0 (Gene Codes, Ann Arbor, MI).

Tests of Topology

For each of the six lineages, we used the following approach to test the concordance of the gene trees with hypothesized population histories for each of the three hypotheses. We selected a model of sequence evolution using DT-MODSEL (Minin et al. 2003), a method that incorporates fit, a penalty for overparameterization, and performance into model selection. We then conducted heuristic searches on each dataset using maximum likelihood (ML) methods implemented in PAUP* (Swofford 2002), including TBR branch swapping, 20 random-addition replicates, and no limit to the maximum number of trees compared. Furthermore, we applied successive approximations and repeated searches by reoptimizing model parameters on the initial ML tree and iterated until the same tree was obtained in successive searches (e.g., Sullivan et al. 2005). This provided estimates of the ML tree for each dataset. Nodal support was assessed using bootstrap analysis with 1000 replicates (Felsenstein 1985).

To approximate the posterior probability distribution of the topology parameter, we conducted eight independent runs for each dataset with MRBAYES 3.0 (Huelsenbeck and Ronquist 2001). Each run used the likelihood model selected with DT-MODSEL, was run for 5.1×10^6 generations, and trees were sampled every 1000 generations. Because the topology parameter may be particularly susceptible to nonstationarity (Huelsenbeck et al. 2002), we conducted an analysis of variance on the symmetric-difference distance between each of the last 5000 sampled trees of the Bayesian run and the optimal ML tree identified above (following Carstens et al. 2004). To complete the hypothesis test, we combined the last 5000 trees from each of the eight independent Bayesian runs into a posterior distribution of 40,000 trees. We then used tree filters in PAUP* to assess the proportion of the trees in the sample that are consistent with the predicted population histories for each hypothesis (Fig. 1A–C). This proportion is the posterior probability of concordance between the gene tree and the hypothesized population history, conditional on the data, priors, and model of sequence evolution (Huelsenbeck et al. 2002).

We also tested the topological concordance of the gene trees with hypothesized population histories using parametric bootstraps (Goldman 1993; Sullivan et al. 2000) in three lineages for which there was an appropriate outgroup. For *Microtus*, we used *M. guatemalensis* (AF410262; Conroy et al. 2001), for *Plethodon* we used *E. eschscholtzii* (AY572108; Carstens et al. 2004), and for *Salix melanopsis* we used the lowland race of the species (DQ060270, DQ060277). For each of these datasets, we constrained ML searches to fit the topological predictions of each of the hypotheses (Fig. 1A–C), which allowed us to determine the best tree consistent with each hypothesis for each of the three datasets. We used the models of sequence evolution selected above (optimized on the best constrained trees) and SEQ-GEN (Rambaut and Grassly 1997) to simulate 100 datasets on the best phylogeny consistent with the hypothesis being tested. We then used PAUP* (with ML as an optimality criterion) to search each simulation replicate for the best unconstrained phylogeny and the best phylogeny constrained to meet the predictions of the hypothesis. This same difference ($\ln L_{constrained} - \ln L_{unconstrained}$) in the actual data formed the test statistic, and the null distribution allowed us to evaluate the significance of the observed test statistic.

The Bayesian hypothesis-testing method and the parametric bootstrap test the topology parameter in different ways. The former relies on Markov chain Monte Carlo (MCMC) methods to generate an approximation of the posterior distribution of trees with high posterior probabilities given the data, prior probabilities, and model. The proportion of trees consistent with the topology predicted by the hypothesis is taken as the probability that the hypothesis is correct, given the priors, data, and model. Conversely, the parametric boot-

TABLE 2. Description of sequence data. From the left, columns show the mesic forest lineage, sample size, gene(s) used, the model of sequence evolution selected with DT-MODSEL (Minin et al., 2003) and the parameters of this model.

Lineage	*n*	gene(s)	Model	Parameters
Ascaphus	80	cyt *b* ND-2	GTR + I	$\pi_A = 0.297$, $\pi_C = 0.247$, $\pi_G = 0.132$, $\pi_T = 0.323$, $r_{AC} = 1.67$, $r_{AG} = 35.63$, $r_{AT} = 2.57$, $r_{CG} = 4.72$, $r_{CT} = 17.89$, $r_{GT} = 1.0$, *pinv* = 0.7659
Dicamptodon	92	cyt *b*	HKY + I + Γ	$\pi_A = 0.311$, $\pi_C = 0.194$, $\pi_G = 0.123$, $\pi_T = 0.371$, *Trat* = 3.698, *pinv* = 0.754; $\alpha = 1.57$
Plethodon	229	cyt *b*	HKY + Γ	$\pi_A = 0.305$, $\pi_C = 0.235$, $\pi_G = 0.14$, $\pi_T = 0.32$, *Trat* = 1.336, *pinv* = 0.716
Microtus	58	cyt *b*	GTR + I	$\pi_A = 0.315$, $\pi_C = 0.275$, $\pi_G = 0.131$, $\pi_T = 0.279$, $r_{AC} = 2.85 \times 10^5$, $r_{AG} = 2.71 \times 10^6$, $r_{AT} = 2.85 \times 10^5$, $r_{CG} = 1.75 \times 10^{-8}$ $r_{CT} = 2.71 \times 10^6$, $r_{GT} = 1.0$, *pinv* = 0.726
Salix	89	*mat*K *rpl*16	F84	$\pi_A = 0.337$, $\pi_C = 0.158$, $\pi_G = 0.156$; $\pi_T = 0.279$, *Trat* = 0.833
Pinus	114	*nad5*	F81	$\pi_A = 0.363$, $\pi_C = 0.115$, $\pi_G = 0.160$, $\pi_T = 0.323$

strap relies on simulation under the chosen, fully defined model to quantify the phylogenetic uncertainty around the topology parameter; the resulting probability (*P*-value) measures the probability of observing a test statistic as large as the empirical value if the hypothesis under examination were true.

Test of the Divergence between Populations

The three hypotheses also make testable predictions about the degree of divergence between populations in the NRM and populations in the Cascades and coastal ranges. The isolation-with-migration model of Nielsen and Wakeley (2001), implemented in the program MDIV (http://ser-loop.tc.cornell.edu/cbsu/mdiv.htm), was used to approximate the posterior distribution of three parameters: the divergence time between populations in generations ($T = T_{div}/2N_e$), the migration rate between populations ($M = 2N_e \times$ migrants per generation), and a measure of genetic diversity ($\theta = 2N_e\mu$, where μ is the mutation rate). We used the results of the topological tests to guide the MDIV analysis in two ways. First, for taxa in which we could not reject one of the dispersal hypotheses based on topology, we estimated the divergence between the NRM populations and the putative source populations from the coast (e.g., for taxa consistent with the IDN hypothesis, we compared divergence between the NC and NRM populations). Secondly, we used the results of the topology test to guide the selection of priors for *M* and *T*. After analyzing each dataset with the default priors ($M = 10$, $T = 5$), we set the prior on $M = 1$ for taxa consistent with the ancient vicariance hypothesis, and experimented with values for *T* following suggestions in Nielsen and Wakeley (2001). Conversely, for taxa consistent with a recent dispersal, we set the maximum $T = 1$ and experimented with truncation of priors for *M*. Once priors were identified that appeared to result in well-behaved posterior distributions (following Nielsen and Wakeley 2001), we reran the MDIV analyses for 5.0×10^6 generations at least twice to assure that the parameter estimates were consistent across runs. Comparisons among lineages require that the coalescent time estimated with MDIV be converted into years, and this conversion requires a mutation rate for each lineage. Because no direct genomic estimates are available for the focal taxa, we assumed a range of mutation rates ($\mu = 1.0 \times 10^{-6}$ to $\mu = 1.0 \times 10^{-8}$ mutations per site per generation) approximately an order of magnitude higher and lower than a direct estimate of μ from *C. elegans* (Denver et al. 2000). In animals, μ is expected to be higher in endothermic lineages (Adachi et al. 1993) and lower in ectotherms (Martin and Palumbi 1993), and we therefore expect μ in *Microtus* to be higher than μ in the amphibian lineages. For the purposes of hypothesis testing, the 95% credibility boundaries are the important aspect of the posterior distribution of the divergence time because, to differentiate between the dispersal and vicariance hypotheses, we must be able to exclude post-Pleistocene divergence between coastal and inland populations to reject the recent IDN and IDS hypotheses and pre-Pleistocene divergence to reject the AV hypothesis.

RESULTS

Sequencing and Data Collection

We sequenced 747 bases of cyt *b* in 58 *M. richardsoni* (deposited in GenBank under accession nos. AY973753–AY973809), and identified 29 unique haplotypes. We selected a variant of the GTR + I model of sequence evolution, with $r_{AC} = r_{AT}$ and $r_{AG} = r_{CT}$ (Table 2). The greatest corrected genetic distance within *Microtus* was 0.0340 substitutions per site. The estimated ML topology ($-\ln L = 1759.46401$) contains two clades (Fig. 3), one composed of samples from the SC and the second composed of samples from the NC and the NRM. Furthermore, the haplotypes from the NC are nested within those from the NRM, with the most basal clades containing only NRM haplotypes. Additionally, we sequenced five *P. vandykei* from the Willapa Hills and five *P. vandykei* from the Olympic Peninsula and identified three new haplotypes (deposited in GenBank under accession numbers AY962289–AY962291). Models of sequence evolution chosen for all lineages are given in Table 2.

Hypothesis Testing of Topology

Maximum-likelihood trees for the other mesic forest lineages (Fig. 4) had the following likelihood scores: *Ascaphus* $-\ln L = 3366.7242$, *Dicamptodon* $-\ln L = 3189.8281$ (Steele et al. 2005), *Plethodon* $-\ln L = 2560.33110$, *Salix* $-\ln L = 1659.49651$, and *Pinus* $-\ln L = 350.67867$. For each dataset, the replicate MCMC runs all appeared to have

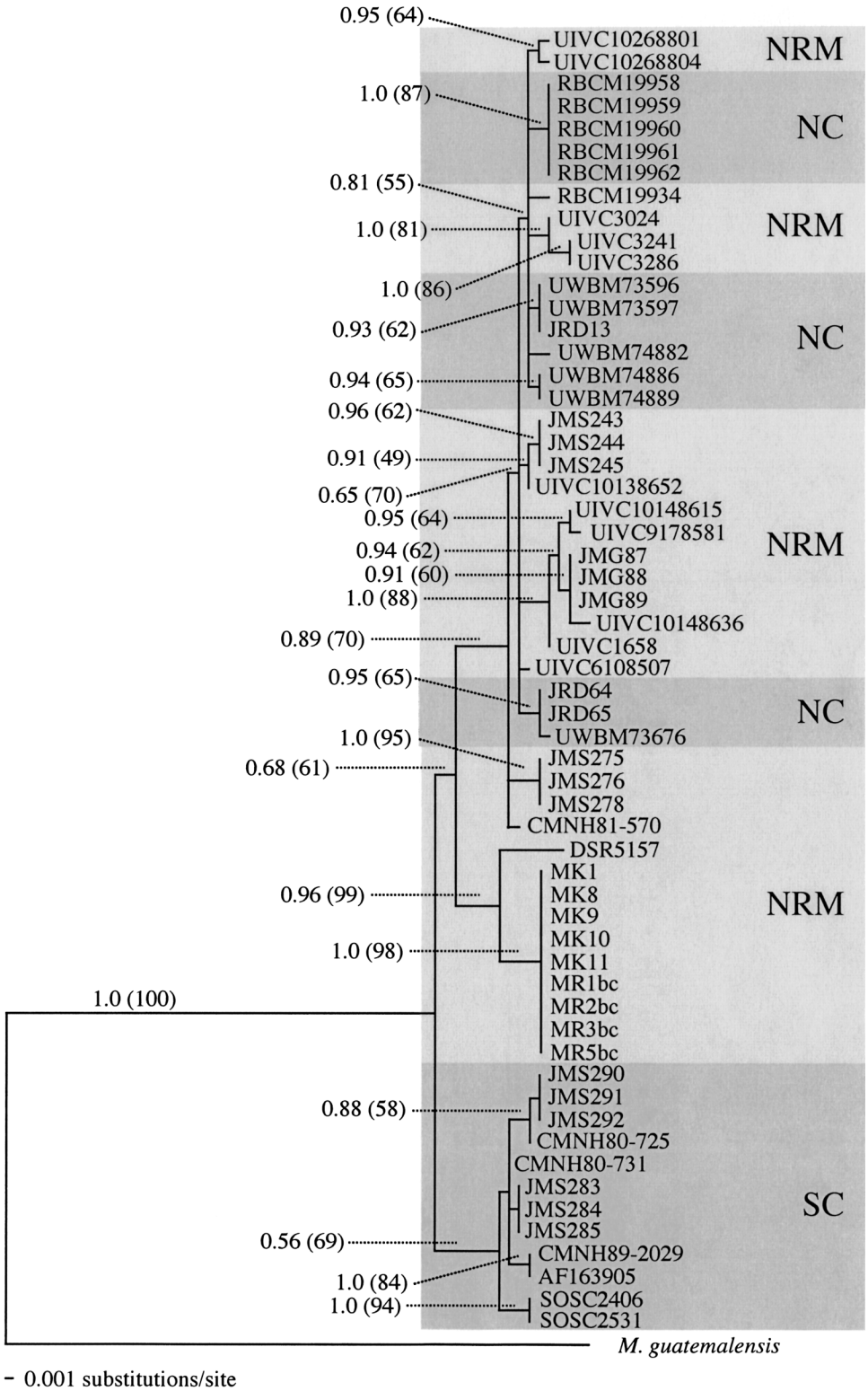

FIG. 3. Maximum likelihood phylogeny estimate ($-\ln L = 1759.46401$) for the *Microtus* data. A variant of the GTR + I model of sequence evolution, with $r_{AC} = r_{AT}$ and $r_{AG} = r_{CT}$, was used. Nodal support is shown with Bayesian posterior probabilities and maximum likelihood bootstrap values (in parentheses). Clades containing samples from the North Cascades (NC), South Cascades (SC), and northern Rocky Mountains (NRM) are labeled.

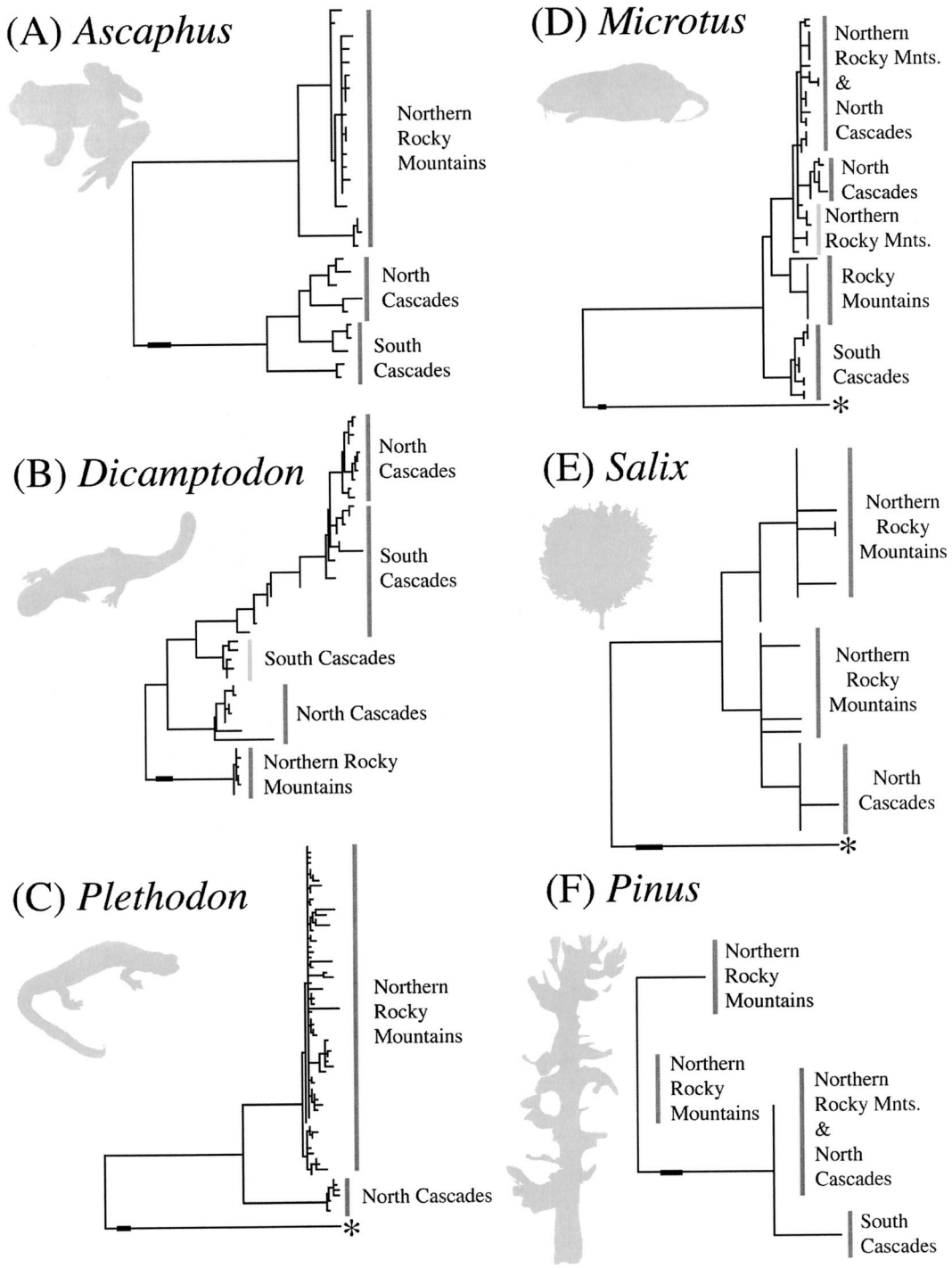

FIG. 4. Maximum likelihood phylogeny estimates for each lineage examined. The phylogenies of *Ascaphus* (A), *Dicamptodon* (B), *Plethodon* (C), *Microtus* (D), *Salix* (E), and *Pinus* (F) are shown. Clades containing samples from the North Cascades (NC), South Cascades (SC), and northern Rocky Mountains (NRM) are labeled and outgroups are marked with an asterisk. Scale bars for branch-lengths are shown as black boxes superimposed on the basal branch in the phylogeny estimates, and represent 0.005 substitutions per site in *Ascaphus*, *Dicamptodon*, and *Plethodon* and 0.001 substitutions per site in *Microtus*, *Salix* and *Pinus*.

sampled from the same posterior distribution of topologies (*Ascaphus* F_{OBS} = 1.2745, *Dicamptodon* F_{OBS} = 1.9358, *Plethodon* F_{OBS} = 2.2109, *Microtus* F_{OBS} = 1.7707, *Salix* F_{OBS} = 0.6618, *Pinus* F_{OBS} = 0.252). Each sample of trees was therefore combined into a posterior distribution of trees for each dataset. For the amphibians, both the IDS and IDN hypotheses have very low posterior probabilities (Table 3), whereas the AV hypothesis has a high posterior probability (*Ascaphus* $Bpp_{AV} > 0.999$, *Dicamptodon* $Bpp_{AV} = 1.0$, *Plethodon* $Bpp_{AV} > 0.999$). Conversely, in *Microtus* the IDN hy-

TABLE 3. Phylogenetic tests of hypotheses related to the formation of the northern Rocky Mountain ecosystem. From the left, columns show: the Pacific Northwest lineage that was used to test the hypotheses, the Bayesian posterior probability (*Bpp*) of the ancient vicariance (AV) hypothesis, the *Bpp* for the inland dispersal north (IDN) hypothesis, the *Bpp* for the inland dispersal south (IDS) hypothesis, the result of the parametric bootstrap tests of the AV hypothesis, the IDN hypothesis, and the IDS hypothesis.

Lineage	Bpp_{AV}	Bpp_{IDN}	Bpp_{IDS}	$pboot_{AV}$	$pboot_{IDN}$	$pboot_{IDS}$
Ascaphus	>0.999	<0.001	<0.001	n/a	n/a	n/a
Dicamptodon	1.0	0.0	0.0	n/a	n/a	n/a
Plethodon	>0.999	<0.001	n/a	>0.99	<0.01	n/a
Microtus	<0.001	>0.999	<0.01	<0.01	>0.99	<0.01
Salix	<0.001	0.40815	n/a	0.01	<0.01	n/a
Pinus	0.338	0.338	n/a	n/a	n/a	n/a

pothesis has a high posterior probability ($Bpp_{IDN} > 0.999$) but both the IDS and AV hypotheses have very low posteriors (Table 3). In the *Salix* data, there was little support for either the IDN or the AV hypotheses (Table 3). In the *Pinus* lineage, there was approximately equal support for the IDN and AV hypotheses (Table 3), a result attributable to the low degree of sequence variation (e.g., four haplotypes) and the fact that one haplotype was present in both the NC and the NRM. Results for the parametric bootstrap were similar in that we could not reject the AV hypothesis in *Plethodon*, could not reject the IDN hypothesis in *Microtus* and found little support for either hypothesis in *Salix* (Table 3).

Hypothesis Testing of Divergence Time

We used MDIV to approximate the posterior distributions of θ, *M*, and *T* for all lineages except *Pinus*, for which there was insufficient variation in the sequence data. The shapes of these posterior probability distributions differed in a manner that was consistent with the results of the topology tests. For example, in lineages with topologies consistent with the AV hypothesis, we could exclude post-Pleistocene divergence with the lower bound of the 95% credibility interval (*Ascaphus* lower bound of $T_{div95Low} = 1.4 \times 10^6$ years, *Dicamptodon* $T_{div95Low} = 5.4 \times 10^5$ years, *Plethodon* $T_{div95Low} = 1.3 \times 10^6$ years; assuming $\mu = 1.0 \times 10^{-7}$), thereby rejecting the recent dispersal hypotheses. Similarly, the mean values of the posterior distribution of population divergence dates are in the Pliocene in *Ascaphus* ($T_{div} = 3.1 \times 10^6$ years) and *Plethodon* ($T_{div} = 4.1 \times 10^6$ years), and in the early Pleistocene in *Dicamptodon* ($T_{div} = 1.2 \times 10^6$ years). In *Dicamptodon*, we had some difficulty approximating the posterior distribution of population divergence, which we attribute to the use of data from three coastal species. While the lower 95% credibility boundary was consistent across multiple MDIV runs, the upper 95% credibility boundary was not. Nevertheless, due to the consistency in the lower range of the estimate of population divergence, we can reject the recent inland dispersal hypotheses for *Dicamptodon* and post-Pleistocene dispersal can be rejected in all amphibian lineages. This result is robust across a large range in the assumed mutation rate (Table 4).

Lineages with topology estimates consistent with the IDN hypothesis have distributions of *T* consistent with more recent divergence between coastal and inland populations and had migration rates between these populations that were greater than zero. In *Microtus*, the upper bound of the posterior distribution of population divergence excludes pre-Pleistocene divergence ($T_{div95Hi} = 1.7 \times 10^4$ years; assuming $\mu = 1.0 \times 10^{-6}$), and pre-Pleistocene divergence can be rejected even if a lower rate of $\mu = 1.0 \times 10^{-8}$ is assumed. As was the case with the phylogenetic tests, results from coalescent tests in *Salix* are inconclusive. We can reject pre-Pleistocene di-

TABLE 4. Tests of hypotheses related to the formation of the northern Rocky Mountain ecosystem using the population divergence parameter. From the left, columns show: the Pacific Northwest lineage that was used to test the hypotheses, θ estimated with MDIV, the generation length (*L*), the assumed mutation rate (μ in units of mutations per site per generation), the mean of the posterior distribution of population divergence time (T_{div}; converted to years), and the lower and upper 95% credibility boundaries of this posterior distribution. Rows in bold are the mutation rates assumed in the text and in Figure 4.

Lineage	θ_{mdiv}	L	μ	N_e	T_{div}	$95LoT_{div}$	$95HiT_{div}$
Ascaphus	0.0076	6	10^{-6}	3800	3.1×10^5	1.4×10^5	4.4×10^5
			10^{-7}	**38,000**	**3.1×10^6**	**1.4×10^6**	**4.4×10^6**
			10^{-8}	380,000	3.1×10^7	1.4×10^7	4.4×10^7
Dicamptodon	0.0093	4	10^{-6}	4650	1.2×10^5	5.4×10^4	n/a
			10^{-7}	**46,500**	**1.2×10^6**	**5.4×10^5**	**n/a**
			10^{-8}	465,000	1.2×10^7	5.4×10^6	n/a
Plethodon	0.0359	5	10^{-6}	17,950	4.1×10^5	1.3×10^5	8.2×10^5
			10^{-7}	**179,500**	**4.1×10^6**	**1.3×10^6**	**8.2×10^6**
			10^{-8}	1,794,365	4.1×10^7	1.3×10^7	8.2×10^7
Microtus	0.0118	1	**10^{-6}**	**5900**	**9.4×10^3**	**2.3×10^3**	**1.7×10^4**
			10^{-7}	59,000	9.4×10^4	2.3×10^4	1.7×10^5
			10^{-8}	590,000	9.4×10^5	2.3×10^5	1.7×10^6
Salix	0.0007	4	10^{-6}	350	1.5×10^4	3.8×10^3	2.6×10^4
			10^{-7}	3500	1.5×10^5	3.8×10^4	2.6×10^5
			10^{-8}	35,000	1.5×10^6	3.8×10^5	2.6×10^6

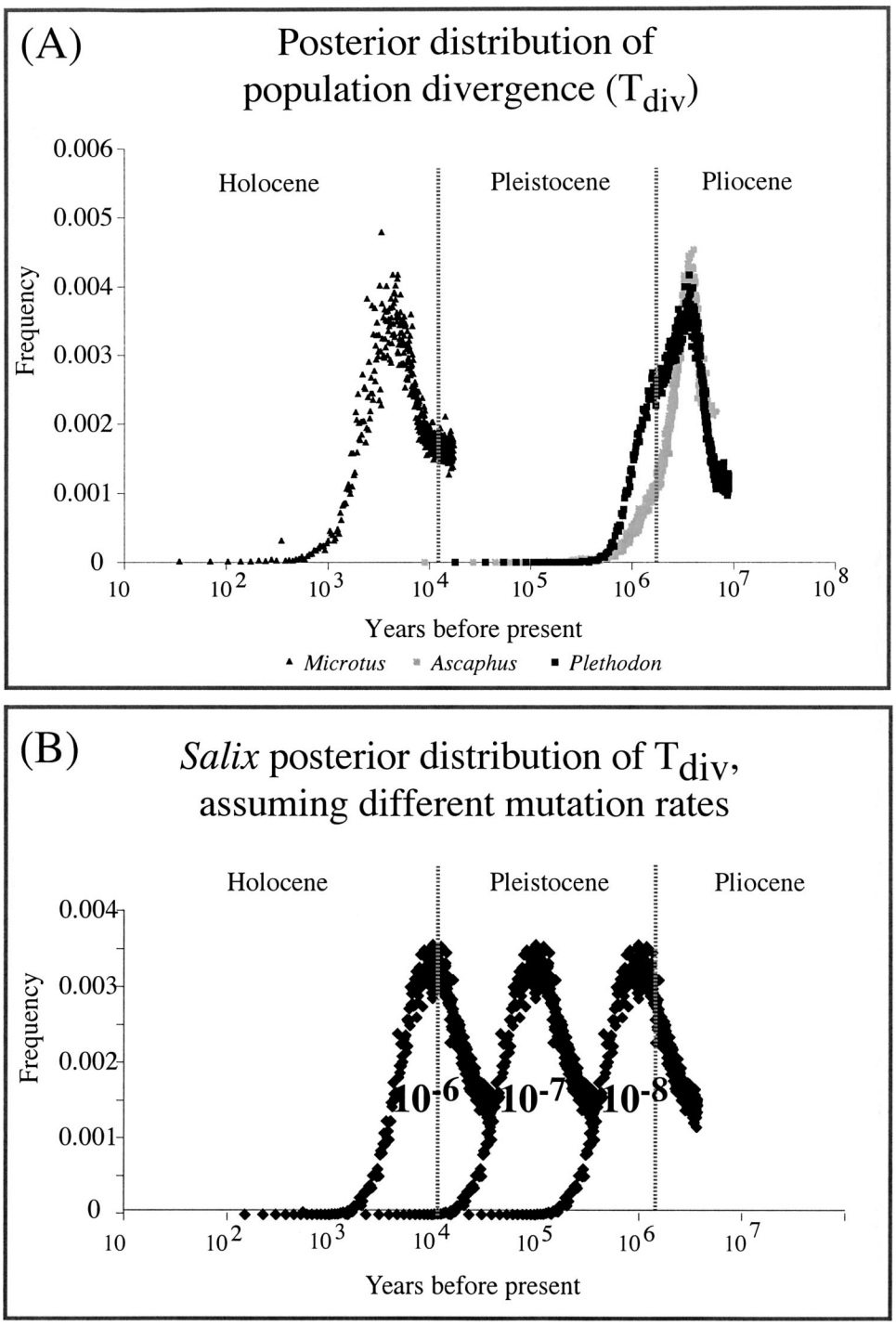

FIG. 5. Examples of posterior distributions of population divergence resulting from the MDIV analysis. Population divergence is shown in years (in log scale) on the x-axis. Approximate boundaries of the geological epochs are shown with dashed vertical lines. (A) Posterior distributions are shown for *Microtus*, *Ascaphus*, and *Plethodon*. The distribution is not shown for *Dicamptodon* due to the difficulties associated with estimating this distribution (described in the text). (B) Posterior distributions assuming three different mutation rates ($\mu = 1.0 \times 10^{-6}$, 1.0×10^{-7}, 1.0×10^{-8} mutations per site per generation) are shown for *Salix*.

vergence if mutation rates of $\mu = 1.0 \times 10^{-6}$ to $\mu = 1.0 \times 10^{-7}$ are assumed (Table 4), but these values result in small effective population sizes that may not be credible. In the animal lineages, the results from the MDIV estimates of T are consistent with the tests of topology, regardless of the mutation rate used in the calculations. The difference between the distributions of T in lineages consistent with the IDN/IDS hypotheses and those consistent with the AV hypothesis are shown (Fig. 5A), as are the ambiguous results from the *Salix* lineage (Fig. 5B).

Discussion

Evolution of the Northern Rocky Mountain Mesic Forest Ecosystem

The patterns of genetic variation contained within the six lineages examined here suggest that a combination of ancient vicariance and more recent northern dispersal events are responsible for the formation of this disjunct ecosystem. In *Ascaphus*, *Dicamptodon*, and *Plethodon* we were able to reject the recent dispersal hypotheses using topological tests (Table 3), suggesting that the establishment of these lineages predates the Pleistocene glaciation. We also used a coalescent-based method (MDIV) to exclude post-Pleistocene divergence (Table 4). Furthermore, the estimates of population divergence in *Plethodon* and *Ascaphus* are similar (4.1×10^6 and 3.6×10^6 years ago, respectively) and the credibility intervals overlap, indicating that these lineages may well have responded to xerification of the Columbian Basin at about the same time during the late Pliocene. Population divergence within *Dicamptodon* appears to be more recent (1.2×10^6 years ago) but is still well before the end of the Pleistocene. However, due to the difficulties associated with using MDIV with data from more than two species, we regard the *Dicamptodon* estimate as tentative. Additionally, in each lineage there is evidence of post-Pleistocene expansion from refugial populations (Nielson et al. 2001; Carstens et al. 2004, 2005), as predicted if these taxa persisted in NRM glacial refugia during the Pleistocene.

In contrast to the amphibians, we can reject the AV hypothesis in *Microtus* and *Salix*, and it appears that post-Pleistocene dispersal along a northern route was the mechanism responsible for the formation of the disjunct distribution in these lineages. However, contrary to our a priori predictions, it appears that dispersal occurred from the NRM to the coastal populations because haplotypes from the NC are nested within clades formed by haplotypes from the NRM in the phylogeny estimates (Fig. 4D, E). In *Microtus*, the upper and lower population divergence estimates from MDIV suggest that the northern dispersal event occurred within the last 20,000 years (Table 4). This estimate is consistent with possible late Pleistocene/ early Holocene westward dispersal along the Cordilleran glacial front followed by subsequent disjunction of water vole populations as a continuous habitat corridor was eroded by Columbia Basin/Okanogan Highlands expansion. The most recent Pleistocene glaciation has been suggested to have played a major role in shaping the present distribution of *M. richardsoni* (Dalquest 1948; Hoffmann and Koeppl 1985), and our results support such a timetable. Data from *Salix* are similar to those of *Microtus* in that there is tentative support for the IDN hypothesis in the topology tests, but with variation considerably lower than observed in the water vole. This dispersal event was apparently earlier than the dispersal within *M. richardsoni*, suggesting that *S. melanopsis* may have dispersed during a Pleistocene interglacial period (Fig. 5). Alternatively, the disjunction could have been caused by ancient vicariance with subsequent gene flow obscuring the signal of this earlier event. Estimates of θ in the *Salix* data are small in comparison to the vertebrates, and assuming values of μ that are sufficiently high to lead to the rejection of the IDN hypothesis implies relatively small N_e sizes (Table 4). A long-term effective population size of less than 3500 seems reasonable in *S. melanopsis*, given a high rate of vegetative propagation and the potential for past population bottlenecks. The small amount of variation in the *Pinus* data, as well as the presence of a haplotype shared between the NC and NRM, suggests that recent dispersal can also account for the presence of *P. albicaulis* in the NRM, but more data are needed to establish the direction of this dispersal. The MDIV analyses illustrate one important property of the hypotheses that we have tested here. The IDN/IDS and AV hypotheses can be clearly differentiated, a property that is most clearly illustrated by comparing the posterior distributions of population divergence times (Fig. 5A).

Our analyses were restricted to taxa that exhibit a clear disjunction in their current distribution and are restricted to the mesic forests of the PNW. The taxonomic status of the lineages also differs; the amphibian lineages contain two to four species and *Microtus* and the plant lineages contain a single species. While the taxonomy would seem to predict the results presented here, each of the amphibian lineages were described as a single species until genetic studies demonstrated cryptic diversity (Daugherty et al. 1983; Howard et al. 1993; Nielson et al. 2001). The fact that our restricted examination of only six mesic forest lineages supports different underlying biogeographic hypotheses is not necessarily surprising, given the numerous climatic and geologic fluctuations the PNW has experienced over the past 2.5 million years. It is apparent that vicariance and dispersal have played different temporal roles in shaping what appears to be, at a coarse scale, phylogeographic congruence based on similar, disjunct distributions.

The abiotic event with the greatest influence on the evolutionary history of organisms inhabiting the PNW mesic forest ecosystem was the xerification of the Columbia Basin during the Pleistocene. By 2 million years ago, the transformation of the Columbia Basin into a xeric sage-shrub habitat was complete (Brunsfeld et al. 2001), and the PNW mesic forest was effectively divided into coastal and inland ecosystems. Many taxa, such as the amphibians studied here, were able to persist in both the coastal ranges and the NRM throughout repeated Pleistocene glaciations and expanded into deglaciated regions as the glaciers retreated. Other lineages may have gone extinct in either the coastal or NRM regions as glaciers advanced during the Pleistocene. Periodically throughout this time, dispersal corridors connected the NRM and coastal mesic forests, and the water vole (and probably the whitebark pine) apparently used these corridors to disperse from the NRM into the NC. Although our analyses were focused on six disjunct taxa restricted to mesic forests in the PNW, it should be noted that multiple examples of cryptic molecular divergence have been observed in different mammalian taxa that coexist in the same region (Arbogast and Kenagy 2001; Cook et al. 2001). Many of these taxa are found in the mesic forest ecosystem, have probably responded to Columbia Basin/Great Basin xerification events (Demboski and Sullivan 2003), and have since experienced secondary contact following rapid northward/westward expansion as ice sheets retreated (Lessa et al. 2003). These otherwise cryptic molecular lineages now meet to form relatively discrete zones of parapatry and sympatry in previously gla-

ciated regions (Cook et al. 2001). Some of these occur in the region that Remington (1968) proposed as a suture zone, and recent comparative analyses suggest that the PNW is a hotspot for cryptic phylogeographic structure and hybrid zone formation (Swenson and Howard 2005).

Investigating the Evolutionary History of Ecosystems

The approach used here is substantially different from previous comparative phylogeographic studies. First, while syntheses of phylogeographic work have been conducted in the PNW (e.g., Soltis et al. 1997; Cook et al. 2001), southeastern North America (reviewed in Avise 2000), and Europe (Taberlèt et al. 1998), these studies have looked for genealogical concordance across focal taxa and have not explicitly investigated the processes that may be ultimately responsible for this concordance. This approach does not account for phylogenetic uncertainty (Sullivan et al. 2000), and it does not consider life-history parameters, such as the effective population size and generation length, that can differ between organisms and subsequently obscure congruence among genealogies. Second, the majority of comparative phylogeography to date has been conducted on a restricted taxonomic scale (e.g., DaSilva and Patton 1998; Smith et al. 2000; Weisrock and Janzen 2000; Lindstrom 2001; Schauble and Mortiz 2001; Costa 2003; Hoffman and Baker 2003), and these studies have typically used a descriptive approach characterized by the estimation of a phylogeny from one taxon and the subsequent comparison of this estimate to those from other taxa.

Our approach has several advantages for comparative studies that use phylogeographic data to investigate the history of an ecosystem. In spite of differences in life history, ecological habitat requirements, and mutation rates, any lineage that inhabits a given ecosystem can be included in a study of this nature by gathering genetic data and using it to test the predictions of shared a priori hypotheses. Like most statistical approaches, the accuracy of the parameter estimates, and consequently the hypothesis tests, are expected to increase with added data. While the estimates presented here would be improved by gathering additional data, the statistical approach nevertheless allows for a quantification of the uncertainty in the results. Ambiguous results, such as those in the *Pinus* and *Salix* lineages, are an indication either that the hypotheses require additional data to be tested adequately (e.g., *Pinus*) or that the hypotheses themselves may need refinement (e.g., *Salix*; Miller 2004). For example, while we can reject the AV hypothesis for the *Microtus* and *Salix* datasets in favor of more recent northern dispersal events, the data suggest possible coastal, not inland, directions of dispersal. One difference between the approach used here and those that rely on identifying genealogical concordance is that we use the tree topology as a starting point for other analyses. Tree topologies, while a critical component of the hypothesis tests used here, are by themselves insufficient to test phylogeographic hypotheses accurately. When tests of population divergence are incorporated into comparisons, differences in parameters such as the effective population sizes and generation lengths are reduced in importance and become more of an issue to the collection of data than to the analysis of the data.

Contributions to Community Genetics

Phylogenetic data are increasingly recognized as an important source of information for community ecology and act as a unifying influence between the fields of ecology and evolutionary biology (Webb et al. 2002). In a similar way, comparative phylogeographic studies can contribute valuable data to community genetics (Antonovics 1992). For example, the hypothesis that coevolved interactions among members of an ecological community may result in extended phenotypes (i.e., expressed traits that serve as the basis for selection at the community level; Whitham et al. 2003), assumes that these members share a common evolutionary history because this provides the opportunity for members to coevolve. One prediction of this model is topological and temporal congruence among community members. This prediction can be tested using the approach taken here. Another potential contribution of comparative phylogeography involves the assembly of ecological communities and ecosystems (Ricklefs 2003). Investigations into the evolutionary history of organisms that interact ecologically will allow biologists to address several questions. Are some types of ecological interactions likely to be stable over long periods of time? Are other interactions relatively transient? Are there processes analogous to ecological succession that occur over evolutionary time? These questions can only be addressed by estimating both temporal and topological phylogeographic parameters.

Direct investigations into the evolutionary history of an ecosystem are now possible, and these investigations offer tremendous potential to biologists in a wide range of fields. Evolutionary processes across entire landscapes can be considered; hypotheses and tests of the predictions of these hypotheses offer an approach to investigate ecological communities and relationships within a given ecosystem. Regardless of the differences among organisms, all share two characteristics, genetic variation within lineages and interactions with other organisms. When statistical phylogeographic methods are used, the first provides the means by which to investigate the evolution and stability of the second.

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APPENDIX 1

Plethodon vandykei collection localities. The source (i.e., person who collected the sample), sample number, and collection locality are given.

	Source	Number	Locality
1	B. Carstens	430	Ellsworth Creek Nature Preserve, Willapa Hills, WA 46.646°N 123.702°W
2	B. Carstens	431	Ellsworth Creek Nature Preserve, Willapa Hills, WA 46.646°N 123.702°W
3	B. Carstens	432	Ellsworth Creek Nature Preserve, Willapa Hills, WA 46.646°N 123.719°W
4	B. Carstens	433	Ellsworth Creek Nature Preserve, Willapa Hills, WA 46.646°N 123.719°W
5	B. Carstens	434	Ellsworth Creek Nature Preserve, Willapa Hills, WA 46.646°N 123.719°W
6	S. Wagner	MJE4	Connelly Creek, Olympic Peninsula, WA
7	S. Wagner	MJE5	Connelly Creek, Olympic Peninsula, WA
8	S. Wagner	APM4	Connelly Creek, Olympic Peninsula, WA
9	S. Wagner	ERB4	Connelly Creek, Olympic Peninsula, WA
10	S. Wagner	ERB5	Connelly Creek, Olympic Peninsula, WA

APPENDIX 2

Microtus richardsoni collection information. Specimens were obtained from the following institutions or individuals: University of Idaho Vertebrate Collection (UIVC); Royal British Columbia Museum, Victoria (RBCM); Connor Museum of Natural History, Washington State University (CMNH); Southern Oregon University (SOSC); Monte L. Bean Life Science Museum, Brigham Young University (MLBLS); Burke Museum of Natural History and Culture, University of Washington (UWBM); and Marion Klaus Sheridan College, (MK). The following initials and associated field numbers denote specimens collected during fieldwork: JRD (John R. Demboski), JMG (Jeffrey M. Good), and JMS (Jack M. Sullivan).

	Source and number	Locality
1	UIVC10268801	British Columbia, 4 mi N Kaslo, Shulty Bench
2	UIVC10268804	British Columbia, 4 mi N Kaslo, Shulty Bench
3	RBCM19958	British Columbia, Cayoosh Creek
4	RBCM19959	British Columbia, Cayoosh Creek
5	RBCM19960	British Columbia, Cayoosh Creek
6	RBCM19961	British Columbia, Cayoosh Creek
7	RBCM19962	British Columbia, Cayoosh Creek
8	RBCM19934	British Columbia, Kootenai Pass
9	JMS275	Idaho, Adams Co., Boulder Creek
10	JMS276	Idaho, Adams Co., Boulder Creek
11	JMS278	Idaho, Adams Co., Boulder Creek
12	UIVC3024	Idaho, Bonner Co., Gold Creek
13	UIVC3241	Idaho, Bonner Co., Gold Creek
14	UIVC3286	Idaho, Bonner Co., Gold Creek
15	JMS243	Idaho, Clearwater Co., 14 mi N Kelly Creek, Long Creek
16	JMS244	Idaho, Clearwater Co., 14 mi N Kelly Creek, Long Creek
17	JMS245	Idaho, Clearwater Co., 14 mi N Kelly Creek, Long Creek
18	UIVC10138652	Idaho, Idaho Co., Bench Lake
19	UIVC10148615	Idaho, Idaho Co., Boulder Creek, near Lochsa River, Rt 12
20	JMG87	Idaho, Idaho Co., Elk Summit, Hoodoo Lake
21	JMG88	Idaho, Idaho Co., Elk Summit, Hoodoo Lake
22	JMG89	Idaho, Idaho Co., Elk Summit, Hoodoo Lake
23	UIVC1658	Idaho, Idaho Co., Red River Hot Springs
24	UIVC10148636	Idaho, Idaho Co., Seven Lakes
25	UIVC917858	Idaho, Lemhi Co., Horse Creek
26	UIVC6108507	Montana, Missoula Co., 5 mi SE of Missoula, Patee Canyon
27	JMS290	Oregon, Deschutes Co., Sparks Lake, Goose Creek
28	JMS291	Oregon, Deschutes Co., Sparks Lake, Goose Creek
29	JMS292	Oregon, Deschutes Co., Sparks Lake, Goose Creek
30	CMNH80–725	Oregon, Deschutes Co., Head of Falls Creek, SE of South Sister
31	CMNH80–731	Oregon, Deschutes Co., Head of Falls Creek, SE of South Sister
32	JMS283	Oregon, Hood River Co., 7 mi E, 3.8 mi S Government Camp, Bonney Meadows
33	JMS284	Oregon, Hood River Co., 7 mi E, 3.8 mi S Government Camp, Bonney Meadows
34	JMS285	Oregon, Hood River Co., 7 mi E, 3.8 mi S Government Camp, Bonney Meadows
35	SOSC2406	Oregon, Jackson Co., Spring Creek
36	SOSC2531	Oregon, Klamath Co.
37	CMNH89–2029	Oregon, Linn Co., 7 mi N, 8 mi E of Blue River
38	AF163905 (GenBank)	Oregon, Linn Co., Big Lake, Hayrick Butte
39	CMNH81–570	Oregon, Wallowa Co., SE side Jarett(?) Lake
40	DSR5157 (MLBLS)	Utah, Emery Co., Spring, Mill Fork Canyon
41	UWBM73596	Washington, Chelan Co., off Hwy 2, 7 mi. on FS6700, onto FS6704
42	UWBM73597	Washington, Chelan Co., off Hwy 2, 7 mi. on FS6700, onto FS6704
43	JRD64	Washington, Columbia Co., 26 km SE Dayton, FR64 along Touchet River
44	JRD65	Washington, Columbia Co., 26 km SE Dayton, FR64 along Touchet River
45	UWBM73676	Washington, Columbia Co., Touchet River, 5 mi on FR64 from USF entrance
46	JRD13	Washington, Kittitas Co., Milk Creek, 3 mi N of Cliffdell, FR1708
47	UWBM74882	Washington, Okanogan Co., S of Meadows Campground on FR500
48	UWBM74886	Washington, Whatcom Co., N of FR5400, Harts Pass
49	UWBM74889	Washington, Whatcom Co., W of FR700, 0.80 mi from junction of FR700 and FR5400
50	MK1	Wyoming, Big Horn Co., Bald Mountain Creek
51	MK8	Wyoming, Big Horn Co., Bald Mountain Creek
52	MK9	Wyoming, Big Horn Co., Bald Mountain Creek
53	MK10	Wyoming, Big Horn Co., Bald Mountain Creek
54	MK11	Wyoming, Big Horn Co., Bald Mountain Creek
55	MK1bc	Wyoming, Big Horn Co.
56	MK2bc	Wyoming, Big Horn Co.
57	MK3bc	Wyoming, Big Horn Co.
58	MK5bc	Wyoming, Big Horn Co.