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Comparing range evolution in two western *Plethodon* salamanders: glacial refugia, competition, ecological niches, and spatial sorting

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ABSTRACT

Aim *Plethodon dunni* and *P. vehiculum* are sister species with widely overlapping ranges, yet the distribution of *P. vehiculum* extends almost 400 km farther north than its sister species. We explore Pleistocene refugial structure, competition, physiological tolerances and dispersal ability as contributing factors to the range difference between these two species.

Location The Pacific Northwest of North America, including Oregon, Washington and British Columbia – the full range of both species.

Methods We used genetic, environmental and morphological data to test hypotheses that explain this range difference. Genetic data were used to explore range expansion dynamics and population structure. Species distributional models were used to compare current niches to mid-Holocene and Pleistocene distributions. Morphological data were used to assess phenotypic differences between the species and test for evidence of spatial sorting.

Results Both species underwent rapid range expansions since the Pleistocene and share similar population structure. Species distributional models are different in distribution at all times periods between species. The species are significantly different in all morphological measurements taken. Only *P. vehiculum* shows evidence of spatial sorting.

Main conclusions Physiological tolerance and dispersal ability best explain the distributional difference between these species. Spatial sorting most likely plays a key role in the range expansion of *P. vehiculum*.

Keywords

Pacific Northwest, phylogeography, *Plethodon*, range expansion, spatial sorting, species distributional modelling

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INTRODUCTION

Current distributions of species are influenced by a combination of factors, including ancestral distributions, physiological tolerances, biotic interactions and dispersal ability (Gaston, 2009). While the relative influence of each factor can be difficult to determine, understanding the contributions of each facilitates a deeper knowledge about the evolutionary history of any organism. This is particularly true for species in temperate zones, where climatic fluctuations associated with Pleistocene glaciations (Pielou, 1991) add complexity in determining species distributions because species ranges must shift accordingly (Shafer *et al.*, 2010).

The Pacific Northwest of North America (PNW) is a geographically and climatically dynamic region that has undergone dramatic changes including repeated glaciation (Pielou, 1991). During the Last Glacial Maximum (LGM; *c.* 21Ka), ice sheets covered much of Washington (WA) and extended into southern Oregon (OR) along the Cascade Mountains, shifting species habitat by the presence of ice, and associated changes in temperature and water availability. The LGM has been implicated as an important factor that influenced the phylogeographical history of many species from the PNW, including salamanders (Carstens *et al.*, 2004; Kuchta & Tan, 2005; Wagner *et al.*, 2005; Steele & Storfer, 2007; Pelletier *et al.*, 2011, 2015). These investigations concluded that glacial refugia were available during the LGM south of the ice

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sheets, either in the foothills of the Cascades or in pockets along the coast, and demonstrated that range expansion occurred as the glaciers retreated (Brunsfeld *et al.*, 2001; Shafer *et al.*, 2010). Thus, salamanders in the PNW represent an effective model for investigating factors that influence geographical range shifts. We focus on developing an understanding of how the geographical range of a pair of sister species of *Plethodon* salamanders has shifted through evolutionary time, in response to climatic changes of the Pleistocene.

In order to understand the factors that influence the evolution of geographical range, it is important to avoid being misled by phylogenetic differences among clades; comparing co-distributed sister taxa can control for non-shared histories of lineages (Dawson, 2012). *Plethodon dunni* Bishop, 1934 and *P. vehiculum* Cooper, 1860 are sister species that diverged *c.* 10 Ma (Kozak *et al.*, 2009) during the warmer climate of the Pliocene and late-Miocene, long before the cyclic glaciations of the Pleistocene (Robinson *et al.*, 2008). Like all *Plethodon* salamanders, *P. dunni* and *P. vehiculum* inhabit moist, cool environments and are generally assumed to be dispersal limited due to dependence on moist conditions for respiration (Ovaska, 1988). These species are largely sympatric and widespread throughout the PNW, though the distribution of *P. vehiculum* extends much farther north than that of *P. dunni* (Fig. 1). Multiple reasons may explain the different distributions between this pair, specifically the more northern distribution of *P. vehiculum*.

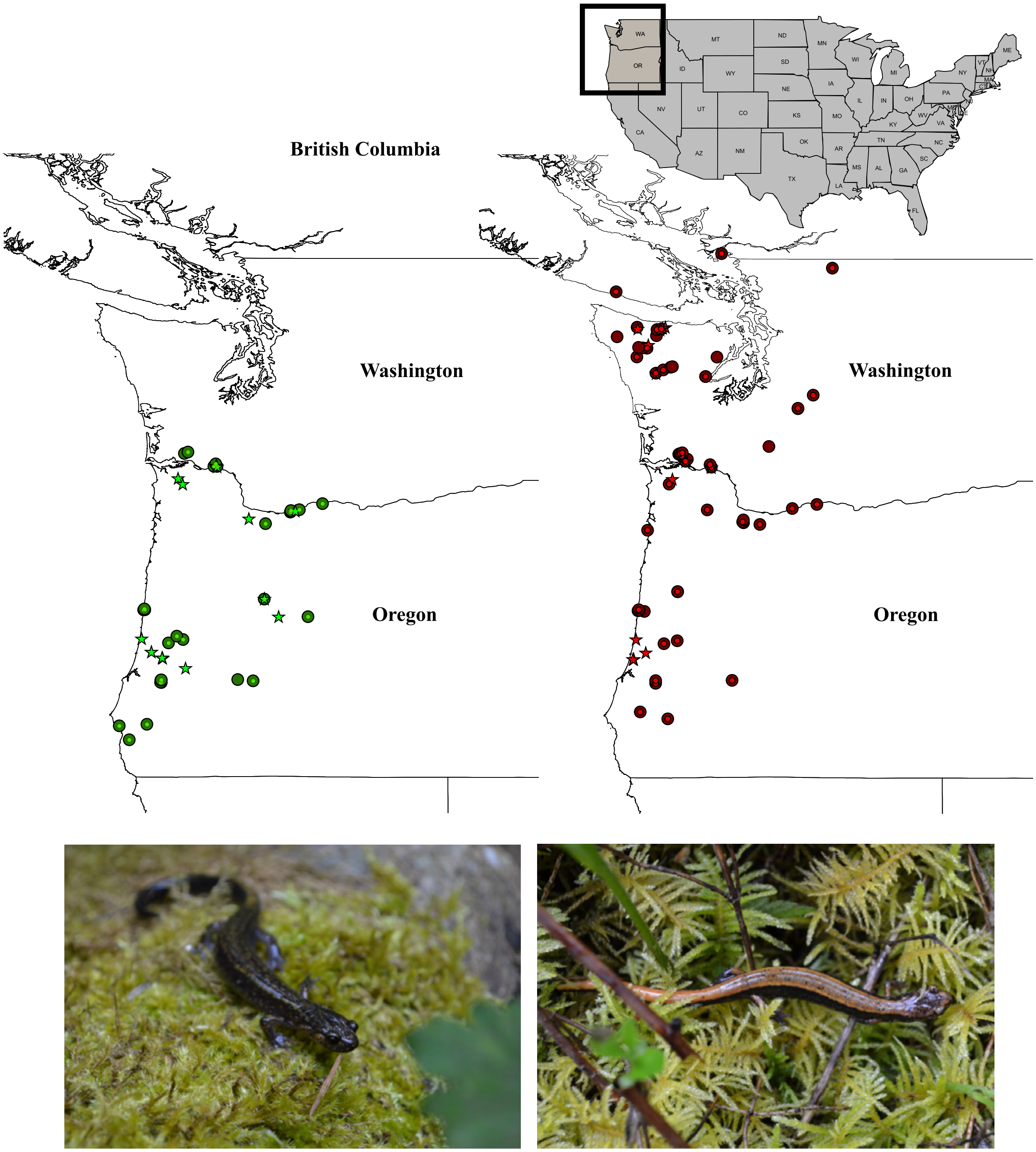
First, the range differences between *P. dunni* and *P. vehiculum* might be the result of differences in refugial structure of the species during Pleistocene glaciations (Stewart *et al.*, 2010). If ancestral ranges were larger, more numerous or located farther north for *P. vehiculum* during the LGM, then its larger current range may result from these factors. Phylogeographical breaks are commonly detected around the Oregon-California boundary (Swenson & Howard, 2005), as habitat between glaciated mountaintops was probably suitable for many amphibian species. Additionally, the southern WA and OR Cascade Mountains and coastal regions of the PNW may have provided areas of suitable habitat for terrestrial organisms (Steele & Storfer, 2006; Pelletier *et al.*, 2011), and this may be the case for *P. vehiculum*. If so, we expect haplotypic data to suggest multiple glacial refugia or higher haplotype diversity in the north, and species distributional models (SDM) at the LGM (*c.* 21 ka) and the mid-Holocene (*c.* 6 ka) to indicate multiple, larger and/or more northern refugia during these times.

Second, competition could account for differential distributional patterns, which could manifest in multiple ways. *Plethodon dunni* may be a stronger competitor, thereby forcing *P. vehiculum* into adjacent, less optimal habitat. Lab experiments propose that *P. vehiculum* will be found in less optimal conditions (i.e. temperature and humidity extremes) when in the presence of *P. dunni*, but the opposite pattern does not occur (Dumas, 1956). We observed *P. dunni* in high numbers by the splash zones of streams and waterfalls, while *P. vehiculum* is often less abundant or non-existent in some of these areas. Body size may play a role, as *P. dunni* (50–55 mm SVL) is generally a larger salamander than *P. vehiculum* (42–44 mm SVL) (Brodie, 1970); body size and behaviour in eastern *Plethodon* salamanders drive resource use and ecological interactions (Hairston *et al.*, 1987; Adams, 2007). If differential competitive ability is present, we expect to see significant morphological differences in body size between *P. dunni* and *P. vehiculum* and also potentially a difference in morphological characters within species where they are and are not sympatric.

However, *P. vehiculum* could play a different role ecologically than other *Plethodon* salamanders in the PNW allowing it to occupy a larger range because it is not in direct competition with other *Plethodon* species. There are two other *Plethodon* salamander species that inhabit regions north of the geographical range of *P. dunni*, and *P. dunni* could be excluded from the northern range of *P. vehiculum* by *P. vandykei* and *P. larselli*, salamanders that are also more restricted to splash zones and overall wetter habitat (Dumas, 1956; Aubry *et al.*, 1987; Corkran & Thoms, 2006). If *P. dunni* is outcompeted by other *Plethodon* salamanders, then we expect the SDMs of *P. dunni* to be similar with these other species, even though the distribution of *P. dunni* does not currently extend that far north.

Finally, the difference in distribution may be explained by physiological tolerances and dispersal ability. Niche models based on the current species distributions will predict larger niche space for *P. vehiculum* than that for *P. dunni* if there is simply more suitable habitat available for *P. vehiculum*. Although both species occur in densely forested areas of the PNW, they may differ in their habitat tolerances. For example, temperature and precipitation have been highly supported as edge limits in some eastern *Plethodon* salamanders (Cahill *et al.*, 2014). *Plethodon dunni* appears to have a higher affinity to moist substratum and lower temperatures, while *P. vehiculum* appears to be more tolerant of lower humidity and higher temperatures (Dumas, 1956). We observe *P. vehiculum* distributed throughout the forest floor as well as alongside streams, but we observe *P. dunni* almost exclusively alongside splash zones of streams and waterfalls. Additionally, if climatic tolerances are the main determinant of a species range, then the observed distribution of both should be almost identical to that of the estimated SDMs (Baselga *et al.*, 2012).

Dispersal limitations that differ between these species may suggest that *P. vehiculum* has undergone a more dramatic expansion than *P. dunni*. If so, we expect to see less haplotypic diversity in the north in *P. vehiculum*, an indication of northern range expansion, as well as genetic evidence of a stronger or earlier demographical expansion. Furthermore, we might identify phenotypic traits associated with dispersal ability to a greater extent in *P. vehiculum* than in *P. dunni*. This may be observed as spatial sorting – assortative mating over geographical space and the subsequent evolution of morphological characteristics associated with dispersal ability



(a) *Plethodon dunni* **(b)** *Plethodon vehiculum*

Figure 1 Sampling localities for (a) *Plethodon dunni* in green and (b) *Plethodon vehiculum* in red from Northwest North America. Dark solid circles are all sampling localities. Light coloured circles within the larger dark coloured circles are sampling localities from which the subset of DNA data was sequenced. Stars represent sampling sites for morphological measurements. Localities are sampled from throughout the distributions of both species. We were unable to sample for morphological measurements at the far south edge of the distribution for both species due to large forest fires and destruction of habitat during the 2013 season. A map of all GPS points used for SDMs can be found in Appendix S2.

at range edges (Simmons & Thomas, 2004; Shine *et al.*, 2011; Lindström *et al.*, 2013). Spatial sorting suggests that faster-dispersing individuals at the leading edge of a species range will breed with each other, therefore producing more faster-moving individuals. In this capacity, individuals with phenotypes allowing for more efficient dispersal will be found at the edge of a species range. For example, lodgepole pine migrated northward from south of the ice sheets at the

LGM into Canada, *c.* 2200 km in *c.* 12,000 years, whereas fossil evidence suggests this species is still migrating north; seed mass and wind loading, two morphological characters that contribute to dispersal ability, are positively correlated with time since founding (Cwynar & MacDonald, 1987). This suggests that temperate systems are ideal for detecting spatial sorting.

Here, we explore three general explanations that could be shaping the difference in distributional pattern between *P. dunni* and *P. vehiculum*: (1) Glacial refugial structure: the range differences between the two species are the result of differences in the refugial structure of the species during Pleistocene glaciations. (2) Competitive exclusion: the two species are in direct competition with one another. (3) Dispersal/physiological differences: the range of *P. vehiculum* is larger than *P. dunni* because there is more suitable habitat available for *P. vehiculum* than *P. dunni* and *P. vehiculum* is a more capable disperser. We use multiple lines of evidence to test these varying hypotheses and elucidate the phylogeographical patterns within *P. dunni* and *P. vehiculum*.

MATERIALS AND METHODS

Genetic data

Tissue samples were collected from 136 *P. dunni* individuals from 30 localities and 218 *P. vehiculum* individuals from 58 localities throughout both of their ranges (Fig. 1; see Appendix S1). One mtDNA locus, cytochrome b (*Cyt b*), was sequenced for all individuals. We sequenced a subset of individuals for a number of nuclear loci (Appendix S1). One of these loci was the Recombination Activating Gene (RAG1). Other primers were designed with genomic DNA libraries constructed from *P. dunni/vehiculum*, using restriction digest and subcloning using the Qiagen PCR cloning kit. To determine the most efficient number of individuals and nuclear loci to sequence to effectively detect population divergence with range expansion, we first conducted a power analysis using approximate Bayesian computation (ABC) on models with and without expansion (Appendix S1). We sequenced 72 individuals for *P. dunni* (28 of the 30 localities) and 96 individuals for *P. vehiculum* (39 of the 58 localities) to ensure a comparable level of sampling across their ranges. We were able to confidently sequence six loci in each species (Appendix S1). PCR clean-up and Sanger sequencing were carried out at Beckman Coulter Inc. (Danvers, MA, USA). Sequence editing and alignment were conducted using Geneious 8.0.4 (2014). Sequence data were phased to alleles using SeqPHASE (Flot, 2010) and PHASE 2.1 (Stephens *et al.*, 2001).

We first calculated nucleotide diversity (π), the number of segregating sites (ss), the number of haplotypes (h), Tajima's *D*, Fu's F_s, Fay and Wu's θ_H, and the difference between θ_H and π (H) for each locus in DnaSP 5 (Rozas *et al.*, 2003). Tajima's *D* and Fu's F_s are used to distinguish population growth from a constant population size because population expansion generates an excess of mutations in external branches (i.e. single mutations; Ramos-Onsins & Rozas, 2002). Significant negative numbers can indicate population expansion.

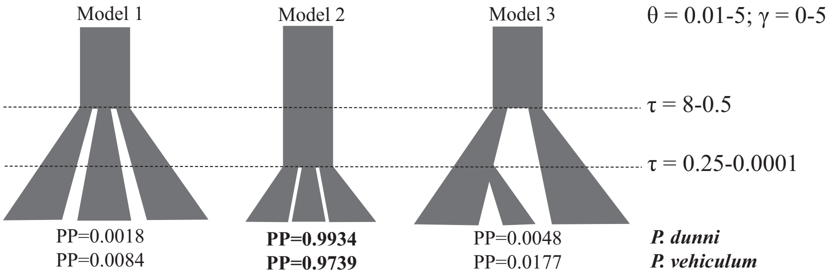
Haplotype distributions were plotted geographically for each locus in both species. Areas with higher levels of haplotype diversity are suspected to be areas of glacial refugia, whereas those with little haplotype diversity are most likely the result of an expanding population. We also calculated mismatch distributions, the distribution of the number of differences between pairs of DNA sequences, in DnaSP for each locus to visualize the distribution of sequence variation. The mismatch distribution for each locus is plotted compared to a model of constant population size (Ramos-Onsins & Rozas, 2002).

Extended Bayesian skyline plots infer effective population size (N_e) through time and were estimated in *BEAST 1.7.1 (Drummond & Rambaut, 2007). Models of sequence evolution were chosen using jModeltest 2.0 (Darriba *et al.*, 2012; see Appendix S1). Strict clocks were used for all loci. We ran the analysis using both linear and stepwise models. Default settings were used except that clock priors were set to uniform between 1 and 100, the demographical population mean initial value was set to 1, the kappa weights were set to 1, and the demographical population mean was unchecked, following the *BEAST Bayesian skyline tutorial. Runs were inspected using Tracer 1.5 (Rambaut & Drummond, 2009) to assess MCMC mixing, and 500,000,000 generations were run sampling every 100,000 generations, using a burn-in of 10%. We assumed a mutation rate of 1.0×10^{-9} (Herrick & Sclavi, 2013; Pelletier & Carstens, 2014) to calculate N_e.

We used Structurama2 (Huelsenbeck *et al.*, 2011) to identify the number of populations for downstream analyses to infer the structure of glacial refugia. One population within a species might indicate better dispersal, while multiple populations could indicate that dispersal is hindered in some way, or multiple glacial refugia existed. The number of populations (*K*) was treated as random variable under the Dirichlet process prior, and for reliability, multiple runs were conducted using a series of gamma priors: (0.1, 1), (1, 1), (0.1, 10), (1, 10). Once *K* was determined, we ran the analysis fixing *K* to allow for admixture. All runs consisted of 10,000,000 generations with a sample frequency of 1000 and 10% burn-in. Plots were produced using DISTRUCT 1.1 (Rosenberg, 2004).

Based on the results from Structurama2, we used ABC to determine the best model of divergence and expansion for each species, assuming three populations (Fig. 2): (1) three populations split deep in time ($\tau = 8$–0.5), and all populations persisted throughout the LGM; (2) one glacial refuge at the start of the LGM with a more recent split ($\tau = 0.25$–0.0001) among the three populations; (3) one population split deep in time ($\tau = 8$–0.5) and the other split more recently ($\tau = 0.25$–0.0001), so two populations persisted throughout the LGM. Differentiating among these models

Figure 2 Demographical models for ABC analysis of two *Plethodon* species from Northwest North America. PP = posterior probability. Model priors: $\theta = 4N_e\mu$, γ = exponential rate of expansion, τ = time in *N* generations.



will elucidate whether populations persisted throughout the Pleistocene, formed during the LGM, or a combination of these.

All populations were allowed to expand (γ = 0–5) and theta ($\theta = 4N_e\mu$) was set between 0.01 and 5. Priors were based on previous *Plethodon* studies (Pelletier & Carstens, 2014; Pelletier *et al.*, 2015) and past climatic fluctuations (Webb & Bartlein, 1992). A total of 6,000,000 simulations were conducted using MS (Hudson, 2002), and the following five summary statistics were calculated: $\{\pi, \text{ss}, D, \theta_H, H\}$. The 'abc' package (Csilléry *et al.*, 2011) in R 3.1.1 (R Core Team, 2014) was used to conduct the rejection step. We first used the leave-one-out cross-validation step to determine which threshold, vector of summary statistics and type of rejection step to use (Appendix S1). For the final analysis, we chose to present results based on the summary statistics vector $\{\pi, D, \theta_H, H\}$, threshold 0.01 and the 'mnlogistic' rejection for model selection and 'loclinear' rejection for parameter estimation.

Species distributional models

We estimated current and historical (21 and 6 ka) niches for both species to determine where the habitat was suitable in the past, how their distributions have changed since the LGM and whether *P. dunni* and *P. vehiculum* could potentially inhabit the same current geographical space. GPS coordinates were obtained from sampling localities of this study and the HerpNet database (http://herpnet.org), totalling 202 *P. dunni* and 346 *P. vehiculum* points (Appendix S2). A total of 19 bioclimatic variables are available at 1-km resolution (Bio1-19) from http://worldclim.org/bioclim. We only used bioclimatic variables also found in the historical datasets making the models directly comparable and removed any variables that were highly correlated ($-0.9 \geq r \geq 0.9$; we also evaluated models at $-0.7 \geq r \geq 0.7$). Our final analyses included 11 bioclimatic variables for *P. dunni* and eight bioclimatic variables for *P. vehiculum* (Appendix S2).

ArcMap 10.2.2 (ESRI, 2011) was used for data layer manipulation and maps were clipped at 51° N, 40° S, −128° W and −120° E. Niche models were created using Maxent 3.3.3 (Phillips *et al.*, 2006b) with 80% of the localities used to predict the model and 20% of the localities used in a jackknife analysis to test the models. Given some of the critiques of this model (Royle *et al.*, 2012; Merow *et al.*, 2013), models were also evaluated using the 'biomod2' package (Thuiller *et al.*, 2014) in R 3.2.1 (R Core Team, 2014), to add pseudo-absence data and test different parameters of the Maxent method (Appendix S2). The models were evaluated using area under the curve (AUC) and true skill statistic (TSS) scores. We also projected models for *P. vandykei* and *P. larselli* with data from Pelletier *et al.* (2015) onto the Mid-Holocene bioclimatic data and compared the niches of *P. dunni* to the current and LGM models of these two species from Pelletier *et al.* (2015).

Morphological data

Total length (TL), snout–vent length (SVL), femur length (FL) and body weight (W) were taken in the field, along with photographs including a colour card from 38 *P. vehiculum* and 42 *P. dunni* individuals (Fig. 1; Appendix S3). Salamanders were subjected to 1–5 g L^{-1} of tricaine-S in distilled H_2O for 5–15 min to anaesthetize the animals for accurate measurements in the field (Mitchell, 2009). Once the data were recorded, the salamanders recovered in fresh distilled water for up to 1 hour and were released where found. Costal groove count (CG) was taken from photographs. FL was scaled to body weight (FL/W) and SVL (FL/SVL) as additional morphological variables. These variables were chosen because body size and leg length have been associated with dispersal ability (Phillips *et al.*, 2006a). We expect body size could be an especially important factor for dispersal in amphibians because desiccation tolerance can be quite variable among closely related amphibian species (Hillman *et al.*, 2009), and smaller amphibians have a greater tolerance to water loss than larger ones (Thorson, 1955). Although we are aware that other phenotypic characteristics, such as behaviour and physiology, may be under selection for dispersal, we focused on morphological characters for this study because they are easily collected in the field.

All statistical analyses were conducted using R 3.1.1 (R Core Team, 2014). No variables deviated substantially from normality. We tested whether each variable was significantly different between species using two-tailed *t*-tests. Differences in certain morphological characters may be indicative of differential dispersal ability and physiological tolerances. We conducted multiple regression analyses for each morphological variable against latitude, longitude, and altitude to identify associations with any of these geographical components. We expect to see a linear relationship with latitude for morphological characters if they are associated with a northern range expansion. Finally, we ran a principal components analysis (PCA) on both species to look for any pattern in the morphological measurements that could be explained by sympatry. Specifically, we expect to see some difference in morphological measurements in *P. vehiculum* where they are or are not in sympatry with *P. dunni* if competitive exclusion was a possibility.

RESULTS

Genetic data

Six loci were sequenced for each species (Table 1). Sequences are deposited in GenBank (KX433187-KX434428).

In *P. dunni,* two of the six and three of the six loci were significantly negative, for Tajima's D and Fu's F_s, respectively, indicating expansion. In *P. vehiculum*, one locus was significantly negative for Tajima's D, but all loci were significantly negative for Fu's F_s. Most haplotypic diversity in both species is found in the southern portion of its distribution (Appendix S1). In *P. dunni*, for most loci, there is one dominant haplotype that can be found from the most southern portion of its range through WA. A similar pattern is evident in *P. vehiculum*, and a single dominant haplotype can be found from the south/central portion of its range all the way into British Columbia (BC) and is the only haplotype found in many northern localities. Such patterns are indicative of range expansion. Results from the mismatch distributions are similar, where three loci in *P. dunni* and five loci in *P. vehiculum* indicated population expansion (Appendix S1). Bayesian skyline plots demonstrate that both species underwent a very recent rapid population expansion, although the expansion was about six times greater in *P. dunni* than in *P. vehiculum* (Fig. 3) and the current N_e differed among species (*c.* 500,000 for *P. dunni* and *c.* 100,000 for *P. vehiculum*).

Results from Structurama2 indicate that both species contain similar population genetic structure, with the highest posterior probability at $K = 3$. *Plethodon vehiculum* is highly geographically structured, while *P. dunni* is less so, particularly in the northern part of its range, and there is little admixture among groups in both species (Fig. 4). The posterior probabilities across runs were similar, so only probabilities from run with prior (1, 10) are provided for simplicity (Appendix S1). Populations within *P. vehiculum* cluster geographically, while two of the populations from *P. dunni* do not.

The ABC results indicate that a model with one glacial refuge at the start of the LGM with a more recent split ($\tau = 0.25–0.0001$) among the three populations was the best fit for the data in both species (Model 2). The posterior probability for this model was 0.9934 in *P. dunni* and 0.9739 in *P. vehiculum* (Fig. 2; Appendix S1). Parameter estimates (Appendix S1) for exponential population growth in all three populations are similar for both species, though slightly higher in *P. dunni* (mean $\gamma_1 = 2.5418$, $\gamma_2 = 2.4708$, $\gamma_3 = 3.0364$) than in *P. vehiculum* (mean $\gamma_1 = 2.4427$, $\gamma_2 = 2.4267$, $\gamma_3 = 3.0163$). Theta (θ) is also higher in *P. dunni* (0.4857) than in *P. vehiculum* (0.3218), and the timing of divergence (τ) is slightly earlier in *P. dunni* (0.1108) than in *P. vehiculum* (0.1189). Using the mutation rate 10^{-9} and a generation time of 3 and 5 years (Herrick & Sclavi, 2013; Pelletier & Carstens, 2014), these estimates range from *c.* 17 to 28 ka for *P. dunni* and from *c.* 12 to 20 ka for *P. vehiculum*.

Species distributional models

To provide insight into the ancestral distributions of each species, we estimated SDMs at the LGM, the mid-Holocene and the present from bioclimatic data that pertain to temperature and precipitation (Fig. 5). These models were a good fit to the

Table 1 Population genetic statistics of two *Plethodon* species from Northwest North America. n = number of alleles; bp = base pairs; π = nucleotide diversity; ss = segregating sites; h = number of haplotypes; θ_H = Wu's theta$H$; H = difference between π and θ_H; $^{**}P < 0.01$; $^{*}P < 0.05$; ns = not significant.

	Locus	n	bp	Tajima's D	Fu's Fs	π	ss	h	θ_H	H
Plethodon dunni	Pl1	120	213	1.10433ns	0.367ns	0.00305	2	4	0.00175	0.0013
	Pl69	136	401	−1.36933ns	1.108ns	0.00357	16	6	0.00727	−0.0037
	Pl110	132	436	−2.15627**	−1.116ns	0.00800	12	4	0.00511	0.00289
	MYL6	116	272	1.32678ns	7.651**	0.01348	13	5	0.00897	0.00451
	RAG1	122	599	−1.58327ns	−6.887**	0.00097	9	10	0.00279	−0.00182
	Cyt *b*	116	510	−1.84118*	−8.121**	0.00253	19	15	0.00728	−0.00475
	Total bp		2431							
Plethodon vehiculum	Pl16	184	387	−1.08576ns	−5.575**	0.00241	10	12	0.00446	−0.00205
	Pl69	182	404	−0.6333ns	−2.521*	0.00382	12	12	0.00514	−0.00132
	Pl110	188	436	−0.61797ns	−5.968**	0.00354	12	16	0.00474	−0.0012
	MYL6	184	316	0.45928ns	−8.593**	0.00527	8	19	0.00437	0.0009
	RAG1	158	599	−2.32316**	−11.299**	0.00025	12	8	0.00355	−0.0033
	Cyt *b*	184	540	−0.02794ns	−2.227*	0.01058	47	26	0.01535	−0.00477
	Total bp		2682							

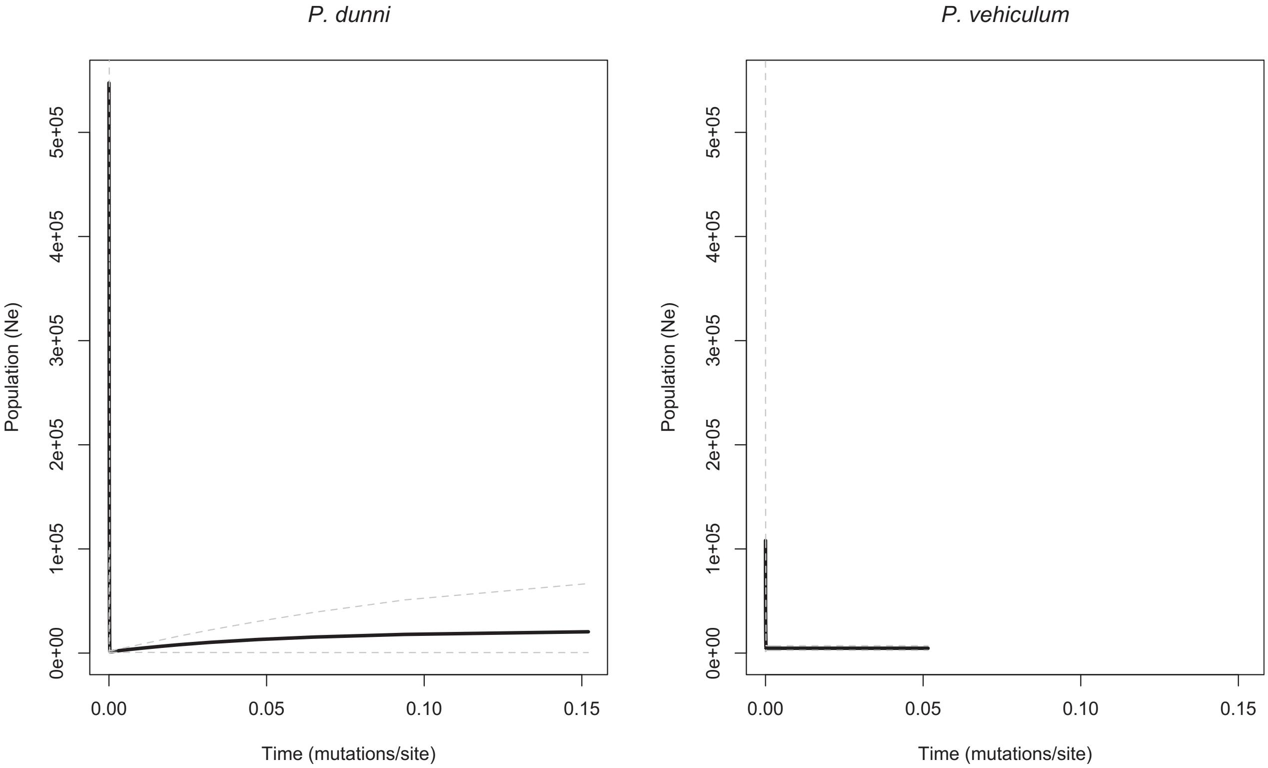


Figure 3 Extended Bayesian skyline plots with 95% HDP for two *Plethodon* species from Northwest North America.

data (*P. dunni*: AUC = 0.97, TSS = 0.83; *P. vehiculum*: AUC = 0.92, TSS = 0.68). Modelling with pseudo-absence data, different Maxent parameters and the different cut-off for variable correlations did not significantly change the models or our interpretations (Appendix S2). The modelling suggests that the potential historical range of these species was not the same: *P. vehiculum* had a larger estimated distribution than *P. dunni* at the LGM and habitat for *P. dunni* was more southern. Both species distributions shift north later during the mid-Holocene, but at this time point, the distribution of *P. vehiculum* becomes smaller than *P. dunni*. Again, there is a shift in distributions for the current SDM; *P. dunni* has a much smaller potential distribution than *P. vehiculum*. Both distributions extend the same distance south, but the model for *P. vehiculum* suggests suitable habitat for geographical space much farther north than *P. dunni*, a pattern that matches their current known distributions.

These models closely match their current known distributions, even to the extent that *P. dunni* appears to have a more patchy distribution. There is overlap in the variables contributing the most to the models between the two species, but *P. dunni* appears to be more affected by cold temperature and precipitation in the dry months, while *P. vehiculum* appears to be more affected by temperature in the dry and wet months (Table 2).

There is little similarity among the current, mid-Holocene and LGM SDMs between *P. vandykei* and *P. dunni*, or *P. larselli* and *P. dunni* (fig. 3a,b in Pelletier *et al.*, 2015; Appendix S2), except for the suitable habitat for all species below the extent of glaciation. Overall, the SDMs of *P. larselli* and *P. vandykei* do not show very similar ecological niches with *P. dunni*.

Morphological data

Plethodon dunni and *P. vehiculum* were significantly different for all variables (Table 3; Appendix S3). The only morphological character in *P. dunni* that co-varied with spatial measures was CG with latitude, while controlling for longitude and altitude ($P = 0.0466$). In contrast, *P. vehiculum* had many such characters: SVL ($P = 0.0276$), TL ($P < 0.001$), FL ($P = 0.0407$), W ($P = 0.0022$) and FL/W ($P = 0.0263$) were all associated with latitude when controlling for longitude and altitude. This indicates that *P. vehiculum* has multiple morphological characteristics that can be associated with dispersal that change in a north–south fashion. In both *P. dunni* and *P. vehiculum*, PC1 + PC2 explained 99% of the variation within species with the highest loading for these axes being SVL and TL (Appendix S3). Neither species showed any clustering, so it does not appear that there are differences within species that group according to whether the species are sympatric or not.

DISCUSSION

Many factors can contribute to the current geographical distribution of organisms, including the size and location of ancestral populations (Stewart *et al.*, 2010), competition

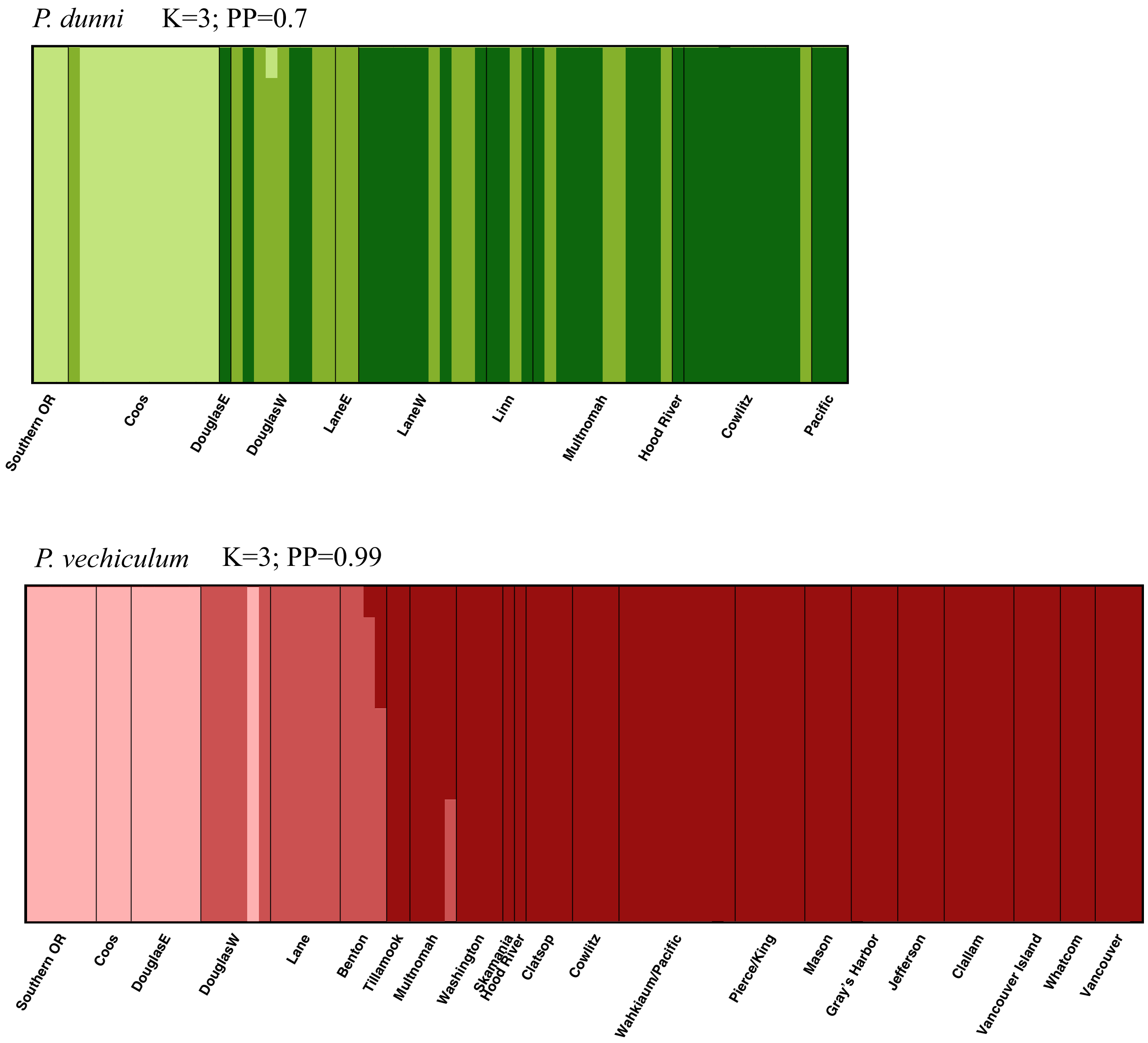


Figure 4 Structurama2 population assignment plots for two *Plethodon* species from Northwest North America. K = number of populations. PP = posterior probability. Group names are county names arranged in a south-to-north fashion. Those that are next to each other in this plot are located next to each other geographically.

(Kozak *et al.*, 2009), and physiological tolerances and dispersal ability (Baselga *et al.*, 2012). Our study examines these factors in two sister species of terrestrial salamander in the PNW. *Plethodon dunni* and *P. vehiculum* are diverged *c.* 10 Ma and sympatric, though *P. vehiculum* has a much larger northern distribution. The combined results from the genetic, environmental and morphological data suggest that temperature and humidity physiological tolerances, and dispersal ability most adequately explain the difference in distribution between these two species.

Genetic data suggest that both species underwent a recent rapid range expansion, as evident by summary statistics, mismatch distributions and Bayesian skyline plots. Population size in *P. dunni* is larger and grew faster than that of *P. vehiculum*. This is an interesting difference because it means that although the population size and demographical expansion in *P. dunni* is substantially greater than *P. vehiculum*, the magnitude of geographical expansion does not coincide with this result.

Population genetic structure in both species has developed since the LGM. In other words, there were no more glacial refugia in *P. vehiculum* than *P. dunni*. The parameter estimates from the ABC analysis are similar to those from the Bayesian skyline plots, suggesting that *P. dunni* has a larger N_e than *P. vehiculum*, and the rate of demographical expansion was higher in *P. dunni* than in *P. vehiculum*. The ABC results suggest that the expansion and divergence of *P. dunni* occurred earlier than it did in *P. vehiculum*, so the idea that

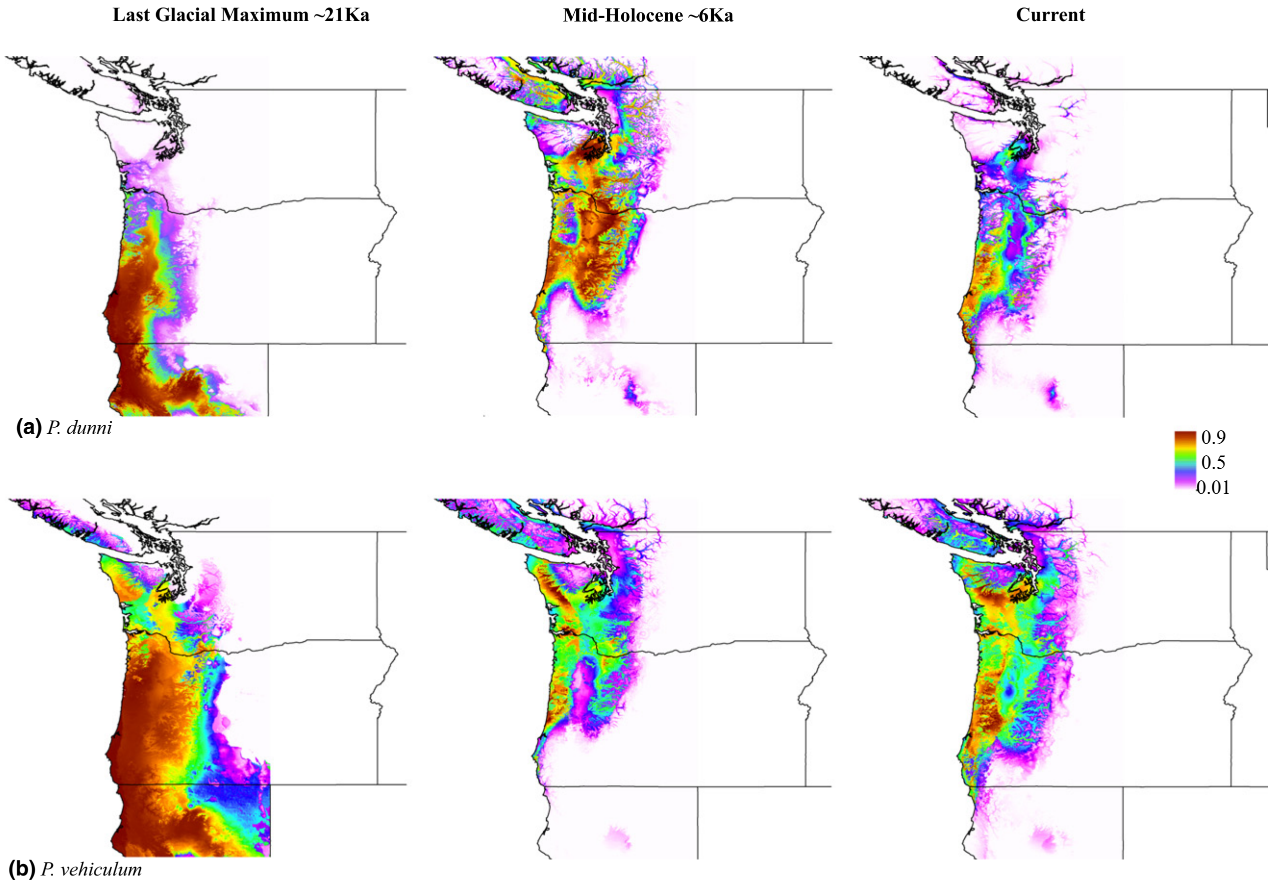


Figure 5 Species distributional models for (a) *Plethodon dunni* and (b) *P. vehiculum* from Northwest North America. Warmer colours show areas with higher probability of suitable habitat.

P. vehiculum has a larger distribution because it has been expanding for a longer period of time is rejected.

Haplotype distributions are consistent with a rapid northern range expansion in both species due to the paucity of haplotypic diversity in the north, whereas almost all haplotypic diversity is found in south/central OR. In *P. vehiculum* in particular, there is almost no haplotypic diversity found in northern OR, WA and BC, suggesting no northern refugia existed in this species. In total, analyses of the genetic data support a recent demographical population expansion, but that differences in the refugial structure between species cannot explain the larger and more northerly distribution of *P. vehiculum*.

Species distributional models indicate that both species persisted throughout the most recent glaciation in a single refugium. In each species, the SDMs from the LGM predict that suitable habitat existed south of the glacial extent. While the extent of suitable habitat in *P. vehiculum* is farther to the north than *P. dunni*, the genetic data suggest that northern areas did not contain glacial refugia. Interestingly, the mid-Holocene model for *P. dunni* is larger than *P. vehiculum*, suggesting this warmer time period might present a more suitable climate for *P. dunni* than for *P. vehiculum* and the larger distribution of *P. vehiculum* is more recent. This result emphasizes the importance of considering climatic conditions during the Hypsithermal Interval (Mathews & Heusser, 1981) when considering ancestral distributions in range expansion studies.

Direct competition between *P. dunni* and *P. vehiculum* also does not explain the differing extent of their geographical ranges. We would expect to see SDMs at all time periods that are nearly identical in both species if they were not limited in range by environmental factors (Baselga *et al.*, 2012). Instead, the current SDMs closely match the known species distributions, which differ in their extent, so for *P. dunni* there is little suitable habitat in the regions that were more recently deglaciated, and there are differences between the LGM and mid-Holocene SDMs between the two species. Furthermore, their morphological differences are not influenced by sympatry, indicating that there is no morphological displacement in these species (Adams & Rohlf, 2000; Adams, 2004). Similarly, the lack of overlap between the SDMs of *P. dunni* and either *P. vandykei* or *P. larselli* in WA indicate that additional northern expansion in *P. dunni* is not being prevented by these species. Overall, there is little evidence that competitive exclusion prevents *P. dunni* from additional northern expansion.

Table 2 Bioclimatic variable contributions from Maxent species distributional models. Mean temperature of the coldest quarter, annual mean temperature, precipitation seasonality and precipitation of the driest quarter contribute the most to the *Plethodon dunni* models. Temperature seasonality, mean temperature of the wettest quarter, precipitation seasonality and mean temperature of the driest quarter contribute the most to the *P. vehiculum* models.

	Variable	Percent contribution
P. dunni		
BIO11	Mean temperature of coldest quarter	27.5
BIO1	Annual mean temperature	23
BIO15	Precipitation seasonality	20.3
BIO17	Precipitation of driest quarter	18.2
BIO13	Precipitation of wettest month	4.4
BIO16	Precipitation of wettest quarter	3.7
BIO4	Temperature seasonality	1.8
BIO10	Mean temperature of warmest quarter	0.6
BIO19	Precipitation of coldest quarter	0.3
BIO8	Mean temperature of wettest quarter	0.2
BIO9	Mean temperature of driest quarter	0
P. vehiculum		
BIO4	Temperature seasonality	38.1
BIO8	Mean temperature of wettest quarter	22
BIO15	Precipitation seasonality	11.4
BIO9	Mean temperature of driest quarter	10.4
BIO17	Precipitation of driest quarter	7.3
BIO18	Precipitation of warmest quarter	4.3
BIO16	Precipitation of wettest quarter	3.5
BIO14	Precipitation of driest month	2.9

Plethodon dunni and *P. vehiculum* are morphologically divergent, which may be an indication that they occupy different ecological niches. Smaller amphibians have a greater tolerance to desiccation than larger ones because they uptake water much faster and are associated with greater oxygen uptake (Thorson, 1955; Feder, 1983; Hillman *et al*., 2009). *Plethodon dunni* is larger than *P. vehiculum* and is, therefore, likely more prone to desiccation (Dumas, 1956). In general, amphibians are strongly constrained by water and temperature (Buckley & Jetz, 2007), and water loss has been found to be a significant predictor of spatial distribution and abundance in *P. albagula* (Peterman, 2013); this constraint would be exacerbated by dramatic climate changes associated with Pleistocene glacial cycles. These characteristics suggest that *P. vehiculum* may be less dispersal limited than *P. dunni* because it is more tolerant of suboptimal habitat.

In *P. vehiculum*, we observe a significant linear relationship between latitude and morphological variables that can be associated with dispersal in amphibians (Phillips *et al*., 2006a). Although body size clines have been observed in eastern *Plethodon* species, they do not appear to be associated with temperature (Olalla-Tárraga *et al*., 2010), making clines observed in *P. vehiculum* difficult to explain. We suspect that *P. vehiculum* is a better disperser than *P. dunni*, although information on dispersal in *Plethodon* salamanders is almost non-existent (but see Smith & Green, 2005). The maximum-recorded distance travelled by *P. vehiculum* is 8.5 m over 2 years with extremely high site fidelity (Ovaska, 1988). Given that the LGM was at *c*. 18–21 ka (Pielou, 1991), *P. dunni* will have had to move at least 23 m year^{-1}, and *P. vehiculum* will have had to move at least 43 m year^{-1}, to reach their current locations, *c*. 470 and 890 km, respectively, from their most likely southern glacial refuge. This observation is consistent with the specific epithet (*vehiculum* translated to vehicle, conveyance, or a means of transport). Clearly, the range expansion of *P. vehiculum* was more successful than its sister taxon, especially given its lower demographical expansion, and is likely ongoing. The latitudinal morphological clines and correspondence with a rapid and ongoing northern range expansion suggest that spatial sorting is a reasonable hypothesis for range evolution in *P. vehiculum*. Limited studies have explored spatial sorting, but it is suspected in several lineages from insects to amphibians (Hanski *et al*., 2002; Simmons & Thomas, 2004).

CONCLUSIONS

Factors such as ancestral population dynamics, range expansions, physiology and dispersal are vital factors that shape species distributions. Identifying these factors from complex scenarios is an ongoing challenge; however, combining multiple data types can make distinguishing among hypotheses possible. The best explanation for the difference in distribution between these two species is physiological tolerances and dispersal ability instead of competition or a difference in glacial refugia, a global pattern found in many taxa

Table 3 Morphological measurements of two *Plethodon* species from Northwest North America. Measurements taken in mm. SVL = snout–vent length; TL = total length; FL = femur length; W = weight in g; FL.SVL = FL scaled by SVL; FL.W = FL scaled by W; CG = costal groove count; SD = standard deviation.

	SVL	TL	FL	W	FL.SVL	FL.W	CG
P. dunni							
Mean	54.6026	104.7829	5.5314	2.9643	0.1020	2.3163	13.1
SD	10.8468	20.4113	1.0014	1.4750	0.0087	1.1294	0.261
P. vehiculum							
Mean	43.8995	79.0076	4.1875	1.5000	0.0960	3.4098	14.2
SD	6.0549	13.8009	0.5007	0.6346	0.0093	1.6931	0.132
t-test *P*-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.004	0.0014	< 0.0001

(Stewart *et al.*, 2010; Baselga *et al.*, 2012; Cahill *et al.*, 2014). This is important because *Plethodon* salamanders are thought to be extremely ecologically and morphologically constrained (Mueller *et al.*, 2004). Conversely, we argue that even small differences in these characteristics can have large impacts on distributional patterns and will certainly play a large role in response to future climate changes (Thomas *et al.*, 2004; Rapacciuolo *et al.*, 2014). Furthermore, we propose that spatial sorting is underexplored in range expansion analyses. In *P. vehiculum*, no other explanation is consistent with the total (genetic, environmental and morphological) evidence.

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REFERENCES

Adams, D.C. (2004) Character displacement via aggressive interference in Appalachian salamanders. *Ecology*, **85**, 2664–2670.

Adams, D.C. (2007) Organization of *Plethodon* salamander communities: guild-based community assembly. *Ecology*, **88**, 1292–1299.

Adams, D.C. & Rohlf, F.J. (2000) Ecological character displacement in *Plethodon*: biomechanical differences found from a geometric morphometric study. *Proceedings of the National Academy of Sciences USA*, **97**, 4106–4111.

Aubry, K.B., Senger, C.M. & Crawford, R.L. (1987) Discovery of Larch Mountain salamanders *Plethodon larselli* in the central cascade range of Washington. *Biological Conservation*, **42**, 147–152.

Baselga, A., Lobo, J.M., Svenning, J.-C. & Araújo, M.B. (2012) Global patterns in the shape of species geographical ranges reveal range determinants. *Journal of Biogeography*, **39**, 760–771.

Brodie, E.D., Jr (1970) Western salamanders of the genus *Plethodon*: systematics and geographic variation. *Herpetologica*, **26**, 468–516.

Brunsfeld, S., Sullivan, J., Soltis, D. & Soltis, P. (2001) Comparative phylogeography of northwestern North America: a synthesis. *Special publication-British Ecological Society*, **14**, 319–340.

Buckley, L.B. & Jetz, W. (2007) Environmental and historical constraints on global patterns of amphibian richness. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 1167–1173.

Cahill, A.E., Aiello-Lammens, M.E., Fisher-Reid, M.C., Hua, X., Karanewsky, C.J., Ryu, H.Y., Sbeglia, G.C., Spagnolo, F., Waldron, J.B. & Wiens, J.J. (2014) Causes of warm-edge range limits: systematic review, proximate factors and implication for climate change. *Journal of Biogeography*, **41**, 429–442.

Carstens, B.C., Stevenson, A.L., Degenhardt, J.D. & Sullivan, J. (2004) Testing nested phylogenetic and phylogeographic hypotheses in the *Plethodon vandykei* species group. *Systematic Biology*, **53**, 781–792.

Corkran, C.C. & Thoms, C. (2006) *Amphibians of Oregon, Washington, and British Columbia*. New York, NY Lone Pine Publishing. p. 1–176.

Csilléry, K., Francois, O. & Blum, M.G.B. (2011) *abc: an R package for approximate Bayesian computation (ABC)*. Available at: http://arXiv:1106.2793 [q-bio.PE] Last accessed: 22 June 2016.

Cwynar, L.C. & MacDonald, G.M. (1987) Geographical variation of Lodgepole pine in relation to population history. *The American Naturalist*, **129**, 463–469.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, **9**, 772.

Dawson, M.N. (2012) Parallel phyogeogrpahic structure in ecologically similar sympatric sister taxa. *Molecular Ecology*, **21**, 987–1004.

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

Dumas, P.C. (1956) The ecological relations of sympatry in *Plethodon dunni* and *Plethodon vehiculum*. *Ecology*, **37**, 484–495.

ESRI (2011) *ArcGIS Desktop: Release 10*. Environmental Systems Research Institute, Redlands, CA.

Feder, M.E. (1983) Integrating the ecology and physiology of plethodontid salamanders. *Herpetologica*, **39**, 291–310.

Flot, J.-F. (2010) SeqPHASE: a web tool for interconverting PHASE input/output files and FASTA sequence alignments. *Molecular Ecology Resources*, **10**, 162–166.

Gaston, K.J. (2009) Geographic range limits: achieving synthesis. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 1395–1406.

Geneious version 8.0.4. (2014) *Created by Biomatters development team*. Available at: http://www.geneious.com/.

Hairston, N.G., Nishikawa, K.C. & Stenhouse, S.L. (1987) The evolution of competing species of terrestrial salamanders: niche partitioning or interference? *Evolutionary Ecology*, **1**, 247–262.

Hanski, I., Breuker, C.J., Schöps, K., Setchfield, R. & Nieminen, M. (2002) Population history and life history influence the migration rate of Glanville fritillary butterflies. *Oikos*, **98**, 87–97.

Herrick, J. & Sclavi, B. (2013) *Lineage specific reductions in genome size in salamanders are associated with increased rates of mutation*. Available at: http://arXiv:1308.0798 [q-bio.GN] Last accessed: 22 June 2016.

Hillman, S.S., Withers, P.C., Drewes, R.C. & Hillyard, S.D. (2009) *Ecological and environmental physiology of amphibians*. Oxford University Press, New York, NY.

Hudson, R.R. (2002) Generating samples under a Wright-Fisher neutral model of genetic variation. *Bioinformatics*, **18**, 337–338.

Huelsenbeck, J.P., Andolfatto, P. & Huelsenbeck, E.T. (2011) Structurama: Bayesian inference of population structure. *Evolutionary Bioinformatics Online*, **7**, 55.

Kozak, K.H., Mendyk, R.W. & Wiens, J.J. (2009) Can parallel diversification occur in sympatry? Repeated patterns of body-size evolution in coexisting clades of North American salamanders. *Evolution*, **63**, 1769–1784.

Kuchta, S.R. & Tan, A.M. (2005) Isolation by distance and post-glacial range expansion in the rough-skinned newt, *Taricha granulosa*. *Molecular Ecology*, **14**, 225–244.

Lindström, T., Brown, G.P., Sisson, S.A., Phillips, B.L. & Shine, R. (2013) Rapid shifts in dispersal behavior on an expanding range edge. *Proceedings of the National Academy of Sciences USA*, **110**, 13456.

Mathews, R.W. & Heusser, L.E. (1981) A 12,000 year palynological record of temperature and precipitation trends in southwestern British Columbia. *Canadian Journal of Botany*, **59**, 707–710.

Merow, C., Smith, M.J. & Silander, J.A., Jr (2013) A practical guide to MaxEnt for modelling species' distributions: what it does and why inputs and settings matter. *Ecography*, **36**, 1058–1069.

Mitchell, M.A. (2009) Anesthetic considerations for Amphibians. *Journal of Exotic Pet Medicine*, **18**, 40–49.

Mueller, R.L., Macey, J.R., Jaekel, M., Wake, D.B. & Boore, J.L. (2004) Morphological homoplasy, life history evolution, and historical biogeography of plethodontid salamanders inferred from complete mitochondrial genomes. *Proceedings of the National Academy of Sciences USA*, **101**, 13820–13825.

Olalla-Tárraga, M.Á., Bini, L.M., Diniz-Filho, J.A. & Rodríguez, M.Á. (2010) Cross-species and assemblage-based approaches to Bergmann's rule and the biogeography of body size in *Plethodon* salamanders of eastern North America. *Ecography*, **33**, 362–368.

Ovaska, K. (1988) Spacing and movements of the salamander *Plethodon vehiculum*. *Herpetologica*, **44**, 377–386.

Pelletier, T.A. & Carstens, B.C. (2014) Model choice in phylogeography using a large set of models. *Molecular Ecology*, **23**, 3028–3043.

Pelletier, T.A., Duffield, D.A. & DeGrauw, E.D. (2011) Rangewide phylogeography of the western Red-backed salamander (*Plethodon vehiculum*). *Northwestern Naturalist*, **92**, 200–2010.

Pelletier, T.A., Crisafulli, C.M., Wagner, R.S., Zellmer, A.J. & Carstens, B.C. (2015) Historical species distribution models predict species limits in western *Plethodon* salamanders. *Systematic Biology*, **64**, 909–925.

Peterman, W.E. (2013) *Factors affecting abundance, physiology, and fine-scale genetic differentiation of the western slimy salamander (Plethodon albagula)*. University of Missouri, Columbia, MO.

Phillips, B.L., Brown, G.P., Webb, J.K. & Shine, R. (2006a) Invasion and the evolution of speed in toads. *Nature*, **439**, 803.

Phillips, S.J., Anderson, R.P. & Schapire, R.E. (2006b) Maximum entropy modelling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.

Pielou, E. (1991) *After the ice age: the return of glaciated life to North America*. The University of Chicago Press, Chicago, IL.

R Core Team (2014) *R: A language and environment for statistical computing*. R foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org Last accessed: 22 June 2016.

Rambaut, A. & Drummond, A.J. (2009) *Tracer v1.5 MCMC trace analysis tool*. Available at: http://beast.bio.ed.ac.uk/Tracer.

Ramos-Onsins, S.E. & Rozas, J. (2002) Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, **19**, 2092–2100.

Rapacciuolo, G., Maher, S.P., Schneider, A.C., Hamond, T.T., Jabis, M.D., Walsh, R.E., Iknayan, K.J., Walden, G.K., Oldfather, M.F., Ackerly, D.D. & Belssinger, S.R. (2014) Beyond a warming fingerprint: individualistic biogeographic responses to heterogeneous climate change in California. *Global Change Biology*, **20**, 2841–2855.

Robinson, M., Dowsett, H.J. & Chandler, M.A. (2008) Pliocene role in assessing future climate impacts. *Eos, Transactions American Geophysical Union*, **89**, 501–502.

Rosenberg, N.A. (2004) Distruct: a program for the graphical display of population structure. *Molecular Ecology Notes*, **4**, 137–138.

Royle, A.J., Chandler, R.B., Yackulic, C. & Nichols, J.D. (2012) Likelihood analysis of species occurrence probability from presence-only data for modelling species distributions. *Methods in Ecology and Evolution*, **3**, 545–554.

Rozas, J., Sánchez-Delbarrio, J.C., Messeguer, X. & Rozas, R. (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496–2497.

Shafer, A.B., Cullingham, C.I., Côté, S.D. & Coltman, D.W. (2010) Of glaciers and refugia: a decade of study sheds new light on the phylogeography of northwestern North America. *Molecular Ecology*, **19**, 4589–4621.

Shine, R., Brown, G.P. & Phillips, B.L. (2011) An evolutionary process that assembles phenotypes through space rather than through time. *Proceedings of the National Academy of Sciences USA*, **108**, 5708–5711.

Simmons, A.D. & Thomas, C.D. (2004) Changes in dispersal during species' range expansions. *The American Naturalist*, **164**, 378–395.

Smith, A.M. & Green, D.M. (2005) Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography*, **28**, 110–128.

Steele, C.A. & Storfer, A. (2006) Coalescent-based hypothesis testing supports multiple Pleistocene refugia in the Pacific

Northwest for the Pacific giant salamander (*Dicamptodon tenebrosus*). *Molecular Ecology*, **15**, 2477–2487.

Steele, C.A. & Storfer, A. (2007) Phylogeographic incongruence of codistributed amphibian species based on small differences in geographic distribution. *Molecular Phylogenetics and Evolution*, **43**, 468–479.

Stephens, M., Smith, N.J. & Donnelly, P. (2001) A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics*, **68**, 978–989.

Stewart, J.R., Lister, A.M., Barnes, I. & Dalén, L. (2010) Refugia revisted: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 661–671.

Swenson, N.G. & Howard, D.J. (2005) Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. *The American Naturalist*, **166**, 581–591.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., Ferreira de Siqueira, M., Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Townsend Peterson, A., Phillips, O.L. & Williams, S.E. (2004) Extinction risk from climate change. *Nature*, **427**, 145–148.

Thorson, T.B. (1955) The relationship of water economy to terrestrialism in amphibians. *Ecology*, **36**, 100–116.

Thuiller, W., Georges, D. & Engler, R. (2014) *biomod2: Ensemble platform for species distribution modelling*. R package version 2013. https://pbil.univ-lyon1.fr/CRAN/index.html. Last accessed: 22 June 2016.

Wagner, R.S., Miller, M.P., Crisafulli, C.M. & Haig, S.M. (2005) Geographic variation, genetic structure, and conservation unit designation in the Larch Mountain salamander (*Plethodon larselli*). *Canadian Journal of Zoology*, **83**, 396–406.

Webb III, T. & Bartlein, P.J. (1992) Global changes during the last 3 million years: climatic controls and biotic responses. *Annual Review of Ecology and Systematics*, **23**, 141–173.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Additional information for genetic data.
Appendix S2 Additional species distributional modelling information.
Appendix S3 Additional information for morphological data.

BIOSKETCH

Tara Pelletier is a postdoctoral researcher at The Ohio State University. Her research investigates the evolutionary processes shaping current species distributional patterns. **Bryan Carstens** is an Associate Professor at The Ohio State University. His research investigates evolutionary processes acting at the population and species level, using a combination of empirical, bioinformatic and theoretical approaches.

Author contributions: T.A.P. conducted the molecular labwork and fieldwork, participated in design of the study, performed statistical analysis and drafted the manuscript. B.C.C. participated in the design of the study and supervised the research. Both authors read and approved the final manuscript.

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