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RESEARCH ARTICLE

# The influence of history, geography and environment on patterns of diversification in the western terrestrial garter snake

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## Abstract

**Aim:** A central aim of biogeography is to understand how biodiversity is generated and maintained across landscapes. Here, we establish phylogenetic and population genetic patterns in a widespread reptile to quantify the influence of historical biogeography and current environmental variation on patterns of genetic diversity.

**Location:** Western North America.

**Taxon:** Western terrestrial garter snake, *Thamnophis elegans*.

**Methods:** We used double-digest RADseq to estimate phylogenetic relationships and characterize population genetic structure across the three widespread subspecies of *T. elegans*: *T. e. vagrans* (wandering garter snake), *T. e. elegans* (mountain garter snake) and *T. e. terrestris* (coast garter snake). We assessed patterns of dispersal and vicariance across biogeographic regions using ancestral area reconstruction (AAR) and deviations from isolation-by-distance across the landscape using estimated effective migration surfaces (EEMS). We identified environmental variables potentially shaping local adaptation in regional lineages using genetic-environment association (GEA) analyses.

**Results:** We recovered three well-differentiated genetic groups that correspond to the three subspecies. AAR analyses inferred the eastern Cascade Range as the ancestral area, with dispersal to both the east and west across western North America. Populations of *T. e. elegans* displayed a latitudinal gradient in genetic variation across the Sierra Nevada and northern California, while populations of *T. e. terrestris* show discrete genetic breaks consistent with well-known biogeographic barriers. Lastly, GEA analyses identified allele frequency shifts at loci associated with a common set of environmental variables in both *T. e. elegans* and *T. e. terrestris*.

**Main Conclusion:** *T. elegans* is composed of distinct evolutionary lineages, each with its own geographic range and history of diversification. *T. e. elegans* and *T. e. terrestris* show unique patterns of diversification as populations dispersed from east to west and while adapting to the new environments they colonized. Historical events, landscape features and environmental variation have all contributed to patterns of differentiation in *T. elegans*.

**KEYWORDS**

ancestral area reconstruction, California Floristic Province, ddRADseq, dispersal, genotype-environment association, phylogeography, population genetics, *Thamnophis elegans*, vicariance, Western North America

## 1 | INTRODUCTION

Western North America, and the California Floristic Province (CFP) in particular, has been a region of considerable interest for understanding the forces that influence patterns of diversity (Brunsfeld et al., 2001; Gottscho et al., 2014; Myers et al., 2000; Schierenbeck, 2014; Stebbins, 1949). This region is home to complex topography and climates, which have given rise to diverse environmental gradients and ecological communities. In addition, the region has a dynamic geological history that includes marine embayments, orogeny, volcanic activity and repeated glacial cycles (Dupré et al., 1991; Hall, 2002). Indeed, phylogeographic work in this region reveals striking patterns of congruence in a variety of terrestrial species, including invertebrates (Bryson et al., 2013; Chatzimanolis & Caterino, 2007; Emata & Hedin, 2016; Peterson et al., 2017; Schoville & Roderick, 2010; Starrett et al., 2018), amphibians (Jockusch et al., 2020; Kuchta et al., 2009; Lind et al., 2011; Moritz et al., 1992; Reilly et al., 2015; Reilly & Wake, 2015), mammals (Conroy & Neuwald, 2008; Matocq, 2002; Rubidge et al., 2014) and reptiles (Bouzid et al., 2021; Feldman & Spicer, 2006; Lavin et al., 2018; Leaché et al., 2009; Myers et al., 2013; Spinks et al., 2010). Common features proposed to have shaped concordant biogeographic patterns include the Monterey embayment and historic Pajaro River (i.e., Monterey Bay), Sacramento–San Joaquin River Delta (i.e., San Francisco Bay), uplift of the Transverse Ranges, and deep river canyons of the Sierra Nevada which were subjected to glaciation (especially the Kern and American Rivers; Figure 1). In addition, steep environmental and ecological gradients separate the biota of the Central Valley and Mojave and Great Basin Deserts from that of the rest of the CFP (Schoenherr, 2017).

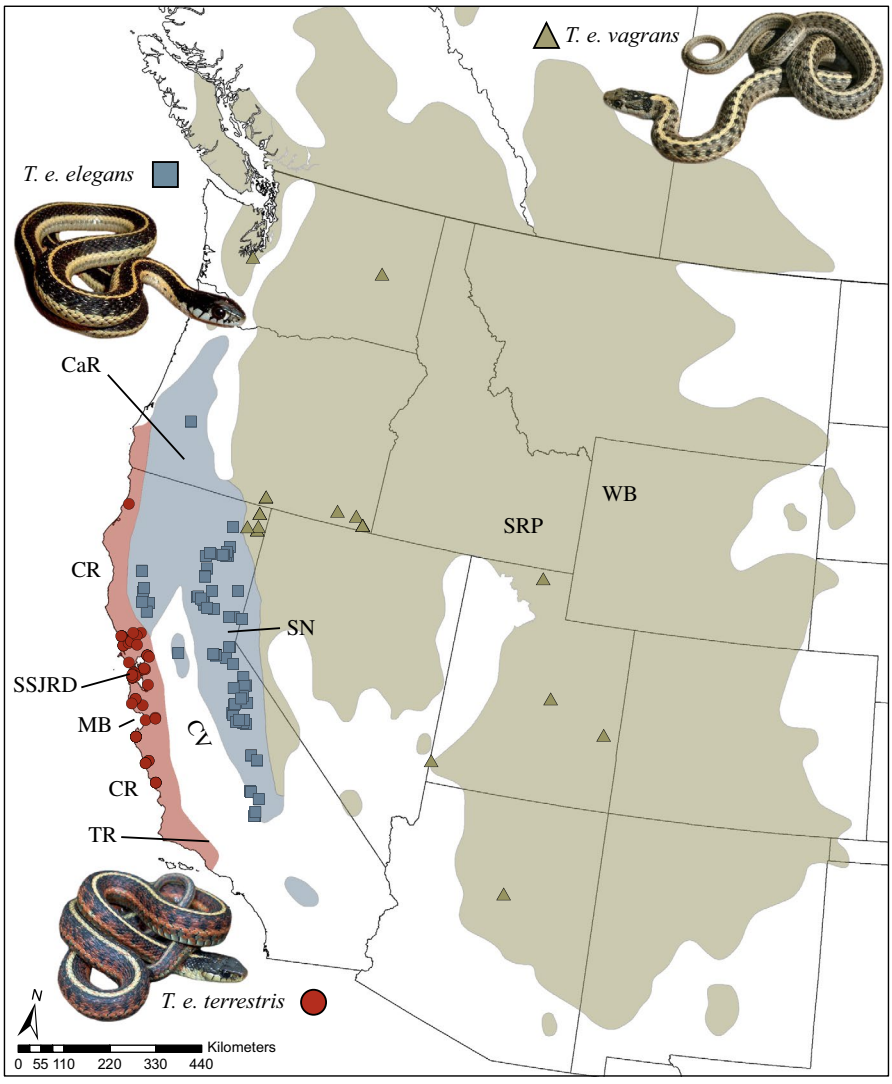


**FIGURE 1** Map of the geographic distribution of the western terrestrial garter snake, *Thamnophis elegans* (after Rossman et al., 1996; Stebbins, 2003), and sample localities used in population genetic analyses. The ranges and samples of the three subspecies colour coded: mountain garter snake *T. e. elegans*—blue square; coast garter snake *T. e. terrestris*—red, circle; wandering garter snake *T. e. vagrans*—tan, triangle. Samples are labelled based on genetic clusters from principal component analysis (PCA). Common biogeographic barriers are labelled (CaR, Cascade Range; CR, Coast Range; CV, Central Valley; MB, Monterey Bay; SN, Sierra Nevada; SSJRD, Sacramento–San Joaquin River Delta; TR, Transverse Range; SRP, Snake River Plain; WB, Wyoming Basin)

Species with broad ranges are particularly well-suited for detecting biogeographic patterns because their distributions often span multiple geographic barriers, ecological communities and environments. The western terrestrial garter snake (*Thamnophis elegans*: Natricinae: Colubridae) encompasses much of western North America, extending from western British Columbia eastward to Manitoba and south through California on the west and to central New Mexico on the east (Rossman et al., 1996; Stebbins, 2003; Figure 1). *T. elegans* also occupies a broad array of ecoregions, from coastal rainforest in the Pacific Northwest, to alpine communities in the high Sierra Nevada, to shrublands of the Columbia Plateau and high deserts of the Great Basin (Rossman et al., 1996; Stebbins, 2003). Not surprisingly, *T. elegans* displays substantial variation across its range, including differences in behaviour, morphology, physiology and life history across populations (Arnold, 1977; Britt et al., 2006; Bronikowski & Arnold, 1999; Drummond & Burghardt, 1983; Fitch, 1983; Fox, 1951; Rossman et al., 1996; Stebbins, 2003). Some of this geographic variation is encompassed by three currently recognized subspecies: *T. e. elegans* (mountain garter snake), *T. e. terrestris* (coast garter snake) and *T. e. vagrans* (wandering garter snake; Figure 1). Several other subspecies have been erected to describe local or isolated variants, such as *T. e. arizonae* (Arizona garter snake), *T. e. hueyi* (San Pedro Mártir garter snake) and *T. e. vascotanneri* (Upper Basin garter snake); however, morphological and genetic evidence cast doubt on the validity of these subspecies (Bronikowski & Arnold, 2001; Fitch, 1983; Hammerson, 1999; Johnson, 1947).

*T. elegans* is also one of the most well-studied snakes in North America, both in the field and lab, in part because of its extensive phenotypic variation, broad range, often local abundance, and ease of care and captive breeding (e.g. Bronikowski & Arnold, 1999; Kelley et al., 1997; Kephart & Arnold, 1982). For example, *T. elegans* has been a useful model for addressing questions of feeding ecology (Arnold, 1977; Britt & Bennett, 2008; Britt et al., 2006; Drummond & Burghardt, 1983; Gregory et al., 1980; Kephart & Arnold, 1982) and for analysing the genetic basis and lability of traits associated with feeding (Arnold, 1981a, 1981b, 1988; Arnold & Phillips, 1999; Manier et al., 2007). Some populations are even known to crudely constrict prey (de Queiroz & Groen, 2001; Gregory et al., 1980) or contain simple venom (Kardong & Luchtel, 1986), providing a model of the early stages of the evolution of these more complex forms of prey acquisition (Chiszar et al., 2013; Hill & Mackessy, 2000). The species has also been used to understand how aspects of ecophysiology influence behaviour and performance (Garland & Arnold, 1983; Huey et al., 1989; Stevenson et al., 1985), and the role the endocrine system plays in mediating physiology and life history (Bronikowski, 2000; Bronikowski & Arnold, 1999; Gangloff et al., 2015; Robert & Bronikowski, 2010; Robert et al., 2009). Despite the remarkable biology and decades long interest in *T. elegans*, we still lack a clear understanding of the regional lineages and their relationships, as well as how genetic variation underlies the phenotypic variation seen across populations.

Previous work on the phylogenetic relationships among *T. elegans* lineages suggested broad geographic regionality among clades of *T. e. elegans*, *T. e. terrestris* and *T. e. vagrans* (Bronikowski & Arnold, 2001; de Queiroz & Lawson, 1994). However, all three subspecies were paraphyletic with respect to each other, and the regional clusters lacked obvious geographic or ecological concordance, as well as any fine-scale spatial structure. These studies were based on modest numbers of traditional molecular markers (mtDNA and allozymes) and may have simply lacked sufficient variation to characterize differentiation across the geographical and environmental gradients inhabited by *T. elegans*. More recently, genome-wide high-throughput sequencing data have been leveraged to resolve phylogenetic relationships across recently diverged lineages as well as to uncover fine-scale structure of populations across spatial and environmental gradients (e.g. Dupuis et al., 2020; McCartney-Melstad et al., 2018; Nunziata et al., 2017; Parham et al., 2020). Here, we use a reduced representation double-digest RADseq (ddRADseq) approach to improve our understanding of the biogeographic factors associated with subspecific differentiation, as well as the geographic and environmental factors shaping spatial genetic structure across *T. elegans*.

We first sought to clarify range-wide patterns of differentiation by using phylogenetic and population genetic approaches. We determine whether the three *T. elegans* subspecies form monophyletic clades and/or cohesive genetic clusters. In addition, we sampled near two contact zones, one between *T. e. elegans* and *T. e. vagrans* along a well-known suture zone in northeastern California and the second near the southern end of the contact zone between *T. e. elegans* and *T. e. terrestris* in the Coast Ranges. These samples allow us to examine the integrity of subspecies in proximity (at least in two locations). Next, we identify the mechanisms associated with diversification in *T. elegans* by reconstructing the history of vicariance and colonization events across ecoregions. Third, we investigated the pattern and extent of spatial genetic structure across populations of *T. e. elegans* and *T. e. terrestris* occupying the CFP. Because all members of *Thamnophis* are tightly associated with mesic habitats, we expect movement within and dispersal between major watersheds through riparian corridors. However, long-term disruption to riparian conduits of dispersal should result in vicariant evolution, leading to patterns of genetic subdivision within *T. elegans*. We therefore consider how previously proposed barriers to migration may explain contemporary patterns of spatial genetic structure, particularly the Monterey embayment, the vast estuarine Sacramento–San Joaquin River Delta and the Central Valley. Lastly, we explored whether local adaptation to environmental variation might also contribute to patterns of differentiation. Our results indicate a hierarchical pattern of divergence among and within subspecies that has been jointly influenced by history, biogeographic barriers and environmental variation.

## 2 | MATERIALS AND METHODS

### 2.1 | Specimen collection

We obtained tissues from 237 *T. elegans*, collected throughout western North America from our own field efforts ($n$ = 111) and from the tissue collections ($n$ = 126) of the California Academy

of Sciences (CAS), Museum of Vertebrate Zoology (MVZ), and University of Nevada, Reno, Museum of Natural History (UNR). We sampled snakes from across the ranges of the three widespread subspecies (*T. e. elegans*, *n* = 104; *T. e. terrestris*, *n* = 112; and *T. e. vagrans*, *n* = 21), focusing on acquiring samples that spanned ecoregions and well-known biogeographic barriers (e.g., Sacramento–San Joaquin River Delta and Central Valley). We preserved our field-collected specimens and deposited them as vouchers in the CAS and UNR herpetology collections. We also included representatives of other closely related *Thamnophis* species (*T. atratus*, *T. brachystoma*, *T. butleri* and *T. radix*) for phylogenetic analyses (de Queiroz & Lawson, 1994; de Queiroz et al., 2002; Guo et al., 2012; McVay et al., 2015).

## 2.2 | ddRADseq, quality cleaning and demultiplexing

We extracted DNA from liver, muscle or tail tips using Qiagen Dneasy Blood & Tissue kits (Qiagen, Inc.) and employed a reduced representation double-digest RADseq approach (ddRADseq; Parchman et al., 2012; Peterson et al., 2012) to characterize genomic variation. We digested genomic DNA with *Eco*RI and *Mse*I restriction enzymes and then ligated modified Illumina adaptors containing barcodes that were 8, 9 or 10 bp in length to the *Eco*RI restriction sites using T4 ligase. Standard Illumina adaptors were ligated to the *Mse*I sites. Adaptor ligated fragments were PCR-amplified using Illumina primers and a high-fidelity proofreading polymerase (iProof polymerase, BioRAD), then pooled into a single library for sequencing. We size-selected fragments ranging from 350 to 450 bases using a PippinPrep device (Sage Science, Inc.), performed quality screening on a BioAnalyzer (Agilent, Inc.) and sequenced the library on three Illumina HiSeq 2500 lanes at the University of Wisconsin Madison Biotechnology Center. We first used `bowtie2` v2.2.5 (Langmead & Salzberg, 2012) and a series of scripts designed for filtering Illumina data (https://github.com/ncgr/tapioca) to discard sequences representing common contaminants (i.e., PhiX, *Escherichia coli* or Illumina oligos). We demultiplexed reads by individual using a Perl script that additionally corrected barcodes with one or two base errors. Fastq files containing data for each individual are publicly available (DRYAD: https://doi.org/10.5061/dryad.wh70rxwks).

## 2.3 | Phylogenetic analyses and ancestral area reconstruction

To examine geographic regionality among *T. elegans* lineages, we estimated phylogenetic relationships among a subset of individuals (*n* = 92) with relatively high sequencing depth and representing the geographic range of each subspecies. We conducted independent assembly and variant calling for phylogenetic analyses using `ipyrad` v0.9.54 (Eaton & Overcast, 2020). Based on levels of genetic divergence among subspecies, we were moderately conservative with our parametrization of `ipyrad`, with most parameters set to default. First, we employed a de novo clustering step using `VSEARCH` (Rognes et al., 2016) with a clustering similarity threshold of 0.85. To account for uneven sequence depth across loci, statistical base calling was set to five, and majority-rule base calling was set to four. We set the maximum shared heterozygous sites per locus to 0.08. We tested the effect of missing data on our phylogenetic estimate by excluding loci if they had more than 20% and 30% missing data. Because this parameter didn't affect downstream phylogenetic analyses, we excluded the former dataset from further analyses.

We estimated phylogenetic relationships among *T. elegans* subspecies, including other *Thamnophis* species as outgroups, using a maximum likelihood (ML) approach with `RAxML` v8.2.12 (Stamatakis, 2006) and a quartet sampling algorithm under a multispecies coalescent (MSC) model (reviewed in Edwards et al., 2016; Liu et al., 2019) using `tetrad` v0.9.13. Both of these methods are well suited for large single nucleotide polymorphism (SNP) datasets and allowed us to compare topologies under different phylogenetic searches. For the ML analysis, we simultaneously conducted a rapid bootstrapping analysis and best-scoring ML tree search utilizing the GTRCAT model of sequence evolution and the multiple alignments of concatenated loci from `ipyrad`. We determined the number of sufficient bootstrap replicates by implementing a posteriori bootstopping analysis that uses the majority-rule consensus tree criterion to determine convergence of bootstrap analyses with the `autoMRE` option. Bootstopping analysis determined 400 replicates to be sufficient for the inferred `RAxML` phylogeny. For `tetrad` analysis, we evaluated all possible quartets under a MSC model using unlinked SNPs from `ipyrad`. These quartets were then joined using the `wQMC` algorithm, and nodal support was assessed with 400 non-parametric bootstrap replicates.

We estimated ancestral area ranges (AARs) in `RASP` v4.0 (Reconstruct Ancestral State in Phylogenies; Yu et al., 2015) using the Bayesian binary Markov chain Monte Carlo (BBM) approach to examine the dispersal scenario of *T. elegans* across western North America. We conduced AAR using both phylogenetic estimates (ML and MSC) because their topologies differed slightly (see Section 3). We pruned outgroup taxa from each phylogeny using the R package `APE` v5.2 (Paradis & Schliep, 2019). We then assigned terminal taxa to areas they occupy, using predefined biogeographic regions defined at two broad scales: Level I ecoregions (biomes); Level III ecoregions (Olson et al., 2001; Omernik & Griffith, 2014) with California ecological subsections (Goudey & Smith, 1994). We then conducted two separate AAR analyses using these distinct biogeographic scales to define our areas. We based analyses on these regions because they describe broad abiotic (e.g., climate, physiography and soils, hydrology) and biotic (e.g., vegetation) variation and have been useful for ecological, conservation, and management research (Di Marco et al., 2018; Dinerstein et al., 2017; Olson & Dinerstein, 2002; Olson et al., 2001).

We ran all AAR analyses with 10 MCMC chains for $1 \times 10^7$ steps sampling every 1000 steps with parameters set to default (temperature = 0.1; model = JC; and among-site rate variation = equal),

allowed for three ancestral areas to incorporate uncertainty into our analysis, and discarded the first 2500 steps as burn-in.

## 2.4 | Population genetic analyses

### 2.4.1 | Assembly and variant calling

We aligned reads, identified, called and filtered variants using an alternative workflow for population genetic analyses. Due to pronounced genetic divergence among *T. e. vagrans* and the other subspecies (see Section 3), we conducted analyses separately for two datasets: one including the three subspecies, and a second that included only *T. e. elegans* and *T. e. terrestris*. Reads were first aligned to the *T. sirtalis* reference genome (NCBI assembly accession: GCF_001077635.1; Castoe et al., 2011) using the `aln` and `samse` algorithms in `bwa` v0.7.17 (Li & Durbin, 2009) with maximum edit distance set to 4. We then identified SNPs and generated genotype likelihoods using `samtools` v1.9 and `bcftools` v1.9 (Li et al., 2009). We used `mpileup` in `samtools` to identify SNPs by setting maximum depth to 100, adjusted mapping quality to 50, minimum base quality to 20, and minimum mapping quality to 20. Using `vcftools` v0.1.16 (Danecek et al., 2011), we retained only bi-allelic SNPs with a minor allele frequency >0.05 and variants where 60% of individuals had at least one read at the locus. We excluded individuals with >50% missing data and loci with mean coverage depth >10× to guard against calling genotypes in misassembled paralogous regions. Full details on variant calling and bioinformatic processing are available at DRYAD (https://doi.org/10.5061/dryad.wh70rxwks).

### 2.4.2 | Spatial genetic structure

We characterized genetic differentiation among subspecies and also investigated the influence of geographic and environmental variation on spatial genetic structure within *T. e. elegans* and *T. e. terrestris*. To make full use of the inherently variable coverage data, we simultaneously calculated genotype probabilities and ancestry coefficients (*q*) using a hierarchical Bayesian model (`entropy`; Gompert et al., 2014) that incorporates genotype uncertainty from sequencing error, mapping error and stochastic variability in coverage across loci and individuals. `entropy` estimates ancestry proportions using a model similar to that implemented in `structure` (Falush et al., 2003; Pritchard et al., 2000), and similarly estimates parameters without a priori information regarding sample origin for models representing each of *k* ancestral populations. To speed the mixing and convergence of MCMC chains, we seeded `entropy` with estimated starting values of cluster assignment for all individuals for models representing each value of *k*. To do this, we first ran a linear discriminant analysis (`lda` package in R; Jombart et al., 2010) on the principal components generated from a genotype likelihood covariance matrix. We then used `k-means` clustering to generate cluster assignment probabilities for all individuals. We ran five independent chains for each value of $k = 2–8$ for 100,000 iterations following a 40,000 iteration burn-in. We saved every 10th iteration and used deviance information criterion (DIC) for model comparison. We summarized posterior distributions to obtain ancestry proportion estimates as well as individual genotype probabilities used in additional downstream analyses.

We summarized genetic structure across the entire dataset as well as the subset of *T. e. elegans* and *T. e. terrestris* using both ancestry coefficients estimated in `entropy` and principal component analyses (PCAs) based on genotype probabilities, using the `prcomp` function in R. As metrics of genetic differentiation among subspecies, we used custom R scripts to calculate pairwise estimates of Hudson's $F_{ST}$ (Hudson et al., 1992) and Nei's genetic distance (Nei's *D*; Nei, 1972). These metrics were also calculated among counties where at least three *T. e. elegans* were sampled and among the geographic clusters of *T. e. terrestris* identified by PCA (see Section 3).

As metrics of genetic diversity for each *T. elegans* subspecies, we calculated nucleotide diversity ($\theta_\pi$) and the Watterson estimator ($\theta_w$) using methods in the program `ANGSD` v0.923 that incorporate genotype uncertainty (Korneliussen et al., 2013, 2014). Folded site allele frequency likelihoods were first estimated with `realsfs` (Fumagalli et al., 2013). Then, we calculated genotype likelihoods using the setting `GL 1`, which generated the folded site frequency spectrum (SFS) likelihood. We used the function `doThetas 1` to estimate $\theta_\pi$ and $\theta_w$ parameters separately at each locus across the reference genome and then averaged each value per population.

### 2.4.3 | Spatial connectivity

In order to consider geography-associated genetic differentiation and potential variation in gene flow across the landscape, we used estimated effective migration surfaces (`EEMS`; Petkova et al., 2016) for *T. e. elegans* and *T. e. terrestris* separately as well as together in a combined analysis. `EEMS` identifies regions of genetic differentiation that deviate from the expectation of isolation by distance and infers them as potential corridors or barriers to gene flow. It does this by implementing the stepping-stone model across a predefined landscape that is divided into demes, with variable gene flow possible between demes (reviewed in House & Hahn, 2018; Petkova et al., 2016). We first generated an individual level matrix of pairwise Euclidean distances based on genotype probabilities. For our individual analyses of *T. e. elegans* and *T. e. terrestris*, we set the number of demes to 600 in order to capture finer scale patterns of gene flow suggested by the tight clustering in PCAs (see Section 3). To increase proper mixing and convergence among chains for our combined *T. e. elegans* and *T. e. terrestris* analysis, we set the number of demes to 400 and expanded the area of analysis across the CFP. For both analyses, we conducted preliminary runs to optimize model parameters to ensure the acceptance of proposal rates roughly ranged from 20% to 30%. Once suitable model parameters were selected, we ran and combined three independent chains for $3 \times 10^7$ iterations with a burn-in of $3 \times 10^6$ and sampling every 5000 iterations. We then checked for convergence among chains and visualized results using the R package `reemsplotsZ`.

### 2.4.4 | Genetic-environment association

We conducted genetic-environment association (GEA) analyses using partial redundancy analysis (pRDA) to identify environmental variables that covary with genetic differentiation among populations as well as candidate loci exhibiting allele frequency shifts consistent with local adaptation to the environmental variables (Forester et al., 2018). Unlike traditional genome scans that do not interrogate environmental variation (e.g., $F_{ST}$ outlier scans), GEA analyses incorporate spatial variation in allele frequencies and environmental variables. As a result, GEA analyses have reduced incidence of false positives arising from neutral processes and are relatively effective at detecting more dispersed multilocus selection (Lotterhos & Whitlock, 2015; Rellstab et al., 2015). While diverse GEA methods exist, multiple recent studies have illustrated that pRDA performs favourably as it is less prone to false negatives and positives and is reasonably robust to demographic and historical variation (Capblancq et al., 2018; Forester et al., 2018). We used pRDA to assess the contribution of specific environmental variables to variation in local adaptation across space and to identify outlier loci potentially linked to genomic regions responding to selection. We downloaded all 19 of the bioclimatic variables at a of resolution of 2.5 (minutes of a degree) for each individual specimen available from WorldClim (Hijmans et al., 2005) using `dismo` v1.1-4 (Hijmans, Philips, et al., 2019) and `raster` v3.1-5 (Hijmans, van Etten, et al., 2019) packages in R. Elevation data was acquired from `elevatr` v0.2.0 package (Hijmans & Shah, 2017). To reduce multicollinearity, we removed environmental variables from each analysis that had a Pearson's correlation of >0.60 (Dormann et al., 2013) and variance inflation factor (VIF) values exceeding 10.

We performed pRDA using the `rda` function in the `vegan` v2.4-5 R package (Oksanen et al., 2015). To account for spatial genetic structure in this analysis, both *T. e. elegans* and *T. e. terrestris* models were conditioned on PC1. The $r^2$ value of each model was used to evaluate proportion of variation explained by the constrained ordinations, and the significance of models and RDA axes were tested with an analysis of variance (ANOVA) and permutation ($n$ = 999). We used loadings of SNPs from the first two constrained ordination axes to identify outlier loci that were 3 standard deviations (two-tailed $p$ = 0.0027) from the mean loading value. We then checked for duplicate candidate loci that were associated with more than one RDA axis and calculated correlations of candidate SNPs to predictor variables.

## 3 | RESULTS

### 3.1 | Phylogenetic estimate and ancestral area reconstruction

After assembly and filtering in `ipyrad`, we retained 16,382 loci spanning 1,427,787 characters, 26.03% missing sites, and 37,321 parsimony informative sites across the subset of 92 individuals. ML and MSC phylogenetic estimates recovered mostly congruent topologies with the same overall relationships among subspecies, with only a few discordances of certain tips (Figure 2a and b). *T. elegans* was a monophyletic clade in both analyses with strong bootstrap support (ML = 100; MSC = 95). This clade bifurcates into a well-supported *T. e. vagrans* clade (ML = 100; MSC = 100) sister to a clade containing both *T. e. elegans* and *T. e. terrestris* (ML = 100; MSC = 100). Within *T. e. vagrans*, two strongly supported subclades corresponded to individuals from the Pacific Northwest (ML = 100; MSC = 100) and Southwest (ML = 100; MSC = 86). We also note that a single specimen (CAS 206466) identified as a putative intergrade based on location and morphology (dorsal pattern and colouration) between *T. e. vagrans* and *T. e. elegans* (often referred to as *T. e. biscutatus*) was nested within *T. e. vagrans*. The second major *T. elegans* clade depicts a monophyletic *T. e. terrestris* (ML = 100; MSC = 76) nested within a clinal grouping of *T. e. elegans*. The deepest split in the *T. e. elegans* lineage occurs within northern California, and the group is characterized by a stepwise pattern of diversification with a large Sierra Nevada clade. Thus, the more derived *T. e. terrestris* clade was recovered as sister to *T. e. elegans* from western California, rendering *T. e. elegans* paraphyletic. See Appendix S1 in supporting Information for all bootstrap support values from our ML (Figure S1.1) and MSC (Figure S1.2) estimates.

AAR analyses based on both ML and MSC topologies recovered strong geographic concordance among the three subspecies and subclades of *T. elegans* associated with geographic regions (Figure 3 and Figure S1.3). The analysis based on ecoregion III recovered movement across the CFP, and inferred the Eastern Cascades to be the ancestral area for *T. elegans* (all three subspecies) with multiple dispersal events from this ecoregion (Figure 3). This analysis also suggests a dispersal scenario within *T. e. vagrans* out of the Eastern Cascades and into the northwest and southwest. Within *T. e. elegans*, there were some regional groupings among the Cascades, northern and southern Sierra Nevada, and Northern California Coast Mountain Range, though groupings are non-monophyletic or were characterized by low bootstrap support. Derived populations of *T. e. elegans* along the Inner Coast Range (e.g., sites in Colusa, Lake, Mendocino Counties) are sister to populations of *T. e. terrestris* in Outer Coast Range (e.g., sites in Sonoma, Napa, Marin Counties), suggesting that *T. e. terrestris* descended from snakes that were isolated from those of the Inner Coast Range. In addition, AAR suggests both vicariance and dispersal in the coastal populations of *T. e. terrestris*, with those from the Northern California Coast ecoregion founding populations further south in the Central California Coast ecoregion. Even though there were similar patterns recovered between both our ML and MSC topologies, dispersal scenarios differed. The AAR analysis of our ML estimate recovered dispersal from the Cascades to Northern California Coast Mountain Range, while our MSC estimate suggested dispersal to the Northern California Coast Mountain Range from Northern Sierra Nevada region (Figure 3).

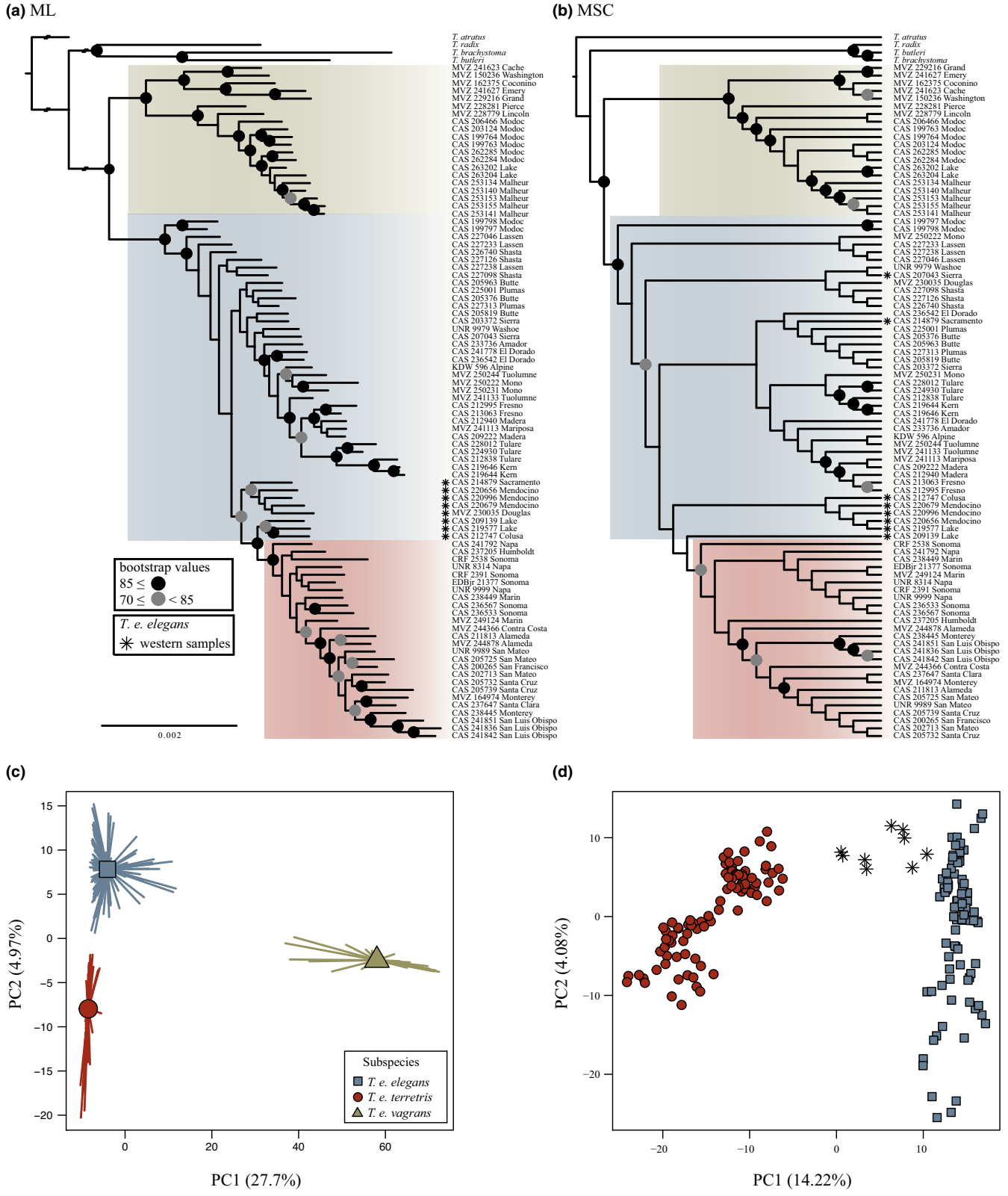


FIGURE 2 Phylogenetic and principal component analyses (PCAs) of *T. elegans*. (a) `RAxML` maximum likelihood (ML) approach and (b) multispecies coalescent (MSC) model employed using `tetrad` recovered largely congruent topologies from a subset of *T. elegans* ($n$ = 92). Black circles represent bootstrap support (bs) values ≥85 and grey labels represent values between 85 and 70. (c) PCA of 8460 single nucleotide polymorphisms (SNPs) of all three subspecies ($n$ = 204) and (d) PCA representing 10,121 SNPs from only *T. e. elegans* and *T. e. terrestris* ($n$ = 187) recovered distinct genetic clustering among subspecies. (c) PCA plot of *T. elegans* subspecies, represented by symbols for the mean PCA score (square: *T. e. elegans*; circle: *T. e. terrestris*; triangle: *T. e. vagrans*) that are linked to individual samples by line segments. Proportion of variation labelled on each axis. Asterisks represent western samples of *T. e. elegans*. Colored shading reflects groups of samples for each subspecies (blue: *T. e. elegans*; red: *T. e. terrestris*; tan: *T. e. vagrans*)

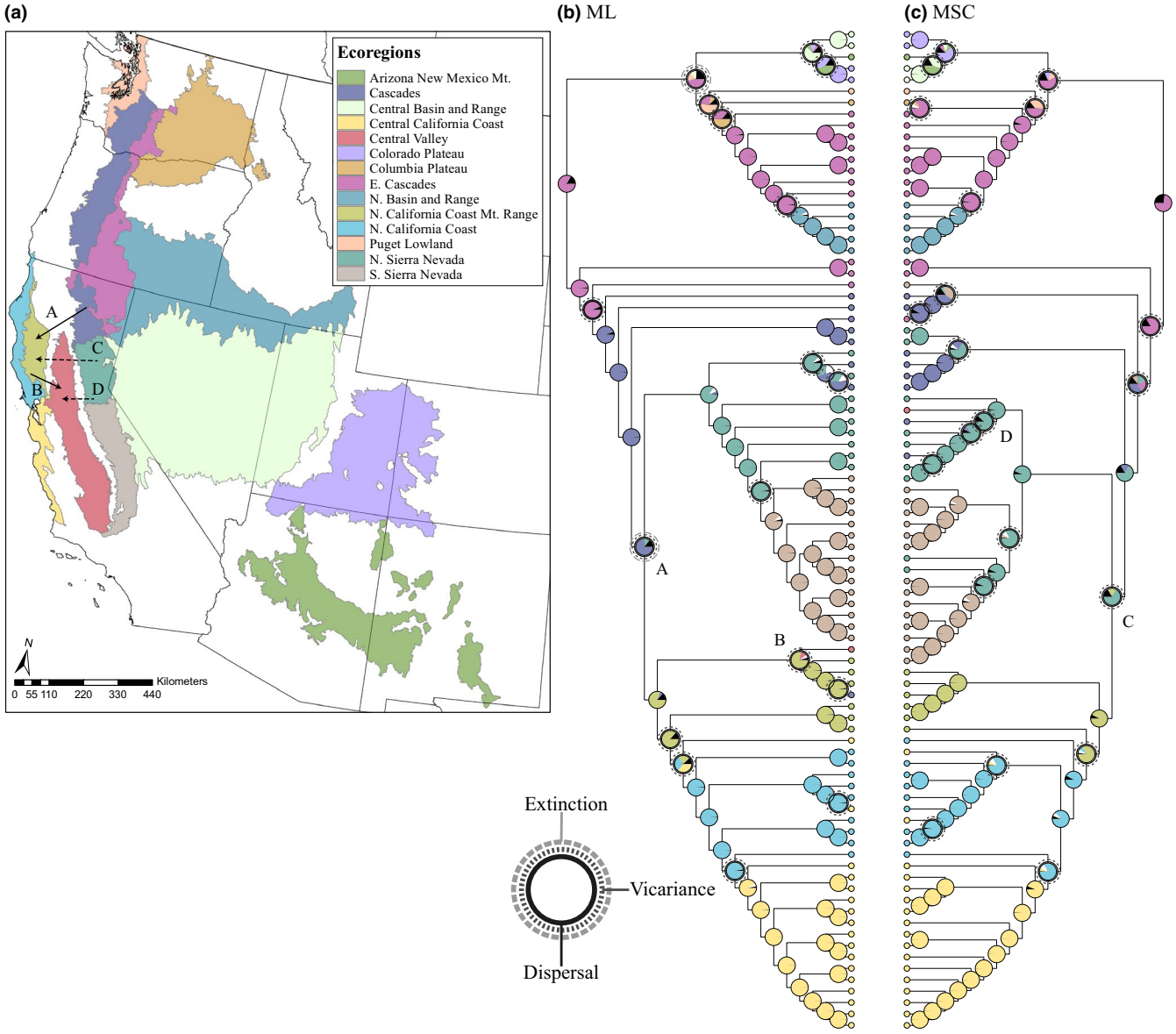


FIGURE 3 Ancestral area reconstruction (AAR) using (a) Level III ecoregions and California ecological subsections on (b) ML and (c) MSC estimates using Bayesian binary Markov chain Monte Carlo (BBM) in RASP (Reconstruct Ancestral State in Phylogenies). AAR recovered strong regionality based on ecoregions among subclades of *T. elegans* subspecies. For simplicity, only probabilities of individual ranges are represented. Circles depict dispersal, extinction or vicariant events recovered from our AAR analyses. Letters depict different dispersal scenarios across northern California recovered from the independent AAR analyses: A and B under ML; C and D under MSC

## 3.2 | Spatial genetic structure

For the full set of individuals used in population genetic analyses, we retained 354,540,830 reads after initial quality screening and demultiplexing with an average of 1,508,684 reads per individual (SD = 638,633). After variant calling and filtering, we retained a dataset of 8460 SNPs across 208 individuals (see Appendix S2; Table S2.1) with a mean coverage depth of 4.89× per individual per locus (SD = 1.81; Table S2.2). For the subset of samples representing *T. e. elegans* and *T. e. terrestris*, we retained a set of 10,121 SNPs across 187 individuals with 4.43× mean coverage depth (SD = 1.57; Table S2.3).

Population genetic analyses recovered distinct genetic clusters of *T. elegans* subspecies in PC space (Figure 2c), revealing differentiation at a finer scale than phylogenetic analyses. PC1 explained a high fraction of variation (27.7%) and clearly differentiated *T. e. vagrans* from the other subspecies, while PC2 explained less variation (4.97%), yet clearly distinguished *T. e. elegans* and *T. e. terrestris*. Differentiation between *T. e. elegans* and *T. e. terrestris* was clearer when analysed independent of *T. e. vagrans* (Figure 2d).

PC analyses also illustrated spatial genetic differentiation across the CFP (Figure 4a) within both *T. e. elegans* (Figure 4b) and *T. e. terrestris* (Figure 4c). *T. e. elegans* exhibited clinal structuring of populations latitudinally, across the Sierra Nevada and northern California

along PC1 (8.29%). In addition, *T. e. elegans* individuals sampled at the southern extent of the Sierra Nevada represented a distinct cluster distinguished along PC2. In contrast, three distinct clusters of *T. e. terrestris* were recovered in PCA (PC1 = 7.79%; PC2 = 3.45%), defined by the Sacramento–San Joaquin River Delta, San Francisco Peninsula and Monterey Bay region. There was a strong relationship between PC score and latitude in both *T. e. elegans* ($r^2$ = 0.824, $p$ < 0.001) and *T. e. terrestris* ($r^2$ = 0.792, $p$ < 0.001).

The $k$ = 2 `entropy` model had the lowest DIC value; however, the $k$ = 5 model also produced biologically meaningful patterns of clustering for both subspecies (Figure 4d). Ancestry coefficient plots depicted patterns of spatial genetic clustering similar to those illustrated with PCA. In addition, evidence of mixed ancestry was recovered between *T. e. elegans* and *T. e. terrestris* in northern California where their ranges intersect (Figure 4d). Ancestry coefficients are reported for $k$ = 2 (Table S2.4) and $k$ = 5 (Table S2.5).

Genetic differentiation was strong between *T. e. vagrans* and the other subspecies (*T. e. elegans*: $F_{ST}$ = 0.2031, Nei's $D$ = 0.0909; *T. e. terrestris*: $F_{ST}$ = 0.2277, $D$ = 0.1139; Table 1), while relatively low between *T. e. elegans* and *T. e. terrestris* ($F_{ST}$ = 0.0377, $D$ = 0.0114). There were moderate levels of genetic differentiation within subspecies *T. e. elegans* (Table S2.6) and *T. e. terrestris* (Table S2.7). In addition, measures of nucleotide diversity were fairly similar across the three subspecies, with *T. e. terrestris* showing slightly higher

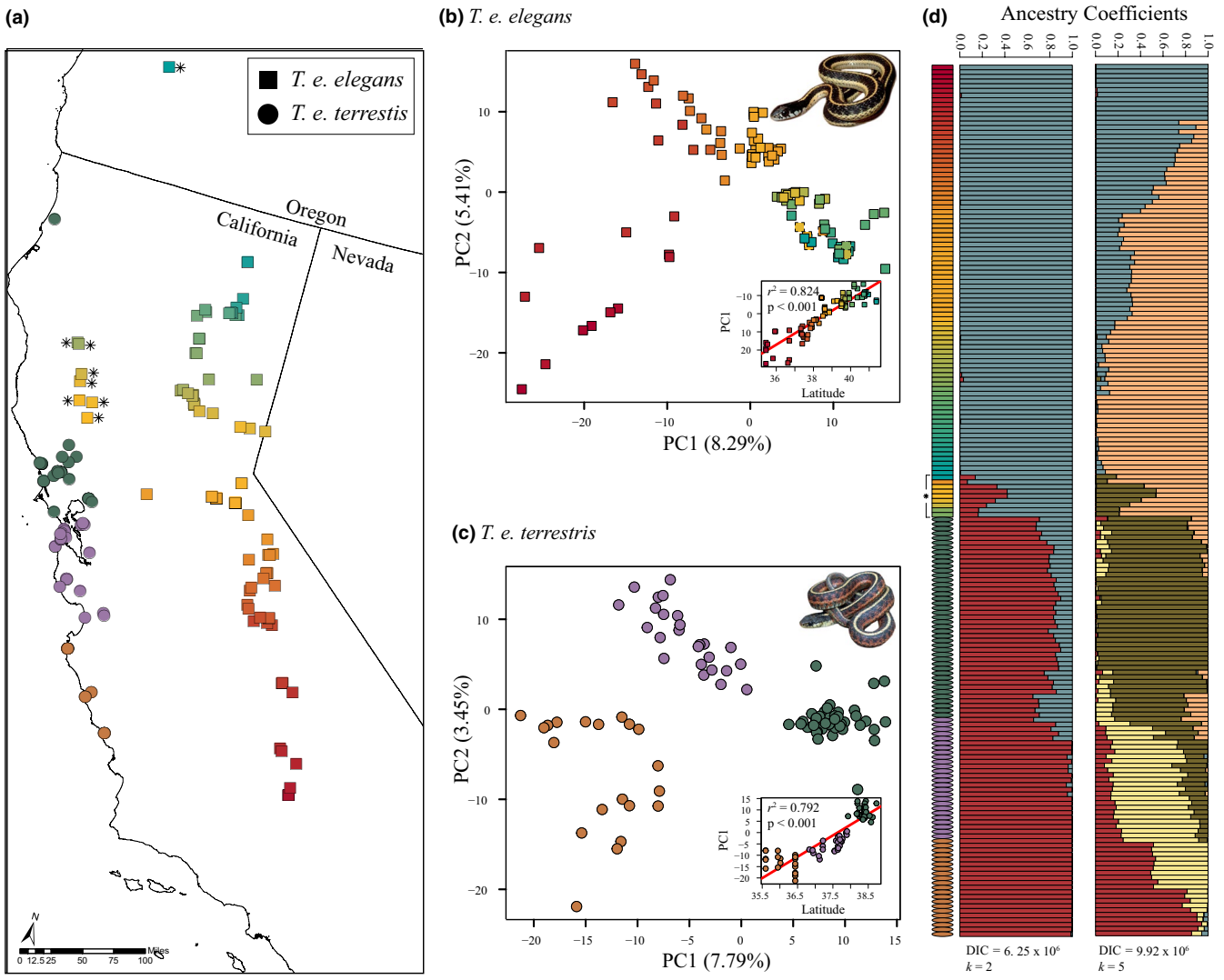


FIGURE 4 Principal component analyses (PCAs) of 10,121 SNPs from our two well-sampled lineages reveals fine-scale genetic structure across the (a) California Floristic Province for both (b) *T. e. elegans* ($n$ = 97) and (c) *T. e. terrestris* ($n$ = 90). Genetic variation in *T. e. elegans* appears more clinal than that of *T. e. terrestris*, which displays discrete breaks across the Sacramento–San Joaquin River Delta and Monterey Bay regions. These patterns are also represented by inset plots of latitude versus PC1 for both subspecies. Plots for *T. e. elegans* are colour coded by latitude, while *T. e. terrestris* represents clusters recovered from PCA. (d) Plots of ancestry coefficients also recovered the clinal variation in *T. e. elegans* and discrete breaks of *T. e. terrestris*. Asterisks represent western samples of *T. e. elegans* with potentially admixed genomes ($n$ = 9). *Note*: we excluded two individuals from these analyses because they represented single samples from very distant sites (*T. e. elegans*, MVZ 230035, Douglas County, OR; *T. e. terrestris* CAS 237205, Humboldt County, CA)

levels ($\theta_\pi$ = 0.0032) than *T. e. elegans* ($\theta_\pi$ = 0.0029) and *T. e. vagrans* ($\theta_\pi$ = 0.0028; Table 2).

### 3.3 | Spatial connectivity

EEMS analyses of *T. e. elegans* and *T. e. terrestris* highlighted several regions in California that may serve as barriers to gene flow (Figure 5a and b). The Central Valley region may represent a major barrier to movement for *T. e. elegans*. A barrier was also inferred in the southern Sierra Nevada, as well as a potential area of limited gene flow in the central Sierra Nevada. Interestingly, potential corridors were detected along the coastal and northern extents of *T. e. elegans*. EEMS analysis of *T. e. terrestris* recovered two regions that appear to be strong barriers to movement: the Sacramento–San Joaquin River Delta and a region south of Monterey Bay. EEMS analysis including both subspecies showed similar patterns as the independent analyses. However, the contact region of northern California between *T. e. elegans* and *T. e. terrestris* appeared as a barrier to gene flow (Figure S1.4).

### 3.4 | Genetic-environment association

pRDA models provided evidence that specific environmental variables influence spatial patterns of genetic variation via local adaptation in both *T. e. elegans* (Figure 6a and b) and *T. e. terrestris* (Figure 6c and d). Our models were significant for both *T. e. elegans* (ANOVA $F_5$ = 1.9347, $p$ = 0.001) and *T. e. terrestris* (ANOVA $F_6$ = 1.4493, $p$ = 0.001). The first two constrained RDA axes for both subspecies were significant ($p$ < 0.001) explaining 33.06% and 24.66% of the total variation for *T. e. elegans* (adjusted $r^2$ = 0.045), 25.33% and 19.80% of the total variation for *T. e. terrestris* (adjusted $r^2$ = 0.029). After excluding environmental variables that were highly correlated, pRDA for both *T. e. elegans* and *T. e. terrestris* included identical predictor variables (Table 3), with the exception that the *T. e. terrestris* model also included elevation. Genetic variation in populations of *T. e. elegans* from western California was strongly associated with mean annual temperature and isothermality. Variation in northern and southern populations of *T. e. elegans* was positively associated with annual temperature range and negatively associated with total annual precipitation (Figure 6a and b). Genetic variation in the San Francisco Peninsula population of *T. e. terrestris* was negatively associated with precipitation seasonality. Mean annual temperature was both positively and negatively associated with genetic variance in populations of *T. e. terrestris* north of the Sacramento–San Joaquin Delta (Figure 6c and d). We identified 49 and 58 outlier loci (Table S2.8) across the environmental variables associated with the first two RDA axes for *T. e. elegans* and *T. e. terrestris*, respectively (Table 4). However, no outlier loci were shared between the two subspecies.

**TABLE 1** Measures of genetic differentiation among the three major genetic clusters (subspecies) of *T. elegans* we recovered

| | *T. e. elegans* | *T. e. terrestris* | *T. e. vagrans* |
|---|---|---|---|
| *T. e. elegans* | – | 0.0377 | 0.2031 |
| *T. e. terrestris* | 0.0114 | – | 0.2277 |
| *T. e. vagrans* | 0.0909 | 0.1139 | – |

*Note:* $F_{ST}$ values are in upper triangle, Nei's $D$ in lower triangle.

## 4 | DISCUSSION

We examined biogeographic patterns in the widely distributed and ecologically variable snake, *T. elegans*. Our analyses of ddRADseq data characterizing populations of *T. elegans* revealed strong genetic differentiation among regional lineages. In addition, each of these groups possesses unique patterns of dispersal, vicariance and regional diversification. History, geological features and adaptation to local environmental variation appear to jointly influence the genetic structure of *T. elegans* lineages.

### 4.1 | Distinctiveness among *T. elegans* lineages

We recovered three distinct genetic groups of *T. elegans* that correspond to the three described subspecies: *T. e. elegans*, *T. e. terrestris* and *T. e. vagrans* (Figure 2). Both phylogenetic and population genetic analyses suggest *T. e. vagrans* is quite distinct from *T. e. elegans* and *T. e. terrestris*. Phylogenetic analysis revealed *T. e. vagrans* is a monophyletic clade sister to a clade comprised of a monophyletic *T. e. terrestris* nested within a paraphyletic *T. e. elegans*. Previous phylogeographic work on *T. elegans* also recovered regional clades, but these did not represent monophyletic groupings consistent with taxonomic classifications (Bronikowski & Arnold, 2001). For example, Bronikowski and Arnold (2001) found that populations of *T. e. elegans* from the northern Sierra Nevada grouped with populations of *T. e. terrestris* from the Monterey Bay region and recovered a large clade that encompassed most of the range of *T. e. vagrans*. However, the lack of robust topological patterns made further biogeographic analyses or hypotheses about diversification and taxonomy within *T. elegans* difficult. Our increased genomic and geographic sampling helped clarify the evolutionary relationships of these regional groups and allowed us to test more complex biogeographic hypotheses.

**TABLE 2** Measures of nucleotide diversity in the three major lineages (subspecies) of *T. elegans* we recovered

| Subspecies | *n* | $\theta_\pi$ | $\theta_\omega$ |
|---|---|---|---|
| *T. e. elegans* | 99 | 0.002902 | 0.00322 |
| *T. e. terrestris* | 90 | 0.003186 | 0.003877 |
| *T. e. vagrans* | 19 | 0.00276 | 0.002841 |

*Note:* Nucleotide diversity is reported as both $\theta_\pi$ and $\theta_\omega$ (Watterson estimator).

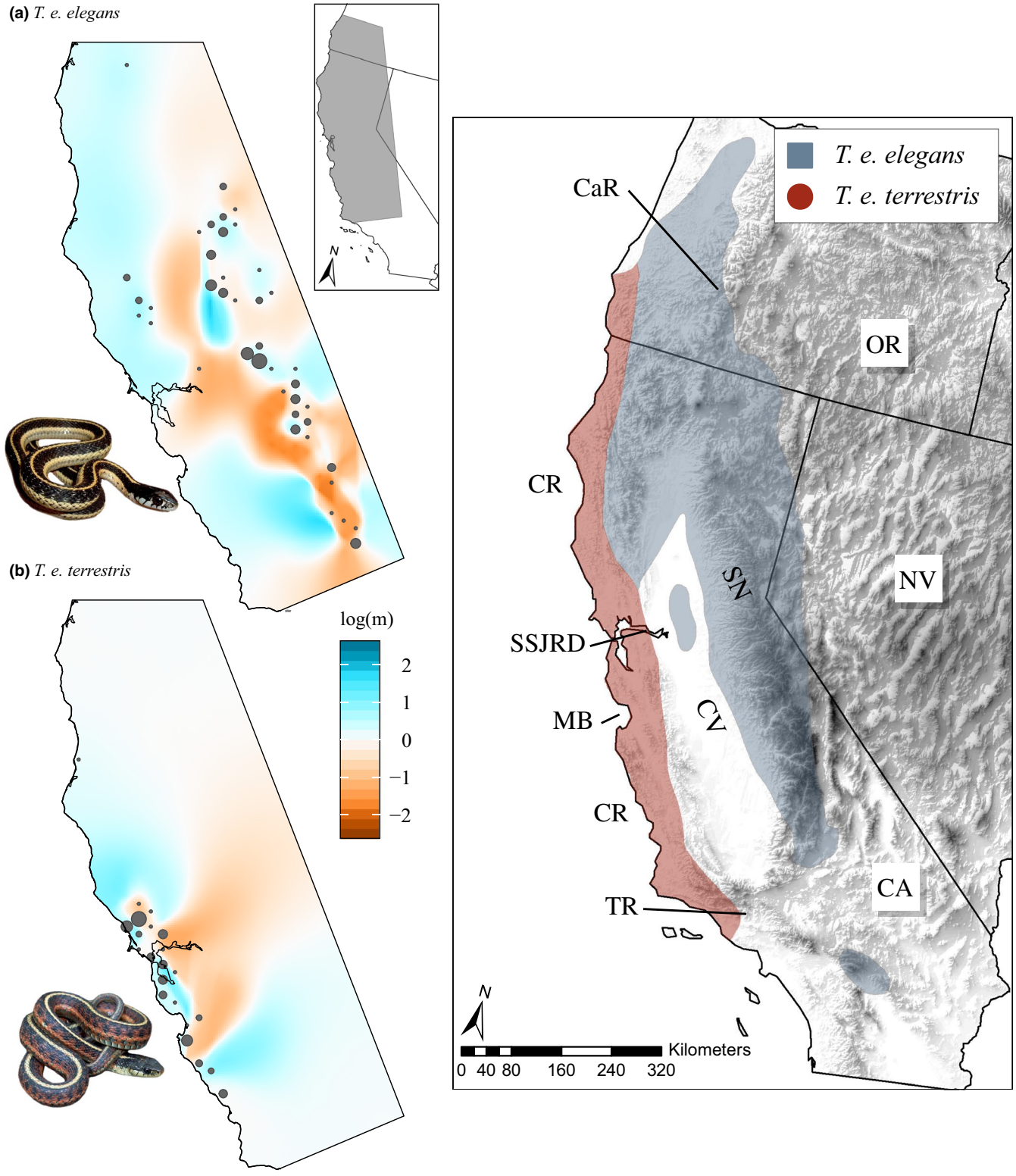


FIGURE 5 Estimated effective migration surface (EEMS) plots of (a) *T. e. elegans* and (b) *T. e. terrestris* displaying estimated rates of migration that deviate from isolation-by-distance expectations. Warmer colours represent barriers to gene flow while cooler colours represent genetic corridors. A number of regions that appear to limit migration were recovered in both species, and are associated with well-known biogeographic barriers such as those in central and southern Sierra Nevada, the Central Valley, Sacramento–San Joaquin River Delta and Monterey Bay. Circles represent the demes ($n$ = 600) that sample localities were assigned (scaled to sample size). Migration estimates given in $\log_{10}$ scale relative to the overall migration rate across the region, which has been scaled to 0 (see Petkova et al., 2016). Common biogeographic barriers are labelled (CaR, Cascade Range; CR, Coast Range; CV, Central Valley; MB, Monterey Bay; SN, Sierra Nevada; SSJRD, Sacramento–San Joaquin River Delta; TR, Transverse Range)

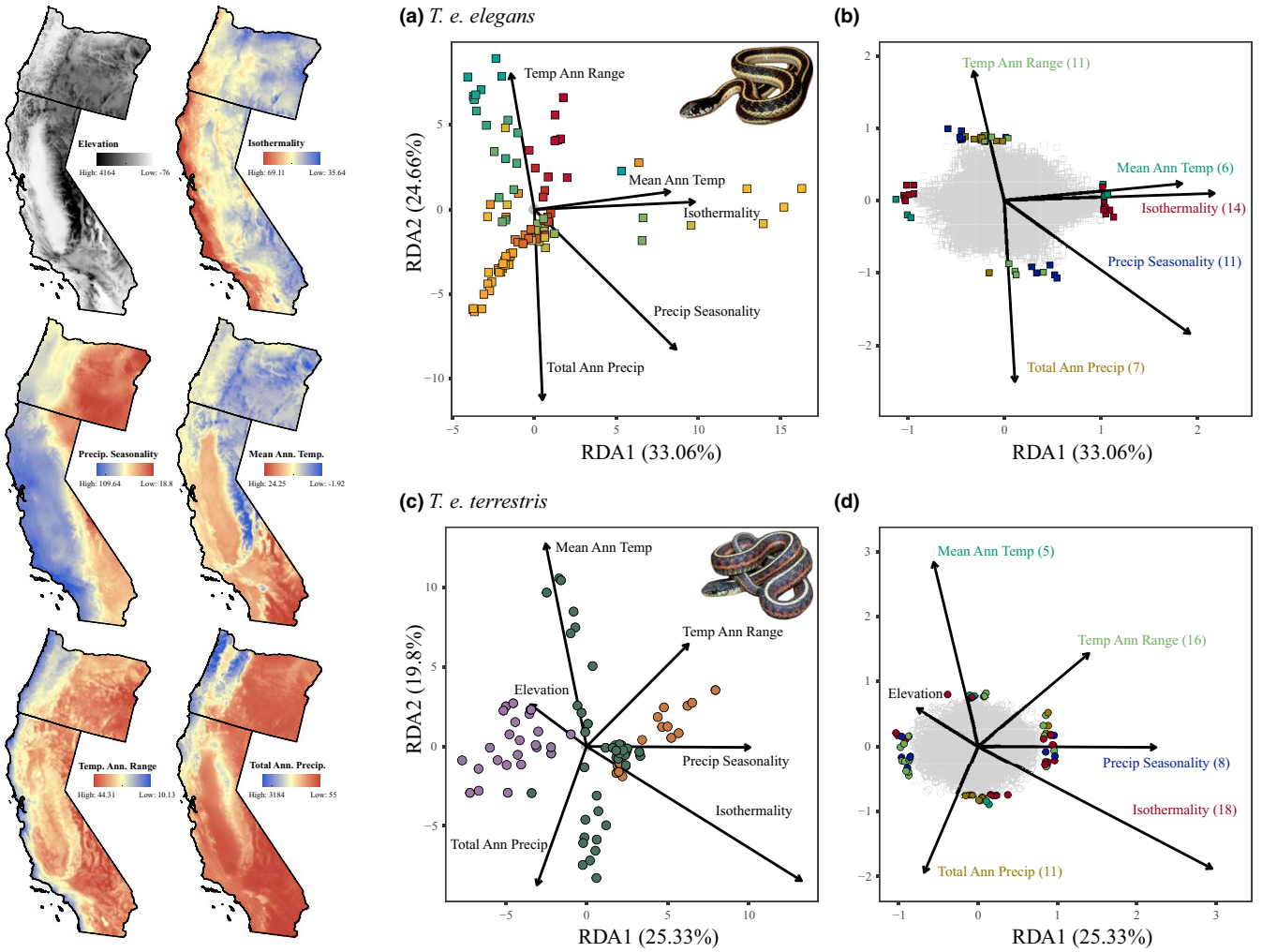


**FIGURE 6** Partial redundancy analyses (pRDA) of 10,121 SNPs depict the environmental variables (isothermality, mean annual temperature, precipitation seasonality, temperature annual range, total annual precipitation) which were significantly associated with genetic variation in (a) *T. e. elegans* and (c) *T. e. terrestris* for the first two constrained ordination axes, after correcting for spatial autocorrelation (PC1). Colours represent populations recovered from PC analyses for each subspecies (see Figure 4). Loadings from candidate loci under selection that are either positively or negatively associated with environmental variables for (b) *T. e. elegans* ($n$ = 49) and (d) *T. e. terrestris* ($n$ = 58). Outliers were detected based on locus scores ±3 SD from the mean loading on each same RDA axes. Loci are labelled based on the environmental variable and the number of loci associated with each variable. Significant environmental variables and elevation used in our pRDA models are depicted across the CFP

**TABLE 3** Set of climatic variables used in both partial redundancy analyses (pRDAs) of spatial genetic variation in *T. e. elegans* and *T. e. terrestris*

| Bioclimatic variable | Description |
|---|---|
| BIO1 | Mean annual temperature |
| BIO3 | Isothermality (mean diurnal range/temp annual range) (×100) |
| BIO7 | Temperature annual range (max temp of warmest month - min temp of coldest month) |
| BIO12 | Total annual precipitation |
| BIO15 | Precipitation seasonality (coefficient of variation) |

*Note*: Environmental variables come from WorldClim database (Hijmans et al., 2005), and are not significantly correlated with each other.

The subspecies rank is useful for describing morphologically distinct 'historical sublineages' that occupy a part of a species range, and that intergrade at zones of contact (Hillis, 2020; Mayr, 1969). Important elements of this concept are that the subspecies are morphologically diagnosable, but still reproductively compatible with other subspecies, such that admixture occurs where lineages meet (Hillis, 2020). Morphological traits such as dorsal and ventral colouration and patterns of blotching and striping, as well

as certain meristic characters (scale counts), have been used to distinguish the geographic races of *T. elegans* (Fitch, 1980, 1983; Fox, 1951; Johnson, 1947; Lawson & Dessauer, 1979; Rossman, 1979; Stebbins, 2003). Unfortunately, these traits can be highly variable, even across small spatial scales or within populations (reviewed in Rossman et al., 1996). In addition, many of these morphological treatments precede more rigorous statistical analyses. Efforts are needed to help clarify the morphological variation within and among groups, as well as the traits that faithfully distinguish the subspecies.

Determining how exclusive these geographic lineages (subspecies) are will also require further effort. Here, we sampled two regions of possible secondary contact. In the first contact zone, the ranges of *T. e. elegans* and *T. e. vagrans* intersect along a portion of northeastern California and southcentral Oregon (an area long considered an important suture zone for multiple hybridizing taxa; Forister et al., 2006; Remington, 1968; Swenson & Howard, 2005). The *T. elegans* of this region (e.g., Modoc County) have been the focus of debate because many appear morphologically unique. They have been proposed to represent their own subspecies, *T. e. biscutatus* (Fitch, 1980, 1983), or instead possible intergradation between *T. e. vagrans* and *T. e. elegans* (Rossman, 1979). Surprisingly, our results do not support either of these hypotheses. The *T. elegans* of this area do not form a unique cluster or lineage, as expected if *T. e. biscutatus* were a distinct regional group (Figure 2). Likewise, we found no evidence of intergradation between *T. e. vagrans* and *T. e. elegans* in any region. The second contact zone is where the narrow coastal range of *T. e. terrestris* meets the western extent of *T. e. elegans* from northwestern California to southwestern Oregon. As with the contact zone between *T. e. elegans* and *T. e. vagrans*, our results suggest that *T. e. elegans* and *T. e. terrestris* form distinct genomic clusters (Figure 2c), with only minimal admixture between groups (Figure 4d). However, the ranges of these two subspecies abut for hundreds of kilometres (Rossman et al., 1996; Stebbins, 2003), where intergradation is thought to be extensive (Fitch, 1983). Greater sampling of proximate populations is needed along both contact zones to establish the extent of potential admixture between the subspecies.

Further work is needed to reappraise the subspecies arrangement in *T. elegans*. The three subspecies may represent distinct species: independent lineages (de Queiroz, 1998, 2007; Wiley, 1978) that are discrete along multiple axes (morphological, molecular, ecological, behavioural, etc.) and display group cohesion (Templeton, 1989) suggesting limited interbreeding between lineages (Hey, 2006; Templeton, 1989). For example, differences in feeding strategies between *T. e. elegans* and *T. e. terrestris* appear linked to morphological and physiological variation (Arnold, 1977, 1981a, 1981b; Britt & Bennet, 2008; Britt et al., 2006; Drummond & Burghardt, 1983) that could function as potential isolating mechanisms between these taxa. Regardless, we hope our results provide a framework for future morphological, genetic and ecological work that might further clarify the taxonomic status of the *T. elegans* complex.

### 4.2 | Biogeographic patterns of genetic variation within lineages

We recovered strong patterns of genetic differentiation across the three subspecies that can be explained by dispersal and vicariance scenarios and which are largely consistent with patterns in other groups that share similar distributions across the CFP (e.g. Feldman & Spicer, 2006; Kuchta et al., 2009; Lavin et al., 2018; Rissler et al., 2006). Geographic features underlying the biogeographic structure of these groups include the Central Valley, Sacramento–San Joaquin River Delta, glacial cycles across the Sierra Nevada, and ancient marine embayments (e.g. Feldman & Spicer, 2006; Martínez-Solano et al., 2007; Reilly et al., 2015; Spinks et al., 2010). AAR analysis recovered the most likely ancestral area as the Eastern Cascades ecoregion, followed by a general westward dispersal scenario associated with *T. e. elegans* and *T. e. terrestris* (Figure 3).

Despite relatively sparse sampling of *T. e. vagrans* across its large distribution, we still recovered noteworthy phylogeographic patterns. Two clades of *T. e. vagrans* were associated with well-known ecoregions spanning the Pacific Northwest and the Southwest (Figure 3). In general, a similar phylogeographic break between northern and southern regions has been recovered in both *Rana luteiventris* (Funk et al., 2008) and *Sorex monticolus* (Demboski & Cook, 2001). These patterns have been attributed to long-standing barriers, such as the Snake River Plain, which bifurcates the distribution of many taxa across southern Idaho (Demboski & Cook, 2001), or the Wyoming Basin which divides the northern and southern Rocky Mountains (Albach et al., 2006; DeChaine & Martin, 2005).

*T. e. elegans* and *T. e. terrestris* also exhibit clear spatial genetic structure across eastern and western California. As a clade, the grouping of *T. e. elegans* and *T. e. terrestris* appears to have originated in the Eastern Cascades and shows a dispersal pattern south into the Sierra Nevada and into the Coast Ranges. The Central Valley divides the distribution into western and eastern ranges, as in other terrestrial animals of the CFP (Matocq et al., 2012). Historically, the Central Valley was environmentally and ecologically distinct from the elevated woodland and forest communities that ring the valley, including vast grasslands and also seasonally flooded wetlands (Garone, 2020; Schoenherr, 2017) that are still occupied by the highly aquatic *T. gigas* and generalist *T. sirtalis* (Rossman et al., 1996; Stebbins, 2003). The Central Valley is now largely inhospitable, having been heavily altered for agricultural use and degraded by urban sprawl (Garone, 2020). Despite this potential barrier to gene flow, phylogeographic estimates in other herpetofauna have recovered lineages on either side of the northern Central Valley to be closely related (Feldman & Hoyer, 2010; Kuchta et al., 2009; Lapointe & Rissler, 2005; Martínez-Solano et al., 2007; Myers et al., 2013; Olson et al., 2021; Reilly et al., 2015; Wake et al., 1986). These observations support the hypothesis that corridors still link, or once linked, western and eastern regions of northern California through the Central Valley, referred to as the 'trans-valley leak' (Stebbins, 1949).

Interestingly, our AAR analyses recover differing scenarios regarding the likelihood of a trans-valley leak (Figure 3). The dispersal

pattern from the ML AAR analysis did not suggest a trans-valley leak in *T. elegans*. Instead coastal populations of *T. e. elegans* and the individual from the Sacramento–San Joaquin River Delta are closely related to individuals from western California. This suggests a different dispersal scenario for *T. e. elegans* where the group split around the northern end of the Central Valley into coastal California and Sierra Nevada. In contrast, the AAR analysis using our MSC phylogeny does suggest a trans-valley leak. Here, the tree suggests a connection between the northern Sierra Nevada and the northern Coast Ranges. However, we note that this relationship was not supported by our population genetic analyses, which placed the individual from the Sacramento–San Joaquin River Delta with coastal populations of *T. e. elegans*. In addition, EEMS results suggest the Central Valley represents a strong barrier to gene flow between the Sierra Nevada and the California coast (Figure 5a).

Population differentiation within *T. e. elegans* appears largely clinal, with a latitudinal gradient in genetic variation across the Sierra Nevada and northwestern California (Figure 4b and d). As mentioned above, these data, and the stepwise tree topology (Figure 3b and c), suggest a southern progression of *T. e. elegans* into the Sierra Nevada and northern California, as seen in other herpetofauna (Kuchta et al., 2009; Moritz et al., 1992; Rodríguez-Robles et al., 2001). Nevertheless, EEMS analysis also suggested barriers to gene flow in the central and southern Sierra Nevada. Multiple glaciation events (Gillespie & Clark, 2004; Moore & Moring, 2013) are thought to have driven genetic differentiation in taxa that span the Sierra Nevada (Feldman & Spicer, 2006; Kuchta et al., 2009; Kuchta & Tan, 2006; Lavin et al., 2018; Lind et al., 2011; Maier et al., 2019; Maldonado et al., 2001; Matocq, 2002; Rubidge et al., 2014).

In *T. e. terrestris*, population differentiation appears more structured than that of *T. e. elegans*. Both PCA and clustering approaches recovered distinct spatial groups (Figure 4c and d), whose boundaries are associated with the Sacramento–San Joaquin River Delta (Martínez-Solano et al., 2007; McCartney-Melstad et al., 2018; Richmond et al., 2014; Rodríguez-Robles et al., 1999; Spinks et al., 2014) and the Monterey Bay (e.g., Reilly et al., 2015; Spinks et al., 2010; reviewed in Rissler et al., 2006). Interestingly, the genetic breaks near Monterey Bay appear to be the result of a series of marine embayments from the Miocene through Pleistocene which isolated most of the central California coast from the northern coast (Dupré et al., 1991; Hall, 2002).

## 4.3 | Local adaptation

Spatial genetic structure within *T. e. elegans* and *T. e. terrestris* has clearly been shaped by geographic features and historical processes, but also appears to have been influenced by local adaptation to environmental variation. Local adaptation to environmental variation can influence spatial genetic structure if migrants are maladapted to local conditions, thereby reducing gene flow (Isolation by Environment or IBE; Nosil et al., 2009; Wang & Bradburd, 2014). Adaptation to local environments will also directly influence variation in the genomic regions responding to selection. Despite the potential pitfalls of using reduced representation data for naïve genome scans to detect selection (Hoban et al., 2016; Lowry et al., 2017, but see Catchen et al., 2017; McKinney et al., 2017), GEA analyses have been robust under a wide range of spatial and demographic conditions (Capblancq et al., 2018; Forester et al., 2018; Lotterhos & Whitlock, 2015). Moreover, they allow analyses to evaluate the contribution of specific environmental variables to local adaptation (Forester et al., 2018; Rellstab et al., 2015).

We found that genetic differentiation within *T. e. elegans* and *T. e. terrestris* is influenced by a consistent set of environmental variables (isothermality, mean annual temperature, precipitation seasonality, temperature annual range, total annual precipitation; Table 3). Interestingly, mean annual temperature was associated with genetic variation in populations from both subspecies from northwest California. Given the pivotal role that temperature plays in ectotherms, these results are perhaps unsurprising. Variation in thermal regimes can have a variety of effects on the behaviour and physiology of reptiles and might drive local adaption among populations by affecting the ability to thermoregulate (Angilletta et al., 2003; Kingsolver & Huey, 1998; Taylor et al., 2020), remain active (Adolph & Porter, 1993; Taylor et al., 2020) and allocate energy (Angilletta, 2001; Bronikowski, 2000; Gangloff et al., 2015). Beyond the direct influence of these environmental variables, we expect that factors such as temperature and precipitation impose

**TABLE 4** Bioclimatic variables from WorldClim database with their associated variance inflation factor (VIF) and RDA loadings for axes one and two

| | *T. e. elegans* | | | | *T. e. terrestris* | | | |
|---|---|---|---|---|---|---|---|---|
| **Bioclimatic variable** | **VIF** | **RDA1** | **RDA2** | **# Loci (%)** | **VIF** | **RDA1** | **RDA2** | **# Loci (%)** |
| PC1 (condition) | 4.1175 | – | – | – | 4.8539 | – | – | – |
| Elevation | – | – | – | – | 4.1663 | −0.1969 | 0.1500 | – |
| Isothermality | 2.1017 | 0.7280 | 0.0337 | 14 (28.6%) | 6.6978 | 0.7545 | −0.4805 | 18 (31.0%) |
| Mean annual temperature | 3.2615 | 0.6156 | 0.0787 | 6 (12.2%) | 9.6744 | −0.1435 | 0.7259 | 5 (8.6%) |
| Precipitation seasonality | 5.7940 | 0.6433 | −0.6159 | 11 (22.4%) | 2.0764 | 0.5738 | −0.0033 | 8 (13.8%) |
| Temperature annual range | 4.9463 | 0.1065 | 0.5985 | 11 (22.4%) | 4.9260 | 0.3574 | 0.3666 | 16 (27.6%) |
| Total annual precipitation | 2.9846 | 0.0369 | −0.8383 | 7 (14.3%) | 5.8846 | −0.1740 | −0.4959 | 11 (19.0%) |

selection indirectly by determining key aspects of the ecological communities or resources these snakes require. For example, total annual precipitation and precipitation seasonality should impact not just the dominant plant communities these snakes occupy but also water availability required by their prey. Precipitation is known to influence prey availability and abundance in *T. sirtalis*, which then impacts reproductive success (Seigel & Fitch, 1985). Because *T. elegans* also exploit prey that are sensitive to available moisture, such as amphibians, earthworms and slugs (Arnold, 1977, 1981a; Drummond & Burghardt, 1983), we expect similar ecological dynamics and selective regimes.

Despite the fact that the same set of environmental variables was associated with genetic variance in *T. e. elegans* and *T. e. terrestris*, completely independent sets of outlier loci exhibited allele frequency shifts consistent with local adaptation in the two species. Such a result is likely influenced by marker density limits of our data, variation in linkage disequilibrium (LD) among populations and species, and a polygenic basis of local adaptation to environmental variation. Our marker density (roughly 1 SNP per 150 KB) is underpowered for detecting genomic regions influenced by selection, and RDA outlier loci presumably reflect some level of LD with causal variants. Given that LD often varies among lineages and populations, the detection of independent sets of loci is perhaps unsurprising. In addition, adaptation to environmental variation is likely to be polygenic, in which case common selective pressures may rarely lead to parallel genetic responses (Csilléry et al., 2018; Losos, 2011; Pritchard et al., 2010).

## 5 | CONCLUSION

By coupling genome-scale data with population genetic and phylogenetic approaches, we revealed previously undocumented differentiation within *T. elegans* at multiple spatial scales. This diversity appears to be the result of spatial, historical, and adaptive processes operating across regional clusters of *T. elegans*. First, we recovered three well- differentiated groups consistent with the three currently recognized subspecies, with *T. e. vagrans* distinct from a *T. e. elegans* and *T. e. terrestris* group. The centre of origin of these lineages appears to be the eastern Cascades, with subsequent dispersal into the Columbia and Great Basins by *T. e. vagrans*, and south into the Sierra Nevada by *T. e. elegans* as well as west into the Coast Ranges, eventually giving rise to *T. e. terrestris*. Genetic differentiation within *T. e. elegans* and *T. e. terrestris* can be explained by both isolation-by-distance and by population fragmentation caused by well-established biogeographic barriers (i.e., Sacramento–San Joaquin River Delta and marine embayments). These biogeographic patterns are congruent with other many other fauna (e.g. Feldman & Spicer, 2006; Rissler et al., 2006; Schierenbeck, 2014), suggesting parallel genetic responses to shared landscape features and historical events. Lastly, we also detected non-neutral patterns of genetic variation associated with environmental variation (especially mean annual temperature, isothermality, total annual precipitation). Overall, our analyses illustrate that genetic structure within *T. elegans* has been shaped across multiple spatial scales and may involve a diverse array of ecological and evolutionary influences.

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## DATA AVAILABILITY STATEMENT

Demultiplexed ddRADseq data of individual specimen fastq files used in analyses are deposited at the DRYAD (https://doi.org/10.5061/dryad.wh70rxwks).

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## BIOSKETCH

**Joshua M. Hallas** is PhD candidate in the EECB program at UNR, whose research is focused on the understanding how environmental variation and natural histories mediate population structure, local adaptation, and genetic differentiation. This work represents a portion of his PhD work on the coevolutionary interactions between *Thamnophis* and *Taricha*.

Author contributions: C.R.F., J.M.H. and T.L.P. conceived of the study; C.R.F. conducted fieldwork and specimen collection; J.M.H. conducted molecular lab work; J.M.H. and T.L.P. analysed genomic data; all authors contributed to drafting the manuscript and approve the final version.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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