SYSTEMATICS AND PHYLOGENY

Genotyping-by-sequencing resolves relationships in Polygonaceae tribe Eriogoneae

Peter B. Pearman,[1,2,3,4] Tyler S. Alioto,[5,6] Jean-Remi P. Trotta[5,6] & J. Travis Columbus[4,7]

1 *Department of Plant Biology and Ecology, University of the Basque Country UPV/EHU, Barrio Sarriena s/n, 48940 Leioa, Bizkaia, Spain*
2 *BC3-Basque Center for Climate Change, Sede Building 1, Scientific Campus of the University of the Basque Country, 48940 Leioa, Bizkaia, Spain*
3 *IKERBASQUE, Basque Foundation for Science, Plaza Euskadi 5, 48009 Bilbao, Bizkaia, Spain*
4 *California Botanic Garden, 1500 N College Ave, Claremont, California 91711, U.S.A.*
5 *CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Baldiri i Reixac 4, 08028 Barcelona, Spain*
6 *Universitat Pompeu Fabra (UPF), Barcelona, Spain*
7 *Claremont Graduate University, 150 E 10th St, Claremont, California 91711, U.S.A.*
Address for correspondence: *Peter B. Pearman, peter.pearman@ehu.es*

DOI https://doi.org/10.1002/tax.12535

Abstract The resolution of cryptic diversity is essential for understanding the evolutionary diversification of lineages and establishing conservation priorities. We examine relationships in Eriogoneae (Polygonaceae), a diverse lineage in western North America. We ask whether *Eriogonum umbellatum*, a morphologically and ecologically diverse species, is monophyletic and whether its varieties represent evolutionary lineages. We use genotyping-by-sequencing to assemble a SNP dataset for 51 species in the genera *Chorizanthe*, *Eriogonum* and *Sidotheca*. We report a hierarchical phylogenetic analysis using maximum likelihood to estimate the evolutionary history of Eriogoneae. We illustrate admixture components for 21 populations of *E. umbellatum*, representing four varieties, and test for lineage structure using TreeMix. We identify strongly supported clades within Eriogoneae. Many relationships in the Eucycla + Oregonium and Latifolia clades are supported, while most relationships within the *Eriogonum* subg. *Oligogonum* clade and a clade with most *Chorizanthe* remain unresolved. *Eriogonum congdonii* resolves within the main *E. umbellatum* clade, while populations of three varieties of *E. umbellatum* are closely related to *E. ursinum* and are associated with serpentine soils. ADmixture and TreeMix analyses suggest *E. umbellatum* varieties represent evolutionary lineages. These results from SNP data are largely consistent with previous phylogenetic studies of Eriogoneae based on sequence variation. Structure within *Oligogonum* suggests consistent environmental association and radiation after initial colonization of serpentine. Morphology is unreliable for the infraspecific taxonomy of *E. umbellatum*. Additional molecular studies are needed to resolve the evolutionary relationships and ecological diversification within this species, in *Oligogonum*, and in Eriogoneae.

Keywords cryptic species; endangered plants; species circumscription; sulphur flower; taxon limits; taxonomic uncertainty; within-species diversity; wild buckwheat

Supporting information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

Understanding taxon limits, evolutionary relationships and within-species diversity is essential for planning for the conservation of biological diversity. Accurate species-level taxonomy is crucial because much of conservation planning and legislation for biodiversity protection is based on lists of species in potential reserves and management areas (Mace, 2004; Costello & al., 2015). Similarly, understanding of lineage structure and geographic distribution of intraspecific diversity is important because focusing on the level of species alone likely misses biological variation that is important to the conservation of species and ecological systems (Rojas, 1992; Pearman, 2001). Intraspecific genetic variation can underlie plant functional diversity (Medrano & al., 2014), and impact plant community structure and ecological processes (Whitlock, 2014). Functional variation among diverging intraspecific lineages suggests the importance of incorporating lineage identity in efforts to model climate impacts on species and on taxa below the species level (A.B. Smith & al., 2019). When lineage structure remains

Article history: Received: 5 Oct 2020 | returned for (first) revision: 3 Dec 2020 | (last) revision received: 28 Jan 2021 | accepted: 23 Feb 2021 | published online: 28 Jun 2021 | **Associate Editor:** Levent Can
This article was published online on the 28th June 2021. It was subsequently noticed that "Columbus6056" was erroneously given as "Columbus6065" in Fig. 3. This notice is included to indicate that it was corrected on 10th July 2021. | © 2021 The Authors.
TAXON published by John Wiley & Sons Ltd on behalf of International Association for Plant Taxonomy.
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

unidentified, prediction of climate change impacts may be compromised, in turn misleading efforts to develop conservation policy (Pauls & al., 2013). This is notably true of cryptic variation that is associated with environmental variability (Theodoridis & al., 2019). Such cryptic variation can arise from lineage divergence, potentially followed by plasticity, parallel evolution during local adaptation (Trucchi & al., 2017), or through morphological convergence of closely related species to similar environments (Kostikova & al., 2014). Unfortunately, our understanding of the occurrence of cryptic diversity in many genera and within species of conservation interest is often lacking.

One plant group that is the subject of substantial systematic and conservation interest is Polygonaceae tribe Eriogoneae (sensu Reveal, 2005, excluding Pterostegieae), a New World and primarily western North American lineage of 340 currently recognized species and 548 minimum-rank taxa (varieties, subspecies). Most of the 18 genera within the tribe are small, with 1–3 species, whereas 93% of the species are placed in *Eriogonum* (252 spp.) or *Chorizanthe* (63 spp.). In fact, *Eriogonum* (wild buckwheat) is one of the largest genera in North America, where it is broadly distributed across a wide range of elevations, soil types, and plant communities. As of December, 2020, 16 Eriogoneae taxa appear listed under the United States Endangered Species Act, including the genera *Eriogonum* (two species, five additional varieties), *Chorizanthe* (three species, four varieties), *Dodecahema* (one species) and *Oxytheca* (one variety; U.S. Fish & Wildlife Service, 2020). Numerous species and varieties of Eriogoneae appear in state, regional and U.S. government agency watch lists of sensitive species and taxa of conservation concern (e.g., Anonymous, 2017; California Native Plant Society Rare Plant Program, 2020; California Natural Diversity Database, 2020; Nevada Division of Natural Heritage, 2021). However, aspects of the taxonomy of Eriogoneae, based almost entirely on comparative morphology, and its evolution have come into question with analyses of DNA sequence data, which reveal that neither *Eriogonum* nor *Chorizanthe* is monophyletic (Sanchez & Kron, 2008). Further, in the most densely sampled phylogenetic estimate of the tribe to date (Kempton, 2012), based on three markers (ITS and two chloroplast loci) and representing all 18 genera and 146 (43%) species, only one subgenus having two or more samples within *Eriogonum* and *Chorizanthe* appears as monophyletic (*C.* subg. *Chorizanthe*).

Eriogoneae exhibit morphological convergence when in similar climates (Kostikova & al., 2014), which suggests that morphological similarity due to adaptation or phenotypic plasticity may obscure species-level variation, hinder development of an accurate taxonomy, and complicate the discovery of intraspecific lineages. For example, assessment based on morphological characters has resulted in relegating some species to the varietal rank within *Eriogonum umbellatum*, a morphologically and ecologically diverse species with 41 currently recognized varieties (Reveal, 1968, 2005). Some varieties of this species are distinguished partially based on geography, suggesting it may be a catchall taxon for morphologically similar ecotypes and lineages. We consider the morphology-based classification of varieties as a hypothesis for lineage structure within the species, the monophyly of which has not been tested. Further, restricted ecological distribution and narrow geographic endemicity of varieties may be insufficient to alert conservationists to potentially important variability in this species and other Eriogoneae in the absence of genetic evidence (e.g., Reveal, 1981). These observations indicate a need for expanded molecular analysis to clarify evolutionary relationships and support conservation needs in Eriogoneae in general, and especially in taxa with high morphological and ecological diversity, such as *E. umbellatum*.

Sequence-based phylogenies of Eriogoneae have been complemented by studies of population genetic structure of some species of conservation concern. Variation at allozyme loci has not provided a genetic basis to distinguish rare and widely distributed varieties of *Eriogonum ovalifolium* (Archibald & al., 2001). In contrast, allozyme and morphological data indicate that *E. robustum* is a species and not a variety of *E. lobbii* (Kuyper & al., 1997). Grady & Reveal (2011) and Grady (2012) suggest that one variety of *E. ochrocephalum* represents a species-level lineage (*E. calcareum*). Nonetheless, inter simple sequence repeat (ISSR) variation does not support designation of rare and common varieties of *E. shockleyi*, despite substantial morphological differences (J.F. Smith & Bateman, 2002). In comparison, plastid and nuclear sequence variation provide evidence of negligible levels of gene flow among endemic *Eriogonum* populations on the California Channel Islands (Riley & al., 2016), and analysis of microsatellite variation indicates low genetic variation and population bottlenecks in a variety of *E. giganteum* on one of these islands (Riley & al., 2019). These results suggest that population genetic variation from several kinds of data do not consistently support the existence of evolutionary lineages that coincide with recognized taxa. It remains unclear whether morphology in Eriogoneae is an indicator of lineage structure at the varietal level, although morphologically similar species of other taxa can often be distinguished with sequence data from multiple loci (Yang & Rannala, 2010). With the exception of Lemon & Wolf (2018; see below), the kinds of loci principally employed so far in the study of Eriogoneae may be unable to resolve genetic variation among varieties when varietal variation in morphology involves little genetic divergence and incomplete lineage sorting.

Single nucleotide polymorphisms (SNPs) at many loci are advantageous for examining evolutionary relationships and lineage structure (Brumfield & al., 2003) because they provide a larger number of markers than do microsatellite loci, and are subject to lower error rates (Ball & al., 2010; Gompert & Buerkle, 2013; Jeffries & al., 2016). In non-model groups with no genomic resources, like Eriogoneae, thousands of SNPs can be obtained with reduced representation sequencing, such as restriction site-associated DNA sequencing (RADseq) and genotyping-by-sequencing (GBS; Andrews & al., 2016). These markers provide useful information for constructing species phylogenies (Cariou & al., 2013) and studying introgression and intraspecific lineage structure in plants (Lexer & al., 2014; Kim & al., 2018). GBS data and phylogenetic

analyses can resolve taxonomic uncertainties among currently recognized species, investigate population structure within species, and identify loci that are associated with environmental gradients and may be targets for local adaptation (Klimova & al., 2018). Recent work reports the use of ddRADseq to examine levels of genetic diversity and admixture in two closely related *Eriogonum* species, confirming their species status (Lemon & Wolf, 2018).

The main objectives of this project are to (1) assess the utility of GBS for phylogenetics at different scales within Eriogoneae—tribe, genus, species, and variety; (2) test the monophyly of the widespread, variable *Eriogonum umbellatum*; and (3) reveal the pattern of intraspecific genetic variation for a large sample of *E. umbellatum* varieties and populations and determine whether the pattern coincides with the current varietal delimitations. We report analysis of GBS data for a sample of species within the genera *Eriogonum* and *Chorizanthe* from California, Nevada and Oregon. We use these data in phylogeny estimation and identification of ancestral groups and admixture of lineages. We find further support for existing phylogenetic hypotheses demonstrating the non-monophyly of *Eriogonum* and *Chorizanthe*. We also identify samples representing three varieties of *E. umbellatum* that resolve apart from the other varieties and likely represent distinct species, and we provide evidence for additional cryptic variation within *E. umbellatum*.

■ MATERIALS AND METHODS

Tissue collecting, sequencing, and bioinformatics. — Plant leaf tissue was collected in the course of targeted sampling during September and October 2014 and June and July 2015. Two of us (PBP, JTC) identified the likely location of populations of *Eriogonum umbellatum* using georeferenced herbarium records. We concentrated collecting in the states of California, Nevada and Oregon because of the large number of varieties of *E. umbellatum* and other Eriogoneae in this combined area. Several initial attempts to use occurrence data lacking original GPS coordinates (i.e., retrospectively georeferenced) presented difficulties for finding small, spatially restricted populations. Thus, we focused on locating populations of *E. umbellatum* from records that had at least 30 arc sec precision. From each plant we collected approximately 0.5 g green leaves into number 1 manila "coin" envelopes, sealed these, and placed them immediately into tripled plastic zip-closure bags with silica desiccant. These bags were kept in a cooler on ice for 48 h. We collected *E. umbellatum* leaves from 30 plants along an informal transect through each population, oriented through the longest dimension of the population and starting with a plant at an arbitrary population edge. We sampled subsequent plants that were at least 2 m apart, unless populations were so small as to make this infeasible. In this case, neighbors were omitted from collection whenever possible to reduce the collection of siblings and better span the extent of the population. At each site we noted whether the soil appeared to be serpentine, relying on soil color, exposed parent material, sparse vegetation, and serpentine indicator species (Brady & al., 2005). While we may have misclassified soil with little serpentine character, serpentine soils are widely distributed in northern California, and the associated vegetation differs distinctly from that on non-serpentine areas (Damschen & al., 2011). We sampled as many varieties of *E. umbellatum* as possible. We collected tissue from single individuals of additional Eriogoneae species as they were encountered, especially *E.* subg. *Oligogonum*, in which *E. umbellatum* is classified. For each population we pressed specimens to serve as vouchers and deposited these in the herbarium (RSA) of the California Botanic Garden, Claremont, California, U.S.A. Additional desiccated samples and voucher specimens were contributed ad hoc by additional botanists. All samples of Eriogoneae were determined to species and variety by JTC, and several species and most varieties were not sampled in previous studies. The taxon names, samples, localities, and vouchers are provided in Appendix 1 and supplementary Appendix S1.

Whole genomic DNA was isolated with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) by SGIker genomic services at the University of the Basque Country, Leioa, Spain. Samples were first homogenized using 1.4 mm ceramic beads in a Percellys 24 (Bertin Instruments, Montigny-le-Bretonneux, France) and then extracted following kit instructions. Libraries for GBS (Elshire & al., 2011) were prepared at the Centro Nacional de d-Anàlisi Genòmica (CNAG, Barcelona, Spain). A single restriction enzyme, ApeKI, was used to cut genomic DNA during library construction. ApeKI recognizes a 5-base pair degenerative sequence (GCWGC). Barcodes were designed to allow for two sequencing errors without confusion of samples. Paired-end sequencing of 678 samples representing the genera *Eriogonum*, *Chorizanthe* and *Sidotheca* was conducted on Illumina HiSeq machines with a read length of 101 base-pairs.

Sequences from each run were parsed based on presence of the enzyme remnant cut site and in-line barcodes with GBS-SNP-CROP v.4.0 (Melo & al., 2016), and trimming based on quality and adapters was performed with GBS-SNP-CROP and Trimmomatic v.0.36 (Bolger & al., 2000). We accepted reads with a minimum Phred quality score of 20. These parsed and quality-filtered reads were demultiplexed according to their in-line barcode, and a pair of FASTQ files were produced for each sample with GBS-SNP-CROP. A mock reference was built with a de novo assembly method based on sequence similarity using Pear v.0.9.6 and Vsearch v.1.1.3 (Zhang & al., 2014; Rognes & al., 2016). Reads were aligned against this reference using BWA-MEM v.0.7.12 and mapped reads were filtered with SAMtools v.1.2 (Li & Durbin, 2009; Li & al., 2009). The properly paired, primary aligned reads were kept to produce an mpileup file for each sample. Variant calling was done using GBS-SNP-CROP pipeline, including a series of filters. We discarded SNPs with more than two alleles and all loci with read depths less than 6 and greater than 100 (to reduce the potential for confounding of non-orthologous loci). The resulting data on 699,331 SNPs

and 678 samples were exported to PLINK v.1.9 (Chang & al., 2015) files for further filtering. SNPs with greater than 50% missingness across samples were removed, as were individuals with greater than 50% missing genotype data at the remaining SNPs. Data from reduced representation sequencing, such as GBS and RADseq, display increasing rates of missing homologous loci (dropout) as the phylogenetic distance among samples increases (Pante & al., 2015). In order to mitigate this effect, we subsequently filtered SNP datasets for each analysis separately.

Phylogenetic analysis. — We conducted a hierarchical phylogenetic analysis of GBS SNP data in order to (1) compare Eriogoneae phylogenetic estimates made from a small number of loci in previous studies to those from many GBS loci in this study, (2) determine whether any of the sampled varieties of *Eriogonum umbellatum* resolve elsewhere in the phylogeny, and (3) determine whether any closely related species in *E*. subg. *Oligogonum* resolve as sister to *E. umbellatum*. All trees were constructed with the maximum likelihood (ML) algorithm RAxML v.8.2.12 (Stamatakis, 2014), using generalized time reversible (GTR) substitution with gamma distributed substitution rates and correction for the removal of invariant sites (ascertainment bias). In each case we assessed node support with 1000 bootstrapped trees and generated a best ML tree. At the broadest phylogenetic level, we included 53 samples representing 50 ingroup species in *Eriogonum* and *Chorizanthe* in a SNP alignment. Eight samples represented *Eriogonum* species not previously included in phylogenetic analyses, which were *E. callistum*, *E. douglasii*, *E. gracillimum*, *E. incanum*, *E. latens*, *E. libertini*, *E. nervulosum*, and *E. polypodum*. Two varieties of *E. umbellatum* were represented and two samples of *E. caespitosum* were also included because of taxonomic uncertainty. We included one sample of *Sidotheca trilobata* as the outgroup. This dataset was filtered to remove SNPs with greater than 50% missing genotypes across sites, as well as SNPs with minor allele frequency (MAF) less than 0.05. Subsequently, all heterozygous genotypes were converted to missing because this has little effect on estimation of topology and we were not estimating divergence times (Lischer & al., 2014). The VCF file was converted to PHYLIP format with a python script (Oritz, 2019), removing invariant sites.

With a goal of identifying the sister clade to *Eriogonum umbellatum*, we then constructed a second SNP alignment with only those species forming a strongly supported clade in the previous analysis and corresponding to members of *E*. subg. *Oligogonum*. Again we included the two *E. umbellatum* samples, and used *E. wrightii* from *E*. subg. *Eucycla* as the outgroup. This alignment contained 16 ingroup samples, representing 15 species. We generated a PHYLIP format file and the ML tree with RAxML as above. Finally, we constructed a third ML tree to further test the monophyly of *E. umbellatum*. We expanded the previous analysis to include all 23 samples of *E. umbellatum*, representing 18 recognized varieties and 10 additional species, and again used *E. wrightii* as the outgroup. We filtered for missingness, heterozygosity and invariant sites, as above.

Varietal delimitation and admixture. — After examining the monophyly of *Eriogonum umbellatum*, we sought to address the genetic relationships among the varieties that were represented by the populations we collected of this species, constituting potentially as many as 580 sequenced samples, 10 per population. We removed samples that resolved outside of *E. umbellatum* in the previous phylogenetic analyses. We then filtered the data to remove SNPs with over 50% missingness and then imported the PLINK file into R (R Core Team, 2020) with BEDMatrix v.2.0.1 and assigned genotype values of 1 or 0 to represent genotype presence or absence. We conducted principal coordinates analysis (PCoA, Legendre & Legendre, 1998) on this matrix using the functions "dist" and "cmdscale". Because we observed structure in plots of individuals on the first three PCoA axes, we clustered the samples with "Mclust" in the mclust R package v.5.4.7 (Scrucca & al., 2016) and determined the most-supported number of clusters with the Bayesian information criterion (BIC). We selected 21 populations with eight or more individuals in the largest cluster for population-level analysis of varieties, using genotypes at all SNPs from all 10 sequenced individuals in these populations. Once reduced to only these individuals, the PLINK file was filtered to remove invariant sites, SNPs with MAF <5%, and ones with >50% missingness. We chose one SNP per locus at random for further analysis. We used the program ADmixture v.1.3.0 (Alexander & al., 2009) to identify groups of populations with common ancestry and identify the degree of genetic admixture among varieties. By using a sample of 10 individuals from all populations, we avoided admixture groups that may be called due to differences in sample sizes among populations (Kalinowski, 2011). We employed TreeMix v.1.13 (Pickrell & Pritchard, 2012) to obtain an ML estimate of relationships among populations, identify putative migration events, and provide an analysis for comparison with population admixture. Preliminary TreeMix runs with 1 to 30 migration events indicated that tree topology did not change substantially with five or more migration events, so we chose this number of migrations to produce a tree and conduct 100 bootstrap replicates in order to examine support for the final topology and putative geneflow. The SNP datasets for the phylogenetic analysis, and the ADmixture and TreeMix analyses can be downloaded from Dryad (https://doi.org/10.5061/dryad.7h44j0zs7).

■ RESULTS

Overall the mean number of reads per sample is 1.48×10^6 (s.d. = 6.35×10^5) and the mean yield is 0.292 gigabases/sample (s.d. = 0.124 gigabases). A total of 16,647 biallelic SNPs have rates of missingness less than 0.5 and are retained. A total of 581 samples have missingness rates less than 0.5 for the retained SNPs and are included in the initial VCF file. Samples for further analysis come from this file and sets of re-filtered SNPs. The number of species and varieties varies among the hierarchical levels of the phylogenetic analyses.

Phylogenetic analysis. — Filtering of the SNP matrix for the 53 samples representing 50 ingroup species and 1 outgroup

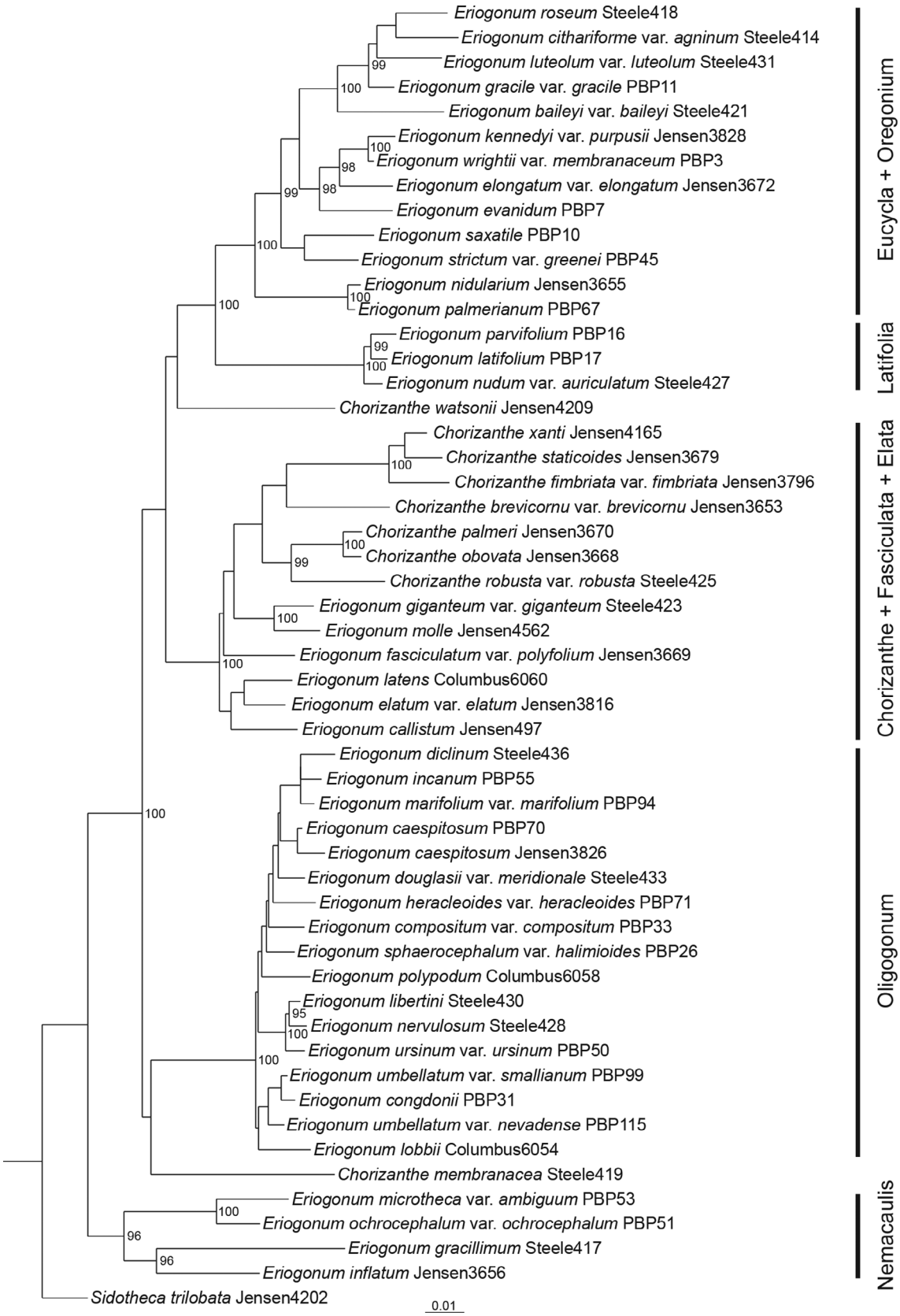

Fig. 1. Best maximum likelihood tree produced with RAxML for 51 taxa in *Eriogonum* and *Chorizanthe*, with *Sidotheca trilobata* as the outgroup. A matrix of 2062 SNPs was used. Bootstrap support values are shown and are based on 1000 trees assembled with the rapid bootstrap option in RAxML. Clade names correspond to those in Kempton (2012).

species (suppl. Appendix S1) results in 2062 SNPs available for analysis after filtering SNPs for less than 50% missing genotypes and to remove heterozygous genotypes and invariant sites. Clade support is mixed (Fig. 1). As discussed below, the topology is largely consistent with previous studies, with no hard conflicts involving well-supported clades. To facilitate comparisons, we employ the provisional names used by Kempton (2012) to identify major lineages within Eriogoneae. The Nemacaulis clade, including *E. microtheca* (Applequist, 2014: 1370) and three additional species, is well supported and sister to a strongly supported clade comprising the remaining sampled species of *Eriogonum* and *Chorizanthe*. This clade comprises four strongly supported clades and two *Chorizanthe* species whose interrelationships, however, are uncertain (i.e., low bootstrap support): (a) Chorizanthe + Fasciculata + Elata clade, (b) Eucycla/Oregonium + Latifolia clade (these two clades in turn are strongly supported as sister), (c) *Chorizanthe membranacea*, (d) *Chorizanthe watsonii*, and (e) Oligogonum clade. Of these, the Oligogonum clade stands out by having many short branches and little internal support. In this analysis, the only well-supported relationships within Oligogonum are among *E. libertini*, *E. nervulosum*, and *E. ursinum*.

Reduction of the original dataset to focus exclusively on 16 samples of 15 species in the Oligogonum clade, with *Eriogonum wrightii* as the outgroup, and re-filtering for genotype missingness less than 50% and invariant sites, yields 1522 SNPs available for analysis (suppl. Appendix S1). The resulting tree (Fig. 2) is better supported than the larger-scale analysis (Fig. 1), but many relationships remain uncertain. However, in addition to the *E. libertini* + *E. nervulosum* + *E. ursinum* clade, the dioecious species *E. diclinum*, *E. incanum*, and *E. marifolium* are strongly supported as a clade, and supported as sister to this clade is *E. polypodum*. Marginal support exists for the placement of *E. congdonii* within *E. umbellatum*.

Expanding the analysis of the Oligogonum clade to include at least one representative of each sampled variety of *Eriogonum umbellatum*, and filtering out invariant sites and those with missing genotypes greater than 50%, provides an alignment of 1503 SNPs with 33 ingroup samples representing 31 taxa (Fig. 3, suppl. Appendix S1). Most samples of *E. umbellatum* form a strongly supported clade. The exceptions are the samples representing *E. umbellatum* var. *glaberrimum* and var. *goodmanii*, and one of the two samples of var. *argus*. These three samples form a clade that is strongly supported as sister to *E. ursinum* + *E. nervulosum* + *E. libertini*. Also, as forecast by the prior analyses (Figs. 1, 2), *E. congdonii* resolves in the main *E. umbellatum* clade. Besides the foregoing relationships, the only other relationship garnering high bootstrap support is that

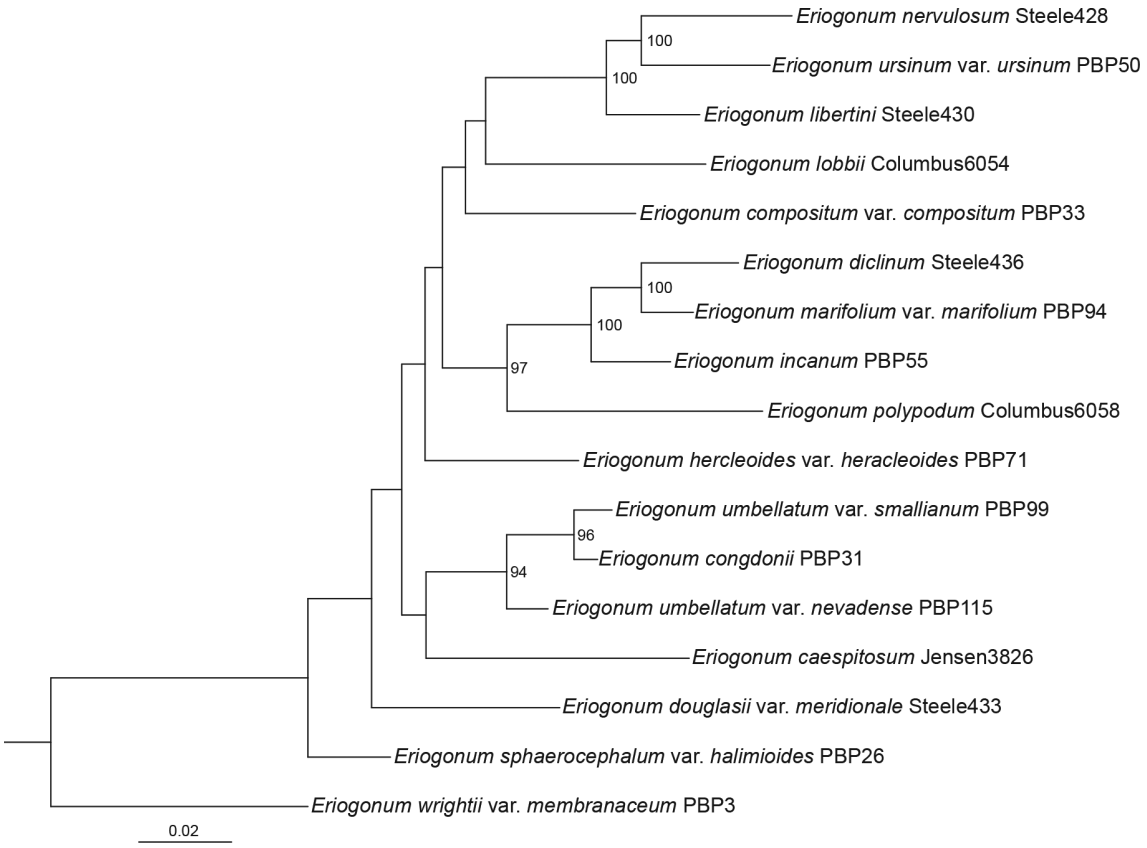

Fig. 2. Maximum likelihood tree for the sampled taxa in the Oligogonum clade, using *Eriogonum wrightii* as the outgroup. Bootstrap support values are based on 1000 trees produced by the rapid bootstrap algorithm in RAxML. A clade with *E. umbellatum* samples is weakly supported.

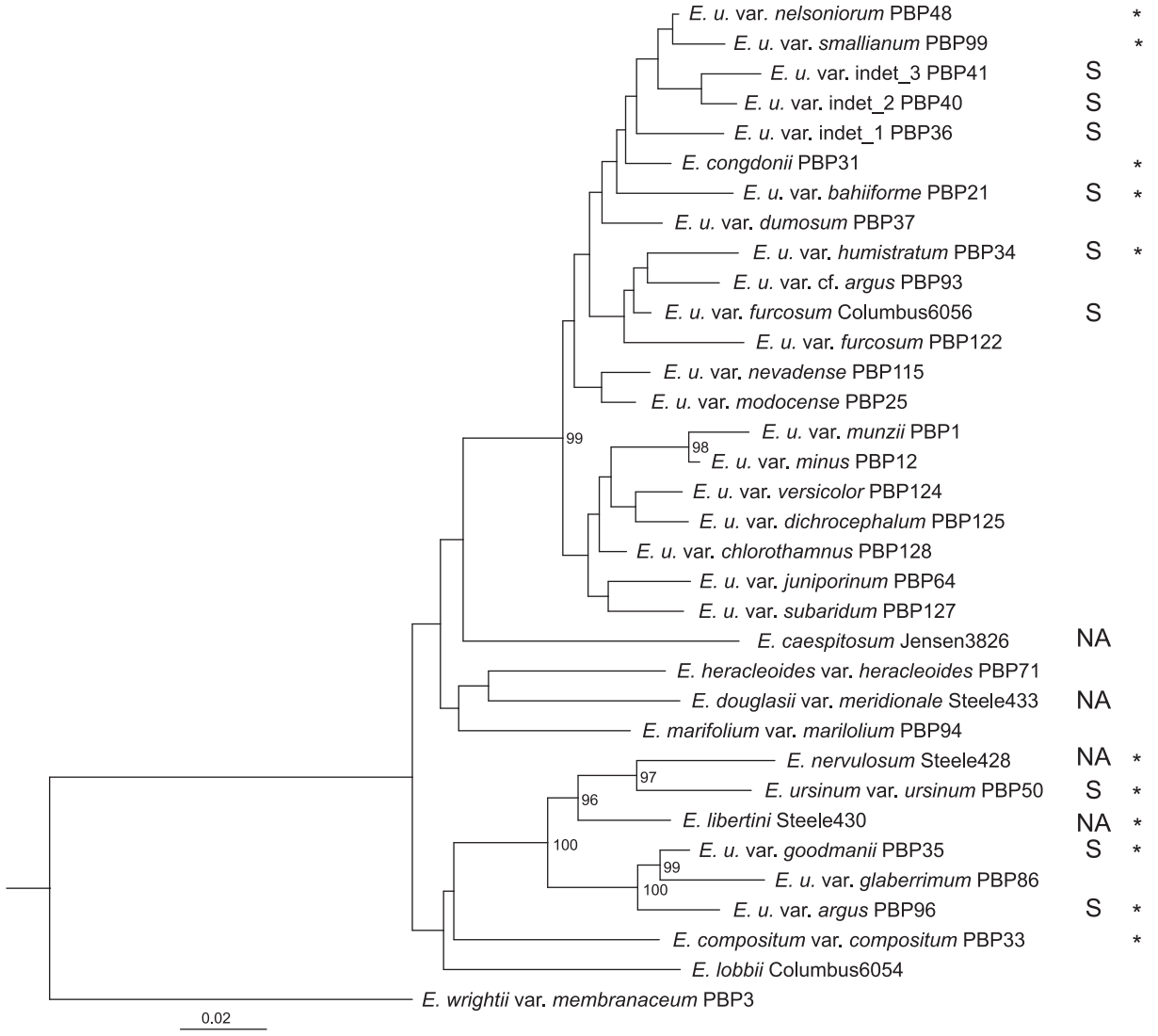

Fig. 3. Maximum likelihood tree of sampled taxa in *Eriogonum umbellatum*, rooted with *E. wrightii*, showing non-monophyly of *E. umbellatum*, including *E. congdonii* positioned in the main *E. umbellatum* clade. Samples representing three varieties of *E. umbellatum* fall outside of the highly supported *E. umbellatum* clade. "S" indicates sample was collected from serpentine or strongly ultramafic soils; "NA", no soil data, "*", reported from serpentine elsewhere in the literature.

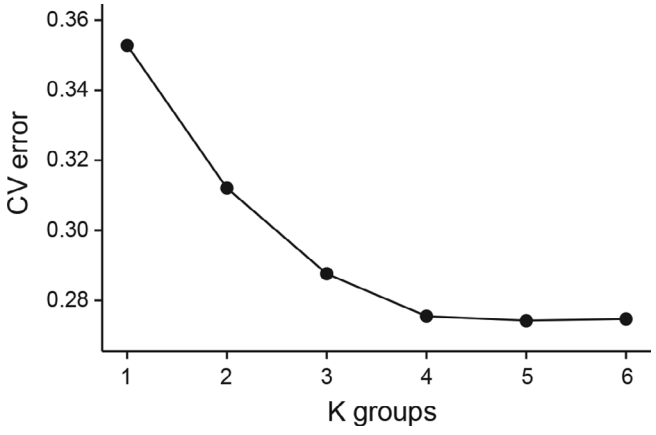

Fig. 4. Cross-validation error as a function of inferred number of ancestral groups in analysis with ADmixture.

between *E. umbellatum* var. *minus* and var. *munzii*, both distributed in the mountains of southern California.

Varietal delimitation and admixture in *E. umbellatum*. — Principal coordinates analysis resolves the structure in missingness of genotypes in a SNP matrix with 560 *Eriogonum umbellatum* samples, representing 56 populations and 16 varieties. Subsequent clustering of the first 100 PCoA eigenvectors with the "Mclust" function reveals that four clusters of populations receive maximal support under the BIC criterion. Of these populations, 21 have eight or more samples included in the largest cluster (suppl. Appendix S2). We focus on these 21 populations because differing patterns of missingness renders incorporation of samples from multiple mclust groups susceptible to artifactual results. The 21 populations represent four named varieties. Filtering the SNP dataset to include only these

samples, then filtering SNPs as described above, with additional reduction of SNPs to only one per locus, produces a dataset with 3608 SNPs and no missing genotypes for the 210 samples.

Pairwise Wier-Cockerham F_{st} values among the 21 populations average 0.196 (s.e. = 0.00416, max = 0.349, min = 0.0356). ADmixture analysis and comparison of cross-validation error indicate that the 21 populations represent four to five putative ancestral populations (Figs. 4, 5). When $K = 5$, four of the five groups coincide with the varietal assignment of the sample and fully 15 populations show less than 10% admixture with other varieties. Populations that were identified as *Eriogonum umbellatum* var. *nevadense* based on morphology tended to have higher levels of admixture than did the other varieties (Fig. 5). Two populations that were determined based on morphology as *E. umbellatum* var. *furcosum* arise from a distinct ancestral population when $K = 5$. These two populations were collected from serpentine soils, while the other samples of *E. umbellatum* var. *furcosum* were found on granitic soils. We refer to these as *E. umbellatum* var. *furcosum* 1 (from non-serpentine soils) and var. *furcosum* 2 (from serpentine soils).

A TreeMix analysis (Fig. 6) nests the two populations of *Eriogonum umbellatum* var. *furcosum* 2 as a clade within *E. umbellatum* var. *nevadense*. Most TreeMix trees with increasing numbers of migration events also resolve the var. *furcosum* 2 clade within var. *nevadense* (suppl. Fig. S1). Bootstrap values within the var. *nevadense* clade are generally low, with the exception of the var. *furcosum* 2 clade and one clade of two var. *nevadense* populations. The position of the var. *furcosum* 2 clade within var. *nevadense* is not strongly supported. The other populations form three well-supported clades that correspond to varietal designations (Fig. 6). However, no particular relationship among these varieties is supported. Further, there is no bootstrap support for particular migration events. Computational demand prevented us from conducting bootstrapping of trees with higher numbers of migration events.

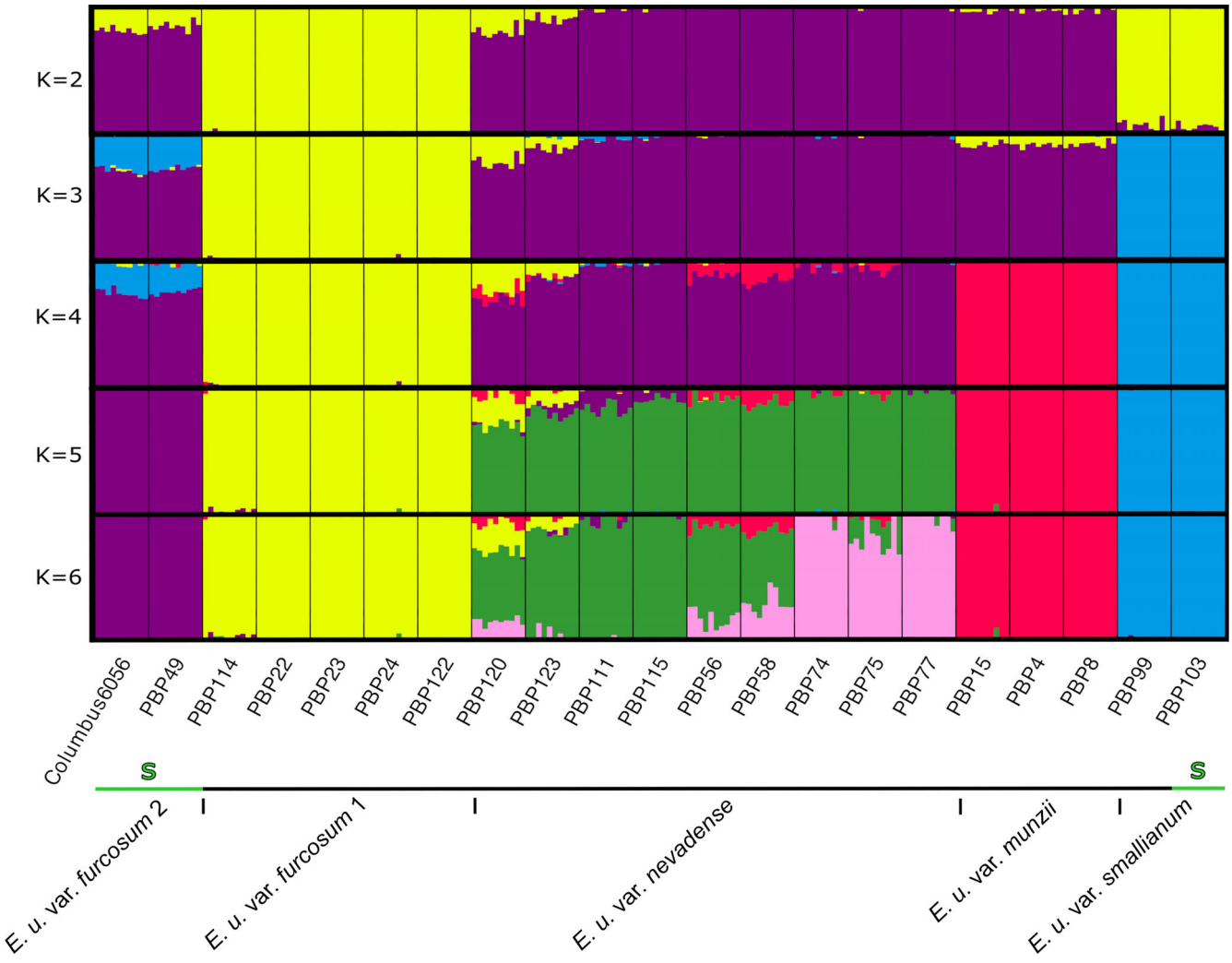

Fig. 5. Admixture diagram of ancestral groups for 21 populations of *Eriogonum umbellatum* as produced with the program ADmixture, for the number of groups shown by *K*. Each population is represented by 10 individuals. "S" indicates populations found on serpentine soils. Two populations of *E. umbellatum* var. *furcosum*, designated with "2", are distinct from other populations of this variety ("1"), and were collected from serpentine soils.

■ DISCUSSION

General patterns. — In the present study, SNP data from GBS prove useful in resolving evolutionary relationships within Eriogoneae at different scales, from relationships among more distantly related species to those among populations of a single species, *Eriogonum umbellatum*. Phylogenetic analyses of these data are largely consistent with lineages identified in previous molecular phylogenies (Grady, 2012; Kempton, 2012; Kostikova & al., 2013, 2014). Our data and analysis with ADmixture provide evidence that genetic differences among populations of *E. umbellatum* partially coincide with published taxonomic designations (Reveal, 2005a, 2012). Phylogenetic analysis, however, provides little support for most relationships among varieties within *E. umbellatum*. Nonetheless, within *E. umbellatum*, we observe a well-supported relationship involving a taxon that has previously been considered a distinct species (*E. congdonii*). As well, samples representing three varieties (*E. umbellatum* var. *argus*, var. *glaberrimum*, var. *goodmanii*) resolve apart from the other samples of *E. umbellatum*. The results also suggest that additional intraspecific variation may exist within this species. (e.g., in *E. umbellatum* var. *furcosum*).

Clarification of interspecific evolutionary relationships and detection of intraspecific lineage structure have been possible using single SNP datasets produced using GBS and RADseq. For example, Schilling & al. (2018) report GBS analyses to clarify evolutionary relationships among the six species of the *Boechera puberula* complex (Brassicaceae) that are distributed broadly across western North America and have undergone hybridization and admixture. They find evidence for monophyly of this group and lineage structure at the subspecific level within *B. puberula*. Similarly, in a case study of California oaks, Kim & al. (2018) examine phylogenetic relationships and population history, finding directional introgression that varies in two tree oak species. In the current study, Eriogoneae present challenges for discerning evolutionary relationships and lineage structure without genetic data because many species-level and infraspecific taxa are distinguished on fine morphological differences and divergent geographic distributions. The analysis of GBS data here improves our understanding of both inter- and intraspecific relationships in Eriogoneae.

Phylogeny and taxonomy. — That neither of the large genera *Eriogonum* and *Chorizanthe* is monophyletic first appears in analyses of sequences from three chloroplast regions and the nuclear gene *LEAFY* (Sanchez & Kron, 2008). This finding is supported in a larger study of Eriogoneae (Kempton, 2012) based on variation at one nuclear region (ITS) and two chloroplast loci. The lack of concordance between generic delimitation and phylogeny also holds true at the rank of subgenus, where all eight subgenera of *Eriogonum* except for one

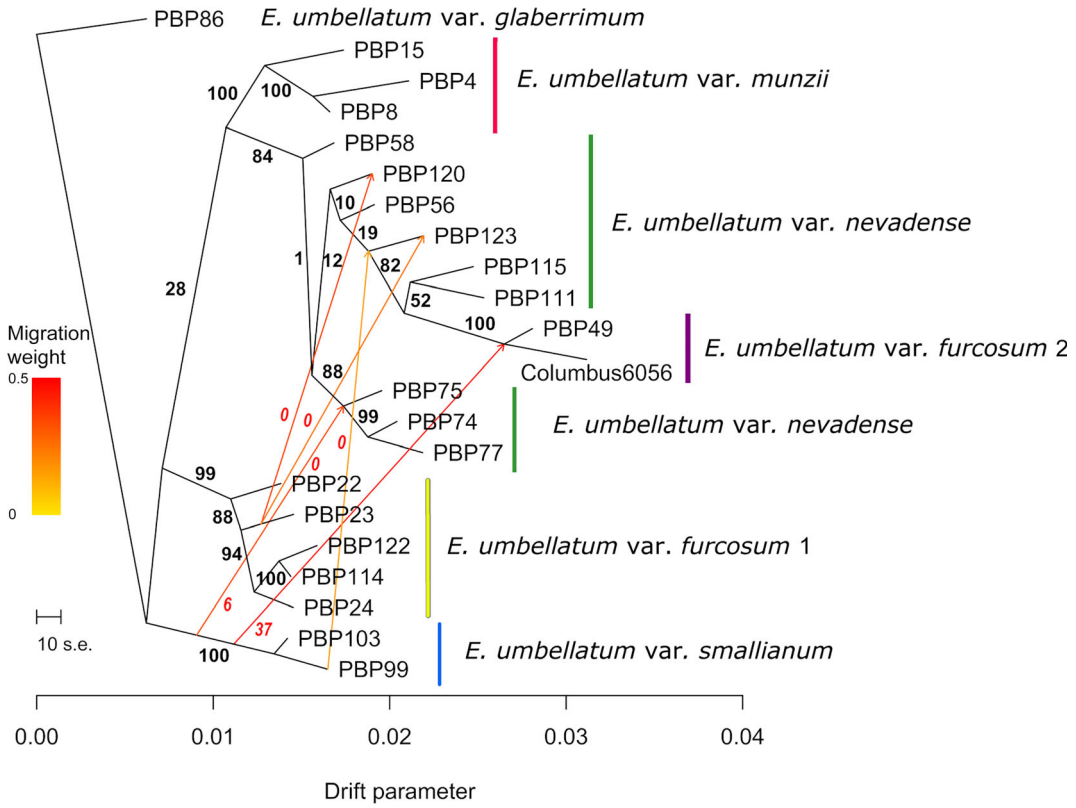

Fig. 6. TreeMix diagram showing relationships and five putative migration events among 21 populations of *Eriogonum umbellatum* of five varieties, plus *E. umbellatum* var. *glaberrimum* as an outgroup. Bootstrap support out of 100 replicates is shown for nodes and migration events. Varieties are supported, with the exception of *E. umbellatum* var. *nevadense*, which here is paraphyletic and includes a serpentine lineage.

monotypic subgenus do not resolve as monophyletic, as is the case for the largest subgenus (*C*. subg. *Amphietes*) of four (two monotypic) in *Chorizanthe* (Kempton, 2012). In the Kempton study, the 15 sampled species of *E*. subg. *Oligogonum* form a strongly supported clade with *E. tomentosum* (*E*. subg. *Eriogonum*), but poor resolution and branch support within the clade leave open the possibility that *E*. subg. *Oligogonum* may be monophyletic. In general, many morphological characters that have been used to delimit genera and subgenera appear to be unreliable based on the phylogenetic estimates.

In addition to Kempton (2012), three other molecular phylogenetic studies of Eriogoneae—Grady (2012) and Kostikova & al. (2013, 2014)—overlap sufficiently with our taxon sample to allow for comparison. Although we have sampled far fewer species across the Eriogoneae phylogeny compared to Kempton (2012), and eight species in our study are not sampled by Kempton or others, our results are largely consistent with previous findings while also offering new insights. The major clades resulting from our analysis of GBS data are well supported and employ Kempton's (2012) provisional clade names (Fig. 1). In addition to Kempton, these clades appear in Grady's (2012) trees based on sequences from three chloroplast and two nuclear loci. Most but not all of these clades are recovered in Kostikova & al.'s (2013, 2014) analyses of sequences from a combined five chloroplast and two nuclear loci. In Kostikova & al. (2013), the four sampled species of *E*. subg. *Oligogonum* do not form a clade (*E. jamesii* resolves elsewhere), and *E. fasciculatum* resolves outside of the Chorizanthe + Fasciculata + Elata clade. In Kostikova & al. (2014), the Eucycla + Oregonium clade is not consistent with their earlier study in that, for example, *E. gracile* resolves elsewhere. Comparing all five studies and taking taxon sampling into account, the position of the major lineages is consistent with regard to the Nemacaulis clade except in Kostikova & al. (2014), where it forms a clade with *Chorizanthe spinosa* and *Sidotheca emarginata*. As well, the Latifolia clade (plus *E. eastwoodianum* in Kempton, 2012) is sister to the Eucycla + Oregonium clade in all studies except Kostikova & al. (2014). Differences in taxon sampling among the studies and often poor resolution and clade support limit further insights into relationships among the major lineages. However, it is noteworthy that the Oligogonum clade is strongly supported as sister to the Latifolia and Eucycla + Oregonium clades in Grady (2012) and Kostikova & al. (2014). Also deserving mention is that the relationships of *Chorizanthe membranacea* and *C. watsonii* are uncertain in our study (Fig. 1). These species, currently classified in different subgenera, resolve apart and outside a clade of seven other species of *Chorizanthe*, and neither species forms a well-supported sister relationship with other lineages. This result mirrors Kempton (2012). However, *C. watsonii* forms a poorly supported clade with *Hollisteria lanata* (not sampled in our study) in Kempton (2012), a relationship that is strongly supported in Kostikova & al. (2014).

We turn now to examining interspecific relationships within each of the major clades. Relationships in our sparsely sampled Nemacaulis clade (Fig. 1) are consistent with other studies. We sample for the first time the annual species *Eriogonum gracillimum* (*E*. subg. *Ganysma*); its position in the Nemacaulis clade is not surprising as most sampled species of *E*. subg. *Ganysma* resolve there (Kempton, 2012). The small Latifolium clade allows for direct comparison, as perennials *E. latifolium*, *E. nudum*, and *E. parvifolium* were all sampled in the foregoing studies except Grady (2012; *E. latifolium* unsampled). In our study, the coastal species *E. latifolium* and *E. parvifolium* are strongly supported as sisters, and this clade in turn receives strong support as being sister to the widespread *E. nudum* (Fig. 1). The species form a polytomy in Kempton (2012). The topology is the same as ours in Kostikova & al. (2013, 2014), although the sister relationship between the coastal species is here well supported.

Sister to the Latifolium clade in most studies, including ours but not Kostikova & al. (2014), is the Eucycla + Oregonium clade. In this clade, except in Kostikova & al. (2014), the annuals *Eriogonum nidularium* and/or *E. palmerianum* (both sampled by us and Grady, 2012) are sister to the remaining species. Another area of consensus is the clade containing the perennials *E. elongatum*, *E. kennedyi*, and *E. wrightii*, sampled in each study. As in Kempton (2012), the annual *E. evanidum* is also a member of the clade in our study, where it receives strong support as being sister to the clade of perennial species (Fig. 1). A clade of morphologically similar annuals in *E*. subg. *Oregonium*, represented here by *E. baileyi* and its sister clade of four species (Fig. 1), is also recovered in Kempton (2012; seven species) and Grady (2012; three species). This conflicts, however, with Kostikova & al. (2013), wherein two perennial species in *E*. subg. *Eucycla* also resolve in the clade, including *E. saxatile*, which we sample as do Kempton (2012) and Grady (2012). As well, these annual species do not form a clade in Kostikova & al. (2014).

In our study, the Chorizanthe + Fasciculata + Elata clade includes the remaining seven sampled species of *Chorizanthe*, which form a clade lacking bootstrap support, and six species of *Eriogonum* (Fig. 1). We include two previously unsampled species, *E. callistum* and *E. latens*. Reveal (2006) speculates that *E. callistum* may be most closely related to *E. latifolium*, which is not supported by our analysis (Fig. 1). As for *E. latens*, Reveal (2005) places the species in *E*. subg. *Oligogonum*, which is also not supported here (Fig. 1). Unlike the other species of *E*. subg. *Oligogonum* we sample, the flowers of *E. latens* we examined lack a conspicuously attenuate (stipe-like) base, a character that has been used to circumscribe the subgenus. However, owing to low clade support, the position of these two newly sampled species in the clade is uncertain. Most of the species we sample are included in Kempton (2012) and, considering clade support, our results do not conflict with hers. Comparison with Grady (2012) is problematic because only three of the six species that appear in this clade in his trees are common to ours, including only one of four *Chorizanthe* species. Likewise, comparison with Kostikova & al. (2013) is limited to *Chorizanthe* because, as noted above, in their study *E. fasciculatum* resolves outside the main Chorizanthe clade, as do all other species of *Eriogonum*. However, in Kostikova & al. (2014), three *Eriogonum* species, including *E. fasciculatum*,

resolve in the clade, and we sample all three. The position of these three species conflicts in the studies: *E. giganteum* and *E. molle* are sisters in our tree (Fig. 1), whereas *E. molle* and *E. fasciculatum* are sisters in Kostikova & al. (2014), both with strong support. In *Chorizanthe*, our study and Kempton (2012) each have a (*C. fimbriata* (*C. staticoides* + *C. xanti*)) clade, and in our tree *C. obovata* and *C. palmeri* form a strongly supported clade that in Kempton (2012) also includes *C. biloba*, sampled neither here nor in Kostikova & al. (2013, 2014). In the Kostikova & al. (2014) analyses the relationships differ: (*C. fimbriata* (*C. palmeri* (*C. staticoides* (*C. xanti* + *C. leptotheca*)))). Neither we nor Kempton (2012) sample *C. leptotheca*, so the conflict involves the position of *C. palmeri*.

Collectively, 25 of the 37 species in *Eriogonum* subg. *Oligogonum* have been sampled for molecular phylogenetics. Here we sample 16 species, including 6 not previously sampled. In all studies besides Kostikova & al. (2014), which sampled only 3 species, *E.* subg. *Oligogonum* is not monophyletic. The presence of *E. tomentosum* (*E.* subg. *Eriogonum*) in the Oligogonum clade in Kempton (2012) and Grady (2012) renders *E.* subg. *Oligogonum* paraphyletic, although poor branch support leaves open the possibility that *E.* subg. *Eriogonum* and subg. *Oligogonum* could be sisters. Likewise, the position of *E. jamesii* in the Kostikova & al. (2013) tree and the position of *E. latens* (Latifolia clade) in our study (Fig. 1) render the subgenus non-monophyletic. Despite multiple studies and the number of *E.* subg. *Oligogonum* species sampled, a characteristic of all these studies is uncertain relationships owing to poor branch support. In Kempton (2012), where 15 species are included, the sole relationship that is strongly supported is that between *E. congdonii* and *E. siskiyouense*, the latter of which is unsampled in other studies including ours. Eight species are reported in Grady (2012), and there is moderate to strong support for the following relationships: ((*E. lobbii* + *E. robustum*) (*E. caespitosum* (*E. diclinum* + *E. umbellatum*))). Of these species, *E. robustum* is not sampled in our study. Two papers, Kostikova & al. (2013, 2014), sampled only a few *E.* subg. *Oligogonum* species, four and three respectively. As noted above, *E.* subg. *Oligogonum* is not monophyletic in their 2013 study, wherein *E. jamesii* does not form a clade with the other three species. Nonetheless, these three species resolve with support as follows: (*E. flavum* (*E. marifolium* + *E. umbellatum*)). Relationships in Kostikova & al. (2014) lack support. As seen in Fig. 2, a few clades in our study receive strong support, including a clade of three dioecious species (*E. diclinum*, *E. incanum*, *E. marifolium*) and three species that grow on serpentine soils (*E. libertini*, *E. nervulosum*, *E. ursinum*). Because sampling among the studies differs appreciably and clade support is limited, there are no supported conflicts among the topologies.

By expanding the representation of *E. umbellatum* varieties in the *E.* subg. *Oligogonum* phylogeny, our analysis also clarifies several relationships in regard to *E. umbellatum*. Our results for several varieties of this species suggest that most samples form a clade even though *E. umbellatum* is not monophyletic due to the placement of *E. congdonii* (Figs. 2, 3). This must be considered preliminary as we include only 18 of 41 currently recognized varieties. As well, several specimens, representing *E. umbellatum* var. *goodmanii*, var. *glaberrimum*, and a sample of var. *argus*, fall outside of this species and are more closely related to *E. libertini*, *E. nervulosum*, and *E. ursinum* (hereafter the *E. ursinum* clade; Figs. 2, 3). With the exception of *E. umbellatum* var. *glaberrimum*, these taxa grow on serpentine soils, as observed by us and others (Fig. 3; Safford & al., 2005; Safford & Miller, 2020), which suggests that these varieties are not products of independent radiations to serpentine conditions. *Eriogonum umbellatum* var. *glaberrimum*, originally described as a distinct species (Gandoger, 1906), has been considered conspecific with *E. umbellatum* beginning with Reveal (1968). Our GBS SNP data support Gandoger's (1906) classification. On the other hand, *E. umbellatum* var. *goodmanii*, which grows on serpentine soils (Reveal, 1989), has never been treated as a distinct species. Indeed, this variety may constitute a species distinct from *E. umbellatum* (Fig. 3). Additional study is needed to determine whether additional *E. umbellatum* varieties that are typical of serpentine soils are, in fact, *E. umbellatum*. This will help to clarify the evolution of serpentine affinity in Eriogoneae.

Emerging patterns involving soil and geography. — Reveal (1989) describes *Eriogonum umbellatum* var. *argus* as "mainly" occurring on serpentine soils and being a northerly "expression" of the *E. umbellatum* var. *stellatum* complex, the Sierra Nevada expression of which is *E. umbellatum* var. *furcosum*. We find that one *E. umbellatum* var. *argus* sample (PBP96) from ultramafic soil groups with the *E. ursinum* clade, while a sample from another population (PBP93), determined as "var. cf. *argus*", resolves in the main *E. umbellatum* clade (Fig. 3). Notably, this latter specimen comes from a population on granitic soils and has simple umbels, unlike the compound umbels that Reveal (1989) describes. Similarly, we find that some populations from serpentine soils that are morphologically indistinguishable from *E. umbellatum* var. *furcosum* are distinguishable genetically from var. *furcosum* populations from granitic soils (Figs. 5, 6), a pattern that does not arise in analyses of single samples (Fig. 3). The internal structure of the *E. umbellatum* clade, while not strongly supported, does reflect both ecological and geographic similarities. With the exception of *E. umbellatum* var. *humistratum*, the taxa from the northern portion of the species range are generally or exclusively on serpentine (*E. umbellatum* var. *smallianum*, var. *nelsoniorium*, three undetermined samples, and *E. congdonii*) and form a clade, and pairs of varieties from the White Mountains (*E. umbellatum* var. *versicolor* and var. *dichrocephalum*) and Transverse Ranges (*E. umbellatum* var. *munzii* and var. *minus*) are placed as sisters (Fig. 3). Overall, these results suggest the hypothesis that ecological and geographic factors have influenced the diversification of *E. umbellatum* lineages, and that additional cryptic variation at the varietal and species levels may exist within this taxon.

Morphological similarity of some plant traits in Eriogoneae, including leaf length, width, and rosette diameter (Kostikova & al., 2014), may converge as a function of shared climate.

However, substantial morphological variability, consistent with varietal-level variation, can exist among neighboring populations of *Eriogonum umbellatum* at serpentine sites (L. Janeway, pers. comm.; J.T. Columbus, pers. obs.). Other edaphic specialist *Eriogonum* species can demonstrate substantial morphological variation across edaphic gradients (McClinton & al., 2020). Extensive hybridization and introgression can occur between congeners of other families on serpentine soils, and among their sympatric populations, and be detected in SNP datasets (Kay & al., 2018). This might also occur among co-occurring varieties, since reproductive isolation is likely weaker than at the species level. Further study that takes advantage of a large number of SNP polymorphisms is needed to determine how morphology and occurrence on serpentine soils maps to lineage diversity within *E. umbellatum* and to resolve uncertainties at the species level.

Intraspecific variation and conservation. — Ancestral group analysis with ADmixture shows ancestral groups largely coincide with varietal designation (Fig. 5), but suggests that additional varietal-level variation may exist within *Eriogonum umbellatum*. This analysis, the TreeMix analysis (Fig. 6), and previous observations (Reveal, 1989) indicate that varietal variation may coincide with edaphic characteristics, similar to the repeated evolution of ecotypes of *Heliosperma pusillum* on distinct soils (Trucchi & al., 2017) and the association of varieties of *E. calcareum* with edaphic variation (Brown & Mansfield, 2017). However, particular patterns of divergence and subsequent geneflow in *E. umbellatum* are not supported here (Fig. 6). This may be because the populations most influenced by such geneflow are not represented in our samples. The low support for particular relationships among populations of *E. umbellatum* var. *nevadense* (Fig. 6) is consistent with generally higher levels of admixture in this variety (Fig. 5). Both admixture, resulting from incomplete lineage sorting, and subsequent hybridization and introgression can blur the boundaries between recognized species-level taxa (Eaton & al., 2015), and such lack of resolution is even more likely among recently diverged intraspecific lineages. The relative contributions of these two processes may be clarified with development of new SNP datasets that can be used to analyze a larger sample of *E. umbellatum* var. *nevadense* populations, as well as populations of additional varieties.

A clear understanding of the extent and structure of genetic variation, and a stable taxonomy that reflects it, are key components for developing effective policy for biodiversity conservation (Samper, 2004). We observed genetic structure in *Eriogonum umbellatum* that is not recognized in the current taxonomy of the species (Figs. 3, 5). Other narrowly restricted varieties of the species have not been recognized as having conservation importance (Reveal, 1981), but study of genetic variation at the level of variety within Eriogoneae is generally lacking. One exception is the support for designated varieties within *E. corymbosum* that is provided by AFLP loci (Ellis & al., 2009). Other genetic analyses have revealed low genetic variation in narrowly restricted varieties of some *Eriogonum* species, suggesting special conservation measures may be necessary (Riley & al., 2019). The greater sensitivity attained with thousands of SNP loci compared to other marker systems may also provide resolution of lineage structure where work with other markers has not, as with the lack of clear genetic support for lineage structure among morphologically distinguishable varieties in two species, *E. ovalifolium* and *E. shockleyi* (Archibald & al., 2001; J.F. Smith & Bateman, 2002). Further, molecular data support the species status of *E. calcareum*, previously a variety within *E. ochrocephalum* (Grady & Reveal, 2011). Additional varietal variation within *E. calcareum* is supported by differences in ecology and distribution (Brown & Mansfield, 2017), which depending on the level of threat and genetic diversity within those populations could also have conservation ramifications.

Kempton (2012) finds *Eriogonum congdonii* strongly supported as a species that is closely related to *E. siskiyouense*. Nonetheless, in that work the placement of these taxa in relationship to *E. umbellatum* is unclear. In our phylogenetic estimate, *E. congdonii* appears strongly supported as intraspecific variation within *E. umbellatum* (Figs. 1–3). This leaves open the question as to whether *E. siskiyouense* also constitutes variation within *E. umbellatum*, as we were unable to include this species in our analysis. While a wealth of methodologies are available to delimit morphologically similar species, it is better to err on the conservative side to avoid delimiting as species entities that are not principally distinct evolutionary lineages (Carstens & al., 2013). Nonetheless, narrowly restricted varieties within *Eriogonum* species can also attract substantial conservation interest and should be supported by genetic evidence.

Identification of species-level and intraspecific variation, and accurate taxonomy are important because of the use of lists of taxa in the development of conservation priorities (Mace, 2004). Accurate identification of lineage structure within species is also important for management planning in the light of ongoing climate change. The inclusion of environmental preferences and geographic variation among intraspecific lineages can influence niche quantification and models of the potential impacts of ongoing climate change on the future distributions of species (Oney & al., 2013; A.B. Smith & al., 2019). In *Eriogonum*, niche optimum tends to evolve more quickly in lineages of *Eriogonum* with annual species while niche breadth tends to evolve more rapidly in perennial lineages (Kostikova & al., 2013). Further, some perennial Eriogoneae species have broad niches and dozens of varieties, many of which are locally distributed or narrowly endemic, and some evidence for climate change impacts on *Eriogonum* species exists (Kopp & Cleland, 2014). Incorporation of accurate lineage structure could improve models of potential climate impacts on *Eriogonum* species, helping to refine analyses of taxa in need of focused conservation action.

■ CONCLUSIONS

Eriogoneae species constitute one of the most diverse groups in western North America, yet our understanding of

their evolutionary relationships is still a work in progress. Cryptic variation likely remains within widely distributed Eriogoneae species, and probably consists of both species-level and varietal variation. Our analysis of SNP datasets derived from GBS sequencing substantiates the non-monophyly of *Eriogonum* and *Chorizanthe*, and suggests the need for a new classification of Eriogoneae. Our results suggest that *E. umbellatum* is not monophyletic but that most samples resolve in the core *E. umbellatum* clade. Most relationships among the varieties of *E. umbellatum* remain unclear, although *E. umbellatum* var. *minus* and var. *munzii* are closely related. The GBS data support both the placement of *E. congdonii* within *E. umbellatum*, and the assessment that at least three varieties of *E. umbellatum* represent lineages more closely related to the *E. ursinum* clade, composed of three serpentine specialists. Expanded sampling of these varieties and *E. congdonii* is necessary to confirm their phylogenetic positions and make taxonomic recommendations. Recognition of some *E. umbellatum* varieties as representing species-level variation likely will have conservation implications. Additional development of SNP datasets with reduced representation sequencing and other approaches, and construction of additional genetic resources, would help clarify evolutionary relationships and entities for conservation prioritization among the Eriogoneae taxa.

■ AUTHOR CONTRIBUTIONS

PBP and JTC designed the study and conducted field collecting. JTC conducted taxon determination. TSA and J-RPT trimmed and assembled GBS sequence data. PBP produced the phylogenetic analysis with JTC. PBP and JTC interpreted the data and wrote the manuscript with contributions by TSA and J-RPT. — PBP, https://orcid.org/0000-0002-0794-101X; TSA, https://orcid.org/0000-0002-2960-5420; J-RPT, https://orcid.org/0000-0001-9548-8165; JTC, https://orcid.org/0000-0001-6949-0245

■ ACKNOWLEDGMENTS

We thank SGIker research support services at the University of the Basque Country, Leioa, Spain for DNA extraction and quality control, and acknowledge Centro Nacional de d-Anàlisi Genòmica in Barcelona, Spain for GBS sequencing. We thank J. Andre, N.J. Jensen and J. Steele for contributing samples and D. Marino for collaboration in nucleic acid extractions. We acknowledge the contributions of O. Lao Grueso to the ADmixture and TreeMix analyses. We thank U.S. Forest Service employees D. Austin, J. Fedorchuk, M. Friend, J. Haas, D. Ikeda, L. Janeway, J. Nelson, D. Netz, A. Sanger, and S. Weis among others for facilitating permitting and collecting. This work was supported by funds from the Basque Government in support of the Terrestrial Plant Diversity group of the Department of Plant Biology and Ecology, University of the Basque Country, and an ERC Advanced Grant, FP7-IDEAS-ERC, 'ADAPT', project 339941 awarded to T. Brown.

■ LITERATURE CITED

Alexander, D.H., Novembre, J. & Lange, K. 2009. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* 19: 1655–1664. https://doi.org/10.1101/gr.094052.109

Andrews, K.R., Good, J.M., Miller, M.R., Luikart, G. & Hohenlohe, P.A. 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. *Nat. Rev. Genet.* 17: 81–92. https://doi.org/10.1038/nrg.2015.28

Anonymous 2017. *2017 final BLM NV sensitive and special species status list* [as of 1 Oct 2017; pdf]. https://www.blm.gov/policy/nv-im-2018-003 (accessed 12 May 2020).

Applequist, W.L. 2014. Report of the Nomenclature Committee for Vascular Plants: 66. *Taxon* 63: 1358–1371. https://doi.org/10.12705/636.20

Archibald, J.K., Wolf, P.G., Tepedino, V.J. & Bair, J. 2001. Genetic relationships and population structure of the endangered Steamboat buckwheat, *Eriogonum ovalifolium* var. *williamsiae* (Polygonaceae). *Amer. J. Bot.* 88: 608–615. https://doi.org/10.2307/2657059

Ball, A.D., Stapley, J., Dawson, D.A., Birkhead, T.R., Burke, T. & Slate, J. 2010. A comparison of SNPs and microsatellites as linkage mapping markers: Lessons from the zebra finch (*Taeniopygia guttata*). *B. M. C. Genomics* 11: 218. https://doi.org/10.1186/1471-2164-11-218

Bolger, D.T., Suarez, A.V., Crooks, K.R., Morrison, S.A. & Case, T.J. 2000. Arthropods in urban habitat fragments in southern California: Area, age, and edge effects. *Ecol. Applic.* 10: 1230–1248. https://doi.org/10.1890/1051-0761(2000)010[1230:Aiuhfi]2.0.Co;2

Brady, K.U., Kruckeberg, A.R. & Bradshaw, H.D. 2005. Evolutionary ecology of plant adaptation to serpentine soils. *Annual Rev. Ecol. Evol. Syst.* 36: 243–266. https://doi.org/10.1146/annurev.ecolsys.35.021103.105730

Brown, B.J. & Mansfield, D.H. 2017. Edaphic and geographic origins of varietal differentiation in *Eriogonum calcareum*. *Madroño* 64: 22–31. https://doi.org/10.3120/0024-9637-64.1.22

Brumfield, R.T., Beerli, P., Nickerson, D.A. & Edwards, S.V. 2003. The utility of single nucleotide polymorphisms in inferences of population history. *Trends Ecol. Evol.* 18: 249–256. https://doi.org/10.1016/s0169-5347(03)00018-1

California Native Plant Society Rare Plant Program 2020. Inventory of rare and endangered plants of California (online edition, v8-03 0.39). http://www.rareplants.cnps.org/ (accessed 7 May 2020).

California Natural Diversity Database 2020. *Special vascular plants, bryophytes, and lichens list*, September 2020. Sacramento: California Department of Fish and Wildlife. https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=109383&inline (pdf, accessed 1 Oct 2020).

Cariou, M., Duret, L. & Charlat, S. 2013. Is RAD-seq suitable for phylogenetic inference? An in silico assessment and optimization. *Ecol. Evol.* 3: 846–852. https://doi.org/10.1002/ece3.512

Carstens, B.C., Pelletier, T.A., Reid, N.M. & Satler, J.D. 2013. How to fail at species delimitation. *Molec. Ecol.* 22: 4369–4383. https://doi.org/10.1111/mec.12413

Chang, C.C., Chow, C.C., Tellier, L., Vattikuti, S., Purcell, S.M. & Lee, J.J. 2015. Second-generation PLINK: Rising to the challenge of larger and richer datasets. *GigaScience* 4(1): s13742-015-0047-8. https://doi.org/10.1186/s13742-015-0047-8

Costello, M.J., Vanhoorne, B. & Appeltans, W. 2015. Conservation of biodiversity through taxonomy, data publication, and collaborative infrastructures. *Conservation Biol.* 29: 1094–1099. https://doi.org/10.1111/cobi.12496

Damschen, E.I., Harrison, S., Going, B.M. & Anacker, B.L. 2011. Climate change and plant communities on unusual soils. Pp. 359–381 in: Harrison, S. & Rajakaruna, N. (eds.), *Serpentine: The evolution and ecology of a model system*. Berkeley: University of California Press.

Eaton, D.A.R., Hipp, A.L., Gonzalez-Rodriguez, A. & Cavender-Bares, J. 2015. Historical introgression among the American live oaks and the comparative nature of tests for introgression. *Evolution* 69: 2587–2601. https://doi.org/10.1111/evo.12758

Ellis, M.W., Roper, J.M., Gainer, R., Der, J.P. & Wolf, P.G. 2009. The taxonomic designation of *Eriogonum corymbosum* var. *nilesii*

(Polygonaceae) is supported by AFLP and cpDNA analyses. *Syst. Bot.* 34: 693–703. https://doi.org/10.1600/036364409790139736

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S. & Mitchell, S.E. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE* 6: e19379. https://doi.org/10.1371/journal.pone.0019379

Gandoger, M. 1906. Le genre *Eriogonum* (Polygonaceae). *Bull. Soc. Roy. Bot. Belgique* 42: 183–200.

Gompert, Z. & Buerkle, C.A. 2013. Analyses of genetic ancestry enable key insights for molecular ecology. *Molec. Ecol.* 22: 5278–5294. https://doi.org/10.1111/mec.12488

Grady, B.R. 2012. *From molecular phylogenetics to the evolution of life history and edaphic endemism*: *A comprehensive appraisal of evolution in Eriogonum (Polygonaceae)*. Dissertation. University of Wisconsin-Madison, U.S.A.

Grady, B.R. & Reveal, J.L. 2011. New combinations and a new species of *Eriogonum* (Polygonaceae: Eriogonoideae) from the Great Basin Desert, United States. *Phytotaxa* 24: 33–38. https://doi.org/10.11646/phytotaxa.24.1.5

Jeffries, D.L., Copp, G.H., Handley, L.L., Olsen, K.H., Sayer, C.D. & Hanfling, B. 2016. Comparing RADseq and microsatellites to infer complex phylogeographic patterns, an empirical perspective in the Crucian carp, *Carassius carassius*, L. *Molec. Ecol.* 25: 2997–3018. https://doi.org/10.1111/mec.13613

Kalinowski, S.T. 2011. The computer program STRUCTURE does not reliably identify the main genetic clusters within species: Simulations and implications for human population structure. *Heredity* 106: 625–632. https://doi.org/10.1038/hdy.2010.95

Kay, K.M., Woolhouse, S., Smith, B.A., Pope, N.S. & Rajakaruna, N. 2018. Sympatric serpentine endemic *Monardella* (Lamiaceae) species maintain habitat differences despite hybridization. *Molec. Ecol.* 27: 2302–2316. https://doi.org/10.1111/mec.14582

Kempton, E.A. 2012. Systematics of Eriogonoideae s.s. (Polygonaceae). *Syst. Bot.* 37: 723–737. https://doi.org/10.1600/036364412x648698

Kim, B.Y., Wei, X.Z., Fitz-Gibbon, S., Lohmueller, K.E., Ortego, J., Gugger, P.F. & Sork, V.L. 2018. RADseq data reveal ancient, but not pervasive, introgression between Californian tree and scrub oak species (*Quercus* sect. *Quercus*: Fagaceae). *Molec. Ecol.* 27: 4556–4571. https://doi.org/10.1111/mec.14869

Klimova, A., Ortega-Rubio, A., Vendrami, D.L.J. & Hoffman, J.I. 2018. Genotyping by sequencing reveals contrasting patterns of population structure, ecologically mediated divergence, and long-distance dispersal in North American palms. *Ecol. Evol.* 8: 5873–5890. https://doi.org/10.1002/ece3.4125

Kopp, C.W. & Cleland, E.E. 2014. Shifts in plant species elevational range limits and abundances observed over nearly five decades in a western North America mountain range. *J. Veg. Sci.* 25: 135–146. https://doi.org/10.1111/jvs.12072

Kostikova, A., Litsios, G., Salamin, N. & Pearman, P.B. 2013. Linking life-history traits, ecology, and niche breadth evolution in North American Eriogonoids (Polygonaceae). *Amer. Naturalist* 182: 760–774. https://doi.org/10.1086/673527

Kostikova, A., Litsios, G., Burgy, S., Milani, L., Pearman, P.B. & Salamin, N. 2014. Scale-dependent adaptive evolution and morphological convergence to climatic niche in Californian eriogonoids (Polygonaceae). *J. Biogeogr.* 41: 1326–1337. https://doi.org/10.1111/jbi.12294

Kuyper, K.F., Yandell, U. & Nowak, R.S. 1997. On the taxonomic status of *Eriogonum robustum* (Polygonaceae), a rare endemic in western Nevada. *Great Basin Naturalist* 57: 1–10.

Legendre, P. & Legendre, L. 1998. *Numerical ecology*, 2nd ed. Amsterdam: Elsevier.

Lemon, J.B. & Wolf, P.G. 2018. Genetic differentiation between endemic *Eriogonum soredium* and its common relative *E. shockleyi* (Polygonaceae). *Syst. Bot.* 43: 901–909. https://doi.org/10.1600/036364418x697797

Lexer, C., Wueest, R.O., Mangili, S., Heuertz, M., Stoelting, K.N., Pearman, P.B., Forest, F., Salamin, N., Zimmermann, N.E. & Bossolini, E. 2014. Genomics of the divergence continuum in an African plant biodiversity hotspot, I: Drivers of population divergence in *Restio capensis* (Restionaceae). *Molec. Ecol.* 23: 4373–4386. https://doi.org/10.1111/mec.12870

Li, H. & Durbin, R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25: 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R. & 1000 Genome Project Data Processing Subgroup 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25: 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Lischer, H.E.L., Excoffier, L. & Heckel, G. 2014. Ignoring heterozygous sites biases phylogenomic estimates of divergence times: Implications for the evolutionary history of *Microtus* voles. *Molec. Biol. Evol.* 31: 817–831. https://doi.org/10.1093/molbev/mst271

Mace, G.M. 2004. The role of taxonomy in species conservation. *Philos. Trans., Ser. B* 359: 711–719. https://doi.org/10.1098/rstb.2003.1454

McClinton, J.D., Parchman, T.L., Torrence, K.L., Verburg, P.S. & Leger, E.A. 2020. How specialized is a soil specialist? Early life history responses of a rare *Eriogonum* to site-level variation in volcanic soils. *Amer. J. Bot.* 107: 1663–1676. https://doi.org/10.1002/ajb2.1582

Medrano, M., Herrera, C.M. & Bazaga, P. 2014. Epigenetic variation predicts regional and local intraspecific functional diversity in a perennial herb. *Molec. Ecol.* 23: 4926–4938. https://doi.org/10.1111/mec.12911

Melo, A.T.O., Bartaula, R. & Hale, I. 2016. GBS-SNP-CROP: A reference-optional pipeline for SNP discovery and plant germplasm characterization using variable length, paired-end genotyping-by-sequencing data. *B. M. C. Bioinf.* 17: 29. https://doi.org/10.1186/s12859-016-0879-y

Nevada Division of Natural Heritage 2021. At-Risk Plant and Animal Tracking List, last updated January, 2021, http://dcnr.nv.gov/uploads/heritage/2021-01-Track-List-Jan-2021.pdf (accessed 17 May 2021).

Oney, B., Reineking, B., O'Neill, G. & Kreyling, J. 2013. Intraspecific variation buffers projected climate change impacts on *Pinus contorta*. *Ecol. Evol.* 3: 437–449. https://doi.org/10.1002/ece3.426

Oritz, E.M. 2019. vcf2phylip v2.0: Convert a VCF matrix into several matrix formats for phylogenetic analysis. https://doi.org/10.5281/zenodo.2540861 (accessed 28 Apr 2019).

Pante, E., Abdelkrim, J., Viricel, A., Gey, D., France, S.C., Boisselier, M.C. & Samadi, S. 2015. Use of RAD sequencing for delimiting species. *Heredity* 114: 450–459. https://doi.org/10.1038/hdy.2014.105

Pauls, S.U., Nowak, C., Balint, M. & Pfenninger, M. 2013. The impact of global climate change on genetic diversity within populations and species. *Molec. Ecol.* 22: 925–946. https://doi.org/10.1111/mec.12152

Pearman, P.B. 2001. Conservation value of independently evolving units: Sacred cow or testable hypothesis? *Conservation Biol.* 15: 780–783. https://doi.org/10.1046/j.1523-1739.2001.015003780.x

Pickrell, J.K. & Pritchard, J.K. 2012. Inference of population splits and mixtures from genome-wide allele frequency data. *PLoS Genet.* 8(11): e1002967. https://doi.org/10.1371/journal.pgen.1002967

R Core Team 2020. *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Reveal, J.L. 1968. Some nomenclatural changes in *Eriogonum* (Polygonaceae). *Taxon* 17: 531–533. https://doi.org/10.2307/1216054

Reveal, J.L. 1981. Notes on endangered buckwheats (*Eriogonum*, Polygonaceae) with three newly described from the western United States. *Brittonia* 33: 441–448. https://doi.org/10.2307/2806434

Reveal, J.L. 1989. New combinations and novelties in *Eriogonum* (Polygonaceae: Eriogonoideae). *Phytologia* 66: 251–265. https://doi.org/10.5962/bhl.part.27514

Reveal, J.L. 2005. Polygonaceae Jussieu subfam. Eriogonoideae Arnott. Pp. 218–478 in: Flora of North America Editorial Committee (ed.), *Flora of North America North of Mexico*, vol. 5. New York: Oxford University Press.

Reveal, J.L. 2006. *Eriogonum callistum* (Polygonaceae), a new species from the Tehachapi Mountains of California. *Sida* 22: 857–861.

Reveal, J.L. 2012. *Eriogonum*. Pp. 1083–1111 in: Baldwin, B.G. (ed.), *The Jepson manual: Vascular plants of California*, 2nd ed. Berkeley: University of California.

Riley, L., McGlaughlin, M.E. & Helenurm, K. 2016. Narrow water barriers prevent multiple colonizations and limit gene flow among California Channel Island wild buckwheats (*Eriogonum*: Polygonaceae). *Bot. J. Linn. Soc.* 181: 246–268. https://doi.org/10.1111/boj.12415

Riley, L., McGlaughlin, M.E. & Helenurm, K. 2019. Limited genetic variability in native buckwheats (*Eriogonum*: Polygonaceae) on San Clemente Island. *W. N. Amer. Naturalist* 78: 722–738. https://doi.org/10.3398/064.078.0413

Rognes, T., Flouri, T., Nichols, B., Quince, C. & Mahe, F. 2016. VSEARCH: A versatile open source tool for metagenomics. *PeerJ* 4: e2584. https://doi.org/10.7717/peerj.2584

Rojas, M. 1992. The species problem and conservation—What are we protecting? *Conservation Biol.* 6: 170–178. https://doi.org/10.1046/j.1523-1739.1992.620170.x

Safford, H.D. & Miller, J.E.D. 2020. An updated database of serpentine endemism in the California flora. *Madroño* 67: 85–104. https://doi.org/10.3120/0024-9637-67.2.85

Safford, H.D., Viers, J.H. & Harrison, S.P. 2005. Serpentine endemism in the California flora: A database of serpentine affinity. *Madroño* 52: 222–257. https://doi.org/10.3120/0024-9637(2005)52[222:SEITCF]2.0.CO;2

Samper, C. 2004. Taxonomy and environmental policy. *Philos. Trans., Ser. B* 359: 721–728. https://doi.org/10.1098/rstb.2004.1476

Sanchez, A. & Kron, K.A. 2008. Phylogenetics of Polygonaceae with an emphasis on the evolution of Eriogonoideae. *Syst. Bot.* 33: 87–96. https://doi.org/10.1600/036364408783887456

Schilling, M.P., Gompert, Z., Li, F.W., Windham, M.D. & Wolf, P.G. 2018. Admixture, evolution, and variation in reproductive isolation in the *Boechera puberula* clade. *B. M. C. Evol. Biol.* 18: 16. https://doi.org/10.1186/s12862-018-1173-6

Scrucca, L., Fop, M., Murphy, T.B. & Raftery, A.E. 2016. mclust 5: Clustering, classification and density estimation using Gaussian finite mixture models. *R Journal* 8: 289–317.

Smith, A.B., Godsoe, W., Rodriguez-Sanchez, F., Wang, H.-H. & Warren, D. 2019. Niche estimation above and below the species level. *Trends Ecol. Evol.* 34: 260–273. https://doi.org/10.1016/j.tree.2018.10.012

Smith, J.F. & Bateman, T.A. 2002. Genetic differentiation of rare and common varieties of *Eriogonum shockleyi* (Polygonaceae) in Idaho using ISSR variability. *W. N. Amer. Naturalist* 62: 316–326.

Stamatakis, A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Theodoridis, S., Nogues-Bravo, D. & Conti, E. 2019. The role of cryptic diversity and its environmental correlates in global conservation status assessments: Insights from the threatened bird's-eye primrose (*Primula farinosa* L.). *Diversity & Distrib.* 25: 1457–1471. https://doi.org/10.1111/ddi.12953

Trucchi, E., Frajman, B., Haverkamp, T.H.A., Schönswetter, P. & Paun, O. 2017. Genomic analyses suggest parallel ecological divergence in *Heliosperma pusillum* (Caryophyllaceae). *New Phytol.* 216: 267–278. https://doi.org/10.1111/nph.14722

U.S. Fish & Wildlife Service 2020. Endangered species. https://www.fws.gov/endangered/species/us-species.html (accessed 1 Dec 2020).

Whitlock, R. 2014. Relationships between adaptive and neutral genetic diversity and ecological structure and functioning: A meta-analysis. *J. Ecol.* 102: 857–872. https://doi.org/10.1111/1365-2745.12240

Yang, Z.H. & Rannala, B. 2010. Bayesian species delimitation using multilocus sequence data. *Proc. Natl. Acad. Sci. U.S.A.* 107: 9264–9269. https://doi.org/10.1073/pnas.0913022107

Zhang, J.J., Kobert, K., Flouri, T. & Stamatakis, A. 2014. PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* 30: 614–620. https://doi.org/10.1093/bioinformatics/btt593

Appendix 1. Specimen information for samples included in the present study.

Taxon name, collection country (state), collector and collection number. All specimens housed in RSA. Data for all specimens were generated newly for this study. Additional specimen data can be found in supplementary Appendix S1.

Chorizanthe brevicornu Torr. var. ***brevicornu***, U.S.A. (California), *N.J. Jensen*, *3653*; ***Chorizanthe fimbriata*** Nutt. var. ***fimbriata***, U.S.A. (California), *N.J. Jensen*, *3796*; ***Chorizanthe membranacea*** Benth., U.S.A. (California), *J.L. Steele*, *419*; ***Chorizanthe obovata*** Goodman, U.S.A. (California), *N.J. Jensen*, *3668*; ***Chorizanthe palmeri*** S.Watson, U.S.A. (California), *N.J. Jensen*, *3670*; ***Chorizanthe robusta*** Parry, U.S.A. (California), *J.L. Steele*, *425*; ***Chorizanthe staticoides*** Benth., U.S.A. (California), *N.J. Jensen*, *3679*; ***Chorizanthe watsonii*** Torr. & A.Gray, U.S.A. (California), *N.J. Jensen*, *4209*; ***Chorizanthe xanti*** S.Watson, U.S.A. (California), *N.J. Jensen*, *4165*; ***Eriogonum baileyi*** S.Watson, U.S.A. (California), *J.L. Steele*, *421*; ***Eriogonum caespitosum*** Nutt., U.S.A. (California), *N.J. Jensen*, *3826*; U.S.A. (Nevada), *P.B. Pearman*, *70*; ***Eriogonum citharifórme*** var. ***agninum*** (Greene) Reveal, U.S.A. (California), *J.L. Steele*, *414*; ***Eriogonum compositum*** Benth. var. ***compositum***, U.S.A. (California), *P.B. Pearman*, *33*; ***Eriogonum congdonii*** (S.Stokes) Reveal, U.S.A. (California), *P.B. Pearman*, *31*; ***Eriogonum diclinum*** Reveal, U.S.A. (California), *J.L. Steele*, *436*; ***Eriogonum douglasii*** Benth., U.S.A. (California), *J.L. Steele*, *433*; ***Eriogonum elatum*** Benth. var. ***elatum***, U.S.A. (California), *N.J. Jensen*, *3816*; ***Eriogonum elatum*** Douglas ex Benth, U.S.A. (California), *N.J. Jensen*, *497*; ***Eriogonum elongatum*** Benth. var. ***elongatum***, U.S.A. (California), *N.J. Jensen*, *3672*; ***Eriogonum evanidum*** Reveal, U.S.A. (California), *P.B. Pearman*, *7*; ***Eriogonum fasciculatum*** var. ***polifolium*** (Benth.) Torr. & A.Gray, U.S.A. (California), *N.J. Jensen*, *3669*; ***Eriogonum giganteum*** S.Watson, U.S.A. (California), *J.L. Steele*, *423*; ***Eriogonum gracile*** Benth. var. ***gracile***, U.S.A. (California), *P.B. Pearman*, *11*; ***Eriogonum gracillimum*** S.Watson, U.S.A. (California), *J.L. Steele*, *417*; ***Eriogonum heracleoides*** Nutt. var. ***heracleoides***, U.S.A. (Nevada), *P.B. Pearman*, *71*; ***Eriogonum incanum*** Torr. & A.Gray, U.S.A. (California), *P.B. Pearman*, *55*; ***Eriogonum inflatum*** Torr. & Frém., U.S.A. (California), *N.J. Jensen*, *3656*; ***Eriogonum kennedyi*** var. ***purpusii*** (Brandegee) Reveal, U.S.A. (California), *N.J. Jensen*, *3828*; ***Eriogonum latens*** Jeps., U.S.A. (California), *J.T. Columbus*, *6060*; ***Eriogonum latifolium*** Sm., U.S.A. (California), *P.B. Pearman*, *17*; ***Eriogonum libertini*** Reveal, U.S.A. (California), *J.L. Steele*, *430*; ***Eriogonum lobbii*** Torr. & A.Gray, U.S.A. (California), *J.T. Columbus*, *6054*; ***Eriogonum luteolum*** M.E.Jones, U.S.A. (California), *J.L. Steele*, *431*; ***Eriogonum marifolium*** (Torr.) A.Gray var. ***marifolium***, U.S.A. (California), *P.B. Pearman*, *94*; ***Eriogonum microtheca*** var. ***ambiguum*** (M.E.Jones) Reveal, U.S.A. (California), *P.B. Pearman*, *53*; ***Eriogonum molle*** Green, U.S.A. (California), *N.J. Jensen*, *4562*; ***Eriogonum nervulosum*** (S.Stokes) Reveal, U.S.A. (California), *J.L. Steele*, *428*; ***Eriogonum nidularium*** Coville, U.S.A. (California), *N.J. Jensen*, *3655*; ***Eriogonum nudum*** Douglas ex Benth., U.S.A. (California), *J.L. Steele*, *427*; ***Eriogonum ochrocephalum*** S.Watson var. ***ochrocephalum***, U.S.A. (California), *P.B. Pearman*, *51*; ***Eriogonum palmerianum*** Reveal, U.S.A. (California), *P.B. Pearman*, *67*; ***Eriogonum parvifolium*** Sm., U.S.A. (California), *P.B. Pearman*, *16*; ***Eriogonum polypodum*** Small, U.S.A. (California), *J.T. Columbus*, *6058*; ***Eriogonum roseum*** Durand & Hilg., U.S.A. (California), *J.L. Steele*, *418*; ***Eriogonum saxatile*** S.Watson, U.S.A. (California), *P.B. Pearman*, *10*; ***Eriogonum***

Appendix 1. Continued.

sphaerocephalum var. ***halimioides*** (Gand.) S.Stokes, U.S.A. (California), *P.B. Pearman*, *26*; ***Eriogonum strictum*** var. ***greenei*** (A.Gray) Reveal, U.S.A. (California), *P.B. Pearman*, *45*; ***Eriogonum umbellatum*** var. ***argus*** Reveal, U.S.A. (California), *P.B. Pearman*, *96*; ***Eriogonum umbellatum*** var. cf. ***argus*** Reveal, U.S.A. (California), *P.B. Pearman*, *93*; ***Eriogonum umbellatum*** var. ***bahiiforme*** (Torr. & A.Gray) Jeps., U.S.A. (California), *P.B. Pearman*, *21*; ***Eriogonum umbellatum*** var. ***chlorothamnus*** Reveal, U.S.A. (California), *P.B. Pearman*, *128*; ***Eriogonum umbellatum*** var. ***dichrocephalum*** Gand., U.S.A. (California), *P.B. Pearman*, *125*; ***Eriogonum umbellatum*** var. ***dumosum*** Reveal, U.S.A. (California), *P.B. Pearman*, *37*; ***Eriogonum umbellatum*** var. ***furcosum*** Reveal, U.S.A. (California), *J.T. Columbus*, *6056*, *P.B. Pearman 22*, *23*, *24*, *49*, *114*, *122*; ***Eriogonum umbellatum*** var. ***glaberrinum*** (Gand.) Reveal, U.S.A. (California), *P.B. Pearman*, *86*; ***Eriogonum umbellatum*** var. cf. ***goodmanii*** Reveal, U.S.A. (California), *P.B. Pearman*, *35*; ***Eriogonum umbellatum*** var. ***humistratum*** Reveal, U.S.A. (California), *P.B. Pearman*, *34*; ***Eriogonum umbellatum*** var. **indet.**, U.S.A. (California), *P.B. Pearman*, *36*, *40*, *41*; ***Eriogonum umbellatum*** var. ***juniporinum*** Reveal, U.S.A. (California), *P.B. Pearman*, *64*; ***Eriogonum umbellatum*** var. ***minus*** I.M.Johnst., U.S.A. (California), *P.B. Pearman*, *12*; ***Eriogonum umbellatum*** var. cf. ***modocense*** (Greene) S.Stokes, U.S.A. (California), *P.B. Pearman*, *25*; ***Eriogonum umbellatum*** var. ***munzii*** Reveal, U.S.A. (California), *P.B. Pearman*, *1*, *4*, *8*; ***Eriogonum umbellatum*** var. ?***munzii*** Reveal, U.S.A. (California), *P.B. Pearman*, *15*; ***Eriogonum umbellatum*** var. cf. ***nelsoniorum*** Reveal, U.S.A. (California), *P.B. Pearman*, *48*; ***Eriogonum umbellatum*** var. ***nevadense*** Gand., U.S.A. (California), *P.B. Pearman*, *56*, *58*, *111*, *115*, *120*, *123*; U.S.A. (Nevada), *P.B. Pearman*, *74*, *75*, *77*; ***Eriogonum umbellatum*** var. ***smallianum*** (A.Heller) S.Stokes, U.S.A. (California), *P.B. Pearman*, *99*, *103*; ***Eriogonum umbellatum*** var. ***subaridum*** S.Stokes, U.S.A. (California), *P.B. Pearman*, *127*; ***Eriogonum umbellatum*** var. ***versicolor*** S.Stokes, U.S.A. (California), *P.B. Pearman*, *124*; ***Eriogonum ursinum*** S.Watson var. ***ursinum***, U.S.A. (California), *P.B. Pearman*, *50*; ***Eriogonum wrightii*** var. ***membranaceum*** Jeps., U.S.A. (California), *P.B. Pearman*, *3*; ***Sidotheca trilobata*** (A.Gray) Reveal, U.S.A. (California), *N.J. Jensen*, *4202*.