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Diversity among rare and common congeneric plant species from the Garry oak and Okanagan shrub-steppe ecosystems in British Columbia: implications for conservation

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Abstract

It is often assumed that the northern peripheral populations of species' ranges are genetically depauperate due in part to founder effects from postglacial colonization. The majority of federally protected plant species are peripheral in Canada, yet we have little information about their patterns of genetic diversity and structure. In British Columbia, the majority of these protected plant species occur in two threatened habitats: the Garry oak and Okanagan shrub-steppe ecosystems. Using universal noncoding chloroplast DNA markers, we investigated genetic diversity and genetic structure in four rare and common plant species pairs inhabiting these two ecosystems. We found that rare species had lower genetic diversity than their common congeners, and detected contrasting patterns of regional diversity and structure based on ecosystem. Species from the Garry oak ecosystem showed lower genetic diversity in the northern deglaciated region and significant differentiation between regions, likely due to limited dispersal between Vancouver Island and the mainland. Species from the Okanagan shrub-steppe, however, tended to have uniform diversity across their range and lack regional structure. This study provides an important first look at the phylogeographic patterns of four rare plant species in British Columbia.

Key words: conservation genetics, British Columbia, rare plants, chloroplast DNA, phylogeography, genetic diversity, Garry oak ecosystem, shrub-steppe ecosystem

Introduction

Genetic diversity is critical to the long-term viability of wild plant and animal species (Frankham 1996; Hughes et al. 2008) and is considered a prerequisite for adaptation to changing environmental conditions such as those anticipated with climate change (Barrett and Schluter 2008). In previously glaciated landscapes, including those of western North America, a long-held assumption is that populations at the northern extreme of the geographic range of species are genetically depauperate due to founder effects after colonization from southern populations or glacial refugia that followed the retreat of the Cordilleran ice sheet (Soltis et al. 1997; Hewitt 2000; Shafer et al. 2010). The central-marginal hypothesis (Eckert et al. 2008; Pironon et al. 2017) predicts that populations at the edges of species distributions will have lower genetic diversity and higher differentiation relative to central populations. To the extent that the rate of adaptation is limited by genetic diversity, low genetic diversity may render peripheral northern populations in western North America less able to respond to climate change and habitat modification. The factors influencing patterns of genetic diversity in core vs. peripheral populations are complex, but investigating trends across glacial divides can provide clues as to how genetic variation in different species and ecosystems has been shaped by historical shifts in climate and geography.

Roughly 80% of federally protected plant species are considered peripheral, with less than 20% of their global range in Canada (Caissy et al. 2020). This pattern is especially notable for at-risk plants in British Columbia (BC) and Ontario,

which together represent roughly two-thirds of Canada's peripheral plants at risk (Caissy et al. 2020). In BC, many rare plant species that occur in the coastal Garry oak and interior Okanagan shrub-steppe ecosystems are peripheral (occurring at the northern margin of their ranges, with the centres of their ranges typically situated further south). Caissy et al. (2020) found that 67% of Canada's at-risk plants are considered globally secure and that this rank disparity is greater among plants that are peripheral in Canada as compared with those that are not. Although the reasons for rarity or decline in species are varied (Caughley 1994), there have been dramatic losses of habitat in both Garry oak and Okanagan shrub-steppe ecosystems due to human activities, including agriculture, urbanization, and alteration of fire regimes (Blackstock and McAllister 2004; Gedalof et al. 2006). The large decline in the extent of high-quality oak savannah and shrub-steppe habitat has led to population losses and population fragmentation, resulting in less common species becoming threatened with extirpation from Canada.

Understanding the structure of genetic diversity and its relationship to the landscape is important for the sustainable management of rare plants. However, conservation practitioners have identified patterns of genetic diversity as a knowledge gap in many federal recovery strategies developed under the Canadian Species at Risk Act (S.C. 2002, c.29, https://laws.justice.gc.ca/eng/acts/S-15.3/) and species assessments produced by the Committee on the Status of Endangered Wildlife in Canada (www.cosewic.ca).

Addressing a lack of understanding of the patterns of genetic diversity in rare plants is essential for species assessments, recovery planning, and implementation of phases of species recovery. For example, northern peripheral populations of rare species may exhibit reduced genetic diversity as a result of founder effects and only represent a subset of the genetic diversity found in the southern portions of species range. Rare species may also exhibit lower genetic diversity relative to common species due to genetic drift and population bottlenecks associated with population declines and fragmentation (Gitzendanner and Soltis 2000), compounding the challenges faced by northern populations. For species that normally outcross, these population processes can result in a loss of heterozygosity and an expression of inbreeding depression (Reed and Frankham 2003), which can then contribute to further declines in the number of individuals, populations, or geographic range (i.e., the extinction vortex; Gilpin and Soule 1986; Fagan and Holmes 2006). Furthermore, the lack of genetic diversity can exacerbate the vulnerability of these populations to climate change and habitat loss and lead to local extirpation (Ellstrand and Elam 1993).

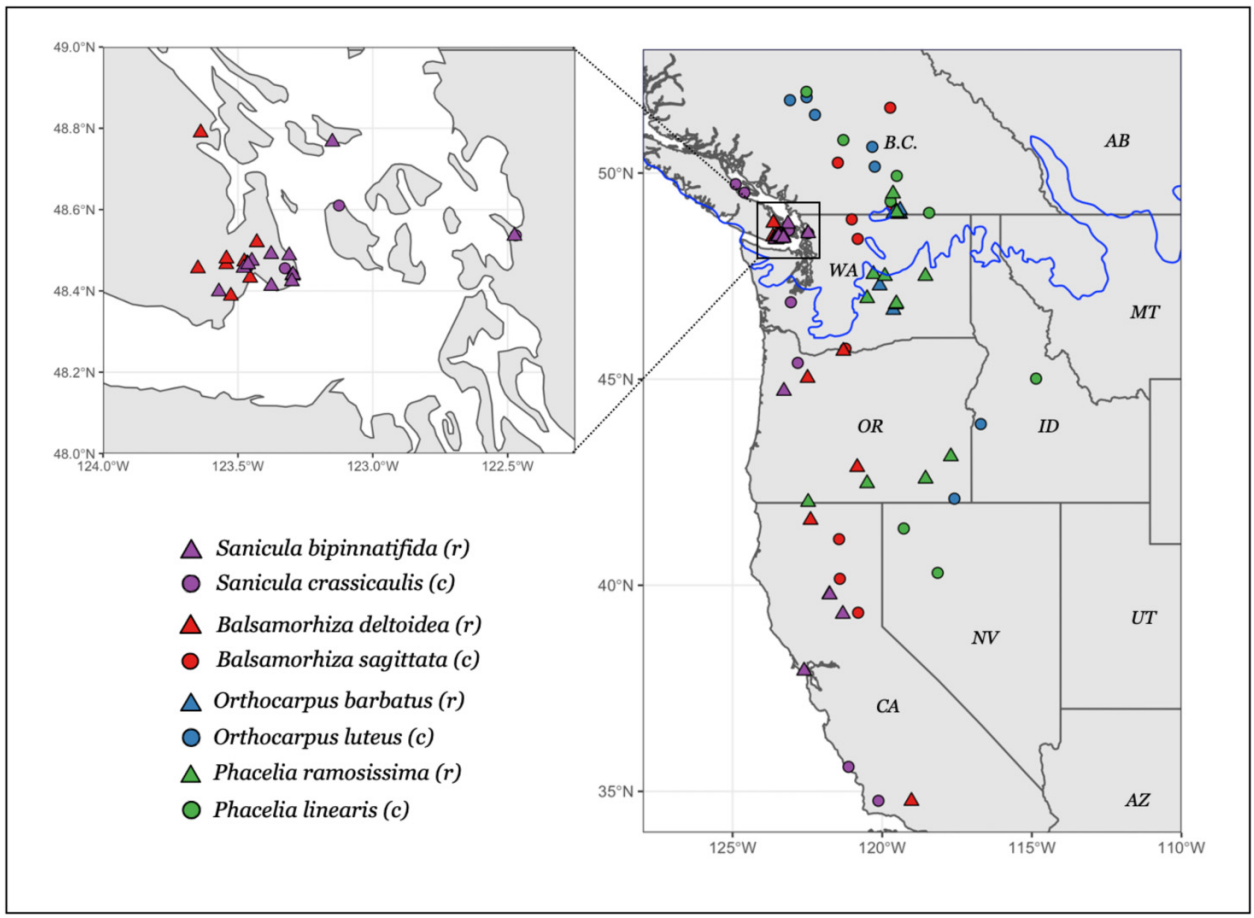
Practically, there are substantial logistical and financial barriers to collecting genetic data for nonmodel organisms that make the analysis of genetic diversity inaccessible to most conservation practitioners. Travel throughout species ranges to collect material for analysis is expensive and time consuming, and though the development of hypervariable genetic markers (e.g., microsatellites) has become cheaper and more efficient, they still require nontrivial amounts of time, money, and expertise (Kalia et al. 2011; Xia et al. 2018). Universal markers do exist for angiosperms (e.g., next-generation sequencing (NGS) of protein-coding plastid genes; Ruhfel et al. 2014), but these are typically prohibitive in cost. To address these challenges, Shaw et al. (2014) recommended a set of eight noncoding chloroplast DNA (cpDNA) regions that are rapidly evolving and sufficiently variable to capture snapshots of intraspecific diversity. The primer sequences targeting these regions are highly conserved across angiosperm families, so are broadly applicable taxonomically. While the maternal inheritance and overall low recombination rate of the chloroplast can lead to poor resolution in low-level studies (Yan et al. 2018), the use of several cpDNA regions is expected to increase the discriminatory power of the plastome (Shaw et al. 2014).

It is important to note that these noncoding cpDNA regions are assumed to be selectively neutral, though they are part of the non-recombining plastid genome, whose genes are largely under purifying selection. While the importance of neutral genetic variation in conservation is currently under debate, with some arguing that the identification of adaptive variation should be prioritized for conservation (Teixeira and Huber 2021), others maintain that conserving genome-wide genetic variation (much of which is presumably neutral) provides a reasonable proxy that can be used to assess genetic risks such as inbreeding depression and loss of adaptive potential (Fernandez-Fournier et al. 2021; Kardos et al. 2021).

We used cpDNA loci identified by Shaw et al. (2014) to conduct an initial range-wide survey of neutral genetic variation in select plant species that are of conservation concern in BC, but considered secure in other parts of their range. Our particular aim was to test the prediction that these species are genetically depauperate and distinct at their northern range edge. To assess whether cpDNA loci were broadly informative at this geographical scale, we chose two plant species from each of four different eudicot families (Fig. 1). We chose species from two distinct hotspots for rare species in western North America: the coastal Garry oak savannah ecosystem and the interior Okanagan shrub-steppe ecosystem (see ecosystem map in Fig. S1). Because reduced genetic diversity in the north may be due to founder effects after postglacial expansion or genetic drift and bottleneck effects in small populations, we included a common congener of each rare species to act as a control (Gitzendanner and Soltis 2000). This strategy provides a comparison of the distribution of genetic variation between closely related rare and common species. Because common species have larger population sizes and less fragmented distributions, we expect that they will be less affected by bottlenecks and drift than their rare relatives, but equally influenced by founder effects.

Although noncoding cpDNA markers have been available for over a decade, they have not been used to investigate the distribution of genetic diversity within and among lineages and ecosystems in Canada. Despite the ongoing debate about the importance of conserving peripheral populations, no genetic or phylogenetic studies of any kind have been conducted for most of Canada's rare plants (Caissy et al. 2020). By employing methods that are generally accessible to conservation practitioners, and including tissue samples from herbarium specimens, our findings may provide a template for a

Fig. 1. Map of sampling locations for each species included in this study. The blue line indicates the extent of the last glacial maximum of the Cordilleran ice sheet, which was used to group samples into "north" and "south" regions for analysis. Species names are appended with their abundance in British Columbia (BC), with (r) indicating rare and (c) indicating common. The map was made in R using the packages SF and GGPLOT2.



broader comparative study that could help set the direction for future recovery and restoration activities.

Methods

Study ecosystems

Coastal Garry oak ecosystem

Garry oak (*Quercus garryana*) is a species of white oak found from southwestern BC to southern California in the USA (Douglas et al. 1999; Nixon 2020), where it is also known as Oregon white oak. In Canada, ecosystems that include Garry oak trees are found within the Georgia Depression Ecoprovince (Demarchi 2011), along the southeast coast of Vancouver Island, adjacent to Gulf Islands, and two sites on the southwestern BC mainland (Lea 2006). Garry oak ecosystems are endangered in Canada with less than 10% remaining and less than 5% undisturbed relative to their abundance at European settlement (Lea 2006). Declines are largely due to agricultural conversion and residential development, but the introduction of invasive plant species, fire suppression, and the subsequent encroachment of coniferous trees have degraded Garry oak habitats and remain key threats (Fuchs 2001; Lea 2006). The loss of traditional First Nations cultural practices is likely a key driver of these changes (Barlow et al. 2021).

Many species associated with Garry oak ecosystems are "peripheral" species in Canada, reaching the northern limit of their range in southern BC (Pojar 1980). There are almost 700 plant species within Garry oak ecosystems, and many are listed provincially and nationally of conservation concern, including at least 60 plant species (Fuchs 2001; British Columbia Conservation Data Centre 2022).

Okanagan shrub-steppe ecosystem

Grasslands in the southern Okanagan Valley in BC are the northernmost extension of the Pacific Northwest Bunchgrass grasslands, which extend south to northeastern Oregon and western Idaho (Tisdale 1947). In BC, this ecoregion contains the remainder of grasslands, shrub-steppe, and low-elevation dry forests (Pryce et al. 2006) and is a hotspot of biodiversity in Canada (Scudder 2003) supporting nationally rare plant species (Pryce et al. 2006). The lower elevations of the Okanagan and Similkameen rivers' dry climates

and pocket desert habits are northern extensions of the Great Basin arid habitats (Pryce et al. 2006). Two plant communities in the shrub-steppe ecosystems—bunchgrass and shrub-steppe—are at risk subnationally (British Columbia Conservation Data Centre 2022).

Study species

The eight species in this study comprise four congeneric pairs, each consisting of one rare and one common species (Table 1). Two rare species occur in the coastal Garry oak ecosystem, and two occur in the interior Okanagan shrub-steppe ecosystem in BC. The species found in the Garry oak ecosystem are *Sanicula bipinnatifida* (purple sanicle; Apiaceae; rare; Fig. S2), *Sanicula crassicaulis* (Pacific sanicle; Apiaceae; common), and *Balsamorhiza deltoidea* (deltoid balsamroot; Asteraceae; rare; Fig. S3). The species found in the Okanagan shrub-steppe ecosystem are *Balsamorhiza sagittata* (arrowleaf balsamroot; Asteraceae; common), *Orthocarpus barbatus* (Grand Coulee owl-clover; Orobanchaceae; rare; Fig. S4), *Orthocarpus luteus* (yellow owl-clover; Orobanchaceae; common), *Phacelia ramosissima* (branching phacelia; Hydrophyllaceae; rare; Fig. S5), and *Phacelia linearis* (thread-leaved phacelia; Hydrophyllaceae; common).

Three of these rare species are considered imperiled (rank S2) in BC, and one (*P. ramosissima*) is considered critically imperiled to imperiled (rank S1S2) in BC (British Columbia Conservation Data Centre 2022), with their Canadian populations lying at the northern edge of their geographic distributions (Pojar 1980; Ceska 1993). Each of the common species is considered secure (rank S5) in BC. We chose species based on their occurrence in threatened ecosystems in BC, but their ranges are not necessarily limited to these ecosystems. For example, *B. deltoidea* and *B. sagittata* occur in and were sampled from different ecosystems in BC (Garry oak and Okanagan shrub-steppe, respectively), but *B. deltoidea* can be found in both habitat types in Washington (Fig. S1). Based on occurrence data from the Global Biodiversity Information Facility (www.gbif.org), common species' ranges also reached their northern edge in southern BC, and these typically had larger ranges than their rare congeners (see Figs. S6–S13 for species range maps). All species are diploid except for *S. crassicaulis*, which is putatively allopolyploid with reports of tetraploid, hexaploid, and octoploid populations (Vargas et al. 1999). A summary of life history and mating system information for each species can be found in Table S1. Additional information about each of the rare species and what is known about their populations in BC is included in the Supplementary Materials.

Sampling strategy

We sampled single individuals from a total of 95 populations (Fig. 1; Table S2). We sampled between 7 (*O. luteus*) and 17 (*S. bipinnatifida* and *B. deltoidea*; Table 2) individuals of each species. Seventy-three samples were leaf excisions, taken with permission from herbarium specimens representing populations in western North America including the provinces/states BC, California, Idaho, Oregon, Nevada, and Washington. Herbarium specimens were

Table 1. General information for the eight species included in this study, including ecosystems in which they are found in British Columbia, abundance, status, mating system, and life history.

Species name	Ecosystem	Abundance	Provincial conservation status	National conservation status	Family	Mating system	Life history	Flowering
Sanicula bipinnatifida	GOE	Rare	Imperiled (S2)	Threatened (May 2001)	Apiaceae	Self-compatible/obligate outcrosser	Perennial	Hermaphroditic
Sanicula crassicaulis	GOE	Common	Secure (S5)	N/A		Self -compatible/obligate outcrosser	Perennial	Androdioecy
Balsamorhiza deltoidea	GOE	Rare	Imperiled (S2)	Endangered (April 2009)	Asteraceae	Self-incompatible	Perennial	Hermaphroditic
Balsamorhiza sagittata	OSSE	Common	Secure (S5)	N/A		Self-compatible	Perennial	Hermaphroditic
Orthocarpus barbatus	OSSE	Rare	Imperiled (S2)	Endangered (May 2005)	Orobanchaceae	Putative outcrosser	Annual	Chasmogamous
Orthocarpus luteus	OSSE	Common	Secure (S5)	N/A		Putative outcrosser	Annual	Hermaphroditic
Phacelia ramosissima	OSSE	Rare	Critically imperiled–imperiled (S1S2)	Endangered (May 2005)	Boraginaceae	Self-incompatible	Perennial	Hermaphroditic
Phacelia linearis	OSSE	Common	Secure (S5)	N/A		Self-incompatible	Annual	Low gynodioecious

Note: In the Ecosystem column, GOE indicates that this species is found in the Garry oak ecosystem and OSSE indicates that this species is found in the Okanagan shrub-steppe ecosystem.

Table 2. Sample sizes and diversity metrics for the eight species included in this study.

Species	Sample size			Private alleles			Private alleles/individual			Nucleotide diversity (π)			Total genetic diversity (Ht)		
	North	South	Total	North	South	Total	North	South	Total	North	South	Total	North	South	Total
S. bipinnatifida (r) (GOE)	13	4	17	19	19	38	1.46	4.75	4.06	0.00217	0.00419	0.00290	0.1370	0.2675	0.219
S. crassicaulis (c) (GOE)	7	4	11	17	22	39	2.43	5.50	4.18	0.00307	0.00585	0.00420	0.1872	0.3333	0.267
B. deltoidea (r) (GOE)	11	6	17	5	23	28	0.45	3.83	3.47	0.00065	0.00416	0.00260	0.0617	0.3943	0.280
B. sagittata (c) (OSSE)	5	4	9	10	28	38	2.00	7.00	4.89	0.00232	0.00583	0.00410	0.1675	0.4231	0.308
O. barbatus (r) (OSSE)	7	3	10	13	4	17	1.86	1.33	2.00	0.00157	0.00083	0.00130	0.2482	0.1765	0.224
O. luteus (c) (OSSE)	5	2	7	25	5	30	5.00	2.50	6.86	0.00351	0.00134	0.00400	0.4593	0.1429	0.399
P. ramosissima (r) (OSSE)	7	9	16	3	26	29	0.43	2.89	1.88	0.00090	0.00427	0.00300	0.0667	0.2111	0.141
P. linearis (c) (OSSE)	5	3	8	27	2	29	5.40	0.67	4.00	0.00486	0.00161	0.00360	0.4806	0.1905	0.330

Note: Results are organized by the regional groupings (north vs. south) of samples within species based on the last glacial maximum of the Cordilleran ice sheet, as well as totals. In the Species column, (r) indicates rare, (c) indicates common, (GOE) indicates that they are found in the Garry oak ecosystem, and (OSSE) indicates that they are found in the Okanagan shrub-steppe ecosystem.

collected between 1963 and 2014, with the majority being collected after 1991 (see Table S2 for sample metadata and herbarium accession numbers). In addition, we collected 22 fresh leaf samples from well-documented and previously vouchered populations of rare species in regional, provincial, and national parks in BC. We placed fresh leaf tissue in paper envelopes inside a sealed plastic bag containing silica gel desiccant and stored them at room temperature (sample numbers are appended with "F" in Table S2). We assigned each of the 95 samples to northern or southern regional categories based on the location of the population sampled in relation to the last glacial maximum of the Cordilleran ice sheet (Dalton et al. 2020) (Table S2; Fig. 1).

DNA extraction, PCR amplification, and sequencing

We ground 10 mg of dried leaf tissue (herbarium) or lyophilized fresh tissue of each sample using a Qiagen TissueLyser II (Qiagen, Valencia, CA, USA), and we extracted DNA with a NucleoSpin® Plant II kit (Macherey-Nagel, Bethlehem, PA, USA) with an extended incubation time of 1 h at 65 °C, recommended for dry herbarium samples. We analyzed DNA concentration and quality with a NanoDrop 2000c (Fisher Scientific, Toronto, ON, Canada) and visualized using agarose gel electrophoresis.

We amplified eight noncoding cpDNA regions for all samples using primer sequences published by Shaw et al. (2014) (Table S3). We conducted polymerase chain reactions (PCRs) in 50 μL reaction volumes consisting of 0.2 mmol/L dNTP (New England Biolabs, Ipswich, MA, USA), 1× PCR buffer (Stratagene, La Jolla, CA, USA), 2.5 units of Paq5000 (Stratagene), 50 pmol each forward and reverse primers (Integrated DNA Technologies, Skokie, IL, USA), 0.5 μg/μL BSA, and 1 mmol/L $MgCl_2$ with 10 ng DNA template. We used the following PCR protocol for amplification: 80 °C for 5 min, 30 cycles of 95 °C for 1 min, 50 °C for 1 min (ramp + 0.3 °C/s), and 65 °C for 5 min followed by a final extension of 65 °C for 5 min. We purified PCR products using Wizard® PCR Preps DNA Purification System (Promega) and sequenced with Thermo Sequenase cycle sequencing kit (Thermo Fisher Scientific, Waltham, MA, USA) on a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) with the following conditions: 95 °C for 3 min, cycled 24 times at 95 °C for 30 s, 58 °C for 30 s, and 70 °C for 30 s. We loaded sequencing products on a 5% polyacrylamide gel (UBC Genetic Data Centre protocol) and ran electrophoresis for 10 h on an LI-COR 4200 automated sequencer (LI-COR Inc., Lincoln, NE, USA). We visualized sequences using eSeq DNA Sequencing and Analysis software version 2.0 (LI-COR Inc., NE, USA).

Sequence assembly and alignment

We inspected chromatograms for quality and trimmed them using Geneious v. 10.2.6 (Biomatters, Inc., San Diego, CA, USA). Herbarium samples generally produced shorter reads and lower sequence quality than fresh samples and required extensive trimming. As a result, it was often not possible to assemble our forward and reverse sequences into a single consensus sequence. Our initial analysis of

sequences from herbarium samples showed that unidirectional sequences generated with forward primers were polymorphic; therefore, to reduce sequencing costs due to limited budget we used only forward primers to generate sequence data for fresh tissue specimens and subsequent analysis of all samples (the exception is *rpl32-trnL* for which we used reverse primers).

We aligned sequences within each species and cpDNA region using the MUSCLE alignment algorithm (Edgar 2004) in Geneious with default parameters. We inspected alignments for ambiguous base calls, with special attention to polymorphic sites, and trimmed to the length of the shortest sequence for each region within each species to minimize missing data. We concatenated sequence data for the eight primer regions for each individual prior to analysis.

Sequence analysis

We exported concatenated sequence alignments for each species in FASTA format and imported them as "DNAbin" objects into R v. 4.0.4 (R Core team 2019) using the *read.dna()* function from the APE package (Paradis and Schliep 2019). We excluded insertions and deletions (indels) from analysis, as the majority were present in only a single individual within each species and may have been the result of inaccurate base calls due to low sequence quality generated from herbarium samples. Unless otherwise specified, missing data were handled using the default options in the R functions described below; in most cases, R functions use pairwise deletion of sites with missing values. We extracted single nucleotide polymorphisms (SNPs) from DNAbin objects and stored them as "genind" objects for use with the analysis packages ADEGENET (Jombart 2008), POPPR (Kamvar et al. 2014), and HIERFSTAT (Goudet 2005).

To describe patterns of genetic diversity in our data set, we calculated nucleotide diversity (π) for each species using the PEGAS package (Paradis 2010) and total gene diversity (Ht) using the *basic.stats()* function (with "diploid = FALSE" setting) from the HIERFSTAT package. Both metrics account for the number of samples sequenced to produce unbiased estimates of diversity. To assess whether our results were biased by unequal sampling, we used linear models to test for a relationship between diversity measures and sample size within species using the *lm()* function in R. To estimate the uniqueness of each species and differences between northern and southern populations, we calculated the number of private alleles using the *private_alleles()* function in POPPR and by calculating the proportion of private alleles per individual in each species and each geographic region.

We used paired *t* tests to determine whether rare species had lower diversity (proportion of polymorphism, proportion of private alleles per individual, π, and Ht) than their common congeners. We also tested whether populations in the north had lower diversity (proportion of private alleles per individual, π, and Ht) than populations in the south within Garry oak and Okanagan shrub-steppe ecosystems separately. We relied on the probability from a one-tailed distribution as we hypothesized that diversity might only be lower (not higher) in the northern populations or among rare species. Means are presented with standard errors.

We further assessed the degree of genetic differentiation between northern (recently glaciated) and southern regional categories within species, using analysis of molecular variance (AMOVA). We performed AMOVA using the *poppr.amova()* function in POPPR and tested for significant differentiation using the *randtest()* function (with 1000 randomizations). We applied the additional options "within = FALSE" (disable calculation of within-individual variation as cpDNA data are haploid) and "cutoff = 0.1" (allow loci with 10% missing data) to *poppr.amova()*.

To visualize relatedness among samples and genetic structure within species, we constructed haplotype networks from DNAbin objects using the *haplotype()* and *haploNet()* functions in PEGAS.

To help us better understand the effects of geographically limited dispersal on the degree of genetic differentiation, we assessed isolation by distance (IBD) within species. We calculated pairwise genetic and geographic distances between all individuals within species using the base R *dist()* function, and tested for significance of IBD using Mantel tests with the *mantel.randtest()* function (1000 replicates) in ADE4.

Results

Diversity in the aligned data set

The average alignment length of the 5′ end of each cpDNA region ranged from 320 to 432 base pairs (bp) among species after quality trimming (Table 3). All regions had SNPs within species, but regions were not equally variable; the *rpl16* intron showed no variation in four of eight species and an average of 2.125 SNPs (0.0065 polymorphic sites) across species, whereas *ndhC-trnV* (UAC) was variable in all species with an average of 6.38 SNPs (0.0162 polymorphic loci).

Diversity within species

All individuals sampled within species had unique haplotypes (based on the concatenated data set) with the exception of two individuals of *B. deltoidea*, BD24F and BD27F, which shared the same haplotype. The number of intraspecific SNPs found in a single cpDNA region ranged from zero in eight cases to 17 (0.056 polymorphic sites) in the case of the *ndhC-trnV* (UAV) region in *P. ramosissima*. Because of variation in sequence quality, the length of concatenated sequence for each species ranged from 1477 to 3448 bp, with *P. ramosissima* being the shortest and *P. linearis* the longest. Across the concatenated data set, the number of SNPs ranged from 17 (0.005 polymorphic sites) in *O. barbatus* to 44 (0.018 polymorphic sites) in *S. crassicaulis*. The species with the highest proportion of SNPs was *P. ramosissima* with 31 SNPs (0.021 polymorphic sites).

Comparisons of diversity between rare and common congeners

Common species had higher nucleotide diversity (π, 0.0040 ± 0.0001 vs. 0.0025 ± 0.0004, $t_{[3]} = 3.5$, $p_{\text{one tail}} < 0.02$)

Table 3. Sequencing results indicating the trimmed length of and number of single nucleotide polymorphisms (SNPs) found in each chloroplast DNA (cpDNA) region in each species used in this study.

Region	*psbE-petL*		*petA-psbJ*		*ndhC-trnV* (UAC)		*trnT* (GGU) -*psbD*		*5′rps16-trnQ* (UUG)		*ndhF-rpl32*		*rpl32-trnL*		*rpl16* intron		Concatenated sequence		Proportion polymorphic loci
Species	Length	SNPs	Length	SNPs	Length	SNPs	Length	SNPs	Length	SNPs	Length	SNPs	Length	SNPs	Length	SNPs	Length	SNPs	
S. bipinnatifida (r) (GOE)	394	8	365	6	343	6	NA	NA	336	4	435	3	400	13	171	1	2504	38	0.015
S. crassicaulis (c) (GOE)	375	13	361	2	290	10	NA	NA	381	2	317	2	394	4	299	11	2477	44	0.018
B. deltoidea (r) (GOE)	309	3	311	8	400	4	314	2	464	11	326	1	184	0	213	0	2591	29	0.011
B. sagittata (c) (OSSE)	214	6	416	14	422	4	253	6	399	8	347	1	234	1	524	0	2879	40	0.014
O. barbatus (r) (OSSE)	340	7	301	5	522	3	671	3	445	0	393	2	207	0	469	0	3418	17	0.005
O. luteus (c) (OSSE)	409	5	512	2	430	1	537	3	294	1	427	4	315	9	197	2	3191	27	0.008
P. ramosissima (r) (OSSE)	341	5	368	8	306	17	433	1	349	6	417	4	501	2	228	3	1477	31	0.021
P. linearis (c) (OSSE)	235	0	367	4	435	6	478	1	600	7	411	6	327	12	525	0	3448	36	0.010
Average	**327.125**	**5.875**	**375.125**	**6.125**	**393.500**	**6.375**	**447.667**	**2.667**	**408.500**	**4.875**	**384.125**	**2.875**	**320.250**	**5.125**	**328.250**	**2.125**	**2748.125**	**32.750**	**0.013**

Note: Results are based on forward primers of each cpDNA region. The concatenated length of all cpDNA regions, total number of SNPs in all cpDNA regions, proportion of polymorphic loci (concatenated sequence length/total number of SNPs), and averages are also included. In the Species column, (r) indicates rare, (c) indicates common, (GOE) indicates that they are found in the Garry oak ecosystem, and (OSSE) indicates that they are found in the Okanagan shrub-steppe ecosystem.

and total gene diversity (Ht, 0.33 ± 0.03 vs. 0.22 ± 0.03, $t_{[3]} = 2.6$, $p_{\text{one tail}} < 0.04$) than their rare congeners (Table 2). However, a difference in the proportion of private alleles was only marginally supported (4.9 ± 0.7 vs. 2.9 ± 0.5, $t_{[3]} = 2.1$, $p_{\text{one tail}} = 0.061$) and the total proportion of polymorphism did not differ between the two groups (0.013 ± 0.002 vs. 0.013 ± 0.003, $t_{[3]} = 0.1$, $p_{\text{one tail}} > 0.44$, Table 3). Ht was negatively correlated with sample size within species ($R^2 = 0.51$, $F_{[1,6]} = 6.14$, $p < 0.05$), though π was not correlated with sample size ($R^2 = 0.144$, $F_{[1,6]} = 1.01$, $p > 0.35$).

Diversity and uniqueness of the northern and southern geographic regions

Northern populations had lower mean nucleotide diversity (π, 0.0020 ± 0.0007 vs. 0.0047 ± 0.0006, $t_{[2]} = 6.4$, $p_{\text{one tail}} < 0.02$), total gene diversity (Ht, 0.13 ± 0.04 vs. 0.33 ± 0.04, $t_{[2]} = 3.1$, $p_{\text{one tail}} < 0.05$), and proportion of private alleles per individual (1.4 ± 0.6 vs. 4.7 ± 0.5, $t_{[2]} = 35.3$, $p_{\text{one tail}} < 0.001$) than southern populations within the Garry oak ecosystem (Table 2). However, we did not find any difference in mean genetic diversity between northern and southern populations within the Okanagan shrub-steppe ecosystem (all $p_{\text{one tail}} > 0.32$, Table 2).

Genetic and geographic structure

AMOVA results indicated significant genetic differentiation between geographic regions in all three species from the Garry oak ecosystem but in only one of five species (*O. luteus*) from the Okanagan shrub-steppe ecosystem (Table 4). This structure was not readily apparent when viewing the haplotype networks except for in *O. luteus*, where northern and southern individuals were grouped separately in the network (Fig. 2). Both mean geographic and genetic distances, represented as Euclidean distances, were higher for common congeners than rare congeners (Table S4). IBD analysis indicated a positive correlation (r values > 0.2) between genetic and geographic distances in all species from the Garry oak ecosystem, with randomization tests indicating significant IBD ($p < 0.05$) in *B. deltoidea* and *S. bipinnatifida* (Table 5; Fig. S14). The species from the shrub-steppe ecosystem displayed no significant IBD except for *B. sagittata*, which showed marginally significant IBD ($p < 0.1$).

Discussion

Our aim was to provide insight into patterns of genetic diversity across the ranges of four rare plant species in BC using universal noncoding cpDNA markers. Our results show that these low-cost markers are an effective tool for broad surveys of genetic diversity in species of conservation concern. We detected variation in each of the eight plastid regions that were sequenced in at least one of the species surveyed and generated enough data to investigate regional genetic trends within species. Our results indicate divergent patterns of regional genetic diversity and differentiation depending on the rarity of the species and ecosystems they inhabit.

In each of the four species pairs, we found increased genetic variation and diversity in the common species relative

Table 4. Analysis of molecular variance (AMOVA) results for each species included in this study, including the percentage (%) of variance attributed to between- vs. within-region components and *p* values based on 1000 randomizations.

Species	Abundance	Ecosystem	% variation between regions	% variation within regions	*p*
S. bipinnatifida	Rare	GOE	25.80259	74.19741	0.032
S. crassicaulis	Common	GOE	14.20559	85.79441	0.037
B. deltoidea	Rare	GOE	43.75651	56.24349	0.001
B. sagittata	Common	OSSE	11.76471	88.23529	0.113
O. barbatus	Rare	OSSE	−10.81187	110.81187	0.753
O. luteus	Common	OSSE	43.78194	56.21806	0.039
P. ramosissima	Rare	OSSE	−3.18787	103.18787	0.797
P. linearis	Common	OSSE	−12.82284	112.82284	0.937

Note: AMOVA was conducted on allele frequencies of single nucleotide polymorphisms of concatenated chloroplast DNA regions found within each species. *p* values <0.05 are considered statistically significant, and negative % variation values are considered effectively zero. In the Ecosystem column, GOE indicates that this species is found in the Garry oak ecosystem and OSSE indicates that this species is found in the Okanagan shrub-steppe ecosystem.

to rare congeners. When comparing intraspecific genetic diversity and differentiation between northern and southern regions, contrasting patterns emerged between oak savannah and shrub-steppe ecosystems. While mean diversity was higher in the south for species from the Garry oak ecosystem, mean diversity generally did not vary between northern and southern populations of species from the Okanagan shrub-steppe ecosystem (except perhaps for *O. luteus*). In addition, there was a signal of IBD between regions among Garry oak species, but little indication of IBD and little regional differentiation among shrub-steppe species. This suggests Garry oak ecosystems are more vulnerable to geographic effects on population connectivity and genetic diversity than Okanagan shrub-steppe ecosystems, and this aligns with evidence from Garry oak trees of declining diversity northwards, IBD, and genetic structure even across short distances (Marsico et al. 2009; Catherall et al. 2018). While our results are somewhat preliminary, they provide a valuable first step towards assessing patterns of genetic diversity within plant species at risk in Canada, about which very little is known.

Utility of noncoding cpDNA regions for intraspecific studies of genetic variation in southern BC

Shaw et al. (2014) recommended the use of 4–8 noncoding cpDNA regions to access the low-level discriminatory power of the chloroplast genome. We used all eight regions in an effort to maximize the probability of detecting intraspecific variation in angiosperm species inhabiting recently glaciated landscapes. Each of the regions contained SNPs that could be used to assess genetic diversity and genealogical relationships in four or more of the species studied, but three of the eight regions (*trnT* (GGU) - *psbD*, *ndhF-rpl32*, and *rpl16* intron; Table 3) were about 50% less variable than the others. For example, the *rpl16* intron was invariant in four out of eight species and had the lowest average number of SNPs. However, in this same region, there were 11 SNPs in *S. crassicaulis*, among the highest number of SNPs found across all cpDNA regions. No single cpDNA region could reliably be used across all plant taxa tested, but a combination of several regions increased the number of potentially informative characters. Based on these limited results, we recommend that future studies screen all eight cpDNA regions to maximize detection of intraspecific variation in taxa of interest.

As is often the case for initial genetic studies for species of conservation concern, we used sampling and sequencing approaches that were constrained by limited resources and limited sample availability. These choices led to key trade-offs that we were not aware of at the outset of the work. Because the costs of collecting fresh plant tissue across the range of all eight species would have been prohibitive, we relied primarily on herbarium specimens for this survey. This proved somewhat costly, because lower DNA quality from herbarium specimens led to lower quality DNA sequences that required extensive trimming. This resulted in a reduction in the overall amount of data available for analysis when compared with the results reported by Shaw et al. (2014) (table 1 in their study). Despite these limitations, our methods recovered enough polymorphic sites to draw broad preliminary conclusions about the regional diversity of the rare plant species in our study. Almost every individual in our data set had a unique haplotype (we only found one case where two individuals shared a haplotype; Fig. 2), which highlights the potential discriminatory power of this screening approach.

Comparison of genetic diversity between rare and common congeneric species pairs

We expected that common species would have higher levels of genetic diversity than their rare congeners due to processes such as genetic drift and bottlenecks associated with population declines, and greater impacts of anthropogenic habitat destruction (Frankham 1996; Gustafsson and Sjögren-Gulve 2002; Leimu et al. 2006; Gibson et al. 2008). As predicted, we found that the common species in this study harboured higher mean levels of genetic diversity (π and Ht) relative to rare species in both ecosystems (Tables 2 and 3). Similarly, we found that common species had a marginal tendency to harbour a higher proportion of private alleles than

Fig. 2. Haplotype networks based on concatenated sequence alignments for each species included in the study. Circles represent haplotypes, and crosshatched lines indicate the number of nucleotide differences between each haplotype. Haplotype circles are coloured by the "north" (blue) and "south" (red) regional groupings of individuals, which are based on the last glacial maximum of the Cordilleran ice sheet. The size of haplotype circles corresponds to the number of individuals represented by each haplotype, and haplotypes are labelled with the sample codes of the individuals they represent. Species names are appended with their abundance in BC, with (r) indicating rare and (c) indicating common.

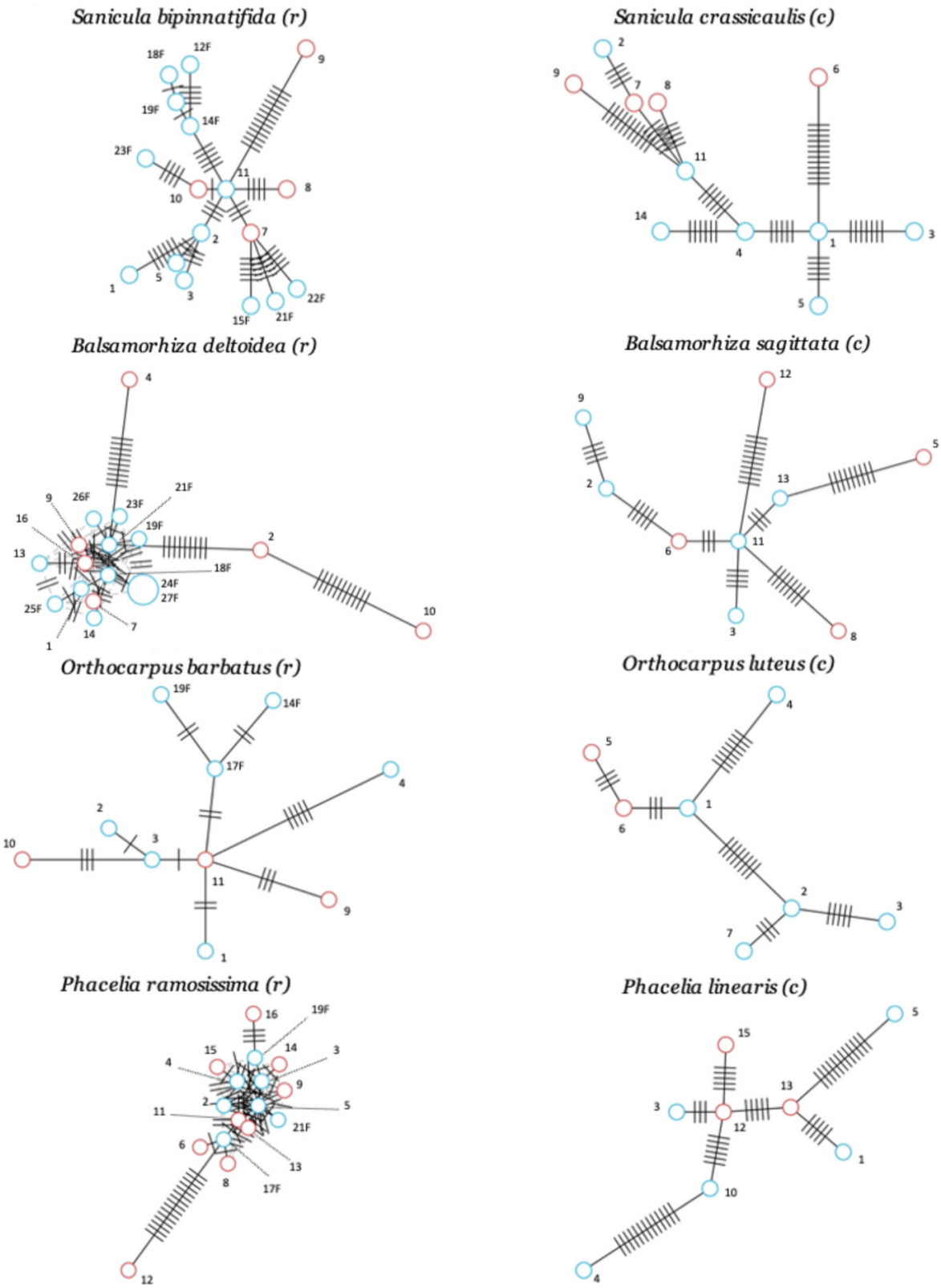


Table 5. Results based on isolation by distance (IBD) analysis.

Species	Abundance	Ecosystem	*r*	*p*
S. bipinnatifida	Rare	GOE	0.445	0.006
S. crassicaulis	Common	GOE	0.266	0.138
B. deltoidea	Rare	GOE	0.5	0.015
B. sagittata	Common	OSSE	0.227	0.084
O. barbatus	Rare	OSSE	−0.103	0.607
O. luteus	Common	OSSE	0.07	0.337
P. ramosissima	Rare	OSSE	0.007	0.378
P. linearis	Common	OSSE	−0.358	0.97

Note: IBD analysis was based on pairwise Euclidean genetic distances (derived from allele frequencies of single nucleotide polymorphisms found in concatenated chloroplast DNA regions) and pairwise Euclidian geographic distances (derived from latitudes and longitudes of each sample) within each species. *r* values indicate the slope of IBD, and *p* values were based on Mantel tests with 1000 replicates. *p* values <0.05 are considered statistically significant, and <0.1 are considered marginally significant. In the Ecosystem column, GOE indicates that this species is found in the Garry oak ecosystem and OSSE indicates that this species is found in the Okanagan shrub-steppe ecosystem.

rare species (Table 2). The common species *S. crassicaulis* had the highest nucleotide diversity (Table 2), as well as the highest number of SNPs and second highest proportion of polymorphic loci (Table 3). The relatively high diversity found in this species may be influenced by multiple origins of polyploidy (Vargas et al. 1999), which has been shown to generate haplotype polymorphism in other polyploid systems (Chen et al. 2021).

Despite the consistency in the patterns that we found, differences in diversity metrics between congeners were often small, and should be treated as preliminary and potentially impacted by some of the limitations of our study. In particular, our samples sizes within species were generally small and biased towards rare species (Table 2), which complicated comparisons between rare and common species, though we attempted to control for this using unbiased estimates of diversity. While we found that total Ht was negatively correlated with sample size, this may have been influenced by the fact that the average geographic distance between samples of common species was greater than their rare congeners in every case (Table S4). Though it is possible that the greater distances among sampled populations contribute to the higher levels of genetic variation that we detected in common congeners, the lack of a significant signature of IBD in the common species (except marginal significance in *B. sagittata*) suggests this is not likely. The absence of significant relationships between genetic and geographic distances in common species suggests that the increased diversity found within common species is driven by processes other than geographic isolation between localities, and perhaps due to other factors associated with being more common (e.g., larger average population sizes; Gitzendanner and Soltis 2000).

Comparisons of regional genetic diversity and structure within species

An interesting pattern that emerged from this study is that we found opposing trends between samples from the two threatened ecosystems when comparing intraspecific genetic structure and diversity. In the Garry oak ecosystem, AMOVA provided evidence for differentiation between northern and southern regions in all three species (Table 4). We also found that genetic diversity was higher in the south and that southern individuals harboured a higher proportion of private alleles than in the north. These results suggest founder effects, in which plant populations suffer from reduced genetic diversity after colonizing previously glaciated areas (Soltis et al. 1997; Hewitt 2004; Shafer et al. 2010; Chung et al. 2018), but anthropogenic habitat loss may have contributed to loss of diversity as well (Schlaepfer et al. 2018). In the case of the Garry oak species, we also note that the Garry oak habitat in BC is situated on Vancouver Island and the southern Gulf Islands, separated from the mainland by the Strait of Georgia and the Salish Sea. This habitat discontinuity poses additional barriers to postglacial dispersal, and contributes to greater isolation from populations in the more diverse mainland region (Alsos et al. 2015). This is supported by our finding that all species from the Garry oak ecosystem displayed a positive signal of IBD, though Mantel tests indicated that this relationship was not significant in the common species *S. crassicaulis* (Table 5). The combination of postglacial colonization, island biogeography, and anthropogenic habitat loss has likely contributed to the patterns found in the rare species from Garry oak ecosystem.

The patterns that we found among Okanagan shrub-steppe species contrast in multiple ways from those described above in Garry oak ecosystem species. We found no mean difference in levels of diversity between northern and southern shrub-steppe populations, except for the common species *O. luteus* that displayed higher diversity in the northern region. This general lack of geographic structure is further supported by the absence of IBD among most of the species from this ecosystem (Table 5). These results are contrary to predictions about northern populations in regions that were recently deglaciated. The divergent trends found in the two ecosystems could reflect differences in habitat connectivity. The Okanagan shrub-steppe species occur in the more or less continuous desert plateaus and basins east of the Coast and the Sierra Nevada Mountain ranges, and are less likely to be constrained by geographic barriers to dispersal and gene flow seen in the island populations of the Garry oak ecosystem.

While the differences between ecosystems are notable, there are some important caveats to consider. Our sampling effort was broadly spread across the range of each species, but we only sampled one individual per population. This prevented us from assessing patterns of genetic diversity within populations, which would be essential in future studies. Instead, we based our analyses on regional groupings of individuals, which may have introduced biases related to our sampling scheme. For example, we sampled more individuals from the north than the south in all species except one (*P. ramosissima*), and northern samples tended to be more tightly grouped geographically than southern ones (particularly in Garry oak species; Fig. 1). In addition, many of our species only had a small number of individuals representing the southern region (Table 2), which makes it difficult to

generalize about diversity and structure in those cases. Our results are best viewed as a broad but shallow view of phylogeographic diversity within species that help identify worthwhile candidates for in-depth future studies.

Managing rare plant species in southern BC

Our results provide guidance for the conservation and management of the four jurisdictionally rare species included in this study. In particular, patterns of diversity, unique variation, and regional structure within species point to habitats worth prioritizing. We explore these patterns in each of the two rare ecosystems below.

Coastal Garry oak ecosystem

Both of the rare species from this ecosystem (*B. deltoidea* and *S. bipinnatifida*) showed patterns of divergence consistent with reduced gene flow from the mainland to the south, as well as reduced levels of diversity and fewer private alleles in comparison to the southern region. Because all but one of the BC populations of the rare species are on Vancouver Island or adjacent islands, gene flow from the more diverse mainland populations is less likely. Several populations of both species are under threat from development, habitat fragmentation, and competition from invasive plants (Supplemental species information), and are therefore likely to experience population bottlenecks or inbreeding depression as the number of individuals dwindles.

The situation seems particularly pronounced for *B. deltoidea* that is known from only eight extant natural populations in BC, all of which are under threat. This species also exhibits sporophytic self-incompatibility (Table 1; Table S1), which may lead to reduced mating success when population sizes are small (Byers and Meagher 1992; Ellstrand and Elam 1993; Aguilar et al. 2008). *Balsamorhiza deltoidea* also showed the strongest signal of genetic differentiation (43% of variation attributed to differences between regions in AMOVA; Table 4) and IBD ($r = 0.5$; Table 5) out of all eight species. *Sanicula bipinnatifida* on the other hand has 20 known populations in BC (9 of which are considered to have good viability; Supplemental species information).

Okanagan shrub-steppe ecosystem

Our results indicate that the two rare species from this ecosystem (*O. barbatus* and *P. ramosissima*) show less evidence of reduced gene flow than the rare species from the Garry oak ecosystem. Contrary to expectations, northern populations in the shrub-steppe ecosystem did not have reduced mean genetic diversity compared with southern populations. More surprisingly, our AMOVA suggested *O. luteus* had higher diversity in the north though we interpret this conservatively as we only sequenced two samples from the southern region (Table 2). While our metrics of diversity account for sample size, we are cautious on how much diversity can be represented in only two samples.

Recommendations for future studies

Peripheral populations may harbour unique genetic variation as a result of multiple processes such as adaptation to ecological conditions at the geographic extreme of the species range, fixation of nonadaptive variation due to initial founder effects, or subsequent bottlenecks or drift associated with isolation and (or) declining populations sizes. The dilemma for conservation practitioners in Canada is that it is not clear whether the many rare species that occur in Canada at their range periphery are likely to be positively or negatively impacted by management strategies such as translocations or population augmentations. We know that protecting locally adapted and divergent genotypes is likely to be important for population persistence (Lesica and Allendorf 1995), but as populations continue to decline, or as conditions change (e.g., in the face of climate change; Vitt et al. 2010; Aitken and Whitlock 2013), the rationale for bolstering genetic diversity to help arrest or reverse declines becomes stronger. Studies have shown that introducing new alleles can increase population growth rates (i.e., genetic rescue) and bolster population resilience (Whiteley et al. 2015), though these strategies can only be so effective in the face of large-scale habitat destruction due to human development. While it would be desirable to have information on adaptive divergence and shifting patterns of selection to help guide management decisions, the slow pace of relevant research on rare plants in Canada suggests that we might benefit from approaches that can be applied to a diversity of rare species, with the aim of identifying shared or divergent patterns of differentiation among species or ecosystems.

This study highlights the utility of direct sequence data from hypervariable cpDNA markers identified by Shaw et al. (2014) to provide insights into genetic structure within and among species, including detecting differences among individuals from different populations. While NGS technologies such as RADSeq may be preferred to address questions requiring sampling of thousands of genomic markers across the genome (e.g., signals of local adaptation or hybridization), the markers used in this study provide a cost-effective first tool for conservation biologists (McKain et al. 2018). These cpDNA markers are particularly useful in nonmodel species for which population genetic tools (i.e., microsatellite markers) have not been developed. Despite the ease of use, we suggest that, when possible, researchers should prioritize the use of fresh tissue collections made from several individuals per population, sampled systematically from throughout the species' ranges. Nevertheless, optimized DNA extraction protocols for herbarium specimens exist (Särkinen et al. 2012) and may be desired if the aim is to investigate changes in genetic patterns over time (Bieker and Martin 2018).

Another benefit of the cpDNA markers used in this study is that they facilitate the comparisons of unrelated species. Broadly applicable plastid markers like these allow for more or less direct comparisons of diversity metrics among species within habitats of conservation concern. As conservation in Canada shows signs of shifting away from protecting individual species and towards prioritizing the protection of

threatened habitats (Martin et al. 2018), study designs like ours provide an important first step towards assessing phylogeographic patterns in a set of species that co-occur in managed regions. When multiple species from the same ecosystem point towards a similar pattern (e.g., regional differentiation and reduced diversity in the Garry oak ecosystems), this provides evidence for genetic processes acting at the geographic scale of entire ecosystems and reinforces the need to reprioritize conservation efforts.

Our results provide a broad but shallow view of patterns of genetic diversity in four species that are rare in southern BC. While we acknowledge that our results are preliminary, they provide a starting point for species that we have little (if any) prior information on genetic diversity. Of the rare species included in this study, our results suggest that *B. deltoidea* from the coastal Garry oak ecosystem and *P. ramosissima* from the Okanagan shrub-steppe ecosystem may be at particular risk due to low levels of genetic diversity and small population sizes. Our preliminary results highlight the need for additional studies with in-depth sampling within species to evaluate population-level genetic diversity attributes (i.e., effective population size, inbreeding, and gene flow among populations) at the northern range periphery.

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Data availability

All individual sequences (unaligned and labelled as "SampleName_PrimerCode") have been submitted to GenBank and assigned accession numbers ON983991–ON985174. Concatenated sequence alignments, metadata files, and R scripts used for analysis have been submitted to Dryad (Hersh et al. 2022) and can be found at https://doi.org/10.5061/dryad.12jm63z1h.

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Author contributions

EWH and BC conceived of and designed the study with contributions from CR and AEM. EWH and BC coordinated herbarium and field sampling, and AEM conducted the DNA sequencing laboratory work. AEM and APR conducted preliminary data analysis. EWH, JW, JCG, and WH refined the data set and expanded the analyses in the final version. EWH, EJW, BC, and AEM drafted sections of the manuscript text and tables; EWH and WH made all figures; and EWH, JW, JCG, and WH crafted the final narrative. All authors provided critical review and revision of the draft manuscript.

Competing interests

The authors state that there are no competing interests.

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Supplementary material

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