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MICROHABITAT DIFFERENCES IMPACT PHYLOGEOGRAPHIC CONCORDANCE OF CODISTRIBUTED SPECIES: GENOMIC EVIDENCE IN MONTANE SEDGES (*CAREX* L.) FROM THE ROCKY MOUNTAINS

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By selecting codistributed, closely related montane sedges from the Rocky Mountains that are similar in virtually all respects but one—their microhabitat affinities—we test predictions about how patterns of genetic variation are expected to differ between *Carex nova*, an inhabitant of wetlands, and *Carex chalciolepis*, an inhabitant of drier meadows, slopes, and ridges. Although contemporary populations of the taxa are similarly isolated, the distribution of glacial moraines suggests that their past population connectedness would have differed. Sampling of codistributed population pairs from different mountain ranges combined with the resolution provided by over 24,000 single nucleotide polymorphism loci supports microhabitat-mediated differences in the sedges' patterns of genetic variation that are consistent with their predicted differences in the degree of isolation of ancestral source populations. Our results highlight how microhabitat preferences may interact with glaciations to produce fundamental differences in the past distributions of presently codistributed species. We discuss the implications of these findings for generalizing the impacts of climate-induced distributional shifts for communities, as well as for the prospects of gaining insights about species-specific deterministic processes, not just deterministic community-level responses, from comparative phylogeographic study.

KEY WORDS: **Comparative phylogeography, Cyperaceae, Last Glacial Maximum, SNPs.**

Concordance in phylogeographic structure across species provides a comparative context critical for testing hypotheses regarding the temporal influence of environmental factors on communities (Avise 1992; Soltis et al. 2006; Shafer et al. 2010; Ribas et al. 2012). If membership in a specific biological community is the primary factor that determines population connectedness through time, it follows that species with similar life histories and morphological traits should show concordant phylogeographic patterns. Although the impact of historical and regional processes is often emphasized in comparative phylogeographic studies by identifying and quantifying the extent of concordance in patterns of genetic variation among species (Hickerson et al. 2010), there could also be deterministic processes that would produce discord.

Deterministic processes associated with the interaction between species' microhabitat affinities and environmental perturbations may be key contributors to phylogeographic discord. Microhabitat preferences are widely recognized as important to a variety of ecological and evolutionary phenomena. They influence the richness of biological communities and partitioning of resources therein (Hixon and Beets 1993; Ackerly 2003; Cavender-Bares et al. 2004), lead to diversification through character displacement dictated by local environmental variables (Schluter and Grant 1983; Losos et al. 1997; Rosenblum 2006), and even facilitate speciation (Feder et al. 1988; Chunco et al. 2007). It follows that microhabitat may also affect species' phylogeographic structure (Hugall et al. 2002; Whiteley et al. 2004; Hodges

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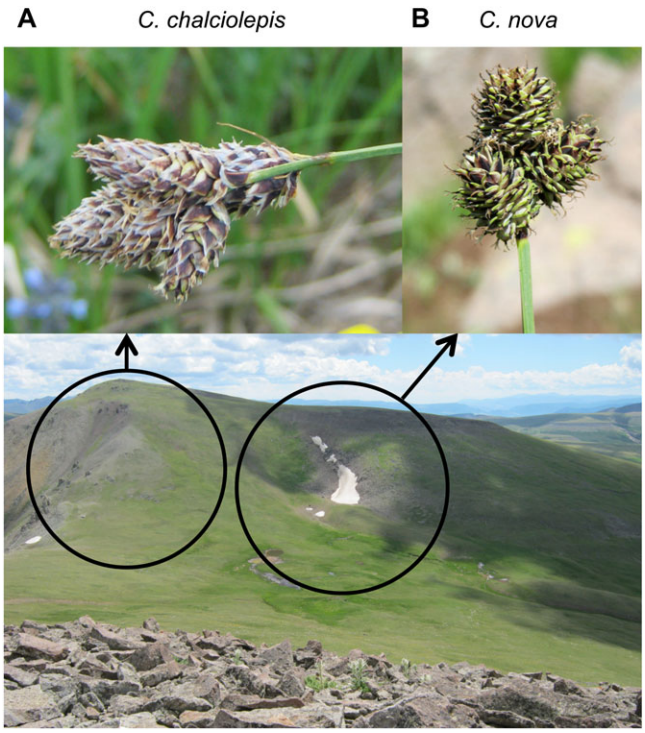

Figure 1. ***Carex chalciolepis*** **(A) and** ***C. nova*** **(B) inflorescence morphologies and representative habitats.**

et al. 2007). For example, an interaction between Australian funnel web spiders' preferences for wood or ground-dwelling microhabitats and Pleistocene climatic cycles has been proposed as an explanation for phylogeographic disparities (Beavis et al. 2011). Even at large geographic scales, patterns of genetic variation may differ across taxa in a manner consistent with species-specific habitat affinities, as suggested by correlations between species' preferences for particular forest canopy strata and genetic differentiation among codistributed South American birds (Burney and Brumfield 2009). Such studies highlight how the deterministic effects of species-specific traits on patterns of genetic variation may be dissected from a comparative phylogeographic perspective.

Here, we test for phylogeographic concordance in two montane sedge species, *Carex nova* and *C. chalciolepis* (*Carex* section *Racemosae*, Cyperaceae; Fig. 1). There are many reasons to expect concordance between these taxa. They are endemic to high-elevation montane habitat in western North America and are common and codistributed throughout the southern Rocky Mountains (from the Sangre de Cristo Mountains, NM, to the Medicine Bow Mountains, WY). They are not distantly related, instead belonging to a clade of exclusively montane taxa that share a common ancestor during the Pleistocene (R. Massatti, unpubl. ms.). With many shared life-history characteristics, the inherent dispersal capabilities do not differ between the species (e.g., they are long-lived perennials with locally dispersed seeds). Both *C. nova* and *C. chalciolepis* flower as soon as environmental conditions are appropriate for plant growth, reflecting the seasonal constraints on the time to seed maturity imposed by high-elevation environments. In these respects, both taxa are representative of many montane plants not only in terms of shared life-history characteristics, but also as members of montane plant communities that respond to shifts in climatic conditions. Yet, there is one primary difference between these species—their microhabitat affinities. Within alpine tundra and unforested subalpine habitats, *C. nova* is restricted to wetlands and mesic meadows, whereas *C. chalciolepis* occurs on drier montane slopes, meadows, and ridges (Fig. 1). The question is as follows: are microhabitat differences of significant evolutionary consequence? That is, instead of phylogeographic concordance, do species-specific differences have the potential to affect phylogeographic structure?

Montane plants are adapted to the strenuous physiological demands of high-elevation habitats. In addition, as inhabitants of areas directly impacted by glacial cycles, they experienced repeated displacements from their current distributions (Pierce 2003). This includes tracking suitable habitat to lower elevations during glacial periods (in addition to potentially surviving in situ, see below), followed by recolonization from ancestral populations to distributions established during interglacials (as established by palynological records and macrofossils; e.g., Betancourt et al. 1990; Thompson et al. 1993; Thompson and Anderson 2000). Despite the similar adaptations that all montane plants share, their microhabitat affinities may have facilitated disparate responses to glaciations. Plants inhabiting montane microhabitats that dry out relatively quickly (e.g., ridges and south-facing slopes) are among the earliest flowering plants in montane ecosystems because wind redistributes snow to places such as drainages and leeward slopes (Holway and Ward 1965). Although the cooler climates of glacial periods likely shortened the growing season throughout montane habitats (Pierce 2003), plants on slopes and ridges, such as *C. chalciolepis*, may have persisted in situ because they are adapted to initiate growth as soon as environmental conditions permit (e.g., Stehlik et al. 2002).

In contrast to ridge and slope microhabitats, drainages and landscape depressions preferentially accumulate snow and ice. Consequently, the majority of plants in wetter microhabitats, such as *C. nova*, flower later compared to plants in other montane microhabitats (Holway and Ward 1965) when meltwater subsides and the substrate warms (Körner 2003). Glacial periods impacted the majority of wetland microhabitats in the southern Rocky Mountains by facilitating the growth of valley glaciers down drainages, as evidenced by the distribution of moraines and glacial till (Ehlers and Gibbard 2004). Furthermore, wetland microhabitats adjacent to glaciers would have had a shortened

growing season due to an increased volume of meltwater resulting from the increased accumulation of snow and ice during glacial periods. Therefore, it is unlikely that wetland plants persisted locally, instead being disproportionately displaced to lower elevations in drainages.

Based on the general assumption that codistributed montane species with similar inherent dispersal capabilities will respond similarly to historical events, we test the null hypothesis of concordant phylogeographic structure between *C. nova* and *C. chalciolepis*. Alternatively, if microhabitat differences are of significant consequence, we predict the species will show discordant patterns of genetic variation. Moreover, we predict that *C. nova* populations will be more differentiated from one another compared to *C. chalciolepis* populations, in line with expectations based on the interactions between species' microhabitat affinities and climatic oscillations. Specifically, populations of *C. nova* from unconnected drainages should have experienced prolonged disjunctions during the glaciations, especially given the relative brevity of interglacial compared to glacial periods (Clark et al. 1999), when they were displaced to lower elevations in drainages around the margins of mountain ranges. Prolonged disjunctions are not expected to be characteristic of *C. chalciolepis* populations, where population differentiation is expected to reflect colonization from within-glacier refugial ridge and slope microhabitats, as well as from lower elevation populations (which were not restricted to drainages). With fewer expected differences between the current and past distributions of *C. chalciolepis*, the genetic signature of distinct ancestral refugia is not predicted to be as strong as in *C. chalciolepis*, as in *C. nova.* Note that unlike other comparative phylogeographic studies where inferences about causality rely on repeated patterns observed across taxa (e.g., Hugall et al. 2002; Burney and Brumfield 2009), our sampling design is structured to assess repeated genetic patterns at the population level (see also Grahame et al. 2006; Rosenblum 2006; Ryan et al. 2007). Specifically, by sampling two populations per mountain range across multiple mountain ranges in each taxon, we can evaluate whether the patterns of genetic variation differ consistently between the species.

Phylogeographic concordance (or lack thereof) was assessed in *C. nova* and *C. chalciolepis* using a genomic dataset of more than 24,000 anonymous single nucleotide polymorphism (SNP) loci sequenced in seven pairs of sympatric populations across five mountain ranges. We used environmental niche modeling to confirm similar projected distributions of the species during the Last Glacial Maximum (LGM). We then investigated the impact of glaciations on genomic differentiation using STRUCTURE and principal component analysis (PCA). Our results show that the sedges have similar magnitudes and ranges of genetic differentiation; however, the gene pool of *C. nova* displays hierarchical structure whereas that of *C. chalciolepis* does not, as predicted under our hypothesis that microhabitat does influence a species' response to climatic cycling. These results highlight how difficult it might be to predict the consequences of future climate change on communities as a whole. We discuss the implications of our results for interpreting previously documented, but difficult to interpret, patterns of community assemblages from regions impacted by glacial cycles (e.g., Soltis et al. 2006), as well as generalizations that have been made from comparative phylogeographic study.

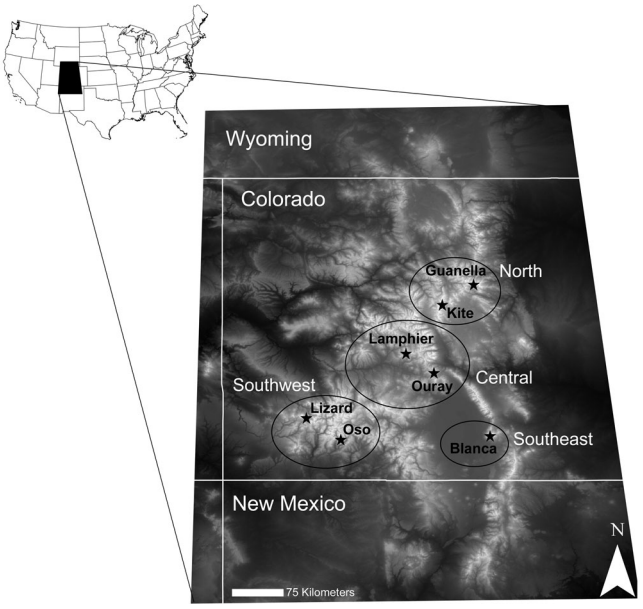

Figure 2. Map of the southern Rocky Mountains showing the distribution of sampled populations (marked by stars) of *C. chalciolepis* and *C. nova* in each of the four geographic regions referred to in the text; whiter colors indicate higher elevations (elevations range from 1200 to 4350 m). Populations are located in the San Juan Mountains (Oso and Lizard), Sangre de Cristo Mountains (Blanca), Sawatch Mountains (Ouray and Lamphier), Mosquito Mountains (Kite), and Front Range (Guanella).

Methods

SAMPLE COLLECTION AND DNA EXTRACTION

To minimize the collection of related individuals, tissue samples (i.e., 2 cm of leaf material) were collected from well-dispersed *C. chalciolepis* and *C. nova* individuals (average distance between samples of 300 m, and a minimum distance of 35 m) at each sampling locality during the summer of 2011 (Fig. 2). A total of 40 individuals were sampled per species; five individuals were sampled per population except for Blanca, where 10 individuals were sampled (see Table 1 for details). Leaf material was stored in silica gel until DNA was extracted with DNeasy Plant Mini Kits (Qiagen, Venlo, Netherlands) following the manufacturer's protocol.

Table 1. **Details for the sampled populations in both *C. chalciolepis* and *C. nova*, including population name, geographic region within the southern Rocky Mountains (see Fig. 2), the number of individuals collected per species, collection site coordinates (latitude, longitude), and the range of elevations (*m*) over which plants were sampled.**

Population	Geographic region	Number of individuals per species	GIS Coordinates	Elevation (*m*)
Oso	Southwest (SW)	5	37.5357, –107.4817	3500–3900
Lizard	Southwest (SW)	5	37.8256, –107.9391	3200–3700
Blanca	Southeast (SE)	10	37.5991, –105.4782	3250–3800
Ouray	Central (C)	5	38.4329, –106.2407	3400–3800
Lamphier	Central (C)	5	38.6772, –106.6069	3600–3900
Kite	North (N)	5	39.3279, –106.1360	3700–4000
Guanella	North (N)	5	39.5941, –105.7117	3600–3900

ENVIRONMENTAL NICHE MODELING

Environmental niche models (ENMs) were used to confirm the similarity of the sedges' responses to glaciations, given that there are areas within their respective ranges where they do not co-occur. ENMs were generated from bioclimatic variables for the present and the LGM for each species with MAXENT version 3.3.3e (Phillips et al. 2006) using the following parameters: regularization multiplier = 1, maximum number of background points = 10,000, replicates = 50, replicated run type = cross-validate. Georeferenced distribution points used in the modeling were representative of species' entire ranges throughout western North America and were collected from personal fieldwork and validated voucher specimens housed at the Rocky Mountain Herbarium (species distribution points are available at http://doi.org/10.5061/dryad.4158c). We used 19 bioclimatically informative variables to model present-day distributions (WorldClim version 1.4; Hijmans et al. 2005) and LGM distributions (PMIP2-CCSM; Braconnot et al. 2007). Full details on ENM modeling procedures are available in the Supporting Information.

RAD LIBRARY PREPARATION AND SEQUENCE ANALYSIS

Extracted genomic DNA was individually bar coded and processed into a reduced complexity library using a restriction fragment based procedure (for details see Gompert et al. 2010). Briefly, DNA was doubly digested with *Eco*RI and *Mse*I restriction enzymes, followed by the ligation of Illumina adaptor sequences and unique bar codes. Ligation products were pooled among samples and the fragments were amplified by PCR. Gel purification was used to size select fragments between 300 and 400 base pairs. The library was sequenced in one lane on the Illumina HiSeq2000 platform (San Diego, CA) according to manufacturer's instructions to generate 50 base pair, single-end reads. Sequences were demultiplexed using custom scripts and only reads with Phred scores ≥32 and that had an unambiguous bar code and restriction cut site were retained (scripts are available at http://doi.org/10.5061/dryad.4158c). Potential chloroplast and mitochondrial sequences were filtered from the processed dataset using Bowtie 0.12.8 (see Supporting Information for more details; Langmead et al. 2009).

SNPs were determined from loci formed from overlapping sets of homologous fragments and genotypes were assigned using a maximum-likelihood statistical model (Catchen et al. 2011; Hohenlohe et al. 2012) with the Stacks version 1.03 pipeline (Catchen et al. 2013); default settings were used except where noted below. Specifically, loci and polymorphic nucleotide sites were identified in each individual using the USTACKS program, which groups reads with a minimum coverage depth (*m*) into a "stack." The data were processed with $m = 3$; increasing the minimum depth helps to avoid erroneously calling convergent sequencing errors as stacks. A catalog of consensus loci among individuals was constructed with the CSTACKS program from the USTACKS output files for each species, where loci were merged together across individuals if the distance between them (*n*) was ≤ 2. This catalog was used to determine the allele(s) present in each individual at each homologous locus using the SSTACKS program. Our choice of parameters was determined with consideration of avoiding both over- and under-merging of homologous loci in the focal taxa, as well as with reference to other studies (e.g., Renaut et al. 2014). Similarity of the number of loci identified in the species for different parameter values used in USTACKS (*m*) and CSTACKS (*n*) suggests that the properties of the genomic libraries were similar (i.e., that the potential errors associated with over- or under-merged homologous loci did not differ substantially between the species). The close relatedness of the taxa and the short reads makes an $n \leq 2$ reasonable (also see Renaut et al. 2014), although we acknowledge this could be conservative if catalogs were assembled for taxa that were more distantly related, and/or for read lengths larger than 50 base pairs.

Population genetics statistics, including major allele frequency, nucleotide diversity (π), and Wright's *F*-statistics, F_{IS} and F_{ST}, were calculated for each SNP using the POPULATIONS

program in the Stacks pipeline (Catchen et al. 2013). For biallelic SNP markers, π is a measure of expected heterozygosity, and is therefore a useful measure of genetic diversity in populations; furthermore, F_{IS} measures the reduction in observed heterozygosity as compared to expected heterozygosity for an allele in a population, and positive values indicate nonrandom mating or cryptic population structure (Nei and Kumar 2000; Hartl and Clark 2006; Holsinger and Weir 2009). One SNP with two alleles was identified in every available homologous locus for each sedge species; this resulted in 24,574 and 25,670 SNPs available for analyses in *C. nova* and *C. chalciolepis*, respectively. Although we could have included SNPs with more than two segregating alleles or additional SNPs segregating at a locus, we opted for the conservative approach of excluding these SNPs as they may disproportionately represent sequencing errors, given the close relatedness of the taxa and short read lengths of 50 base pairs (see also Renaut et al. 2014). Note that SNPs with more than two segregating alleles were uncommon, representing less than 1% of the loci.

Only loci present in at least two populations ($p = 2$) and genotyped in at least 50% or 75% of the individuals of each population ($r = 0.50$ or $r = 0.75$) were used to create molecular summary statistics for each species; in instances where 50% or 75% did not result in a round number of individuals in a population, the number of individuals required before the locus was processed was rounded up (e.g., a locus would need to be genotyped in three out of five individuals with $r = 0.50$, and four out of five individuals with $r = 0.75$). In addition, per locus F_{ST}-values were calculated only for loci with minimum minor allele frequencies of 5% or higher ($a = 0.05$), the lowest possible minor allele frequency given our sampling effort within populations. Isolation by distance was assessed within each species by graphing populations' pairwise F_{ST} against Euclidean distance (km) in the Isolation By Distance Web Service (Jensen et al. 2005); one-sided *P*-values were computed by randomizing the data 30,000 times, and both geographic distance and genetic distance were log-transformed to determine their effect on results.

CHARACTERIZATION OF POPULATION GENETIC STRUCTURE

Population genetic structure was characterized within *C. chalciolepis* and *C. nova* using STRUCTURE 2.3.4 (Pritchard et al. 2000). SNP data were exported from the POPULATIONS program in Stacks into a STRUCTURE file using the parameter settings $r = 0$, $p = 2$, and $a = 0$ (see above for parameter descriptions). We created different datasets using more inclusive parameters to take advantage of variation in individuals within populations that was filtered out when calculating population-level summary statistics; this variation may be important for assessing genetic similarity between individuals from different populations when population membership is not assumed a priori. *K*-values ranging from 1 to 9 (two more than the total number of populations in each species) were analyzed in STRUCTURE. Ten independent runs per *K* were conducted, each with 100,000 burn-in and 150,000 MCMC iterations, using the "Admixture Model" and "Correlated Allele Frequency Model" with default settings. Results were not different using more burn-in or MCMC iterations. STRUCTURE HARVESTER (Earl and vonHoldt 2012) and DISTRUCT (Rosenberg 2004) were used to visualize results, and the most probable *K* was chosen based on ΔK (Evanno et al. 2005).

To explore genetic structure that might be present within initial clusters identified by STRUCTURE (i.e., hierarchical structuring of genetic variation), an iterative approach was used (e.g., Ryan et al. 2007). This involved the analysis of subsets of the data that corresponded to the respective genetic clusters identified in previous runs. For the analyses of data subsets, values of *K* ranging from 1 to 5 were explored using the same parameter settings as those used in the initial STRUCTURE analyses. As with the analyses of the entire dataset across all populations, only loci with SNPs were included in the analyses.

In addition to the analyses described above, all the analyses were repeated with a dataset in which three putative hybrid individuals were removed to confirm the robustness of the results. These individuals were identified using a STRUCTURE and PCA of the pooled datasets of *C. chalciolepis* and *C. nova*, along with a third closely related sedge species (*C. nelsonii*), which was not included in this study because of limited sampling. The genomic composition of these three individuals contained significant heterospecific contributions; one *C. nova* individual was clearly admixed with *C. chalciolepis* and two *C. chalciolepis* individuals were hybridized with *C. nelsonii* (Fig. S1).

To visualize the major axes of population genetic variation, a PCA was performed using the "adegenet" package (Jombart 2008) in R (R Core Team 2012). PCA is a natural companion to STRUCTURE because it is free from many of the population genetics assumptions underlying STRUCTURE (Gao et al. 2007; Jombart et al. 2009), and it can be more useful with continuous patterns of differentiation (e.g., isolation by distance; Engelhardt and Stephens 2010). The datasets requiring either 50% or 75% completeness within populations' loci (see above) were used for PCA; in addition, analyses were repeated excluding the putative hybrids, similar to the method described for STRUCTURE analyses (above).

Results

ECOLOGICAL NICHE MODELING

Present-day and LGM ENMs were generated using four bioclimatic variables: temperature seasonality (Bio4), maximum temperature of the warmest quarter (Bio5), precipitation of the driest

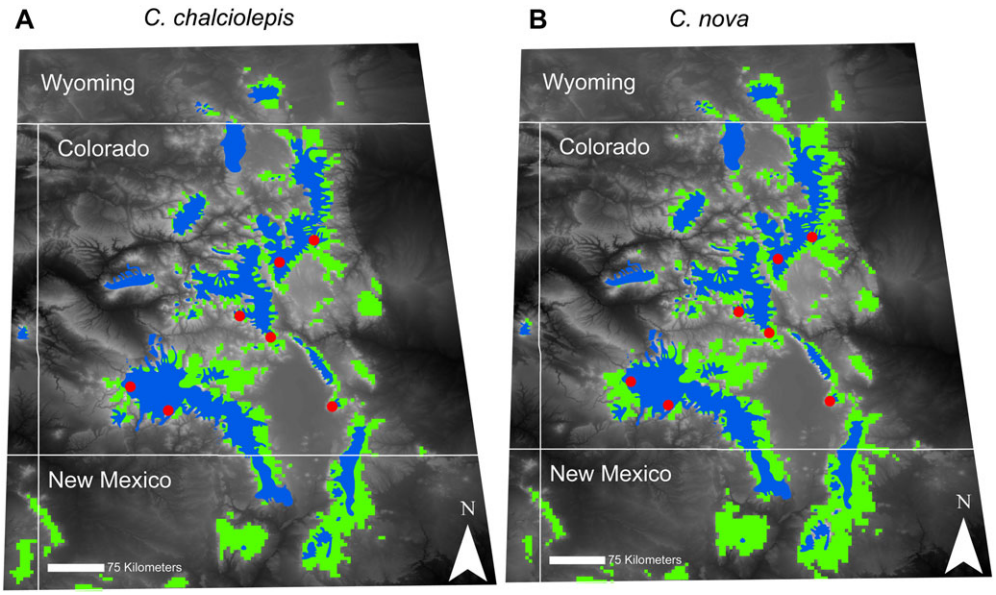

Figure 3. The predicted LGM distributions of *C. chalciolepis* (A) and *C. nova* (B) are shown in green using the maximum training sensitivity plus specificity threshold. Glaciers (i.e., potentially nonsuitable habitat) are shown in blue (data extracted from Glaciers of the American West, http://glaciers.us), and red dots depict the sampling locations.

month (Bio14), and precipitation of the warmest quarter (Bio18). Not only are the present-day ENMs of *C. chalciolepis* and *C. nova* nearly identical within the southern Rocky Mountains (Fig. S2), but their distributions are also predicted to be largely congruent during the LGM (Fig. 3). The similarity of the ENMs between the species confirms the predicted phylogeographic concordance of the two sedge taxa under the assumption that montane taxa respond similarly to past climatic cycles. The maximum training sensitivity plus specificity (MTSS) threshold was among the most overpredictive of the thresholds calculated by MAXENT, deeming habitat with ≥18% and ≥11% probability of being suitable as suitable habitat for *C. nova* and *C. chalciolepis*, respectively. Models created using more overfitted thresholds only decreased the area surrounding predicted regions using MTSS and never identified regions as no longer habitable, suggesting the approach used was appropriate given our intentions to assess the overall similarity of *C. chalciolepis*' and *C. nova*'s LGM distributions.

SEQUENCE DATA QUALITY AND PROCESSING

One lane of Illumina sequencing produced more than 132 million reads derived from 80 individuals (average of 1,659,473 ± 481,727), of which more than 127 million reads had Phred scores ≥32 and an unambiguous bar code (Table 2, Fig. S3). On average, an additional 6% of reads per individual were discarded because they aligned with either chloroplast or mitochondrial genomes. Based on low coverage or low quality of reads, five individuals were excluded from further analyses, including four from *C. nova* and one from *C. chalciolepis* (Table 2, Fig. S3). Filtering loci containing one SNP and two alleles from the species' MySQL databases resulted in 24,574 loci for *C. nova* and 25,670 loci for *C. chalciolepis* (data are available at http://doi.org/10.5061/dryad.4158c), with all populations well represented (Fig. S4).

GENETIC DIVERSITY WITHIN AND AMONG SOUTHERN ROCKY MOUNTAIN *CAREX* POPULATIONS

Characterization of genetic diversity within and among populations was conducted on the two datasets that differed in the tolerance levels for missing data, requiring either 50% or 75% of individuals within a population to have a locus to be included in the dataset. The results were qualitatively similar and therefore we only present data for the dataset restricted to loci present in 50% or more of the individuals within a population. *Carex nova* and *C. chalciolepis* are quite comparable with respect to common measures of genetic diversity (e.g., average major allele frequencies and observed heterozygosities; see Tables 3 and S1 for details). Likewise, their populations are characterized by similar ranges of genetic diversity, π (0.390–0.581 and 0.359–0.504 in *C. nova* and *C. chalciolepis*, respectively), with the lowest diversity in the most isolated population (Blanca) among the sites collected. Observed heterozygosities are lower than π in each population for both species, resulting in positive F_{IS}-values within all populations (Table 3).

Genetic relatedness between population pairs within *C. chalciolepis* and *C. nova* was measured using F_{ST}. The range of

Table 2. Summary of genomic data collected in each population, presented as averages across individuals for a given population in each species. Shown are the raw count of reads from the Illumina run and the number of reads after processing for quality control (i.e., after excluding reads with low-quality scores, ambiguous bar codes, and that aligned with a haploid genome), as well as the number of reads analyzed with Stacks to identify homologous loci.

Populations	*n**	Raw read count	Postquality control	Analyzed reads	Percentage of raw reads used
C. chalciolepis					
Oso	4	1,631,473	1,485,349	1,411,438	86.51
Lizard	5	1,378,608	1,281,107	1,225,386	88.89
Blanca	10	1,492,198	1,416,754	1,359,250	91.09
Ouray	5	1,898,460	1,747,426	1,691,626	89.11
Lamphier	5	1,904,632	1,791,184	1,735,826	91.14
Kite	5	1,606,399	1,504,082	1,444,351	89.91
Guanella	5	1,728,853	1,628,912	1,569,360	90.77
C. nova					
Oso	5	2,301,651	2,126,856	2,054,335	89.25
Lizard	5	2,355,473	2,075,661	2,021,638	85.83
Blanca	9	1,602,688	1,484,440	1,411,294	88.06
Ouray	4	1,779,119	1,656,305	1,580,144	88.82
Lamphier	5	1,466,146	1,359,203	1,282,636	87.48
Kite	5	1,922,637	1,774,684	1,707,109	88.79
Guanella	3	1,447,511	1,038,007	971,188	67.09

* The number of individuals analyzed, *n*, differed among populations because five individuals were excluded due to low coverage and/or low quality of reads.

Table 3. Summary statistics for the sampled populations of *C. chalciolepis* and *C. nova*. Results are presented only for polymorphic nucleotide positions. Shown are the number of loci, average frequency of the major allele (*P*), average observed heterozygosity per locus (H_{obs}), average nucleotide diversity (π), and Wright's inbreeding coefficient (F_{IS}). See Table S1 for summary statistics including polymorphic + fixed nucleotide positions.

	Population	Loci	*P*	H_{obs}	π	F_{IS}
C. chalciolepis	Oso	8129	0.662	0.262	0.504	0.4470
	Lizard Head	4471	0.717	0.238	0.434	0.4251
	Blanca	5493	0.744	0.271	0.359	0.2645
	Ouray	3547	0.674	0.305	0.474	0.3773
	Lamphier	3560	0.679	0.327	0.455	0.3198
	Kite	7298	0.728	0.353	0.428	0.1484
	Guanella	4694	0.699	0.262	0.440	0.4062
C. nova	Oso	5041	0.694	0.225	0.448	0.5269
	Lizard Head	2753	0.667	0.289	0.503	0.4227
	Blanca	5265	0.719	0.204	0.390	0.4910
	Ouray	7160	0.743	0.336	0.411	0.1493
	Lamphier	3949	0.689	0.274	0.454	0.4125
	Kite	4895	0.688	0.232	0.456	0.5060
	Guanella	1947	0.614	0.431	0.581	0.2517

F_{ST}-values was similar between the two species (0.053–0.088 in *C. chalciolepis*, and 0.041–0.071 in *C. nova*). However, a significant pattern of isolation by distance was only apparent in *C. chalciolepis* (*P*-value = 0.0419; Table S2). Log transformations of the geographic distance or genetic distance had no impact on the significance of the results.

POPULATION STRUCTURE OF SOUTHERN ROCKY MOUNTAIN *CAREX*

Analyses with STRUCTURE across different values of *K* identified $K = 2$ as the most probable number of genetic groups (the *K* with the highest ΔK, hereafter referred to as the most probable *K*) in both *C. chalciolepis* and *C. nova* (Table 4). Groups

Table 4. Summary of results for STRUCTURE analyses for each species. Each row represents a separate analysis with the first and second most probable *K*-value and their associated ΔK shown. The number of individuals, the geographic regions represented by those individuals, and the number of SNPs used in the respective analyses are also given. Note the putative hybrid individuals were not included in these analyses (see Methods and Fig. S1). Inclusion of the two putative hybrid individuals did not affect the most probable *K*-value in *C. chalciolepis* (i.e., the analyses were robust). However, inclusion of the putative hybrid in *C. nova* from the Ouray population (Central region) affected the most probable *K*-value inferred by STRUCTURE in the two analyses of regional subsets of the data (i.e., those containing individuals from the Central region). Specifically, the hybrid individual was distinguished from the other individuals from the Ouray population, thereby making the most probable *K*-value = 3.

Species	Number of loci	Number of individuals	Geographic region(s)	First *K*	ΔK	Second *K*	ΔK
C. chalciolepis	24,566	37	SW, SE, C, N	2	14.6	4	6.4
C. nova	23,405	35	SW, SE, C, N	2	675.1	3	2.4
	22,950	19	SW, SE	2	503.9	3	71.6
	22,223	10	SW	2	68.3	3	2.5
	22,565	16	C, N	2	67.3	4	2.7
	21,579	8	C	2	10.9	4	0.6
	21,365	8	N	2	8.6	4	2.2

identified at $K = 2$ showed a strong correspondence with geography in *C. nova* (separating the North and Central populations from the Southeast and Southwest populations), but were not interpretable geographically in *C. chalciolepis* (Fig. 4). Moreover, in contrast to the large difference in ΔK between the first and second most probable *K*-values in *C. nova* ($K = 2$ and $K = 3$), the small difference in ΔK between the most probable *K*-values in *C. chalciolepis* ($K = 2$ and $K = 4$), indicates the lack of equivalent distinctiveness of genetic clusters in *C. chalciolepis* compared to *C. nova* (Table 4). Considering the second most probable *K*-value in *C. chalciolepis* ($K = 4$), there is more of a geographical correspondence than at $K = 2$ (Fig. 4), suggesting that genetic variation in *C. chalciolepis* is structured. However, this structuring is less pronounced than the hierarchical nature of *C. nova*'s genetic variation (see below).

In contrast with *C. chalciolepis*, not only was there structuring of genetic variation at the regional scale within *C. nova*, but the series of STRUCTURE analyses on regional subsets of the data identified significant structuring of genetic variation within mountain ranges (Fig. 4). Moreover, *C. nova* populations were repeatedly delimited across multiple mountain ranges. The pronounced difference in ΔK separating the most probable *K* ($K = 2$) and the second most probable *K* (either $K = 3$ or 4; Table 4) in most analyses confirms that the major axes of genetic variation within subsequent subsets of data are strictly concordant with geography in *C. nova*.

The genetic composition of *C. chalciolepis* individuals generally differed from that of *C. nova* individuals. For example, the probability of mixed ancestry was very low in *C. nova* individuals at both the regional scale of mountain ranges and the local scale of populations within mountain ranges (Fig. 4B). The only exception was the mixed ancestry of some individuals from Ouray within the Central region (i.e., the Sawatch Mountains; Fig. 2). In contrast, *C. chalciolepis* not only displays a consistent lack of clear genetic differences between individuals from different populations within each of the mountain ranges, but it also shows evidence of mixed ancestry that traces to populations from different mountain ranges (i.e., regions; Fig. 4A).

Results from the PCA analyses parallel results from STRUCTURE. Individuals from *C. chalciolepis* exhibited some geographic structuring of genetic variation (Fig. 5A). However, with the exception of individuals from Blanca, the populations were not separated along the PCA axes to the same degree as *C. nova* populations (Fig. 5B). *Carex nova* populations were separated on both PC1 and PC2 (Fig. 5B) into clusters at the regional level (i.e., between mountain ranges from the Southwest, Southeast, and Central + North regions), as well as at the population level for most, but not all, populations. Specifically, populations from the Central region (i.e., the Ouray and Lamphier populations) are overlapping with those from the North region (i.e., the Kite and Guanella populations). The two individuals from Oso that are separated from the other *C. chalciolepis* individuals along PC1 (i.e., the two left-most red points in Fig. 5A) correspond to those that are suspected to be putative hybrids based on species-level analyses with the third closely related species, *C. nelsonii* (as discussed in the methods, *C. nelsonii* was not included in this study because of limited geographic sampling). Analyses without these putative admixed individuals, or allowing different amounts of missing data, did not change the overall patterns of genetic variation among individuals or populations of *C. chalciolepis* or *C. nova* (results not shown).

Discussion

This study highlights how comparative phylogeography may be used to provide insights about the interaction between species'

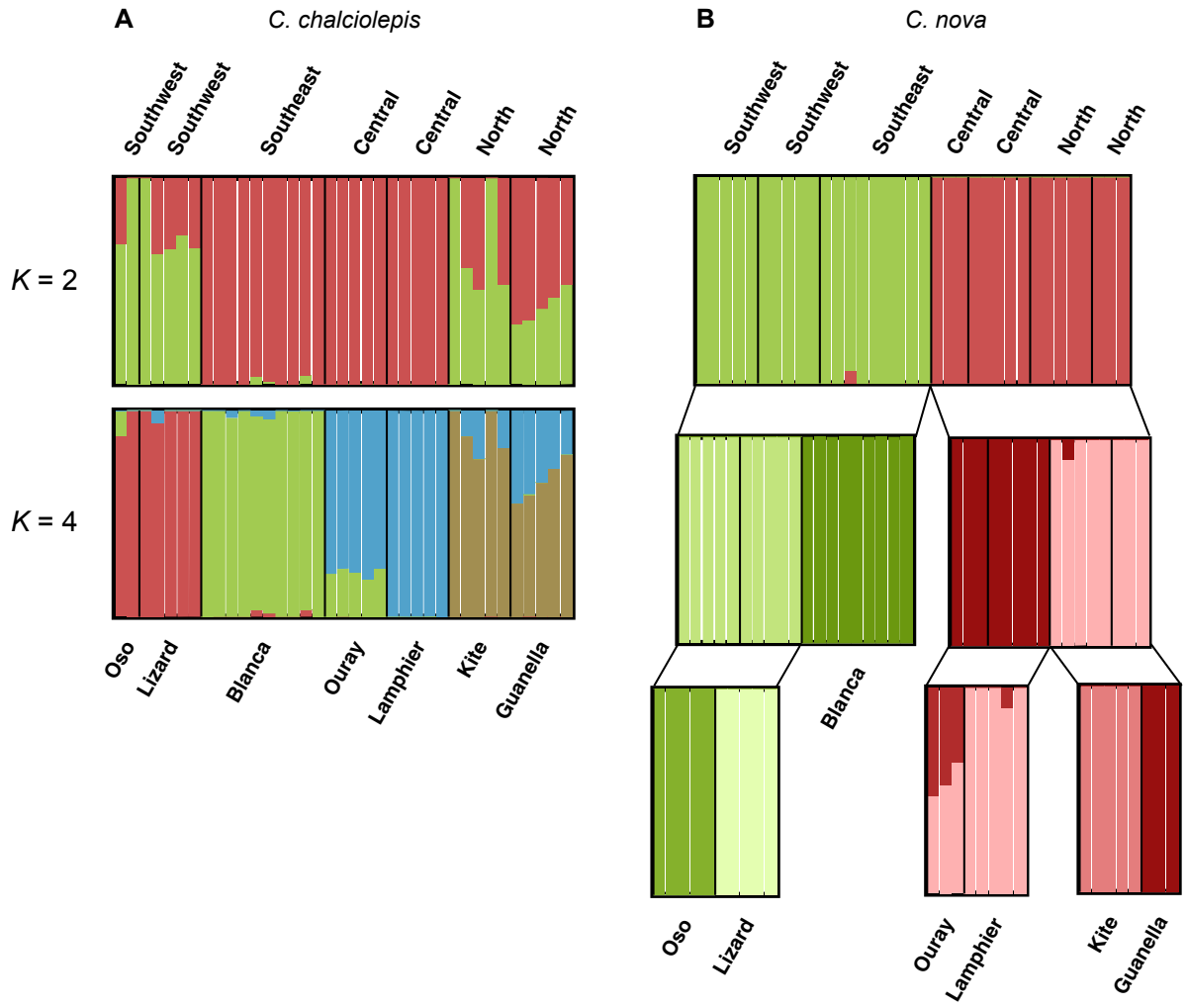

Figure 4. Plots of posterior probabilities for individuals assigned to *K* groups from STRUCTURE analyses (each separate block corresponds to one analysis). Each of the *K* groups within an analysis is shown as a different color for *C. chalciolepis* (A) and *C. nova* (B), and thin black lines delimit populations, whose names are listed along the bottom. The regional membership of populations (see Fig. 2) is listed along the top-most analysis. The two most probable values of *K* are shown for analyses of all the individuals of *C. chalciolepis*, whereas the results from a series of hierarchical analyses of subsets of data with $K = 2$ are shown for *C. nova* (slanted lines show the subsets of data analyzed, starting from the entire dataset depicted at the top, down to individuals within regions, shown at the bottom, excluding the Southeast region because of the lack of multiple sampled populations). Note that no hierarchical geographic structuring was detected in *C. chalciolepis* (see text and Table 4 for details).

traits (e.g., morphological traits or life-history characteristics) and historical environmental perturbations. Our approach—that is, selecting closely related species with many similarities, thereby controlling for the phylogeographic disparity that may be due to suites of trait differences, and sampling codistributed populations from multiple mountain ranges—provides an opportunity to explore whether patterns of genetic variation differ between taxa in a predictable fashion. Below we discuss the relative strengths and weaknesses of this approach, and argue that the manner in which phylogeographic study is pursued can contribute to a bias in both our perceptions about the factors structuring patterns of genetic variation and the tendency to interpret discordant patterns of genetic variation as reflecting the vagaries of history rather than deterministic processes.

SPECIES-SPECIFIC TRAITS STRUCTURING PATTERNS OF GENETIC VARIATION

Phylogeographic concordance of codistributed species can provide important details about the impact of environmental changes on communities over time. For example, investigation of disparate taxa distributed throughout Europe has elucidated refugia, migration routes, and suture zones resulting from Pleistocene glacial cycles (Hewitt 2004). Similar scenarios have been reconstructed for the Pacific Northwest in North America (reviewed

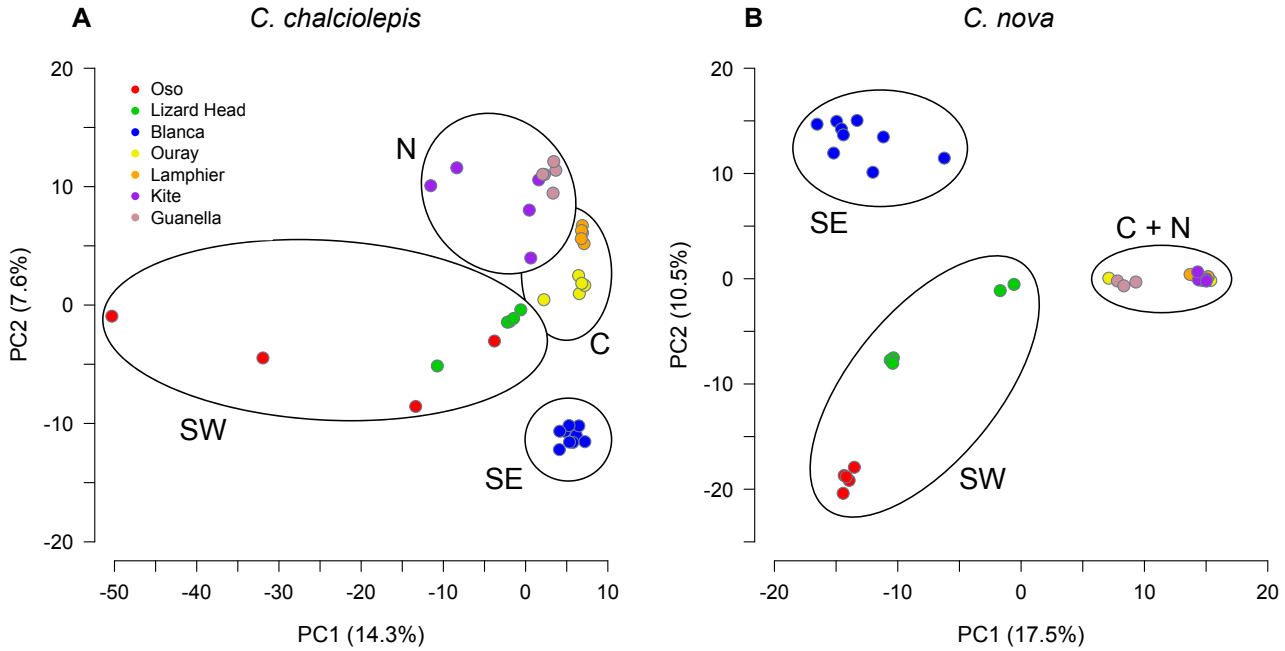

Figure 5. Distribution of individuals along PC1 and PC2 axes of genetic variation for *C. chalciolepis* (A) and *C. nova* (B), with the amount of variation explained by each axis given in parentheses. The pattern among *C. nova*'s Central and North individuals is shown in detail in Figure S5. Colors indicate population identity in each species, and ellipses demarcate regions (SW, Southwest; SE, Southeast; C, Central; and N, North; see Fig. 2).

in Shafer et al. 2010), and to a limited extent for the Rocky Mountains (Brunsfeld and Sullivan 2005; DeChaine and Martin 2005a; Spellman et al. 2007). In addition, concordance among species may help elucidate the factors that shaped past refugia, as in the Brazilian Atlantic Coastal Forest (Carnaval et al. 2009).

As may be expected based on the many studies of montane taxa (Galbreath et al. 2010; Knowles and Alvarado-Serrano 2010), including tests of phylogeographic concordance within montane regions of North America (Soltis et al. 1997; Carstens et al. 2005), there are similarities in the geographic patterns of genetic variation of *C. chalciolepis* and *C. nova*. For example, within both species, populations are regionally differentiated, and they exhibit similar ranges of genetic diversity (Fig. 5, Table 3). In additional, relatedness is generally correlated with geographic proximity (Fig. 5), with the geographically isolated Blanca population from the Southeast region (Fig. 2) well differentiated from other populations in both species. These similarities are evidence of common processes that structure patterns of genetic variation. However, they are not unexpected given that the sedges have similar dispersal capabilities and are codistributed across the southern Rocky Mountains. For example, similar results were found for codistributed butterfly species sampled across the Rocky Mountains (DeChaine and Martin 2005b). The similarities in patterns of genetic variation not only reinforce the commonalities between the species as montane taxa with limited dispersal, but they also provide a compelling framework for interpreting the potential genetic consequences of microhabitat differences.

The proposed differences in past population connectedness based on their microhabitats are supported by differences in the extent of geographic structuring of molecular variation in *C. nova* compared to *C. chalciolepis* (Fig. 4). For example, in contrast to the regional correspondence of a Central and North genetic cluster and a Southeast and Southwest genetic cluster at $K = 2$ in *C. nova*, there are no regional groups at $K = 2$ in *C. chalciolepis* (in fact, the delimited clusters display no obvious geographic pattern). Only at $K = 4$ does the signature of regional structuring become apparent in *C. chalciolepis*. However, in contrast to the distinctiveness of regional groups in *C. nova* (i.e., there is little evidence of admixture between individuals from different mountain ranges), there is clear evidence of admixture among regional groups in *C. chalciolepis* (Fig. 4). This is also reflected in the PCA, where *C. chalciolepis* populations are continuously distributed along PC2 from south to north, whereas *C. nova* populations are regionally distinct (Fig. 5). Only among populations within the Central region (i.e., Ouray and Lamphier populations) is there a lack of genetic distinctiveness in *C. nova* (Fig. 4). These are the only two *C. nova* populations sampled in this study that may have been recolonized by ancestral populations that shared a common drainage during Pleistocene glaciations, consequently experiencing less isolation compared to other regional population pairs.

Paleoclimatic modeling provides additional evidence that *C. chalciolepis* potentially persisted within suitable habitat exposed in glaciated areas (i.e., ridges and slopes). Four environmental variables had comparable ranges during the LGM compared to the present for the southern Rocky Mountains: temperature seasonality (Bio4), maximum temperature of the warmest quarter (Bio5), precipitation of the driest month (Bio14), and precipitation of the warmest quarter (Bio18). Relative stability of these climatic variables suggests that a montane growing season may have persisted within and adjacent to glaciers. This supports our hypothesis that montane plants adapted to ridges and slopes may have persisted not only at lower elevations, but also within the margins of glaciers (for a detailed perspective of the interaction of ridges, slopes, and valley glaciers, see Fig. S6). Although survival within high elevation refugia (i.e., nunataks) has been supported in other montane systems (e.g., Schönswetter et al. 2005), the additional example represented by *C. chalciolepis* (but not *C. nova*) is informative to ongoing debates about the generality of nunataks for in situ survival within mountains and their contribution to geographic patterns of genetic variation (i.e., Brochmann et al. 2003). In particular, the contrast between the likelihood of survival in the mountains during glacial periods between the sedge species highlights the importance of considering microhabitat when evaluating the proposed role for nunataks.

In contrast to many phylogeographic studies that are motivated by seeking concordance among taxa to elucidate historical processes, our work highlights how considering species-specific traits may facilitate investigations focusing on phylogeographic disparities. Our work complements past studies that have shown how genetic variation can differ in a fashion consistent with differences in the species' ecological traits. For example, in darters (Turner and Trexler 1998), differences in life-history strategies among taxa from headwater habitats are associated with differing levels of gene flow and subsequently disparate phylogeographic patterns (i.e., species with small clutches and large eggs are characterized by low gene flow compared to species with high fecundity and small eggs), whereas phylogeographic discord has been linked to differences in spawning locations in Bull trout and mountain whitefish from the northern Rocky Mountains (Whiteley et al. 2004). Instead of reflecting intrinsic characteristics of the taxa (e.g., differences in inherent dispersal capabilities or other life-history traits; see Knowles and Alvarado-Serrano 2010), aspects of the habitats themselves might also contribute to predictable discordant patterns of genetic variation across taxa. For example, the degree of similarity in the structuring of genetic variation among codistributed darkling beetle taxa is associated with whether the taxa inhabit ephemeral versus stable habitats (Papadopoulou et al. 2009). As with the work presented herein, such studies suggest that deterministic processes may indeed underlie some of the differences observed in patterns of genetic variation across taxa (but see below).

Although the results we present are consistent with the predicted patterns of genetic variation, there are certainly limitations to our analyses. The evaluation of the data with respect to the predictions based on the effects of microhabitat differences is correlative, and in this respect, our study is not unlike many phylogeographic studies (see references in Shafer et al. 2010). Specifically, without an explicit model capable of generating predictions that reflect species-specific microhabitat differences, we cannot distinguish the statistical support for alternative hypotheses. At this point, no such model exists (see review in Alvarado-Serrano and Knowles 2014). There have been promising advances for testing hypotheses that capture biological phenomena that cannot be accommodated in more generic modeling approaches (see Knowles 2009). Fortunately, with the potential power provided by the genomic dataset presented here, we will be poised to take advantage of new methodologies for generating species-specific predictions as they develop (e.g., Neuenschwander et al. 2008; He et al. 2013; Martinkova et al. 2013). Moreover, by identifying the potential importance of microhabitat in structuring patterns of genetic variation, our study can serve to motivate and guide future methodological developments. Such work could also be extended to additional codistributed *Carex* species within the same clade as *C. nova* and *C. chalciolepis* (i.e., there are four other taxa that overlap geographically; www.rmh.uwyo.edu/data/search.php), which would both complement the aforementioned modeling approaches and provide a means for evaluating the impact of species-specific attributes through tests of phylogeographic concordance among taxa with similar microhabitats (e.g., see Whiteley et al. 2004; Burney and Brumfield 2009).

DETERMINISTIC PROCESSES REFLECTED IN DISCORDANT PHYLOGEOGRAPHIC PATTERNS

When both deterministic and stochastic events contribute to observed patterns of genetic variation, how do we recognize their relative contributions? Phylogeographic concordance has been the primary method to identify deterministic processes, whether to identify biogeographic processes structuring genetic variation of whole communities (Avise 1992; Soltis et al. 2006), to identify how past climate changes have impacted codistributed species (Hewitt 2004), or to prioritize areas for biological conservation (Moritz 1994). Notwithstanding the merit of such study, focusing on concordance obviously limits the types of deterministic processes that can be investigated. For example, tests of concordance across disparate organisms (e.g., Carstens and Richards 2007) do not allow for tests about the genetic consequences of a trait that varies in a species-specific fashion (like microhabitat affinity) because of the lack of a control (i.e., more than the particular trait of interest differs across taxa). Moreover, focusing

on concordant patterns of genetic variation may also introduce a bias in our general perception of the relative predominance of factors structuring genetic variation. For example, biogeographic barriers may shape gene flow patterns, as a vast number of phylogeographic studies attest (Avise 2000). The importance of factors that act in a species-specific manner, such as the impact of habitat specialization on patterns of genetic variation (Neuenschwander et al. 2008; Knowles and Alvarado-Serrano 2010), may go undetected because of a study's design (e.g., when taxa differ in many characteristics, as discussed above).

Even if there is a signal of species-specific traits on the patterns of genetic variation, it may go overlooked, mistakenly assigned to chance because of the tendency to interpret the lack of concordance as being symptomatic of the vagaries of history (e.g., Taberlet et al. 1998; DeChaine and Martin 2005b; Kropf et al. 2003). This is in some ways not surprising given the historical focus of the field on geologic and environmental influences that affect taxa in a similar, deterministic fashion, when they predominate (Avise 2000). Alternatively, and as our results support, the lack of concordance may also reflect deterministic processes associated with species-specific traits. Elucidating how phylogeographic processes are influenced by these traits, whether the traits encompass microhabitat requirements, species interactions, or differing degrees of habitat specialization, will lead to a better resolution of the interactions of taxa with their environments and the resulting consequences for divergence and diversification processes. The only difference is the practical challenges associated with their study.

Research into the effects of climate change on communities is one area where phylogeographic biases may have a profound impact. Our study suggests that it may be quite difficult to make generalizations about the effects of climate-induced distributional shifts on whole communities. In particular, despite taxa sharing the strenuous physiological demands of montane environments and the disturbance regime imposed by glaciations on suitable habitat, microhabitat preferences may interact with glaciations to result in fundamental differences in the past distributions of presently codistributed species. This is not to deny that observed concordance in past studies (Carstens and Richards 2007) reflects the common effects of climate change. However, given the broad geographic scales of such phylogeographic studies (e.g., montane taxa and the disjunction between populations in the Rocky Mountains and the Cascade Range in the Pacific Northwest), the conclusions may not be applicable at local geographic scales, which are arguably the most relevant to the persistence of populations across a taxon's range (Hanski 1998; Saccheri et al. 1998).

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DATA ARCHIVING

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LITERATURE CITED

Ackerly, D. D. 2003. Community assembly, niche conservatism, and adaptive evolution in changing environments. Int. J. Plant Sci. 164:S165–S184.

Alvarado-Serrano, D. F., and L. L. Knowles. 2014. Ecological niche models in phylogeographic studies: applications, advances, and precautions. Mol. Ecol. Res. 14:233–248.

Avise, J. C. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63:62–76.

———. 2000. Phylogeography: the history and formation of species. Havard Univ. Press, Cambridge, MA.

Beavis, A. S., P. Sunnucks, and D. M. Rowell. 2011. Microhabitat preferences drive phylogeographic disparities in two Australian funnel web spiders. Biol. J. Linn. Soc. 104:805–819.

Betancourt, J. L., T. R. Van Devender, and P. S. Martin, eds. 1990. Packrat middens: the last 40,000 years of biotic change. University of Arizona Press, Tucson, AZ.

Braconnot, P., B. Otto-Bliesner, S. Harrison, S. Joussaume, J.-Y. Peterchmitt, A. Abe-Ouchi, M. Crucifix, E. Driesschaert, T. Fichefet, C. D. Hewitt, et al. 2007. Results of PMIP2 coupled simulations of the Mid-Holocene and Last Glacial Maximum–Part 1: experiments and large-scale features. Clim. Past 3:261–277.

Brochmann C., T. M. Gabrielsen, I. Nordal, J. Y. Landvik, and R. Elven. 2003. Glacial survival or *tabula rasa*? The history of North Atlantic biota revisited. Taxon 52:417–450.

Brunsfeld, S. J., and J. Sullivan. 2005. A multi-compartmented glacial refugium in the northern Rocky Mountains: evidence from the phylogeography of *Cardamine constancei* (Brassicaceae). Conserv. Genet. 6:895–904.

Burney, C. W., and R. T. Brumfield. 2009. Ecology predicts levels of genetic differentiation in neotropical birds. Am. Nat. 174:358–368.

Carnaval, A. C., M. J. Hickerson, C. F. B. Haddad, M. T. Rodrigues, and C. Moritz. 2009. Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. Science 323:785–789.

Carstens B. C., and C. L. Richards. 2007. Integrating coalescent and ecological niche modeling in comparative phylogeography. Evolution 61:1439–1454.

Carstens, B. C., S. J. Brunsfeld, J. R. Demboski, J. M. Good, and J. Sullivan. 2005. Investigating the evolutionary history of the Pacific Northwest mesic forest ecosystem: hypothesis testing within a comparative phylogeographic framework. Evolution 59:1639–1652.

Catchen, J. M., A. Amores, P. Hohenlohe, W. Cresko, and J. H. Postlethwait. 2011. *Stacks*: building and genotyping loci *de novo* from short-read sequences. Genes Genomes Genet. 1:171–182.

Catchen, J., P. Hohenlohe, S. Bassham, A. Amores, and W. A. Cresko. 2013. Stacks: an analysis tool set for population genomics. Mol. Ecol. 22:3124–3140.

Cavender-Bares J., D. D. Ackerly, D. A. Baum, and F. A. Bazzaz. 2004. Phylogenetic overdispersion in Floridian oak communities. Am. Nat. 163:823–843.

Chunco, A. J., J. S. McKinnon, and M. R. Servedio. 2007. Microhabitat variation and sexual selection maintain male color polymorphisms. Evolution 61:2504–2515.

Clark, P. U., R. B. Alley, and D. Pollard. 1999. Northern Hemisphere ice-sheet influences on global climate change. Science 286:1104–1111.

DeChaine, E. G., and A. P. Martin. 2005a. Marked genetic divergence among sky island populations of *Sedum lanceolatum* (Crassulaceae) in the Rocky Mountains. Am. J. Bot. 92:477–486.

———. 2005b. Historical biogeography of two alpine butterflies in the Rocky Mountains: broad-scale concordance and local-scale discordance. J. Biogeogr. 32:1943–1956.

Earl, D. A., and B. M. vonHoldt. 2012. Structure Harvester: a website and program for visualizing Structure output and implementing the Evanno method. Conserv. Genet. Res. 4:359–361.

Ehlers, J., and P. L. Gibbard, eds. 2004. Quaternary glaciations—extent and chronology II: North America. Elsevier, Lond.

Engelhardt, B. E., and M. Stephens. 2010. Analysis of population structure: a unifying framework and novel methods based on sparse factor analysis. PLoS Genet. 6:e1001117.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software Structure: a simulation study. Mol. Ecol. 14:2611–2620.

Feder, J. L., C. A. Chilcote, and G. L. Bush. 1988. Genetic differentiation between sympatric host races of the apple maggot fly *Rhagoletispomonella*. Nature 336:61–64.

Galbreath, K. E., D. J. Hafner, and K. R. Zamudio. 2010. Isolation and introgression in the Intermountain West: contrasting gene genealogies reveal the complex biogeographic history of the American pika (*Ochotona princeps*). J. Biogeogr. 37:344–362.

Gao, H., S. Williamson, and C. D. Bustamante. 2007. A Markov chain Monte Carlo approach for joint inference of population structure and inbreeding rates from multilocus genotype data. Genetics 176: 1635–1651.

Gompert, Z., M. L. Forister, J. A. Fordyce, C. C. Nice, R. J. Williamson, and C. A. Buerkle. 2010. Bayesian analysis of molecular variance in pyrosequences quantifies population genetic structure across the genome of Lycaeides butterflies. Mol. Ecol. 19:2455–2473.

Grahame, J. W., C. S. Wilding, and R. K. Butlin. 2006. Adaptation to a steep environmental gradient and an associated barrier to gene exchange in *Littorina saxatilis*. Evolution 60:268–278.

Hanski, I. 1998. Metapopulation dynamics. Nature 396:41–49.

Hartl, D. L., and A. G. Clark. 2006. Principles of population genetics. 4th ed. Sinauer Associates, Sunderland, MA.

He, Q., D. L. Edwards, and L. L. Knowles. 2013. Integrative testing of how environments from the past to the present shape genetic structure across landscapes. Evolution 67:3386–3402.

Hewitt, G. 2004. Genetic consequences of climatic oscillations in the Quaternary. Philos. Trans. R. Soc. B 359:183–195.

Hickerson, M. J., B. C. Carstens, J. Cavender-Bares, K. A. Crandall, C. H. Graham, J. B. Johnson, L. Rissler, P. F. Victoriano, and A. D. Yoder. 2010. Phylogeography's past, present, and future: 10 years after. Mol. Phylogenet. Evol. 54:291–301.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25:1965–1978.

Hixon, M. A., and J. P. Beets. 1993. Predation, prey refuges, and the structure of coral-reef fish assemblages. Ecol. Monogr. 63:77–101.

Hodges, K. M., D. M. Rowell, and J. S. Keogh. 2007. Remarkably different phylogeographic structure in two closely related lizard species in a zone of sympatry in south-eastern Australia. J. Zool. 272:64–72.

Hohenlohe, P. A., J. Catchen, and W. A. Cresko. 2012. Population genomic analysis of model and nonmodel organisms using sequenced RAD tags. Methods Mol. Biol. 888:235–260.

Holsinger, K. E., and B. S. Weir. 2009. Genetics in geographically structured populations: defining, estimating and interpreting F_{ST}. Nat. Rev. Genet. 10:639–650.

Holway, J. G., and R. T. Ward. 1965. Phenology of alpine plants in northern Colorado. Ecology 46:73–83.

Hugall A., C. Moritz, A. Moussalli, and J. Stanisic. 2002. Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). Proc. Natl. Acad. of Sci. USA 99:6112–6117.

Jensen, J. L., A. J. Bohonak, and S. T. Kelley. 2005. Isolation by distance, web service (v.3.23). BMC Genet. 6:13. Available at http://ibdws.sdsu.edu/

Jombart, T. 2008. *adegenet*: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405.

Jombart, T., D. Pontier, and A. B. Dufour. 2009. Genetic markers in the playground of multivariate analysis. Heredity 102:330–341.

Knowles, L. L. 2009. Statistical phylogeography. Annu. Rev. Ecol. Evol. Syst. 40:593–612.

Knowles, L. L., and D. F. Alvarado-Serrano. 2010. Exploring the population genetic consequences of the colonization process with spatio-temporally explicit models: insights from coupled ecological, demographic and genetic models in montane grasshoppers. Mol. Ecol. 19:3727–3745.

Körner, C. 2003. Alpine plant life: functional plant ecology of high mountain ecosystems. 2nd ed. Springer-Verlag, Berlin.

Kropf, M., J. W. Kadereit, and H. P. Comes. 2003. Differential cycles of range contraction and expansion in European high mountain plants during the Late Quaternary: insights from *Pritzelago alpina* (L.) O. Kuntze (Brassicaceae). Mol. Ecol. 12:931–949.

Langmead, B., C. Trapnell, M. Pop, and S. L. Salzberg. 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 10:R25.

Losos, J. B., K. I. Warheit, and T. W. Schoener. 1997. Adaptive differentiation following experimental island colonization in *Anolis* lizards. Nature 387:70–73.

Martinkova, N., R. Barnett, T. Cucchi, R. Struchen, M. Pascal, M. Pascal, M. C. Fischer, T. Higham, S. Brace, S. Y. W. Ho, et al. 2013. Divergent evolutionary processes associated with colonization of offshore islands. Mol. Ecol. 22:5205–5220.

Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Mol. Ecol. 3:401–411.

Nei, M., and S. Kumar. 2000. Molecular evolution and phylogenetics. Oxford Univ. Press, New York.

Neuenschwander, S., C. R. Largiader, N. Ray, M. Currat, P. Vonlanthen, and L. Excoffier. 2008. Colonization history of the Swiss Rhine basin by the bullhead (*Cottusgobio*): inference under a Bayesian spatially explicit framework. Mol. Ecol. 17:757–772.

Papadopoulou, A., I. Anastasiou, B. Keskin, and A. P. Vogler. 2009. Comparative phylogeography of tenebrionid beetles in the Aegean archipelago: the effect of dispersal ability and habitat preference. Mol. Ecol. 18:2503–2517.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model. 190:231–259.

Pierce, K. L. 2003. Pleistocene glaciations of the Rocky Mountains. Dev. Quat. Sci. 1:63–76.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

R Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Renaut, S., G. L. Owens, and L. H. Rieseberg. 2014. Shared selective pressure and local genomic landscape lead to repeatable patterns of genomic divergence in sunflowers. Mol. Ecol. 23:311–324.

Ribas, C. C., A. Aleixo, A. C. R. Nogueira, C. Y. Miyaki, and J. Cracraft. 2012. A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. Philos. Trans. R. Soc. B22:681–689.

Rosenberg, N. A. 2004. distruct: a program for the graphical display of population structure. Mol. Ecol. Notes 4:137–138.

Rosenblum, E. B. 2006. Convergent evolution and divergent selection: lizards at the White Sands ecotone. Am. Nat. 167:1–15.

Ryan, P. G., P. Bloomer, C. L. Moloney, T. J. Grant, and W. Delport. 2007. Ecological speciation in South Atlantic Island finches. Science 315:1420–1423.

Saccheri, I., M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392:491–494.

Schluter, D., and P. R. Grant. 1983. Determinants of morphological patterns in communities of Darwin's finches. Am. Nat. 123:175–196.

Schönswetter, P., I. Stehlik, R. Holderegger, and A. Tribsch. 2005. Molecular evidence from glacial refugia of mountain plants in the European Alps. Mol. Ecol. 14:3547–3555.

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

Soltis, D. E., M. A. Gitzendanner, D. D. Strenge, and P. S. Soltis. 1997. Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. Plant Syst. Evol. 206:353–373.

Soltis, D. E., A. B. Morris, J. S. McLachlan, P. S. Manos, and P. S. Soltis. 2006. Comparative phylogeography of unglaciated eastern North America. Mol. Ecol. 15:4261–4293.

Spellman, G. M., B. Riddle, and J. Klicka. 2007. Phylogeography of the mountain chickadee (*Poecilegambeli*): diversification, introgression, and expansion in response to Quaternary climate change. Mol. Ecol. 16:1055–1068.

Stehlik, I., F. R. Blattner, R. Holderegger, and K. Bachmann. 2002. Nunatak survival of the high Alpine plant *Eritrichum nanum* (L.) Gaudin in the central Alps during the ice ages. Mol. Ecol. 11:2027–2036.

Taberlet, P., L. Fumagalli, A. G. Wust-Saucy, and J. F. Cosson. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Mol. Ecol. 7:453–464.

Thompson, R. S., and K. H. Anderson. 2000. Biomes of western North America at 18,000, 6000, and 0 ^{14}C yr $_{BP}$ reconstructed from pollen and packrat midden data. J. Biogeogr. 27:555–584.

Thompson, R. S., C. Whitlock, P. J. Bartelin, S. P. Harrison, and W. G. Spaulding. 1993. Climatic changes in the western United States since 18,000 yr B.P. Pp. 468–513 *in* H. E. Wright Jr., J. E. Kutzbach, W. F. Ruddiman, F. A. Street-Perrott, and T. Webb III, eds. Global climates since the last glacial maximum. University of Minnesota Press, Minneapolis, MN.

Turner, T. F., and J. C. Trexler. 1998. Ecological and historical associations of gene flow in darters (Teleostei: Percidae). Evolution 52: 1781–1801.

Whiteley, A. R., P. Spruell, and F. W. Allendorf. 2004. Ecological and life history characteristics predict population genetic divergence of two salmonids in the same landscape. Mol. Ecol. 13:3675–3688.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. PCA results (A) and STRUCTURE results (B) identifying three *Carex* individuals with significant contributions of heterospecific genomic material.

Figure S2. Present-day ENMs for *C. chalciolepis* (A) and *C. nova* (B).

Figure S3. The number of reads per individual, where individuals 1 through 40 are *C. nova* and individuals 41 through 80 are *C. chalciolepis*.

Figure S4. The number of SNPs present in each population of *C. chalciolepis* (in black) and *C. nova* (in gray).

Figure S5. PCA detail of *C. nova*'s Central and North populations (see Fig. 5B).

Figure S6. Detail of the North region (see Fig. 2) to illustrate the interaction of topography, glaciers, and predicted habitat.

Table S1. Summary statistics for the sampled populations of *C. chalciolepis* and *C. nova*.

Table S2. Population pairwise F_{ST} values (below diagonal) and Euclidean distances (above diagonal) for *C. chalciolepis* (A) and *C. nova* (B).