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Environmental gradients shape genetic variation in the desert moss, *Syntrichia caninervis* Mitt. (Pottiaceae)

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The moss *Syntrichia caninervis* Mitt. is distributed throughout drylands globally, and often anchors ecologically significant communities known as biological soil crusts (biocrusts). The species occupies a variety of dryland habitats with varying levels of drought and temperature stress, suggesting the potential for ecological specialization within *S. caninervis*. Here, we sampled *S. caninervis* from sites along two elevation gradients and used restriction site associated DNA sequencing to compare the relative impacts of environmental factors and geospatial distances on genetic differentiation in *S. caninervis* populations. While we found no evidence of isolation by distance in our data, one environmental variable, mean annual precipitation (MAP), was found to be a positive predictor of F_{ST}. An ecological association analysis identified 32 SNP alleles that covary significantly with MAP, 15 of which fall within the exonic regions of genes with annotations suggesting diverse roles in response to dehydration stress. Understanding the degree to which genetic variation in *S. caninervis* is associated with environmental factors is key to predicting its potential for persistence in the face of global climate change, which is predicted to be especially detrimental to desert organisms already living at their physiological limits.

Keywords Bryophyte, Desiccation tolerant, Biocrust, Mojave Desert, Colorado Plateau, Population genomics

A changing global climate of increased dry spells, more intense droughts, and heavier rainfall events is predicted to exert a disproportionately negative effect on deserts and drylands, which are already subject to degradation through land use activities[1,2]. In this context, physiological or life history adaptations for survival under environmental extremes may be critical for the persistence of dryland species[3]. Bryophytes – comprising mosses, liverworts, and hornworts – are important contributors to ecosystem services worldwide[4–8]. Bryophytes are poikilohydric in their water relations, with tissues that equilibrate rapidly to ambient relative humidity[9]. When external moisture is lacking in the environment, bryophytes rely on the complex trait of desiccation tolerance to suspend metabolism and enter a state of quiescence between periods of hydration[10–12]. Desiccation tolerant bryophytes, especially mosses, are abundant in drylands globally, where they impact soil formation, stability, fertility, pH, hydrology, and the successful establishment of adjacent plants[13,14].

Syntrichia caninervis Mitt. is a dominant moss species in drylands globally and serves as an important component of biological soil crusts (biocrusts)[15]. Biocrusts are communities of diverse bacteria, including cyanobacteria, and often include additional eukaryotic members such as fungi, green algae, lichens, and mosses[16]. In deserts and drylands, biocrusts perform critical ecosystem functions such as nutrient cycling and soil stabilization[4,5,17]. Changes in species composition due to changing climate are particularly critical for biocrusts, as important ecological functions, such as soil stability, carbon, and nitrogen capture are diminished when larger biocrust organisms such as mosses are eliminated[18]. These ecological functions are tightly linked to species composition and resulting morphology of biocrusts, as biocrust structure impacts the manner in which materials (plant litter, soil, water, dust) travel across or are absorbed by the surface[19–21]. With climate change, the intensity with which environmental stressors limit the survival or reproduction of local populations rises, particularly for dryland species such as *S. caninervis,* threatening their long-term persistence[22,23]. The presence of genetic variation becomes necessary for survival when populations are exposed to new conditions[24–26],

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so understanding the factors influencing genetic diversity and genetic structure in *S. caninervis* is critical for predicting the fate of this species and the biocrust community it frequently supports.

Local adaptation is hypothesized to be widespread in dryland plant species and influences long-term population establishment[3]. While *S. caninervis* inhabits arid environments that would prove too stressful for more sensitive moss species, it still exhibits a broad distribution across different habitat types within western North America[27], including, from low to high elevations, creosote (*Larrea tridentata*) scrub, blackbrush (*Coleogyne ramosissima*) scrub, Joshua tree woodland, and piñon-juniper woodland[28]. Local adaptation can facilitate the breadth of conditions that species occupy[29], so this distribution across varied elevations and habitats in western North America suggests the potential for ecological specialization within *S. caninervis*.

The life history and reproductive ecology of *S. caninervis* may also contribute to genetic structure within the species. Successful fertilization in bryophytes requires standing water[30,31]. In dry conditions, mosses such as *S. caninervis* primarily rely on asexual reproduction and clonal growth for local persistence[32,33]. Additionally, in water-limited habitats, the energy that mosses produce during limited windows of photosynthetic activity is primarily allocated to survival as opposed to sexual reproduction[34,35], so both sex expression and sporophyte production become increasingly rare in *S. caninervis* as environmental stress increases[36]. Because mosses rely on spores for dispersal between populations, and spore production is contingent on successful sexual reproduction and sporophyte development, attenuation of sexual reproduction may also reduce potential for gene flow between populations[37,38]. Thus, heavy reliance on asexual reproduction and clonal growth in stressful environments could amplify isolation by distance in *S. caninervis* and lead to structuring of populations through reduced gene flow[37,39,40].

Environmental factors such as temperature and hydration regimes are expected to influence the allocation of energy to growth, reproduction, and physiological tolerance in *S. caninervis*; the degree to which these energetic trade-offs contribute to the processes of differentiation and diversification can be examined by investigating the population structure of this species across the different environments it occupies. This study explores genetic structure in *S. caninervis* within the context of environmental variables and geospatial distances. Although manipulative experiments are a classical approach to understanding plant ecotypic diversity[2,41,42], the accessibility of methods for genotyping individuals now allows for direct characterization of population structure and ecotypic adaptation in natural environments[43–47]. Studying natural gradients is particularly useful, as they serve as an analog of both community and population structure responses to long-term climate change, particularly when short-term and manipulative experimental climate factors are treated as variables[48]. Abiotic factors, including but not limited to precipitation, soil formation processes, disturbances, temperature, wind velocity, and seasonality, can change significantly over relatively short distances along elevation gradients[49,50]. Within a single study system, geographic isolation is minimized while important environmental factors covary with elevation[51]. In contrast, geographic patterns are expected to play an important role in the differentiation of populations through physical barriers to dispersal and reduced gene flow over large distances; thus, isolation by distance (drift) is often contrasted with differentiation along environmental axes (selection) when investigating potential sources of population genetic structure within species[52,53].

The goal of this study was to compare the relative importance of geographic distance versus environmental distance in shaping genotypic diversity within *S. caninervis*. Using elevation gradients as a proxy for modulated environmental stress levels, we sampled *S. caninervis* populations from two gradients in the Mojave and Colorado Deserts in western North America. We then used restriction site associated DNA sequencing (RADseq) and conducted both reference-based and de novo assemblies of the RADseq data to generate SNPs. The SNP data sets were analyzed to compare the relative impacts of environmental factors and geospatial distances on genetic differentiation to investigate the potential for local adaptation in *S. caninervis* populations. With this information, we will be better equipped to predict the impact of climate change on genetic diversity and local persistence of *S. caninervis* across North America.

Methods

Sample collection

In October and November, 2018, *Syntrichia caninervis* populations were sampled along elevation gradients at two locations: within the Desert National Wildlife Refuge (Mojave Desert, Nevada); and near the Canyonlands Research Center (Colorado Plateau, Utah), separated by a Euclidean distance of 335.97 miles (540.70 km). At each location, *S. caninervis* was collected within three sites along an elevation gradient (Low / Mid / High) (Table 1; Fig. 1). At each site along a gradient, 10 individual moss patches approximately 1 cm in diameter were sampled along three parallel transects. Each transect was 30 m in length, and samples of *S. caninervis* were collected approximately three meters apart. The three parallel transects were separated by at least 10 m, on different slopes when feasible to capture the full scope of microhabitat conditions, for a total of 30 samples collected per site. These steps were repeated for each location, for a total of 18 transects across two elevation gradients (90 samples per gradient; 180 samples in total). The samples were collected using a scoopula and were placed in small paper bags in the field. The samples were transported to the Bryophyte Ecology Lab at California State University, Los Angeles where they were allowed to air-dry in the paper bags for several days prior to preparation for DNA extraction and sequencing.

Sample preparation and sequencing

Once dried, individual stems (one stem per collection) were extracted from the samples under a dissecting microscope and placed into 96-well plates using forceps and scalpels. The dry samples were sent to the University of Missouri DNA Core Facility where they underwent DNA extraction and double-digestion using the restriction enzymes MseI and PstI prior to RADseq. Library construction proceeded through several PCR amplifications

Geographic Region	*Site*	*Elevation*	*Latitude (N)*	*Longitude (W)*
Mojave Desert	Sheep Mountain Creosote (NV Low)	893 m	36.435345°	115.35585°
	Sheep Mountain Joshua Tree (NV Mid)	1680 m	36.519724°	115.16330°
	Sheep Mountain Pinyon-Juniper (NV High)	2272 m	36.590255°	115.21416°
Colorado Plateau	Rio Mesa (UT Low)	1280 m	38.799467°	109.18159°
	Canyonland Research Center (UT Mid)	1633 m	38.070451°	109.56645°
	Semi-Arid Juniper (UT High)	1815 m	37.987836°	109.61722°

Table 1. Collection localities for samples used in this study. $N = 30$ for each site.

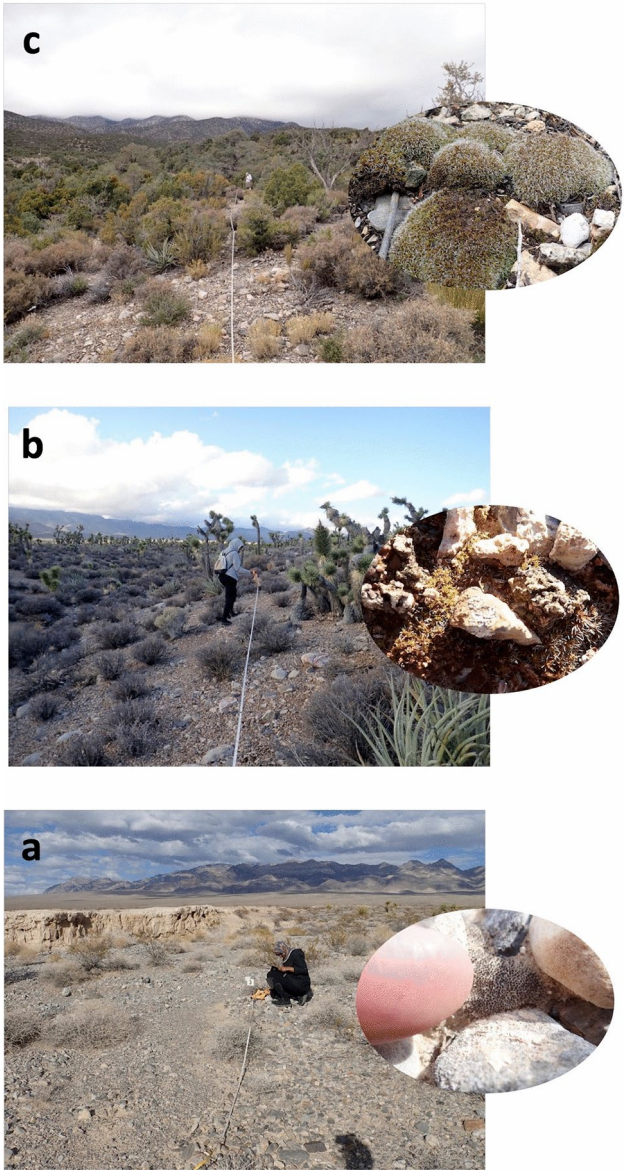

Fig. 1. Representative habitats along Sheep Mountain gradient in the Desert National Wildlife Refuge, Nevada: (**a**) low elevation creosote scrub; (**b**) mid elevation Joshua tree woodland; (**c**) high elevation pinyon-juniper woodland.

and ligations; first to attach barcodes and then to attach the Illumina sequence adaptors to these products. Once libraries were constructed for the samples, they were multiplexed and sequenced in two lanes of a 1 × 150 Illumina NextSeq 500 run. Raw fastq sequence data for each sample were submitted to the NCBI Sequence Read Archive and are available under BioProject accession number PRJNA1002376.

De Novo Assembly and SNP Filtering

Raw fastq sequence data were demultiplexed, trimmed to a consistent length of 60 bp, filtered for quality, and assembled de novo in STACKS v2[54,55]. Initial filtering was performed to retain loci present in at least 60% of individuals across populations and with a minimum minor allele count (mac) of 6 (i.e., minor allele shared by at least three individuals). Identical multi-locus genotypes (MLGs) were identified in *poppr* [56], and the data set was subsequently filtered in VCFtools[57] to retain one representative genotype per clone and to remove individual samples with more than 20% missing data.

Reference-based assembly and SNP filtering

Raw fastq sequence data were demultiplexed, trimmed, filtered for quality, and assembled to the *S. caninervis* reference genome[58] using the iPyrad pipeline[59]. iPyrad was also used to generate consensus loci across samples and identify SNPs. Parameters were set to retain only biallelic loci (max SNPs per locus = 2), and to enforce the expectation of haploidy in the samples (max heterozygotes per consensus sequence = 0; max heterozygous sites per locus = 0). VCFtools[57] was used to iteratively filter SNP loci. First, loci missing from more than 33% of the samples, or with a mac less than 4 (i.e., not present in at least two individuals) were removed, as were individual samples with more than 50% missing data (122 samples, 18,067 sites remaining). Next loci with more than 30% missing data in any of the populations were identified and filtered out. In *poppr* [56], identical multi-locus genotypes (MLGs) were identified and identical genotypes were subsequently filtered from the .vcf file to leave a single representative individual per genotype. Individuals from the NV high elevation site were also filtered from the data set, as too few (< 5) individuals from this site remained for meaningful population genetic inference. Finally, the data set was filtered to remove sites on the sex chromosome (n = 14), thinned so that all loci were separated by at least 2 Kb, and filtered to enforce a minimum minor allele frequency of 0.05 (i.e., minor alleles present in at least three individuals).

Population structure

For all analyses of population structure, the packages dartR, *poppr*, adegenet, and StAMPP[56,60–62] were used in R version 3.6.2[63]. In adegenet and *poppr*, the de novo assembled SNP data were first transformed using principal components analysis (PCA), and 100 PCs were retained to run the K-means clustering algorithm for values of K between 1 and 20. The Bayesian Information Criterion (BIC) was calculated and compared for each value of K to find the number of clusters that best described the data. Discriminant analysis of principal components (DAPC) was run with 40 PCs retained (representing 76% of conserved variance between groups). For the de novo assembled SNP data, ancestry proportions were estimated using sparse nonnegative matrix factorization (snmf)[64]. For the reference assembled SNP data, population pairwise Euclidean distances, F_{ST}, and G_{ST} were calculated using dartR and StAMPP.

Genetic differentiation by geospatial and environmental distance

Latitude and longitude coordinates from each of the collection sites were transformed to a Mercator projection (packages dartR, dismo) to calculate geospatial distances between populations[60]. For each of the collection sites, environmental data were obtained from the software ClimateWNA, which comprises a data set that covers western North America[65]. Environmental variables obtained from ClimateWNA included: monthly (January – December) mean, maximum mean, and minimum temperatures (°C), monthly (January – December) precipitation (mm); seasonal (winter, spring, summer, and autumn) mean, mean maximum, and mean minimum temperature (°C) and precipitation (mm); and annual mean temperature (°C), precipitation (mm), and heat-moisture index. In addition to these individual variables, an ordination (PCA) was performed on the data and the first principal component was extracted as a variable summarizing environmental distances between the populations. Pairwise distance tables for F_{ST}, environmental, and geospatial distances were standardized (mean = 0, var = 1) in the vegan package[66]. Standardized distances were used in linear model regression and Mantel tests to look for relationships between genetic, geospatial, and environmental distances.

Ecological association analysis

The reference-based assembly data were investigated for association of alleles with mean annual precipitation (MAP) in the R package LEA[64]. Sparse nonnegative matrix factorization was used to produce least-squares estimates of ancestry proportions and to compute entropy criteria for different values of K to evaluate the number of ancestral populations that best fit the data. This estimate of K was then used to set the range of latent factor values to use in subsequent latent factor mixed model (LFMM)-based genome-wide association analysis[67]. Twenty replicate LFMMs were run for each value of K from 3 to 6, with 10,000 cycles and a burnin of 5,000. Histograms of test significance values at each value of K were compared, and a latent factor value of 4 was selected for further analysis. Median z-scores from 20 LFMM runs with K = 4 were re-calibrated using a genomic inflation factor of 0.6 to generate a uniform distribution of p-values under the null hypothesis, and the Benjamini–Hochberg algorithm was applied to correct for multiple testing and control for false positives[64]. Loci found to significantly co-vary with environmental variables were mapped back to the *S. caninervis* genome assembly, and gene annotations were recorded for intragenic SNPs. Finally, gene annotations were used to

identify those genes that exhibited significantly altered (compared to hydrated control) transcript abundance in a previous transcriptomic analysis of *S. caninervis* stress responses[58].

Results

The final de novo assembled SNP data set contained 147 individual genotypes from six populations and 1,004 bi-allelic SNP loci with 9.4% missing data. The final reference-based SNP data set comprised 84 unique genotypes from five populations and 1095 bi-allelic loci with 7.9% missing data. Final filtered SNP data sets derived from the de novo and reference-based assemblies along with associated population and sample metadata are available in Dryad (DOI: https://doi.org/https://doi.org/10.5061/dryad.kprr4xhfs).

Population structure – For the de novo assembled SNP dataset, K-means clustering and BIC calculations identified K = 4 as the best fit for the data (Fig. 2a). Discriminant analysis of principle components for the de novo SNP data using K = 4 and the ancestry group assignments of samples based on DAPC posterior probabilities and snmf ancestry proportions suggested two very divergent groups within our de novo assembled samples (indicated in green and yellow, Fig. 2b,c). Morphological re-evaluation of representative samples from each of the four clusters and manual inspection of SNP genotypes strongly supported the presence of non-canonical *S. caninervis* samples in our data set. Based on morphology, both of the non-canonical groups contain samples that fall within the range of *S. ruralis* phenotypic variation (J. Brinda, *pers. comm*). Apparent heterozygosity at several SNP loci in multiple samples within these groups suggested that they comprise admixed allopolyploid individuals and/or as-of-yet undetermined species. Since inclusion of these off-target samples (which were associated with higher elevation sites) could confound our results, further analyses of population structure and environmental associations were restricted to the SNP data set derived from the reference-based assembly. We ensured that this data set excluded the non-canonical *S. caninervis* samples through several mechanisms: morphology of the included samples was re-inspected; RADseq loci from each sample were aligned to both the *S. caninervis*[58] and *S. ruralis*[68] genome assemblies to verify identity as *S. caninervis*; and the reference-based assembly parameters we utilized in iPyrad were sufficiently stringent to exclude any off-target samples.

Cross-entropy calculations from snmf of the reference-assembled SNP data suggested either two or four ancestral populations (Fig. 3a). Ancestry proportions of samples estimated from snmf runs at K = 2 suggested some minimal geographic structuring of the two groups, but no clear pattern was evident (Fig. 3b). Diversity statistics for the five populations included in the reference-based SNP set were at their highest for the low elevation Utah population and at their lowest for the high elevation Utah population (Table 2). Genotypic diversity was generally higher in the low and mid elevation sites than their Nevada counterparts (Table 2). Pairwise F_{ST} revealed relatively low levels of differentiation between the populations, with the highest fixation index (0.220) between the high elevation Utah and low elevation Nevada populations (Table 3).

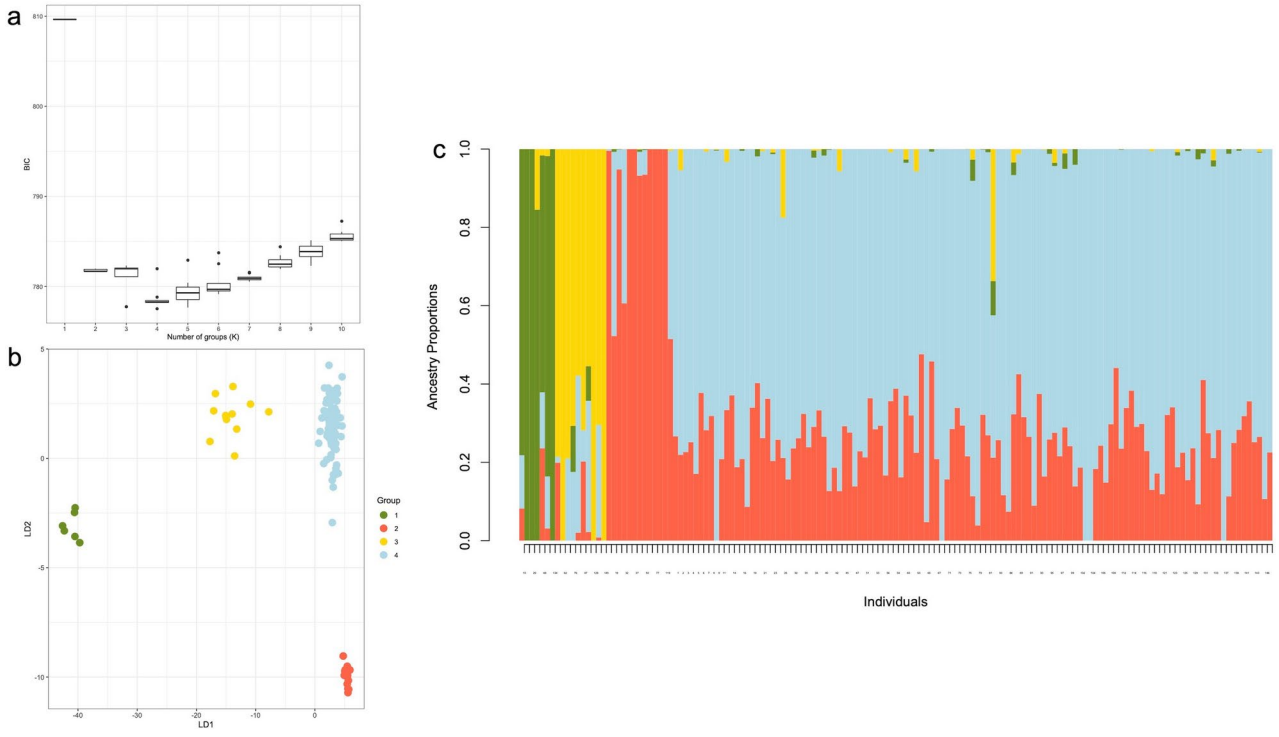

Fig. 2. Population structure in the de novo assembled SNP data set. (**a**) BIC scores for K-means clustering of K values from 1–10. (**b**) Discriminant analysis of principle components at K = 4; green cluster and yellow clusters later identified as putative allopolyploids involving *S. ruralis* and/or undescribed *Syntrichia* species. (**c**) Ancestry group proportions estimated by sparse nonnegative matrix factorization at K = 4.

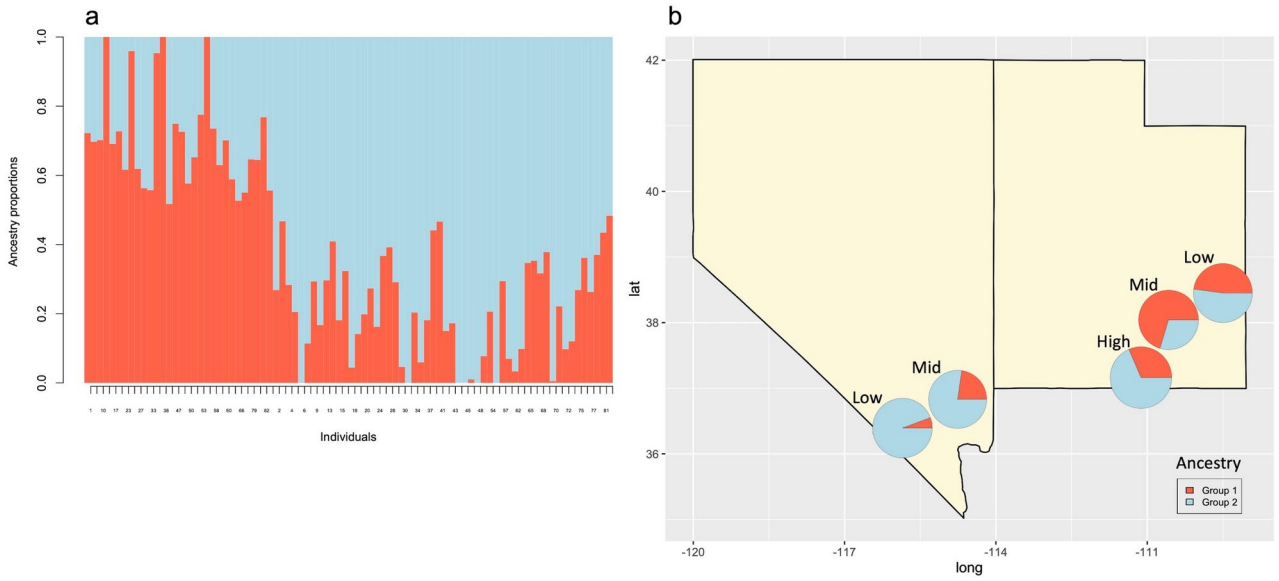

Fig. 3. Genetic structure in samples from the refence assembled SNP data set. (**a**) Sparse nonnegative matrix factorization ancestry proportion estimates for K = 2. (**b**) Proportions of the two ancestry groups in each of the populations from Nevada and Utah. Geographic placement of pie diagrams is not to scale and is intended for illustrative purposes only.

Population	*n*	*Shannon's Index*	*Inverse Simpson's Index*	*Simpson's Index*	*Evenness*	*Clonal Diversity*
NV Low	16	2.467	8.321	0.881	0.679	0.714
NV Mid	16	2.513	11.636	0.914	0.938	0.813
UT Low	26	3.205	24.143	0.959	0.979	0.962
UT Mid	19	2.698	13.370	0.925	0.893	0.842
UT High	7	1.474	3.273	0.694	0.676	0.500

Table 2. Diversity statistics for the five populations included in the reference-assembled SNP data set. All statistics excluding clonal diversity were calculated after removal of identical MLGs.

	NV Low	NV Mid	UT Low	UT Mid
NV Mid	0.106			
UT Low	0.118	0.057		
UT Mid	0.159	0.082	0.028	
UT High	0.220	0.184	0.154	0.195

Table 3. Pairwise F_{ST} estimates for the five populations included in the reference-assembled SNP data set.

Genetic differentiation by geospatial and environmental distance – Linear regression indicated that geospatial distance is not predictive of genetic differentiation in the five study populations ($R^2 = 0.005$, $F(1, 8) = 0.037$, $p = 0.853$). Among all the environmental predictor variables we explored, only mean annual precipitation (MAP) was a significant (positive) predictor of F_{ST} ($R^2 = 0.513$, $F(1, 8) = 8.371$, $p = 0.020$). A Mantel test of MAP x F_{ST} controlling for geospatial distance was not significant (p-value 0.057, 9999 replicates).

Ecological association – Based on the potential relationship between MAP and population differentiation in our data, we performed LFMM on the reference-assembled SNP data to further investigate associations between specific alleles and MAP at the level of individual samples[64]. Our LFMM analysis identified 32 SNP loci that covaried significantly with MAP. Of these loci, 22 mapped to intragenic regions of the *S. caninervis* genome and 15 occurred in exons. Ten of the 15 genes containing exonic SNPs associated with MAP showed significant changes in transcript abundance with drought and heat stress treatments[58] (Table 4).

Discussion

Syntrichia caninervis frequently anchors the biocrust communities of western North America and is an ecologically important plant in dryland ecosystems[69,70]. With this study, we aimed to assess whether ecological

Locus	*Gene*	*Annotation*	*Significant Changes in Transcript Abundance*							
			SD +	SD-	SD/RH +	SD/RH-	30C +	30C-	35C +	35C-
Sc_s1_2483052	Sc_g15720	DUF561 containing protein		X						
Sc_s1_5988534	Sc_g15991	L-Ala-D/L-amino acid epimerase	X							
Sc_s12_29672215	Sc_g02243	galactan beta-1,4-galactosyltransferase GALS1-like	X					X		X
Sc_s3_1252758	Sc_g13412	importin beta-like SAD2 (in tandem repeat)								X
Sc_s6_5216177	Sc_g10167	BTB/POZ domain-containing protein At5g48130	X		X			X		X
Sc_s7_7797113	Sc_g08979	pentatricopeptide repeat-containing protein At2g41720			X					
Sc_s7_11186579	Sc_g09236	ATP-binding cassette transporter	X		X			X		
Sc_s7_19494845	Sc_g09763	aspartic proteinase-like protein 2			X					
Sc_s8_2206296	Sc_g07366	probable trehalase	X		X					X
Sc_s9_1958556	Sc_g05753	hypothetical protein PHYPA_013517					X		X	

Table 4. Exonic SNP loci that covary significantly with mean annual precipitation and occur in genes with significant transcript abundance shifts ("+", increased abundance; "-", decreased abundance) during dehydration and heat stress in *Syntrichia caninervis*. Treatments: "SD", slow-dried; "SD/RH", slow-dried/rehydrated; "30C", elevated temperature; "35C", heat shock.

specialization might contribute to the observed distribution of *S. caninervis* across diverse dryland habitats within this region[27]. We did so by comparing the relative roles of geographic vs. environmental distance in shaping patterns of genotypic diversity in *S. caninervis*. While they should be interpreted cautiously due to low sample size after removal of ambiguous samples, the results of this study do not support a strong role for isolation and drift. Instead, they suggest that ecological specialization associated with variation in precipitation patterns may be more important in shaping the observed patterns of genetic diversity in *S. caninervis*.

Contrary to the expectations of isolation by distance, pairwise F_{ST} estimates failed to increase or decrease consistently with spatial distance between populations. The high elevation population from Utah exhibited the highest F_{ST} estimates in pairwise comparisons with all the other populations. The population from the opposite environmental extreme, the low elevation creosote scrub site in Nevada, displayed the next highest pairwise F_{ST} values relative to the other populations. In our characterization of genetic structure in the reference-assembled SNP data, a plot of cross-entropy calculations from snmf analyses contained two 'elbows' indicative of potential number of ancestral groups at K = 2 and K = 4. Based on the assignment of individual samples to ancestry groups under K = 4, we suspect that the higher K reflects the presence of clonal lineage structure within some of the populations, which persisted despite our removal of identical and nearly identical MLGs from our data set. Thus, we conducted our LFMM with the higher number of latent factors to account for this underlying structure in our SNP data but visualized the relative proportions of the two ancestral groups assigned to our population samples under K = 2, which may reflect deeper genetic structure in *S. caninervis*. While not a striking pattern, each of the two putative ancestry groups is proportionally more dominant in either Nevada or Utah. The notable lack of any relationship between F_{ST} and geographic distance between populations suggests that this underlying genetic structure may reflect processes other than genetic isolation and drift.

A study of *S. ruralis* populations across the Colorado Plateau uncovered a similar lack of evidence for isolation by distance[71], and an earlier study of *S. caninervis* population structure in the Mojave Desert using microsatellite markers found relatively low population structure among sites[37]. Recent phylogenetic work in *Syntrichia* has shown that both *S. caninervis* and *S. ruralis* are members of a northern hemisphere clade within the genus that originated 15.4 – 11.8 mya and diversified rapidly during the Late Miocene, as drier habitats emerged within the northern hemisphere[72,73]. Both the lack of clear geographic structure in our study populations and the observed prevalence of clonality could be consistent with the relatively young age of the species coupled with its life history, which exemplifies the 'perennial stayer'[74]. This life history strategy is characterized by long-lived, slow-growing gametophytes with low rates of sexual reproduction that primarily rely on spores for long-distance dispersal as opposed to local persistence, which is instead accomplished through clonal growth[74].

The emergence of MAP distance as the only environmental variable that positively predicted pairwise F_{ST} is consistent with previous work in *S. ruralis*[71]. Populations of *S. ruralis* sampled across the Colorado Plateau were found to show genetic structure along a monsoonal precipitation gradient, and, to a lesser degree, with elevation, despite evidence of gene flow[71]. For highly desiccation tolerant mosses such as *S. caninervis*, rehydration from a dried and quiescent state involves extensive cellular repair that is energetically costly and creates a carbon deficit that must be re-balanced by a sustained period of photosynthetic activity[70,75]. Thus, in addition to MAP, the seasonal timing and duration of precipitation is critically important for survival, and brief rainfall events during warmer summer months can lead to high mortality in populations not adapted to such conditions[76]. While F_{ST} in combination with our environmental data was not sufficiently granular to resolve the effects of seasonal variation in precipitation at different elevations along the two gradients, this variation in timing of precipitation events is likely an important driver of local adaptation.

Our ecological association analysis identified several loci with alleles that covary significantly with MAP. Subsequent mapping of these loci to the *S. caninervis* genome revealed that a majority (22/32) fall within introns or exons of transcriptome-validated gene regions[58]. Of these loci, one fell within an exon of Sc_g13412, a gene that occurs in a tandem repeat on chromosome 3[58] and is annotated as an importin beta-like SAD2. In

Arabidopsis, SAD2 is implicated in the UV-B response and is thought to function in UV-B protection through mediation of nuclear MYB4 trafficking[77]. In *S. caninervis*, UV exposure acts as a signal to trigger pathways necessary for the desiccation tolerance response[78]. Notably, *S. caninervis* plants grown without UV in the field displayed much slower recovery from desiccation than plants with full UV exposure[79]. Relevant to MAP, UV exposure may be a critical cue necessary for non-photochemical quenching[79], a process that could protect *S. caninervis* from oxidative damage and carbon deficit by preventing the initiation of photosynthesis during brief or unseasonal (i.e., summer) rain events.

Based on annotations, two other loci significantly associated with MAP fall within genes that may be related to abiotic stress signaling in plants: Sc_g12518, a LRR receptor-like protein kinase[80]; and Sc_g07366, a probable trehalase[81]. While Sc_g12518 is not itself part of a tandem repeat, the LRR receptor-like protein kinase genes contribute to the longest tandem repeats within the *S. caninervis* genome and display transcript abundance patterns under stress treatments that suggest an important role in the desiccation tolerance response of this species[58].

Also consistent with previous work on *S. caninervis*, annotations for several genes containing MAP-associated loci are related to plasma membranes and membrane transport: Sc_g01044, similar to nucleobase-ascorbate transporter 12, which is located in the plasma membrane[82]; Sc_g09236, an ATP-binding cassette transporter; and Sc_g06378, an AP-2 complex subunit alpha-1-like gene involved in endocytosis[83]. Analyses relating genomic structure to transcriptomic responses of *S. caninervis* under abiotic stress noted a probable role for membrane-associated proteins in both the stabilization of membranes during drying and rehydration as well as the repair of membranes during rehydration[58].

Additionally, two genes recovered in our association analysis highlight the potential role for cell wall synthesis in the adaptation of *S. caninervis* to precipitation regimes, a result consistent with previous studies investigating the relationship between desiccation tolerance and cell wall traits such as thickness, porosity, and elasticity[11,84]. Sc_g02243 is similar to the galactan beta-1,4-galactosyltransferase-1 (GALS1) gene in *Arabidopsis*, which is necessary for the synthesis of β-1,4-galactan, a major component of pectin that has viscoelastic properties and likely affects the mechanics of cell walls[85]. Sc_g15062 is annotated as cellulose synthase interactive protein 1 (CSI1), which is responsible for coordinating the orientation of cellulose microfibril synthesis so that microfibrils are correctly aligned with the microtubules of the cytoskeleton[86].

To our knowledge, the role of cell wall traits in the desiccation tolerance phenotype of *S. caninervis* has not been investigated, but the recovery of genes related to cell wall synthesis in our association study suggests that this might be a fruitful area for future study. Pectin composition in cell walls influences traits such as hydrophobicity, elasticity, and porosity of plant cells and may play an important role during water deficit[87,88]. Additionally, cell wall thickness may directly impact photosynthetic rates in mosses and other plants that lack stomata, since CO_2 diffusion through cell walls (mesophyll conductance) is thought to be a rate-limiting factor for photosynthesis[84,89]. Pectin content correlates positively with cell wall thickness compared to other cell wall constituents, suggesting that pectin composition in cell walls may be a key factor influencing photosynthetic rates in mosses[89]. Cell wall elasticity has also been shown to have a positive relationship with degree of desiccation tolerance in several moss species[84]. The physical properties of the cell wall and the impact of these traits on processes such as water retention and gas diffusion are likely to be especially important for the highly desiccation tolerant *S. caninervis,* which must survive rapid drying and rehydration events and their accompanying mechanical and physiological challenges[12].

An unanticipated outcome of our study was the detection of possible hybridization between *S. ruralis* and *S. caninervis* in higher elevation sites where they co-occur. Inspection of individual SNP alleles in the putative hybrid samples from the de novo assembly revealed apparent heterozygosity, with one allele matching those in the *S. ruralis* samples and one allele characteristic of *S. caninervis*. The samples from this putative hybrid group failed to make it through our iPyrad reference-based assembly, presumably because our strict intra-individual homozygosity requirement for SNP loci resulted in too much missing data for these samples to pass through subsequent filtering steps. The presence of heterozygosity in moss gametophytes, which should be haploid, suggests that the samples are either recently derived *S. ruralis* X *S caninervis* hybrids or an older allopolyploid lineage that is found at higher elevations. *Syntrichia ruralis* taxonomy is notoriously difficult, and studies of *S. ruralis* and related taxa in Scandinavia found similar evidence of introgression between *S. ruralis* and other co-occurring *Syntrichia* species, suggesting that hybridization and allopolyploidy may be a common phenomenon within the Northern Hemisphere *Syntrichia* clade containing *S. ruralis* and *S. caninervis*[90]. Further work is now underway to resolve the history and identity of these samples.

While not the focus of our study, our diversity statistics revealed the potential influence of anthropogenic disturbance and environmental stress on clonality and genotypic diversity in *S. caninervis*. We expected lower diversity indices and higher levels of clonality at lower elevations, where sexual reproduction is rarely observed and populations rely heavily on asexual propagation[32,35]. Conversely, we predicted the less stressful sites at higher elevations that support sexual reproduction and/or the establishment of more spores would have fewer clones and higher diversity metrics. The levels of clonality and genotypic diversity metrics for the low and mid elevation Nevada sites were consistent with this pattern; however, our removal of the high elevation Nevada site from the analyses weakens our ability to draw any confident inferences about clonality and diversity patterns within the Nevada gradient. The Utah gradient showed the opposite pattern, with the low elevation site containing the highest level of genotypic diversity, and an attenuation of genotypic diversity in the mid and high elevation sites. Both the Utah mid and high elevation sites experience periodic disturbance in the form of cattle grazing (Canyonlands Research Center, the Nature Conservancy), so their lower genotypic diversity and higher levels of clonality may reflect the disproportionately strong negative effect of physical disturbance on biocrust organisms, including mosses, which are adapted to survive extreme physiological stressors but are highly vulnerable to physical disturbance[16].

Data availability

The RADseq data sets generated during and/or analyzed during the current study are available in the NCBI Sequence Read Archive (SRA) and are available under BioProject accession number PRJNA1002376. Filtered SNP files derived from the de novo and reference-based assemblies of the RADseq loci and associated population and sample metadata files are deposited in Dryad: DOI https://doi.org/https://doi.org/10.5061/dryad.kprr4xhfs.

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Author contributions

Both authors conceived and designed the project. UF Collected samples in the field, prepared samples for sequencing, analyzed data, and drafted the first version of the manuscript. KF performed assemblies and SNP filtering, analyzed data, and created figures. Both authors contributed to the ideas and editing of the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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