



## **Differing Patterns of Genetic Diversity and Inbreeding in Two Rare Serpentine Monardellas in the Northern Sierra Nevada**

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DIFFERING PATTERNS OF GENETIC DIVERSITY AND INBREEDING IN TWO RARE SERPENTINE MONARDELLAS IN THE NORTHERN SIERRA NEVADA

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ABSTRACT

*Monardella follettii* (Jeps.) Jokerst and *M. stebbinsii* Hardham and Bartel are two rare endemic mints restricted to patchy ultramafic (serpentine) soil exposures in the northern Sierra Nevada. These species are rare because of small population sizes, low numbers of total populations, and limited availability of their specialized habitat. We collected samples from populations across the range of both species, and assessed genetic diversity, inbreeding, and genetic distance among populations. In the relatively more widespread *M. follettii*, we found low genetic diversity, little differentiation among populations, and no evidence of inbreeding. In contrast, we found significant inbreeding, higher genetic diversity, and high population differentiation over short distances in *M. stebbinsii*. We suggest continued protection and monitoring for *M. follettii*, but do not recommend any action intended solely for genetic management. To alleviate inbreeding in *M. stebbinsii*, we suggest pollen transfer from other conspecific populations.

Key Words: conservation, endemism, genetic diversity, genotyping by sequencing (GBS), inbreeding, *Monardella*, serpentine.

Small populations of endemic plants face an elevated risk of extinction from climate change and anthropogenic disturbance (Jump and Peñuelas 2005; Harrison 2013). With rising temperatures, plant populations are predicted to move higher in altitude and latitude, creating especially challenging circumstances for edaphic endemic plants with a low potential to shift their ranges if their specialized habitat is local and rare (Dullinger et al. 2012). These external threats faced by small plant populations may be compounded by the internal genetic challenges of inbreeding, low genetic diversity, and disruptive or insufficient amounts of gene flow that can reduce a population's ability to adapt (Ellstrand and Elam 1993). As land managers and conservationists strategize for the future, they can attempt to mitigate these genetic problems as part of multifaceted conservation plans for threatened plants.

Molecular population genetic analyses allow for the quantification of genetic diversity, inbreeding, and gene flow to elucidate the evolutionary histories and population dynamics of groups of populations. In population genetics, inbreeding appears as an increased level of homozygosity of a population, a state that has been shown to reduce fitness and increase extinction risk in experimental (Frankham 1995), natural (Saccheri et al. 1998), and simulated populations (O'Grady et al. 2006). As genetic diversity decreases, so too does the raw material on which evolution can act, potentially lowering population fitness in a changing environment (Reed and Frankham 2003). Gene flow among populations of a species can augment genetic variation and increase the fitness of a population low in genetic diversity

(Willi et al. 2007; Sexton et al. 2011). However, gene flow also can introduce maladaptive alleles and lower fitness if the populations are in divergent habitats (Fischer and Matthies 1997; Oakley et al. 2015). Conservation geneticists seek to leverage these genetic measures to guide management strategies for the preservation of rare and threatened species. In formulating management guidelines for conservation of endemic plants under sustained climate change, population genetic measures are an essential part of comprehensive conservation plans that include demographic assessments, a thorough understanding of an organism's ecology, and modeling of the geographic factors influencing a species' distribution (Schierenbeck 2017).

*Monardella follettii* (Jeps.) Jokerst and *M. stebbinsii* Hardham & Bartel are two rare, strict serpentine soil endemic mints in the northern Sierra Nevada of California. The species occur throughout the same serpentine soil belt, but their habitats are distinct. *Monardella stebbinsii* occurs on steep, exposed scree slopes and cliff ledges of serpentinite-derived soil in a small geographic range around the mountain known as Red Hill. Populations of *M. follettii* occur on less extreme slopes of peridotite-derived soil across a larger range in Lassen and Plumas Counties (Coppoletta and Woolhouse 2010). The plants are easily morphologically distinguishable. *Monardella follettii* exhibits a mat-like growth and pale-green leaves, whereas *M. stebbinsii* grows as a subshrub with purple-green leaves densely covered in glandular hairs. The reproductive structures of the species are similar in size, phenology, and color, but *M. stebbinsii* has larger inflorescences with more

flowers (Sanders et al. 2013). Both species exhibit significant decreases in seed production when prevented from outcrossing, and *M. stebbinsii* shows lower seed set than *M. follettii*, despite having more pollinator visits (Woolhouse 2012). *Monardella stebbinsii* is reported to be a diploid of  $n = 21$  with some individuals occasionally exhibiting aneuploidy (Hardham and Bartel 1990). No chromosome counts have been explicitly reported for *M. follettii*; however the base number of chromosomes in *Monardella* is thought to be  $n = 21$  (Raven et al. 1965; Hardham and Bartel 1990).

The patchy, limited habitat restricts both species to a small number of populations across Plumas and Lassen National Forests. This equates to about 15 populations and fewer than 1500 individuals of *M. stebbinsii* and 25 populations and 5000–10,000 individuals of *M. follettii*. The California Native Plant Society lists both plants as status 1B.2, signifying moderate threats to 20–80% of the populations. NatureServe ranks *M. stebbinsii* and *M. follettii* as status G2 and G1, respectively, because of the small numbers of populations and individuals. The United States Forest Service, which manages most of the land on which these species occur, lists both taxa as critically imperiled. The loss of a single population could be a major detriment to the survival of the species.

In addition to the genetic consequences of small population size, these species face a number of anthropogenic threats, including logging operations, increasing frequency of wildfires, firefighting activities (e.g., the construction of fire lines), and road construction. Populations of *M. stebbinsii* are especially susceptible to erosion and anthropogenic disturbance because of the steep habitat. A recent conservation assessment recommends that people take extreme care when surveying *M. stebbinsii*, as a minor disturbance can cause the soil underneath the plants to completely slide away (Woolhouse 2012). Further, both species are difficult to propagate in the greenhouse and growing large numbers of individuals for restoration would be challenging.

Phylogenetic relationships within *Monardella* are poorly understood, but some have hypothesized relationships based on morphology and geographic distribution. Elvin and Sanders (2009) placed *M. stebbinsii* in an alliance of relictual mountaintop species with similar morphology. However, Hardham and Bartel (1990) argued that *M. stebbinsii* is not closely related to any other member of the genus. Elvin and Sanders (2009) further suggested *M. follettii* belongs in the *Odoratissimae* alliance defined by glabrous leaves and a suffrutescent habit. The historic population sizes of these species, their progenitors and the extent to which they are reproductively isolated are unknown. However, these factors may influence the amount of genetic diversity in populations, as leaky reproductive barriers could allow for gene flow between these two species and other nearby members of *Monardella*.

Here we present the results of a population genetic survey of the two rare, serpentine-endemic *Monardella* species of Plumas and Lassen National Forests and use the data to construct conservation recommendations for land managers. We sample plants from six populations of *M. follettii* and four populations of *M. stebbinsii*, representing the range of both species through Plumas and Lassen National Forests. We use a genotyping by sequencing (GBS) approach to discover single nucleotide polymorphisms (SNPs) in each species and estimate population genetic parameters. We ask to what extent populations of the two species are genetically diverse, isolated from each other across the landscape, and/or inbred. We then synthesize management strategies to guide assisted gene flow and seed banking.

## METHODS

### Collections

We collected plant tissue from six *M. follettii* and four *M. stebbinsii* Forest Service-described occurrences (hereafter “populations”) from across Plumas and Lassen National Forests, spanning the entire geographic range of both species (Table 1, Fig. 1). We chose our collection sites to match previous work completed as part of an ecological and demographic assessment of the two taxa (Coppoletta and Woolhouse 2010). These populations are abbreviated with the prefixes “MOFO” for *M. follettii* and “MOST” for *M. stebbinsii*, and we have adopted this Forest Service nomenclature for consistency. MOFO3003, MOST005, and MOST003 have Forest Service suboccurrences that were lumped into single populations, as we assumed they were close enough to allow frequent and consistent gene flow. These suboccurrences spanned up to 200 m for MOFO3003 and <15 m for the *M. stebbinsii* populations. At each site, we sampled small amounts of leaf or flower bud tissue from 20–30 individuals, or fewer samples that represented every individual in the population. We vouchered entire plants from most populations for which herbarium accessions did not exist, but did not harvest from populations with very small numbers of individuals. We deposited the specimens into the herbarium at the University of California, Santa Cruz (UCSC).

### DNA Extractions

We extracted total DNA (i.e., nuclear, plastid, and mitochondrial DNA) from all individuals using a modified CTAB protocol (Doyle and Doyle 1987). We tested all DNA samples for purity with a NanoDrop spectrophotometer (ThermoFisher Scientific, Wilmington, Delaware), evaluated degradation and shearing with agarose gel electrophoresis, and quantified concentrations with a Qubit fluorometer (Invitrogen, Carlsbad, California). For samples that were insufficiently clean or slightly degraded, we

TABLE 1. *MONARDELLA* COLLECTION LOCATIONS. GPS coordinates in WGS84 of locations of the populations sampled in this study. Population names correspond to Coppoleta and Woolhouse (2010) and Woolhouse (2012). Because of very small population sizes, herbarium specimens were only taken if existing accessions did not exist and sufficient individuals were present in the population.

Population	Species	Latitude	Longitude	Altitude (m)	Herbarium accession
LFO	<i>M. follettii</i>	40.085904	-121.276602	1347	UCSC8318
MOFO 3009	<i>M. follettii</i>	39.924422	-121.033577	1219	UCSC8316
MOFO 3005	<i>M. follettii</i>	39.925070	-121.077590	1158	JEPS63417
MOFO 3003	<i>M. follettii</i>	40.050508	-121.236500	1463	UCSC8310
MOFO 3002	<i>M. follettii</i>	40.043186	-121.179569	1767	CAS886559
MOFO 3001Nn	<i>M. follettii</i>	39.991851	-121.105293	1584	UCSC8312
MOST 005	<i>M. stebbinsii</i>	40.013566	-121.192917	792	UCSC8311
MOST 004	<i>M. stebbinsii</i>	40.046765	-121.218556	822	CHSC34000
MOST 003	<i>M. stebbinsii</i>	40.023904	-121.166290	853	UCSC8314
MOST 001	<i>M. stebbinsii</i>	40.052640	-121.208145	762	N/A

further cleaned the extractions with a sodium acetate-ethanol precipitation protocol. We chose to genotype the twenty individuals from each population with the highest DNA quality. Once we had genotyped our samples, we simulated population genetic analyses under different numbers of individuals and genetic markers with SPOTG, a conservation genetics planning tool (Laval and Excoffier 2004; Excoffier and Lischer 2010; Hoban et al. 2013),

and found we sampled appropriately with sufficient marker numbers, individuals, and populations for robust analysis.

Library Construction and Sequencing

We sent DNA samples to the Institute for Genomic Diversity at Cornell University (Ithaca, New York) for GBS library construction (Elshire et

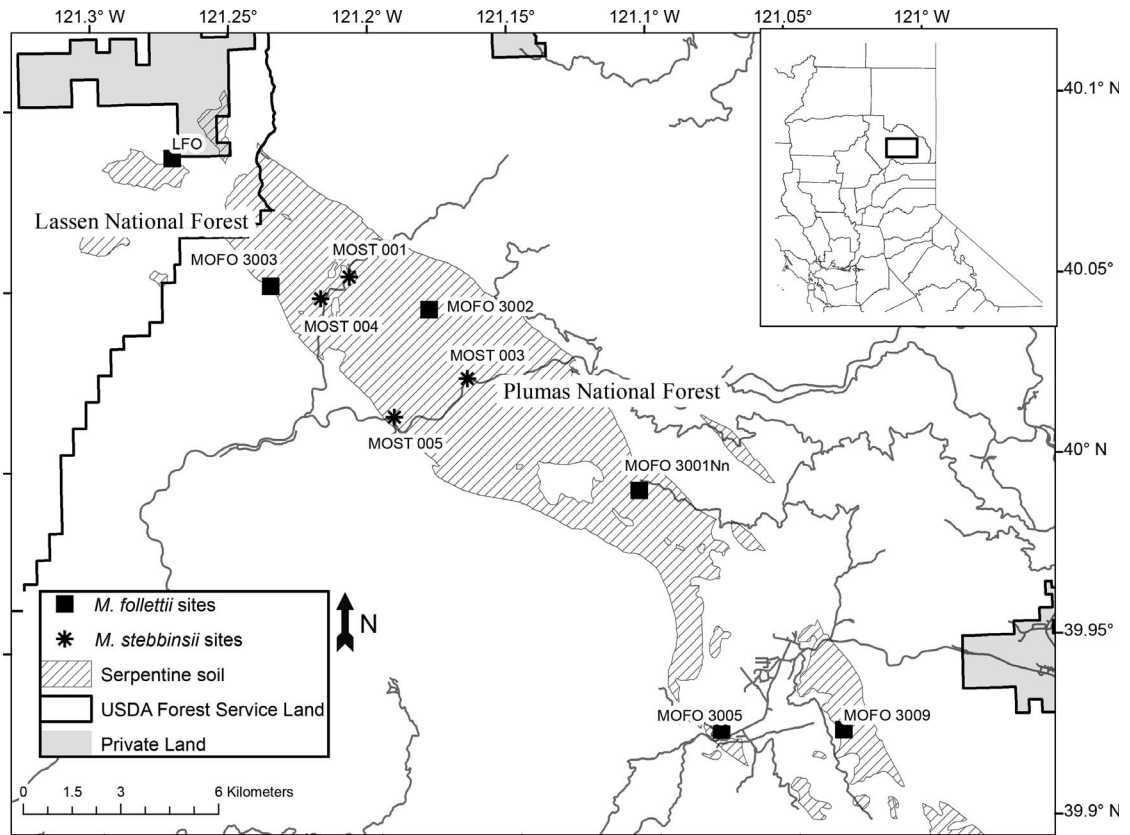


FIG. 1. Map of the study area showing all populations sampled. Species are represented by different shapes. Inset shows expanded map of northern California with a box indicating the approximate area shown in the main map.

al. 2011) and Illumina HiSeq 2500 sequencing (San Diego, California). To construct the libraries (i.e., collections of DNA fragments with known sequences to facilitate sequencing), individual DNA samples were digested with the restriction enzyme *Pst*I, barcoded with adapters of known sequence, and pooled into groups of ninety-five individuals and one negative control. Next, each library was amplified by polymerase chain reaction (PCR) and sequenced in a single lane, returning about two hundred million 100-base-pair sequences per library.

### Sequence Analyses and SNP Calling

We analyzed the raw sequence data with the TASSEL/UNEAK bioinformatics pipeline to generate biallelic SNP calls from the raw sequence data (Bradbury et al. 2007; Lu et al. 2013a). Briefly, the Universal Network Enabled Analysis Kit (UNEAK) pipeline sorts the raw sequences by individual barcode, trims the sequences to 64 base pairs, compiles exactly matching reads as tags, aligns the sequences among individuals to find tags differing at only one base, creates networks of these nearly matching tags, and filters networks that are too complex (i.e., tags with too many SNPs). We employed strict sequence-quality filtering parameters and a minimum coverage threshold of 3 to call a SNP (Lu et al. 2013b). We ran the pipeline separately for each species to maximize the number of loci suitable for within-species analysis. We removed individuals missing more than 20% of the data and loci that were not present in at least 80% of individuals. After this process, we obtained for each species a final matrix that contains a row for each individual with columns for its nucleotide identity at a given locus.

### Population Genetic Analyses

To understand genetic diversity and inbreeding within species, we calculated summary statistics for each species using the software GenAlEx 6.5 (Peakall and Smouse 2012). These summary statistics include the number of private alleles, private allele frequency, expected heterozygosity ( $H_E$ ), and observed heterozygosity ( $H_O$ ). Expected heterozygosity indicates the genetic variability in a population, and the difference between expected and observed values indicates inbreeding. The count and frequency of private alleles give a simple indication of the extent of gene flow between populations. We derived the summary statistics per individual and averaged across all loci. We tested for significant differences between  $H_E$  and  $H_O$  with Bartlett tests of homogeneity of variances in the *adegenet* (Jombart and Ahmed 2011) and *stats* (R Core Team 2016) packages in R.

To evaluate genetic structuring among individuals and populations, we assigned individuals to genetic clusters based on similarities in patterns of genetic variation with a Bayesian assignment analysis implemented in the software STRUCTURE (Pritch-

ard et al. 2000). For both datasets, we ran STRUCTURE using the admixture model with 50,000 burn-in steps, which allows the algorithm to stabilize around realistic values before collecting data from simulations, followed by 100,000 steps. We estimated the hyperparameter  $\lambda$  for each dataset before running the simulations, and subsequently fixed it at the estimated value, as suggested for SNP data sets by Pritchard et al. (2000). For the *M. follettii* and *M. stebbinsii* data sets, we set the prior for most likely number of clusters ( $K$ ) as 1–6 and 1–4, respectively, based on the number of populations sampled. We ran fifteen replicates for each  $K$ . We examined the rate of change of probability in successive numbers of  $K$  to determine the most likely number of genetic clusters for each species (Evanno et al. 2005) with the software STRUCTURE HARVESTER (Earl and von Holdt 2012).

We used Analysis of Molecular Variance (AMOVA) and F-statistics to further examine heterozygosity at the individual, population, and species level. We ran a locus-by-locus AMOVA (Excoffier et al. 1992) using the codominant allelic input with 9999 permutations, and calculated pairwise F-statistics with 999 permutations for significance testing (Wright 1969). We corrected P-values for multiple comparisons for F-statistics using a Holm-Bonferroni adjustment (Holm 1979).  $F_{IS}$ ,  $F_{ST}$ , and  $F_{IT}$  make up the F-statistics and are known as the inbreeding coefficient, fixation index, and overall fixation index, respectively.  $F_{IS}$  represents the reduction of heterozygosity of a population due to inbreeding,  $F_{ST}$  represents the differentiation among populations as a reduction in heterozygosity due to genetic drift within a population, and  $F_{IT}$  represents the total expected reduction in heterozygosity of an individual, i.e., the sum of  $F_{IS}$  and  $F_{ST}$ . We chose to interpolate missing data in the calculation of F-statistics to avoid biased sources of variation.

In order to visualize genetic distances among individuals and how those relate to population membership, we calculated codominant genetic distances among individuals (Peakall et al. 1995) and summarized the results in a principal coordinates analysis (PCoA). Like a principal components analysis (PCA), a PCoA is a method to summarize multivariate data, but a PCoA looks for dissimilarities in the data set as opposed to the similarities sought in a PCA. Finally, we tested for isolation by distance using a paired Mantel Test of linearized pairwise F-statistics and their corresponding pairwise geographic distances to determine if populations that are geographically farther from each other are more genetically distinct than populations closer to each other.

## RESULTS

### SNP Calling

The Illumina sequencing returned about 600 million reads for *M. stebbinsii* and *M. follettii*. After

TABLE 2. SUMMARY STATISTICS (MEAN ± SE) FOR *MONARDELLA FOLLETTII*. Population names correspond to Coppoleta and Woolhouse (2010) and Woolhouse (2012).

	Population						Mean
	MOFO 3001Nn	MOFO 3 002	MOFO 3003	MOFO 3005	MOFO 3009	LFO	
Observed heterozygosity ( $H_O$ )	0.124 (±0.009)	0.157 (±0.01)	0.181 (±0.011)	0.157 (±0.01)	0.135 (±0.009)	0.153 (±0.010)	0.151 (±0.004)
Expected heterozygosity ( $H_E$ )	0.136 (±0.008)	0.149 (±0.008)	0.160 (±0.008)	0.149 (±0.008)	0.135 (±0.008)	0.149 (±0.008)	0.146 (±0.003)
Private allele frequency	0.005 (±0.004)	0.008 (±0.005)	0.008 (±0.005)	0.011 (±0.005)	0.005 (±0.004)	0.008 (±0.005)	
Number of private alleles	2	3	3	4	2	3	

filtering for quality and coverage, we identified 675 SNP loci in 78 individuals and 365 SNP loci in 100 individuals for the *M. stebbinsii* and *M. follettii* data sets, respectively. Before filtering, these totals were 5693 loci and 3318 loci in *M. stebbinsii* and *M. follettii*, respectively.

Genetic Diversity

Populations of *M. follettii* exhibit a mean  $H_E$  of 0.146, and values are consistent across the range of the species (Table 2). Populations of *M. stebbinsii* show a mean  $H_E$  of 0.209 and a significantly lower mean  $H_O$  of 0.165 (Bartlett test of homogeneity of variances,  $P < 0.001$ ) (Table 3), a pattern indicative of genetic drift or inbreeding. Private allele frequencies, which indicate the extent to which populations have differentiated, are about four times higher in *M. stebbinsii* compared to *M. follettii*.

Genetic Structure

The assignment of individuals in the STRUCTURE analysis reveals how the populations cluster based on genotypes alone. Using the  $\Delta K$  method to examine our STRUCTURE analysis, we find  $K = 3$  to be the most likely number of clusters for *M. follettii* (Fig. 2) and  $K = 2$  to be the most likely number of clusters for *M. stebbinsii* (Fig. 3). In *M. follettii*, all individuals are assigned mainly to one major cluster. After this first major assignment, the populations vary based on their assignment to one of the remaining two clusters, with individuals within each population showing fairly consistent assignment to the same proportion of the same clusters. For *M. stebbinsii*, MOST004 and MOST001 individuals

show assignment almost entirely to one of the two genetic clusters, and MOST005 exhibits assignment to the other genetic cluster. The fourth population, MOST003, splits across both clusters, and individuals generally show more variable assignment than individuals in other populations. Clustering in *M. stebbinsii* mirrors the geographic locations of the populations, with MOST001 and MOST004 geographically close together on the northwest side of Red Hill whereas MOST003 and MOST005 are separated from each other by a few kilometers on the south side of Red Hill.

AMOVA and F-statistics reveal how genetic variation is partitioned across the hierarchical levels of individual and population. In *M. follettii*, among-population differences are only responsible for 2% of the genetic variation, and the remaining 98% arises from within individuals (Table 4). The slight negative numbers simply indicate a lack of genetic structure at this level (Excoffier 2000). The overall  $F_{ST}$  values indicate very little genetic structure among populations of *M. follettii* (Table 5), and only one pairwise  $F_{ST}$  (MOFO3002 & MOFO3009) is significant ( $F_{ST} = 0.038$ ,  $P < 0.05$  after Bonferroni adjustment). These low  $F_{ST}$  levels suggest little differentiation among populations of *M. follettii*. In *M. stebbinsii*,  $F_{IT}$  and  $F_{IS}$ , do not differ significantly from zero, suggesting little inbreeding in the populations. The *M. stebbinsii* data show 8% of variation among populations, and the rest of the variation partitioned within and among individuals (Table 6). In contrast with *M. follettii*, we find significant structure among populations in *M. stebbinsii* with an overall  $F_{ST}$  of 0.082 (Table 7). Moreover, *M. stebbinsii* exhibits a large, significant  $F_{IS}$  of 0.210, suggesting substantial inbreeding across the species. All pairwise  $F_{ST}$

TABLE 3. SUMMARY STATISTICS (MEAN ± SE) FOR *MONARDELLA STEBBINSII*. Population names correspond to Coppoleta and Woolhouse (2010) and Woolhouse (2012).

	Population				Mean
	MOST 001	MOST 003	MOST 004	MOST 005	
Observed heterozygosity ( $H_O$ )	0.17 (±0.007)	0.159 (±0.006)	0.15 (±0.006)	0.179 (±0.007)	0.165 (±0.003)
Expected heterozygosity ( $H_E$ )	0.208 (±0.007)	0.218 (±0.007)	0.202 (±0.007)	0.208 (±0.007)	0.209 (±0.003)
Private allele frequency	0.053 (±0.009)	0.03 (±0.007)	0.037 (±0.007)	0.012 (±0.004)	
Number of private alleles	36	20	25	28	

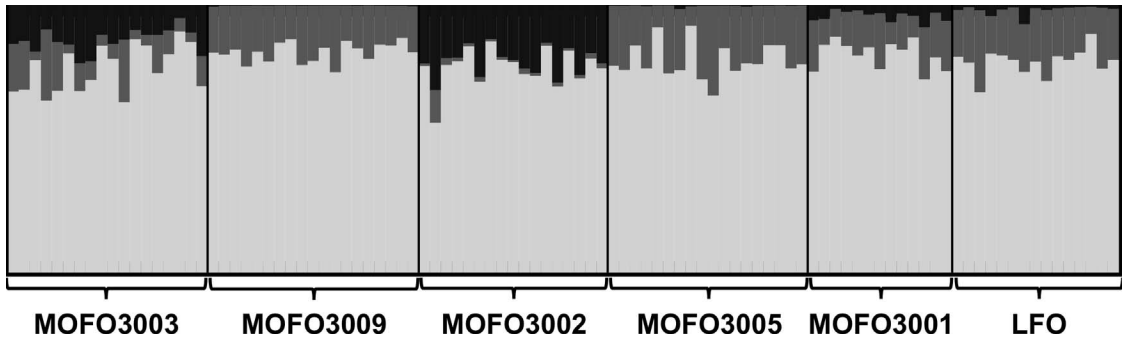


FIG. 2. Assignment of *M. follettii* individuals to genetic clusters as determined by the software STRUCTURE. Each stacked bar represents one individual and different shades show the proportion of its assignment to each of  $K = 3$  genetic clusters. Population names are noted below groups of bars. All individuals are mostly assigned to one cluster with populations differing based on their assignment to the second or third cluster.

comparisons are significant in *M. stebbinsii* (Table 8), and their geographic pattern mirrors the cluster assignment of the STRUCTURE analysis. Mantel tests for isolation by distance using linearized pairwise  $F_{ST}$  values were not significant for either species.

The principal coordinate analyses show how individual pairwise genetic distances correlate with population identity. Our analyses mirror patterns of genetic structure as determined by STRUCTURE and  $F_{ST}$ . In *M. follettii*, we see little discernable clustering of individuals in populations in the first two coordinates (Fig. 4), suggesting that genetic distances between individuals are not correlated with an individual's geographic location. Thus little differentiation seems to have occurred at the population level in *M. follettii*. The MOST001 and MOST004 populations of *M. stebbinsii* show tight clustering on the first coordinate and some differentiation along the second coordinate (Fig. 5). About half of the individuals from MOST003 cluster tightly with MOST001 and MOST004, but the remaining individuals are highly differentiated from MOST001 and MOST004 on the first coordinate and spread

diffusely on the second coordinate. Individuals in MOST005 show the same pattern as this latter half of the MOST003 individuals.

#### DISCUSSION

We set out to determine the extent of inbreeding, the level of genetic diversity, and the connectedness of populations of two rare, serpentine-endemic *Monardella* species in the northern Sierra Nevada. We found different population genetic patterns in the two species. In *M. follettii* we found no evidence of inbreeding within populations with  $H_E$  and  $H_O$  of 0.146 and 0.151, respectively, and little differentiation among populations. Populations of *M. stebbinsii* showed significant inbreeding within populations with  $H_E$  and  $H_O$  of 0.209 and 0.165, respectively, and significant population differentiation over very short distances. These differing patterns tell two different stories for these two, rare congeners. *Monardella follettii*, the species with greater numbers of individuals and populations, appears to have lower genetic diversity as quantified by  $H_E$  than the very rare, restricted *M. stebbinsii*. Though *M.*

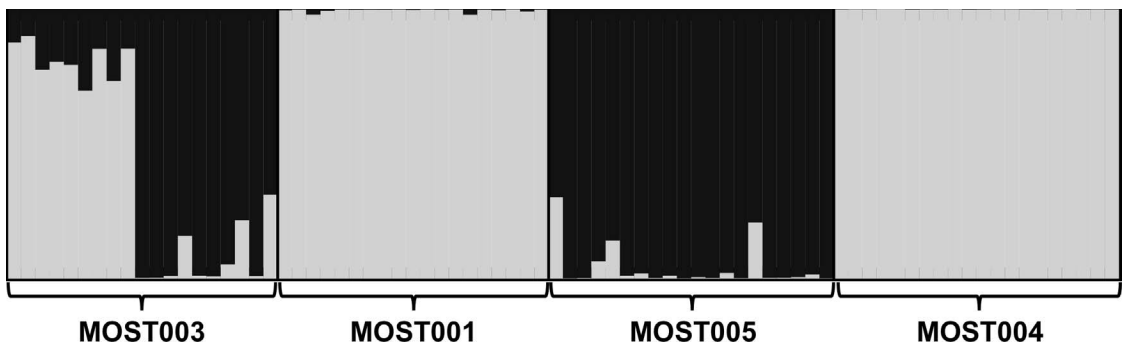


FIG. 3. Assignment of *M. stebbinsii* individuals to genetic clusters as determined by the software STRUCTURE. Each stacked bar represents one individual and different shades show the proportion of its assignment to each of  $K = 2$  genetic clusters. Three of four populations are assigned almost entirely to one or the other cluster. The fourth population splits assignment between the two clusters.

TABLE 4. ANALYSIS OF MOLECULAR VARIANCE (AMOVA) FOR *MONARDELLA FOLLETTII*.

Source of genetic variation	Degrees of freedom	Sum of squares	Estimated variance	Percent variance
Among Populations	5	225.714	0.584	2.1%
Among Individuals	94	2323.238	-0.098	-0.3%
Within Individuals	100	2552.000	27.796	98.3%
Total	199	5100.952	28.283	100%

*stebbinsii* has a higher measure genetic diversity, its populations exhibit extensive inbreeding. Further, populations of *M. stebbinsii* appear to have differentiated to a much greater extent than populations of *M. follettii*, despite its much smaller range and shorter distances between populations. Below, we examine each of these patterns in more depth and offer conservation recommendations based on our interpretations of these data.

Patterns of genetic diversity can elucidate recent and past evolutionary histories of the species under examination, and our data tell diverging stories for *M. stebbinsii* and *M. follettii*. To understand the levels of  $H_E$  in the context of other plants, it is important to know that  $H_E$  varies based on molecular marker type, and SNPs generally show lower diversity than microsatellites (e.g., Ryyänänen et al. 2007). Using GBS to evaluate rare plant populations remains a new area of conservation genetics, and few data sets exist to compare our data to other similar species using these markers. From the available data, large populations of angiosperms typically exhibit  $H_E$  in the range of 0.18–0.30 when examined with SNP markers (e.g., Vandepitte et al. 2012; Saxena et al. 2014; Schilling et al. 2014). Levels of  $H_E$  in populations of *M. stebbinsii* fit in this range, suggesting the rare species has genetic diversity equal to species with many times the numbers of populations and individuals. The level of genetic diversity in *M. stebbinsii* could mean the species was once more widespread, and it has only recently become rare. Such events are known as population bottlenecks and eventually result in very low  $H_E$  as alleles are lost to genetic drift (Young et al. 1996). Although *M.*

TABLE 5. ANALYSIS OF MOLECULAR VARIANCE (AMOVA) F-STATISTICS FOR *MONARDELLA FOLLETTII*. P-values determined by randomization.  $F_{IS}$  represents the reduction of heterozygosity of a population due to inbreeding,  $F_{ST}$  represents the differentiation among populations as a reduction in heterozygosity due to genetic drift within a population, and  $F_{IT}$  represents the total expected reduction in heterozygosity of an individual.

F-statistic	Value	P-value
$F_{ST}$	0.021	<0.001
$F_{IS}$	-0.012	0.745
$F_{IT}$	0.010	0.292

TABLE 6. ANALYSIS OF MOLECULAR VARIANCE (AMOVA) FOR *MONARDELLA STEBBINSII*.

Source of genetic variation	Degrees of freedom	Sum of squares	Estimated variance	Percent variance
Among Populations	3	1024.598	6.495	8%
Among Individuals	74	6532.429	15.315	19%
Within Individuals	78	4496.366	57.646	73%
Total	155	12053.393	79.456	100%

*stebbinsii* is currently restricted to a rare habitat type, this could be through paleoendemism, in which a once widespread species becomes relegated to marginal habitat, such as serpentine barrens, because of low competitive ability (Raven and Axelrod 1978). We would need a resolved phylogeny to evaluate this hypothesis, given that there is no detailed fossil record. *Monardella follettii* shows lower  $H_E$  despite greater numbers of individuals and a more widespread distribution of habitat and populations. In general, low  $H_E$  as seen in *M. follettii* is concerning for rare, threatened plants, but it is not necessarily unexpected given the small population sizes in these species (Ellstrand and Elam 1993; Paschke et al. 2002).

A primary goal of this study was to quantify genetic diversity in order to strategize for the maintenance of evolutionary potential of *M. follettii* and *M. stebbinsii*. Only *M. follettii* shows low  $H_E$  compared to populations of other species of widespread angiosperms (Gitzendanner and Soltis 2000). A paucity of genetic diversity can increase a population's susceptibility to a number of extinction-inducing events. Low genetic diversity can decrease a population's resistance to disease and may leave it less capable of persisting through rapid or sustained changes in climate or other environmental conditions (Spielman et al. 2004; Jump et al. 2009), though it is impossible to directly relate our measures of neutral genetic variation to adaptive genetic variation (Holderegger et al. 2006).

Under current climate projections, populations of both species will have to either adapt to higher temperatures or migrate higher in elevation or northward to track their current climate envelope.

TABLE 7. F-STATISTICS FOR *MONARDELLA STEBBINSII*. P-values determined by randomization.  $F_{IS}$  represents the reduction of heterozygosity of a population due to inbreeding,  $F_{ST}$  represents the differentiation among populations as a reduction in heterozygosity due to genetic drift within a population, and  $F_{IT}$  represents the total expected reduction in heterozygosity of an individual.

F-statistic	Value	P-value
$F_{ST}$	0.082	<0.001
$F_{IS}$	0.210	<0.001
$F_{IT}$	0.274	<0.001



TABLE 8. PAIRWISE  $F_{ST}$  VALUES FOR *MONARDELLA STEBBINSII* POPULATIONS. Population names correspond to Coppoleta and Woolhouse (2010) and Woolhouse (2012). Asterisks indicate significance at  $P < 0.01$ .

	MOST 001	MOST 003	MOST 004	MOST 005
MOST 001				
MOST 003	0.073*			
MOST 004	0.069*	0.071*		
MOST 005	0.118*	0.026*	0.125*	

Uphill migration potential for some populations of *M. follettii* may be limited, because many populations are already located along ridges and mountaintops. Likewise the steep slopes of *M. stebbinsii* habitat may make upward migration to higher altitudes difficult for the species, and the genetic structure seen over short distances suggests that *M. stebbinsii* generally does not disperse even small distances. Looking forward over many decades, both species may face severe challenges from climate change. *Monardella stebbinsii* and *M. follettii* occur in some of the northernmost mountains in the Sierra Nevada, and the volcanic bedrock of the Cascades to the north does not give rise to many serpentine soils. With assisted migration there may be suitable habitat in the Klamath Mountains, but these *Monardella* exhibit fine-scale partitioning in their specific serpentine habitats and may not survive in other serpentine soils (Woolhouse 2012; Kay et al. unpublished data). In addition to human disturbance, climate change, and the genetic challenges of small population sizes, *M. follettii* is threatened by wildfire. Several wildfires have scorched populations of *M. follettii* over the last 15 yr, including the LFO and MOFO3003 populations described here. Wildfires are expected to increase in frequency under sustained climate change towards a warmer and drier climate (Westerling et al. 2006), and even fire-adapted species can be suscep-

tible to a low fire-return interval that exhausts seed banks and allows establishment of competing invasive species (Whisenant 1990; Jacobsen et al. 2004).

In outbreeding plants, low genetic diversity can reduce viable seed set when fewer unrelated mates are available (Byers and Meagher 1991; Young and Pickup 2010). Though mechanisms for self-incompatibility remain unknown for *Monardella*, our focal species are visited by a wide variety of insect pollinators (primarily bees) and show large decreases in seed set when pollinators are prevented from accessing the flowers in the field. Compared to open-pollinated controls, bagged inflorescences of *Monardella follettii* and *M. stebbinsii* show 75 and 73 percent lower seed set, respectively (Woolhouse 2012). Moreover, the open-pollinated control inflorescences show much lower seed set for the relatively inbred *M. stebbinsii* (45%) compared to *M. follettii* (77%), even though pollinator visitation is slightly higher, suggesting that *M. stebbinsii* may be having problems accessing appropriate mates (Woolhouse 2012). High homozygosity and significant  $F_{IS}$  indicate significant inbreeding throughout *M. stebbinsii* that does not occur in *M. follettii*. The sustained inbreeding and persistently small population sizes in *M. stebbinsii* likely reduce fitness through the accumulation of deleterious mutations in populations of the species (Frankham 1995). The larger population sizes of *M. follettii*, usually hundreds of individuals compared with tens of individuals in *M. stebbinsii*, likely enable more random mating in *M. follettii*.

In conservation planning it is important to understand population genetic structure, which reveals the interpopulation dynamics of a species. Such information can indicate which populations may be examined for local adaptation before conservation action is taken (McKay et al. 2005). The two rare *Monardella* species of Plumas and Lassen National Forests exhibit different patterns of genetic structure. The STRUCTURE, PCoA, and

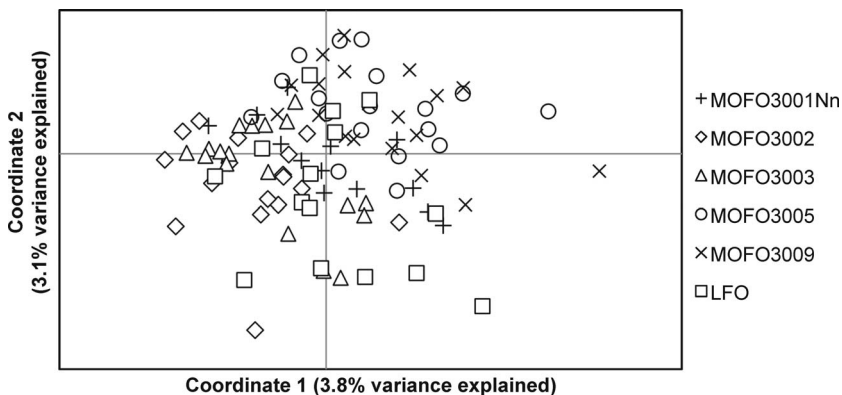


FIG. 4. Principal coordinates analysis of genetic distances for all individuals of *M. follettii*. Individuals are positioned in space according to the first two coordinates from a summarized transformation of a pairwise genetic distance matrix. Little separation between the populations is evident, with only MOFO3002 and MOFO3009 showing no immediate overlap. Coordinate 3 (not shown) explained an additional 2.9% of the variation.

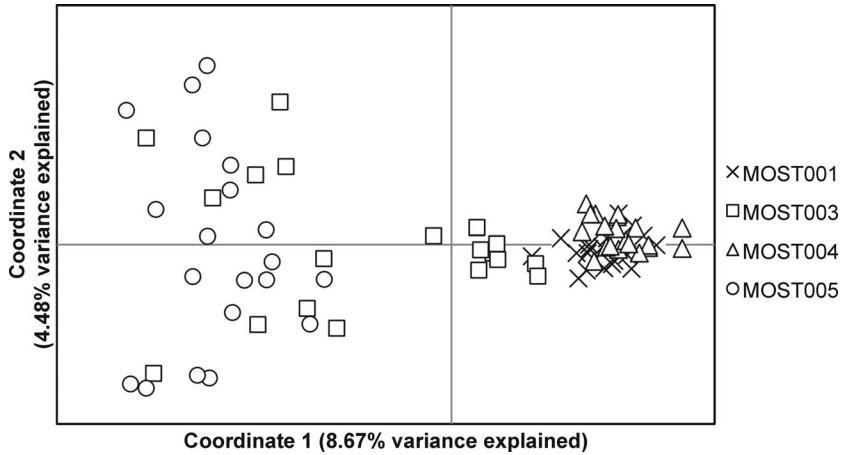


FIG. 5. Principal coordinates analysis of genetic distances for all individuals of *M. stebbinsii*. Individuals are positioned in space according to the first two coordinates from a summarized transformation of a pairwise genetic distance matrix. MOST001 and MOST 004 overlap almost entirely in the first and second coordinate, with almost no overlap of the other two populations. Coordinate 3 (not shown) explains an additional 4.12% of the variation.

$F_{ST}$  analyses reveal little genetic structure among populations of *M. follettii* suggesting frequent gene flow or little separation in time between populations. Conversely, our results show little gene flow and high structure between *M. stebbinsii* populations, even though they occur around a single mountain. In small populations, genetic drift can rapidly fix alleles and contribute to differentiation among populations. Others have shown  $F_{IS}$  to be correlated with genetic structure among populations (Barrett and Kohn 1991; Duminil et al. 2007) suggesting the patterns seen in *M. stebbinsii* likely result from a combination sustained inbreeding and rapid genetic drift, as opposed to long time periods of isolation. In contrast, the larger populations of *M. follettii* populations do not exhibit any detectable inbreeding. Differences in the habitat may further explain some of the differing patterns seen in the two species, as *M. stebbinsii* inhabits a much rarer, patchier, and more extreme landscape than *M. follettii*.

Prior to this conservation assessment, no genetic resources had been developed for *Monardella*, and GBS proved to be an effective choice. With no genomic resources, we were able to develop hundreds of sequence-based markers for each species. Still, the number of SNPs derived from our analysis was lower than anticipated given the thousands described in other systems (Allendorf et al. 2010; Elshire et al. 2011). The lower numbers in our system may be due in part to incomplete digestion of the *Monardella* DNA, which tended to be full of terpenoids and other secondary plant metabolites, despite many refinements to the DNA extraction and cleanup protocols. The difference in the amount of SNPs between the two species is likely in part a result of generally lower genetic variation in *M. follettii* as the TASSEL/UNEAK pipeline requires polymorphism to call a locus. Nevertheless, the number of SNPs in

our analysis were more than sufficient to quantify genetic diversity, inbreeding, and structure of the two species.

#### Conservation Considerations

*Monardella stebbinsii* and *M. follettii* are two of the rarest plant species in Plumas and Lassen National Forests, and the genetic parameters derived from this study can inform management policy. Our recommendations assume that genetic diversity is essential for the long-term evolutionary potential of the populations (Honnay and Jacquemyn 2007), inbreeding can increase extinction risk (O'Grady et al. 2006), and maximizing genetic diversity through transplantation should not compromise local adaptation (McKay et al. 2005). *Monardella* congeners likely hybridize (Sanders et al. 2013; Kay unpublished data), so we advise caution in moving genetic material from any population in which two species of *Monardella* co-occur. However, these two rare species of concern exhibit very different specialized habitats and may be unable to grow in each other's habitats (Woolhouse 2012).

With little inbreeding, high apparent gene flow, and approximately equal genetic diversity in most populations, *Monardella follettii* would likely not benefit from genetic supplementation via pollen or seed movement. Likewise, low genetic distance among populations indicates managers likely do not need to be concerned with the source of plant material if a catastrophic event requires reestablishing a population. In some species in wildfire-prone environments, fire induces seed germination and recruitment of new individuals, which might reveal genetic diversity in a dormant seed bank (Menges and Dolan 1998). Conversely, frequent burn can act as a bottleneck that greatly reduces a population's size and encourages genetic drift and a resulting low

genetic diversity (England et al. 2002). Of the two species examined, *M. follettii* populations are more likely to burn given their more densely occupied habitat. No simulated burn or scarification is needed to germinate seeds in the greenhouse, therefore it is unlikely that the plants require wildfire for recruitment (Woolhouse 2012). In *M. follettii* populations that have recently burned (e.g., MOFO3003, LFO), we found no evidence fire has negatively or positively influenced genetic diversity compared to populations that have not recently burned. Therefore, we cannot recommend the use of artificial burn as a management strategy for *M. follettii* to increase genetic diversity. However, we also think large populations that have not recently burned will not be greatly affected by artificial burns designed to manage other co-occurring species. Ultimately we do not think this species would benefit from any strategies targeted at genetic management, but populations will surely benefit from continued protection from human and environmental threats.

The high inbreeding coefficient in *M. stebbinsii* suggests the species could benefit from population genetic management. Pollen movement could likely relieve inbreeding in populations of *M. stebbinsii*. In the scarlet gilia (*Ipomopsis aggregata*, Polemoniaceae) pollen transfers resulted in increased seed size and count in small, inbred populations (Heschel and Paige 1995). Such transfers would need to be undertaken carefully, since the *M. stebbinsii* habitat is very fragile, and even careful walking around these plants can cause extensive erosion. Therefore, we suggest managers place inflorescences from disparate populations in vases at the periphery of the steep, unstable *M. stebbinsii* habitat, in a location that will not cause erosion. Due to the very low population sizes in *M. stebbinsii*, we suggest managers only take inflorescences from a small number of individuals, as any clipping of flowers represents a substantial reduction in the reproductive potential of the source population. Pollen supplementation would be especially appropriate for the populations along Caribou Road (MOST001 and MOST004), since they show the lowest genetic diversity. In the case that pollen transfers are ineffective, managers could transfer seeds among populations as prescribed above. However, our genetic data suggest that the seeds could be inbred, which could lead to lower germination rates and reproductive success.

In addition to the genetic maintenance strategies described above, we encourage detailed follow-up demographic monitoring and further basic study to better understand the life history of these rare plants. To be specific, studies of the following would be especially relevant to conservation: seed bank quantification and longevity, seed dispersal, and response to natural and anthropogenic disturbance.

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