ORIGINAL ARTICLE

Sympatric serpentine endemic *Monardella* (Lamiaceae) species maintain habitat differences despite hybridization

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Funding information
U.S. Department of Agriculture, Grant/Award Number: FS # 08-JV-11272150-052; Michael Lee Environmental Foundation; California Native Plant Society; Directorate for Biological Sciences; Northern California Botanists; Department of Biological Sciences at SJSU; Department of Ecology and Evolutionary Biology at UCSC

Abstract

Ecological differentiation and genetic isolation are thought to be critical in facilitating coexistence between related species, but the relative importance of these phenomena and the interactions between them are not well understood. Here, we examine divergence in abiotic habitat affinity and the extent of hybridization and introgression between two rare species of *Monardella* (Lamiaceae) that are both restricted to the same serpentine soil exposure in California. Although broadly sympatric, they are found in microhabitats that differ consistently in soil chemistry, slope, rockiness and vegetation. We identify one active hybrid zone at a site with intermediate soil and above-ground characteristics, and we document admixture patterns indicative of extensive and asymmetric introgression from one species into the other. We find that genetic distance among heterospecific populations is related to geographic distance, such that the extent of apparent introgression is partly explained by the spatial proximity to the hybrid zone. Our work shows that plant species can maintain morphological and ecological integrity in the face of weak genetic isolation, intermediate habitats can facilitate the establishment of hybrids, and that the degree of apparent introgression a population experiences is related to its geographic location rather than its local habitat characteristics.

KEYWORDS
edaphic endemic, gene flow, habitat partitioning, hybridization, serpentine, sympatry

1 | INTRODUCTION

The sympatric coexistence of closely related species is a common feature of diverse lineages and species-rich communities (Abbott et al., 2013; Anacker & Strauss, 2014; Grossenbacher, Veloz, & Sexton, 2014). For example, Lakes Victoria, Tanganyika and Malawi each host hundreds of species of cichlid fish (Meyer, 1993), and the small forest reserve at La Selva Biological Station in Costa Rica hosts 44 species in the plant genus *Piper* (Gentry, 1993). Understanding the ecological and genetic factors that enable coexistence among relatives bears directly on such fundamental biological processes as speciation (Barraclough & Vogler, 2000; Fitzpatrick & Turelli, 2006) and community assembly (Briscoe Runquist, Grossenbacher, Porter, Kay, & Smith, 2016; Chesson, 2000; HilleRisLambers, Adler, Harpole, Levine, & Mayfield, 2012). For closely related species, shared ancestral traits and habitat affinities can translate into stronger competition and sharing of pests, pathogens and mutualists compared to distantly related species, potentially leading to competitive exclusion, character displacement, facilitation or convergent evolution (Beans, 2014; Moeller, 2004; Parker et al., 2015; Sargent & Ackerly, 2008; Yguel et al., 2011). Closely related species may also hybridize if reproductive isolation is incomplete, leading to diverse possible outcomes such as the decline or extinction of one or both parental species through genetic or demographic swamping (Holt & Gomulkiewicz, 1997; Levin, Francisco-Ortega, & Jansen, 1996; Wolf, Takebayashi, & Rieseberg, 2001), reinforcement of reproductive

barriers (Dobzhansky, 1940; Kay & Schemske, 2008), establishment of new polyploid or recombinational species (Rieseberg et al., 2003; Stebbins, 1940) or the transfer of adaptive alleles (Arnold et al., 2016; Morjan & Rieseberg, 2004). Documenting the ecological and genetic interactions (or lack thereof) between closely related sympatric species can help establish the relative importance of these diverse outcomes.

Moreover, ecological and genetic interactions between closely related species are not independent, as ecological divergence can reduce gene flow and genetic isolation can allow for ecological divergence. Niche differentiation between coexisting relatives can be implicated in both pre- and postzygotic reproductive barriers, such as selection against migrants between habitats, phenological divergence, pollinator and mating system isolation, and extrinsic selection against hybrids (reviewed in Coyne & Orr, 2004; Lowry, Modliszewski, Wright, Wu, & Willis, 2008). Although ecologically based barriers can be strong (e.g., Kay, 2006; Ramsey, Bradshaw, & Schemske, 2003), they may also be reversible under certain conditions, resulting in ecologically dependent hybridization (Chase & Raven, 1975). Ecological divergence may also shape the extent of introgression across populations and across the genome, if mating patterns of hybrids are influenced by ecological factors or there is selection against particular loci involved in divergent adaptation (Mallet, 2005). Conversely, genetic isolation, caused by either pre- or postzygotic isolation, may facilitate greater ecological divergence by reducing maladaptive gene flow (Hendry & Taylor, 2004; Nosil & Crespi, 2004; Riechert, 1993). In these cases, we expect a relationship between the ecological similarity of heterospecific populations and the extent of hybridization and introgression (isolation by ecology; IBE). Within species, genetic distance commonly increases with environmental or phenotypic distance, supporting the importance of ecological divergence in shaping gene flow within species and initiating speciation (Sexton, Hangartner, & Hoffman, 2014; Shafer & Wolf, 2013). However, any pattern of IBE is less clear among heterospecific populations.

Plants and their edaphic (soil) habitats provide a compelling system to examine ecological and genetic isolation between coexisting species. Unlike many animals, for which vicariant allopatric speciation is the norm (Fitzpatrick & Turelli, 2006; but see Turelli, Lipkowitz, & Brandvain, 2014), plants often speciate at small spatial scales (Kisel & Barraclough, 2010) with abundant opportunities for sympatric contact (Anacker & Strauss, 2014; Grossenbacher et al., 2014). Moreover, their sessile nature makes plants relatively straightforward to characterize ecologically. Edaphic endemism is an especially common and important form of habitat specialization in plants (Rajakaruna, 2018). The striking effects of unusual and often extreme substrates (e.g., serpentine, limestone, dolomite, shale, gypsum and guano) on plants are found even within distances of a few metres (Rajakaruna & Boyd, 2008; Yost, Barry, Kay, & Rajakaruna, 2012), and floras rich with edaphic endemics make up a majority of the earth's biodiversity hot spots (Damschen, Harrison, Anacker, & Going, 2011; Mittermeier et al., 2005). Edaphic factors can contribute to genetic isolation by selecting against migrants, causing genetic or plastic differences in flowering phenology, influencing coflowering plant communities and/or pollinator assemblages, directly altering pollen–pistil interactions or selecting against hybrids (MacNair & Christie, 1983; Meindl, Bain, & Ashman, 2013; Searcy & Macnair, 1990; Yost et al., 2012). Serpentine-adapted plants, which inhabit nutrient-deficient soils with low Ca:Mg and high heavy metal concentrations, have long been a model system for studies of plant speciation (reviewed in Kruckeberg, 1986; Kay, Ward, Watt, & Schemske, 2011; O'Dell & Rajakaruna, 2011).

Here, we study ecological divergence and genetic isolation between a pair of broadly sympatric serpentine soil endemics in the coyote mint genus *Monardella*. We first address two primary questions. First, are the species ecologically divergent? We characterize their habitats and soil element uptake strategies, and we ask whether there are consistent species-level differences. Second, are the species genetically isolated? We use genome-wide genetic markers to identify whether hybridization and subsequent introgression have occurred. We then use our ecological and genetic data to test a series of explicit hypotheses about their genetic isolation. First, the species may be fully isolated and either not hybridize or not experience introgression following hybridization. Second, the species may hybridize and experience introgression through spatial proximity. Third, the species may hybridize in intermediate habitats and introgression may be facilitated by ecological similarity among sites regardless of spatial proximity. Finally, the species may hybridize so extensively that neutral loci are essentially homogenized and only a few key morphological traits are kept distinct by selection. To test these hypotheses, we relate genetic distance to both ecological and geographic distances.

2 | MATERIALS AND METHODS

2.1 | Study area and taxa

We studied two sympatric species of *Monardella* occurring on the Feather River complex in California. The Feather River complex (Lat 39°59′56″N, Lon 121°7′26″W) is a belt of ultramafics approximately 53 km long and three to six km wide consisting of serpentinite, peridotite and other ultramafic rocks. This belt occurs mainly in Plumas County (Northern California, USA) although parts stretch into Sierra, Placer and El Dorado Counties. Elevation ranges from 762 to 1,920 m, the latter being the peak of Red Hill, the only place where both study taxa have been found growing in close sympatry.

The genus *Monardella* (Lamiaceae) is found throughout western North America, with a centre of diversity in California, and comprises over 30 annual and perennial species representing 50 recognized taxa at the species, subspecies, and varietal level (Baldwin et al., 2012; Elvin & Sanders, 2009). It is a taxonomically difficult genus, with putative hybrids commonly reported (Baldwin et al., 2012; Shevock, Ertter, & Jokerst, 1989) and a poor understanding of phylogenetic relationships. *Monardella stebbinsii* and *M. follettii* are strict serpentine endemics (Safford, Viers, & Harrison, 2005), with a short, woody, and rhizomatously spreading growth form. The two are

distinguished morphologically by their leaves, which are glabrous and lanceolate to elliptic in *M. follettii* and narrowly ovate and covered in a dense coat of white hairs in *M. stebbinsii* (Baldwin et al., 2012). Both are self-compatible but predominantly outcrossing and dependent on a variety of insect pollinators for seed set (Woolhouse, 2012). Whereas both taxa are restricted to the Feather River complex, *M. follettii* is more common and widespread than *M. stebbinsii* across the belt, with 25 known occurrence sites and an estimated total of 5,000–10,000 individuals. *Monardella stebbinsii* is only found on and around Red Hill (Figure 1), with 15 known occurrences and <1,500 individuals (CNPS 2014). Both species are state-listed and globally ranked (Faber-Langendoen, Tart, & Crawford, 2009). Phylogenetic relationships between *M. follettii* and *M. stebbinsii* are unknown, and taxonomic treatments have not considered them particularly closely related (Elvin & Sanders, 2009; Hardham & Bartel, 1990). A recent conservation genetics assessment of these two species found contrasting patterns of genetic diversity (Smith & Kay, 2018). The more widespread *M. follettii* have lower genetic diversity, little differentiation among populations and no evidence of inbreeding. In contrast, the more rare *M. stebbinsii* have higher genetic diversity, high population differentiation over extremely short distances and significant inbreeding.

We used a two-stage approach for this study. We first characterized ecological attributes for five sites per species, avoiding any plants that were morphologically unassignable to species. We recorded a set of site-level characteristics and sampled soil and tissue from individual plants for chemical analysis. We then returned the following 2 years to genotype individuals from these and additional sites to characterize patterns of hybridization and genetic differentiation and to relate these patterns to ecological attributes. Our sampling encompassed the full geographic ranges of both species, and we chose sites based on their relatively even spacing, accessibility and plant numbers (Table S1).

2.2 | Are *Monardella follettii* and *M. stebbinsii* ecologically divergent?

To answer this question, we examined general habitat characteristics for five sites per species, sampled soil and plant tissue from five plants per site, and related plant tissue composition to the soil environment.

2.2.1 | Ecological sampling

Site-level habitat characteristics included per cent slope, aspect, per cent canopy cover and per cent cover of rocks, boulders, duff, shrubs and herbaceous species. Canopy cover was averaged over five plants per site, using a convex spherical crown densitometer (Forestry Suppliers, Inc., Jackson, Mississippi, Hinds County). Per cent cover measurements were made by eye, allowing for overlap of layers of coverage. We also recorded all vascular plant species present at each site and generated vegetation ordinations using detrended correspondence analysis in Jmp Pro version 11. All further analyses of plant community composition used the primary axis score of the vegetation ordination.

For each of five plants per site, we analysed both soil chemistry and tissue chemistry. We collected two hundred grams of soil from the rhizosphere using a stainless steel hand trowel. We air-dried soil samples, removed rocks by hand and sent the samples to A & L Western Agricultural Laboratory (Modesto, California, Stanislaus County) for analysis of cation exchange capacity, organic matter, estimated nitrogen release, pH, exchangeable K, Mg, Zn, Mn, Fe, Ni, Cu, B, Ca, SO_4^{2} , NH^{4+}, Na and Bray P. To determine whether *Monardella follettii* and *M. stebbinsii* differ in uptake processes in response to soil chemistry, we also collected 45 g (dry mass) of leaf tissue from the upper portion of each plant, avoiding any soil contamination. Leaf samples were washed in the field in 0.01M HCl, rinsed three times in distilled water and then oven-dried for 48 hr at 70°F. We sent tissue to the University of Maine (Orono, ME) Analytical Laboratory for analysis of 11 elements (Ca, K, Mg, P, B, Cu, Fe, Mn, Zn, Na and Ni). Elemental concentrations were determined by the dry-ashing method and detected by ICP-OES (Thermo Scientific, Tewksbury, MA).

2.2.2 | Ecological analyses

We used principal components analysis (PCA) in R (R Core Team, 2014) to investigate the covariance structure of soil elements and site characteristics (slope, aspect, vegetation cover, etc.) and to reduce these interrelated factors to a small number of orthogonal variables. All soil elemental variables were log-transformed and scaled to a variance of one, because single-unit changes at small concentrations will likely have larger biological impacts than single-unit changes at high concentrations. We performed three PCAs: one on soil elements that were sampled under individual plants, one on characteristics that were sampled for each site, and one on soil elements and site characteristics combined, in which the site characteristics were identical for each soil sample from that site. The latter analysis provided a comprehensive measure of ecological distance among sites for downstream analyses. To relate site characteristics and soil chemistry to the identity of the occupying species, the principal components axes were used as predictors in binomial generalized linear (mixed) models with species as a response and with the PCA axes as a predictor. Random slopes across sites were included for each PCA axis. These GLMMs were fit using package MCMCGLMM in R (Hadfield, 2010), and a weakly informative prior was used for each to prevent variance inflation in the posterior distributions of regression coefficients due to complete separation (Gelman, Jakulin, Pittau, & Su, 2008).

We investigated whether plant ion uptake differed between our focal species by relating foliar tissue chemistry to soil chemistry in a regression framework. Elemental concentrations in a plant's tissue can be influenced directly by soil elemental concentrations, by the bioavailability of elements in the soil, which is strongly affected by soil pH (Rajakaruna & Boyd, 2008) and by intrinsic physiological differences in ion uptake, exclusion and

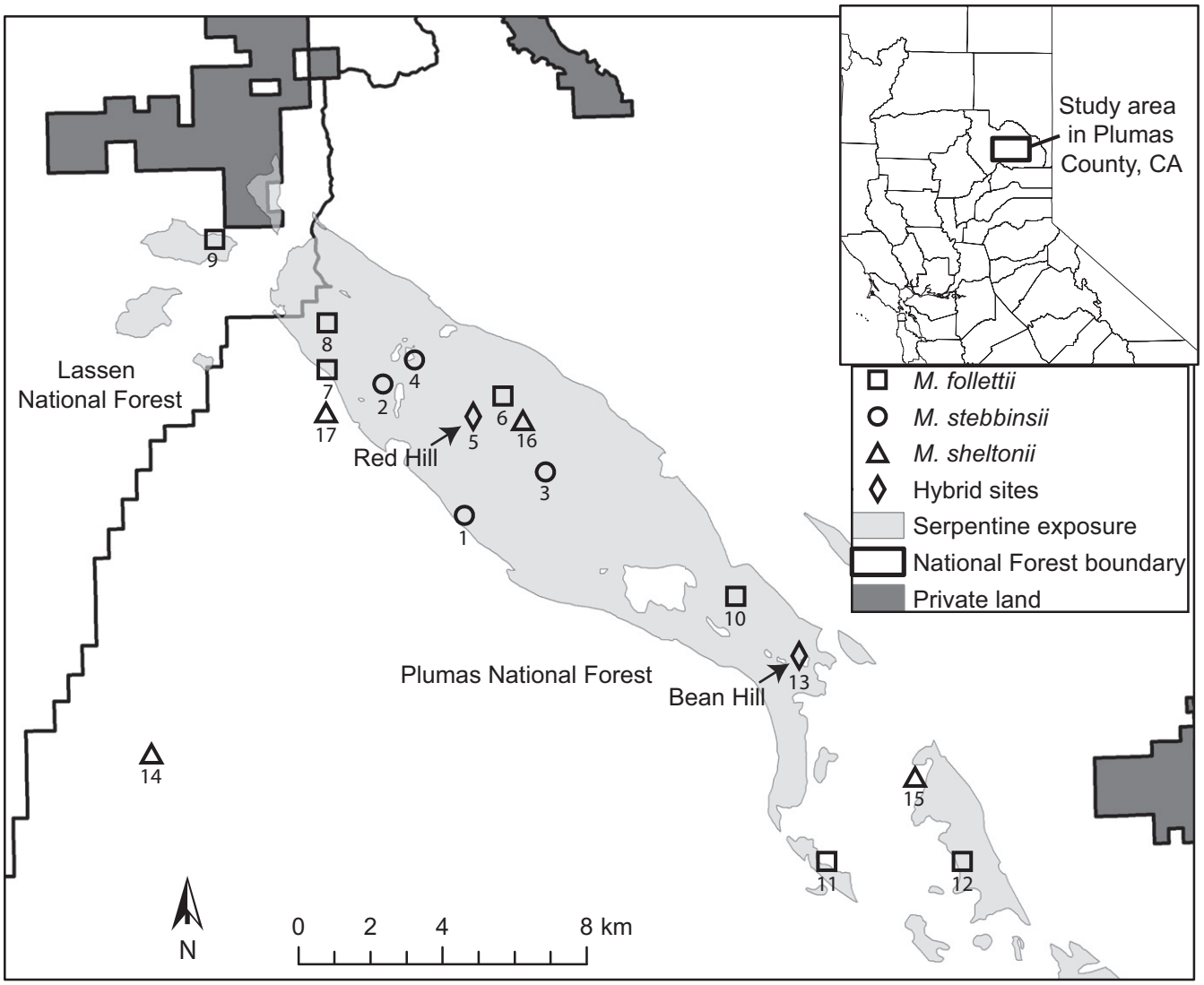

FIGURE 1 Map of study sites. Numbers below symbols correspond to Figures 2 and 4. Red Hill is the putative hybrid zone between *Monardella follettii* and *M. stebbinsii*, whereas Bean Hill is the putative hybrid zone between *M. follettii* and *M. sheltonii*

translocation (Rajakaruna, Siddiqi, Whitton, Bohm, & Glass, 2003). We fit Gaussian regression models for the log-transformed foliar concentrations of Ca, K, Mg, P, B, Cu, Fe, Mn, Zn, Na and Ni across 45 individuals in nine populations. One population of *M. follettii* was excluded from this analysis because of a sample processing error. We included a random intercept per site in each model and used the log-transformed soil concentration of the element, soil pH, species and a species-by-soil concentration interaction as fixed effects. We used AIC to select among the ten possible models that result from various combinations of these fixed effects (Table S2) and averaged model coefficients across the top models (e.g., those with $\Delta AIC < 2$; Burnham & Anderson, 2003). Models were fit using the LME4 package (Bates, Maechler, Bolker, & Walker, 2014) in R statistical language. We were specifically looking for evidence that species or a species-by-soil concentration interaction would best predict tissue concentration, indicating species-specific differences in uptake processes.

2.3 | Are *Monardella follettii* and *M. stebbinsii* genetically isolated?

2.3.1 | Genetic sampling

We sampled plants for genetic analysis from sites throughout the range of *M. follettii* and *M. stebbinsii*, including the 10 sites described above, and additional *M. follettii* sites to fully encompass its geographic range (Figure 1; Table S1). During field sampling, we suspected hybridization between both focal species and a widespread serpentine-tolerating congener, *M. sheltonii*, and thus added limited sampling of this third species. At each of seven sites for *M. folletii*, four sites for *M. stebbinsii* and four sites for *M. sheltonii*, we sampled young leaf tissue from approximately 20 individuals (or a smaller number representing every individual found at the site). One of the five *M. stebbinsii* sites sampled for ecological characteristics (Red Hill) also had *M. follettii* present, and both species, along with an array of

morphologically intermediate plants, were sampled for genotyping. Additionally, one of the five *M. folletii* sites sampled for ecological characteristics (Bean Hill) also had *M. sheltonii* present, and both were sampled for genotyping. At Bean Hill, individuals that key to *M. follettii* display some *M. sheltonii*-like morphology; they have inflorescences with more flowers, are paler green in colour, and are generally larger than other *M. follettii* individuals. Thus, we suspected some hybridization at this site.

We isolated genomic DNA using a modified CTAB protocol (Doyle & Doyle, 1987), and we assessed quality and concentration by NanoDrop (Thermo Fisher Scientific, Wilmington, Delaware), agarose gel electrophoresis and Qubit (Invitrogen, Carlsbad, California). Most extractions were further cleaned with a sodium acetate–ethanol precipitation. We used genotyping by sequencing (GBS; Davey et al., 2011; Elshire et al., 2011) to identify and characterize single nucleotide polymorphisms (SNPs). We sent DNA samples to the Institute for Genomic Diversity (IGD) at Cornell University (Ithaca, New York) for library construction using the *Pst*I restriction enzyme followed by sequencing on the Illumina HiSeq platform (San Diego, California). Ninety-five samples plus one negative control were multiplexed in each sequencing lane, for a total of 285 samples.

We then used the TASSEL/UNEAK bioinformatics pipeline to generate bi-allelic SNP calls from the raw sequence data (Lu, Lipka, et al., 2013). The Universal Network Enabled Analysis Kit (UNEAK) pipeline sorts raw data into files for each barcoded individual, trims the reads to 64 bp, compiles exactly matching reads as tags, pairwise aligns sequences to find tags differing by only 1 bp, creates networks of these nearly matching tags and filters networks that are too complex (Lu, Lipka, et al., 2013). This is a conservative pipeline appropriate for species lacking a reference genome. We employed strict filtering parameters on sequence quality and a minimum coverage threshold of 3 to call a SNP (Lu, Glaubitz, et al., 2013). We recovered a set of loci shared among all three species. We removed any locus that was not sequenced in at least 90% of individuals and any individual missing more than 20% of the data. We tried several alternative values for these filtering cut-offs; however, our results were not qualitatively sensitive to these changes, and therefore, we only report results for these filtering levels. We calculated basic summary statistics of genetic diversity (π and H_e) and divergence (D_{xy}) in the POPGENOME (Pfeifer, Wittelsbürger, Ramos-Onsins, & Lercher, 2014) and ADEGENET (Jombart, 2008) R packages.

2.3.2 | Genetic analyses

We first assessed evidence for hybridization and admixture using a Bayesian assignment analysis, implemented as a Markov chain Monte Carlo algorithm in the software STRUCTURE (Pritchard, Stephens, & Donnelly, 2000) that identifies genetic clusters (*K*) within the SNP data set and infers the genomic composition of each individual in terms of genetic clusters. We ran 15 replicate analyses for each level of *K* using the admixture model with 50,000 burn-in steps followed by 100,000 steps. We estimated the hyperparameter λ, which controls average cluster size, before running the simulations, and subsequently fixed it at the estimated value, as suggested for SNP data sets by Pritchard et al. (2000). We defined the possible range of *K* as 1–14 to cover the total number of sites sampled (but assuming sampling of *M. sheltonii* was not deep enough to separate sites) and determined the most likely *K* by considering the known biology of our study organisms, as recommended by Pritchard et al. (2000) and by looking at the rate of change in the probability of successive numbers of clusters (Evanno, Regnaut, & Goudet, 2005) in *StructureHarvester* (Earl & vonHoldt, 2012).

To determine whether the *M. stebbinsii* × *M. follettii* hybrid zone at Red Hill is solely composed of F1 hybrids or whether there is mating among hybrids and/or backcrossing to either parental species, we used maximum likelihood to estimate the proportion of alleles in each individual plant inherited from each parental species, using the software hindex (Buerkle, 2005) as implemented in the R package INTROGRESS (Gompert & Buerkle, 2010). For the hindex analysis, we used all *M. stebbinsii* and *M. follettii* individuals found away from hybrid zones as parental populations.

To assess support for specific scenarios of historical migration between *M. stebbinsii* and *M. follettii*, we inferred a phylogeny of populations with admixture events using site-level allele counts in the program TREEMIX (Pickrell & Pritchard, 2012). Although sparsely sampled, *M. sheltonii* has high absolute divergence from all *M. follettii* and *M. stebbinsii* sites except Bean Hill (see Results). We therefore chose to pool the four *M. sheltonii* sites as an outgroup in the TREEMIX analyses, although its relatively distant position may be because of hybridization, and not more recent speciation, between the focal species. We first inferred a topology without admixture and then sequentially added six admixture events. We excluded the two hybrid zones from this analysis, as ongoing hybridization in a highly admixed population is not well captured by the TREEMIX model. As we do not know the species tree and did not sample a nonhybridizing outgroup, more formal tests of introgression (e.g., D-statistics; Green et al., 2010) were precluded.

2.4 | How is genetic differentiation related to ecological divergence and geographic distance?

To assess whether hybridization and introgression are promoted by habitat similarity, spatial proximity or both, we examined relationships between genetic distance and both ecological and geographic distances. We modelled these relationships by type of comparison (intraspecific, interspecific and those involving the hybrid zone). We used a regression framework with the maximum-likelihood population effects (MLPE) correlation structure described in Clarke, Rothery, and Raybould (2002), which models the interdependencies in a set of pairwise distances by including two random effects in the linear predictor of each observation (i.e., one for each population in the pairwise comparison). Code implementing the MLPE correlation structure within the R package NLME is available from NSP at github.com/nspope/corMLPE. We used linearized pairwise F_{ST} values (Slatkin, 1995) as our measure of genetic distance, the Euclidean distance between population mean PC1 and PC2 values from the combined

soil chemistry and site characteristics PCA as our measure of ecological distance, and the natural log of linear kilometres as our measure of geographic distance. We regressed genetic isolation against the type of comparison, ecological distance, geographic distance and interactions between each of the two distances and the type of comparison. Both independent variables were centred prior to analysis so that the intercepts could be interpreted as the expected genetic distance at the mean value of the continuous predictor variables. We assessed significance of main effects and interactions with a marginal (Type III) *F* test and employed Wald tests for single regression coefficients under the null hypothesis of zero IBD/IBE slopes. We used partial correlations to check for collinearity between the explanatory variables; additionally, to determine whether ecological distance is related to geographic distance, we fit a second regression model with the MLPE correlation structure with ecological distance as the response and geographic distance and comparison type as the predictor. To assess whether each type of comparison exhibited significant isolation by distance or isolation by ecology, we parameterized each model so that the slopes for each type of comparison (interspecific pairings, intraspecific pairings, Red Hill v. *M. follettii* and Red Hill v. *M. stebbinsii*) were estimated independently. Because we found no significant relationship between genetic and ecological distance within any comparison type, or any relationship between ecological and geographic distance (see Results), we also regressed genetic distance against comparison type and geographic distance for the full set of sites for which we had genetic data. This geography-only analysis included three additional *M. follettii* sites (7, 9 and 10 in Figure 1) at intermediate distances from Red Hill from which we did not sample ecological data.

The distance regression analyses provide a phenomenological description of spatial genetic structure that is easily visualized, but do not provide a generative description of the data; that is, one cannot take a fitted model and simulate new allele frequencies that can be compared to observed allele frequencies. Thus, it is difficult to attribute observed patterns to particular scenarios of gene flow and introgression. To model covariance in genotypes across populations and species, we adopted the approach of Bradburd, Ralph, and Coop (2013), which treats observed allele frequencies at a locus as beta-binomial random variables and (unobserved, logit-transformed) true allele frequencies as a Gaussian random field across geographic and ecological space.

We modified the spatial beta-binomial model of Bradburd et al. (2013; see also Wang & Bradburd, 2014) to accommodate two species with spatially and/or ecologically varying admixture. Specifically, we modelled the (logit) allele frequencies of each species across geographic and/or environmental space as separate Gaussian random fields. At a given spatial location (e.g., a population), we assumed that the genotypes are a combination of the two species. Thus, we modelled the observed allele frequencies as a mixture of two beta-binomial distributions, each associated with the underlying random field of allele frequencies of a species. The mixture weights are unknown quantities that represent the degree of admixture of the two species within each population. Each of the two random fields has its own stationary mean (per locus) and covariance function (shared across loci). To compare among different covariance models (described below), we used the Watanabe-Akaike information criterion (WAIC; Watanabe, 2010). Mathematical and computational details are provided in Appendix S1.

Each of the covariance models that we considered represents a hypothesis about gene flow between populations. We divided the populations into three "blocks": populations that are phenotypically *M. stebbinsii*, phenotypically *M. follettii* and a single population in the putative Red Hill hybrid zone. The models were constructed by fixing the covariance between and within certain blocks to zero: (i) zero covariance within and between blocks, representing the lack of any type of genetic structure; (ii) positive covariance within and between blocks, representing genetic structure of all populations, regardless of phenotype; (iii) positive covariance within blocks, representing genetic structure of both species within species and no gene flow from the hybrid zone; (iv) positive covariance within blocks and between hybrid and phenotypically *M. follettii* populations; and (v) positive covariance within blocks and between hybrid and phenotypically *M. stebbinsii* populations. Note that we made a distinction between the phenotypic identity of populations and the underlying allele frequencies that are associated with the species: for example, under (iv), the allele frequencies for both *M. stebbinsii* and *M. follettii* are spatially correlated between the hybrid zone and the phenotypically *M. follettii* populations, but not between the hybrid zone and the phenotypically *M. stebbinsii* populations. Thus, this covariance model represents the formation of hybrid genotypes within a single population that have subsequently migrated to *M. follettii* populations but not to *M. stebbinsii* populations. We fit this series of five models three times: once for ecological covariance only, once for geographic covariance only and once for both geographic and ecological covariance.

3 | RESULTS

3.1 | Ecological divergence

Monardella folettii and *M. stebbinsii* show significant differentiation in both soil and site characteristics. The first three axes of a PCA of 19 soil variables represent highly correlated soil variables and explain 62% of the variance (Figure S1a); subsequent axes represent single variables or marginal correlations between variables and are not discussed further. Letting +/ denote the sign of the loading of variables onto PCA axes; soil organic matter ( ), ENR ( ), pH (+), K (–), Ca (–), H (–), CEC (–) and Ni (–) are highly correlated and load strongly on PC1 (37% variance explained); Ca:Mg (+), Mg (–), Zn (+) and CEC (–) correlate strongly on PC2 (15% variance); and Cu (–) and Fe (–) load strongly onto PC3 (11% variance). A binomial GLMM with species as a response and fixed predictors and random slopes (per population) for all three PC axes reveals that only PC1 influences species membership (fit via MCMC; log-odds favouring *M. stebbinsii* = 1.48, $p < .001$, 95% CI = 0.68–2.35, $N = 45$).

The first two axes of our PCA of site characteristics (various types of ground cover, slope and aspect) explain 73% of the

variation in nine variables among 10 sites (Figure S1b). The first axis represents interspecific differences in site characteristics (binomial GLM via MCMC; log-odds favouring *M. follettii* = 9.9, $p < .001$, $N = 10$) and distinguishes steep, boulder-filled sites (negative loadings; *M. stebbinsii*) from flatter, more vegetated sites (positive loadings; *M. follettii*), each with substantially different co-occurring plant communities. The second axis represents intraspecific variation in site characteristics, such as aspect, shrubbiness and rock cover.

Finally, the first two axes of our combined PCA of soil and site variables explain 36% and 12% of the variance, with PC1 representing interspecific differences and distinguishing steep, boulder-filled sites with high pH from flatter, more vegetated sites with high soil organic matter, Ca, ENR, Ni, K and CEC (Figure 2). Euclidean distances between mean population scores for PC1 and PC2 from the combined soil and site PCA were used as a measure of ecological distance among populations for further analyses. Although only phenotypically *M. stebbinsii*-like plants were sampled for ecological characteristics at Red Hill, this site is intermediate. It shows *M. folletti*-like soil chemistry and *M. stebbinsii*-like site characteristics (Figure S1).

Monardella folettii and *M. stebbinsii* show evidence for differential uptake of three elements. Regardless of the soil concentration, foliar Fe is higher in *M. stebbinsii*, while foliar Ni is higher in *M. follettii* (Figure 3, Tables S3 and S4). The influence of soil Zn on foliar Zn differed between species; with increasing soil concentrations, foliar concentrations increased in *M. stebbinsii* but decreased in *M. follettii* (Figure 3, Tables S3 and S4). Foliar concentrations of other elements were predicted by soil concentrations and/or soil pH, but not by species identity per se. Ca, K and Zn decreased with soil pH, while Mg increased with soil pH (Figure S2, Tables S3 and S4). Foliar K, Mn and Ni increased while foliar Cu decreased with increasing soil concentrations (Figure S2, Tables S3 and S4).

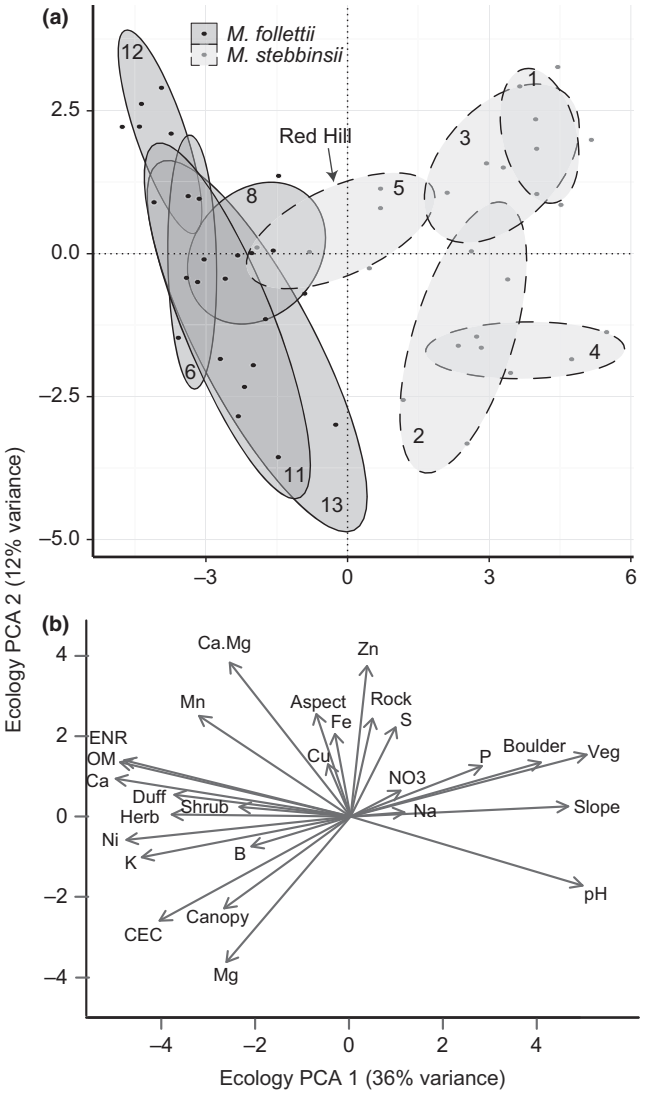

FIGURE 2 Principal components analysis of soil chemistry and site characteristics showing 95% confidence ellipses (a) and a biplot of loading factors (b). Numbers inside each ellipse corresponds to Figures 1 and 4. Euclidean distances between mean population scores were used as a measure of ecological distance in further analyses. For this data set, only plants that morphologically resembled *Monardella stebbinsii* were sampled from Red Hill

3.2 | SNP genotyping

The Illumina sequencing returned about 600 million reads for *M. stebbinsii* and *M. follettii*. After filtering for sequence quality and minimum read depth, we recovered 3,615 loci, each containing a single bi-allelic SNP. After filtering for coverage across individuals and loci, we identified 158 SNP loci shared among 215 individuals, including 77 *M. stebbinsii*, 100 *M. follettii*, 12 *M. sheltonii*, 17 individuals from Red Hill and nine individuals from Bean Hill (Table S1). The average read depth across all loci was 2.3× prefiltering and 84× postfiltering. Summary statistics show similar levels of genetic diversity within each or our focal species, but higher diversity at Red Hill, as expected for a hybrid zone. They also show low absolute divergence within species compared to between species (Table S5).

3.3 | Genetic isolation

The STRUCTURE analysis suggests a complex pattern of hybridization and admixture across the three species, with the most extensive admixture within *M. follettii* (Figure 4). The $K = 3$ model shows three clusters that approximately correspond to the three species. Excluding the hybrid zones at Red Hill and Bean Hill, *M. stebbinsii* individuals have no more than 3% of their genome (mean 0.3%) assigned to the *M. follettii*-like cluster. Yet *M. follettii* individuals, outside of the Red Hill and Bean Hill hybrid zones, show 3%–40% assignment (mean 18%) to the *M. stebbinsii*-like cluster. Across all individuals at Red Hill, the site at which *M. stebbinsii* and *M. follettii* occur in close sympatry assignment to the *M. stebbinsii*-like cluster ranges from 35% to 96%, with the rest of the genome assigned almost entirely to the *M. follettii*-like cluster. Our detailed study of hybrid indices at Red Hill using hindex shows that most individuals exhibit significant hybridity between *M. follettii* and *M. stebbinsii* and that hybrid scores form a continuum between the parent species, consistent with backcrosses and/or mating among hybrids (Figure 5).

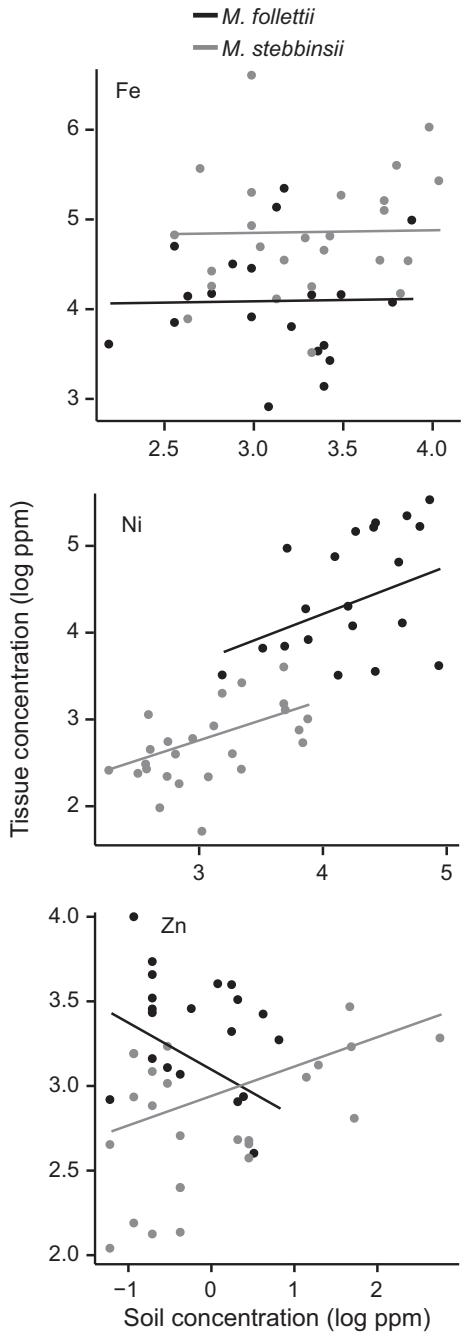

FIGURE 3 Three elements show differences in uptake independent of soil elemental concentrations or soil pH. For Fe, neither soil pH nor soil Fe predicts tissue Fe, but *Monardella stebbinsii* has higher tissue Fe. Tissue Ni is positively related to soil Ni, and *M. follettii* has more tissue Ni for a given soil Ni. For Zn, the effect of soil Zn on tissue Zn differs between species (positive for *M. stebbinsii* and negative for *M. follettii*). Model selection, coefficients and significance levels are detailed in Tables S3 and S4

Both *M. stebbinsii* and *M. follettii* also show some assignment to the *M. sheltonii*-like cluster (Figure 4). Approximately 13% of the genome for all *M. stebbinsii* individuals from one site and half the individuals from another site are assigned to the *M. sheltonii*-like cluster. The former site is adjacent to a population of *M. sheltonii* that was not sampled for this study, but could be a source of admixture. In contrast, *Monardella sheltonii* individuals show essentially no assignment to the *M. stebbinsii*-like cluster (range 0.1%–0.5%), but a consistent 14% assignment (range 13%–15%) to the *M. follettii*-like cluster. Individuals from *M. follettii* sites, excluding Bean Hill, show 0.1%–11% assignment to the *M. sheltonii*-like cluster (mean 1.6%). At Bean Hill, where both *M. follettii* and *M. sheltonii* are present, assignment to the *M. follettii*-like cluster varies from 18%–98% (mean 80%), with the rest of the genome assigned almost entirely to the *M. sheltonii*-like cluster. At Bean Hill, the *M. stebbinsii*-like cluster composes only 0.5%–11% of the genome (mean 3%).

TREEMIX indicates that a simple bifurcating tree with no admixture is a poor fit to the data (Figure S3). Adding admixture events to the tree improves the model fit, with ln(likelihood) scores levelling off after the addition of three admixture events. The placement of these admixture events on the tree indicates introgression from *M. stebbinsii* into *M. follettii* populations in the order expected based on their geographic distance from Red Hill (Figure S3). In all trees, *M. stebbinsii* shows more neutral divergence among sites due to genetic drift, consistent with its higher inbreeding coefficient and F_{ST} values (Smith & Kay, 2018).

3.4 | Isolation by ecology and geographic distance

Within comparison types (intraspecific, pairings involving either species and Red Hill, and interspecific) gene flow does not show isolation by ecology (IBE; Figure S4a), although across comparison types there is progressively more genetic and ecological distance, as indicated by significantly different intercepts ($F_{4,33} = 5.52$, $p = .001$ for genetic distance; $F_{4,37} = 94.97$, $p < .0001$ for ecological distance). For isolation by distance (IBD), we find similar patterns for the combined model that includes ecological distance (Figure S4b) and for the geography-only model that includes additional *M. follettii* sites (Figure 6). There is no significant IBD for intraspecific comparisons (combined model: $\beta = 0.009$, $p = .16$; geography-only model: $\beta = 0.002$, $p = .4775$). However, genetic distance is positively correlated with geographic distance for interspecific comparisons (combined model: $\beta = 0.030$, $p < .0001$; geography-only model: $\beta = 0.031$, $p < .0001$) and for comparisons between Red Hill and *M. follettii* (combined model: $\beta = 0.018$, $p = .001$; geography-only model: $\beta = 0.021$, $p < .0001$). Thus, admixture between species and between the Red Hill hybrid zone and *M. follettii* can be partially explained by the geographic distance of those pairings. There is no significant relationship between ecological distance and geographic distance ($F_{4,37} = 0.23668$, $p = .916$), which facilitates our ability to separate the effects of these factors on genetic distance.

Our modelling of genetic covariance between populations and species shows that covariance between species is best explained by geographic distance, as opposed to ecological distance or geographic and ecological distance combined (Table S6). The best-fit covariance model for ecological distance (as measured by WAIC) includes covariance only within each species and provides relatively little improvement in model fit from a null model of no covariance

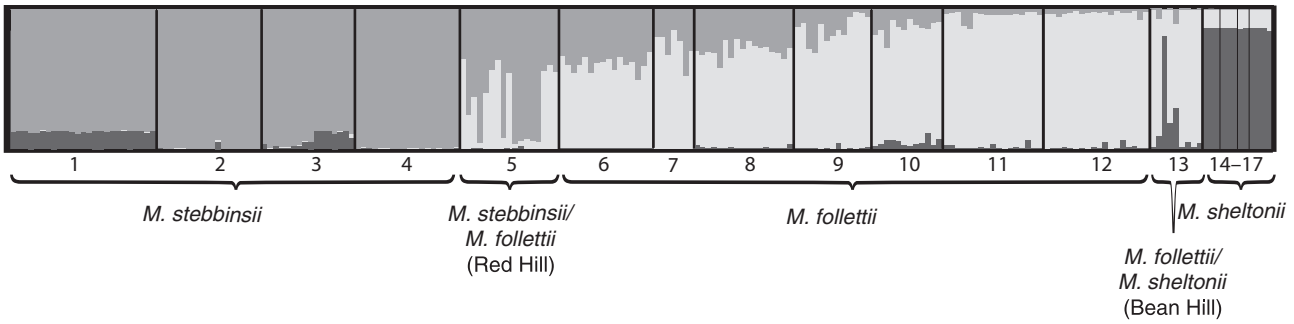

FIGURE 4 Genetic structure of all individuals in the $K = 3$ model as estimated by Bayesian assignment. Each vertical bar represents one individual, and the proportion of each colour within each bar corresponds to the proportion of the individual's genome assigned to each of the three genetic clusters. Vertical black bars separate different geographic sampling sites. The morphological species sampled at each site are indicated below the bars, and numbers correspond to Figures 1 and 2. *Monardella follettii* populations are arrayed ordinally according to their geographic distance from the Red Hill site to highlight the decreasing assignment to the *M. stebbinsii*-like cluster with geographic distance from the hybrid zone

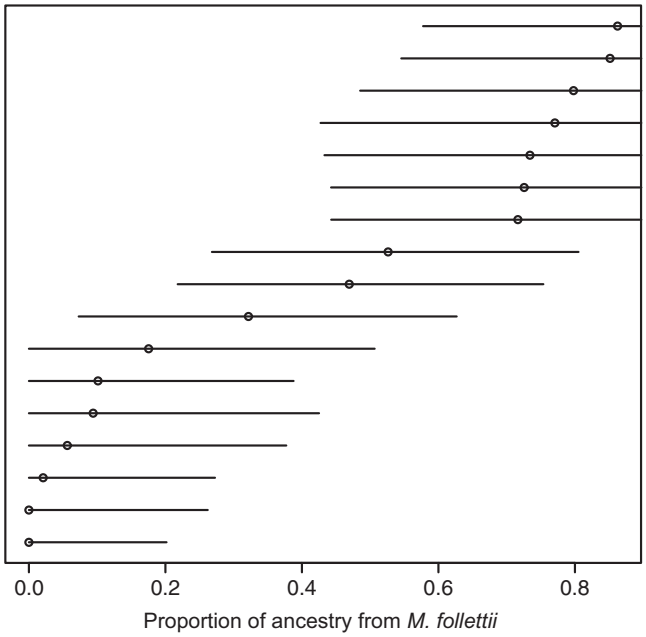

FIGURE 5 Maximum-likelihood estimates of ancestry with 95% confidence intervals for individual plants at the Red Hill hybrid zone. Zero values indicate complete ancestry from *Monardella stebbinsii*

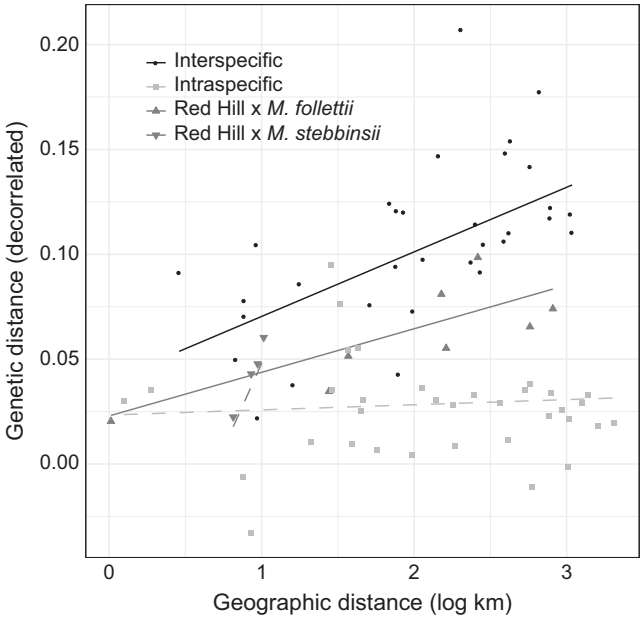

FIGURE 6 Isolation-by-distance (IBD) relationships for different types of pairings. Pairings between species and between Red Hill and *Monardella follettii* show significant IBD (solid lines), but pairings within species or between Red Hill and *M. stebbinsii* do not (dashed lines)

(Table S6a). In contrast, the two best-fit covariance models for geographic distance substantially improve the model fit over the null model and include a model with spatial covariance between all populations, and a model with spatial covariance within species and between *M. folletii* and the hybrid zone (Table S6b). The model with covariance between all populations has the lowest WAIC and effective number of parameters (the "complexity penalty" component of WAIC), while the model where the only cross-species gene flow occurs between the hybrid zone and *M. follettii* had a greater log pointwise predictive density (the "goodness-of-fit" component of WAIC; Table S6b). Examination of pointwise WAIC scores (i.e., the contributions of each data point to the overall score) shows that the difference in WAIC between the two top models is strongly influenced by a single locus (Figure S5) that is fixed in all populations of all species except for three geographically disparate populations of *M. follettii*. Removal of this locus from the analysis swaps the order of the WAIC scores of these two models, but leaves the order of the remaining models unchanged (Table S6). The ordering of the models is invariant to removal of any other single locus. Modelling genetic covariance based on both ecological and geographic distances provides no additional improvement in model fit over geography only (Table S6c).

From this analysis, the species mixture weights agree with the results of STRUCTURE and are consistent across all covariance models: the allele frequencies in the Red Hill hybrid zone are a mixture of the two species at nearly equal proportions, the phenotypically

M. stebbinsii populations show little cross-species admixture, and the phenotypically *M. follettii* populations range from little to some cross-species admixture (Figure S6).

4 | DISCUSSION

4.1 | Species differ in soil chemistry, site characteristics and mineral uptake

Despite the substantial similarities between *M. follettii* and *M. stebbinsii*—both rare perennials with similar stature, nearly identical flowers and restricted to the same ultramafic exposure—we find striking differences in habitat affinity and biogeochemical niche. *Monardella stebbinsii* occupies extremely steep, open and boulder-filled slopes that are unstable for most plant growth. These physical characteristics lead to extremely thin soils with minimal organic matter, soil moisture and nutrient content. In contrast, the gently sloping *M. follettii* habitat is more favourable for substantial plant growth as the relatively deeper soils accumulate more organic matter, nutrients and moisture. Although all sites fall under the broad spectrum of what nongeologists call serpentine, they also differ in their parent material, with *M. stebbinsii* found on serpentinite-derived soil and *M. follettii* on peridotite-derived soil (Coppoletta & Woolhouse, 2010), a difference that likely underlies some of the differences in soil chemistry and slope stability (Alexander & DuShey, 2011). For both above- and below-ground abiotic factors, the *M. stebbinsii* habitat is much harsher for typical plant growth than the *M. folletii* habitat, yet it may also be a less competitive environment, as there are fewer co-occurring trees, shrubs and herbs.

Two lines of evidence suggest the habitat differences we identified reflect divergent adaptation. The entire serpentine exposure is only a few km^2 of nearly contiguous habitat, and populations of the two species grow interspersed, with distances between heterospecific populations similar to distances between conspecific populations (Figure 6). Thus, the species are within easy dispersal distance of each other, yet maintain substantial ecological differences, with only the *M. stebbinsii* adjacent to the Red Hill hybrid zone exhibiting a somewhat intermediate habitat (Figure 2). This pattern likely results from strong selection against migrants, although only a reciprocal transplant in the field would definitively test that hypothesis. Because of the species' conservation status, the fragility of the *M. stebbinsii* habitat and the longevity of the plants, field transplants are not possible in this system. We attempted a common garden study in the glasshouse, in which both plant species were sown into both types of field-collected soil. All plants died within the first couple months on *M. stebbinsii* soil. In contrast, *M. stebbinsii* had lower survival and slower growth than *M. follettii* on *M. follettii* soils (Woolhouse, 2012). It is unclear how these results can be generalized to the field because several habitat characteristics were not replicated in the glasshouse, such as water availability and the competitive environment. Second, our leaf tissue analyses suggest that the species have physiological differences in uptake for several soil elements, including Zn, Fe and Ni. Future work could use hydroponic solutions that manipulate elemental concentrations, perhaps singly and in combination with pH (e.g., Rajakaruna et al., 2003) to better understand the physiological differences tentatively identified here.

Our results highlight the wide range of serpentine habitats present even in small geographic areas and show that serpentine adaptation can be multifaceted. In general, serpentine soils are shallow, retain little water and are characterized by low Ca:Mg molar ratios, high levels of toxic metals and low concentrations of essential plant nutrients (Brady, Kruckeberg, & Bradshaw, 2005; Rajakaruna, Harris, & Alexander 2009). However, within the broad category of serpentine, there are habitats ranging from barrens to chaparral, grassland and forest; soils derived from a range of ultramafic parent material and exposed to weathering for varying amounts of time; and a wide range of soil chemistries, water availability and spatial heterogeneity (Alexander & DuShey, 2011; Baythavong & Stanton, 2010; Kruckeberg, 1984; Rajakaruna & Boyd, 2014). Physiological mechanisms of serpentine adaptation are also diverse and encompass ion exclusion, selective translocation, sequestration, tolerance and hyperaccumulation as well as drought tolerance and physiognomic changes such as increased root systems and smaller stature (reviewed in Kay et al., 2011; Palm & Van Volkenburgh, 2014). Treating adaptation to serpentine as a binary characteristic, as is often done (but see Safford et al., 2005), is clearly a simplification. This study, along with other studies documenting fine-scale habitat specialization within serpentine among close relatives (e.g., Jurjavcic, Harrison, & Wolf, 2002; Pope, Fong, Boyd, & Rajakaruna, 2013; Yost et al., 2012), is important for better characterizing the nature of serpentine adaptation and for understanding distinct pathways to edaphic specialization in a heterogeneous landscape.

4.2 | Genetic isolation is incomplete among species

Despite the substantial differences in habitat affinity, we find evidence of extensive hybridization and introgression among our two serpentine endemic focal species. Red Hill represents an ecologically intermediate site and an active hybrid zone between *M. follettii* and *M. stebbinsii*, with a nearly continuous distribution of hybrid indices indicating advanced generations of hybrids and backcrosses. Outside this site, however, introgression appears asymmetrical, occurring from *M. stebbinsii* into *M. follettii*, but not in the reverse direction. Mixed assignment in a STRUCTURE analysis can be caused by factors besides introgression, including lineage sorting from a polymorphic ancestral species, bottlenecks affecting a derived species in which a subset of genetic diversity is lost, and the presence of unsampled introgressing species in the area. However, introgression between our focal species, specifically from *M. stebbinsii* to *M. follettii*, is the most parsimonious explanation of our results because there are obvious morphological hybrids; the measures of absolute genetic diversity and divergence do not support a budded origin of *M. stebbinsii* from within *M. follettii*; the TREEMIX, distance regression and covariance modelling results all support introgression from *M. stebbinsii* into *M. follettii* according to geographic distance (both north and south) from Red Hill; and we include genotypic data from the third

Monardella species on the Feather River serpentine complex (*M. sheltonii*). Our results strongly support a history of gene flow from *M. stebbinsii* and/or the Red Hill hybrid zone into *M. follettii*, although we cannot clearly distinguish these two possible scenarios. There is little support for either widespread homogenization of neutral loci between the species or a singular admixture event in their evolutionary history.

We also find STRUCTURE evidence consistent with an active hybrid zone between *M. follettii* and *M. sheltonii* at Bean Hill (Figure 4), possible introgression from *M. follettii* into *M. sheltonii* and possible introgression from *M. sheltonii* into *M. stebbinsii*, although we would need more extensive sampling of *M. sheltonii* to substantiate these patterns. Nevertheless, our results suggest that genetic barriers among these three species are porous, but only in certain directions and locations.

The hybridization and introgression among these species are especially striking, as they are not thought to be particularly closely related, relative to the rest of the *Monardella* genus. Elvin and Sanders (2009) place *M. follettii* in the Odoratissimae alliance, in which species share glabrous (smooth) leaves and suffrutescent habit (erect stems woody near the base and herbaceous at the top). In contrast, they fit *M. stebbinsii* in the Australae alliance, a group of putatively relictual mountaintop dwellers that appear similar despite their allopatric distribution. Others have argued that *M. stebbinsii* is not closely related to any other member of the genus (Hardham & Bartel, 1990). The widespread congener, *M. sheltonii*, occurs prolifically throughout Plumas and Lassen National Forests and is occasionally sympatric on serpentine soils with the rare species. Elvin and Sanders (2009) assign *M. sheltonii* to the Villosae alliance on the basis of its wide distribution in western North America. In our study, the low number of shared GBS loci recovered also supports more distant relationships among the species. Unfortunately, standard species-level molecular phylogenetic tools, such as chloroplast or nuclear ribosomal DNA, have not proven useful in this genus, and we have a poor understanding of phylogenetic relationships or phylogeography. Thus, it is unclear whether or how long these species have been geographically isolated from each other before experiencing sympatry.

4.3 | Hybrids occur on an ecologically intermediate site, but gene flow is primarily related to geographic distance

Our data best support a scenario in which intermediate habitat supports an active hybrid zone and spatial proximity determines the extent of introgression away from the hybrid zone. Red Hill is essentially a hybrid habitat, with the soil clustering more closely with *M. follettii* (Figure S1a), but the site characteristics clustering with *M. stebbinsii* (Figure S1b). Thus, it appears that the hybrid habitat facilitates the establishment of genetic hybrids that span a wide range of hybrid indices. Nevertheless, outside of Red Hill, geographic proximity of sites, not habitat similarity, predicts the amount of introgression from *M. stebbinsii* into *M. follettii*. This pattern is suggested by our STRUCTURE results, in which *M. follettii* populations with the highest assignment to the *M. stebbinsii*-like cluster are near Red Hill, the ones with intermediate assignment are further north and south of Red Hill, and the ones with very little assignment are on relatively distant and disjunct serpentine outcrops to the south (Figure 4). This pattern is corroborated by the TREEMIX, isolation-by-distance regressions and covariance modelling results. The Red Hill hybrid zone could provide fruitful sampling for a future detailed study of genotype–environment associations to understand whether hybrid indices, or even particular SNP alleles, are associated with particular habitat variables (e.g., Coop, Witonsky, Di Rienzo, & Pritchard, 2010). In our current study, we did not find any SNP loci with significant departures from neutrality that would indicate selection for or against introgression (data not shown), yet this is not surprising with our limited set of shared loci, our averaging of ecological PC scores across all individuals at a site and our lack of ecological data from the full set of sites sampled in the genetic study.

At a mechanistic level, we speculate that hybridization among these species is promoted by a lack of strong prezygotic reproductive isolating mechanisms. The flowers of all three species are nearly identical, and at least *M. follettii* and *M. stebbinsii* are primarily outcrossing and attract a partially overlapping range of insect pollinators, including many bee species and a few flies (Woolhouse, 2012). They differ in flowering phenology, with peak *M. follettii* flowering in June and peak *M. stebbinsii* flowering in July and August, but there is some overlap, and the species are seen coflowering at Red Hill (Woolhouse, 2012). Postzygotic isolation, in terms of intrinsic hybrid viability and fertility or hybrid performance in the field, may also be weak and would need to be assessed in experimental crosses.

The apparent asymmetry of introgression is a striking feature of this system and has several potential explanations. There may be asymmetry in the strength of prezygotic barriers such as pollinator visitation, the mechanics of pollen transfer by pollinators, flowering time overlap or pollen–pistil interactions. These barriers show asymmetries in many plant systems in which they have been examined (reviewed in Lowry et al., 2008; Yost & Kay, 2009) and could be independent of the edaphic habitat characteristics we measured. There also could be asymmetric postzygotic isolation, such as cytonuclear incompatibility in one direction (Chou & Leu, 2010; Fishman & Willis, 2006). One species could receive more heterospecific pollen simply as a result of being more rare; however, in this case, we would expect the exceedingly rare *M. stebbinsii* to experience more introgression, which is the opposite of what we found. It could be that there has been selection on introgressed alleles; for example, perhaps *M. stebbinsii* alleles are beneficial in allowing *M. follettii* to persist in certain habitats or *M. follettii* alleles are deleterious in *M. stebbinsii* habitats, as has been found for introgressed alleles involved in serpentine adaptation in *Arabidopsis* species (Arnold et al., 2016), although we did not have the appropriate design to test this hypothesis. Finally, patterns of inbreeding and migration may play an important role in facilitating introgression from *M. stebbinsii* to *M. follettii* but not the reverse. *Monardella stebbinsii* is characterized by lower heterozygosity, a higher inbreeding coefficient

and higher F_{ST} than *M. follettii* (Smith & Kay, 2018); this overall isolation of *M. stebbinsii* populations may slow the spread of *M. follettii* alleles away from any hybrid zone.

Factors allowing the coexistence of congeners have generated substantial interest for their importance in both speciation and community assembly processes. Our work provides compelling evidence that coexisting congeners are ecologically distinct and robust to the potentially homogenizing effects of hybridization and introgression. Although our focal taxa show broadly similar habitat affinities, co-occurring on the same local serpentine exposure, they partition habitats in a fine-grained and consistent way. *Monardella follettii* is especially striking in having essentially no genetically pure individuals according to STRUCTURE, yet maintaining its morphological integrity and ecological integrity as a species. Although there is a long history of comparing close relatives on and off serpentine, this study is one of the first to document divergent specialization between close relatives within serpentine soil and highlights the complexities of edaphic adaptation.

ACKNOWLEDGEMENTS

We would like to thank Dr. Earl Alexander for his wealth of knowledge and help with soil classification. Janis Vieto, Rachel Hussey, Julia Lang, Kari Olandese, Annette Bieger and Teri Barry provided field assistance and Yann Surget-Groba provided laboratory assistance. Erica J. Case also provided much logistical support, and the first season of data collection could not have been carried out without her. Jim Belsher-Howe and Michelle Coppoletta of Plumas National Forest assisted with permitting, field operations and general support. The funding for this project was provided primarily by the USDA Forest Service Pacific Southwest Region through a joint agreement (USDA FS # 08-JV-11272150-052) between Dr. Susan Harrison (University of California, Davis), NR, KMK and Dr. Hugh Safford (USDA Forest Service). Additional funding was provided by the Department of Biological Sciences at SJSU, the Department of Ecology and Evolutionary Biology at UCSC, the Michael Lee Environmental Foundation, the Santa Clara Valley Chapter of the California Native Plant Society and a Northern California Botanists Research Scholarship. NSP was supported by an NSF Graduate Research Fellowship.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository at https://doi.org/10.5061/dryad.n0vf77m and include the following:

- Soil, tissue and site characteristics;
- R code for PCA of ecological data;
- R code for leaf tissue regressions;
- STRUCTURE input file;
- TREEMIX input file;
- Ecological, geographic and genetic distances;
- R code for MLPE regressions of distances;
- R and Stan code for beta-binomial covariance models.

AUTHOR CONTRIBUTION

N.R. and K.K. conceived the project. S.W. collected the ecological data. B.S. collected the genetic data. N.P. performed the genetic covariance modeling. All authors performed the analyses. K.K. wrote the manuscript with input from all authors.

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