



# Comparison of phylogeographical structures of a lichen-forming fungus and its green algal photobiont in western North America

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## ABSTRACT

**Aim** Lichens comprise a symbiosis of two separate taxa that share geographical distribution but not necessarily the same evolutionary history. Comparison of phylogeographical structures of lichen symbionts provides valuable insight about the processes shaping the lichen's biogeographical pattern. In this study, we tested the extent to which the shared distribution of the widespread lichen-forming fungus *Ramalina menziesii* and its photobiont *Trebouxia decolorans* across six major ecoregions reflect parallel genetic structure and gene flow.

**Location** Western North America.

**Methods** Based on DNA sequences from multiple genes, we employed distance-based methods to assess co-divergence of symbiosis partners. To assess historical opportunity for co-evolution, we compare migrations among six ecoregions.

**Results** Tests of genetic concordance between the mycobiont and the photobiont genetic variation revealed an overall significant congruent genetic structure across ecoregions. However, the photobiont and the mycobiont do not have high congruencies within ecoregions and the two taxa have different histories of migration among ecoregions.

**Main conclusions** Congruent phylogeographical patterns in several clades between the mycobiont and the photobiont on a large spatial scale can be explained either by parallel isolation due to ecological and geographical discontinuities or by similar selective pressures on the symbionts due to common environmental conditions within each ecoregion leading to co-divergence. At the regional spatial scale, the two taxa share some degree of evolutionary history but the weak phylogeographical congruence within ecoregions and the lack of shared migration history both indicate a flexible association between mutualists. This flexibility may facilitate the widespread geographical distribution of the lichen.

## Keywords

co-divergence, lichen, photobiont, *Ramalina menziesii*, symbiosis, *Trebouxia decolorans*, western North America

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## INTRODUCTION

Lichens are an example of a highly integrated symbiotic association formed by a fungus (mycobiont) with at least one photosynthetic partner, such as green algae and/or cyanobacteria, referred to as 'photobionts' (Nash, 2008). This association has been hypothesized to be coevolved at the species scale (Ahmadjian, 1993). Previous phylogenetic studies of

lichen symbionts at high taxonomic levels have revealed many fungal genera associated only with specific photobiont genera (DePriest, 2004). Similarly, at the species level, some lichen symbionts show species-to-species congruent phylogenies (Kroken & Taylor, 2000; Paulsrud *et al.*, 2001). Although the fungi and their green algal partners occur in tight association, the lack of phylogeographical congruence found in several studies between the two taxa would suggest

low specificity at the intra-species level (Guzow-Krzeminska, 2006; Piercey-Normore, 2006). Yet, because the two symbionts must have some degree of compatibility to become a lichen and the two mutualists share a common geographical distribution, individuals or populations of each partner species must specialize on the other to some extent and that degree of specialization will shape the geographical range of the lichen.

To understand the extent of intraspecific co-evolution between lichen symbionts, we need to assess the degree of co-phylogeographical structure, that is, the geographical concordance between genealogies across multiple co-distributed species and the degree of shared migratory history (Fernández-Mendoza *et al.*, 2011; Werth, 2011; Widmer *et al.*, 2012). It appears that the greatest likelihood for co-evolution in lichen symbionts will occur when the two taxa are co-dispersed (Büdel & Scheidegger, 1996; Bongaerts *et al.*, 2010). Nonetheless, some degree of co-evolution might be possible for symbionts that are dispersed independently because they share niches and spatial distributions and many lichen fungi associate with only a small spectrum of algal clades and strains (Buckley *et al.*, 2014). Phylogeographical congruence may reflect co-evolution or it may simply be due to common geographical or ecological barriers subjecting both symbionts to the parallel actions of genetic differentiation. For example, two symbionts could show co-divergence when the population of one symbiont splits at the same time as that of its partner's population (Parker & Spoerke, 1998; Althoff & Thompson, 1999; Thompson & Cunningham, 2002; de Vienne *et al.*, 2013). Unique local or regional environmental conditions can create selection pressures that favour parallel specialization and possible selection against non-local genotypes, thus leading to congruent genetic structure of both symbionts (Werth, 2010; Werth & Sork, 2010; del Campo *et al.*, 2013; Rodelo-Urrego *et al.*, 2013; Buckley *et al.*, 2014). In addition, historical climatic oscillations, such as those during the Quaternary (Comes & Kadereit, 1998; Hewitt, 2004), could result in co-migration to new sites with suitable environmental conditions (Taberlet *et al.*, 1998; Arbogast & Kenagy, 2001; Soltis *et al.*, 2006).

Here, we study *Ramalina menziesii* Taylor (Ramalinaceae), a common, epiphytic lichen found in coastal western North America ranging from Baja California in Mexico to south-eastern Alaska (Rundel, 1974). These localities can be grouped into six major ecogeographical regions (ecoregions), which are defined by climate, physical landscape features and vegetation, for example, fog desert (BI) and coastal chaparral (BC) in Baja California, Mexico; inland California oak savanna (CS) and deciduous woodland habitats (CN); coastal California chaparral (CC), and coastal coniferous forest ranging throughout the Pacific Northwest of North America (PN) (Sork & Werth, 2014). The reproductive strategy of the lichen-forming fungus *R. menziesii* is primarily sexual, mediated by fungal ascospores, with negligible vegetative reproduction (Werth & Sork, 2008, 2014). A previous phylogeographical study on the lichen-forming fungus *R.*

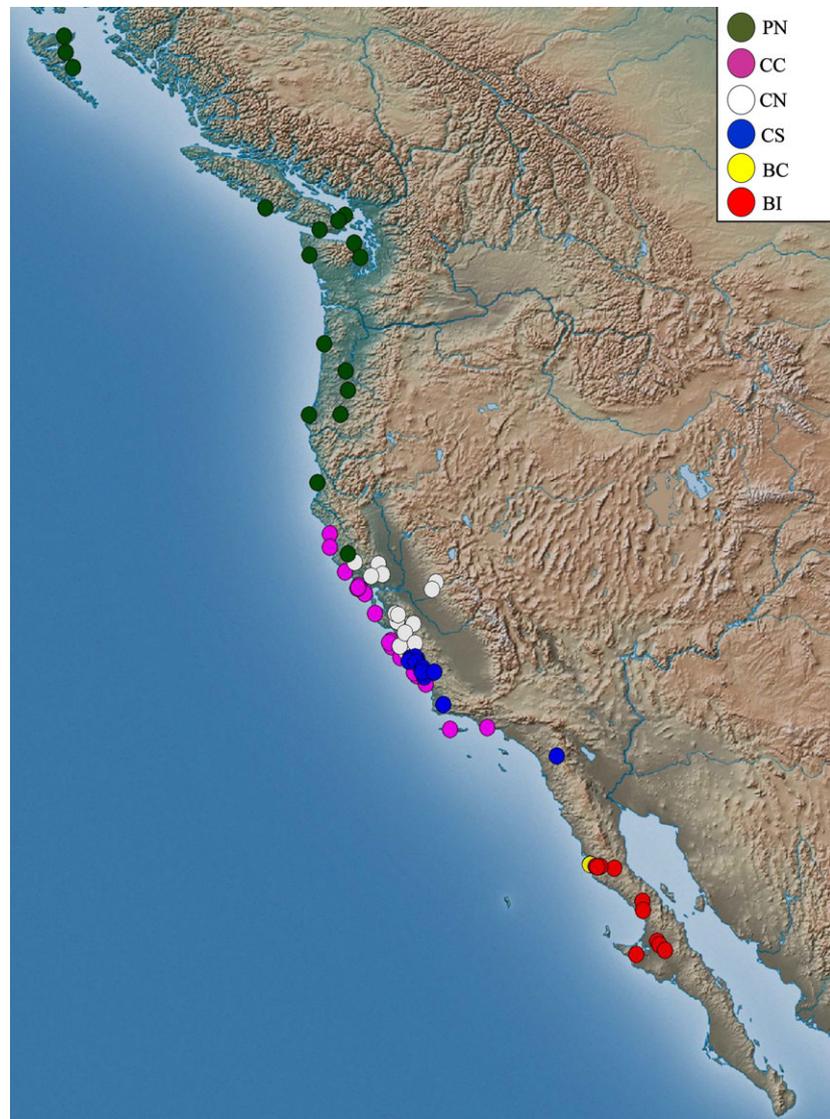
*menziesii* showed that the phylogeographical structure is highly structured and is concordant with some of six major ecoregions in western North America (Sork & Werth, 2014). Moreover, a population genetic study on the photobiont of this lichen documented significant geographical structure across ecoregions in the *Trebouxia* photobionts, which were dominated by one green alga related to *Trebouxia decolorans* Ahmadjian (Werth & Sork, 2014). In fact, 94% of the lichens sampled were associated with *T. decolorans* while 6% were associated with strains related to *T. jamesii* (Hildreth & Ahmadjian) Gärtner in restricted regions of the southern part of the species range (Werth & Sork, 2014). Thus, some local populations of the fungus exhibit some flexibility in its association with species of *Trebouxia*.

The overall goal of this study is to assess the extent to which the lichen-forming fungus *R. menziesii* and its main photobiont *T. decolorans* share common intra-specific genetic structures and migration histories. The mycobiont and the photobiont have been studied separately (Sork & Werth, 2014; Werth & Sork, 2014), but we have not yet directly tested the extent of their co-evolution. Many studies have used phylogeographical comparisons to understand evolutionary processes of multiple species sharing the same geographical distribution (e.g. Poelchau & Hamrick, 2013) or even sharing the same geography and host plant (e.g. Thompson & Rich, 2011). Here, using the algal species that is most widely found in *R. menziesii* and would have had extensive opportunity for shared evolutionary history, we investigate different types of evidence at decreasing geographical and temporal scales. First, to test for co-diversification at the largest spatial scale, we compare the phylogeographical structure of the two symbiotic species so that we can determine the extent to which specific clades of the mycobionts and the photobionts associate with each other, whether such associations show congruence within each ecoregion, or whether their phylogeographical structures are completely independent. Second, to determine whether the two taxa share similar histories of migration among ecoregions, we compare directional pairwise migration among the six ecoregions for each symbiont.

## MATERIALS AND METHODS

### Sampling and DNA sequence data

DNA sequences from the mycobiont (*R. menziesii*) and the photobiont (*T. decolorans*) used in this study were constructed from 226 *R. menziesii* lichen thalli sampled from 73 sampling localities out of a larger data set described elsewhere (Sork & Werth, 2014; Werth & Sork, 2014; see Fig. 1; see Appendix S1: Table S1 in Supporting Information). The predominant and widespread photobiont *T. decolorans* co-occurred with the mycobiont *R. menziesii* in each of the sampled localities, while the other photobiont, *T. jamesii*, found in only a limited part of the lichen's range (Werth & Sork, 2014), will not be included in these analyses.



**Figure 1** Map of collecting sites for western North American lace lichen, *Ramalina menziesii*. Populations from the same ecoregion are shown in the same color. BI: Baja California inland region; BC: Baja California coastal region; CC: California coastal region; CS: southern California inland region; CN: northern California inland region; PN: Pacific Northwest region. Note that one locality from BC is hidden and that due to scaling of the coloured circles, the inland locations of BI do not seem inland.

DNA sequences for the mycobionts comprised four nuclear genes: betatubulin (*bet*), elongation factor 1- $\alpha$  (*efa*), glyceraldehyde 3-phosphate dehydrogenase (*gpd*), and an unidentified locus similar (e-score  $5 \times 10^{-117}$ ) to glycine dehydrogenase (*uid*) (Werth & Sork, 2008). For the algal photobionts, we used the internal transcribed spacer region of the nuclear ribosomal DNA (ITS) and the chloroplast *rbcL* gene (ribulose-1,5-bisphosphate carboxylase oxygenase, RuBisCO). DNA extraction, PCR amplification and sequencing of four nuclear genes of the mycobiont *R. menziesii* and the ITS and *rbcL* regions of the photobiont *T. decolorans* have been previously described in Sork & Werth (2014) and Werth & Sork (2008, 2010, 2014).

The sequences of four nuclear genes (*bet*, *efa*, *gpd* and *uid*) of the mycobiont *R. menziesii* and the ITS and *rbcL* sequences of the photobiont *T. decolorans* were aligned independently using the program Clustal W (Thompson *et al.*, 1994) and then adjusted manually. Gaps and missing data for the photobiont's ITS region and the mycobiont's nuclear

genes were removed in subsequent analyses. The photobiont *rbcL* locus had no gaps or missing data. Haplotypes of the mycobiont and the photobiont were determined from nucleotide substitutions of the combined and aligned sequences.

### Analyses of population structure

To infer the population genetic structure of the mycobiont *R. menziesii* and the photobiont *T. decolorans* in western North America, previous studies based on larger data sets analysed the molecular variance among the six ecoregions (BC, BI, CS, CC, CN and PN) (Sork & Werth, 2014; Werth & Sork, 2014). In the present study, we repeated the analyses of molecular variance (AMOVA) using ARLEQUIN 3.1 (Excoffier *et al.*, 2006) to estimate genetic structure among the six ecoregions for the reduced data set and to ensure we obtained similar patterns to our earlier work. To facilitate comparison among subpopulations within ecoregions, we

created subpopulations by pooling individuals from proximal localities within a 200 km distance threshold, which occupy similar habitats, and which are not separated by topographical barriers. This yielded a total of 21 subpopulations with 6–18 individuals per subpopulation (except for one subpopulation in the BC ecoregion with only two individuals) and 3–5 subpopulations per ecoregion (ecoregion BC only consisted of one subpopulation). Significance of variance components was tested using 10,000 permutations.

To infer the phylogeographical pattern of the mycobiont *R. menziesii* and the photobiont *T. decolorans* in western North America, Sork & Werth (2014) and Werth & Sork (2014) studied the phylogenies of the symbionts based on larger data sets. In the present study, to test whether the symbionts showed parallel phylogenies, we also performed a Bayesian phylogenetic analysis of both the reduced mycobiont and the photobiont sequence data sets using MRBAYES 3.1.2 (Huelsenbeck & Ronquist, 2005) (see the detailed methods and additional references in Appendix S2).

### Tests of co-divergence

To test whether the photobiont *T. decolorans* and the mycobiont *R. menziesii* exhibited co-divergence, we used the distance-based methods through the COPYCAT program (Meier-Kolthoff *et al.*, 2007), which incorporates a wrapper for the programs AXPAREFIT and AXPCOORDS (Stamatakis *et al.*, 2007). The AXPCOORDS is a procedure that converts distance matrices into principal coordinate matrices, and AXPAREFIT is a permutation procedure that uses distance matrices to test for congruence between host and parasite phylogenies (Stamatakis *et al.*, 2007). The distance-based method has an advantage over tree-based methods because it can accommodate uncertainty in tree topologies. In this study, genetic distance matrices for the photobiont and the mycobiont were derived from pairwise genetic distances between localities based on Kimura's two-parameter model (Kimura, 1980) calculated using the program MEGA 3.1 (Kumar *et al.*, 2004). For each symbiont, pairwise genetic distances between individuals collected from the same locality were averaged. Statistical significance of the co-divergence was evaluated using COPYCAT program by performing 9999 permutations. We considered a significant ( $P < 0.01$ ) contribution to the overall level of congruence between the topologies, under both the ParaFitLink1 and ParaFitLink2 statistics (Legendre *et al.*, 2002).

### Test of co-migration among ecoregions

To test for co-migration among the mycobiont *R. menziesii* and its photobiont *T. decolorans*, we used the coalescent software MIGRATE-N 3.5.1 (Beerli & Palczewski, 2010) and obtained Bayesian nonequilibrium estimates of effective population sizes and bidirectional rates of migration among ecoregions (BC, BI, CC, CS, CN and PN). For the mycobiont *R. menziesii*, MIGRATE-N was run on separately for each

locus and then the parameters were estimated jointly. To ensure a consistent interpretation of migrate output in the northern ecoregion analyses, we conducted multiple runs – some with all samples, and others using different randomly selected subsamples. In one set of runs, we randomly selected 60 individuals per three ecoregions and all 58 individuals for the fourth. In another set of runs, we randomly selected 100 individuals per three ecoregions and used all individuals in the fourth, which contained fewer individuals. We report the results using all of the samples for both the northern ecoregion and the southern ecoregion models because the findings were qualitatively the same, regardless of sample sizes. For the photobiont *T. decolorans*, MIGRATE-N was run separately on the ITS data set and *rbcL* data set. Because MIGRATE-N does not allow for recombination within a locus, which may cause an over-estimation of variability leading to biased parameter estimates (Beerli & Palczewski, 2010), we determined the longest non-recombining block of DNA sequence of each locus for both the mycobiont *R. menziesii* and the photobiont *T. decolorans* with the PERL SCRIPT IMGIC (Woerner *et al.*, 2007) (see Appendix S1: Table S2). All analyses of MIGRATE-N were based on the non-recombined blocks of sequence data. MIGRATE-N was run with ten replicates to estimate migration rates among ecoregions (all parameters free to vary) with one long Markov chain Monte Carlo (MCMC) chain and a long increment of 100 and 5000 samples after a burn-in of 1,000,000 steps in the chain. Starting parameters for population size  $\theta$  and migration rates ( $M$ ) were inferred from  $F_{ST}$  values; mutation rate modifiers were deduced from the data using Watterson's  $\theta$ . A uniform prior on  $\theta$  was assumed with a minimum of 0, a maximum of 0.1 and a  $\Delta$  of 0.01. The bidirectional gene flow ( $Nm$ ) among ecoregions were estimated according to the formula  $Nm = \theta \times M/x$  (Beerli & Palczewski, 2010), where  $x$  is the inheritance parameter ( $x = 4$  for nuclear data and  $x = 1$  for chloroplast data in our estimations). For the migration rate, we used a uniform prior (minimum 0; maximum 1000;  $\Delta$  100). The MCMC chain was run using a heating scheme with the temperatures 1.0, 1.5, 3.0 and 1,000,000, allowing swapping of chains.

## RESULTS

### Haplotype distribution

From the 226 sequences of each of the aligned nuclear genes (*bet*: 810 bp, *efa*: 486 bp, *gpd*: 586 bp, and *uid*: 789 bp; excluding gaps and missing data) of the mycobiont *R. menziesii*, we found 15 haplotypes in *bet*, 43 in *efa*, 18 in *gpd* and 39 in *uid*, yielding a total of 151 haplotypes using the combination of all four loci. Most of the identified four-locus haplotypes (124; 82%) were unique to a particular locality and only 27 haplotypes were found in two or more localities. Among the widely distributed haplotypes, only 11 were shared among ecoregions (see Appendix S1: Table S3).

For the photobiont *T. decolorans*, the length after multiple alignments of the combined ITS and *rbcl* sequences was 1,209 bp (ITS: 615/569 bp, *rbcl*: 594/594 bp; numbers refer to the number of sites including/excluding gaps and missing data). In total, 58 haplotypes (ITS: 49 haplotypes, *rbcl*: 17 haplotypes) were identified from these combined ITS and *rbcl* sequences (excluding gaps and missing data). Among the 58 identified haplotypes based on the combined ITS and *rbcl* sequence data set, 37 haplotypes were unique to a locality. The remaining 21 haplotypes were found widely distributed in two or more localities and most of them (12 haplotypes) were shared among ecoregions (one haplotype was widely distributed across the six ecoregions) (see Appendix S1: Table S3).

Among the 58 photobiont two-locus haplotypes and 151 mycobiont four-locus haplotypes, 24 photobiont haplotypes (41%) were shared by two or more mycobiont haplotypes forming different lichen individuals (i.e. one photobiont haplotype associated with many mycobiont haplotypes). Seventeen of 151 mycobiont haplotypes (11%) associated with more than one photobiont haplotype (i.e. one mycobiont haplotype was found in association with several photobiont haplotypes). Only 17 of the photobiont and mycobiont haplotypes represented specific one-to-one associations.

### Genetic structure analyses

In the photobiont *T. decolorans*, based on the ITS data set, the hierarchical AMOVA revealed significant structure among ecoregions ( $F_{CT} = 0.4134$ ) and among subpopulations within

ecoregions ( $F_{SC} = 0.2370$ ); in the nonhierarchical AMOVA model, we estimated  $F_{ST} = 0.5229$  (Table 1A). Based on *rbcl* data set, the hierarchical AMOVA revealed significant structure among ecoregions ( $F_{CT} = 0.3245$ ) and among subpopulations within ecoregions ( $F_{SC} = 0.2021$ ); in the nonhierarchical AMOVA model, the  $F_{ST}$  value was estimated to be 0.4335 (Table 1A). In the mycobiont *R. menziesii*, the hierarchical AMOVA revealed significant genetic structure among ecoregions ( $F_{CT} = 0.6479$ ) and much less structure among subpopulations within ecoregions ( $F_{SC} = 0.1101$ ). The nonhierarchical genetic structure among subpopulation yielded  $F_{ST} = 0.6530$  (Table 1B).

Our phylogeny reconstructions revealed that both the mycobiont and the photobiont included at least three phylogenetic clades: in inland California (CS and CN ecoregions), California coast and Pacific Northwest region (CC and PN ecoregions), and Baja California inland (BI ecoregion) (see Appendix S2: Figs. S1 and S2).

### Analyses of co-divergence

Global tests of co-phylogeny using AXPARAFIT implemented in the program COPYCAT resulted in rejection of random association between the mycobiont *R. menziesii* and the photobiont *T. decolorans* (ParaFitGlobal = 0.00005,  $P = 0.0010$ ). However, tests of location links indicated that not all mycobiont-photobiont associations contribute to the global fit between the two data sets. The results show that a total of 27 out of 73 associations were significantly associated (see Appendix S1: Table S4). The significant associations mostly

**Table 1** Analysis of molecular variance (AMOVA) for subpopulations of photobiont *Trebouxia decolorans* (A) and mycobiont *Ramalina menziesii* (B). The table gives the source of variance, the degrees of freedom (d.f.), the sum of squares (SSD), the variance component (Var.) and the percentage of variance (%), as well as  $F$ -statistics for differentiation among ecoregions ( $F_{CT}$ ), among subpopulations within ecoregion ( $F_{SC}$ ), and among all subpopulations ( $F_{ST}$ ). All  $F$ -statistics were significant ( $P < 0.001$ ).

A. Photobiont						
Source of variation	d.f.	SSD	Var.	%	$F$ -statistic	
a. ITS data set						
Among ecoregions	5	688.38	3.342	41.34	$F_{CT}$	0.4134
Among subpopulations within ecoregions	15	230.49	1.125	13.90	$F_{SC}$	0.2370
Within subpopulations	205	741.73	3.618	44.76		
Among subpopulations	20	918.87	3.965	52.29	$F_{ST}$	0.5229
Among individuals within subpopulations	205	741.73	3.618	47.71		
b. <i>rbcl</i> data set						
Among ecoregions	5	51.32	0.240	32.45	$F_{CT}$	0.3245
Among subpopulations within ecoregions	15	21.84	0.101	13.65	$F_{SC}$	0.2021
Within subpopulations	205	81.79	0.399	53.90		
Among subpopulations	20	73.16	0.305	43.35	$F_{ST}$	0.4335
Among individuals within subpopulations	205	81.79	0.399	56.65		
B. Mycobiont						
Source of variation	d.f.	SSD	Var.	%	$F$ -statistic	
Among ecoregions	5	1280.17	6.834	64.79	$F_{CT}$	0.6479
Among subpopulations within ecoregions	15	113.67	0.409	3.88	$F_{SC}$	0.1101
Within subpopulations	205	677.47	3.305	31.33		
Among subpopulations	20	1393.85	6.219	65.30	$F_{ST}$	0.6530
Among individuals within subpopulations	205	677.47	3.305	34.70		

occurred in localities from ecoregions of BI (eight localities), CC (14 localities) and PN (five localities). We found no significant associations in the three ecoregions of BC, CS and CN (see Appendix S1: Table S4).

### Comparative analysis of migration patterns

For the mycobiont *R. menziesii*, our analysis with MIGRATE-N indicated that migration among ecoregions was uneven (Table 2). In the northern ecoregion analysis, the mean values of the effective number of migrants were small for most pairs of populations. Three pairs of populations showed high rates of migration (Table 2A): migration from the PN ecoregion into the adjacent California ecoregion ( $Nm = 6.7$ ); from CN ecoregion into the CS ecoregion ( $Nm = 22.5$ ); and from the CC ecoregion into the CS ecoregion ( $Nm = 8.0$ ). Our overall model found no gene exchange between California and Baja California populations (data not shown) and negligible gene exchange among three adjacent southern ecoregions in California and Baja California (Table 2B), where the lower 2.5% of the posterior distribution overlapped with zero migration. Thus, substantial gene movement is mainly directed towards the CC and CS ecoregions (Fig. 2a).

The migration pathways of the photobiont *T. decolorans* among the six ecoregions differed from that of the mycobiont *R. menziesii* (Fig. 2). Based on the ITS data set, considerable levels of gene flow were inferred among the Baja California coastal ecoregion BC and the California coastal ecoregion CC, the BC and the California inland ecoregion CS and CN, and the BC and the Baja California inland ecoregion BI; the migration was bidirectional between BC and CS; there was negligible gene flow between the coastal ecoregions PN and CC, considering the lower 2.5% of the posterior distribution overlapped with zero migration (Fig. 2b; Table 3). Based on the *rbcL* data set, considerable levels of gene flow were inferred from the California inland ecoregions CN, CS and

the California coastal ecoregion CC to the Baja California coastal ecoregion BC (Fig. 2c; Table 3).

### DISCUSSION

In this comparative study, of the mycobiont *R. menziesii*, and its predominant photobiont *T. decolorans*, we found evidence that indicates some degree of co-evolution but we also find evidence that explains why tight co-evolution was unlikely. On the one hand, our findings demonstrate co-phylogeographical structure across the lichen's range in coastal western North America: the large proportion of genetic variation resided among ecoregions in both mycobiont and photobiont and each symbiont included at least three congruent phylogenetic clades. Tests of concordance between mycobiont and photobiont genetic variation also revealed an overall congruent genetic structure: the mycobiont and the photobiont genetic distance matrices were significantly associated at large spatial scales. On the other hand, we did not observe specialized associations between haplotypes, but instead observed low fungal specificity. For example, the mycobiont *R. menziesii* formed different associations with multiple algal genotypes. Moreover, migration patterns among ecoregions differed among symbionts. Thus, despite the large-scale congruence in genetic structure that would create an opportunity for local selection pressures by each symbiont for mutual or even unilateral specialization that would enhance the symbiosis, the symbiotic relationship is very generalized at the haplotype level. Below we discuss the evidence and consequences of this type of symbiotic association.

### High clade-level specificity of the lichen-forming fungus

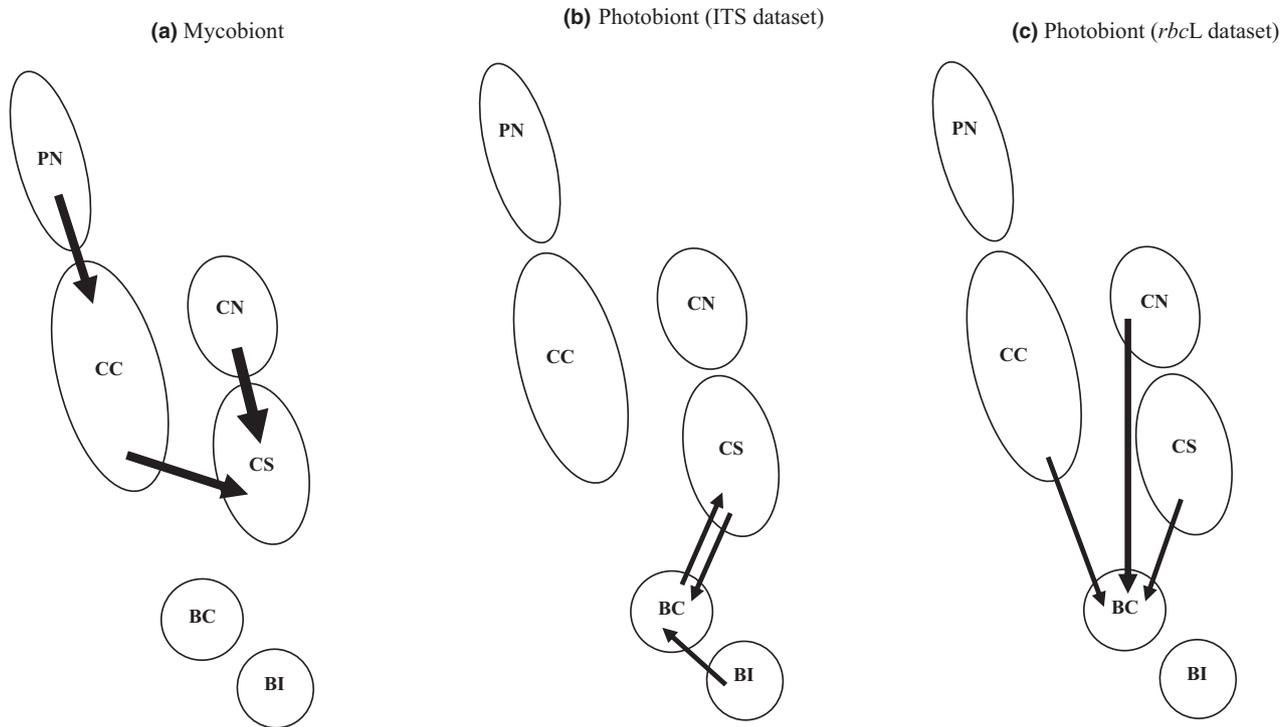
Previously, it was shown that the mycobiont specializes mainly on one predominant species of *Trebouxia* (Werth &

**Table 2** MIGRATE-N Bayesian means of effective population size ( $\theta$ ) and bidirectional gene flow ( $Nm$ ) among ecoregions using average on sequences of four fungal nuclear genes in the western North American lace lichen, *Ramalina menziesii*. Numbers in parentheses are estimates associated with lower 2.5% and upper 97.5% of the posterior distribution.

A. Four northern ecoregions					
Source population	$\theta$	$Nm$ into recipient population			
		CC	CN	CS	PN
CC	0.0395		0.7 (0,4.6)	8.0 (0.1, 18.4)	1.1 (0, 9.0)
CN	0.0183	1.4 (0, 10.8)		22.5 (0.4, 51.6)	0.5 (0, 3.6)
CS	0.0345	1.1 (0, 9.5)	1.1 (0,11.1)		0.6 (0, 3.9)
PN	0.0220	6.7 (0.3, 39.2)	0.5 (0, 3.8)	4.5 (0, 11.6)	

B. Three southern ecoregions				
Source population	$\theta$	$Nm$ into recipient population		
		CS	BC	BI
CS	0.0390		1.5 (0, 7.5)	0.3 (0, 2.8)
BC	0.0073	0.7 (0, 3.7)		0.4 (0, 3.1)
BI	0.0065	0.4 (0, 3.0)	0.6 (0, 3.8)	



**Figure 2** Diagram of distinctly high inter-region migration, based on bi-directional analysis in MIGRATE-N (Beerli & Palczewski, 2010). Arrows represent gene flow ( $Nm$ ) estimates within 2.5% and 97.5% confidence interval. a: Migration among six ecoregions of the lichen-forming fungus, *Ramalina menziesii*; b: Migration among six ecoregions of the green algal photobiont, *Trebouxia decolorans*, based on ITS sequences; c: Migration among six ecoregions of the green algal photobiont, *T. decolorans*, based on *rbcL* sequences.

**Table 3** MIGRATE-N Bayesian means of effective population size ( $\theta$ ) and bidirectional gene flow ( $Nm$ ) among ecoregions using ITS and *rbcL* sequences of the green algal photobionts of *Trebouxia decolorans* in the western North America. Numbers in parentheses are estimates associated with lower 2.5% and upper 97.5% of the posterior distribution.

Source population	$\theta$	$Nm$ into recipient population					
		BC	BI	CC	CN	CS	PN
<b>a. ITS data set</b>							
BC	0.0503		0.11 (0, 0.63)	3.49 (0, 12.12)	0.15 (0, 0.67)	7.38 (0.32, 24.47)	1.58 (0, 0.98)
BI	0.0008	7.26 (0.4, 5.57)		3.34 (0, 11.5)	0.14 (0, 0.79)	5.16 (0, 20.78)	1.34 (0, 6.06)
CC	0.0270	6.43 (0.42, 5.47)	0.1 (0, 0.63)		0.15 (0, 0.81)	8.82 (0.32, 24.88)	1.52 (0, 8.36)
CN	0.0013	7.86 (0.57, 5.58)	0.11 (0, 0.65)	4.53 (0, 12.13)		8.22 (0.67, 24.88)	1.76 (0, 8.52)
CS	0.0575	6.26 (0.33, 0.15)	0.11 (0, 0.65)	3.5 (0, 12.03)	0.15 (0, 0.78)		1.47 (0, 7.52)
PN	0.0132	5.6 (0, 4.85)	0.09 (0, 0.53)	3.86 (0, 12.13)	0.16 (0, 0.80)	8.89 (0, 24.88)	
<b>b. <i>rbcL</i> data set</b>							
BC	0.0511		0.14 (0, 0.65)	0.21 (0, 0.99)	0.13 (0, 0.66)	0.1 (0, 0.56)	0.19 (0, 1.01)
BI	0.0009	6.25 (0, 3.1)		0.19 (0, 0.99)	0.12 (0, 0.66)	0.09 (0, 0.58)	0.11 (0, 0.85)
CC	0.0016	6.32 (0.1, 19.28)	0.13 (0, 0.65)		1.48 (0, 0.77)	0.11 (0, 0.58)	0.16 (0, 0.85)
CN	0.0012	6.6 (0.25, 19.3)	0.09 (0, 0.56)	0.18 (0, 0.82)		0.14 (0, 0.67)	0.17 (0, 1.02)
CS	0.0009	6.4 (0.14, 18.66)	0.09 (0, 0.54)	0.18 (0, 0.91)	2.05 (0, 0.77)		0.20 (0, 1.01)
PN	0.0013	4.8 (0, 16.69)	0.09 (0, 0.54)	0.17 (0, 0.89)	1.64 (0, 0.77)	0.11 (0, 0.59)	

Sork, 2014), but here we examine specialization within the species and find little evidence. The lack of any congruence at the level of haplotype may be expected given that the fungus reproduces sexually and ascospores take up local photobionts after dispersal (Werth & Sork, 2008). However, if the two taxa specialized on local genetic strains, then we would find some degree of congruence among clades even at

neutral loci. The incongruence indicates a lack of discrimination by the fungus for specific genetic strains of the photobiont as long as it is the right species, which would allow the lichen to associate with a locally adapted photobiont genetic strain and thus persist even when the fungus disperses to new environmental conditions (Romeike *et al.*, 2002; Piercey-Normore, 2006; Wolfe & Pringle, 2011; Werth & Sork,

2014). Across environments, it would be beneficial to switch to a different genotype of alga (Piercey-Normore, 2006). Thus, these non-specific associations among haplotypes may promote novel combinations of fungi and algae and create the opportunity for adaptation to new conditions (Wornik & Grube, 2010; Wolfe & Pringle, 2011). Following the initial contacts and establishment, site-specific selective forces would retain the successful thalli and remove unfit symbiotic combinations (Yahr *et al.*, 2006). For example, Yahr *et al.* (2006) found no overall associations between the lichenized fungus *Cladonia subtenuis* and its associated *Asterochloris* algae across a broad geographical range, but they did find that one fungal clade was associated with only one algal clade. Thus, the lack of complete specialization does not necessarily lead to an overall lack of specificity.

The geographical patterns of the genetic variations found in current and previous studies (Sork & Werth, 2014; Werth & Sork, 2014) indicate that *R. menziesii* is an example of local clade-level specificity of a lichen-forming fungus. The phylogenetic trees of the mycobiont and photobiont haplotypes showed several distinct genetic clusters corresponding to different ecoregions, for example, fog desert in Baja California, Mexico (ecoregion: BI), and inland California oak savanna and oak woodland habitats (ecoregions: CS and CN). In addition, some distinct clusters were found in two ecoregions (e.g. CC and PN). In this study, the tests of co-divergence between mycobionts and photobionts among different localities using genetic distance-based methods revealed that most of the significant congruence among symbiont haplotypes occurred within the ecoregions of BI, CC and PN, but not within the CS and CN ecoregions. Thus, ecological conditions common to BI, CC, and PN may have influenced the distribution of symbiont haplotypes in the mycobiont *R. menziesii*, suggesting that the phylogeographical congruence observed results from the joint selective environment of the two taxa rather than their specialization in symbiosis.

We propose that the reason we observe common phylogeographical patterns at a large spatial scale is that parallel long-term population persistence and isolation promote concordant patterns and local adaptation. For example, such patterns have been found among an epiphytic lichen-forming fungus *Lobaria pulmonaria* and its photobiont *Dictyochloropsis reticulata* in European glacial refugia (Widmer *et al.*, 2012). Similarly, we observed genetic structure among clades in both the mycobionts and the photobionts of the lichen *R. menziesii*, which is consistent with multiple ancient lineages in the mycobiont that could have evolved in separate refugia across western North America (Sork & Werth, 2014). In addition, the large spatial scale might result in limited availability of one or both partners relative to the other, which could also drive patterns in phylogenetic congruence (Buckley *et al.*, 2014). This pattern has been evidenced by examples from comparative phylogenetic studies in *Ramalina* (Buckley *et al.*, 2014) and in *Caloplaca* (Vargas Castillo & Beck, 2012). Spatial scales might also be important for

explaining the co-phylogeographical structure revealed in this study. For example, in our case, we found an overall significant congruent genetic structure occurred in the mycobiont *R. menziesii* and its photobiont *T. decolorans* on a large spatial scale (e.g. across the ecoregions) in western North America, but not on a small spatial scale (e.g. within ecoregions).

Symbiont co-dispersal, the vertical transmission of the mycobiont and the photobiont, is another mechanism that could create similar phylogeographical structures. In most clonally reproducing lichens, asexual propagules are often specialized structures that facilitate the co-dispersal of the mycobiont and the photobiont (Büdel & Scheidegger, 1996), resulting in the vertical transmission of the photobiont from one generation to the next. Strictly vertical transmission maintains tight associations between symbionts over generations through co-dispersal and leads to similar genetic structures in symbionts (Bongaerts *et al.*, 2010). For example, congruent genetic structures of symbionts in *Cetraria aculeata* and *Lobaria pulmonaria* have been attributed to their mostly vegetative dispersal by thallus fragments that contain both the photobiont and the mycobiont (Werth *et al.*, 2006; Scheidegger & Werth, 2009; Fernández-Mendoza *et al.*, 2011; Dal Grande *et al.*, 2012; Werth & Scheidegger, 2012). However, this mechanism is likely less important in our study system because *R. menziesii* disperses predominantly (but not exclusively) with fungal spores that need to associate with compatible algae for establishment (Werth & Sork, 2008, 2010). Consistent with a predominantly sexual dispersal mode, we found that one mycobiont haplotype can associate with more than one photobiont haplotype, forming different symbiont associations. This finding implies that horizontal transmission of symbionts was frequent, and that the partners were not co-dispersed. Thus, co-dispersal of symbionts is an unlikely mechanism for the congruent genetic patterns of mycobionts and photobionts of lichen *R. menziesii* across ecoregions.

### No co-migration between mycobionts and photobionts

Co-migration is a potential mechanism for shaping common phylogeographical structures between two symbiotic taxa, if they were to respond in a similar manner to climate changes across glacial periods. In the previous phylogeographical study of the lichen-forming fungus *R. menziesii*, Sork & Werth (2014) assessed the migration pattern among the same six ecoregions. Those analyses indicated that the lowest migration was found between populations in BI and BC, the highest rate of migration from populations in PN and CC into CN and CS, whereas populations in CN and CS have intermediate migration rates. In this study, to facilitate a robust test of fungal and algal co-migration, we used subsamples from each ecoregion and found the same pattern for the fungus. We then compared the patterns of fungal migration with those of the photobiont *T. decolorans*, and found that the two symbionts had different histories of movement. In

fact, they often migrated in opposite directions, which would make co-migration between these two species unlikely.

It is not obvious why the migration patterns of the photobiont *T. decolorans* and the mycobiont *R. menziesii* do not coincide more closely, given that they share common habitats and their co-occurring populations should have been impacted in a similar manner by historical climatic oscillations. However, perhaps the two taxa have different environmental requirements for persistence. The lichen-forming fungus *R. menziesii* requires moist microclimates, as provided by coastal fog, evaporation from water bodies, valley fog and dew, or winter rain (Rundel, 1978; Matthes-Sears *et al.*, 1986). Fog has been suggested to be a major driver of its expansions, contractions, and migrations (Sork & Werth, 2014). In contrast, the green alga *T. decolorans* that is much more broadly distributed than the lichen-forming fungus *R. menziesii*, and this suggests that this algal species has very different environmental tolerances. *T. decolorans* has been reported from several continents and in association with diverse fungal species (Helms *et al.*, 2001; Beck & Mayr, 2012; Werth, 2012; Muggia *et al.*, 2013; Nyati *et al.*, 2013a, b). The wider distributional range and low specificity of *T. decolorans* imply that this photobiont may have persisted under more diverse environmental conditions and experienced a population history distinct from the mycobiont *R. menziesii*.

## CONCLUSIONS

This comparative analysis of the phylogeographical structure in the mycobiont *R. menziesii* and its photobiont *T. decolorans* in western North America demonstrates that the environment matters at a broad ecogeographical scale but not at the specific haplotype level. The congruent phylogeographical patterns observed at the large scale could be explained by isolation among ecoregions, similar demographic histories within ecoregions, and/or similar selective pressures on the symbionts due to common environmental conditions leading to co-divergence. However, the independent migration histories and lack of specificity at the haplotype level show that sexual reproduction in this fungal species and independent dispersal of the two taxa result in loose associations that may facilitate the wide geographical distribution of this lichen across major ecosystems and climate zones.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Supplementary tables.

**Appendix S2** Details of phylogenetic tree analyses and supplementary figures.

## DATA ACCESSIBILITY

DNA sequences of each gene from each haplotype and their corresponding accession nos. are deposited in GenBank (**bet**: FJ004897–FJ004899, FJ004900–FJ004911, FJ004913, EF377538; **efa**: FJ656371, FJ656372, FJ656384, FJ656385, FJ656387–FJ656389, FJ656391–FJ656395, FJ656397, FJ656402, FJ656406–FJ656409, FJ656411, FJ656416–FJ656421, FJ656423, FJ656424, EF377548, EF377550–58, EF377561–4, EF377569, EF37736970; **gpd**: FJ705149, FJ705150, FJ705152, FJ705153, FJ705155, FJ705156, FJ705158, FJ705159, FJ705162, FJ705163, FJ705165, FJ705167–FJ705169, FJ705174, EF377544, EF377545, EF377547; **uid**: FJ656310–FJ656313, FJ656315–FJ656319, FJ656322, FJ656324–FJ656328, FJ656330–FJ656333, FJ656336, FJ656338, FJ656339, FJ656341–FJ656343, FJ656345, FJ656346, FJ656348, FJ656351, FJ656352, FJ656354, FJ656358, FJ656360, FJ656362, FJ656363, FJ656365, EF377571, EF377578, EF377580; **ITS**: KF549535, KF549540, KF549545, KF549547, KF549550, KF549555, KF549556, KF549558, KF549561–KF549577, KF549580, KF549584, KF549585, KF549592, KF549598, KF549599, KF549603, KF549607, KF549613, KF549614, KF549616, KF549621–KF549624, KF549632, KF549633, KF549636, KF549640, KF549641, KF549647, KF549651, KF549652, KF549658; **rbcl**: KF549515–KF549525, KF549527–KF549531, KF549533).

## BIOSKETCHES

**Jin-Ming Chen** studies population genetics, molecular systematics and phylogeography of plant species.

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**Victoria L. Sork** studies the ecology, evolution and conservation biology of trees using landscape and population geno-

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Author contributions: J.M.C. was lead author on the manuscript, developed the focus with V.L.S., subsampled existing data sets, and conducted the data analysis. V. L. S. and S. W. designed the larger project, collected samples, conducted background phylogeographical and data analysis, and collab-

orated on manuscript preparation. In addition, S.W. conducted laboratory and data analysis that yielded gene sequences for the final data sets. All authors read and approved the final manuscript.

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