Short Communication: Co-occurring *Lobaria pulmonaria* and *Ricasolia quercizans* share green algal photobionts: Consequences for conservation

Jessica L. Allen[1,2,3] and Christoph Scheidegger[2]

[1]*Eastern Washington University, Biology Department, Cheney, WA 99004, U.S.A.;* [2]*Swiss Federal Institute for Forest, Snow, and Landscape Research, WSL, Biodiversity and Conservation Biology, Birmensdorf, 8903, Switzerland*

Photobiont sharing among lichens within communities is a well-documented phenomenon, especially among closely related fungal species that form photobiont-mediated guilds (Dal Grande et al. 2014; Rikkinen et al. 2002). Species of *Lobaria* and other closely related genera that associate with *Symbiochloris reticulata* are one example of a photobiont-mediated guild that have been the subject of extensive research (Dal Grande et al. 2014). In their study, Dal Grande et al. (2014) compared algal multi-locus genotypes (MLGs) at one site in Taiwan and one site in Madeira among four species of *Dendriscosticta*, 16 species of *Lobaria*, and one species of *Ricasolia*, and, using pairwise FST values, found the species did not associate with significantly different green algal partners when they co-occurred. Green algal symbiont sharing has also been documented between the predominantly sexually reproducing *Umbilicaria spodochroa* and predominantly vegetatively reproducing *Lasallia pustulata* (Hestmark et al 2016). Werth (2012) recovered a more complex pattern of compartmentalization in *Ramalina menziesii* and adjacent lichen species. Photobiont sharing may also occur with co-occurring non-lichen organisms, such as liverworts (Cornejo & Scheidegger 2016).

◆ ◆ ◆

Here we aimed to test the photobiont sharing capacity of one iconic eastern North American species, *Ricasolia quercizans*, with *Lobaria pulmonaria* (**Fig. 1**). We sampled 41 individuals of *R. quercizans* and 66 individuals of co-occurring *L. pulmonaria* at five sites in the southeastern United States (**Table 1**). We amplified and sequenced nine microsatellite markers (LPh1, LPh2, LPh3, LPh4, LPh5, LPh6, LPh7, LPh8 and LPh9; **Supplementary Table S1**) for *S. reticulata* from both species using established protocols (Dal Grande et al. 2010; Widmer et al. 2010). The R packaged poppr was used to analyze multi-locus genotypes (MLGs) among the samples (Kamvar et al. 2014). There were no shared identical MLGs among any of the samples within four of the five populations sampled. In one population (CRG) four individuals, two *L. pulmonaria* and two *R. quercizans*, shared photobionts with identical MLGs. To test if genetic distances among photobionts associated with co-occurring individuals of *L. pulmonaria* and *R. quercizans* were significantly different within or between each site, a PERMANOVA was performed using the adonis function in the vegan package with 999 permutations (Oksanen et al. 2019). The results suggest that genetic distances of photobionts associated with co-occurring *L. pulmonaria* and *R. quercizans* were not significantly different at the site level ($R^2 = 0.0131$, $p = 0.184$). Our finding that *S. reticulata* associated with co-occurring *L. pulmonaria* and *R. quercizans* are not significantly genetically distinct in southeastern North America concurs with results from Dal Grande at al. (2014) on other *Ricasolia* species.

Our study provides an additional example of a photobiont mediated guild of *Symbiochloris reticulata* where *Lobaria pulmonaria* is considered the core species of this guild, actively spreading the photobiont by means of vegetative propagules. In *Ricasolia quercizans* the fungal partner reproduces sexually with ascospores and relichenization depends on available *S. reticulata* which is dispersed only by

[3] Corresponding author's e-mail: jallen73@ewu.edu

DOI: 10.1639/0007-2745-125.2.219

0007-2745/22/$0.45/0

Figure 1. *Ricasolia quercizans* and *Lobaria pulmonaria* growing together with overlapping lobes from Rough Butt Bald, North Carolina, U.S.A. Both individuals were sampled in this study from site RBB (see Table 1).

means of vegetative propagules of *L. pulmonaria*. Most of the remaining populations of *L. pulmonaria* in the southeastern United States are very small and genetically depauperate (Allen et al. 2021). Selective logging practices have led to significant declines in both abundance and fertility of both *L. pulmonaria* and *R. quercizans* in eastern North America (Edman et al. 2007). A continued decline of *L. pulmonaria* is expected to result in an even more dramatic decline of *R. quercizans* in this region because of the declining availability of the shared photobiont, leading to an extinction vortex of lichen species and its shared photobiont. In Switzerland the continuous decline of *L. pulmonaria* (Vulnerable) over at least two centuries (Scheidegger et al. 2002) resulted in a reported dramatic decline of the two fringe species *R. amplissima* (Endangered) and *R. virens* (Regionally Extinct) (Scheidegger et al. 2015).

Table 1. Sampling locations and sizes of *Symbiochloris reticulata* from co-occurring *Lobaria pulmonaria* (*Sr – Lp*) and *Ricasolia quercizans* (*Sr – Rq*).

Identifier	Locality	Lat.	Long.	*Sr - Lp*	*Sr - Rq*
CRG	U.S.A., West Virginia, Pocahontas County, Monongahela National Forest	38.2025	-80.2768	9	5
POT	U.S.A., West Virginia, Pocahontas County, Monongahela National Forest	38.1707	-80.2747	6	3
RBB	U.S.A., North Carolina, Jackson County, Pisgah National Forest	35.3171	-82.9697	31	19
RMT	U.S.A., North Carolina, Mitchell County, Pisgah National Forest	36.1049	-82.1105	14	10
WIR	U.S.A., West Virginia, Pocahontas County, West Virginia, Monongahela National Forest	38.3279	-80.2132	6	4

Acknowledgments

An NSF Graduate Research Fellowship to JLA supported field work in the Unites States. The Genetic Diversity Centre of ETH Zürich (GDC) provided facilities for genetic data collection. This research was funded by grant 31003A-105830 from the Swiss National Science Foundation to CS.

Literature Cited

Allen, J. L., R. T. McMullin, Y. Weirsma & C. Scheidegger. 2021. Population genetics and biogeography of the Lungwort Lichen in North America support distinct eastern and western gene pools. American Journal of Botany DIO: https://doi.org/10.1002/ajb2.1774.

Cornejo, C. & C. Scheidegger. 2016. Cyanobacterial gardens: the liverwort *Frullania asagrayana* acts as a reservoir of lichen photobionts. Environmental Microbiology Reports 8: 352–357.

Dal Grande, F., I. Widmer, A. Beck & C. Scheidegger. 2010. Microsatellite markers for *Dictyochloropsis reticulata* (Trebouxiophyceae), the symbiotic alga of the lichen *Lobaria pulmonaria* (L.). Conservation Genetics 11: 1147–1149.

Dal Grande F., A. Beck, C. Cornejo, G. Singh, S. Cheenacharoen, M. P. Nelsen & C. Scheidegger. 2014. Molecular phylogeny and symbiotic selectivity of the green algal genus *Dictyochloropsis* s.l. (Trebouxiophyceae): a polyphyletic and widespread group forming photobiont-mediated guilds in the lichen family Lobariaceae. New Phytologist 202: 455–470.

Edman, M., A.-M. Eriksson & M. A. Villard. 2007. Effects of selection cutting on the abundance and fertility of indicator lichens *Lobaria pulmonaria* and *Lobaria quercizans*. Journal of Applied Ecology 45: 26–33.

Hestmark, G., F. Lutzoni & J. Miadlikowska. 2016. Photobiont associations in co-occurring umbilicate lichens with contrasting modes of reproduction in coastal Norway. Lichenologist 48: 545–557.

Kamvar, Z. N., J. F. Tabima & N. J. Grünwald. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2: e281.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs & H. Wagner. 2019. vegan: Community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

Rikkinen, J., I. Oksanen & K. Lohtander. 2002. Lichen guilds share related cyanobacterial symbionts. Science 297: 357.

Scheidegger, C., P. Clerc, M. Dietrich, M. Frei, U. Groner, C. Keller, I. Roth, S. Stofer & M. Vust. 2002. Rote Liste der gefährdeten baum- und erdbewohnenden Flechten der Schweiz. WSL, CJB, BUWAL, Bern.

Scheidegger, C., S. Stofer & B. Senn-Irlet. 2015. 4.8. Gefährdete Arten. Pages 86–87. In: A. Rigling & H. P. Schaffer (eds.), Waldbericht 2015. Zustand und Nutzung des Schweizer Waldes. Bundesamt für Umwelt. Birmensdorf, Eidgenössische Forschungsanstalt WSL, Bern und Birmensdorf.

Werth, S. 2012. Fungal-algal interactions in *Ramalina menziesii* and its associated epiphytic lichen community. Lichenologist 44: 543–560.

Widmer, I., F. Dal Grande, C. Cornejo & C. Scheidegger. 2010. Highly variable microsatellite markers for the fungal and algal symbionts of the lichen *Lobaria pulmonaria* and challenges in developing biont-specific molecular markers for fungal associations. Fungal Biology 114: 538–544.

manuscript received October 28, 2021; accepted January 22, 2022.

Supplementary documents online:

Supplementary Table S1. All data underlying the analyses conducted in this research. Data includes both microsatellite marker data for all photobionts and locality data.