

Modeling the Coevolutionary Dynamics of the *Lobaria pulmonaria* Lichen Symbiosis

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Abstract

1 Introduction

Lichens are a hyperdiverse symbiotic group spread across the globe in widely different *niche* and climates. This partnership between fungi and a photobiont (cyanobacteria, algae or both) allows the fungi to obtain carbohydrate-rich resources directly from their photosynthetic partner [1] while the fungus protects the photobiont from desiccation, allowing for coevolution and adaptive radiation into new environments [2]. Lichenization is an evolutionarily and ecologically successful strategy (>20% of fungi are lichenized), resulting in approximately 14,000 lichen species known to date [1, 5]. Although the nature of the lichen symbiosis is still widely debated, many sources agree that the lichen system represents an ecologically obligate mutualistic interaction whereby the net fitness of all partners is maximized [3, 4, 5].

Lichens can reproduce sexually via fungal spores (horizontal transmission) and asexually via vegetative propagules and thallus fragmentation (vertical transmission). In the sexual mode of reproduction, the fungal spores must interact with a compatible free-living algae and/or cyanobacterium in order to reconstitute the lichen thallus. In the asexual mode of reproduction, mycobionts and photobionts are co-dispersed via fragmentation of the main thallus body and specialized asexual propagules.

These peculiar mode of reproduction strongly influences the genetic structure of lichen populations [10, 11], affecting dispersal and evolutionary rates. This was evidenced by recent articles in the most studied lichen system: *L. pulmonaria*, where thalli from 62 populations in forests throughout Europe, North America, Asia, and Africa were genotyped at several hypervariable microsatellite loci [10]. These studies concluded that the *L. pulmonaria*-*S. reticulata* symbiosis showed significant within-population genetic structure due to restricted gene flow and vertical transmission (i.e. co-dispersal of vegetative propagules).

With the same dataset ([?]), we used different complex systems approaches such as network theory and agent-based models to better understand the structural genetic diversity existing within the lichen populations as well as reproduce general features of this system. First, we analyzed the fungal-algal population network structures of the data (8 fungal-specific and 7 algal-specific microsatellites) from the fungal-algal partnership. We then reconstructed the empirical bipartite genetic network, and obtained common network metrics (e.g., nestedness and modularity). Moreover, we introduced the particularities of the fungal-algal interactions in a continuous evolutionary algorithm based on the widely used ECHO framework [18]. With these analyses, we obtained similar patterns of diversification as well as ecological interactions, allowing us to better understand the mechanisms driving the evolution of symbionts in the *L. pulmonaria* system. Determining the co-evolutionary relationships and dynamics in this particular system will help us to better understand the role that symbiotic interactions play in the generation and maintenance of biodiversity in forest communities.

2 Method

2.1 Empirical Network Analysis.

We used the [?] dataset to create a bipartite network of the relationships between the symbionts. We took the algae and the fungal lists of microsatellites separately. For each algae A_n (in symbiosis with a fungus F_n , we looked for the existence of another fungus F_m in symbiosis with a similar algae A_m and thus created a link between A_n and F_m . As a preliminary test to check the validity of our methods, we considered two symbionts as similar when they shared at least six out of seven microsatellites (for the algae) and seven out of eight microsatellites for the fungi. The assumption behind this is that symbionts that are closer genetically have a higher probability to come from the same ancestor (Fig. 2).

2.2 ECHO Model.

In order to better understand the interplay between the peculiar mode of reproduction associated to the *L. pulmonaria* system and the underlying population structure and evolutionary dynamics, we constructed an agent-based model based on the widely used ECHO framework [18, 19]. The ECHO model typically consists of a collection of entities living in a simplified spatial domain, which can move around and interact with one another and with their environment. The interactions among agents can be used to model different kinds of processes -such as mating-, and are driven by locality as well as agent-specific properties, namely the agents' genotypes. The ECHO model is also a continuous genetic algorithm [20]; upon reproduction old genotypes are copied with slight mutations, giving rise to quantifiable evolutionary dynamics.

In our case, we used the tag system of ECHO to model the molecular recognition (receptors and physical embedding) between algae and fungi necessary

to create the lichen. We considered two different lichenization functions based on similarity, sigmoid (hill function with $n = 2$) and michaelis-menten (saturation dynamics). Additionally, other ecologically relevant features such as dispersal rates (introduced here as random walks) and the ratio between sexual and asexual reproduction were included in the model. Simulations were carried out assuming a wide range of ecological relations between the algae and fungi: competition (both algae and fungi are better off on their own than forming a lichen), parasitism (only one type of agent benefits from the partnership) and mutualism (both agents benefit).

Model Network Analysis. For the bipartite networks retrieved from the ECHO model, we unraveled the modular structure of the lichen population to reveal the coevolution of the symbionts. We analyzed nestedness and modularity of the bipartite networks retrieved from the ECHO model and compared it to various null models via the BiMAT package in MATLAB [21]. For ecological networks (dealing with species? interactions), nestedness occurs when specialist species tend to interact with subsets of species that interact with more generalist species [22]. Although there is relative consensus on the meaning of nestedness, there are several distinct metrics by which it can be measured. The biMAT package implements the widely used NODF measure ($0 \leq \text{NODF} \leq 1$) of nestedness [23]. The modularity measure ($0 \leq Q \leq 1$) we used detects communities including both types of nodes [24], as opposed to the measure where communities are formed by nodes of the same type [25].

3 Results

3.1 Empirical Network Analysis.

3.2 ECHO Model Network Analysis.

From the different ecological interactions implemented in ECHO, no significant modular structure was found. However, the conditions of mutualism, with saturated and sigmoid lichenization functions showed statistically significant nestedness values. The z-score determines how different our measured value is from the distribution calculated from the randomized networks (null model), and the percentile tells us the percentage of random network distributions that have values inferior to the one obtained from our network. We did not retrieve any nestedness value higher than 0.5. We obtained a nestedness value of 0.18 (the maximum nestedness is 1). Although our value of 0.18 is significantly more nested than the null models, we cannot say definitively that it is a nested network. Regarding the modularity metric, no case was found to be statistically significant. The mean modularity of the randomized networks was practically equal to the one observed in our network.

Acknowledgements

This work was initiated at the 2016 Complex Systems Summer School (CSSS) at the Sante Fe Institute (SFI) and was supported by grants from the British Lichen

Society and the British Ecological Society. The authors would like to thank all SFI resident faculty members, SFI external faculty members, SFI Omidyar Post-doctoral Fellows, and 2016 CSSS course participants for productive discussions as well as SFI staff during the 2016 CSSS. The authors would also like to thank Dr. Christoph Scheidegger for access to multiple datasets and Dr. Francesco Dal Grande for providing detailed knowledge of the *L. pulmonaria* system.