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Polish Academy of Sciences

Plant and Fungal Systematics 64(2): 163–172, 2019
DOI: 10.2478/pfs-2019-0017

ISSN 2544-7459 (print)
ISSN 2657-5000 (online)

Scale-dependent co-occurrence patterns of closely related genotypes in a lichen species complex

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Article info

Received: 21 Mar. 2019
Revision received: 19 Aug. 2019
Accepted: 22 Aug. 2019
Published: 2 Dec. 2019

Associate Editor

Adam Flakus

Abstract. The 'competition-relatedness' hypothesis postulates that co-occurring taxa should be more distantly related, because of lower competition. This hypothesis has been criticized for its dependence on untested assumptions and its exclusion of other assembly forces beyond competition and habitat filtering to explain the co-existence of closely related taxa. Here we analyzed the patterns of co-occurring individuals of lichenized fungi in the *Graphis scripta* complex, a monophyletic group of species occurring in temperate forests throughout the Northern Hemisphere. We generated sequences for three nuclear ribosomal and protein markers (nuLSU, *RPB2*, *EF-1*) and combined them with previously generated sequences to reconstruct an updated phylogeny for the complex. The resulting phylogeny was used to determine the patterns of co-occurrences at regional and at sample (tree) scales by calculating standard effect size of mean pairwise distance (SES.MPD) among co-occurring samples to determine whether they were more clustered than expected from chance. The resulting phylogeny revealed multiple distinct lineages, suggesting the presence of several phylogenetic species in this complex. At the regional and local (site) levels, SES.MPD exhibited significant clustering for five out of six regions. The sample (tree) scale SES.MPD values also suggested some clustering but the corresponding metrics did not deviate significantly from the null expectation. The differences in the SES.MPD values and their significance indicated that habitat filtering and/or local diversification may be operating at the regional level, while the local assemblies on each tree are interpreted as being the result of local competition or random colonization.

Key words: Assembly, community phylogeny, crustose lichens, cryptic species, mean pairwise distance (MPD)

Introduction

Combining the competitive exclusion principle (Gause 1934) with Darwin's (1859) hypothesis that closely related species are unlikely to coexist, the competitive relatedness hypothesis (CRH) postulates that closely related species should not co-occur persistently in the same community, because they are likely to be ecologically equivalent, so that competitive interactions would lead to exclusion of less competitive species (Cahill et al. 2008). This theoretical conjecture has stimulated numerous empirical studies, including the development of computational tools for hypothesis testing within a phylogenetic framework (e.g., Webb 2000; Webb et al. 2002; Kembel et al. 2010; Smith et al. 2013). With increasing access to molecular data, phylogenetics has become one of the principal tools for ecologists to study patterns and infer processes of community assembly, allowing them to estimate the 'relatedness' among co-existing species through phylogenetic distance (Webb et al. 2008). If competition shapes community assembly and closely related species are ecologically equivalent, competing for niche space, we would expect phylogenetic distance among co-occurring species to be greater than random, and co-occurring species would be dispersed across the phylogeny. If abiotic factors are more critical for community assembly or if community dynamics prevent species from outcompeting others, we would expect phylogenetic 'clustering' of lineages including species with similar

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ecological traits (Webb et al. 2002). An example for the latter is from tropical foliicolous lichens, in which multiple closely related species often grow together on the same leaf, due to the fact that leaves are a highly dynamic substrate and their longevity is usually shorter than the time required to reach competitive exclusion between species (Lücking 2001; Lücking & Bernecker-Lücking 2002).

While phylogenetic frameworks have been widely used to infer the assembly processes of various communities including lichens (e.g., Horner-Devine & Bohannan 2006; Kembel & Hubbell 2006; Verdú & Pausas 2007; Burns & Strauss 2011; Lücking et al. 2016), this approach relies heavily on the assumption that relatedness can be used as a proxy for the degree of competition and that competition is a primary factor in community assembly (Gerhold et al. 2015). This notion has been tested in various study systems, many of which showed no correlation between phylogenetic relatedness and strength of competition (Alexandrou et al. 2014; Naughton et al. 2015). Furthermore, phylogenetic community structure may be the result of processes other than competition (Vamosi et al. 2009). Therefore, with the appropriate spatial and temporal scale, these patterns could be used to describe or test hypotheses about broader macroevolutionary processes such as dispersal and diversification of co-occurring species (Gerhold et al. 2015).

Besides the problem of relating community assembly and competition to phylogeny, testing the CRH is challenging for other reasons. The scale of competition depends on the type of organism (Cavender-Bares, Keen & Miles 2006; Swenson et al. 2006; Slingsby & Verboom 2006; Cooper, Rodriguez & Purvi 2008). While large mammals may compete at landscape (regional or meta-community) scale, trees largely compete at habitat scale, and small organisms such as lichens at microhabitat scale (Peterson et al. 1998; Bowker & Maestre 2012; Genet et al. 2014). Niche dimensions along which organisms compete are often insufficiently known, and proxies of ecological equivalency may be inaccurate. Lichens do not seem to exhibit specific biotic interactions such as found in plant pollination and seed dispersal, and the niche dimensions along which competition acts are presumed to be largely limited to the availability of space and to abiotic growth factors such as nutrients and microclimate (Lawrey 1981; John & Dale 1989). In lichens, a commonly used proxy to determine niche overlap or ecological equivalency, and hence competition, is the nearest neighbour approach, the observation of thalli growing side by side (Lawrey 1981; John 1989; Armstrong & Welch 2007).

Since competition acts at the individual level, lichens that frequently grow side by side should undergo strong competition. The CRH proposes that phylogenetic divergence results in phenotypic divergence, going along with changing ecological preferences (Violle et al. 2011; Herben & Goldberg 2014), so that closely related lineages are likely to be ecologically equivalent, whereas distantly related species are not. Provided that the ecologically similar species cannot remain in stable coexistence, members in an observed community should be ecologically different enough to reduce the interspecific competition. Such a mechanism can lead to the phenomena of 'limiting similarity' among coexisting species (MacArthur & Levins 1967).

The *Graphis scripta* complex, commonly known as 'script lichen', presents a useful study system to test the CRH in a phylogenetic framework and to grapple with the challenges relating to the definition of niche overlap and ecological equivalence. As one of a few extratropical members of the predominantly tropical family Graphidaceae (Lücking et al. 2014), this species complex is found on the bark of trees across North America and Eurasia, in particular on smooth-barked trees of the genera *Fagus*, *Carpinus*, *Betula* and *Prunus* (Otte 1999; Bollinger et al. 2007; Neuwirth 2013; Wirth, Hauck & Schultz 2013; Gnüchtel 2014). It is one of the few lichens for which possible mechanisms for competitive exclusion have been studied, focusing on allelopathy (Whiton & Lawrey 1984). Thalli of these lichens frequently grow side by side (Neuwirth & Aptroot 2011), facilitating the use of the nearest neighbour approach.

The *Graphis scripta* complex is characterized by elongated ascomata with a black margin (labia) and a laterally carbonized excipulum, a clear hymenium, small, transversely septate ascospores, and the lack of secondary substances (Lücking et al. 2009). Other characters, such as branching of the ascomata, visibility of the disc, and the presence of pruina, vary within this complex, which has led to a large number of nomenclatural novelties since the first description of *Lichen scriptus* by Linnaeus (1753), particularly at the infraspecies level (Acharius 1809; Zahlbruckner 1923). Zahlbruckner (1923) adopted a broad concept of *G. scripta*, subsuming all other taxa under a single name, a concept accepted until recently, when Neuwirth & Aptroot (2011) attempted to structure the observed morphological and anatomical variation by recognizing various species. Molecular data indeed suggest that the *Graphis scripta* complex contains a number of phylogenetic lineages representing distinct species (Kraichak et al. 2015).

Putative species within the *Graphis scripta* complex often co-occur spatially at regional and local (tree) levels. Two or more morphologically different but well-demarcated thalli of this complex can grow next to each other on the same bark (Neuwirth & Aptroot 2011). In a survey of epiphytic lichens in Styria in Austria, multiple thalli of the complex were found on single trees even though they were not strongly correlated with species delimitation (Obermayer, pers. comm.; Kraichak et al. 2015). According to the CRH, these co-occurring thalli either should belong to the same species or, if representing various lineages, should differ somewhat in their niche space and be more distantly related than expected by chance. On the other hand, competitive effects are not expected at local and regional scales. Hence, lichens in this complex should be phylogenetically dispersed (or show no phylogenetic diversity) at tree scale but appear phylogenetically clustered at habitat and landscape scales, due to similar habitat preferences.

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Materials and methods

Molecular methods

Data matrices of 238 sequences from specimens of *Graphis scripta* were generated, comprising nuclear large subunit rDNA (nuLSU), RNA polymerase II second largest subunit (*RPB2*), and translation elongation factor (*EF-1*) from a previous study (Kraichak et al. 2015); new sequences were generated from additional samples from North America and Europe (Table 1). *Graphis implicata* and *G. librata* were used as outgroups (Rivas Plata et al. 2013; Kraichak et al. 2015). Nuclear internal transcribed spacer (ITS) and mitochondrial small subunit (mtSSU) data were not included, due to low amplification success. DNA extraction, PCR reactions, product purification, and sequencing followed the protocol outlined in Kraichak et al. (2015). DNA extracts are housed at the Pritzker Laboratory of Molecular Systematics at the Field Museum,

Table 1. List of samples, with their collection data and GenBank accession numbers for the sequences used in this study. New sequences are bolded.

Locality	Specimen Voucher	DNA Voucher	nuLSU	*RPB-2*	*EF-1*
Austria	Neuwirth 11834 (F)	6814	KF875544	KF875527	KJ441077
Austria, Steirmark	Obermayer 12305_10A (GZU)	8994	**MN612583**	—	**MN612635**
Austria, Steirmark	Obermayer 12305_12 (GZU)	8997	**MN612584**	**MN612607**	**MN612636**
Austria, Steirmark	Obermayer 12305_1A (GZU)	8978	**MN612576**	**MN612601**	—
Austria, Steirmark	Obermayer 12305_20 (GZU)	9005	**MN612585**	—	**MN612637**
Austria, Steirmark	Obermayer 12305_2A (GZU)	8983	**MN612578**	**MN612603**	—
Austria, Steirmark	Obermayer 12305_3 (GZU)	8982	**MN612577**	**MN612602**	—
Austria, Steirmark	Obermayer 12305_3A (GZU)	8984	**MN612579**	**MN612604**	—
Austria, Steirmark	Obermayer 12305_4 (GZU)	8986	**MN612580**	**MN612605**	—
Austria, Steirmark	Obermayer 12305_5 (GZU)	8987	**MN612581**	**MN612606**	**MN612633**
Austria, Steirmark	Obermayer 12305_6A (GZU)	8989	**MN612582**	—	**MN612634**
Austria, Steirmark	Obermayer 13060_a (GZU)	8968	**MN612568**	**MN612593**	**MN612624**
Austria, Steirmark	Obermayer 13060_b (GZU)	8969	**MN612569**	**MN612594**	—
Austria, Steirmark	Obermayer 13060_c (GZU)	8970	**MN612570**	**MN612595**	**MN612625**
Austria, Steirmark	Obermayer 13060_d (GZU)	8971	**MN612571**	**MN612596**	**MN612626**
Austria, Steirmark	Obermayer 13060_e (GZU)	8972	**MN612572**	—	**MN612627**
Austria, Steirmark	Obermayer 13060_f (GZU)	8973	**MN612573**	—	**MN612628**
Austria, Steirmark	Obermayer 13060_g (GZU)	8974	**MN612574**	**MN612597**	**MN612629**
Austria, Steirmark	Obermayer 13060_h (GZU)	8975	—	**MN612598**	**MN612630**
Austria, Steirmark	Obermayer 13061_1 (GZU)	8976	**MN612575**	**MN612599**	**MN612631**
Austria, Steirmark	Obermayer 13061_2 (GZU)	8977	—	**MN612600**	**MN612632**
Canada, British Columbia	Tønsberg 42518 (BG)	8611	KJ440922	KJ441017	KJ441061
Canada, British Columbia	Tønsberg 42519 (BG)	8612	KJ440923	—	KJ441062
Canada, British Columbia	Tønsberg 42520 (BG)	8613	KJ440924	KJ441018	KJ441063
Canada, British Columbia	Tønsberg 42522 (BG)	8615	KJ440925	KJ441019	KJ441064
Canada, British Columbia	Tønsberg 42525 (BG)	8618	**MN612559**	—	**MN612616**
Canada, Ontario	Lendemer 28278A (NY)	8252	**MN612555**	—	**MN612611**
China	Sohrabi 16429 (F)	6464	KF875541	KF875524	—
China	Sohrabi 16579 (F)	6454	KF875540	KF875523	KJ441030
China,	Sohrabi 16438 (F)	6450	KF875542	KF875525	KJ441072
Costa Rica	Lücking 16103 (F)*	3194	DQ431939	JF828947	KJ441071
El Salvador	Lücking 28001 (F)**	3188	HQ639636	JF828945	KJ441070
France, Lorraine	Stapper F14-1472	8948	**MN612560**	—	**MN612617**
Germany	Bachmann 8.208 (POLL)	7505	KJ440893	KJ440993	KJ441039
Germany	Bachmann 8.21 (POLL)	7507	KJ440894	KJ440994	KJ441028
Germany, Baden-Wérttemberg	Dornes 21304.006 (M)	8286	KJ440920	KJ441015	KJ441059
Germany, Baden-Wérttemberg	Dornes 21304.007 (M)	8287	KJ440921	KJ441016	KJ441060
Germany, Baden-Württemberg	Dornes 21207.36 (M)	8285	**MN612557**	—	**MN612614**
Germany, Baden-Württemberg	Dornes 21208.03 (M)	8290	**MN612558**	—	**MN612615**
Germany, Baden-Württemberg	Dornes 21212.136 (M)	8274	KJ440911	KJ441006	KJ441053
Germany, Baden-Württemberg	Dornes 21212.151 (M)	8275	KJ440912	KJ441007	—
Germany, Baden-Württemberg	Dornes 21304.008 (M)	8276	KJ440913	KJ441008	KJ441054
Germany, Baden-Württemberg	Dornes 21304.012 (M)	8277	KJ440914	KJ441009	KJ441055
Germany, Baden-Württemberg	Dornes 21304.015 (M)	8278	KJ440915	KJ441010	KJ441056
Germany, Bayern	Dornes K_OA4410 (M)	8271	KJ440909	KJ441004	KJ441051
Germany, Bayern	Dornes K_OA4411 (M)	8272	KJ440910	KJ441005	KJ441052
Germany, Bayern	Dornes K_OA4413 (M)	8280	KJ440916	KJ441011	—
Germany, Bayern	Dornes K_OA4433 (M)	8281	KJ440917	KJ441012	KJ441057

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Table 1. Continued.

Locality	Specimen Voucher	DNA Voucher	nuLSU	*RPB-2*	*EF-1*
Germany, Bayern	Dornes K_OA4442 (M)	8282	KJ440918	KJ441013	KJ441074
Germany, Bayern	John 8.051 (POLL)	6999	KJ440889	—	KJ441035
Germany, Bayern	Werth K_OA9237 (M)	8284	KJ440919	KJ441014	KJ441058
Germany, Rheinland-Pfalz.	John 8.14 (POLL)	6991	KJ440881	KJ440984	KJ441026
Germany, Rheinland-Pfalz.	John 8.144 (POLL)	6992	KJ440882	—	KJ441031
Germany, Rheinland-Pfalz.	John 8.149 (POLL)	6993	KJ440883	KJ440985	KJ441027
Germany, Rheinland-Pfalz.	John 8.15 (POLL)	6994	KJ440884	KJ440986	KJ441032
Italy, Trentino	Kalb 39883 (WIS)	8952	**MN612564**	**MN612590**	**MN612621**
Netherlands,	Aptroot 11808 (ABL)	8018	KJ440895	—	KJ441040
Switzerland, Schweiz Tessin	John 8.172 (POLL)	7000	KJ440890	KJ440990	KJ441036
Switzerland, Schweiz Tessin	John 8.173 (POLL)	7001	KJ440891	KJ440991	KJ441037
Switzerland, Schweiz Tessin	John 8.174 (POLL)	7002	KJ440892	KJ440992	KJ441038
Switzerland, Schweiz Tessin	John 8.175 (POLL)	6995	KJ440885	KJ440987	KJ441033
Switzerland, Schweiz Tessin	John 8.176 (POLL)	6996	KJ440886	KJ440988	—
Switzerland, Schweiz Tessin	John 8.177 (POLL)	6997	KJ440887	—	KJ441029
Switzerland, Schweiz Tessin	John 8.178 (POLL)	6998	KJ440888	KJ440989	KJ441034
USA, Alaska	Spribille 38023 (GZU)	8961	**MN612566**	**MN612592**	**MN612622**
USA, Alaska	Spribille 38060 (GZU)	8954	**MN612565**	**MN612591**	—
USA, Alaska	Spribille 39075 (GZU)	8963	**MN612567**	—	**MN612623**
USA, Delaware	Harris 57956 (NY)	8236	KJ440900	KJ440999	KJ441044
USA, Delaware	Harris 57982 (NY)	8237	KJ440901	KJ441000	KJ441045
USA, Delaware	Hodkinson 18892 (NY)	8238	—	**MN612587**	**MN612609**
USA, Delaware	Lendemer 32055 (NY)	8239	KJ440902	—	KJ441046
USA, Delaware	Lendemer 32104 (NY)	8240	KJ440903	—	KJ441075
USA, Delaware	Lendemer 32130 (NY)	8241	—	**MN612588**	**MN612610**
USA, Delaware	Lendemer 32154 (NY)	8242	KJ440904	KJ441001	KJ441047
USA, Delaware	Lendemer 33748 (NY)	8234	—	**MN612586**	**MN612608**
USA, Delaware	Lendemer 35766 (NY)	8233	KJ440898	KJ440997	KJ441042
USA, Delaware	Lendemer 35829 (NY)	8235	KJ440899	KJ440998	KJ441043
USA, Illinois	Nelsen MN503 (F)	MN503	KJ440936	KJ441023	KJ441067
USA, Illinois	Nelsen MN507 (F)	MN507	KJ440937	KJ441024	KJ441068
USA, Illinois	Nelsen MN559 (F)	MN559	KJ440938	KJ441025	KJ441069
USA, Maine	Lendemer 32300 (NY)	8226	KJ440896	KJ440995	KJ441041
USA, Maryland	Harris 57934 (NY)	8270	KJ440908	KJ441003	KJ441050
USA, Maryland	Lendemer 31990 (NY)	8266	—	**MN612589**	**MN612613**
USA, Maryland	Lendemer 33481 (NY)	8268	KJ440907	KJ441002	KJ441049
USA, Michigan	Nelsen MN184 (F)	MN184	KJ440933	KJ441020	KJ441065
USA, North Carolina	Harris 57037 (NY)	8264	KJ440906	—	KJ441048
USA, North Carolina	Lendemer 32154 (NY)	8263	**MN612556**	—	**MN612612**
USA, Pennsylvania	Lendemer 37782 (NY)	8232	KJ440897	KJ440996	—
USA, Washington	Tonsberg s.n. (BG)	8949	**MN612561**	—	**MN612618**
USA, Washington	Tonsberg s.n. (BG)	8950	**MN612562**	—	**MN612619**
USA, Washington	Tonsberg s.n. (BG)	8951	**MN612563**	—	**MN612620**
USA, Wisconsin	Nelsen MN498 (F)	MN498	KJ440934	KJ441021	KJ441066
USA, Wisconsin	Nelsen MN499 (F)	MN499	KJ440935	KJ441022	KJ441076
Number of new sequences			0	0	0
Total number of sequences			87	70	81
Adjusted alignment length (bp)			483	966	357
Number of identical sites			176	603	169

Asterisked specimens represent *Graphis imbricata* (*) and *G. librata* (**), use as outgroup.

and specimen vouchers in the corresponding herbaria (Table 1). New sequences were deposited in GenBank (Table 1).

Sequence Alignments and Phylogenetic Analysis

Alignments of individual genes were performed using Muscle (Edgar 2004) and manually adjusted with Geneious 8.1.6 (Kearse et al. 2012; File S1 and File S2). Individual gene trees were reconstructed under maximum likelihood (ML) to examine potential conflict. The concatenated data of the three loci were then subjected to phylogenetic analyses using ML and Bayesian approaches (B/MCMC).

The ML analysis was performed on a partitioned alignment with RAxML-HPC2 (version 7.3.1) on Xsede (Stamatakis 2006), using the default settings and the Gtr-Gamma model of nucleotide substitution. Rapid bootstrap estimates were carried out for 1000 pseudoreplicates (Stamatakis et al. 2008).

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For B/MCMC analysis the dataset was also partitioned for each locus and analyzed using MrBAYES 3.1.2 (Huelsenbeck & Ronquist 2001). The GTR+I+G model was chosen for all loci as the appropriate substitution model. Two parallel runs with 20,000,000 generations were executed, starting with a random tree and four simultaneous chains. Heating of chains was set to 0.2. Posterior probabilities were estimated by sampling every 1000th tree, using a variant of the Markov chain Monte Carlo (MCMC) method, to avoid sample autocorrelation. The first 4,000 trees were discarded as burn-in. A 50% majority-rule consensus tree with average branch lengths was computed from the remaining trees, using the sumt command. Only clades with bootstrap support equal to or above 70% under ML and a posterior probability equal to or above 0.95 under B/MCMC were considered as supported. Both analyses were performed using the CIPRES online computing facility (www.phylo.org) (Miller, Pfeiffer & Schwartz 2010). Phylogenetic trees were visualized using FigTree 1.4.0 (Rambaut 2012) and the R-package 'ape' (Paradis et al. 2004).

Phylogenetic clustering of co-occurring genotypes

To determine whether specimens from the same community at various scales were more distantly or more closely related than expected from chance, we calculated the standardized effect size of mean pairwise distance (SES.MPD), using the R-package 'picante' (Kembel et al. 2010). For this calculation we treated each sequence as an operational taxonomic unit (OTU) that occurred in each locality (or 'community' in the sense of phylogenetic community). The algorithm first calculated the actual phylogenetic distance from the resulting ML tree between each pair of sequences in the same locality and computed the mean for all observed distance values (mean pairwise distance: MPD). The program then ran 9,999 simulations by randomly drawing from the sequence pool the same number of sequences for each community ('phylogeny pool' option for the simulation method). The mean pairwise phylogenetic distance was calculated for each simulation and became a 'null' value. The observed MPD value was compared against the distribution of the null values, using the standardized score [z-score: mean(observed MPD) – mean(null MPD) / SD (null MPD)], which is known as standardized effect of mean pairwise distance (SES.MPD). The P-value for this analysis was calculated by dividing the number of null values that are more extreme than the observed value by the total number of cases (1 observed + 9,999 values from simulations) (Webb et al. 2008). We considered P-values of 0.05 or lower to be a significant departure from the simulated null values.

An MPD lower than expected from chance suggests that co-occurring specimens are more closely related (phylogenetic clustering), whereas a value higher than expected from chance suggests that co-occurring species are more distantly related (phylogenetic dispersion). A SES.MPD value of (close to) zero indicates that the assembly of species in each community is not different from a random pattern (Webb et al. 2008).

We performed this analysis at two different scales: regional (landscape) scale and local (tree) scale. For the regional scale we chose a region equivalent to state-level in each studied country that had at least six specimens in the dataset; we treated it as a metacommunity for the calculations. These specimens were collected from at least three different sites within the regions, according to the collectors' label information. For the tree scale we focused on a particularly dense sampling of *Graphis scripta* on two European Hornbeam trees (*Carpinus betulus*), each

Figure 1. Co-occurrence of two genotypes of *Graphis scripta* on bark with a distinct boundary.

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containing ten specimens in our dataset, from Styria in Austria. At this scale the lichen thalli of *G. scripta* were found growing side-by-side, with distinct boundaries and variable morphologies (Fig. 1).

Results

Phylogenetic Analysis

The analyzed data matrix contained a total of 238 nucleotide sequences, of which 83 new sequences were generated for this study, while the remaining 155 sequences were obtained from a previous study (Kraichak et al. 2015) (Table 1). A matrix of 1,761 unambiguously aligned nucleotide position characters was produced, of which 948 were identical (Table 1). Inspection of individual gene trees from the ML analysis did not show any significant incongruence among the gene trees, and therefore the concatenated data matrix of three loci was used. Because of similarity of topologies between the best ML and 50% majority-rule consensus trees from the B/MCMC analysis, we used the best ML tree for the subsequent analyses. The resulting phylogenetic tree revealed a similar set of several distinct clades that were previously recovered (Kraichak et al. 2015) (Fig. S1). All of the additional samples from Austria and North America were placed among already existing clades and did not form additional lineages at species level.

Phylogenetic clustering of co-occurring genotypes

At regional scale we identified six areas with at least six samples in the dataset (Fig. 2). The SES.MPD values for all areas were negative (–4.41 to –1.15) and significantly deviated from the null distribution, with the exception of Bavaria, Germany (P = 0.068), suggesting phylogenetic clustering. At tree scale (Fig. 3) the SES.MPD values for each tree were also negative (–1.79 to –0.98) but did not deviate significantly from the null distribution (P > 0.05). When compared across the two scales, the SES.MPD values at regional scale were generally more negative and significantly deviated from the null distribution, whereas the values at local scale were less negative and did vary significantly from the null distribution.

Discussion

Our phylogenetic reconstruction of the *Graphis scripta* complex revealed several distinct lineages, in accordance with a previous study (Kraichak et al. 2015). The geographic distribution of these lineages, combined with branch length patterns, support the notion that they are to

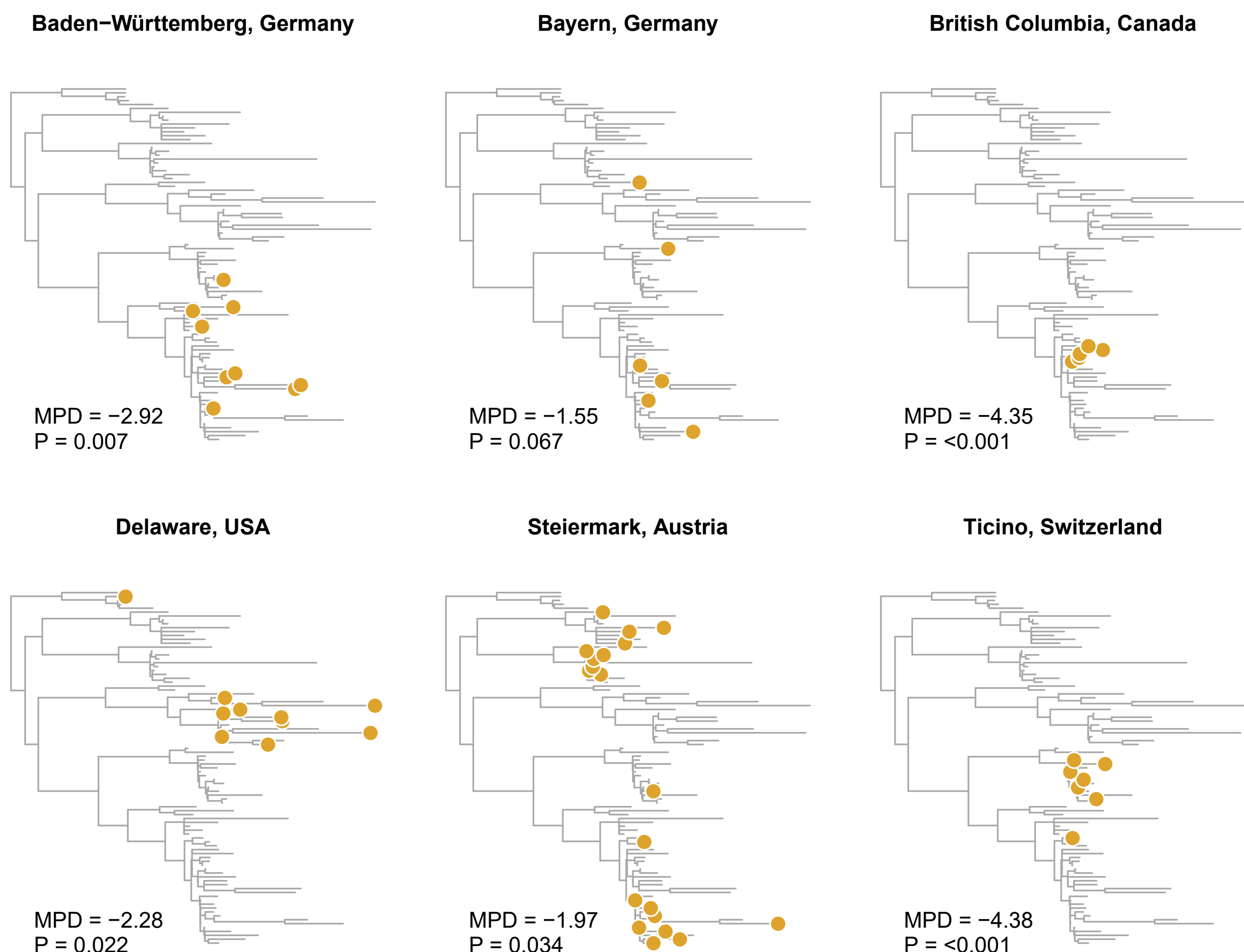

Figure 2. Phylogenetic distribution of the *Graphis scripta* complex specimens from six localities, and their associated SES.MPD and P-values.

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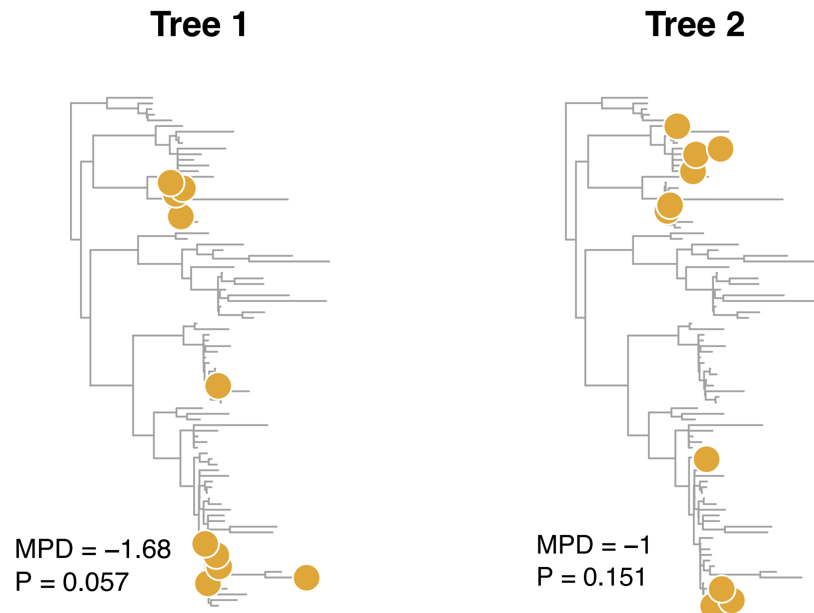

Figure 3. Phylogenetic distribution of the *Graphis scripta* complex specimens from two host trees found in Styria, Austria, and their associated SES.MPD and P-values.

be interpreted as distinct species and not as genetically variable, geographically isolated populations of a single taxon. The analyses of co-occurrence patterns showed that at the regional level, the specimens in the same region were significantly more closely related than expected from chance, while the clustering pattern was less clear at local level. The discrepancy in the phylogenetic patterns at two spatial scales points to the potential differences in the processes by which these specimens have come to occur (Fig. 4).

At the broad geographical scale considered in this study, the co-occurring specimens exhibited the clustering pattern in the comparison to the global phylogeny pool. Under the traditional community framework this pattern is interpreted to be a result of habitat filtering that selects similar individuals into the same habitat due to their similarity of ecological requirements (Webb et al. 2002). A study of Amazonian plant communities also showed that phylogenetic clustering is more common at a larger scale (25–10,000 m^2; Kraft & Ackerly 2010). For lichens, however, it is unlikely that the habitats across the landscape at this broad scale would be uniform enough to exert such a strong habitat-filtering effect on the species pool (Kraft & Ackerly 2010). One of the few studies of the distribution dynamics of lichenized fungi showed that environmental filtering is significant to a local assemblage but also is much constrained by local dispersal dynamics (Schei et al. 2012). We would need functional traits of these lichens to validate the assumption that the more genetically similar individuals would have a similar set of traits associated with habitat filtering; that is beyond the scope of this study. Moreover, studies of the correlations between traits and genetic distance in the context of community assembly have shown mixed results (Cahill et al. 2008; Alexandrou et al. 2014; Naughton et al. 2015). Without more concrete evidence on the functional traits of these lichens, habitat filtering cannot be confirmed as a main assembly mechanism here.

We can offer two alternative mechanisms (Fig. 4). First, the phylogenetic clustering of the specimens can be the result of dispersal events, where only certain clades from the global phylogenetic pool disperse to an area. In this case the genetically similar individuals in the region are simply the result of an initial dispersal event and its subsequent in situ propagation. Second, local diversification of a lineage can also lead to a clustering pattern. Within a region, a lineage may diverge genetically and give rise to genetically similar populations across the landscape, as has been shown in several other lichen groups (Printzen & Ekman 2003; Walser et al. 2005; Lindblom & Ekman 2006). It should be noted that these two explanations are not mutually exclusive, as local diversification can be followed by dispersal of the resulting lineages to another area (also known as secondary contact). High dispersal ability has been widely recognized in lichenized fungi (Sillett et al. 2000; Yemets et al. 2014). This would appear to be the case for the *Graphis scripta* complex as well, because, despite the clustering pattern, each of the genetic groups contains specimens from various geographic areas, suggesting that dispersal limitation is unlikely for this complex (Kraichak et al. 2015). However, because we observed the clustering patterns in this study, it is also possible that long-range dispersal events might be rare and do not contribute in the assembly process. Additional molecular markers, such as microsatellite or RADseq data, will be needed at population level to illustrate the relative importance of these processes.

At local scale the co-occurring specimens from Styria did not exhibit significant phylogenetic clustering. The standardized values of MPD, while still negative, showed less deviation from the null expectation. This result showed that each individual tree hosted multiple distantly related genotypes, which is consistent with the patterns we would expect from the competition-relatedness hypothesis

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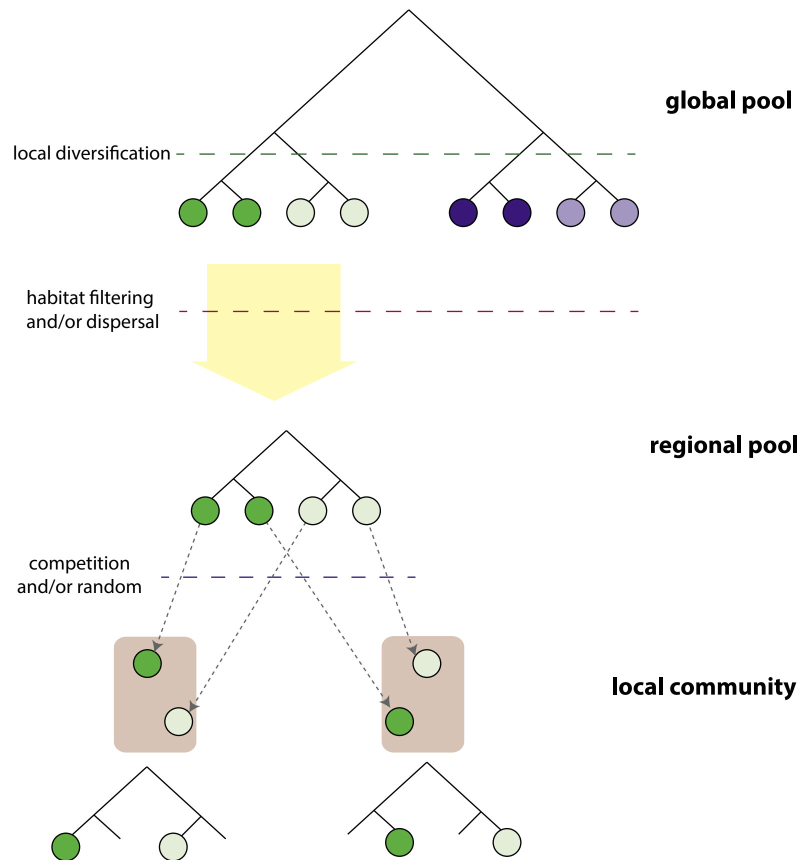

Figure 4. Schematic diagram illustrating an assembly model of the *Graphis scripta* complex community, based on the current findings.

(Cahill et al. 2008). Several studies showed that competition between lichen thalli does occur at local level, as they are limited by the availability of space on the substrate (Armstrong 1986; Armstrong & Welch 2007; Pastore et al. 2014). However, crustose lichens such as *Graphis scripta* are also known for their slow growth, which prevents their use in a manipulative experiment to illustrate competitive interactions (Lange 1990; Armstrong & Bradwell 2010). However, since SES.MPD at this scale did not significantly deviate from the null distribution (standardized zero), we can attribute a random process such as random colonization as a possible mechanism for the local assemblage of multiple genotypes within the *Graphis scripta* complex, with little or no influence of competition (Webb et al. 2008).

Scale-dependent community phylogenetic patterns have been discovered in several empirical and theoretical works (Swenson et al. 2006; Kraft & Ackerly 2010). In many cases such sensitivity to the scale of analysis leads to problems in inferring biological processes from the observed pattern, especially when the species pool and local taxa are not completely sampled (Swenson et al. 2006; Cavender-Bares et al. 2009). However, it is also suggested that scale-sensitive patterns can be used as a way to gain a more comprehensive understanding of assembly processes which will encompass local and regional processes as well as the evolutionary dynamics of studied taxa (Swenson et al. 2006; Gerhold et al. 2015). In this study we integrated phylogenetic data with their locality information to analyze co-occurrences at two spatial scales and to propose an assembly of this species complex of lichenized fungi. To our knowledge, this is one of the first studies employing the community phylogenetic approach to examine the co-occurrence of closely related taxa in lichenized fungi. We hope that our approach will stimulate the use of interdisciplinary tools to study the ecology and evolution of lichens.

Acknowledgements

We would like to thank A. Aptroot, A. Beck, P. Dornes, V. John, K. Kalb, J. C. Lendemer, B. McCune, M. P. Nelsen, G. Neuwirth, M. Sohrabi, T. Sprillbe and T. Tønsberg for providing material for the current and previous studies. A. Nutakki, S. Parnmen and L. Strozier are thanked for their help in generating part of the molecular data. The project was funded by the U.S. National Science Foundation (NSF; DEB-1025861). The preparation and presentation of the manuscript were funded in part by grants from the Field Museum of Natural History, USA, and the Faculty of Science and Graduate School of Kasetsart University, Thailand.

Supplementary electronic material

Figure S1. Phylogram from maximum likelihood analyses of nuLSU, *EF-1*, *RPB2* sequences from specimens in the *Graphis scripta* complex. Blue lettering refers to samples new to this study. Download file

File S1. Concatenate alignment of nuLSU, *EF-1* and *RPB2* sequences from specimens in the *Graphis scripta* complex, in nexus format. Download file

File S2. Maximum likelihood tree from the concatenated alignment of nuLSU, *EF-1*, *RPB*2 sequences from specimens in the *Graphis scripta* complex, in newick format. Download file

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