Molecular Phylogenetics and Evolution 61 (2011) 45–63

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

# Parallel evolution and phenotypic divergence in lichenized fungi: A case study in the lichen-forming fungal family Graphidaceae (Ascomycota: Lecanoromycetes: Ostropales)

Eimy Rivas Plata [a,b,*], H. Thorsten Lumbsch [b]

[a] Biological Sciences Department, University of Illinois at Chicago, 845 W. Taylor St., Chicago, IL 60607, USA
[b] Department of Botany, The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA

ARTICLE INFO

*Article history:*
Received 12 October 2010
Revised 14 March 2011
Accepted 30 April 2011
Available online 14 May 2011

*Keywords:*
Ancestral character state reconstruction
Character evolution
Convergent evolution
Homoplasy
Thelotremataceae

ABSTRACT

A molecular phylogeny of combined mtSSU, nuLSU, and *RPB2* data revealed previously unrecognized levels of parallel evolution and phenotypic divergence in the lichen family Graphidaceae. Five clades were supported within the family: the *Fissurina*, *Ocellularia*, *Graphis*, *Topeliopsis*, and *Thelotrema* clades, containing 33 of the 42 currently accepted genera within the family. The results for the first time provide a fully resolved phylogeny of this family and confirm the synonymy of Graphidaceae and Thelotremataceae. Ancestral character state reconstruction using likelihood, Bayesian, and parsimony approaches indicate that lirellate ascomata evolved independently in each of the five clades. Carbonized ascomata evolved independently in at least four of the five clades. An unexpected result was the independent evolution of columella structures in the *Fissurina* and *Ocellularia* clades. Besides these more general findings, we document several cases in which evolution of several traits in parallel resulted in striking look-alikes within unrelated lineages, such as *Topeliopsis muscigena* and *Chapsa meridensis* in the *Topeliopsis* and *Thelotrema* clades, *Leptotrema wightii*, *Myriotrema laeviusculum*, and *Leucodecton phaeosporum* in the *Ocellularia* and *Thelotrema* clades, *Ocellularia stylothecia* and *Melanotrema meiosporum* in the *Fissurina* and *Ocellularia* clades, and *Myriotrema pycnoporellum*, *Myriotrema clandestinum* and *Wirthiotrema glaucopallens* in the *Fissurina*, *Ocellularia*, and *Topeliopsis* clades. Pagel's test of independent character evolution suggested that at least for some of the traits involved in these cases, ecological constraints may have caused their evolution in parallel. The most intriguing find is the correlation between gall-forming thalli and vertical columns of calcium oxalate crystals, suggesting that these crystals do not function as light distributors, as previously assumed, but instead stabilize the thalli which are usually hollow beneath, similar to a dome-shaped structure. Ancestral character state reconstruction together with an approach to visualize the phenotype of putative ancestral lineages suggested the alpha-Graphidaceae to resemble some of the extant species currently classified in *Myriotrema* s.lat., with pore-like ascomata, and non-amyloid ascospores with lens-shaped lumina.



## 1. Introduction

One of the most intriguing evolutionary phenomena is the independent evolution of similar or identical traits from a distant ancestor that did not have the traits in question. This phenomenon is variously termed 'parallel' or 'convergent' evolution, or 'homoplasy', but these terms address very specific and conceptually different evolutionary phenomena (Moore and Willmer, 1997; Zhang and Kumar, 1997; Olson and Hall, 2003; Futuyma, 2005; Stearns and Hoekstra, 2005; Barton et al., 2007). Convergent evolution denotes the independent evolution of functionally similar traits based on different structural elements in unrelated or distantly related lineages. These traits involve different structural elements and their underlying body plan shows anatomical and ontogenetic differences; such traits are called 'analogous'. A classical example for convergent evolution are the wings of bats and pterodactyls: being phylogenetically unrelated, they represent a similar solution to biological 'engineering', but use different parts of the vertebrate forelimb (Stearns and Hoekstra, 2005). The opposite of convergent would be divergent evolution, in which related lineages acquire very different traits due to radiation, but based on homologous body parts. Divergent evolution is common in organisms with an open body plan, such as plants and fungi, in which closely related lineages often exhibit phenotypic divergence (Blanco et al., 2004a,b; Tehler and Irestedt, 2007; Mugambi and Huhndorf, 2009; Lumbsch et al., 2010; Parnmen et al., 2010). A prominent

* Corresponding author at: Department of Botany, The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA.
*E-mail address:* erivasplata@fieldmuseum.org (E. Rivas Plata).

1055-7903/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2011.04.025

example of convergent and divergent evolution in lichenized fungi are the hyphophore anamorphs of the family Gomphillaceae (Lücking et al., 2008). These exhibit remarkably divergent evolution in different lineages whereas some forms convergently evolved to resemble the campylidia anamorphs of the Pilocarpaceae (Lücking et al., 2008).

Parallel evolution occurs when related lineages evolve similar or identical traits independently but based on the same ancestral trait or body parts. A spectacular example is the evolution of similar life forms in the two branches of mammals, placentals and marsupials, such as the extinct European sabre-tooth tiger (*Smilodon*) and the South American marsupial sabre-tooth (*Thylacosmilus*). Other well-known examples are the neotropical poison dart frogs (Dendrobatidae) versus the Malagasy poison frogs (Mantellidae) (Schaefer et al., 2002). In general, the underlying structures that evolved in parallel are homologous, but their evolutionary change occurs independently in each lineage. In fungi, an interesting case of parallel evolution is found in termite-egg mimicry in unrelated lineages (Matsuura and Yashiro, 2010). Parallel evolution often occurs repeatedly within a lineage, then referred to as evolutionary trend. Disentangling phenomena such as divergent, convergent and parallel evolution is crucial for our understanding of evolutionary processes.

Fungi, including lichens, are a prime example of how the lack of understanding of divergent, convergent, and parallel evolution caused instable classification schemes over time. Traditional characters used to define major lineages within Ascomycota and Basidiomycota, such as fruiting body type and development, have been shown to have evolved multiple times even among closely related lineages (Berbee and Taylor, 1992; Gargas and Taylor, 1995; Berbee, 1996; Hibbett et al., 2007; Larsson and Jeppson, 2008; Mugambi and Huhndorf, 2009). Striking examples among lichenized fungi are the order Ostropales in the Ascomycota and the family Hygrophoraceae (Agaricales) in the Basidiomycota, which now include lineages that previously had been placed in different classes due to their different fruiting body types: Porinaceae with perithecia in the otherwise apothecioid Ostropales and *Dictyonema* with resupinate basidiocarps in the otherwise mushroom-forming Hygrophoraceae (Grube et al., 2004; Lawrey et al., 2009; Schmitt et al., 2009; Baloch et al., 2010).

While divergent and parallel evolution of fruiting body types in fungi has been repeatedly documented in molecular studies (Lutzoni et al., 2004; Hibbett et al., 2007; Schoch et al., 2009), emerging phylogenies of selected clades reveal that divergent and parallel evolution occurred at unexpected levels. Phylogenetic studies in the lichen family Graphidaceae, the largest family of tropical lichens with possibly close to 2000 species, suggested that monophyletic genera shared thallus and fruiting body morphotypes (Staiger, 2002; Frisch et al., 2006), contrary to previous classifications which defined genera based on singular taxonomic characters such as ascospores (Staiger, 2002; Frisch et al., 2006; Lücking, 2009; Rivas Plata et al., 2010). However, a larger taxon sampling showed that these revised generic concepts can be equally misleading and that identical morphotypes representing an entire suite of phenotypic traits evolved several times independently within the family (Rivas Plata et al., 2011).

In the present paper, we document and analyze specific cases of parallel evolution and evolutionary divergence of fruiting body and thallus types in the lichen family Graphidaceae. We used a molecular phylogeny based on sequences of ribosomal nuclear and mitochondrial genes and the protein-coding *RPB2* gene to reconstruct the phylogeny and better understand the evolution of particular phenotypes in this group of fungi. To that end, we also employed ancestral character state reconstruction and tested evolutionary models of independent character evolution.

## 2. Material and methods

### 2.1. Taxon selection and DNA extraction

Ingroup taxa were selected based on variation of phenotype characters, including thallus, ascoma morphology, anatomy, and secondary chemistry. The molecular dataset included 33 of the 42 currently recognized genera. The data matrix from which the phenotype characters were extracted contained a total of 130 characters in multistate coding and 215 characters in binary coding. For our analyses, we used a subset of 128 binary-coded characters (Appendix A). As outgroup taxa we used representatives of three lineages shown to be close to Graphidaceae (Porinaceae, Coenogoniaceae, Gyalectaceae), plus *Ramonia valenzueliana* (Baloch et al., 2010).

We assembled a molecular dataset of three genes for this study: mitochondrial small subunit ribosomal DNA (mtSSU), nuclear large subunit ribosomal DNA (nuLSU), and the second largest subunit of the nuclear RNA polymerase II (*RPB2*). Relevant sequences were downloaded from GenBank (Table 1). New sequences were generated for this study using the Sigma REDExtract-N-Amp Plant PCR Kit (St. Louis, Missouri, SA) for DNA extraction following the manufacturer's instructions, except that we extracted from small samples of fruiting individual bodies and therefore only 40 μl of extraction buffer and 40 μl dilution buffer were used. DNA dilutions (5×) were used in PCR reactions of the genes coding for the nuLSU, mtSSU and *RPB2*, respectively. Primers for amplification were: (a) for nuLSU: AL2R (Mangold et al., 2008), and nu-LSU-1125-3′ (=LR6) (Vilgalys and Hester, 1990), (b) for mtSSU: mr-SSU1 (Zoller et al., 1999) and Mr-SSU3R (Zhou and Stanosz, 2001), and (c) for *RPB2*: fRPB2-7cF and fRPB2-11aR (Liu et al., 1999). PCR reactions contained 5.0 μl R4775 Sigma REDExtract-N-Amp™ PCR ReadyMix, 0.5 μl of each primer (10 μM), 2 μl genomic DNA extract and 2 μl distilled water for a total of 10 μl. Thermal cycling parameters were: (1) for nuLSU: initial denaturation for 5 min at 94 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 58 °C, 1 min at 72 °C, and a final elongation for 10 min at 72 °C; (2) for mtSSU: initial denaturation for 5 min at 95 °C, followed by 35 cycles of 45 s at 94 °C, 1 min at 50 °C, 1 min 30 s at 72 °C, and a final elongation for 10 min at 72 °C; and (3) for *RPB2*: initial denaturation for 3 min at 95 °C, then 1 min at 95 °C, and 37 cycles of 1 min at 57 °C, 1 min at 58 °C, 1 min at 59 °C, 1 min at 60 °C, 1 min at 61 °C, 1 min at 62 °C, 1 min at 63 °C, 1 min at 64 °C and 1.5 min at 72 °C, and a final elongation for 10 min at 72 °C. Samples were visualized on a 1% ethidium bromide-stained agarose gel under UV light and bands were gel extracted, heated at 70 °C for 5 min, cooled to 45 °C for 10 min, treated with 1 μl GELase (Epicentre Biotechnologies, Madison, WI, USA) and incubated at 45 °C for at least 24 h. The 10 μl cycle sequencing reactions consisted of 1–1.5 μl of Big Dye version 3.1 (Applied Biosystems, Foster City, California, USA), 2.5–3 μl of Big Dye buffer, 6 μM primer, 0.75–2 μl GELased PCR product and water. Samples were sequenced with PCR primers. The cycle sequencing conditions were as follows: 96 °C for 1 min, followed by 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. Samples were precipitated and sequenced using Applied Biosystems 3730 DNA Analyzer (Foster City, California, USA), sequences were assembled in SeqMan 4.03 (DNASTAR) and submitted to GenBank (Table 1).

### 2.2. Alignment and phylogenetic analyses

Sequences were arranged into multiple alignments for each gene using BioEdit 7.09 (Hall, 1999) and automatically prealigned with CLUSTAL W2 (Larkin et al., 2007) to sort out problematic