

Lichen Guilds Share Related Cyanobacterial Symbionts

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Lichen symbioses represent a major way of life among the Fungi. Almost one-fifth of all known fungal species are lichenized, and about 1500 species of lichens contain cyanobacterial photobionts, most of which belong to the genus *Nostoc*. Despite extensive studies, many basic aspects of their biology remain poorly known, especially cyanobiont diversity and specificity (1).

Cyanolichen specimens were collected from northern Europe, western North America, and central China. Epiphytic lichen communities in two old-growth forests in central Finland were studied in detail (tables S1 and S2). Lichens were identified, and free-living cyanobacteria were cultured from substrate samples. The small subunit (16S) of the ribosomal gene repeat and the tRNA^{Leu} (UAA) intron were sequenced from cyanobacterial DNA. The 16S rDNA sequences were used to resolve phylogenetic relationships, and the tRNA^{Leu} intron sequences were used for identifying *Nostoc* strains (2).

Cyanobacterial 16S rDNA sequences were obtained from 16 species of cyanolichens. Additional sequences were acquired from cyanobacterial cultures and GenBank (table S1). A phylogenetic analysis of these sequences resulted in a consensus tree in which all *Nostoc* formed a monophyletic group with 100% support (Fig. 1). The *Nostoc* clade was divided into two subgroups; the first only included cyanobionts of epiphytic lichen species (*Nephroma* guild). Further subdivisions within this group did not correlate with the geographical origin or generic identity of the lichen specimens. The second subgroup (*Peltigera* guild) included cyanobionts of terrestrial lichens, free-living *Nostoc* strains, and a symbiotic *Nostoc* from the roots of a cycad (Fig. 1).

Most epiphytic cyanolichens in central Finland contained similar tRNA^{Leu} intron genotypes (table S2). One intron genotype was found from all six sample plots, and it was shared extensively by four different species of *Nephroma* and *Parmeliella*. Two other intron genotypes differed from the most frequent intron genotype by only one base change each; these genotypes were found from several epiphytic lichens on different sample plots. These and a fourth Finnish intron genotype shared the same repeat motif in their P6b element (3). Four North American and two Chinese intron genotypes also had this repeat motif. As in Finland, all these sequences were obtained from epiphytic cyanolichens (table S2).

A different set of tRNA^{Leu} intron genotypes was obtained from *Peltigera* thalli and cultured *Nostoc* strains (table S2). These sequences had a different repeat motif in the P6b element and could not be readily aligned with the sequences from epiphytic cyanolichens (3). Species of *Peltigera* are predominately terricolous, and when growing epiphytically, they are usually confined to mossy basal trunks. Two intron

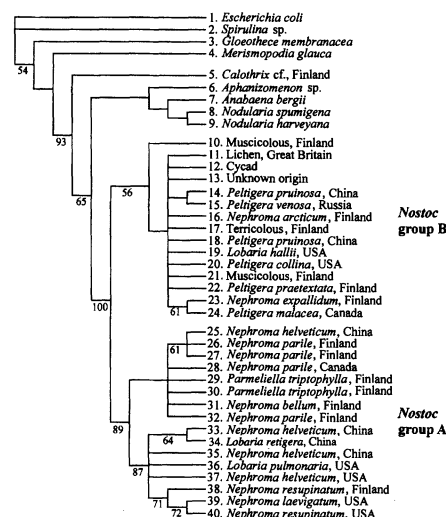


Fig. 1. A strict consensus tree showing phylogenetic relationships among symbiotic and free-living cyanobacteria based on 16S rDNA sequence data (table S1). Bootstrap support >50% is shown at nodes. All *Nostoc* strains form a monophyletic group. The cyanobionts of the *Nephroma* guild form a well-supported subgroup within the *Nostoc* clade. The cyanobionts of the *Peltigera* guild group together with free-living *Nostoc* strains. These groupings are congruent with data from tRNA^{Leu} (UAA) intron sequences (table S2).

genotypes were obtained by culturing thallus fragments of *Lobaria pulmonaria*. Two North American and two Chinese intron genotypes had the same P6b repeat motif as Finnish *Peltigera* cyanobionts. As in Finland, these sequences were amplified from different species of *Peltigera* and *Lobaria* (table S2).

Although lichen mycobionts are selective in their choice of *Nostoc* symbionts, several fungi may often share identical cyanobiont strains (1, 3, 4). Our results demonstrate the full extent of this phenomenon and indicate that cyanolichens express their *Nostoc* specificity on a community scale. Many cyanolichens associated with old-growth forests depend on a specific group of *Nostoc* strains that have not been found in

other types of cyanolichens. These epiphytes exploit a common pool of cyanobacteria and form a horizontally linked system, the *Nephroma* guild. Conversely, many terrestrial cyanolichens share a different group of related *Nostoc* strains, thus forming the *Peltigera* guild. The guild membership of Finnish *Lobaria pulmonaria* specimens remained unclear because of inherent uncertainties in identifying symbiotic *Nostoc* strains by using cultures. North American *L. pulmonaria* specimens belonged to the *Nephroma* guild (Fig. 1).

The dispersal ecology of cyanolichen guilds may center around "core species," such as *N. parile* and *P. triptophylla*, that produce massive amounts of symbiotic diaspores. "Fringe species," such as *N. bellum* and *N. resupinatum*, produce only fungal spores and may largely depend on the core species for the dispersal of appropriate cyanobionts. Only a small proportion of symbiotic propagules can develop into mature lichen thalli. Many diaspores land on suboptimal substrates, eventually disintegrate, and release their cyanobionts. These cyanobionts may be salvaged by the mycobionts of fringe species. Core species may also benefit from this activity, as their cyanobionts are deposited into other guild members rather than being completely lost. Some of the cyanobionts can potentially be reclaimed because, without the ability to produce symbiotic diaspores, fringe species cannot "grab the cyanobionts and run." These phenomena may help to explain why the existence of competition is often difficult to demonstrate in lichen communities (5).

In addition to the two cyanolichen guilds discussed here, we have preliminarily identified several other cyanolichen guilds. Similar systems may also operate among green algal lichens and other symbiotic systems, such as corals.

References and Notes

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2. Supporting material is available on Science Online.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/297/5580/357/DC1
Materials and Methods
Tables S1 and S2

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