BIODIVERSITY OF FUNGI

Inventory and Monitoring Methods

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LICHENIZED FUNGI

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Lichens are composite organisms consisting of a fungal partner (the mycobiont) and a photosynthetic partner (the photobiont) that grow symbiotically with one another to form a coherent structure (the thallus). Through their participation in the lichen symbiosis, lichenized fungi pursue a lifestyle different from that of most other fungi but similar to that of plants, in that they operate as autotrophic, photosynthetic units. These units are more physically compact, spatially circumscribed, and distinguishable from the substratum than are units of other fungi. As a consequence, lichenized fungi have been subjects of far more studies involving quantitative sampling than have other fungi.

In this chapter we briefly review the ecology of lichenized fungi, summarize current knowledge of their taxonomy and patterns of diversity, and discuss important variables and criteria for designing inventories of their biodiversity. Nonquantitative biotic surveys can make important contributions to our knowledge of biodiversity, especially in poorly collected areas. This chapter will focus, however, on organized biodiversity surveys with at least minimum levels of quantification and repeatability. Information on the biodiversity of lichenized fungi can also contribute to the development of management strategies for their conservation in nature reserves and to efforts to retard loss of biodiversity and ecosystem function on lands managed for human use and resource consumption (Hawksworth 1991; Galloway 1992a; Rose 1992; Rosentreter 1995).

LICHEN CHARACTERISTICS

The relationship between the fungal partner and the photosynthetic partner of lichens has been variously described

(Nash 1996). It sometimes is considered a mutualism in which both partners benefit; the fungus gains carbohydrates, and the photosynthetic partner is protected from desiccation. Alternatively, the association is considered to be a controlled parasitism in which the fungal partner benefits and the photobiont loses by growing more slowly than when alone. From an ecological perspective, the relationship is usually mutualistic because the integrated lichen thallus survives in more habitats and is more abundant than is either partner when alone.

An individual thallus usually is composed of one species of fungus and one species of either alga or cyanobacterium. Many thousands of fungal species, but only about 150–200 species of photobiont, have been identified as participants in lichen symbioses. Only the fungal partner reproduces sexually while in the association.

In contrast to most other fungi, the thallus of a lichen usually has a definable boundary; a distinctly layered structure; and a characteristic, often species-specific morphology. Most lichen thalli fall into one of five general morphological categories (Fig. 9.1): leprose, composed of mealy particles of intertwined fungal hyphae and algal cells; crustose, embedded on or in the surface of the substratum; squamulose, composed of small flakes of thallus; foliose, leaflike, with distinct upper and lower surfaces; and fruticose, shrubby or stringlike (Fig. 9.2). Although the fungal partner produces the characteristic structure of the lichen thallus, the morphological form of the composite thallus is a result of the interaction; isolated mycobionts on agar plates form colonies similar to nonlichenized fungi (and without typical "lichen" structures), although they grow more slowly than most fungi and are very compact. Scientific names given to the lichen thallus are based on and refer to the fungal partner (see "Identification," under "Taxonomy, Diversity, and Distribution," later in this chapter).

A number of functional characteristics are common to most lichens. These include autotrophic nutrition via photosynthesis, (mostly) slow growth, small size, long life, long-lasting (nonseasonal) vegetative morphology and reproductive structures, mineral nutrition mostly from airborne sources, and greater tolerance of desiccation than most other photosynthetic organisms in the same habitat.

Clearly, the partners have independent phylogenies, with the integration represented by the lichen thallus best described as a product of coevolution. In addition, the fungus and/or the photobiont cells of a single lichen thallus are not necessarily products of a single gene line, even when they are from a single species (see "Basis for Taxonomic Distinctions," under "Taxonomy," later in this chapter). The concepts of "organism" and "individual" for the lichen thallus thus do not necessarily have the same implications of genetic coherence as they do when applied to humans or to vascular plants (Allen and Hoekstra 1992).

The lichen habit is remarkably widespread among fungi (>13,500 species, or about 20% of all described fungal species; Sipman and Aptroot 2001; Hawksworth et al. 1995). Lichenized fungi belong to several distantly related orders and families and are not a cohesive taxonomic group (see "Taxonomy," later). Rather, they represent a biological strategy that has evolved on separate occasions in different groups of fungi. Their cohesiveness as a group thus relates to their similar ecological and functional roles and the common constraints of the lichen habit. For more information on lichen biology, see Brodo and colleagues (2001), Galun (1988), Hale (1983), Hawksworth and Hill (1984), Lawrey (1984), and Nash (1996).

The roles of lichenized fungi in communities and ecosystems are best understood by studying the lichen thallus as a photosynthetic unit that in many ways is equivalent to plants. For this reason, methods for assessing the diversity of lichenized fungi can be more similar to those for plants than for other fungi. To study the biogeographic and taxonomic relationships of lichenized fungi, however, one needs to investigate the phylogenetic relationships of the fungi and, to a lesser extent, of their photobiont partners.

LICHEN ECOLOGY

Lichenized fungi are found in every terrestrial habitat capable of supporting photosynthesis, and a few lichens occur in aquatic habitats as well. The photosynthesizing lichen units compete with plants for light, space, and possibly other resources. Because of their small stature and extremely slow growth, lichens are poor competitors, perhaps best described when compared to vascular plants as extreme examples of Grime's (1977) stress-tolerator lifestyle. The only plants with which lichens sometimes appear to compete on an equal footing are bryophytes, with which they often are compared in community studies (Nash and Egan 1988). Major factors affecting the presence and abundance of lichenized fungi are the following: (1) substratum chemistry, stability, and longevity; (2) light availability, with effects often mediated through competition with faster growing, larger plants and other lichens; and (3) moisture availability. Common substrata that support lichen growth include rock surfaces, woody plant bark and wood, soil and dead organic matter in low productivity environments and microhabitats, and broad evergreen leaves in the humid tropics. Most species have at least some substratum and habitat preferences, although individual species vary widely in substratum and habitat specificity.

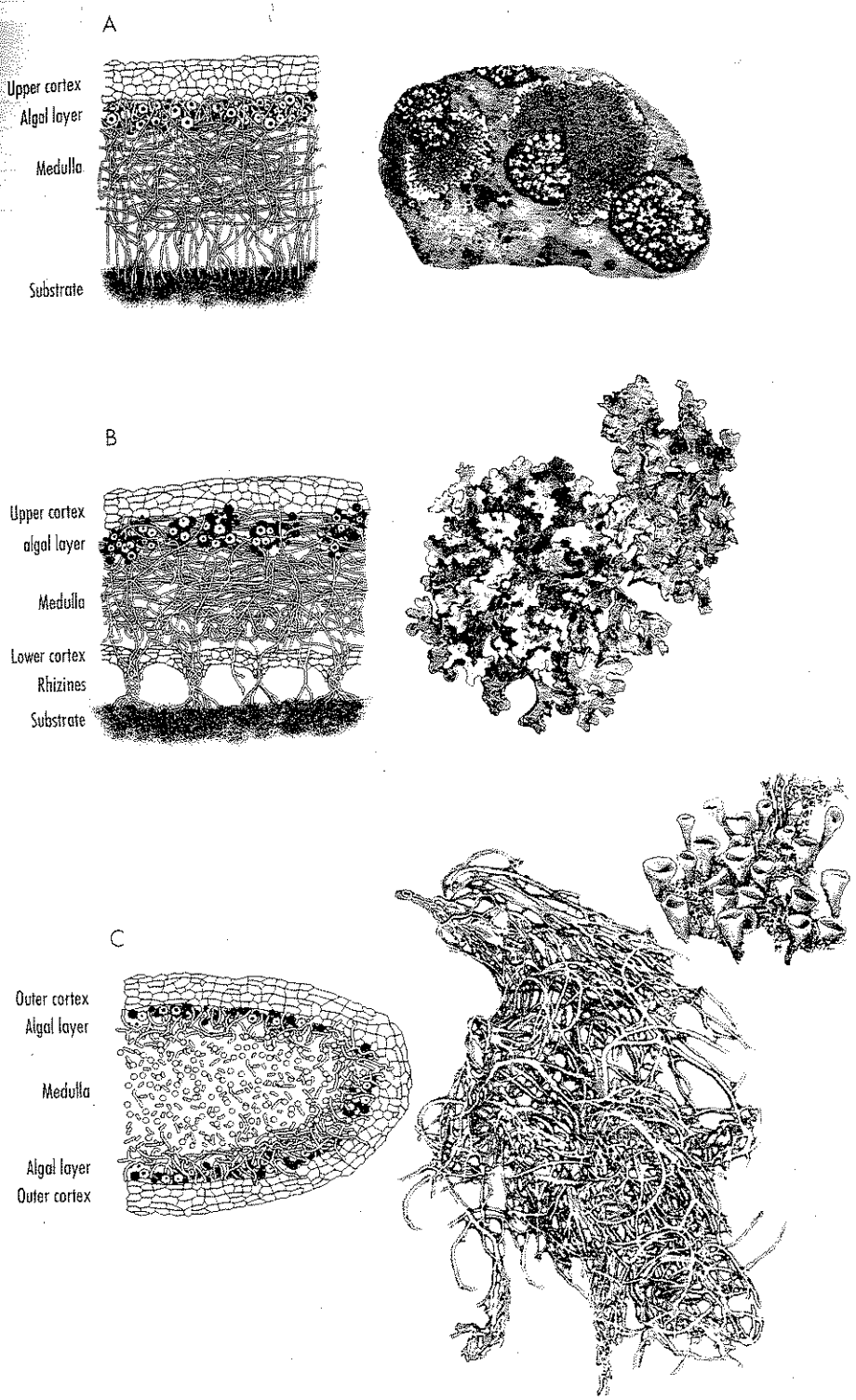

FIGURE 9.1 Three of five common growth forms of lichens: surface views and cross-sections. **A.** Crustose lichens usually have several layers, with the medullary fungal hyphae attached directly to the substratum. **B.** Foliose lichens have distinct upper and lower cortex layers and usually are attached to the substratum by special groups of rootlike fungal hyphae called rhizines. **C.** Fruticose lichens are stalked, tufted, or pendulous and usually have a uniform outer layer. (Redrawn with permission from fig. 2 of Ahmadjian 1993 by Kandis Elliot.)

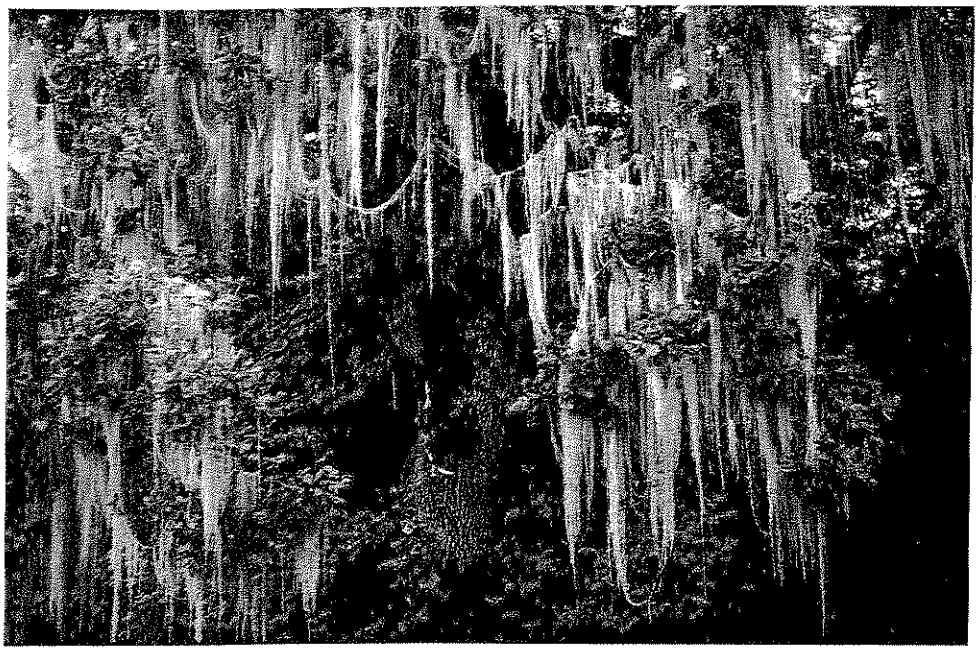

FIGURE 9.2 Hanging fruticose lichens such as *Ramalina menziesii*, growing on a *Quercus garryana* in the Willamette Valley of Oregon, United States, are often most abundant in habitats with much moisture available. Many lichen species with this growth form are quite sensitive to air pollution. (Photo by Bruce McCune)

In general, lichens interact minimally with their substrata, although some species penetrate the bark surface or cause changes in rock surface chemistry. Some bark lichens may obtain at least some carbohydrates from the surface of the host tree, but additional studies of this phenomenon are needed (Hawksworth 1988a). Lichens with thalli above or outside their substrata usually harbor minicommunities of invertebrates and serve as food for some.

Terrestrial and arboreal lichens are important regular winter foods for large mammal herbivores, including reindeer, caribou, and deer, in boreal forest and arctic tundra (Stevenson and Rochelle 1984; Seaward 1988) and for some small mammals (Maser et al. 1986). Lichens are an important emergency food for many other large mammalian herbivores during particularly hard winters (Fox and Smith 1988; Thomas and Rosentreter 1992). They also are used regularly as nest material by birds and mammals in some habitats (Seaward 1988; Hayward and Rosentreter 1994).

Industrial melanism in peppered moths in England (Kettlewell 1973; Majerus 1989; Cook et al. 1990) is a famous example of indirect impacts of lichens on animals. Moth populations whose color patterns mimicked patterns of lichens on trees ("cryptic coloration") were affected first by loss of lichens because of air pollution, and second by the lichens' subsequent return with improving air quality. Relationships of lichens on trees or rocks to other instances of cryptic coloration in insects, reptiles, and amphibians are largely unstudied, and possible indirect effects of loss of lichens on those animals are unknown.

In some desert and tundra ecosystems, lichens constitute a significant proportion of the biomass of autotrophs (Kappen 1988). In some low-nutrient habitats, such as conifer forests of the Pacific Northwest of the United States, lichens may fix a significant proportion of nitrogen for the system (Nash 1996). In temperate and boreal forests, they may alter the availability of nutrients and buffer (by sequestration) heavy metals entering the system from atmospheric deposition via canopy throughfall and stemflow (Seaward 1988). Summaries of the ecological roles of lichens can be found in most books on lichen biology; more extensive literature reviews can be found in Armstrong (1988), Galun (1988), Gilbert (2000), Nash (1996), and Slack (1988).

TAXONOMY, DIVERSITY, AND DISTRIBUTION

TAXONOMY

Lichenization appears to have evolved independently several times during the history of fungi (Gargas et al. 1995; Nash 1996). A few Basidiomycota form lichens, particularly some genera in Agaricales (*Omphalina*),

Cantharellales (*Multiclavula*), and Stereales (*Dictyonema*), but mostly fungi that form lichen associations[16] belong to the Ascomycota. Of the 46 orders of that phylum (Kirk et al. 2001), 14 include lichen-forming representatives, and five of those comprise lichenized species exclusively. Molecular studies have shown that many species in the former order Caliciales actually fall in the Lecanorales (Wedin and Tibell 1997; Wedin et al. 1999). Some lichenologists place the Peltigerales, Pertusariales, and Teloschistales within the Lecanorales; we list them separately. No consensus exists yet on assignment of 15 additional lichen-forming families to an order, although Harris (1995) suggested placing the Aspidotheliaceae (as part of Thelenellaceae) and Strigulaceae in the order Melanotommatales. Most of the unassigned families are exclusively lichenized, generally with green algae. In Table 9.1, we list the 14 orders and their families that include lichen-forming fungi, the unassigned families, and estimates of the number of lichenized species in each.

Proposed changes in classification of Ascomycota were summarized twice per year in *Systema Ascomycetum* (1982–1998), and a revised "Outline of the Ascomycetes" now appears regularly on Myconet (see Appendix III). The latest revision is by Eriksson and colleagues (2002).

Many lichenized Ascomycota also form conidial structures. In contrast to most other fungi, however, the conidial forms (anamorphs) of lichenized species are not given independent scientific names. In some cases conidia act as spermatia; in others they act as dispersal propagules. Some lichen-forming genera are either completely sterile or mitosporic; those forms are given holomorphic names (Vobis and Hawksworth 1981; Hawksworth and Poelt 1986). Most are probably states of Ascomycota, but in some the sexual phase now may have been completely lost (e.g., *Blarneya, Cheiromycina, Flakea, Lepraria, Siphula, Thamnolia*).

Basis for Taxonomic Distinctions

Currently orders, families, and usually genera of lichen-forming fungi are delimited primarily by characters of the fruiting bodies (Henssen and Jahns 1974; Hafellner 1984, 1988). As a result, lichens whose thalli are quite

TABLE 9.1
Orders of Fungi in the Phylum Ascomycota and Their Families That Include Lichen-forming Species

AGYRIALES. 3 families: Agyriaceae, Anamylosporaceae, Elixiaceae; 100 species; mostly lichenized but some saprobic on wood.
ARTHONIALES (including OPEGRAPHALES). 4 families: Arthoniaceae, Chrysothricaceae, Melaspileaceae, Roccellaceae (= Opegraphaceae); 1000 species* (of 1200); mostly lichenized with the green algae *Trentepohlia* species, but some lichenicolous or saprobic.
DOTHIDEALES *sensu lato*.† 3 families* (of 58): Arthopyreniaceae, Microtheliopsidaceae, Pyrenothricaceae, 120 species (of 4770); the biological status of many members of Arthopyreniaceae is unclear; the order also includes some families primarily with lichenicolous species (e.g., Dacampiaceae).
GYALECTALES. 1 family: Gyalectaceae; 100 species; all lichen-forming, especially with the green algae, *Trentepohlia* species.
LECANORALES. 34 families (of 40): the larger families include Acarosporaceae, Bacidiaceae, Catillariaceae, Cladoniaceae, Collemataceae, Lecanoraceae, Lecideaceae, Pannariaceae, Parmeliaceae, Physciaceae, Ramalinaceae, and Stereocaulaceae. Most former CALICIALES belong here, but family assignments are uncertain; 7150 species (of 7250); the majority of lichen-forming fungi belong here; those that are lichenicolous or saprobic probably evolved from lichenized species; mainly forming lichens with green algae, especially Chlorococcales and Pleurastrales (e.g., *Trebouxia* species).
LICHINALES. 3 families: Gloeoheppiaceae, Lichinaceae, Peltulaceae; 280 species all lichen-forming with cyanobacteria.
OSTROPALES (including GRAPHIDALES). 6 families (of 7): Asterothyriaceae, Graphidaceae, Heppiaceae, Solorinellaceae, Stictidaceae Thelotrematoceae; 1600 species (of 1800); mainly lichen-forming with *Trentepohlia* species; some lichenicolous species in Odontotremaceae in this order.
PATELLARIALES. 1 family (of 2): Arthrorhaphidaceae; 5 species (of 50); lichen-forming with green algae, or lichenicolous.
PELTIGERALES.‡ 4 families: Lobariaceae, Nephromataceae, Peltigeraceae, Placynthiaceae; 510 species; all lichenized with either green algae or cyanobacteria, sometimes with more than one photobiont in the same thallus, or forming morphologically different thalli according to the photobiont present.
PERTUSARIALES.‡ 1 family: Pertusariaceae; 300 species; all lichenized with green algae.
PYRENULALES. 4 families (of 5): Megasporaceae, Monoblastiaceae, Pyrenulaceae, Trypetheliaceae; 500 species; mainly lichenized with the green algae *Trentepohlia* species.
TELOSCHISTALES.‡ 3 families: Fuscideaceae, Letrouitiaceae, Teloschistaceae; 570 species; almost all species lichenized with green algae, commonly *Trebouxia* species, but some lichenicolous species in a few genera.
TRICHOTHELIALES. 1 family: Trichotheliaceae; 240 species; all lichenized with *Phycopeltis* species or other trentepohlioid green algae.
VERRUCARIALES. 1 family (of 2): Verrucariaceae (= Dermatocarpaceae), 700 species; mainly lichenized with green algae; some genera with all or a few species lichenicolous; the other family of the order, Adelococcaceae, exclusively lichenicolous.
FAMILIES OF UNCERTAIN POSITION. 15 families: Aphanopsidaceae, Aspidotheliaceae, Baeomycetaceae, Epigloeaceae, Gomphillaceae, Icemadophillaceae, Mastodiaceae, Pachyascaceae, Phlyctidaceae, Protothelenellaceae, Strigulaceae, Thelenellaceae, Thelocarpaceae, Thrombiaceae, and Umbilicariaceae.

* Estimated number of lichenized species or families in the order.
† Now used in a restricted sense; the three families of uncertain ordinal placement.
‡ Often included in LECANORALES by recent authors.

different morphologically often are united in the same genus, family, or order. For example, the family Teloschistaceae includes lichens with thalli that are endolithic (completely embedded within a rock surface), leprose (granular), crustose, placodioid (crustose with a lobed margin), squamulose, foliose, and fruticose. Recent taxonomic revisions based on fungal reproductive structures have led to major changes in generic delimitations (e.g., in *Lecidea, sensu lato*; Purvis et al. 1992).

Genera (e.g., *Arthonia, Arthrorhaphis, Buellia, Caloplaca, Catillaria, Mycomicrothelia, Rhizocarpon, Toninia*), as well as some orders and families, can include both lichenized and nonlichenized fungal species. The nonlichenized species are most commonly lichenicolous or more rarely saprobic on wood. In some cases the biology of a single species may vary during its life cycle; for example, several species of *Diploschistes* and *Rhizocarpon* are initially lichenicolous, parasitizing another lichen and then taking over its photobiont to form an independent lichen thallus.

Molecular approaches bring significant modifications to classical morphological and chemical species concepts in lichenized fungi (Grube and Kroken 2000). Secondary chemistry is used extensively for identification (see "Identification," later in this chapter); morphologically identical species differing only in their secondary chemistry have been recognized as separate species by some workers, generating considerable debate (Lumbsch 1998). Studies of morphological, chemical, and genetic variation within a species used to be uncommon (Culberson et al. 1988; DePriest 1993, 1994) but are moving ahead rapidly with the advent of molecular approaches. Morphologically defined species may include two or more cryptic genetic species (Kroken and Taylor 2001; Crespo et al. 2002). Lichens that are identical in most features (including secondary chemistry) but differ in that one reproduces by ascospores and the other by vegetative means (usually isidia or soredia) have been considered "primary" (sexually reproducing) and "secondary" (vegetatively reproducing) species pairs (Mattsson and Lumbsch 1989). However, molecular data suggest that some "species pairs" may be better treated as single species (Articus et al. 2002).

Occasionally different propagules, perhaps genotypically distinct, grow together to form a single lichen thallus. Although the propagules are generally of the same species, that is not always the case, and both interspecific and intergeneric "mechanical" hybrids have been postulated (Hawksworth 1994), the latter being similar to the presence of parasymbionts in lichens. Such hybrid thalli can exhibit features of both fungal partners as their hyphae intertwine in the lichen tissues and around the shared photobiont cells, and they can confound the unwary. Luckily they are uncommon.

Identification

Scientific names given to lichens refer to the fungal partner (mycobiont); the photobiont(s) has an independent scientific name. Officially, the lichen association itself has no name, and under the current International Code of Botanical Nomenclature (Greuter et al. 2000), it is not possible to "identify" or "name" a lichen, only the lichen-forming fungus (and/or the photobiont, Hawksworth 1998). In practice the name assigned to a specimen is the name of the lichen-forming fungus. A consequence of this restriction is that when more than one species of photobiont occurs with the same fungus (either in the same or in separate thalli), the same fungal name is used for all resulting lichen thalli even if they have different morphologies.

Keys to families are included in the *Dictionary of the Fungi* (Kirk et al. 2001). No world key to lichenized genera has appeared since that of Clements and Shear (1931), but several major regional treatments include keys to genera—for example, those of Awasthi (1988, 1991), Brodo and colleagues (2001), Clauzade and Roux (1985), Foucard (1990), Nash and colleagues (2002), Poelt (1969), Poelt and Vězda (1977, 1981), Purvis and colleagues (1992), Swinscow and Krog (1988), Thomson (1984, 1997), and Wirth (1995a, b).

Catalogues of names of lichen-forming fungi are provided by Zahlbruckner (1921–1940); Lamb (1963); Hawksworth (1972); since 1970, in the twice yearly *Index of Fungi* (begun 1940), and the free Index Fungoram web database (see Append III) with some 340,000 species names of fungi, including lichens. The *Bibliography of Systematic Mycology* (also twice yearly, since 1946) indexes all fungal systematic literature, including that on lichens, down to the rank of genus, and data since 1980 are available as a searchable CD-ROM. The "Recent Literature on Lichens" series begun by W. L. Culberson in 1951 and currently compiled by T. L. Esslinger in the journal *The Bryologist* (including a searchable reference database on the web page of University of Oslo, Lichen Herbarium) notes new and principal species treated in each listed paper.

Monographs and key regional or national treatments, which often include keys to species, are listed under family and generic entries in the *Dictionary of the Fungi* (Hawksworth et al. 1995), and major modern national compilations are listed by country. Another recent bibliographic guide organized by geography (Hawksworth and Ahti 1990) includes about 1390 literature citations. Many Internet sources for keys and information about lichens are now available, including, for example, the LIAS web site and H. J. M. Sipman's *Lichen Determination Keys* web site (Appendix III). In addition, the web pages (see Appendix III) of the American Bryological

and Lichenological Society and the British Lichen Society include numerous links to Internet resources for lichens.

Lichen chemistry is a valuable tool for identifying and separating species, particularly for sterile material. Spot tests or color reactions can be used on the thallus surface, on internal tissues exposed with a razor or scalpel blade, or on compound microscope sections of fruiting bodies or thallus tissue. The chemicals most commonly used are known to lichenologists as K (10% solution of potassium hydroxide in water); C (undiluted commercial bleach); P or PD (saturated alcohol [95% ethanol] solution of p-phenylenediamine, or Steiner's Stable Solution); I (mainly Lugol's Iodine; sometimes Melzer's Iodine is used); N (10–50% solution of nitric acid), and the staining compound LCB (lactophenol–cotton blue). Most lichen identification guides (e.g., Hale 1979; Purvis et al. 1992; Malcolm and Galloway 1997; Brodo et al. 2001) include summaries of how to make and use these chemicals. Wright (1996) has provided hints on interpreting test results.

The identification of secondary metabolites in lichen thalli, including those responsible for color changes with spot tests, generally is performed by thin-layer chromatography using standardized solvent and spray systems, and such equipment routinely is present in lichenology laboratories (Arup et al. 1993; Orange et al. 2001). Culberson and others have compiled the available data on the chemistry of each species (Culberson 1969, 1970; Culberson et al. 1977); Hunech and Yoshimura (1996) have compiled structural information. Computer programs to aid in the use of chemical contents for species identification are available also (Mietzsch et al. 1993).

Discussion of identification of photobionts in lichens is beyond the scope of this chapter. Identification to species often requires isolation of the organism into unialgal cultures. For information on identification procedures, see Ahmadjian (1993) and Tschermak-Woess (1988).

Major Reference Collections

Lichens have been collected extensively for hundreds of years. Most major botanical collections (herbaria) have old lichen material and also serve as depositories for voucher specimens from fresh inventories. Air-dried samples of lichens can be maintained as reference collections for extended periods when they are kept in a stable, dry atmosphere and thus provide a rich source of background material for inventory studies. They also add a historical dimension to a survey and provide comparative material for identifications. In addition, metabolites in appropriately maintained material (some dating as far back as the 1690s) still can be detected with thin-layer chromatography for identification of specimens.

The largest dried-specimen collections (Appendix III) are those of the University of Uppsala, Sweden (UPS), with about 500,000 lichen specimens and the Natural History Museum in London, United Kingdom, with about 400,000 specimens. The Smithsonian Institution in Washington, DC, with about 250,000 specimens is the largest collection in the United States of America. Hall and Minter (1994) and Holmgren and colleagues (1990) listed institutions maintaining dried collections of lichens, and lichen-related Internet sites often have lists of and links to such herbaria (e.g., New York Botanical Garden; Appendix III).

Living cultures of lichen-forming fungi (without photobionts) are difficult to maintain for extended periods, mainly because of their slow growth and susceptibility to contamination. Therefore, few of these fungi are available from the main collections of microbial cultures. The American Type Culture Collection (Appendix III) has the largest number publicly available. About one-third of those lichenized fungal species tested have been cultured successfully on the first attempt (Crittenden et al. 1995). Growth rates are very slow on solid media, so shaken liquid cultures are preferred. Sophisticated technology now allows individualized freezing and thawing protocols for preserving cultures of lichen-forming fungi in liquid nitrogen (Smith and Onions 1994).

Diversity and Distribution

The count of lichenized fungal species accepted by Hawksworth and colleagues (1995) and Sipman and Aptroot (2001) is just less than 13,500, a number unchanged from the 1930s. This somewhat surprising result indicates that the numbers of newly described lichenized fungi have been roughly balanced by recognition of synonymy. Larger figures have been claimed during this period by some workers (e.g., 17,000 species by Nash and Egan 1988 using rough estimation rather than a precise count). The complete world inventory is expected to total about 18,000 species (Sipman and Aptroot 2001). Thus, lichenized fungi are much better known than most other fungal groups, with perhaps 60–80% of the species already described, as opposed to the now generally accepted 5% for fungi overall (Hawksworth 1991; Heywood 1995).

Taxa of lichenized fungi tend to be found over larger geographic areas than do equivalent taxa of vascular plants. For example, a significant proportion of lichen genera occur on all continents, and several individual species have worldwide distributions (e.g., *Chrysothrix candelaris, Parmelia sulcata, Placopsis gelida, Thamnolia vermicularis, Xanthoria elegans*). Others are bipolar (e.g., *Pseudephebe minuscula:* Galloway and Aptroot

1995) or pantropical (e.g., *Strigula smaragdula*, Santesson 1952, as *S. elegans*). Regional distribution patterns also have been and continue to be strongly influenced by human activity, especially sulfur dioxide air pollution, habitat destruction, loss of ecological continuity, the introduction of new substrata such as buildings, and the spread of exotic lichen species.

In contrast to diversity patterns for vascular plants and many other fungi, the world's richest areas for lichen-forming fungal species currently appear not to be the humid tropics, but rather the southern temperate rain forests (Galloway 1992a, 1992b, 1995), northern temperate forests, and high-latitude zones. This may be an artifact of limited knowledge; the richest lichen biota known for a single tree is reported from an Asian tropical forest (Aptroot 1997). When whole countries, including all available habitats and biomes, are compared, the boreal zone currently appears to be the richer of the two high-latitude zones. For Sweden and Norway combined, 2271 lichen species are listed, whereas for New Zealand, a southern hemisphere area of comparable latitude, size, and climatic range, only 1378 species are known (Table 9.2). This difference may exist partly because the lichens of New Zealand are currently less completely known than those of Sweden and Norway.

Distribution patterns may differ when biodiversity is measured at the level of family or order rather than species because several exclusively lichenized families are primarily tropical (e.g., Gomphillaceae). Sipman (1995) reported a significant contribution of Ostropales (including Graphidales) and Pyrenulales to the lowland lichen mycobiota of Colombia, whereas in the high Andes more than 90% of all species belong to the Lecanorales, a percentage similar to that in the temperate zones of the world. Aptroot and Sipman (1997) reported that the diversity centers of three of the principal orders (Ostropales, Pyrenulales, Arthoniales; 3100 species combined) of lichenized fungi are in the tropics. Accurate

TABLE 9.2
Numbers of Species of Lichens Reported from Large (≥5–10 Million ha) Geographic Areas

Location	Number of species*	Citation
Africa		
East Africa	628 (macrolichens)	Swinscow and Krog 1988
Antartica and South Georgia	427	Øvstedal and Smith 2001
Asia		
China	1766 (macrolichens)	Wei 1991
India, Nepal, and Sri Lanka	1850	Awasthi 1988, 1991
Japan	1557	Kurokawa 2003
Papua New Guinea and Irian Jaya	1500+	Aptroot and Sipman 1997†; Streimann 1986
Thailand	554	Wolseley et al. 2002
Australasia		
Australia	2500+	Filson 1996
New Zealand	1378	Malcolm and Galloway 1997
Europe		
Austria	2101	Hafellner and Türk 2001
Belgium and Luxembourg	930	Diederich and Sérusiaux 2000
Germany	1835	Scholz 2000
Great Britain and Ireland	1660	Coppins 2002
Italy	2145	Nimis 1993
Netherlands	706	Aptroot et al. 1999
Spain and Portugal	2426	Llimona and Hladun 2001
Sweden and Norway	2271	Santesson 1993
North America		
British Columbia	1100	Goward et al. 1994†
California	1000	Hale and Cole 1988†
North of Mexico	3580	Esslinger and Egan 1995
American Arctic	996	Thomson 1984, 1997
Pacific Islands		
Hawaiian Islands	723	Smith 1991†
South America		
The Guyanas	800+	Aptroot and Sipman 1997†
Colombia	1000–1500	Sipman 1995†

* Species numbers represent the combined efforts of many collectors over many years, rather than the efforts of any single person or project.
† Authors provide number but do not list all species in the publication.

estimation of the diversity of lichens in most tropical areas is currently impossible because of inadequate collections and taxonomic knowledge (Galloway 1991).

Estimation of diversity by substratum also provides different patterns. For instance, foliicolous (living on leaves) lichens (Fig. 9.3) are almost exclusively tropical (Lücking 1995). Rocky substrata (Fig. 9.4), however, which support much of the diversity in the temperate and cold zones of the world, are rare in the lowland tropics. The wide variety of habitats, substrata, and climates available in temperate areas of the world probably explain why they are so rich in lichens (Galloway 1992a). In general, the number of lichenized fungi present in an area of a particular size depends heavily on the range of substrata available.

Despite the differences in broad patterns of distribution, the proportion of common to rare genera and species among lichenized fungi in a particular area appears to be generally similar to that of vascular plants. Patterns relating to the number of species per area have long been discussed in ecology, but the comparability of particular numbers is often in question (see Chapter 5). For ecologists, species recorded as present in fixed areas provide the basis for calculating frequency values (see "Designing Sampling Protocols for Biodiversity Inventory," later in this chapter), which have long been recognized as scale-dependent (Grieg-Smith 1983). Palmer and White (1994) have shown that species-area relationships at small scales (<1 km^2) depend on the grain (size of the smallest sample unit) and other aspects of field study design. We present species/area figures for lichenized fungi in Tables 9.2 and 9.3. The data come either from knowledge accumulated by many investigators over many years or from studies with many different sample designs, so we present them with minimal discussion of general relationships.

Both the regional species pool and the diversity of available microhabitats are important contributors to the diversity of lichenized fungi in a particular area. Rose (1992) compiled extensive surveys of lichenized fungi in deciduous temperate woodlands in the British Isles and found that up to 227 species can be expected per square km (100 ha) on bark and wood alone, depending on the degree of air pollution and other human influences. The epiphytic (growing on plants) lichen community in the tropics, however, can be more diverse than in temperate

FIGURE 9.3 There may be 10 to 30 species of foliicolous lichens on each leaf and, perhaps, 40 to 60 species on the whole twig of this rain forest plant (Bignoniaceae) in Costa Rica. (Photo by Robert Lücking)

areas. Lücking (1995) reported 177 foliicolous (growing on leaves) lichen species from a single forest plot in Costa Rica, a number similar to that reported for corticolous (growing on bark) species in a forest studied by Montfoort and Ek (1990) in French Guyana (Table 9.3). Together, these two studies suggest that a local forested area (<200 ha) in old-growth tropical lowland forest may have more than 400 species of epiphytic lichens, more than in a local area of old-growth temperate forest in the United Kingdom (Table 9.3).

Cumulative lichen species numbers from published checklists and regional treatments (Table 9.2) provide

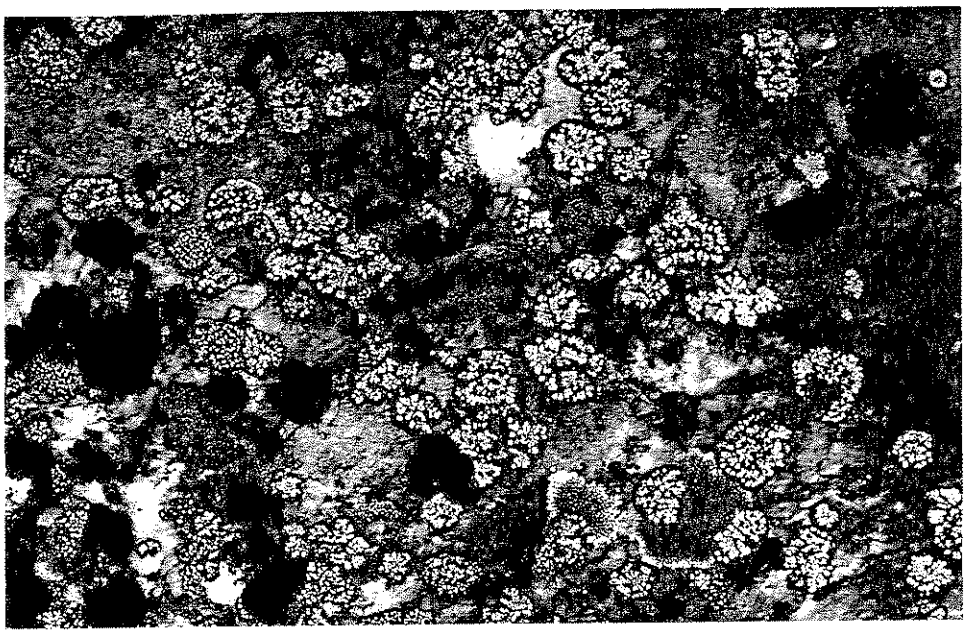

FIGURE 9.4 This section of a granite boulder in the northern Rocky Mountains of Alberta, Canada, supports 10 to 15 species of crustose saxicolous lichens. (Photo by John Wolf)

TABLE 9.3
Numbers of Species of Lichens Reported from Local and Regional Areas of Various Sizes[a]

Location	Biome	Area (ha)	Number of species	Topography	Reference
Regional geographic areas[b]					
NE Unites States (US)	Conifer-deciduous forest	6272	126	Hilly	Wetmore 1995
N Central US	Conifer-deciduous forest	2560	190	Rolling	Wetmore 1993
Western US	Alpine-tundra forest	31,200	137	Mountainous	Hale 1982
	Conifer forest-savanna	1.3 million	404	Mountainous	Wetmore 1967
	Savanna-grassland	98,550	171	Hilly	Will-Wolf 1998
SE Alaska, US	Conifer forest	6.8 million	381	Mountainous	Geiser et al. 1994a
Venezuela	Tropical forest, scrub	40,000	216[c]	Mountainous	Sipman 1992
Greek Islands	Mediterranean scrub, rocks	20,000	295	Hilly	Sipman and Raus 2002
Hong Kong	Tropical forest	107,000	261	Hilly	Aptroot and Seaward 1999
Local geographic areas[d]					
Great Britain	Deciduous forest	211	323 (of 2500 fungi)	Rolling	D. L. Hawksworth, unpublished data[e]
French Guyana	Tropical rain forest	<100	209[f]	Flat	Montfoort and Ek 1990

[a] Based on comprehensive surveys of all possible substrata, except as noted.
[b] Each report represents the results of a single survey project with moderate survey effort.
[c] Total lichen mycobiota estimated to be twice as many species (H. J. M. Sipman, personal communication).
[d] Results of exceptionally intensive survey efforts.
[e] D. L. Hawksworth's Slapton NNR study is discussed elsewhere in this volume (see Chapter 11).
[f] Does not include leaf epiphyte lichens, of which >100 species may occur in the area.

some idea of the available species pool for large geographic areas. Species found per area for smaller areas (Table 9.3) in single surveys give rough estimates of the variation in species per area to be expected at smaller scales. From comparison of numbers of species in Tables 9.2 and 9.3, one could suggest that in a single biodiversity survey of a 50,000-hectare area, one might find between 5% and 20%, depending on survey intensity, of the species in the large regional species pool for a 5- to 10-million-hectare (or larger) area.

DESIGNING SAMPLING PROTOCOLS FOR BIODIVERSITY INVENTORY

In this section we address the design of biodiversity studies with at least minimal levels of quantification and repeatability. We do not address the design of strictly qualitative studies. For a broad review of monitoring lichens and using lichens as monitors see Nimis and colleagues (2003).

Resources Required

Personnel

It is important that trained lichenologists participate in the design of sampling protocols for lichen surveys, and it is essential that they identify or confirm identifications for all voucher specimens. It is desirable to have lichenologists conduct the field work; a team of specialists in different lichen groups will maximize species capture in high-intensity inventories. Nevertheless, the use of nonspecialist field personnel trained to distinguish between species and collect them without necessarily identifying them often allows much larger areas to be inventoried for the same costs. Species capture, however, especially for crustose lichens, will be much less complete in the latter case.

For low-intensity inventories, the ability of a properly trained observer to distinguish species in the field maximizes species capture with limited field time. Nonspecialist field personnel can be appropriate for moderate-intensity inventories of large areas, for example, regionwide status and trend inventories that form part of a national program (e.g., for the United States, see McCune 2000), or as team members in high-intensity inventories.

Training for nonspecialist field personnel should be designed and conducted by a lichenologist familiar with the region of the proposed survey. Both habitat selection criteria and morphological variation of lichens differ from the norms for vascular plants. Nonspecialist field technicians trained by an expert(s) can, with continued experience, become quite proficient, especially with macrolichens, and those having fruticose, foliose, and squamulose growth forms (McCune et al. 1997b).

Collecting Equipment

Field equipment needed to collect lichen voucher specimens is minimal, including the following items: hand lens; rock hammer and chisel for hard mineral substrata; stout knife or wood chisel for woody and soft substrata; hand pruning shears for twigs, paper packets, or bags; and small card boxes for fragile soil-surface specimens. Individuals surveying special habitats, such as vertical cliff faces or tree canopies, may require special equipment and training (e.g., Pike et al. 1975; ter Steege and Cornelissen 1988).

Field Procedures

Lichens should not be placed in watertight or water-resistant containers because molds can overgrow specimens confined under warm, humid conditions in as little as a few hours. Specimens should be stored in the field one to a paper bag or packet, with substratum, location, and date recorded on the bag in pencil or waterproof ink. Abundance data should be linked to individual field specimens when they are collected. Wet or damp specimens must be air-dried thoroughly, ideally within a few hours of collection, and should never be stored damp.

Both for conservation reasons and for efficient processing, the field-sampling protocol should require collection of the minimum numbers of specimens needed to ensure accurate identifications, while achieving an appropriate level of quantification. The number of specimens to be collected should be decided partly based on the expertise of the collector. A minimum of one specimen for each species encountered in the whole study area should be collected as a voucher. Nonspecialist technicians can be trained to distinguish some species accurately in the field; an expert familiar with the study region should decide which species can be treated this way. Other species may be identifiable, or at least unambiguously distinguishable, in the field by an expert. Presence and abundance of species distinguished in the field, along with pertinent information, can be recorded on data sheets or in an electronic data recorder. For species distinguished in the field, two to four specimens from sites widely separated within the study area and linked to specific field data sheets should be collected to provide multiple vouchers and to confirm correct field charac-

terization. At least one specimen per site should be collected of species for which field distinction is relatively difficult. Most crustose lichens fall into the latter category for most field personnel. We recommend that one specimen per species per site be collected in poorly surveyed regions.

Sampling protocols also must take into account conservation issues. Protocols requiring massive collection of specimens from small areas may result in overcollection of uncommon species and thus be inappropriate for many species in some areas. Field etiquette for lichenologists dictates that if only one specimen is seen during a search, only half of the specimen is collected. This practice should be adopted for all biodiversity sampling protocols. Overcollection is usually not a problem for most species in general surveys on large plots and when several years elapse between repeat surveys (McCune et al. 1997a). Collecting epiphytic lichens from recent litterfall has no impact on current or future abundance because these thalli will die even if not collected.

Field personnel should learn to identify any threatened or endangered species suspected to occur in an area by sight and should record but not collect them. When collecting is not allowed or is limited severely, inventory goals and sampling design must be planned carefully, and field surveys should be carried out only by lichen experts. For material on historical buildings, monuments, or similar structures, microscope preparations made in the field directly from specimens can facilitate identifications. Finally, it is important to recognize that many lichens simply cannot be identified to species with certainty unless they are collected and subsequently studied in the laboratory.

Information Processing and Archiving

Most lichen taxa must be identified to species for an inventory to be meaningful. A minimum of one voucher specimen per species reported should be housed in an accessible permanent collection. At minimum the scientific name, latitude and longitude of the collection locality, specimen substratum, date of collection, name(s) of the collector and identifier, and a unique collection number should accompany each voucher specimen. Voucher specimens are critical for assessing the validity and reliability of data. Species reports not supported by voucher specimens generally are considered unreliable. Arrangements with a lichenologist to identify voucher specimens, provision for time and expense to curate specimens, and agreement with a herbarium to house voucher specimens permanently should be completed as part of the planning for a biodiversity survey.

When field collecting and preliminary sorting are done by nonspecialist personnel, several specimens of each "species" (sorted group) should be identified by expert lichenologists. Resorting of "species" groups then also involves reassigning associated abundance values.

Voucher specimens are archived dry in acid-free paper packets. Most lichen field guides include instructions for proper curation of lichen specimens (e.g., Hale 1979; Purvis et al. 1992; Wirth 1995a; Brodo et al. 2001; Chapter 2 of this volume). To reduce fragmentation, the substratum of soil specimens (not the lichen specimen itself) can be dipped in a solution of water-soluble glue as soon as is practical, dried, and then glued to a stiff card (Rosentreter et al. 1988).

Reports of inventory and monitoring studies should include descriptions of sampling protocols that are described in sufficient detail to allow other professionals to duplicate the study methods. Lists of species should report the names of experts consulted for identifications, the taxonomic authorities followed (e.g., published checklists, keys), and the location(s) of voucher specimens. Summary statements and conclusions should be supported by data included in the report or cited from published sources.

Both hard copy and electronic versions of raw data and data summaries from biodiversity inventories should be archived in some standard format (ASCII is a commonly accepted minimal standard format for electronic data) with the institute or organization responsible for managing the area surveyed (or funding the survey) as well as at other appropriate locations. Comparison with future surveys is usually an important goal for biodiversity inventory and monitoring projects. Electronic technology can be expected to continue to change at a fast pace in the foreseeable future, so translatability should be a major criterion in choice of electronic formats for archiving data.

Estimated ratios of field time (including travel) to laboratory and office time for lichen biodiversity studies range from approximately 1:4 to 1:10. Some time estimates for biodiversity studies are given in Table 9.4.

Criteria for Design of Sampling Protocols

Three aspects of designing sampling protocols are critical: (1) matching the sampling protocol to the goals of the survey, (2) selecting sites for sampling, and (3) selecting a within-site sampling protocol. As we use the terms, a sample site is an independently chosen geographic location. The number of these independently chosen sites is the sample size for the inventory as a whole and the basis for all between-site comparisons. The within-site sampling protocol designates the size of the area to be searched at a site and specifies how within-site subsampling will be carried out. It has no effect on the sample

TABLE 9.4
Characteristics of Biodiversity Survey Protocols with Different Intensities of Effort

Size of sample site (ha)	No. of sites sampled	Area covered (ha)	Site placement	Subplot placement	Lichen groups	Abundance measure
Species presence inventory						
~0.8, varied	27 (21)*	2,560 (31,000)*	Stratified by habitat, vegetation	None (permanent on rocks)*	All lichens	None
Rapid, semiquantitative inventory						
0.4, fixed	79	9 million (estimated)	Regular grid, forest only	None	Macrolichens, standing woody substrata	4 abundance classes
Semiquantitative inventory						
0.05, fixed	188 (+77 partial)*	6.8 million	Stratified by habitat, vegetation	None	All macrolichen, some crustose	5 density classes
Intensive site inventory, proposed†						
0.1, fixed	At least 2/ site-class	unknown	Stratified by habitat, vegetation	Stratified by tree species, forest layer, microhabitat	All lichens	Frequency from presence in samples

Field personnel	Time field	Time lab, reports	Data Products: Within-site	Data Products: Between-site	References
[Species presence inventory]					
Specialist	14 person-days	6 person-months	Species richness, notes on abundance, substrata	190 species (137 spp.); species frequency by site class	Wetmore 1993, Wisconsin USA (Hale 1982, Colorado, USA)
[Semi-quantitative inventory, rapid]					
Nonspecialist	40 person-days	7 person-months	Abundance by species; diversity indices	155 species; species response to climate/air-quality gradients; correlations with vegetation variables; critique of methods	McCune et al. 1997a, 1997b, SE United States
[Semi-quantitative inventory]					
Nonspecialist	20 person-months (much travel time)	35 person-months	Abundance by species; diversity indices	381 species; species abundance by site class; multivariate community analyses; correlations with habitat/ vegetation variables; critique of methods	Geiser et al. 1994a, 1994b, SE Alaska, USA
[Intensive site inventory, proposed]					
Teams of specialists and nonspecialists	4–10 person-days/site	3–5 person-months/site	Species frequency and diversity indices by subplot class	Proposed, no species numbers available; species abundance by site and subplot class; correlations with vegetation/ habitat variables	Rossman et al. 1998, Costa Rica; Wolseley et al. 1995, Thailand; for methodology, Lücking and Lücking 1996, Sipman 1996b

* Refer to study by Hale (1982).
† Several reports pertain to this proposed inventory design.

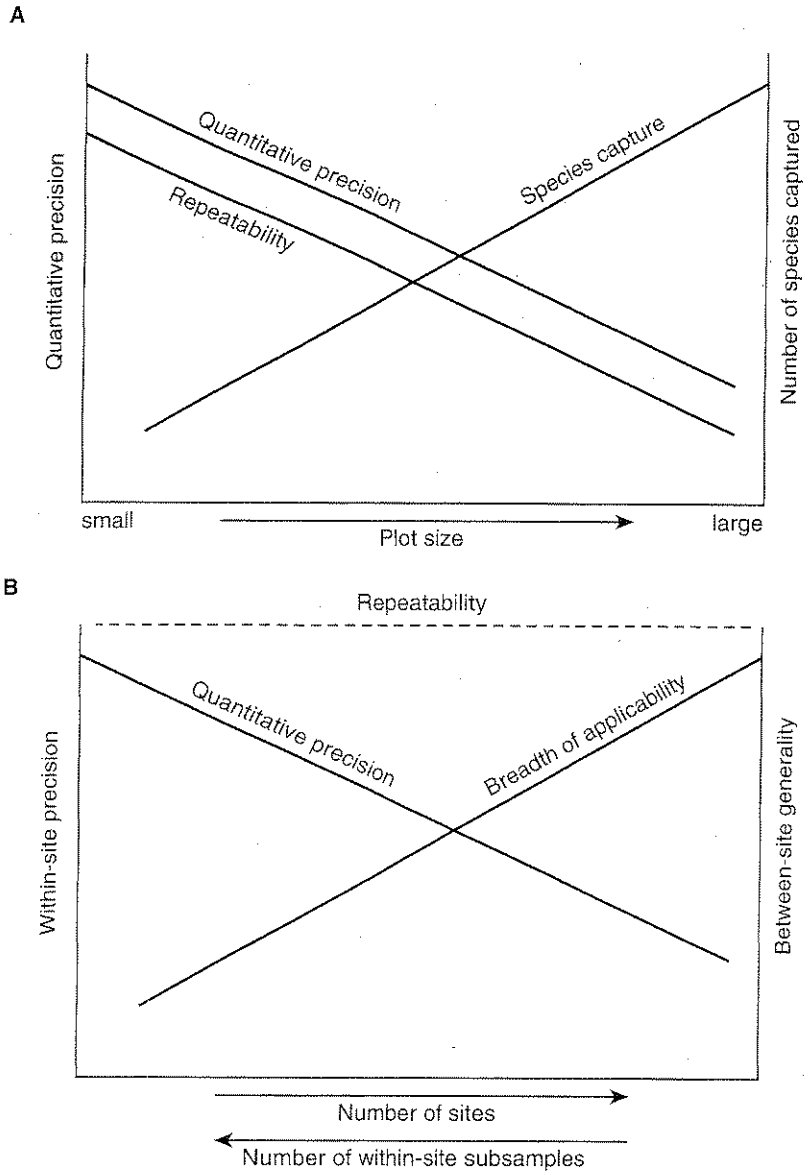

FIGURE 9.5 Trade-offs involved in allocation of effort for different biodiversity sampling designs. **A.** Allocation of a fixed level of survey resources per plot for a range of sample plot sizes involves a trade-off between species capture and quantitative accuracy. **B.** Allocation of a fixed level of resources for an entire survey involves trade-offs between inclusion of many independent sites for generality and elaboration of within-site sample protocol.

size for the inventory as a whole (Hurlbert 1984; Will-Wolf 1988; Will-Wolf et al. 2002). Variations in within-site sampling protocols affect the precision (closeness of repeated measurements of the same entity) of descriptions of the lichen community at that site. When the available resources for a project are fixed, design of the protocol involves trade-offs such as (1) sampling many sites with low within-site quantitative precision for assessment at broad geographic scales versus fewer sites but with greater within-site quantitative precision for assessment of smaller, less diverse areas or (2) using many small sample subplots for quantitative precision versus fewer large subplots for greater species capture at a site (Fig. 9.5).

Matching Survey Goals with Sampling Protocols

The first step in designing a biodiversity survey is to state clearly the overall purpose and specific objectives of the survey. If maximizing species capture for small areas is

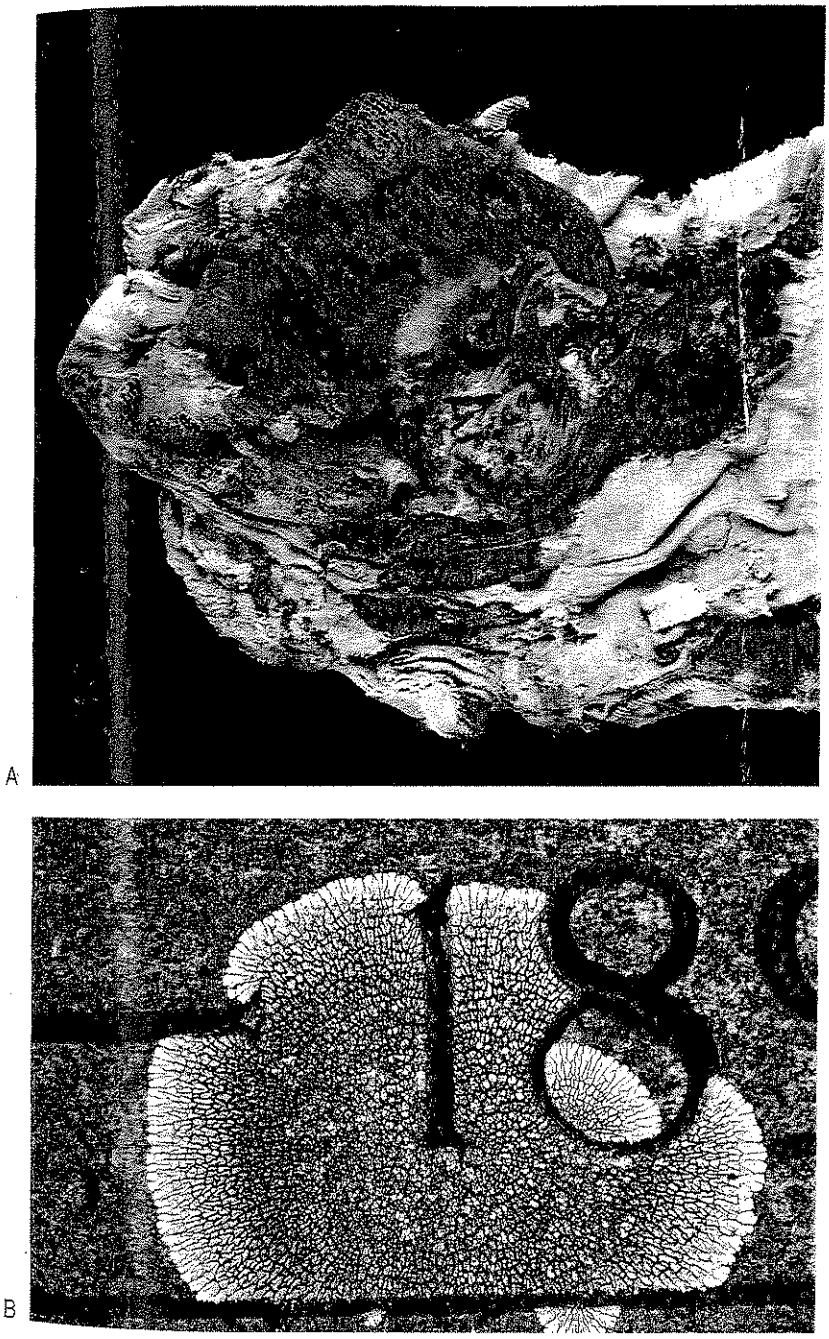

FIGURE 9.6 Human activities can introduce unusual substrata into regions. **A.** Five species of lichens and two species of nonlichenized fungi colonized these discarded cotton briefs, which were exposed for perhaps 15 to 20 years in the arid environment of Badlands National Park, South Dakota, United States. Such small patches of unusual substratum have little impact on regional lichen biodiversity. (Photo by Claudia Lipke) **B.** In other situation, such as buildings and grave markers erected in regions otherwise poor in rocklike substrata, the impact on lichen diversity could be very large. *Dimelaena oreina* has colonized this granite tombstone in a rural cemetery in central Wisconsin, United States. (Photo by John Wolf)

the main goal of the survey, one can focus on completeness of coverage within sites with a minimum of replicate sample sites to optimize use of time and resources. If monitoring for trends in change across time or space is a major goal, adequate replication of appropriate sample sites at the expense of some completeness of coverage within sites is preferred if time and resources are limiting.

Next, to focus the design of a sampling protocol, three factors should be considered: (1) size and overall diversity (topographic, climatic, vegetation, habitat, degree and diversity of human influence (Fig. 9.6) or other disturbance) of the area to be characterized; (2) time and resources available for the project; and (3) the need to select multiple independent sample sites per class so that results can be generalized across that class of sites (e.g., a type of vegetation or habitat).

One further practical constraint on the design of sampling protocols is the need to search slowly, intensively, and at very close range (often with a hand lens) in all potential microhabitats of an area to maximize the number of lichenized fungi found. This means that a

careful survey of a discrete site of fixed size will give more repeatable results than a superficial inventory of a large, diffuse area. Many species are likely to be overlooked unless each microhabitat is searched carefully.

The two major attributes of sampling protocols that can vary with resource availability and required sampling intensity are (1) the number of independent sites to be sampled in the area being inventoried and (2) the degree to which within-site search strategy is quantified and subdivided. To assess variation between site classes, or changes in a site class over time, one must have a valid estimate of variation between sites within each class (at each time), which requires replicate sites within each class (at each time). The number of sites needed to achieve the desired precision in estimating differences or changes for the survey as a whole should be decided first, followed by a decision on how detailed the within-site sample protocol can be and still allow completion of the whole survey within the allotted time. Increased precision (reduction in variance of measurements) at the scale of within-site sample protocol does not automatically improve the accuracy (closeness of an estimate to the "true" value) of comparisons at the scale of between-site comparisons (Sokal and Rohlf 1995).

An extensive literature, including hundreds of citations going back more than 100 years, exists on monitoring air quality by surveying lichen communities (see Ferry et al. 1973; Nash and Wirth 1988; Stolte et al. 1993; Nimis et al. 2002; and the recurrent feature "Literature on Air Pollution and Lichens" in the journal *The Lichenologist*). Much of the discussion of sample design in that literature is pertinent to biodiversity inventory. However, one goal of biodiversity inventory is as complete a representation of the lichenized fungi of an area as possible at the chosen degree of effort, so all major substrata and habitats should be sampled. In contrast, air-quality monitoring (as well as other studies of lichen response to specific factors) often targets a relatively homogeneous subset of lichen habitats for convenience and comparability. The goal of choosing the subset is to reduce or constrain variation over time and space as a result of all factors other than the air-quality gradient or other target factor. Literature on air-quality monitoring should be consulted with this difference in mind. Lichen-community composition can be thought of as a composite response to constraints operating at many different spatial and temporal scales (Allen and Hoekstra 1990). Seasonal variation in community composition usually is negligible. Variation in species response and community composition along successional and disturbance gradients, in contrast, is important. The greatest range of variation in species composition among lichenized fungi can be related to microhabitat and habitat differences, which vary at spatial scales from centimeters to hundreds of kilometers. Variation as a result of habitat differences and macrovegetation successional and disturbance gradients is addressed with location of sample sites. Variation as a result of microhabitat differences is addressed through within-site sampling protocol.

Many topics pertaining to the design of biodiversity studies in general are discussed in greater detail in Chapter 5. Chapters in Stolte and colleagues (1993) provide extensive reviews of lichen survey methods, including all aspects of planning and implementing the sampling protocol. Although the authors focus on use of lichens for air-quality monitoring, their coverage is comprehensive and pertinent to biodiversity studies.

Sample Site Location

In many cases in which an organized biodiversity survey of lichenized fungi is planned, classifications of the landscape, habitats, and/or vegetation of the area either already exist or are developed concurrently. Stratification of samples by classification units (selecting sample sites to represent classification units, rather than locating them at random within a survey area; Grieg-Smith 1983) is useful for relating lichenized fungi to other organisms and to the environment (Fig. 9.7) and will encourage the use of the information for management of the area (McCune and Antos 1981a, b; Wolseley and Aguirre-Hudson 1991; Wolseley et al. 1995). Another advantage to stratifying by classification units is that one sometimes can choose readily accessible sites that adequately represent more remote areas.

Spatial autocorrelation (the tendency for geographically closer samples from a single habitat to be more similar to one another than to more distant samples from the same habitat) has been shown to be an important aspect of ecological pattern in nature at many scales (Legendre and Fortin 1989). To represent the biodiversity of an area fairly, sample units should be dispersed over the area they are to represent, whether it be sample sites within the whole survey area or subsample plots within a site (Hurlbert 1984). Whatever spatial autocorrelation exists then is represented in the samples, allowing characteristics of the samples to be extrapolated to the whole study area from which they are drawn.

Investigators should not assume that variation among lichen communities will be represented adequately by classification units of an area when the latter are based on macrovegetation composition or on environmental and habitat attributes important to macrovegetation. Just as worldwide distribution and diversity patterns of lichenized fungi do not match those of vascular plants (see "Diversity and Distribution," earlier), local diversity patterns (scale of 0.1–1.0 ha) of lichenized fungi also often diverge from those of vascular plants (McCune and

FIGURE 9.7 The 6 to 8 species of lichens on this section of an *Acer saccharum* trunk grew in the well-illuminated mid-canopy of a large tree in an old-growth forest (Sylvania Wilderness Area, Michigan, United States). Biodiversity of epiphytic lichens is strongly affected by forest management and other human land-use practices. (Photo by Susan Will-Wolf)

Antos 1981a, 1981b). There is no particular reason to expect that diversity patterns of lichenized fungi always should match those of vascular plants at spatial scales between those extremes. For that reason, it is likely that some macrovegetation class distinctions are not important for lichens and vice versa. Macrovegetation classes with similar species compositions but different ages (e.g., old-growth versus young forest) may or may not be classified separately, but the communities of lichenized fungi differ (Lesica et al. 1991; Wolseley and Aguirre-Hudson 1991; Tibell 1992; Selva 1994; Wolseley et al. 1995). The well-known sensitivity of lichens to air pollution also means that diversity patterns of lichenized fungi may differ markedly from those of less pollution-sensitive organisms in an area affected by local air pollution.

Many survey designs call for the sampling of lichens and macrovegetation at the same sites to provide a strong basis for relating lichen and macrovegetation variables. Surveying for lichenized fungi in preexisting, permanently marked, macrovegetation sample sites offers many of the same advantages. If placement of individual sample sites and subplots for macrovegetation does not match needs for lichen surveys, then additional sites for sampling lichens alone can be selected to supplement the joint survey. Habitats that contribute much to the diversity of lichenized fungi, such as rock outcrops, talus piles, coarse woody debris, and desert pavements, are often less important for vascular plant communities. The advantages of being able to relate patterns of lichens to data from a large sample of other organisms outweigh the problems presented by data analysis for such a mixed-strategy survey.

In practice, statistical aspects of sample site selection often are subordinated to the particular conservation goals of an inventory. For example, an agency may want to monitor an area that is being considered for development (e.g., logging, urban, mining), so sample sites are not selected randomly. Inventories and monitoring programs also often are requested for protected areas that are expected to remain intact for long periods and can serve as benchmarks for comparison with human-dominated areas.

Within-Site Sampling Protocols

A within-site sampling protocol designates the size of a site, whether and how to subsample that site, and what information to record. Within-site sampling protocols can vary from time-constrained qualitative surveys of the whole site to intensive, quantitative surveys of subsamples within the site. Field time can range from 1 to 2 hours to several days. Choices within this range should be firmly constrained to meet goals of the whole project with available resources. The kinds of information obtained with different sampling protocols differ significantly (Fig. 9.5b; Smith et al. 1993). Contrast, for example, the results of a rapid survey of many sites representing widely differing habitats with the results of intensive surveys of a few sites that replicate a narrow range of habitats. There is no one best way to inventory lichens, and even with the most intensive survey protocol discussed in this chapter, it is unlikely that all species in an entire site will be found in a single survey. Repeated sampling by different observers and with different methods can be used as a basis for estimating "species capture" rates of different sampling designs (McCune and Lesica 1992). Smith et al. (1993) discussed in detail many aspects of within-site sampling design, including few-and-large versus many-and-small subsample units;

they also compared various abundance estimates and types of data analysis.

Selecting the Size of the Sample Plot. Lichens occupy surfaces at scales corresponding to microhabitat variation. Microhabitats are distributed discontinuously so that in most cases at spatial scales larger than the 0.01–0.25 m^2 range, the distribution of lichenized fungi will be heterogeneous. Above this very small scale, heterogeneity of lichen habitats at many different scales can contribute to the lichen diversity and community composition of an area; there may be no "best" scale (plot or microplot size) for measuring lichen diversity. Microhabitat variables known to be important to lichens include light regime; moisture status; and type, hardness, chemistry (especially pH and calcium content), and age of substratum.

We strongly recommend surveying a fixed-area plot at each site to facilitate comparability of site data, especially when diversity indices or species frequency are used. Sites corresponding to habitat or macrovegetation classes typically occur in the 0.1–5 hectare (0.25–12 acres) range, with sites larger than 0.5 hectares usually subsampled in some way. Sizes of plots for surveys of lichenized fungi at independently chosen sites have been at the lower end of that range (0.05–1 ha). One hectare probably represents a practical upper limit for a circumscribed search plot for lichenized fungi; the largest plot used in a study we cite was about 0.8 hectares (Wetmore 1993, 1995). Larger sample sites (>1 ha) can be inventoried by locating more than one plot at a site, but these plots are not independent of one another and do not increase the sample size for between-site comparisons. Most published lists of lichen species have been generated from strictly qualitative surveys of areas of no fixed size.

The relative advantages and disadvantages of sample plots of different shape, and the effects of shape on species capture, also should be considered. Compact shapes, such as squares or circles, are more likely to be homogeneous within, whereas extended shapes like rectangles are more likely to capture heterogeneity within (Grieg-Smith 1983). Compact shapes often are faster to lay out accurately in field conditions, and circular quadrants can be relocated precisely from a single permanent center marker, so they can be recommended for field efficiency. Also, because compact shapes are more likely to be homogeneous within, correlation between lichen community composition and other plot-level ecological factors can be estimated more efficiently for them. However, because capturing and representing heterogeneity is a goal for a biodiversity study, extended shapes may be preferred.

Within-Site (Within-Plot) Sampling Strategies. An investigator can search an entire plot to determine an average microhabitat heterogeneity or can search subsamples of the plot to represent this heterogeneity (stratified sample design). McCune and Lesica (1992) explicitly considered the trade-offs involved in these alternative strategies for estimating lichen diversity in three forest layers (ground, tree trunks, and branches), as well as the more general problem of subsample size, for 0.08-hectare plots in a conifer forest. They found that whole-plot sampling was more accurate at measuring species richness and less accurate at estimating cover (see "Estimating Abundance," in the next section) than quantitative subsampling strategies. Whole-plot sampling was much quicker for branches but took the same time or longer than quantitative subsampling of tree trunks and the ground. Because repeatable estimates of species richness for whole site samples are desirable for comparing sites with one another and for comparing diversity of lichenized fungi with other site variables, a whole-plot qualitative search at each site visited should be a part of any sampling protocol. More quantitative subsampling strategies, if included, will provide improved assessment of abundance for the more common species (Fig. 9.5; Will-Wolf 1988).

The degree to which lichen sampling is stratified by microhabitat again will depend on the level of intensity of the survey. Lichen species often have strong substratum/habitat preferences, but relatively few species are completely substratum/habitat specific. Major differences in species composition of lichens on rocks or soil are related to differences in the calcareous composition of the substratum (Hale 1982). Occurrences of lichen species on trees vary with bark pH and surface texture and less often with tree species (Oksanen 1988; Schmitt and Slack 1990). Community composition, which reflects abundance as well as presence of lichen species, may differ strongly among tree species (Schmitt and Slack 1990). The number of subplots, stratified by microhabitat within site, that are needed to represent differences between microhabitats adequately depends on within-microhabitat variability and the repeatability desired (Mueller-Dombois and Ellenberg 1974). Some estimates from studies of temperate-zone lichens are as follows: for tree trunks, 10–25 trees per tree species (e.g., Schmitt and Slack 1990; McCune and Lesica 1992); for tree branches, 25–60 branches for abundance and 100 or more branches for species capture (e.g., McCune and Lesica 1992; Geiser et al. 1994a; both constructed species-area curves to estimate number of sample units needed); and for ground and rock surfaces, 40–60 subplots (Lawrey 1991; McCune and Lesica 1992). Sipman (1997) observed in Guyana that two adjacent *Licania densiflora* trees shared only about 50% of their foliicolous lichen species, suggesting that several trees per species should be sampled in tropical forests also.

Shape and size of the subsample unit should be tailored to the specific habitat or microhabitat being sampled (Goldsmith et al. 1986; Longman and Jenik 1987; Wolf 1993; Lücking and Lücking 1996; Sipman 1996b; Nimis et al. 2002). Rock or ground surfaces, shrub branches, tree trunks, and tree canopies all have particular characteristics that influence the choice of an appropriate sampling unit. For trees, trunk plots from 0.1 to 0.5 m^2 in area and branch lengths of 0.25 to 1 m are recommended (Johansson 1974; Will-Wolf 1980, 1988; Cornelissen and ter Steege 1989; McCune 1990; McCune and Lesica 1992). For rock and ground surfaces, plots from 0.2 to 1 m^2 in area are recommended (Anderson et al. 1982; Rosentreter 1986; James and Wolseley 1992; McCune and Lesica 1992); ground plots often are located along transects.

Much field time and effort can be spent sampling microhabitats that are difficult to reach (e.g., upper tree trunks, tree canopy branches, cliff faces), so they should be included only in very intensive surveys. Investigators should take advantage of low-effort opportunities to collect data on such microhabitats. For example, samples from recently fallen ("recent" means lichens still look healthy) or felled trees and branches can represent the compositions of tree-canopy lichen communities, yielding important information even if the data acquired must be analyzed separately from the rest of the inventory data. Tree canopies are known to harbor lichen species different from (and with abundances different from) those of the more accessible lower trunks (Yarranton 1972; Pike et al. 1975; Lang et al. 1980; Oksanen 1988; McCune and Lesica 1992; McCune et al. 2000). This technique may be less successful in moist tropical forests, where thalli decay rapidly when they die.

Estimating Abundance. Abundance of lichens can be estimated or measured in a variety of ways to ascertain "how much" or "how many." Field assessment of abundance of lichens is constrained in practice by limits on the ability of even the best lichenologists to identify and distinguish species of some lichenized fungi in the field. If one cannot distinguish a species group in the field, one must make complete collections and estimate abundance in the laboratory from proportions of specimens of different species in the collections. The latter practice quickly generates massive numbers of specimens when subplots within sites are grouped by microhabitat. A much higher proportion of macrolichens (squamulose, foliose, and fruticose growth forms) than microlichens can be readily distinguished in the field. Usually, lichenologists only attempt to assess abundance of macrolichen species in the field.

Another factor affecting abundance estimates is the surveyor's ability to delineate individuals, which is sometimes easy and sometimes impossible. For general community inventory purposes, the investigator usually records abundance in the field based on easily countable units, whether they be individuals, clones, or multi-individual complexes. At a minimum, a surveyor can record qualitative notes about the apparent abundance of species or species complexes distinguishable in the field. A further step in increased effort would be to assign species to abundance classes. For example, McCune and colleagues (1997b) used two density classes combined with two abundance classes, whereas Geiser and colleagues (1994a) used five density classes (Scenarios 2 and 3 in Table 9.4). Mueller-Dombois and Ellenberg (1974) compared several abundance class schemes for ecological sampling. Alternatively, an investigator can record species presence in subplots, then use frequency across subplots as the estimate of abundance. The known problems of using frequency as an estimator of abundance (see "Data Analysis," later) must be weighed against the ease and efficiency of collecting presence data in the field.

Yet another approach to assessing abundance is to assign abundance classes to morphological groups of lichens (e.g., all small foliose rock lichens, or all long, hanging fruticose bark lichens; McCune 1993); the usefulness of data for particular groups is likely to vary by habitat (Rosentreter 1986, 1995; Eldridge and Rosentreter 1999). For example, vagrant (unattached) macrolichens are an important group in grasslands and steppes (Rosentreter 1993), whereas they are unimportant in forests. Cover classes or percent cover of morphological groups often are recorded in subplots for studies of terricolous lichens in grassland and steppe habitats, where even macrolichens are difficult to distinguish in the field (Kaltenecker et al. 1999). In some cases, those morphological groups may be surrogates for functional groups (Pike 1978; Rosentreter 1995). Gelatinous ground lichens in steppe communities, for example, include *Collema* species, *Leptogium* species, and *Polychidium* species, all of which fix nitrogen and protect the soil surface (Anderson et al. 1982; Brotherson et al. 1983; Nash 1996).

For completely quantitative estimates of abundance of species in replicated fixed-area subplots stratified by microhabitats within a site, cover classes or percent cover of lichen species provide simpler and more accurate measures of abundance than counts of individuals (Will-Wolf 1988; James and Wolseley 1992; Marcelli 1992). Cover estimation protocols are probably too labor-intensive both in the field and for data analysis to be recommended for general biodiversity inventories and monitoring programs (McCune and Lesica 1992). Measurements of lichen biomass require similarly labor-intensive, rigorous subsampling and weighing of lichens

(Lang et al. 1980; McCune 1993; Hayward and Rosentreter 1994; Rosso and Rosentreter 1999). Biomass of canopy macrolichens has been estimated efficiently by analysis of litterfall (McCune 1994) and lichens on lower canopy branches (Esseen et al. 1996) in conifer forests.

Because many lichen thalli, unlike most other fungi, are reliably visible, an alternative to assessing abundance in the field is to establish permanent photoplots (sizes as for small subplots) to monitor trends in abundance over time (e.g., Will-Wolf 1980; Hale 1982; Bureau of Land Management 1996). Species may or may not be recognizable in photographs, but morphological types are, and identifications of voucher specimens as well as cover estimates for recognizable lichen groups can be made in the laboratory. Use of photoplots for monitoring lichen abundance has been simplified by recent advances in digital camera technology and development of more sophisticated computer programs for spatial analysis.

Biodiversity Indicators. The use of individual species as indicators for the condition of a whole guild or community of species presupposes detailed knowledge of what the species indicate and how reliably they do it. Such detailed knowledge of ecological and physiological relationships for many lichenized fungi in an area currently exists only in Europe (Seaward 1988), and interpretation of relationships there is still subject to discussion. Also, even common lichen species seldom are present at all sites one might wish to monitor. We, therefore, do not recommend the indicator-species approach for baseline biodiversity studies. We also do not recommend exclusive use of single species as indicators of air pollution (or other specific factors affecting lichen biodiversity), based on the extensive literature review of Smith and colleagues (1993).

Use of groups of species to indicate the condition of whole communities has much more promise, although again, some knowledge of relationships among species must precede acceptance of groups as indicators. The identification of an easily quantifiable subset of lichen species (including both rare and common species) to serve as monitors of biodiversity for repeat sampling is an appropriate goal for a baseline biodiversity survey. Communities of macrolichens on tree bark, for example, are now widely accepted as good indicators of the response of all lichens to sulfurous air pollutants (Ferry et al. 1973; Nash and Wirth 1988; Bates and Farmer 1992; Smith et al. 1993) and as indicators of forest ecosystem biodiversity and function (McCune et al. 1997b; Will-Wolf et al. 2002). Nitrogen-fixing lichens (lichenized fungi associated with cyanobacteria photobionts) have been shown in several regions to be more common and more diverse in older, less disturbed forests (Fig. 9.8) and grasslands than in more disturbed areas and also in areas with cleaner air (Eldridge and Rosentreter 1999; Gauslaa 1995; Kondratyuk and Coppins 1998; Sillett et al. 2000). "Pin-lichens," species in the former order Caliciales, have been recommended for use as indicators of long, continuous occupation of a site by mature ("old-growth") forest in northern temperate and boreal regions (Tibell 1992; Selva 1994). Selva (1994) developed a group of forest continuity (old-growth) indicator species, including pin-lichens, for northeastern North America. Ground lichens have been used as indicators of ecosystem function for grassland and steppe habitats (Anderson et al. 1982; Brotherson et al. 1983; Rosentreter 1986; Eldridge and Tozer 1996).

Habitat Information

Minimum habitat information required for each sample site includes location; general vegetation description/classification, including life forms and structure; brief vegetation history (e.g., old, young, disturbed); landform; topography; and rock or soil type(s). As much as possible, this information should be acquired from the literature and previous studies rather than as part of the sampling protocol. If no background information about a specific site is available, then investigators need to collect information in each of the previously mentioned categories as part of the sampling protocol (Stolte et al. 1993; Nimis et al. 2002) rather than relying solely on generalized map information or general landscape unit, habitat type, or vegetation class descriptions.

Detailed habitat information should be recorded at a site as needed to characterize subplots at the spatial scale used for collecting lichen data. For instance, instead of compiling a list of common or dominant plant species, an investigator might estimate the relative importance of those species. Likewise, quantitative estimates of structure, such as shrub or ground cover, percent standing dead stems, cover of dead wood on the ground, and canopy cover in several forest layers, may provide more useful data than just a brief characterization of vegetation structure (shrubland, old forest). Notes on rock type and ground habitats can be augmented with more detailed information such as estimates of cover of different types of rock and ground surface, patch sizes, percent of aspect exposures available, and percent in sun or shade. Information should be collected by categories that match lichen microhabitat subdivisions. Lichenologists with little ecological training should consult an ecologist familiar with the region for help in designing appropriate sampling protocols.

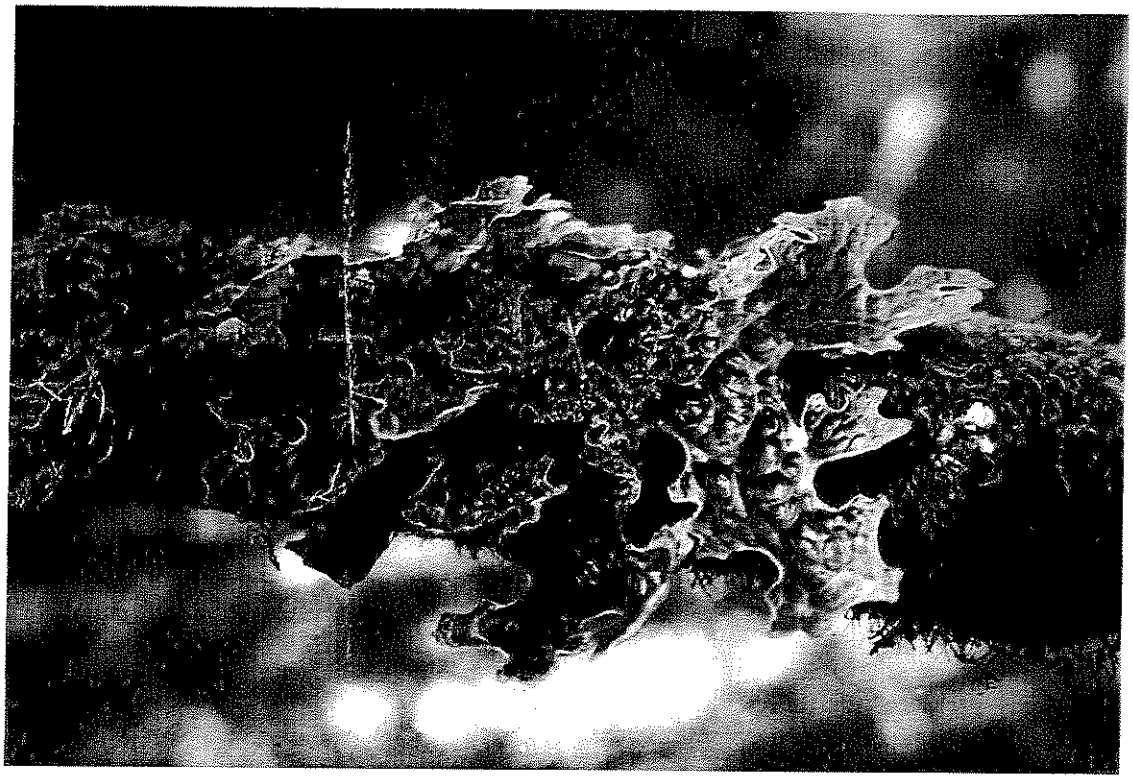

FIGURE 9.8 *Lobaria pulmonaria*, the lung lichen, has both green alga and cyanobacteria photobionts and thus fixes atmospheric nitrogen. It is found in northern hemisphere temperate zone moist forests, where it is considered to be a bioindicator of old-growth forests. It also has been shown in several regional studies to be sensitive to air pollution. (Photo by Bruce McCune)

DATA ANALYSIS

An investigator must analyze his or her data in a way appropriate to the survey design protocol. Species richness will be available for each site from all survey protocols we discussed, as will at least qualitative estimates of commonness or rarity and habitat and microhabitat affinity. More complex within-site sampling protocols will support more complex data analyses. Relative abundance for species or groups can be estimated with varying degrees of precision from cover-class or abundance-class data. Relative abundance estimates, although often only semiquantitative, can provide repeatable measures of community composition useful for monitoring change over time in one area and for comparing areas, if methods of data collection are the same.

Basic Summary Statistics

Species frequency, the proportion of fixed-area sample units in which a species is found, is probably the most commonly used estimator of species abundance because the data are so easy to collect, but it is somewhat difficult to interpret and compare among areas. Frequency values for the same site vary with the size of the sample unit (Mueller-Dombois and Ellenberg 1974; Grieg-Smith 1983; see "Collection Effort Curves," Chapter 5), and frequency estimates have nonlinear relationships with quantitative abundance estimates such as density or cover.

Absolute measures of density (counts of individuals per unit area) and dominance (e.g., cover or biomass) for whole sites are difficult to calculate for lichens. They are not readily attainable using the sampling designs recommended here.

For any single within-site sampling protocol, data aggregated from single sites into site classes and from site classes into all sites can be subjected to increasingly detailed data analyses. For example, if the chosen within-site protocol is species presence in fixed-area sites with abundance classes for macrolichens, one could summarize the data at different spatial scales as follows:

1. Within-site: presence list for all species; abundance and relative abundance for macrolichens
2. Within-site class (e.g., habitat or vegetation class, climate zone): frequency and relative frequency for all species; average abundance and variation in abundance for macrolichens
3. All sites combined: average frequency and variation in frequency among site classes for all species; comparisons of average abundances and variation among site classes for macrolichens; frequency estimates for all species and abundance comparisons for macrolichens for the whole survey area, including any nonreplicated sites.

Diversity Indices

Common diversity indices can be calculated from any semiquantitative data, but such numbers are strongly scale-dependent (see "Spatial Scale of Biodiversity," in Chapter 5) and therefore difficult to compare using numbers from studies with different sample designs. Species richness (the total number of species in a sample unit) is a simple, effective, and easily communicated measure of alpha diversity (Whittaker 1972). Diversity indices for equal-area sample sites within the survey can readily be compared with one another and can be compared over time. McCune and associates (1997b) used the total species pool across sites in a region or subregion as an estimator of gamma, or landscape diversity, and average alpha diversity (number of species/site) divided by gamma diversity as an estimator of beta diversity, or turnover rate across environmental gradients or between habitats (Whittaker 1972). Measures of beta and gamma diversity are not as standardized as are the familiar indices for alpha diversity (e.g., Stoms and Estes 1993).

Species-Area Curves

Species-area curves can be constructed, but they have complex interpretations and can differ notably for the same area depending on the method of data aggregation used to produce the curves (Palmer and White 1994) and the size of the smallest sample unit used. They should be extrapolated only cautiously to predict diversity for areas larger than, or other than, the area represented in the survey. Differences between species-area curves constructed with data on lichenized fungi obtained using the same methods in similar habitats that differ in air quality have been interpreted to indicate alteration of community function (Lawrey 1991, 1992). Comparisons of such curves for lichenized fungi of similar microhabitats in different parts of a survey area or in the same areas over time deserve investigation as a potential tool for monitoring biodiversity changes.

Partitioning Data by Morphological or Functional Groups

During analysis, partitioning data *a posteriori* by important groups of lichens that differ in function or morphology may help to clarify the nature of lichen community response to habitat and other environmental variables. Correlations of such groups of similar lichen species with explanatory variables often differ. For example, cyanolichens, alectorioid lichens, and green-alga foliose lichens in Pacific Northwest forests of the United States respond differently to such variables as position in canopy, forest age, tree density, and habitat heterogeneity (McCune 1993; Sillett and Neitlich 1996; Peck and McCune 1997; McCune et al. 2000).

Correlation with Environmental Variables

All recommended sampling protocols support investigation of relationships between patterns of lichen data and habitat and macrovegetation variables. Nonstatistical, but quantitative, presentations of patterns of species diversity often are used to interpret survey results (Geiser et al. 1994a, 1994b; Vitt and Belland 1997). Single-factor statistical analysis can include correlation, regression, and contingency table analyses. Multivariate quantitative analyses of species data, including ordination (Will-Wolf 1980; Rosentreter 1986; Oksanen 1988; Marcelli 1992; Geiser et al. 1994a; Wolseley et al. 1995; McCune et al. 1997a, 1997b), classification (Tibell 1992; Geiser et al. 1994a), and gradient analysis (Oksanen 1988; McCune et al. 1997b), can facilitate description of differences between communities, highlight habitats and microhabitats with high diversity, and help define relationships between communities and habitats.

Implementation of Biodiversity Inventories

In this chapter we have summarized the current status of classification of lichenized fungi and have discussed in detail the important design elements for surveys of their biodiversity with examples (Table 9.4) to show how these elements are combined in practical situations.

Within-site survey protocols can be grouped into four categories: (1) Species presence at sites; all species, sites grouped into classes (Table 9.4: example 1). (2) Abundance at sites, for an easily surveyed subset of species (indicator species); sites grouped into classes or placed along gradients (Table 9.4: example 2). (3) Species presence at sites, all species; plus abundance at sites for a subset of species; sites grouped into classes (Table 9.4: example 3). (4) Species presence or abundance in subplots within site, all species; sites grouped into classes (Table 9.4, example 4).

Each category of within-site protocol is useful for biodiversity surveys with particular sets of goals:

1. Rapid (1-year) inventory of large regions. Protocols 1 and 2 are appropriate; 1 results in greater species capture; 2 provides better quantification and repeatability for monitoring trends.
2. Medium-term (3–5-year) inventory of regions. Protocol 3 results in good species capture plus more precise-abundance estimates than protocol 2; variation in number of sites affects accuracy and time to completion.
3. Intensive inventory of selected sites. Variations on protocol 4 give very accurate single-site inventories but require considerable time. For a large region, an inventory may take many years to complete.

Each study used as an example in Table 9.4 relied primarily on one within-site sampling protocol. This need not be the case, however, for an effective biodiversity survey. For example, a two-phase inventory might first use a protocol with a low-intensity effort per site to obtain an areawide survey that can be completed within a year or two. That would be followed by high-intensity sampling of a selected subset of sites to be completed over a longer period. Results from the first-phase inventory would form the basis for selecting the subset of sites to be emphasized in the high-intensity inventory and at the same time would provide a completed inventory to be used for making management decisions before the more intensive surveys were finished. Another strategy to make information available for reference before the completion of a several-year survey is to distribute sites across a survey area or between site classes in an area for each field season. Then preliminary areawide summaries can be made available before the completion of the entire inventory.

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